UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Departamento de Genética, Ecologia e Evolução
Programa de Pós-graduação em Genética

Luís Cláudio Lima de Jesus

PROBIOGENÔMICA DE *Lactobacillus delbrueckii* CIDCA 133

Belo Horizonte
2022

Luís Cláudio Lima de Jesus

PROBIOGENÔMICA DE *Lactobacillus delbrueckii* CIDCA 133

Versão Final

Tese apresentada ao Programa de Pós-Graduação em Genética da Universidade Federal de Minas Gerais como requisito parcial para obtenção do título de Doutor em Genética.

Orientador: Prof. Dr. Vasco Ariston de Carvalho Azevedo

Coorientadora: Profª. Drª. Flávia Figueira Aburjaile

Belo Horizonte

2022

043 Jesus, Luís Cláudio Lima de.
Probiogenômica de Lactobacillus delbrueckii CIDCA 133 [manuscrito] / Luís Cláudio Lima de Jesus. – 2022.
176 f. : il. ; 29,5 cm.

Orientador: Prof. Dr. Vasco Ariston de Carvalho Azevedo. Coorientadora: Profª. Drª. Flávia Figueira Aburjaile.
Tese (doutorado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Genética.

1. Genômica. 2. Probióticos. 3. Imunomodulação. 4. Anti-Infecciosos. 5. Resistência Microbiana a Medicamentos. I. Azevedo, Vasco Ariston de Carvalho. II. Aburjaile, Flávia Figueira. III. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. IV. Título.

CDU: 575

Ficha catalográfica elaborada pela bibliotecária Fabiane C M Reis – CRB 6 – 2680

UNIVERSIDADE FEDERAL DE MINAS GERAIS
Programa de Pós-Graduação em Genética
Instituto de Ciências Biológicas

ATA DE DEFESA DE TESE

ATA DA DEFESA DE TESE	**159/2022** **Entrada 1º/2018**
LUÍS CLÁUDIO LIMA DE JESUS	**CPF: 603.145.253-78**

Às nove horas do dia **28 de julho de 2022**, reuniu-se, a Comissão Examinadora de Tese, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho intitulado: **"Probiogenômica de Lactobacillus delbrueckii CIDCA 133"**, requisito para obtenção do grau de Doutor em **Genética.** Abrindo a sessão, o Presidente da Comissão, **Vasco Ariston de Carvalho Azevedo**, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra ao candidato, para apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa do candidato. Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	**Instituição**	**CPF**	**Indicação**
Vasco Ariston de Carvalho Azevedo	UFMG	283.171.225-49	APROVADO
FLÁVIA FIGUEIRA ABURJAILE	UFMG/Escola de Veterinária	100.981.496-60	APROVADO
Hilário Mantovani	University of Wisconsin-Madison	773.767.496-91	APROVADO
Flaviano dos Santos Martins	UFMG	043.684.026-00	APROVADO

Anderson Miyoshi	UFMG	034.357.036-01	APROVADO
Ulisses de Pádua Pereira	UEL	066.898.566-66	APROVADO

Pelas indicações, o candidato foi considerado: APROVADO

O resultado final foi comunicado publicamente ao candidato pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 28 de julho de 2022.

Vasco Ariston de Carvalho Azevedo - Orientador

FLÁVIA FIGUEIRA ABURJAILE - Coorientadora

Hilário Mantovani

Flaviano dos Santos Martins

Anderson Miyoshi

Ulisses de Pádua Pereira

Documento assinado eletronicamente por **Anderson Miyoshi**, **Membro de comissão**, em 28/07/2022, às 12:35, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Ulisses de Padua Pereira**, **Usuário Externo**, em 28/07/2022, às 14:14, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Vasco Ariston de Carvalho Azevedo**, **Professor do Magistério Superior**, em 28/07/2022, às 14:24, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Flávia Figueira Aburjaile**, **Professora do Magistério Superior**, em 28/07/2022, às 14:57, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Flaviano dos Santos Martins**, **Professor do Magistério Superior**, em 28/07/2022, às 15:25, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Hilario Cuquetto Mantovani**, **Usuário Externo**, em 02/08/2022, às 10:16, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

A autenticidade deste documento pode ser conferida no site https://sei.ufmg.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **1622056** e o código CRC **BA76A02C**.

Referência: Processo nº 23072.243646/2022-55 SEI nº 1622056

UNIVERSIDADE FEDERAL DE MINAS GERAIS
Programa de Pós-Graduação em Genética
Instituto de Ciências Biológicas

FOLHA DE APROVAÇÃO

"Probiogenômica de Lactobacillus delbrueckii CIDCA 133"

Luís Cláudio Lima de Jesus

Tese aprovada pela banca examinadora constituída pelos Professores:

Vasco Ariston de Carvalho Azevedo - Orientador
UFMG

FLÁVIA FIGUEIRA ABURJAILE - Coorientadora
UFMG/Escola de Veterinária

Hilário Mantovani
University of Wisconsin-Madison

Flaviano dos Santos Martins
UFMG

Anderson Miyoshi
UFMG

Ulisses de Pádua Pereira
UEL

Belo Horizonte, 28 de julho de 2022.

Documento assinado eletronicamente por **Anderson Miyoshi**, **Membro de comissão**, em 28/07/2022, às 12:35, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Ulisses de Padua Pereira**, **Usuário Externo**, em 28/07/2022, às 14:14, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Vasco Ariston de Carvalho Azevedo**, **Professor do Magistério Superior**, em 28/07/2022, às 14:24, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Flaviano dos Santos Martins**, **Professor do Magistério Superior**, em 28/07/2022, às 15:24, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Hilario Cuquetto Mantovani**, **Usuário Externo**, em 02/08/2022, às 10:15, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

A autenticidade deste documento pode ser conferida no site https://sei.ufmg.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **1622175** e o código CRC **34111186**.

Referência: Processo nº 23072.243646/2022-55 SEI nº 1622175

Com AMOR, aos meus pais Maria da Paz
e Elisvaldo de Jesus.

AGRADECIMENTOS

Agradeço a DEUS, por ter me concedido o dom da vida e da sabedoria, me guiando, me levantando e dando forças em mais esta etapa da minha vida. O caminho é árduo, mas a chegada será grandiosa!

A toda minha família, em especial aos meus pais Maria da Paz e Elisvaldo, por serem meu porto seguro e meu maior exemplo de superação de vida.

Aos meus irmãos Angélica e Edson, que aos seus modos contribuíram para que eu alcançasse este objetivo.

Ao meu orientador, Prof. Vasco Azevedo, pela credibilidade, incentivo, direcionamento, pela liberdade concedida, pela confiança e pelo meu crescimento científico.

À coorientadora, Prof. Flávia Aburjaile, pela supervisão do trabalho, oportunidades, e auxílio nas mais variadas circunstâncias.

Aos meus anjos da guarda Bárbara e Walfrido (e seus familiares) pela hospitalidade, amizade, incentivo, imenso apoio nas mais diversas situações e por abrirem a porta do seu aconchego familiar para que eu pudesse realizar um dos maiores desafios da minha vida. Meu imenso e eterno “Obrigado”.

A minha professora de Francês, Prof. Elizabeth, pelo exemplo de profissionalismo, incentivo, palavras de conforto, por me mostrar que eu posso fazer muito mais daquilo que me proponho, e pelo entusiasmo que me fizeram perseverar até o fim.

Aos meus amigos de laboratório Fernanda Lima, Raquel, Juliana, Viviane, Laísa, Nina, Andria, Monique, Gabriela, Rafael, Lucas Jorge, Joyce, Tales, Thais, Wilklin, Rhayane Viegas, Rhayane Ávila, Octávio, Ludmilla, Arthur, Marina, Evandro, pelo apoio, e credibilidade quando eu mesmo já não estava mais acreditando em mim e na minha capacidade de superar desafios.

A minha mãe científica, Prof. Silma Regina, pela amizade, apoio, profissionalismo e críticas sempre construtivas essenciais ao meu desenvolvimento científico.

Aos meus amigos da “Terra das Palmeiras”, André Luiz, Lia, Malu, Vanessinha, minha

prima Kátia, Prof. Sônia, pela amizade e pelo apoio incondicional durante estes 4 anos.

Às secretárias, Natália, Fernanda e Sheilla pelo apoio incondicional, pela simpatia, e disponibilidade em me atender e me ajudar sempre que precisei.

Aos colaboradores, Kátial Vital, Prof. Enio Ferreira e Prof. Flaviano, pelo apoio e contribuição na execução dos nossos projetos.

Ao CNPq pela bolsa concedida, e que me possibilitou permanecer em BH e continuar o Doutorado.

A todo o LGCM e outros profissionais que, direta ou indiretamente, participaram e me ajudaram na concretização de mais uma etapa da minha vida.

A todos, meus sinceros agradecimentos!

“Admiremos os grandes mestres, mas não os imitemos” (Victor Hugo).

RESUMO

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 é uma bactéria que tem propriedades probióticas e terapêuticas relatadas apenas por estudos *in vitro*. Não há evidências científicas sobre o potencial probiótico desta linhagem *in vivo*, assim como quais as proteínas e mecanismos moleculares estariam relacionados aos seus efeitos benéficos e ao seu status de segurança para o consumo. Análises ao nível genômico, associadas com estudos *in vitro* e *in vivo*, vêm sendo utilizadas para a caracterização de novos candidatos probiótico, e fornecendo novas perspectivas sobre os principais fatores genéticos e mecanismos moleculares associados às características funcionais e de segurança destes microrganismos. Deste modo, com a abordagem probiogenômica, este estudo caracterizou o perfil probiótico de CIDCA 133 *in vivo*, utilizando um modelo de inflamação intestinal, e investigou os possíveis genes associados aos efeitos benéficos e de segurança para futuras aplicações probióticas. O estudo *in vivo* demonstrou que CIDCA 133 é uma potencial linhagem probiótica capaz de melhorar danos inflamatórios histopatológicos na mucosa intestinal induzida pelo agente quimioterápico 5-Fluorouracil. CIDCA 133 apresenta também propriedades imunoestimulatórias capazes de aumentar a expressão gênica de citocinas anti-inflamatórias *Il10* e *Tgfb1* e inibição de marcadores associados à ativação da via inflamatória NF-κB. Esses efeitos podem estar associados a proteínas secretadas, de membrana/expostas à superfície bacteriana identificadas *in silico* e ratificados *in vivo*, no qual foi observado que, mesmo após inativação pelo calor, CIDCA 133 mantém efeitos anti-inflamatórios, demonstrando que esta propriedade pode ser atribuida aos componentes protéicos e não-protéicos presentes na superficie celular. Muitos desses fatores genéticos foram também identificados, por meio de genômica comparativa, em outras linhagens probióticas da espécie, sendo que a proteína PrtB parece ser a candidata alvo responsável pelas propriedades anti-inflamatórias das linhagens probióticas da espécie *L. delbrueckii*. Os ensaios fenotípicos demonstraram também que CIDCA 133 sobrevive ao estresse ácido, osmótico e térmico. Além disso, esta linhagem apresenta atividade antibacteriana contra bactérias patogênicas, possivelmente por meio de bacteriocinas e produção de ácidos orgânicos como lactato, cujos genes foram identificados *in silico*. Por outro lado, em relação à segurança, a análise genômica mostrou que CIDCA 133 contém genes associados a virulência, metabólitos tóxicose resistência a antimicrobianos. No entanto, nenhum desses genes está inserido em regiões de profagos e plasmídeo. Ao nível fenotípico foi observada resistência antimicrobiana de CIDCA 133 aos antibióticos estreptomicina e gentamicina, mas nenhuma atividade hemolítica e degradação da mucina foi exibida pela linhagem. Além disso, nenhum efeito adverso foi observado na análise clínica e histopatológica de camundongos saudáveis após o consumo da linhagem (5×10^7 UFC/mL) por 13 dias consecutivos. No geral, os achados do presente trabalho mostram que CIDCA 133 apresenta características funcionais benéficas *in vivo* e revelou que esta linhagem apresenta determinado nível de segurança para ser explorada para oconsumo e futuras aplicações probióticas.

Palavras-chave: Genômica; Probiótico; Paraprobiótico; Imunomodulação; Atividade antimicrobiana; Segurança; Resistência aos Antibióticos.

ABSTRACT

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 is a bacterium that has probiotic and therapeutic properties reported only by *in vitro* studies. There is no scientific evidence about the probiotic potential of this strain *in vivo*, as well as which proteins and molecular mechanisms would be related to its beneficial effects and its safe status for consumption. Genomic-level analyses, coupled with *in vitro* and *in vivo* studies, have been used to characterize new probiotic candidates, and provide new insights into the key genetic factors and molecular mechanisms associated with the functional and safety characteristics of these microorganisms. Thus, with the probiogenomic approach, this study characterized the probiotic profile of CIDCA 133 *in vivo*, using a model of intestinal inflammation, and investigated the possible genes associated with beneficial and safety effects for future probiotic applications. The *in vivo* study demonstrated that CIDCA 133 is a potential probiotic strain capable of ameliorating histopathological inflammatory damage in the intestinal mucosa induced by the chemotherapeutic agent 5-Fluorouracil. CIDCA 133 also exhibits immunostimulatory properties capable of increasing gene expression of anti-inflammatory cytokines *Il10* and *Tgfb1* and inhibition of markers associated with activation of the NF-κB inflammatory pathway. These effects may be associated with secreted, membrane/bacterial surface proteins identified *in silico* and ratified *in vivo*, in which it was observed that even after heat inactivation, CIDCA 133 maintains anti-inflammatory effects, demonstrating that this property can be attributed to proteinaceous and non-proteinaceous components present on the cell surface. Many of these genetic factors were also identified, through comparative genomics, in other probiotic strains of the species, and the PrtB protein seems to be the target candidate responsible for the anti-inflammatory properties of the probiotic strains of *L. delbrueckii*. Phenotypic assays also demonstrated that CIDCA 133 survives acid, osmotic, and heat stress. Furthermore, this strain exhibits antibacterial activity against pathogenic bacteria, possibly through bacteriocins and the production of organic acids such as lactate, whose genes were identified *in silico*. On the other hand, regarding safety, the genomic analysis showed that CIDCA 133 contains genes associated with virulence, toxic metabolites, and antimicrobial resistance. However, none of these genes is inserted in prophage and plasmid regions. At the phenotypic level, antimicrobial resistance of CIDCA 133 to the antibiotics streptomycin and gentamicin was observed, but no hemolytic activity and mucin degradation was exhibited by the strain. Furthermore, no adverse effects were observed in the clinical and histopathological analysis of healthy mice after consumption of the strain (5×10^7 CFU/mL) for 13 consecutive days. Overall, the findings of the present workshow that CIDCA 133 exhibits beneficial functional characteristics *in vivo* and reveals that this strain presents a certain level of safety to be explored for consumption and future probiotic applications.

Keywords: Genomics; Probiotic; Paraprobiotic; Immunomodulation; Antimicrobial Activity; Safety; Antibiotic Resistance

LISTA DE ILUSTRAÇÕES

REVISÃO BIBLIOGRÁFICA

Figura 1. Representação esquemática da barreira intestinal e seus componentes.....25

Figura 2. Distribuição dos principais grupos de bactérias ao longo do TGI humano.....26

Figura 3. Representação esquemática da ativação do sistema imune de mucosa pela microbiota.....28

Figura 4. Principais mecanismos de ação propostos para bactérias probióticas no Trato Gastrointestinal humano.....30

Figura 5. Representação esquemática da via de homo e heterofermentação.....37

CAPÍTULO I

Figure 1. Experimental protocol of mucositis induced in a murine model.....48

Figure 2. Food, milk intake and body weight of animals.....50

Figure 3. Effect of CIDCA 133 fermented milk in mouse small intestines with induced mucositis by 5-fluorouracil (5-FU) 51

Figure 4. Representative photomicrographs from mucosal histopathology, histopathological scores, morphometrical analysis of villus height, crypt depth and villus height-to-crypt depth ratio of the ileum of animals with mucositis and administrated with strain CIDCA 133 fermented milk.....52

Figure 5. Representative photomicrographs from ileum section stained with PAS.....53

CAPÍTULO II

Figure 1. Schematic representation of the plasmid circular map present in *Lactobacillus delbrueckii* CIDCA 133 and restriction enzyme cut sites.....65

Figure 2. Distribution of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 (CIDCA 133) genes among the Gene Ontology (GO) categories predicted in Gene Ontology Functional Enrichment Annotation Tool (GO FEAT) 66

Figure 3. Heatmap representing the degree of similarity between *Lactobacillus delbrueckii* genomes based on the Average Nucleotide Identity (ANI) 67

Figure 4. *L. delbrueckii* CIDCA 133 tolerates different stressors.....68

Figure 5. Circular schematic representation of Metabolic (MI) and Symbiotic (SI) islands predicted with GIPSy software in CIDCA 133 genome and its comparison with others *L. delbrueckii* complete genomes.....69

Figure 6. Schematic representation of bacteriocin gene operon (A–C) present in *L. delbrueckii* CIDCA 133 genome predicted by BAGEL4. (D) Antibacterial activity spectrum of bacterial supernatants from *L. delbrueckii* CIDCA 133 against pathogenic and non-pathogenic bacteria.....70

Figure 7. Distribution of subcellular localization of CIDCA 133 proteins predicted by SurfG+ software (A). Several proteins with high probably adhesion capacity predicted by Vaxign software (B). Protein–protein interaction network mapping to the KEGG toll-like receptor/nuclear factor κappa B (TLR/NF-κB) signaling pathway.....71

Figure 8. Relative gene expression of (A–L) Tlr2, Tlr4, Myd88, Nfkb1, Tgfb1, Il10, Il6, Il12, Il17a, Il1b, Tnf, and Muc2 in the ileum section of animals that received oral administration of *L. delbrueckii* CIDCA 133 for 13 consecutive days.....72

CAPÍTULO III

Figure 1. Experimental design protocol.....87

Figure 2. Protective effect of heat-killed (LDi) and cell-free supernatant (LDs) of *L. delbrueckii* CIDCA 133 on 5-FU-induced body weight loss and recruitment of polymorphonuclear cells.....89

Figure 3. Heat-killed (LDi) *L. delbrueckii* CIDCA 133 modulates gene expression of inflammatory cytokines.....90

Figure 4. Cell-free supernatant ((LDs) of *L. delbrueckii* CIDCA 133 downregulated 5-FU-induced gene expression of pro-inflammatory cytokines.....91

Figure 5. Heat-killed (LDi) *L. delbrueckii* CIDCA 133 modulates mRNA expression of markers involved in epithelial barrier function.....92

Figure 6. Cell-free supernatant (LDs) of *L. delbrueckii* CIDCA 133 modulates mRNA expression of markers involved in epithelial barrier function.....93

Figure 7. Heat-killed (LDi) and supernatant (LDs) of *L. delbrueckii* CIDCA 133 ameliorate intestinal architecture damage induced by 5-FU administration.....94

Figure 8. Proposed mechanism action of paraprobiotics and postbiotics of *L.* delbrueckii CIDCA 133 in intestinal mucositis induced by 5-FU chemotherapy.....97

CAPÍTULO IV

Figure 1. Schematic representation of insertion site of prophages regions (black arrows), CRISPR-Cas system (red arrow), and distribution of transposases along of the *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 chromosome.....108

Figure 2. Hemolysis (a) and mucin degradation activity (b) of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133.....110

Figure 3. Food intake (a), body weight (b), and spleen and thymus index (c) after *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 consumption for 13 consecutive days.....111

Figure 4. Relative gene expression of oxidative stress marker (a) and tight junction's proteins occludin, claudin 2, and zonulin (b–d) in the ileum section of animals that received oral administration of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 for 13 consecutive days.....112

Figure 5. Effects of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 consumption on inflammatory cell infiltration (a), morphometric (b), and histopathological (c-d) analysis……113

CAPÍTULO V

Figure 1. Pan-genome of probiotics *L. delbrueckii* strains.....123

Figure 2. Functional analysis of gene families of probiotics *L. delbrueckii* strains.....124

Figure 3. Bacteriocins profile across probiotics *L. delbrueckii* strains.....126

Figure 4. Core proteins of *L. delbrueckii* strains interact with human immune proteins involved in inflammatory pathways.....127

LISTA DE TABELAS

CAPÍTULO II

Table 1. Complete genomes of *Lactobacillus delbrueckii* strains obtained from NCBI used in comparative analysis……...61

Table 2. Quantitative Polymerase Chain Reaction (qPCR) primers used in this study.....64

Table 3. Gene's prediction involved with stress tolerance of *Lactobacillus delb*rueckii CIDCA 133…….....68

CAPÍTULO III

Table 1. qPCR primers sequences.....88

CAPÍTULO IV

Table 1. Quantitative polymerase chain reaction (qPCR) primers used in this study……..107

Table 2. Putative virulence factors and harmful metabolites-encoding genes predicted on *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 genome…….....109

Table 3. Antimicrobial susceptibility profile of *L. delbrueckii* subsp. *lactis* CIDCA 133……...110

CAPÍTULO V

Table 1. Genome features of *Lactobacillus delbrueckii* strains used in this study……..122

Table 2. Predicted proteins identified in the core genome of probiotics *Lactobacillus delbrueckii* strains involved in acid and bile tolerance…….....125

LISTA DE SIGLAS E ABREVIATURAS

EFSA	*European Food Safety Authority*
EPO	*Eosinophil peroxidase*
FDA	*Food and Drug Administration*
GALT	*Gut-Associated Lymphoid Tissue*
GEI	*Genomic Islands*
GRAS	*Generally Recognized as Safe*
HSP	*Heat Shock Protein*
IBD	*Inflammatory Bowel Diseases*
IL	*Interleukin*
IEC	*Intestinal Epithelial cells*
LPS	*Lipopolysaccharide*
MAMP	*Microbe-Associated Molecular Pattern*
MPO	*Myeloperoxidase*
MUC	*Mucin*
NCBI	*National Center for Biotechnology Information*
NF-κB	*Nuclear Factor kappa B*
NGS	*Next Generation Sequencing*
PRR	*Pattern Recognition Receptor*
ROS	*Reactive Oxygen Species*
rRNA	*Ribosomal Ribonucleic Acid*
SCFA	*Short Chain Fatty Acids*
SLP	*Surface Layer protein*
sIgA	*Secretory immunoglobulin A*
TLR	*Toll like Receptor*
Treg	*T regulatory cell*
5-FU	*5-Fluourouracil*

SUMÁRIO

1. INTRODUÇÃO GERAL.....22

2. REVISÃO BIBLIOGRÁFICA.....24

2.1. Trato Gastrointestinal e Microbiota.....24

2.2. Probióticos.....29

2.3. Bactérias Lácticas.....30

2.4. Probiogenômica de Bactérias Lácticas.....32

2.5. Mecanismos de Adaptação/ Probiose 33

2.5.1. Sobrevivência a Agentes Estressores do TGI.....33

2.5.2. Interação com a Mucosa Intestinal e Estímulo do Sistema Imune.....34

2.5.3. Exclusão Competitiva e Antagonismo Bacteriano.....35

2.5.4. Fermentação por Bactérias Lácticas e Produção de Metábolitos.....36

2.6. Aspectos de Segurança.....38

2.7. Caracterização probiótica de *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133.....40

3. RELEVÂNCIA DA TESE 42

4. OBJETIVOS DA TESE..... 43

4.1. Geral..... 43

4.2. Específicos..... 43

***5.* CAPÍTULO I: Protective effect of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 in a model of 5 Fluorouracil-Induced intestinal mucositis… 45**

***6.* CAPÍTULO II: Probiogenomics of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: In Silico, In Vitro, and In vivo Approaches 58**

7. CAPÍTULO III: Paraprobiotics and Postbiotics of *Lactobacillus delbrueckii* CIDCA 133 Mitigate 5-FU-induced Intestinal Inflammation..82

8. CAPÍTULO IV: Safety Evaluation of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: a Health-promoting Bacteria..... 103

***9.* CAPÍTULO V: Genomic Characterization of *Lactobacillus delbrueckii* Strains with Probiotics Properties..... 119**

10. CONCLUSÕES GERAIS..... 132

11. PERSPECTIVAS..... 133

12. REFERÊNCIAS..... 134

APÊNDICE A - Produção Científica (2018-2022) 148

ANEXOS 174

PREFÁCIO

A fim de facilitar a compreensão e organização do trabalho, esta Tese foi dividida nas seguintes seções:

I - Introdução Geral: Apresenta uma breve descrição sobre as propriedades benéficas de microrganismos probióticos e o uso da genômica para a identificação de fatores genéticos associados a estas propriedades.

II - Revisão Bibliográfica: Descreve a interação entre a microbiota e o trato gastrointestinal no contexto da homeostase intestinal e inflamação, e o uso de bactérias lácticas como probiótico e seus mecanismos de ação para promover efeitos benéficos ao hospedeiro. A seção também discute a utilização da genômica como ferramenta para a identificação de fatores genéticos e elucidação de possíveis mecanismos moleculares envolvidos tanto aos efeitos benéficos à saúde do hospedeiro bem como ao status de segurança para o consumo humano atribuídos a linhagens probióticas. Além disso, são descritas as principais caracteristicas probióticas reportadas para a linhagem *Lactobacillus delbrueckii* CIDCA 133.

III - Relevância do Projeto de Tese: Diferentes linhagens de bactérias lácticas isoladas de diferentes fontes, vêm sendo investigadas quanto a efeitos benéficos ao hospedeiro (potencial probiótico) e aos fatores genéticos envolvidos com essas propriedades. Nesse contexto, buscamos realizar a caracterização funcional e genômica de *L. delbrueckii* CIDCA 133 de forma a identificar os possíveis genes e mecanismos moleculares que sustentem sua aplicação probiótica como agente anti-inflamatório, e também seu perfil de segurança para o consumo humano e veterinário.

IV - Objetivos Da Tese: Caracterização do potencial probiótico de *L. delbrueckii* CIDCA 133 *in vivo*, e a identificação de fatores genéticos associados às suas propriedades probióticas e de segurança para o consumo.

V - Capítulo I: Apresentado em forma de artigo científico, caracteriza as propriedades probióticas de *L. delbrueckii* CIDCA 133 *in vivo*, usando um modelo de inflamação intestinal induzida pelo quimioterápico 5-Fluorouracil (5-FU).

VI - Capítulo II: Apresentado em forma de artigo científico, descreve os principais fatores genéticos e mecanismos moleculares associados com a probiose de *L. delbrueckiii* CIDCA 133, incluindo seus efeitos anti-inflamatórios, confirmados fenotipicamente por meio de análises *in*

vitro e *in vivo*.

VII - Capítulo III: Apresentado em forma de artigo científico, avalia o efeito anti-inflamatório de *L. delbrueckii* CIDCA 133 após a inativação pelo calor, de forma a elucidar se os efeitos imunomodulatórios dessa linhagem são atribuidos a componentes protéicos e não-protéicos presentes na sua superfície celular.

VIII - Capítulo IV: Apresentado em forma de artigo científico, descreve os principais fatores genéticos que possam sustentar o status de segurança de *L. delbrueckiii* CIDCA 133 para o consumo humano e veterinário, avaliados por análises *in vitro* e por meio de um estudo de toxicidade *in vivo*.

IX - Capítulo V: Apresentado em forma de artigo científico, realiza uma caracterização genômica das propriedades probióticas compartilhadas por linhagens da espécie *Lactobacillus delbrueckii* cujos efeitos benéficos foram reportados fenotipicamente na literatura.

X - Conclusões Gerais: Sumariza os principais achados desta Tese.

XI - Perspectivas: Descreve as próximas etapas a serem desenvolvidas a partir dos dados obtidos nesta Tese.

XII - Anexos: Lista o material suplementar desta Tese.

XIII - Apêndice: Produção Cientifica (2018-2022): Lista as principais publicações científicas obtidas durante o doutorado.

1. INTRODUÇÃO GERAL

As bactérias lácticas representam um grupo taxonômico altamente diverso tanto no nível fenotípico e ecológico quanto genotípico (Salvetii et al., 2012; Pot et al., 2014). Grande parte desses microrganismos, especialmente linhagens de *Lactobacillus*, apresentam características funcionais benéficas ao hospedeiro, tornando-lhes de alta relevância no setor biotecnológico e industrial alimentício (Sun et al., 2015; Plaza-Diaz et al., 2019).

No que diz respeito às propriedades benéficas ao hospedeiro, muitas linhagens de bactérias lácticas vêm sendo caracterizadas como probióticos, e demonstrando excelentes propriedades tanto em estudos *in vitro* (Presti et al., 2015; Rocha-Ramírez et al., 2017) quanto *in vivo* (Rocha et al., 2014; Gosmann et al., 2017). Estes benefícios têm sido extensivamente demonstrados em dados que utilizam modelos animais e evidências clínicas como, por exemplo, sua efetividade como adjuvante no tratamento de desordens inflamatórias relacionadas ao trato gastrointestinal (TGI), incluindo doença de Crohn e retocolite ulcerativa (Fedorak et al., 2015; Bjarnason et al., 2019), mucosite intestinal (De Jesus et al., 2019), infecções entéricas (Acurcio et al., 2017), dentre outras.

Muitos dos efeitos dos probióticos são originados a partir de sua relação simbiótica com o hospedeiro, uma vez que este fornece um habitat rico em nutrientes, enquanto os microrganismos podem desempenhar inúmeras funções benéficas, tais como desenvolvimento da arquitetura da mucosa intestinal, metabolismo de compostos não digeríveis, exclusão de patógenos, produção de metabólitos bioativos como ácidos graxos de cadeia curta (SCFA, do inglês *Short-Chain Fatty Acid*) e vitaminas, e modulando a barreira epitelial e o sistema imunológico que, consequentemente, favorece a homeostase intestinal (Plaza-Diaz et al., 2019). Uma vez que as propriedades probióticas das bactérias lácticas assim como de outros microrganismos são linhagem específicas, cada característica de uma linhagem candidata a ser um probiótico precisa ser testada.

Propriedades biológicas individuais de linhagens probióticas podem estar relacionadas a um alto grau de variação em seu conteúdo genômico. Assim, estudos em nível genômico podem fornecer informações sobre os principais fatores genéticos e mecanismos moleculares associados às características probióticas desses microrganismos, como sobrevivência aos agentes estressores do TGI, inibição de patógenos e imunomodulação (Ventura et al., 2012; Castro-López et al., 2021). Nesse sentindo, com o desenvolvimento das plataformas de sequenciamento de nova geração (NGS, do inglês *Next-Generation Sequencing*), análises *in silico* que utilizam tecnologias baseadas em ômicas associadas, quando possível, aos estudos

moleculares *in vitro* e *in vivo* (análises probiogenômicas) vêm sendo realizados. Esta abordagem visa caracterizar novos probióticos, e identificar fatores genéticos e elucidar os complexos mecanismos que promovem a sobrevivência, adaptação ao TGI e promoção de efeitos benéficos desses microrganimos à saúde do hospedeiro (Lebeer et al., 2008; Ventura et al., 2012). Além disso, têm se buscado cada vez mais identificar fatores genéticos relacionados à segurança de novas linhagens com perfil probiótico em relação à aplicação para o consumo humano, devido alguns relatos de infecções após o consumo de alguns desses microrganismos (Yelin et al. 2019; Gore et al., 2020).

A abordagem probiogenômica vem sendo aplicada em diferentes microrganismos com potencial probiótico como *Lactobacillus helveticus* (Fontana et al., 2019), *Lactiplantibacillus plantarum* (Zhang et al., 2018), *Pediococcus* sp. (Wanna et al., 2021), *Bifidobacterium* sp. (Duar et al., 2020), *Enterococcus* sp. (Hussein et al., 2020), *Lactococcus lactis* (Oliveira et al., 2017), dentre outros. No entanto, apesar da importância de linhagens de *L. delbrueckii* na indústria alimentícia, são escassos os dados relacionados à caracterização de novos candidatos probióticos da espécie *L. delbrueckii* bem como estudos genômicos destas linhagens, relacionados aos efeitos benéficos à saúde do hospedeiro e segurança para uso em aplicações probióticas.

2. REVISÃO BIBLIOGRÁFICA

2.1. Trato Gastrointestinal e Microbiota

O trato gastrointestinal (TGI) de mamíferos é um complexo sistema biológico que, além de possuir papel digestivo e de absorção de nutrientes, atua como uma barreira física, química, microbiológica e imunológica, tendo a capacidade de defender o organismo contra agentes potencialmente prejudiciais e evitando que microrganismos, antígenos e fatores pró-inflamatórios atinjam o ambiente celular do intestino (Salvo-Romero et al., 2015; Konig et al., 2016). Essa barreira intestinal é categorizada de acordo com os diversos níveis de proteção, bem como pela localização e natureza dos seus componentes celulares e extracelulares (Rescigno, 2013; Salvo-Romero et al., 2015).

A primeira linha de defesa na barreira epitelial contra a invasão de microrganismos patogênicos e antígenos é a camada de muco (produzido pelas células caliciformes) que recobre a superfície intestinal. Essa camada evita que agentes nocivos ao organismo entrem em contato diretamente com as células epiteliais intestinais.As mucinas são os principais componentes da camada de muco, sendo a mucina 2 (MUC2) a proteína mais abundante na superfície intestinal (Camilleri et al., 2012; Vancamelbeke e Vermeire, 2017) (**Figura 1**).

Abaixo da camada de muco tem-se a camada epitelial, constituída principalmente por enterócitos. Esta camada de células é selada por proteínas denominadas junções firmes (do inglês, *tight junctions*), tais como claudina, occludina e zonulina, que funcionam como uma vedação entre as células epiteliais adjacentes, impedindo a passagem paracelular de microrganismos e antígenos (Pastorelli et al., 2013; Zihni et al., 2016) (**Figura 1**).

Além dos enterócitos, os diversos papéis desempenhados pelo epitélio intestinal derivam da presença de outras células especializadas como as células caliciformes (células secretoras de mucinas e peptídeos necessários à função de crescimento epitelial e reparo da mucosa), células de Paneth (células secretoras de peptídeos antimicrobianos,tais como defensinas e lisozimas que são produzidas para eliminar microrganismos potencialmente patogênicos que eventualmente penetram na camada de muco) e células enteroendócrinas (células produtoras de hormônios peptídicos que regulam o intestino, tais como gastrina, e neurotransmissores como o ácido gama-aminobutírico-GABA, que promove a interação do eixo cérebro-intestino) (Salvo-Romero et al., 2015) (**Figura 1**).

Outras células que também merecem destaque são as células B, produtoras de imunoglobulina A secretória (sIgA), capazes de inibir a penetração, a aderência e colonização de micróbios prejudiciais ao lúmen intestinal. Além dessas células, deve-se destacar também o papel das células dendríticas, os linfócitos intraepiteliais e as células M (do inglês, *Microfold cells*) responsáveis, respectivamente, pelo reconhecimento e apresentação de antígenos às células especializadas do sistema imune, e pelo transporte de microrganismos e partículas através da camada de células epiteliais do lúmen intestinal até a lâmina própria, onde as interações com células imunes podem ocorrer (Peterson e Artis, 2014; Vancamelbeke e Vermeire, 2017) (**Figura 1**).

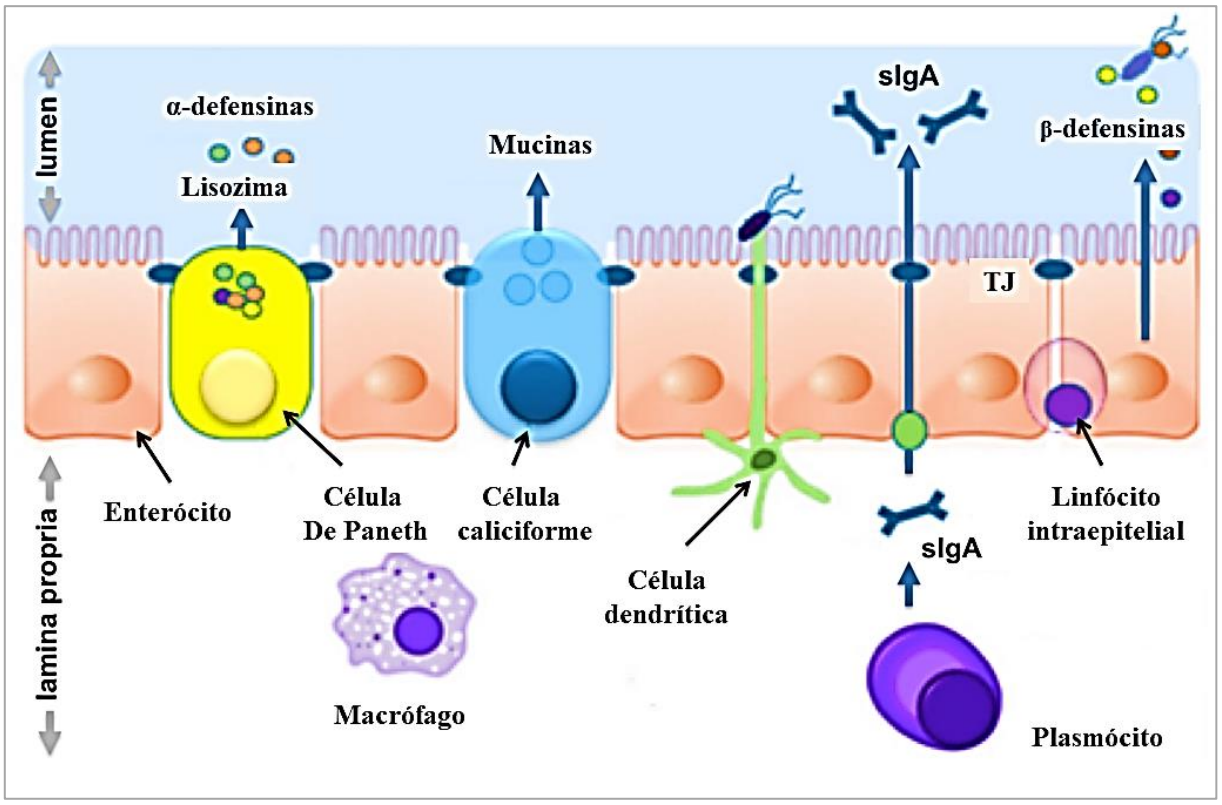


Figura 1. Representação esquemática da barreira intestinal e seus componentes. A mucosa intestinal compreende uma camada de células epiteliais e uma região subepitelial que contém a lâmina própria. O epitélio inclui enterócitos, células caliciformes, células de Paneth, e células enteroendócrinas. Acima da barreira epitelial está a camada de muco, a microbiota e IgA secretória. Abaixo da camada epitelial está a lâmina própria constituída por células imunes como macrófagos, células dendríticas, plasmócitos, linfócitos, entre outros. **Fonte:** Adaptado de Bischof et al. (2014).

Deve-se ressaltar que o intestino é o ambiente com maior nível populacional de microrganismos comensais do hospedeiro e está em constante interação com a comunidade bacteriana, que em condições normais mantém a homeostase do organismo e promove benefícios ao hospedeiro (Shreiner et al., 2015; Lane et al., 2017). Esta comunidade bacteriana é denominada de microbiota intestinal.

A composição e a densidade da microbiota intestinal humana na fase adulta variam consideravelmente ao longo do TGI (Mowat e Agace, 2014), devido a determinadas condições fisiológicas como pH ácido (1,5-4), disponibilidade de O_2, sais biliares, secreções pancreáticas do hospedeiro, dentre outros (Rinninella et al., 2019). Esses fatores contribuem para que baixas concentrações e diversidade bacteriana (até 10^3UFC/mL) sejam encontradas no TGI superior (estômago, duodeno, jejuno e íleo proximal), de forma a evitar competição nutricional entre a microbiota e o hospedeiro (Walter e Ley, 2011). Por outro lado, um maior número de bactérias (10^9-10^{12} UFC/mL) reside nos compartimentos inferiores do TGI (íleo distal e cólon), que constituem o hábitat com a maior densidade microbiana conhecida até então (Mowat e Agace, 2014; Thursby e Juge, 2017) (**Figura 2**).

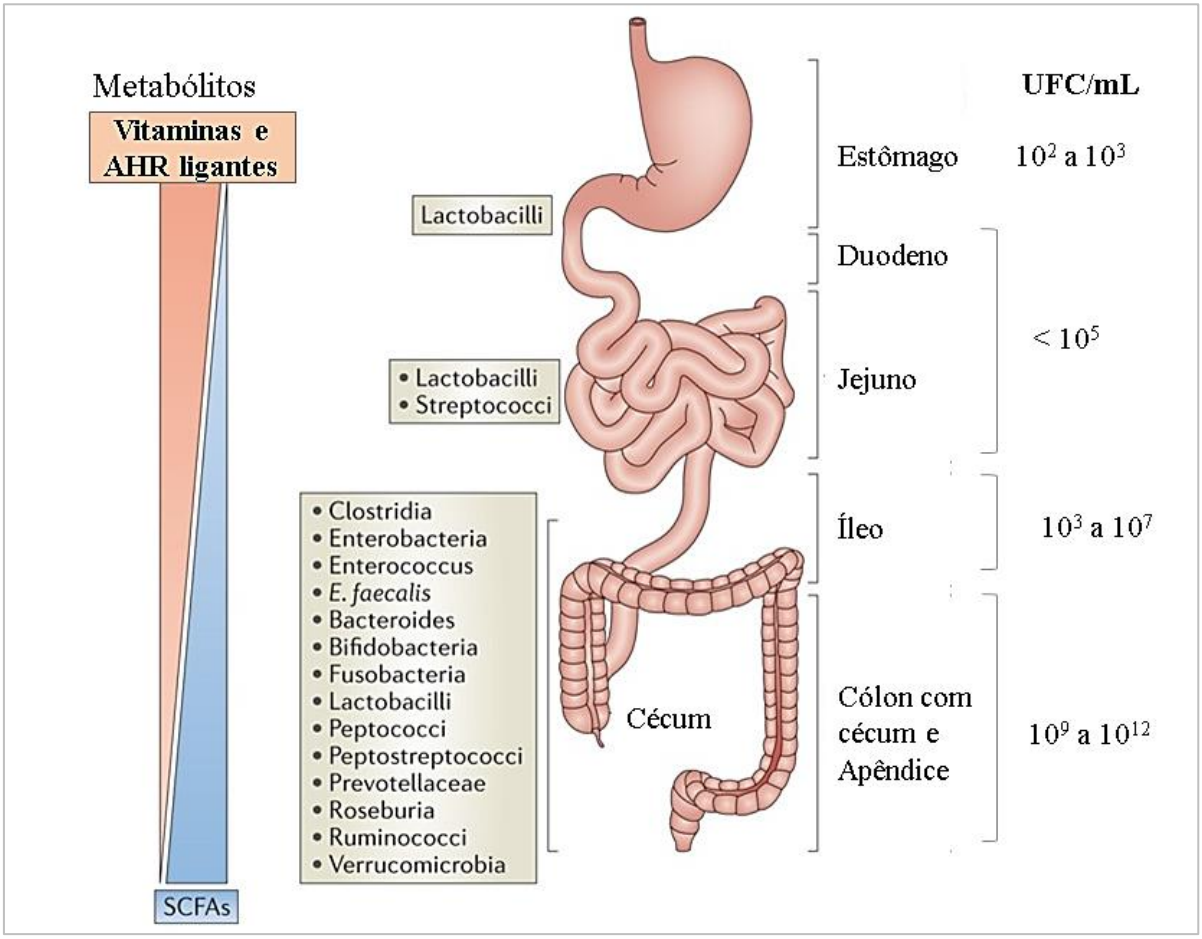


Figura 2. Distribuição dos principais grupos de bactérias ao longo do TGI humano. Baixa diversidade bacterianav è encontrada na parte superior do TGI. Alta diversidade bacteriana é encontrada no íleo distal e no cólon. **Fonte**: Adaptado de Mowat e Agace (2014).

A microbiota intestinal co-evoluiu com seu hospedeiro e essa intrínseca relação mútua fornece benéficios essenciais à saúde do hospedeiro de muitas maneiras, particularmente ao regular a homeostase imunológica da mucosa intestinal (Wu e Wu, 2012). No entanto, quando esta relação mutualista é comprometida e ocorre uma interação alterada entre células imunes

intestinais e microrganismos, a microbiota intestinal pode causar ou contribuir para o estabelecimento de doenças infecciosas, bem como para o desencadeamento de doenças inflamatórias crônicas e autoimunes (Dieterich et al., 2017; Zheng et al. 2020). Portanto, a interlocução entre a microbiota e o sistema imunológico intestinal é essencial para a manutenção da homeostase do intestino.

Para que essa homeostase ocorra, estruturas imunológicas especializadas conhecidas como tecido linfóide associado ao intestino (GALT, do inglês, *Gut-AssociatedLymphoid Tissue*), devem ser capazes de reconhecer e eliminar especificamente patógenos enquanto toleram as espécies comensais. Em condições normais, o GALT gera tolerância aos microrganismos comensais principalmente por meio da ação das células T reguladoras (Treg) e produção das citocinas interleucina IL10 e o fator de crescimento transformador beta (TGF-β) (Chistiakov et al., 2014; Neish et al., 2014). Além disso, a própria microbiota intestinal propicia o desenvolvimento intestinal normal devido à sua capacidade de influenciar a proliferação das células epiteliais e apoptose das células hospedeiras por meio da produção de SCFA (como butirato, acetato e propionato). A produção desses compostos possui um importante papel imunomodulador e auxilia no reparo intestinal por meio da promoção da proliferação e diferenciação celular (Corrêa-Oliveira et al., 2016; Morrison et al., 2016).

O reconhecimento diferencial de microrganismos da microbiota pelo epitélio intestinal ocorre por meio de receptores de reconhecimento de padrão (PRRs, do inglês, *Pattern Recognition Receptors*), como os receptores do tipo Toll (TLRs, do inglês, *Toll like receptors*) presentes em células epiteliais (caliciformes e enterócitos) e células imunes (macrófagos e as células dendríticas). Estes receptores são especializados no reconhecimento de padrões moleculares associados a micróbios (MAMPs, do inglês, *Microbe-Associated Molecular Pattern*) como lipoproteínas, peptidoglicanos, ácidos lipotecóicos e lipopolissacarídeo (LPS), entre outros, presente na superfície celular das bactérias (Kawasaki e Kawai, 2014) (**Figura 3**).

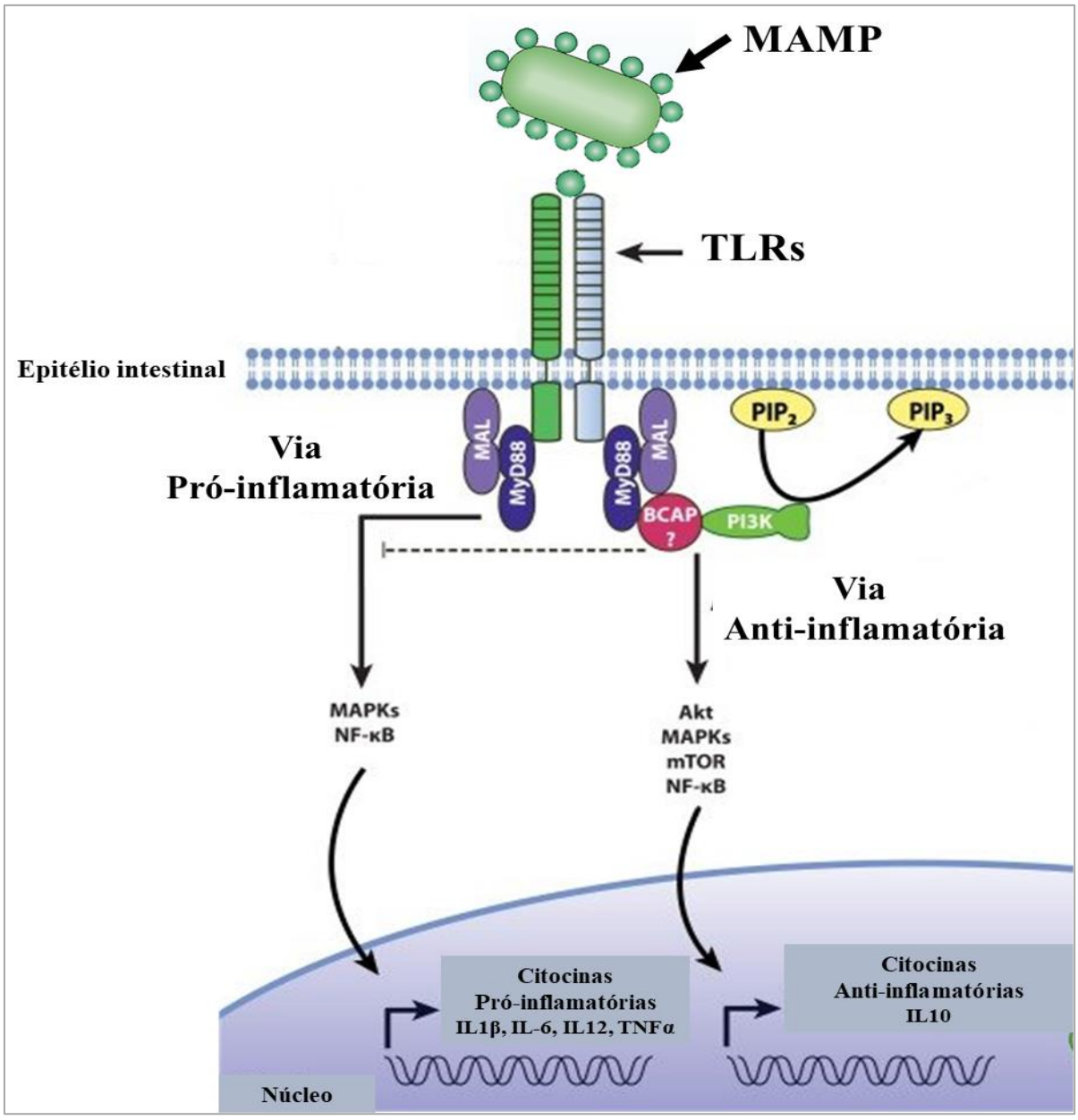


Figura 3.Representação esquemática da ativação do sistema imune de mucosa pela microbiota. As célulasepiteliais e linfóides detectam micróbios por meio de receptores de reconhecimento de padrões, como os receptores do tipo Toll (TLRs). A ligação dos TLRs aos componentes microbianos (MAMPs) leva a ativação de cascatas de sinalização, como a via de sinalização NF-κB, que culminam na produção de mediadores inflamatórios, como as citocinas IL1β, IL6, IL10, entre outras. **Fonte**. Adaptado de Li et al. (2013).

O reconhecimento dos MAMPs por PRRs induz a ativação de diferentes vias de sinalização como, por exemplo, a do fator nuclear κappa B (NF-κB), por meio doenvolvimento de proteínas adaptadoras, como o gene de resposta primária da diferenciação mielóide 88 (Myd88). A ativação da via de sinalização NF-κB por este complexo (TLR-Myd88) pode resultar na produção de citocinas, quimiocinas e outras moléculas efetoras, ativando a resposta imune inata com subsequente ativação do sistema imune adaptativo no hospedeiro (Kawasaki e Kawai, 2014; Burgueño e Abreu, 2020). Todo esse processo ocorre de forma controlada, sendo

um mecanismo necessário para manter as células intestinais em homeostase com a microbiota comensal (Burgueño e Abreu, 2020) (**Figura 3**).

Quando ocorre uma ativação exacerbada dos receptores TLRs ou há uma alteração na microbiota intestinal em virtude de variados eventos, como o uso de antibióticos, microrganismos patogênicos tendem a predominar no intestino e podem degradar, invadir a camada de muco intestinal e translocar, atingindo outros órgãos via circulação. Consequentemente, esse processo ativa exacerbadamente a resposta imune do hospedeiro por meio de mediadores pró-inflamatórios, como as citocinas IL1β, IL6, IL12, IL23, entre outras (Hooper et al., 2012; Belkaid e Hand, 2014). Essas citocinas, por sua vez, acabam estimulando o desenvolvimento de células Th1 e células Th17 e assim, potencializando a produção de mais citocinas pró-inflamatórias, resultandoem inflamação crônica (Rooks e Garrett, 2016; Oliveira et al., 2017) o que, muitas vezes, contribui para o surgimento de doenças inflamatórias intestinas (IBDs, do inglês, *Inflammatory Bowel Diseases*). Assim, como forma de regular esse processo disbiótico, a modulação da microbiota do indivíduo por meio da administração oral de microrganismos benéficos (probióticos) vem ganhando destaque como alternativa terapêutica adjuvante para inflamações intestinais.

2.2. Probióticos

Os probióticos são definidos como "*microrganismos vivos que, quando administrados em quantidades adequadas, conferem benefícios à saúde do hospedeiro*" (Hill et al., 2014).

Os mecanismos de ação propostos para os efeitos das bactérias e outros microrganismos probióticos são diversos, específicos da linhagem e dependem da quantidade de microrganismos ingeridos (Plaza-Diaz et al., 2019; Batista et al., 2020). Dentre os mecanismos de ação descritos para estes microrganismos destacam-se: (***i***) regulação da microbiota intestinal disbiótica (Shi et al., 2017); (***ii***) proteção da barreira epitelial por meio da manutenção das proteínas de junção firme (Blackwood et al., 2017); (***iii***) indução da produção de mucinas pelas células caliciformes (Aliakbarpour et al., 2012); (***iv***) interação com a mucosa intestinal e exclusão competitiva de microrganismos patogênicos como *Escherichia coli* patogênicas e *Salmonella enterica* serovar Typhimurium (Halder et al., 2017; Plaza-Diaz et al., 2019); (***v)*** produção de substâncias antimicrobianas, como bacteriocinas, ácidos orgânicos e peróxido de hidrogênio, e consequente inibição de patógenos (Collado et al., 2010; Monteagudo-Mera et al. 2019; Castilho et al., 2019); (***vi***) produção e secreção de metabólitos (como SCFA) com propriedades anti-inflamatórias (Maslowski et al., 2009); (***vii***) interação com o eixo intestino-cérebro via produção de metabólitos como SCFA e GABA (Carabotti et al., 2015); (***viii***)

imunomodulação por meio da inibição da via de sinalização NF-κB (Kaci et al., 2011; GAO et al., 2015), (***ix***) indução da produção de IgA e (***x***) ativação de células T reguladoras (Treg) (Hemarajata e Versalovic, 2013; Underwood et al., 2016) (**Figura 4**).

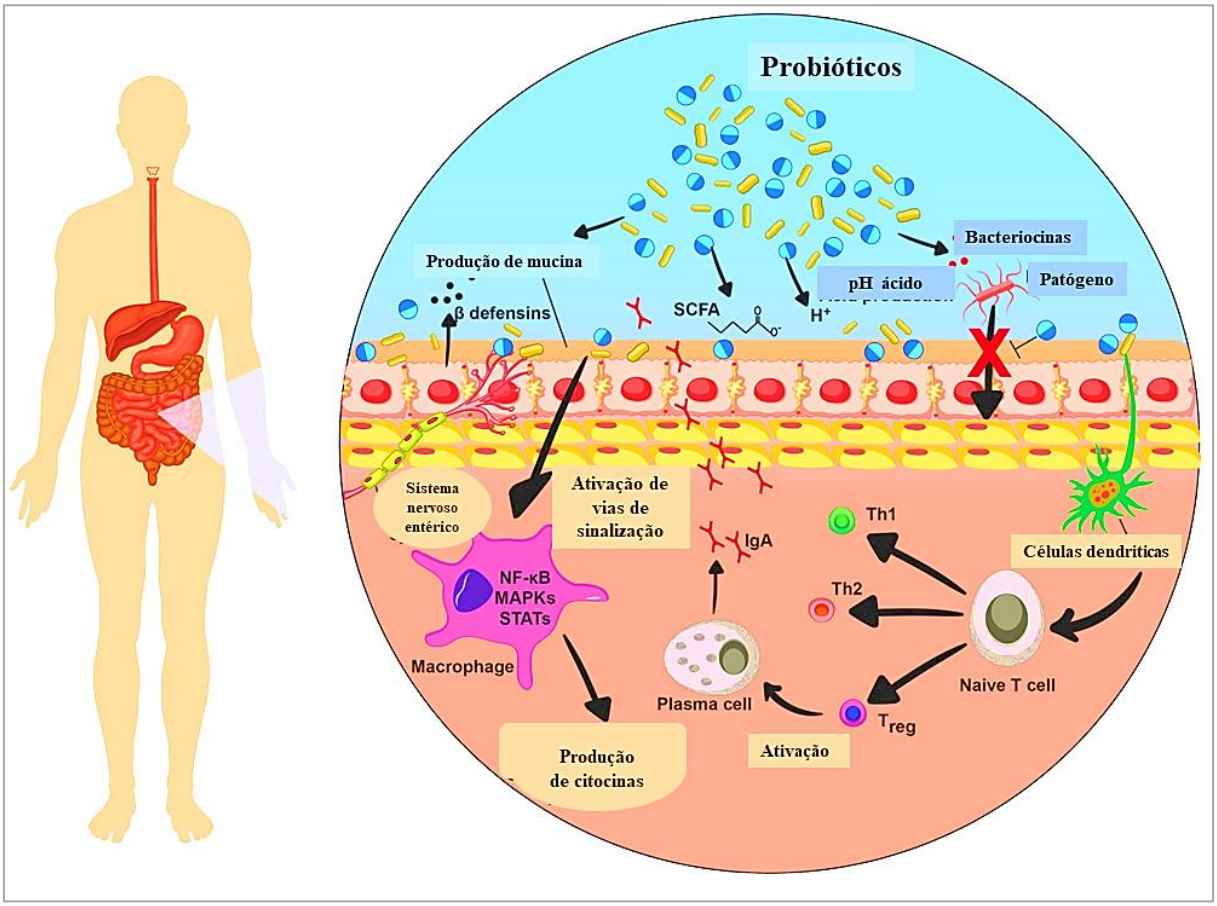


Figura 4. Principais mecanismos de ação propostos para bactérias probióticas no trato gastrointestinal humano. Os principais mecanismos de ação dos probióticos incluem aumento da barreira epitelial por meio da manutenção das proteínas *tight junctions* e produção de mucinas pelas células caliciformes, interação com a mucosa intestinal e inibição concomitante da adesão de patógenos, exclusão competitiva de microrganismos patogênicos através da produção de substâncias antimicrobianas e modulação do sistema imunológico. **Fonte:** Adaptado de Batista et al. (2020).

A maioria dos estudos relacionados à utilização de probióticos diz respeito às bactérias lácticas.

2.3. Bactérias Lácticas

As bactérias lácticas consistem em um grupo heterogêneo de microrganismos Gram-positivo, catalase-negativo, anaeróbicos facultativos ou microaerofilos, não formadores de esporos e não móveis, altamente resistentes ao pH ácido, e que possuem a capacidade de produzir ácido láctico, bem como outros compostos, como produto final da fermentação de carboidratos utilizando vias metabólicas específicas (Tamang et al., 2016; Mokoena, 2017).

O grupo das bactérias lácticas inclui espécies bacterianas do gênero: *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Sporolactobacillus, Tetragenococcus, Vagococcus* e *Weissella* (Burgain et al., 2014; Plavec e Berlec, 2019), e estes microrganismos podem ser isolados de diferentes fontes no ambiente, como produtos alimentícios, solo, água, bem como o TGI e o trato urogenital de seres humanos e outros animais (Liu et al., 2014; George et al., 2018), entre outros.

As bactérias lácticas vêm sendo utilizadas em uma variedade de processos, principalmente na indústria alimentícia, uma vez que estes microrganismos são utilizados na produção e preservação de produtos fermentados, como iogurte e alguns tipos de queijos e vinhos. Devido a capacidade de acidificar o meio, estes microrganismos melhoram as caracteristicas organolépticas (textura, sabor e aroma) e valor nutricional desses produtos (Widyastuti et al., 2014; Tamang et al., 2016). Por outro lado, estes microrganismos são funcionais como probióticos e vêm sendo explorados como sistemas vivos para entrega de vacinas gênicas e bioterapêuticos, além da produção de proteínas heterólogas com potencial aplicação no tratamento e prevenção de diferentes condições patológicas tanto humanas como veterinárias (Hazebrouck et al. 2006; Cristofori et al., 2021).

No que diz respeito à segurança para o consumo humano, grande parte das linhagens de bactérias lácticas são consideradas seguras (GRAS, do inglês, *Generally Recognized as Safe*) pelo FDA (do inglês, *Food and Drug Administratio*n), apresentando importante papel na manutenção da saúde e na prevenção/tratamento de diferentes condições patológicas humanas. Tais achados têm sido extensivamente demonstrados em estudos que utilizam modelos animais e evidências clínicas, como sua efetividade como adjuvante no tratamento da doença de Crohn e de retocolite ulcerativa (Fedorak et al., 2015; Bjarnason et al., 2019), mucosite intestinal (De Jesus et al., 2019), inflamações entéricas (Acurcio et al., 2017), câncer colorretal (Górska et al., 2019), diabetes (Hsieh et al., 2018), artrite (Lei et al., 2017), dentre outros.

Entre as linhagens de bactérias lácticas, deve-se destacar que espécies de *Lactobacillus* são as linhagens bacterianas mais bem estudadas como probiótico. Uma nova reclassificação taxonômica do gênero *Lactobacillus* em 25 novos gêneros foi realizada baseada na posição filogenética dos microrganismos associada com propriedades ecológicas e metabólicas compartilhadas entre eles. Assim, estes novos gêneros são: *Lactobacillus* (que compreende o grupo das *L. delbrueckii, L. crispatus, L. gasseri, L. paragasseri, L. helveticus*, entre outras), *Paralactobacillus* (grupo das *L. selangorensis*), *Holzapfelia* (grupo das *L. florum*), *Amylolactobacillus* (grupo das *L.amylophilus*), *Bombilactobacillus* (grupo das *L. mellis*), *Companilactobacillus* (grupo das *L. alimentarius*), *Lapidilactobacillus* (grupo das *L.*

dextrinicus e L. convacus), *Agrilactobacillus* (grupo das *L. composti*), *Schleiferilactobacillus* (grupo das *L. perolens*), *Loigolactobacilus* (grupo das *L. coryniformis*), *Lacticaseibacillus (*grupo das *L. casei* e *L. paracasei*), *Latilactobacillus* (grupo das *L. sakei*), *Dellaglioa* (grupo das *L. algidus*), *Liquorilactobacillus* (grupo das *Lactobacillus mali*, mas que é formado também em parte por *L. salivarius*) e *Ligilactobacillus* (grupo das *L. salivarius*), *Lactiplantibacillus* (grupo das *L. plantarum*), *Furfurilactobacillus* (grupo d*as L. rossiae*),*Paucilactobacillus* (grupo das *L. vaccinostercus*), *Limosilactobacillus* (grupo das *L. reuteri*), *Fructilactobacillus* (grupo das *L. fructivorans*), *Acetilactobacillus* (grupo das *Acetilactobacillus jinshanensis*), *Apilactobacillus* (grupo das *L. kunkeei*), *Levilactobacillus* (grupo das *L. brevis*), *Secundilactobacillus* (grupo das *L. collinoides*) e *Lentilactobacillus* (grupo das *L. buchneri*) (Zheng et al., 2020). No presente trabalho, o termo "*Lactobacillus*" será utilizado para esses microrganismos a fim de facilitar a compreensão das informações.

Em virtude do seu potencial comercial, biotecnológico e seus benefícios atribuídos à saúde do hospedeiro, nos últimos anos o número de genomas sequenciados e depositados de diferentes espécies de bactérias lácticas, bem como de outras bactérias probióticas, têm aumentado.

2.4. Probiogenômica de Bactérias Lácticas

Historicamente, do ponto de vista taxonômico, a distinção primária entre os microrganismos pertencentes ao grupo das bactérias lácticas, assim como outros microrganismos, era realizada somente por meio de características fenotípicas e bioquímicas (morfologia, padrão de fermentação de carboidratos, resistência ao pH ácido e bile, ensaio de catalase, entre outros) (Angelis e Gobbetti, 2016). No entanto, este cenário mudou com a introdução da análise comparativa genômica via sequências do gene ribossomal 16S (16S rRNA) e o avanço das tecnologias de sequenciamento, como as plataformas NGS (Ion Torrent, Illumina, PacBio e NanoPore), que possibilita o sequenciamento do genoma total desses e de outros microrganismos de forma rápida e com menos custos (Zhang et al., 2011; Slatko et al., 2018; Alexandraki et al., 2019).

O aprimoramento das plataformas de sequenciamento e a grande quantidade de dados gerados por estas plataformas influenciaram também no surgimento de programas capazes de processar de forma eficaz esses dados, como os softwares de montagens (ex: Edena, Spades, pipeline Unicycler) e anotação de genomas (ex: RAST, PROKKA) (Rice e Green, 2019; Segerman, 2020). Além disso, houve uma influência positiva no desenvolvimento e disponibilidade de banco de dados que facilitam a análise funcional dos produtos gênicos desses

genomas, como o banco de dados do BioCyc, que fornece informações de vias metabólicas para diferentes bactérias (Caspi et al., 2019).

Genes relacionados a cada uma das características/mecanismos atribuídos ao potencial probiótico de diferentes espécies de bactérias lácticas, bem como de outras bactérias probióticas, vêm sendo identificados por meio da associação de abordagens ômicas (genômica, transcriptômica, proteômica e metabolômica) em conjunto com análises tanto *in vitro* quanto *in vivo* (Kazou et al., 2018; Fontana et al., 2019; Zhanget al., 2019). Essas abordagens culminaram no surgimento e desenvolvimento da probiogenômica, uma área da genômica que vem fornecendo uma quantidade considerável de informações genéticas, filogenéticas, e sobre a capacidade metabólica e a base molecular dos efeitos benéficos à saúde do hospedeiro proporcionados pelas bactérias comensais/probióticas, como espécies de *Lactobacillus* e *Bifidobacterium* (Ventura et al., 2012).

O estudo de bactérias probióticas no nível genômico vem identificando e elucidando mecanismos moleculares envolvidos com aspectos relacionados à: (*i*) capacidade de sobrevivência e adaptação da bactéria ao TGI; (*ii*) mecanismos de probiose (adesão à mucosa intestinal, estímulo do sistema imune de mucosa intestinal, antagonismo bacteriano, produção de metabólitos bioativos) e, (*iii*) segurança do probiótico para consumo humano e veterinário (atividade hemolítica, produção de aminas biogênicas, genes associados à virulência e resistência aos antibióticos) (Papadimitrou et al., 2015; Castro-López et al., 2021).

2.5. Mecanismos de Adaptação/ Probiose

2.5.1. Sobrevivência a Agentes Estressores do TGI

Para produzir efeitos benéficos à saúde do hospedeiro, duas propriedades fundamentais para os microrganismos probióticos devem ser destacadas: capacidade de resistir e sobreviver à acidez estomacal, aos sais biliares e secreções pancreáticas durante o trânsito pelo TGI, e capacidade em aderir à mucosa intestinal (Vélez et al., 2007; Papadimitriou et al., 2016).

Os principais mecanismos desencadeados por espécies de bactérias lácticas, assim como outras bactérias, para lidar com agentes estressores do TGI são: reparos do DNA, mudança na composição de peptideoglicano e ácidos graxos da membrana celular, indução de chaperonas e proteases, acúmulo de solutos, e desintoxicação de espécies reativas de oxigênio (ROS, do inglês *Reactive Oxygen Species*) (Gaucher et al., 2019). Esses mecanismos dependem da expressão ou supressão coordenada de genes que atuam em conjunto para melhorar a tolerância a diferentes tipos de estresses (Papadimitriou et al., 2016).

As bactérias lácticas também codificam sistemas regulatórios de 2 componentes (2CRS,

do inglês *Two-component regulatory system*) que promovem respostas rápidas a estresses ambientais. Esses sistemas geralmente consistem em uma histidina quinase (HPK) e um gene regulador de resposta citoplasmática (RR), os quais trabalham para detectar e reagir às mudanças no ambiente (Yu et al., 2014; Monedero etal., 2017), facilitando a resistência a estresses.

Com o auxílio das ômicas, muitos genes envolvidos na resposta a diferentes tipos de estresses vêm sendo identificados em diferentes espécies de *Lactobacillus* (*L. reuteri, L. rhamnosus, L. johnsonii, L. casei, L. delbrueckii*, e outras). Entre esses genes incluem-se: hidrolases de sais biliares (*bsh*), chaperonas moleculares (*dnaK, hsp, cpl* e *groEL)* (Ferreira et al., 2013; Arnold et al., 2018; Zhang et al., 2019), enolase (*eno*), glicina betaína (*gb*); arginina desaminase (*arcA*), gliceraldeído-3-fosfato desidrogenase (*gap*) (Papadimitriou et al., 2016; Chen et al., 2019), entre outros, e que estão envolvidos diretamente em processos de estresse no TGI, como acidez e sais biliares.

2.5.2. Interação com a Mucosa Intestinal e Estímulo do Sistema Imune

As bactérias probióticas devem também possuir a habilidade de aderir à mucosa intestinal, promovendo interações diretas na modulação da resposta imune do hospedeiroe inibindo o crescimento de patógenos por meio dos mecanismos de exclusão competitiva e antagonismo bacteriano (Vélez et al., 2007; Plaza-Diaz et al., 2019).

A interação entre probióticos e a mucosa do hospedeiro ocorre por meio de diferentes proteínas relacionadas à adesão (adesinas) que reconhecem, se ligam ou interagem com regiões receptoras específicas da célula do hospedeiro ou de outros componentes do lúmen intestinal, como a camada de muco e a matriz extracelular, por exemplo. Dentre as proteínas com propriedades de adesão identificadas *in silico* nos genomas de diferentes espécies de *Lactobacillus* incluem-se as de ligação ao muco (Mubs), a adesina específica para manose (Msa), o fator de ligação ao muco (MBF), a sortase A-LPXTG (srtA), as da camada S (Slps), a de ligação à fibronectina (FbpA), a de ligação ao colágeno (CnPB), a promotora da adesão ao muco (MapA), o fator de alongamento Tu (EF-Tu), a de choque térmico GroEL, entre outras (Turpin et al., 2012; Sengupta et al., 2013; Houeix et al., 2019).

A capacidade de adesão à mucosa intestinal por bactérias probióticas ou pela microbiota permite, por meio de receptores como os TLRs, a interação desses microrganismos com células epiteliais, células dendríticas, macrófagos e linfócitos, com subsequente estimulação do sistema imunológico do hospedeiro via produção de moléculas com funções imunomoduladoras e anti-inflamatórias (Plaza-Diaz et al., 2019).

Entre os fatores que promovem interação desses microrganismos com a mucosa intestinal, as proteínas da camada S (Slps), como SlpA, SlpB, SlpE, SlpH vêm sendo identificadas em bactérias probióticas como capazes de estimular o sistema imune do hospedeiro, conforme já reportado para *L. acidophilus* (Li et al., 2011), *L. helveticus* (Taverniti et al., 2013) e *P. freudenreichii* (Le Maréchal et al. 2015; Deutsch etal., 2017). Além disso, outros componentes da membrana celular bacteriana merecem destaque nesse processo, incluindo exopolissacarideos, peptideoglicanos, ácido teicóico e lipoteicóico, entre outros (Sengupta et al., 2013).

2.5.3. Exclusão Competitiva e Antagonismo Bacteriano

A exclusão competitiva refere-se à situação em que uma espécie de um determinado microrganismo (bactéria ou fungo, por exemplo) compete pelos nutrientes disponíveis e pelos locais de adesão à mucosa com outros microrganismos. Para obter uma vantagem competitiva, as bactérias também podem modificar seu ambiente para torná-lo menos adequado para outros microrganismos que ocupem seu nicho ecológico, em um processo denominado de antagonismo bacteriano. Um dos exemplos desse tipo de modificação é a produção de compostos orgânicos como lactato, peróxido de hidrogênio, entre outros, que diminuem o pH do lúmen intestinal, criando um microambiente hostil para o crescimento de bactérias patogênicas (Bermudez-Brito et al., 2012; Plaza-Diaz et al., 2019). Além disso, deve-se destacar o papel de moléculas antimicrobianas produzidas por estes microrganismos, como as bacteriocinas.

As bacteriocinas formam um grupo heterogêneo de peptídeos antimicrobianos contendo cerca de 30-60 aminoácidos, produzidos por diferentes espécies bacterianas, cuja principal função é matar e/ou inibir o crescimento de outras bactérias (Mokoena et al., 2017). Elas atuam via formação de poros na membrana citoplasmática das bactérias alvos, o que altera a permeabilidade da membrana, aumenta o fluxo de íons transmembranares, ocasionando uma queda no pH intracelular, inibindo processos enzimáticos e levando a célula à morte (Todorov, 2009; Mokoena et al., 2017).

Baseado em suas estruturas primárias, pesos moleculares, modificações pós- traducionais e características genéticas, as bacteriocinas são categorizadas em três principais classes: *i*) Classe I, peptídeos catiônicos e hidrofóbicos, <5 kDa (nisina e lactocina); *ii*) Classe II, peptídeos hidrofóbicos não modificados, estáveis ao calor, catiônicos, <10 kDa. A depender do seu modo de ação, esta classe é subdividida em classe IIa (pediocina PA1 e leucocina) e classe IIb (plantaricina A e enterocina X); e por fim *iii*) Classe III, peptídeos hidrofílicos e pouco estáveis ao calor, >30 kDa (helveticina J, enterolisina) (Preciado etal., 2016; Mokoena et al., 2017).

Muitas dessas bacteriocinas são produzidas por diferentes espécies de bactérias lácticas, e alguns estudos já identificaram tanto genes relacionados à produção desses antimicrobianos por estes microrganismos como também vêm demonstrando a sua capacidade em inibir diversas bactérias patogênicas, incluindo *Gardnerella vaginalis*(Gaspar et al., 2018), *Staphylococcus aureus, Listeria monocytogenes* (Wang et al., 2018), *Salmonella enterica* subsp. *enterica* serovar Thyphi, *Enterococcus faecalis, Pseudomonas aeruginosa* (Prabhurajeshwar et al., 2019), bem como outros *Lactobacillus* (Oliveira et al., 2017), entre outras.

2.5.4. Fermentação por Bactérias Lácticas e Produção de Metábolitos

Outro aspecto importante relacionado à probiose de bactérias lácticas é a capacidade metabólica e fermentativa com subsequente produção de metabólitos bioativos.

As bactérias lácticas têm uma alta importância industrial, principalmente no setor de produtos fermentados, devido à elevada capacidade de fornecer características organolépticas desejáveis (Cruz et al., 2010), produzir nutrientes (Rakhmanova et al., 2018) e aumentar o valor nutricional desses produtos (Oliveira et al., 2017; Dimitrellou et al., 2019).

Muitas destas caracteristicas são produzidas durante a proteólise das proteínas do leite ou de seus derivados, por meio da utilização de proteinases de ligação à membrana, peptidases (aminopeptidases, endopetidases, prolina peptidase) e transportadores de peptídeos, o que consequentemente acaba gerando a formação de peptídeos e compostos bioativos que conferem benéficios ao hospedeiro (Liu et al., 2010; Broadbent et al., 2011;Raveschot et al., 2018).

Além de serem capazes de quebrar as proteínas do leite, estes microrganismos são capazes também de metabolizar diferentes tipos de carboidratos como lactose, fruto-oligossacarídeo (FOS), galactose, frutose, manose, arabinose, xilose, ribose, entre outros, o que lhes fornecem vantagens competitivas para colonizar ecossistemas com diferentes fontes de carboidratos (Watson et al., 2012; Pot et al. 2014). O uso dessa variedade de substratos permite a utilização de diferentes vias metabólicas, o que torna as bactérias lácticas tão destacadas em termos de potencial de fermentação (Saulnier et al., 2011; Buron-Moles et al., 2019).

Os açúcares são fontes primárias de carbono e energia para diversas bactérias lácticas cultivadas em substratos usados para fermentação, bem como em meios de cultivo laboratorial. De acordo com o tipo de via metabólica utilizada durante a fermentação de carboidratos, o gênero *Lactobacillus* pode ser dividido em 2 principais grupos: (***i***) obrigatoriamente homofermentativo, que produz apenas ácido lático como produto final do metabolismo de carboidratos pela via glicolítica (também conhecida como via EMP-Embden-Meyerhof-Parnas) (**Figura 5**). Dentro desse grupo se incluem as espécies como: *L. acidophilus, L. delbrueckii, L.*

helveticus, L. salivarius (Kandler, 1983)e (***ii***) obrigatoriamente heterofermentativo, que produz ácido láctico e acético, ou etanol e CO_2 como produtos finais do metabolismo de carboidratos pela via da fosfocetolase (PK). As principais espécies incluídas nesse grupo são *L. brevis, L. buchneri, L. fermentum, L. reuteri* (Kandler, 1983) (**Figura 5**).

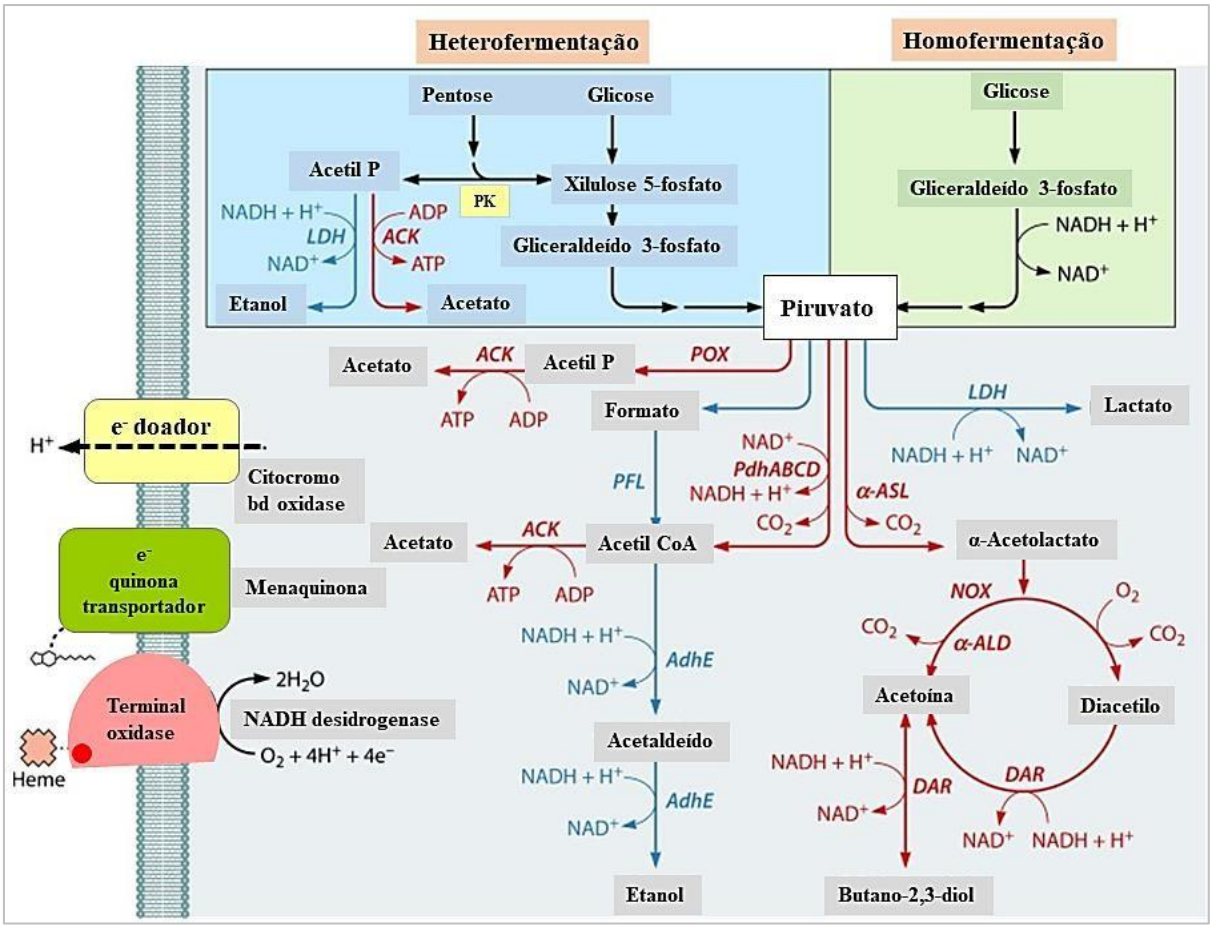


Figura 5. Representação esquemática da via de homo e heterofermentação. Glicose dentro da célula passa por diversas etapas de metabolização até formar o subproduto piruvato, que ao ser utilizado como substratopela enzima lactato desidrogenase conduz à produção de lactato (homofermentação) e outros compostos. Por outro lado, glicose ou outro açucar (pentose) dentro da célula passa por diversas etapas de metabolização até formar o subproduto Xilulose-5-fosfato. A metabolização deste subproduto leva à produção de acetato, etanol e lactato por meio da via fosfocetolase, utilizando a enzima xilulose-5-fosfato fosfocetolase. **Fonte**: Adaptado de Papadimitrou et al. (2016).

Durante o processo de fermentação, as espécies de bactérias lácticas, como *L. delbrueckii* subsp. *bulgaricus*, utilizam vias metabólicas especificas (a via glicolítica e a fosfocetolase) para a degradação dos carboidratos. Como resultado final desse processo, essas bactérias são capazes de produzir compostos orgânicos como lactato e SCFA (Le Blanc et al., 2017; Venegas et al., 2019).

Os SCFAs possuem variadas funções. Estudos têm demonstrado a capacidade desses compostos em modular a função das células imunes no intestino e em outros tecidos, apresentando principalmente efeito anti-inflamatório (Tan et al., 2014; Luu e Visekruna, 2019),

o que os tornam uma ferramenta biotecnológica potencial para o tratamento de IBDs, mucosite intestinal, entre outras doenças que afetam o TGI.

É importante salientar que determinadas espécies de bactérias lácticas também são capazes de produzir vitaminas, principalmente as do complexo B, tais como tiamina (B1), riboflavina (B2), biotina (B7), folato (B9) e cobalamina (B12) (Le Blanc et al., 2011; Yoshii et al., 2019). As vitaminas são micronutrientes essenciais à saúde e que participam de inúmeras reações bioquímicas. A falta desses compostos está associada a diferentes condições patológicas e estados fisiológicos, como desnutrição, doenças cardiovasculares, beribéri, dermatite, osteoporose, má formação do tubo neural, entre outras (Smith e Garg, 2017; Wang et al., 2019).

Uma vez que a espécie humana é incapaz de sintetizar as vitaminas do complexo B, estas devem ser adquiridas de fontes exógenas. Assim, o consumo de microrganismos probióticos capazes de produzí-las naturalmente seria uma solução para os problemas decorrentes de sua deficiência no organismo. Nesse contexto, diferentes *Lactobacillus*(*L. casei*; *L. gasseri, L. reuteri, L. fermentum, L. rhamnosus, L. plantarum*, e outras) vêm sendo reportadas quanto à capacidade em produzir diferentes tipos de vitaminas (Russo et al., 2014; Torres et al., 2016).

2.6. Aspectos de Segurança

Apesar de muitas linhagens probióticas comerciais serem geralmente reconhecidas como seguras (GRAS, do inglês, *Generally Recognized as Safe*), bactérias candidatas a serem probiótico devem ser avaliadas quanto a características relacionadas a segurança para o consumo humano e veterinário (Zhang et al., 2012; Pariza et al., 2015), devido alguns estudos reportarem associação entre surgimento de infecções e o consumo de probióticos (Kunz et al., 2004; Darbro et al., 2009; Yelin et al., 2019).

Muitas dessas características vêm sendo identificadas por meio de análises genômicas em conjunto com ensaios *in vitro* e *in vivo*. Nesse contexto, entre as principais análises realizadas para avaliação do perfil de segurança de novos probióticos destacam-se: (i) identificação de fatores de virulência (Senan et al., 2015); (ii) genes relacionados à resistência aos antibióticos de relevância clínica e produção de aminas biogênicas (Alayande et al., 2020); e (iii) identificação de fatores genéticos relacionados com a capacidade desses microrganismos promoverem a degradação de mucina, acidose e atividade hemolítica (Casarotti et al., 2017; Pradhan et al., 2010).

A principal preocupação quanto à segurança dos probióticos é a resistência aos antibióticos, pois essas linhagens podem transferir genes de resistência de diferentes

antimicrobianos para bactérias patogênicas na microbiota intestinal, o que pode representar um sério risco para o tratamento de pacientes com infecções bacterianas (Ventola et al., 2015). Além disso, é importante destacar a necessidade de se identificar a presença de elementos genéticos responsáveis por promover a disseminação desses genes, como plasmídeos, elementos de inserção (transposons) (Partridge et al., 2018), regiões de fagos e ilhas genômicas (GEIs, do inglês *Genomic Islands*) (Rahimi et al., 2012; Jani et al., 2017), bem como regiões de sistema CRISPR-Cas, que conferem imunidade a essas bactérias contra a inserção desses elementos genéticos móveis ao seu genoma (Alexandraki et al., 2019).

Além da presença de genes relacionados à resistência a antibióticos, genes relacionados à produção de metabólitos secundários (por exemplo, D-lactato), toxinas (como hemolisinas) e compostos tóxicos (como aminas biogênicas) também vêm sendo estudados por meio de análises ômicas em linhagens de bactérias lácticas (Surachat et al., 2017; Chokesajjawatee et al., 2020).

A hemólise é um processo em que ocorre a lise de células vermelhas sanguíneas (eritrócitos). Este é um reconhecido processo de virulência utilizado por muitos patógenos, ocasionando ao hospedeiro tanto anemia quanto edema (Vesterlund et al., 2007). A depender do grau de hemólise das células sanguíneas, esse processo pode ser divido em: α-hemólise (hemólise parcial do sangue, representada por um halo de coloração esverdeada em torno das colônias bacterianas), β-hemólise (hemólise total do sangue, representada por um halo de coloração clara em torno das colônias bacterianas) e γ-hemólise (não há lise das células do sangue) (Xu et al., 2019).

Em relação às aminas biogênicas produzidas por esses microrganismos, como por exemplo putrescina, tiramina, histamina, tem sido reportado que esses compostos podem ser tóxicos quando consumidos em altas concentrações (50-100 mg/L), podendo ocasionar perda das características organolépticas de produtos fermentados, intoxicação manifestada na forma de diarreia, náuseas e vômito, e serem precursores na produção de agentes carcinorgênicos (Alvarez e Moreno-Arribas, 2014).

Além dos fatores acima reportados, a produção de enzimas capazes de degradar a mucina ou produzir D-lactato também foi proposta como fatores determinantes de virulência para alguns enteropatógenos (Pradhan et al., 2010). Assim, degradar mucina não é considerada uma característica desejável para algumas linhagens probióticas, pois pode contribuir para alterações na barreira da mucosa intestinal, favorecendo a translocação de patógenos e outros agentes tóxicos para o sangue e outros órgãos e, assim, causando sepsia, principalmente em pacientes imunocomprometidos (MacFIE, 2005). Por sua vez, a produção metabólica de D-lactato pode

resultar em acidose sanguínea, o que pode promover problemas neurológicos, respiratórios e renais ao hospedeiro (Pohanka, 2020).

Muitos genes envolvidos nos processos acima vêm sendo identificados em linhagens probióticas por meio de análises ômicas, como os genes: *hemolisina A* (envolvido no processo de hemólise) (Chokesajjawatee et al., 2020) , *tet (M*) (envolvido no processode resistência a antibiótico tetraciclina) (Campedelli et al., 2019), *ornitina descarboxilase* (envolvido no processo de produção da amina biogênica putrescina) (LI et al., 2018), *D-lactato desidrogen*ase (envolvido na produção de D-lactato) (Pohanka, 2020) e *α-N-acetilgalactosaminidases* (envolvido na degradação de mucina) (Tailford et al., 2019).

