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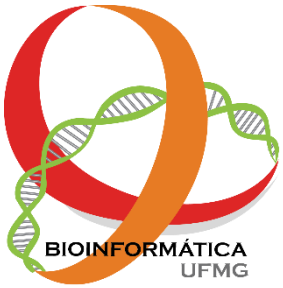

Roselane Gonçalves dos Santos

CARACTERIZAÇÃO DO PRIMEIRO GENOMA DE *GLUTAMICIBACTER CREATINOLYTICUS* ISOLADA DE ABSCESSO DE UMA ÉGUA

Belo Horizonte
Julho – 2019

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Dissertação apresentada ao Programa Interunidades de Pós-Graduação em Bioinformática, do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como parte dos requisitos para obtenção do título de Mestre em Bioinformática.

Orientador: Prof. Dr. Vasco Azevedo

Coorientadora: Dra. Núbia Seyffert

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Roselane Gonçalves dos Santos

Às quatorze horas do dia **18 de julho de 2019**, reuniu-se, no Instituto de Ciências Biológicas da UFMG, a Comissão Examinadora de Dissertação, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho intitulado: **"Caracterização do primeiro genoma de *Glutamicibacter creatinolyticus* isolada de abscesso de uma égua"**, requisito para obtenção do grau de Mestre em **Bioinformática.** Abrindo a sessão, o Presidente da Comissão, **Dr. Vasco Ariston de Carvalho Azevedo**, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presença da candidata e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	Instituição	CPF	Indicação
Dr. Vasco Ariston de Carvalho Azevedo	UFMG	28317225-49	APROVADA
Dra. Núbia Seyffert	UFBA	00083 4780-86	APROVADA
Dr. José Miguel Ortega	UFMG	059501268-04	Aprovado
Dr. Siomar de Castro Soares	UFTM	056 9578 2618	APROVADA
Dr. Rodrigo Bentes Kato	UFMG	366971972-00	APROVADA

Pelas indicações, a candidata foi considerada: APROVADA
O resultado final foi comunicado publicamente à candidata 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, 18 de julho de 2019.

Dr. Vasco Ariston de Carvalho Azevedo - Orientador

Dra. Núbia Seyffert - Coorientadora Núbia Seyffert

Dr. José Miguel Ortega

Dr. Siomar de Castro Soares

Dr. Rodrigo Bentes Kato Rodrigo Bentes Kato

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"E quando comecei a redigir, cheio de confiança, verifiquei logo em que terreno escorregadio me aventurava. (…) Mas um pensamento me consola. Quem fez a experiência de pensar em outro domínio sobrepuja sempre aquele que não pensa de modo algum ou muito pouco."

*Albert Einstein (*14 março de 1879 †18 de abril de 1955)*

Como Vejo o Mundo (Mein Weltbild), Zurich, 1953.

Resumo

Glutamicibacter creatinolyticus é um coco Gram-positivo, imóvel, que não esporula e habita vários nichos ecológicos. Essa bactéria é utilizada em processos biotecnológicos e pode ser considerada um patógeno oportunista por ter sido isolada de humanos. Poucas informações foram encontradas na literatura relacionadas aos estudos genômicos de linhagens do gênero e das espécies de *G. creatinolyticus.* Neste estudo, sequenciamos o primeiro genoma de uma linhagem de *G. creatinolyticus*, denominada LGCM 259, que foi isolada de nódulos subcutâneos de uma égua em Régio de Calábria na Itália. As sequências de DNA obtidas da linhagem LGCM 259 foram montadas usando uma abordagem *ab initio*, resultando em um cromossomo de 3,3 Mb. Análises genômicas comparativas foram realizadas entre a linhagem LGCM 259, e outras espécies do gênero *Glutamicibacter* isoladas de diferentes ambientes, incluindo: planta, queijo, solo, cujas sequências completas estavam disponíveis no *National Center for Biotechnology Information* (NCBI). A linhagem LGCM 259 apresenta um cromossomo circular com 3,3 Mb, um conteúdo de GC de 66,4%, 2882 CDSs, 4 clusters de rRNAS (5S, 16S e 23S) e 61 tRNA genes, respectivamente. Alguns locus_tag (LGCM259_1698, LGCM259_0905) foram encontradas, e podem estar envolvidos na resistência múltipla a medicamentos para rifampicina, elfamicina e fluoroquinolona. O genoma de *G. creatinolyticus* apresentou genes de tolerância ao cobre, arsênico, cobalto-zinco-cádmio e compostos de cromo que são sérios contaminantes ambientais. O genoma de LGCM 259 também apresentou 23 ilhas genômicas, que podem contribuir para sua adaptação no hospedeiro. A análise comparativa de busca por genes de resistência revelou que todas as linhagens selecionadas contêm pelo menos um gene codificador para a classe de antibiótico rifampicina e algumas ilhas genômicas são compartilhadas entre os todos os genomas, mas muitas delas são exclusivas para cada espécie. Estudos do genoma de *G. creatinolyticus* podem contribuir para uma melhor compreensão das suas ilhas genômicas, genes de resistências a antibióticos, ganho/ perda de genes que possam estar envolvidos na especialização pelo hospedeiro, na identificação dos genes adquiridos por transferência horizontal, no papel destes genes na adaptação ao hospedeiro e virulência bacteriana. Além disso, estudos dessas espécies podem contribuir para novas estratégias de tratamento e diagnóstico desses tipos de infecções causadas em égua.

Palavra Chaves: *Glutamicibacter creatinolyticus;* genoma; resistência; LGCM 259; sequenciamento; análises comparativas.

Abstract

Glutamicibacter creatinolyticus is a Gram-positive, non-motile coccus, no spore forming and inhabits various ecological niches. This bacterium is used in a variety of biotechnological processes, and may be regarded as an opportunistic pathogen, once it was isolated from humans isolated from humans. Very little data has been found in written literature regarding the study of the genomes of both its genus and the *G. creatinolyticus* species itself. In this work, we have sequenced the first genome of a *G. creatinolyticus* strain, named LGCM 259, which has been isolated from the subcutaneous nodules of a mare in Reggio di Calabria, in Italy. The DNA sequences obtained from strain LGCM 259 were assembled through an *ab initio* approach, resulting in a 3,3 Mb chromosome. Comparative genomic analyses were performed between LGCM 259 and other species from the *Glutamicibacter* genus, isolated from different environments, including plants, cheese and soil. These species' complete sequences were available in the *National Center for Biotechnology Information* (NCBI) database. The LGCM 259 strain has a circular chromosome, 3,3 Mb in size, a GC content of 66,4%, 2882 CDSs, 4 rRNA clusters (5S, 16S and 23S), and 61 tRNA genes, respectively. The LGCM259_1698, LGCM259_0905 were found, which may be involved in the strain's multi-drug resistance to rifampicin, elfamycin and fluoroquinolone. The *G. creatinolyticus* genome presented genes encoding for copper, arsenic, cobalt-zinc-cadmium, and chrome compost resistance, which are all severe environmental contaminants. The strain's genome also displayed 23 genomic islands, which may contribute to its host adaptation. A comparative analysis seeking resistance genes showed that all selected strains harbor at least one gene encoding for rifampicin resistance and that some genomic islands are shared between all genomes, but many of them were unique for each species. Studying the *G. creatinolyticus* genome may lead to a better understanding of its genomic islands, antibiotic resistance genes, acquisition/loss of genes that may be involved in host adaptation, acquisition of genes through Horizontal Gene Transfer events, and the role of these genes in host adaptation and bacterial virulence. Additionally, studying these species may lead to new treatment and diagnosis strategies.

Keywords: *Glutamicibacter creatinolyticus*; genome; resistance; LGCM 259; sequencing; comparative analyses

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BHI agar	(Brain Heart Infusion Agar)
BLASTn	Nucleotide-nucleotide BLAST
BLASTp	Protein-protein BLAST.
BRIG	BLAST Ring Image Generator.
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CDS	Coding Sequence
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
DNA	Ácido desoxirribonucleico
FAPEMIG	Fundação de Amparo à Pesquisa do Estado de Minas Gerais
G+C	Guanina + Citosina
kDa	kilodalton
LGCM	Laboratório de Genética Celular e Molecular
Lys-Ala-Glu	Lisina - Alanina - Acido glutâmico
MALDI-TOF	Matrix-assisted laser desorption ionization (MALDI)- time of flight (TOF)
Mb	Megabases
MI	Metabolic island
NCBI	National Center of Biotechnology Information
NGS	Next-Generation Sequencing
PAI	Pathogenicity Island
Paired- end	Sequenciamento de ambas as extremidades da fita de DNA
pb	Pares de base
PCR	Polymerase Chain Reaction
Reads	Leituras provenientes do sequenciamento
RI	Resistance Island
RNA	Ácido ribonucleico
rRNA	Ácido ribonucleico ribossomal
SI	Symbiotic island
Single read	Sequenciamento de apenas uma extremidade da fita de DNA

tRNA	Ácido ribonucleico transportador

APRESENTAÇÃO

Colaboradores

Este trabalho foi realizado no Laboratório de Genética Celular e Molecular (LGCM), na Universidade Federal de Minas Gerais (UFMG) em colaboração com a Universidade de Camerino (Itália).