Além da identificação *in silico* e avaliação do fenótipo *in vitro* de genes associados a diferentes processos de segurança para o consumo de bactérias probióticas, deve-se enfatizar que estudos *in vivo* também vêm sendo realizados a fim de avaliar a capacidade de infectividade/toxicidade de bactérias probióticas. Nesse contexto, são avaliados alguns parâmetros como: perda de peso, translocação bacteriana, aumento do tamanho de órgãos como o baço e fígado, razão entre o peso do orgão e o peso corporal do animal, contagem de leucócitos e aumento de inflamação intestinal (Sanders et al., 2010).

A identificação de genes relacionados à segurança de diferentes linhagens bacterianas com perfil probiótico e avaliação das características fenotípicas após utilização desses microrganismos se torna de extrema importância na caraterização sobre funções biológicas e segurança de linhagens bacterianas utilizadas em aplicações probióticas. Além disso, deve-se ressaltar que os efeitos imunomodulatórios e anti-inflamatórios reportados cientificamente até então para os *Lactobacillus*, assim como outros probióticos, são espécie e linhagem dependentes (McFarland et al., 2018). Portanto, existe a necessidade de se identificar e caracterizar novas espécies e linhagens com potencial probiótico, bem como investigar seus efeitos em diferentes modelos de doenças e seu nível de segurança em animais sadios, de forma a avaliar possíveis efeitos adversos ao hospedeiro.

2.7. Caracterização probiótica de *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 é uma promissora linhagem com potencial probiótico que foi isolada de leite de vaca cru na região de La Plata, Argentina. Estudos *in vitro* mostraram que esta linhagem é capaz de resistir aos sais biliares (Kociubinski et al., 1999), e inibir o crescimento de *Pseudomonas aeruginosa* (Kociubinski et al., 1996) envolvida no processo de contaminação e deterioração de alimentos, e *Escherichia coli* enterohemorrágica (Hugo et al., 2008), um importante patógeno causador de diarréia

sanguinolenta que pode evoluir para síndrome hemolítico-urêmica, levando o paciente a óbito.

Além disso, CIDCA 133 também foi capaz de sobreviver e crescer na presença de moléculas antimicrobianas derivadas de enterócitos, como β-defensinas (Hugo et al., 2010; Hugo et al., 2012), e estimular o sistema imune inato e adaptativo de células eucarióticas (macrofágos e celulas dendriticas) infectadas com *Bacillus cereus* e *Citrobacter rodentium* (Rolny et al., 2016,Hugo et al., 2017), apresentando, portanto, propriedades imunomodulatórias potenciais para controlar a infecção em células eucarióticas gerada por agentes patogênicos.

Apesar de CIDCA 133 apresentar caracteristicas funcionais de um microrganismo probiótico, estas propriedades foram observadas somente em estudos *in vitro*. Não há dados na literatura avaliando o potencial probiótico dessa linhagem, principalmente seu efeito anti-inflamatório, usando modelos *in vivo*. Diante disso, nosso grupo de pesquisa é pioneiro na caracterização das propriedades funcionais dessa linhagem bacteriana, investigando fatores genéticos e mecanismos pelos quais CIDCA 133 exerce efeitos benéficos ao hospedeiro, e o nível de segurança que esta linhagem apresenta para ser utilizada em futuras formulações e aplicações probióticas em humanos e na área veterinária.

3. RELEVÂNCIA DA TESE

Muitas linhagens de bactérias lácticas caracterizadas como probióticas, com amplo potencial comercial, biotecnológico, e com grande quantidade de benefícios atribuídos à saúde do hospedeiro, estão sendo estudadas no âmbito genômico (Oliveira et al., 2017; Zhang et al., 2019; Chokesajjawatee et al., 2020). Dentro deste contexto, as ciências ômicas contribuem para uma caracterização mais detalhada no nível estrutural e funcional bacteriano e auxilia na descoberta de mecanismos moleculares e metabólicos relacionados aos efeitos probióticos destes microrganismos e ao nível de segurança para o consumo (Ventura et al., 2012; Salvetii et al., 2018). Além disso, a genômica comparativa tem permitido comparar o genoma de bactérias com perfil probiótico com novas linhagens candidatas, permitindo assim observar o grau de similaridade e diferenças no nível de conteúdo gênico entre essas linhagens que possa auxiliar, juntamente com estudos *in vitro* e *in vivo*, na caracterização probiótica e no direcionamento da possivel aplicação (indústria alimentícia, terapêutica e /ou biotecnológica) desses microrganismos (Fontana et al., 2019; Castro-López et a., 2021).

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 é uma bactéria que tem propriedades probióticas/terapêuticas relatadas apenas por estudos *in vitro* (Hugo et al., 2008, Rolny et al., 2016; Hugo et al., 2017). Não há evidências científicas sobre o potencial probiótico desta linhagem *in vivo*, bem como quais proteínas e mecanismos moleculares estariam relacionados aos seus efeitos benéficos. Nesse sentido, nesta pesquisa de Tese buscou-se caracterizar a linhagem *L. delbrueckii* CIDCA 133 quanto às suas propriedades probióticas *in vivo*, e investigar, por meio de análises de bioinformática, quais os possíveis genes e vias metabólicas estariam associados aos mecanismos moleculares que possam atuar na capacidade probiótica e imunomoduladora desta linhagem, bem como fatores genéticos, com posterior ratificação por modelos experimentais *in vitro* e *in vivo*, capazes de dar informações sobre o nível de segurança desta linhagem para o consumo. Além disso, investigamos se os possíveis fatores genéticos associados à probiose de CIDCA 133 são compartilhados com outras linhagens probióticas da espécie *Lactobacillus delbruecki* por meio de estudos pangenômicos.

Os dados obtidos a partir deste trabalho darão suporte a outros estudos direcionados a caracterizar, e identificar fatores genéticos e mecanismos relacionados aos efeitos benéficos e segurança para o consumo humano e veterinário de novos candidatos probióticos, que possam sustentar futuramente sua aplicação na área clinica e bioterapêutica . Além disso, os dados desta pesquisa de Tese darão suporte informacional para que novos bancos de dados, relacionados às caracteristicas funcionais e de segurança de microrganismos probióticos, sejam desenvolvidos.

4. OBJETIVOS DA TESE

4.1. Geral

- Caracterizar *in vitro, in vivo* e *in silico* a linhagem *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 quanto às suas propriedades probióticas e de segurança para o consumo.

4.2. Específicos

- Caracterizar o perfil probiótico de CIDCA 133 em modelo *in vivo*;

- Identificar possíveis genes envolvidos na adaptação, atividade metabólica, e anti-inflamatória de CIDCA 133;

- Avaliar o efeito imunomodulatório de CIDCA 133 após inativação e por meio de seus produtos secretados;

- Avaliar aspectos de segurança para o consumo e futuras aplicações probióticas de CIDCA 133;

- Comparar o conteúdo gênico de CIDCA 133 com outras linhagens probióticas da espécie *L. delbrueckii*.

CAPÍTULO I

5. CAPÍTULO I: Protective effect of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 in a model of 5 Fluorouracil-Induced intestinal mucositis

Este artigo cientifico foi publicado na revista *Journal of Functional Foods* (ISSN: 1756-4646) em fevereiro de 2019. Fator de impacto atual: 5.223.

Lactobacillus delbrueckii é uma espécie amplamente utilizada na indústria de laticínios. No entanto, alguns estudos vêm caracterizando como probiótico algumas linhagens dessa espécie com base em sua capacidade de resistir a estressores do trato gastrointestinal, inibição de patógenos e ação anti-inflamatória focado principalmente no tratamento de doenças do TGI, como câncer colorretal, colite ulcerativa, mucosite intestinal, entre outros. No entanto, deve-se ressaltar que estes efeitos são linhagem dependentes, o que mostra que novas linhagens probióticas dessa espécie devem ser identificadas e caracterizadas quanto à sua funcionalidade benéfica ao hospedeiro. O perfil probiótico *L. delbrueckii* CIDCA 133 tem sido relatado unicamente por meio de estudos *in vitro*. Assim, neste capítulo, avaliou-se o potencial probiótico (anti-inflamatório) dessa linhagem usando um modelo *in vivo* de inflamação induzida pelo quimioterápico 5-Fluorouracil. Para realizar o estudo, camundongos BALB/c foram inflamados com uma única dose do quimioterápico 5-FU (300 mg/kg) e tratados com CIDCA 133 (7.5×10^7 CFU/mL) através de uma formulação de leite fermentado durante 13 dias. O efeito protetor de CIDCA 133 foi investigado sob parâmetros clínicos (peso, consumo hidrico e alimentar dos animais, contagem de leucócitos), inflamatórios (atividade enzimática de MPO e EPO para avaliação do infiltrado inflamatório de neutrófilos e eosinófilos), histopatológicos (avaliação da arquitetura do epitélio intestinal, contagem de células produtoras de muco, e comprimento das vilosidades) e função de barreira (produção de IgA secretória e permeabilidade intestinal). Este trabalho foi pioneiro na caracterização funcional das propriedades probióticas de CIDCA 133 *in vivo*. Os resultados obtidos demostraram que o tratamento com leite fermentado por CIDCA 133 foi capaz de prevenir os danos histopatológicos e inflamatórios à mucosa intestinal induzidos pela administração de 5-FU, confirmando suas propriedades funcionais benéficas previamente relatadas em estudos *in vitro*, e abrindo perspectivas do uso dessa bactéria e das formulações terapêuticas derivadas dela na rotina da terapia oncológica.

Journal of Functional Foods 53 (2019) 197–207

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Protective effect of *Lactobacillus delbrueckii* subsp. *Lactis* CIDCA 133 in a model of 5 Fluorouracil-Induced intestinal mucositis

Luís Cláudio Lima De Jesus[a], Mariana Martins Drumond[a,b], André de Carvalho[a], Spencer S. Santos[c], Flaviano S. Martins[c], Ênio Ferreira[d], Renata Salgado Fernandes[e], André Luís Branco de Barros[e], Fillipe L.R. do Carmo[a], Pablo F. Perez[f], Vasco Azevedo[a,1], Pamela Mancha-Agresti[a,*,1]

[a] *Laboratório de Genética Celular e Molecular (LGCM), Instituto de Ciências Biológicas, Departamento de Biologia Geral, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil*
[b] *Centro Federal de Educação Tecnológica de Minas Gerais (CEFET/MG), Coordenação de Ciências, Belo Horizonte, Minas Gerais, Brazil*
[c] *Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*
[d] *Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[e] *Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*
[f] *Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA-CCT CONICET), UNLP, La Plata, Argentina and Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Cátedra de Microbiología, UNLP, La Plata, Argentina*

ARTICLE INFO

Keywords:
Probiotics
Cancer chemotherapy
5-Fluorouracil
Intestinal mucositis
Inflammation
Permeability

ABSTRACT

Mucositis is a cytotoxic side effect caused by chemotherapy drugs, such as 5-Fluorouracil (5-FU), being a serious clinical issue. *Lactobacillus* spp. could be a helpful strategy to alleviate 5-FU chemotherapy-caused intestinal damage, due to their ability to contribute to intestinal homeostasis through improvement of microbiota balance and immunomodulation. In this work we evaluated the effect of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 fermented milk in 5-FU-induced experimental mucositis. Intestinal histology, permeability and biochemical parameters showed that animals administrated with 5-FU and treated with CIDCA 133 fermented milk presented reduced intestinal IgA secretion and lower permeability in the small bowel. We showed that this strain preserves villus/crypt ratio, reduces the loss of goblet cells and inflammatory infiltration in ileum sections of 5-FU-treated animals. In conclusion, CIDCA 133 is able to prevent the intestinal mucosa damage caused by 5-FU revealing to be a promising strategy for intestinal mucositis treatment.

1. Introduction

Chemotherapy and radiotherapy are the principal treatments used in several types of cancer (Longley, Harkin, & Johnston, 2003; Sonis, 1998). The 5-Fluorouracil (5-FU) is one of the chemotherapeutic agents used in the clinical oncology practice. This drug acts on the proliferation of cancer cells through the inhibition of the thymidylate synthase (TS) enzyme (leading to unbalance of the nucleotide pool), as well as the incorporation of its metabolites into the DNA and/or RNA of these cells (Sonis, 2004), which impedes its normal functioning and induces apoptosis (Miura et al., 2010; Pinedo & Peters, 1988). However, this treatment, apart from destroying neoplastic cells, affect different cell populations of healthy tissue situated throughout the human body (Duncan & Grant, 2003), being mucositis one of the most prevalent adverse effects.

The mucositis is characterized by three phases (inflammation, epithelial degradation and ulceration) leading to quick loss of intestinal structure and functionality (Duncan & Grant, 2003) including damage

Abbreviations: 5-FU, 5-fluorouracil; CTL, control; CIDCA 133, *Lactobacillus delbrueckii* ssp. *lactis* CIDCA 133; CD, *crypt depths*; DMSO, dimethyl sulfoxide; DTPA, diethyleneaminopentacetic acid; EPO, eosinophil peroxidase; HTAB, hexadecyltrimethylammonium bromide; HE, hematoxylin and eosin; MPO, myeloperoxidase; MUC, mucositis; NAG, N-acetyl-beta-D-glucosaminidase; NO, nitric oxide; GIT, gastrointestinal tract; PBS, phosphate buffered saline; PMSF, phenylmethanesulfonyl fluoride; ROS, reactive oxygen species; SCFAs, short chain fatty acids; TS, thymidylate synthase; TER, trans, epithelial resistance; PAS, periodic acid schiff; VH, villus heights

* Corresponding author.
E-mail address: pamemancha@hotmail.com (P. Mancha-Agresti).
[1] These authors have contributed equally to senior authorship.

https://doi.org/10.1016/j.jff.2018.12.027
Received 24 October 2018; Received in revised form 18 December 2018; Accepted 18 December 2018

of the epithelial wall, increased intestinal permeability and bacterial translocation; disbalance of intestinal microbiota and reduced levels of mucin secretion (Stringer et al., 2009). Above effects lead to compromised food intake that in turn compromises cancer therapy (Logan, Gibson, Sonis, & Keefe, 2007; Soveri, Hermunen, de Gramont, Poussa, Quinaux, Bono, & Österlund, 2014).

Local anesthetics, analgesics and/or antibiotics are some proposed treatments for this disease. However, these palliative approaches present several side effects as short and temporary relief (Herbers, de Haan, van der Velden, Donnelly, & Blijlevens, 2014). Thus, the use of bacteria/yeast strains with probiotic potential constitute a promising perspective for the treatment of mucositis due to their ability to modify the composition of the intestinal microbial community, as well as their capability to improve the epithelial barrier function and the immunomodulating effect (Bastos et al., 2016; Carvalho et al., 2017; Justino et al., 2015; Oh, Lee, Lee, Lee, & Kim, 2017).

The probiotics are "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*" (Hill et al., 2014). In this context, several studies conducted with probiotic microorganisms have demonstrated strain-dependent effects in the prevention/treatment of intestinal disorders. Indeed, beneficial effects have been demonstrated in (i) maintenance of the epithelial barrier in dextran sulphate-induced intestinal inflammation in mice (Mennigen et al., 2009), (ii) protection of the intestinal function in a model of hydrogen peroxide-induced epithelial barrier disruption (Seth, Yan, Polk, & Rao, 2008), (iii) increase in trans-epithelial resistance (TER), IL-10 deficient mice (Ewaschuk et al., 2008). The mechanisms behind these probiotics effects are related or to the reduction in IL-12 and IFN-γ levels, and increased of IL-10 (Martins et al., 2009; Sokol et al., 2008), diminution of cell apoptosis by inhibition of caspase 3 activation (Dalmasso et al., 2006; Mennigen et al., 2009), prevention of oxidative damage by induction of mucosal glutathione biosynthesis (Lutgendorff et al., 2009), enhancing of mucine gene expression (Caballero-Franco, Keller, De Simone, & Chadee, 2007) among others. For all these reasons, the probiotics represent a promising adjuvant strategy for mucositis amelioration.

Pioneering studies using *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 strain (hereafter CIDCA 133) have shown its resistance to high acid and bile concentrations and the ability to inhibit the growth of food contaminants (Kociubinski, Pérez, & De Antoni, 1999; Kociubinski, Pérez, Añón, & De Antoni, 1996) and pathogenic microorganisms such as entero-hemorragic *E. coli* (Hugo, De Antoni, & Pérez, 2006). In addition, the CIDCA 133 strain, was able to decrease harmful bacterial enzymatic activities such as nitrate reductase (Hugo, Kakisu, De Antoni, & Pérez, 2008). Furthermore, CIDCA 133 strain has demonstrated to resist the inhibitory cationic effectors of the innate immune system in cultured human enterocytes (Hugo, De Antoni, & Pérez, 2010) and human ß-defensins (Hugo, Tymczyszyn, Gómez-Zavaglia, & Pérez, 2012). Another *in vitro* study on the interaction between CIDCA 133 strain with cultured murine macrophages (RAW 264.7 cells) infected with *C. rodentium* revealed that the presence of probiotic bacteria is able to stimulate phagocytosis, induces reactive oxygen species (ROS and NO), and promotes expression of surface markers related to antigen presentation (Hugo, Rolny, Romanin, & Pérez, 2017).

Altogether, these studies support the potential probiotic effect of CIDCA 133 strain and the promising activities that could be studied in different diseases models and their resulting response. Therefore, the aim of the present study was to investigate the effect of oral administration of *L. delbrueckii* subsp. *lactis* strain CIDCA 133 fermented milk in an *in vivo* model of 5-FU induced mucositis in BALB/c mice.

2. Materials and methods

2.1. Probiotic strain

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 strain was isolated from raw cow milk (Kociubinski et al., 1996), with probiotics characteristics described as was mentioned in previous section. This strain belongs to the culture collection of the Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina).

2.2. Dairy formulation and growth conditions

Frozen bacterial suspension (−80 °C) were reactivated in de Man, Rogosa and Sharpe (MRS) broth (Kasvi, Italia) at 37 °C for 16 h. Next, 50 µL of bacterial culture were inoculated into 15 mL of reconstituted skim milk (12% w/v) supplemented with glucose (2% w/v), yeast extract (1,2% w/v) (milk broth) for overnight growth at 37 °C. Afterwards, the culture was diluted 100 times in sterilized milk broth and administrated *ad libitum* for 12 h. Bacterial suspension were renewed every 12 h to avoid bacterial decantation and clogging. This beverage and standard chow diet were administered for 13 days *ad libitum* access. To confirm the administrated CFU to the mice, the number of colonies (viable bacteria) were counted by pour plate method (MRS-agar medium) by counting the colony forming unit (CFU) after incubation of 16 h.

2.3. Animal trial and experimental design

All experiments described were conducted on male BALB/c mice (4–6 weeks old, weight 21–24 g) obtained from Centro de Bioterismo (CEBIO) of the Federal University of Minas Gerais (Belo Horizonte, Minas Gerais, Brazil). Animals were kept in polycarbonate boxes under controlled conditions: temperature around 21 ± 2 °C, humidity of 55 ± 10%, photoperiod of 12 h light/dark. All procedures were in compliance with the Brazilian College of Animal Experimentation (COBEA) and were approved by the Local Animal Experimental Ethics Committee (CEUA-UFMG, Protocol no. 366/2012).

Mice were randomly split into 4 groups (8 animals per group): Control (CTL), Control + probiotic (CIDCA 133), Mucositis (MUC), and mucositis + probiotic (MUC + CIDCA 133). Animals were orally fed on a daily basis with non-fermented milk (CTL and MUC) or fermented milk by CIDCA 133 (7.5×10^7 CFU/mL) (CIDCA 133 and MUC + CIDCA 133) (the water was substituted by fermented milk or only milk broth) over a period of 13 days. In order to induce gastrointestinal mucositis on day 10, mice (MUC and MUC + CIDCA 133) received a single intraperitoneal injection (*i.p.*) of 5-FU (300 mg/kg) (Fauldfluor®, Libbs, São Paulo, Brazil) as previously reported (Carvalho et al., 2017). Control groups (CTL and CIDCA 133) received NaCl 0.9% (w/v) instead of 5-FU. At 72 h after *i.p.* administration either 5-FU or saline solution, the animals were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg) mixture (Agener União) (Fig. 1). The blood and the small intestine were collected for analysis. Body weight, milk and feed intake were assessed daily.

2.4. Intestinal histology, morphology and goblet cells analysis

After euthanasia, the entire small intestine was removed and its length was measured, ileum sections (approximately 5 cm) were longitudinally opened, carefully washed and rolls were excised and handled for histological analysis. Tissue samples were placed in 10% buffered formaldehyde for 24 h. Then, samples were embedded in paraffin wax and slides of 4 µm sections were mounted on glass slides and stained with hematoxylin and eosin (HE) or Periodic Acid Schiff (PAS). In HE-stained slides, alterations of the mucosal architecture and polymorphonuclear cells infiltrate were analyzed using a histopathological grading system (Soares et al., 2008). Ten images per specimen were captured with a BX41 optical microscope (Olympus, Tokyo, Japan) and digital images were processed using *ImageJ 1.51j.8* software (NIH, Bethesda, MD, USA), for morphological examination. Image acquisition was performed with a 40× magnification objective. Villus heights (VH)

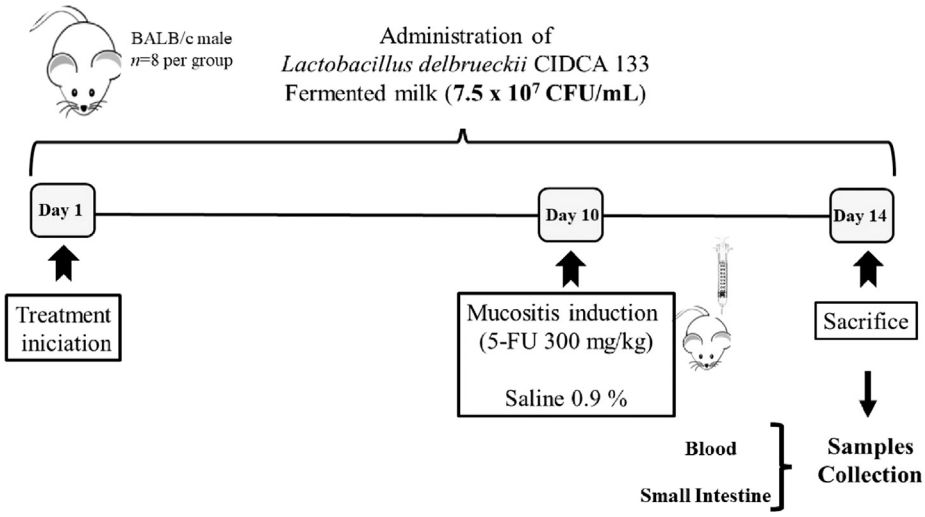


Fig. 1. Experimental protocol of mucositis induced in a murine model. BALB/c mice (n = 8 animals per group) were treated with *L. delbrueckii* subsp. *lactis* CIDCA 133 fermented milk for 13 days. 300 mg/kg of 5-FU by intraperitoneal injection was administrated, on day 10, to induce the mucositis. Control groups received 0.9% (w/v) NaCl (*i.p.*).

measurements (from villus tip to villus- crypt junction) and crypt depths (CD) (defined as invagination depth between adjacent villi) per small intestinal tissue section (10 sections/mouse) were measured. The values were averaged. The ratio of villus height/crypt depth from the intestinal epithelium was also acquired. The study of goblet cells was done in PAS-stained samples.

2.5. Intestinal permeability

In order to study the epithelial permeability from lumen to blood, intestinal permeability was determined by blood radioactivity of diethylenetriamine pentaacetic acid (DTPA) labelled with technecium-99 m (^{99m}Tc). The DTPA probe is larger enough (molecular weight: 500–700 Da) to allow for the study of intestinal permeability through the paracellular pathway (Jørgensen, Nielsen, Espersen, & Perner, 2006). Briefly, at the end of the experimental design, all mice received 0.1 mL of ^{99m}Tc-DTPA solution containing 18.5 MBq of activity by gavage. After 4 h, mice were anesthetized and blood samples were collected, weighted, and placed in appropriate tubes for radioactivity determination using a Wallac Wizard 1470-020 automated gamma counter (Perkin Elmer, Waltham, MA, USA). A standard dosage containing the same injected amount was counted simultaneously in a separate tube, which was defined as 100% radioactivity. The results were expressed as the percentage of injected dose per gram (%ID/g) of blood.

2.6. Leukocyte count

Blood samples were collected by axial plexus. The total number of white cells was measured by an automatic hematological counter (Bio-2900 Vet, Bioeasy, EUA). Results were expressed as number of leukocytes per μL of sample.

2.7. Enzyme assay: Intestinal myeloperoxidase (MPO) and eosinophil peroxidase (EPO) activity

To evaluate the extent of neutrophil and eosinophil accumulation in the intestinal mucosa the MPO and EPO activity assays were performed (Bradley, Christensen, & Rothstein, 1982; Vieira et al., 2012). Enzyme activity values are considered appropriate and reliable markers for neutrophil and eosinophil infiltration, respectively (Vieira et al., 2009). Briefly, 100 mg of tissue was homogenized in 1.9 mL phosphate buffered saline (PBS, pH 7.4) using a tissue homogenizer. The homogenate was centrifuged (3000*g* for 10 min), then the pellets were subjected to hypotonic lysis (1.5 mL of 0.2% NaCl) and osmolarity was restored with 1.5 mL of a 1.6% NaCl solution containing 5% glucose. Then, samples were centrifuged (3000*g* for 10 min) and the pellet was resuspended in 0.5% hexadecyltrimethylammonium bromide (HTAB-Sigma-Aldrich, USA) in phosphate buffer. The tissue suspension was homogenized, freeze-thawed three times in liquid nitrogen and centrifuged for 15 min at 3000*g*. The resulting supernatant was used in the colorimetric assay to measure EPO and MPO activities.

For EPO assessment, 75 μL of supernatant was added to 75 μL of 1.5 mM *o*-phenylenediamine (OPD-Sigma-Aldrich, USA), diluted in 0.075 mM Tris–HCl and 6.6 mM H_2O_2 and incubated at 37 °C during 30 min. For MPO quantification, 25 μL of supernatant was added to 25 μL of 1.6 mM 3,3,5,5′-Tetramethylbenzidine (TMB-Sigma-Aldrich, USA) in dimethyl sulfoxide (DMSO-Sigma-Aldrich, USA). After the addition of 100 μL 0.5 mM H_2O_2, the solution was incubated at 37 °C for 5 min. Both reactions were stopped by adding 50 μL of 1 M H_2SO_4. Absorbance was measured at 492 nm (EPO) and 450 nm (MPO) on a microplate spectrophotometer (Bio-Rad 450 model, Bio-Rad Laboratories, Hercules, CA, USA). Results were reported as MPO or EPO arbitrary units/mg of tissue.

2.8. Intestinal secretory IgA (sIgA)

Levels of sIgA were determined by enzyme-linked immunosorbent assay (ELISA), as described by Martins et al. (2009). To this end, the small intestine of each mouse was removed by an incision at the gastroduodenal and ileocecal junctions. The content was withdrawn, weighed, and supplemented with PBS 0.1 M (pH 7.2) with protease inhibitors cocktails [1 μM aprotinin, 25 μM leupeptin, 1 μM pepstatin and 1 mM Phenylmethanesulfonyl fluoride (PMSF)], this mixture was added in the ratio of 500 mg of intestinal contents per 2.0 mL PBS. Afterwards, samples were centrifuged (5.000 rpm for 30 min at 4 °C), and the supernatant was collected and frozen at −80 °C for further dosage of the immunoglobulin.

To determinate total sIgA, microtiter plates (Nunc-Immuno Plates, MaxiSorp) were coated with goat anti-mouse IgA antibody (M-8769, Sigma, St. Louis, USA) in coat buffer (1 M Na_2CO_3; 0,1M $NaHCO_3$; pH 9.6) for 18 h at 4 °C. Plates were washed 5 times with washing solution (0.1 M PBS containing 0.05% Tween 20) and blocked with 200 μL of blocking solution (1% albumin in PBS Tween 20) for 1 h at room temperature. The plate was then washed 5 times and the pre-diluted intestinal fluids (1:1000) in 0.1 M PBS containing 0.05% Tween 20 were added and incubated at room temperature for 1 h. After this time, the plates were washed 5 times and biotin-conjugated anti-mouse IgA antibody (A 4789-Sigma, St. Louis, MO, USA) in PBS-0.05% Tween 20 (1:1000) was added and incubated for 1 h. After washing 100 μL/well of OPD (1 mg/mL) and 0.04% H_2O_2 substrates were added and

incubated for 10 min at room temperature. The reaction was stopped by the addition of 20 µL/well of 1 M H_2SO_4. The absorbance was determined at 492 nm using on a microplate spectrophotometer (Bio-Rad model 450, Bio-Rad Laboratories, Hercules, CA, USA). The results of the concentration of sIgA were expressed in µg/mL of intestinal contents.

2.9. Statistical analysis

Data normality was assessed by Kolmogorov-Smirnov test. Normal data (body weight loss, small bowel length, MPO activity, leukocytes count, intestinal permeability, villus/crypt ratio, histological score and globet cells count) were evaluated by analysis of variance (ANOVA) followed by the Bonferroni post-test (parametric distribution). Non-normal data (non-parametric distribution) (food and milk intake, EPO activity and sIgA levels) were evaluated by Kruskal-Wallis test followed by the Dunn's post-test. Two-ANOVA followed by the Bonferroni test was performed to compare the variation of body weight between all experimental groups. Mann Whitney test was performed to compare food and milk intake before and after mucositis induction. All data were analyzed using GraphPad Prism 5.0 software, and $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Weight loss, shortening intestinal length and leucopenia was relieved in 5-FU induced mucositis mice by CIDCA 133 fermented milk treatment

Total milk and food intake was similar in all analyzed groups before the induction of mucositis. Animals of all groups consumed on average the same amount of food and drink (3 g and 5 mL/day/animal respectively). There were no significant statistical differences between the experimental groups. After induction of mucositis, the MUC group reduced food and milk intake, as expected (2.18 ± 1.29 g, and 2.81 ± 1.48 mL, $p < 0.01$). Nevertheless, administration of CIDCA 133 strain (7.5×10^7 CFU/mL) did not modify food and milk intake (1.90 ± 1.14 g and 2.65 ± 1.41 mL) (Fig. 2A and B). Accordingly, the analysis of time-course weight of mice showed a significant reduction in the 5-FU-treated variation from day 12 (MUC and MUC + CIDCA 133 groups). However, body weight variation was significantly lower in CIDCA 133-treated mice (Fig. 2C). In contrast, body weight of mice injected with 5-FU were significantly lower ($p < 0.001$). Noteworthy, the body weight loss of the mice in MUC + CIDCA 133 group was significantly lower (approximately 3%) than those in the MUC group (about 9%) ($p < 0.001$) (Fig. 2D). These results showed that treatment with CIDCA 133 fermented milk prevents the loss of body mass induced by chemotherapy accompanied by less intake of food and milk. It is also observed that treatment with CIDCA 133 without mucositis induction does not have any influence on the weight of the animals during the experimental period (Fig. 2C and D). It is worth noting that no mortality was observed during the experiment.

Intestinal shortening, was observed in MUC group (~46.00 ± 1.38 cm). Interestingly, we observed that animals treated with CIDCA 133 fermented milk were able to prevent the shortening of the intestinal length caused by the 5-FU (50.75 ± 2.31 cm, $p < 0.01$) as shown in Fig. 3A. As expected, the negative control groups (CTL and CIDCA 133) showed normal intestinal length (~55.00 ± 2.43 cm).

Significant leukopenia was observed after 5-FU administration (0.627 ± 0.13 cells × 10^3/µL) compared to the negative control (CTL) (4.350 ± 1.06 cells × 10^3/µL, $p < 0.001$) (Fig. 3B). However, this effect of 5-FU was minimized in animals treated with CIDCA 133 fermented milk (MUC + CIDCA 133 group) (2.043 ± 0.93 cells × 10^3/µL), which significantly ($p < 0.05$) prevented the reduction of the total leukocyte blood rate induced by chemotherapy. No statistical differences were observed between CTL and CICDA 133 groups (4.769 ± 1.49 cells × 10^3/µL) that were not treated for mucositis induction (Fig. 3B).

3.2. CIDCA 133 fermented milk treatment reduces inflammatory parameters in intestinal mucosa

To verify whether treatment with CIDCA 133 fermented milk could have an effect on reducing the infiltration of neutrophils and eosinophils in the intestinal mucosal layer, the activity of myeloperoxidase (MPO) and eosinophil peroxidase (EPO) were measured in ileum cell lysates. As shown in Fig. 3(C and D), mucositis induced significant recruitment of intestinal neutrophils and eosinophils. These parameters were significantly reduced (1.02 ± 0.20 U/mg for MPO, $p < 0.001$; 0.40 ± 0.07 U/mg for EPO, $p < 0.01$) by administration of CIDCA 133 fermented milk in inflamed animals.

3.3. Reduced IgA levels and intestinal permeability in mice with mucositis treated with CIDCA 133 fermented milk

Secretory IgA (sIgA) in the small intestine was investigated. As shown in Fig. 3E, 5-FU induced significant increase of sIgA levels in the intestinal fluid (MUC group: 3.684 ± 303.9 µg/mL) as compared with negative controls (CTL group: 2.402 ± 230.0 µg/mL, $p < 0.001$) and CIDCA 133 group (without mucositis induction: 2.439 ± 296.0 µg/mL, $p < 0.001$). The treatment with CIDCA 133 fermented milk (MUC + CIDCA 133 group) had significantly reduced levels of sIgA (2.347 ± 247 µg/mL) ($p < 0.001$) in the intestinal fluid of the animals which received the chemotherapy treatment.

Alteration of mucosal permeability is another side effect of 5-FU treatment. To determine whether the administration of CIDCA 133 fermented milk prevents mucosal damage, the intestinal permeability was evaluated. At 72 h after the 5-FU injection, intestinal permeability was significantly increased in the MUC group (0.13 ± 0.021 %ID/g) compared to the CTL (0.019 ± 0.005 %ID/g) and CIDCA 133 (0.014 ± 0.006 %ID/g) groups (without mucositis induction) ($p < 0.001$). Interestingly the animals that received CIDCA 133 fermented milk (MUC + CIDCA 133) group were able to reduce significantly the effect of 5-FU treatment on intestinal permeability (0.036 ± 0.015 %ID/g) as compared to the MUC group mice ($p < 0.001$) (Fig. 3F).

3.4. Mucosal damage and loss of goblet cells were reduced by CIDCA 133 fermented milk administration

Alterations such as villus shortening, increase in crypt depth, intense inflammatory cell infiltrate in the villi, *lamina propria* and submucosa, ulceration, edema, vacuolization, and decrease in goblet cells number, were clearly observed in inflamed animals (Fig. 4A), which correlated with histological scores (Fig. 4B). Our results showed that feeding of inflamed mice with CIDCA 133 fermented milk (MUC + CIDCA 133group) was able to ameliorate 5-FU-induced intestinal mucosal damage. Indeed, crypts depth, villus height, as well as villus/crypt ratio were significantly restored. In addition, inflammatory infiltrate was also decreased when compared with inflamed group (MUC) ($p < 0.05$) (Fig. 4C and D). Consequently, the villus/crypt ratio was increased (Fig. 4E). The histological scores correlated with above results demonstrating a reduction in scores in the MUC + CIDCA 133 group as compared with the MUC group (Fig. 4B and E).

As expected the MUC group presented a significant decrease in goblet cells (11.80 ± 3.47 cell/field). However, feeding of 5-FU-treated animals with milk containing CIDCA 133 strain (MUC + CIDCA 133) was able to prevent the loss of goblet cells (23.85 ± 5.53 cell/field) ($p < 0.05$) as compared with MUC group (Fig. 5). In addition, no histopathological nor morphological changes were observed for the CTL and CIDCA 133 groups (without mucositis induction) (Fig. 4).

4. Discussion

Intestinal mucositis, as a side effect of chemotherapy or

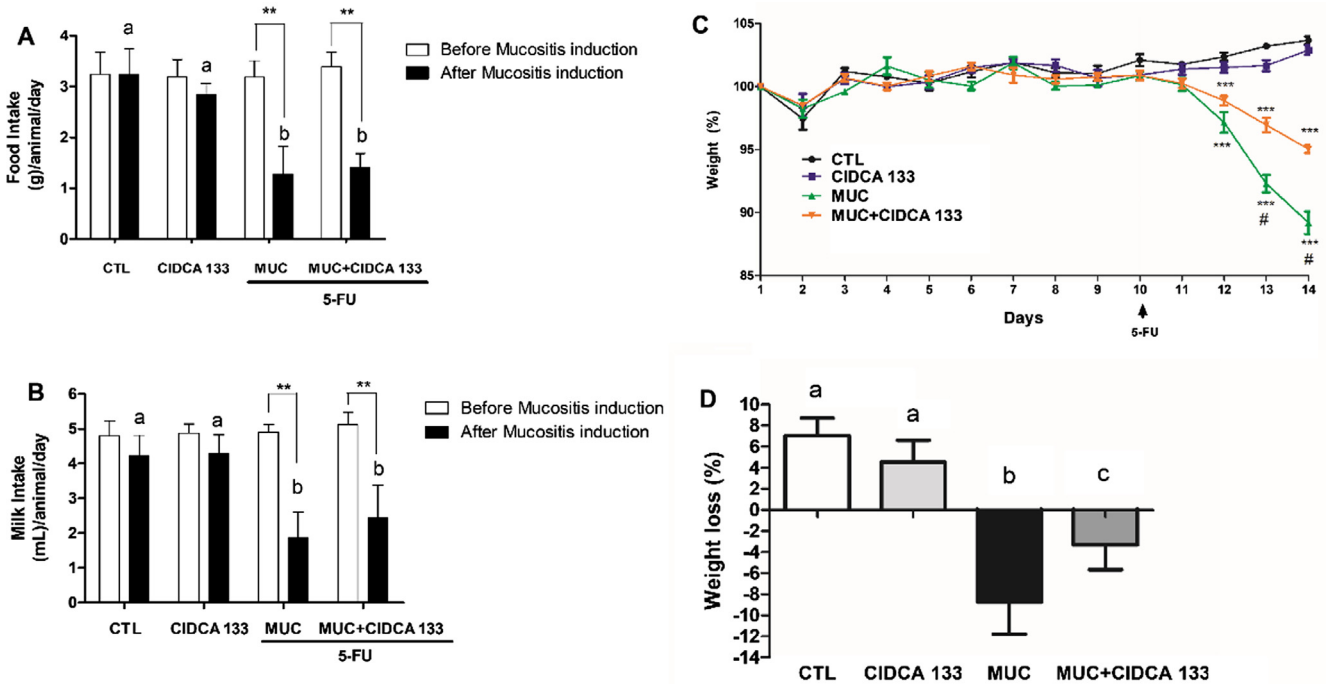


Fig. 2. Food, milk intake and body weight of animals: (A) Food and (B) Milk intake, (C) Body weight variation and (D) (%) Body weight loss measured daily. Mice received intraperitoneal 5-FU (300 mg/kg) (MUC and MUC + CIDCA 133 group) or 0.9% saline solution (CTL and CIDCA 133 groups) and were treated with non-fermented milk or *L. delbrueckii* subsp. *lactis* CIDCA 133 fermented milk (n = 8 animals per group). * indicates a statistically significant difference ($p < 0.05$) between MUC and MUC + CIDCA 133 group before and after mucositis induction by Mann Whitney test (A and B).* indicates a statistically significant difference ($p < 0.05$) between CTL, CIDCA 133, MUC and MUC + CIDCA 133 groups by Two-ANOVA followed by the Bonferroni post-test (C). # indicates a statistically significant difference ($p < 0.05$) between MUC and MUC + CIDCA 133 group by Two-ANOVA followed by the Bonferroni post-test (C). Different letters (a–c) indicate statistically significant differences ($p < 0.05$) by Kruskal-Wallis test followed by the Dunn's post-test (A and B), and ANOVA followed by the Bonferroni post-test (D).

radiotherapy for cancer treatment, is one of the most relevant gastrointestinal inflammatory conditions in humans. This disease leads a considerable reduction in doses of chemotropic drugs administrated (45%) and delays in therapy (71%) of patients undergoing treatment, and statistically, 3% of them discontinue the therapy (Arnold et al., 2005; Lalla & Peterson, 2006). The 5-FU is one of the most commonly used chemotherapy drugs in oncological practice (Fata et al., 1999). To prevent, reduce and/or treat the intestinal mucosal damage caused by anticancer drugs and/or radiotherapy, many investigations about therapeutic alternatives are being considered.

Fermented milk products are considered as a "functional foods" (foods with health-promoting properties) (Balthazar et al., 2018; Lollo et al., 2013) having high relevance for the industrial and food sector. During the fermentation process the microorganisms generate metabolites, products coming from the hydrolysis of the components of the food matrix, capable to modify the organoleptic characteristics of the foods, as well as promote the conservation of nutrients and consequently improve their shelf-life (Champagne, Gomes da Cruz, & Daga, 2018; Silva et al., 2018).

The probiotic potential of *L. delbrueckii* subsp. *lactis* CIDCA 133 has been analyzed *in vitro*, where authors showed its ability to resist high concentrations of acids and bile salts (Kociubinski et al., 1999), and its capability to resist antimicrobial peptides derived from enterocytes (Hugo et al., 2010) and human ß -defensins (Hugo et al., 2012). It was also described that this strain exhibits immunomodulatory properties by stimulating TNF-α production in dendritic cells (DC) in a co-culture system with *B. cereus*-infected epithelial cells (Rolny, Tiscornia, Racedo, Pérez, & Bollati-Fogolín, 2016), as this cytokine promotes the recruitment of immune cells helps to control of pathogens multiplication (Ramadan, Moyer, & Callegan, 2008). Furthermore, this strain was able to activate murine macrophages (RAW 264.7) infected with *C. rodentium* through increase of phagocytic activity, expression of surface markers associated to antigen presentation, and also induction of ROS activity (Hugo et al., 2017).

Taking into account the immunomodulatory potential of lactobacilli and particularly the characteristics of CIDCA 133 strain, we hypothesized that the orally administration of *L. delbrueckii* subsp. *lactis* CIDCA 133 fermented milk (7.5×10^7 CFU/mL) could alleviate the intestinal damage associated to the administration of 5-FU in a murine model of mucositis.

In the present study, it was demonstrated that the daily administration of the probiotic strain CIDCA 133 significantly reduced the harshness of 5-FU intestinal induced mucositis in a murine model. One of the mucositis features is the shortening of small intestine length, as was demonstrated in animals of MUC group that presented 16% shortening of the small intestine. A lower percentage of bowel shortening was observed in mice treated with the probiotic CIDCA 133 fermented milk (7.2%). This finding is important because a larger area of the intestine provides enough absorption surface for nutrient uptake, as well as lower compromise of the loss of water and electrolytes which, consequently, acts positively on the energetic balance of the animals, which makes attractive the use of CIDCA 133 fermented milk. The shortening of the small intestine correlates with the decrease in body weight observed in mucositis disease (de Barros et al., 2018; Kato et al., 2017; Maioli et al., 2014; Vieira et al., 2012). In agreement with these authors, our work shows that animals which received 5-FU (MUC group) exhibit a considerable weight loss (around 9%). These findings are related to lower food consumption since the inflamed groups (MUC and MUC + CIDCA 133) showed significantly less food and liquid intake than the controls groups. In contrast, animals treated with the probiotic CIDCA 133 were able to recover around 6% of this weight loss. The same effect was reported by Maioli et al. (2014). Interestingly, the administration of probiotic to 5-FU-treated mice reduces weight loss and this finding can be related to the prevention of intestine shortening.

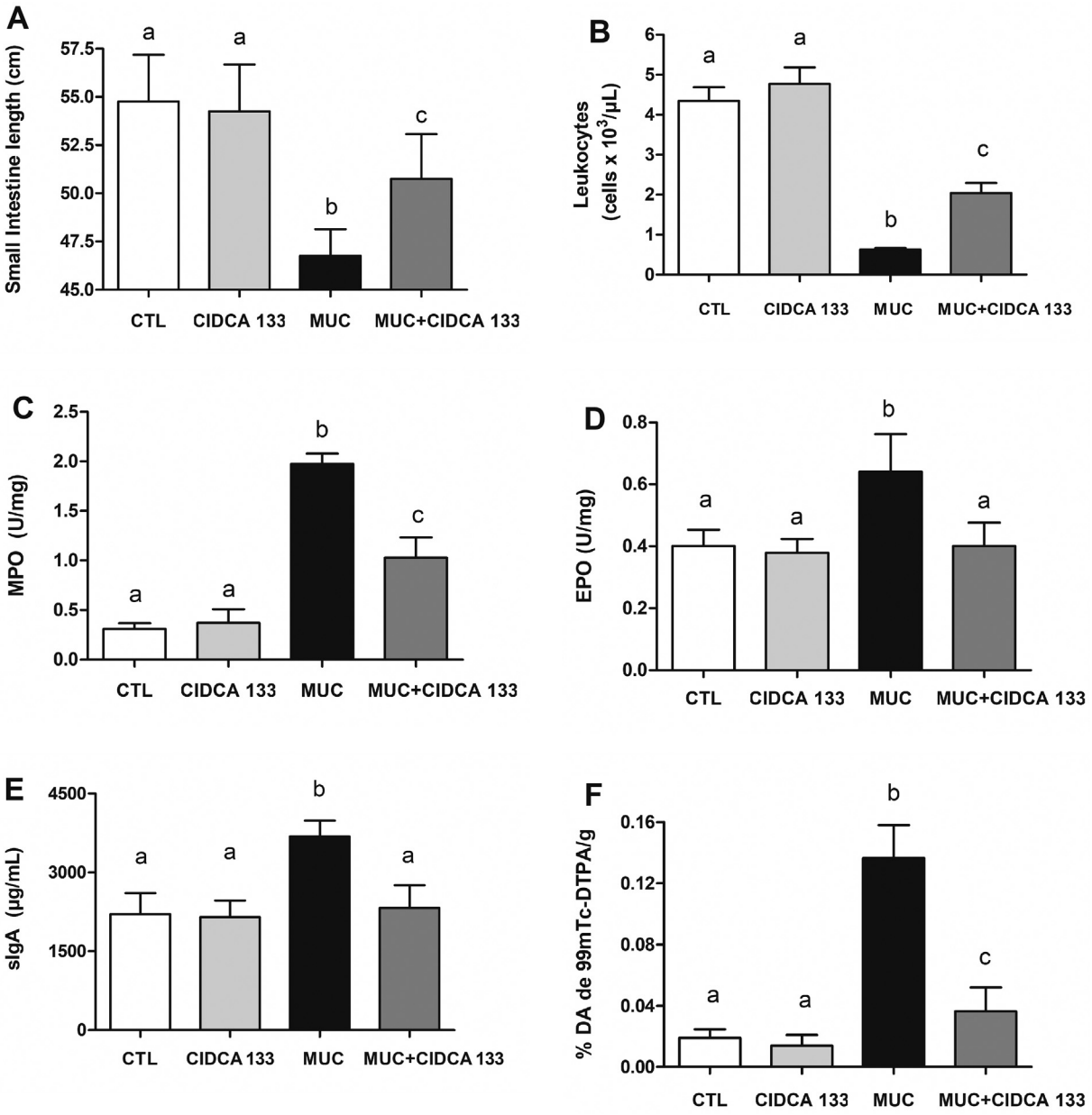


Fig. 3. Effect of CIDCA 133 fermented milk in mouse small intestines with induced mucositis by 5-fluorouracil (5-FU): (A) small intestine length, (B) number of leukocytes (cells $\times 10^3/\mu L$), study of (C) MPO activity and (D) EPO activity, (E) levels of sIgA (µg/mL) and (F) percentage of injected dose per gram (%ID/g) of blood to measure the intestinal permeability. Mice received intraperitoneal 5-FU (300 mg/kg) (MUC and MUC + CIDCA 133 group) or 0.9% (w/v) NaCl (CTL and CIDCA 133 group) injection and were fed daily with milk or *L. delbrueckii* subsp. *lactis* CIDCA 133 fermented milk (n = 8 animals per group). Different letters (a, b, and c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by the Bonferroni post-test (A, C, E), and Kruskal-Wallis test followed by the Dunn's post-test (B, D, F).

Another important feature of intestinal mucositis is the alteration of the small intestine architecture and integrity, causing villus flattening, inflammatory cell infiltrates in the *lamina propria* and cell damage. These effects let to diminished villus/crypt ratio (Duncan & Grant, 2003; Lee, Ryan, & Doherty, 2014; Soares et al., 2013; Sonis, 2004), and also to an increased production of pro-inflammatory cytokine. In the present study, animals injected with 5-FU without probiotic administration showed loss of architecture of the ileum mucosa. Interestingly, mice inflamed with 5-FU and given *L. delbrueckii* CIDCA 133 fermented milk were able to circumvent mucosa inflammation, showing decrease of mucosal inflammation scores, with maintenance of the length villus and the depth crypt and also, preserving mucosal architecture and total thickness when compared to those that only received 5-FU. These findings also are correlated with signs of improvement of the intestinal dysbiosis induced by 5-FU. Thus, CIDCA 133 fermented milk administration was able to attenuate the mucosal damage in inflamed mice.

The pathogenesis of 5-FU induced intestinal mucositis involves intestinal injury which is associated with inflammatory infiltration in the small intestine (Soares et al., 2008), which can be verified by increase in myeloperoxidase (indirect neutrophil infiltration), eosinophils peroxidase (indirect eosinophils infiltration), and NAG (macrophage infiltration) activity. In the present study, the administration of 300 mg/kg of 5-FU induces increased MPO and EPO activities in the mouse ileum mucosa. Of note, animals which received the probiotic bacteria showed reduced levels of neutrophil and eosinophil infiltration. Moreover, these inflammatory parameters are intrinsically related to our results referring to the increased leukocytes number in the blood of animals with intestinal mucositis treated with CIDCA 133, showing that

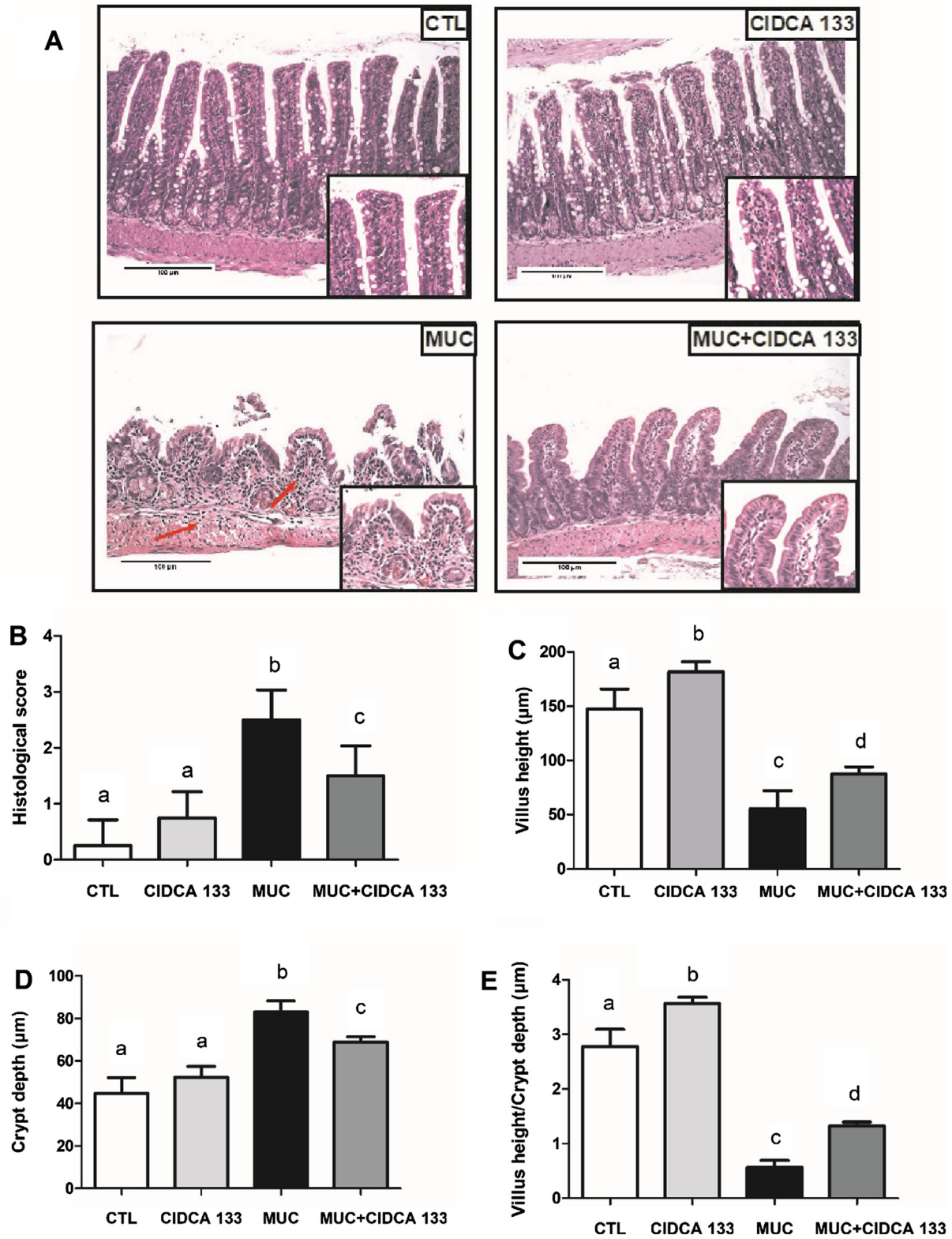


Fig. 4. Representative photomicrographs from: (A) mucosal histopathology, (B) Histopathological scores, (C) morphometrical analysis of villus height, (D) crypt depth and (E) villus height-to-crypt depth ratio of the ileum of animals with mucositis and administrated with strain CIDCA 133 fermented milk (objective: ×20, scale 100 µm). Mice received 5-FU (300 mg/kg) (MUC and MUC + CIDCA 133 group) or 0.9% saline (CTL and CIDCA 133 group) *i.p.* injection, (n = 8 animals per group). Different letters (a–d) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by the Bonferroni post-test (B–E).

the lower migration of these cells to the affected tissues is a result of the administration of this probiotic strain improving the severity of intestinal mucositis.

As reported by Sonis (2004), late manifestations of intestinal mucositis encompass an increase in intestinal permeability, villus and crypts atrophy, thus leading to severe loss of function of the epithelial barrier (Daniele et al., 2001; Song, Park, & Sung, 2013). One of the causes of these effects could be attributed to the reduction in the

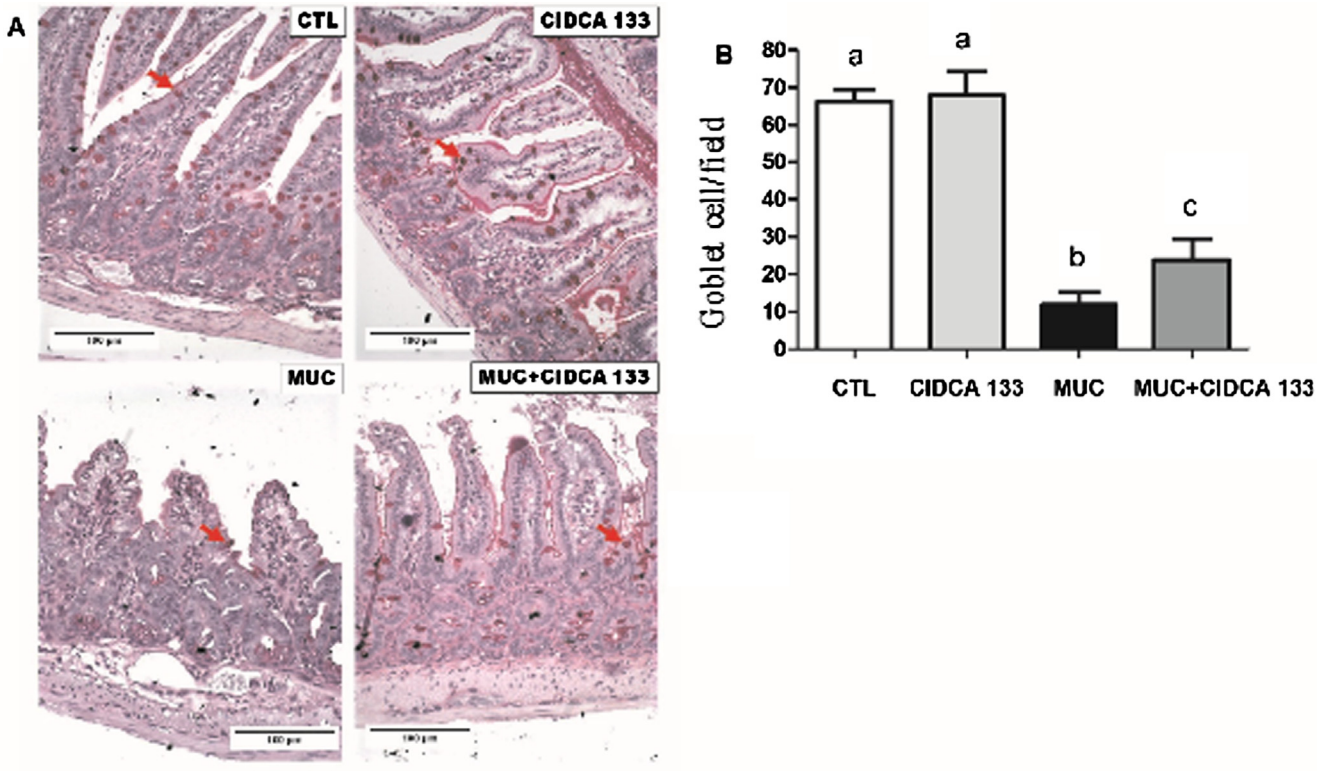


Fig. 5. Representative photomicrographs from: (A) ileum section stained with PAS: the arrows shows the Goblet cells (objective: ×20, scale 100 μm), (B) Number of Goblet cells/field obtained for experimental groups: Mice received 5-FU (300 mg/kg) (MUC and MUC + CIDCA 133 group) or 0.9% saline (CTL and CIDCA 133 group) *i.p* injection and were fed daily either with milk or with CIDCA 133 fermented milk (n = 8 animals per group). Different letters (a, b, and c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by the Bonferroni post-test (B).

expression of genes encoding tight junction proteins (Youmba, Belmonte, Galas, Boukhettala, Bôle-Feysot, Déchelotte, Coëffier, 2012). In our work, as in other reports (Ferreira et al., 2012; Li et al., 2017; Song et al., 2013) it was demonstrated the 5-FU drug increases the intestinal permeability. However, it was observed that mice injected with 5-FU and treated with CIDCA 133 fermented milk greatly reduced the intestinal permeability as it was detected by the ^{99m}Tc-DTPA administration. This is in agreement with other reports which related the probiotics administration with the reduction of the intestinal permeability induced by chemotherapy drugs (Bastos et al., 2016; Favaro-Trinidade & Grosso, 2000; Justino et al., 2014).

According to Yu, Yuan, Deng, and Yang (2015), microorganisms belonging to the genus *Lactobacillus* are able to improve the integrity of epithelia due to its ability antagonize the adhesion of pathogens to intestinal cells. Therefore, these pathogens cannot cause damage to the cellular junctions (Yu et al., 2015), and also might reduce the paracellular permeability by modulating the gene expression of tight junction proteins (Ahrne & Hagslatt, 2011).

It is also know that administration of 5-FU lead to a marked decrease in goblet cell number (Ciobanu et al., 2016; Stringer et al., 2009; Yeung et al., 2015). These cells are responsible of mucins secretion that along with trefoil factor are important components for the protection of the epithelium (van Vliet, Harmsen, de Bont, & Tissing, 2010). Our results revealed that the number of goblet cells dramatically decrease in 5-FU-treated mice. However, mice administrated with CIDCA 133 (MUC + CIDCA 133) presented significantly higher goblet cell numbers that mice administrated only with 5-FU (MUC group).

Our results with CIDCA 133 fermented milk are in agreement with previous reports that showed that probiotic bacteria and yeast are beneficial for both the structure and the function of the intestinal epithelium (Bastos et al., 2016; Oh et al., 2017; Yeung et al., 2015). We can hypothesize that the effect of strain CIDCA 133 fermented milk in preventing loss of goblet cells number could be related to its ability to protect the cells from intestinal crypts that contain stem cells that in turn differentiate into cells with different functions in the intestinal epithelium, including goblet cells.

Level of secretory IgA is another parameter related to the intestinal barrier which have relevant importance in the maintenance of mucosal homeostasis (Mantis, Rol, & Corthésy, 2011; Monteiro, 2014) both controlling harmful effects of intestinal pathogens and containing potential inflammatory processes (Schmucker, Owen, Outenreath, & Thoreux, 2003). Mucositis is characterized by high levels of sIgA and this pattern was observed in animals that did not receive CIDCA 133 strain (MUC group). Interesting, the administration of probiotic fermented milk was able to decrease the levels of sIgA to similar values found in the negative controls, demonstrating a reduced severity of 5-FU-induced intestinal mucositis in the mice ileum. Similar results were observed in mice administrated with *L. lactis* NZ9000 in a model of experimental mucositis (Carvalho et al., 2017). In different experimental models, it has been demonstrated that probiotics are able to increase sIgA production and this ability is considered a positive effect (Kawashima et al., 2018; Kikuchi et al., 2014; Santos Rocha et al., 2014). Interestingly, in our model of severe 5-FU-induced intestinal inflammation, the strain CIDCA 133 normalized levels of sIgA that were increased in the MUC group. Also, our model shows that reduced levels of sIgA as compared with inflamed animals, could be related to the improvement of intestinal mucosal barrier that in turns reduces to the improvement in the general state of inflammation.

Many researches are being done to clarify the underlying mechanisms related to the beneficial effect of probiotic bacteria in the bowel in different IBDs models. *L. acidophilus* (Justino et al., 2015; Oh et al., 2017), *L. fermentum* (Smith et al., 2008), *Saccharomyces boulardii* (Justino et al., 2014) and *L. plantarum CRL 2130* (Levit, Savoy de Giori, de Moreno de LeBlanc, & LeBlanc, 2018) are able to modulate the

inflammatory infiltrate generated by chemotherapy drugs. Furthermore, the capacity of some probiotics to prevent production of pro-inflammatory cytokines (Justino et al., 2015; Yeung et al., 2015) could be related to the inhibition of the NF-κB pathway (Dai et al., 2013) and the restoration of the Th17/Treg cells balance (Jeong, Lee, Jang, Han, & Kim, 2018). In addition, as suggested by other studies, probiotic bacteria are able to produce anti-oxidant compounds that contribute to ameliorate oxidative stress associated to inflammatory bowel disease (Juarez del Valle, Laiño, Savoy de Giori, & LeBlanc, 2014; Levit, Savoy de Giori, de Moreno de LeBlanc, & LeBlanc, 2018).

It is also known that fermented milk by Lactic Acid Bacteria (LAB) could produce extracellular factors. In addition it has been demonstrated that bacterial grow in milk are able to hydrolyze the large proteins contained in milk (LeBlanc, Matar, Valdéz, LeBlanc, & Perdigon, 2002), that could contribute to their immunomodulating activity (Cordeiro et al., 2018). Thus, we can hypothesize that soluble factors present in suspensions of CIDCA 133 fermented milk, contribute to the effects reported in our study. Indeed, it is important to highlight that even short chain fatty acids produced during growth of lactic acid bacteria have demonstrated beneficial effects to the host (Garrote, Abraham, & Rumbo, 2015; Iraporda et al., 2015).

Additionally, it is important to highlight that the immunomodulatory/regulatory effects reported for CIDCA 133 fermented milk can be enhanced with the use of prebiotic, because these compounds stimulate growth activating bacteria metabolism and promote protection of bacteria beneficial to the host organism, as reported in previous studies (Galdino et al., 2018; Trindade et al., 2018).

5. Conclusion

The present work demonstrates for the first time the protective effect of *L. delbrueckii* subsp. *lactis* CIDCA 133 on the damage of the intestinal mucosa in a murine model of inflammation induced by a chemotherapeutic drug. Our findings showed that fermented milk produced in this work serve as efficient matrix for delivery of CIDCA 133 in GIT, and enlarge the perspectives of application of this strain and support novel probiotic-based treatments for gastrointestinal toxicity associated with anticancer therapy.

Funding

This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Ahrne, S., & Hagslatt, M.-L. J. (2011). Effect of lactobacilli on paracellular permeability in the gut. *Nutrients, 3*(1), 104–117. https://doi.org/10.3390/nu3010104.

Arnold, R. J. G., Gabrail, N., Raut, M., Kim, R., Sung, J. C. Y., & Zhou, Y. (2005). Clinical implications of chemotherapy-induced diarrhea in patients with cancer. *The Journal of Supportive Oncology, 3*(3), 227–232. http://www.ncbi.nlm.nih.gov/pubmed/15915825.

Balthazar, C. F., Silva, H. L. A., Esmerino, E. A., Rocha, R. S., Moraes, J., Carmo, M. A. V., ... Cruz, A. G. (2018). The addition of inulin and Lactobacillus casei 01 in sheep milk ice cream. *Food Chemistry, 246*, 464–472. https://doi.org/10.1016/j.foodchem.2017.12.002.

Bastos, R. W., Pedroso, S. H. S. P., Vieira, A. T., Moreira, L. M. C., França, C. S., Cartelle, C. T., ... Martins, F. S. (2016). Saccharomyces cerevisiae UFMG A-905 treatment reduces intestinal damage in a murine model of irinotecan-induced mucositis. *Beneficial Microbes, 7*(4), 549–557. https://doi.org/10.3920/BM2015.0190.

Bradley, P. P., Christensen, R. D., & Rothstein, G. (1982). Cellular and extracellular myeloperoxidase in pyogenic inflammation. *Blood, 60*(3), 618–622. http://www.ncbi.nlm.nih.gov/pubmed/6286012.

Caballero-Franco, C., Keller, K., De Simone, C., & Chadee, K. (2007). The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 292*(1), G315–G322. https://doi.org/10.1152/ajpgi.00265.2006.

Carvalho, R. D., Breyner, N., Menezes-Garcia, Z., Rodrigues, N. M., Lemos, L., Maioli, T. U., ... de Azevedo, M. S. (2017). Secretion of biologically active pancreatitis-associated protein I (PAP) by genetically modified dairy Lactococcus lactis NZ9000 in the prevention of intestinal mucositis. *Microbial Cell Factories, 16*(1), 27. https://doi.org/10.1186/s12934-017-0624-x.

Champagne, C. P., Gomes da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science, 22*, 160–166. https://doi.org/10.1016/j.cofs.2018.04.008.

Ciobanu, L., Tantau, M., Valean, S., Parau, A., Bedecean, I., Mîrleneanu, R., ... Taulescu, M. (2016). Rifaximin modulates 5-fluorouracil-induced gastrointestinal mucositis in rats. *European Review for Medical and Pharmacological Sciences, 20*(23), 4993–5001. http://www.ncbi.nlm.nih.gov/pubmed/27981532.

Cordeiro, B. F., Oliveira, E. R., da Silva, S. H., Savassi, B. M., Acurcio, L. B., Lemos, L., ... do Carmo, F. L. R. (2018). Whey protein isolate-supplemented beverage, fermented by lactobacillus casei BL23 and propionibacterium freudenreichii 138, in the prevention of mucositis in mice. *Frontiers in Microbiology, 9*. https://doi.org/10.3389/fmicb.2018.02035.

Dai, C., Zheng, C.-Q., Meng, F.-J., Zhou, Z., Sang, L.-X., & Jiang, M. (2013). VSL#3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-κB pathway in rat model of DSS-induced colitis. *Molecular and Cellular Biochemistry, 374*(1–2), 1–11. https://doi.org/10.1007/s11010-012-1488-3.

Dalmasso, G., Loubat, A., Dahan, S., Calle, G., Rampal, P., & Czerucka, D. (2006). Saccharomyces boulardii prevents TNF-alpha-induced apoptosis in EHEC-infected T84 cells. *Research in Microbiology, 157*(5), 456–465. https://doi.org/10.1016/j.resmic.2005.11.007.

Daniele, B., Secondulfo, M., De Vivo, R., Pignata, S., De Magistris, L., Delrio, P., ... Carratù, R. (2001). Effect of chemotherapy with 5-fluorouracil on intestinal permeability and absorption in patients with advanced colorectal cancer. *Journal of Clinical Gastroenterology, 32*(3), 228–230. http://www.ncbi.nlm.nih.gov/pubmed/11246350.

de Barros, P. A. V., Rabelo Andrade, M. E., de Vasconcelos Generoso, S., Mendes Miranda, S. E., dos Reis, D. C., Lacerda Leocádio, P. C., ... Cardoso, V. N. (2018). Conjugated linoleic acid prevents damage caused by intestinal mucositis induced by 5-fluorouracil in an experimental model. *Biomedicine & Pharmacotherapy, 103*, 1567–1576. https://doi.org/10.1016/j.biopha.2018.04.133.

Duncan, M., & Grant, G. (2003). Oral and intestinal mucositis - causes and possible treatments. *Alimentary Pharmacology & Therapeutics, 18*(9), 853–874. http://www.ncbi.nlm.nih.gov/pubmed/14616150.

Ewaschuk, J. B., Diaz, H., Meddings, L., Diederichs, B., Dmytrash, A., Backer, J., ... Madsen, K. L. (2008). Secreted bioactive factors from Bifidobacterium infantis enhance epithelial cell barrier function. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 295*(5), G1025–G1034. https://doi.org/10.1152/ajpgi.90227.2008.

Fata, F., Ron, I. G., Kemeny, N., O'Reilly, E., Klimstra, D., & Kelsen, D. P. (1999). 5-fluorouracil-induced small bowel toxicity in patients with colorectal carcinoma. *Cancer, 86*(7), 1129–1134. http://www.ncbi.nlm.nih.gov/pubmed/10506695.

Favaro-Trinidade, C., & Grosso, C. (2000). The effect of the immobilization of L. acidophilus and B. lactis in alginate on their tolerance to gastrointestinal secretions. *Milchwissenschaft, 55*, 496–499.

Ferreira, T. M., Leonel, A. J., Melo, M. A., Santos, R. R. G., Cara, D. C., Cardoso, V. N., ... Alvarez-Leite, J. I. (2012). Oral supplementation of butyrate reduces mucositis and intestinal permeability associated with 5-Fluorouracil administration. *Lipids, 47*(7), 669–678. https://doi.org/10.1007/s11745-012-3680-3.

Galdino, F. M. P., Andrade, M. E. R., de Barros, P. A. V., de Generoso, S. V., Alvarez-Leite, J. I., Almeida-Leite, C. M., ... de Cardoso, V. N. (2018). Pretreatment and treatment with fructo-oligosaccharides attenuate intestinal mucositis induced by 5-FU in mice. *Journal of Functional Foods, 49*, 485–492. https://doi.org/10.1016/j.jff.2018.09.012.

Garrote, G. L., Abraham, A. G., & Rumbo, M. (2015). Is lactate an undervalued functional component of fermented food products? *Frontiers in Microbiology, 6*, 629. https://doi.org/10.3389/fmicb.2015.00629.

Herbers, A. H. E., de Haan, A. F. J., van der Velden, W. J. F. M., Donnelly, J. P., & Blijlevens, N. M. A. (2014). Mucositis not neutropenia determines bacteremia among hematopoietic stem cell transplant recipients. *Transplant Infectious Disease : An Official Journal of the Transplantation Society, 16*(2), 279–285. https://doi.org/10.1111/tid.12195.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., ... Sanders, M. E. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*(8), 506–514. https://doi.org/10.1038/nrgastro.2014.66.

Hugo, A. A., De Antoni, G. L., & Pérez, P. F. (2006). Lactobacillus delbrueckii subsp lactis strain CIDCA 133 inhibits nitrate reductase activity of Escherichia coli. *International Journal of Food Microbiology, 111*(3), 191–196. https://doi.org/10.1016/j.ijfoodmicro.2006.04.024.

Hugo, A. A., De Antoni, G. L., & Pérez, P. F. (2010). Lactobacillus delbrueckii subsp lactis (strain CIDCA 133) resists the antimicrobial activity triggered by molecules derived from enterocyte-like Caco-2 cells. *Letters in Applied Microbiology, 50*(4), 335–340. https://doi.org/10.1111/j.1472-765X.2010.02796.x.

Hugo, A. A., Kakisu, E., De Antoni, G. L., & Pérez, P. F. (2008). Lactobacilli antagonize biological effects of enterohaemorrhagic Escherichia coli in vitro. *Letters in Applied Microbiology, 46*(6), 613–619. https://doi.org/10.1111/j.1472-765X.2008.02363.x.

Hugo, A. A., Rolny, I. S., Romanin, D., & Pérez, P. F. (2017). Lactobacillus delbrueckii subsp. lactis (strain CIDCA 133) stimulates murine macrophages infected with Citrobacter rodentium. *World Journal of Microbiology & Biotechnology, 33*(3), 48. https://doi.org/10.1007/s11274-017-2219-4.

Hugo, A. A., Tymczyszyn, E. E., Gómez-Zavaglia, A., & Pérez, P. F. (2012). Effect of human defensins on lactobacilli and liposomes. *Journal of Applied Microbiology, 113*(6), 1491–1497. https://doi.org/10.1111/j.1365-2672.2012.05433.x.

Iraporda, C., Errea, A., Romanin, D. E., Cayet, D., Pereyra, E., Pignataro, O., ... Rumbo, M. (2015). Lactate and short chain fatty acids produced by microbial fermentation downregulate proinflammatory responses in intestinal epithelial cells and myeloid cells. *Immunobiology, 220*(10), 1161–1169. https://doi.org/10.1016/j.imbio.2015.06.004.

Jeong, J.-J., Lee, H.-J., Jang, S.-E., Han, M. J., & Kim, D.-H. (2018). Lactobacillus plantarum C29 alleviates NF-κB activation and Th17/Treg imbalance in mice with TNBS-induced colitis. *Food and Agricultural Immunology, 29*(1), 577–589. https://doi.org/10.1080/09540105.2017.1418841.

Jørgensen, V. L., Nielsen, S. L., Espersen, K., & Perner, A. (2006). Increased colorectal permeability in patients with severe sepsis and septic shock. *Intensive Care Medicine, 32*(11), 1790–1796. https://doi.org/10.1007/s00134-006-0356-6.

Juarez del Valle, M., Laiño, J. E., Savoy de Giori, G., & LeBlanc, J. G. (2014). Riboflavin producing lactic acid bacteria as a biotechnological strategy to obtain bio-enriched soymilk. *Food Research International, 62*, 1015–1019. https://doi.org/10.1016/j.foodres.2014.05.029.

Justino, P. F. C., Melo, L. F. M., Nogueira, A. F., Costa, J. V. G., Silva, L. M. N., Santos, C. M., ... Soares, P. M. G. (2014). Treatment with Saccharomyces boulardii reduces the inflammation and dysfunction of the gastrointestinal tract in 5-fluorouracil-induced intestinal mucositis in mice. *The British Journal of Nutrition, 111*(9), 1611–1621. https://doi.org/10.1017/S0007114513004248.

Justino, P. F. C., Melo, L. F. M., Nogueira, A. F., Morais, C. M., Mendes, W. O., Franco, A. X., ... Soares, P. M. G. (2015). Regulatory role of Lactobacillus acidophilus on inflammation and gastric dysmotility in intestinal mucositis induced by 5-fluorouracil in mice. *Cancer Chemotherapy and Pharmacology, 75*(3), 559–567. https://doi.org/10.1007/s00280-014-2663-x.

Kato, S., Hamouda, N., Kano, Y., Oikawa, Y., Tanaka, Y., Matsumoto, K., ... Shimakawa, M. (2017). Probiotic Bifidobacterium bifidum G9–1 attenuates 5-fluorouracil-induced intestinal mucositis in mice via suppression of dysbiosis-related secondary inflammatory responses. *Clinical and Experimental Pharmacology & Physiology, 44*(10), 1017–1025. https://doi.org/10.1111/1440-1681.12792.

Kawashima, T., Ikari, N., Kouchi, T., Kowatari, Y., Kubota, Y., Shimojo, N., & Tsuji, N. M. (2018). The molecular mechanism for activating IgA production by Pediococcus acidilactici K15 and the clinical impact in a randomized trial. *Scientific Reports, 8*(1), 5065. https://doi.org/10.1038/s41598-018-23404-4.

Kikuchi, Y., Kunitoh-Asari, A., Hayakawa, K., Imai, S., Kasuya, K., Abe, K., ... Hachimura, S. (2014). Oral administration of Lactobacillus plantarum strain AYA enhances IgA secretion and provides survival protection against influenza virus infection in mice. *PloS One, 9*(1), e86416. https://doi.org/10.1371/journal.pone.0086416.

Kociubinski, G. L., Pérez, P. F., Añón, M. C., & De Antoni, G. L. (1996). A method of screening for highly inhibitory lactic acid bacteria. *Journal of Food Protection, 59*(7), 739–745. https://doi.org/10.4315/0362-028X-59.7.739.

Kociubinski, G., Pérez, P., & De Antoni, G. (1999). Screening of bile resistance and bile precipitation in lactic acid bacteria and bifidobacteria. *Journal of Food Protection, 62*(8), 905–912. http://www.ncbi.nlm.nih.gov/pubmed/10456745.

Lalla, R. V., & Peterson, D. E. (2006). Treatment of mucositis, including new medications. *Cancer Journal (Sudbury, Mass.), 12*(5), 348–354. http://www.ncbi.nlm.nih.gov/pubmed/17034671.

LeBlanc, J. G., Matar, C., Valdéz, J. C., LeBlanc, J., & Perdigon, G. (2002). Immunomodulating effects of peptidic fractions issued from milk fermented with Lactobacillus helveticus. *Journal of Dairy Science, 85*(11), 2733–2742. https://doi.org/10.3168/jds.S0022-0302(02)74360-9.

Lee, C. S., Ryan, E. J., & Doherty, G. A. (2014). Gastro-intestinal toxicity of chemotherapeutics in colorectal cancer: The role of inflammation. *World Journal of Gastroenterology, 20*(14), 3751–3761. https://doi.org/10.3748/wjg.v20.i14.3751.

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A., & LeBlanc, J. G. (2018). Protective effect of the riboflavin-overproducing strain Lactobacillus plantarum CRL2130 on intestinal mucositis in mice. *Nutrition, 54*, 165–172. https://doi.org/10.1016/j.nut.2018.03.056.

Li, H.-L., Lu, L., Wang, X.-S., Qin, L.-Y., Wang, P., Qiu, S.-P., ... Wu, X.-J. (2017). Alteration of gut microbiota and inflammatory cytokine/chemokine profiles in 5-fluorouracil induced intestinal mucositis. *Frontiers in Cellular and Infection Microbiology, 7*, 455. https://doi.org/10.3389/fcimb.2017.00455.

Logan, R. M., Gibson, R. J., Sonis, S. T., & Keefe, D. M. K. (2007). Nuclear factor-kappaB (NF-kappaB) and cyclooxygenase-2 (COX-2) expression in the oral mucosa following cancer chemotherapy. *Oral Oncology, 43*(4), 395–401. https://doi.org/10.1016/j.oraloncology.2006.04.011.

Lollo, P. C. B., de Moura, C. S., Morato, P. N., Cruz, A. G., de Castro, W. F., Betim, C. B., ... Amaya-Farfan, J. (2013). Probiotic yogurt offers higher immune-protection than probiotic whey beverage. *Food Research International, 54*(1), 118–124. https://doi.org/10.1016/j.foodres.2013.06.003.

Longley, D. B., Harkin, D. P., & Johnston, P. G. (2003). 5-fluorouracil: Mechanisms of action and clinical strategies. *Nature Reviews. Cancer, 3*(5), 330–338. https://doi.org/10.1038/nrc1074.

Lutgendorff, F., Nijmeijer, R. M., Sandström, P. A., Trulsson, L. M., Magnusson, K.-E., Timmerman, H. M., ... Söderholm, J. D. (2009). Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. *PloS One, 4*(2), e4512. https://doi.org/10.1371/journal.pone.0004512.

Maioli, T. U., de Melo Silva, B., Dias, M. N., Paiva, N. C., Cardoso, V. N., Fernandes, S. O., ... de Vasconcelos Generoso, S. (2014). Pretreatment with Saccharomyces boulardii does not prevent the experimental mucositis in Swiss mice. *Journal of Negative Results in Biomedicine, 13*, 6. https://doi.org/10.1186/1477-5751-13-6.

Mantis, N. J., Rol, N., & Corthésy, B. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunology, 4*(6), 603–611. https://doi.org/10.1038/mi.2011.41.

Martins, F. S., Silva, A. A., Vieira, A. T., Barbosa, F. H. F., Arantes, R. M. E., Teixeira, M. M., & Nicoli, J. R. (2009). Comparative study of Bifidobacterium animalis, Escherichia coli, Lactobacillus casei and Saccharomyces boulardii probiotic properties. *Archives of Microbiology, 191*(8), 623–630. https://doi.org/10.1007/s00203-009-0491-x.

Mennigen, R., Nolte, K., Rijcken, E., Utech, M., Loeffler, B., Senninger, N., & Bruewer, M. (2009). Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 296*(5), G1140–G1149. https://doi.org/10.1152/ajpgi.90534.2008.

Miura, K., Kinouchi, M., Ishida, K., Fujibuchi, W., Naitoh, T., Ogawa, H., ... Sasaki, I. (2010). 5-fu metabolism in cancer and orally-administrable 5-fu drugs. *Cancers, 2*(3), 1717–1730. https://doi.org/10.3390/cancers2031717.

Monteiro, R. C. (2014). Immunoglobulin A as an anti-inflammatory agent. *Clinical and Experimental Immunology, 178*(Suppl), 108–110. https://doi.org/10.1111/cei.12531.

Oh, N. S., Lee, J. Y., Lee, J. M., Lee, K. W., & Kim, Y. (2017). Mulberry leaf extract fermented with Lactobacillus acidophilus A4 ameliorates 5-fluorouracil-induced intestinal mucositis in rats. *Letters in Applied Microbiology, 64*(6), 459–468. https://doi.org/10.1111/lam.12741.

Pinedo, H. M., & Peters, G. F. (1988). Fluorouracil: Biochemistry and pharmacology. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology, 6*(10), 1653–1664. https://doi.org/10.1200/JCO.1988.6.10.1653.

Ramadan, R. T., Moyer, A. L., & Callegan, M. C. (2008). A role for tumor necrosis factor-alpha in experimental Bacillus cereus endophthalmitis pathogenesis. *Investigative Ophthalmology & Visual Science, 49*(10), 4482–4489. https://doi.org/10.1167/iovs.08-2085.

Rolny, I. S., Tiscornia, I., Racedo, S. M., Pérez, P. F., & Bollati-Fogolín, M. (2016). Lactobacillus delbrueckii subsp lactis CIDCA 133 modulates response of human epithelial and dendritic cells infected with Bacillus cereus. *Beneficial Microbes, 7*(5), 749–760. https://doi.org/10.3920/BM2015.0191.

Santos Rocha, C., Gomes-Santos, A. C., Garcias Moreira, T., de Azevedo, M., Diniz Luerce, T., Mariadassou, M., ... van de Guchte, M. (2014). Local and systemic immune mechanisms underlying the anti-colitis effects of the dairy bacterium Lactobacillus delbrueckii. *PloS One, 9*(1), e85923. https://doi.org/10.1371/journal.pone.0085923.

Schmucker, D. L., Owen, R. L., Outenreath, R., & Thoreux, K. (2003). Basis for the age-related decline in intestinal mucosal immunity. *Clinical and Developmental Immunology, 10*(2–4), 167–172. https://doi.org/10.1080/10446670310001642168.

Seth, A., Yan, F., Polk, D. B., & Rao, R. K. (2008). Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 294*(4), G1060–G1069. https://doi.org/10.1152/ajpgi.00202.2007.

Silva, H. L. A., Balthazar, C. F., Silva, R., Vieira, A. H., Costa, R. G. B., Esmerino, E. A., ... Cruz, A. G. (2018). Sodium reduction and flavor enhancer addition in probiotic prato cheese: Contributions of quantitative descriptive analysis and temporal dominance of sensations for sensory profiling. *Journal of Dairy Science, 101*(10), 8837–8846. https://doi.org/10.3168/jds.2018-14819.

Smith, C. L., Geier, M. S., Yazbeck, R., Torres, D. M., Butler, R. N., & Howarth, G. S. (2008). Lactobacillus fermentum BR11 and fructo-oligosaccharide partially reduce jejunal inflammation in a model of intestinal mucositis in rats. *Nutrition and Cancer, 60*(6), 757–767. https://doi.org/10.1080/01635580802192841.

Soares, P. M. G., Mota, J. M. S. C., Gomes, A. S., Oliveira, R. B., Assreuy, A. M. S., Brito, G. A. C., ... Souza, M. H. L. P. (2008). Gastrointestinal dysmotility in 5-fluorouracil-induced intestinal mucositis outlasts inflammatory process resolution. *Cancer Chemotherapy and Pharmacology, 63*(1), 91–98. https://doi.org/10.1007/s00280-008-0715-9.

Soares, P. M. G., Mota, J. M. S. C., Souza, E. P., Justino, P. F. C., Franco, A. X., Cunha, F. Q., ... Souza, M. H. L. P. (2013). Inflammatory intestinal damage induced by 5-fluorouracil requires IL-4. *Cytokine, 61*(1), 46–49. https://doi.org/10.1016/j.cyto.2012.10.003.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L. G., Gratadoux, J.-J., ... Langella, P. (2008). Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences of the United States of America, 105*(43), 16731–16736. https://doi.org/10.1073/pnas.0804812105.

Song, M.-K., Park, M.-Y., & Sung, M.-K. (2013). 5-Fluorouracil-induced changes of intestinal integrity biomarkers in BALB/c mice. *Journal of Cancer Prevention, 18*(4), 322–329. http://www.ncbi.nlm.nih.gov/pubmed/25337561.

Sonis, S. T. (1998). Mucositis as a biological process: A new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncology, 34*(1), 39–43. http://www.ncbi.nlm.nih.gov/pubmed/9659518.

Sonis, S. T. (2004). The pathobiology of mucositis. *Nature Reviews. Cancer, 4*(4), 277–284. https://doi.org/10.1038/nrc1318.

Soveri, L. M., Hermunen, K., de Gramont, A., Poussa, T., Quinaux, E., Bono, P., ... Österlund, P. (2014). Association of adverse events and survival in colorectal cancer patients treated with adjuvant 5-fluorouracil and leucovorin: Is efficacy an impact of toxicity? *European Journal of Cancer (Oxford, England : 1990), 50*(17), 2966–2974. https://doi.org/10.1016/j.ejca.2014.08.017.

Stringer, A. M., Gibson, R. J., Logan, R. M., Bowen, J. M., Yeoh, A. S. J., Hamilton, J., & Keefe, D. M. K. (2009). Gastrointestinal microflora and mucins may play a critical role in the development of 5-Fluorouracil-induced gastrointestinal mucositis.

Experimental Biology and Medicine (Maywood, N.J.), 234(4), 430–441. https://doi.org/10.3181/0810-RM-301.

Trindade, L. M., Martins, V. D., Rodrigues, N. M., Souza, E. L. S., Martins, F. S., Costa, G. M. F., ... Generoso, S. V. (2018). Oral administration of Simbioflora® (synbiotic) attenuates intestinal damage in a mouse model of 5-fluorouracil-induced mucositis. *Beneficial Microbes, 9*(3), 477–486. https://doi.org/10.3920/BM2017.0082.

van Vliet, M. J., Harmsen, H. J. M., de Bont, E. S. J. M., & Tissing, W. J. E. (2010). The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. *PLoS Pathogens, 6*(5), e1000879. https://doi.org/10.1371/journal.ppat.1000879.

Vieira, A. T., Fagundes, C. T., Alessandri, A. L., Castor, M. G. M., Guabiraba, R., Borges, V. O., ... Teixeira, M. M. (2009). Treatment with a novel chemokine-binding protein or eosinophil lineage-ablation protects mice from experimental colitis. *The American Journal of Pathology, 175*(6), 2382–2391. https://doi.org/10.2353/ajpath.2009.090093.

Vieira, E. L. M., Leonel, A. J., Sad, A. P., Beltrão, N. R. M., Costa, T. F., Ferreira, T. M. R., ... Alvarez-Leite, J. I. (2012). Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis. *The Journal of Nutritional Biochemistry, 23*(5), 430–436. https://doi.org/10.1016/j.jnutbio.2011.01.007.

Yeung, C.-Y., Chan, W.-T., Jiang, C.-B., Cheng, M.-L., Liu, C.-Y., Chang, S.-W., ... Lee, H.-C. (2015). Amelioration of Chemotherapy-Induced Intestinal Mucositis by Orally Administered Probiotics in a Mouse Model. *PloS One, 10*(9), e0138746. https://doi.org/10.1371/journal.pone.0138746.

Youmba, S. B., Belmonte, L., Galas, L., Boukhettala, N., Bôle-Feysot, C., Déchelotte, P., & Coëffier, M. (2012). Methotrexate Modulates Tight Junctions Through NF-κB, MEK, and JNK Pathways. *Journal of Pediatric Gastroenterology and Nutrition, 4*(54), 463–470.

Yu, Q., Yuan, L., Deng, J., & Yang, Q. (2015). Lactobacillus protects the integrity of intestinal epithelial barrier damaged by pathogenic bacteria. *Frontiers in Cellular and Infection Microbiology, 5*, 26. https://doi.org/10.3389/fcimb.2015.00026.

CAPÍTULO II

6. CAPÍTULO II: Probiogenomics of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: In Silico, In Vitro, and In vivo Approaches

Este artigo cientifico foi publicado na revista *Microorganisms* (ISSN: 2076-2607) em março de 2021. Fator de impacto atual: 4.926.

Apesar da importância das linhagens de *L. delbrueckii* na indústria alimentícia, poucos estudos se concentraram em estudos genômicos em linhagens probióticas dessa espécie relacionados aos seus efeitos benéficos à saúde do hospedeiro. A linhagem *Lactobacillus delbrueckii* CIDCA 133 vem sendo descrita como uma potencial linhagem probiótica, apresentando propriedades imunomoduladoras. No entanto, os mecanismos moleculares relacionados às suas propriedades benéficas não são conhecidos. Nesse contexto, por meio de análises genômicas associadas a avaliações *in vitro* e *in vivo*, neste capítulo buscou-se caracterizar o genoma de *L. delbrueckii* CIDCA 133 e investigar os possíveis fatores genéticos envolvidos com o perfil probiótico da linhagem. Para realizar o estudo, o DNA da linhagem foi sequenciado pela plataforma Illumina HiSeq2500. A fim de ratificar a identificação microbiológica da linhagem realizada pela via clássica, realizamos a análise filogenômica incluindo 26 genomas completos de *L. delbrueckii* disponíveis na plataforma NCBI. Buscou-se também identificar genes relacionados à atividade proteolítica da bactéria, que podem estar associados à produção de compostos bioativos durante o processo de fermentação. A identificação da sublocalização celular das proteínas de *L. delbrueckii* CIDCA 133 e sua função na adesão e interação com o sitema imune foi realizada, a fim de correlacionar estes dados aos efeitos imunoestimulatórios da linhagem observados *in vivo*. Outro dado importante avaliado neste trabalho foi a busca por fatores genéticos que corroborassem a capacidade de *L. delbrueckii* CIDCA 133 resistir ao estresse biliar, ácido, térmico e osmótico, e seu efeito inibitório sobre o crescimento de bactérias patogênicas, observados *in vitro*. Os achados deste trabalho, além de descrever os genes e mecanismos moleculares utilizados por *L. delbrueckii* CIDCA 133 para produzir seus efeitos benéficos, reforçam a utilização da linhagem em aplicações probióticas.

microorganisms

MDPI

Article

Probiogenomics of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: In Silico, In Vitro, and In Vivo Approaches

Luís Cláudio Lima de Jesus [1], Mariana Martins Drumond [2], Flávia Figueira Aburjaile [1,3], Thiago de Jesus Sousa [1], Nina Dias Coelho-Rocha [1], Rodrigo Profeta [1], Bertram Brenig [4], Pamela Mancha-Agresti [5] and Vasco Azevedo [1,*]

1 Laboratório de Genética Celular e Molecular (LGCM), Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte 31270-901, Brazil; lc.luiis@yahoo.com.br (L.C.L.d.J.); faburjaile@gmail.com (F.F.A.); thiagojsousa@gmail.com (T.d.J.S.); ninadias008@gmail.com (N.D.C.-R.); profeta.biotec@gmail.com (R.P.)
2 Centro Federal de Educação Tecnológica de Minas Gerais (CEFET/MG), Departamento de Ciências Biológicas, Belo Horizonte 31421-169, Brazil; mmdrumond@gmail.com
3 Laboratório de Flavivírus, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro 21040-360, Brazil
4 Institute of Veterinary Medicine, University of Göttingen, D-37077 Göttingen, Germany; bbrenig@gwdg.de
5 Faculdade de Minas-Faminas-BH, Medicina, Belo Horizonte 31744-007, Brazil; p.mancha.agresti@gmail.com
* Correspondence: vasco@icb.ufmg.br

Abstract: *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 (CIDCA 133) has been reported as a potential probiotic strain, presenting immunomodulatory properties. This study investigated the possible genes and molecular mechanism involved with a probiotic profile of CIDCA 133 through a genomic approach associated with in vitro and in vivo analysis. Genomic analysis corroborates the species identification carried out by the classical microbiological method. Phenotypic assays demonstrated that the CIDCA 133 strain could survive acidic, osmotic, and thermic stresses. In addition, this strain shows antibacterial activity against *Salmonella* Typhimurium and presents immunostimulatory properties capable of upregulating anti-inflammatory cytokines *Il10* and *Tgfb1* gene expression through inhibition of *Nfkb1* gene expression. These reported effects can be associated with secreted, membrane/exposed to the surface and cytoplasmic proteins, and bacteriocins-encoding genes predicted in silico. Furthermore, our results showed the genes and the possible mechanisms used by CIDCA 133 to produce their beneficial host effects and highlight its use as a probiotic microorganism.

Keywords: genomic characterization; phylogenomic; probiotic; *Lactobacillus delbrueckii*; acid and bile tolerance; *Nfkb1* gene expression; surface proteins; bacteriocin

Citation: de Jesus, L.C.L.; Drumond, M.M.; Aburjaile, F.F.; Sousa, T.d.J.; Coelho-Rocha, N.D.; Profeta, R.; Brenig, B.; Mancha-Agresti, P.; Azevedo, V. Probiogenomics of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: In Silico, In Vitro, and In Vivo Approaches. *Microorganisms* **2021**, *9*, 829. https://doi.org/10.3390/microorganisms9040829

Academic Editor: Mariantonietta Succi

Received: 10 March 2021
Accepted: 30 March 2021
Published: 14 April 2021

1. Introduction

Lactobacillus is a highly diverse taxonomic group of Gram-positive microorganisms, rod or coccobacilli-shaped, members of lactic acid bacteria (LAB), facultatively anaerobic [1,2], and able to produce lactic acid as the primary metabolic end product of carbohydrate fermentation [2,3]. These microorganisms can be found and isolated from different ecological niches (e.g., vegetables, fermented products, gastrointestinal and vaginal tracts of humans and animals) where there is a high carbohydrate availability [4].

Many *Lactobacillus* strains have a probiotic profile and, thus, present functional characteristics beneficial to the host, such as their immunomodulatory and anti-inflammatory properties [5,6], and its effectiveness on the treatment of Crohn's disease and ulcerative colitis [7,8], intestinal mucositis [9,10], and enteric infections [11,12]. However, it should be emphasized that the beneficial effects of probiotics on the host are strain-dependent [13,14] and cannot be generalized.

The *Lactobacillus* strains described as probiotics, with many potential benefits attributed to the host health, and general commercial and biotechnological potential, are

now being studied in the genomic field [15–17]. The *Lactobacillus* genome analysis has contributed to more detailed characterization in terms of the identification and function of gene products and possible molecular mechanisms related to the probiotic effects attributed to these bacteria [18], as well as their animal and human consumption-related safety [16,19]. However, unlike other LAB species, few studies have focussed on *Lactobacillus delbrueckii* probiogenomics, and the few genomic data are mainly obtained from bulgaricus subspecies [20,21].

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 (CIDCA 133) is a new potential probiotic strain of *Lactobacillus delbrueckii* subsp. *lactis* species isolated from raw cow's milk [22]. According to previous in vitro studies, this strain has shown the ability to tolerate high concentrations of bile salts [23] and antagonistic action against the pathogenic enterohemorrhagic *Escherichia coli* (EHEC) [24] and bacteria causing food contamination, such as *Pseudomonas aeruginosa* [22]. Furthermore, this probiotic strain modulated cell response in both monocyte-derived dendritic cells and murine macrophages (RAW 264.7 cells) infected with *Bacillus cereus* [25] and *Citrobacter rodentium* [26] through enhancing the TNF-α and ROS production, respectively. This probiotic strain was also able to survive and grow in the presence of enterocyte-derived antimicrobial molecules, such as β-defensins [27,28]. Additionally, it was also reported that the administration of fermented milk by CIDCA 133 to BALB/c mice prevented the inflammatory response and histopathological damage caused to the intestinal mucosa after 5-Fluorouracil (300 mg/Kg) chemotherapy administration [10].

Although promising results have been previously obtained in pre-clinical studies regarding this strain, there is little information about the genetic factors related to its protective action mechanisms; therefore, this work aims to characterize the *L. delbrueckii* CIDCA 133 strain through a probiogenomics approach. The genomic data (in silico) will allow the potential molecular mechanisms involved with the probiotic properties and immunomodulatory capacity for this strain to be known and reported in vitro and in vivo studies.

2. Materials and Methods

2.1. Bacteria Strain and Growth Conditions

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 belongs to the culture collection of the Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) of the Universidad Nacional de La Plata, Argentina. This strain was deposited at the Bacteria Collection from Environment and Health (CBAS) of the Oswaldo Cruz Foundation (FIOCRUZ) (Accession number: CBAS 815). CIDCA 133 was cultivated in de Man, Rogosa, and Sharpe (MRS) broth (Kasvi, São José dos Pinhais, Brazil) for 16 h at 37 °C.

2.2. CIDCA 133 Identification by MALDI-TOF Biotyper®

After growth, CIDCA 133 was plated using a sterile plastic loop on MRS agar plates (São José dos Pinhais, Brazil) and incubated at 37 °C for 48 h. he colonies' identification using the MALDI-TOF Biotyper® Mass Spectrometry (Brukker Daltoniks, Billerica, MA, USA) was performed according to the manufacturer's instructions.

2.3. Genomic and Plasmid DNA Extraction

CIDCA 133 genomic DNA extraction was performed through mechanical lysis followed by purification with a phenol solution (phenol:chloroform:isoamyl alcohol 25:24:1, v/v, respectively), precipitation with ethanol 70% and sodium acetate 3 M, and suspension in DNase and RNase-free water, according to the protocol established by Sachinandan et al. [29]. According to the manufacturer's instructions, the plasmid extraction was performed using the Pure Link™ Quick Plasmid Miniprep Kit (Invitrogen, Carlsbad, CA, USA).

2.4. Genome Sequencing, Assembly, and Annotation

CIDCA 133 whole genome sequence was performed using the HiSeq 2500 platform (Illumina, San Diego, CA, USA), paired-end libraries (2 × 150 bp). The quality assessment

of the reads was performed using FastQC [30]. De novo genome assembly was performed using the Edena assembler (v. 3.13) [31]. The assembly quality was verified using QUAST (Quality assessment tool) [32]. The contigs were ordered and oriented through the *CONTIGUATOR* (v. 2.74) [33], using the whole genome of *L. delbrueckii* subsp. *bulgaricus* ND02 (RefSeq: NC_014727.1) as a reference. The remaining gaps were closed using GapBLASTER (v. 1.1.2) [34], GenomeFinisher (v.1.4) [35] and QIAGEN CLC Genomics Workbench 20 (v. 20.0.4) [36].

The protein encoding-ORFs were automatically annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) from the National Center for Biotechnology (NCBI) [37]. The genome and plasmid sequences were deposited in the NCBI (Access Number: CP065513 and CP065514, respectively).

2.5. In Silico Analysis

2.5.1. Plasmid Identification

The presence of plasmids was searched using the PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) (accessed on 15 December 2020) [38]. The circular map of the plasmid was obtained by SnapGene® software (v. 5.1.3.1).

2.5.2. Phylogenomic Analysis

For comparative genomic analysis, 26 complete genomes of *Lactobacillus delbrueckii* strains from the NCBI database were used (Table 1). The taxonomic analysis to compare whether or not the strains belonged to the same species was carried out by calculating the Average Nucleotide Identity (ANI) by Blast (ANIb) performed within the JSpecies Web Server [39]. ANIb values were visualized as a heatmap. Genomes with ANI > 95% were considered the same species.

Table 1. Complete genomes of *Lactobacillus delbrueckii* strains obtained from NCBI used in comparative analysis.

Nº	Bacteria Strain	Genome Access	Size (Mb)	GC%
1	*L. delbrueckii* P3MRA	NZ_CP045604.1	1.87	49.70
2	*L. delbrueckii* TS1-06	NZ_CP046390.1	1.85	49.80
3	*L. delbrueckii* subsp. *bulgaricus* LJJ	NZ_CP049052.1	1.89	49.50
4	*L. delbrueckii* subsp. *bulgaricus* KLDS1.1011	NZ_CP041280.1	1.89	49.80
5	*L. delbrueckii* subsp. *bulgaricus* MN-BM-F01	NZ_CP013610.1	1.88	49.70
6	*L. delbrueckii* subsp. *bulgaricus* KLDS1.0207	NZ_CP032451.1	1.87	49.80
7	*L. delbrueckii* subsp. *bulgaricus* DSM 20080	NZ_CP019120.1	1.87	49.80
8	*L. delbrueckii* subsp. *bulgaricus* ND04	NZ_CP016393.1	1.86	49.60
9	*L. delbrueckii* subsp. *bulgaricus* ACA-DC 87	NZ_LT899687.1	1.86	49.80
10	*L. delbrueckii* subsp. *bulgaricus* L99	NZ_CP017235.1	1.85	49.70
11	*L. delbrueckii* subsp. *bulgaricus* 2038	NC_017469.1	1.87	49.70
12	*L. delbrueckii* subsp. *bulgaricus* ATCC 11842	NC_008054.1	1.86	49.70
13	*L. delbrueckii* subsp. *bulgaricus* ATCC BAA-365	NC_008529.1	1.86	49.70
14	*L. delbrueckii* subsp. *bulgaricus* ND02	NC_014727.1	2.13	49.59
15	*L. delbrueckii* subsp. *delbrueckii* NBRC 3202	NZ_AP019750.1	1.91	50.10
16	*L. delbrueckii* subsp. *delbrueckii* TUA4408L	NZ_CP021136.1	2.01	49.90
17	*L. delbrueckii* subsp. *delbrueckii* KCTC 13731	NZ_CP018216.1	1.91	50.00
18	*L. delbrueckii* subsp. *indicus* JCM 15610	NZ_CP018614.1	2.02	49.37
19	*L. delbrueckii* subsp. *jakobsenii* DSM 26046	NZ_CP018218.1	1.89	50.10
20	*L. delbrueckii* subsp. *lactis* KCCM 34717	NZ_CP018215.1	2.26	49.10
21	*L. delbrueckii* subsp. *lactis* KCTC 3034	NZ_CP023139.1	2.24	49.00
22	*L. delbrueckii* subsp. *lactis*1	NZ_LS991409.1	2.05	49.60
23	*L. delbrueckii* subsp. *lactis* KCTC 3035	NZ_CP018156.1	1.97	50.00
24	*L. delbrueckii* subsp. *lactis* NWC_1_2	CP029250.1	2.26	48.58
25	*L. delbrueckii* subsp. *lactis* DSM 20072	NZ_CP022988.1	2.17	49.00
26	*L. delbrueckii* subsp. *sunkii* JCM 17838	NZ_CP018217.1	2.00	50.10

2.5.3. Subcellular Localization of CIDCA 133 Proteins

The prediction of subcellular localization of CIDCA 133 proteins was performed using the SurfG+ software, which classifies proteins based on the presence (secreted proteins) or absence (cytoplasmic proteins) of a signal peptide, transmembrane helices (membrane proteins), and signal retention (proteins that are covalently or transiently bound to the cell wall) [40].

2.5.4. Functional Annotation of CIDCA 133 Proteins

For functional characterization, the protein sequences predicted in the CIDCA 133 genome were submitted to the GO FEAT (Gene Ontology Functional Enrichment Annotation Tool) (http://computationalbiology.ufpa.br/gofeat/) (accessed on 8 January 2021) [41].

2.5.5. Cell Adhesion-Related Genes

The CIDCA 133 genes' prediction involved in the adhesion mechanisms was evaluated through Vaxign (Vaccine Design) (v.2beta) (http://www.violinet.org/vaxign/) (accessed on 17 January 2021) [42]. A score of 0.6 was selected as the criteria to analyze which CIDCA 133 proteins (sub-located in the membrane, surface-exposed (PSE), or secreted) have a high adhesion capacity.

2.5.6. Proteolytic Activity and Stress Tolerance-Related Genes

The CIDCA 133 stress tolerance (acid, bile, thermal, and osmotic) and proteolytic system-related genes' prediction were based on literature data revision [43–48] for previously reported genes identified in probiotic bacteria involved with the above processes.

2.5.7. Metabolic and Symbiotic Islands Prediction

The Metabolic (MI) and Symbiotic (SI) Islands prediction in the CIDCA 133 genome was performed with GIPSy software (Genomic Island Prediction Software) (v.1.1.2) [49], using the *Lacticaseibacillus rhamnosus* GG (*L. rhamnosus*) genome (NZ_CP031290.1) as subjects. The Genomic Islands (GEIs) map was visualized using BRIG (BLAST Ring Image Generator) software (v. 0.95) [50].

2.5.8. Bacteriocins Prediction

Gene's prediction related to bacteriocins synthesis was performed by BAGEL4 (BActeriocin GEnome mining tooL) (http://bagel4.molgenrug.nl/index.php) (accessed on 22 December 2020) [51].

2.5.9. Protein–Protein Interactions Prediction

For the potential biological functions of CIDCA 133 on human immunology, the prediction of interactions between CIDCA 133 and human proteins was carried out. The human protein sequence was mapped to KEGG pathways (toll-like receptor 2/4 nuclear factor κappa B (TLR2/4-NF-κB) pathway) and obtained from UniProt (UP000005640) (Table S1). The CIDCA 133 proteins with a high likelihood of adherence predicted by Vaxign (>0.6 scores) were used. The protein–protein interaction was performed in InterSPPI [52]. The resulting interactions were filtered according to the 0.9765 score prediction (specificity of 0.99). The graphical interaction results were achieved by Cytoscape software [53].

2.6. In Vitro Analysis

2.6.1. Simulated Gastric Juice and Heat Stress Tolerance

The CIDCA 133 tolerance to acidic gastric juice simulated with pepsin solution (pH 3.0) was performed according to Singhal et al. [54]. Briefly, 3 g/L of pepsin (Sigma–Aldrich, St. Louis, MO, USA) was diluted in 0.5% sterile NaCl (pH 3.0) (Vetec, Rio de Janeiro, Brazil). Subsequently, the cell pellet (10^8 CFU/mL) was washed twice with sterile and cold PBS 0.1 M (pH 7.0) and suspended with 400 µL of sterile NaCl 0.5% (pH 7.0). One hundred

microliters of the culture was inoculated in 900 µL of the pepsin solution (pH 3.0) and incubated at 37 °C for 4 h with shaking (200 rpm) in a shaker (Labnet, Edison, NJ, USA)

For heat stress, the CIDCA 133 culture (10^8 CFU/mL) was centrifuged (5000 rpm for 10 min at 4 °C), washed twice with sterile and cold PBS 0.1 M (pH 7.0), suspend with 1 mL of MRS broth, and incubated for 30 min to 65 °C (a temperature of the simulated pasteurization process) [55]. As a control, 1 mL of CIDCA 133 was not submitted to heat stress.

Then, 100 µL of each sample was collected after 0, 2, and 4 h (acid stress) and 30 min (heat stress) of incubation, and serially diluted (1:10) (acid stress: 10^{-8}; heat stress: 10^{-7}) in sterile-cold PBS 0.1 M (pH 7.0), plated on MRS agar (Kasvi, São José dos Pinhais, Brazil) and incubated at 37 °C for 48 h. The number of viable bacteria was determined by counting colony-forming units (CFU/mL) after incubation period.

2.6.2. Osmotic Stress Tolerance

For CIDCA 133 ability to tolerate different concentrations of sodium chloride (NaCl), 150 µL of the culture was inoculated in 15 mL of MRS broth containing different concentrations of NaCl (1%, 2%, 3%, 4%, and 5%) [56]. As a control, 150 µL of CIDCA 133 was inoculated in 15 mL of MRS broth without NaCl supplementation. After 24 h of growth at 37 °C, the samples' absorbance was measured at $O.D._{600}$ nm.

2.6.3. Antibacterial Activity

For this analysis, the indicator strains *Shigella sonnei* ATCC® 9290, *Salmonella enterica* serovar Typhimurium ATCC® 29630, *Enterococcus faecalis* ATCC® 19433, *Listeria monocytogenes* ATCC® 15313 were obtained from the American Type Culture Collection (ATCC) (Manassas, Virginia, EUA). *Lactobacillus delbrueckii* CNRZ327 and *Lacticaseibacillus paracasei* BL23 (*L. paracasei* BL23) belongs to the culture collection of the Institute Nacional de la Recherche Agronomique (INRA, Jouy-en-Josas, France). These strains were cultivated in MRS broth (Kasvi, São José dos Pinhais, Brazil) or BHI (Brain Heart Infusion) (Sigma–Aldrich, St. Louis, MO, USA) at 37 °C for 24 h.

Antibacterial activity of CIDCA 133 against these indicator strains was evaluated using CIDCA 133 cells-free supernatant (CFS), according to the method described by Somashekaraiah et al. [57], with some modifications. For this purpose, 100 mL of CIDCA 133 culture grown in MRS broth at 37 °C for 24 h was centrifuged (5000 rpm for 15 min at 4 °C). Part of the cell-free supernatants (CFS) was kept with their initial acid pH. Another was neutralized (nCFS) (pH 6.5) with 1.0 M NaOH (Vetec, Rio de Janeiro, Brazil). The CFS and nCFS aliquots were sterilized through a 0.22 µm filter (Kasvi, São José dos Pinhais, Brazil). Then, 200 µL of the indicator strains, previously grown in BHI broth at 37 °C for 24 h, was inoculated in 2 mL of the CIDCA 133 supernatant (CFS or nCFS). As a control, the indicator strains were grown in MRS broth. After 24 h incubation at 37 °C, the $O.D._{600}$ nm was measured.

2.7. In Vivo Analysis

2.7.1. Gene Expression of Cytokines in Mice Ileum

The experiments were conducted on male BALB/c mice (weight 25–30 g, six weeks old) obtained from Centro de Bioterismo (CEBIO) of the Institute of Biological Sciences at the Federal University of Minas Gerais (UFMG). The animals were kept in polycarbonate-ventilated cages under controlled conditions: temperature around 21 ± 2 °C with a 12-h light/dark cycle, and ad libitum access to water and standard chow diet 24 h before experiments. All procedures followed the Brazilian College of Animal Experimentation (COBEA), and the Local Animal Experimental Ethics Committee (CEUA-UFMG) approved the project (Protocol n° 112/2020).

2.7.2. CIDCA 133 Administration

Mice were randomized into two experimental groups (n = 6 animals per group): I-NC (negative control) and II- CIDCA 133. These groups were administered by continuous feeding with 100 mL/cage of MRS broth (CTL group) or CIDCA 133 (5×10^7 CFU/mL) for 13 consecutive days. After the experimentation period, the animals were euthanized by a single intraperitoneal injection of anesthetic overdose (30 mg/kg of xylazine and 300 mg/kg of ketamine mixture) (Ceva, São Paulo, Brazil) and samples of the intestine (ileum section) were collected and stored in RNAlater® solution (Invitrogen, Carlsbad, CA, USA).

2.7.3. RNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)

According to the manufacturer's instructions, the total RNA of ileum sections (~20 mg) was obtained using the RNeasy Mini Kit (QIAGEN, Hilden, Germany). The RNA quality and concentration were evaluated on 1.5% agarose gel electrophoresis and through the NanoDrop® 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), respectively. Residual DNA was digested with DNAse I from the TURBO DNA-free™ Kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The complementary deoxyribonucleic acid (cDNA) synthesis was produced with the Applied Biosystems™ High-Capacity cDNA Reverse Transcription kit (ThermoFisher, Waltham, MA, USA), according to the manufacturer's instructions.

Quantitative PCR (qPCR) was performed using the PowerUp™ SYBR® Green Master Mix (ThermoFisher, Waltham, MA, USA) and the genes-specific primers for *Tlr2*, *Tlr4*, *Myd88*, *Nfkb1*, *Tnf*, *Il1b*, *Il6*, *Il12*, *Il10*, *Il17a*, *Tgfb1*, and *Muc2* (Table 2). Amplification reactions were performed on the Applied Biosystems 7900HT Fast Real-Time PCR System under the following conditions: initial denaturation at 95 °C for 10 min, 95 °C for 15 seg, annealing/extension at 60 °C for 1 min, 40 cycles followed by a dissociation stage for recording the melting curve. The expression of target genes was analyzed by the $2^{-\Delta\Delta Ct}$ method using housekeeping genes encoding β-actin (*actb*) and GAPDH (*gapdh*) as endogenous references.

Table 2. Quantitative Polymerase Chain Reaction (qPCR) primers used in this study.

Gene	Primer Forward	Primer Reverse	Amplicon Size (bp)	Reference
Actb	GCTGAGAGGGAAATCGTGCGTG	CCAGGGAGGAAGAGGATGCGG	100	[58]
Gapdh	TCACCACCATGGAGAAGGC	GCTAAGCAGTTGGTGGTGCA	168	[59]
Il6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA	141	[59]
Il10	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT	191	[59]
Il12p40	GGAAGCACGGCAGCAGAATA	AACTTGAGGGAGAAGTAGGAATGG	180	[59]
Tnf	ACGTGGAACTGGCAGAAGAG	CTCCTCCACTTGGTGGTTTG	236	[60]
Il1b	CTCCATGAGCTTTGTACAAGG	TGCTGATGTACCAGTTGGGG	245	[60]
Il17a	GCTCCAGAAGGCCCTCAGA	AGCTTTCCCTCCGCATTGA	142	[59]
Tgfb1	TGACGTCACTGGAGTTGTACGG	GGTTCATGTCATGGATGGTGC	170	[59]
Muc2	GATGGCACCTACCTCGTTGT	GTCCTGGCACTTGTTGGAAT	246	[58]
Myd88	ATCGCTGTTCTTGAACCCTCG	CTCACGGTCTAACAAGGCCAG	199	[61]
Tlr2	ACAATAGAGGGAGACGCCTTT	AGTGTCTGGTAAGGATTTCCCAT	149	[61]
Tlr4	ATGGCATGGCTTACACCACC	GAGGCCATTTTTGTCTCCACA	129	[61]
Nfkb1 (p105)	GTGGAGGCATGTTCGGTAGTG	TCTTGGCACAATCTTTAGGGC	195	[62]

2.8. Statistical Analysis

The experiments were done in triplicate (gastric juice, osmotic and thermal stress tolerance, bacterial antagonism) or duplicate (qPCR analysis). The results were presented as mean and standard deviation (SD). Statistical differences between the two groups were performed by the Student's *t*-test (thermal stress tolerance, qPCR, and bacterial antagonism analysis). Stress experiments (gastric juice and osmotic stress tolerance) were performed by analyzing variance (ANOVA) followed by Tukey's post hoc test. All data were analyzed using the GraphPad Prism 8.0 software, and a p-value < 0.05 was considered significant.

3. Results

3.1. L. delbrueckii CIDCA 133 General Genomic Features

Genome sequencing of *L. delbrueckii CIDCA 133* strain revealed a single circular chromosomal DNA of 2,127,785 bp, with a GC% content of 49.57%, 27 rRNA, 98 tRNA, 153 pseudogenes, 2132 genes, and a total of 2004 protein-coding sequences (CDS). Additionally, the presence of one plasmid sequence was detected in CIDCA 133 (Figure 1). This plasmid had 6224 bp, a GC content of 44.67%, and six CDSs.

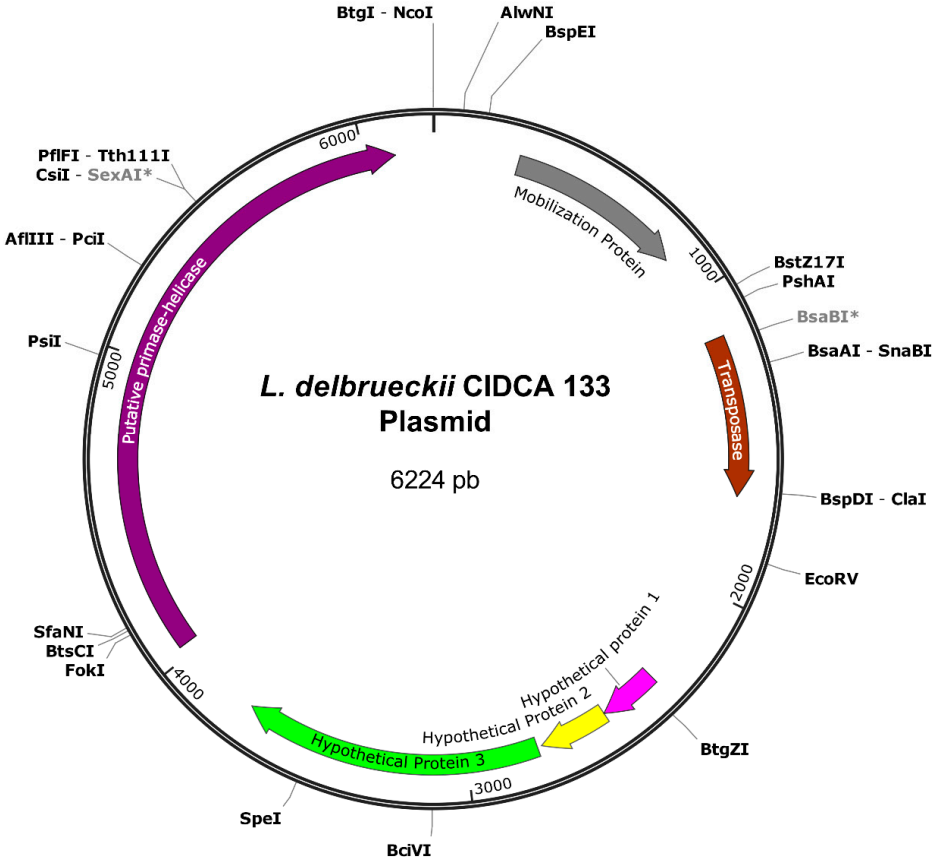


Figure 1. Schematic representation of the plasmid circular map present in *Lactobacillus delbrueckii* CIDCA 133 and restriction enzyme cut sites. Asterisk (*) indicates site blocked by methylation.

3.2. Gene Ontology (GO) Annotation

A total of 1590 genes of CIDCA 133 exhibited results in the GO FEAT platform's functional annotation. The GO terms were represented in three categories: molecular function (50.94% hits), biological process (27.06% hits), and cell component (22% hits) (Figure 2A).

The cellular component category contained GO terminologies involved in membrane function (integral components of the membrane, plasm membrane) and cytoplasmic function (ribosome), among others (Figure 2B). For molecular function, it was identified that

the main GO terminologies functions referred to protein binding (DNA, ATP, and metal-binding) and catalytic activity (ATPase and hydrolase activity), among others (Figure 2C).

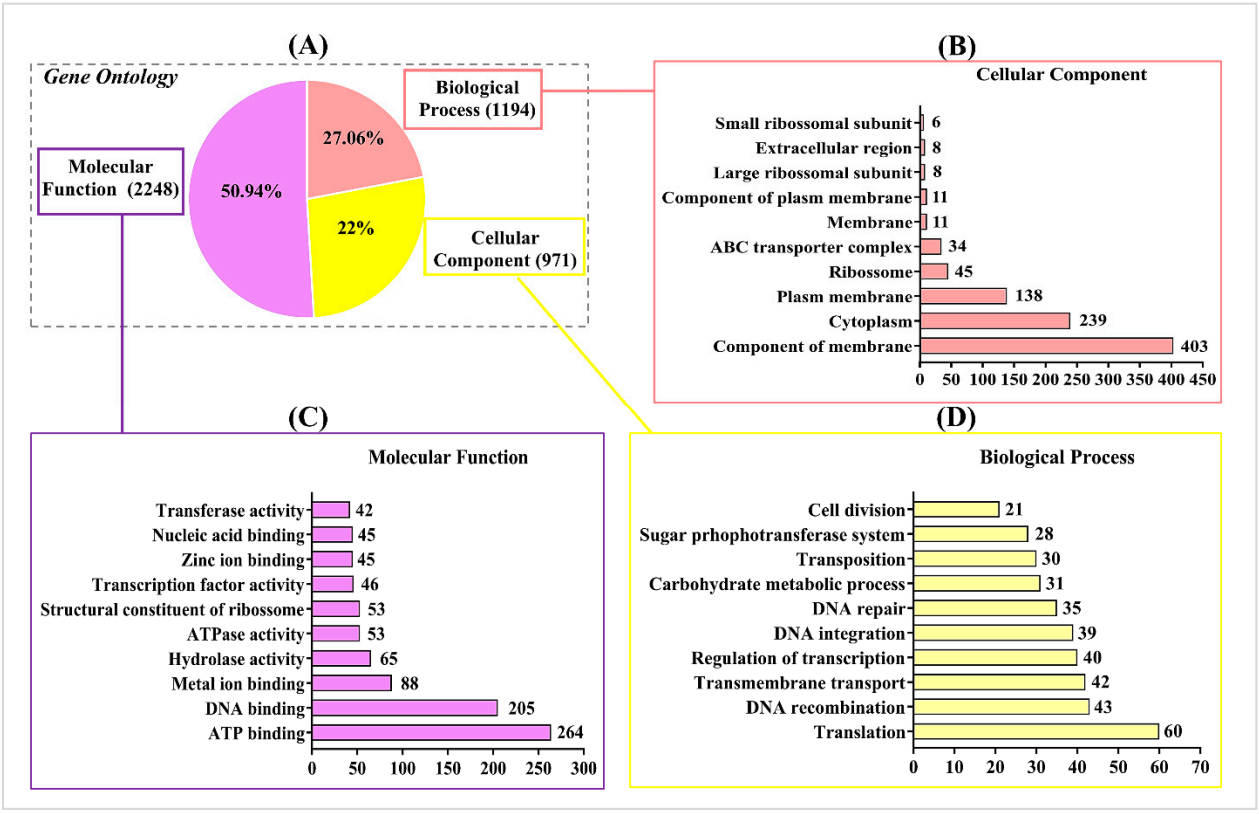


Figure 2. Distribution of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 (CIDCA 133) genes among the Gene Ontology (GO) categories predicted in Gene Ontology Functional Enrichment Annotation Tool (GO FEAT) (**A**). The genes were distributed in Cellular component (**B**), Molecular function (**C**), and Biological process (**D**) categories.

For the biological process category, the most representative GO terms were translation, transmembrane transport, DNA repair, and carbohydrate metabolism (Figure 2D).

3.3. Species Identification

The CIDCA 133 identification by MALDI-TOF Biotyper® classified this strain as belonging to the *L. delbrueckii* species, but with a certain degree of uncertainty (score < 2.2). However, pairwise comparisons of the Average Nucleotide Identity based on BLAST (ANIb) indicate that CIDCA 133 genome presented an identity threshold > 97% with 26 *L. delbrueckii* genomes (Figure 3), consistent with their identification as members of the same species.

ANIb distance between the strains indicated the formation of two main clades: one represented by strains of *L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp. *jakobsenii* and *L. delbrueckii* subsp. *lactis* (red region upper right), and the other included strains of *L. delbrueckii* subsp. *bulgaricus* (red region, lower left). This phylogenomic analysis shows that *L. delbrueckii* CIDCA 133 is closely related to the clade of the *L. delbrueckii* subsp. *lactis* species (ANIb > 98%).

3.4. CIDCA 133 Tolerates Acid, Osmotic and Thermal Stresses

Genes coding for proteins involved in acid, thermal, osmotic and bile salt resistance were identified in CIDCA 133 genome. These genes encode proteins as ornithine decarboxylase, F0F1-ATP synthase (acid stress), Na (+)/H (+) antiporter NhaC, aquaporin family

protein (osmotic stress), choloylglycine hydrolase, S-ribosylhomocysteine lyase (bile salt stress), chaperones (GroEL, DnaK) (heat stress), among others (Table 3).

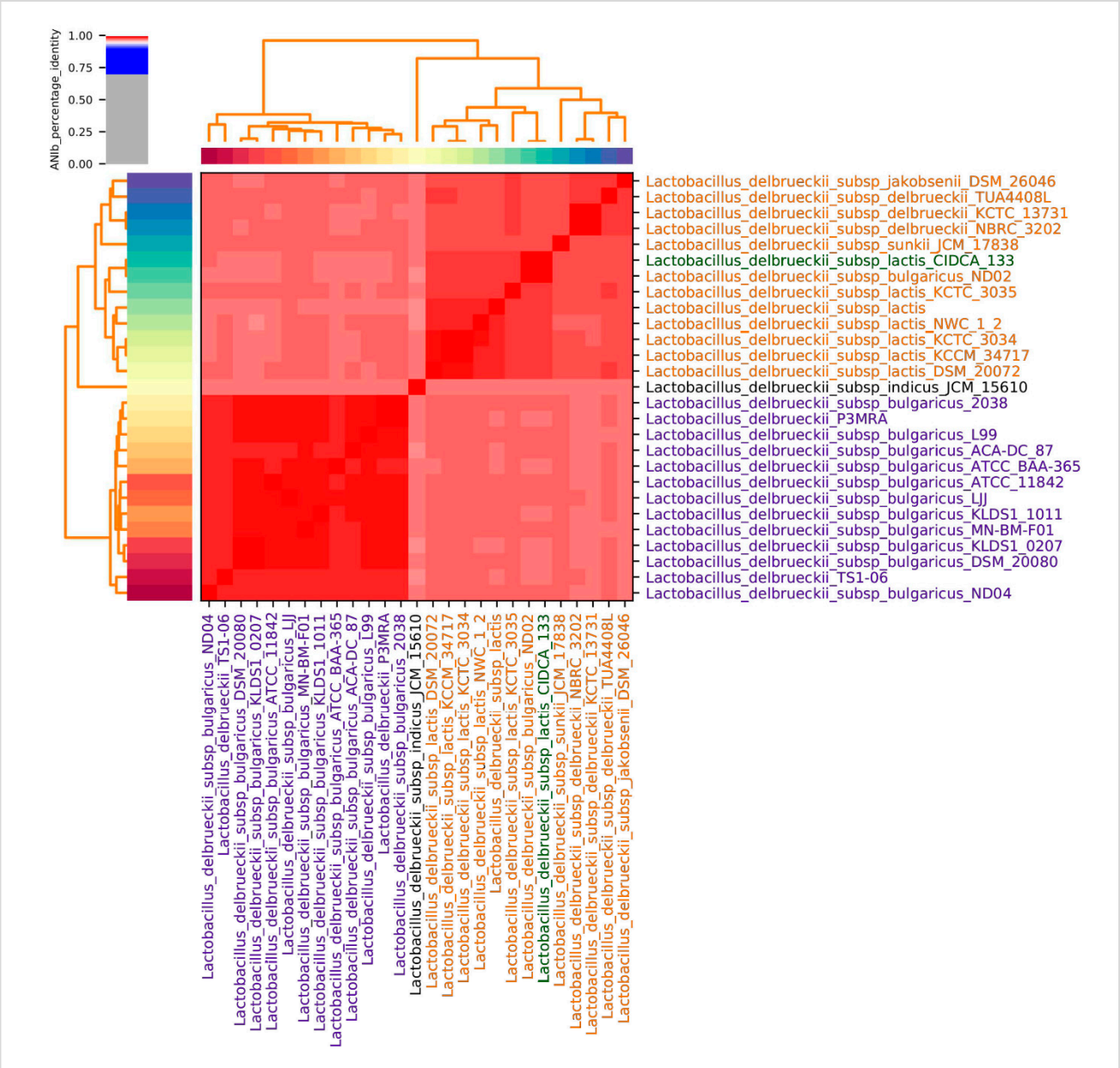


Figure 3. Heatmap representing the degree of similarity between *Lactobacillus delbrueckii* genomes based on the Average Nucleotide Identity (ANI). Red = more similar, light red = less similar.

Additionally, the capacity of CIDCA 133 to tolerate these stressors agents was evaluated. For acid stress, it was observed that compared to the initial time (0 h), the viability of CIDCA 133 decreased after 2 h and 4 h in contact with artificial gastric juice, but the strain continued to maintain a high survival rate: 77.7% (2 h) and 67.4% (4 h), thus being able to grow after acid pH challenge (Figure 4A).

For osmotic stress, no change in the growth of CIDCA 133 was observed in the presence of 1%, 2%, and 3% NaCl. The strain showed a growth rate of 99.5%, 98.1%, and 87.6%, respectively. These results are like bacteria that were not submitted to osmotic stress (NaCl 0%; 100% in the growth rate). However, when the NaCl concentration was increased

to 4% and 5%, the strain had a growth rate of 48.1% and 31.7%, respectively, revealing that these high concentrations of NaCl reduce the CIDCA 133 growth (Figure 4B).

Table 3. Gene's prediction involved with stress tolerance of *Lactobacillus delbrueckii* CIDCA 133.

Locus Tag	Gene	Protein	Stress Condition
HR078_02445	*Odcl*	Ornithine decarboxylase	Acid
HR078_03205	*atpD*	F0F1-ATP synthase subunit beta	Acid
HR078_03195	*atpA*	F0F1- ATP synthase subunit alpha	Acid
HR078_03180	*atpE*	F0F1-ATP synthase subunit C	Acid
HR078_03185	*atpF*	F0F1-ATP synthase subunit B	Acid
HR078_03210	*atpC*	F0F1-ATP synthase epsilon	Acid
HR078_03190	*atpH*	F0F1-ATP synthase delta	Acid
HR078_03200	*atpG*	F0F1-ATP synthase gamma	Acid
HR078_03175	*atpB*	F0F1-ATP synthase subunit A	Acid
HR078_03560	*clpX*	ATP-dependent ClpX protease	Acid
HR078_00335	*ark*	Aldo/keto reductase	Osmotic
HR078_09455	*glpF*	Aquaporin family protein	Osmotic
HR078_10525	*nagB*	Glucosamine-6-phosphate deaminase	Biliar
HR078_01470	*pyrG*	CTP synthase	Biliar
HR078_09705	*pepF*	Oligoendopeptidase F	Biliar
HR078_04350	*cbh*	Choloylglycine hydrolase family protein	Biliar
HR078_07785	*groel*	chaperonin GroEL	Heat
HR078_06405	*hcrA*	Heat-inducible transcription repressor HrcA	Heat
HR078_06390	*dnaJ*	Molecular chaperone DnaJ	Acid, Biliar, Osmotic, Heat
HR078_06395	*dnaK*	Molecular chaperone DnaK	Acid, Biliar, Osmotic, Heat
HR078_06400	*grpE*	Nucleotide exchange factor GrpE	Acid, Biliar, Osmotic, Heat
HR078_00560	*YyclC*	Two-component system regulatory protein	Acid, Biliar, Osmotic, Heat
HR078_08640	*nhaC*	Na+/H+ antiporter NhaC	Acid, Osmotic
HR078_06090	*clpP*	Clp protease ClpP	Acid, Biliar
HR078_06270	*clpE*	AAA family ATPase	Acid, Biliar
HR078_06320	*eno*	Phosphopyruvate hydratase	Acid, Biliar
HR078_00380	*luXs*	S-ribosylhomocysteine lyase	Osmotic, Biliar

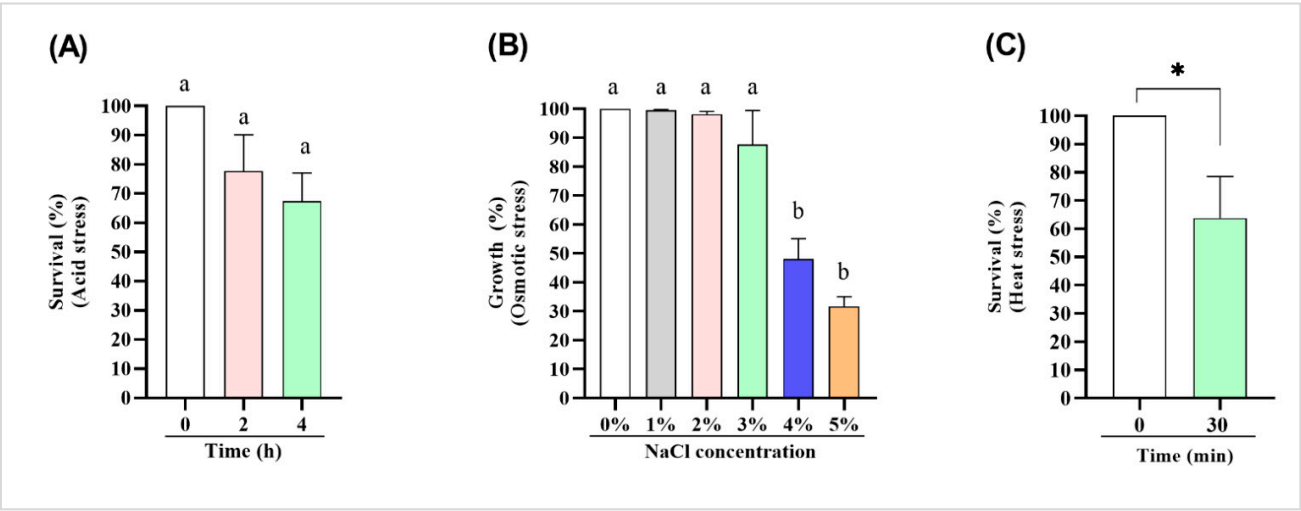


Figure 4. *L. delbrueckii* CIDCA 133 tolerates different stressors. (**A**) Survival percentage in acid stress (0.3% pepsin solution pH 3.0). (**B**) The growth rate in different concentrations of NaCl (1–5%). (**C**) Survival percentage in heat stress. Different letters and * indicate statistically significant differences ($p < 0.05$) by ANOVA followed by Tukey's post hoc test (acid and osmotic stress) and Student's *t*-test (thermal stress).

After heat stress, it was observed that CIDCA 133 presented 63.75% of viability, revealing that the strain can tolerate high temperature (Figure 4C).

3.5. Proteolytic System, Symbiotic, and Metabolic Genomic Islands

Based on data from the literature, through manual inspection of the CIDCA 133 genome annotation, it was possible to identify CDS possibly related to the strain proteolytic activity. CIDCA 133 genome encodes genes related to cell-wall bound proteinase (*PrtB*, *PrtM*), different classes of peptidases (*pepN*, *pepC*, *pepV*, *pepT*, *pepO*, *pepX*), and peptide transporters (*oppA*, *oppC*, *dppB*, *dppE*) (Table S2).

Additionally, twelve genomic islands (GEIs) were identified: seven symbiotic (SI) and five metabolic islands (MI), respectively (Figure 5). All CDS of CIDCA 133 GEIs are described in Table S3.

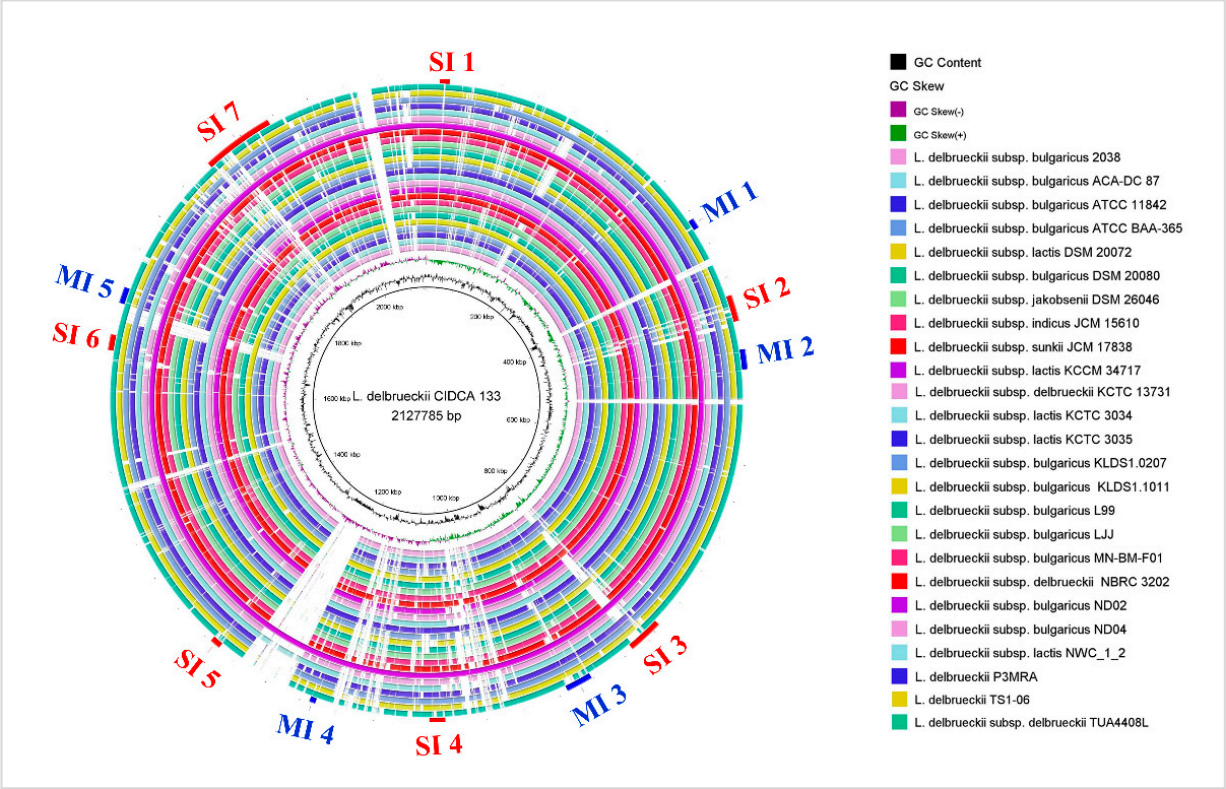


Figure 5. Circular schematic representation of Metabolic (MI) and Symbiotic (SI) islands predicted with GIPSy software in CIDCA 133 genome and its comparison with others *L. delbrueckii* complete genomes. Each ring of the circle corresponds to a specific *L. delbrueckii* whole genome, represented by different colors in the legend (right).

3.6. Putative Bacteriocins and Antibacterial Activity

In CIDCA 133 genome, the BAGEL4 web server predicted three bacteriocins belonging to class III: two helveticin-J (Figure 6A,C) (330 and 331 amino acids, respectively) and enterolysin-A (Figure 6B) (269 amino acids).

Additionally, to determine whether *L. delbrueckii* CIDCA 133 exhibited antibacterial activity, a bacterial inhibition assay was performed based on inhibitory compounds present in its culture supernatant. The bacterial culture supernatant (CFS) had an approximate pH of 3.8 after 24 h of growth and was able to inhibit the growth of pathogens, such as *L. monocytogenes* (90.9% ± 1.70), *E. faecalis* (88.4% ± 7.5), *S. sonnei* (91.6% ± 2.6), and *S. enterica* Typhimurium (84% ± 11.1). In addition, the CIDCA 133 supernatant effect was evaluated in other *Lactobacillus* species, such as *L. delbrueckii* CNRZ327 and *L. paracasei* BL23, in which it was possible to observe an inhibition rate against these bacteria of 77.7% ± 4.9 and 88.8% ± 0.14, respectively (Figure 6D). After the neutralization of supernatant (nCFS) (pH = 6.5), it was possible to observe a reduction in the inhibition rate of *L. monocytogenes* (35.9% ± 1.12; $p = 0.0007$), *E. faecalis* (30.95% ± 0.3; $p = 0.0086$), *S. sonnei*

(39.7% ± 6.6; $p = 0.0094$), *S. enterica* Typhimurium (34.71% ± 3.3; $p = 0.0267$), *L. paracasei* BL23 (29.4% ± 4.1; $p = 0.0024$), and *L. delbrueckii* CNRZ327 (42.41% ± 8.7; $p < 0.05$) (Figure 6D).

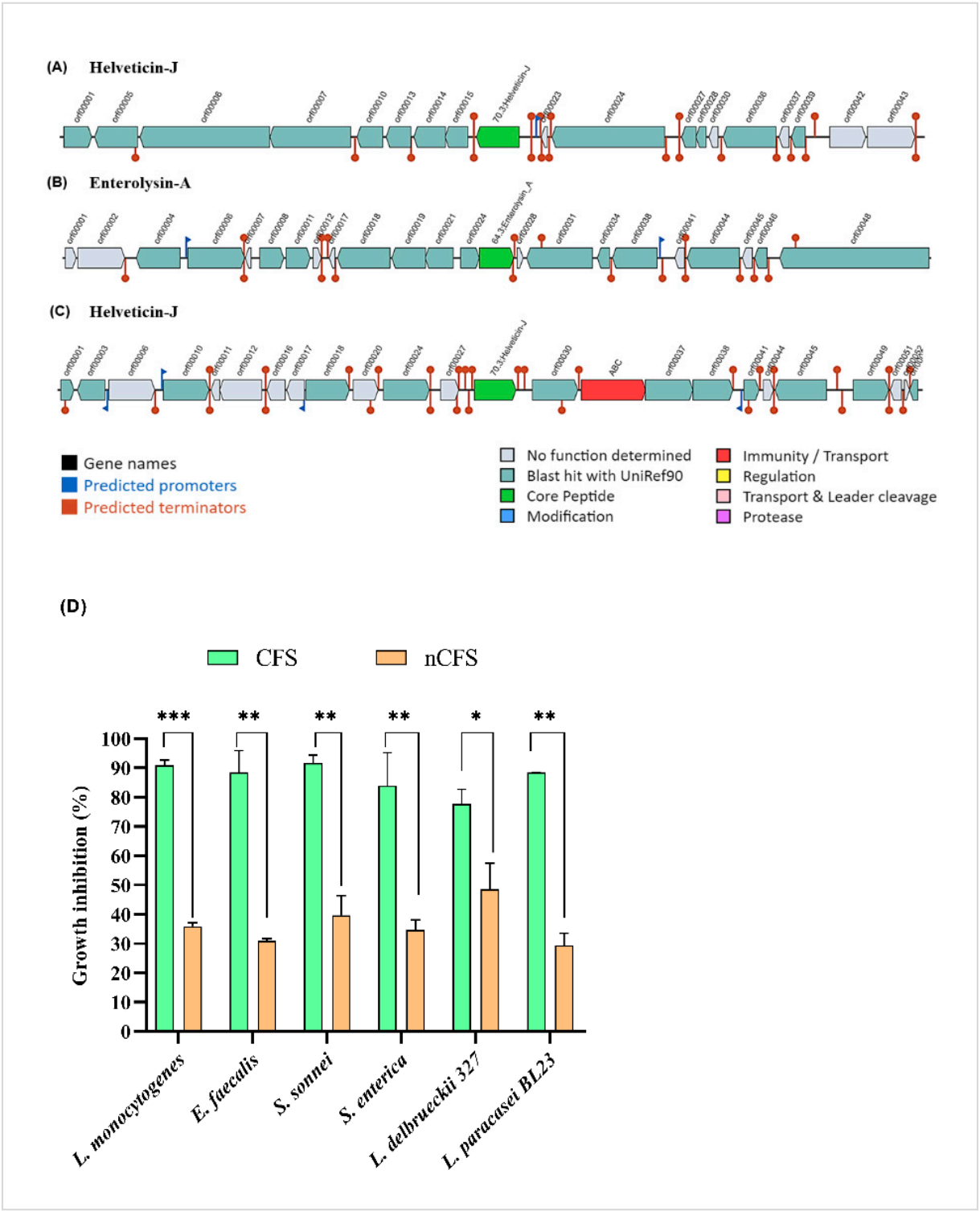


Figure 6. Schematic representation of bacteriocin gene operon (**A**–**C**) present in *L. delbrueckii* CIDCA 133 genome predicted by BAGEL4. (**D**) Antibacterial activity spectrum of bacterial supernatants from *L. delbrueckii* CIDCA 133 against pathogenic and non-pathogenic bacteria. Asterisk indicates statistical difference by Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.7. Cell Adhesion-Related Genes

Using SurfG+ software, it was found that 1606 proteins of CIDCA 133 are cytoplasmic (CYT), 312 membrane (ME), 156 protein surfaces exposed (PSE), and 58 secreted (SE) (Figure 7A; Table S4). According to the Vaxign web server, 16 of the predicted proteins sub located on the membrane, 48 PSE and 38 secreted had high cell adhesion probability (Figure 7B; Table S5). These proteins-encoding genes include the SLAP domain-containing protein (*SLAP*), peptidase S8 (*PrtB*), MucBP domain protein (*MucB*), aggregation promoting factor (*Apf*), lipoteichoic acid synthase family protein (*LtaS*), trypsin-like serine protease (*HtrA*), among others.

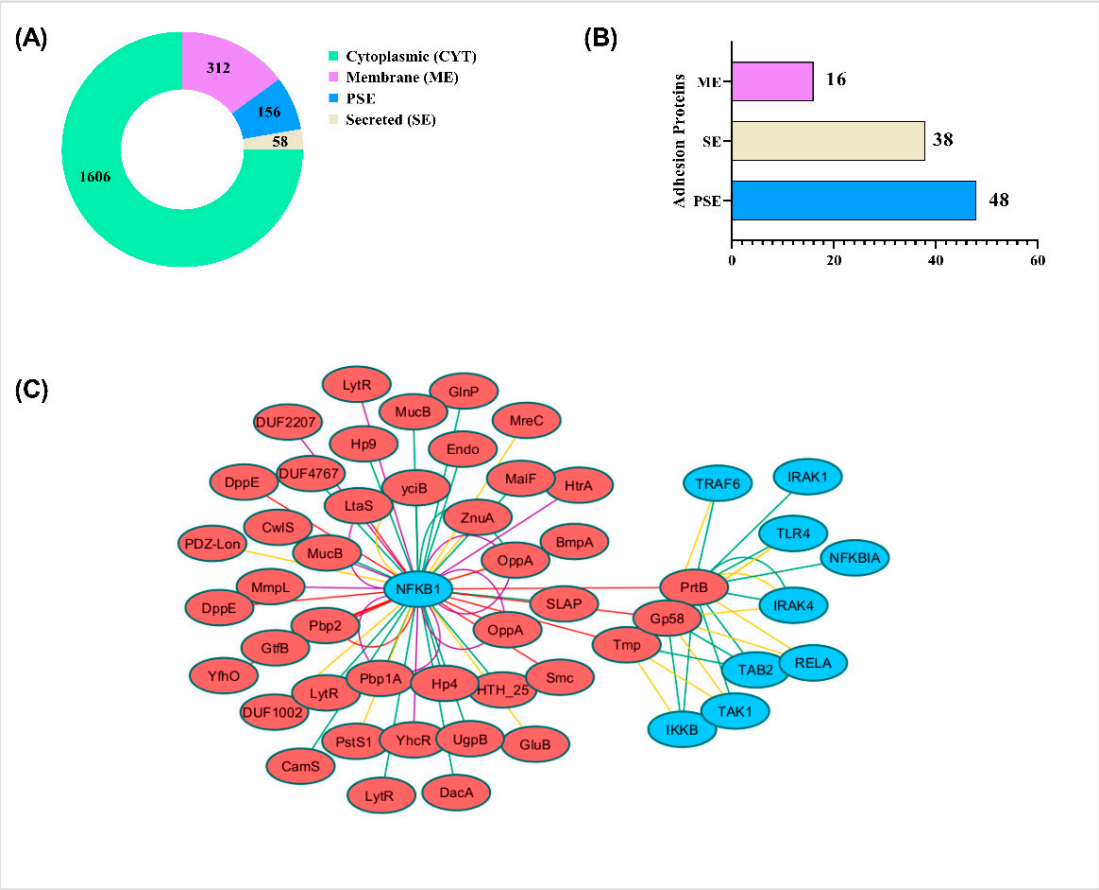


Figure 7. Distribution of subcellular localization of CIDCA 133 proteins predicted by SurfG+ software (**A**). Several proteins with high probably adhesion capacity predicted by Vaxign software (**B**). Protein–protein interaction network mapping to the KEGG toll-like receptor/nuclear factor κappa B (TLR/NF-κB) signaling pathway. Red circle nodes represent bacteria proteins, and blue circle nodes represent human proteins. The strongest associations are represented with different colors line. Interaction score: 0.97 (yellow line), 0.98 (green line), 0.99 (purple line), 1.0 (red line) (**C**).

3.8. Protein-Protein Interaction

The CIDCA 133 and human protein–protein interaction by InterSPPI predicted 74 interactions (Table S6). The nuclear factor NF-κB p105 subunit (NFKB1) was the most frequent interacting human protein. On the other hand, the PrtB protein (a cell surface proteinase) was the most frequent interacting CIDCA 133 protein. CIDCA 133 proteins also interacted with other human proteins involved with the TLR/NF-κB signaling pathway activation, such as TLR4, IRAK4, IRAK1, TRAF6, TAB2, TAK1, IKKB, RELA, and NFKBIA (Figure 7C).

3.9. CIDCA 133 Influences on Intestinal Mucosa Immune System

Consumption of CIDCA 133 was also reported to modulate ileal expression of cytokines genes in mice. After oral CIDCA 133 administration, it was possible to observe a downregulation in the mRNA expression of inflammatory cytokines *Tnf* (0.79 ± 0.12), *Il6* (0.67 ± 0.11), *Il12* (0.42 ± 0.16), *Il1b* (0.56 ± 0.14), and *Il17a* (0.31 ± 0.25) when compared to those exhibited in the NC group: *Tnf* (1.00 ± 0.18; $p = 0.0406$), *Il6* (1.00 ± 0.20; $p = 0.0064$), *Il12* (1.00 ± 0.19; $p = 0.0004$), *Il1b* (1.00 ± 0.18; $p = 0.0011$), and *Il17a* (1.00 ± 0.30; $p = 0.0017$) (Figure 8).

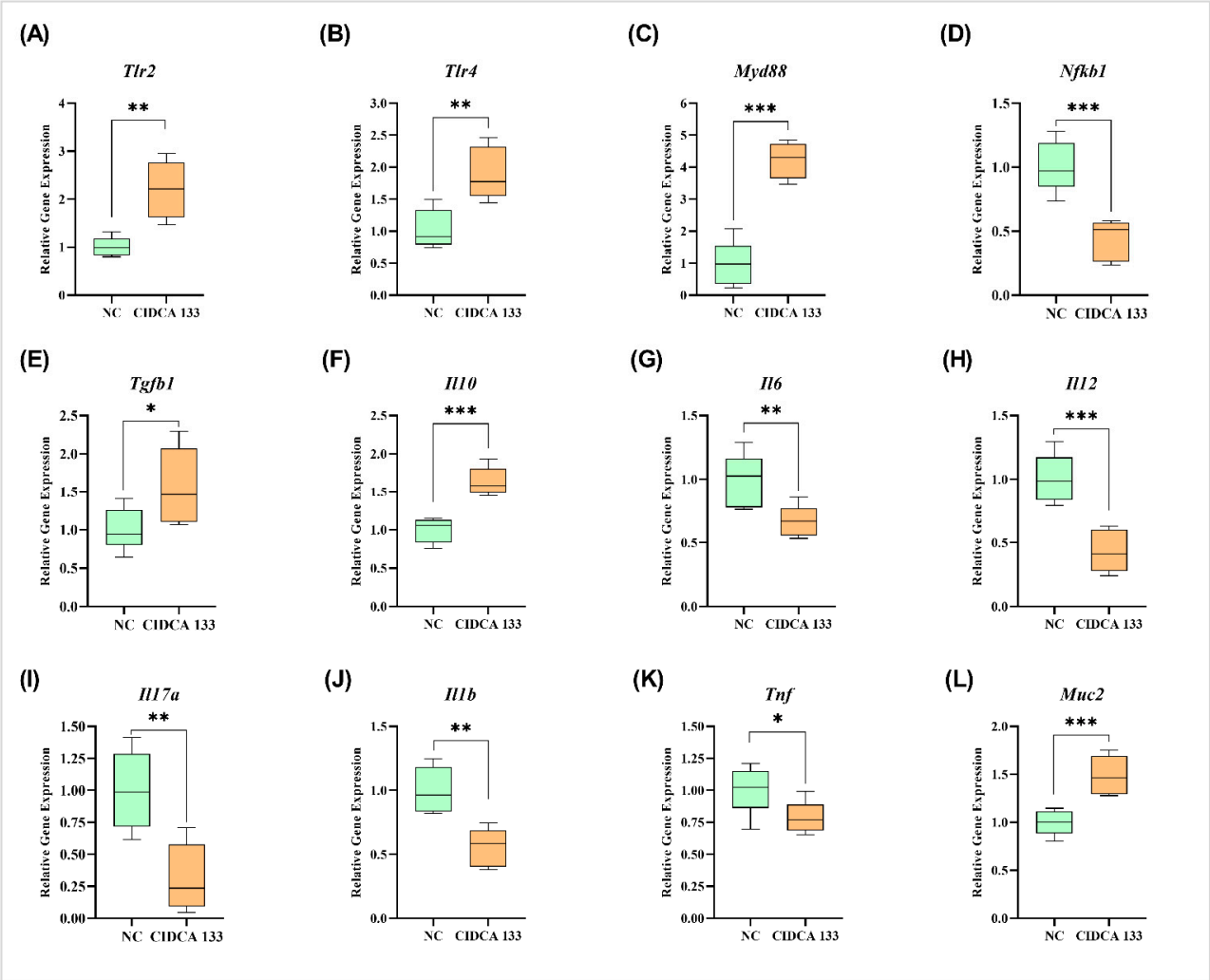


Figure 8. Relative gene expression of (**A**–**L**) *Tlr2*, *Tlr4*, *Myd88*, *Nfkb1*, *Tgfb1*, *Il10*, *Il6*, *Il12*, *Il17a*, *Il1b*, *Tnf*, and *Muc2* in the ileum section of animals that received oral administration of *L. delbrueckii* CIDCA 133 for 13 consecutive days. Asterisk indicates statistical difference by Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

On the other hand, the mRNA expression of *Muc2* (1.48 ± 0.19), *Tlr2* (2.19 ± 0.59), *Tlr4* (1.90 ± 0.41), *Myd88* (4.22 ± 0.57), *Nfkb1* (0.44 ± 0.15), *Tgfb1* (1.56 ± 0.48), and *Il10* (1.63 ± 0.18) were upregulated after oral administration of CIDCA 133 in relation to the NC group: *Muc2* (1.00 ± 0.12; $p = 0.0010$), *Tlr2* (1.00 ± 0.20; $p = 0.0028$), *Tlr4* (1.00 ± 0.30; $p = 0.0052$), *Myd88* (1.00 ± 0.67; $p < 0.0001$), *Nfkb1* (1.00 ± 0.19; $p = 0.0003$), *Tgfb1* (1.00 ± 0.27; $p = 0.0325$), and *Il10* (1.00 ± 0.15; $p = 0.0002$) (Figure 8).

4. Discussion

Lactobacillus strains have functional characteristics beneficial to the host, such as an anti-inflammatory effect and resistance and adaptation mechanisms to the GIT conditions. These features lead these microorganisms to have high relevance in the biotechnological and industrial food sector [5,63] for their use as a probiotic supplement. Due to these properties, specific mechanisms of action of these microorganisms have been elucidated through omics investigations [18].

L. delbrueckii CIDCA 133 has emerged as a potential probiotic strain [10,22,25]. Based on its beneficial effects, species identification, gene product function, and potential molecular mechanisms associated with these strain's probiotic effects were investigated in this work through a genome and phenotype-scale analysis.

CIDCA 133 had its identification performed by classical microbiological methods. Both MALDI-TOF Biotyper® and Average Nucleotide Identity (ANI) analysis supported this classification, which showed that this strain presents a high similarity with the others belonging to the *L. delbrueckii* subsp. *lactis* species [64]. The CIDCA 133 genome had about 2.2 Mb and 2004 protein-coding sequences. In addition, this strain had one plasmid sequence (6224 bp). According to Lee et al. [65], the presence of plasmids in *L. delbrueckii* strains is rare, unlike other Lactic Acid Bacteria (LAB) species. The low number of plasmid sequences of this species deposited in the NCBI corroborates this fact, with only four plasmid sequences deposited (Access Number: CP002342.1; CP018612.1; CP018613.1 and CP029251.1).

Probiotic microorganisms must resist stress in both product matrices and during their passage through the GIT to produce many beneficial effects on the host's health. CIDCA 133 harbored many genes encoded for stress-related proteins, such as a two-component system sensor, F0F1 ATP synthase, ornithine decarboxylase, phosphopyruvate hydratase, and choloylglycine hydrolase. These proteins respond to specific stress stimulus and generate a broad range of response results [45]. Furthermore, these genetic factors can be associated with CIDCA 133 survival capacity to simulated gastric juice reported in this study and to the data found by Kociubinski et al. [23], which demonstrated for the first time the ability of CIDCA 133 to resist bile salt (0.1% and 0.5%). These genes were also previously shown to be involved with the capacity of *L. rhamnosus* [66], *Limosilactobacillus reuteri* (*L. reuteri*) [67], and *L. helveticus* [47] strain to survive to pH 3 and 0.3% bile salt for 2–3 h.

CIDCA 133 also carried genes, such as Na+/H+ antiporter (*NhaC*), S-ribosylhomocysteine lyase (*luXs*), aquaporin family protein (*glpF*), and heat shock proteins (*DnaK, DnaJ, GroEL*), which may be related to its ability to tolerate different concentrations (1%–3%) of sodium chloride (osmotic stress) and heat stress, respectively. These findings corroborate with other studies that demonstrated that different *Lactobacillus* species could tolerate different NaCl concentrations, such as *Lactiplantibacillus plantarum* (*L. plantarum*) [68] that tolerates up to 5% of NaCl, *L. paracasei* [56] and *L. delbrueckii* subsp. *bulgaricus* [69], which tolerated up to 4% NaCl.

Sodium chloride is generally used in the fermented food industry, such as cheese [70]. However, varying concentrations of sodium chloride present in these products and the high temperature used for their production can compromise probiotic bacteria's viability and activity [71,72]. Therefore, the ability of CIDCA 133 to resist acid, bile, different concentrations of NaCl (1–3%), and pasteurization temperature allows this strain to perform better at its health-promoting site of action and makes it promising for application in the food sector for the development of dairy fermented products with functional characteristics.

When consumed, probiotic bacteria must also have the ability to interact with intestinal epithelial cells, which is a crucial factor for their interaction activation with the host [73,74]. Several studies have demonstrated the involvement of extracellular and surface-bound proteins identified in the bacteria/host interaction, leading to biological processes, such as cell adhesion, competitive exclusion of pathogens, and mucosal immune regulation. These proteins include SlpB, slpE, htrA4, and hsdM3 of *P. freudenreichii* CIRM-BIA 129

and CIRM-BIA 121 [75–77], and SlpA of *L. acidophilus* [78] and *L. helveticus* MIMLh5 [79], among others.

In the CIDCA 133 genome, 312 membrane proteins, 58 secreted and 156 surfaces exposed (PSE) were predicted. Of these, 102 were identified with a high probability of cell adhesion, such as the SLAP domain-containing protein, MucBP domain-containing protein, lipoteichoic acid synthase family protein, proteinase PrtB, and aggregation promoting factor. These proteins can be involved in the protective effects of CIDCA 133 against *Bacillus cereus* [25] and *Citrobacter rodentium* [26] infection. This bacteria stimulated immune cell responses (macrophages and dendritic cells derived from human monocytes) infected with these pathogens to reduce the infection by producing co-stimulatory and effector molecules (TNF-α, IL-6, IL-8, and iNOS).

Lactobacillus strains can modulate the host's immune response through their interaction with intestinal epithelial cells [80] mainly conducted by toll-like receptors (TLRs), which when activated can stimulate the activation of signaling pathways, such as the nuclear factor κappa B (NF-κB) and mitogen-activated protein kinase (MAPK), with subsequent production of cytokines [81].

The immunostimulatory capacity of CIDCA 133 in vivo was evaluated in this work. It was possible to observe an increase in the gene expression of *Tlr2*, *Tlr4* and *Myd88* after CIDCA 133 strain consumption. These findings are supported by other studies, which observed that administration of the probiotics *Lacticaseibacillus casei* (*L. casei*) and *Saccharomyces boulardii* could also stimulate the mucosal immune system of healthy mice and broilers, respectively, by increasing gene expression of *Tlr2*, *Tlr4,* and *Myd88* [82,83].

The NF-κB pathway leads to the upregulation of pro-inflammatory genes that, if not controlled at homeostatic levels, can lead to the onset and progression of inflammatory bowel diseases (IBDs) [84–86]. Several probiotics can downregulate the expression of pro-inflammatory cytokines. *L. acidophilus* was able to decrease the intestinal damage caused by 5-Fluorouracil (5-FU) (450 mg/kg) by inhibiting the signaling of the NF-κB pathway and observing low levels of pro-inflammatory cytokines TNF-α and IL-1β [87]. *L. gasseri* 4M13 inhibited the release of inflammatory mediators, such as TNF-α, IL-6, IL-1β, and induced IL-10, in LPS-stimulated RAW 264.7 macrophages [88]. In addition, *L. helveticus* SBT2171 induces A20 gene expression for inhibiting the activation of NF-κB/MAPKs and IL-6 and IL-1β production in macrophages cell [89].

Knowing the reduction in pro-inflammatory cytokines expression is also reported as a positive effect of probiotic microorganisms [82,83], in this work, a reduction in pro-inflammatory (*Tnf*, *Il6*, *Il12*, *Il17a*, and *Il1b*) and an increase in anti-inflammatory (*Tgfb1* and *Il10*) cytokines gene expression in health mice was observed after oral CIDCA 133 administration. This modulation can be related to the downregulation of *Nfkb1* gene expression. This result is following the InterSPPI prediction since the nuclear factor NF-κB p105 subunit (NFKB1) was the most frequent interacting human protein with CIDCA 133 proteins, suggesting these proteins are possibly involved with its immunomodulatory property. However, other studies must be performed to validate this finding, such as CIDCA 133 knockout genes or heterologous production of these proteins, and their phenotypic evaluation on inflammation models.

Some studies have also demonstrated that epithelial activation of TLR2/TLR4 is associated with the development and maturation of mucus-producing goblet cells [90,91]. This finding supports the results reported in this work, in which it was observed that oral administration of CIDCA 133 also increased the gene expression of the MUC2 protein (*mucin 2*), one of the main components of the intestinal mucus layer.

Based on these findings, it can also be inferred that the modulation of the epithelial barrier markers and immune system to an anti-inflammatory profile by CIDCA 133 in healthy mice can be associated with its protective effect against intestinal mucosa damage caused by 5-FU chemotherapy [10]. Thus, this property further highlights the anti-inflammatory effect that the CIDCA 133 strain can exert on the host.

The commensal and probiotic bacteria must also act in symbiosis with the host to promote its beneficial effects. The host provides a stable habitat for these microorganisms while providing them with beneficial nutrients [92,93]. In this context, the presence of five metabolic islands (MI), seven symbiotic Islands (SI), and genes related to proteolytic activity in the CIDCA 133 genome (e.g., *OppA, pepC pepI, pepA, PrtB*) highlights the ability of this strain to capture and metabolize dairy proteins during the fermentation process. An organized proteolytic system has also been identified in other *Lactobacillus* species, such as *L. reuteri* [94,95], *L. helveticus* [96], and *Lactiplantibacillus pentosus* (*L. pentosus*) [97].

The proteolytic activity of probiotic bacteria during the fermentation process is much responsible for bioactive peptides production [98] and other compounds, such as vitamins [99] and Short Chain Fatty Acids (SCFA) [100,101], which, besides improving the sensory characteristics of dairy products [102,103], promote beneficial effects to the host due to its antioxidant and immunomodulatory activity. The beneficial effects of fermented formulations by probiotic bacteria, such as *L. rhamnosus* GG [104], *L. delbrueckii* CNRZ327 [105], *L. plantarum* [11], *L. paracasei* BL23, and *P. freudenreichii* 138 [9], has been reported due to their effectiveness for preventing enteric infection, and the intestinal inflammation and histological damage in murine models of colitis and mucositis disease. The beneficial effects of dairy fermented product by CIDCA 133 were previously reported in a murine model of mucositis [10], evidencing, therefore, the intrinsic and healthy symbiotic relationship between the administration of this probiotic strain and the host.

Another relevant property attributed to CIDCA 133 is its ability to inhibit enteropathogenic and other probiotic bacteria, an effect previously reported by Kociubinski et al. [22] and Hugo et al. [24] for other pathogens. The authors observed inhibition of CIDCA 133 against food spoilage and pathogenic bacteria *B. subtilis*, *B. cereus*, *P. aeruginosa*, and enterohemorrhagic *E. coli* O157:H7, and attributed all above inhibitory effects to the probiotic strain's capacity to produce organic compounds, such as lactate.

The inhibitory effects of probiotics against pathogenic bacteria are also related to the production of bacteriocins. This property, as previously demonstrated by Oliveira et al. [106], showed that *L. rhamnosus* L156.4 inhibits the growth of pathogenic bacteria and other *Lactobacillus* by both the production of organic acids present in the strain supernatant and to the antibacterial activity of the bacteriocin enterocin A, whose gene was identified in its genome through BAGEL3 web server [106]. These findings support the present study results due to identifying the gene encoding the bacteriocins helveticin J and enterolysin A, and CIDCA 133's ability to inhibit acid-resistance bacteria with a probiotic profile, such as *L. delbrueckii* CNRZ327 and *L. paracasei* BL23.

In conclusion, the genome-scale analysis of health-promoting probiotic CIDCA 133 elucidated many important functional roles of this strain. CIDCA 133 showed a broader repertoire of genes involved with molecular mechanisms related to its interaction with host, survival, adaptation, and immunostimulatory ability. The molecular bases attributed to the anti-inflammatory profile of CIDCA 133 can be associated with secreted and membrane/exposed to surface proteins. This is the first probiogenomics study of CIDCA 133, validated with in vitro and in vivo experiments, reinforcing that this strain is a highly effective probiotic, providing valuable benefits to the host.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/microorganisms9040829/s1, Table S1: List of human proteins used to protein–protein interaction; Table S2: Proteolytic system predicted in *L. delbrueckii* CIDCA 133 genome; Table S3: Genomic Islands identified in the *L. delbrueckii* CIDCA 133 genome by Gipsy software; Table S4: Proteins of *L. delbrueckii* CIDCA 133 sublocated on Membrane, surface exposed (PSE) and Secreted predicted by SurfG+ software; Table S5: Predicted proteins of *L. delbrueckii* CIDCA 133 with high probably adhesion capacity predicted by Vaxign software; Table S6: InterSPPI human and *L. delbrueckii* CIDCA 133 protein-protein interaction.

Author Contributions: Conceptualization, L.C.L.d.J. and F.F.A.; methodology, L.C.L.d.J., F.F.A., T.d.J.S., N.D.C.-R., R.P., B.B., data curation, T.d.J.S.; Writing—Original draft preparation, L.C.L.d.J.; Writing—Review and editing, F.F.A., T.d.J.S., M.M.D., P.M.-A., V.A.; supervision, V.A.; funding acquisition, V.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant number 312045/2020-4.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Brazilian College of Animal Experimentation (COBEA), and approved by the Local Animal Experimental Ethics Committee of Universidade Federal de Minas Gerais (CEUA-UFMG) (Protocol nº 112/2020; 08 August 2020).

Informed Consent Statement: Not applicable.

Data Availability Statement: Complete genome sequences used for these analyses are available from the GenBank, NCBI.