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Estrutura da dissertação

Essa dissertação está organizada em três seções, anexo e apêndice:

Na seção (I) Introdução, Justificativa e Objetivos;

Na seção (II) Manuscrito completo em língua inglesa formatado de acordo com as normas do periódico científico internacional *GENE*;

Na seção (III) Considerações finais e Perspectivas.

O anexo está constituído de um case *report* submetido a revista *BMC Veterinary Research*, relacionado a infecção do animal e identificação da espécie deste trabalho.

E por fim, o apêndice contém trabalhos desenvolvidos durante o mestrado e um resumo de cada análise que foi realizada nos devidos trabalhos, e o *currículo lattes* dos últimos dois anos relatando as atividades realizadas durante o mestrado (como certificado de cursos, eventos, apresentação de pôster, monitoria de cursos de verão).

SUMÁRIO

1. INTRODUÇÃO GERAL

1.1.1. Aspectos microbiológicos do gênero *Glutacimibacter*

O gênero *Arthrobacter*, foi proposto por Conn Dimmick (1947) e inclui as espécies, *Arthrobacter globiformis* (CONN; DIMMICK, 1947; SKERMAN,'; MCGOWAN; SNEATH', 1980), *Arthrobacter tumerens* (COLLINS; DORSCH; STACKEBRANDT, 1989) e *Arthrobacter helvolum* e pertencem a família *Micrococcaceae* (BUSSE et al 2012). Em 2016, houve uma proposta de reclassificação de espécies selecionadas do gênero *Arthrobacter* em 5 novos gêneros: *Glutamicibacter*, *Paeniglutamicibacter*, *Pseudoglutamicibacter*, *Paenarthrobacter* e *Pseudarthrobacter* (BUSSE, 2016). Essa mudança se deve ao fato de que as análises filogenéticas empregando o algoritmo *neighbor-joining* e abrangendo grande parte das espécies do gênero *Arthrobacter* não indicam uma estrutura interna estável do gênero. Isso é indicado pelo fato de que muitos nós ramificados não são suportados por altos valores de inicialização (> 70%) e vários nós não foram encontrados com o algoritmo de máxima verossimilhança ou máxima-parcimônia (DING; HIROSE; YOKOTA, 2009; GANZERT et al., 2011; YASSIN et al., 2011). Da mesma forma, quando representantes de membros adicionais da família *Micrococcaceae* são incluídos em reconstruções de árvores, além de espécies do gênero *Arthrobacter*, as relações filogenéticas tornam-se mais incertas (BUSSE, 2016). Dessa forma, *Arthrobacter creatinolyticus* como era conhecido, a partir de 2016 foi renomeada para *Glutamicibacter creatinolyticus* permanecendo com as mesmas características descritas para a espécie na literatura.

O gênero *Glutamicibacter* foi proposto por Busse (2016) e a nomenclatura refere-se às características celulares relacionadas à presença de ácido glutâmico na ponte interpeptídica de peptidoglicano. O gênero está agrupado na família *Micrococcaceae*, pertencente à classe das *Actinobacterias* e contém espécies de importância médica, ambiental e biotecnológica. O gênero apresenta características em comum que incluem: (i) conteúdo G+C do DNA genômico na faixa de 55-67%; (ii) o tipo do peptidoglicano é A4α (Lys-Ala-Glu; A11.35); (iii) o sistema quinona que contém menaquinonas exclusivamente insaturadas (MK-8 e / ou MK-9); (iv) o perfil lipídico polar composto pelos difosfatidilglicerol, fosfatidilglicerol e dimanossildiacilglicerol; (v) o monogalactosildiacilglicerol e quantidades menores de trimanossilditilglicerol podem estar presentes, sendo que o fosfatidilinositol pode estar ausente (BUSSE, 2016).

Os membros do gênero *Glutamicibacter* são consideradas ubíquas, pois podem ser encontrados no solo, na água e no ar. Foram isolados de diferentes habitats, incluindo diferentes tipos de solos, amostras clínicas, queijos e plantas como *G. nictoninae*, *G. creatinolyticus*, *G. arilaitensis e G. halophytocola,* respectivamente (FENG et al., 2017; HOU et al., 1998; MONNET et al., 2010; WANG et al., 2015; YAMAMOTO et al., 2017).

1.1.2. *Glutamicibacter creatinolyticus*

Glutamicibacter creatinolyticus é uma bactéria Gram-positiva, que possui um crescimento favorável aerobicamente a 37°C, se apresenta em formas de hastes irregulares com um comprimento de cerca 1.4 - 3.6 µm. As células dessas bactérias podem mudar para cocos em culturas mais antigas e seu diâmetro é 0,5 - 0,8 µm. As colônias são circulares, lisas e amarelas pigmentadas em meio *Brain Heart Infusion* (BHI) Agar, seu diâmetro mede cerca de 1-1.5 mm após 24 h de cultura, não esporula e é imóvel. Os componentes e proporção de aminoácidos da parede celular são Glu: Ala: Lys, 1,9: 3,1: 1, o diaminoácido é a lisina. Em relação ao seus testes bioquímicos, catalase é produzido, creatinina e xantina são hidrolisadas, nitrato é reduzido, ácido é produzido a partir de glicerol, e seu conteúdo G + C é 66-67 %, suas principais menaquinones são MK-8 e MK-9 (HOU et al., 1998) e além disso apresenta ampla resistência a antibióticos.

G. creatinolyticus revelou sua patogenicidade em humanos pela primeira vez em 1998, quando foi isolado da urina, com concentrações de creatinina baixas, devido à sua capacidade de hidrolisar a creatinina (HOU et al., 1998). Em 2017 foi isolada de uma paciente diabética com colangite aguda que apresentou um quadro de bacteremia (YAMAMOTO et al., 2017) .

Segundo Vila et al. (2012), o sistema API Coryne (bio Mérieux, Marcy l'Etoile, França), complementado com testes fenotípicos convencionais, são mais comumente usados em laboratórios de rotina para identificação desse microrganismo. No entanto, esse método é demorado e nem sempre fornece uma identificação confiável em nível de espécie (VILA et al., 2012). Consequentemente, a infecção por *Glutamicibacter* pode ser subestimada porque a identificação correta da linhagem de *G. creatinolyticus* só foi possível através da aplicação de métodos de identificação por sequenciamento, do gene 16S rRNA e MALDI Biotyper (HOU et al., 1998; YAMAMOTO et al., 2017, RIFICI et al., 2019). *Glutamicibacter creatinolyticus* LGCM 259 foi isolada de nódulos subcutâneos de uma égua de 12 anos de idade proveniente da cidade Reggio Calabria na Itália. Pelas manifestações

clínicas do animal, análises morfológicas e pelo procedimento de identificação bioquímica, realizado pelo painel RapID™ CB Plus System (ThermoFisher, Itália), a linhagem foi identificada primeiramente como *Corynebacterium pseudotuberculosis* com um teste (urease) atípico (RIFICI et al., 2019).

Após essa identificação e com finalidade de confirmação da espécie, o nosso grupo do Laboratório de Genética Celular e Molecular (LGCM) realizou o cultivo da linhagem, a reação em cadeia da polimerase (PCR) multiplex e o MALDI Biotyper. O resultado da PCR multiplex foi negativo para *C. pseudotuberculosis* e o MALDI Biotyper identificou a linhagem como *Arthrobacter creatinolyticus*, com um valor superior ou similar a 2,3 o que indica que as identificações do gênero e espécie são confiáveis (SINGHAL et al., 2015, RIFICI et al., 2019).

1.1.3. Importância biotecnológica de *Glutamicibacter creatinolyticus*

A fitorremediação é uma tecnologia promissora que utiliza plantas para despoluir metais tóxicos e contaminantes orgânicos do ambiente, especialmente do solo (KONG; GLICK, 2017). Para tornar essa tecnologia mais eficaz, os cientistas verificaram a possibilidade de usar várias bactérias do solo junto com as plantas. Essas bactérias incluem bactérias biodegradativas, bactérias promotoras de crescimento de plantas e bactérias que facilitam a fitorremediação por outros meios, o que torna a fitorremediação amplamente aceita como uma tecnologia de restauração ambiental econômica (LASAT, 2002). Em um estudo de Prum (2018), *G. creatinolyticus* foi capaz de melhorar as plantas para remover o arsênico das águas residuais melhor do que *Bacillus subtilis* através de interações com *Echinodorus cordifolius*. A interação endofítica de plantas-micróbio, mostraram que raízes de *E. cordifolius* mergulhadas em suspensão de *G. creatinolyticus* por 5 min, proporcionou as melhores condições para remoção de arsênio. Isso se deve ao fato de que a capacidade dos microrganismos de sintetizar ácido indol-3-acético (AIA) e reduzir as espécies reativas do oxigênio **(**EROs) nos tecidos vegetais pode melhorar a planta para tolerar a toxicidade por arsênico e aumentar a sua remoção (PRUM; DOLPHEN; THIRAVETYAN, 2018).