Acknowledgments: The authors would like to acknowledge the Pró-Reitoria de Pesquisa—Universidade Federal de Minas Gerais, Rede de Ciências Ômicas (RECOM), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for their financial support and fellowships. We would also like to acknowledge the Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) and Pablo F. Pérez of Universidad Nacional de La Plata-Argentina for bacteria strain supply.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. De Angelis, M.; Gobbetti, M. Lactobacillus SPP.: General Characteristics. In *Reference Module in Food Science*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 72–90.
2. Salvetti, E.; Torriani, S.; Felis, G.E. The Genus *Lactobacillus*: A Taxonomic Update. *Probiotics Antimicrob. Proteins* **2012**, *4*, 217–226. [CrossRef]
3. Kandler, O. Carbohydrate metabolism in lactic acid bacteria. *Antonie Van Leeuwenhoek* **1983**, *49*, 209–224. [CrossRef] [PubMed]
4. Pot, B.; Felis, G.E.; Bruyne, K. De; Tsakalidou, E.; Papadimitriou, K.; Leisner, J.; Vandamme, P. The genus *Lactobacillus*. In *Lactic Acid Bacteria*; John Wiley & Sons, Ltd.: Chichester, UK, 2014; pp. 249–353.
5. Santos Rocha, C.; Gomes-Santos, A.C.; Garcias Moreira, T.; de Azevedo, M.; Diniz Luerce, T.; Mariadassou, M.; Longaray Delamare, A.P.; Langella, P.; Maguin, E.; Azevedo, V.; et al. Local and Systemic Immune Mechanisms Underlying the Anti-Colitis Effects of the Dairy Bacterium *Lactobacillus delbrueckii*. *PLoS ONE* **2014**, *9*, e85923. [CrossRef]
6. Galdeano, C.M. Stimulation of Innate Immune Cells Induced by Probiotics: Participation of Toll-Like Receptors. *J. Clin. Cell. Immunol.* **2015**, *6*. [CrossRef]
7. Fedorak, R.N.; Feagan, B.G.; Hotte, N.; Leddin, D.; Dieleman, L.A.; Petrunia, D.M.; Enns, R.; Bitton, A.; Chiba, N.; Paré, P.; et al. The Probiotic VSL#3 Has Anti-inflammatory Effects and Could Reduce Endoscopic Recurrence After Surgery for Crohn's Disease. *Clin. Gastroenterol. Hepatol.* **2015**, *13*, 928–935.e2. [CrossRef] [PubMed]
8. Bjarnason, I.; Sission, G.; Hayee, B. A randomised, double-blind, placebo-controlled trial of a multi-strain probiotic in patients with asymptomatic ulcerative colitis and Crohn's disease. *Inflammopharmacology* **2019**, *27*, 465–473. [CrossRef] [PubMed]
9. Cordeiro, B.F.; Oliveira, E.R.; da Silva, S.H.; Savassi, B.M.; Acurcio, L.B.; Lemos, L.; Alves, J.d.L.; Carvalho Assis, H.; Vieira, A.T.; Faria, A.M.C.; et al. Whey Protein Isolate-Supplemented Beverage, Fermented by *Lactobacillus casei* BL23 and *Propionibacterium freudenreichii* 138, in the Prevention of Mucositis in Mice. *Front. Microbiol.* **2018**, *9*. [CrossRef]
10. De Jesus, L.C.L.; Drumond, M.M.; de Carvalho, A.; Santos, S.S.; Martins, F.S.; Ferreira, Ê.; Fernandes, R.S.; de Barros, A.L.B.; do Carmo, F.L.R.; Perez, P.F.; et al. Protective effect of *Lactobacillus delbrueckii* subsp. Lactis CIDCA 133 in a model of 5 Fluorouracil-Induced intestinal mucositis. *J. Funct. Foods* **2019**, *53*, 197–207. [CrossRef]
11. Acurcio, L.B.; Bastos, R.W.; Sandes, S.H.d.C.; Guimarães, A.C.d.C.; Alves, C.G.; Reis, D.C.d.; Wuyts, S.; Nunes, Á.C.; Cassali, G.D.; Lebeer, S.; et al. Protective effects of milk fermented by *Lactobacillus plantarum* B7 from Brazilian artisanal cheese on a *Salmonella* enterica serovar Typhimurium infection in BALB/c mice. *J. Funct. Foods* **2017**, *33*, 436–445. [CrossRef]
12. Arreguin-Nava; Hernández-Patlán; Solis-Cruz; Latorre; Hernandez-Velasco; Tellez; El-Ashram; Hargis; Tellez-Isaias Isolation and Identification of Lactic Acid Bacteria Probiotic Culture Candidates for the Treatment of *Salmonella* enterica Serovar Enteritidis in Neonatal Turkey Poults. *Animals* **2019**, *9*, 696. [CrossRef]
13. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. The International Scientific Association for Probiotics and *Prebiotics consensus* statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **2014**, *11*, 506–514. [CrossRef]

14. McFarland, L.V.; Evans, C.T.; Goldstein, E.J.C. Strain-Specificity and Disease-Specificity of Probiotic Efficacy: A Systematic Review and Meta-Analysis. *Front. Med.* **2018**, *5*. [CrossRef]
15. Douillard, F.P.; Ribbera, A.; Järvinen, H.M.; Kant, R.; Pietilä, T.E.; Randazzo, C.; Paulin, L.; Laine, P.K.; Caggia, C.; von Ossowski, I.; et al. Comparative Genomic and Functional Analysis of *Lactobacillus casei* and *Lactobacillus rhamnosus* Strains Marketed as Probiotics. *Appl. Environ. Microbiol.* **2013**, *79*, 1923–1933. [CrossRef]
16. Zhang, W.; Wang, J.; Zhang, D.; Liu, H.; Wang, S.; Wang, Y.; Ji, H. Complete Genome Sequencing and Comparative Genome Characterization of *Lactobacillus johnsonii* ZLJ010, a Potential Probiotic With Health-Promoting Properties. *Front. Genet.* **2019**, *10*. [CrossRef] [PubMed]
17. Verce, M.; De Vuyst, L.; Weckx, S. Comparative genomics of *Lactobacillus fermentum* suggests a free-living lifestyle of this lactic acid bacterial species. *Food Microbiol.* **2020**, *89*, 103448. [CrossRef]
18. Ventura, M.; Turroni, F.; van Sinderen, D. Probiogenomics as a tool to obtain genetic insights into adaptation of probiotic bacteria to the human gut. *Bioengineered* **2012**, *3*, 73–79. [CrossRef]
19. Arellano, K.; Vazquez, J.; Park, H.; Lim, J.; Ji, Y.; Kang, H.-J.; Cho, D.; Jeong, H.W.; Holzapfel, W.H. Safety Evaluation and Whole-Genome Annotation of *Lactobacillus plantarum* Strains from Different Sources with Special Focus on Isolates from Green Tea. *Probiotics Antimicrob. Proteins* **2020**, *12*, 1057–1070. [CrossRef] [PubMed]
20. Gilbert, C.; Atlan, D.; Blanc, B.; Portailer, R.; Germond, J.E.; Lapierre, L.; Mollet, B. A new cell surface proteinase: Sequencing and analysis of the prtB gene from *Lactobacillus delbruekii* subsp. bulgaricus. *J. Bacteriol.* **1996**, *178*, 3059–3065. [CrossRef] [PubMed]
21. El Kafsi, H.; Binesse, J.; Loux, V.; Buratti, J.; Boudebbouze, S.; Dervyn, R.; Kennedy, S.; Galleron, N.; Quinquis, B.; Batto, J.-M.; et al. *Lactobacillus delbrueckii* ssp. lactis and ssp. bulgaricus: A chronicle of evolution in action. *BMC Genom.* **2014**, *15*, 407. [CrossRef] [PubMed]
22. Kociubinski, G.L.; Pérez, P.F.; Añón, M.C.; de Antoni, G.L. A Method of Screening for Highly Inhibitory Lactic Acid Bacteria. *J. Food Prot.* **1996**, *59*, 739–745. [CrossRef] [PubMed]
23. Kociubinski, G.; Pérez, P.; de Antoni, G. Screening of Bile Resistance and Bile Precipitation in Lactic Acid Bacteria and Bifidobacteria. *J. Food Prot.* **1999**, *62*, 905–912. [CrossRef]
24. Hugo, A.A.; Kakisu, E.; De Antoni, G.L.; Pérez, P.F. Lactobacilli antagonize biological effects of enterohaemorrhagic *Escherichia coli* in vitro. *Lett. Appl. Microbiol.* **2008**, *46*, 613–619. [CrossRef]
25. Rolny, I.S.; Tiscornia, I.; Racedo, S.M.; Pérez, P.F.; Bollati-Fogolín, M. *Lactobacillus delbrueckii* subsp *lactis* CIDCA 133 modulates response of human epithelial and dendritic cells infected with *Bacillus cereus*. *Benef. Microbes* **2016**, *7*, 749–760. [CrossRef]
26. Hugo, A.A.; Rolny, I.S.; Romanin, D.; Pérez, P.F. *Lactobacillus delbrueckii* subsp. lactis (strain CIDCA 133) stimulates murine macrophages infected with Citrobacter rodentium. *World J. Microbiol. Biotechnol.* **2017**, *33*, 48. [CrossRef]
27. Hugo, A.A.; De Antoni, G.L.; Pérez, P.F. *Lactobacillus delbrueckii* subsp *lactis* (strain CIDCA 133) resists the antimicrobial activity triggered by molecules derived from enterocyte-like Caco-2 cells. *Lett. Appl. Microbiol.* **2010**, *50*, 335–340. [CrossRef]
28. Hugo, A.A.; Tymczyszyn, E.E.; Gómez-Zavaglia, A.; Pérez, P.F. Effect of human defensins on lactobacilli and liposomes. *J. Appl. Microbiol.* **2012**, *113*, 1491–1497. [CrossRef]
29. De, S.; Kaur, G.; Roy, A.; Dogra, G.; Kaushik, R.; Yadav, P.; Singh, R.; Datta, T.K.; Goswami, S.L. A Simple Method for the Efficient Isolation of Genomic DNA from Lactobacilli Isolated from Traditional Indian Fermented Milk (dahi). *Indian J. Microbiol.* **2010**, *50*, 412–418. [CrossRef] [PubMed]
30. Simon Andrews FastQC: A Quality Control Tool for High Throughput Sequence Data. Available online: http://www.bioinformatics.babraham.ac.uk/projects/fastqc (accessed on 2 December 2020).
31. Hernandez, D.; Francois, P.; Farinelli, L.; Osteras, M.; Schrenzel, J. De novo bacterial genome sequencing: Millions of very short reads assembled on a desktop computer. *Genome Res.* **2008**, *18*, 802–809. [CrossRef] [PubMed]
32. Gurevich, A.; Saveliev, V.; Vyahhi, N.; Tesler, G. QUAST: Quality assessment tool for genome assemblies. *Bioinformatics* **2013**, *29*, 1072–1075. [CrossRef]
33. Galardini, M.; Biondi, E.G.; Bazzicalupo, M.; Mengoni, A. CONTIGuator: A bacterial genomes finishing tool for structural insights on draft genomes. *Source Code Biol. Med.* **2011**, *6*, 11. [CrossRef] [PubMed]
34. de Sá, P.H.C.G.; Miranda, F.; Veras, A.; de Melo, D.M.; Soares, S.; Pinheiro, K.; Guimarães, L.; Azevedo, V.; Silva, A.; Ramos, R.T.J. GapBlaster—A Graphical Gap Filler for Prokaryote Genomes. *PLoS ONE* **2016**, *11*, e0155327. [CrossRef] [PubMed]
35. Guizelini, D.; Raittz, R.T.; Cruz, L.M.; Souza, E.M.; Steffens, M.B.R.; Pedrosa, F.O. GFinisher: A new strategy to refine and finish bacterial genome assemblies. *Sci. Rep.* **2016**, *6*, 34963. [CrossRef] [PubMed]
36. QIAGEN CLC Genomics Workbench. Available online: www.clcbio.com/products/clc-genomics-workbench (accessed on 2 December 2020).
37. Tatusova, T.; DiCuccio, M.; Badretdin, A.; Chetvernin, V.; Nawrocki, E.P.; Zaslavsky, L.; Lomsadze, A.; Pruitt, K.D.; Borodovsky, M.; Ostell, J. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* **2016**, *44*, 6614–6624. [CrossRef]
38. Carattoli, A.; Zankari, E.; García-Fernández, A.; Voldby Larsen, M.; Lund, O.; Villa, L.; Møller Aarestrup, F.; Hasman, H. In Silico Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. *Antimicrob. Agents Chemother.* **2014**, *58*, 3895–3903. [CrossRef]
39. Richter, M.; Rosselló-Móra, R.; Oliver Glöckner, F.; Peplies, J. JSpeciesWS: A web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* **2016**, *32*, 929–931. [CrossRef]

40. Barinov, A.; Loux, V.; Hammani, A.; Nicolas, P.; Langella, P.; Ehrlich, D.; Maguin, E.; van de Guchte, M. Prediction of surface exposed proteins in *Streptococcus pyogenes*, with a potential application to other Gram-positive bacteria. *Proteomics* **2009**, *9*, 61–73. [CrossRef]
41. Araujo, F.A.; Barh, D.; Silva, A.; Guimarães, L.; Ramos, R.T.J. Go Feat: A rapid web-based functional annotation tool for genomic and transcriptomic data. *Sci. Rep.* **2018**, *8*, 1794. [CrossRef] [PubMed]
42. He, Y.; Xiang, Z.; Mobley, H.L.T. Vaxign: The First Web-Based Vaccine Design Program for Reverse Vaccinology and Applications for Vaccine Development. *J. Biomed. Biotechnol.* **2010**, *2010*, 297505. [CrossRef]
43. Liu, M.; Bayjanov, J.R.; Renckens, B.; Nauta, A.; Siezen, R.J. The proteolytic system of lactic acid bacteria revisited: A genomic comparison. *BMC Genom.* **2010**, *11*, 36. [CrossRef]
44. Broadbent, J.R.; Cai, H.; Larsen, R.L.; Hughes, J.E.; Welker, D.L.; De Carvalho, V.G.; Tompkins, T.A.; Ardö, Y.; Vogensen, F.; De Lorentiis, A.; et al. Genetic diversity in proteolytic enzymes and amino acid metabolism among *Lactobacillus helveticus* strains. *J. Dairy Sci.* **2011**, *94*, 4313–4328. [CrossRef]
45. Papadimitriou, K.; Alegría, Á.; Bron, P.A.; de Angelis, M.; Gobbetti, M.; Kleerebezem, M.; Lemos, J.A.; Linares, D.M.; Ross, P.; Stanton, C.; et al. Stress Physiology of Lactic Acid Bacteria. *Microbiol. Mol. Biol. Rev.* **2016**, *80*, 837–890. [CrossRef]
46. Oliveira, L.C.; Saraiva, T.D.L.; Silva, W.M.; Pereira, U.P.; Campos, B.C.; Benevides, L.J.; Rocha, F.S.; Figueiredo, H.C.P.; Azevedo, V.; Soares, S.C. Analyses of the probiotic property and stress resistance-related genes of *Lactococcus lactis* subsp. lactis NCDO 2118 through comparative genomics and in vitro assays. *PLoS ONE* **2017**, *12*, e0175116. [CrossRef]
47. Fontana, A.; Falasconi, I.; Molinari, P.; Treu, L.; Basile, A.; Vezzi, A.; Campanaro, S.; Morelli, L. Genomic Comparison of *Lactobacillus helveticus* Strains Highlights Probiotic Potential. *Front. Microbiol.* **2019**, *10*. [CrossRef]
48. Wu, P.; An, J.; Chen, L.; Zhu, Q.; Li, Y.; Mei, Y.; Chen, Z.; Liang, Y. Differential Analysis of Stress Tolerance and Transcriptome of Probiotic *Lacticaseibacillus casei* Zhang Produced from Solid-State (SSF-SW) and Liquid-State (LSF-MRS) Fermentations. *Microorganisms* **2020**, *8*, 1656. [CrossRef] [PubMed]
49. Soares, S.C.; Geyik, H.; Ramos, R.T.J.; de Sá, P.H.C.G.; Barbosa, E.G.V.; Baumbach, J.; Figueiredo, H.C.P.; Miyoshi, A.; Tauch, A.; Silva, A.; et al. GIPSy: Genomic island prediction software. *J. Biotechnol.* **2016**, *232*, 2–11. [CrossRef]
50. Alikhan, N.-F.; Petty, N.K.; Ben Zakour, N.L.; Beatson, S.A. BLAST Ring Image Generator (BRIG): Simple prokaryote genome comparisons. *BMC Genom.* **2011**, *12*, 402. [CrossRef]
51. van Heel, A.J.; de Jong, A.; Song, C.; Viel, J.H.; Kok, J.; Kuipers, O.P. BAGEL4: A user-friendly web server to thoroughly mine RiPPs and bacteriocins. *Nucleic Acids Res.* **2018**, *46*, W278–W281. [CrossRef] [PubMed]
52. Lian, X.; Yang, S.; Li, H.; Fu, C.; Zhang, Z. Machine-Learning-Based Predictor of Human–Bacteria Protein–Protein Interactions by Incorporating Comprehensive Host-Network Properties. *J. Proteome Res.* **2019**, *18*, 2195–2205. [CrossRef]
53. Shannon, P. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res.* **2003**, *13*, 2498–2504. [CrossRef] [PubMed]
54. Singhal, N.; Maurya, A.K.; Mohanty, S.; Kumar, M.; Virdi, J.S. Evaluation of Bile Salt Hydrolases, Cholesterol-Lowering Capabilities, and Probiotic Potential of Enterococcus faecium Isolated From Rhizosphere. *Front. Microbiol.* **2019**, *10*. [CrossRef]
55. Farias, N.; Soares, M.; Gouveia, E. Enhancement of the viability of *Lactobacillus rhamnosus* ATCC 7469 in passion fruit juice: Application of a central composite rotatable design. *LWT-Food Sci. Technol.* **2016**, *71*, 149–154. [CrossRef]
56. Xu, Y.; Tian, Y.; Cao, Y.; Li, J.; Guo, H.; Su, Y.; Tian, Y.; Wang, C.; Wang, T.; Zhang, L. Probiotic Properties of *Lactobacillus paracasei* subsp. paracasei L1 and Its Growth Performance-Promotion in Chicken by Improving the Intestinal Microflora. *Front. Physiol.* **2019**, *10*. [CrossRef] [PubMed]
57. Somashekaraiah, R.; Shruthi, B.; Deepthi, B.V.; Sreenivasa, M.Y. Probiotic Properties of Lactic Acid Bacteria Isolated From Neera: A Naturally Fermenting Coconut Palm Nectar. *Front. Microbiol.* **2019**, *10*. [CrossRef]
58. Volynets, V.; Rings, A.; Bárdos, G.; Ostaff, M.J.; Wehkamp, J.; Bischoff, S.C. Intestinal barrier analysis by assessment of mucins, tight junctions, and α-defensins in healthy C57BL/6J and BALB/cJ mice. *Tissue Barriers* **2016**, *4*, e1208468. [CrossRef] [PubMed]
59. Giulietti, A.; Overbergh, L.; Valckx, D.; Decallonne, B.; Bouillon, R.; Mathieu, C. An Overview of Real-Time Quantitative PCR: Applications to Quantify Cytokine Gene Expression. *Methods* **2001**, *25*, 386–401. [CrossRef] [PubMed]
60. Song, M.-K.; Park, M.-Y.; Sung, M.-K. 5-Fluorouracil-Induced Changes of Intestinal Integrity Biomarkers in BALB/C Mice. *J. Cancer Prev.* **2013**, *18*, 322–329. [CrossRef] [PubMed]
61. Chang, C.-W.; Lee, H.-C.; Li, L.-H.; Chiang Chiau, J.-S.; Wang, T.-E.; Chuang, W.-H.; Chen, M.-J.; Wang, H.-Y.; Shih, S.-C.; Liu, C.-Y.; et al. Fecal Microbiota Transplantation Prevents Intestinal Injury, Upregulation of Toll-Like Receptors, and 5-Fluorouracil/Oxaliplatin-Induced Toxicity in Colorectal Cancer. *Int. J. Mol. Sci.* **2020**, *21*, 386. [CrossRef] [PubMed]
62. Zheng, L.; Zhang, Y.-L.; Dai, Y.-C.; Chen, X.; Chen, D.-L.; Dai, Y.-T.; Tang, Z.-P. Jianpi Qingchang decoction alleviates ulcerative colitis by inhibiting nuclear factor-κB activation. *World J. Gastroenterol.* **2017**, *23*, 1180. [CrossRef]
63. Sun, Z.; Harris, H.M.B.; McCann, A.; Guo, C.; Argimón, S.; Zhang, W.; Yang, X.; Jeffery, I.B.; Cooney, J.C.; Kagawa, T.F.; et al. Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. *Nat. Commun.* **2015**, *6*, 8322. [CrossRef] [PubMed]
64. Inglin, R.C.; Meile, L.; Stevens, M.J.A. Clustering of Pan- and Core-genome of *Lactobacillus* provides Novel Evolutionary Insights for Differentiation. *BMC Genom.* **2018**, *19*, 284. [CrossRef] [PubMed]
65. Lee, J.-H.; Halgerson, J.S.; Kim, J.-H.; O'Sullivan, D.J. Comparative Sequence Analysis of Plasmids from *Lactobacillus delbrueckii* and Construction of a Shuttle Cloning Vector. *Appl. Environ. Microbiol.* **2007**, *73*, 4417–4424. [CrossRef] [PubMed]

66. Arnold, J.W.; Simpson, J.B.; Roach, J.; Kwintkiewicz, J.; Azcarate-Peril, M.A. Intra-species Genomic and Physiological Variability Impact Stress Resistance in Strains of Probiotic Potential. *Front. Microbiol.* **2018**, *9*. [CrossRef]
67. Chen, L.; Gu, Q.; Li, P.; Chen, S.; Li, Y. Genomic analysis of *Lactobacillus reuteri* WHH 1689 reveals its probiotic properties and stress resistance. *Food Sci. Nutr.* **2019**, *7*, 844–857. [CrossRef]
68. Yao, W.; Yang, L.; Shao, Z.; Xie, L.; Chen, L. Identification of salt tolerance-related genes of *Lactobacillus plantarum* D31 and T9 strains by genomic analysis. *Ann. Microbiol.* **2020**, *70*, 10. [CrossRef]
69. Shehata, M.G.; El Sohaimy, S.A.; El-Sahn, M.A.; Youssef, M.M. Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. *Ann. Agric. Sci.* **2016**, *61*, 65–75. [CrossRef]
70. Albarracín, W.; Sánchez, I.C.; Grau, R.; Barat, J.M. Salt in food processing; usage and reduction: A review. *Int. J. Food Sci. Technol.* **2011**, *46*, 1329–1336. [CrossRef]
71. Bautista-Gallego, J.; Arroyo-López, F.N.; Durán-Quintana, M.C.; Garrido-Fernández, A. Individual Effects of Sodium, Potassium, Calcium, and Magnesium Chloride Salts on *Lactobacillus pentosus* and Saccharomyces cerevisiae Growth. *J. Food Prot.* **2008**, *71*, 1412–1421. [CrossRef] [PubMed]
72. Gandhi, A.; Shah, N.P. Effect of salt on cell viability and membrane integrity of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium longum* as observed by flow cytometry. *Food Microbiol.* **2015**, *49*, 197–202. [CrossRef] [PubMed]
73. Vélez, M.P.; De Keersmaecker, S.C.J.; Vanderleyden, J. Adherence factors of *Lactobacillus* in the human gastrointestinal tract. *FEMS Microbiol. Lett.* **2007**, *276*, 140–148. [CrossRef] [PubMed]
74. Turpin, W.; Humblot, C.; Noordine, M.-L.; Thomas, M.; Guyot, J.-P. *Lactobacillaceae* and Cell Adhesion: Genomic and Functional Screening. *PLoS ONE* **2012**, *7*, e38034. [CrossRef] [PubMed]
75. Le Maréchal, C.; Peton, V.; Plé, C.; Vroland, C.; Jardin, J.; Briard-Bion, V.; Durant, G.; Chuat, V.; Loux, V.; Foligné, B.; et al. Surface proteins of Propionibacterium freudenreichii are involved in its anti-inflammatory properties. *J. Proteom.* **2015**, *113*, 447–461. [CrossRef]
76. Deutsch, S.-M.; Mariadassou, M.; Nicolas, P.; Parayre, S.; Le Guellec, R.; Chuat, V.; Peton, V.; Le Maréchal, C.; Burati, J.; Loux, V.; et al. Identification of proteins involved in the anti-inflammatory properties of Propionibacterium freudenreichii by means of a multi-strain study. *Sci. Rep.* **2017**, *7*, 46409. [CrossRef]
77. do Carmo, F.L.R.; Rabah, H.; Cordeiro, B.F.; Silva, S.H. da; Pessoa, R.M.; Fernandes, S.O.A.; Cardoso, V.N.; Gagnaire, V.; Deplanche, M.; Savassi, B.; et al. Probiotic Propionibacterium freudenreichii requires SlpB protein to mitigate mucositis induced by chemotherapy. *Oncotarget* **2019**, *10*, 7198–7219. [CrossRef] [PubMed]
78. Ashida, N.; Yanagihara, S.; Shinoda, T.; Yamamoto, N. Characterization of adhesive molecule with affinity to Caco-2 cells in *Lactobacillus acidophilus* by proteome analysis. *J. Biosci. Bioeng.* **2011**, *112*, 333–337. [CrossRef] [PubMed]
79. Taverniti, V.; Stuknyte, M.; Minuzzo, M.; Arioli, S.; De Noni, I.; Scabiosi, C.; Cordova, Z.M.; Junttila, I.; Hämäläinen, S.; Turpeinen, H.; et al. S-Layer Protein Mediates the Stimulatory Effect of *Lactobacillus helveticus* MIMLh5 on Innate Immunity. *Appl. Environ. Microbiol.* **2013**, *79*, 1221–1231. [CrossRef] [PubMed]
80. Rocha-Ramírez, L.M.; Pérez-Solano, R.A.; Castañón-Alonso, S.L.; Moreno Guerrero, S.S.; Ramírez Pacheco, A.; García Garibay, M.; Eslava, C. Probiotic *Lactobacillus* Strains Stimulate the Inflammatory Response and Activate Human Macrophages. *J. Immunol. Res.* **2017**, *2017*, 4607491. [CrossRef]
81. Kawai, T.; Akira, S. Signalling to NF-κB by Toll-like receptors. *Trends Mol. Med.* **2007**, *13*, 460–469. [CrossRef] [PubMed]
82. Galdeano, C.M.; Perdigón, G. The Probiotic Bacterium *Lactobacillus casei* Induces Activation of the Gut Mucosal Immune System through Innate Immunity. *Clin. Vaccine Immunol.* **2006**, *13*, 219–226. [CrossRef]
83. Rajput, I.R.; Ying, H.; Yajing, S.; Arain, M.A.; Weifen, L.; Ping, L.; Bloch, D.M.; Wenhua, L. Saccharomyces boulardii and Bacillus subtilis B10 modulate TLRs and cytokines expression patterns in jejunum and ileum of broilers. *PLoS ONE* **2017**, *12*, e0173917. [CrossRef]
84. Schreiber, S.; Nikolaus, S.; Hampe, J. Activation of nuclear factor kappa B in inflammatory bowel disease. *Gut* **1998**, *42*, 477–484. [CrossRef]
85. Burkitt, M.D.; Hanedi, A.F.; Duckworth, C.A.; Williams, J.M.; Tang, J.M.; O'Reilly, L.A.; Putoczki, T.L.; Gerondakis, S.; Dimaline, R.; Caamano, J.H.; et al. NF-κB1, NF-κB2 and c-Rel differentially regulate susceptibility to colitis-associated adenoma development in C57BL/6 mice. *J. Pathol.* **2015**, *236*, 326–336. [CrossRef] [PubMed]
86. Han, Y.M.; Koh, J.; Kim, J.W.; Lee, C.; Koh, S.-J.; Kim, B.; Lee, K.L.; Im, J.P.; Kim, J.S. NF-kappa B activation correlates with disease phenotype in Crohn's disease. *PLoS ONE* **2017**, *12*, e0182071. [CrossRef] [PubMed]
87. Justino, P.F.C.; Melo, L.F.M.; Nogueira, A.F.; Morais, C.M.; Mendes, W.O.; Franco, A.X.; Souza, E.P.; Ribeiro, R.A.; Souza, M.H.L.P.; Soares, P.M.G. Regulatory role of *Lactobacillus acidophilus* on inflammation and gastric dysmotility in intestinal mucositis induced by 5-fluorouracil in mice. *Cancer Chemother. Pharmacol.* **2015**, *75*, 559–567. [CrossRef] [PubMed]
88. Oh, N.S.; Joung, J.Y.; Lee, J.Y.; Kim, Y. Probiotic and anti-inflammatory potential of *Lactobacillus rhamnosus* 4B15 and *Lactobacillus gasseri* 4M13 isolated from infant feces. *PLoS ONE* **2018**, *13*, e0192021. [CrossRef]
89. Kawano, M.; Miyoshi, M.; Miyazaki, T. *Lactobacillus helveticus* SBT2171 Induces A20 Expression via Toll-Like Receptor 2 Signalling and Inhibits the Lipopolysaccharide-Induced Activation of Nuclear Factor-kappa B and Mitogen-Activated Protein Kinases in Peritoneal Macrophages. *Front. Immunol.* **2019**, *10*. [CrossRef] [PubMed]
90. Podolsky, D.K.; Gerken, G.; Eyking, A.; Cario, E. Colitis-Associated Variant of TLR2 Causes Impaired Mucosal Repair Because of TFF3 Deficiency. *Gastroenterology* **2009**, *137*, 209–220. [CrossRef] [PubMed]

91. Dheer, R.; Santaolalla, R.; Davies, J.M.; Lang, J.K.; Phillips, M.C.; Pastorini, C.; Vazquez-Pertejo, M.T.; Abreu, M.T. Intestinal Epithelial Toll-Like Receptor 4 Signalling Affects Epithelial Function and Colonic Microbiota and Promotes a Risk for Transmissible Colitis. *Infect. Immun.* **2016**, *84*, 798–810. [CrossRef] [PubMed]
92. Buron-Moles, G.; Chailyan, A.; Dolejs, I.; Forster, J.; Mikš, M.H. Uncovering carbohydrate metabolism through a genotype-phenotype association study of 56 lactic acid bacteria genomes. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 3135–3152. [CrossRef] [PubMed]
93. Visconti, A.; Le Roy, C.I.; Rosa, F.; Rossi, N.; Martin, T.C.; Mohney, R.P.; Li, W.; de Rinaldis, E.; Bell, J.T.; Venter, J.C.; et al. Interplay between the human gut microbiome and host metabolism. *Nat. Commun.* **2019**, *10*, 4505. [CrossRef] [PubMed]
94. Saulnier, D.M.; Santos, F.; Roos, S.; Mistretta, T.-A.; Spinler, J.K.; Molenaar, D.; Teusink, B.; Versalovic, J. Exploring Metabolic Pathway Reconstruction and Genome-Wide Expression Profiling in *Lactobacillus reuteri* to Define Functional Probiotic Features. *PLoS ONE* **2011**, *6*, e18783. [CrossRef]
95. Kristjansdottir, T.; Bosma, E.F.; Branco dos Santos, F.; Özdemir, E.; Herrgård, M.J.; França, L.; Ferreira, B.; Nielsen, A.T.; Gudmundsson, S. A metabolic reconstruction of *Lactobacillus reuteri* JCM 1112 and analysis of its potential as a cell factory. *Microb. Cell Fact.* **2019**, *18*, 186. [CrossRef]
96. Griffiths, M.W.; Tellez, A.M. *Lactobacillus helveticus*: The proteolytic system. *Front. Microbiol.* **2013**, *4*. [CrossRef] [PubMed]
97. Abriouel, H.; Pérez Montoro, B.; Casimiro-Soriguer, C.S.; Pérez Pulido, A.J.; Knapp, C.W.; Caballero Gómez, N.; Castillo-Gutiérrez, S.; Estudillo-Martínez, M.D.; Gálvez, A.; Benomar, N. Insight into Potential Probiotic Markers Predicted in *Lactobacillus pentosus* MP-10 Genome Sequence. *Front. Microbiol.* **2017**, *8*. [CrossRef] [PubMed]
98. Singh, B.P.; Vij, S. Growth and bioactive peptides production potential of *Lactobacillus plantarum* strain C2 in soy milk: A LC-MS/MS based revelation for peptides biofunctionality. *LWT* **2017**, *86*, 293–301. [CrossRef]
99. De Angelis, M.; Bottacini, F.; Fosso, B.; Kelleher, P.; Calasso, M.; Di Cagno, R.; Ventura, M.; Picardi, E.; van Sinderen, D.; Gobbetti, M. *Lactobacillus rossiae*, a Vitamin B12 Producer, Represents a *Metabolically versatile* Species within the Genus *Lactobacillus*. *PLoS ONE* **2014**, *9*, e107232. [CrossRef] [PubMed]
100. Sun, M.; Wu, W.; Chen, L.; Yang, W.; Huang, X.; Ma, C.; Chen, F.; Xiao, Y.; Zhao, Y.; Ma, C.; et al. Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. *Nat. Commun.* **2018**, *9*, 3555. [CrossRef]
101. Nagpal, R.; Wang, S.; Ahmadi, S.; Hayes, J.; Gagliano, J.; Subashchandrabose, S.; Kitzman, D.W.; Becton, T.; Read, R.; Yadav, H. Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mice and human gut microbiome. *Sci. Rep.* **2018**, *8*, 12649. [CrossRef] [PubMed]
102. Cruz, A.G.; Cadena, R.S.; Walter, E.H.M.; Mortazavian, A.M.; Granato, D.; Faria, J.A.F.; Bolini, H.M.A. Sensory Analysis: Relevance for Prebiotic, Probiotic, and Synbiotic Product Development. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 358–373. [CrossRef]
103. Dimitrellou, D.; Salamoura, C.; Kontogianni, A.; Katsipi, D.; Kandylis, P.; Zakynthinos, G.; Varzakas, T. Effect of Milk Type on the Microbiological, Physicochemical and Sensory Characteristics of Probiotic Fermented Milk. *Microorganisms* **2019**, *7*, 274. [CrossRef]
104. Yoda, K.; Miyazawa, K.; Hosoda, M.; Hiramatsu, M.; Yan, F.; He, F. *Lactobacillus* GG-fermented milk prevents DSS-induced colitis and regulates intestinal epithelial homeostasis through activation of epidermal growth factor receptor. *Eur. J. Nutr.* **2014**, *53*, 105–115. [CrossRef]
105. Plé, C.; Breton, J.; Richoux, R.; Nurdin, M.; Deutsch, S.-M.; Falentin, H.; Hervé, C.; Chuat, V.; Lemée, R.; Maguin, E.; et al. Combining selected immunomodulatory Propionibacterium freudenreichii and *Lactobacillus delbrueckii* strains: Reverse engineering development of an anti-inflammatory cheese. *Mol. Nutr. Food Res.* **2016**, *60*, 935–948. [CrossRef] [PubMed]
106. Oliveira, L.d.C.; Silveira, A.M.M.; Monteiro, A.d.S.; dos Santos, V.L.; Nicoli, J.R.; Azevedo, V.A.d.C.; Soares, S.d.C.; Dias-Souza, M.V.; Nardi, R.M.D. In silico Prediction, in vitro Antibacterial Spectrum, and Physicochemical Properties of a Putative Bacteriocin Produced by *Lactobacillus rhamnosus* Strain L156.4. *Front. Microbiol.* **2017**, *8*. [CrossRef] [PubMed]

CAPÍTULO III

7. CAPÍTULO III: Paraprobiotics and Postbiotics of *Lactobacillus delbrueckii* CIDCA 133 Mitigate 5-FU-induced Intestinal Inflammation

Este artigo cientifico foi publicado na revista *Microorganisms* (ISSN: 2076-2607) em julho de 2022. Fator de impacto atual: 4.926.

Muitos dos efeitos anti-inflamatórios dos probióticos são derivados de componentes presentes na membrana celular desses microrganismos ou à compostos/proteínas secretados por eles, como proteínas associadas à camada S (Slp), proteínas extracelulares e SCFA, que são capazes de interagir com receptores celulares específicos do hospedeiro, e assim desencadear a ativação/inativação de diferentes vias de sinalização. Nesse contexto, alguns estudos demonstram que os efeitos anti-inflamatórios de bactérias benéficas não dependem intrisicamente da sua viabilidade. O uso de probióticos não viáveis/inativados, conhecidos como Paraprobióticos (por exemplo, probióticos mortos pelo calor ou raios UV) ou moléculas secretadas ou derivadas destes microrganismos, conhecidos como Posbióticos (por exemplo, sobrenadante sem células, proteínas da camada superficial, lisados celulares, entre outros), vêm sendo usados em terapias anti-inflamatórias. Assim, baseado nos achados dos capítulos I e II, neste capítulo, investigou-se o efeito anti-inflamatório de CIDCA 133 após sua inativação pelo calor (paraprobiótico) ou uso do seu sobrenadante (posbiótico), de forma a ratificar a hipótese de que os efeitos imunomodulatórios reportados para esta linhagem advém de componentes presentes na superficie de sua membrana e/ou a proteínas secretadas. Para realizar o estudo, camundongos BALB/c foram inflamados com uma única dose do quimioterápico 5-FU (300 mg/kg) e tratados com CIDCA 133 inativada pelo calor (10^9 CFU/mL) ou seu sobrenadante durante 13 dias. O efeito anti-inflamatório de CIDCA 133 foi investigado sob parâmetros inflamatórios (análise de expressão gênica de marcadores inflamatórios e atividade enzimática de células inflamatórias como neutrófilos e eosinófilos), histopatológicos (avaliação da arquitetura do epitélio intestinal, comprimento das vilosidades) e função de barreira (análise da expressão gênica de mucina 2 e proteínas de junção firme). Os resultados obtidos demonstraram que o tratamento com a forma paraprobiótica e ou posbiótica de CIDCA 133 melhorou os danos inflamatórios e histopatológicos ocasionados à mucosa intestinal pela administração de 5-FU, demonstrando, assim, que os efeitos anti-inflamatórios dessa linhagem são oriundos de componentes protéico e não protéicos presentes na membrana/parede celular da bactéria e ou produtos secretados por ela, sendo capazes de interagir com receptores específicos do hospedeiro, e assim reduzir a ativação de vias de sinalização inflamatória (como a NF-κB). Os resultados obtidos neste trabalho mostram que, além de ser uma alternativa terapêutica promissora contras danos inflamatórios e

histopatológicos induzidos pelo agente quimioterápico 5-FU, CIDCA 133 na sua forma paraprobiótica e posbiótica demonstra ser uma abordagem alternativa aos problemas decorrentes da utilização de microrganismos benéficos vivos na prática clínica, uma vez que vêm sendo relatados riscos de infecções sistêmicas por probióticos, especialmente em pacientes imuno-comprometidos, na sua forma viável.

microorganisms

MDPI

Article

Paraprobiotics and Postbiotics of *Lactobacillus delbrueckii* CIDCA 133 Mitigate 5-FU-Induced Intestinal Inflammation

Viviane Lima Batista [1,†], Luís Cláudio Lima De Jesus [1,†], Laísa Macedo Tavares [1], Fernanda Lima Alvarenga Barroso [1], Lucas Jorge da Silva Fernandes [1], Andria dos Santos Freitas [1], Monique Ferrary Americo [1], Mariana Martins Drumond [2], Pamela Mancha-Agresti [2], Enio Ferreira [3], Juliana Guimarães Laguna [1], Luiz Carlos Júnior Alcantara [1,4,*] and Vasco Azevedo [1,*]

1 Department of Genetics, Ecology and Evolution, Federal University of Minas Gerais, Belo Horizonte 31270-901, Brazil; vivianelimabio@gmail.com (V.L.B.); lc.luiis@yahoo.com.br (L.C.L.D.J.); macedolaisa@gmail.com (L.M.T.); fernanda_alima@hotmail.com (F.L.A.B.); lucazjorge@hotmail.com (L.J.d.S.F.); andria.sfreitas@gmail.com (A.d.S.F.); moniquefamerico@gmail.com (M.F.A.); jujulaguna@gmail.com (J.G.L.)
2 Department of Biological Sciences, Federal Center for Technological Education of Minas Gerais, Belo Horizonte 30421-169, Brazil; mmdrumond@gmail.com (M.M.D.); p.mancha.agresti@gmail.com (P.M.-A.)
3 Department of General Pathology, Federal University of Minas Gerais, Belo Horizonte 31270-901, Brazil; enioferreira@icb.ufmg.br
4 Flavivirus Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro 21040-900, Brazil
* Correspondence: luiz.alcantara@ioc.fiocruz.br (L.C.J.A.); vasco@icb.ufmg.br (V.A.)
† These authors contributed equally to this work.

check for updates

Citation: Batista, V.L.; De Jesus, L.C.L.; Tavares, L.M.; Barroso, F.L.A.; Fernandes, L.J.d.S.; Freitas, A.d.S.; Americo, M.F.; Drumond, M.M.; Mancha-Agresti, P.; Ferreira, E.; et al. Paraprobiotics and Postbiotics of *Lactobacillus delbrueckii* CIDCA 133 Mitigate 5-FU-Induced Intestinal Inflammation. *Microorganisms* **2022**, *10*, 1418. https://doi.org/10.3390/microorganisms10071418

Academic Editor: Luis G. Bermudez Humaran

Received: 21 May 2022
Accepted: 1 July 2022
Published: 14 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Intestinal mucositis is a commonly reported side effect in oncology practice. Probiotics are considered an excellent alternative therapeutic approach to this debilitating condition; however, there are safety questions regarding the viable consumption of probiotics in clinical practice due to the risks of systemic infections, especially in immune-compromised patients. The use of heat-killed or cell-free supernatants derived from probiotic strains has been evaluated to minimize these adverse effects. Thus, this work evaluated the anti-inflammatory properties of paraprobiotics (heat-killed) and postbiotics (cell-free supernatant) of the probiotic *Lactobacillus delbrueckii* CIDCA 133 strain in a mouse model of 5-Fluorouracil drug-induced mucositis. Administration of paraprobiotics and postbiotics reduced the neutrophil cells infiltrating into the small intestinal mucosa and ameliorated the intestinal epithelium architecture damaged by 5-FU. These ameliorative effects were associated with a downregulation of inflammatory markers (*Tlr2*, *Nfkb1*, *Il12*, *Il17a*, *Il1b*, *Tnf*), and upregulation of immunoregulatory *Il10* cytokine and the epithelial barrier markers *Ocln*, *Cldn1*, *2*, *5*, *Hp* and *Muc2*. Thus, heat-killed *L. delbrueckii* CIDCA 133 and supernatants derived from this strain were shown to be effective in reducing 5-FU-induced inflammatory damage, demonstrating them to be an alternative approach to the problems arising from the use of live beneficial microorganisms in clinical practice.

Keywords: mucositis; chemotherapy; intestinal damage; heat-killed bacteria; cell-free supernatant; immunomodulation; epithelial barrier markers

1. Introduction

Mucositis is characterized as a gastrointestinal (GIT) mucosal inflammation that frequently occurs in patients with cancer disease submitted to radiotherapy [1,2] and/or chemotherapy treatments (5-Fluorouracil, irinotecan, oxaliplatin, etc.) [3,4]. This debilitating condition is associated with pain and difficulty eating, leading to malnutrition, increasing the infection risk, alteration in the patient's clinical status, and therapy delays [5–7].

Many strategies have been investigated to prevent and/or treat mucositis [5,8]. According to the pathobiology phases described by Sonis [3], the chemotherapy-induced dysbiotic intestinal microbiota has an essential role in the progression and severity of mucositis [9].

In this context, some probiotic microorganisms (e.g., *Lactobacillus* sp., *Bifidobacterium* sp., *Saccharomyces* sp.) have been intentionally explored as a therapeutic tool against the intestinal mucositis caused by chemotherapy treatment due to their ability to regulate the dysbiotic microbiota and their well-known anti-inflammatory properties, which mainly occur through the inhibition of the nuclear factor kappa B (NF-κB) signaling pathway with subsequent cellular and humoral immunomodulation [10–13]. Other positive effects attributed to some probiotic strains include the production of beneficial secreted metabolites such as short-chain fatty acids (SCFA) (e.g., acetate, propionate, and butyrate), inhibition of pathogenic bacterial growth by antimicrobial compounds (e.g., acetic and lactic acids and bacteriocins), and reinforcement of the intestinal barrier by mucus production and induced expression of tight junction proteins [14,15]. Probiotics can use these mechanisms in a strain-specific manner to protect and improve the intestinal mucosa healing process altered by chemotherapy.

Although some preclinical studies report the host's beneficial effects related to "live" probiotics consumption [16–20], there are many questions regarding their safety concerns in clinical practice, especially in premature infants or immune-compromised patients. In these conditions, probiotics can translocate into the blood system and increase the risk of systemic infections [21–24]. The use of non-viable/inactivated probiotics, known as paraprobiotics (e.g., heat-killed probiotics) or products/molecules secreted or derived from these microorganisms, known as postbiotics (e.g., cell-free supernatant, surface layer proteins, cell lysates), besides minimizing or avoiding these possible adverse effects, has also been evaluated in anti-inflammatory therapies [25,26]. Paraprobiotics or postbiotics are an effective therapeutic alternative to gastrointestinal inflammation since they can also stimulate the host's immune system to form an anti-inflammatory profile [15,27,28]. However, like probiotics, these biotics' safety evaluations must also be performed before use for therapeutic purposes [25].

The anti-inflammatory properties and the intestinal barrier function conferred by the potential probiotic bacterium *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 in a murine model of 5-FU-induced mucositis was previously demonstrated by our research group [29]. Ameliorative effects promoted by this strain were mainly associated with the reduction of inflammatory cells, improvement in mucus-producing goblet cell count, secretory IgA levels and intestinal permeability reduction, modulation of markers involved in NF-κB signaling pathway activation (*Tlr2*, *Nfkb1*, *Il6*, and *Il1b*), and regulation of tight junction protein expression [29,30]. Additionally, a probiogenomics study of this strain showed that its anti-inflammatory profile could be related to genes encoding secreted and membrane surface proteins able to interact with immune proteins involved in the NF-κB signaling pathway [31]. Regarding its safety concerns, it was reported that CIDCA 133 (10^7 CFU/mL) did not cause blood hemolysis, mucin degradation, or epithelial damage in healthy mice, evidencing that this strain presents safety levels for probiotic application [32].

Few studies have evaluated the effects of heat-inactivated or cell-free supernatant on intestinal mucositis, mainly studies focused on the *Lactobacillus delbrueckii* species. The *L. delbrueckii* CIDCA 133 strain is a potential health-promoting bacterium. However, no beneficial effects have been reported with either the inactivation or extractable/secreted bioactive products derived from this strain. Thus, this work evaluated the anti-inflammatory properties of heat-killed and cell-free supernatants of *L. delbrueckii* CIDCA 133 in a mouse inflammation model induced by 5-Fluorouracil.

2. Materials and Methods

2.1. Bacterial Culture Conditions

The strain *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 (culture collection of Center for Research and Development in Food Cryotechnology of the National University of La Plata, Argentina) was grown on de Man, Rogosa and Sharpe (MRS) broth (Kasvi, São José dos Pinhais, Brazil) at 37 °C for 16 h. The strain was deposited at the Bacteria Collection

from Environment and Health (CBAS) of the Oswaldo Cruz Foundation (FIOCRUZ) (access number: CBAS 815).

2.2. Lactobacillus delbrueckii CIDCA 133 Preparation (Viable, Heat-Inactivated, and Cell-Free Supernatant)

Viable bacteria were centrifuged (1520× *g* for 15 min at 4 °C), washed two times with sterile phosphate-buffered saline (PBS) 0.1 M (pH 7.2), and adjusted to a concentration of 10^9 CFU/mL. The cell-free supernatant (CFS) was collected and buffered to a pH of 7.2 using TRIS 1 M (Sigma-Aldrich, St. Louis, MO, USA), as previously described by Prisciandaro et al. [33]. The CFS (15 mL) was sterilized with a 0.22 µm filter (Kasvi, São José dos Pinhais, Brazil), concentrated with Vivaspin 20 (weight cut-off 10,000 Kilodalton (kDa) (Sartorius, Gottingen, Germany), and stored at −80 °C until administration in mice. The heat-killed CIDCA 133 (10^9 CFU/mL) was prepared by heat treatment (15 min, 121 °C). The inactivation process and CFS sterilization were confirmed after plating 100 µL of these biotics in MRS agar (Kasvi, São José dos Pinhais, Brazil).

2.3. Animals

Six-week-old conventional BALB/c mice (males) weighing 20–24 g were obtained from the Bioterism Center (CEBIO) at the Federal University of Minas Gerais (UFMG, Belo Horizonte, Brazil). This study was approved by the Local Animal Experimentation Ethics Committee (CEUA-UFMG, Protocol n○ 112/2020). Mice were housed in ventilated polycarbonate cages under controlled room conditions: 12 h light/dark cycle, temperature 25 ± 2 °C, and ad libitum access to standard chow and water. All procedures were conducted according to Brazilian Association for Laboratory Animal Science (SBCAL) guidelines.

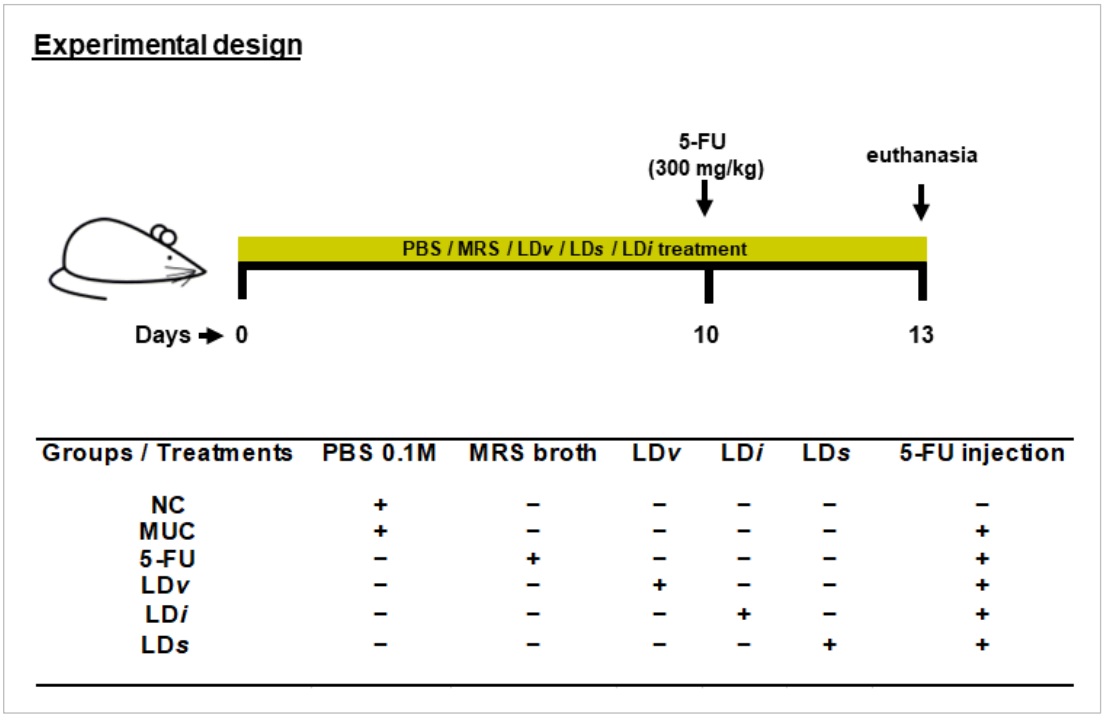
2.4. Experimental Design

Mice were split into six experimental groups (*n* = six animals per group): negative control (NC), inflamed (MUC), inflamed (5-FU), inflamed and treated with viable CIDCA 133 (LD*v*), heat-killed inactivated CIDCA 133 (LD*i*), and CIDCA 133 cell-free supernatant (LD*s*). Mice received doses of 300 µL containing 10^9 CFU/mL of CIDCA 133 (LD*v* and LD*i* groups) or 300 µL of CIDCA 133 supernatants (LD*s* group) for 13 days by intragastric gavage. Control groups received 300 µL of PBS 0.1M solution (NC and MUC groups) or MRS broth (5-FU group) by the same route. On the 10^{th} day, mice (MUC, 5-FU, LD*v*, LD*i*, and LD*s* groups) were inflamed intraperitoneally with a single injection of 5-Fluorouracil (Fauldfluor®) (300 mg/kg) (Libbs, São Paulo, Brazil) [29]. Sterile saline solution injection (NaCl 0.9%) (Vetec, Rio de Janeiro, Brazil) (Figure 1) was administered to the negative control group. At the end of the experimental procedure, 72 h after mucositis induction, mice were euthanized by an anesthetic overdose (ketamine 300 mg/kg and xylazine 30 mg/kg solution) (Syntec, Tamboré, Brazil), and afterward, ileum sections were collected for analysis.

2.5. Inflammatory Cell Infiltration

The recruitment of neutrophils and eosinophils to intestinal mucosa was performed by myeloperoxidase (MPO) [34] and eosinophil peroxidase (EPO) [35] enzymatic activity assays, respectively. For MPO, ileum tissues (100 mg) were homogenized with 1.9 mL of buffered solution (pH 4.7) (NaCl 0.1 M (LabSynth, Diadema, Brazil), NaH_2PO_4 0.02 M (LabSynth, Diadema, Brazil), and Na_2EDTA 0.015 M (LabSynth, Diadema, Brazil)) and centrifuged (9500× *g* for 10 min at 4 °C). After a hypotonic process (0.2% NaCl solution plus 1.6% NaCl solution containing glucose 5% (LabSynth, Diadema, Brazil)) and centrifugation (9500× *g* for 10 min at 4 °C), the pellet was homogenized in a NaH_2PO_4 (0.05 M) solution (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide (Sigma-Aldrich, St. Louis, MO, USA) and submitted to lysis process with three cycles of freeze-thaw in liquid nitrogen. After the second centrifugation (9500× *g* for 15 min at 4 °C), the supernatant

was collected for colorimetric assay. Thus, 25 µL of supernatant was added to 25 µL of 3,3,5,5′-Tetramethylbenzidine 1.6 mM (Sigma-Aldrich, St. Louis, MO, USA) previously diluted in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA). After adding 100 µL of H_2O_2 0.5 mM (Vetec, Rio de Janeiro, Brazil), the plates were incubated at 37 °C for 5 min.



Groups / Treatments	PBS 0.1M	MRS broth	LD*v*	LD*i*	LD*s*	5-FU injection
NC	+	−	−	−	−	−
MUC	+	−	−	−	−	+
5-FU	−	+	−	−	−	+
LD*v*	−	−	+	−	−	+
LD*i*	−	−	−	+	−	+
LD*s*	−	−	−	−	+	+

Figure 1. Experimental design protocol. Mice received gavage with 300 µL of PBS 0.1 M (NC and MUC group), MRS broth (5-FU group), viable CIDCA 133 (LD*v* group), heat-inactivated CIDCA 133 (LD*i* group), or cell-free supernatant (LD*s*) for 13 days. Mice were inflamed with a single dose of 5-Fluorouracil (300 mg/kg) on the 10th day. Symbols indicate (+) presence or (−) absence of treatment.

For EPO, 100 mg of ileum tissues were homogenized in 1.9 mL of PBS 0.1 M (pH 7.4). After centrifugation (9500× *g* for 15 min at 4 °C), a hypotonic process (0.2% NaCl solution, plus 1.6% NaCl solution containing glucose 5%) and second centrifugation (9500× *g* for 15 min at 4 °C), the pellet was homogenized in a PBS 0.1 M solution (pH 7.4) containing 0.5% hexadecyltrimethylammonium bromide and submitted to lysis process with three cycles of freeze-thaw in liquid nitrogen. After another centrifugation (9500× *g* for 15 min at 4 °C), the supernatant was collected for colorimetric assay. For this purpose, 75 µL of supernatant were added to 75 µL of O-phenylenediamine 1.5 mM (Sigma-Aldrich, St. Louis, MO, USA), diluted in Tris–HCl 0.075 mM (pH 8) plus H_2O_2 6.6 mM, and incubated at 20 °C for 30 min. Both enzymes' reactions were stopped by adding 50 µL of H_2SO_4 1 M (Vetec, Rio de Janeiro, Brazil). The absorbance of the products of the enzymatic assays was measured at 450 nm (MPO) and 492 nm (EPO) on a microplate spectrophotometer (Bio-Rad 450 model, Bio-Rad Laboratories). The results were expressed as MPO or EPO arbitrary units/mg of tissue based on absorbance.

2.6. Gene Expression of Cytokines and Epithelial Barrier Markers

2.6.1. Total RNA Isolation

The profile of cytokines and genetic markers involved with epithelial barrier function was evaluated through gene expression. For this, ileum total RNA isolation was carried out using Pure Link™ RNA Mini Kit (Invitrogen, Carlsbad, CA, USA), according to the recommended protocol. Samples were analyzed using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and 1.5 % agarose gel to verify the total RNA con-

centration and quality. Residual genomic DNA was removed using the Turbo DNA-free™ Kit (Invitrogen, Carlsbad, CA, USA). Complementary DNA was produced with 2 µg of RNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems™, ThermoFisherC, Waltham, MA, USA), according to the recommended protocol.

2.6.2. Quantitative PCR (qPCR)

Quantitative PCR was carried out with the PowerUp™ SYBR® Green Master Mix (ThermoFisher) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems™) under the following steps: 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The Toll-like receptor 2 (*Tlr2*), nuclear factor-kappa B p105 subunit (*Nfkb1*), interleukins b (*Il1b*), 10 (*Il10*), 12 (*Il12*), 17-alfa (*Il17a*), tumor necrosis factor (*Tnf*), transforming growth factor beta-1 (*Tgfb1*), mucin 2 (*Muc2*), claudin 1 (*Cldn1*), 2 (*Cldn2*), and 5 (*Cldn5*), zonulin (*Hp*), and occludin (*Ocln*) were used as gene-specific primers (Table 1) [36–40]. Gene expression results were analyzed following the $2^{-\Delta\Delta CT}$ method using GAPDH (*Gapdh*) and β-actin (*Actb*) as endogenous references.

Table 1. qPCR primer sequences.

Gene	Primer Forward	Primer Reverse	Reference
Gapdh	TCACCACCATGGAGAAGGC	GCTAAGCAGTTGGTGGTGCA	[36]
Actb	GCTGAGAGGGAAATCGTGCGTG	CCAGGGAGGAAGAGGATGCGG	[38]
Tlr2	ACAATAGAGGGAGACGCCTTT	AGTGTCTGGTAAGGATTTCCCAT	[39]
Nfkb1	GTGGAGGCATGTTCGGTAGTG	TCTTGGCACAATCTTTAGGGC	[40]
Il12p40	GGAAGCACGGCAGCAGAATA	AACTTGAGGGAGAAGTAGGAATGG	[36]
Il17a	GCTCCAGAAGGCCCTCAGA	AGCTTTCCCTCCGCATTGA	[36]
Tgfb1	TGACGTCACTGGAGTTGTACGG	GGTTCATGTCATGGATGGTGC	[36]
Il10	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT	[36]
Tnf	ACGTGGAACTGGCAGAAGAG	CTCCTCCACTTGGTGGTTTG	[37]
Il1b	CTCCATGAGCTTTGTACAAGG	TGCTGATGTACCAGTTGGGG	[37]
Muc2	GATGGCACCTACCTCGTTT	GTCCTGGCACTTGTTGGAAT	[38]
Cldn1	TCCTTGCTGAATCTGAACA	AGCCATCCACATCTTCTG	[38]
Cldn2	GTCATCGCCCATCAGAAGAT	ACTGTTGGACAGGGAACCAG	[38]
Cldn5	GCTCTCAGAGTCCGTTGACC	CTGCCCTTTCAGGTTAGCAG	[38]
Ocln	ACTCCTCCAATGGACAAGTG	CCCCACCTGTCGTGTAGTCT	[38]
Hp	CCACCTCTGTCCAGCTCTTC	CACCGGAGTGATGGTTTTCT	[38]

2.7. Histological and Morphometric Analysis

The ileum sections were collected for histological analysis, washed with PBS 0.1 M, longitudinally opened, rolled up, and immersed in 10% buffered formaldehyde solution (Neon, Suzano, Brazil) for 24 h until tissue fixation. This material was embedded in paraffin, and sections of 4 µm thickness were stained with hematoxylin and eosin (HE). The histological inflammation score was examined by a pathologist and evaluated according to the Soares et al. [41] method. Histological images were captured by a BX41 optical microscope (Olympus, Tokyo, Japan) with a 20× magnification objective, and the morphometric parameters were evaluated by measuring 20 villi height and 20 crypt depth with the ImageJ 1.51j.8 software (National Institutes of Health, Bethesda, MD, USA).

2.8. Statistical Analysis

The results are presented as the mean ± standard deviation. Data were evaluated by one-way ANOVA followed by Tukey's post hoc test (parametric data) or by the Kruskal–Wallis test and post-tested by Dunn's test (nonparametric data). Graphs were generated and data analysis performed using GraphPad Prism 8.0 software (GraphPad Software) and a p-value < 0.05.

3. Results

3.1. Heat-Killed Lactobacillus delbrueckii CIDCA 133 Improved Weight Loss in Chemotherapy-Inflamed Mice

Body weight loss was higher in the MUC (−9.9 ± 0.85%) (Figure 2A) and 5-FU (−7.0 ± 0.68%) (Figure 2D) groups when compared to the NC group (0.00 ± 0.5%) ($p < 0.0001$) (Figure 2A,D). Treatment with viable (LD*v*) (−6.6 ± 1.1%) and heat-killed CIDCA 133 (LD*i*) (−6.5 ± 0.97%) (Figure 2A) improved the weight loss ($p < 0.001$). However, the cell-free supernatant (LDs) (−5.95 ± 1.51%) had no protective effect on this parameter ($p = 0.26$) (Figure 2D).

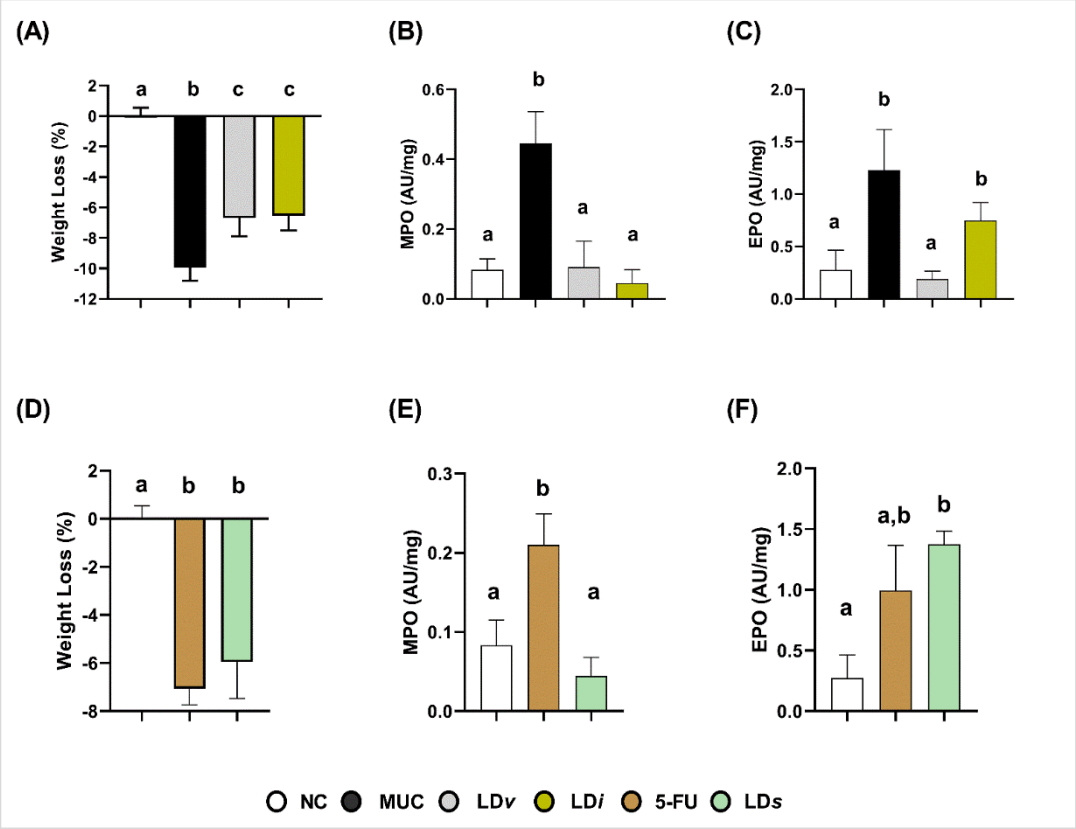


Figure 2. Protective effect of heat-killed (LD*i*) and cell-free supernatant (LDs) of *L. delbrueckii* CIDCA 133 on 5-FU-induced body weight loss and recruitment of polymorphonuclear cells. (**A**–**C**) Viable and heat-killed CIDCA 133. (**D**–**F**) CIDCA 133 supernatant. Different letters (a, b, c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by Tukey's (**A**–**E**) or by Kruskal–Wallis test followed by Dunn's post hoc test (**F**).

3.2. Heat-Killed and Cell-Free Supernatant of Lactobacillus delbrueckii CIDCA 133 Reduced Levels of Myeloperoxidase Activity

Inflammatory infiltrates of neutrophils and eosinophils in ileum mucosa were assessed by levels of their respective myeloperoxidase (MPO) and eosinophil peroxidase (EPO) enzymatic activities. High levels of MPO (MUC: 0.44 ± 0.09 AU/mg, Figure 2B; 5-FU: 0.21 ± 0.03 AU/mg, Figure 2E) and EPO (MUC: 1.23 ± 0.38 AU/mg, Figure 2C; 5-FU: 0.99 ± 0.37 AU/mg, Figure 2F) enzymatic activities were detected in the ileum mucosa of inflamed mice when compared to the control group (MPO: 0.08± 0.03 AU/mg; EPO: 0.27 ± 0.19 AU/mg) (Figure 2B,C,E) ($p < 0.0001$). Treatment with viable CIDCA 133 (LD*v*) reduced the level activity of both enzymes (MPO: 0.09± 0.07 AU/mg, $p < 0.0001$; EPO: 0.18 ± 0.07 AU/mg, $p < 0.0001$) (Figure 2B,C). Positive effects of heat-killed (LD*i*) (0.09 ± 0.07 AU/mg, $p < 0.0001$) and cell-free supernatant (LDs) (0.04 ± 0.02 AU/mg, $p < 0.0001$) were only observed against MPO enzyme levels (Figure 2B,E). No protective

effect of heat-killed (LD*i*) (0.74 ± 0.17 AU/mg, $p = 0.06$) (Figure 2C) and cell-free supernatant of CIDCA 133 (LD*s*) (1.37 ± 0.10 AU/mg, $p = 0.7130$) (Figure 2F) was observed with respect to EPO levels.

3.3. Heat-Killed and Cell-Free Supernatant of Lactobacillus delbrueckii CIDCA 133 Modulated the Gene Expression of Inflammatory Cytokines

The gene expression of pro-inflammatory (*Il1b*, *Il12*, *Il17a*, and *Tnf*) and anti-inflammatory (*Il10* and *Tgfb1*) cytokines was performed to evaluate the immunoregulatory effects of heat-killed and cell-free supernatant of *L. delbrueckii* CIDCA 133. Compared to the control group, mice in the MUC group presented an upregulation in the gene expression of *Tlr2* ($p < 0.001$) (Figure 3A), *Nfkb1* ($p < 0.001$) (Figure 3B), *Il12* ($p < 0.01$) (Figure 3C), *Il17a* ($p < 0.001$) (Figure 3D), *Tnf* ($p < 0.01$) (Figure 3E), *Il1b* ($p < 0.01$) (Figure 3F), and *Tgfb1* ($p < 0.05$) (Figure 3H), and a downregulation of the immunoregulatory cytokine *Il10* ($p < 0.0001$) (Figure 3G).

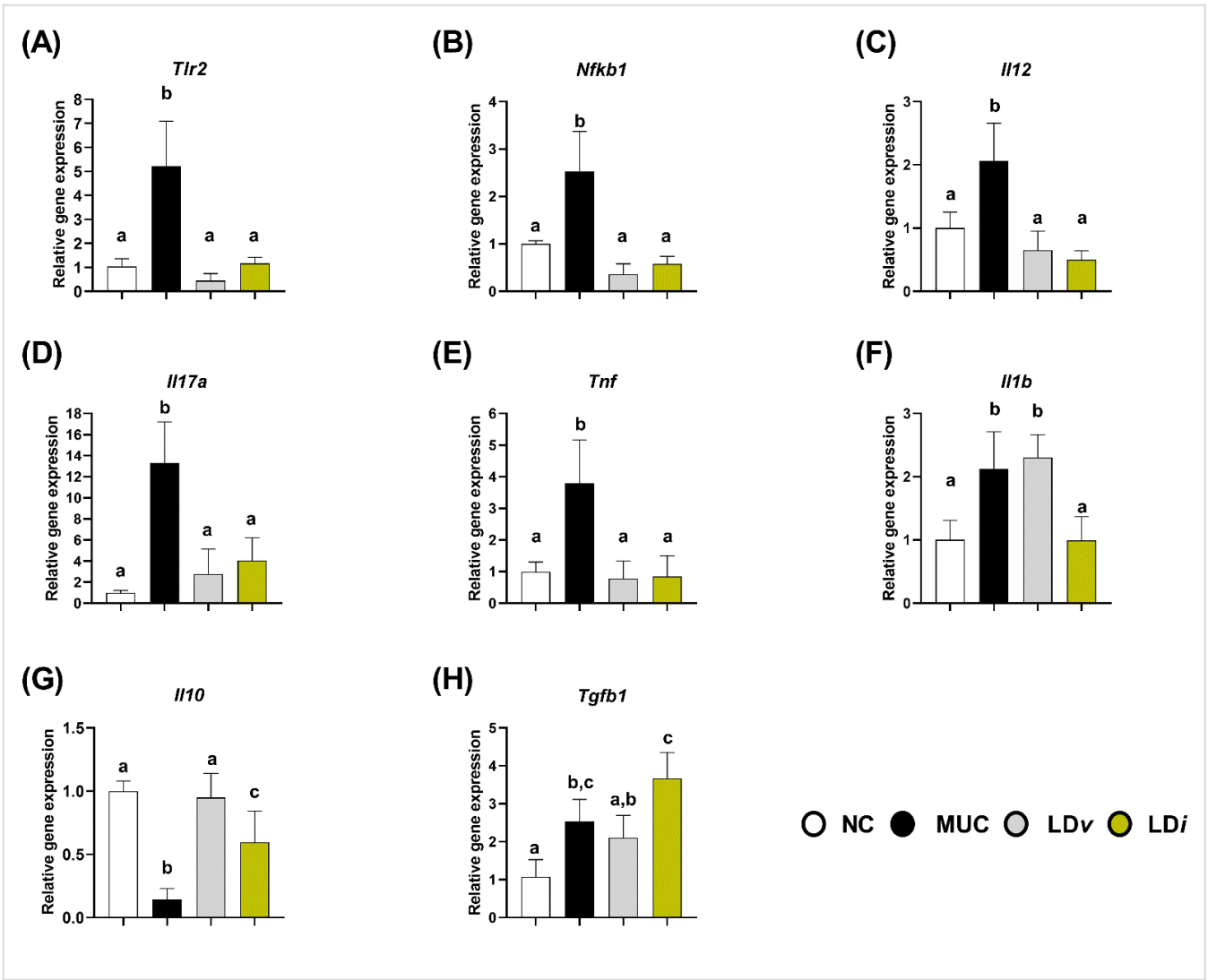


Figure 3. Heat-killed (LD*i*) *L. delbrueckii* CIDCA 133 modulated gene expression of inflammatory cytokines. (**A**) *Tlr2*, (**B**) *Nfkb1*, (**C**) *Il12*, (**D**) *Il17a*, (**E**) *Tnf*, (**F**) *Il1b*, (**G**) *Il10* and (**H**) *Tgfb1*. Different letters (a, b, c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by Tukey's post hoc test.

Modulation of these inflammatory markers was observed after treatment with CIDCA 133. Consumption of viable bacteria (LD*v*) reduced the mRNA expression of *Tlr2* ($p < 0.0001$) (Figure 3A), *Nfkb1* ($p < 0.0001$) (Figure 3B), *Il12* ($p < 0.001$) (Figure 3C), *Il17a* ($p < 0.001$)

(Figure 3D), *Tnf* ($p < 0.0001$) (Figure 3E), and increased *Il10* ($p < 0.0001$) (Figure 3G) when compared to the MUC group. Heat-killed (LD*i*) treatment downregulated *Tlr2* ($p < 0.0001$) (Figure 3A), *Nfkb1* ($p < 0.0001$) (Figure 3B), *Il12* ($p < 0.0001$) (Figure 3C), *Il17a* ($p < 0.01$) (Figure 3D), *Tnf* ($p < 0.0001$) (Figure 3E), and *Il1b* ($p < 0.01$) (Figure 3F), and upregulated the gene expression of *Il10* ($p < 0.01$) (Figure 3G). No differences were observed between the MUC and LD*i* groups in *Tgfb1* expression ($p = 0.112$) (Figure 3H).

On the other hand, the 5-FU group exhibited upregulation of *Tlr2* ($p < 0.0001$) (Figure 4A), *Nfkb1* ($p < 0.001$) (Figure 4B), *Il17a* ($p < 0.05$) (Figure 4D) and *Il1b* ($p < 0.0001$) (Figure 4F), and downregulation of *Il10* ($p < 0.01$) (Figure 4E). Treatment with the cell-free supernatant of CIDCA 133 (LD*s*) downregulated the gene expression of *Tlr2* ($p < 0.0001$) (Figure 4A), *Nfkb1* ($p < 0.0001$) (Figure 4B), pro-inflammatory cytokines *Il12* ($p < 0.01$) (Figure 4C), and *Il17a* ($p < 0.05$) (Figure 4D), and upregulated anti-inflammatory cytokine *Il10* ($p < 0.01$) (Figure 4E) when compared to the 5-FU group. No differences were observed in *Il1b*, *Tnf*, and *Tgfb1* gene expression between 5-FU and LD*s* groups ($p > 0.05$) (Figure 4F–H).

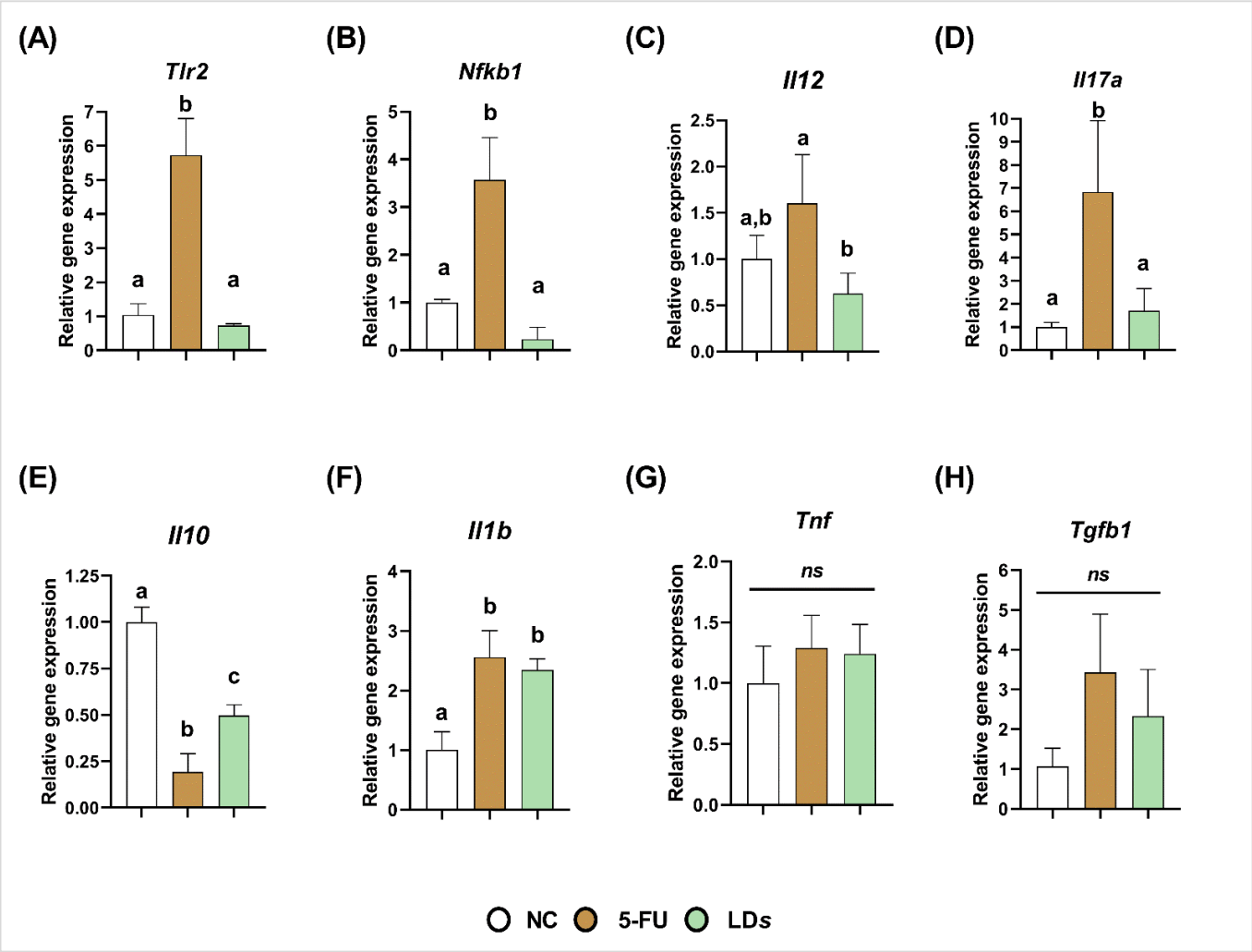


Figure 4. Cell-free supernatant ((LD*s*) of *L. delbrueckii* CIDCA 133 downregulated 5-FU-induced gene expression of pro-inflammatory cytokines. (**A**) *Tlr2*, (**B**) *Nfkb1*, (**C**) *Il12*, (**D**) *Il17a*, (**E**) *Il10*, (**F**) *Il1b*, (**G**) *Tnf* and (**H**) *Tgfb1*. Different letters (a, b, c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by Tukey's posttest. ns indicates no statistically significant differences.

3.4. Heat-Killed and Cell-Free Supernatant of Lactobacillus delbrueckii CIDCA 133 Regulated Genes Related to Intestinal Epithelial Barrier

The regulatory effect of heat-killed and cell-free supernatant of *L. delbrueckii* CIDCA 133 on epithelial barrier protection was evaluated through the gene expression of mucin 2 and

tight junction proteins (*Cldn1*, *Cldn2*, *Cldn5*, *Hp* and *Ocln*). The MUC group exhibited a downregulation in the mRNA expression of *Muc2* ($p < 0.01$) (Figure 5A), *Hp* ($p < 0.0001$) (Figure 5B), and *Cldn1* ($p < 0.0001$) (Figure 5D) when compared to the control group. The treatment with viable *L. delbrueckii* CIDCA 133 (LD*v*) only upregulated the *Cldn5* ($p < 0.0001$) (Figure 5F) gene expression; however, upregulation of *Hp* ($p < 0.05$) (Figure 5B), *Ocln* ($p < 0.01$) (Figure 5C), *Cldn1* ($p < 0.05$) (Figure 5D), *Cldn2* ($p < 0.01$) (Figure 5E), and *Cldn5* ($p < 0.01$) (Figure 5F) was observed after heat-killed CIDCA 133 treatment (LD*i*). No differences were observed in the mRNA expression of *Muc2* (Figure 5A) after heat-killed CIDCA 133 treatment and in *Hp* (Figure 5B), *Ocln* (Figure 5C), *Cldn1* (Figure 5D), and *Cldn2* (Figure 5E) after viable CIDCA 133 (LD*v*) treatment ($p > 0.05$).

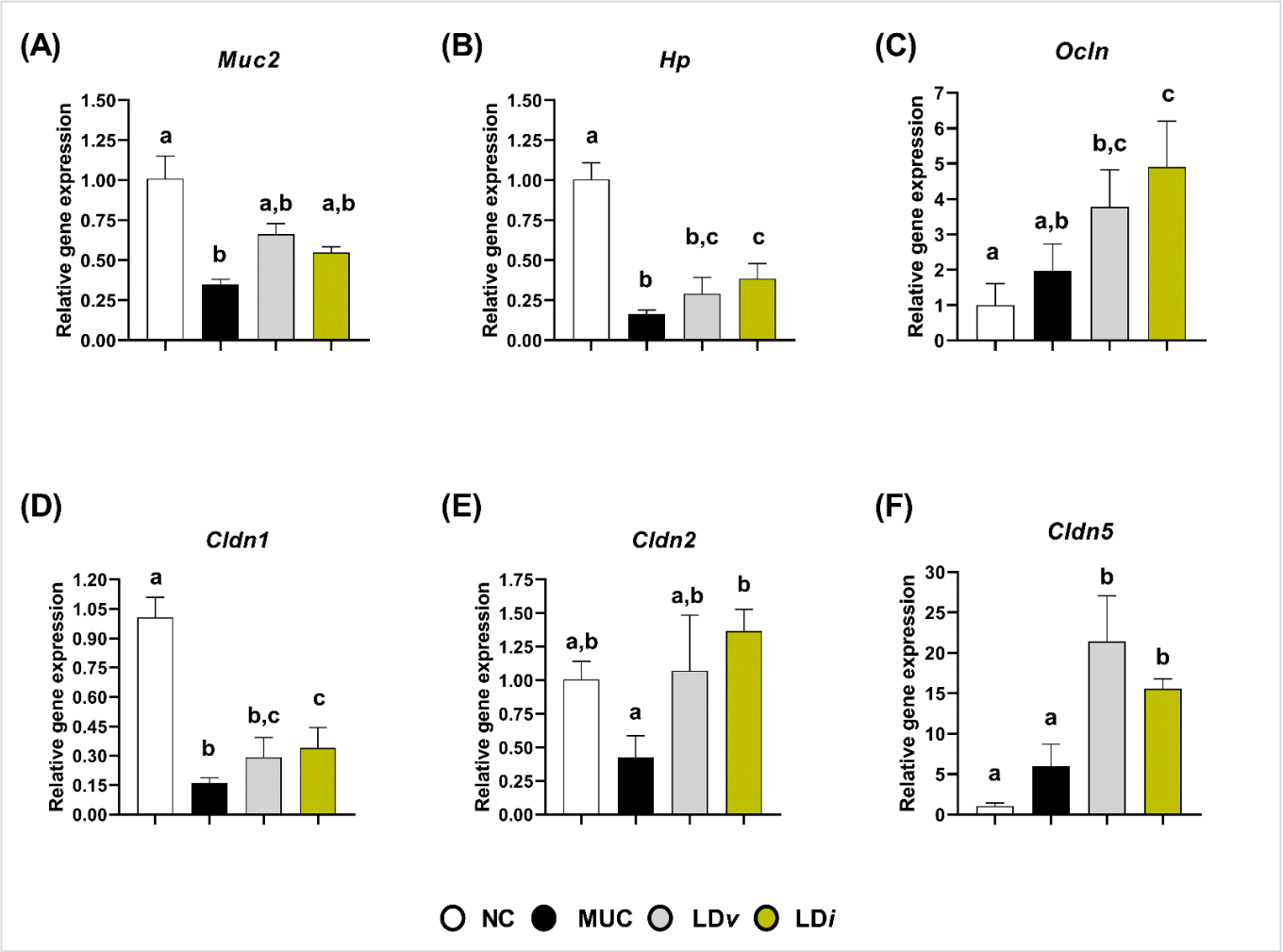


Figure 5. Heat-killed (LD*i*) *L. delbrueckii* CIDCA 133 modulated mRNA expression of markers involved in epithelial barrier function. (**A**) mucin 2, (**B**) zonulin, (**C**) occludin (**D**) claudin 1, (**E**) claudin 2 (**F**) claudin 5. Different letters (a, b, c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by Tukey's posttest (**B**–**D**,**F**) or by Kruskal–Wallis test followed by Dunn's post hoc test (**A**,**E**).

The 5-FU group also exhibited a downregulation in the mRNA expression of *Muc2* ($p < 0.01$) (Figure 6A), *Hp* ($p < 0.0001$) (Figure 6B), and *Cldn1* ($p < 0.0001$) (Figure 6D), but upregulated expression of *Cldn5* ($p < 0.05$) (Figure 6F) when compared to the control group. However, after treatment with cell-free supernatant of CIDCA 133 (LD*s*), an upregulation of mRNA expression of *Muc2* ($p < 0.01$) (Figure 6A), *Hp* ($p < 0.05$) (Figure 6B), *Ocln* ($p < 0.01$) (Figure 6C), *Cldn1* ($p < 0.05$) (Figure 6D), *Cldn2* ($p < 0.01$) (Figure 6E), and *Cldn5* ($p < 0.01$) (Figure 6F) was observed.

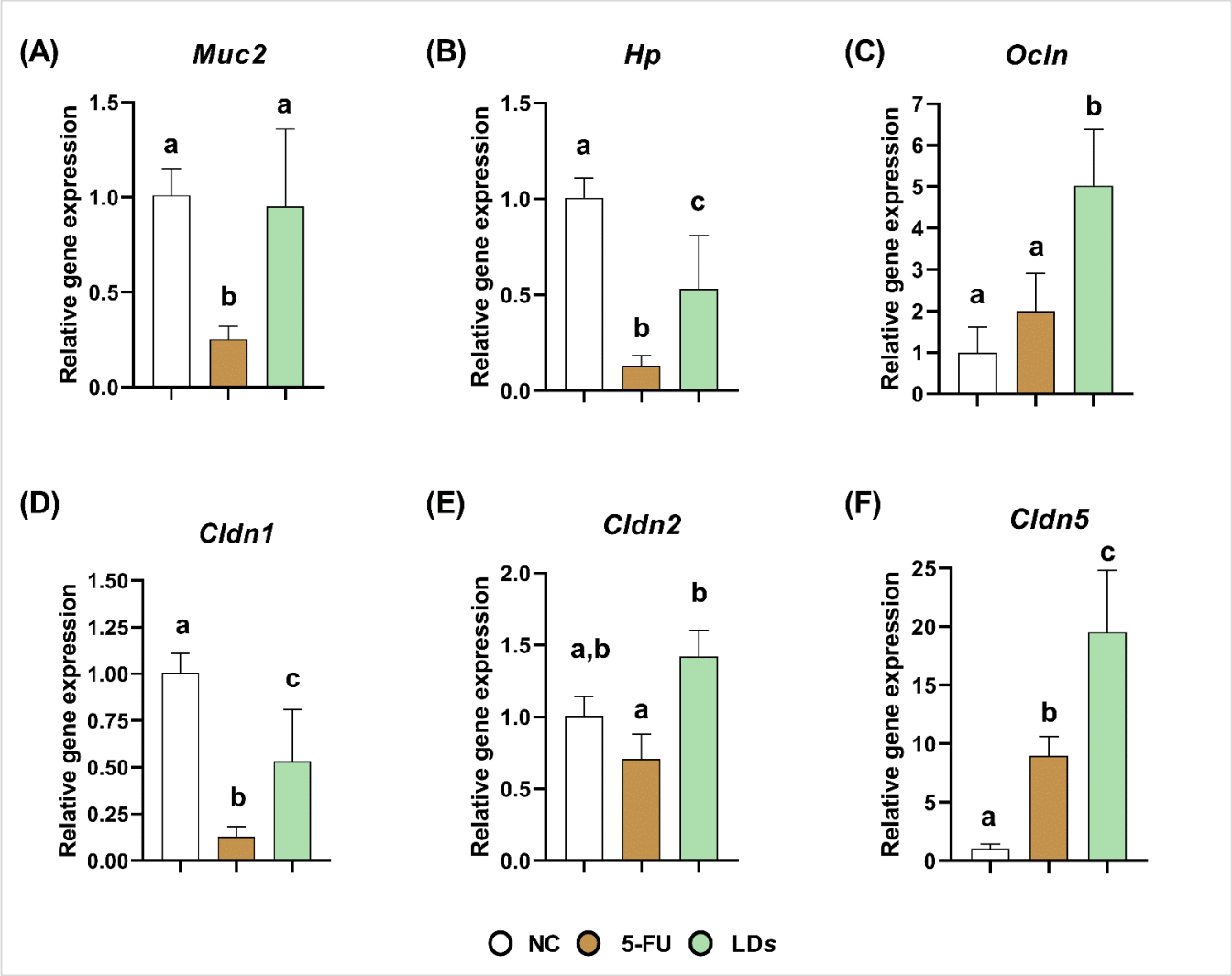


Figure 6. Cell-free supernatant (LD*s*) of *L. delbrueckii* CIDCA 133 modulated mRNA expression of markers involved in epithelial barrier function. (**A**) mucin 2, (**B**) zonulin, (**C**) occludin (**D**) claudin 1, (**E**) claudin 2 (**F**) claudin 5. Different letters (a, b, c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by Tukey's posttest (**A**–**D**,**F**) or by Kruskal–Wallis test followed by Dunn's post hoc test (**E**).

3.5. Heat-Killed and Cell-Free Supernatant of Lactobacillus delbrueckii CIDCA 133 Improved Epithelium Intestinal Architecture

Histopathological analysis demonstrated that mice inflamed with 5-Fluorouracil chemotherapy presented intestinal architecture alterations characterized by intense inflammatory cell infiltration into the lamina propria and villi, degeneration of goblet cells, villus shortening, and crypt necrosis when compared to the control group (Figure 7A), corroborating the histopathological score (Figure 7B,C) and morphometric analysis (Figure 7D–I).

Treatment with viable (LD*v*), heat-killed (LD*i*) (Figure 7D), and cell-free supernatant (LD*s*) (Figure 7E) of CIDCA 133 had a protective effect on villus shortening and inflammatory cell infiltration into the lamina propria and villus, improving the epithelium architecture of the ileum section (Figure 7A). After consumption of these biotic agents, no beneficial effects were observed in the mice´s histopathological score, crypt depth, or villus/crypt ratio (Figure 7B,C,F–I).

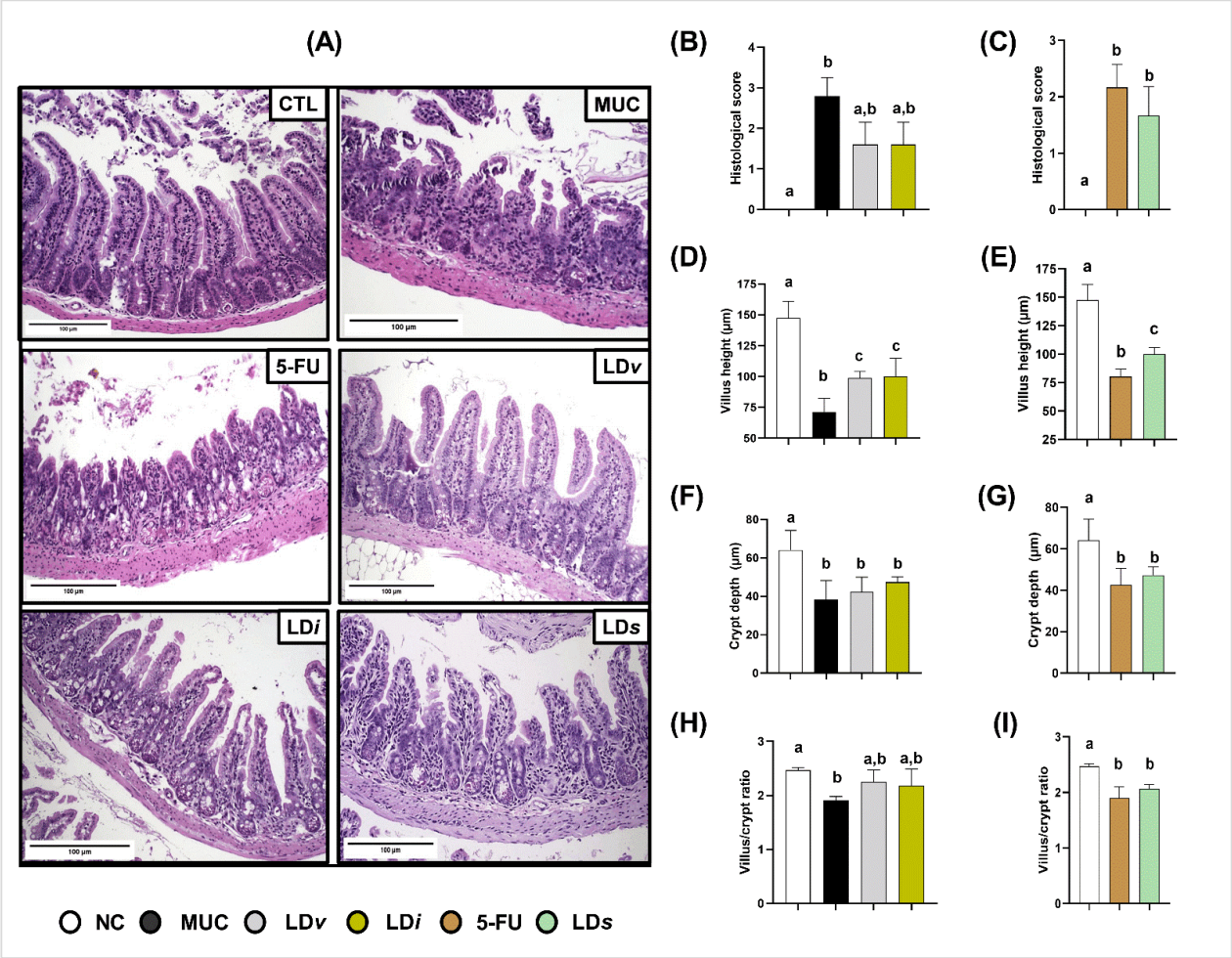


Figure 7. Heat-killed (LD*i*) and cell-free supernatant (LDs) of *L. delbrueckii* CIDCA 133 ameliorated intestinal architecture damage induced by 5-FU administration. (**A**) Ileum mucosa histopathology images (objective: ×20, scale 100 μm); (**B**,**F**) histopathological score; (**C**,**G**) villus height; (**D**,**H**) crypt depth; and (**E**,**I**) villus height to crypt depth ratio, respectively. Different letters (a, b, c) indicate statistically significant differences ($p < 0.05$) by Kruskal–Wallis test followed by Dunn's post hoc test (**B**,**F**) or ANOVA followed by Tukey's post hoc test (**C**–**E**,**G**–**I**).

4. Discussion

The anti-inflammatory properties of viable *L. delbrueckii* CIDCA 133 have been previously reported [29–31]. This study evaluated the anti-inflammatory properties of the bacterial inactivation or secreted bioactive products derived from this strain in a 5-Fluorouracil drug-induced mouse intestinal inflammation model.

The mucosal destruction by chemotherapy leads to lower absorption of nutrients, influencing body weight loss [3,41,42]. Our results showed no protective effect against weight loss with the cell-free supernatant of *L. delbrueckii* CIDCA 133. This lack of protection was possibly due to the removal of small molecules derived from CIDCA 133 after supernatant concentration with Vivaspin 20, thus reducing their interaction with intestinal epithelial cells to provide beneficial properties to the host. These data are similar to the findings of Prisciandaro et al. [33], demonstrating that *Escherichia coli* Nissle 1917 and *Limosilactobacillus fermentum* BR11 supernatant did not improve weight loss in mice with intestinal mucositis [33]. On the other hand, the consumption of heat-killed *L. delbrueckii* CIDCA 133 ameliorated the weight loss in BALB/c mice after 5-FU administration, similarly to viable bacteria, as previously reported by De Jesus et al. [29], and

also corroborating with Trindade et al. [43], who reported that heat-killed *Lacticaseibacillus rhamnosus* CGMCC1.3724 strain influenced weight recovery of 5-FU inflamed mice.

The intestinal architecture alteration resulting from the inflammatory process is one of the most critical features in the pathobiology of chemotherapy-induced mucositis [44,45]. Our results showed that the heat-killed and cell-free supernatants of *L. delbrueckii* CIDCA 133 improved the ileum epithelium architecture destroyed by 5-FU administration. This protective property can be linked to the ability of these biotics to attenuate villus shortening, upregulate the immunoregulatory cytokine *Il10* at the mRNA level, and reduce the inflammatory markers (e.g., *Tlr2*, *Nfkb1*, pro-inflammatory cytokines *Il1b*, *Il12*, *Il17a*, *Tnf*, and neutrophil infiltrates). These findings are supported by other studies in which administration either of inactivated *Lacticaseibacillus rhamnosus* CGMCC1.3724 [43], *Lactiplantibacillus plantarum* Zhang-LL [28], and VSL#3 therapy composed of *Bifidobacterium breve*, *B. longum*, *B. infantis*, *L. plantarum*, *Lb. bulgaricus*, *L. casei*, *L. acidophilus*, and *Streptococcus salivarius* [46], or secreted products derived from beneficial microorganisms, such as supernatant of mulberry leaf extract fermented by *L. acidophilus* A4 [47] and supernatant of *Faecalibacterium prausnitzii* [27], ameliorated the intestinal inflammatory process through modulation of inflammatory markers, such as myeloperoxidase activity levels, Tregs $CD4^+$ $Foxp3^+$ cells, pro-inflammatory cytokines IL1β, IFNγ, IL17A, TNFα, and anti-inflammatory IL10 and IL4 cytokines, thus restoring the intestinal homeostasis.

Interestingly, our results show that the transcripts levels of immunoregulatory cytokine TGFβ1 were upregulated after 5-FU administration and maintained after viable and heat-inactivated CIDCA 133 administration. Furthermore, we only observed a reduction in eosinophil inflammatory infiltrate with viable CIDCA 133 treatment. The protective effect on this parameter was not observed with the paraprobiotic and postbiotic forms of CIDCA 133.

TGFβ1 is an abundant cytokine in the intestinal mucosa with pleiotropic effects. The anti-inflammatory or pro-inflammatory property of this immunoregulatory factor occurs through cellular and environmentally dependent contexts [48,49]. Increased TGFβ levels were observed in the 5-FU-induced inflammation model [50–52]. On the other hand, IL10 cytokine levels were reduced [30,53–55]. Evidence shows that in the absence/reduction of IL10 cytokine in the intestinal inflammation context, levels of TGFβ1 are increased [19,56]. Thus, we suggest that, due to the reduction of IL10 transcript levels, paraprobiotics and postbiotics of CIDCA 133 maintain the mRNA level of the TGFβ1 cytokine as a compensatory anti-inflammatory mechanism to control tissue damage and restore gut homeostasis disrupted by 5-Fluorouracil chemotherapy.

Regarding infiltrating eosinophils, these cells reside in the intestine, in both normal and inflammatory states [57]. Pieces of evidence show that eosinophil activation and migration can be regulated by microbiota metabolites (e.g., SCFA) [58]. Thus, we believed that SCFA production and microbiota interaction might be a mechanism used by CIDCA 133 in its beneficial effect, suggesting that its viability is necessary to reduce 5-FU-induced eosinophil infiltration in the ileum mucosa.

The anti-inflammatory profile of heat-inactivated and cell-free supernatant of *L. delbrueckii* CIDCA 133 was, at least in part, similar to that of its viable form. We believe that these results can be linked to extracellular molecules and cell surface-associated components (e.g., LtaS, SLAP, HtrA, MucBP, and PrtB) identified in the strain genome [31] since these genetic factors interacted the most with human immune protein receptors associated with NF-κB signaling pathway regulation, including NFKB1 protein. These molecular markers were also associated as possibly genetic factors of viable CIDCA 133 involved with its anti-inflammatory properties through the upregulation of immunoregulatory *Tgfb1* and *Il10* cytokines and downregulation of *Nfkb1* gene expression in vivo [30,31].

Cell surface-associated components or extracellular molecules (e.g., surface layer proteins, lipoteichoic acids, exopolysaccharides) can stimulate the immune system of the host through their interaction with intestinal epithelial cells via specific receptors such as Toll-like (TLRs) and NOD receptors (NLRs), thus regulating the balance between the anti-

inflammatory and pro-inflammatory responses induced by different signaling pathways, such as NF-κB and MAPK [9,59]. Immunoregulation promoted by these bacterial factors has been previously reported by Chandhni et al. [60], who showed that the extractable surface proteins derived from *Lactiplantibacillus plantarum* MTCC 5690, *Lactobacillus acidophilus* NCFM and *Limosilactobacillus fermentum* MTCC 5689 ameliorated DSS or TNBS-induced acute intestinal inflammation by increasing immunoregulatory IL10 cytokine and decreasing leukocyte infiltration, TNFα, and IFNγ pro-inflammatory markers [60]. Similar findings were also reported by Deutsch et al. [61] when demonstrating that surface proteins, such as SlpB and SlpE, are the biological agents responsible for the anti-inflammatory properties of *Propionibacterium freudenreichii* strains. These proteins increased the production of the immunoregulatory cytokine IL10 and modulated pro-inflammatory cytokine TNFα, IFNγ, and IL6 responses of human PBMCs cells [61]. Anti-inflammatory properties of extracellular polysaccharides of *L. delbrueckii* TUA4408L were also reported. These bacterial components attenuated enterotoxigenic *Escherichia coli*-induced inflammatory response in porcine intestinal epitheliocytes by regulating TLR2/4 receptors and MAPK or NF-κB pathway activation and decreasing IL6, IL8, and monocyte chemoattractant protein-1 (MCP-1) levels [59].

It is also interesting to note that, despite the literature demonstrating an anti-inflammatory profile attributed to the probiotic-derived immune molecular effectors, there is evidence that some of these bioactive factors, such as the B7 peptides derived from the probiotic *Bifidobacterium longum*, can exacerbate the intestinal inflammatory process by increasing pro-inflammatory markers and immune cell activation (e.g., circulating antigen-presenting cells) [62]. Thus, these findings show that bioactive bacterial factors can elicit differential immune mechanisms depending on the context of the progression stage (early or late) of the inflammation [62]. In this context, despite surface-exposed components or secreted bioactive molecules appearing to be essential for leading the anti-inflammatory process in the host by CIDCA 133, further studies should be conducted to characterize the cytotoxic/pro-inflammatory profiles of these CIDCA 133-derived molecular components or metabolites, especially in severe inflammation models.

The 5-FU chemotherapy-induced intestinal inflammation also leads to loss of epithelial barrier integrity via the depletion of tight junction proteins (TJs), reduction in goblet cell numbers, and increased intestinal permeability [37,45,53]. TJs proteins are part of an interconnected network of adhesion complexes that act as selective barriers between internal and external cellular environments, thus controlling the passage of pathogens and other foreign molecules [63,64]. The reduction in mRNA expression of mucin 2 and tight junction proteins zonulin, claudin 1, and claudin 2 observed in the inflamed mice was attenuated by both heat-killed and cell-free supernatants of *L. delbrueckii* CIDCA 133. These results are consistent with data obtained by De Jesus et al. [29] and Barroso et al. [30], who demonstrated that viable *L. delbrueckii* CIDCA 133 reduced intestinal permeability and goblet cell degeneration and upregulated the gene expression of tight junction proteins (*Cldn1, Fr11, Hp*) altered by 5-FU chemotherapy, thus being essential to reduce the translocation of toxins and pathogenic bacteria, and consequently the inflammation amplification process in the intestinal mucosa [45].

The improvement in levels of mucins and tight junction proteins after treatment with heat-inactivated beneficial microorganisms and their supernatants has been previously reported. For example, mulberry leaf extract supernatant fermented by *L. acidophilus* upregulated the gene expression of *Muc2* and *Muc5AC* in 5-FU-inflamed mice [47]. Furthermore, secreted factors of *E. coli* Nissle 1917 attenuated the epithelial barrier disruption induced by enteropathogenic *E. coli* in Caco-2 cells by enhancing the gene expression of tight junction proteins zonulin-1, claudin-14, and claudin-2 [65]. Trindade et al. [43] also reported that paraprobiotics *L. rhamnosus* CGMCC1.3724 reduced intestinal permeability induced by 5-FU and enhanced *Muc2* gene expression. Promising results were also reported with inactivated *B. longum* K2-21-4, which modulated the gene expression of claudin-1, zonulin-1, and occludin disrupted by lipopolysaccharide (LPS) in colon epithelial cells [66].

Claudins are the essential transmembrane proteins participating in complex strand networks that regulate the paracellular permeability and maintain the intestinal mucosal barrier function [63,67,68]. The discrepancies in these proteins' expressions may be related to their different interactions with other membrane compartments [63]. Surprisingly, our results showed that 5-FU administration increased the gene expression of claudin-5, contrary to other studies [9,30,37,45,53,69,70]. One possible explanation for this outcome is that this protein was upregulated as a compensatory response due to decreased expression of other tight junction proteins caused by 5-FU. A similar outcome was observed by Li et al. [45], who demonstrated that 5-FU enhanced the expression levels of tight junction ZO-1 and adhesion molecules such as JAM-A while reducing colonic occludin levels. Another piece of evidence indicates that upregulation of claudin-5 can be a mechanism to accelerate intestinal epithelial differentiation to maintain epithelial homeostasis [71].

Based on our results and the pathobiology of mucositis proposed by Sonis [3], we believe that a proposed mechanism used for paraprobiotics CIDCA 133 and its postbiotics to reduce intestinal mucosal inflammation and reinforce the epithelial barrier occurs due to surface-associated components or secreted molecules able to interact with intestinal epithelial cells via specific pattern recognition receptors (PRRs), mainly TLR receptors. This process would control the dysbiotic intestinal microbiota and stimulate goblet cells to secrete mucin, reinforcing the epithelial barrier. On the other hand, the activation of these epithelial cell receptors by these biotics would induce intestinal immune cells to produce the immunoregulatory IL10 cytokine to control activation of the NF-κB signaling pathway and, consequently, regulate the balance between anti-inflammatory and pro-inflammatory immune responses. This process would reduce the migration of inflammatory cells to the mucosa, with subsequent reduction of oxidative stress generated by these cells. All the above processes would decrease tissue damage, reduce the disruption of the tight junction proteins, improve villus length due to migration of enterocytes produced by stem cells in crypts, and, thus, contribute to intestinal architecture recovery and homeostasis (Figure 8). However, we emphasize that more studies should be carried out to better elucidate the immunological, molecular, and cellular mechanisms of action of paraprobiotics and postbiotics from CIDCA 133 in intestinal mucositis and other inflammation models, including knock-out gene mouse models, proteomics, SCFA analysis, microbiota regulation, evaluation of cytokine levels, and activation of immune regulatory cells.

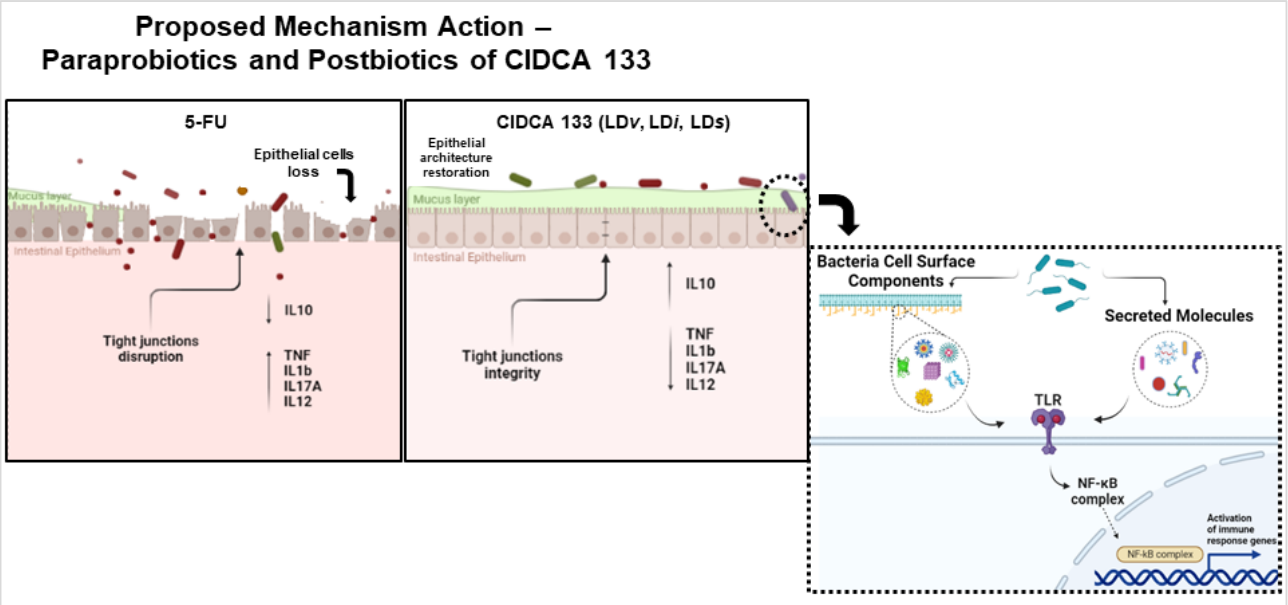


Figure 8. Proposed mechanism of action of paraprobiotics and postbiotics of *L. delbrueckii* CIDCA 133 in intestinal mucositis induced by 5-FU chemotherapy.

5. Conclusions

Altogether, our results demonstrated that heat-killed and cell-free supernatants of the *Lactobacillus delbrueckii* CIDCA 133 strain protected the intestinal mucosa from epithelial damage caused by the 5-FU drug. These ameliorative effects were detectable morphologically and had a profile similar to that of the viable form of CIDCA 133. Modulation of inflammatory parameters and epithelial barrier dysfunction was the primary mechanism used by these biotics to protect the intestinal mucosa from epithelial destruction caused by the 5-FU drug, suggesting them to be an attractive alternative therapeutic approach against intestinal damage induced by chemotherapy and to the problems arising from the use of live beneficial microorganisms in clinical practice.

Author Contributions: Conceptualization, V.L.B., L.C.L.D.J., P.M-A., M.M.D. and V.A.; methodology, V.L.B., L.C.L.D.J., L.M.T., F.L.A.B., L.J.d.S.F., A.d.S.F., J.G.L., M.F.A. and E.F.; formal analysis, V.L.B., L.C.L.D.J., E.F; data curation, V.L.B. and L.C.L.D.J.; writing—original draft preparation, V.L.B., L.C.L.D.J. and J.G.L.; writing—review and editing, L.C.L.D.J., P.M.-A., M.M.D., L.C.J.A. and V.A.; funding acquisition, V.A.; supervision, V.A; All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant number 312045/2020-4.

Institutional Review Board Statement: The animal study protocol was approved by the Local Animal Experimentation Ethics Committee of Federal University of Minas Gerais (CEUA-UFMG, Protocol n○ 112/2020).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available by contacting the corresponding author.

Acknowledgments: We would like to acknowledge the CIDCA 133 center (Center for Research and Development in Food Cryotechnology) and Pablo F. Pérez of the National University of La Plata, Argentine, for bacterial strain supply.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Maria, O.M.; Eliopoulos, N.; Muanza, T. Radiation-Induced Oral Mucositis. *Front. Oncol.* **2017**, *7*, 89. [CrossRef] [PubMed]
2. Lu, L.; Li, W.; Chen, L.; Su, Q.; Wang, Y.; Guo, Z.; Lu, Y.; Liu, B.; Qin, S. Radiation-Induced Intestinal Damage: Latest Molecular and Clinical Developments. *Futur. Oncol.* **2019**, *15*, 4105–4118. [CrossRef] [PubMed]
3. Sonis, S.T. The Pathobiology of Mucositis. *Nat. Rev. Cancer* **2004**, *4*, 277–284. [CrossRef]
4. Bowen, J.; Al-Dasooqi, N.; Bossi, P.; Wardill, H.; Van Sebille, Y.; Al-Azri, A.; Bateman, E.; Correa, M.E.; Raber-Durlacher, J.; Kandwal, A.; et al. The Pathogenesis of Mucositis: Updated Perspectives and Emerging Targets. *Support. Care Cancer* **2019**, *27*, 4023–4033. [CrossRef] [PubMed]
5. Peterson, D.E.; Jones, J.B.; Petit, R.G. Randomized, Placebo-Controlled Trial of Saforis for Prevention and Treatment of Oral Mucositis in Breast Cancer Patients Receiving Anthracycline-Based Chemotherapy. *Cancer* **2007**, *109*, 322–331. [CrossRef]
6. Elad, S.; Cheng, K.K.F.; Lalla, R.V.; Yarom, N.; Hong, C.; Logan, R.M.; Bowen, J.; Gibson, R.; Saunders, D.P.; Zadik, Y.; et al. MASCC/ISOO Clinical Practice Guidelines for the Management of Mucositis Secondary to Cancer Therapy. *Cancer* **2020**, *126*, 4423–4431. [CrossRef]
7. Rosenthal, D.I. Consequences of Mucositis-Induced Treatment Breaks and Dose Reductions on Head and Neck Cancer Treatment Outcomes. *J. Support. Oncol.* **2007**, *5*, 23–31.
8. Dahlgren, D.; Sjöblom, M.; Hellström, P.M.; Lennernäs, H. Chemotherapeutics-Induced Intestinal Mucositis: Pathophysiology and Potential Treatment Strategies. *Front. Pharmacol.* **2021**, *12*, 681417. [CrossRef]
9. Van Vliet, M.J.; Harmsen, H.J.M.; de Bont, E.S.J.M.; Tissing, W.J.E. The Role of Intestinal Microbiota in the Development and Severity of Chemotherapy-Induced Mucositis. *PLoS Pathog.* **2010**, *6*, e1000879. [CrossRef]
10. Ashaolu, T.J.; Fernández-Tomé, S. Gut Mucosal and Adipose Tissues as Health Targets of the Immunomodulatory Mechanisms of Probiotics. *Trends Food Sci. Technol.* **2021**, *112*, 764–779. [CrossRef]
11. Santos Rocha, C.; Lakhdari, O.; Blottière, H.M.; Blugeon, S.; Sokol, H.; Bermu'dez-Humara'n, L.G.; Azevedo, V.; Miyoshi, A.; Doré, J.; Langella, P.; et al. Anti-Inflammatory Properties of Dairy Lactobacilli. *Inflamm. Bowel Dis.* **2012**, *18*, 657–666. [CrossRef] [PubMed]

12. Petrof, E.O.; Kojima, K.; Ropeleski, M.J.; Musch, M.W.; Tao, Y.; De Simone, C.; Chang, E.B. Probiotics Inhibit Nuclear Factor-KB and Induce Heat Shock Proteins in Colonic Epithelial Cells through Proteasome Inhibition. *Gastroenterology* **2004**, *127*, 1474–1487. [CrossRef] [PubMed]
13. Justino, P.F.C.; Franco, A.X.; Pontier-Bres, R.; Monteiro, C.E.S.; Barbosa, A.L.R.; Souza, M.H.L.P.; Czerucka, D.; Soares, P.M.G. Modulation of 5-Fluorouracil Activation of Toll-like/MyD88/NF-KB/MAPK Pathway by *Saccharomyces boulardii* CNCM I-745 Probiotic. *Cytokine* **2020**, *125*, 154791. [CrossRef] [PubMed]
14. Plaza-Diaz, J.; Ruiz-Ojeda, F.J.; Gil-Campos, M.; Gil, A. Mechanisms of Action of Probiotics. *Adv. Nutr.* **2019**, *10*, S49–S66. [CrossRef] [PubMed]
15. Batista, V.L.; da Silva, T.F.; de Jesus, L.C.L.; Coelho-Rocha, N.D.; Barroso, F.A.L.; Tavares, L.M.; Azevedo, V.; Mancha-Agresti, P.; Drumond, M.M. Probiotics, Prebiotics, Synbiotics, and Paraprobiotics as a Therapeutic Alternative for Intestinal Mucositis. *Front. Microbiol.* **2020**, *11*, 544490. [CrossRef]
16. Cereda, E.; Caraccia, M.; Caccialanza, R. Probiotics and Mucositis. *Curr. Opin. Clin. Nutr. Metab. Care* **2018**, *21*, 399–404. [CrossRef]
17. Bian, X.; Wu, W.; Yang, L.; Lv, L.; Wang, Q.; Li, Y.; Ye, J.; Fang, D.; Wu, J.; Jiang, X.; et al. Administration of *Akkermansia muciniphila* Ameliorates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice. *Front. Microbiol.* **2019**, *10*, 2259. [CrossRef]
18. Sokol, H.; Pigneur, B.; Watterlot, L.; Lakhdari, O.; Bermúdez-Humarán, L.G.; Gratadoux, J.-J.; Blugeon, S.; Bridonneau, C.; Furet, J.-P.; Corthier, G.; et al. *Faecalibacterium prausnitzii* Is an Anti-Inflammatory Commensal Bacterium Identified by Gut Microbiota Analysis of Crohn Disease Patients. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 16731–16736. [CrossRef]
19. Santos Rocha, C.; Gomes-Santos, A.C.; Garcias Moreira, T.; de Azevedo, M.; Diniz Luerce, T.; Mariadassou, M.; Longaray Delamare, A.P.; Langella, P.; Maguin, E.; Azevedo, V.; et al. Local and Systemic Immune Mechanisms Underlying the Anti-Colitis Effects of the Dairy Bacterium *Lactobacillus delbrueckii*. *PLoS ONE* **2014**, *9*, e85923. [CrossRef]
20. Quintanilha, M.F.; Miranda, V.C.; Souza, R.O.; Gallotti, B.; Cruz, C.; Santos, E.A.; Alvarez-Leite, J.I.; Jesus, L.C.L.; Azevedo, V.; Trindade, L.M.; et al. *Bifidobacterium longum* Subsp. longum 51A Attenuates Intestinal Injury against Irinotecan-Induced Mucositis in Mice. *Life Sci.* **2022**, *289*, 120243. [CrossRef]
21. Koyama, S.; Fujita, H.; Shimosato, T.; Kamijo, A.; Ishiyama, Y.; Yamamoto, E.; Ishii, Y.; Hattori, Y.; Hagihara, M.; Yamazaki, E.; et al. Septicemia from *Lactobacillus rhamnosus* GG, from a Probiotic Enriched Yogurt, in a Patient with Autologous Stem Cell Transplantation. *Probiotics Antimicrob. Proteins* **2019**, *11*, 295–298. [CrossRef] [PubMed]
22. Yelin, I.; Flett, K.B.; Merakou, C.; Mehrotra, P.; Stam, J.; Snesrud, E.; Hinkle, M.; Lesho, E.; McGann, P.; McAdam, A.J.; et al. Genomic and Epidemiological Evidence of Bacterial Transmission from Probiotic Capsule to Blood in ICU Patients. *Nat. Med.* **2019**, *25*, 1728–1732. [CrossRef] [PubMed]
23. Pasala, S.; Singer, L.; Arshad, T.; Roach, K. *Lactobacillus* Endocarditis in a Healthy Patient with Probiotic Use. *IDCases* **2020**, *22*, e00915. [CrossRef]
24. D'Agostin, M.; Squillaci, D.; Lazzerini, M.; Barbi, E.; Wijers, L.; Da Lozzo, P. Invasive Infections Associated with the Use of Probiotics in Children: A Systematic Review. *Children* **2021**, *8*, 924. [CrossRef]
25. Salminen, S.; Collado, M.C.; Endo, A.; Hill, C.; Lebeer, S.; Quigley, E.M.M.; Sanders, M.E.; Shamir, R.; Swann, J.R.; Szajewska, H.; et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) Consensus Statement on the Definition and Scope of Postbiotics. *Nat. Rev. Gastroenterol. Hepatol.* **2021**, *18*, 649–667. [CrossRef] [PubMed]
26. Teame, T.; Wang, A.; Xie, M.; Zhang, Z.; Yang, Y.; Ding, Q.; Gao, C.; Olsen, R.E.; Ran, C.; Zhou, Z. Paraprobiotics and Postbiotics of Probiotic Lactobacilli, Their Positive Effects on the Host and Action Mechanisms: A Review. *Front. Nutr.* **2020**, *7*, 570344. [CrossRef] [PubMed]
27. Zhou, Y.; Xu, H.; Xu, J.; Guo, X.; Zhao, H.; Chen, Y.; Zhou, Y.; Nie, Y.F. *prausnitzii* and Its Supernatant Increase SCFAs-Producing Bacteria to Restore Gut Dysbiosis in TNBS-Induced Colitis. *AMB Express* **2021**, *11*, 33. [CrossRef]
28. Jin, J.; Wu, S.; Xie, Y.; Liu, H.; Gao, X.; Zhang, H. Live and Heat-Killed Cells of *Lactobacillus plantarum* Zhang-LL Ease Symptoms of Chronic Ulcerative Colitis Induced by Dextran Sulfate Sodium in Rats. *J. Funct. Foods* **2020**, *71*, 103994. [CrossRef]
29. De Jesus, L.C.L.; Drumond, M.M.; de Carvalho, A.; Santos, S.S.; Martins, F.S.; Ferreira, Ê.; Fernandes, R.S.; de Barros, A.L.B.; do Carmo, F.L.R.; Perez, P.F.; et al. Protective Effect of *Lactobacillus delbrueckii* Subsp. lactis CIDCA 133 in a Model of 5 Fluorouracil-Induced Intestinal Mucositis. *J. Funct. Foods* **2019**, *53*, 197–207. [CrossRef]
30. Barroso, F.A.L.; de Jesus, L.C.L.; da Silva, T.F.; Batista, V.L.; Laguna, J.; Coelho-Rocha, N.D.; Vital, K.D.; Fernandes, S.O.A.; Cardoso, V.N.; Ferreira, E.; et al. *Lactobacillus delbrueckii* CIDCA 133 Ameliorates Chemotherapy-Induced Mucositis by Modulating Epithelial Barrier and TLR2/4/Myd88/NF-KB Signaling Pathway. *Front. Microbiol.* **2022**, *13*, 858036. [CrossRef]
31. De Jesus, L.C.L.; Drumond, M.M.; Aburjaile, F.F.; de Jesus Sousa, T.; Coelho-Rocha, N.D.; Profeta, R.; Brenig, B.; Mancha-Agresti, P.; Azevedo, V. Probiogenomics of *Lactobacillus delbrueckii* Subsp. lactis CIDCA 133: In Silico, In Vitro, and In Vivo Approaches. *Microorganisms* **2021**, *9*, 829. [CrossRef] [PubMed]
32. De Jesus, L.C.L.; de Jesus Sousa, T.; Coelho-Rocha, N.D.; Profeta, R.; Barroso, F.A.L.; Drumond, M.M.; Mancha-Agresti, P.; Ferreira, Ê.; Brenig, B.; Aburjaile, F.F.; et al. Safety Evaluation of *Lactobacillus delbrueckii* Subsp. *lactis* CIDCA 133: A Health-Promoting Bacteria. *Probiotics Antimicrob. Proteins* **2021**, *in press*. [CrossRef] [PubMed]
33. Prisciandaro, L.D.; Geier, M.S.; Butler, R.N.; Cummins, A.G.; Howarth, G.S. Probiotic Factors Partially Improve Parameters of 5-Fluorouracil-Induced Intestinal Mucositis in Rats. *Cancer Biol. Ther.* **2011**, *11*, 671–677. [CrossRef]

34. Souza, D.G.; Cara, D.C.; Cassali, G.D.; Coutinho, S.F.; Silveira, M.R.; Andrade, S.P.; Poole, S.P.; Teixeira, M.M. Effects of the PAF Receptor Antagonist UK74505 on Local and Remote Reperfusion Injuries Following Ischaemia of the Superior Mesenteric Artery in the Rat. *Br. J. Pharmacol.* **2000**, *131*, 1800–1808. [CrossRef] [PubMed]
35. Strath, M.; Warren, D.J.; Sanderson, C.J. Detection of Eosinophils Using an Eosinophil Peroxidase Assay. Its Use as an Assay for Eosinophil Differentiation Factors. *J. Immunol. Methods* **1985**, *83*, 209–215. [CrossRef]
36. Giulietti, A.; Overbergh, L.; Valckx, D.; Decallonne, B.; Bouillon, R.; Mathieu, C. An Overview of Real-Time Quantitative PCR: Applications to Quantify Cytokine Gene Expression. *Methods* **2001**, *25*, 386–401. [CrossRef]
37. Song, M.-K.; Park, M.-Y.; Sung, M.-K. 5-Fluorouracil-Induced Changes of Intestinal Integrity Biomarkers in BALB/C Mice. *J. Cancer Prev.* **2013**, *18*, 322–329. [CrossRef]
38. Volynets, V.; Rings, A.; Bárdos, G.; Ostaff, M.J.; Wehkamp, J.; Bischoff, S.C. Intestinal Barrier Analysis by Assessment of Mucins, Tight Junctions, and α-Defensins in Healthy C57BL/6J and BALB/CJ Mice. *Tissue Barriers* **2016**, *4*, e1208468. [CrossRef]
39. Chang, C.-W.; Lee, H.-C.; Li, L.-H.; Chiang Chiau, J.-S.; Wang, T.-E.; Chuang, W.-H.; Chen, M.-J.; Wang, H.-Y.; Shih, S.-C.; Liu, C.-Y.; et al. Fecal Microbiota Transplantation Prevents Intestinal Injury, Upregulation of Toll-Like Receptors, and 5-Fluorouracil/Oxaliplatin-Induced Toxicity in Colorectal Cancer. *Int. J. Mol. Sci.* **2020**, *21*, 386. [CrossRef]
40. Zheng, L.; Zhang, Y.-L.; Dai, Y.-C.; Chen, X.; Chen, D.-L.; Dai, Y.-T.; Tang, Z.-P. Jianpi Qingchang Decoction Alleviates Ulcerative Colitis by Inhibiting Nuclear Factor-KB Activation. *World J. Gastroenterol.* **2017**, *23*, 1180. [CrossRef]
41. Soares, P.M.G.; Mota, J.M.S.C.; Gomes, A.S.; Oliveira, R.B.; Assreuy, A.M.S.; Brito, G.A.C.; Santos, A.A.; Ribeiro, R.A.; Souza, M.H.L.P. Gastrointestinal Dysmotility in 5-Fluorouracil-Induced Intestinal Mucositis Outlasts Inflammatory Process Resolution. *Cancer Chemother. Pharmacol.* **2008**, *63*, 91–98. [CrossRef] [PubMed]
42. Gan, Y.; Ai, G.; Wu, J.; Luo, H.; Chen, L.; Huang, Q.; Wu, X.; Xu, N.; Li, M.; Su, Z.; et al. Patchouli Oil Ameliorates 5-Fluorouracil-Induced Intestinal Mucositis in Rats via Protecting Intestinal Barrier and Regulating Water Transport. *J. Ethnopharmacol.* **2020**, *250*, 112519. [CrossRef] [PubMed]
43. Trindade, L.M.; Torres, L.; Matos, I.D.; Miranda, V.C.; de Jesus, L.C.L.; Cavalcante, G.; de Souza Oliveira, J.J.; Cassali, G.D.; Mancha-Agresti, P.; de Carvalho Azevedo, V.A.; et al. Paraprobiotic *Lacticaseibacillus rhamnosus* Protects Intestinal Damage in an Experimental Murine Model of Mucositis. *Probiotics Antimicrob. Proteins* **2021**, *in press*. [CrossRef] [PubMed]
44. Chang, C.-T.; Ho, T.-Y.; Lin, H.; Liang, J.-A.; Huang, H.-C.; Li, C.-C.; Lo, H.-Y.; Wu, S.-L.; Huang, Y.-F.; Hsiang, C.-Y. 5-Fluorouracil Induced Intestinal Mucositis via Nuclear Factor-KB Activation by Transcriptomic Analysis and In Vivo Bioluminescence Imaging. *PLoS ONE* **2012**, *7*, e31808. [CrossRef]
45. Li, H.-L.; Lu, L.; Wang, X.-S.; Qin, L.-Y.; Wang, P.; Qiu, S.-P.; Wu, H.; Huang, F.; Zhang, B.-B.; Shi, H.-L.; et al. Alteration of Gut Microbiota and Inflammatory Cytokine/Chemokine Profiles in 5-Fluorouracil Induced Intestinal Mucositis. *Front. Cell. Infect. Microbiol.* **2017**, *7*, 455. [CrossRef]
46. Sang, L.-X.; Chang, B.; Dai, C.; Gao, N.; Liu, W.-X.; Jiang, M. Heat-Killed VSL#3 Ameliorates Dextran Sulfate Sodium (DSS)-Induced Acute Experimental Colitis in Rats. *Int. J. Mol. Sci.* **2013**, *15*, 15–28. [CrossRef]
47. Oh, N.S.; Lee, J.Y.; Lee, J.M.; Lee, K.W.; Kim, Y. Mulberry Leaf Extract Fermented with *Lactobacillus acidophilus* A4 Ameliorates 5-Fluorouracil-Induced Intestinal Mucositis in Rats. *Lett. Appl. Microbiol.* **2017**, *64*, 459–468. [CrossRef]
48. Li, M.O.; Wan, Y.Y.; Sanjabi, S.; Robertson, A.-K.L.; Flavell, R.A. Transforming Growth Factor-β Regulation of Immune Responses. *Annu. Rev. Immunol.* **2006**, *24*, 99–146. [CrossRef]
49. Konkel, J.E.; Chen, W. Balancing Acts: The Role of TGF-β in the Mucosal Immune System. *Trends Mol. Med.* **2011**, *17*, 668–676. [CrossRef]
50. Al-Khrashi, L.A.; Badr, A.M.; AL-Amin, M.A.; Mahran, Y.F. Thymol Ameliorates 5-fluorouracil-induced Intestinal Mucositis: Evidence of Down-regulatory Effect on TGF-β/MAPK Pathways through NF-κB. *J. Biochem. Mol. Toxicol.* **2022**, *36*, e22932. [CrossRef]
51. Kim, H.J.; Kim, J.H.; Moon, W.; Park, J.; Park, S.J.; Song, G.A.; Han, S.H.; Lee, J.H. Rebamipide Attenuates 5-Fluorouracil-Induced Small Intestinal Mucositis in a Mouse Model. *Biol. Pharm. Bull.* **2015**, *38*, 179–183. [CrossRef] [PubMed]
52. Barbosa, M.M.; de Araújo, A.A.; de Araújo Júnior, R.F.; Guerra, G.C.B.; de Castro Brito, G.A.; Leitão, R.C.; Ribeiro, S.B.; de Aragão Tavares, E.; Vasconcelos, R.C.; Garcia, V.B.; et al. Telmisartan Modulates the Oral Mucositis Induced by 5-Fluorouracil in Hamsters. *Front. Physiol.* **2018**, *9*, 1204. [CrossRef] [PubMed]
53. Barroso, F.A.L.; de Jesus, L.C.L.; de Castro, C.P.; Batista, V.L.; Ferreira, Ê.; Fernandes, R.S.; de Barros, A.L.B.; Leclerq, S.Y.; Azevedo, V.; Mancha-Agresti, P.; et al. Intake of *Lactobacillus delbrueckii* (PExu:Hsp65) Prevents the Inflammation and the Disorganization of the Intestinal Mucosa in a Mouse Model of Mucositis. *Microorganisms* **2021**, *9*, 107. [CrossRef] [PubMed]
54. Wu, J.; Gan, Y.; Li, M.; Chen, L.; Liang, J.; Zhuo, J.; Luo, H.; Xu, N.; Wu, X.; Wu, Q.; et al. Patchouli Alcohol Attenuates 5-Fluorouracil-Induced Intestinal Mucositis via TLR2/MyD88/NF-KB Pathway and Regulation of Microbiota. *Biomed. Pharmacother.* **2020**, *124*, 109883. [CrossRef]
55. Zhang, L.; Jin, Y.; Peng, J.; Chen, W.; Lisha, L.; Lin, J. Qingjie Fuzheng Granule Attenuates 5-Fluorouracil-Induced Intestinal Mucosal Damage. *Biomed. Pharmacother.* **2019**, *118*, 109223. [CrossRef]
56. Gomes-Santos, A.C.; de Oliveira, R.P.; Moreira, T.G.; Castro-Junior, A.B.; Horta, B.C.; Lemos, L.; de Almeida, L.A.; Rezende, R.M.; Cara, D.C.; Oliveira, S.C.; et al. Hsp65-Producing *Lactococcus lactis* Prevents Inflammatory Intestinal Disease in Mice by IL-10- and TLR2-Dependent Pathways. *Front. Immunol.* **2017**, *8*, 30. [CrossRef]

57. Rosenberg, H.F.; Masterson, J.C.; Furuta, G.T. Eosinophils, Probiotics, and the Microbiome. *J. Leukoc. Biol.* **2016**, *100*, 881–888. [CrossRef]
58. Theiler, A.; Bärnthaler, T.; Platzer, W.; Richtig, G.; Peinhaupt, M.; Rittchen, S.; Kargl, J.; Ulven, T.; Marsh, L.M.; Marsche, G.; et al. Butyrate Ameliorates Allergic Airway Inflammation by Limiting Eosinophil Trafficking and Survival. *J. Allergy Clin. Immunol.* **2019**, *144*, 764–776. [CrossRef]
59. Wachi, S.; Kanmani, P.; Tomosada, Y.; Kobayashi, H.; Yuri, T.; Egusa, S.; Shimazu, T.; Suda, Y.; Aso, H.; Sugawara, M.; et al. *Lactobacillus delbrueckii* TUA4408L and Its Extracellular Polysaccharides Attenuate Enterotoxigenic *Escherichia coli*- Induced Inflammatory Response in Porcine Intestinal Epitheliocytes via Toll-like Receptor-2 and 4. *Mol. Nutr. Food Res.* **2014**, *58*, 2080–2093. [CrossRef]
60. Chandhni, P.R.; Pradhan, D.; Sowmya, K.; Gupta, S.; Kadyan, S.; Choudhary, R.; Gupta, A.; Gulati, G.; Mallappa, R.H.; Kaushik, J.K.; et al. Ameliorative Effect of Surface Proteins of Probiotic Lactobacilli in Colitis Mouse Models. *Front. Microbiol.* **2021**, *12*, 679773. [CrossRef]
61. Deutsch, S.-M.; Mariadassou, M.; Nicolas, P.; Parayre, S.; Le Guellec, R.; Chuat, V.; Peton, V.; Le Maréchal, C.; Burati, J.; Loux, V.; et al. Identification of Proteins Involved in the Anti-Inflammatory Properties of *Propionibacterium freudenreichii* by Means of a Multi-Strain Study. *Sci. Rep.* **2017**, *7*, 46409. [CrossRef] [PubMed]
62. Fernández-Tomé, S.; Marin, A.C.; Ortega Moreno, L.; Baldan-Martin, M.; Mora-Gutiérrez, I.; Lanas-Gimeno, A.; Moreno-Monteagudo, J.A.; Santander, C.; Sánchez, B.; Chaparro, M.; et al. Immunomodulatory Effect of Gut Microbiota-Derived Bioactive Peptides on Human Immune System from Healthy Controls and Patients with Inflammatory Bowel Disease. *Nutrients* **2019**, *11*, 2605. [CrossRef] [PubMed]
63. Zihni, C.; Mills, C.; Matter, K.; Balda, M.S. Tight Junctions: From Simple Barriers to Multifunctional Molecular Gates. *Nat. Rev. Mol. Cell Biol.* **2016**, *17*, 564–580. [CrossRef] [PubMed]
64. Chelakkot, C.; Ghim, J.; Ryu, S.H. Mechanisms Regulating Intestinal Barrier Integrity and Its Pathological Implications. *Exp. Mol. Med.* **2018**, *50*, 1–9. [CrossRef]
65. Alvarez, C.-S.; Giménez, R.; Cañas, M.-A.; Vera, R.; Díaz-Garrido, N.; Badia, J.; Baldomà, L. Extracellular Vesicles and Soluble Factors Secreted by *Escherichia coli* Nissle 1917 and ECOR63 Protect against Enteropathogenic *E. coli*-Induced Intestinal Epithelial Barrier Dysfunction. *BMC Microbiol.* **2019**, *19*, 166. [CrossRef]
66. Guan, J.; Liu, F.; Zhao, S.; Evivie, S.E.; Shi, J.; Li, N.; Zhao, L.; Yue, Y.; Xie, Q.; Huo, G.; et al. Effect of *Bifidobacterium longum* Subsp. longum on the Proliferative and Tight-Junction Activities of Human Fetal Colon Epithelial Cells. *J. Funct. Foods* **2021**, *86*, 104715. [CrossRef]
67. Tsukita, S.; Furuse, M. Occludin and Claudins in Tight-Junction Strands: Leading or Supporting Players? *Trends Cell Biol.* **1999**, *9*, 268–273. [CrossRef]
68. Findley, M.K.; Koval, M. Regulation and Roles for Claudin-Family Tight Junction Proteins. *IUBMB Life* **2009**, *61*, 431–437. [CrossRef]
69. Yue, X.; Wen, S.; Long-kun, D.; Man, Y.; Chang, S.; Min, Z.; Shuang-yu, L.; Xin, Q.; Jie, M.; Liang, W. Three Important Short-Chain Fatty Acids (SCFAs) Attenuate the Inflammatory Response Induced by 5-FU and Maintain the Integrity of Intestinal Mucosal Tight Junction. *BMC Immunol.* **2022**, *23*, 19. [CrossRef]
70. Carvalho, P.L.A.; Andrade, M.E.R.; Trindade, L.M.; Leocádio, P.C.L.; Alvarez-Leite, J.I.; dos Reis, D.C.; Cassali, G.D.; de Sales Souza e Melo, É.L.; dos Santos Martins, F.; Fernandes, S.O.A.; et al. Prophylactic and Therapeutic Supplementation Using Fructo-Oligosaccharide Improves the Intestinal Homeostasis after Mucositis Induced by 5- Fluorouracil. *Biomed. Pharmacother.* **2021**, *133*, 111012. [CrossRef]
71. Dahan, S.; Rabinowitz, K.M.; Martin, A.P.; Berin, M.C.; Unkeless, J.C.; Mayer, L. Notch-1 Signaling Regulates Intestinal Epithelial Barrier Function, Through Interaction With $CD4^+$ T Cells, in Mice and Humans. *Gastroenterology* **2011**, *140*, 550–559. [CrossRef] [PubMed]

CAPÍTULO IV

8. CAPÍTULO IV: Safety Evaluation of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: a Health-promoting Bacteria

Este artigo científico foi publicado na revista *Probiotics and Antimicrobial Proteins* (ISSN: 1867-1314) em agosto de 2021. Fator de impacto atual: 5.265.

Microrganismos probióticos, além de promoverem efeitos benéficos, devem apresentar níveis de segurança para serem consumidos ou utilizados em aplicações probióticas. Embora alguns probióticos comerciais sejam geralmente reconhecidos como seguros (GRAS), a avaliação de segurança para consumo humano e veterinário de novos candidatos probióticos deve ser realizada, devido alguns estudos relatarem a associação de algumas infecções após o consumo desses microrganismos, especialmente espécies de *Lactobacillus*. *Lactobacillus delbrueckii* CIDCA 133 é uma bactéria probiótica cujos efeitos benéficos ao hospedeiro vêm sendo reportados tanto na sua forma viável quanto inativada. No entanto, para evitar questões futuras sobre os efeitos adversos e prejudiciais do consumo de CIDCA 133 ao hospedeiro, neste capítulo avaliou-se o status de segurança da linhagem por meio de análise genômica e fenotípica. Assim, buscou-se por genes relacionados à resistência aos antibióticos, à fatores de virulência de bactérias patogênicas e à produção de metabólitos tóxicos, bem como elementos móveis (plasmídeos, fagos e elementos de inserção) responsáveis pela disseminação dessas características danosas ao organismo. Atividade hemolítica, degradação de mucina e resistência a antimicrobianos também foram investigados *in vitro*. No mais, um estudo *in vivo* foi realizado com a finalidade de avaliar se o consumo de CIDCA 133 ($5x10^7$ CFU/mL) durante 13 dias consecultivos causaria alguma alteração clínica ou histopatológica em animais saudáveis. Os resultados demonstram que CIDCA 133 apresenta nível seguro para ser utilizada em formulações e futuras aplicações probióticas.

Safety Evaluation of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: a Health-Promoting Bacteria

Luís Cláudio Lima de Jesus[1] · Thiago de Jesus Sousa[1] · Nina Dias Coelho-Rocha[1] · Rodrigo Profeta[1] · Fernanda Alvarenga Lima Barroso[1] · Mariana Martins Drumond[2] · Pamela Mancha-Agresti[3] · Ênio Ferreira[4] · Bertram Brenig[5] · Flávia Figueira Aburjaile[1] · Vasco Azevedo[1]

Accepted: 22 July 2021

Abstract
Lactobacillus delbrueckii subsp. *lactis* CIDCA is a new potential probiotic strain whose molecular basis attributed to the host's benefit has been reported. This study investigated the safety aspects of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 based on whole-genome sequence and phenotypic analysis to avoid future questions about the harmful effects of this strain consumption. Genomic analysis showed that *L. delbrueckii* subsp. *lactis* CIDCA 133 harbors virulence, harmful metabolites, and antimicrobial resistance-associated genes. However, none of these genetic elements is flanked or located within prophage regions and plasmid sequence. At a phenotypic level, it was observed *L. delbrueckii* subsp. *lactis* CIDCA 133 antimicrobial resistance to aminoglycosides streptomycin and gentamicin antibiotics, but no hemolytic and mucin degradation activity was exhibited by strain. Furthermore, no adverse effects were observed regarding mice clinical and histopathological analysis after the strain consumption (5×10^7 CFU/mL). Overall, these findings reveal the safety of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 for consumption and future probiotic applications.

Keywords Genomic analysis · *Lactobacillus delbrueckii* · Epithelial barrier · CRISPR-Cas system · Safety assessment

Introduction

Lactobacillus is one of the main microorganisms of the lactic acid bacteria (LAB) group with high importance in the dairy industry [1, 2] and a broad spectrum of beneficial effects, mainly focused on gastrointestinal tract (GIT) diseases treatment [3, 4], diabetes [5], and foodborne pathogens control [6], among others. A new taxonomic classification was proposed for the genus *Lactobacillus*, with *Lactobacillus delbrueckii* being the type species [7]. Among the species, *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 is a new potential probiotic strain isolated from raw cow's milk in La Plata, Argentina [8].

The beneficial effects of *L. delbrueckii* subsp. *lactis* CIDCA 133 have been reported, such as its ability to inhibit the growth of pathogenic and spoilage bacteria (e.g., *Pseudomonas aeruginosa* and *Escherichia coli*) [8, 9], to tolerate stress conditions, as bile salts [10] and acidity [11], and survive to enterocyte-derived antimicrobial molecules such as β-defensins [12, 13]. Furthermore, this strain stimulated immune cells like dendritic cells and murine macrophage infected with *Bacillus cereus* and *Citrobacter rodentium* [14, 15] to control the infection generated by these pathogens. It was also reported that *L. delbrueckii* subsp. *lactis* CIDCA 133 protected the mice's intestinal mucosa from inflammatory and histopathological damage caused by chemotherapy [3]. The molecular basis attributed to stress tolerance,

Luís Cláudio Lima de Jesus, Thiago de Jesus Sousa, Flávia Figueira Aburjaile, and Vasco Azevedo contributed equally to this work.

✉ Vasco Azevedo
vasco@icb.ufmg.br

1 Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

2 Departamento de Ciências Biológicas, Centro Federal de Educação Tecnológica de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

3 Faculdade de Minas-Faminas-BH, Medicina, Belo Horizonte, Minas Gerais, Brazil

4 Departamento de Patologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

5 Institute of Veterinary Medicine, University of Göttingen, Göttingen, Germany

Published online: 17 August 2021

pathogens inhibition, and anti-inflammatory effects of *L. delbrueckii* subsp. *lactis* CIDCA 133 were associated with genes encoding proteins as Na (+)/H (+) antiporter NhaC, choloylglycine hydrolase, chaperones (GroEL, DnaK), and helveticin J bacteriocin, as well as its ability to inhibit the expression of *Nfkb1* (p105), and upregulation the gene expression of *Il10* and *Tgfb* anti-inflammatory cytokines [11], respectively.

Although some commercial probiotic strains are generally recognized as safe (GRAS) and qualified presumption of safety (QPS), safety assessment for human and veterinary consumption of new probiotic candidates should be evaluated [16], due to some studies reporting the association of some infections after probiotics consumption [17–20].

Phenotypic assays (in vitro and in vivo) have been used for the comprehensive safety evaluation of new potential probiotic strains [21, 22]. With advances in next-generation sequencing (NGS) technologies, the genomic approach has been shown essential for strain identification and genetic basis study related to the beneficial effects of probiotics. It is also used as a complementary tool for evaluating desired and undesired factors of promising probiotic bacteria [23, 24]. The safety assessment based on the whole-genome sequences has been carried out for potential probiotic bacteria, such as *Lactobacillus helveticus* KLDS1.8701 [25], *Enterococcus durans* KLDS6.0930 [26], *Lactobacillus crispatus* YIT 12,319 [27], *Lactiplantibacillus plantarum* strains [28], *Blautia producta* DSM 2950 [29], and *Limosilactobacillus reuteri* IDCC 3701 [30].

Our research group has previously performed the genetic basis related to probiotic properties of *L. delbrueckii* subsp. *lactis* CIDCA 133. Thus, to avoid future questions about the adverse and harmful effects of this strain consumption, this work investigated the safety status of *L. delbrueckii* subsp. *lactis* CIDCA 133, a health-promoting bacteria, through a whole-genome sequence and phenotypic-scale analysis.

Materials and Methods

Bacteria Strain and DNA Sequences

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 (hereafter CIDCA 133) was obtained from the culture collection of the CIDCA center (Center for Research and Development in Food Cryotechnology) of the National University of La Plata, Argentina. The bacteria was grown in de Man, Rogosa, and Sharpe (MRS) broth (Kasvi, São José dos Pinhais, Brazil) for 16 h at 37 °C.

CIDCA 133 genome and plasmid sequences were obtained from National Center for Biotechnology (NCBI) (BioProject: PRJNA636695; BioSample: SAMN15082156; GenBank: CP065513 and CP065514), published in the previous work [11].

Genome Analysis

Insertion Elements, Prophages, and CRISPR-Cas System of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

The annotation of insertion sequences (IS) was performed using the ISsaga (Insertion Sequence Semi-Automatic Genome Annotation) (http://issaga.biotoul.fr/) [31]. The prediction of prophage arrangements was carried out with PHASTER (PHAge Search Tool Enhanced Release) (https://phaster.ca/) [32]. The CRISPR-Cas system was predicted using the CRISPRCasFinder (https://crisprcas.i2bc.paris-saclay.fr/) [33]. Finally, the insertion sites of these genetic elements on the CIDCA 133 genome were visualized using BRIG (BLAST Ring Image Generator) software [34].

Antibiotic Resistance-Related Genes of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

The genes identification related to antibiotic resistance on CIDCA 133 plasmid and chromosome was performed using BLASTp against the CARD (The Comprehensive Antibiotic Resistance Database) database (https://card.mcmaster.ca/) [35]. A coverage > 70%, similarity > 30%, and E-value < e^{-2} were used as criteria to identify the possible antibiotic resistance genes [25].

Pathogenicity of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

PathogenFinder web server (https://cge.cbs.dtu.dk/services/PathogenFinder/) [36] was used to determine the pathogenic potential of CIDCA 133 towards the human host. The CIDCA 133 virulence-associated genes were evaluated through an alignment via BLASTp against the VFDB (Virulence Factor Database) (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi) [37]. A coverage and identity > 60% were used as the criteria for this analysis [37].

Harmful Metabolite-Associated Genes of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

The genes prediction related to bacterial toxins and harmful metabolites production were investigated through both functional annotations from the PGAP pipeline (NCBI), based on literature data revision [21, 23, 28, 38–41], and using the KEGG Mapper/BLASTKOALA tool (https://www.kegg.jp/blastkoala/) [42].

Mucin Degradation-Related Genes of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

Genes involved with the mucin degradation process by CIDCA 133 were manually investigated through functional annotation from the PGAP pipeline (NCBI), based on literature data revision [43–45].

In Vitro Safety Assessment of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

Antibiotic Susceptibility Testing

The antibiotic susceptibility profile of CIDCA 133 was determined by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [46], with some modifications. For this purpose, 100 µL of CIDCA 133 culture (10^8 CFU/mL) was grown in MRS agar plates (Kasvi, São José dos Pinhais, Brazil) containing clinical relevance antibiotics disks (Cecon®, São Paulo, Brazil): clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), streptomycin (10 µg), tetracycline (30 µg), vancomycin (30 µg), ampicillin (10 µg), and chloramphenicol (30 µg). After incubation at 37 °C for 24 h, the inhibition zone diameter was measured using a millimeter ruler, and the results were expressed as sensitive (S) and resistant (R), according to the interpretative criteria described by CLSI [46]. The assay was performed in triplicate and on two independent occasions.

Mucin Degradation Activity

Mucin degradation was performed according to the method described by Zhou et al. [47], with some modifications, using modified Luria–Bertani (LB) agar plates containing partially purified hog gastric mucin (0.3%) (HGM type III) (Sigma-Aldrich, St. Louis, MO, USA) as only carbon source. For this, 10 µL of CIDCA 133 culture (10^8 CFU/mL) was spotted onto the LB agar plates' surface. *Salmonella enterica* serovar Typhimurium ATCC® 29630 and *Salmonella enterica* serovar Typhi ATCC® 33458 (ATCC®, Manassas, Virginia, EUA) were used as a positive control. After incubation at 37 °C for 48 h, the plates were stained with 0.1% black starch (Sigma-Aldrich, St. Louis, MO, USA) in acetic acid (3.5 M) (Vetec, Rio de Janeiro, Brazil) for 30 min and subsequently washed with acetic acid (1.2 M) until discoloration. A clear area (discolored halo) appearance around the colonies was considered positive for mucin degradation. The assay was performed in triplicate and on two independent occasions.

Hemolytic Activity

The hemolytic activity method was carried out according to Xu et al. [48], with some modifications, using tryptic soybean agar plates (Kasvi, São José dos Pinhais, Brazil) containing 5% (v/v) sheep blood. For this, 10 µL of CIDCA 133 culture (10^8 CFU/mL) was spotted onto the surface of blood agar plates. *Staphylococcus aureus* O46, obtained from INRA collection (INRA, Rennes, France), was a positive control. After incubation at 37 °C for 48 h, the hemolytic reaction was evaluated based on the presence of a clear area around the bacteria colonies (total hemolysis, β-hemolysis), a green zone (partial hemolysis, α-hemolysis) or no reaction (γ-hemolysis). The assay was performed in triplicate and on two independent occasions.

In Vivo Safety Evaluation of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

The body weight loss and daily food consumption, index organ (spleen and thymus), tight junction's proteins and oxidative stress markers gene expression, inflammatory infiltration, and intestinal epithelium damage were analyzed to evaluate the CIDCA 133 capacity for causing infection/toxicity in healthy mice.

Experimental Design

The experiments were carried out with conventional male BALB/c mice (weight 25–30 g, 6 weeks old) obtained from the Federal University of Minas Gerais (UFMG). The mice were kept in polycarbonate ventilated cages under temperature and light/dark cycle-controlled room, with ad libitum access to water and standard chow diet 24 h before experiments. All procedures complied with the Brazilian College of Animal Experimentation (COBEA) and were approved by the Local Animal Experimental Ethics Committee (CEUA-UFMG) (Protocol no. 112/2020).

Mice were randomized into two experimental groups ($n = 6$ animals per group): (I) negative control (NC) and (II) treated group CIDCA 133. These groups were administered by continuous feeding with 100 mL/cage of MRS broth (NC group) or CIDCA 133 (5×10^7 CFU/mL) for 13 consecutive days. After the experimentation period, the animals were euthanized by a single intraperitoneal injection of anesthetic overdose (30 mg/kg of xylazine and 300 mg/kg of ketamine mixture) (Ceva, São Paulo, Brazil) and samples of the small intestine (ileum section), spleen, and thymus were collected for analysis. In addition, body weight and feed intake were assessed daily before euthanasia.

Organ Index

The splenic and thymus index was determined as follows [49]: organ index = (weight of organ/body weight of the mice) × 100.

Histological Analysis

After the euthanasia for histological analysis, the ileum section was collected, washed with PBS 0.01 M, rolled up, and immersed in 10% buffered formaldehyde solution (Labsynth, São Paulo, Brazil) for tissue fixation. This material was embedded in paraffin, and 4-µm-thick slices of samples were placed on a glass slide and stained with hematoxylin and eosin (HE).

The histological score was determined as described by Alvim et al. [50], measuring significant histological changes: (i) intensity of the infiltrate of mononuclear and polymorphonuclear cells in the lamina propria, (ii) epithelial ulceration and erosion, (iii) lamina propria edema, (iv) hyperemia, and (v) alterations in mucosal architecture. In addition, the score was given according to the severity of the lesion in the tissue: absent (0), mild (1), moderate (2), and severe (3). For morphological examination, ten images of each animal's ileum-slides were captured by a BX41 optical microscope (Olympus, Tokyo, Japan), and 20 villus lengths of each animal were measured using ImageJ 1.51j.8 software (NIH, Bethesda, MD, USA).

Inflammatory Cell Infiltration

The polymorphonuclear cell infiltration (neutrophils and eosinophils) in the intestinal mucosa was also determined by myeloperoxidase (MPO) and eosinophil peroxidase (EPO) enzyme activities, respectively. Briefly, ileum tissues were homogenized and centrifuged. After the cells pellet lysis process with three cycles of freezing and thawing in liquid nitrogen, the supernatants were used to quantify enzyme activities as previously described by De Jesus et al. [3]. The absorbance of the enzymatic assay was measured at 492 nm (EPO) and 450 nm (MPO) on a microplate spectrophotometer (Bio-Rad 450 model) (Bio-Rad Laboratories, Hercules, CA, USA). Results were expressed as MPO or EPO arbitrary units (based on absorbance)/100 mg of tissue. The assay was performed in duplicate and on two independent occasions.

Oxidative Stress and Epithelial Barrier's Markers Gene Expression in Mice Ileum

According to the manufacturer's instructions, mice ileum (~ 20 mg) total RNA isolation was carried out using RNeasy Mini Kit (QIAGEN, Hilden, Germany). Then, following the manufacturer's instructions, the RNA samples (2 µg) were purified with DNAse I from the TURBO DNA-free™ Kit (Invitrogen, Carlsbad, CA, USA). Finally, the complementary deoxyribonucleic acid (cDNA) synthesis was performed according to the Applied Biosystems™ High-Capacity cDNA Reverse Transcription kit (Thermo Fisher, Waltham, MA, USA).

Quantitative PCR (qPCR) was performed using the PowerUp™ SYBR® Green Master Mix (Thermo Fisher, Waltham, MA, USA) and the gene-specific primers nitric oxide synthase 2 (*nos2*), claudin 2 (*cldn2*), *zonulin*, and *occludin* (Table 1) [51, 52]. Amplification reactions were performed on the Applied Biosystems 7900HT Fast Real-Time PCR System under the following conditions: initial denaturation at 95 °C for 10 min, 95 °C for 15 s, annealing/extension at 60 °C for 1 min, 40 cycles followed by a dissociation stage for recording the melting curve. In addition, gene expression levels were analyzed by the $2^{-\Delta\Delta Ct}$ method using housekeeping genes encoding β-actin (*actb*) and GAPDH (*gapdh*) as endogenous references. The assay was performed in duplicate and on two independent occasions.

Statistical Analysis

The graphics and statistical analyses were performed by the Student's *t*-test using the GraphPad Prism 8.0 software and p-value < 0.05. All data were presented as mean and standard deviation (SD).

Table 1 Quantitative polymerase chain reaction (qPCR) primers used in this study

Gene	Primer foward	Primer reverse	Amplicon size (bp)	Reference
actb	GCTGAGAGGGAAATCGTGCGTG	CCAGGGAGGAAGAGGATGCGG	100	[52]
gapdh	TCACCACCATGGAGAAGGC	GCTAAGCAGTTGGTGGTGCA	168	[51]
cldn2	GTCATCGCCCATCAGAAGAT	ACTGTTGGACAGGGAACCAG	159	[52]
occludin	ACTCCTCCAATGGACAAGTG	CCCCACCTGTCGTGTAGTCT	249	[52]
zonulin	CCACCTCTGTCCAGCTCTTC	CACCGGAGTGATGGTTTTCT	249	[52]
nos2	CAGCTGGGCTGTACAAACCTT	CATTGGAAGTGAAGCGTTTCG	95	[51]

Results

Genome Analysis

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 Plasmid and Mobile Genetic Elements

As previously reported by De Jesus et al. [11], CIDCA 133 harbors a plasmid sequence (6,224 bp). This plasmid has six CDS, in which three have an unknown function (hypothetical proteins), and the others are associated with the mobilization, transposase, and helicase function, respectively.

Five prophage regions (one intact, one questionable, and three incomplete) were also identified on CIDCA 133 genome. The incomplete prophages have 9.2 kb (phage 1), 22.6 kb (phage 2), and 7.5 kb (phage 3). The questionable prophage (phage 4) and the intact prophage (phage 5) have 10.4 kb and 70.9 kb. All prophage regions belong to the family *Siphoviridae*, except the questionable prophage related to the *Myoviridae* family. The predicted prophages CDS were listed in Table S1.

Furthermore, CIDCA 133 genome contains a type I-E CRISPR-Cas system (13,314 bp), with 64 spacers, 65 direct repetitive sequences (DR) (DR consensus: 5'-GTA TTCCCCACGCAAGTGGGGGTGATCC-3'), and seven CDS related to Cas proteins (*Cas 1*, *Cas 2*, *Cas 3*, *Cas 5*, *Cas 6*, *Cas 7*, and *Cse2*) (Table S2).

Regarding insertion sequences (IS), 58 complete ORFs were annotated as putative transposases identified on CIDCA 133 genome. These ORFs were distributed in ten known IS families: IS607 (34.44%), IS66 (18.96%), IS30 (15.51%), and IS110 (10.34%) were among the most numerous elements in the genome. There were also copies of IS256 (5.17%), IS200_IS605 (5.17%), IS4 (3.44%), ISL3 (3.44%), IS5 (1.72%), and IS630 (1.72%) (Table S3). No insertion sequence was also detected on CIDCA 133 plasmid. The insertion site of prophages regions, CRISPR-Cas system, and distribution of transposases along the CIDCA 133 chromosome are illustrated in Fig. 1.

Antibiotic Resistance-Related Genes of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

The genetic elements involved with antimicrobial susceptibility of CIDCA 133 were searching through the CARD database (coverage > 70%; similarity > 30%). This analysis identified 70 putative antibiotic resistance genes in CIDCA

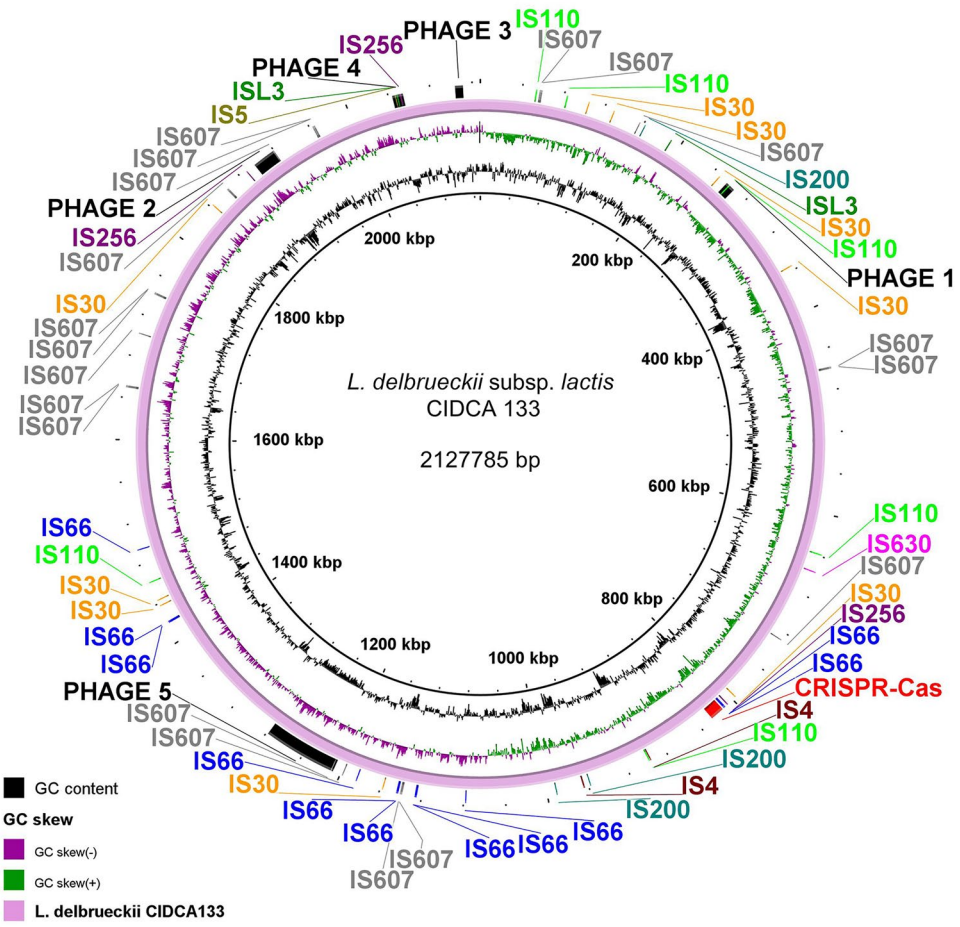


Fig. 1 Schematic representation of insertion site of prophages regions (black arrows), CRISPR-Cas system (red arrow), and distribution of transposases along of the *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 chromosome: IS66 (blue arrow), IS607 (gray arrow), IS110 (lime arrow), IS200 (teal arrow), IS30 (orange arrow), IS4 (maroon arrow), IS256 (purple arrow), IS630 (fuchsia arrow), ISL3 (green arrow), and IS5 (olive arrow)

133 genome. They were drugged transmembrane transport and transcriptional regulators genes, the most common genetic factors associated with fluoroquinolone, macrolides, glycopeptides, tetracyclines, and aminoglycosides antibiotics resistance (Table S4). No antimicrobial resistance genes were located within the prophage regions and CIDCA 133 plasmid sequence.

Virulence Factors-Encoding Genes of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

Based on the VFDB database, six CDS of the CIDCA 133 genome were described as virulence factors*: rfbA*, *groeL*, *ef-Tu*, *galU*, *lisR*, and *uppS* (Table 2; Table S5). However, according to PathogenFinder, CIDCA 133 strain was predicted as a non-human pathogen (probability score = 0.201; matched pathogenic families = 0). Additionally, functional analysis through KEGGMapper/BlastKOALA showed that, in CIDCA 133, these putative virulence-associated factors are involved in the cellular process as protein refolding (*groeL*), environmental adaptation (*ef-Tu*), secondary metabolites biosynthesis (*uppS*, *rfbA*), transcriptional regulation (*lisR*), and carbohydrates metabolism (*galU*).

Harmful Metabolites-Encoding Genes of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133

It was possible to identify genetic elements related to putative ornithine decarboxylase, carbamate kinase, ornithine carbamoyltransferase, and arginine deiminase proteins involved in the metabolic pathway of biogenic amine putrescine production. Furthermore, D-lactate dehydrogenase and genes encoding toxins hemolysin and mucin-degrading enzymes (such as glycoside hydrolases, galactosidases, and glucosidases) were also found in the CIDCA 133 genome (Table 2).

In Vitro Analysis

Antimicrobial Susceptibility Profile of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 Based on antibiogram assay, CIDCA 133 was susceptible to clindamycin (2 µg), tetracycline (30 µg), vancomycin (30 µg), ampicillin

Table 2 Putative virulence factors and harmful metabolites-encoding genes predicted on *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 genome

Locus tag	Gene	Category	Product
Virulence-associated factors			
HR078_09435	*rfbA*		Glucose-1-phosphate thymidylyltransferase
HR078_07785	*groEL*		chaperonin GroEL
HR078_06525	*uppS*		Isoprenyl transferase
HR078_03550	*ef-Tu*		Elongation factor Tu
HR078_07220	*lisR*		Response regulator transcription factor
HR078_02490	*galU*		UTP–Glucose-1-phosphate uridylyltransferase
Undesirable genes			
HR078_02525	*tlyC*		Putative hemolysin
HR078_05915	*hlyIII*		Hemolysin III
HR078_00830	*mapA*		Glycoside hydrolase family 65 protein
HR078_01275	*bglA*		Glycoside hydrolase family 1 protein
HR078_07920	*nagZ*		Glycoside hydrolase
HR078_02035	–		Glycoside hydrolase family 73 protein
HR078_00820	*malZ*		alpha-Galactosidases
HR078_05465	*lacZ*		Beta-Galactosidases
HR078_05730	*bglA*		β-Glucosidases
HR078_04625	*ldhA*		D-Lactate dehydrogenase
HR078_02445	*odc1*		Putative ornithine decarboxylase
HR078_08920	*arcC*		Carbamate kinase
HR078_08925	*argF*		Ornithine carbamoyltransferase
HR078_08915	*arcA*		Arginine deiminase

Table 3 Antimicrobial susceptibility profile of *L. delbrueckii* subsp. *lactis* CIDCA 133

Antimicrobial	Concentration (μg)	Inhibition zone diameter (mm)	Profile
Ampicillin	10	40	S
Chloramphenicol	30	30.5	S
Clindamycin	2	30	S
Erythromycin	15	30.5	S
Gentamicin	10	0	R
Streptomycin	10	0	R
Tetracycline	30	40	S
Vancomycin	30	30	S

R resistant, *S* susceptible

(10 μg), chloramphenicol (30 μg), and erythromycin (15 μg) antibiotics. On the other hand, its resistant profile was observed to gentamicin (10 μg) and streptomycin (10 μg) (Table 3).

Hemolytic and Mucin Degradation Activity of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 No β-hemolytic (total hemolysis) (Fig. 2a) and mucin degradation activity (Fig. 2b) were exhibited by CIDCA 133.

In Vivo Analysis

Effect of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 on Mice Body Weight, Feed Consumption, and Organs Index No abnormal clinical observations and mortality were observed in treated mice throughout the 13 days of experimental period. Also, no significant difference was observed in body weight (1.48 ± 0.21 g; $p = 0.32$) (Fig. 3a) and food intake (4.21 ± 0.56 g/day/animal; $p = 0.32$) (Fig. 3b) after CIDCA 133 consumption when compared with control group (body weight 1.64 ± 0.25 g; food intake 4.03 ± 0.34 g/day/animal). Furthermore, no significant difference in relative organ index among the groups was found, including spleen (NC 0.53 ± 0.07; CIDCA 133: 0.52 ± 0.05; $p = 0.83$) and thymus (NC 0.22 ± 0.06; CIDCA 133: 0.21 ± 0.05; $p = 0.66$) (Fig. 3c).

Gene Expression of Oxidative Stress and Epithelial Barrier Markers After Administration of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 After oral CIDCA 133 administration, it was possible to observe a downregulation in the mRNA expression of *nos2* (0.59 ± 0.11) and an upregulation for *occludin* (1.90 ± 0.32) gene expression when compared to those exhibited in the control group: *nos2* (1.00 ± 0.21; $p = 0.0078$) and *occludin* (1.00 ± 0.30; $p = 0.0036$) (Fig. 4 a and b). No differences in *cldn2* (1.14 ± 0.18) and *zonulin* (0.97 ± 0.17) gene expression were observed between mice that consumed CIDCA 133 and control group: *cldn2* (1.00 ± 0.21; $p = 0.21$) and *zonulin* (1.00 ± 0.17; $p = 0.85$) (Fig. 4c, d).

Inflammatory Cell Infiltration and Histopathological Damage Analysis After *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 Consumption After CIDCA 133 consumption, no inflammatory infiltrate by neutrophils (MPO activity) (0.15 ± 0.05 U/mg) and eosinophils (EPO activity) (0.32 ± 0.08 U/mg) was observed in ileum of treated mice when compared to those exhibited in the control group: MPO (0.12 ± 0.06 U/mg; $p = 0.51$) and EPO (0.43 ± 0.04 U/mg; $p = 0.10$) (Fig. 5a). Moreover, no villus shortening (Fig. 5b), edema, ulceration, hyperemia, and mucosa architecture alteration (Fig. 5c) was observed after the strain consumption ($p > 0.05$), as shown in Fig. 5d. Thus, no his-

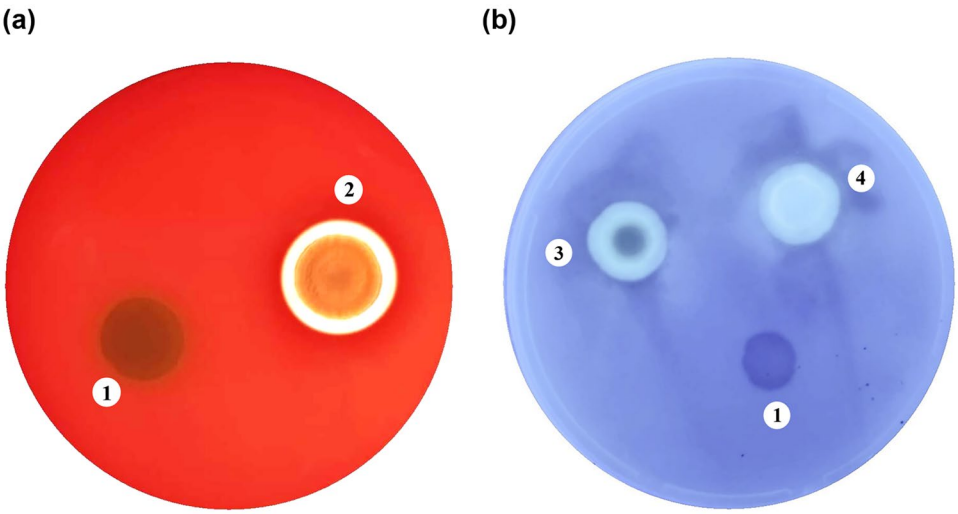


Fig. 2 Hemolysis (**a**) and mucin degradation activity (**b**) of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 (1). Positive control: *Staphylococcus aureus* O46 (2), *Salmonella enterica* serovar Typhi (3), *Salmonella enterica* serovar Typhimurium (4)

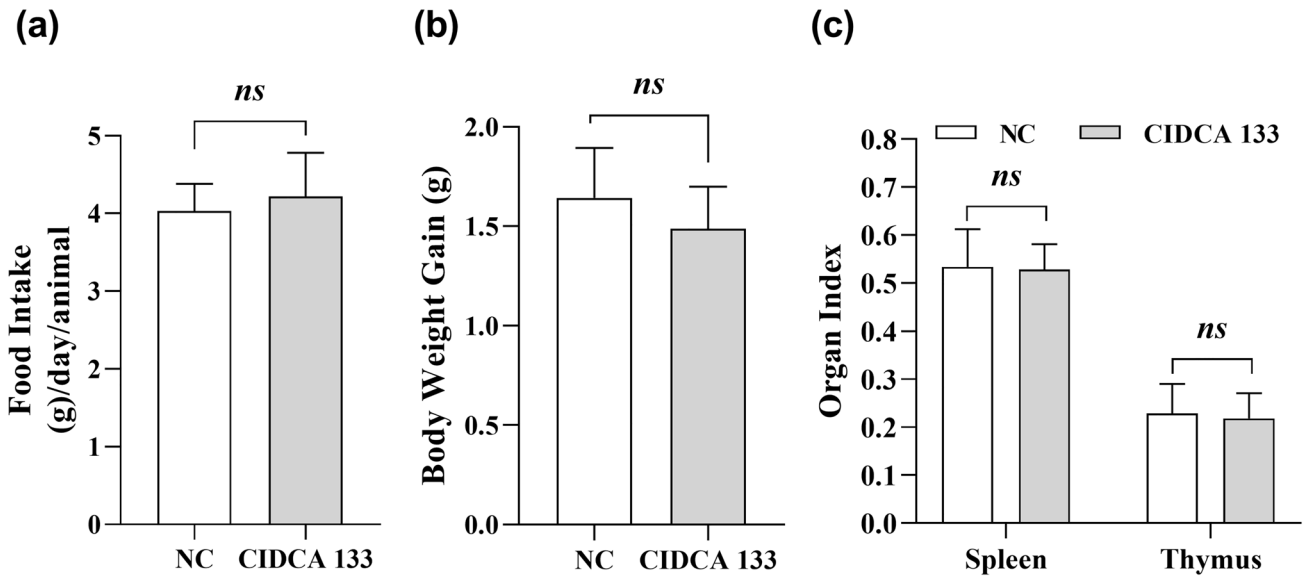


Fig. 3 Food intake (**a**), body weight (**b**), and spleen and thymus index (**c**) after *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 consumption for 13 consecutive days. *ns* indicates no statistical difference by Student's *t*-test ($p < 0.05$)

topathological abnormalities were related to CIDCA 133 consumption, and this strain was likely to be safe for consumption.

Discussion

Newly candidates probiotic strains after correct typing must be studied for their specific health-promoting effects and safety for human and veterinary consumption [39, 53] through both phenotypic analysis (in vitro tests and in vivo toxicity studies) and genomic approach [21, 25, 54]. The correct strain typing, probiotic characteristics, and the molecular basis attributed to beneficial effects of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 consumption was previously reported [3, 11]. Thus, in this study, this new potential probiotic strain was analyzed regarding its safety.

The main concern regarding probiotics safety is unequivocal identification, virulence and antimicrobial resistance genes, and mobile elements that could transfer these last features to commensal and pathogenic bacteria from the gastrointestinal tract [16, 55, 56].

CIDCA 133 harbors a plasmid [11]. Concerning mobile elements, transposases and prophage regions are inserted on its chromosome. However, CIDCA 133 developed a mechanism to cleaving and preventing the entry and integration of exogenous genetic material through their CRISPR-Cas and R/M restriction system, whose functionality may be associated with other incomplete phage regions detected on the strain's chromosome. Others *Lactobacillus delbrueckii* strains also have these anti-phage mechanism, such as *L. delbrueckii* CNRZ 326 [57], *L. delbrueckii* ACA-DC [58], and *L. delbrueckii* TUA4408 [59].

Regarding antibiotic resistance, multidrug-resistant strains can represent a severe risk to patient's treatment with bacterial infections [60]. Therefore, according to EFSA guidelines, new *Lactobacillus* strains candidates for probiotic applications and human consumption should be evaluated for their resistance to clindamycin, chloramphenicol, ampicillin, kanamycin, gentamicin, streptomycin, erythromycin, tetracycline, and vancomycin antibiotics [61]. Based on the antibiogram test, CIDCA 133 was susceptible to all the above antibiotics, except aminoglycosides gentamicin and streptomycin. This phenotypic profile can be associated with predicted drug transmembrane transporters (e.g., ABC transporter ATP-binding protein) genes identified on this strain genome.

Antimicrobial resistance genes have already been identified in many *Lactobacillus* [23, 62, 63], and resistance to aminoglycosides (streptomycin and gentamicin) has been described as one of the best-characterized intrinsic resistance mechanisms in *Lactobacillus* species, such as *L. delbrueckii*, *Lactiplantibacillus plantarum*, *Lactiplantibacillus pentosus*, and *Levilactobacillus brevis*, among others [63–65].

Resistance to aminoglycosides may occur based on several mechanisms, being the most common in *Lactobacillus* associated with aminoglycoside-modifying enzymes [63, 66]. In CIDCA 133 genome, no genes related to these enzymes were predicted, but this strain harboring the ABC transporter genes related to this antibiotic class resistance, which has also been previously identified in *L. delbrueckii*

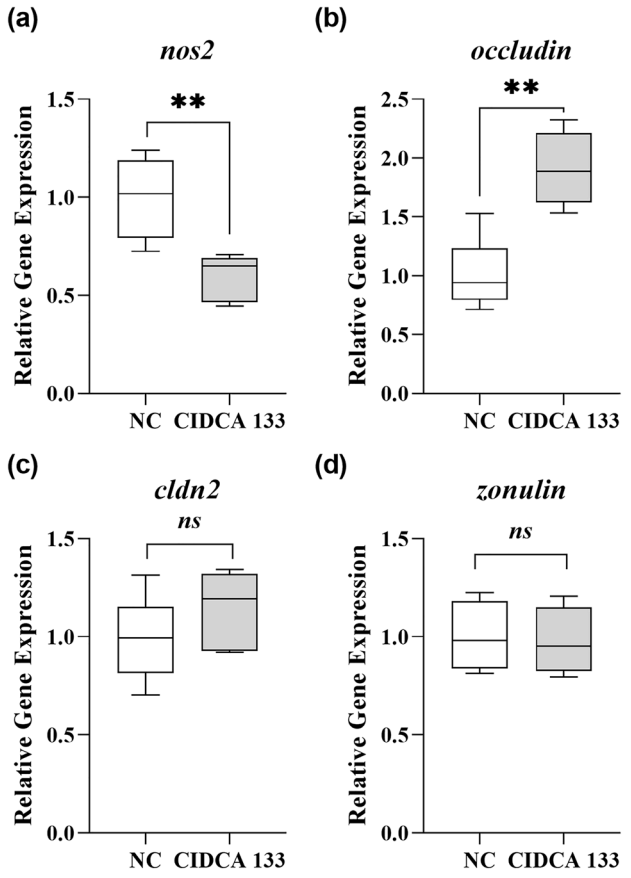


Fig. 4 Relative gene expression of oxidative stress marker (**a**) and tight junction's proteins occludin, claudin 2, and zonulin (**b**–**d**) in the ileum section of animals that received oral administration of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 for 13 consecutive days. Asterisk (*) indicates statistical difference by Student's *t*-test ($p < 0.05$)

subsp. *bulgaricus* LDB-C1 [67], and *Lactiplantibacillus plantarum* BCC9546 [39].

One of the main aspects of antibiotic resistance in probiotics is to separate intrinsic resistance from acquired resistance. According to the EFSA guidelines [68], and supported by other approaches based on whole-genome sequence [39, 56], antibiotic resistance as such is not a safety issue. It only becomes a threat when resistance is transferable. No CIDCA 133 antimicrobial resistance genes were associated with mobile elements (acquired resistance mechanism). Thus, the antimicrobial resistance genes present only on CDICA 133 chromosome do not represent an immediate clinical risk to the organism once this strain does not show properties to disseminate them, suggesting its safety for probiotics applications and consumption.

Pathogenicity is another essential property to be evaluated during the new probiotics screening process. CIDCA 133 chromosome harbors the toxins hemolysin and glucosidases genes related to hemolysis and mucin degradation. The hemolysin-encoding genes have previously been identified in other *Lactobacillus* strains, such as *L. crispatus* [69], *L. helveticus* [23], and *Lacticaseibacillus paracasei* [70], as well as including strains with approved safe status and commercially available, as *Lactiplantibacillus plantarum* BCC9546 [39].

At the phenotypic level, CIDCA 133 no exhibited hemolytic activity. Similar results were observed by Liu et al. [29] and Toropov et al. [71], who reported that both *Blautia producta* DSM 2950 and *Lactobacillus helveticus* D75 have a gene related to hemolysin. However, no presented hemolytic activity, evidencing that the probiotic strains harboring hemolysin genes should not be of safety concern. However, it should also be highlighted that these studies show a discrepancy between the phenotype and the genotype. It suggests that, despite being present in the genome of probiotic strains, it is possible that mucin degradation or hemolysis activity-associated genes were not sufficiently expressed or were modified after transcription (gene regulation).

Despite mucin degradation being an infection mechanism used by pathogenic bacteria, its degradation by commensal bacteria, as carbon source, stimulates goblet cells to increase mucus production. In addition, the mucin-degrading enzymes can be playing a vital role in the synthesis of oligosaccharides that may be selectively used as prebiotics by probiotics bacteria, a mechanism used by *Lactiplantibacillus pentosus* [72] and *Akkermansia muciniphila* [73].

The CIDCA 133 property did not cause blood hemolysis, and mucin degradation confirms the PathogenFinder results, showing that CIDCA 133 has no potential to act as a human pathogen. It is important to emphasize that many proteins that have already been characterized as associated with virulence in pathogenic bacteria were described in probiotic microorganisms. These processes occur when probiotic bacteria are consumed or inhabit the same ecological niche; they suffer the same types of stresses as pathogenic bacteria, and thus, often use the same strategies to colonize, survive, and behave [74, 75]. Therefore, the virulence-associated genes identified on CIDCA 133 genome are only niche/colonization factors used for its adaptation.