Metais são contaminantes ambientais estáveis e persistentes uma vez que não podem ser degradados ou destruídos, sendo eles os agentes tóxicos mais conhecidos. Entre os mais perigosos estão: o cromo, cádmio, cobre, arsênico, mercúrio e zinco (ALTIMIRA et al., 2012; SANJAY et al., 2018). Alguns metais como o zinco em quantidades

pequenas são requeridos como nutrientes essenciais aos seres vivos, podendo atuar na regulação da expressão gênica, atividade de biomoléculas e estabilização estrutural do DNA (BRUINS; KAPIL; OEHME, 2000), já outros como o mercúrio e chumbo mesmo em quantidades bem pequenas são tóxicos aos seres vivos (PAN; WANG, 2012) . *G. creatinolyticus* foi utilizada na triagem e produção de uma potente urease extracelular para determinação de íons de metais pesados (RAMESH et al., 2014). Segundo Ramesh (2014), os resultados desses estudos sugerem que a enzima poderia ser utilizada como sensores para determinar os níveis de íons de Nitrato de cobre (II) (Cu II) em efluentes industriais, solo contaminado e água subterrânea (RAMESH et al., 2015). Além disso, *G. creatinolyticus* foi utilizada em um biossensor potenciométrico para determinação de uréia em leite utilizando urease imobilizada (RAMESH et al., 2015).

1.1.4. Importância dos estudos de multirresistência e fatores de virulência bacteriana

O surgimento de bactérias resistentes aos antimicrobianos tornou-se um problema crítico de saúde pública, uma vez que existem menos agentes antimicrobianos eficazes disponíveis para o tratamento de infecções causadas por essas bactérias. Padrões resistentes a múltiplos fármacos em bactérias Gram-positivas e negativas resultaram em infecções difíceis de tratar ou mesmo intratáveis com antimicrobianos convencionais (FRIERI; KUMAR; BOUTIN, 2017). A resistência antimicrobiana pode ser definida como a capacidade dos microrganismos resistirem aos efeitos das drogas (antibióticos) ou seja, os microrganismos não são mortos e seu crescimento não é interrompido. Os mecanismos de resistência a antibióticos podem ser intrínsecos do microrganismo ou podem ser adquiridos por meio de mutações cromossômicas ou, mais comumente, pela aquisição de um gene de resistência a antibióticos de outra bactéria via plasmídeos ou transposons móveis (transferência horizontal de genes), fagos que podem carrear dna de uma bactéria a outra (LI; WEBSTER, 2018).

Atualmente, a classificação mais comum dos antibióticos baseia-se no seu mecanismo de ação, sendo eles inibição da síntese da parede celular; inibição da síntese ou dano da membrana citoplasmática; inibição da síntese proteica nos ribossomos; alterações na síntese dos ácidos nucleicos; alteração de metabolismos celulares (BAPTISTA, 2013). A resistência a antibióticos pode ser essencialmente adquirida através de quatro diferentes vias (transformação, transdução, conjugação e mutação) e expressas

por quatro mecanismos diferentes (prevenção da penetração celular, expulsão via bombas de efluxo, inativação de proteínas e modificação do alvo) (LI; WEBSTER, 2018).

Alguns dos patógenos resistentes a drogas, com grande problemática relacionada às infecções nosocomiais incluem o *Staphylococcus aureus* resistente *à* meticilina*,* o *Streptococcus pneumoniae* e espécies do gênero *Enterococcus spp* resistente à vancomicina dentre as bactérias Gram-positivas. *Acinetobacter baumannii*, *Klebsiella pneumoniae* , *Escherichia coli* e *Pseudomonas aeruginosa* estão dentre as bactérias Gram-negativas multirresistentes (LISTER; WOLTER; HANSON, 2009). *Pseudomonas aeruginosa* é um patógeno ambiental, ubíquo, oportunista, capaz de causar uma ampla gama de infecções agudas e crônicas, sendo resistentes a uma ampla gama de antibióticos. *P. aeruginosa* possui capacidade metabólica extensa, devido a produção de múltiplos metabólitos e polímeros secundários. A presença onipresente de *P. aeruginosa* bem como a sua prevalência e persistência em situações clínicas, incluindo a resistência intrínseca à terapêutica, são atribuídas à sua extraordinária capacidade de sobrevivência através do recrutamento de um arsenal de mecanismos responsivos. A diversidade desses mecanismos, faz com que *P. aeruginosa* se adapte e prospere em condições desfavoráveis, particularmente durante a patogênese, sendo os principais mecanismos de sua adaptação estratégica, o *quorum sensing* (QS), formação de biofilme (MORADALI; GHODS; REHM, 2017).

G. creatinolyticus LGCM 259 também apresentou resistência à alguns antibióticos o que dificultou o tratamento do equino. Inicialmente a égua apresentou nódulos subcutâneos difusos que variaram de 2-3 à 5-20 cm em tamanho, apresentando-se aderente aos músculos. Os nódulos eram fortemente vascularizados sendo a doença citologicamente diagnosticada como miosite granulomatosa. Miosite granulomatosa se dá pela infecção bacteriana múltipla em equinos (RIFICI et al., 2019) e o tratamento do animal foi realizado através de antibioticoterapia, sendo a penicilina o primeiro medicamento administrado. As penicilinas são antibióticos do grupo dos beta-lactâmicos e estavam entre os primeiros medicamentos a serem eficazes contra várias infecções bacterianas. Atualmente, as penicilinas ainda são amplamente usadas, embora as bactérias tenham desenvolvido resistência após uso extensivo. Após três meses de tratamento com a penicilina, houve um agravamento da condição clínica geral da égua infectada por *G. creatinolyticus* LGCM 259 (KARDOS; DEMAIN, 2011). Posteriormente, foram administradas no equino outras antibioticoterapias como: cefalosporinas de terceira

geração, ceftiofur e tetraciclinas. O ceftiofur é um antibiótico do tipo cefalosporina de terceira geração, liberado para uso em medicina veterinária (YANCEY et al., 1987). As tetraciclinas são um grupo de antibióticos naturais ou semi-sintéticos usados no tratamento da maioria das bactérias Gram-positivas e Gram-negativas, quer anaeróbias como aeróbias (CHOPRA; ROBERTS, 2001). Assim, houve um desaparecimento inicial da maioria das lesões e uma melhora geral da égua, mas a condição clínica piorou novamente apresentando o surgimento de novas lesões (RIFICI et al., 2019). Uma terceira terapia direcionada foi realizada com sulfametoxazol combinado com trimetoprima (10g / 100 kg x no primeiro dia e depois 5 g / 100 kg x 10 dias) resultando em reduções do número e tamanho das lesões detectadas. Sulfametoxazol combinado com trimetoprima é um medicamento sintetizado em laboratório para combater microrganismos, com propriedades bactericidas e duplo mecanismo de ação (ALVAREZ et al., 2005) (http://www.anvisa.gov.br). Após um ano de acompanhamento, e tratamento com diferentes terapias antibióticas, houve uma redução no número dos nódulos, mas não ocorreu o desaparecimento total das lesões (RIFICI et al., 2019).

Os fatores de virulência bacteriana são importantes para a invasão e evasão das defesas do hospedeiro. Alguns dos fatores de virulência bacterianos mais importantes são: (I) fatores de adesão: muitas bactérias patogênicas colonizam as mucosas usando *pili* (fimbriae) para aderir às células, (II) fatores de invasão: os componentes da superfície que permitem que a bactéria invada as células do hospedeiro podem ser codificados em plasmídeos, mas mais frequentemente estão no cromossomo. (III) Cápsulas: muitas bactérias são cercadas por cápsulas que as protegem da opsonização e da fagocitose. (IV) endotoxinas: essas toxinas lipopolissacarídicas em bactérias Gram-negativas causam febre, alterações na pressão sanguínea, inflamação, choque letal e muitos outros eventos tóxicos. (V) exotoxinas: que incluem vários tipos de toxinas proteicas e enzimas produzidas e/ou secretadas por bactérias patogênicas. As principais categorias incluem citotoxinas, neurotoxinas e enterotoxinas. (VI) sideróforos: são fatores que atua na captação de ferro pelos organismos como bactérias (PETERSON, 1996).