The evaluation of probiotics strain's capacity to produce biogenic amines and D-lactate is also required during the safety screen, once these compounds may cause intoxication and acidosis, respectively, when consumed in high concentrations in fermented foods [76, 77].

In CIDCA, 133 were identified genes associated with the production of the putrescine biogenic amine. These genes have previously been identified in other *Lactobacillus* strains, such as *Limosilactobacillus reuteri* [38], *Furfurilactobacillus rossiae* [78], *L. helveticus* [25], and *Latilactobacillus curvatus* [40], including strains present on commercially available products as Pecorino di Farindola cheese, an Italian cheese [79]. According to EFSA guidelines, the presently available

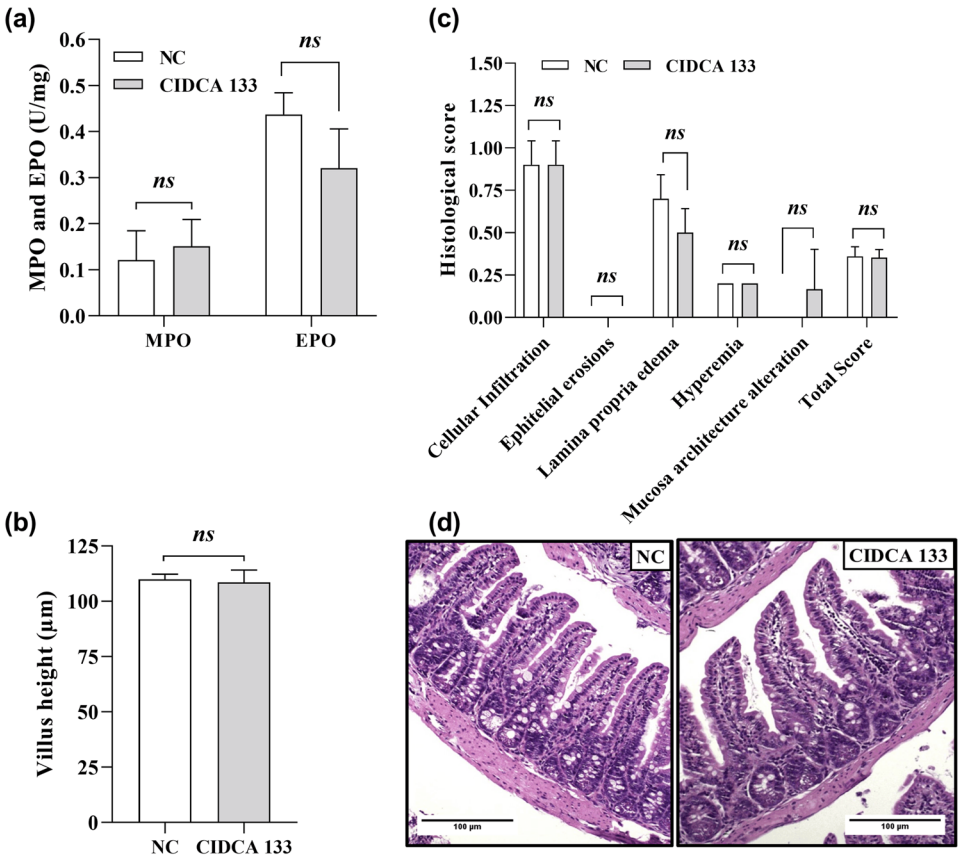


Fig. 5 Effects of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 consumption on inflammatory cell infiltration (**a**), morphometric (**b**), and histopathological (**c**-**d**) analysis. *ns* indicates no statistical difference by Student's *t*-test ($p < 0.05$)

putrescine information is insufficient to identify concentrations that directly cause acute adverse health effects and/or potentiate other biogenic amines' toxic effects [80]). Additionally, although toxic effects are reported to biogenic amine consumption, putrescine accumulation can represent a cellular defense mechanism to withstand acid stress [81], a mechanism previously identified in *Furfurilactobacillus rossiae* [78] and *Levilactobacillus brevis* [82]. The acid tolerance of CIDCA 133 was previously reported by De Jesus et al. [11], which in addition to other genes, attributed this effect to ornithine decarboxylase, identified in the strain's genome.

Despite the D-lactate not being highly toxic, its accumulation in blood might cause health problems. In CIDCA, 133 genomes were identified with the D-lactate dehydrogenase gene. Clinical studies have reported therapeutic efficacy with D-lactate producing *Lactobacillus* strains without making D-lactic acidosis [83–85]. D-Lactic acidosis is a rare metabolic condition in humans, and its occurrence has only been reported in cases with short bowel syndrome [86, 87]. Thus, these findings support the CIDCA 133 safe.

Safety evaluation in vivo can provide more practical information about adverse effects to an organism by probiotic consumption, as previously reported in some studies [25, 41, 88]. Thus, in this study, administration of CIDCA 133 (5×10^7 CFU/mL) for 13 consecutive days was used to evaluate the strain effects on general health mice.

Neither adverse effects on internal organ functions nor clinical alteration, inflammatory, and histopathological damage in healthy mice were observed after CIDCA 133 consumption. These findings corroborating the previous study performed by De Jesus et al. [11], which demonstrated that CIDCA 133 presents beneficial and no adverse effects to the host due to their immunostimulatory properties capable of upregulating anti-inflammatory cytokines (e.g., *Il10* and *Tgfb1*) gene expression through inhibition of *Nfkb1 (p105)* gene expression, maintaining the host's homeostasis [11].

Moreover, it was also observed that CIDCA 133 consumption reduces oxidative stress marker (*nos2*) and increases or not alters epithelial barrier markers (*occludin*, *claudin 2*, and *zonulin*) gene expression. Additionally, through a bacterial translocation method using radiolabeled *E. coli* (^{99m}Tc-*E. coli*) [89], it was observed that mice presenting only physiological levels of radiolabeled bacteria after CIDCA 133 fermented milk consumption (unpublished data; Fig. S1). These results reinforce this strain's epithelial barrier function. Furthermore, they can be associated with the findings previously reported by De Jesus et al. [3], which demonstrated that no intestinal permeability and degeneration of mucus-producing goblet cells increasing were

observed after CIDCA 133 consumption. Thus, all these effects indicate that CIDCA 133 is safe for consumption. However, higher dose, longer chronic, and acute oral toxicity studies must also be performed.

Conclusions

In conclusion, this study identified the safety-related genes of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133. This strain presented antimicrobial resistance to aminoglycosides streptomycin and gentamicin antibiotics. However, this strain did not show properties to disseminate these antibiotic resistance-associated genes. Furthermore, the genes related to virulence and harmful metabolites were already identified in other potential probiotic bacteria, including those with approved safe status and commercially available. No adverse effects also were observed regarding clinical and histopathological analysis after the strain consumption. Thus, these results suggest that *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 has a level of safety for use in future probiotic applications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12602-021-09826-z.

Acknowledgements We would like to acknowledge the Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) and Pablo F. Pérez of Universidad Nacional de La Plata-Argentina for bacteria strain supply.

Author Contribution Conceptualization: Luís Cláudio Lima de Jesus and Flávia Figueira Aburjaile. Methodology: Luís Cláudio Lima de Jesus, Thiago de Jesus Souza, Fernanda Alvarenga Lima Barroso, Nina Dias Coelho-Rocha, Rodrigo Profeta, Ênio Ferreira, Bertram Brenig. Formal analysis and investigation: Luís Cláudio Lima de Jesus. Writing—original draft preparation: Luís Cláudio Lima de Jesus. Writing—review and editing: Flávia Figueira Aburjaile, Thiago de Jesus Souza, Mariana Martins Drumond, Pamela Mancha-Agresti, and Vasco Azevedo. Supervision: Vasco Azevedo. Funding acquisition: Vasco Azevedo. All authors read and approved the final manuscript.

Funding This work was financially supported by the Rede de Ciências Ômicas (RECOM), Pró-Reitoria de Pesquisa—Universidade Federal de Minas Gerais, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for their financial support and fellowships.

Availability of Data and Material CIDCA 133 complete genome and plasmid sequences used for these analyses are available from the GenBank, NCBI.

Declarations

Ethics Approval The study was conducted according to the guidelines of the Brazilian College of Animal Experimentation (COBEA) and approved by the Local Animal Experimental Ethics Committee of Universidade Federal de Minas Gerais (CEUA-UFMG) (Protocol no. 112/2020; 08 August 2020).

Conflict of Interest The authors declare no conflict of interest.

Consent for Publication All authors consent to the publication of this article.

References

1. Heller KJ (2001) Probiotic bacteria in fermented foods: product characteristics and starter organisms. Am J Clin Nutr 73:374s–379s. https://doi.org/10.1093/ajcn/73.2.374s
2. Song Y, Sun Z, Guo C et al (2016) Genetic diversity and population structure of *Lactobacillus delbrueckii* subspecies *bulgaricus* isolated from naturally fermented dairy foods. Sci Rep 6:22704. https://doi.org/10.1038/srep22704
3. De Jesus LCL, Drumond MM, de Carvalho A et al (2019) Protective effect of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 in a model of 5-fluorouracil-induced intestinal mucositis. J Funct Foods 53:197–207. https://doi.org/10.1016/j.jff.2018.12.027
4. Rocha CS, Gomes-Santos AC, Moreira TG et al (2014) Local and systemic immune mechanisms underlying the anti-colitis effects of the dairy bacterium *Lactobacillus delbrueckii*. PLoS ONE 9:e85923. https://doi.org/10.1371/journal.pone.0085923
5. Hallajzadeh J, Eslami RD, Tanomand A (2021) Effect of *Lactobacillus delbrueckii* subsp. *lactis* PTCC1057 on serum glucose, fetuin-a, and sestrin 3 levels in streptozotocin-induced diabetic mice. Probiotics Antimicrob Proteins 13:383–389. https://doi.org/10.1007/s12602-020-09693-0
6. Evivie SE, Abdelazez A, Li B et al (2020) *Lactobacillus delbrueckii* subsp. *bulgaricus* KLDS 1.0207 exerts antimicrobial and cytotoxic effects in vitro and improves blood biochemical parameters in vivo against notable foodborne pathogens. Front Microbiol 11:583070. https://doi.org/10.3389/fmicb.2020.583070
7. Zheng J, Wittouck S, Salvetti E et al (2020) A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* beijerinck 1901, and union of lactobacillaceae and leuconostocaceae. Int J Syst Evol Microbiol 70:2782–2858. https://doi.org/10.1099/ijsem.0.004107
8. Kociubinski GL, Pérez PF, Añón MC, De Antoni GL (1996) A method of screening for highly inhibitory lactic acid bacteria. J Food Prot 59:739–745. https://doi.org/10.4315/0362-028X-59.7.739
9. Hugo AA, Kakisu E, De Antoni GL, Pérez PF (2008) *Lactobacilli* antagonize biological effects of enterohaemorrhagic *Escherichia coli* in vitro. Lett Appl Microbiol 46:613–619. https://doi.org/10.1111/j.1472-765X.2008.02363.x
10. Kociubinski G, Pérez P, De Antoni G (1999) Screening of bile resistance and bile precipitation in lactic acid bacteria and bifidobacteria. J Food Prot 62:905–912. https://doi.org/10.4315/0362-028X-62.8.905
11. De Jesus LCL, Drumond MM, Aburjaile FF et al (2021) Probiogenomics of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: in silico, in vitro, and in vivo approaches. Microorganisms 9:829. https://doi.org/10.3390/microorganisms9040829
12. Hugo AA, De Antoni GL, Pérez PF (2010) *Lactobacillus delbrueckii* subsp. *lactis* (strain CIDCA 133) resists the antimicrobial activity triggered by molecules derived from enterocyte-like caco-2 cells. Lett Appl Microbiol 50:335–340. https://doi.org/10.1111/j.1472-765X.2010.02796.x

13. Hugo AA, Tymczyszyn EE, Gómez-Zavaglia A, Pérez PF (2012) Effect of human defensins on lactobacilli and liposomes. J Appl Microbiol 113:1491–1497. https://doi.org/10.1111/j.1365-2672.2012.05433.x
14. Rolny IS, Tiscornia I, Racedo SM et al (2016) *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 modulates response of human epithelial and dendritic cells infected with *Bacillus cereus*. Benef Microbes 7:749–760. https://doi.org/10.3920/BM2015.0191
15. Hugo AA, Rolny IS, Romanin D, Pérez PF (2017) *Lactobacillus delbrueckii* subsp. *lactis* (strain CIDCA 133) stimulates murine macrophages infected with *Citrobacter rodentium*. World J Microbiol Biotechnol 33:48. https://doi.org/10.1007/s11274-017-2219-4
16. Sanders ME, Akkermans LMA, Haller D et al (2010) Safety assessment of probiotics for human use. Gut Microbes 1:164–185. https://doi.org/10.4161/gmic.1.3.12127
17. Kunz AN, Noel JM, Fairchok MP (2004) Two cases of *Lactobacillus* bacteremia during probiotic treatment of short gut syndrome. J Pediatr Gastroenterol Nutr 38:457–458. https://doi.org/10.1097/00005176-200404000-00017
18. Darbro BW, Petroelje BK, Doern GV (2009) *Lactobacillus delbrueckii* as the cause of urinary tract infection. J Clin Microbiol 47:275–277. https://doi.org/10.1128/JCM.01630-08
19. Yelin I, Flett KB, Merakou C et al (2019) Genomic and epidemiological evidence of bacterial transmission from probiotic capsule to blood in icu patients. Nat Med 25:1728–1732. https://doi.org/10.1038/s41591-019-0626-9
20. Gore S, Tucker K (2020) Bloodstream infections with typical probiotic organisms. Infect Control Hosp Epidemiol 41:s22–s22. https://doi.org/10.1017/ice.2020.498
21. Casarotti SN, Carneiro BM, Todorov SD et al (2017) In vitro assessment of safety and probiotic potential characteristics of *Lactobacillus* strains isolated from water buffalo mozzarella cheese. Ann Microbiol 67:289–301. https://doi.org/10.1007/s13213-017-1258-2
22. Lazarenko LM, Babenko LP, Gichka SG et al (2021) Assessment of the safety of *Lactobacillus casei* IMV B-7280 probiotic strain on a mouse model. Probiotics Antimicrob Proteins. https://doi.org/10.1007/s12602-021-09789-1
23. Senan S, Prajapati JB, Joshi CG (2015) Feasibility of genome-wide screening for biosafety assessment of probiotics: a case study of *Lactobacillus helveticus* MTCC 5463. Probiotics Antimicrob Proteins 7:249–258. https://doi.org/10.1007/s12602-015-9199-1
24. Pradhan D, Mallappa RH, Grover S (2020) Comprehensive approaches for assessing the safety of probiotic bacteria. Food Control 108:106872. https://doi.org/10.1016/j.foodcont.2019.106872
25. Li B, Jin D, Evivie SE et al (2017) Safety assessment of *Lactobacillus helveticus* KLDS1.8701 based on whole genome sequencing and oral toxicity studies. Toxins (Basel) 9:301. https://doi.org/10.3390/toxins9100301
26. Li B, Zhan M, Evivie SE et al (2018) Evaluating the safety of potential probiotic *Enterococcus durans* KLDS6.0930 using whole genome sequencing and oral toxicity study. Front Microbiol 9:1943. https://doi.org/10.3389/fmicb.2018.01943
27. Terai T, Kato K, Ishikawa E et al (2020) Safety assessment of the candidate oral probiotic *Lactobacillus crispatus* YIT 12319: analysis of antibiotic resistance and virulence-associated genes. Food Chem Toxicol 140:111278. https://doi.org/10.1016/j.fct.2020.111278
28. Arellano K, Vazquez J, Park H et al (2020) Safety evaluation and whole-genome annotation of *Lactobacillus plantarum* strains from different sources with special focus on isolates from green tea. Probiotics Antimicrob Proteins 12:1057–1070. https://doi.org/10.1007/s12602-019-09620-y
29. Liu X, Guo W, Cui S et al (2021) A comprehensive assessment of the safety of *Blautia producta* DSM 2950. Microorganisms 9:908. https://doi.org/10.3390/microorganisms9050908
30. Lee BS, Ban O-H, Bang WY et al (2021) Safety assessment of *Lactobacillus reuteri* IDCC 3701 based on phenotypic and genomic analysis. Ann Microbiol 71:10. https://doi.org/10.1186/s13213-021-01622-y
31. Varani AM, Siguier P, Gourbeyre E et al (2011) ISsaga is an ensemble of web-based methods for high throughput identification and semi-automatic annotation of insertion sequences in prokaryotic genomes. Genome Biol 12:R30. https://doi.org/10.1186/gb-2011-12-3-r30
32. Arndt D, Grant JR, Marcu A et al (2016) Phaster: a better, faster version of the phast phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387
33. Couvin D, Bernheim A, Toffano-Nioche C et al (2018) Crisprcasfinder, an update of crisrfinder, includes a portable version, enhanced performance and integrates search for cas proteins. Nucleic Acids Res 46:W246–W251. https://doi.org/10.1093/nar/gky425
34. Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA (2011) Blast ring image generator (brig): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402
35. Alcock BP, Raphenya AR, Lau TTY et al (2019) Card 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res. https://doi.org/10.1093/nar/gkz935
36. Cosentino S, Larsen MV, Møller Aarestrup F, Lund O (2013) Pathogenfinder - distinguishing friend from foe using bacterial whole genome sequence data. PLoS ONE 8:e77302. https://doi.org/10.1371/journal.pone.0077302
37. Liu B, Zheng D, Jin Q et al (2019) Vfdb 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res 47:D687–D692. https://doi.org/10.1093/nar/gky1080
38. Alayande KA, Aiyegoro OA, Nengwekhulu TM et al (2020) Integrated genome-based probiotic relevance and safety evaluation of *Lactobacillus reuteri* PNW1. PLoS ONE 15:e0235873. https://doi.org/10.1371/journal.pone.0235873
39. Chokesajjawatee N, Santiyanont P, Chantarasakha K et al (2020) Safety assessment of a nham starter culture *Lactobacillus plantarum* BCC9546 via whole-genome analysis. Sci Rep 10:10241. https://doi.org/10.1038/s41598-020-66857-2
40. Li L, Wen X, Wen Z et al (2018) Evaluation of the biogenic amines formation and degradation abilities of *Lactobacillus curvatus* from chinese bacon. Front Microbiol 9:1015. https://doi.org/10.3389/fmicb.2018.01015
41. Morovic W, Roper JM, Smith AB et al (2017) Safety evaluation of howaru® restore (*Lactobacillus acidophilus* NCFM, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium animalis* subsp. *lactis* Bl-04 and *B. lactis* Bi-07) for antibiotic resistance, genomic risk factors, and acute toxicity. Food Chem Toxicol 110:316–324. https://doi.org/10.1016/j.fct.2017.10.037
42. Kanehisa M, Sato Y, Morishima K (2016) Blastkoala and ghostkoala: kegg tools for functional characterization of genome and metagenome sequences. J Mol Biol 428:726–731. https://doi.org/10.1016/j.jmb.2015.11.006
43. Papadimitriou K, Zoumpopoulou G, Foligné B et al (2015) Discovering probiotic microorganisms: in vitro, in vivo, genetic and omics approaches. Front Microbiol 6:58. https://doi.org/10.3389/fmicb.2015.00058
44. Derrien M, van Passel MWJ, van de Bovenkamp JHB et al (2010) Mucin-bacterial interactions in the human oral cavity and digestive tract. Gut Microbes 1:254–268. https://doi.org/10.4161/gmic.1.4.12778

45. Tailford LE, Crost EH, Kavanaugh D, Juge N (2015) Mucin glycan foraging in the human gut microbiome. Front Genet 6:81. https://doi.org/10.3389/fgene.2015.00081
46. Clinical and Laboratory Standards Institute (CLSI) (2018) Performance standards for antimicrobial susceptibility testing. 28th ed. CSLI supplement M100. Wayne, PA. https://file.qums.ac.ir/repository/mmrc/CLSI-2018-M100-S28.pdf. Accessed 10 March 2021
47. Zhou JS, Gopal PK, Gill HS (2001) Potential probiotic lactic acid bacteria *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019) do not degrade gastric mucin in vitro. Int J Food Microbiol 63:81–90. https://doi.org/10.1016/S0168-1605(00)00398-6
48. Xu Y, Tian Y, Cao Y et al (2019) Probiotic properties of *Lactobacillus paracasei* subsp. *paracasei* L1 and its growth performance-promotion in chicken by improving the intestinal microflora. Front Physiol 10:937. https://doi.org/10.3389/fphys.2019.00937
49. Ren D, Wang D, Liu H et al (2019) Two strains of probiotic *Lactobacillus* enhance immune response and promote naive T cell polarization to Th1. Food Agric Immunol 30:281–295. https://doi.org/10.1080/09540105.2019.1579785
50. Alvim LB, Sandes SHC, Silva BC et al (2016) *Weissella parameseenteroides* WpK4 reduces gene expression of intestinal cytokines, and hepatic and splenic injuries in a murine model of typhoid fever. Benef Microbes 7:61–73. https://doi.org/10.3920/BM2015.0093
51. Volynets V, Rings A, Bárdos G et al (2016) Intestinal barrier analysis by assessment of mucins, tight junctions, and α-defensins in healthy c57bl/6j and balb/cj mice. Tissue Barriers 4:e1208468. https://doi.org/10.1080/21688370.2016.1208468
52. Giulietti A, Overbergh L, Valckx D et al (2001) An overview of real-time quantitative pcr: applications to quantify cytokine gene expression. Methods 25:386–401. https://doi.org/10.1006/meth.2001.1261
53. Baccouri O, Boukerb AM, Farhat L Ben et al (2019) Probiotic potential and safety evaluation of *Enterococcus faecalis* OB14 and OB15, isolated from traditional tunisian testouri cheese and rigouta, using physiological and genomic analysis. Front Microbiol 10:881. https://doi.org/10.3389/fmicb.2019.00881
54. Zhang Z-Y, Liu C, Zhu Y-Z et al (2012) Safety assessment of *Lactobacillus plantarum* JDM1 based on the complete genome. Int J Food Microbiol 153:166–170. https://doi.org/10.1016/j.ijfoodmicro.2011.11.003
55. Vankerckhoven V, Huys G, Vancanneyt M et al (2008) Genotypic diversity, antimicrobial resistance, and virulence factors of human isolates and probiotic cultures constituting two intraspecific groups of *Enterococcus faecium* isolates. Appl Environ Microbiol 74:4247–4255. https://doi.org/10.1128/AEM.02474-07
56. Pariza MW, Gillies KO, Kraak-Ripple SF et al (2015) Determining the safety of microbial cultures for consumption by humans and animals. Regul Toxicol Pharmacol 73:164–171. https://doi.org/10.1016/j.yrtph.2015.07.003
57. Auad L, Peril MAA, de Holgado AAP, R, Raya RR, (1998) Evidence of a restriction/modification system in *Lactobacillus delbrueckii* subsp. *lactis* CNRZ326. Curr Microbiol 36:271–273. https://doi.org/10.1007/s002849900308
58. Alexandraki V, Kazou M, Pot B et al (2019) Whole-genome sequence data and analysis of *Lactobacillus delbrueckii* subsp. *lactis* ACA-DC 178 isolated from greek kasseri cheese. Data Br 25:104282. https://doi.org/10.1016/j.dib.2019.104282
59. Kanmani P, Albarracin L, Kobayashi H et al (2018) Genomic characterization of *Lactobacillus delbrueckii* TUA4408L and evaluation of the antiviral activities of its extracellular polysaccharides in porcine intestinal epithelial cells. Front Immunol 9:2178. https://doi.org/10.3389/fimmu.2018.02178
60. Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. P T 40:277–283
61. EFSA (2012) Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA J 10:. https://doi.org/10.2903/j.efsa.2012.2740
62. Liu C, Zhang Z-Y, Dong K et al (2009) Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. Biomed Environ Sci 22:401–412. https://doi.org/10.1016/S0895-3988(10)60018-9
63. Campedelli I, Mathur H, Salvetti E et al (2018) Genus-wide assessment of antibiotic resistance in *Lactobacillus* spp. Appl Environ Microbiol 85:e01738-18. https://doi.org/10.1128/AEM.01738-18
64. Muñoz MCC, Benomar N, Lerma LL et al (2014) Antibiotic resistance of *Lactobacillus pentosus* and *Leuconostoc pseudomesenteroides* isolated from naturally-fermented aloreña table olives throughout fermentation process. Int J Food Microbiol 172:110–118. https://doi.org/10.1016/j.ijfoodmicro.2013.11.025
65. Fukao M, Tomita H, Yakabe T et al (2009) Assessment of antibiotic resistance in probiotic strain *Lactobacillus brevis* KB290. J Food Prot 72:1923–1929. https://doi.org/10.4315/0362-028X-72.9.1923
66. Garneau-Tsodikova S, Labby KJ (2016) Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. Medchemcomm 7:11–27. https://doi.org/10.1039/C5MD00344J
67. Guan Y, Cui Y, Qu X, Jing K (2021) Safety and robustness aspects analysis of *Lactobacillus delbrueckii* ssp. *bulgaricus* LDB-C1 based on the genome analysis and biological tests. Arch Microbiol. https://doi.org/10.1007/s00203-021-02383-7
68. EFSA (2008) Technical guidance - update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. EFSA J 6:. https://doi.org/10.2903/j.efsa.2008.732
69. Almeida MO, Carvalho R, Aburjaile FF et al (2021) Characterization of the first vaginal *Lactobacillus crispatus* genomes isolated in brazil. PeerJ 9:e11079. https://doi.org/10.7717/peerj.11079
70. Surachat K, Sangket U, Deachamag P, Chotigeat W (2017) In silico analysis of protein toxin and bacteriocins from *Lactobacillus paracasei* SD1 genome and available online databases. PLoS ONE 12:e0183548. https://doi.org/10.1371/journal.pone.0183548
71. Toropov V, Demyanova E, Shalaeva O et al (2020) Whole-genome sequencing of *Lactobacillus helveticus* D75 and D76 confirms safety and probiotic potential. Microorganisms 8:329. https://doi.org/10.3390/microorganisms8030329
72. Abriouel H, Montoro BP, Casimiro-Soriguer CS et al (2017) Insight into potential probiotic markers predicted in *Lactobacillus pentosus* MP-10 genome sequence. Front Microbiol 8:891. https://doi.org/10.3389/fmicb.2017.00891
73. Shin J, Noh J-R, Chang D-H et al (2019) Elucidation of *Akkermansia muciniphila* probiotic traits driven by mucin depletion. Front Microbiol 10:1137. https://doi.org/10.3389/fmicb.2019.01137
74. Hill C (2012) Virulence or niche factors: what's in a name? J Bacteriol 194:5725–5727. https://doi.org/10.1128/JB.00980-12
75. Wassenaar T, Zschüttig A, Beimfohr C et al (2015) Virulence genes in a probiotic *E. coli* product with a recorded long history of safe use. Eur J Microbiol Immunol 5:81–93. https://doi.org/10.1556/EuJMI-D-14-00039
76. Hove H (1998) Lactate and short chain fatty acid production in the human colon: implications for d-lactic acidosis, short-bowel syndrome, antibiotic-associated diarrhoea, colonic cancer, and inflammatory bowel disease. Dan Med Bull 45:15–33
77. Alvarez MA, Moreno-Arribas MV (2014) The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. Trends Food Sci Technol 39:146–155. https://doi.org/10.1016/j.tifs.2014.07.007
78. Del Rio B, Alvarez-Sieiro P, Redruello B et al (2018) *Lactobacillus rossiae* strain isolated from sourdough produces putrescine from arginine. Sci Rep 8:3989. https://doi.org/10.1038/s41598-018-22309-6

79. Schirone M, Tofalo R, Mazzone G et al (2011) Biogenic amine content and microbiological profile of pecorino di farindola cheese. Food Microbiol 28:128–136. https://doi.org/10.1016/j.fm.2010.09.005
80. EFSA (2011) Scientific opinion on risk based control of biogenic amine formation in fermented foods. EFSA J 9:2393. https://doi.org/10.2903/j.efsa.2011.2393
81. Romano A, Trip H, Lonvaud-Funel A et al (2012) Evidence of two functionally distinct ornithine decarboxylation systems in lactic acid bacteria. Appl Environ Microbiol 78:1953–1961. https://doi.org/10.1128/AEM.07161-11
82. Romano A, Ladero V, Alvarez MA, Lucas PM (2014) Putrescine production via the ornithine decarboxylation pathway improves the acid stress survival of *Lactobacillus brevis* and is part of a horizontally transferred acid resistance locus. Int J Food Microbiol 175:14–19. https://doi.org/10.1016/j.ijfoodmicro.2014.01.009
83. Tomasz B, Zoran S, Jarosław W et al (2014) Long-term use of probiotics *Lactobacillus* and *Bifidobacterium* has a prophylactic effect on the occurrence and severity of pouchitis: a randomized prospective study. Biomed Res Int 2014:1–4. https://doi.org/10.1155/2014/208064
84. Barreto FM, Simão ANC, Morimoto HK et al (2014) Beneficial effects of *Lactobacillus plantarum* on glycemia and homocysteine levels in postmenopausal women with metabolic syndrome. Nutrition 30:939–942. https://doi.org/10.1016/j.nut.2013.12.004
85. Papagaroufalis K, Fotiou A, Egli D et al (2014) A randomized double blind controlled safety trial evaluating d-lactic acid production in healthy infants fed a *Lactobacillus reuteri*-containing Formula. Nutr Metab Insights 7:19–27. https://doi.org/10.4137/NMI.S14113
86. Oh MS, Phelps KR, Traube M et al (1979) D-lactic acidosis in a man with the short-bowel syndrome. N Engl J Med 301:249–252. https://doi.org/10.1056/NEJM197908023010505
87. Kowlgi NG, Chhabra L (2015) D-lactic acidosis: an underrecognized complication of short bowel syndrome. Gastroenterol Res Pract 2015:1–8. https://doi.org/10.1155/2015/476215
88. Shokryazdan P, Faseleh Jahromi M, Liang JB et al (2016) Safety assessment of two new *Lactobacillus* strains as probiotic for human using a rat model. PLoS ONE 11:e0159851. https://doi.org/10.1371/journal.pone.0159851
89. Diniz SO, Resende B, Nunan E et al (1999) 99mtechnetium labelled *Escherichia coli*. Appl Radiat Isot 51:33–36. https://doi.org/10.1016/S0969-8043(98)00185-7

CAPÍTULO V

9. CAPÍTULO V: Genomic Characterization of *Lactobacillus delbrueckii* Strains with Probiotics Properties

Este artigo científico foi publicado na revista *Frontiers in Bioinformatics* (ISSN: 2673-7647) em junho de 2022.

Lactobacillus delbrueckii é uma bactéria láctica altamente utilizada na produção industrial de produtos fermentados, incluindo queijos, iogurtes, leite fermentado, entre outros, e que vêm tendo suas propriedades probióticas caracterizadas. No entanto, comparada à outras espécies pertencentes ao grupo das bactérias lácticas, há poucos estudos demonstrando o potencial probiótico de linhagens de *L. delbrueckii*, principalmente estudos no nível genômico. Com os dados apresentados nos capítulos anteriores desta Tese, CIDCA 133 é a linhagem mais bem caracterizada dentro da espécie *Lactobacillus delbrueckii*. Baseado nessas informações, o objetivo do presente capítulo foi realizar uma caracterização de genes compartilhados por nove linhagens da espécie *L. delbrueckii*, que possuem genoma completo e cujos efeitos probióticos vêm sendo reportados fenotipicamente na literatura, de forma a elucidar os principais fatores genéticos compartilhados por estes microrganismos que possam estar associados as propriedades probióticas. Para realizar este estudo, foi utilizada uma metodologia combinada que inclui vários softwares e banco de dados (BPGA, SPAAN, BAGEL4, BioCyc, KEEG e InterSPPI). Este trabalho foi pioneiro na caracterização genômica de linhagens de *L. delbrueckii* que possuem dados relacionados à sua probiose reportados na literatura. Os dados obtidos neste capítulo mostram um alto grau de variação no conteúdo genômico entre as subespécies *lactis* e *bulgaricus*. Além disso, mostra que estas linhagens compartilham genes essenciais que ratificam os dados da sua probiose reportados por meio de estudos *in vitro* e *in vivo*, principalmente aqueles relacionados aos efeitos anti-inflamatórios, tolerância aos estresses do TGI e atividade antibacteriana. A proteína PrtB, identificada na linhagem CIDCA 133 e compartilhada com as outras oito bactérias probióticas, parece ser a candidata alvo responsável pelas propriedades anti-inflamatórias das linhagens probióticas da espécie *L. delbrueckii*. Os dados do presente trabalho podem ser usados para a caracterização e identificação dos fatores genéticos relacionados à probiose de novas linhagens da espécie *L. delbrueckii*, bem como abre perspectivas para que os candidatos alvos a serem as moléculas anti-inflamatórias das linhagens probióticas de *L. delbrueckii* sejam testados em diferentes modelos de inflamação.

frontiers | Frontiers in Bioinformatics

ORIGINAL RESEARCH
published: 06 June 2022
doi: 10.3389/fbinf.2022.912795

Genomic Characterization of *Lactobacillus delbrueckii* Strains with Probiotics Properties

Luís Cláudio Lima De Jesus[1], *Flávia Figueira Aburjaile*[2], *Thiago De Jesus Sousa*[1], *Andrei Giacchetto Felice*[3], *Siomar De Castro Soares*[3], *Luiz Carlos Junior Alcantara*[1,4]* *and Vasco Ariston De Carvalho Azevedo*[1]*

[1]Department of Genetics, Ecology and Evolution, Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Department of Preventive Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil, [3]Department of Immunology, Microbiology and Parasitology, Federal University of Triângulo Mineiro, Uberaba, Brazil, [4]Flavivirus Laboratory, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Probiotics are health-beneficial microorganisms with mainly immunomodulatory and anti-inflammatory properties. *Lactobacillus delbrueckii* species is a common bacteria used in the dairy industry, and their benefits to hosting health have been reported. This study analyzed the core genome of nine strains of *L. delbrueckii* species with documented probiotic properties, focusing on genes related to their host health benefits. For this, a combined methodology including several software and databases (BPGA, SPAAN, BAGEL4, BioCyc, KEEG, and InterSPPI) was used to predict the most important characteristics related to *L. delbrueckii* strains probiose. Comparative genomics analyses revealed that *L. delbrueckii* probiotic strains shared essential genes related to acid and bile stress response and antimicrobial activity. Other standard features shared by these strains are surface layer proteins and extracellular proteins-encoding genes, with high adhesion profiles that interacted with human proteins of the inflammatory signaling pathways (TLR2/4-MAPK, TLR2/4-NF-κB, and NOD-like receptors). Among these, the PrtB serine protease appears to be a strong candidate responsible for the anti-inflammatory properties reported for these strains. Furthermore, genes with high proteolytic and metabolic activity able to produce beneficial metabolites, such as acetate, bioactive peptides, and B-complex vitamins were also identified. These findings suggest that these proteins can be essential in biological mechanisms related to probiotics' beneficial effects of these strains in the host.

Keywords: comparative genomics, core genome, probiogenomics, GIT stress response, bacteriocins, immunoregulatory proteins

OPEN ACCESS

Edited by:
Richard Allen White III, University of North Carolina at Charlotte, United States

Reviewed by:
Rostyslav V. Bubnov, National Academy of Sciences of Ukraine, Ukraine
Vinod Kumar Gupta, Mayo Clinic, United States

***Correspondence:**
Luiz Carlos Junior Alcantara
luiz.alcantara@ioc.fiocruz.br
Vasco Ariston De Carvalho Azevedo
vasco@icb.ufmg.br

Specialty section:
This article was submitted to Genomic Analysis, a section of the journal Frontiers in Bioinformatics

Received: *04 April 2022*
Accepted: *16 May 2022*
Published: *06 June 2022*

Citation:
De Jesus LCL, Aburjaile FF, Sousa TDJ, Felice AG, Soares SDC, Alcantara LCJ and Azevedo VADC (2022) Genomic Characterization of Lactobacillus delbrueckii Strains with Probiotics Properties. Front. Bioinform. 2:912795. doi: 10.3389/fbinf.2022.912795

INTRODUCTION

Lactobacillus delbrueckii is the type species of *Lactobacillus* genus after a new proposed taxonomic reclassification that divided this genus into 25 new, based on genetic and phylogenetic analysis associated with ecological and metabolic properties (Zheng et al., 2020). This Lactic Acid Bacteria (LAB) member comprises gram-positive, rod-shaped, facultatively anaerobic, and acid-resistant microorganisms, which occupy diverse carbohydrate-rich environments with final fermentative metabolism-derived lactic acid production (Salvetti et al., 2012; Duar et al., 2017). This species

includes mainly two subspecies: *bulgaricus* and *lactis,* both with high importance in industrial fermented dairy products (primarily yogurt and cheeses production) and biotherapeutics approaches (Hao et al., 2011; El Kafsi et al., 2014; Santos Rocha et al., 2014).

Some studies have been characterizing the *L. delbrueckii* strains as probiotics based on their ability to resist gastrointestinal tract (GIT) stressors (Ferreira et al., 2013), pathogens inhibition (De Jesus L. C. L. et al., 2021), and anti-inflammatory effects mainly focused on GIT disease treatment, such as colorectal cancer (Wan et al., 2014), ulcerative colitis (Santos Rocha et al., 2014), and intestinal mucositis (De Jesus et al., 2019). In addition, pre-clinical therapeutical applications of these microorganisms to other pathological conditions, such as arthritis (Kano et al., 2013), depression (Qiu et al., 2021), and diabetes (Hallajzadeh et al., 2021), have also been reported. Among this species, *L. delbrueckii* subsp. *lactis* CIDCA 133 is the best-characterized probiotics strain whose beneficial characteristics and safety aspects have been widely evaluated by *in vitro* and *in vivo*, as well as *in silico* analysis, for example, its ability to inhibit *Escherichia coli*, *Bacillus cereus*, *Citrobacter rodentium*, and *Salmonella* Typhimurium pathogens; immunomodulation by inhibition of NF-κB signaling pathway; tolerance to high concentrations of bile salts; no hemolytic or mucin degradation activity, and no adverse effects to clinical and histopathological mice parameters (Rolny et al., 2016; Hugo et al., 2017; De Jesus et al., 2019; De Jesus L. C. L. et al., 2021; De Jesus LCL. et al., 2021; Barroso et al., 2022).

Although most studies focus on the effect and action mechanism of viable probiotic strains, there is a growing interest in applying probiotics as microbiologically non-viable but immunologically active products. This would be more viable and safer for probiotic applications in clinical practice due to safety concerns regarding this active metabolic form favoring the risk of bacterial translocation (Moradi et al., 2020; Teame et al., 2020). Some studies have evaluated the inactivation of these microorganisms or products derived from them in different inflammation models and obtained similar results to their metabolically active form (Sang et al., 2013; Nakai et al., 2021; Trindade et al., 2021).

According to Hill et al. (2014), probiotics are defined as "live microorganisms that confer a health benefit on the host when administered in adequate amounts." However, it should also be highlighted that most of these beneficial effects attributed to probiotics are strain-dependent, revealing that individual characteristics of the strains provide relevant data for the development of effective probiotic products and facilitate individualized or personalized use for clinical applications (Bubnov et al., 2018; McFarland et al., 2018). This strain-specific property was more substantially related by Rocha et al. (2012) that, when screening 57 dairy *L. delbrueckii* strains, observed that the immunomodulation levels of these bacteria varied depending on the strain. Among the 37 *L. delbrueckii* subsp. *bulgaricus* and 20 *L. delbrueckii* subsp. *lactis* strains tested, the most effective immunomodulators strains belong to the subsp. *lactis* (Rocha et al., 2012), including CNRZ327 and CNRZ333 strains.

Individual biological properties of probiotic strains may be related to a high degree of variation in their genomic content. Thus, studies at the genomic level can provide insights into the main genetic factors and molecular mechanisms associated with the probiotic features of these microorganisms, such as GIT survival, pathogens inhibition, and immunoregulation (Ventura et al., 2012; Salvetti and O,Toole, 2018; Castro-López et al., 2021). Probiotics studies using the genome approach have been performed to identify genetic factors involved with features of different potential probiotics strains, such as *Lactobacillus helveticus* (Fontana et al., 2019), *Lactiplantibacillus plantarum* (Zhang et al., 2018), *Pediococcus sp.* (Wanna et al., 2021), *Bifidobacterium sp*. (Duar et al., 2020), *Enterococcus sp*. (Hussein et al., 2020), *Lactococcus lactis* (Oliveira et al., 2017), among others. In this context, the comparative analysis proves to be an essential tool in probiogenomics, contributing to further exploring the diversity and evolutionary relationship of species (Sun et al., 2015), and identifying and comparing the gene repertoire in different strains (Fontana et al., 2019) and the relationship of these molecules with reported probiotics effects of these bacteria on the host (Papadimitriou et al., 2015; Sun et al., 2015).

Although the importance of *L. delbrueckii* strains in the food industry, few studies have focused on genomic studies of *L. delbrueckii* probiotics strains regarding their host health benefits (El Kafsi et al., 2014; Sun et al., 2015; Kanmani et al., 2018; De Jesus L. C. L. et al., 2021). Thus, this study carried out a comprehensive functional gene characterization of *L. delbrueckii* species with reported probiotics effects, which may be associated with the specific host health benefits of these strains reported phenotypically, and provide a better comprehension of their probiotics features.

MATERIALS AND METHODS

Genome Data

Nine genomes of *L. delbrueckii* strains with reported probiotics properties in the literature (Savino et al., 2011; Santos Rocha et al., 2014; Li et al., 2015, 2017, 2020; Kanmani et al., 2018; Usui et al., 2018; De Jesus et al., 2019; El-Khadragy et al., 2019) were downloaded from the NCBI database (**Table 1**). The genome assemblies were evaluated by QUAST 5.0.2 (Gurevich et al., 2013) and BUSCO v4.0.6 software (Benchmarking Universal Single-Copy Orthologs) (Simão et al., 2015). In addition, all genomes were annotated using the Prokaryotic Genome Annotation System (Prokka) v1.14.5 software (Seemann, 2014).

Pan-Genome Analysis

This study used the BPGA (Bacterial Pan Genome Analysis) pipeline for performance pan-genome (Chaudhari et al., 2016). The genome sequences were submitted in FASTA format to Orthofinder software to predict orthologs genes (Emms and Kelly, 2019), using default parameters with a *p*-value cut-off of $1E^{-5}$. This software bases its inference method on OrthoMCL (Li et al., 2003) through the hybrid Markov Clustering algorithm (Enright, 2002), which computes sequence similarities with BLAST and then uses the MCL clustering algorithm to identify clusters of highly connected sequences. After this

TABLE 1 | Genome features of *Lactobacillus delbrueckii* strains used in this study.

Strain	Genome Access	Size (Mb)	GC %	CDS	Source	Probiotic Property	References
LJJ	NZ_CP049052.1	1.89	49.50	1,604	Dairy products	Acid tolerance mechanism	Li et al. (2020)
KLDS1.0207	NZ_CP032451.1	1.87	49.80	1,607	Dairy products	Alleviation of lead (Pb) toxicity	Li et al. (2017)
DSM 20080	NZ_CP019120.1	1.87	49.80	1,680	Environment	Oxidative stress modulation on *S. mansoni*-infected mice	El-Khadragy et al. (2019)
2038	NC_017469.1	1.87	49.70	1,792	---	Microbiota regulation in aging mice	Usui et al. (2018)
ATCC 11842	NC_008054.1	1.86	49.70	1,683	Bulgarian Yogurt	Osmotic tolerance mechanism	Li et al. (2015)
TUA4408L	NZ_CP021136.1	2.01	49.90	1,801	Sunki-zuke	Immunomodulatory activity on rotavirus infection	Kanmani et al. (2018)
DSM20074	NZ_CP018615.1	1.95	49.60	1,721	Environment	Pathogen inhibition	Savino et al. (2011)
CNRZ327	GCF_000751695.1	2.11	49.60	1,525	Cheese	Anti-inflammatory effect on DSS-induced Colitis	Santos Rocha et al. (2014)
CIDCA 133	CP065513	2.13	49.59	1,921	Raw cow's milk	Anti-inflammatory effect on 5-FU-induced Mucositis	De Jesus et al. (2019)

process of predicting orthologous genes, through in-house scripts, these genes were classified according to the subsets of the pangenome, being divided into the core genome, shared, and singletons. For the development of the pangenome, after the classification process in its subsets, in-house scripts were used to estimate what would be the fixed parameters of the Heap Law (Soares et al., 2013; Guimaraes et al., 2015) and the Utterance of the Least Squares Principle (for core genome subsets and singletons). For genetic contexts, we can represent Heap's Law according to the formula $n = k * N \gamma$, which (n) would be the value for the number of genes for a given number of genomes (N); and then k and γ can be considered as free parameters. By this law, γ can be calculated as $\alpha = 1 - \gamma$, so when $\alpha > 1$ ($\gamma < 0$), the pangenome is called closed, which means that there is no increase, or there is no significant increase, of genes when more genomes of the studied organism are sequenced. If $\alpha < 1$ ($0 < \gamma < 1$), suggests the pan-genome of the probiotic strains is open, which indicates that there is an increase in the number of genes when more genomes are sequenced. The Least Squares Principle Statement can be represented by the formula $n = k * \exp[-x/t] + tg\theta$, where (n) is also the number of genes, and k, t, and $tg\theta$ are considered as parameters free. With the result of this law, we were able to estimate, based on the number of singletons added to each new sequencing, how many genomes are still needed for the core genome of the studied group to reach stability.

Functional Annotation of Pan-Genome

Clusters of Orthologous Groups (COGs) for core genes, accessory genes, and singletons (first unique genes of the strains) were obtained using the eggNOG-mapper v2 web tool (http://eggnog-mapper.embl.de/) (E-value < 0.001) (Cantalapiedra et al., 2021). Furthermore, a complementary functional annotation analysis was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) through the KEGG Mapper/BLASTKOALA tool (https://www.kegg.jp/blastkoala/) (Kanehisa et al., 2016).

Prediction of Genes Related to Antibacterial Activity

Proteins involved in antibacterial activity were also evaluated across the probiotics *L. delbrueckii* genomes. For this purpose, genes coding bacteriocins were predicted through BAGEL4 (http://bagel4.molgenrug.nl/) (van Heel et al., 2018). The bacteriocins-encoding genes' distribution among the genomes was visualized through a heatmap of presence and absence. Furthermore, core proteins producing other antimicrobial compounds, such as hydrogen peroxide and organic acids, were investigated using the KEGG Mapper/BLASTKOALA tool (Almeida et al., 2021).

Identification of Gastrointestinal Tract Stress Response Genes and Proteolytic Enzymes in Core Genome

Identification of core proteins of probiotics *L. delbrueckii* strains related to GIT stress response (acid and bile) and proteolytic activity were manually predicted through Prokka–derived annotation, based on previous studies (Liu et al., 2010; Papadimitriou et al., 2016; De Jesus LCL. et al., 2021).

Prediction of Metabolic Pathway-Related Genes in Core Genome

The presence of genes involved in metabolic pathways related to carbohydrate metabolism, lactate, short-chain fatty acids (SCFAs), and vitamin B biosynthesis was predicted using the BioCyc database (https://biocyc.org/) (Karp et al., 2019). The genomes of *L. delbrueckii* subsp. *lactis* DSM 20072 (Genome access: NZ_CP022988.1) and *L. delbrueckii* subsp. *bulgaricus* ATCC 11842 (Genome access: NC_008054.1) strains were used for this comparative analysis. Furthermore, the carbohydrate-active enzymes (CAZymes) families were predicted through the Carbohydrate-Active Enzyme (CAZy) database (http://www.cazy.org/) (Cantarel et al., 2009).

Interaction of Core Proteins of *Lactobacillus delbrueckii* Strains With Human Immune Proteins

To evaluate the potential biological interaction between core proteins of *L. delbrueckii* probiotic strains and human immune proteins, first, the subcellular localization of proteins identified in the core genome

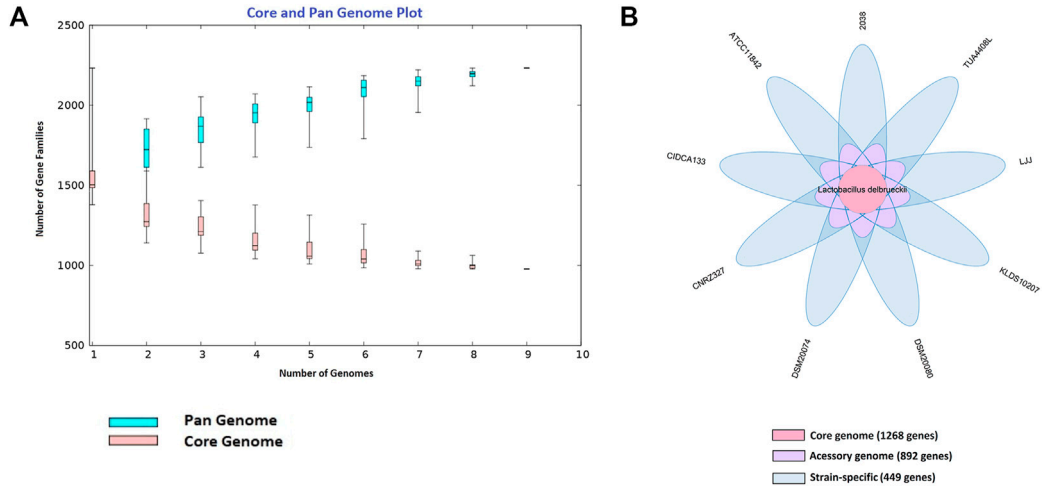


FIGURE 1 | Pan-genome of probiotics *L. delbrueckii* strains. **(A)** Curve development of pan (blue color) and core (salmon color) genome (α = 0.83). The number of gene families is plotted in function of the genome number. **(B)** Venn diagram illustrating the core-genome size (center part), accessory genomes (around the center), and unique genes for each strain (extreme values).

was predicted using SurfG+ software (Barinov et al., 2009). Second, the core proteins were predicted for their ability to be an adhesin calculated by SPAAN software (score >0.7) (Sachdeva et al., 2005). After, immune protein sequences related to the inflammation pathways (TLR2/4-MAPK, TLR2/4-NF-κB, and NOD-like receptor signaling pathways) were mapped and obtained from KEGG pathways and UniProt (UP000005640), respectively (**Supplementary Table S1**). Finally, the protein-protein interaction was conducted in the InterSPPI v2 web server (http://zzdlab.com/InterSPPI/) (Lian et al., 2019). Graphical analysis of resulting interaction networks (minimum score: 0.9765; specificity: 0.99) was performed by Cytoscape v3.9.0 software (Shannon, 2003).

RESULTS

Genome Features of Probiotics *Lactobacillus delbrueckii* Strains

The probiotics group of *Lactobacillus delbrueckii* strains evaluated in this study is mainly formed by the subspecies *bulgaricus* (n = 5) (LJJ, KLDS1.0207, DSM 20080, 2038 and ATCC 11842), *delbrueckii* (n = 2) (TUA4408L, DSM20074), and *lactis* (n = 2) (CNRZ327 and CIDCA 133). The strains were mainly isolated from dairy environments, including cheeses, yogurts, and fermented milk. The genome evaluation of these nine strains revealed a genome size and GC content average of 1,951 ± 0.10 Mb and 49.69 ± 0.12%, with 1,664 ± 0.09 protein-coding sequences (CDS) (**Table 1**).

Pan-Genome Analysis

The pan-genome (total gene repertoire) obtained through BPGA with nine probiotics *L. delbrueckii* strains is composed of a total of 2,609 genes (**Figure 1A**), of which 1,268 (48.60%) belong to the core genome (number of genes shared by all strains), 892 genes (34.18%) to the accessory genome (genes shared by two or more strains), and 449 (17.20%) are strain-specific (uniques) (genes present in a single strain) (**Figure 1B**). Furthermore, the CIDCA 133, DSM20074, and CNRZ327 strains presented the highest exclusive genes, with 102, 76, and 69 genes, respectively, followed by TUA4408L (53 genes), 2038 (47 genes), ATCC11842 (39 genes), KLDS10207 (32 genes), LJJ (16 genes), and DSM20080 (15 genes).

According to the curve generated for these nine genomes based on Heap's Law and leats-square fit of the exponential regression decay, the number of genes families in the pan-genome increased with the addition of each other genome ($n = 1{,}848.134 * n^{0.156}$), suggesting that the pan-genome of probiotics *L. delbrueckii* strains remains open. For the subsets of the core genome and singletons developed by the Utterance of the Least Squares Principle, it can be observed a value of $tg\theta$ of approximately 1,182 genes ($n = 465.995 * \exp[-x/4.839] + 1182.675$) for the core genome, and a value of approximately 24 ($n = 219.676 * \exp[-x/4.356] + 24.813$) for the strain-specific. This result shows that at each new sequencing, 24 new genes are added to this pangenome, and it is expected that the core genome will stabilize when it reaches around 1,182 genes (**Figure 1A**).

Functional Annotation of Gene Families

Analysis of the COG distribution for the pan-genome revealed that many of the proteins in the core genome are related to "translation, ribosomal structure, and biogenesis" (12%), "replication, recombination and repair" (8%), "amino acid transport and metabolism" (7%), "nucleotide transport and metabolism" (7%), and "unknown function" (18%). The accessory genome presented COG terms related to "amino acid transport and metabolism" (14%), "replication, recombination and repair" (10%), "transcription" (8%), "defense mechanisms" (7%), and "unknown function" (13%). Finally, "replication, recombination and repair" (29%), "amino acid transport and metabolism" (16%), "defense mechanisms" (8%), and "unknown function" (18%) were the most common COGs terms related to unique genes (**Figure 2A**).

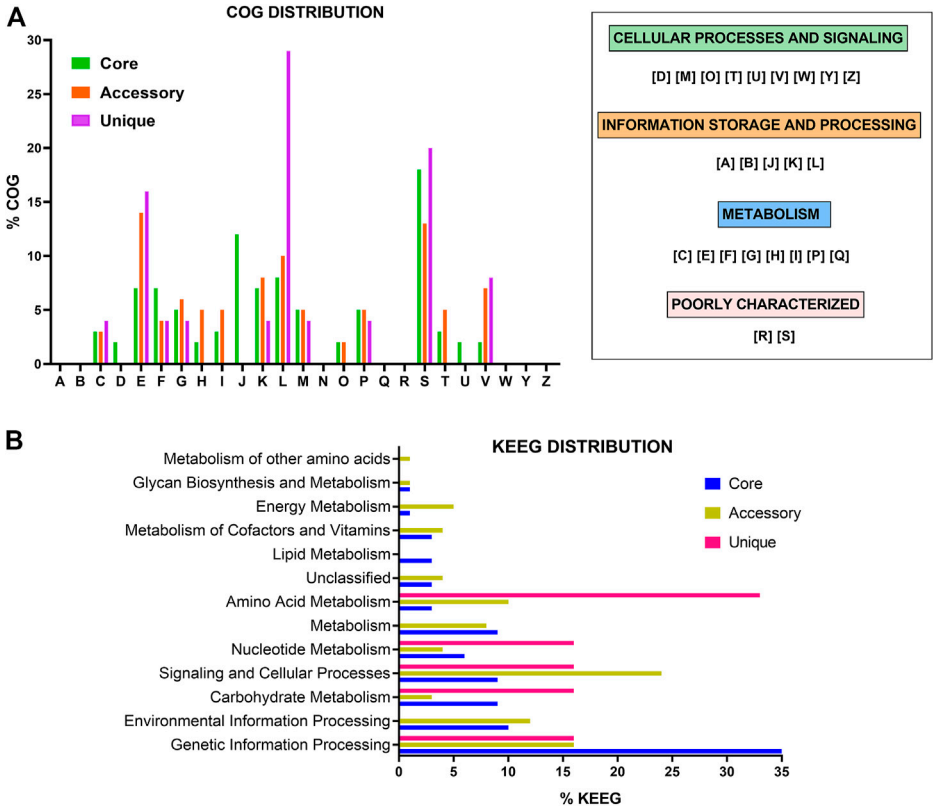


FIGURE 2 | Functional analysis of gene families of probiotics *L. delbrueckii* strains. Distribution of COG **(A)** and KEGG **(B)** functional categories in the core, accessory, and unique genes. COG categories: [A] RNA processing and modification, [B] Chromatin structure and dynamics, [C] Energy production and conversion, [D] Cell cycle control, cell division, chromosome partitioning, [E] Amino acid transport and metabolism, [F] Nucleotide transport and metabolism, [G] Carbohydrate transport and metabolism, [H] Coenzyme transport and metabolism, [I] Lipid transport and metabolism, [J] Translation, ribosomal structure and biogenesis, [K] Transcription, [L] Replication, recombination and repair, [M] Cell wall/membrane/envelope biogenesis, [N] Cell motility, [O] Post-translational modification, protein turnover, and chaperones, [P] Inorganic ion transport and metabolism, [Q] Secondary metabolites biosynthesis, transport, and catabolism, [R] General function prediction only, [S] Function unknown, [T] Signal transduction mechanisms, [U] Intracellular trafficking, secretion, and vesicular transport, [V] Defense mechanisms, [W] Extracellular structures, [Y] Nuclear structure, and [Z] Cytoskeleton.

Furthermore, the KEGG annotation revealed that most genes in the core genome are related to "genetic information processing" (35%), followed by "environmental information processing" (10%), "carbohydrates metabolism" (9%), and "signaling and cellular processes" (9%). In the accessory genome, most of the genes were related to "signaling and cellular processes" (24%), "genetic information processing" (16%), "environmental information processing" (12%), and "amino acid metabolism" (10%) function. To unique genes, "amino acid metabolism" (33%), followed by "genetic information processing" (16%), "signaling and cellular processes" (16%), and "carbohydrates metabolism" (16%) were the most frequent categories (**Figure 2B**).

Core Proteins Involved in Gastrointestinal Tract Stress Responses

In the core genome of *L. delbrueckii* probiotics strains, it was identified some genes encoding proteins that were previously reported to be involved in GIT stress response (acid and bile), including enolase, serine protease HtrA, ornithine decarboxylase, two-component sensor histidine kinase, chaperones (DnaK, DnaJ, GroeL), Na+/H+ antiporter NhaC, F0F1 ATP system genes, S-ribosylhomocysteine lyase, ATP-dependent ClpX protease, glycine/betaine ABC transporter permease, among others (**Table 2**).

Core Genome of *Lactobacillus delbrueckii* Probiotics Strains Have Potential Genes Involved in Proteolytic Activity, Carbohydrates Metabolism, and Secondary Metabolic Product

The core genome of *L. delbrueckii* probiotic strains encodes various proteolytic enzymes essential for their growth, survival, and organoleptic properties of dairy products manufacturing. These enzymes including oligopeptide ABC transporters system (*oppD, oppC, oppF, oppA, oppB*), peptidases (*pepM, pepQ, pepT, pepO, pepR*), and proteinases (*PrtB, PrtM*). The peptidases mainly cleave substrates containing casein, methionine, proline, cysteine, leucine, serine, asparagine, and glutamate-derived peptides (**Supplementary Table S2**).

TABLE 2 | Predicted proteins identified in the core genome of probiotics *Lactobacillus delbrueckii* strains involved in acid and bile tolerance.

Locus Tag	Predicted Protein	Gene
OHNDKLAL_00510	Putative ornithine decarboxylase	*odcI*
OHNDKLAL_01838	UDP-galactopyranose mutase	*glf*
OHNDKLAL_02062	Pyruvate oxidase	*pox1*
OHNDKLAL_00195	Peptidase M13	*pepO*
OHNDKLAL_01467	Two-component sensor histidine kinase	*arlS*
OHNDKLAL_01763	Na+/H+ antiporter NhaC	*nhaC*
OHNDKLAL_00075	L-lactate dehydrogenase	*ldh*
OHNDKLAL_00088	S-ribosylhomocysteine lyase	*luxS*
OHNDKLAL_00120	Serine protease HtrA	*htrA*
OHNDKLAL_00179	Universal stress protein	*usp5*
OHNDKLAL_00196	Potassium transporter Kup	*kup*
OHNDKLAL_00262	Glutamine-hydrolyzing GMP synthase	*guaA*
OHNDKLAL_00321	CTP synthetase	*pyrG*
OHNDKLAL_00365	30S ribosomal protein S19	*rpsS*
OHNDKLAL_00482	Exopolyphosphatase	*ppx3*
OHNDKLAL_00483	Polyphosphate kinase	*ppk*
OHNDKLAL_00531	ATP-dependent Clp protease	*clpE*
OHNDKLAL_00534	Phosphoenolpyruvate--protein phosphotransferase	*ptsI*
OHNDKLAL_00540	GTP pyrophosphokinase	*yjbM*
OHNDKLAL_00557	Recombinase recA	*recA*
OHNDKLAL_00587	Glyceraldehyde-3-phosphate dehydrogenase	*gap*
OHNDKLAL_00588	Phosphoglycerate kinase	*pgk*
OHNDKLAL_00595	Phosphate acetyltransferase	*pta*
OHNDKLAL_00636	Acetate kinase	*ackA*
OHNDKLAL_00656	F0F1 ATP synthase subunit A	*atpB*
OHNDKLAL_00657	F0F1 ATP synthase subunit C	*atpE*
OHNDKLAL_00658	F0F1 ATP synthase subunit A	*atpF*
OHNDKLAL_00659	F0F1 ATP synthase subunit B	*atpH*
OHNDKLAL_00660	F0F1 ATP synthase subunit Alfa	*atpA*
OHNDKLAL_00661	F0F1 ATP synthase subunit gamma	*atpG*
OHNDKLAL_00662	F0F1 ATP synthase subunit beta	*atpD*
OHNDKLAL_00663	F0F1 ATP synthase subunit epsilon	*atpC*
OHNDKLAL_00733	ATP-dependent ClpX protease	*clpX*
OHNDKLAL_00759	Arginyl-tRNA synthetase	*argS*
OHNDKLAL_00785	Pyruvate kinase	*pyk*
OHNDKLAL_00833	ppGpp (guanosine 3'-diphosphate 5-' diphosphate) synthetase	*relA*
OHNDKLAL_00840	3-oxoacyl-ACP synthase	*fabH*
OHNDKLAL_01171	Glycine/betaine ABC transporter permease	*opuB*
OHNDKLAL_01283	Enolase	*eno*
OHNDKLAL_01301	Molecular chaperone DnaJ	*dnaJ*
OHNDKLAL_01302	Molecular chaperone DnaK	*dnaK*
OHNDKLAL_01303	Heat shock protein GrpE	*grpE*
OHNDKLAL_01333	30S ribosomal protein S2	*rpsB*
OHNDKLAL_01377	Asp23/Gls24 family envelope stress response protein	*yloU*
OHNDKLAL_01506	Dihydroorotate dehydrogenase	*pyrD*
OHNDKLAL_01584	Chaperonin GroEL	*groL*
OHNDKLAL_01585	Chaperonin GroES	*groS*
OHNDKLAL_01700	Phosphoglycerate mutase family protein	*pgm*
OHNDKLAL_01903	Oligoendopeptidase F	*pepF*
OHNDKLAL_02047	Glucosamine-6-phosphate deaminase	*nagB*

Italics represents the gene ID of predicted proteins related to GIT stress response.

Carbohydrate metabolism was identified as essential enzymes related to glucose, fructose, sucrose, mannose, chitobiose, and galactose. These proteins include 6-phospho-beta-glucosidase, glucokinase, mannose-6-phosphate isomerase, and phosphoglucomutase. Furthermore, some genes related to the transport of cellobiose, mannose, fructose, and glucose carbohydrates were also identified, mainly related to the PTS system, the main carbohydrate active-transport system in bacteria (**Supplementary Table S3**). It was also determined that the most abundant carbohydrate-active enzymes (CAZy) gene families in the core genome of *L. delbrueckii* probiotics strains belong to glycosyltransferases (GTs) families (GT1, GT2, GT4, GT26, GT28, GT51) (n = 10), followed by glycoside hydrolases (GHs) (GH4, GH13, GH31, GH32, GH73) (n = 7), and carbohydrate-binding modules (CBMs) (CBM48) (n = 1), respectively.

Genes encoding proteins such as glucokinase, glucose-6-phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, ribulose-phosphate 3-epimerase, pyruvate

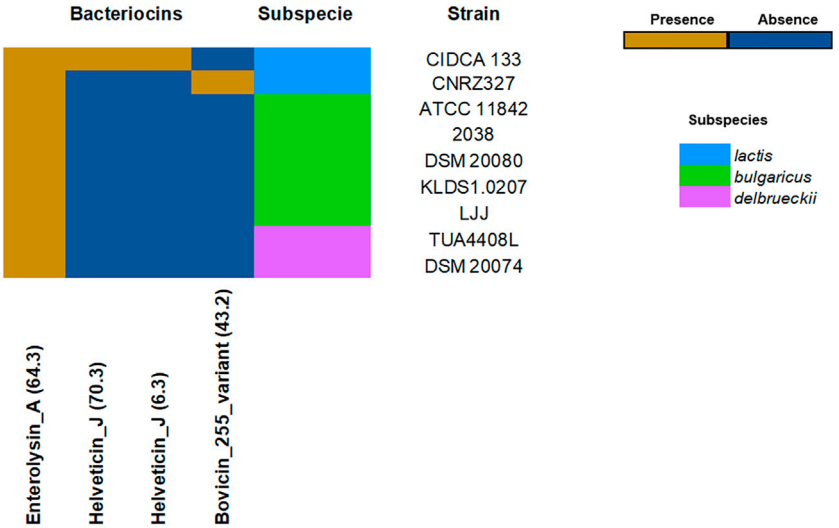


FIGURE 3 | Bacteriocins profile across probiotics *L. delbrueckii* strains. The heatmap indicates the presence (dark yellow) or absence (dark blue) of bacteriocins-encoding genes.

kinase, phosphoketolase, lactate dehydrogenase, and acetate kinase were also identified in the core genome of *L. delbrueckii* probiotics strains. These essential proteins are involved in the homofermentative or heterofermentative pathways, producing lactate or acetate. Genes involved in the biosynthesis of complex B vitamins were also predicted, including thiamine pyrophosphokinase (thiamine or vitamin B1), a riboflavin kinase (riboflavin or vitamin B2), dihydrofolate reductase (folate or vitamin B9), and cob(I)alamin adenosyltransferase (cobalamin or vitamin B12). No propionate or butyrate-related gene was identified in the core genome (**Supplementary Table S4**).

Probiotics *L. delbrueckii* Strains Harbors Genes Related to Antibacterial Profile

The *L. delbrueckii* strains showed different profiles in terms of bacteriocins. Among all strains, subspecies *lactis* showed a greater diversity of bacteriocins in their genome, including enterolysin A, helveticin J, and bovicin_255. Few bacteriocins were found for the subspecies *bulgaricus*. The bacteriocins enterolysin A appears to be conserved in the species (**Figure 3**). Furthermore, it was identified in the core genome D-lactate dehydrogenase, L-lactate dehydrogenase, acetate kinase, L-lactate oxidase, glycolate oxidase, and pyruvate oxidase genes, which acts like crucial enzymes in the biosynthesis of organic acids (lactate and acetate), and hydrogen peroxide, respectively (**Supplementary Table S5**).

Core Proteins With High Adhesin Profile Potentially Interacts With Human Immunomodulatory Proteins

A total of 1,268 proteins identified in the core genome of *L. delbrueckii* probiotics strains were classified by SurfG+ software as cytoplasmic (CYT) (n = 918), membrane (ME) (n = 204), protein surfaces exposed (PSE) (n = 105), and secreted (SE) (n = 41) (**Figure 4A**). Of these proteins, 22 classified as secreted, 17 PSE, eight cytoplasmic, and two membranes were predicted by SPAAN with a high probability of being an adhesin (score >0.7) (**Figure 4B**), including LysM peptidoglycan-binding domain-containing protein (LysM), aggregation promoting protein (Apf), proteinase B (PrtB), penicillin-binding protein (Pbp1A), oligopeptide ABC transporter (OppA), lipoteichoic acid synthase (LtsA), phosphoglycerate mutase (Pgm1), peptide methionine sulfoxide reductase (MsrA), fluoride efflux transporter CrcB (CrcB), among others (**Supplementary Table S6**).

The functional characteristics of these 49 predicted adhesin-like proteins were determined using COG analysis. These proteins were spread over 13 COGs related to "cellular processes and signaling" (38.7%) (e.g., cell wall/membrane/envelope biogenesis—28.6%; cell motility—2%; intracellular trafficking, secretion, and vesicular transport—2%; cell cycle control, cell division, chromosome partitioning—4.1%; and post-translational modification, protein turnover, and chaperones—2%), "metabolism" (20.4%) (*e.g.,* amino acid transport and metabolism-10.2%; nucleotide transport and metabolism—2%; carbohydrate transport and metabolism—6.2%; and inorganic ion transport and metabolism—2%), and "information, storage and processing" (6.1%) (*e.g.,* transcription—4.1%; and translation, ribosomal structure, and biogenesis—2%). Furthermore, 33.3% of adhesin-like proteins were assigned to the "poorly characterized" category (function unknown) (**Figure 4C**).

The core proteins with a high adhesion profile were also evaluated to interact with human immune proteins. InterSPPI software predicted 44 interactions (**Supplementary Table S7**). The proteinase PrtB was the most frequent interaction among the core proteins. Other immunomodulatory proteins were also

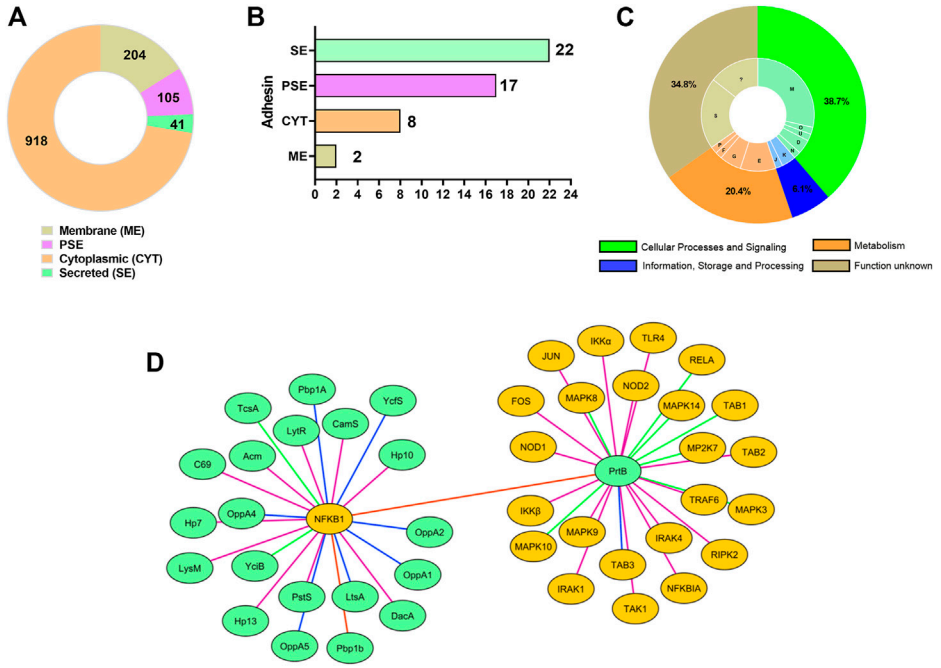


FIGURE 4 | Core proteins of *L. delbrueckii* strains interact with human immune proteins involved in inflammatory pathways. **(A)** Subcellular localization of core proteins. **(B)** The number of core proteins with adhesin profile. **(C)** COG categories are assigned to core proteins with adhesin profiles. **(D)** Subnetwork mapping of *L. delbrueckii* core proteins (green circles nodes) interacting with human immune proteins (yellow, dark circle nodes). Different line colors indicates the interaction degree between the proteins, based on interaction score: 0.97 (green line), 0.98 (purple line), 0.99 (blue light line), 1.0 (red line).

predicted, such as LysM peptidoglycan-binding domain-containing protein (LysM), lipoteichoic acid synthase (LtsA), penicillin-binding protein (Pbp1b), N-acetylmuramidase (Acm), putative lipoprotein A-antigen (TcsA), among others (**Figure 4D**), demonstrating that these proteins can be involved with immunoregulatory ability of the *L. delbrueckii* probiotics strains. Regarding human proteins, the nuclear factor NF-κB p105 subunit (NFKB1), engaged in TLR/NF-κB signaling pathway, was the most frequent interaction. However, other human immune proteins involved in TLR2/4-MAPK, TLR2/4-NF-κB, and NOD-like receptor signaling pathways also interacted with immunoregulatory proteins of *L. delbrueckii* strains, such as TLR4, TRAF6, RELA, NFKBIA, NOD1, NOD2, FOS, JUN, and MAPK10, among others (**Figure 4D**; **Supplementary Table S7**).

DISCUSSION

Comparative genomics revealed a high variation level in the genome of nine *L. delbrueckii* probiotics strains, with the subspecies *lactis* presenting a larger genome size (Mb) than subspecies *bulgaricus*, corroborating the findings of El Kafsi et al. (2014). This genomic variation can be related to the differences in the number of unique genes observed across the strains, in which the subspecies *lactis* had the highest number. The pangenome analysis of *Lactobacillus delbrueckii* species has already been carried out by Inglin et al. (2018) and Kim et al. (2021). However, the above authors did not perform a functional analysis related to the probiosis of these strains. Thus, in our work, the performance of a combined analysis of pan-genome data of nine potential *L. delbrueckii* probiotics strains allowed us to obtain more robust data related to the most relevant characteristics of the probiose of these strains, mainly related to their ability to survive the TGI, adhesion, antibacterial activity, and immunomodulation.

Functional analysis of the core genome revealed that the proteins of nine *L. delbrueckii* probiotic strains are mainly involved in genetic and environmental information processing and metabolic activities, which suggests the importance of these genes in conserved cellular processes of these microorganisms to survive and adapt to specific environments or host.

One of the first adaptation steps of probiotics to the host involved molecular/cellular mechanisms related to their response to GIT stressors (stomach acidity and bile salt) (Papadimitriou et al., 2016). The core genome of *L. delbrueckii* probiotics strains harbors genes related to these stress response mechanisms, mainly including transcriptional regulators expression (e.g., two-component sensor histidine kinase), proton extrusions, and bile efflux (e.g., Na+/H+ antiporter, F0F1 ATPase genes, glycine/betaine ABC transporter permease), metabolic response (e.g., acetate kinase, pyruvate oxidase, ornithine decarboxylase), and heat shock/chaperones proteins production (e.g., GroEL, GroES, DnaK, DnaJ, ClpX). The expression of these genetic factors can be essential to the survival strategy of these bacteria on the GIT, allowing them to arrive in viable

amounts sufficient to promote their interactions and beneficial effects with the specific-host sites of action. Genome and phenotype-scale studies demonstrated that these survival and adaptation mechanisms were observed in *L. delbrueckii* LJJ (Li et al., 2020) strain and are also shared with others, such as UFV H2b20 (Ferreira et al., 2013), 2038 (Hao et al., 2011), ATCC 11842, and CNRZ327 (El Kafsi et al., 2014), and CIDCA 133 (De Jesus LCL. et al., 2021).

This study´s probiotics *L. delbrueckii* strains were mainly isolated from dairy products, supporting the prediction of core genome enzymes related to a conserved proteolytic and metabolic sugar system. This high metabolic property enhances the fermentation ability of these strains with the production of essential metabolites (e.g., bioactive peptides, lactate, SCFA, and vitamins). These compound's synthesis requires specific enzymes (e.g., proteinases and peptidases, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, phosphoketolase, acetate kinase, lactate dehydrogenase, riboflavin kinase, thiamine pyrophosphokinase, among others) of these strains, involved in proteolysis, and both phosphoketolase or Embden-Meyerhof (EMP) metabolic pathways (Kandler, 1983; Ye et al., 2021).

A vital feature derived from the fermentation process by probiotic strains is their antimicrobial activity due to organic acids, hydrogen peroxide, and bacteriocins production. The genome of *L. delbrueckii* probiotics strains has genes coding for these antimicrobial compounds (e.g., bacteriocin enterolysin A, D-lactate dehydrogenase, L-lactate dehydrogenase, acetate kinase, L-lactate oxidase, glycolate oxidase, and pyruvate oxidase), which makes them highly relevant in the food industry, since when used in the fermentation of food dairy products, it can control and preserve these products against the food spoilage of pathogens. The antibacterial effect associated with these *L. delbrueckii*-producing compounds against some pathogens, such as *Salmonella sp.*, *Enterococcus faecalis*, *Escherichia coli*, *Gardnerella vaginalis*, *Listeria monocytogens*, *Pseudomonas aeruginosa*, has been previously reported (Evivie et al., 2020; De Jesus LCL. et al., 2021; Qiu et al., 2021).

It is essential to identify genes/metabolic pathways and characterize bioproducts produced by probiotic bacteria with high fermentative capacity since studies have demonstrated the beneficial effects of fermented products derived from these microorganisms in GIT inflammatory diseases. For example, milk fermented by *L. delbrueckii* CNRZ327 (2×10^9 CFU/mL) attenuated TNBS-induced colitis in a murine model, improving the epithelial architecture, and reducing inflammatory parameters (IL6, TNFα, MPO) and oxidative markers (COX2 and Hmox) (Plé et al., 2016). Similar effects were reported in *L. delbrueckii* CIDCA 133, whose milk fermented by the strain preserved the intestinal epithelium from the inflammatory damage caused by the chemotherapy drug 5-FU (300 mg/kg) (De Jesus et al., 2019). Another study demonstrated that intake of yogurt fermented with *L. delbrueckii* 2038 improves aging by metabolites production and microbiota and intestinal epithelial regulation (Usui et al., 2018). It is suggested that these effects can be associated with the production of organic acids (lactate, SCFA) and bioactive metabolites (vitamins) produced by these bacteria, although these studies did not assess their concentration. However, it is important to highlight that the ability of *L. delbrueckii* species to produce SCFA or vitamins with host health benefits has been previously reported (Laiño et al., 2012; Levit et al., 2018; Dan et al., 2019), makes them promise to be used as an adjuvant for the treatment of inflammatory GIT diseases and other pathological conditions due to their reported antioxidants, anti-inflammatory, and immunomodulatory properties.

Immunomodulatory and anti-inflammatory properties or probiotics bacterial can also be related to the surface layer proteins or extracellular proteins (Hidalgo-Cantabrana et al., 2020; Chandhni et al., 2021) due to the ability of these proteins to interact with the host cells via pattern recognition receptors (e.g., Toll-like receptors-TLR, NOD-like receptors-NLR) inducing specific signalization pathways responses, as nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) (Delgado et al., 2020). This hypothesis is corroborated by Rocha et al. (2012) when they showed that surface-exposed proteins of the *L. delbrueckii* CNRZ333 strain played a role in NF-κB immune modulation (Rocha et al., 2012).

L. delbrueckii strains characterized as probiotics shared genes with potential interaction with inflammatory pathways-related human immune proteins, including proteinase B (PrtB), penicillin-binding protein (Pbp1A), and lipoteichoic acid synthase (LtsA), among others, with PrtB being the most interacting protein. PrtB is a cell envelope-associated serine protease essential to milk casein degradation (Gilbert et al., 1996). The expression of this protein and its analogs producing bioactive health-beneficial peptides has been suggested to be crucial to the immunomodulatory properties of *L. delbrueckii* strains (De Jesus LCL. et al., 2021). For example, De Jesus LCL. et al. (2021) showed that predicted proteins of *L. delbrueckii* CIDCA 133 with a high adhesin profile, including PrtB protein, interacted with human immune proteins involved with NF-κB signaling pathway activation. These findings can be related to their *in vivo* results. It was demonstrated that consumption of this probiotic strain presented an anti-inflammatory profile by activating TLRs receptors (*Tlr2, Tlr4*), decreasing *Nfkb1* and enhancing immunoregulatory markers *Il10* and *Tgfb* gene expression (De Jesus LCL. et al., 2021). These results are also supported by Espeche Turbay et al. (2012). They demonstrated that milk β-casein degradation by *L. delbrueckii* CRL581 ameliorates TNBS-induced acute intestinal inflammation by increasing immunoregulatory IL10 and decreasing leukocytes infiltrate and the IFNγ pro-inflammatory marker (Espeche Turbay et al., 2012). It is believed that these effects can be attributed to its cell envelope-associated proteinase PrtL activity (Villegas et al., 2015).

Anti-inflammatory properties of other surface layer components of *L. delbrueckii* strains have also been reported as extracellular polysaccharides of *L. delbrueckii* TUA4408L, which presented antiviral activity against rotavirus infection in porcine cells by modulating TLR2/4, interferon regulatory factor (IRF)-3, and the antiviral factors IFN-β, MxA, and RNase L expression (Kanmani et al., 2018). Altogether, these findings

reveal that these bacteria factors are essential to leading the biological process of the host, mainly immune regulation. Therefore, based on these findings, the knowledge at the genomic level of the individual characteristics of probiotics, as well as the genetic factors associated with their immunoregulatory capacity, can facilitate individualized or personalized use of them for clinical applications, thus being an alternative approach to the problems arising from the use of live beneficial microorganisms in clinical practice. Furthermore, the exploration of genetic factors can contribute to validating the role of these probiotics-derived bioactive molecules in different pathological conditions, including their beneficial effects on those that affect distant sites and organs (e.g., skin, respiratory and urogenital tracts, brain, bones, among others) (Reid et al., 2017; Bubnov et al., 2018). Thus, we reinforce that further studies, including knockout genes or heterologous production of these proteins, must be performed to validate these genotypic findings with the phenotypic reported results described for these strains and elucidate the underlying mechanisms involved in their immunomodulatory activities.

In summary, this first probiotic genomic characterization study for potential *L. delbrueckii* probiotics species shows that these bacteria share a broad gene repertoire that functionally may be responsible for phenotypic features attributed to these strains on the host. The data presented support other studies that aim to identify genetic factors and mechanisms related to the beneficial effects of new probiotic targets from the *Lactobacillus* species with high commercial and biotechnological relevance. Furthermore, these data open perspectives for new studies to be carried out to evaluate the predicted interacting bacteria proteins with human immune proteins as possible anti-inflammatory molecules to be tested in therapeutic approaches to different inflammatory conditions.

AUTHOR CONTRIBUTIONS

Conceptualization: LJ and FA; Methodology: LJ, TS, AF, and FA; Formal analysis and investigation: LJ and FA; Writing-original draft preparation: LJ; Writing-review and editing: FA, TS, SS, LA, and VA. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Council for Scientific and Technological Development (CNPq) (Grant number 312045/2020-4).