1.1.5. Estudos de genômica bacteriana

Os sequenciamentos genômicos podem ser realizados com o intuito de identificar e caracterizar um determinado organismo, bem como analisar questões evolutivas (VERLI, 2014). O sequenciamento bacteriano aumentou significativamente o conhecimento de sua estrutura, função, variação e sua relação com os mecanismos genéticos envolvidos na relação entre patógeno e hospedeiro (LAND *et al*., 2015; LOMAN; PALLEN, 2015). O primeiro genoma de bactéria patogênica inteiramente sequenciado foi da espécie *Haemophilus influenzae* (FLEISCHMANN *et al*., 1995). Desde então, a genômica tem transformado o entendimento da interação bactéria-bactéria e bactéria-hospedeiro (LOMAN; PALLEN, 2015). A primeira técnica de sequenciamento foi desenvolvida por Sanger e colaboradores em 1977 através do sequenciamento por dideoxinucleotídeos, concomitante com a técnica de Maxam-Gilbert (SANGER *et al*., 1977). O avanço contínuo das tecnologias de sequenciamento possibilitou o desenvolvimento dos sequenciadores de nova geração (NGS, do inglês *Next-generation sequencing*), tais como, Ion Torrent (Thermo Fisher Scientific) e Illumina (Illumina Inc., San Diego, CA, USA), que são considerados sequenciadores de segunda geração e PacBio e NanoPore, considerados sequenciadores de terceira geração. O NGS refere-se às tecnologias de sequenciamento de DNA que surgiram após o método de Sanger. Essas novas tecnologias permitiram a análise massiva e paralela de um número muito maior de amostras a um menor custo e tempo quando comparado com este método. As tecnologias ditas de segunda geração (Ion Torrent e Illumina) caracterizam-se pela necessidade de amplificar o material genético via reação em cadeia da polimerase (PCR) antes do sequenciamento em si. Já as tecnologias de terceira geração (PacBio e NanoPore) caracterizam-se pelo sequenciamento de uma única molécula de DNA (BUERMANS; DEN DUNNEN, 2014).

A plataforma de sequenciamento Illumina utiliza nucleotídeos marcados por fluoróforos que são inseridos a um *chip* de sequenciamento (*flow cell*), que contém adaptadores fixos (sequências universais específicas de cada plataforma de sequenciamento), e uma DNA polimerase. Os nucleotídeos são terminadores reversíveis, o que garante que apenas um nucleotídeo seja inserido por vez na fita crescente de DNA prevenindo assim problemas com homopolímeros (MARDIS, 2013). Além disso, essa plataforma utiliza a tecnologia *paired-end*, que consiste na leitura de ambas as direções dos fragmentos de DNA (*forward* e *reverse*), o que produz grande vantagem em comparação ao dado *single-read*, pois as sequências geradas têm maior acurácia e capacidade de

detectar inserções e deleções (BERGLUND; KIIALAINEN; SYVÄNEN, 2011)(Illumina, 2016).

Os genomas do gênero *Glutamicibacter* consistem em um cromossomo circular variando aproximadamente de 2,5 a 4,0Mb, possuindo uma quantidade de proteínas que variam de 2.776 a 3.600 e conteúdo GC de cerca de 60-67 % (www.ncbi.nlm.nih.gov)(FENG et al., 2017; MONNET et al., 2010). O primeiro genoma desse gênero foi sequenciado no ano de 2010, e pertence à espécie *G. arialitensis* Re117, isolada de queijo (MONNET et al., 2010). Atualmente, dezessete genomas do gênero *Glutamicibacter* estão depositados na base de dados do *National Center for Biotechnology Information* (NCBI) e, destes, apenas 3 estão depositados como completos. Apesar disso, dentre os genomas completos de linhagens com informações sobre o hospedeiro de origem ou nicho específico, não há informações desses genomas associados à casos de infecções em animais. Os genomas completos são de extrema importância para a genômica comparativa, pois contribuem para a correta representação dos genes (PURANIK et al., 2015).

1.1.6. Genômica comparativa

A genômica comparativa é uma ferramenta que permite avaliar as relações evolutivas entre diferentes indivíduos, através da comparação entre os genomas de dois ou mais organismos (COSTA, 2015). Com as crescentes tecnologias de sequenciamento de nova geração, estudos direcionados à composição do genoma e à provável função dos genes que se diferenciam entre as linhagens, nos permite identificar mecanismos importantes para a adaptação e sobrevivência de microrganismos nos seus respectivos hospedeiros. Além disso, pode contribuir para o conhecimento de novos determinantes de virulência bacterianos, resistência a antimicrobianos, e funções atribuídas a determinados segmentos do genoma, como regiões gênicas regulatórias (COSTA, 2015; METZKER, 2010).

Nesse contexto, a filogenética é o estudo das relações evolucionárias entre espécies (CHOUDHURI, 2014). A filogenômica consiste na análise comparativa em escala genômica para o estudo dos relacionamentos evolutivos entre espécies buscando uma melhor compreensão dos mecanismos de evolução molecular (CHAN; RAGAN, 2013; EISEN, 1998). A filogenômica é indispensável na avaliação de diversas hipóteses biológicas, por exemplo, a distribuição e disseminação da patogenicidade bacteriana, a convergência ou divergência da função gênica, a origem das organelas ou a resolução da árvore (ou rede)

da vida. Relações entre taxa são representadas por um diagrama ramificado, ou árvore, em que os ramos representam o tempo de divergência, os ancestrais hipotéticos são representados pelos nós, e as unidades taxonômicas operacionais (OTU) são representadas pelos nós terminais (folhas). Um clado ou grupo monofilético é formado por um grupo composto de um ancestral comum e todos os seus descendentes, já um grupo parafilético não contém todos os descendentes (CHAN; RAGAN, 2013). De modo geral, utilizar o genoma inteiro ou uma grande quantidade de genes torna a filogenia mais confiável do que utilizando um ou alguns genes. Podendo assim, obter informação detalhada das diferenças entre os genomas e não simplesmente uma classificação geral destes (ÅGREN *et al*., 2012).

Estudos de genômica comparativa têm auxiliado para uma melhor compreensão de características como a patogenicidade e evolução bacteriana. O genoma bacteriano é um mosaico de regiões estáveis e instáveis. A variação na estrutura do genoma, ou plasticidade genômica, se deve a processos de aquisição (inserção) e perda (deleção) de sequências, além de duplicações, inversões e translocações (PATEL, 2016). O tamanho do genoma e estilo de vida estão diretamente relacionados, e pode influenciar no estilo de vida bacteriano. O genoma bacteriano tem a tendência à redução, como por exemplo organismos patogênicos podem ter genomas menores que organismos não patogênicos devido a sua habilidade de utilizar metabólitos produzidos pelo hospedeiro (BARBOSA et al., 2014; OCHMAN; DAVALOS, 2006; TOFT; ANDERSSON, 2010) .

Outra característica que auxilia no estudo da plasticidade genômica são a predição de Ilhas genômicas (GEIs), as quais são formadas pela incorporação de grandes blocos de sequências genômicas através da transferência horizontal de genes (SOARES *et al*., 2015). GEIs podem ser classificadas em quatro diferentes categorias: ilhas de patogenicidade (PAIs), que possuem genes de fatores de virulência (DOBRINDT et al., 2000); ilhas metabólicas (MIs) que possuem genes associados à biossíntese de metabólicos (TUMAPA et al., 2008); ilhas de resistência (RIs); com genes que codificam a resistência, tipicamente contra antibióticos (KRIZOVA; NEMEC, 2010) e ilhas simbióticas (SIs); facilitando associações simbióticas do hospedeiro com outros microrganismos (BARCELLOS et al., 2007).

As abordagens de genômica citadas acima são muito utilizadas para o estudo de diversos organismos (COSTA, 2015; SOARES et al., 2013). No presente trabalho, essas abordagens foram utilizadas a fim de estudar o genoma da linhagem LGCM 259 de *G.*

creatinolyticus e buscar compreender seu repertório de virulência e resistência a antibióticos bem como compreender os determinantes genéticos envolvidos em suas adaptações. Entretanto, estudos adicionais são necessários para que os resultados deste trabalho sejam mais bem compreendidos para que novos métodos de tratamento e identificação possam ser desenvolvidos.

2. JUSTIFICATIVA

Glutamicibacter creatinolyticus recentemente tem sido utilizada em processos biotecnológicos (PRUM; DOLPHEN; THIRAVETYAN, 2018; RAMESH et al., 2014, 2015) e vem sendo relatada como um patógeno de importância médica que pode causar infecções em animais e humanos (HOU et al., 1998; YAMAMOTO et al., 2017). Neste trabalho apresentamos o primeiro isolado desta espécie causadora de infecção em uma égua, que até o momento da realização desse trabalho informações sobre a infecção nesses animais estava ausente na literatura. A identificação dessa espécie enfrenta dificuldades, uma vez que o sistema API é demorado e nem sempre fornece uma identificação confiável em nível da espécie. A infecção por bactérias do gênero *Glutamicibacter* pode ser subestimada, porque a identificação correta das linhagens de *G. creatinolyticus* só tem sido possível através da aplicação de métodos de identificação de sequenciamento do gene 16S rRNA e\ou MALDI Biotyper (HOU et al., 1998; YAMAMOTO et al., 2017). O tratamento das infecções urinárias em humanos e dos nódulos causados na égua, provocadas por *G. creatinolyticus* se deu principalmente com o uso de antibióticos, o que pode levar ao aumento da resistência bacteriana e pode ocasionar a reincidência da infecção (HOU et al., 1998) (RIFICI et al., 2019 artigo submetido para publicação).