ACKNOWLEDGMENTS

The authors would like to acknowledge the Pró-Reitoria de Pesquisa—UFMG, Instituto Oswaldo Cruz-FIOCRUZ, and funding agencies FAPEMIG, CAPES, and CNPq.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbinf.2022.912795/full#supplementary-material

REFERENCES

Barinov, A., Loux, V., Hammani, A., Nicolas, P., Langella, P., Ehrlich, D., et al. (2009). Prediction of Surface Exposed Proteins in *Streptococcus pyogenes*, with a Potential Application to Other Gram-Positive Bacteria. *Proteomics* 9, 61–73. doi:10.1002/pmic.200800195

Barroso, F. A. L., de Jesus, L. C. L., da Silva, T. F., Batista, V. L., Laguna, J., Coelho-Rocha, N. D., et al. (2022). *Lactobacillus delbrueckii* CIDCA 133 Ameliorates Chemotherapy-Induced Mucositis by Modulating Epithelial Barrier and TLR2/4/Myd88/NF-Kb Signaling Pathway. *Front. Microbiol.* 13, 858036. doi:10.3389/fmicb.2022.858036

Bubnov, R. V., Babenko, L. P., Lazarenko, L. M., Mokrozub, V. V., and Spivak, M. Y. (2018). Specific Properties of Probiotic Strains: Relevance and Benefits for the Host. *EPMA J.* 9, 205–223. doi:10.1007/s13167-018-0132-z

Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P., and Huerta-Cepas, J. (2021). eggNOG-Mapper V2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. *Mol. Biol. Evol.* 38, 5825–5829. doi:10.1093/molbev/msab293

Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., and Henrissat, B. (2009). The Carbohydrate-Active EnZymes Database (CAZy): an Expert Resource for Glycogenomics. *Nucleic Acids Res.* 37, D233–D238. doi:10.1093/nar/gkn663

Castro-López, C., García, H. S., Guadalupe Martínez-Ávila, G. C., González-Córdova, A. F., Vallejo-Cordoba, B., and Hernández-Mendoza, A. (2021). Genomics-based Approaches to Identify and Predict the Health-Promoting and Safety Activities of Promising Probiotic Strains - A Probiogenomics Review. *Trends Food Sci. Technol.* 108, 148–163. doi:10.1016/j.tifs.2020.12.017

Chandhni, P. R., Pradhan, D., Sowmya, K., Gupta, S., Kadyan, S., Choudhary, R., et al. (2021). Ameliorative Effect of Surface Proteins of Probiotic Lactobacilli in Colitis Mouse Models. *Front. Microbiol.* 12, 679773. doi:10.3389/fmicb.2021.679773

Chaudhari, N. M., Gupta, V. K., and Dutta, C. (2016). BPGA- an Ultra-fast Pan-Genome Analysis Pipeline. *Sci. Rep.* 6, 24373. doi:10.1038/srep24373

Dan, T., Ren, W., Liu, Y., Tian, J., Chen, H., Li, T., et al. (2019). Volatile Flavor Compounds Profile and Fermentation Characteristics of Milk Fermented by *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Front. Microbiol.* 10, 2183. doi:10.3389/fmicb.2019.02183

De Jesus, L. C. L., Drumond, M. M., Aburjaile, F. F., Sousa, T. J., Coelho-Rocha, N. D., Profeta, R., et al. (2021b). Probiogenomics of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: *In Silico*, *In Vitro*, and *In Vivo* Approaches. *Microorganisms* 9, 829. doi:10.3390/microorganisms9040829

De Jesus, L. C. L., de Jesus Sousa, T., Coelho-Rocha, N. D., Profeta, R., Barroso, F. A. L., Drumond, M. M., et al. (2021a). Safety Evaluation of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: a Health-Promoting Bacteria. *Probiotics Antimicro. Prot.* doi:10.1007/s12602-021-09826-z

De Jesus, L. C. L., Drumond, M. M., de Carvalho, A., Santos, S. S., Martins, F. S., Ferreira, Ê., et al. (2019). Protective Effect of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 in a Model of 5 Fluorouracil-Induced Intestinal Mucositis. *J. Funct. Foods* 53, 197–207. doi:10.1016/j.jff.2018.12.027

Delgado, S., Sánchez, B., Margolles, A., Ruas-Madiedo, P., and Ruiz, L. (2020). Molecules Produced by Probiotics and Intestinal Microorganisms with Immunomodulatory Activity. *Nutrients* 12, 391. doi:10.3390/nu12020391

Duar, R. M., Casaburi, G., Mitchell, R. D., Scofield, L. N. C., Ortega Ramirez, C. A., Barile, D., et al. (2020). Comparative Genome Analysis of *Bifidobacterium longum* subsp. *infantis* Strains Reveals Variation in Human Milk

Oligosaccharide Utilization Genes Among Commercial Probiotics. *Nutrients* 12, 3247. doi:10.3390/nu12113247

Duar, R. M., Lin, X. B., Zheng, J., Martino, M. E., Grenier, T., Pérez-Muñoz, M. E., et al. (2017). Lifestyles in Transition: Evolution and Natural History of the Genus *Lactobacillus*. *FEMS Microbiol. Rev.* 41, S27–S48. doi:10.1093/femsre/fux030

El Kafsi, H., Binesse, J., Loux, V., Buratti, J., Boudebbouze, S., Dervyn, R., et al. (2014). *Lactobacillus delbrueckii* ssp. *lactis* and ssp. *bulgaricus*: a Chronicle of Evolution in Action. *BMC Genomics* 15, 407. doi:10.1186/1471-2164-15-407

El-Khadragy, M. F., Al-Olayan, E. M., Elmallah, M. I. Y., Alharbi, A. M., Yehia, H. M., and Abdel Moneim, A. E. (2019). Probiotics and Yogurt Modulate Oxidative Stress and Fibrosis in Livers of *Schistosoma mansoni*-infected Mice. *BMC Complement. Altern. Med.* 19, 3. doi:10.1186/s12906-018-2406-3

Emms, D. M., and Kelly, S. (2019). OrthoFinder: Phylogenetic Orthology Inference for Comparative Genomics. *Genome Biol.* 20, 238. doi:10.1186/s13059-019-1832-y

Enright, A. J., Van Dongen, S., and Ouzounis, C. A. (2002). An Efficient Algorithm for Large-Scale Detection of Protein Families. *Nucleic Acids Res.* 30, 1575–1584. doi:10.1093/nar/30.7.1575

Espeche Turbay, M. B., de Moreno de LeBlanc, A., Perdigón, G., Savoy de Giori, G., and Hebert, E. M. (2012). β-Casein Hydrolysate Generated by the Cell Envelope-Associated Proteinase of *Lactobacillus delbrueckii* ssp. *lactis* CRL 581 Protects against Trinitrobenzene Sulfonic Acid-Induced Colitis in Mice. *J. Dairy Sci.* 95, 1108–1118. doi:10.3168/jds.2011-4735

Evivie, S. E., Abdelazez, A., Li, B., Lu, S., Liu, F., and Huo, G. (2020). *Lactobacillus delbrueckii* subsp. *bulgaricus* KLDS 1.0207 Exerts Antimicrobial and Cytotoxic Effects *In Vitro* and Improves Blood Biochemical Parameters *In Vivo* against Notable Foodborne Pathogens. *Front. Microbiol.* 11, 583070. doi:10.3389/fmicb.2020.583070

Ferreira, A. B., De Oliveira, M. N., Freitas, F. S., Alfenas-Zerbini, P., Da Silva, D. F., De Queiroz, M. V., et al. (2013). Increased Expression of Clp Genes in *Lactobacillus delbrueckii* UFV H2b20 Exposed to Acid Stress and Bile Salts. *Benef. Microbes* 4, 367–374. doi:10.3920/BM2013.0022

Fontana, A., Falasconi, I., Molinari, P., Treu, L., Basile, A., Vezzi, A., et al. (2019). Genomic Comparison of *Lactobacillus helveticus* Strains Highlights Probiotic Potential. *Front. Microbiol.* 10, 1380. doi:10.3389/fmicb.2019.01380

Gilbert, C., Atlan, D., Blanc, B., Portailer, R., Germond, J. E., Lapierre, L., et al. (1996). A New Cell Surface Proteinase: Sequencing and Analysis of the prtB Gene from *Lactobacillus delbruekii* subsp. *bulgaricus*. *J. Bacteriol.* 178, 3059–3065. doi:10.1128/jb.178.11.3059-3065.1996

Guimarães, L. C., Florczak-Wyspianska, J., de Jesus, L. B., Viana, M. V., Silva, A., Ramos, R. T., et al. (2015). Inside the Pan-Genome - Methods and Software Overview. *Curr. Genomics* 16, 245–252. doi:10.2174/1389202916666150423002311

Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. (2013). QUAST: Quality Assessment Tool for Genome Assemblies. *Bioinformatics* 29, 1072–1075. doi:10.1093/bioinformatics/btt086

Hallajzadeh, J., Eslami, R. D., and Tanomand, A. (2021). Effect of *Lactobacillus delbrueckii* subsp. *lactis* PTCC1057 on Serum Glucose, Fetuin-A ,and Sestrin 3 Levels in Streptozotocin-Induced Diabetic Mice. *Probiotics Antimicrob. Proteins* 13, 383–389. doi:10.1007/s12602-020-09693-0

Hao, P., Zheng, H., Yu, Y., Ding, G., Gu, W., Chen, S., et al. (2011). Complete Sequencing and Pan-Genomic Analysis of *Lactobacillus delbrueckii* subsp. *bulgaricus* Reveal its Genetic Basis for Industrial Yogurt Production. *PLoS One* 6, e15964. doi:10.1371/journal.pone.0015964

Hidalgo-Cantabrana, C., Moro-García, M. A., Blanco-Míguez, A., Fdez-Riverola, F., Oliván, M., Royo, L. J., et al. (2020). The Extracellular Proteins of *Lactobacillus acidophilus* DSM 20079T Display Anti-inflammatory Effect in Both in Piglets, Healthy Human Donors and Crohn's Disease Patients. *J. Funct. Foods* 64, 103660. doi:10.1016/j.jff.2019.103660

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). Expert Consensus Document. The International Scientific Association for Probiotics and Prebiotics Consensus Statement on the Scope and Appropriate Use of the Term Probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11, 506–514. doi:10.1038/nrgastro.2014.66

Hugo, A. A., Rolny, I. S., Romanin, D., and Pérez, P. F. (2017). *Lactobacillus delbrueckii* subsp. *lactis* (Strain CIDCA 133) Stimulates Murine Macrophages Infected with *Citrobacter rodentium*. *World J. Microbiol. Biotechnol.* 33, 48. doi:10.1007/s11274-017-2219-4

Hussein, W. E., Abdelhamid, A. G., Rocha-Mendoza, D., García-Cano, I., and Yousef, A. E. (2020). Assessment of Safety and Probiotic Traits of *Enterococcus durans* OSY-EGY, Isolated from Egyptian Artisanal Cheese, Using Comparative Genomics and Phenotypic Analyses. *Front. Microbiol.* 11, 608314. doi:10.3389/fmicb.2020.608314

Inglin, R. C., Meile, L., and Stevens, M. J. A. (2018). Clustering of Pan- and Core-Genome of *Lactobacillus* Provides Novel Evolutionary Insights for Differentiation. *BMC Genomics* 19, 284. doi:10.1186/s12864-018-4601-5

Kandler, O. (1983). Carbohydrate Metabolism in Lactic Acid Bacteria. *Ant. Van Leeuwenhoek* 49, 209–224. doi:10.1007/BF00399499

Kanehisa, M., Sato, Y., and Morishima, K. (2016). BlastKOALA and GhostKOALA: KEGG Tools for Functional Characterization of Genome and Metagenome Sequences. *J. Mol. Biol.* 428, 726–731. doi:10.1016/j.jmb.2015.11.006

Kanmani, P., Albarracin, L., Kobayashi, H., Hebert, E. M., Saavedra, L., Komatsu, R., et al. (2018). Genomic Characterization of *Lactobacillus delbrueckii* TUA4408L and Evaluation of the Antiviral Activities of its Extracellular Polysaccharides in Porcine Intestinal Epithelial Cells. *Front. Immunol.* 9, 2178. doi:10.3389/fimmu.2018.02178

Kano, H., Kita, J., Makino, S., Ikegami, S., and Itoh, H. (2013). Oral Administration of *Lactobacillus delbrueckii* subspecies *bulgaricus* OLL1073R-1 Suppresses Inflammation by Decreasing Interleukin-6 Responses in a Murine Model of Atopic Dermatitis. *J. Dairy Sci.* 96, 3525–3534. doi:10.3168/jds.2012-6514

Karp, P. D., Billington, R., Caspi, R., Fulcher, C. A., Latendresse, M., Kothari, A., et al. (2019). The BioCyc Collection of Microbial Genomes and Metabolic Pathways. *Brief. Bioinform.* 20, 1085–1093. doi:10.1093/bib/bbx085

Kim, E., Cho, E. J., Yang, S. M., and Kim, H. Y. (2021). Identification and Monitoring of *Lactobacillus delbrueckii* Subspecies Using Pangenomic-Based Novel Genetic Markers. *J. Microbiol. Biotechnol.* 31, 280–289. doi:10.4014/jmb.2009.09034

Laiño, J. E., LeBlanc, J. G., and Savoy de Giori, G. (2012). Production of Natural Folates by Lactic Acid Bacteria Starter Cultures Isolated from Artisanal Argentinean Yogurts. *Can. J. Microbiol.* 58, 581–588. doi:10.1139/w2012-026

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A., and LeBlanc, J. G. (2018). Effect of Riboflavin-Producing Bacteria against Chemically Induced Colitis in Mice. *J. Appl. Microbiol.* 124, 232–240. doi:10.1111/jam.13622

Li, B., Jin, D., Yu, S., Etareri Evivie, S., Muhammad, Z., Huo, G., et al. (2017). *In Vitro* and *In Vivo* Evaluation of *Lactobacillus delbrueckii* subsp. *bulgaricus* KLDS1.0207 for the Alleviative Effect on Lead Toxicity. *Nutrients* 9, 845. doi:10.3390/nu9080845

Li, C., Sun, J., Qi, X., and Liu, L. (2015). NaCl Stress Impact on the Key Enzymes in Glycolysis from *Lactobacillus bulgaricus* during Freeze-Drying. *Braz J. Microbiol.* 46, 1193–1199. doi:10.1590/S1517-838246420140595

Li, L., Stoeckert, C. J., and Roos, D. S. (2003). OrthoMCL: Identification of Ortholog Groups for Eukaryotic Genomes. *Genome Res.* 13, 2178–2189. doi:10.1101/gr.1224503

Li, W., Yang, L., Nan, W., Lu, J., Zhang, S., Ujiroghene, O. J., et al. (2020). Whole-genome Sequencing and Genomic-Based Acid Tolerance Mechanisms of *Lactobacillus delbrueckii* subsp. *bulgaricus* LJJ. *Appl. Microbiol. Biotechnol.* 104, 7631–7642. doi:10.1007/s00253-020-10788-5

Lian, X., Yang, S., Li, H., Fu, C., and Zhang, Z. (2019). Machine-Learning-Based Predictor of Human-Bacteria Protein-Protein Interactions by Incorporating Comprehensive Host-Network Properties. *J. Proteome Res.* 18, 2195–2205. doi:10.1021/acs.jproteome.9b00074

Liu, M., Bayjanov, J. R., Renckens, B., Nauta, A., and Siezen, R. J. (2010). The Proteolytic System of Lactic Acid Bacteria Revisited: a Genomic Comparison. *BMC Genomics* 11, 36. doi:10.1186/1471-2164-11-36

McFarland, L. V., Evans, C. T., and Goldstein, E. J. C. (2018). Strain-Specificity and Disease-Specificity of Probiotic Efficacy: A Systematic Review and Meta-Analysis. *Front. Med. (Lausanne)* 5, 124. doi:10.3389/fmed.2018.00124

Moradi, M., Kousheh, S. A., Almasi, H., Alizadeh, A., Guimarães, J. T., Yılmaz, N., et al. (2020). Postbiotics Produced by Lactic Acid Bacteria: The Next Frontier in Food Safety. *Compr. Rev. Food Sci. Food Saf.* 19, 3390–3415. doi:10.1111/1541-4337.12613

Nakai, H., Murosaki, S., Yamamoto, Y., Furutani, M., Matsuoka, R., and Hirose, Y. (2021). Safety and Efficacy of Using Heat-Killed *Lactobacillus plantarum* L-137:

High-Dose and Long-Term Use Effects on Immune-Related Safety and Intestinal Bacterial Flora. *J. Immunotoxicol.* 18, 127–135. doi:10.1080/1547691X.2021.1979698

Oliveira de Almeida, M., Carvalho, R., Figueira Aburjaile, F., Malcher Miranda, F., Canário Cerqueira, J., Brenig, B., et al. (2021). Characterization of the First Vaginal *Lactobacillus crispatus* Genomes Isolated in Brazil. *PeerJ* 9, e11079. doi:10.7717/peerj.11079

Oliveira, L. C., Saraiva, T. D., Silva, W. M., Pereira, U. P., Campos, B. C., Benevides, L. J., et al. (2017). Analyses of the Probiotic Property and Stress Resistance-Related Genes of *Lactococcus lactis* subsp. *lactis* NCDO 2118 through Comparative Genomics and *In Vitro* Assays. *PLoS One* 12, e0175116. doi:10.1371/journal.pone.0175116

Papadimitriou, K., Alegría, Á., Bron, P. A., de Angelis, M., Gobbetti, M., Kleerebezem, M., et al. (2016). Stress Physiology of Lactic Acid Bacteria. *Microbiol. Mol. Biol. Rev.* 80, 837–890. doi:10.1128/MMBR.00076-15

Papadimitriou, K., Zoumpopoulou, G., Foligné, B., Alexandraki, V., Kazou, M., Pot, B., et al. (2015). Discovering Probiotic Microorganisms: *In Vitro*, *In Vivo*, Genetic and Omics Approaches. *Front. Microbiol.* 6, 58. doi:10.3389/fmicb.2015.00058

Plé, C., Breton, J., Richoux, R., Nurdin, M., Deutsch, S. M., Falentin, H., et al. (2016). Combining Selected Immunomodulatory *Propionibacterium freudenreichii* and *Lactobacillus delbrueckii* Strains: Reverse Engineering Development of an Anti-inflammatory Cheese. *Mol. Nutr. Food Res.* 60, 935–948. doi:10.1002/mnfr.201500580

Qiu, X., Wu, G., Wang, L., Tan, Y., and Song, Z. (2021). *Lactobacillus delbrueckii* Alleviates Depression-like Behavior through Inhibiting Toll-like Receptor 4 (TLR4) Signaling in Mice. *Ann. Transl. Med.* 9, 366. doi:10.21037/atm-20-4411

Reid, G., Abrahamsson, T., Bailey, M., Bindels, L. B., Bubnov, R., Ganguli, K., et al. (2017). How Do Probiotics and Prebiotics Function at Distant Sites?. *Benef. Microbes* 8, 521–533. doi:10.3920/BM2016.0222

Rolny, I. S., Tiscornia, I., Racedo, S. M., Pérez, P. F., and Bollati-Fogolín, M. (2016). *Lactobacillus delbrueckii* subsp *lactis* CIDCA 133 Modulates Response of Human Epithelial and Dendritic Cells Infected with *Bacillus cereus*. *Benef. Microbes* 7, 749–760. doi:10.3920/BM2015.0191

Sachdeva, G., Kumar, K., Jain, P., and Ramachandran, S. (2005). SPAAN: a Software Program for Prediction of Adhesins and Adhesin-like Proteins Using Neural Networks. *Bioinformatics* 21, 483–491. doi:10.1093/bioinformatics/bti028

Salvetti, E., Torriani, S., and Felis, G. E. (2012). The Genus *Lactobacillus*: A Taxonomic Update. *Probiotics Antimicrob. Proteins* 4, 217–226. doi:10.1007/s12602-012-9117-8

Salvetti, E., and O'Toole, P. W. (2018). The Genomic Basis of Lactobacilli as Health-Promoting Organisms. *Microbiol. Spectr.* 5, 49–71. doi:10.1128/microbiolspec.BAD-0011-201610.1128/9781555819705.ch2

Sang, L. X., Chang, B., Dai, C., Gao, N., Liu, W. X., and Jiang, M. (2013). Heat-killed VSL#3 Ameliorates Dextran Sulfate Sodium (DSS)-Induced Acute Experimental Colitis in Rats. *Int. J. Mol. Sci.* 15, 15–28. doi:10.3390/ijms15010015

Santos Rocha, C., Gomes-Santos, A. C., Garcias Moreira, T., de Azevedo, M., Diniz Luerce, T., Mariadassou, M., et al. (2014). Local and Systemic Immune Mechanisms Underlying the Anti-colitis Effects of the Dairy Bacterium *Lactobacillus delbrueckii*. *PLoS One* 9, e85923. doi:10.1371/journal.pone.0085923

Santos Rocha, C., Lakhdari, O., Blottière, H. M., Blugeon, S., Sokol, H., Bermúdez-Humarán, L. G., et al. (2012). Anti-inflammatory Properties of Dairy Lactobacilli. *Inflamm. Bowel Dis.* 18, 657–666. doi:10.1002/ibd.21834

Savino, F., Cordisco, L., Tarasco, V., Locatelli, E., Di Gioia, D., Oggero, R., et al. (2011). Antagonistic Effect of *Lactobacillus* Strains against Gas-Producing Coliforms Isolated from Colicky Infants. *BMC Microbiol.* 11, 157. doi:10.1186/1471-2180-11-157

Seemann, T. (2014). Prokka: Rapid Prokaryotic Genome Annotation. *Bioinformatics* 30, 2068–2069. doi:10.1093/bioinformatics/btu153

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res.* 13, 2498–2504. doi:10.1101/gr.1239303

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. (2015). BUSCO: Assessing Genome Assembly and Annotation Completeness with Single-Copy Orthologs. *Bioinformatics* 31, 3210–3212. doi:10.1093/bioinformatics/btv351

Soares, S. C., Silva, A., Trost, E., Blom, J., Ramos, R., Carneiro, A., et al. (2013). The Pan-Genome of the Animal Pathogen *Corynebacterium pseudotuberculosis* Reveals Differences in Genome Plasticity between the Biovar ovis and Equi Strains. *PLoS One* 8, e53818. doi:10.1371/journal.pone.0053818

Sun, Z., Harris, H. M., McCann, A., Guo, C., Argimón, S., Zhang, W., et al. (2015). Expanding the Biotechnology Potential of Lactobacilli through Comparative Genomics of 213 Strains and Associated Genera. *Nat. Commun.* 6, 8322. doi:10.1038/ncomms9322

Teame, T., Wang, A., Xie, M., Zhang, Z., Yang, Y., Ding, Q., et al. (2020). Paraprobiotics and Postbiotics of Probiotic Lactobacilli, Their Positive Effects on the Host and Action Mechanisms: A Review. *Front. Nutr.* 7, 570344. doi:10.3389/fnut.2020.570344

Trindade, L. M., Torres, L., Matos, I. D., Miranda, V. C., de Jesus, L. C. L., Cavalcante, G., et al. (2021). Paraprobiotic *Lacticaseibacillus rhamnosus* Protects Intestinal Damage in an Experimental Murine Model of Mucositis. *Probiotics Antimicro. Prot.* doi:10.1007/s12602-021-09842-z

Usui, Y., Kimura, Y., Satoh, T., Takemura, N., Ouchi, Y., Ohmiya, H., et al. (2018). Effects of Long-Term Intake of a Yogurt Fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* 2038 and *Streptococcus Thermophilus* 1131 on Mice. *Int. Immunol.* 30, 319–331. doi:10.1093/intimm/dxy035

van Heel, A. J., de Jong, A., Song, C., Viel, J. H., Kok, J., and Kuipers, O. P. (2018). BAGEL4: a User-Friendly Web Server to Thoroughly Mine RiPPs and Bacteriocins. *Nucleic Acids Res.* 46, W278–W281. doi:10.1093/nar/gky383

Ventura, M., Turroni, F., and van Sinderen, D. (2012). Probiogenomics as a Tool to Obtain Genetic Insights into Adaptation of Probiotic Bacteria to the Human Gut. *Bioeng. Bugs* 3, 73–79. doi:10.4161/bbug.18540

Villegas, J. M., Brown, L., Savoy de Giori, G., and Hebert, E. M. (2015). Characterization of the Mature Cell Surface Proteinase of *Lactobacillus delbrueckii* subsp. *lactis* CRL 581. *Appl. Microbiol. Biotechnol.* 99, 4277–4286. doi:10.1007/s00253-014-6258-6

Wan, Y., Xin, Y., Zhang, C., Wu, D., Ding, D., Tang, L., et al. (2014). Fermentation Supernatants of *Lactobacillus delbrueckii* Inhibit Growth of Human Colon Cancer Cells and Induce Apoptosis through a Caspase 3-dependent Pathway. *Oncol. Lett.* 7, 1738–1742. doi:10.3892/ol.2014.1959

Wanna, W., Surachat, K., Kaitimonchai, P., and Phongdara, A. (2021). Evaluation of Probiotic Characteristics and Whole Genome Analysis of *Pediococcus pentosaceus* MR001 for Use as Probiotic Bacteria in Shrimp Aquaculture. *Sci. Rep.* 11, 18334. doi:10.1038/s41598-021-96780-z

Ye, H., Zhang, X., Jiang, Y., Guo, M., Liu, X., Zhao, J., et al. (2021). Comparative Peptidomics Analysis of Fermented Milk by *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus delbrueckii* ssp. *lactis*. *Foods* 10, 3028. doi:10.3390/foods10123028

Zhang, W., Ji, H., Zhang, D., Liu, H., Wang, S., Wang, J., et al. (2018). Complete Genome Sequencing of *Lactobacillus plantarum* ZLP001, a Potential Probiotic that Enhances Intestinal Epithelial Barrier Function and Defense against Pathogens in Pigs. *Front. Physiol.* 9, 1689. doi:10.3389/fphys.2018.01689

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., et al. (2020). A Taxonomic Note on the Genus *Lactobacillus*: Description of 23 Novel Genera, Emended Description of the Genus *Lactobacillus* Beijerinck 1901, and Union of Lactobacillaceae and Leuconostocaceae. *Int. J. Syst. Evol. Microbiol.* 70, 2782–2858. doi:10.1099/ijsem.0.004107

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

10. CONCLUSÕES GERAIS

Esta Tese caracterizou o perfil probiótico de *Lactobacillus delbrueckii* CIDCA 133 usando uma abordagem probiogenômica.

L. delbrueckii CIDCA 133 mostrou-se eficaz em reduzir danos inflamatórios e histopatólogicos induzidos pelo quimioterápico 5-FU, demonstrando, assim, propriedades probióticas funcionais *in vivo*. Nossos achados revelaram que muitos dos efeitos benéficos promovidos por CIDCA 133, principalmente seu potencial anti-inflamatório, são atribuidos a proteínas secretadas e ou expostas à superficie celular capazes de possivelmente inibir a sinalização da via inflamatória NF-κB via interação com receptores celulares do hospereiro. A hipótese de que estas proteínas (expostas à superficie bacteriana e /ou secretadas) ditam os efeitos imunomodulatórios de CIDCA 133 foi sustentada após observarmos o efeito anti-inflamatório da linhagem após a sua inativação pelo calor. Além disso, foram identificados possíveis fatores genéticos que possam estar envolvidos com a capacidade da linhagem de tolerar agentes estressores do TGI e seu potencial antibacteriano. Muitos desses fatores genéticos são compartilhados por outras linhagens probióticas da espécie, sendo que a proteína PrtB parece ser a candidata alvo responsável pelas propriedades anti-inflamatórias dessas linhagens, conforme observado através da genômica comparativa.

Por meio de análises genômicas associadas com estudos *in vitro* e *in vivo* foi possível também identificar os elementos genéticos (plasmídeo e regiões de fagos) adquiridos por *L. delbrueckii* CIDCA 133 possivelmente devido à pressão seletiva que atua sobre seu hospedeiro. No entanto, CIDCA 133 demonstra possuir mecanismos de evitar a inserção de material genético exógeno ao seu genoma, por meio do sistema CRISPR-Cas. Em relação aos aspectos associados à segurança da linhagem para o consumo, foi observado que, apesar de possuir genes associados à resistência aos antibióticos, CIDCA 133 não possui propriedades de disseminá-los. Nossos achados mostram também que os genes preditos como associados à virulência são apenas fatores de nicho/colonização utilizados estrategicamente pela linhagem para adaptação e sobrevivência. Portanto, os achados do nosso trabalho mostram que *L. delbrueckii* CIDCA 133 é uma promissora bactéria capaz de promover benéficios à saúde do hospedeiro e apresenta níveis de segurança para ser consumida e utilizada em futuras aplicações probióticas, principalmente aquelas direcionadas ao tratamento de doenças inflamatórias intestinais.

11. PERSPECTIVAS

- Avaliar a produção de metabólitos bioativos (SCFA, vitaminas) preditos no genoma de CIDCA 133 por meio de Cromatografia Líquida de Alta Eficiência;

- Avaliar o efeito de CIDCA 133 sobre a regulação da microbiota intestinal em homeostase e disbiótica.

- Avaliar o efeito das proteínas extraídas da superfície celular de CIDCA 133 em modelo de inflamação intestinal (colite ou mucosite).

- Identificar, por meio de análise proteômica, as proteínas da superficie celular e secretadas de CIDCA 133 que estejam associadas a seu efeito anti-inflamatório.

- Realizar *Knockout* de genes alvos codificantes de proteínas anti-inflamatórias identificadas no genoma de CIDCA 133, e avaliar o perfil fenotípico desses mutantes.

- Identificar e comparar sequências codificantes da proteína PrtB em diferentes linhagens da espécie *Lactobacillus delbrueckii;*

- Clonar o gene codificante da proteína PrtB e avaliar seu efeito anti-inflamatório em modelo de inflamação intestinal (colite ou mucosite);

- Avaliar o efeito de CIDCA 133 sobre a inibição de patógenos causadores de infecções entéricas (como por exemplo *Salmonella* sp.) em modelo *in vivo*;

- Avaliar aspectos de segurança do consumo de CIDCA 133 a longo prazo e com altas doses.

12. REFERÊNCIAS

Acurcio, L. B., Bastos, R. W., Sandes, S. H. de C., Guimarães, A. C. de C., Alves, C. G., Reis, D.C. dos, Wuyts, S., Nunes, Á. C., Cassali, G. D., Lebeer, S., Souza, M. R. de, & Nicoli, J. R. (2017). Protective effects of milk fermented by *Lactobacillus plantarum* B7 from Brazilian artisanal cheese on a *Salmonella enterica* serovar Typhimurium infection in BALB/c mice. Journal of Functional Foods, 33, 436–445. https://doi.org/10.1016/j.jff.2017.04.010

Alayande, K. A., Aiyegoro, O. A., Nengwekhulu, T. M., Katata-Seru, L., & Ateba, C. N. (2020). Integrated genome-based probiotic relevance and safety evaluation of *Lactobacillus reuteri* PNW1. PLOS ONE, 15(7), e0235873. https://doi.org/10.1371/journal.pone.0235873

Alexandraki, V., Kazou, M., Pot, B., Tsakalidou, E., & Papadimitriou, K. (2019). Whole-genome sequence data and analysis of *Lactobacillus delbrueckii* subsp. *lactis* ACA-DC 178 isolated from Greek Kasseri cheese. Data in Brief, 25, 104282. https://doi.org/10.1016/j.dib.2019.104282

Aliakbarpour, H. R., Chamani, M., Rahimi, G., Sadeghi, A. A., & Qujeq, D. (2012). The *Bacillus subtilis* and Lactic Acid Bacteria Probiotics Influences Intestinal Mucin Gene Expression, Histomorphology and Growth Performance in Broilers. Asian-Australasian Journal of Animal Sciences, 25(9), 1285–1293. https://doi.org/10.5713/ajas.2012.12110

Alvarez, M. A., & Moreno-Arribas, M. V. (2014). The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. Trends in Food Science & Technology, 39(2), 146–155. https://doi.org/10.1016/j.tifs.2014.07.007

Arnold, J. W., Simpson, J. B., Roach, J., Kwintkiewicz, J., & Azcarate-Peril, M. A. (2018). Intra- species Genomic and Physiological Variability Impact Stress Resistance in Strains of Probiotic Potential. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.00242

Batista, V. L., da Silva, T. F., de Jesus, L. C. L., Coelho-Rocha, N. D., Barroso, F. A. L., Tavares, L. M., Azevedo, V., Mancha-Agresti, P., & Drumond, M. M. (2020). Probiotics, Prebiotics, Synbiotics, and Paraprobiotics as a Therapeutic Alternative for Intestinal Mucositis. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.544490

Belkaid, Y., & Hand, T. W. (2014). Role of the Microbiota in Immunity and Inflammation. Cell, 157(1), 121–141. https://doi.org/10.1016/j.cell.2014.03.011

Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic Mechanisms of Action. Annals of Nutrition and Metabolism, 61(2), 160–174. https://doi.org/10.1159/000342079

Bischoff, S. C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J.-D., Serino, M., Tilg, H., Watson, A., & Wells, J. M. (2014). Intestinal permeability – a new target for disease prevention and therapy. BMC Gastroenterology, 14(1), 189. https://doi.org/10.1186/s12876-014-0189-7

Bjarnason, I., Sission, G., & Hayee, B. (2019). A randomised, double-blind, placebo-controlled trial of a multi-strain probiotic in patients with asymptomatic ulcerative colitis and Crohn's disease. Inflammopharmacology, 27(3), 465–473. https://doi.org/10.1007/s10787-019-00595-4

Blackwood, B. P., Yuan, C. Y., Wood, D. R., Nicolas, J. D., Grothaus, J. S., & Hunter, C. J. (2017). Probiotic Lactobacillus Species Strengthen Intestinal Barrier Function and Tight Junction Integrity in Experimental Necrotizing Enterocolitis. Journal of Probiotics & Health, 05(01). https://doi.org/10.4172/2329-8901.1000159

Broadbent, J. R., Cai, H., Larsen, R. L., Hughes, J. E., Welker, D. L., De Carvalho, V. G., Tompkins, T. A., Ardö, Y., Vogensen, F., De Lorentiis, A., Gatti, M., Neviani, E., & Steele, J. L. (2011). Genetic diversity in proteolytic enzymes and amino acid metabolism among *Lactobacillus helveticus* strains. Journal of Dairy Science, 94(9), 4313–4328. https://doi.org/10.3168/jds.2010- 4068

Burgain, J., Scher, J., Francius, G., Borges, F., Corgneau, M., Revol-Junelles, A. M., Cailliez-Grimal, C., & Gaiani, C. (2014). Lactic acid bacteria in dairy food: Surface characterization and interactions with food matrix components. Advances in Colloid and Interface Science, 213, 21– 35. https://doi.org/10.1016/j.cis.2014.09.005

Burgueño, J. F., & Abreu, M. T. (2020). Epithelial Toll-like receptors and their role in gut homeostasis and disease. Nature Reviews Gastroenterology & Hepatology, 17(5), 263–278. https://doi.org/10.1038/s41575-019-0261-4

Buron-Moles, G., Chailyan, A., Dolejs, I., Forster, J., & Mikš, M. H. (2019). Uncovering carbohydrate metabolism through a genotype-phenotype association study of 56 lactic acid bacteria genomes. Applied Microbiology and Biotechnology, 103(7), 3135–3152. https://doi.org/10.1007/s00253-019-09701-6

Camilleri, M., Madsen, K., Spiller, R., Van Meerveld, B. G., & Verne, G. N. (2012). Intestinal barrier function in health and gastrointestinal disease. Neurogastroenterology & Motility, 24(6), 503–512. https://doi.org/10.1111/j.1365-2982.2012.01921.x

Campedelli, I., Mathur, H., Salvetii, E., Clarke, S., Rea, M. C., Torriani, S., Ross, R. P., Hill, C., & O'Toole, P. W. (2018). Genus-Wide Assessment of Antibiotic Resistance in *Lactobacillus* spp. Applied and Environmental Microbiology, 85(1). https://doi.org/10.1128/AEM.01738-18

Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (n.d.). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Annals of Gastroenterology, 28(2), 203–209. http://www.ncbi.nlm.nih.gov/pubmed/25830558

Casarotti, S. N., Carneiro, B. M., Todorov, S. D., Nero, L. A., Rahal, P., & Penna, A. L. B. (2017). In vitro assessment of safety and probiotic potential characteristics of *Lactobacillus* strains isolated from water buffalo mozzarella cheese. Annals of Microbiology, 67(4), 289–301. https://doi.org/10.1007/s13213-017-1258-2

Caspi, R., Billington, R., Fulcher, C.A., Keseler, I.M., Kothari, A., Krummenacker, M., Latendresse, M., Midford, P.E., Ong, W.K., Paley, S., Subhraveti, P. and Karp, P.D. (2019), BioCyc: A Genomic and Metabolic Web Portal with Multiple Omics Analytical Tools. The FASEB Journal, 33: 473.2-473.2. https://doi.org/10.1096/fasebj.2019.33.1_supplement.473.2

Castilho, N. P. A., Colombo, M., Oliveira, L. L. de, Todorov, S. D., & Nero, L. A. (2019). *Lactobacillus curvatus* UFV-NPAC1 and other lactic acid bacteria isolated from calabresa, a fermented meat product, present high bacteriocinogenic activity against *Listeria monocytogenes*. BMC Microbiology, 19(1), 63. https://doi.org/10.1186/s12866-019-1436-4

Castro-López, C., García, H. S., Guadalupe Martínez-Ávila, G. C., González-Córdova, A. F., Vallejo-Cordoba, B., & Hernández-Mendoza, A. (2021). Genomics-based approaches to identify and predict the health-promoting and safety activities of promising probiotic strains – A probiogenomics review. Trends in Food Science & Technology, 108, 148–163. https://doi.org/10.1016/j.tifs.2020.12.017

Chen, L., Gu, Q., Li, P., Chen, S., & Li, Y. (2019). Genomic analysis of *Lactobacillus reuteri* WHH 1689 reveals its probiotic properties and stress resistance. Food Science & Nutrition, 7(2), 844–857. https://doi.org/10.1002/fsn3.934

Chistiakov, D. A., Bobryshev, Y. V., Kozarov, E., Sobenin, I. A., & Orekhov, A. N. (2015). Intestinal mucosal tolerance and impact of gut microbiota to mucosal tolerance. Frontiers in Microbiology, 5. https://doi.org/10.3389/fmicb.2014.00781

Chokesajjawatee, N., Santiyanont, P., Chantarasakha, K., Kocharin, K., Thammarongtham, C., Lertampaiporn, S., Vorapreeda, T., Srisuk, T., Wongsurawat, T., Jenjaroenpun, P., Nookaew, I., & Visessanguan, W. (2020). Safety Assessment of a Nham Starter Culture *Lactobacillus plantarum* BCC9546 via Whole-genome Analysis. Scientific Reports, 10(1), 10241. https://doi.org/10.1038/s41598-020-66857-2

Collado, M. C., Gueimonde, M., & Salminen, S. (2010). Probiotics in Adhesion of Pathogens. In Bioactive Foods in Promoting Health (pp. 353–370). Elsevier. https://doi.org/10.1016/B978-0-12- 374938-3.00023-2

Corrêa-Oliveira, R., Fachi, J. L., Vieira, A., Sato, F. T., & Vinolo, M. A. R. (2016). Regulation of immune cell function by short-chain fatty acids. Clinical & Translational Immunology, 5(4), e73. https://doi.org/10.1038/cti.2016.17

Cristofori, F., Dargenio, V. N., Dargenio, C., Miniello, V. L., Barone, M., and Francavilla, R. (2021). Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. Front. Immunol. 12:578386. doi: 10.3389/fimmu.2021.578386

Cruz, A. G., Cadena, R. S., Walter, E. H. M., Mortazavian, A. M., Granato, D., Faria, J. A. F., & Bolini, H. M. A. (2010). Sensory Analysis: Relevance for Prebiotic, Probiotic, and Synbiotic Product Development. Comprehensive Reviews in Food Science and Food Safety, 9(4), 358–373. https://doi.org/10.1111/j.1541-4337.2010.00115.x

Darbro BW, Petroelje BK, Doern G V. (2009) *Lactobacillus delbrueckii* as the Cause of Urinary Tract Infection. Journal of Clinical Microbiology, 47, 275–277. https://doi.org/10.1128/JCM.01630-08

De Jesus, L. C. L., Drumond, M. M., de Carvalho, A., Santos, S. S., Martins, F. S., Ferreira, Ê., Fernandes, R. S., de Barros, A. L. B., do Carmo, F. L. R., Perez, P. F., Azevedo, V., & Mancha-Agresti, P. (2019). Protective effect of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 in a model of 5 Fluorouracil-Induced intestinal mucositis. Journal of Functional Foods, 53, 197–207. https://doi.org/10.1016/j.jff.2018.12.027

de Oliveira, G. L. V., Leite, A. Z., Higuchi, B. S., Gonzaga, M. I., & Mariano, V. S. (2017). Intestinal dysbiosis and probiotic applications in autoimmune diseases. Immunology, 152(1), 1– 12. https://doi.org/10.1111/imm.12765

Deutsch, S.-M., Mariadassou, M., Nicolas, P., Parayre, S., Le Guellec, R., Chuat, V., Peton, V., Le Maréchal, C., Burati, J., Loux, V., Briard-Bion, V., Jardin, J., Plé, C., Foligné, B., Jan, G., & Falentin, H. (2017). Identification of proteins involved in the anti-inflammatory properties of *Propionibacterium freudenreichii* by means of a multi-strain study. Scientific Reports, 7(1), 46409. https://doi.org/10.1038/srep46409

Dieterich, W., Schink, M., & Zopf, Y. (2018). Microbiota in the Gastrointestinal Tract. Medical Sciences, 6(4), 116. https://doi.org/10.3390/medsci6040116

Dimitrellou, D., Salamoura, C., Kontogianni, A., Katsipi, D., Kandylis, P., Zakynthinos, G., & Varzakas, T. (2019). Effect of Milk Type on the Microbiological, Physicochemical and Sensory Characteristics of Probiotic Fermented Milk. Microorganisms, 7(9), 274. https://doi.org/10.3390/microorganisms7090274

Duar, R. M., Casaburi, G., Mitchell, R. D., Scofield, L. N. C., Ortega Ramirez, C. A., Barile, D., et al. (2020). Comparative Genome Analysis of *Bifidobacterium longum* subsp. *infantis* Strains Reveals Variation in Human Milk Oligosaccharide Utilization Genes Among Commercial Probiotics. Nutrients 12, 3247. doi:10.3390/nu12113247

Fedorak, R. N., Feagan, B. G., Hotte, N., Leddin, D., Dieleman, L. A., Petrunia, D. M., Enns, R., Bitton, A., Chiba, N., Paré, P., Rostom, A., Marshall, J., Depew, W., Bernstein, C. N., Panaccione, R., Aumais, G., Steinhart, A. H., Cockeram, A., Bailey, R. J., Madsen, K. (2015). The Probiotic VSL#3 Has Anti-inflammatory Effects and Could Reduce Endoscopic Recurrence After Surgery for Crohn's Disease. Clinical Gastroenterology and Hepatology, 13(5), 928-935.e2. https://doi.org/10.1016/j.cgh.2014.10.031

Ferreira, A. B., De Oliveira, M. . V., Freitas, F. S., Alfenas-Zerbini, P., Da Silva, D. F., De Queiroz, M. V., Borges, A. C., & De Moraes, C. A. (2013). Increased expression of clp genes in *Lactobacillus delbrueckii* UFV H2b20 exposed to acid stress and bile salts. Beneficial Microbes, 4(4), 367–374. https://doi.org/10.3920/BM2013.0022

Fontana, A., Falasconi, I., Molinari, P., Treu, L., Basile, A., Vezzi, A., Campanaro, S., & Morelli, L. (2019). Genomic Comparison of *Lactobacillus helveticus* Strains Highlights Probiotic Potential. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.01380

Gao, K., Wang, C., Liu, L., Dou, X., Liu, J., Yuan, L., Zhang, W., & Wang, H. (2017). Immunomodulation and signaling mechanism of *Lactobacillus rhamnosus* GG and its components on porcine intestinal epithelial cells stimulated by lipopolysaccharide. Journal of Microbiology, Immunology and Infection, 50(5), 700–713. https://doi.org/10.1016/j.jmii.2015.05.002

Gaspar, C., Donders, G. G., Palmeira-de-Oliveira, R., Queiroz, J. A., Tomaz, C., Martinez-de-Oliveira, J., & Palmeira-de-Oliveira, A. (2018). Bacteriocin production of the probiotic *Lactobacillus acidophilus* KS400. AMB Express, 8(1), 153. https://doi.org/10.1186/s13568-018- 0679-z

Gaucher, F., Bonnassie, S., Rabah, H., Marchand, P., Blanc, P., Jeantet, R., & Jan, G. (2019). Review: Adaptation of Beneficial Propionibacteria, Lactobacilli, and Bifidobacteria Improves Tolerance Toward Technological and Digestive Stresses. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.00841

George, F., Daniel, C., Thomas, M., Singer, E., Guilbaud, A., Tessier, F. J., Revol-Junelles, A.-M., Borges, F., & Foligné, B. (2018). Occurrence and Dynamism of Lactic Acid Bacteria in Distinct Ecological Niches: A Multifaceted Functional Health Perspective. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.02899

Górska, A., Przystupski, D., Niemczura, M. J., & Kulbacka, J. (2019). Probiotic Bacteria: A Promising Tool in Cancer Prevention and Therapy. Current Microbiology, 76(8), 939–949. https://doi.org/10.1007/s00284-019-01679-8

Gore S, Tucker K (2020) Bloodstream infections with typical probiotic organisms. Infect Control Hosp Epidemiol 41:s22–s22. https://doi.org/10.1017/ice.2020.498

Gosmann, C., Anahtar, M. N., Handley, S. A., Farcasanu, M., Abu-Ali, G., Bowman, B. A., Padavattan, N., Desai, C., Droit, L., Moodley, A., Dong, M., Chen, Y., Ismail, N., Ndung'u, T., Ghebremichael, M. S., Wesemann, D. R., Mitchell, C., Dong, K. L., Huttenhower, C., Kwon, D. S. (2017). *Lactobacillus*-Deficient Cervicovaginal Bacterial Communities Are Associated with Increased HIV Acquisition in Young South African Women. Immunity, 46(1), 29–37. https://doi.org/10.1016/j.immuni.2016.12.013

Halder, D., Mandal, M., Chatterjee, S., Pal, N., & Mandal, S. (2017). Indigenous Probiotic *Lactobacillus* Isolates Presenting Antibiotic like Activity against Human Pathogenic Bacteria. Biomedicines, 5(4), 31. https://doi.org/10.3390/biomedicines5020031

Hazebrouck, S., Oozeer, R., Adel-Patient, K., Langella, P., Rabot, S., Wal, J.-M., & Corthier, G. (2006). Constitutive Delivery of Bovine β-Lactoglobulin to the Digestive Tracts of Gnotobiotic Mice by Engineered *Lactobacillus casei*. Applied and Environmental Microbiology, 72(12), 7460– 7467. https://doi.org/10.1128/AEM.01032-06

Hemarajata, P., & Versalovic, J. (2013). Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therapeutic Advances in Gastroenterology, 6(1), 39–51. https://doi.org/10.1177/1756283X12459294

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology, 11(8), 506–514. https://doi.org/10.1038/nrgastro.2014.66

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions Between the Microbiota and the Immune System. Science, 336(6086), 1268–1273. https://doi.org/10.1126/science.1223490

Houeix, B., Synowsky, S., Cairns, M. T., Kane, M., Kilcoyne, M., & Joshi, L. (2019). Identification of putative adhesins and carbohydrate ligands of *Lactobacillus paracasei* using a combinatorial in silico and glycomics microarray profiling approach. Integrative Biology. https://doi.org/10.1093/intbio/zyz026

Hsieh, M.-C., Tsai, W.-H., Jheng, Y.-P., Su, S.-L., Wang, S.-Y., Lin, C.-C., Chen, Y.-H., & Chang, W.-W. (2018). The beneficial effects of *Lactobacillus reuteri* ADR-1 or ADR-3 consumption on type 2 diabetes mellitus: a randomized, double-blinded, placebo-controlled trial. Scientific Reports, 8(1), 16791. https://doi.org/10.1038/s41598-018-35014-1

Hugo, A.A., De Antoni, G. L., & Pérez, P. F. (2010). *Lactobacillus delbrueckii* subsp *lactis* (strain CIDCA 133) resists the antimicrobial activity triggered by molecules derived from enterocyte-like Caco-2 cells. Letters in Applied Microbiology, 50(4), 335–340. https://doi.org/10.1111/j.1472- 765X.2010.02796.x

Hugo, A.A., Kakisu, E., De Antoni, G. L., & Pérez, P. F. (2008). Lactobacilli antagonize biological effects of enterohaemorrhagic *Escherichia coli* in vitro. Letters in Applied Microbiology, 46(6), 613–619. https://doi.org/10.1111/j.1472-765X.2008.02363.x

Hugo, A.A., Tymczyszyn, E. E., Gómez-Zavaglia, A., & Pérez, P. F. (2012). Effect of human defensins on lactobacilli and liposomes. Journal of Applied Microbiology, 113(6), 1491–1497. https://doi.org/10.1111/j.1365-2672.2012.05433.x

Hugo, Ayelén A., Rolny, I. S., Romanin, D., & Pérez, P. F. (2017). *Lactobacillus delbrueckii* subsp. *lactis* (strain CIDCA 133) stimulates murine macrophages infected with Citrobacter rodentium. World Journal of Microbiology and Biotechnology, 33(3), 48. https://doi.org/10.1007/s11274-017-2219-4

Hussein, W. E., Abdelhamid, A. G., Rocha-Mendoza, D., García-Cano, I., and Yousef, A. E. (2020). Assessment of Safety and Probiotic Traits of *Enterococcus durans* OSY-EGY, Isolated from Egyptian Artisanal Cheese, Using Comparative Genomics and Phenotypic Analyses. Front. Microbiol. 11, 608314. doi:10.3389/fmicb.2020.608314

Jani, M., Sengupta, S., Hu, K., & Azad, R. K. (2017). Deciphering pathogenicity and antibiotic resistance islands in methicillin-resistant *Staphylococcus aureus* genomes. Open Biology, 7(12), 170094. https://doi.org/10.1098/rsob.170094

Kaci, G., Lakhdari, O., Doré, J., Ehrlich, S. D., Renault, P., Blottière, H. M., & Delorme, C. (2011). Inhibition of the NF-κB Pathway in Human Intestinal Epithelial Cells by Commensal *Streptococcus salivarius*. Applied and Environmental Microbiology, 77(13), 4681–4684. https://doi.org/10.1128/AEM.03021-10

Kandler, O. (1983). Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek, 49(3), 209–224. https://doi.org/10.1007/BF00399499

Kawasaki, T., & Kawai, T. (2014). Toll-Like Receptor Signaling Pathways. Frontiers in Immunology, 5. https://doi.org/10.3389/fimmu.2014.00461

Kazou, M., Alexandraki, V., Blom, J., Pot, B., Tsakalidou, E., & Papadimitriou, K. (2018). Comparative Genomics of *Lactobacillus acidipiscis* ACA-DC 1533 Isolated From Traditional Greek Kopanisti Cheese Against Species Within the *Lactobacillus salivarius* Clade. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.01244

Kociubinski, G. L., Pérez, P. F., Añón, M. C., DE Antoni, G. L. (1996). A Method of Screening for Highly Inhibitory Lactic Acid Bacteria. Journal of Food Protection, 59(7), 739– 745. https://doi.org/10.4315/0362-028X-59.7.739

Kociubinski, G., Pérez, P., De Antoni, G. (1999). Screening of Bile Resistance and Bile Precipitation in Lactic Acid Bacteria and Bifidobacteria. Journal of Food Protection, 62(8), 905–912. https://doi.org/10.4315/0362-028X-62.8.905

König, J., Wells, J., Cani, P. D., García-Ródenas, C. L., MacDonald, T., Mercenier, A., Whyte, J., Troost, F., & Brummer, R.-J. (2016). Human Intestinal Barrier Function in Health and Disease. Clinical and Translational Gastroenterology, 7(10), e196. https://doi.org/10.1038/ctg.2016.54

Kunz AN, Noel JM, Fairchok MP. (2004). Two Cases of *Lactobacillus* Bacteremia During Probiotic Treatment of Short Gut Syndrome. J Pediatr Gastroenterol Nutr 38:457–458. https://doi.org/10.1097/00005176-200404000-00017

Lane, E. R., Zisman, T., & Suskind, D. (2017). The microbiota in inflammatory bowel disease: current and therapeutic insights. Journal of Inflammation Research, Volume 10, 63–73. https://doi.org/10.2147/JIR.S116088

Le Maréchal, C., Peton, V., Plé, C., Vroland, C., Jardin, J., Briard-Bion, V., Durant, G., Chuat, V., Loux, V., Foligné, B., Deutsch, S.-M., Falentin, H., & Jan, G. (2015). Surface proteins of *Propionibacterium freudenreichii* are involved in its anti-inflammatory properties. Journal of Proteomics, 113, 447–461. https://doi.org/10.1016/j.jprot.2014.07.018

Lebeer, S., Vanderleyden, J., & De Keersmaecker, S. C. J. (2008). Genes and Molecules of Lactobacilli Supporting Probiotic Action. Microbiology and Molecular Biology Reviews, 72(4), 728–764. https://doi.org/10.1128/MMBR.00017-08

LeBlanc, J.G., Laiño, J. E., del Valle, M. J., Vannini, V., van Sinderen, D., Taranto, M. P., de Valdez, G. F., de Giori, G. S., & Sesma, F. (2011). B-Group vitamin production by lactic acid bacteria - current knowledge and potential applications. Journal of Applied Microbiology, 111(6), 1297–1309. https://doi.org/10.1111/j.1365-2672.2011.05157.x

LeBlanc, Jean Guy, Chain, F., Martín, R., Bermúdez-Humarán, L. G., Courau, S., & Langella, P. (2017). Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. Microbial Cell Factories, 16(1), 79. https://doi.org/10.1186/s12934-017-0691-z

Lei, M., Guo, C., Wang, D., Zhang, C., & Hua, L. (2017). The effect of probiotic *Lactobacillus casei* Shirota on knee osteoarthritis: a randomised double-blind, placebo-controlled clinical trial. Beneficial Microbes, 8(5), 697–703. https://doi.org/10.3920/BM2016.0207

Li, J., Lee, D. S. W., & Madrenas, J. (2013). Evolving Bacterial Envelopes and Plasticity of TLR2- Dependent Responses: Basic Research and Translational Opportunities. Frontiers in Immunology, 4. https://doi.org/10.3389/fimmu.2013.00347

Liu, M., Bayjanov, J. R., Renckens, B., Nauta, A., & Siezen, R. J. (2010). The proteolytic system of lactic acid bacteria revisited: a genomic comparison. BMC Genomics, 11(1), 36. https://doi.org/10.1186/1471-2164-11-36

Liu, W., Pang, H., Zhang, H., & Cai, Y. (2014). Biodiversity of Lactic Acid Bacteria. In Lactic Acid Bacteria (pp. 103–203). Springer Netherlands. https://doi.org/10.1007/978-94-017-8841-0_2

Luu, M., & Visekruna, A. (2019). Short-chain fatty acids: Bacterial messengers modulating the immunometabolism of T cells. European Journal of Immunology, 49(6), 842–848. https://doi.org/10.1002/eji.201848009

MacFie, J. (2005). Current status of bacterial translocation as a cause of surgical sepsis. British Medical Bulletin, 71(1), 1–11. https://doi.org/10.1093/bmb/ldh029

Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J., Sierro, F., Di Yu, Schilter, H. C., Rolph, M. S., Mackay, F., Artis, D., Xavier, R. J., Teixeira, M. M., & Mackay, C. R. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature, 461(7268), 1282–1286. https://doi.org/10.1038/nature08530

McFarland, L. V., Evans, C. T., & Goldstein, E. J. C. (2018). Strain-Specificity and Disease-Specificity of Probiotic Efficacy: A Systematic Review and Meta-Analysis. Frontiers in Medicine, 5. https://doi.org/10.3389/fmed.2018.00124

Mokoena, M. P. (2017). Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Molecules, 22(8), 1255. https://doi.org/10.3390/molecules22081255

Monedero, V., Revilla-Guarinos, A., & Zúñiga, M. (2017). Physiological Role of Two-Component Signal Transduction Systems in Food-Associated Lactic Acid Bacteria (pp. 1–51). https://doi.org/10.1016/bs.aambs.2016.12.002

Monteagudo-Mera, A., Rastall, R. A., Gibson, G. R., Charalampopoulos, D., & Chatzifragkou, A. (2019). Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Applied Microbiology and Biotechnology, 103(16), 6463–6472. https://doi.org/10.1007/s00253-019-09978-7

Morrison, D. J., & Preston, T. (2016). Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes, 7(3), 189–200. https://doi.org/10.1080/19490976.2015.1134082

Mowat, A. M., & Agace, W. W. (2014). Regional specialization within the intestinal immune system. Nature Reviews Immunology, 14(10), 667–685. https://doi.org/10.1038/nri3738

Neish, A. S. (2014). Mucosal Immunity and the Microbiome. Annals of the American Thoracic Society, 11(Supplement 1), S28–S32. https://doi.org/10.1513/AnnalsATS.201306-161MG

Oliveira, D., Vidal, L., Ares, G., Walter, E. H. M., Rosenthal, A., & Deliza, R. (2017). Sensory, microbiological and physicochemical screening of probiotic cultures for the development of non- fermented probiotic milk. LWT - Food Science and Technology, 79, 234–241. https://doi.org/10.1016/j.lwt.2017.01.020

Oliveira, L. de C., Silveira, A. M. M., Monteiro, A. de S., dos Santos, V. L., Nicoli, J. R., Azevedo, V. A. de C., Soares, S. de C., Dias-Souza, M. V., & Nardi, R. M. D. (2017). In silico Prediction, in vitro Antibacterial Spectrum, and Physicochemical Properties of a Putative Bacteriocin Produced by *Lactobacillus rhamnosus* Strain L156.4. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.00876

Papadimitriou, K., Alegría, Á., Bron, P. A., de Angelis, M., Gobbetti, M., Kleerebezem, M., Lemos, J. A., Linares, D. M., Ross, P., Stanton, C., Turroni, F., van Sinderen, D., Varmanen, P., Ventura, M., Zúñiga, M., Tsakalidou, E., & Kok, J. (2016). Stress Physiology of Lactic Acid Bacteria. Microbiology and Molecular Biology Reviews, 80(3), 837–890. https://doi.org/10.1128/MMBR.00076-15

Papadimitriou, K., Zoumpopoulou, G., Foligné, B., Alexandraki, V., Kazou, M., Pot, B., & Tsakalidou, E. (2015). Discovering probiotic microorganisms: in vitro, in vivo, genetic and omics approaches. Frontiers in Microbiology, 6. https://doi.org/10.3389/fmicb.2015.00058

Parada Venegas, D., De la Fuente, M. K., Landskron, G., González, M. J., Quera, R., Dijkstra, G., Harmsen, H. J. M., Faber, K. N., & Hermoso, M. A. (2019). Short Chain Fatty Acids (SCFAs)- Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.00277

Pariza, M. W., Gillies, K. O., Kraak-Ripple, S. F., Leyer, G., & Smith, A. B. (2015). Determining the safety of microbial cultures for consumption by humans and animals. Regulatory Toxicology and Pharmacology, 73(1), 164–171. https://doi.org/10.1016/j.yrtph.2015.07.003

Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile Genetic Elements Associated with Antimicrobial Resistance. Clinical Microbiology Reviews, 31(4). https://doi.org/10.1128/CMR.00088-17

Pastorelli, L., De Salvo, C., Mercado, J. R., Vecchi, M., & Pizarro, T. T. (2013). Central Role of the Gut Epithelial Barrier in the Pathogenesis of Chronic Intestinal Inflammation: Lessons Learned from Animal Models and Human Genetics. Frontiers in Immunology, 4. https://doi.org/10.3389/fimmu.2013.00280

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nature Reviews Immunology, 14(3), 141–153. https://doi.org/10.1038/nri3608

Plavec, T. V., & Berlec, A. (2019). Engineering of lactic acid bacteria for delivery of therapeutic proteins and peptides. Applied Microbiology and Biotechnology, 103(5), 2053–2066. https://doi.org/10.1007/s00253-019-09628-y

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, A. (2019). Mechanisms of Action of Probiotics. Advances in Nutrition, 10(suppl_1), S49–S66. https://doi.org/10.1093/advances/nmy063

Pohanka, M. (2020). D-Lactic Acid as a Metabolite: Toxicology, Diagnosis, and Detection. BioMed Research International, 2020, 1–9. https://doi.org/10.1155/2020/3419034

Pot, B., Felis, G. E., Bruyne, K. De, Tsakalidou, E., Papadimitriou, K., Leisner, J., & Vandamme, P. (2014). The genus *Lactobacillus*. In Lactic Acid Bacteria (pp. 249–353). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118655252.ch19

Prabhurajeshwar, C., & Chandrakanth, K. (2019). Evaluation of antimicrobial properties and their substances against pathogenic bacteria in-vitro by probiotic Lactobacilli strains isolated from commercial yoghurt. Clinical Nutrition Experimental, 23, 97–115. https://doi.org/10.1016/j.yclnex.2018.10.001

Pradhan, D., Mallappa, R. H., & Grover, S. (2020). Comprehensive approaches for assessing the safety of probiotic bacteria. Food Control, 108, 106872. https://doi.org/10.1016/j.foodcont.2019.106872

Preciado, G. M., Michel, M. M., Villarreal-Morales, S. L., Flores-Gallegos, A. C., Aguirre-

Joya, J., Morlett-Chávez, J., Aguilar, C. N., & Rodríguez-Herrera, R. (2016). Bacteriocins and Its Use for Multidrug-Resistant Bacteria Control. In Antibiotic Resistance (pp. 329–349). Elsevier. https://doi.org/10.1016/B978-0-12-803642-6.00016-2

Presti, I., D'Orazio, G., Labra, M., La Ferla, B., Mezzasalma, V., Bizzaro, G., Giardina, S., Michelotti, A., Tursi, F., Vassallo, M., & Di Gennaro, P. (2015). Evaluation of the probiotic properties of new *Lactobacillus* and *Bifidobacterium* strains and their in vitro effect. Applied Microbiology and Biotechnology, 99(13), 5613–5626. https://doi.org/10.1007/s00253-015-6482-8

Rahimi, F., Bouzari, M., Katouli, M., & Pourshafie, M. R. (2012). Prophage and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* strains in Iran. Archives of Virology, 157(9), 1807–1811. https://doi.org/10.1007/s00705-012-1361-4

Rakhmanova, A., Khan, Z. A., & Shah, K. (2018). A mini review fermentation and preservation: role of Lactic Acid Bacteria. MOJ Food Processing & Technology, 6(5), 414–417. https://doi.org/10.15406/mojfpt.2018.06.197

Raveschot, C., Cudennec, B., Coutte, F., Flahaut, C., Fremont, M., Drider, D., & Dhulster, P. (2018). Production of Bioactive Peptides by *Lactobacillus* Species: From Gene to Application. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.02354

Rescigno, M. (2013). Mucosal immunology and bacterial handling in the intestine. Best Practice & Research Clinical Gastroenterology, 27(1), 17–24. https://doi.org/10.1016/j.bpg.2013.03.004

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G., Gasbarrini, A., & Mele, M. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms, 7(1), 14. https://doi.org/10.3390/microorganisms7010014

Rice, E.S., Green, R.E. (2019). New Approaches for Genome Assembly and Scaffolding. Annu Rev Anim Biosci, 7, 17-40. http://doi: 10.1146/annurev-animal-020518-115344.

Rocha-Ramírez, L. M., Pérez-Solano, R. A., Castañón-Alonso, S. L., Moreno Guerrero, S. S., Ramírez Pacheco, A., García Garibay, M., & Eslava, C. (2017). Probiotic *Lactobacillus* Strains Stimulate the Inflammatory Response and Activate Human Macrophages. Journal of Immunology Research, 2017, 1–14. https://doi.org/10.1155/2017/4607491

Rolny, I. S., Tiscornia, I., Racedo, S. M., Pérez, P. F., & Bollati-Fogolín, M. (2016). *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 modulates response of human epithelial and dendritic cells infected with Bacillus cereus. Beneficial Microbes, 7(5), 749–760. https://doi.org/10.3920/BM2015.0191

Rooks, M. G., & Garrett, W. S. (2016). Gut microbiota, metabolites and host immunity. Nature Reviews Immunology, 16(6), 341–352. https://doi.org/10.1038/nri.2016.42

Rocha, C., Gomes-Santos, A. C., Garcias Moreira, T., de Azevedo, M., Diniz Luerce, T., Mariadassou, M., Longaray Delamare, A. P., Langella, P., Maguin, E., Azevedo, V., Caetano de Faria, A. M., Miyoshi, A., & van de Guchte, M. (2014). Local and Systemic Immune Mechanisms Underlying the Anti-Colitis Effects of the Dairy Bacterium *Lactobacillus delbrueckii*. PLoS ONE, 9(1), e85923. https://doi.org/10.1371/journal.pone.0085923

Russo, P., Capozzi, V., Arena, M. P., Spadaccino, G., Dueñas, M. T., López, P., Fiocco, D., & Spano, G. (2014). Riboflavin-overproducing strains of *Lactobacillus fermentum* for riboflavin-enriched bread. Applied Microbiology and Biotechnology, 98(8), 3691–3700. https://doi.org/10.1007/s00253-013-5484-7

Salvetii, E. (2018). The Genomic Basis of Lactobacilli as Health-Promoting Organisms. In Bugs as Drugs (pp. 49–71). American Society of Microbiology. https://doi.org/10.1128/microbiolspec.BAD-0011-2016

Salvetii, E., Torriani, S., & Felis, G. E. (2012). The Genus *Lactobacillus*: A Taxonomic Update. Probiotics and Antimicrobial Proteins, 4(4), 217–226. https://doi.org/10.1007/s12602-012-9117- 8

Salvo Romero, E., Alonso Cotoner, C., Pardo Camacho, C., Casado Bedmar, M., & Vicario, M. (2015). The intestinal barrier function and its involvement in digestive disease. Revista Española de Enfermedades Digestivas, 108. https://doi.org/10.17235/reed 2015.3846/2015

Sanders, M. E., Akkermans, L. M. A., Haller, D., Hammerman, C., Heimbach, J. T., Hörmannsperger, G., & Huys, G. (2010). Safety assessment of probiotics for human use. Gut Microbes, 1(3), 164–185. https://doi.org/10.4161/gmic.1.3.12127

Saulnier, D. M., Santos, F., Roos, S., Mistretta, T.-A., Spinler, J. K., Molenaar, D., Teusink, B., & Versalovic, J. (2011). Exploring Metabolic Pathway Reconstruction and Genome-Wide Expression Profiling in *Lactobacillus reuteri* to Define Functional Probiotic Features. PLoS ONE, 6(4), e18783. https://doi.org/10.1371/journal.pone.0018783

Senan, S., Prajapati, J. B., & Joshi, C. G. (2015). Feasibility of Genome-Wide Screening for Biosafety Assessment of Probiotics: A Case Study of *Lactobacillus helveticus* MTCC 5463. Probiotics and Antimicrobial Proteins, 7(4), 249–258. https://doi.org/10.1007/s12602-015-9199- 1

Sengupta, R., Altermann, E., Anderson, R. C., McNabb, W. C., Moughan, P. J., & Roy, N. C. (2013). The Role of Cell Surface Architecture of Lactobacilli in Host-Microbe Interactions in the Gastrointestinal Tract. Mediators of Inflammation, 2013, 1–16. https://doi.org/10.1155/2013/237921

Segerman, B. (2020) The Most Frequently Used Sequencing Technologies and Assembly Methods in Different Time Segments of the Bacterial Surveillance and RefSeq Genome Databases. Front Cell Infect Microbiol, 10, 527102. https://doi: 10.3389/fcimb.2020.527102

Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and in disease. Current Opinion in Gastroenterology, 31(1), 69–75. https://doi.org/10.1097/MOG.0000000000000139

Shi, Y., Zhai, Q., Li, D., Mao, B., Liu, X., Zhao, J., et al. (2017). Restoration of cefixime-induced gut microbiota changes by *Lactobacillus* cocktails and fructooligosaccharides in a mouse model. Microbiol. Res. 200, 14–24. doi: 10.1016/j.micres.2017.04.001

Slatko, B. E., Gardner, A. F., & Ausubel, F. M. (2018). Overview of Next-Generation Sequencing Technologies. Current Protocols in Molecular Biology, 122(1). https://doi.org/10.1002/cpmb.59

Smith, L. D., & Garg, U. (2017). Disorders of vitamins and cofactors. In Biomarkers in Inborn Errors of Metabolism (pp. 361–397). Elsevier. https://doi.org/10.1016/B978-0-12-802896-4.00011-0

Sun, Z., Harris, H. M. B., McCann, A., Guo, C., Argimón, S., Zhang, W., Yang, X., Jeffery, I. B., Cooney, J. C., Kagawa, T. F., Liu, W., Song, Y., Salvetii, E., Wrobel, A., Rasinkangas, P., Parkhill, J., Rea, M. C., O'Sullivan, O., Ritari, J., … O'Toole, P. W. (2015). Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. Nature Communications, 6(1), 8322. https://doi.org/10.1038/ncomms9322

Surachat, K., Sangket, U., Deachamag, P., & Chotigeat, W. (2017). In silico analysis of protein toxin and bacteriocins from *Lactobacillus paracasei* SD1 genome and available online databases. PLOS ONE, 12(8), e0183548. https://doi.org/10.1371/journal.pone.0183548

Tailford, L. E., Crost, E. H., Kavanaugh, D., & Juge, N. (2015). Mucin glycan foraging in the human gut microbiome. Frontiers in Genetics, 6. https://doi.org/10.3389/fgene.2015.00081

Tamang, J. P., Shin, D.-H., Jung, S.-J., & Chae, S.-W. (2016). Functional Properties of Microorganisms in Fermented Foods. Frontiers in Microbiology, 7. https://doi.org/10.3389/fmicb.2016.00578

Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L. (2014). The Role of Short-Chain Fatty Acids in Health and Disease (pp. 91–119). https://doi.org/10.1016/B978-0-12-800100-4.00003-9

Taverniti, V., Stuknyte, M., Minuzzo, M., Arioli, S., De Noni, I., Scabiosi, C., Cordova, Z. M., Junttila, I., Hämäläinen, S., Turpeinen, H., Mora, D., Karp, M., Pesu, M., & Guglielmetti, S. (2013). S-Layer Protein Mediates the Stimulatory Effect of *Lactobacillus helveticus* MIMLh5 on Innate Immunity. Applied and Environmental Microbiology, 79(4), 1221–1231. https://doi.org/10.1128/AEM.03056-12

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. Biochemical Journal, 474(11), 1823–1836. https://doi.org/10.1042/BCJ20160510

Todorov, S. D. (2009). Bacteriocins from *Lactobacillus plantarum* production, genetic organization and mode of action: produção, organização genética e modo de ação. Brazilian Journal of Microbiology, 40(2), 209–221. https://doi.org/10.1590/S1517-83822009000200001

Torres, A. C., Vannini, V., Bonacina, J., Font, G., Saavedra, L., & Taranto, M. P. (2016).Cobalamin production by *Lactobacillus coryniformis*: biochemical identification of the synthetized corrinoid and genomic analysis of the biosynthetic cluster. BMC Microbiology, 16(1), 240. https://doi.org/10.1186/s12866-016-0854-9

Turpin, W., Humblot, C., Noordine, M.-L., Thomas, M., & Guyot, J.-P. (2012). Lactobacillaceae and Cell Adhesion: Genomic and Functional Screening. PLoS ONE, 7(5), e38034. https://doi.org/10.1371/journal.pone.0038034

Underwood, M. A. (2016). Probiotics and Innate and Adaptive Immune Responses in Premature Infants. Forum on Immunopathological Diseases and Therapeutics, 7(1–2), 1–15. https://doi.org/10.1615/ForumImmunDisTher.2016018178

Velez, M. P., De Keersmaecker, S. C. J., & Vanderleyden, J. (2007). Adherence factors of *Lactobacillus* in the human gastrointestinal tract. FEMS Microbiology Letters, 276(2), 140–148. https://doi.org/10.1111/j.1574-6968.2007.00908.x

Vancamelbeke, M., & Vermeire, S. (2017). The intestinal barrier: a fundamental role in health and disease. Expert Review of Gastroenterology & Hepatology, 11(9), 821–834. https://doi.org/10.1080/17474124.2017.1343143

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. P & T : A Peer-Reviewed Journal for Formulary Management, 40(4), 277–283. http://www.ncbi.nlm.nih.gov/pubmed/25859123

Ventura, M., Turroni, F., & van Sinderen, D. (2012). Probiogenomics as a tool to obtain genetic insights into adaptation of probiotic bacteria to the human gut. Bioengineered, 3(2), 73–79. https://doi.org/10.4161/bbug.18540

Vesterlund, S., Vankerckhoven, V., Saxelin, M., Goossens, H., Salminen, S., & Ouwehand, A. C. (2007). Safety assessment of *Lactobacillus* strains: Presence of putative risk factors in faecal, blood and probiotic isolates. International Journal of Food Microbiology, 116(3), 325–331. https://doi.org/10.1016/j.ijfoodmicro.2007.02.002

Walter, J., & Ley, R. (2011). The Human Gut Microbiome: Ecology and Recent Evolutionary Changes. Annual Review of Microbiology, 65(1), 411–429. https://doi.org/10.1146/annurev-micro-090110-102830

Wang, Yao, Qin, Y., Xie, Q., Zhang, Y., Hu, J., & Li, P. (2018). Purification and Characterization of Plantaricin LPL-1, a Novel Class IIa Bacteriocin Produced by *Lactobacillus plantarum* LPL-1 Isolated From Fermented Fish. Frontiers in Microbiology, 9.https://doi.org/10.3389/fmicb.2018.02276

Wang, Yuan, Jin, Y., Wang, Y., Li, L., Liao, Y., Zhang, Y., & Yu, D. (2019). The effect of folic acid in patients with cardiovascular disease. Medicine, 98(37), e17095. https://doi.org/10.1097/MD.0000000000017095

Watson, D., O'Connell Motherway, M., Schoterman, M. H. C., van Neerven, R. J. J., Nauta, A., & van Sinderen, D. (2013). Selective carbohydrate utilization by lactobacilli and bifidobacteria. Journal of Applied Microbiology, 114(4), 1132–1146. https://doi.org/10.1111/jam.12105

Widyastuti, Y., Rohmatussolihat, & Febrisiantosa, A. (2014). The Role of Lactic Acid Bacteria in Milk Fermentation. Food and Nutrition Sciences, 05(04), 435–442. https://doi.org/10.4236/fns.2014.54051

Wu, H.-J., & Wu, E. (2012). The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes, 3(1), 4–14. https://doi.org/10.4161/gmic.19320

Xu, Y., Tian, Y., Cao, Y., Li, J., Guo, H., Su, Y., Tian, Y., Wang, C., Wang, T., & Zhang, L. (2019). Probiotic Properties of *Lactobacillus paracasei* subsp. *paracasei* L1 and Its Growth Performance-Promotion in Chicken by Improving the Intestinal Microflora. Frontiers in Physiology, 10. https://doi.org/10.3389/fphys.2019.00937

Wanna, W., Surachat, K., Kaitimonchai, P., and Phongdara, A. (2021). Evaluation of Probiotic Characteristics and Whole Genome Analysis of *Pediococcus pentosaceus* MR001 for Use as Probiotic Bacteria in Shrimp Aquaculture. Sci. Rep. 11, 18334. doi:10.1038/s41598-021-96780-z

Yoshii, K., Hosomi, K., Sawane, K., & Kunisawa, J. (2019). Metabolism of Dietary and Microbial Vitamin B Family in the Regulation of Host Immunity. Frontiers in Nutrition, 6. https://doi.org/10.3389/fnut.2019.00048

Yu, S., Peng, Y., Chen, W., Deng, Y., & Guo, Y. (2014). Comparative Genomic Analysis of Two- Component Signal Transduction Systems in Probiotic *Lactobacillus casei*. Indian Journal of Microbiology, 54(3), 293–301. https://doi.org/10.1007/s12088-014-0456-x

Yelin I, Flett KB, Merakou C, et al (2019) Genomic and epidemiological evidence of bacterial transmission from probiotic capsule to blood in ICU patients. Nature Medicine, 25,1728–1732. https://doi.org/10.1038/s41591-019-0626-9

Zhang, J., Chiodini, R., Badr, A., & Zhang, G. (2011). The impact of next-generation sequencing on genomics. Journal of Genetics and Genomics, 38(3), 95–109. https://doi.org/10.1016/j.jgg.2011.02.003

Zhang, W., Wang, J., Zhang, D., Liu, H., Wang, S., Wang, Y., & Ji, H. (2019). Complete Genome Sequencing and Comparative Genome Characterization of *Lactobacillus johnsonii* ZLJ010, a Potential Probiotic With Health-Promoting Properties. Frontiers in Genetics, 10. https://doi.org/10.3389/fgene.2019.00812

Zhang, W., Ji, H., Zhang, D., Liu, H., Wang, S., Wang, J., et al. (2018). Complete Genome Sequencing of *Lactobacillus plantarum* ZLP001, a Potential Probiotic that Enhances Intestinal Epithelial Barrier Function and Defense against Pathogens in Pigs. Front. Physiol. 9, 1689. doi:10.3389/fphys.2018.01689

Zhang, Z.-Y., Liu, C., Zhu, Y.-Z., Wei, Y.-X., Tian, F., Zhao, G.-P., & Guo, X.-K. (2012). Safety assessment of *Lactobacillus plantarum* JDM1 based on the complete genome. International Journal of Food Microbiology, 153(1–2), 166–170. https://doi.org/10.1016/j.ijfoodmicro.2011.11.003

Zheng, J., Wittouck, S., Salvetii, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O'Toole, P. W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G., & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. International Journal of Systematic and Evolutionary Microbiology, 70(4), 2782–2858. https://doi.org/10.1099/ijsem.0.004107

Zihni, C., Mills, C., Matter, K. et al. (2016). Tight junctions: from simple barriers to multifunctional molecular gates. Nat Rev Mol Cell Biol 17: 564–580 https://doi.org/10.1038/nrm.2016.80

APÊNDICE A - Produção Científica (2018-2022)

Durante o período do Doutorado foram publicados 17 artigos científicos, incluindo os que fazem parte desa Tese: 4 como autor principal e 13 como co-autor. Além disso, foram publicados 8 capítulos de livros, 2 como autor principal e 6 como co-autor, conforme pode ser observado abaixo no currículo lattes resumido e comprovantes.

Curriculum Lattes (Resumido)

Luís Cláudio Lima de Jesus

Curriculum Vitae

Formação acadêmica/titulação

2018 Doutorado em Genética.
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil
Título: Probiogenômica de *Lactobacillus delbrueckii* CIDCA 133
Orientador: Vasco Ariston de Carvalho Azevedo
Co-orientador: Flávia Figueira Aburjaile
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico

2016 - 2018 Mestrado em Genética.
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil
Título: Efeito terapêutico do leite fermentado por Lactobacillus delbrueckii subsp. lactis CIDCA 133 em modelo de mucosite intestinal induzido pelo antineoplásico 5-Fluorouracil, Ano de obtenção: 2018
Orientador: Vasco Ariston de Carvalho Azevedo
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2011 - 2015 Graduação em Ciências Biológicas.
Universidade Federal do Maranhão , UFMA, Sao Luis, Brasil
Título: Efeito modulador do Ácido Ascórbico (vitamina C) sobre danos ao DNA causados pelo antimoniato de meglumina (Glucantime)
Orientador: Silma Regina Ferreira Pereira

2006 - 2008 Ensino Médio (2o grau) .
Centro de Ensino Médio Raimundo Rodrigues, C. E. M. R. R, Brasil

---

Formação complementar

2019 Francês.
Viamundi Idiomas, VIAMUNDI, Brasil

2019 English Live - Inglês.
English Live, EF, Brasil

2022 - 2022 Curso de curta duração em RT-qPCR. (Carga horária: 5h).
Bio-Rad Laboratories, BIO-RAD, Estados Unidos

2021 - 2021 Curso de curta duração em Noções de Biossegurança no Trabalho relacionadas à COVID 19. (Carga horária: 60h).
Universidade Estadual do Maranhão, UEMA, Sao Luis, Brasil

2020 - 2020 Curso de curta duração em Filogenia: pequena-escala (Filogenética) e larga-escala (Filogenômica). (Carga horária: 15h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil

2019 - 2019 Extensão universitária em Lingua francesa: produção escrita e apresentação oral. (Carga horária: 16h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil

2019 - 2019 Extensão universitária em Idiomas sem Fronteiras: interações em Lingua Francesa. (Carga horária: 16h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil

2018 - 2018 Curso de curta duração em Uso de tecnologia de NGS na vigilância genômica de Flavivírus emergentes. (Carga horária: 80h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil

2018 - 2018 Curso de curta duração em RNAs não Codificantes. (Carga horária: 15h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil

2018 - 2018 Curso de curta duração em Medicina e Genômica de Precisão. (Carga horária: 15h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil

2018 - 2018 Curso de curta duração em Microbiologia dos Alimentos. (Carga horária: 60h).
Universidade Estadual do Maranhão, UEMA, Sao Luis, Brasil

Prêmios e títulos

2021 Relevância Acadêmica-Semana do Conhecimento UFMG 2021, Pró-Reitoria de Pesquisa.

2021 Relevância Acadêmica-Semana do Conhecimento UFMG 2021, Pró-Reitoria de Pesquisa.

2019 Melhor Pôster da área de Genética da 71ª Reunião Anual da SBPC.

2019 Menção Honrosa - 2nd Associated International Laboratory Meeting (LIA 2019),

2018 Grande Prêmio Conceição Ribeiro Machado- Semana do Conhecimento da UFMG 2018.

2018 Menção Honrosa - Melhor Pôster da área de Biotecnologia do Gene Time Conference 2018.

2018 Menção Honrosa-XXVII Semana de Iniciação Cientifica / UFMG, 2018.

2018 Relevância Acadêmica- Semana do Conhecimento UFMG 2018.

Produção bibliográfica

Artigos completos publicados em periódicos

1. **DE JESUS, LUÍS CLÁUDIO LIMA**; ABURJAILE, FLÁVIA FIGUEIRA; SOUSA, THIAGO DE JESUS; FELICE, ANDREI GIACCHETTO; SOARES, SIOMAR DE CASTRO; ALCANTARA, LUIZ CARLOS JUNIOR; AZEVEDO, VASCO ARISTON DE CARVALHO. Genomic Characterization of Lactobacillus delbrueckii Strains with Probiotics Properties. Frontiers in Bioinformatics. , v.2, p.912795, 2022.

2. BATISTA, V. L. ; **DE JESUS, LUÍS CLÁUDIO LIMA** ; TAVARES, L. M. ; BARROSO, F. A. L. ; FERNANDES, L. J. S. ; FREITAS, A. S. ; AMERICO, M. ; DRUMMOND, M. M. ; MANCHA-AGRESTI, P. ; FERREIRA, E. ; LAGUNA, JULIANA ; ALCANTARA, LUIZ CARLOS JUNIOR ; AZEVEDO, V. .

Paraprobiotics and Postbiotics of Lactobacillus delbrueckii CIDCA 133 Mitigate 5-FU-Induced Intestinal Inflammation. Microorganisms, v. 10, p. 1418, 2022.

3. BARROSO, FERNANDA ALVARENGA LIMA; **DE JESUS, LUÍS CLÁUDIO LIMA**; DA SILVA, TALES FERNANDO; BATISTA, VIVIANE LIMA; LAGUNA, JULIANA; COELHO-ROCHA, NINA DIAS; VITAL, KÁTIA DUARTE; FERNANDES, SIMONE ODÍLIA ANTUNES; CARDOSO, VALBERT NASCIMENTO; FERREIRA, ENIO; MARTINS, FLAVIANO SANTOS; DRUMOND, MARIANA MARTINS; MANCHA-AGRESTI, PAMELA; BIRBRAIR, ALEXANDER; BARH, DEBMALYA; AZEVEDO, VASCO. Lactobacillus delbrueckii CIDCA 133 Ameliorates Chemotherapy-Induced Mucositis by Modulating Epithelial Barrier and TLR2/4/Myd88/NF-kB Signaling Pathway. Frontiers in Microbiology, v.13, p.858036, 2022.

4. QUINTANILHA, MÔNICA F.; MIRANDA, VIVIAN C.; SOUZA, RAMON O.; GALLOTTI, BRUNO; CRUZ, CLÊNIO; SANTOS, ELANDIA A.; ALVAREZ-LEITE, JACQUELINE I.; **JESUS, LUÍS C.L.**; AZEVEDO, VASCO; TRINDADE, LUÍSA M.; CARDOSO, VALBERT N.; FERREIRA, ENIO; CARVALHO, BÁRBARA A.; SOARES, PEDRO M.G.; VIEIRA, ANGÉLICA T.; NICOLI, JACQUES R.; MARTINS, FLAVIANO S.Bifidobacterium longum subsp. longum 51A attenuates intestinal injury against irinotecan-induced mucositis in mice. LIFE SCIENCES, v.289, p.120243, 2021.

5. GABRIELLE VIDAL DA SILVA, JÉSSICA; THOMAZ VIEIRA, ANGÉLICA; SOUSA, THIAGO J; VINICIUS CANÁRIO VIANA, MARCUS; PARISE, DOGLAS; SAMPAIO, BRUNA; LIMA DA SILVA, ALESSANDRA; **Cláudio Lima de Jesus, Luís**; KÁSSIO RIBEIRO MATOS LOUREIRO DE CARVALHO, PEDRO; DE CASTRO OLIVEIRA, LETÍCIA; FIGUEIRA ABURJAILE, FLAVIA; MARTINS, FLAVIANO S; ROBERT NICOLI, JACQUES; GHOSH, PREETAM; BRENIG, BERTRAM; AZEVEDO, VASCO; CYBELLE PINTO GOMIDE, ANNE. Comparative genomics and in silico gene evaluation involved in the probiotic potential of Bifidobacterium longum 51A. GENE, v.795, p.145781, 2021.

6. CARVALHO, RODRIGO; ABURJAILE, FLAVIA; CANARIO, MARCUS; NASCIMENTO, ANDRÉA M. A.; CHARTONE-SOUZA, EDMAR; **DE JESUS, LUIS**; ZAMYATNIN, ANDREY A.; BRENIG, BERTRAM; BARH, DEBMALYA; GHOSH, PREETAM; GOES-NETO, ARISTOTELES; FIGUEIREDO, HENRIQUE C. P.; SOARES, SIOMAR; RAMOS, ROMMEL; PINTO, ANNE; AZEVEDO, VASCO. Genomic Characterization of Multidrug-Resistant Escherichia coli BH100 Sub-strains. Frontiers in Microbiology, v.11, p.549254, 2021.

7. BARROSO, F. A. L.; **DE JESUS, LUÍS CLÁUDIO LIMA**; PROSPERI, C. C.; BATISTA, V. L.; FERREIRA, E.; FERNANDES, R. S.; BARROS, A. L. B.; LECLERCQ, S.; AZEVEDO, V.; MANCHA-AGRESTI, P.; DRUMMOND, M. M. Intake of Lactobacillus delbrueckii (pExu:hsp65) Prevents the Inflammation and the Disorganization of the Intestinal Mucosa in a Mouse Model of Mucositis. Microorganisms, v.9, p.107, 2021.

8. TRINDADE, LUÍSA MARTINS; TORRES, LÍCIA; MATOS, ISABEL DAVID; MIRANDA, VIVIAN CORREIA; **DE JESUS, LUÍS CLÁUDIO LIMA**; CAVALCANTE, GREGÓRIO; DE SOUZA OLIVEIRA, JONATHAN JÚNIO; CASSALI, GEOVANNI DANTAS; MANCHA-AGRESTI, PAMELA; DE CARVALHO AZEVEDO, VASCO ARISTON; MAIOLI, TATIANI UCELI; CARDOSO, VALBERT NASCIMENTO; MARTINS, FLAVIANO DOS SANTOS; DE VASCONCELOS GENEROSO, SIMONE. Paraprobiotic Lacticaseibacillus rhamnosus Protects Intestinal Damage in an Experimental Murine Model of Mucositis. Probiotics and Antimicrobial Proteins, v.13, p.1, 2021.

9. **DE JESUS, LUÍS CLÁUDIO LIMA**; DRUMMOND, M. M.; ABURJAILE, FLAVIA; SOUSA, T. J.; COELHO-ROCHA, NINA D.; PROFETA, R.; BRENIG, B.; MANCHA-AGRESTI, P.; AZEVEDO, V. Probiogenomics of Lactobacillus delbrueckii subsp. lactis CIDCA 133: In Silico, In Vitro, and In Vivo Approaches. Microorganisms, v.9, p.829, 2021.

10. ABURJAILE, FLAVIA; VIANA, M.; CERQUEIRA, J.; **DE JESUS, LUÍS CLÁUDIO LIMA**; SILVA, T. F.; CARVALHO, RODRIGO; AZEVEDO, V. Probiotic potential of novel Brazilian Lactobacillus crispatus strains. GENETICS AND MOLECULAR RESEARCH, v.20, p.GMR18900, 2021.

11. **DE JESUS, LUÍS CLÁUDIO LIMA**; DE JESUS SOUSA, THIAGO; COELHO-ROCHA, NINA DIAS; PROFETA, RODRIGO; BARROSO, FERNANDA ALVARENGA LIMA; DRUMOND, MARIANA MARTINS; MANCHA-AGRESTI, PAMELA; FERREIRA, ÊNIO; BRENIG, BERTRAM; ABURJAILE,

FLÁVIA FIGUEIRA; AZEVEDO, VASCO. Safety Evaluation of Lactobacillus delbrueckii subsp. lactis CIDCA 133: a Health-Promoting Bacteria. Probiotics and Antimicrobial Proteins, v.13, p.1, 2021.

12. GALLOTTI, B; GALVAO, I; LELES, G; QUINTANILHA, MF; SOUZA, RO; MIRANDA, VC; ROCHA, VM; TRINDADE, LM; **JESUS, LCL**; MENDES, V; ANDRE, LC; D'AURIOL-SOUZA, MM; AZEVEDO, V; CARDOSO, VN; MARTINS, FS; VIEIRA, AT. EFFECTS OF DIETARY FIBER INTAKE IN CHEMOTHERAPY-INDUCED MUCOSITIS IN MURINE MODEL. BRITISH JOURNAL OF NUTRITION, v.124, p.1 - 34, 2020.

13. TAVARES, L. M.; **DE JESUS, LUÍS CLÁUDIO LIMA**; SILVA, T. F.; BARROSO, F. A. L.; BATISTA, V. L.; ROCHA, N. D. C.; AZEVEDO, V.; DRUMMOND, M. M.; MANCHA-AGRESTI, PAMELA. Novel Strategies for Efficient Production and Delivery of Live Biotherapeutics and Biotechnological Uses of Lactococcus lactis: The Lactic Acid Bacterium Model. FRONTIERS IN BIOENGINEERING AND BIOTECHNOLOGY, v.8, p.517166, 2020.

14. BATISTA, VIVIANE LIMA; DA SILVA, TALES FERNANDO; **DE JESUS, LUÍS CLÁUDIO LIMA**; COELHO-ROCHA, NINA DIAS; BARROSO, FERNANDA ALVARENGA LIMA; TAVARES, LAISA MACEDO; AZEVEDO, VASCO; MANCHA-AGRESTI, PAMELA; DRUMOND, MARIANA MARTINS. Probiotics, Prebiotics, Synbiotics, and Paraprobiotics as a Therapeutic Alternative for Intestinal Mucositis. Frontiers in Microbiology, v.11, p.544490, 2020.