Apesar da importância deste patógeno, pouco se conhece sobre os mecanismos moleculares e os fatores de virulência associados a este organismo (HOU et al., 1998; YAMAMOTO et al., 2017). Adicionalmente, novos estudos são necessários para revelar e compreender as características deste organismo, podendo auxiliar na melhor compreensão dos determinantes moleculares de virulência e patogenicidade, o que poderá contribuir para melhores práticas de tratamento.

Até o momento não existe genomas dessa espécie sequenciados e depositados no banco de dados do NCBI. Em relação ao gênero ainda há poucos genomas disponíveis. Dentre os isolados do gênero apenas 3 espécies têm seu genoma completo e depositado, e como ainda não se tem uma reclassificação do gênero *Arthrobacter* no NCBI, pode dizer que não se tem uma certeza na quantidade desses dados. Com isso, pouco se sabe sobre o perfil exclusivos dos genomas *G. creatinolyticus* isoladas de equinos. Diante desse cenário, o presente estudo se propõe realizar estudos genômicos da linhagem isolada de uma égua a fim de analisar características que podem estar associadas à virulência, a

genes de resistência a antibióticos, a especificidade pelo hospedeiro, a fim de contribuir para novos estudos que auxiliam em novas estratégias de diagnóstico e tratamento dessa infecção. A contribuição desse trabalho também pode ir além da genômica estrutural, uma vez que a linhagem, aqui utilizada, está disponível em banco de dados públicos a fim de contribuir para novos estudos envolvendo outras abordagens ômicas (HOU et al., 1998; YAMAMOTO et al., 2017, RIFICI et al., 2019).

3. OBJETIVOS

3.1.1. Objetivo geral

Caracterizar o genoma de *Glutamicibacter creatinolyticus* LGCM259 e realizar uma análise comparativa com o gênero *Glutamicibacter*.

3.1.2. Objetivos específicos

- Sequenciar e montar o primeiro genoma da linhagem *G. creatinolyticus* LGCM 259;
- Identificar o repertório de genes de virulência e resistência a antibióticos presentes na linhagem LGCM 259, em comparação com outras linhagens do mesmo gênero e uma linhagem do gênero *Arthrobacter*.
- Avaliar a presença de ilhas genômicas na linhagem LGCM 259, em comparação com linhagens do gênero *Glutamicibacter*;
- Identificar a sintenia gênica entre a *G. creatinolyticus* LGCM 259 e as quatro linhagens completas do NCBI;

4. MANUSCRITO COMPLETO

Complete genome analysis of *Glutamicibacter creatinolyticus* from mare abscess and comparative genomics provide insight of diversity and adaptation for *Glutamicibacter*.

Roselane Gonçalves dos Santos, Raquel Hurtado Castillo, Lucas Gabriel, Rodrigo Profeta, Rifici Claudia, Attili Anna Rita, De Biase Davide, Scaramozzino Carmelo, Paciello Orlando,Cuteri Vincenzo, Spier Sharon J., Mazzullo Giuseppe, Anne Cybelle Pinto Gomide, Bertram Brenig, Alfonso Gala-García, Thiago Luiz de Paula Castro, Vasco Azevedo, NúbiaSeyffert

O Manuscrito completo referente às análises realizadas neste trabalho, foi preparado para submissão à revista “*GENE*”.

Complete genome analysis of *Glutamicibacter creatinolyticus* from mare abscess and comparative genomics provide insight of diversity and adaptation for *Glutamicibacter*

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Dear Mrs. Roselane Gonçalves dos Santos,

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Abstract

Bacteria of the genus *Glutamicibacter* are considered ubiquitous because they can be found in soil, water and air. They have already been isolated from different habitats, including different types of soil, clinical samples, cheese and plants. *Glutamicibacter creatinolyticus* is a Gram-positive bacterium important to various biotechnological processes, however, as a pathogen it is associated to urinary tract infections and bacteremia. Recently, *Glutamicibacter creatinolyticus* LGCM 259 was isolated from a mare, which displayed several diffuse subcutaneous nodules with heavy vascularization. In this study, sequencing, genomic analysis of *G. creatinolyticus* LGCM 259 and comparative analyses were performed among 4 representatives of different members of genus from different habitats, available in the NCBI database. The LGCM 259 strain's genome carries important factors of bacterial virulence that are essential in cell viability, virulence, and pathogenicity. Genomic islands were predicted for 4 members of genus *Glutamicibacter*, showing a high number of GEIs, which may reflect a high interspecific diversity and a possible adaptive mechanism responsible for the survival of each species in its specific niche. Furthermore, *G. creatinolyticus* LGCM 259 shares syntenic regions, albeit with a considerable loss of genes, in relation to the other species. In addition, *G. creatinolyticus* LGCM 259 presents resistance genes to 6 different classes of antibiotics and heavy metals, such as: copper, arsenic, chromium and cobalt-zinc-cadmium. Comparative genomics analyses could contribute to the identification of mobile genetic elements particular to the species *G. creatinolyticus* compared to other members of genus. The presence of specific regions in *G. creatinolyticus* could be indicative of their roles in host adaptation, virulence, and the characterization of astrain that affects animals.

Keywords: mare[1], pathogenicity[2], resistance[3], virulence factors[4], sequencing[5], genomic islands[6].

Introduction

Glutamicibacter creatinolyticus, previously described as *Arthrobacter creatinolyticus*, belonged to the genus *Arthrobacter* of the family *Micrococcaceae*. The new reclassification proposal occurred in 2016, when the genera of the family *Micrococcaceae* were renamed as *Glutamicibacter*, *Paeniglutamicibacter*, *Pseudoglutamicibacter*, *Paenarthrobacter* and *Pseudarthrobacter* (Busse, 2016).

The environmental prevalence of *Glutamicibacter s*p. strains can be considered to be due to their nutritional versatility and ability to respond to environmental stresses (Yao et al., 2015). It is unsurprising that strains of the genus *Glutamicibacter* are phenotypically heterogeneous and have been isolated from distinct sources, such as soil (Shen et al., 2013), clinical specimens (Hou et al., 1998),(Yamamoto et al., 2017), cheese (Monnet et al., 2010), and plants (Feng et al., 2017). Bacteria of the genus *Glutamicibacter* are thought to play a significant role in many ecosystems and to affect human welfare (Yamamoto et al., 2017). The currently identified *Glutamicibacter* species are *Glutamicibacter nicotianae*, *Glutamicibacter arilaitensis*, *Glutamicibacter halophytocola, Glutamicibacter soli*, *Glutamicibacter mysorens* and *Glutamicibacter creatinolyticus (Information acquired from the NCBI Database).*

The species *G. creatinolyticus* is thought to be associated to biotechnological processes and has been reported in a limited number of clinical cases related to infection processes such as bacteremia and urinary tract infections (Hou et al., 1998, Yamamoto et al., 2017)). The general morphological characteristics of *Glutamicibacter creatinolyticus* have been described as follows: circular colonies, smooth and pigmented yellow when grown on brain heart infusion agar (BHI), the bacteria are endospore negative, non-motile, creatinine hydrolysis positive, shaped like irregular rods, aerobic, catalase-positive, and have a genomic G + C content of 66-67% (Hou et al., 1998). The peptidoglycan composition and quinone system are in accordance with the genus' description, but its polar lipid and fatty acid profiles are unknown (Busse, 2016). *G. creatinolyticus* as a bioremediation treatment, works efficiently in the decontamination of arsenic contaminated water, through the interaction between plants and bacteria (Prum et al., 2018). This species has shown to be a potent producer of extracellular urease for the determination of heavy metal ions (Ramesh et al., 2014) and been deployed as a potentiometric biosensor for the determination of urea content in milk, using immobilized urease (Ramesh et al., 2015).

Likewise, *G. creatinolyticus* strains have been isolated from human urine with unusually low creatinine concentrations, due to their ability to hydrolyze creatinine (Hou et al., 1998). Strains of this species were also isolated from an elderly diabetic man with acute cholangitis, to whom this species caused bacteremia (Yamamoto et al., 2017). *G. creatinolyticus* strain LGCM 259 was recently isolated from diffuse subcutaneous nodules and masses from a mare in Italy (RIFICI et al., 2019).

Due to the inefficiency of classical biochemical methods in the taxonomic classification of this species, better prediction strategies were required, such as 16S rRNA and MALDI Biotyper sequence analyses (Funke et al., 1996). Currently in the genus *Glutamicibacter*, three species have complete genomes and 9 have incomplete genomes without a definite species deposited in the *National Center for Biotechnology Information* (NCBI) database.

In this study, we present the first complete genome of the species *G. creatinolyticus* LGCM 259.The comprehensive analyses of this genome sequence provide better ways of understanding the molecular and genetic basis of this species. Comparative genomic analyses between species could allow for the identification of mobile genetic elements (e.g. pathogenicity islands and bacteriophage sequences) as genetic characteristics determinant of the completely different habitats described for each species of the genus. In addition, phylogenomic analyses allow for the verification of the taxonomic reclassification of all completely sequenced strains of members of genera *Arthrobacter* and *Glutamicibacter*.

Material and Methods

Bacterial culture and DNA extraction from *G. creatinolyticus*

G. creatinolyticus strain LGCM 259 was previously isolated from the abscess of a 12-year-old mare in Italy (RIFICI et al., 2019, article accepted for publication).In order to extract the genomic DNA, this strain was first cultivated on BHI (Brain Heart Infusion) agar and, next, on 30mL of BHI broth, at 37°C, overnight. The culture was centrifuged for bacterial pellet formation and the supernatant was discarded. The pellet was suspended in 600µL of solution (Tris-HCI pH 7.0, 0.5M EDTA pH 8.0, NaCl 5M, and distilled H2O enough to obtain 50 mL) and transferred to a 2mL tube containing glass beads (VK01) (Bertin Technologies) to subject the bacteria to mechanical lysis. Two homogenization cycles of 15sec each, at 6,500rpm, were performed using Precellys 24 (Bertin Technologies). Subsequently, 1mL of phenol: chloroform: isoamyl alcohol (25:24:1) solution was added to the tube and the mixture

was homogenized and centrifuged at 13,000rpm, for 7min. Next, the upper aqueous phase of the mixture was transferred to a new tube, and a second round of phenol: chloroform: isoamyl alcohol purification was performed. Next, the upper aqueous phase was recovered and mixed with 1mL of chloroform. Following centrifugation at 13,000rpm, for 7min, the upper aqueous phase was transferred to a new tube. 1mL of ethyl alcohol, 40µL of 3M NaAc, and 4µL of 20mg/mL glycogen were added. Following gentle inversion, the mixture was placed at -20°C, overnight, for DNA precipitation. Following centrifugation at 13,000rpm, for 15min, the supernatant was discarded. 1mL of 70% ethyl alcohol was added to rehydrate the DNA pellet. A second round of 70% ethyl alcohol wash was performed using centrifugation at 13,000rpm, for 15min. The DNA pellet was placed at 60°C to dry. Finally, the DNA precipitate was suspended in 50 µL of sterilized ultra-pure water. DNA quantity and quality assessments were conducted using NanoDropTM 2000 (Thermo ScientificTM), Qubit Fluorometer (Thermo ScientificTM) and 1% agarose gel electrophoresis (Pacheco et al., 2007).

Genome sequencing, assembly and annotation

Chromosomal sequencing of *G. creatinolyticus* strain was performed using Hiseq technology (Illumina, San Diego, CA, USA), using paired-end libraries (2 x 150 bp). The *ab initio* assembly was performed using the software Spades, version 3.9 (Bankevich et al., 2012). For assembly by reference, was made the choice of the best references, the contigs generated for the strain were submitted to the Basic Local Alignment Search Tool for nucleotides (BLASTn) (Altschul et al., 1990). The assembled contigs were oriented to generate a scaffold using Medusa v.1.3 (Bosi et al., 2015), and the strains *Arthrobacter sp.* YC-RL1 and *Arthrobacter*/*Glutamicibacter arilaitensis* Re117 were used as reference. Gaps resulting from the assemblies were manually filled using the CLC Genomics Workbench software, for visualization, version 7.0 (Qiagen, USA), where the reads were mapped against a reference genome to generate a consensus sequence, which was then used to close the gaps. The genome of the *G. creatinolyticus* LGCM 259 was automatically annotated using PROKKA v3 (Seemann, 2014) and deposited in National Center for Biotechnology Information database (NCBI) (https://www.ncbi.nlm.nih.gov).

Complete genomes of the *Arthrobacter* genus and its new reclassification available for comparative genomics analysis

The sequences of 26 genomes were downloaded from NCBI (**Table 1**). The software Prokka was used to homogenize the genome annotation to perform the comparative analyses (Seemann, 2014).

Phylogenomic analyses

The Taxonomical Revision, a proposal to reclassify the species of the genus *Arthrobacter*, has divided it into 5 different genera (Busse, 2016). Due to this new classification, we performed a phylogenomic analysis, using all complete genomes from the *Arthrobacter* genus, as well as the new genera *Glutamicibacter*, *Pseudoarthrobacter*, and *Paenarthrobacte*r that were available in the NCBI database (**Table 1**). The other two genera (*Paeniglutamicibacter* and *Pseudoglutamacibacter*) have no available complete genome. For that purpose, a phylogenomic tree using a fasta file and amino acid sequences was generated using the Phylogenomic Tree Tool in Pathosystems Resource Integration Center (PATRIC) (http://www.patricbrc.org), version 3.5.17 (Wattam et al., 2014). The Maximum Likelihood method was used, with the Automated Progressive Refinement option selected. *Micrococcus luteus* NCTC 2665 was used as an outgroup. The software MEGA X: Molecular Evolutionary Genetics Analysis was used to construct the Maximum-likelihood tree using the 16S gene sequence (Kumar et al., 2018).

Table 1: Genomes downloaded from the NCBI used in this work.

N°	Strain Name	Accession	Size (bp)
1	*Arthrobacter sp. Rue61a*	PRJNA78011	4,736,495
2	*Arthrobacter sp. FB24*	PRJNA12640	4,698,945
3	*Arthrobacter sp. PAMC 25486*	PRJNA244892	4,593,579
4	*Arthrobacter sp. ERGS1:01*	PRJNA293260	4,030,317
5	*Arthrobacter sp. YC-RL1*	PRJNA302833	3,846,272

6	*Arthrobacter sp. ATCC 21022*	PRJNA306041	4,434,904
7	*Arthrobacter sp. U41*	PRJNA320863	4,386,369
8	*Arthrobacter sp. ZXY-2*	PRJNA341911	4,495,402
9	*Arthrobacter sp. QXT-31*	PRJNA361372	5,041,568
10	*Arthrobacter sp. YN*	PRJNA393603	5,063,552
11	*Arthrobacter sp. Hiyo4*	PRJDB3373	3,779,248
12	*Arthrobacter sp. PGP41*	PRJNA431708	4,270,237
13	*Arthrobacter sp. DCT-5*	PRJNA473399	4,359,122
14	*Arthrobacter sp. Hiyo8*	PRJDB3373	4,698,617
15	*Glutamicibacter arilaitensis Re117*	PRJNA224116	3,859,257
16	*Glutamicibacter halophytocola KLBMP 5180*	PRJNA289022	3,911,798
17	*Glutamicibacter nicotianae OTC-16*	PRJNA490584	3,643,989
18	*Arthrobacter crystallopoietes DSM 20117*	PRJNA357926	5,032,705
19	*Arthrobacter alpinusR3.8*	PRJNA295631	4,046,453
20	*Pseudarthrobacter phenanthrenivorans Sphe3*	PRJNA295631	4,046,453
21	*Paenarthrobacter aurescensTC1*	PRJNA12512	4,597,686

22	*Arthrobacter radiotolerans RSPS-4*	PRJNA241417	3,267,233
23	*Pseudarthrobacter equi*	PRJEB16401	4,459,178
24	*Pseudarthrobacter sulfonivorans Ar51*	PRJNA305788	5,043,757
25	*Pseudarthrobacterc hlorophenolicus A6*	PRJNA20011	4,980,870
26	*Micrococcus luteus NCTC 2665*	PRJNA20655	2,501,097

Average Nucleotide Identity (ANI) and Tetra nucleotide

http://www.patricbrc.org

Average nucleotide identity (ANI) and tetra nucleotide frequencies were estimated by JSpecies Web Server (available at http://imedea.uib-csic.es/jspecies/about.html) (Richter et al., 2016) based on BLAST (Altschul et al., 1990), selecting the ANIb and Tetra nucleotide options. In a given genome pair, the ANI performs pairwise comparisons of 1020bp fragments between a query and a reference genome (Tindall et al., 2010). The tetra nucleotide frequencies perform signatures between paired genome comparisons (Tindall et al., 2010). Typically, the threshold frontier to consider two organisms to belong to the same species could be set at >95% identity for ANI and >99% for Tetra (Busse et al., 2010; Richter et al., 2016).