15. **DE JESUS, LUÍS CLÁUDIO LIMA**; DRUMOND, MARIANA MARTINS; DE CARVALHO, ANDRÉ; SANTOS, SPENCER S.; MARTINS, FLAVIANO S.; FERREIRA, ÊNIO; FERNANDES, RENATA SALGADO; DE BARROS, ANDRÉ LUÍS BRANCO; DO CARMO, FILLIPE L.R.; PEREZ, PABLO F.; AZEVEDO, VASCO; MANCHA-AGRESTI, PAMELA. Protective effect of Lactobacillus delbrueckii subsp. Lactis CIDCA 133 in a model of 5 Fluorouracil-Induced intestinal mucositis. Journal of Functional Foods, v.53, p.197 - 207, 2019.

16. **DE JESUS, LUÍS CLÁUDIO LIMA**; SOARES, ROSSY-ERIC PEREIRA; MOREIRA, VANESSA RIBEIRO; PONTES, RAISSA LACERDA; CASTELO-BRANCO, PATRÍCIA VALÉRIA; PEREIRA, SILMA REGINA FERREIRA. Genistein and Ascorbic Acid Reduce Oxidative Stress-Derived DNA Damage Induced by the Antileishmanial Meglumine Antimoniate. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY (ONLINE), v.62, p.AAC.00456-18, 2018.

17. COELHO-ROCHA, NINA D.; DE CASTRO, CAMILA P.; **DE JESUS, LUIS C. L.**; LECLERCQ, SOPHIE Y.; DE CICCO SANDES, SAVIO H.; NUNES, ALVARO C.; AZEVEDO, VASCO; DRUMOND, MARIANA M.; MANCHA-AGRESTI, PAMELA. Microencapsulation of Lactic Acid Bacteria Improves the Gastrointestinal Delivery and in situ Expression of Recombinant Fluorescent Protein. Frontiers in Microbiology., v.9, p.2398, 2018.

Capítulos de livros publicados

1. LUISVALDEZ-BAEZ, J.; **DE JESUS, LUÍS CLÁUDIO LIMA**; MARQUES, P. H.; PRADO, L. C. S.; FELICE, A. G.; RODRIGUES, T. C. V.; ABURJAILE, FLAVIA; AZEVEDO, V.; SOARES, SIOMAR. Comparative genomics in probiotic bacteria In: Lactic Acid Bacteria in Food Biotechnology.1 ed.: Elsevier, 2022, v.2022, p. 242-278.

2. ABURJAILE, FLAVIA; **DE JESUS, LUÍS CLÁUDIO LIMA**; SILVA, T. F.; DRUMMOND, M. M.; CARVALHO, RODRIGO; AZEVEDO, V.; MANCHA-AGRESTI, P. Lactic acid bacteria in gut microbiota, probiotics and disease prevention In: Lactic Acid Bacteria in Food Biotechnology.1 ed.: Elsevier, 2022, p. 207-219.

3. **DE JESUS, LUÍS CLÁUDIO LIMA**; SILVA, T. F. ; ASSIS, R. ; FREITAS, A. S. ; AMERICO, M. ; FERNANDES, L. J. S. ; CAMPOS, G. M. ; GOMES, G. C. ; SANTOS, R. C. V. ; CARVALHO, RODRIGO; BARH, DEBMALYA ; AZEVEDO, V. Lactic acid bacteria based beverages in the promotion of gastrointestinal tract health. Microbiome, Immunity, Digestive Health and Nutrition. 1 ed.: Academic Press, 2022, p. 373-385.

4. AMERICO, M.; FREITAS, A. S.; SILVA, T. F.; **DE JESUS, LUÍS CLÁUDIO LIMA**; LIMA, F. A.; CARVALHO, RODRIGO; AZEVEDO, V. Uso de Bactérias Lácticas como Vetores de Entrega de

Vacinas Recombinates In: O Estado da Arte nas Pesquisas em Vacinologia.1 ed.: Creative, 2022, p. 102-120.

5. FREITAS, A. S.; FERNANDES, L. J. S.; ROCHA, N. D. C.; **DE JESUS, LUÍS CLÁUDIO LIMA**; RODOVALHO, V.; SILVA, T. F.; CARVALHO, RODRIGO; AZEVEDO, V. Immunomodulatory and antiinflammatory mechanisms of probiotics In: Probiotics Advanced Food and Health Applications.1 ed.: Elsevier, 2021, p. 321-341.

6. BATISTA, VIVIANE LIMA; DA SILVA, TALES FERNANDO; **de Jesus, Luis Cláudio Lima**; Tapia-Costa, Ana Paula; DRUMOND, MARIANA MARTINS; AZEVEDO, VASCO; MANCHA-AGRESTI, PAMELA. Lactic Acid Bacteria as Delivery Vehicle for Therapeutics Applications In: Methods in Molecular Biology.1 ed.: Springer US, 2021, p. 447-459.

7. **Cláudio Lima de Jesus, Luís**; Alvarenga Lima, Fernanda; Dias Coelho-Rocha, Nina; Fernando da Silva, Tales; Paz, Júlia; AZEVEDO, VASCO; MANCHA-AGRESTI, PAMELA; Martins Drumond, Mariana. Recombinant Probiotics and Microbiota Modulation as a Good Therapy for Diseases Related to the GIT In: The Health Benefits of Foods - Current Knowledge and Further Development.1 ed.: IntechOpen, 2020, p. 1-30.

8. LIMA, F. A.; **DE JESUS, LUÍS CLÁUDIO LIMA**; BATISTA, V. L.; TAVARES, L. M.; ASSIS, R.; DRUMMOND, M. M.; AZEVEDO, V.; MANCHA-AGRESTI, P. The Gastrointestinal Disease to the Microbiota Treatment: From the Present to the Future In: INFLAMMATORY BOWEL DISEASE VOLUME 2.2, 2019, v.2, p. 1-32.

Trabalhos publicados em anais de eventos (resumo)

1. SOUZA, RO; MIRANDA, VIVIAN CORREIA; QUINTANILHA, MF; COSTA, B. G.; **JESUS, LCL**; AZEVEDO, V.; VITAL, K. D.; FERNANDES, S. O. A.; MARTINS, F. S. Avaliação da permeabilidade intestinal e expressão gênica de tight junctions em camundongos tratados com Akkermansia muciniphila BAA-835 submetidos a modelo experimental de mucosite intestinal In: 31º Congresso Brasileiro de Microbiologia – CBM 2021, 2021. **Anais do 31º Congreso Brasileiro de Microbiologia 2021 - Evento 100% online**, 2021.

2. **DE JESUS, LUÍS CLÁUDIO LIMA**; ABURJAILE, FLAVIA; SOUSA, T. J.; ROCHA, N. D. C.; PROFETA, R.; DRUMMOND, M. M.; MANCHA-AGRESTI, P.; AZEVEDO, V. Avaliação da segurança de Lactobacillus delbrueckii subsp. lactis CIDCA 133 para o consumo através de análises genômicas In: XXIII Encontro de Genética do Nordeste, 2021, Petrolina. **XXIII ENGENE-Anais do Evento**, 2021.

3. **DE JESUS, LUÍS CLÁUDIO LIMA**; ABURJAILE, FLAVIA; SOUSA, T. J.; ROCHA, N. D. C.; PROFETA, R.; DRUMMOND, M. M.; MANCHA-AGRESTI, P.; AZEVEDO, V. Probiogenômica de Lactobacillus delbrueckii subsp. lactis CIDCA 133: uma promissora bactéria com propriedades probióticas In: XXIII Encontro de Genética do Nordeste, 2021, Petrolina. **XXIII ENGENE-Anais do Evento**, 2021.

4. ASSIS, R.; LIMA, F. A.; **DE JESUS, LUÍS CLÁUDIO LIMA**; BATISTA, V. L.; ROCHA, N. D. C.; SILVA, T. F.; TAVARES, L. M.; SANTOS, E.; PAZ, J.; MOURA, V.; SILVA, C.; TAPIA-COSTA, A.; DURAN, N.; DRUMMOND, M. M.; MANCHA-AGRESTI, P.; AZEVEDO, V. Administração oral da vacina de DNA Hsp65 entregue por Lactococcus lactis em modelo de colite induzida por DSS In: VI SIMPÓSIO DE MICROBIOLOGIA DA UFMG, 2019, Belo Horizonte. **Caderno de Resumos- VI SIMPÓSIO DE MICROBIOLOGIA DA UFMG**, 2019.

5. SILVA, T. F.; MADUREIRA, E.; **DE JESUS, LUÍS CLÁUDIO LIMA**; ASSIS, R.; SANTOS, E.; PAZ, J.; MOURA, V.; COELHO-ROCHA, NINA D.; BATISTA, V. L.; LIMA, F. A.; TAVARES, L. M.; MANCHA-AGRESTI, P.; AZEVEDO, V.; DRUMMOND, M. M. Expressão de mCherry em enterócitos de peixe pós administração oral com a cepa recombinante L. lactis MG1363 (pExu:mCherry) In: VI SIMPÓSIO DE MICROBIOLOGIA DA UFMG, 2019, Belo Horizonte. **Caderno de Resumos- VI SIMPÓSIO DE MICROBIOLOGIA DA UFMG**, 2019.

6. DRUMMOND, M. M.; **DE JESUS, LUÍS CLÁUDIO LIMA**; BATISTA, V. L.; ROCHA, N. D. C.; TAVARES, L. M.; BARROSO, F. A. L.; SILVA, T. F.; MANCHA-AGRESTI, P.; AZEVEDO, V. Fermented

milk by Lactobacillus delbrueckii CIDCA 133 carrying the vaccinal plasmid pExu: Hsp65 ammeliorates intestinal mucositis in murine model In: 7th World Congress on Targeting Microbiota, 2019, Krakow. **Archive of the International Society of Microbiota**. Archives of International Society of Microbiota, 2019. v.6. p.1 - 112

7. PROFETA, R.; **DE JESUS, LUÍS CLÁUDIO LIMA**; VIANA, M.; CERQUEIRA, J.; DRUMMOND, M. M.; MANCHA-AGRESTI, P.; BRENIG, B.; AZEVEDO, V. Genomic characterization of Lactobacillus delbrueckii CIDCA 133: a potential probiotic strain In: X-Meeting 2019: 15th International Conference of the AB3C, 2019, Campos do Jordão. **Proceedings X-Meeting 2019**, 2019.

8. COELHO-ROCHA, NINA D.; BATISTA, V. L.; **DE JESUS, LUÍS CLÁUDIO LIMA**; LIMA, F. A.; SILVA, T. F.; TAVARES, L. M.; PAZ, J.; SANTOS, E.; ASSIS, R.; SILVA, C.; MOURA, V.; TAPIA-COSTA, A.; DURAN, N.; DRUMMOND, M. M.; AZEVEDO, V.; MANCHA-AGRESTI, P. Testes de seleção de bactérias potencialmente probióticas para futuras utilizações terapêuticas ou preventivas In: VI SIMPÓSIO DE MICROBIOLOGIA DA UFMG, 2019, Belo Horizonte. **Caderno de Resumos-VI SIMPÓSIO DE MICROBIOLOGIA DA UFMG**, 2019.

9. BATISTA, V. L.; CARVALHO, A.; **DE JESUS, LUÍS CLÁUDIO LIMA**; ROCHA, N. D. C.; PROSPERI, C. C.; DRUMMOND, M. M.; NUNES, A.; MANCHA-AGRESTI, P.; AZEVEDO, V.
A functional evaluation of a Recombinant Lineage of Lactococcus lactis MG1363 delivering the vaccine plasmid pExu:hsp65: perspectives for colitis in a murine model In: Fourth International Symposium on Inflammatory Diseases - INFLAMMA IV, 2018, Belo Horizonte. **ANNALS OF INFLAMMA IV – 2018**, 2018.

10. ROCHA, N. D. C.; DRUMMOND, M. M.; PROSPERI, C. C.; **DE JESUS, LUÍS CLÁUDIO LIMA**; BATISTA, V. L.; CARVALHO, A.; NUNES, A.; LECLERCQ, SOPHIE Y.; DE CICCO SANDES, SAVIO H.; NUNES, ALVARO C.; AZEVEDO, VASCO; MANCHA-AGRESTI, PAMELA. Encapsulated Lactococcus lactis as an alternative to improve DNA vaccines delivery in Gastrointestinal Tract In: Fourth International Symposium on Inflammatory Diseases - INFLAMMA IV, 2018, Belo Horizonte. **ANNALS OF INFLAMMA IV – 2018**, 2018.

11. DRUMMOND, M. M.; **DE JESUS, LUÍS CLÁUDIO LIMA**; CARVALHO, A.; SANTOS, S. S.; MARTINS, F. S.; FERREIRA, E.; FERNANDES, R. S.; BARROS, A. L. B.; BATISTA, V. L.; NUNES, A.; COELHO-ROCHA, NINA D.; MANCHA-AGRESTI, P.; AZEVEDO, V. Fermented milk by Lactobacillus delbrueckii CIDCA 133 is able to ameliorate the chemoterapy-induced intestinal mucositis in BALB/C mice In: 6th World Congress on Targeting Microbiota Towards Clinical Revolution, 2018, Porto. **Targeting Microbiota**, 2018. v.5.

12. BATISTA, V. L.; **DE JESUS, LUÍS CLÁUDIO LIMA**; ROCHA, N. D. C.; CARVALHO, A.; BARROSO, F. A. L.; SANTOS, S. S.; MARTINS, F. S.; FERREIRA, E.; FERNANDES, R. S.; BARROS, A. L. B.; DRUMMOND, M. M.; PROSPERI, C. C.; MANCHA-AGRESTI, P.; AZEVEDO, V. Protective effect of recombinant strain of Lactobacillus delbrueckii subsp. lactis CIDCA 133 (pExu: hsp65) on intestinal mucositis In: Gene Time Conference 2018, 2018, Belo Horizonte. **Gene Time Conference 2018-Caderno de Resumos**, 2018.

13. NUNES, A.; CARVALHO, A.; **DE JESUS, LUÍS CLÁUDIO LIMA**; ROCHA, N. D. C.; BATISTA, V. L.; MANCHA-AGRESTI, P.; DRUMMOND, M. M.; AZEVEDO, V. Study of the location of the gastrointestinal tract of Lactococcus lactis and Lactobacillus casei through the expression of two recombinant egfp and mCherry proteins encoded by the vector pExu In: Fourth International Symposium on Inflammatory Diseases - INFLAMMA IV, 2018, Belo Horizonte. **ANNALS OF INFLAMMA IV – 2018**, 2018.

14. **DE JESUS, LUÍS CLÁUDIO LIMA**; BATISTA, V. L.; CARVALHO, A.; ROCHA, N. D. C.; BARROSO, F. A. L.; SANTOS, S. S.; MARTINS, F. S.; FERREIRA, E.; FERNANDES, R. S.; BARROS, A. L. B.; DRUMMOND, M. M.; MANCHA-AGRESTI, P.; AZEVEDO, V. Therapeutic effect of milk fermented by Lactobacillus delbrueckii subsp. lactis CIDCA 133 on 5-FU-induced intestinal mucositis. In: Gene Time Conference 2018, 2018, Belo Horizonte. **Gene Time Conference 2018-Caderno de Resumos**, 2018.

15. CARVALHO, A.; **DE JESUS, L.C.L**; BATISTA, V. L.; ROCHA, N. D. C.; SANTOS, S. S.; MARTINS, F. S.; NUNES, A.; FERREIRA, E.; FERNANDES, R. S.; BARROS, A. L. B.; DRUMMOND, M. M.;

PROSPERI, C. C.; MANCHA-AGRESTI, P.; AZEVEDO, V. Therapeutic effect of wild type and recombinant Lactobacillus delbrueckii subsp. lactis CIDCA 133 on 5-FU-induced intestinal mucositis. In: https://www.sbg.org.br/abstract/35704, 2018, Foz do Iguaçu. **Abstracts- 2018 International Congress of Genetics**. , 2018.

Trabalhos publicados em anais de eventos (resumo expandido)

1. SOUZA, R.; MIRANDA, V.; QUINTANILHA, M.; GALLOTTI, B; **DE JESUS, LUÍS CLÁUDIO LIMA**; AZEVEDO, VASCO; MARTINS, F. S. Avaliação de parâmetros clínicos e imunológicos de Akkermansia muciniphila BAA-835 em modelo murino de mucosite intestinal induzida por 5-Fluouracil In: VII SIMPÓSIO DE MICROBIOLOGIA DA UFMG: CONECTA SIM 2020- Microbiologia Interligada, Belo Horizonte. **Caderno de Resumos-Microbiologia Interligada**, 2020.
2. COELHO-ROCHA, NINA DIAS; DRUMMOND, M. M.; **DE JESUS, LUÍS CLÁUDIO LIMA**; BATISTA, V. L.; LIMA, F. A.; FERNANDES, R. S.; AZEVEDO, V.; MANCHA-AGRESTI, P. Administração oral de Lactobacillus delbrueckii CIDCA133 carreando o plasmideo vacinal pExu:HSP65 previne a mucosite intestinal em modelo murino In: 71ª REUNIÃO ANUAL DA SBPC, 2019, Campo Grande. **Anais/Resumos da 71ª Reunião Anual da SBPC**, 2019.

Totais de produção

Produção bibliográfica

Artigos completos publicados em periódico..... 19
Capítulos de livros publicados..... 8
Trabalhos publicados em anais de eventos..... 34
Apresentações de trabalhos (Conferência ou palestra)..... 8
Apresentações de trabalhos (Congresso)..... 8
Apresentações de trabalhos (Seminário)..... 2
Apresentações de trabalhos (Simpósio)..... 8
Apresentações de trabalhos (Outra)..... 14
Demais produções bibliográficas..... 3

Produção técnica

Desenvolvimento de material didático ou instrucional..... 1

Eventos

Participações em eventos (congresso)..... 6
Participações em eventos (seminário)..... 4
Participações em eventos (simpósio)..... 2
Participações em eventos (encontro)..... 4
Participações em eventos (outra)..... 6
Organização de evento (outro)..... 4

Capítulos de Livros

Chapter 26

Lactic acid bacteria–based beverages in the promotion of gastrointestinal tract health

Luís Cláudio Lima de Jesus[1], Tales Fernando da Silva[1], Rafael de Assis Glória[1], Andria dos Santos Freitas[1], Monique Ferrary Américo[1], Lucas Jorge da Silva Fernandes[1], Gabriela Munis Campos[1], Gabriel Camargos Gomes[1], Rhayane Cristina Viegas Santos[1], Rodrigo Dias de Oliveira Carvalho[1], Debmalya Barh[1,2] and Vasco Azevedo[1]

[1]*Laboratório de Genética Celular e Molecular (LGCM), Instituto de Ciências Biológicas, Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil,* [2]*Centre for Genomics and Applied Gene Technology, Institute of Integrative Omics and Applied Biotechnology (IIOAB), Nonakuri, West Bengal, India*

26.1 Introduction

Humanity has known fermented beverages since the beginning of civilizations, and they are recognized as beneficial for therapeutic use. It was an innovation that was introduced in prehistoric agricultural societies, with evidence of fermented beverage production found in China 7000 B.C. and in Mesopotamia 5000 B.C. Fermented milk has been used in the Middle East, Egypt, Greece, and Italy since 7000–5000 B.C., while in Asia, at approximately the same time, the primary consumption was products that were fermented from rice (Gasbarrini et al., 2016). Since then, modern technology has been employed to investigate the health effects that are provided by fermented products, with increasing research in discovering microorganism strains able to confer additional health improvement in daily consumption (Gasbarrini et al., 2016).

Fermented beverages must contain a sugar source and a natural microbiota or surrounding microorganisms that are responsible for the natural fermentation process (Otles and Ozyurt, 2019). *Lactobacillus* and *Bifidobacterium* comprise most species present in dairy fermented beverages and food (Tamang et al., 2016a,b). However, other bacterial genera (*Enterococcus*, *Lactococcus*, *Pediococcus*, *Bacillus*, *Brevibacterium*, *Leuconostoc*, and *Propionibacterium*) and yeasts, such as *Saccharomyces*, were also isolated and are used in fermented beverages worldwide (Hittinger et al., 2018; Tamang et al., 2016a, 2016b).

The health benefits for the gastrointestinal tract (GIT) of fermented beverages were recognized even before the existence of microorganisms was discovered. Fermented beverages provide vital nutrients to a well-balanced diet: calcium, proteins, potassium, phosphorus, vitamins, and lipids. Also, the acid that is produced slowly helps digestion. Besides nutrients, fermented beverages contain microorganisms that transform the raw material properties that enhance sensorial quality, degrade toxic components and nonnutritive factors, fortify and deliver bioactive compounds, and produce antioxidant and antimicrobial components (Hittinger et al., 2018). The consumption of dairy products was associated with a reduction in the risk of type 2 diabetes, better digestion, attenuation of irritable bowel syndrome, fatty acid liver disease through immunomodulation, and even a decrease in cholesterol levels (Chandan et al., 2017). Many of these effects are derived from Lactic Acid Bacteria (LAB), the mainly microorganism group used in the production of fermented products.

26.2 Lactic Acid Bacteria

Lactic Acid Bacteria are a heterogeneous group of gram-positive, facultative anaerobic microorganisms (Pachla et al., 2018), generally nonmotile, nonsporulating, catalase-negative, and able to produce lactic acid as the primary metabolic end product of carbohydrate fermentation (Bintsis, 2018). They can adapt to different environments, which could

Microbiome, Immunity, Digestive Health and Nutrition. DOI: https://doi.org/10.1016/B978-0-12-822238-6.00008-X

Contribuição: Levantamento bibliográfico, escrita e revisão do manuscrito.

Chapter

Recombinant Probiotics and Microbiota Modulation as a Good Therapy for Diseases Related to the GIT

Luís Cláudio Lima de Jesus, Fernanda Alvarenga Lima, Nina Dias Coelho-Rocha, Tales Fernando da Silva, Júlia Paz, Vasco Azevedo, Pamela Mancha-Agresti and Mariana Martins Drumond

Abstract

Many diseases that affect the gastrointestinal tract (GIT) have great influence on the quality of life of the majority of patients. Many probiotic strains are being highly studied as a promising candidate due to their beneficial effect reported in the GIT. With the purpose of increasing the beneficial characteristics of some probiotics strains and, consequently, to improve further the reported results, many probiotic strains expressing or encoding different proteins, with anti-inflammatory activities, have been developed. These recombinant strains have been reported as good candidates for the treatment of different pathological conditions, especially colitis and mucositis disease since they have been shown to have positive results and good perspectives for GIT inflammation. Thus, this chapter will first address the aspects of the gastrointestinal tract in humans as well as its microbiota. In a second moment, it will discuss about chronic diseases, mainly the intestinal ones. Finally, it will discuss about probiotics, especially concerning on lactic acid bacteria (LAB), and its action in the prevention and treatment of these diseases. At the final part, we will point out aspects on the development of recombinant strains and the results found in the literature on disease models.

Keywords: *L. lactis*, *Lactobacillus*, DNA vaccine, heterologous protein

1. The human gastrointestinal tract

The human gastrointestinal tract is formed by a complex ecosystem which includes the gastrointestinal epithelium, immune cells, and resident microbiota [1] and comprehends one of the biggest existent interfaces between the host, environmental factors, and antigens in the human body.

The intestine encompasses a broad variety of microorganisms (bacteria, archaea, eukarya, and viruses) [2] from more than 3500 different species [3, 4] that coevolved with the host in a mutually beneficial relationship [5, 6]. The composition and density of bacterial populations in adult individuals differ considerably over the GIT. The area

Contribuição: Levantamento bibliográfico, escrita e revisão do manuscrito.

CAPÍTULO 06

Uso De Bactérias Lácticas Como Vetores De Entrega De Vacinas Recombinantes

Monique Ferrary Américo [1]; Andria dos Santos Freitas [2]; Tales Fernando da Silva [2]; Luís Lima de Jesus [2]; Fernanda Alvarenga Lima [2]; Rodrigo Dias de Oliveira Carvalho [3]; Vasco Ariston de Carvalho Azevedo [4]

[1] Mestranda em Genética. Programa de Pós-Graduação em Genética. Universidade Federal de Minas Gerais – UFMG
[2] Doutorando(a) em Genética. Programa de Pós-Graduação em Genética. Universidade Federal de Minas Gerais – UFMG
[3] Professor Visitante no Departamento de Bioquímica e Biofísica. Universidade Federal da Bahia - UFBA
[4] Professor Titular no Departamento de Genética Ecologia e Evolução. Universidade Federal de Minas Gerais – UFMG

RESUMO

A vacinologia tem avançado rapidamente apesar de ser um campo relativamente novo da Ciência. Entre as várias estratégias que vem sendo utilizadas para o controle de doenças, o uso da tecnologia do DNA recombinante tem trazido perspectivas animadoras para o desenvolvimento de vacinas mais seguras e mais eficazes, incluindo para novos patógenos emergentes. Neste contexto, destaca-se o uso de bactérias lácticas como vetores e adjuvantes. Este capítulo contém uma revisão descritiva sobre os mais relevantes tópicos envolvendo vacinas recombinantes, incluindo a história da arte, mecanismos, estratégias imunizantes e avanços recentes obtidos em estudos de prova de conceito para o desenvolvimento de novas vacinas.

Palavras-chave: Bactérias Lácticas. Biotecnologia. Vacinas de DNA. Imunologia de mucosas.

https://doi.org/10.53924/vac1.06

Contribuição: Levantamento bibliográfico, escrita e revisão do manuscrito.

Chapter 16

Immunomodulatory and antiinflammatory mechanisms of probiotics

Andria dos Santos Freitas[a], Lucas Jorge da Silva Fernandes[a], Nina Dias Coelho-Rocha[a], Luís Cláudio Lima de Jesus[a], Vinicius de Rezende Rodovalho[a], Tales Fernando da Silva[a], Rodrigo Dias de Oliveira Carvalho[b], and Vasco Azevedo[a]

[a]*Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil,* [b]*Department of Biochemistry and Biophysics, Institute of Health Sciences, Federal University of Bahia, Salvador, Brazil*

16.1 Introduction

The gastrointestinal immune system known as gut-associated lymphoid tissue (GALT) is the largest specialized immunological anatomic structure in the body, and it is constantly exposed to external compounds and microbes, playing a crucial role in the host defense and homeostasis (Ballan, Battistini, Xavier-Santos, & Saad, 2020). It must be able to carry out the dual role of both tolerating the presence of a huge number of commensal microbiota and protecting the intestinal mucosa against pathogenic microorganism and dietary antigens (Allaire et al., 2018; Wershil & Furuta, 2008). In order to maintain the balance in the colonizing bacterial communities, the innate immunity and adaptive immunity work together to recognize a variety of antigens and coordinate protection mechanisms in the intestines (Kato, Shimpei, Mikako, & Sidonia, 2014).

16.1.1 Mucosal immunity and intestinal epithelium

Apart from immune responses, the very first line of defense against pathogens for host protection is the mucosal barrier, which comprises both cellular and enzymatic components (Farhadi, Banan, Fields, & Keshvarzian, 2003). Starting in the mouth and running through the entire upper gastrointestinal (GI) tract, low pH, bile salts, and proteolytic enzymes such as pepsin, trypsin and pancreatic proteases fulfill the role of facilitating the digestion process by breaking down big molecules into small ones. Besides helping digestion, this process allows conversion of potentially immunogenic proteins into small length peptides, therefore less immunogenic (Justin & Dhamoon, 2020).

The intestinal epithelial cells (IECs) are the basis of the mucosal barrier and are generally found in the whole intestines in different specialized forms. The majority of IECs bordering the intestinal lumen are absorptive enterocytes, which are responsible for nutrient and water absorption and provide a physical barrier against pathogens, being joined together by tight junctions, maintaining low intestinal permeability (Mowat & Agace, 2014). Among the main cellular components of the mucus barrier are the goblet cells, specialized IECs, which produce mucin glycoproteins that cover the adjacent epithelium (Kim et al., 2016). This barrier prevents pathogen and antigen invasion, and works as a binding site for secretory IgA (sIgA), which is thereafter directly transported through transcytosis by IECs across the epithelial barrier into the intestinal lumen (Peterson & Artis, 2014). Despite being a component of adaptive immune responses, sIgA is an important immunoglobulin that functions as an inhibitor of microbial attachment to the underlying epithelium, avoiding their translocation to the lumen, thus supporting barrier function (Lamont, 1992).

Barrier function against pathogens is also enhanced by the presence of Paneth cells, specialized IEC type that are found at the base of intestinal crypts and release many antimicrobial peptides, such as α-defensins, lysozyme, and secretory phospholipase (Gallo & Hooper, 2012). These antimicrobial factors, in special α-defensins, have been demonstrating to exert inhibitory activity against Gram-positive and Gram-negative bacteria, fungi, viruses, and protozoa (Vaishnava, Behrendt, Ismail, Eckmann, & Hooper, 2008; Wehkamp & Stange, 2006).

Besides promoting barrier function, specialized IEC lineages also help regulate local and systemic immunity through a cross talk between innate and adaptive cells (Peterson & Artis, 2014), carried out by activating the cell signaling cascade,

Probiotics. https://doi.org/10.1016/B978-0-323-85170-1.00019-1

Contribuição: Levantamento bibliográfico, escrita e revisão do manuscrito.

CHAPTER

12

Lactic acid bacteria in gut microbiota, probiotics and disease prevention

Flavia Figueira Aburjaile[a], *Luís Cláudio Lima de Jesus*[a], *Tales Fernando da Silva*[a], *Mariana Martins Drumond*[b], *Rodrigo Dias de Oliveira Carvalho*[a], *Vasco Azevedo*[a], *and Pamela Del Carmen Mancha-Agresti*[c]

[a]Laboratory of Cellular and Molecular Genetics (LGCM), Department of Genetics, Ecology and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil [b]Departamento de Ciências Biológicas, Centro Federal de Educação Tecnológica de Minas Gerais, Belo Horizonte, MG, Brazil [c]Faculdade de Minas-Faminas-BH, Belo Horizonte, MG, Brazil

1 Introduction: Microbiota: Health and disease

1.1 Intestinal microbiota

Microbiota is the collection of microorganisms in a defined region and/or period. The human microbiota corresponds to approximately 1–2 kg, being weight-like compared to the brain (roughly 1.5 kg). Depending on where in the body they are found, the species of microorganisms vary widely in their functions (Cummings and Macfarlane, 1997). It is estimated that the microbiota population can exceed 10^{14}, surpassing the number of cells from the host itself (Gill et al., 2006). Intestinal microbiota comprehends microorganisms habiting from the duodenum to the rectum producing vitamins, short-chain fatty acids (SCFA), nutrients, and closely interacting with the host, influencing the metabolic and health status of the host (David et al., 2014).

Environmental factors can have a significant impact on the microbiota composition. Since birth or, as some studies have found, since the creation of the womb, the way the host interacts with the environment can determine which groups of organisms will prevail and how they will affect the health of the host (Thursby and Juge, 2017). Babies born by c-section have different microbiota of the ones from a vaginal birth; the same individual has different microbiota compositions in various stages of life, based on the types of food they eat, the beings

Lactic Acid Bacteria in Food Biotechnology
https://doi.org/10.1016/B978-0-323-89875-1.00001-8

Contribuição: Levantamento bibliográfico, escrita e revisão do manuscrito.

eBook on Inflammatory Bowel Disease

Chapter 2

The Gastrointestinal Disease to the Microbiota Treatment: From the Present to the Future

Fernanda Alvarenga Lima[1], Luís Cláudio Lima de Jesus[1], Viviane Lima Batista[1], Laísa Macedo Tavares[1], Rafael Assis[1], Mariana Martins Drumond[1,2], Vasco Azevedo[1], Pamela Mancha-Agresti[1*]

[1]Laboratório de Genética Celular e Molecular (LGCM), Instituto de Ciências Biológicas, Departamento de Biologia Geral, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

[2]Centro Federal de Educação Tecnológica de Minas Gerais (CEFET/MG), Departamento de Ciências Biológicas, Belo Horizonte, Minas Gerais, Brazil.

***Correspondence to: Pamela Mancha-Agresti,** Laboratório de Genética Celular e Molecular (LGCM), Instituto de Ciências Biológicas, Departamento de Biologia Geral, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.*

Email: p.mancha.agresti@gmail.com

1. Introduction

Chronic diseases, especially the inflammatory ones, share several characteristics to each other. In this chapter we will first discuss the main characteristics of inflammatory bowel diseases, afterwards the Metabolic Syndrome and associated diseases and, finally, intestinal mucositis that although is not a disease itlself, produces an inflammatory process with serious problems related to the discontinuation of cancer treatment. Second, we will discuss the effects of probiotics on the control, prevention or attenuation of the symptoms of these diseases and, finally, we will discuss about the changes that these diseases cause in the microbiota, emphasizing the methods and results of the researches involving the microbiota and the next steps of scientific research to elucidate the mechanisms involved in the control of inflammatory processes through its manipulation.

Contribuição: Levantamento bibliográfico, escrita e revisão do manuscrito.

Chapter 24

Lactic Acid Bacteria as Delivery Vehicle for Therapeutics Applications

Viviane Lima Batista, Tales Fernando da Silva, Luis Cláudio Lima de Jesus, Ana Paula Tapia-Costa, Mariana Martins Drumond, Vasco Azevedo, and Pamela Mancha-Agresti

Abstract

Lactic acid bacteria comprise a large group of Gram-positive organisms capable of converting sugar into lactic acid. They have been studied due to their therapeutic potential on the mucosal surface. Among the species, *Lactococcus lactis* is considered the model bacterium and it has been explored as an important vehicle for providing therapeutic molecules and antigens in the mucosa. They can be genetically engineered to produce a variety of molecules as well as deliver heterologous DNA and protein. DNA vaccines consist of the administration of a bacterial plasmid under the control of a eukaryotic promoter encoding the antigen of interest. The resulting proteins are capable of stimulating the immune system, becoming a promising technique for immunization against a variety of tumors and infection diseases and having several advantages compared to conventional nucleic acid delivery methods (such as bioballistic delivery, electroporation, and intramuscular administration).

Key words Lactic acid bacteria, DNA vaccine, DNA delivery, Mucosal administration, *Lactococcus lactis*

1 Introduction

Lactic acid bacteria (LAB) comprises a large group of gram-positive and nonsporulating microorganisms with fermentative properties, having the capacity to convert sugar into lactic acid. The majority of LAB have a "Generally Recognized as Safe" (GRAS) status according to the United States Food and Drug Administration (US FDA), meeting the criteria to be considered safe for human consumption [1].

LAB has been intensively studied due to their potential therapeutic effects on mucosal surfaces and can also be genetically engineered to efficiently produce a large variety of molecules either for the delivery of DNA or heterologous proteins [2–5]. In this context, among the various species of LAB, *Lactococcus lactis* is

Blaine A. Pfeifer and Andrew Hill (eds.), *Vaccine Delivery Technology: Methods and Protocols*, Methods in Molecular Biology, vol. 2183, https://doi.org/10.1007/978-1-0716-0795-4_24,

Contribuição: Levantamento bibliográfico, escrita e revisão do manuscrito.

CHAPTER

14

Comparative genomics in probiotic bacteria

Juan Luis Valdez-Baez[a], *Luís Cláudio Lima De Jesus*[a], *Pedro Henrique Marques*[b], *Ligia Carolina da Silva Prado*[c], *Andrei Giacchetto Felice*[b], *Thaís Cristina Vilela Rodrigues*[a], *Flávia Aburjaile*[a], *Vasco Azevedo*[a], *and Siomar de Castro Soares*[b]

[a]Laboratory of Cellular and Molecular Genetics (LGCM), Department of Genetics, Ecology and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil [b]Department of Microbiology, Immunology, and Parasitology, Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil [c]Interunits Postgraduate Program in Bioinformatics, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil

1 Introduction: Probiotic bacteria

Probiotics are known as live microorganisms, which, when administered in adequate amounts, confer a health benefit upon the host (FAO, 2001). Since 1907, Élie Metchnikoff proposed the relationship between the presence of centenarians in Bulgaria and the consumption of soured milk containing beneficial microorganisms (*Lactobacillus bulgaricus*) capable of influencing the gut microbiota; therefore, Tissier suggested the administration of *Bifidobacterium* isolates to treat persistent diarrhea in children. Continuing with Shirota with the first probiotic product (Yakult) until the present, probiotics have been an object of study in several in vitro and in vivo evaluations that have shown varied beneficial effects on the host (human, animal, and plant) and have acquired relevance as health promoters (Siezen and Wilson, 2010).

Traditionally, probiotics have been isolated from fermented and dairy products; however, several of these bacteria are part of the intestinal microbiota and they were also isolated from feces samples (Cunha et al., 2013; Karami et al., 2017; Mulaw et al., 2019). Other sources of

Lactic Acid Bacteria in Food Biotechnology
https://doi.org/10.1016/B978-0-323-89875-1.00005-5

Contribuição: Levantamento bibliográfico, escrita e revisão do manuscrito.

Artigos Científicos (Colaborações)

- Aplicação de probióticos/paraprobióticos no contexto de inflamação intestinal

Contribuição: Escrita e revisão do manuscrito

frontiers in Microbiology

REVIEW
published: 17 September 2020
doi: 10.3389/fmicb.2020.544490

Probiotics, Prebiotics, Synbiotics, and Paraprobiotics as a Therapeutic Alternative for Intestinal Mucositis

Viviane Lima Batista[1], Tales Fernando da Silva[1], Luís Cláudio Lima de Jesus[1], Nina Dias Coelho-Rocha[1], Fernanda Alvarenga Lima Barroso[1], Laisa Macedo Tavares[1], Vasco Azevedo[1], Pamela Mancha-Agresti[1,2] and Mariana Martins Drumond[1,3]**

[1] Laboratório de Genética Celular e Molecular (LGCM), Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil, [2] Faculdade de Minas, FAMINAS-BH, Belo Horizonte, Brazil, [3] Centro Federal de Educação Tecnológica de Minas Gerais (CEFET/MG), Departamento de Ciências Biológicas, Belo Horizonte, Brazil

OPEN ACCESS

Edited by:
Konstantinos Papadimitriou,
University of Peloponnese, Greece

Reviewed by:
Natasa Golic,
University of Belgrade, Serbia
Eric Claassen,
Vrije Universiteit Amsterdam, Netherlands
Jia Yin,
Hunan Normal University, China

***Correspondence:**
Pamela Mancha-Agresti
p.mancha.agresti@gmail.com
Mariana Martins Drumond
mmdrumond@gmail.com

Specialty section:
This article was submitted to Food Microbiology, a section of the journal Frontiers in Microbiology

Received: 21 March 2020
Accepted: 24 August 2020
Published: 17 September 2020

Citation:
Batista VL, da Silva TF, de Jesus LCL, Coelho-Rocha ND, Barroso FAL, Tavares LM, Azevedo V, Mancha-Agresti P and Drumond MM (2020) Probiotics, Prebiotics, Synbiotics, and Paraprobiotics as a Therapeutic Alternative for Intestinal Mucositis. Front. Microbiol. 11:544490. doi: 10.3389/fmicb.2020.544490

Intestinal mucositis, a cytotoxic side effect of the antineoplastic drug 5-fluorouracil (5-FU), is characterized by ulceration, inflammation, diarrhea, and intense abdominal pain, making it an important issue for clinical medicine. Given the seriousness of the problem, therapeutic alternatives have been sought as a means to ameliorate, prevent, and treat this condition. Among the alternatives available to address this side effect of treatment with 5-FU, the most promising has been the use of probiotics, prebiotics, synbiotics, and paraprobiotics. This review addresses the administration of these "biotics" as a therapeutic alternative for intestinal mucositis caused by 5-FU. It describes the effects and benefits related to their use as well as their potential for patient care.

Keywords: lactic acid bacteria, chemotherapy, intestinal inflammation, treatment, mucosite

INTRODUCTION

Cancer is a disease characterized by uncontrolled proliferation of cells with cellular differentiation properties, having the capacity to invade tissues and organs and spread to other regions of the body, causing metastases (World Health Organization [WHO], 2018). This disease is the second leading cause of death globally, according to the World Health Organization, accounting for an estimated 9.6 million deaths in 2018; lung (1.76 million deaths), colorectal (862,000 deaths), stomach (783,000 deaths), liver (782,000 deaths), and breast cancer (627,000 deaths) are the most common types and have the highest mortality rates (World Health Organization [WHO], 2018).

Despite the high incidence and mortality rates, when identified early, cancer is a potentially curable and treatable disease. Treatment may be done through surgery, chemotherapy, radiotherapy, or bone marrow transplantation, depending on the type of cancer, degree of tumor aggressiveness, as well as the patient's physical and immunological status. It is often necessary to combine more than one type of treatment to achieve satisfactory results (World Health Organization [WHO], 2018).

Antineoplastic chemotherapy consists of the use of drugs that destroy cancer cells, inhibit their growth, and prevent their spread by targeting DNA or critical processes involved in cell division (Guichard et al., 2017; Shields, 2017). The traditional chemotherapeutics are classified according to their mechanisms of action, including antimetabolites, microtubule-targeting agents, topoisomerases, and antibiotics (Shields, 2017). The therapeutic arsenal mostly used in the

Acesso: https://doi.org/10.3389/fmicb.2020.544490

Contribuição: Escrita do manuscrito

GMR
The Original by FUNPEC-RP

Probiotic potential of novel Brazilian *Lactobacillus crispatus* strains

F.F. Aburjaile, M.V.C. Viana, J.C. Cerqueira, L.C.L. de Jesus, T.F. da Silva, R. Carvalho and V. Azevedo

Departamento de Genética, Ecologia e Evolução, Universidae Federal de Minas Gerais, Belo Horizonte, MG, Brasil

Corresponding author: V. Azevedo
E-mail: vasco@icb.ufmg.br

Genet. Mol. Res. 20 (2): gmr18900
Received April 28, 2021
Accepted May 02, 2021
Published May 31, 2021
DOI http://dx.doi.org/10.4238/gmr18900

ABSTRACT. Lactobacilli are the predominant bacterial species colonizing the vaginal surfaces of healthy women, where they play a protective role against opportunistic and polymicrobial infections, such as bacterial vaginosis. Several *Lactobacillus* species, especially *L. crispatus*, have been prospected for probiotic applications due to their potential antimicrobial and anti-inflammatory capacities. During the last decade, several genomic studies have been investigating the genetics of *L. crispatus* strains in an effort to identify novel probiotic strains and evaluate their potential for improving human and animal health. This mini review highlights the main genes associated with *L. crispatus* protective mechanisms in four novel strains of this species that we recently isolated from healthy Brazilian women of reproductive age. Among the probiotic features of these strains, the roles of a pyruvate oxidase-encoding gene, lactate synthesis related enzymes, bacteriocin genes, and genomic islands, are reviewed, and the next steps for confirming their activity are indicated.

INTRODUCTION

First identified in 1894 by a German physician named Doderlein, lactobacilli have been reported as the dominant bacterial species that colonize the vaginal epithelium of women of reproductive age (Tachedjian et al., 2018). *Lactobacillus crispatus* is the most frequently isolated microorganism from this environment; it plays an important role in

Acesso: http://dx.doi.org/10.4238/gmr18900

Contribuição: Auxílio na realização do experimento, análise dos dados, escrita e revisão do manuscrito

frontiers | Frontiers in Microbiology

ORIGINAL RESEARCH
published: 26 April 2022
doi: 10.3389/fmicb.2022.858036

Lactobacillus delbrueckii CIDCA 133 Ameliorates Chemotherapy-Induced Mucositis by Modulating Epithelial Barrier and TLR2/4/Myd88/NF-κB Signaling Pathway

Fernanda Alvarenga Lima Barroso[1], Luís Cláudio Lima de Jesus[1], Tales Fernando da Silva[1], Viviane Lima Batista[1], Juliana Laguna[1], Nina Dias Coelho-Rocha[1], Kátia Duarte Vital[2], Simone Odília Antunes Fernandes[2], Valbert Nascimento Cardoso[2], Enio Ferreira[3], Flaviano Santos Martins[4], Mariana Martins Drumond[1,5], Pamela Mancha-Agresti[1], Alexander Birbrair[3], Debmalya Barh[1,6] and Vasco Azevedo[1]**

[1] Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2] Departamento de Análises Clínicas e Toxicológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [3] Departamento de Patologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [4] Departamento de Microbiologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [5] Departamento de Ciências Biológicas, Centro Federal de Educação Tecnológica de Minas Gerais, Belo Horizonte, Brazil, [6] Centre for Genomics and Applied Gene Technology, Institute of Integrative Omics and Applied Biotechnology (IIOAB), Purba Medinipur, India

OPEN ACCESS

Edited by:
Konstantinos Papadimitriou,
University of Peloponnese, Greece

Reviewed by:
Martinna Bertolini,
UCONN Health, United States
Ayelen Amelia Hugo,
Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA)-CCT La Plata, Argentina

***Correspondence:**
Debmalya Barh
dr.barh@gmail.com
Vasco Azevedo
vasco@ufmg.br;
vascoariston@gmail.com

Specialty section:
This article was submitted to Food Microbiology, a section of the journal Frontiers in Microbiology

Received: 19 January 2022
Accepted: 31 March 2022
Published: 26 April 2022

Citation:
Barroso FAL, de Jesus LCL, da Silva TF, Batista VL, Laguna J, Coelho-Rocha ND, Vital KD, Fernandes SOA, Cardoso VN, Ferreira E, Martins FS, Drumond MM, Mancha-Agresti P, Birbrair A, Barh D and Azevedo V (2022) Lactobacillus delbrueckii CIDCA 133 Ameliorates Chemotherapy-Induced Mucositis by Modulating Epithelial Barrier and TLR2/4/Myd88/NF-κB Signaling Pathway. Front. Microbiol. 13:858036. doi: 10.3389/fmicb.2022.858036

Intestinal mucositis promoted by the use of anticancer drugs is characterized by ulcerative inflammation of the intestinal mucosa, a debilitating side effect in cancer patients undergoing treatment. Probiotics are a potential therapeutic option to alleviate intestinal mucositis due to their effects on epithelial barrier integrity and anti-inflammatory modulation. This study investigated the health-promoting impact of *Lactobacillus delbrueckii* CIDCA 133 in modulating inflammatory and epithelial barrier markers to protect the intestinal mucosa from 5-fluorouracil-induced epithelial damage. *L. delbrueckii* CIDCA 133 consumption ameliorated small intestine shortening, inflammatory cell infiltration, intestinal permeability, villus atrophy, and goblet cell count, improving the intestinal mucosa architecture and its function in treated mice. Upregulation of *Muc2*, *Cldn1*, *Hp*, *F11r,* and *Il10,* and downregulation of markers involved in NF-κB signaling pathway activation (*Tlr2, Tlr4, Nfkb1, Il6,* and *Il1b*) were observed at the mRNA level. This work suggests a beneficial role of *L. delbrueckii* strain CIDCA 133 on intestinal damage induced by 5-FU chemotherapy through modulation of inflammatory pathways and improvement of epithelial barrier function.

Keywords: 5-FU-induced mucositis, probiotics, *Lactobacillus delbrueckii*, anti-inflammatory cytokines, intestinal permeability, tight junction proteins

Acesso: https://doi.org/10.3389/fmicb.2022.858036

Contribuição: Realização de RT-qPCR, análise dos dados, e revisão do manuscrito.

Life Sciences 289 (2022) 120243

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Bifidobacterium longum subsp. *longum* 5^{1A} attenuates intestinal injury against irinotecan-induced mucositis in mice

Mônica F. Quintanilha [a], Vivian C. Miranda [a], Ramon O. Souza [a], Bruno Gallotti [a], Clênio Cruz [b], Elandia A. Santos [b], Jacqueline I. Alvarez-Leite [b], Luís C.L. Jesus [c], Vasco Azevedo [c], Luísa M. Trindade [d], Valbert N. Cardoso [d], Enio Ferreira [e], Bárbara A. Carvalho [e], Pedro M. G. Soares [f], Angélica T. Vieira [b], Jacques R. Nicoli [a], Flaviano S. Martins [a,*]

[a] Department of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[b] Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[c] Department of Genetics, Ecology, and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[d] Department of Clinical and Toxicological Analysis, Faculty of Pharmacy, University Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[e] Department of General Pathology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[f] Department of Morphology, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil

ARTICLE INFO

Keywords:
Bifidobacterium longum
Probiotic
Intestinal mucositis
Irinotecan
Intestinal barrier

ABSTRACT

Intestinal mucositis (IM) is a critical side-effect associated with antineoplastic therapy. Treatment available is only palliative and often not effective. However, alternative therapeutic strategies, such as probiotics, have attracted significant attention due to their immune-modulatory action in several diseases. Thus, the present study aims to elucidate the therapeutic potential of the probiotic strain *Bifidobacterium longum* 5^{1A} in a murine model of mucositis induced by irinotecan. Due to the scarcity of studies on dose-response and viability (probiotic vs paraprobiotic), we first evaluated which dose and cell viability would be most effective in treating mucositis. In this study, the oral pretreatment with viable *B. longum* 5^{1A} at a concentration of 1×10^9 CFU/mL reduced the daily disease activity index ($p < 0.01$), protected the intestinal architecture, preserved the length of the intestine ($p < 0.05$), and reduced intestinal permeability ($p < 0.01$), inflammation, and oxidative damage ($p < 0.01$) induced by irinotecan. Also, treatment with *B. longum* 5^{1A} increased the production of secretory immunoglobulin A ($p < 0.05$) in the intestinal fluid of mice with mucositis. Furthermore, *B. longum* 5^{1A} reversed the mucositis-induced increase in Enterobacteriaceae bacterial group in the gut ($p < 0.01$). In conclusion, these results showed that oral administration of *B. longum* 5^{1A} protects mice against intestinal damage caused by irinotecan, suggesting its use as a potential probiotic in therapy during mucositis.

1. Introduction

Camptothecin (CPT)-11 (irinotecan), a topoisomerase I inhibitor frequently used against solid tumors, has side effects that target the healthy gastrointestinal (GI) tissue and interrupt DNA synthesis, leading to apoptosis [1,2]. The constant damage and the loss of intestinal epithelium's ability to quickly repair cause an inflammatory response that increases intestinal permeability and severe diarrhea. This condition is called Intestinal Mucositis (IM), one of the main adverse effects associated with the use of antineoplastic drugs that inhibit cell growth and/or cell division [3–5].

IM affects more than 520,000 patients undergoing chemotherapy or radiotherapy per year only in the US. This implied severe impairment in patients' quality of life, primarily due to interruptions or premature suspension of treatment, prolonged hospital stay, and increased readmission rates in intensive care units [6]. Despite advances in the development of new therapeutic agents for IM, the treatment is limited to the use of antioxidants, anti-inflammatory drugs, cytokine synthesis inhibitors, cytoprotection, and growth factors. These compounds are used only to reduce the GI symptoms experienced by the patient, being more palliative and often have minimal clinical efficacy [4,7,8].

The gut microbiota plays an essential role in the homeostasis of the

* Corresponding author at: Laboratory of Biotherapeutic Agents, Department of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Av. Antônio Carlos, 6627, 30270-901, MG, Brazil.
E-mail address: flaviano@icb.ufmg.br (F.S. Martins).

https://doi.org/10.1016/j.lfs.2021.120243
Received 28 July 2021; Received in revised form 7 December 2021; Accepted 13 December 2021
Available online 16 December 2021

Acesso: https://doi.org/10.1016/j.lfs.2021.120243

Contribuição: Realização de RT-qPCR e análise dos dados.

British Journal of Nutrition, page 1 of 12 doi:10.1017/S0007114520004924

Effects of dietary fibre intake in chemotherapy-induced mucositis in murine model

B. Gallotti[1,2]†, I. Galvao[1]†, G. Leles[1], M. F. Quintanilha[2], R. O. Souza[2], V. C. Miranda[2], V. M. Rocha[1], L. M. Trindade[3], L. C. L. Jesus[4], V. Mendes[1], L. C. Andre[5], M. M. d'Auriol-Souza[5], V. Azevedo[4], V. N. Cardoso[3], F. S. Martins[2]‡ and A. T. Vieira[1]‡*

[1]*Laboratório de Microbiota e Imunomodulação, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*
[2]*Laboratório de Agentes Bioterapêuticos, Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*
[3]*Laboratório de Radioisótopos, Departamento de Analises Clinicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*
[4]*Laboratório de Genética Celular e Molecular, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*
[5]*Laboratório de Toxicologia Ocupacional, Departamento de Analises Clinicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*

(Submitted 22 May 2020 – Final revision received 27 November 2020 – Accepted 28 November 2020)

Abstract

Mucositis is an inflammation of the gastrointestinal mucosa resulting from high doses of radio/chemotherapy treatment and may lead to interruption of antineoplasic therapy. Soluble fibres, like pectin, increase SCFA production, which play a role in gut homoeostasis and inflammation suppression. Due to the properties of pectin, the aim of the present study was to evaluate the effect of a high-fibre (HF) diet on chemotherapy-induced mucositis in a murine model. C57/BL6 mice received control (AIN93M), HF, low/zero fibre (LF) diets for 10 d prior to mucositis challenging with irinotecan (75 mg/kg), or they were treated with acetate added to drinking water 5 d prior to and during the mucositis induction. Mice that received the HF diet showed decreased immune cells influx and improved histopathological parameters in the intestine, compared with mice that received the normal diet. Furthermore, the HF diet decreased intestinal permeability induced in the mucositis model when compared with the control group. This effect was not observed for acetate alone, which did not improve gut permeability. For instance, mice that received the LF diet had worsened gut permeability, compared with mice that received the normal diet and mucositis. The effects of the HF and LF diets were shown to modulate the intestinal microbiota, in which the LF diet increased the levels of Enterobacteriaceae, a group associated with gut inflammation, whereas the HF diet decreased this group and increased *Lactobacillus* and *Bifidobacterium* (SCFA producers) levels. In conclusion, the results demonstrated the importance of dietary fibre intake in the modulation of gut microbiota composition and homoeostasis maintenance during mucositis in this model.

Key words: **Mucositis: Fibre: Pectin: SCFA: Microbiota modulation**

Chemotherapy is the most common and widely used cancer treatment therapy. It consists of a drug mix that targets the neoplastic cells, promoting their elimination[1]. Unfortunately, chemotherapeutic agents are not specific for cancer cells, they also affect the host's cells, such as the immune and gastrointestinal epithelium cells, with a high proliferation rate[2]. Chemotherapy can trigger some side effects, including nausea, vomiting, inappetence, diarrhoea and, the most common, mucositis[3,4]. The frequency of mucositis is approximately 40 % in patients under antineoplastic therapy and almost 100 % in patients with head and neck cancer undergoing chemotherapy[4,5].

Abbreviations: EPO, eosinophil peroxidase; FITC, fluorescein isothiocyanate; HF, high fibre; LF, low fibre; MPO, myeloperoxidase.

* **Corresponding author:** Angelica Thomaz Vieira, fax +55 31 34092613, email angelicathomazvieira@ufmg.br

† These authors contributed equally to this work.
‡ These authors were co-supervisors and contributed equally to this work.

Acesso: https://doi.org/10.1017/S0007114520004924

Contribuição: Realização de RT-qPCR e análise dos dados.

Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-021-09842-z

Paraprobiotic *Lacticaseibacillus rhamnosus* Protects Intestinal Damage in an Experimental Murine Model of Mucositis

Luísa Martins Trindade[1] · Lícia Torres[2] · Isabel David Matos[3] · Vivian Correia Miranda[4] · Luís Cláudio Lima de Jesus[5] · Gregório Cavalcante[2] · Jonathan Júnio de Souza Oliveira[6] · Geovanni Dantas Cassali[6] · Pamela Mancha-Agresti[5] · Vasco Ariston de Carvalho Azevedo[5] · Tatiani Uceli Maioli[3] · Valbert Nascimento Cardoso[7] · Flaviano dos Santos Martins[4] · Simone de Vasconcelos Generoso[3]

Accepted: 27 August 2021

Abstract
Intestinal mucositis (IM) is a common side effect resulting from cancer treatment. However, the management so far has not been very effective. In the last years, the role of the gut microbiota in the development and severity of mucositis has been studied. Therefore, the use of probiotics and paraprobiotics could have a potential therapeutic effect on IM. The aim of our study was to investigate the impact of the administration of *Lacticaseibacillus rhamnosus* (*L. rhamnosus*) CGMCC1.3724 and the paraprobiotic on IM in mice. For 13 days, male Balb/c mice were divided into six groups: control (CTL) and mucositis (MUC)/0.1 mL of saline; CTL *Lr*V and MUC *Lr*V/0.1 mL of 10^8 CFU of viable *Lr*; CTL *Lr*I and MUC *Lr*I/0.1 mL of 10^8 CFU of inactivated *Lr*. On the 10th day, mice from the MUC, MUC *Lr*V, and MUC *Lr*I groups received an intraperitoneal injection (300 mg/kg) of 5-fluorouracil to induce mucositis. The results showed that the administration of the chemotherapeutic agent increased the weight loss and intestinal permeability of the animals in the MUC and MUC *Lr*V groups. However, administration of paraprobiotic reduced weight loss and maintained PI at physiological levels. The paraprobiotic also preserved the villi and intestinal crypts, reduced the inflammatory infiltrate, and increased the mucus secretion, Muc2 gene expression, and Treg cells frequency.

Keywords Mucositis · 5-Fluorouracil · *Lacticaseibacillus rhamnosus* · Probiotic · Paraprobiotic

Introduction

5-Fluorouracil (5-FU) is a chemotherapeutic agent commonly used for cancer therapy, mainly metastatic colorectal cancer [1]. The drug's mechanism of action leads to cytotoxicity due to the erroneous incorporation of fluoronucleotides, analogous to uracil, into deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) that cause the DNA strand to break, inhibiting the thymidylate synthase enzyme [2]. This process induces apoptosis in fast turnover cells, such as cancerous cells, but also in healthy cells located in tissues as the gastrointestinal (GI) tract mucosa [3]. Nauseas, pain, and

✉ Simone de Vasconcelos Generoso
simonenutufmg@gmail.com

[1] Programa de Pós-Graduação Em Ciência de Alimentos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[2] Programa de Pós-Graduação Em Bioquímica E Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[3] Programa de Pós-Graduação Em Nutrição E Saúde, Departamento de Nutrição, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[4] Programa de Pós-Graduação Em Microbiologia, Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[5] Programa de Pós-Graduação Em Genética, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[6] Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[7] Departamento de Análises Clínicas E Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Published online: 15 September 2021

Acesso: https://doi.org/10.1007/s12602-021-09842-z

- Caracterização genômica de bactérias probióticas e patogênicas

Contribuição: Processamento amostras de DNA para sequenciamento.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 08 January 2021
doi: 10.3389/fmicb.2020.549254

Genomic Characterization of Multidrug-Resistant *Escherichia coli* BH100 Sub-strains

*Rodrigo Carvalho[1], Flavia Aburjaile[2,3], Marcus Canario[2], Andréa M. A. Nascimento[2], Edmar Chartone-Souza[2], Luis de Jesus[2], Andrey A. Zamyatnin Jr.[1,4], Bertram Brenig[5], Debmalya Barh[2,6], Preetam Ghosh[7], Aristoteles Goes-Neto[2], Henrique C. P. Figueiredo[2], Siomar Soares[8], Rommel Ramos[9], Anne Pinto[2] and Vasco Azevedo[2]**

[1] Institute of Molecular Medicine, Sechenov First Moscow State Medical University, Moscow, Russia, [2] Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [3] Departamento de Genética, Universidade Federal de Pernambuco, Recife, Brazil, [4] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia, [5] Institute of Veterinary Medicine, University of Göttingen, Göttingen, Germany, [6] Institute of Integrative Omics and Applied Biotechnology, Purba Medinipur, India, [7] Department of Computer Science, Virginia Commonwealth University, Richmond, VA, United States, [8] Departmento de Microbiologia, Imunologia e Parasitologia, Universidade Federal do Triangulo Mineiro, Uberaba, Brazil, [9] Universidade Federal do Pará, Belém, Brazil

OPEN ACCESS

Edited by:
David W. Ussery, University of Arkansas for Medical Sciences, United States

Reviewed by:
Christopher Morton Thomas, University of Birmingham, United Kingdom
Ulrich Dobrindt, University of Münster, Germany

***Correspondence:**
Vasco Azevedo
vasco@icb.ufmg.br

Specialty section:
This article was submitted to Evolutionary and Genomic Microbiology, a section of the journal Frontiers in Microbiology

Received: *05 April 2020*
Accepted: *09 December 2020*
Published: *08 January 2021*

Citation:
Carvalho R, Aburjaile F, Canario M, Nascimento AMA, Chartone-Souza E, de Jesus L, Zamyatnin AA Jr, Brenig B, Barh D, Ghosh P, Goes-Neto A, Figueiredo HCP, Soares S, Ramos R, Pinto A and Azevedo V (2021) Genomic Characterization of Multidrug-Resistant Escherichia coli BH100 Sub-strains. Front. Microbiol. 11:549254. doi: 10.3389/fmicb.2020.549254

The rapid emergence of multidrug-resistant (MDR) bacteria is a global health problem. Mobile genetic elements like conjugative plasmids, transposons, and integrons are the major players in spreading resistance genes in uropathogenic *Escherichia coli* (UPEC) pathotype. The *E. coli* BH100 strain was isolated from the urinary tract of a Brazilian woman in 1974. This strain presents two plasmids carrying MDR cassettes, pBH100, and pAp, with conjugative and mobilization properties, respectively. However, its transposable elements have not been characterized. In this study, we attempted to unravel the factors involved in the mobilization of virulence and drug-resistance genes by assessing genomic rearrangements in four BH100 sub-strains (BH100 MG2014, BH100 MG2017, BH100L MG2017, and BH100N MG2017). Therefore, the complete genomes of the BH100 sub-strains were achieved through Next Generation Sequencing and submitted to comparative genomic analyses. Our data shows recombination events between the two plasmids in the sub-strain BH100 MG2017 and between pBH100 and the chromosome in BH100L MG2017. In both cases, *IS3* and *IS21* elements were detected upstream of *Tn21* family transposons associated with MDR genes at the recombined region. These results integrated with Genomic island analysis suggest pBH100 might be involved in the spreading of drug resistance through the formation of resistance islands. Regarding pathogenicity, our results reveal that BH100 strain is closely related to UPEC strains and contains many *IS3* and *IS21*-transposase-enriched genomic islands associated with virulence. This study concludes that those IS elements are vital for the evolution and adaptation of BH100 strain.

Keywords: antibiotic resistance, genomic sequencing, mobile genetic elements, urinary tract infection, UPEC

Acesso: https://doi.org/10.3389/fmicb.2020.549254

Contribuição: Processamento amostras de DNA para sequenciamento, análise dados.

Gene 795 (2021) 145781

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Comparative genomics and *in silico* gene evaluation involved in the probiotic potential of *Bifidobacterium longum* 5^{1A}

Jéssica Gabrielle Vidal da Silva [a,1], Angélica Thomaz Vieira [b,1], Thiago J. Sousa [a], Marcus Vinicius Canário Viana [a], Doglas Parise [a], Bruna Sampaio [a], Alessandra Lima da Silva [a], Luís Cláudio Lima de Jesus [a], Pedro Kássio Ribeiro Matos Loureiro de Carvalho [c], Letícia de Castro Oliveira [d], Flavia Figueira Aburjaile [a,*], Flaviano S. Martins [e], Jacques Robert Nicoli [e], Preetam Ghosh [f], Bertram Brenig [g], Vasco Azevedo [a,1], Anne Cybelle Pinto Gomide [a,1]

[a] Laboratory of Cellular and Molecular Genetics, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[c] Department of Computing, Federal Center of Technological Education of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[d] Department of Microbiology, Immunology and Parasitology, Institute of Biology and Natural Sciences, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais Brazil
[e] Department of Microbiology, Institute of Biological Sciences, University Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[f] Department of Computer Science, Virginia Commonwealth University, Richmond, VA, United States
[g] Department of Molecular Biology of Livestock, Institute of Veterinary Medicine, Georg-August Universität Göttingen, Göttingen, Germany

ARTICLE INFO

Keywords:
Acetate
Bifidobacteria
Pangenomics
Probiogenomics

ABSTRACT

The *Bifidobacterium longum* 5^{1A} strain of isolated from feces of a healthy child, has demonstrated probiotic properties by *in vivo* and *in vitro* studies, which may be assigned to its production of metabolites such as acetate. Thus, through the study of comparative genomics, the present work sought to identify unique genes that might be related to the production of acetate. To perform the study, the DNA strain was sequenced using Illumina HiSeq technology, followed by assembly and manual curation of coding sequences. Comparative analysis was performed including 19 complete *B. longum* genomes available in Genbank/NCBI. In the phylogenetic analysis, the CECT 7210 and 157F strains of *B. longum* subsp. *infantis* aggregated within the subsp. *longum* cluster, suggesting that their taxonomic classification should be reviewed. The strain 5^{1A} of *B. longum* has 26 unique genes, six of which are possibly related to carbohydrate metabolism and acetate production. The phosphoketolase pathway from *B. longum* 5^{1A} showed a difference in acetyl-phosphate production. This result seems to corroborate the analysis of their unique genes, whose presence suggests the strain may use different sources of carbohydrates that allow a greater production of acetate and consequently offer benefits to the host health.

1. Introduction

Firmicutes, Bacteroidetes and Actinobacteria constitute the most abundant phyla in the microbiota of the human gastrointestinal tract (GIT). Firmicutes and Bacteroidetes are predominant in adults, and Actinobacteria in breast-fed infants, where the genus *Bifidobacterium* can exceed 90% of the total bacterial population, contributing to the health and well-being of the newborn (Turroni et al., 2009b) *Bifidobacterium* species are comprised of Gram-positive, obligate anaerobic, immobile and generally non-pathogenic bacteria (Hidalgo-Cantabrana et al., 2017).

Well controlled clinical trials showed that, when administered as probiotics, bifidobacteria produce

several beneficial effects, such as prevention of antibiotic associated

Abbreviations: Bp, base pair; CDSs, Coding sequences; COGs, Clusters of Orthologous Groups of proteins; GC, guanosine/cytosine; GIT, Gastrointestinal tract; *glpR*, Glycerol-3-phosphate regulon repressor; *gutQ*, D-arabinose 5-phosphate isomerase GutQ; KEGG, Kyoto Encyclopedia of Genes and Genomes; Mb, mega base; MRS, Man, Rogosa and Sharpe; OF, Operom forward; OR, Operon reverse; *rpe_1*, Ribulose-phosphate 3-epimerase; rRNA, ribosomal RNA; tRNA, transfer RNA.

* Corresponding author.
E-mail address: fabujaile@gmail.com (F.F. Abujaile).
[1] Contributed equally.

https://doi.org/10.1016/j.gene.2021.145781
Received 19 October 2020; Received in revised form 1 June 2021; Accepted 14 June 2021
Available online 19 June 2021

Acesso: https://doi.org/10.1016/j.gene.2021.145781

- Clonagem gênica, construção de bactérias recombinantes e aplicação em modelos de inflamação intestinal

Contribuição: Escrita e revisão do manuscrito

frontiers in Bioengineering and Biotechnology

REVIEW
published: 04 November 2020
doi: 10.3389/fbioe.2020.517166

Novel Strategies for Efficient Production and Delivery of Live Biotherapeutics and Biotechnological Uses of *Lactococcus lactis*: The Lactic Acid Bacterium Model

Laísa M. Tavares[1], Luís C. L. de Jesus[1], Tales F. da Silva[1], Fernanda A. L. Barroso[1], Viviane L. Batista[1], Nina D. Coelho-Rocha[1], Vasco Azevedo[1], Mariana M. Drumond[1,2] and Pamela Mancha-Agresti[1,3]**

[1] Laboratory of Cellular and Molecular Genetics, Federal University of Minas Gerais, Belo Horizonte, Brazil, [2] Departamento de Ciências Biológicas, Centro Federal de Educação Tecnológica de Minas Gerais, Belo Horizonte, Brazil, [3] FAMINAS - BH, Belo Horizonte, Brazil

OPEN ACCESS

Edited by:
Ana Gomes,
Universidade Católica Portuguesa, Portugal

Reviewed by:
Noppol Leksawasdi,
Chiang Mai University, Thailand
José Carlos Andrade,
University Institute of Health Sciences, Portugal

***Correspondence:**
Pamela Mancha-Agresti
p.mancha.agresti@gmail.com
Mariana M. Drumond
mmdrumond@gmail.com

Specialty section:
This article was submitted to Bioprocess Engineering, a section of the journal Frontiers in Bioengineering and Biotechnology

Received: 03 December 2019
Accepted: 09 October 2020
Published: 04 November 2020

Citation:
Tavares LM, de Jesus LCL, da Silva TF, Barroso FAL, Batista VL, Coelho-Rocha ND, Azevedo V, Drumond MM and Mancha-Agresti P (2020) Novel Strategies for Efficient Production and Delivery of Live Biotherapeutics and Biotechnological Uses of Lactococcus lactis: The Lactic Acid Bacterium Model. Front. Bioeng. Biotechnol. 8:517166. doi: 10.3389/fbioe.2020.517166

Lactic acid bacteria (LAB) are traditionally used in fermentation and food preservation processes and are recognized as safe for consumption. Recently, they have attracted attention due to their health-promoting properties; many species are already widely used as probiotics for treatment or prevention of various medical conditions, including inflammatory bowel diseases, infections, and autoimmune disorders. Some LAB, especially *Lactococcus lactis,* have been engineered as live vehicles for delivery of DNA vaccines and for production of therapeutic biomolecules. Here, we summarize work on engineering of LAB, with emphasis on the model LAB, *L. lactis*. We review the various expression systems for the production of heterologous proteins in *Lactococcus* spp. and its use as a live delivery system of DNA vaccines and for expression of biotherapeutics using the eukaryotic cell machinery. We have included examples of molecules produced by these expression platforms and their application in clinical disorders. We also present the CRISPR-Cas approach as a novel methodology for the development and optimization of food-grade expression of useful substances, and detail methods to improve DNA delivery by LAB to the gastrointestinal tract. Finally, we discuss perspectives for the development of medical applications of recombinant LABs involving animal model studies and human clinical trials, and we touch on the main safety issues that need to be taken into account so that bioengineered versions of these generally recognized as safe organisms will be considered acceptable for medical use.

Keywords: ***Lactococcus lactis*, genetic engineering, biotherapeutic molecules, mucosal immunity, safe for consumption**

Acesso: https://doi.org/10.3389/fbioe.2020.517166

Contribuição: Realização de RT-qPCR e análise dos dados, e escrita do manuscrito

frontiers in Microbiology

ORIGINAL RESEARCH
published: 05 October 2018
doi: 10.3389/fmicb.2018.02398

Microencapsulation of Lactic Acid Bacteria Improves the Gastrointestinal Delivery and *in situ* Expression of Recombinant Fluorescent Protein

Nina D. Coelho-Rocha[1], Camila P. de Castro[1,2], Luis C. L. de Jesus[1], Sophie Y. Leclercq[3], Savio H. de Cicco Sandes[4], Alvaro C. Nunes[4], Vasco Azevedo[1], Mariana M. Drumond[1,5]*† and Pamela Mancha-Agresti[1]*†*

[1] Laboratório de Genética Celular e Molecular, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2] Kroton Educacional, Faculdade Pitágoras, Contagem, Brazil, [3] Laboratório de Inovação Biotecnológica, Fundação Ezequiel Dias, Belo Horizonte, Brazil, [4] Laboratório de Genética Molecular de Protozoários Parasitas, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [5] Centro Federal de Educação Tecnológica de Minas Gerais, Coordenação de Ciências, Belo Horizonte, Brazil

OPEN ACCESS

Edited by:
Aleš Berlec,
Jožef Stefan Institute (IJS), Slovenia

Reviewed by:
Jorge Gomez-Gutierrez,
University of Louisville, United States
Pasquale Russo,
University of Foggia, Italy

***Correspondence:**
Vasco Azevedo
vasco@icb.ufmg.br
Mariana M. Drumond
mmdrumond@gmail.com
Pamela Mancha-Agresti
p.mancha.agresti@gmail.com

† *These authors have contributed equally to senior authorship*

Specialty section:
This article was submitted to Infectious Diseases, a section of the journal Frontiers in Microbiology

Received: *09 May 2018*
Accepted: *19 September 2018*
Published: *05 October 2018*

Citation:
Coelho-Rocha ND, de Castro CP, de Jesus LCL, Leclercq SY, de Cicco Sandes SH, Nunes AC, Azevedo V, Drumond MM and Mancha-Agresti P (2018) Microencapsulation of Lactic Acid Bacteria Improves the Gastrointestinal Delivery and in situ Expression of Recombinant Fluorescent Protein. Front. Microbiol. 9:2398. doi: 10.3389/fmicb.2018.02398

The microencapsulation process of bacteria has been used for many years, mainly in the food industry and, among the different matrixes used, sodium alginate stands out. This matrix forms a protective wall around the encapsulated bacterial culture, increasing its viability and protecting against environmental adversities, such as low pH, for example. The aim of the present study was to evaluate both *in vitro* and *in vivo*, the capacity of the encapsulation process to maintain viable lactic acid bacteria (LAB) strains for a longer period of time and to verify if they are able to reach further regions of mouse intestine. For this purpose, a recombinant strain of LAB (*L. lactis* ssp. *cremoris* MG1363) carrying the pExu vector encoding the fluorescence protein mCherry [*L. lactis* MG1363 (pExu:*mCherry*)] was constructed. The pExu was designed by our group and acts as a vector for DNA vaccines, enabling the host cell to produce the protein of interest. The functionality of the pExu:*mCherry* vector, was demonstrated *in vitro* by fluorescence microscopy and flow cytometry after transfection of eukaryotic cells. After this confirmation, the recombinant strain was submitted to encapsulation protocol with sodium alginate (1%). Non-encapsulated, as well as encapsulated strains were orally administered to C57BL/6 mice and the expression of mCherry protein was evaluated at different times (0–168 h) in different bowel portions. Confocal microscopy showed that the expression of mCherry was higher in animals who received the encapsulated strain in all portions of intestine analyzed. These results were confirmed by qRT-PCR assay. Therefore, this is the first study comparing encapsulated and non-encapsulated *L. lactis* bacteria for mucosal DNA delivery applications. Our results showed that the microencapsulation process is an effective method to improve DNA delivery, ensuring a greater number of viable bacteria are able to reach different sections of the bowel.

Keywords: recombinant *Lactococcus lactis*, sodium alginate encapsulation, mCherry reporter protein, pExu vector, DNA delivery

Acesso: https://doi.org/10.3389/fmicb.2018.02398

Contribuição: Clonagem e construção da bactéria recombinante, auxílio na realização do experimento, análise dos dados, escrita e revisão do manuscrito.

microorganisms

MDPI

Article

Intake of *Lactobacillus delbrueckii* (pExu:*hsp65*) Prevents the Inflammation and the Disorganization of the Intestinal Mucosa in a Mouse Model of Mucositis

Fernanda Alvarenga Lima Barroso [1,†], Luís Cláudio Lima de Jesus [1,†], Camila Prosperi de Castro [1], Viviane Lima Batista [1], Ênio Ferreira [2], Renata Salgado Fernandes [3], André Luís Branco de Barros [3], Sophie Yvette Leclerq [4], Vasco Azevedo [1], Pamela Mancha-Agresti [1,5,*] and Mariana Martins Drumond [1,6,*]

[1] Laboratório de Genética Celular e Molecular (LGCM), Departamento de—Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte 31270-901, Brazil; fernanda_alima@hotmail.com (F.A.L.B.); lc.luiis@yahoo.com.br (L.C.L.d.J.); camilaprosperic@gmail.com (C.P.d.C.); vivianelimabio@gmail.com (V.L.B.); vasco@icb.ufmg.br (V.A.)
[2] Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte 31270-901, Brazil; enioferreira@icb.ufmg.br
[3] Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Campus da UFMG, Universidade Federal de Minas Gerais, Cidade Universitária, Belo Horizonte 31270-901, Brazil; renatasalgadof@yahoo.com.br (R.S.F.); brancodebarros@yahoo.com.br (A.L.B.d.B.)
[4] Laboratório de Inovação Biotecnológica, Fundação Ezequiel Dias (FUNED), Belo Horizonte 30510-010, Brazil; sodris2003@gmail.com
[5] Faculdade de Minas-Faminas-BH, Medicina, Belo Horizonte 31744-007, Brazil
[6] Centro Federal de Educação Tecnológica de Minas Gerais (CEFET/MG), Departamento de Ciências Biológicas, Belo Horizonte 31421-169, Brazil
* Correspondence: p.mancha.agresti@gmail.com (P.M.-A.); mmdrumond@gmail.com (M.M.D.); Tel.: +55-31-99817-5004 (P.M.-A.); +55-31-99222-2761 (M.M.D.)
† These authors contributed equally to this work.

check for updates

Citation: Barroso, F.A.L.; de Jesus, L.C.L.; de Castro, C.P.; Batista, V.L.; Ferreira, Ê.; Fernandes, R.S.; de Barros, A.L.B.; Leclerq, S.Y.; Azevedo, V.; Mancha-Agresti, P.; et al. Intake of *Lactobacillus delbrueckii* (pExu:*hsp65*) Prevents the Inflammation and the Disorganization of the Intestinal Mucosa in a Mouse Model of Mucositis. *Microorganisms* **2021**, *9*, 107. https://doi.org/10.3390/microorganisms9010107

Received: 2 November 2020
Accepted: 30 November 2020
Published: 5 January 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: 5-Fluorouracil (5-FU) is an antineoplastic drug that causes, as a side effect, intestinal mucositis, acute inflammation in the small bowel. The Heat Shock Protein (Hsp) are highly expressed in inflammatory conditions, developing an important role in immune modulation. Thus, they are potential candidates for the treatment of inflammatory diseases. In the mucositis mouse model, the present study aimed to evaluate the beneficial effect of oral administration of milk fermented by *Lactobacillus delbrueckii* CIDCA 133 (pExu:*hsp65*), a recombinant strain. This approach showed increased levels of sIgA in the intestinal fluid, reducing inflammatory infiltrate and intestinal permeability. Additionally, the histological score was improved. Protection was associated with a reduction in the gene expression of pro-inflammatory cytokines such as Tnf, Il6, Il12, and Il1b, and an increase in Il10, Muc2, and claudin 1 (Cldn1) and 2 (Cldn2) gene expression in ileum tissue. These findings are corroborated with the increased number of goblet cells, the electronic microscopy images, and the reduction of intestinal permeability. The administration of milk fermented by this recombinant probiotic strain was also able to reverse the high levels of gene expression of Tlrs caused by the 5-FU. Thus, the rCIDCA 133:Hsp65 strain was revealed to be a promising preventive strategy for small bowel inflammation.

Keywords: recombinant probiotics; DNA delivery; intestinal mucositis; inflammation; technetium-99m; bacterial translocation; gene expression

1. Introduction

Radio- and chemotherapy and the combination of both are widely used for cancer treatment. 5-Fluorouracil (5-FU) is one of the main chemotherapeutic drugs used to treat several types of cancer. This drug is responsible for several adverse effects, such as mucositis, an inflammatory process that affects the entire digestive tract, causing abdominal

Acesso: https://doi.org/10.3390/microorganisms9010107

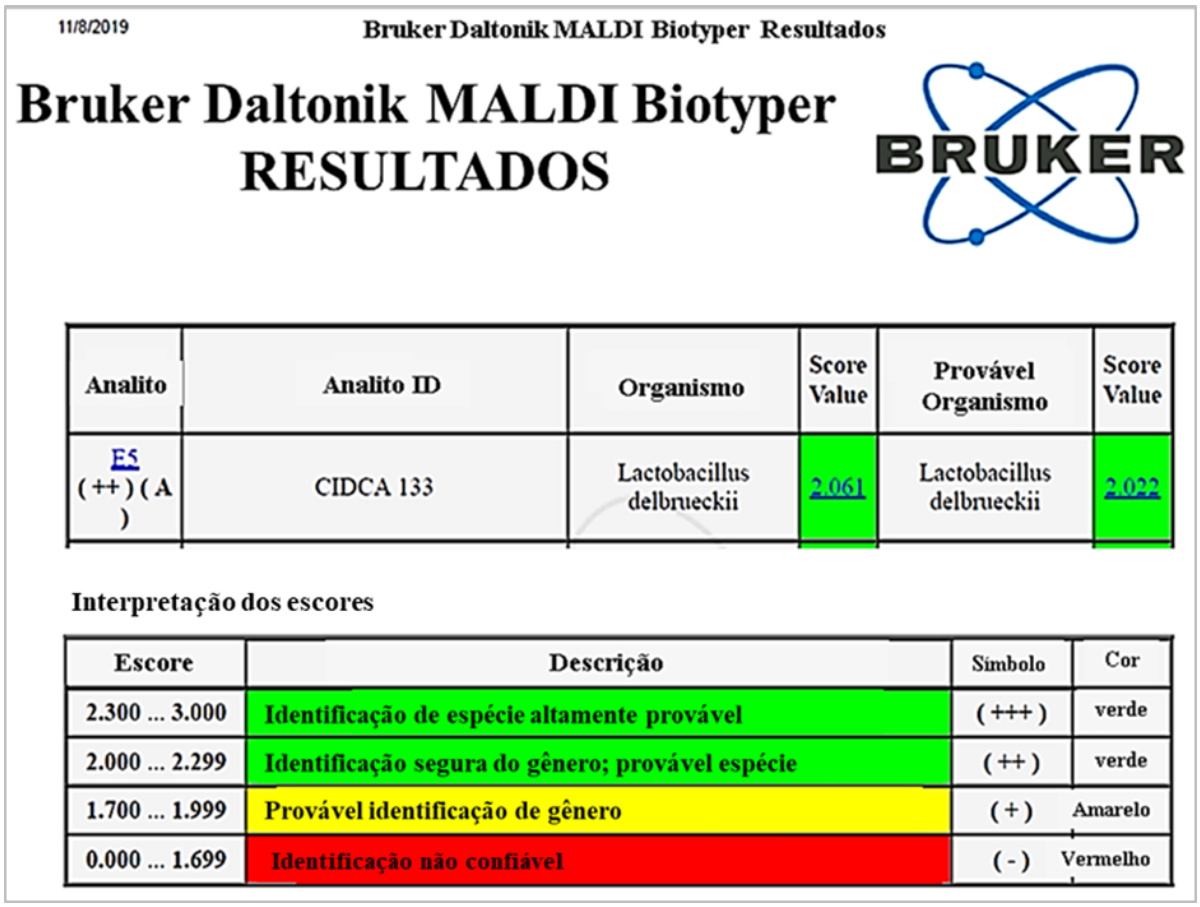
ANEXOS

Esta seção contém informações suplementares da Tese. Os anexos incluem as seguintes subseções:

a) Imagem suplementar: Identificação de CIDCA 133 por MALDITOF-Biotyper (Capítulo II);
b) Tabelas Suplementares (Capítulo II);
c) Imagem suplementar: Avaliação dsa capacidade de Translocação de CIDCA 133 (Capítulo IV);
d) Tabelas suplementares (Capítulo IV);
e) Tabelas suplementares (Capítulo V);

Capítulo II

Figura Suplementar - Identificação microbiológica de *L. delbrueckii* CIDCA 133 por MALDITOF-Biotyper.



11/8/2019 Bruker Daltonik MALDI Biotyper Resultados

Bruker Daltonik MALDI Biotyper RESULTADOS

BRUKER

Analito	Analito ID	Organismo	Score Value	Provável Organismo	Score Value
E5 (++) (A)	CIDCA 133	Lactobacillus delbrueckii	2.061	Lactobacillus delbrueckii	2.022

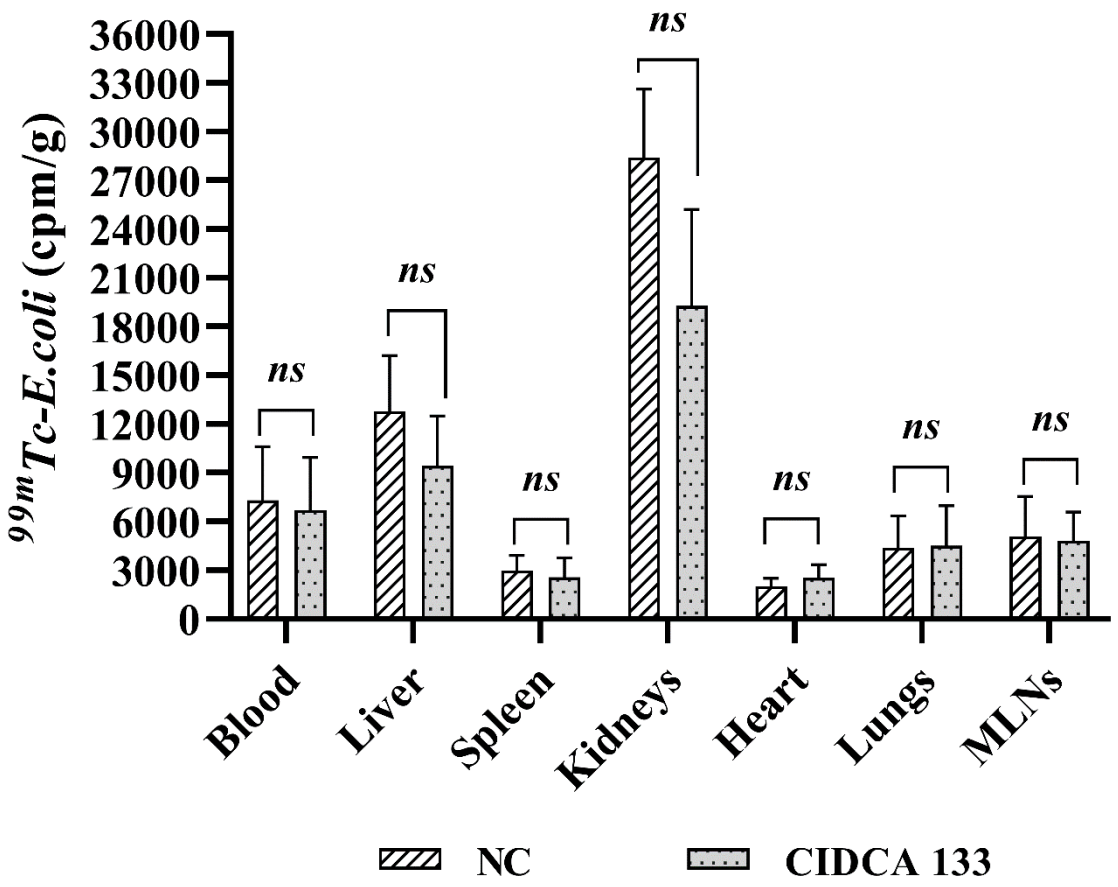
Interpretação dos escores

Escore	Descrição	Símbolo	Cor
2.300 ... 3.000	Identificação de espécie altamente provável	(+++)	verde
2.000 ... 2.299	Identificação segura do gênero; provável espécie	(++)	verde
1.700 ... 1.999	Provável identificação de gênero	(+)	Amarelo
0.000 ... 1.699	Identificação não confiável	(-)	Vermelho

Tabelas suplementares desse capítulo estão disponiveis no seguinte endereço: https://www.mdpi.com/article/10.3390/microorganisms9040829/s

Capítulo IV

Figura Suplementar: Biodistribution of ^{99mTc}-*Escherichia coli* after *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 consumption for 13 consecutive days. ns indicates no statistical difference by Student t-test ($p < 0.05$). Note: cpm, counts per minute; MLN, mesenteric lymph node



Tabelas suplementares desse capítulo estão disponiveis no seguinte endereço: https://doi.org/10.1007/s12602-021-09826-z

Capítulo V

Tabelas suplementares desse capítulo estão disponiveis no seguinte endereço: https://www.frontiersin.org/articles/10.3389/fbinf.2022.912795/full#supplementary-material