Identification of genes encoding virulence factors

The presence of virulence genes in the genome was identified using BLASTp (Altschul et al., 1990)against the Virulence Factor Database (VFDB) (Chen et al., 2004). The parameters considered were an e-value of 1e−5, a minimum identity percentage of 50% and minimum coverage of 70%, between the query and subject sequences (Lindahl and Elofsson, 2000; Yang and Honig, 2000). The functional annotations were obtained from the categories provided by the Virulence Factor Database (Chen et al., 2004).

Genome plasticity analysis of *G. creatinolyticus* compared to other *Glutamicibacter*

Genomic island (GEIs) predictions were performed using Genomic Island prediction Software (GIPSy), version 1.1.2 (Soares et al., 2016), using the genome of *G. arilaitensis* strain Re117 as a reference. Gipsy classifies GEIs into four different categories: (i) pathogenicity islands (PAIs), which carry virulence factor genes (Dobrindt and Hacker, 2001); (ii) metabolic islands (MIs), which harbor genes associated to the biosynthesis of (secondary) metabolites (Tumapa et al., 2008); (iii) resistance islands (RIs), containing genes that code for resistance, typically against antibiotics (Krizova and Nemec, 2010); and symbiotic islands (SIs), facilitating symbiotic associations of the host with other microorganisms (Barcellos et al., 2007). In addition, we evaluated the presence of genomic islands for each species of the genus *Glutamicibacter*. To define homologous GEIs, we considered an identity higher than 70%. Finally, BLAST Ring Image Generator (BRIG), version 0.95 (Alikhan et al., 2011), was used to map the GEIs.

http://imedea.uib-csic.es/jspecies/about.html

To identify conserved genomic regions among species of genus *Glutamicibacter*, including DNA rearrangements and inversions, a synteny analysis was conducted using Mauve (Darling et al., 2004), version 2.4.0, with the most accurate option for alignment selected, Progressive Mauve.

For this analysis, sequences from the chromosome of strains *A. sp.*YCRL1 isolated from petroleum-contaminated soil, *G. halophytocola* KLBM5180, isolated from the roots of the plant *Limonium sinense* (Feng et al., 2017), *G. nicotinae* OTC16 isolated from active sludge around pharmaceutical company (Wang et al., 2015), and *G. arilaitensis* RE117isolated from the surface of cheeses (Monnet et al., 2010).

Results

G. creatinolyticus general genomic features

The *G. creatinolyticus* LGCM 259 chromosome sequence has been deposited in the NCBI database under accession number CP034412. The strain was sequenced and assembled in a circular chromosome, which exhibits a length of 3,3 Mb, with a G+C content of 66.4%, and a total of 2882 CDSs, 4 clusters of rRNAS (5S, 16S, and 23S), and 61 tRNA genes, respectively. To further analyze the genome of *G. creatinolyticus* LGCM 259, functional characterization of genome sequences was realized with Kyoto Encyclopedia of

Genes and Genomes (KEGG) using BLASTKOALA (Kanehisa et al., 2016). Gene clustering analysis (**Supplementary table 1**), revealed the presence of a total of 1487 KEGG-associating genes; the top categories belonged to carbohydrate metabolism (174 genes; 11,58%); protein families: genetic information processing (173 genes; 11.51%), genetic information processing (161 genes; 10.71%); signaling and cellular processes (155 genes; 10.31%); amino acid metabolism (133 genes; 8.85%); environmental information processing (104 genes; 6.92%), unclassified: metabolism (82 genes; 5.46%), metabolism of cofactors and vitamins (81 genes; 5.39%), and other categories (440 genes; 29.27%), respectively (**Figure 1**).

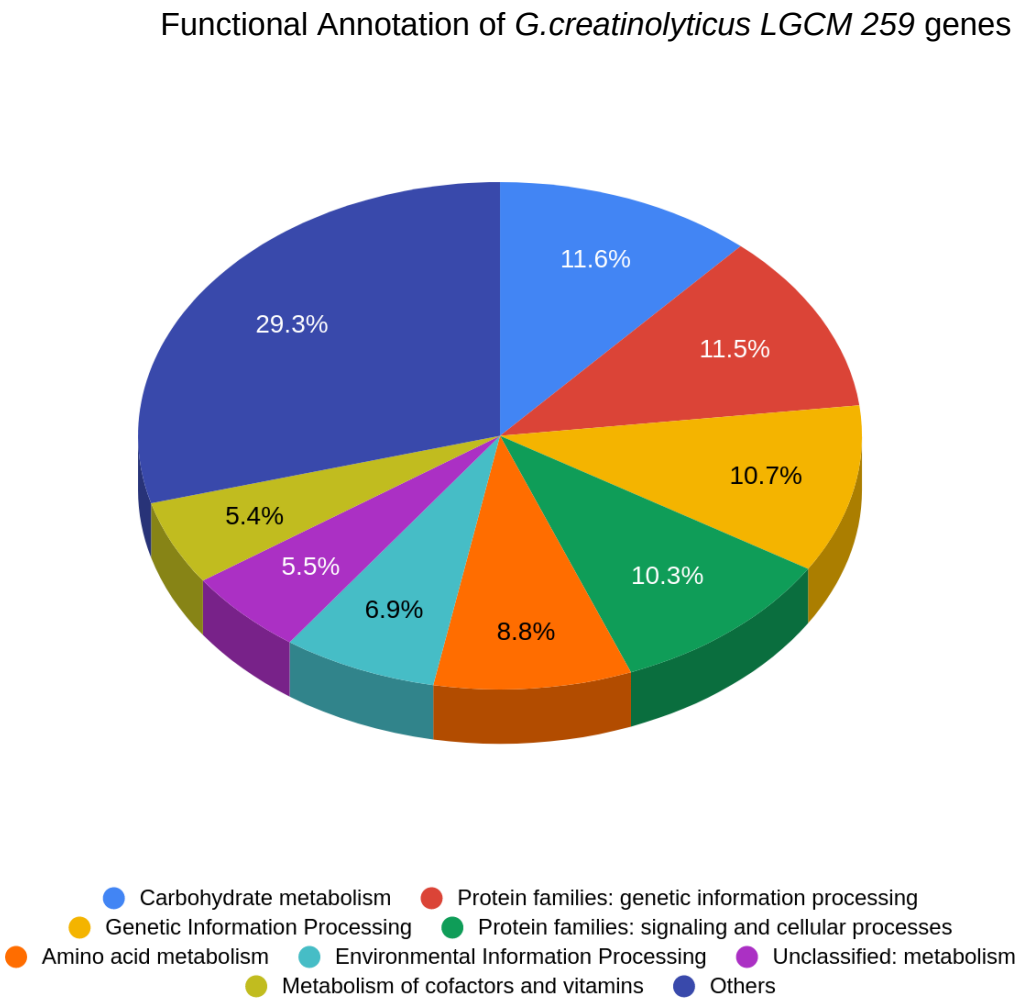

Figure 1: Functional Annotation of *G. creatinolyticus* LGCM 259 genes. This figure was prepared by analysis of the whole genome and relative abundance and distribution of KEGG categories plotted using Microsoft Excel.

Phylogenomic analysis with *G. creatinolyticus*

Phylogenomic analyses were performed between the completed sequence of strain LGCM 259 and twenty-six (**Table 1**) other whole bacterial genomes previously described. The phylogenetic tree based on core genes was constructed using the Maximum Likelihood method (**Figure 2**). Additionally, a phylogenetic analysis using 16S rRNA genes was performed (**Supplementary figure 1**). In both comparisons, the species *Arthrobacter radiotolerans* had the most divergent genome in relation to the closest available complete sequences of the same family found in the database, once this species has already been

reclassified as *Rubrobacter radiotolerans* (Egas et al., 2015). In both approaches, it is possible to see that the *G. creatinolyticus* LGCM 259 forms a clade with its respective genus (**Figure 2**). The genome of *Arthrobacter sp.* YCRL1, was grouped with the *Glutamicibacter* genus, suggesting that this strain may be part of the genus. However, more studies need to be performed, with genomes representing every genus, in order to reclassify all these genomes in accordance to the new classification suggested by Busse, 2016 (Busse, 2016). The resulting Newick tree file was visualized using iTOL v4.261 (Letunic and Bork, 2016). Thus, all comparative analyses were performed using the complete genomes representatives of the members of the genus *Glutamicibacter* and the genome of *A. sp* YCRL1, because it is so close to the genus.

Figure 2: Phylogenomic analysis based in the Maximum Likelihood method. The *Glutamicibacter* genus is highlighted in red, along with the genome of *G. creatinolyticus* LGCM 259, and *A. sp.* YCRL1. *Arthrobacter radiotolerans* is the most divergent strain in the tree. The genome *Micrococcus luteus* NCTC 2665, members of *Microccaceae* family was used as an outgroup. The numbers represent the bootstrap values.

Average Nucleotide Identity (ANI) and Tetra nucleotide

In order to better understand how strain LGCM 259 is genetically correlated with the genomes of other genera, formerly known as *Arthrobacter* and the new genera proposed by Busse, 2016, mean nucleotide identity predictions were conducted, using all completed genomes, encompassing the entire *Arthrobacter* genus and its new classification, that include the 3 different species of *Glutamicibacter* genus downloaded from NCBI (Busse, 2016). All genomes displayed a low degree of similarity with the reference strain, *G. creatinolyticus* LGCM 259 (ANI<80% and tetra nucleotide <96%), thus, we can hypothesize that none of genomes belonged to the same species (**Supplementary figures 2 and 3**).

Virulence factors (VFs) predicted in *G. creatinolyticus* may have a role in infection

We identified 10 virulence factors (VFs) in the genome, detailed in (**Supplementary Table 2**).We identified the gene, isocitrate lyase activity (ICL) (locus tag: LGCM259_0286), that is related the metabolism of fatty acids and necessary for the assimilation of acetates in *Rhodococcus equi* (Wall et al., 2005). Isocitrate lyase activity (ICL) were previously studied in other organisms including *M. tuberculosis* and have been associated with increased virulence (Muñoz-Elías and McKinney, 2005).

Bacterial ABC transporters involved in cell viability, virulence, and pathogenicity were also identified. We identified a cluster of genes coding for enzymes involved in ABC transportation, which includes groEL (locus tag: LGCM259_0465), *groES* (locus tag: LGCM259_ 0460) and *fepG* (locus tag: LGCM2_0733*)*. The *groEL* gene prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions. To function properly, *groEL* requires the lid-like cochaperonin protein complex GroES (*groES* gene) (Maguire et al., 2002). Already *fepG* is part of the genes involved in the iron transport, important elements for growth and bacterial metabolism (Bunet et al., 2006). We also identified the virulence factor involved in two-component signal transduction systems (TCSs) (locus tag: LGCM259_0734), allows bacteria to sense, respond, and adapt to changes in their environment or in their intracellular state (Othmer et al., 2013). TCSs usually consist of a membrane-bound sensor histidine protein kinase (HPK) that perceives environmental stimuli, and a response regulator (RR) that affects gene expression (Yamamoto et al., 2005). This virulence factor is involved in metal ions fulfill, a

plethora of essential roles within bacterial pathogens. Aside of being indispensable for the structure and for the function of proteins, they also fulfill roles in signaling and virulence regulation (Begg, 2019). We identified the *ideR* (locus tag: LGCM259_0465) gene acts as a siderophore biosynthesis repressor and as an iron storage positive modulator (Gold et al., 2001). A protein involved in kinase activity, the GTP pyrophosphokinase RelA (locus tag: LGCM259_1510), a stringent response mediator that coordinates a variety of cellular activities in response to changes in nutritional abundance, was also identified (Edwards et al., 2011). In this analysis we find the UDP-galactose 4-epimerase (GalE) (locus tag: LGCM259_1204) which is also called UDP-glucose 4-epimerase, is an enzyme responsible for interconversion of UDP-galactose and UDP-glucose (Li et al., 2014). GalE is also an important virulence factor in a few bacterial pathogens (Li et al., 2014). Other VFs involved in pentose-phosphate shunt and Peptidyl-prolyl cis-trans isomerase or PPIase (Unal and Steinert, 2014) (cypB gene locus tag: LGCM259_0015) were found. PPIases accelerate the folding of proteins and have also been identified as virulence-associated proteins. The extent of their contribution to virulence is highly variable and dependent on the pleiotropic roles of a single PPIase in the respective pathogen (Unal and Steinert, 2014).

Genome reduction and unique genomic island profile of *G. creatinolyticus* compared to other *Glutamicibacter*

Comparative analysis using MAUVE evidenced an overall structural conservation and collinearity among the chromosomes of the different genomes (**Figure 3**). The multiple alignments showed the existence of locally collinear blocks (LCBs). However, it was observed that specific regions of each genome were present in the LCBs (white segments), which may harbor specific adaptations of each bacterium and infer adaptation by species (Darling et al., 2004). These regions probably represent DNA acquired during Horizontal Gene Transfer (HGT) events. Regions that are inverted in relation to the strain LGCM 259 are shown as dislocated below the central genome axis. Regarding the size of the chromosome, the LGCM 259 strain chromosome presented to be smaller (3.3 Mpb) than the four other species used for this work (3.8, 3.8, 3.9, 3.6 Mbp for *A. sp.*YCRL1, *G. arilaitensis* RE117, *G. halophytocola* KLBM5180, and *G. nicotinae* OTC16, respectively). The genome of *G. halophytocola* KLBM5180 showed the largest chromosome size. This is probably due to the accumulation of genes related to their adaptation to a different ecological niche, since this strain is a bacterium capable of producing a wide range of secondary biological metabolites and their beneficial effects on host plants (Feng et al., 2017). The GC

content of strain LGCM 259 strain is 66.4%, higher than the other genomes (64.26, 59.29, 60.91, 62.92% *A. sp.* YCRL1, *G. arilaitensis* RE117, *G. halophytocola* KLBM5180, and *G. nicotinae* OTC16, respectively).

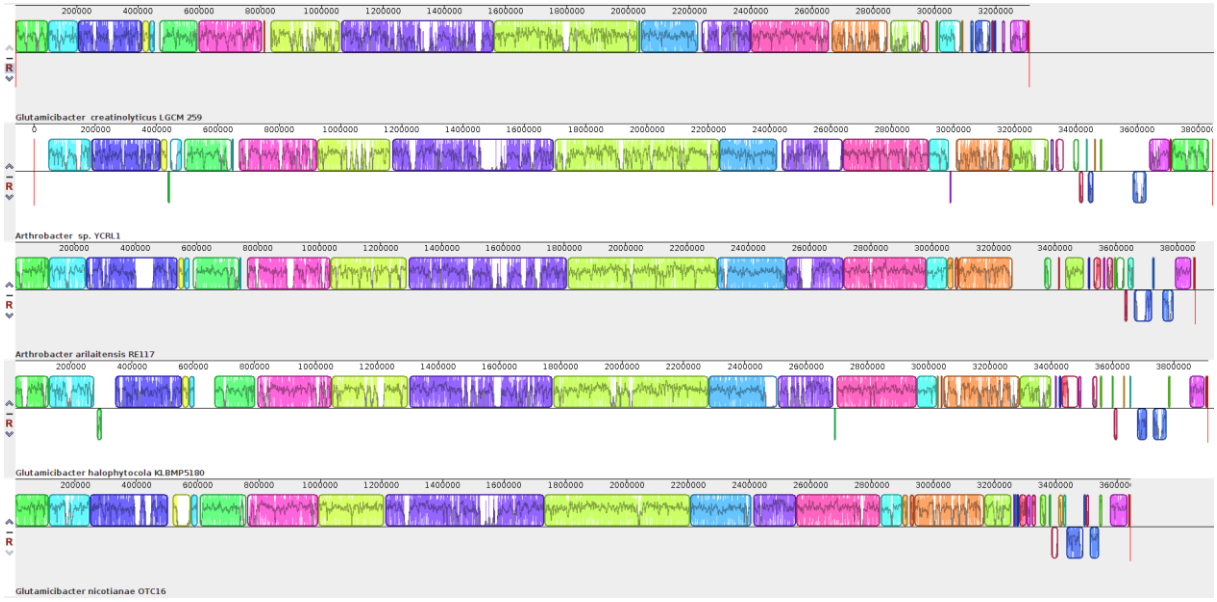

Figure 3: Whole genome comparative alignments between *G. creatinolyticus* LGCM 259 with *A. sp.* YCRL1, *G. arilaitensis* RE117, *G. halophytocola* KLBM5180, and *G. nicotinae* OTC16. Each analyzed genome is displayed horizontally, with each conserved segment represented by colored blocks, connected between each genome, and sequence coordinates are shown in scale. The aligned region is in the forwards orientation in relation to the first genome sequence if a block is above the central line, and blocks below the central line indicate regions aligned in the complementary inverted orientation. Regions not belonging to any blocks are regions that do not show significant homology between the analyzed genomes. Regions in white are unique to each species.

Another interesting trait of the strain LGCM 259 is that the genome presented several genomic islands. The number of predicted GEIs was 23 (**Figure 4**). Of these 23 GEIs, 15 islands were pathogenic, 4 resistance, 2 metabolic and 2 symbiotic islands. As expected, the GEIs displayed a different GC content from the rest of the genome, as well as factor codifying products typically involved in genetic mobility, such as integrases, recombinase and transposases, this suggests that some GEIs were likely recruited through Horizontal Gene Transfer (HGT), to facilitate the bacterium's survival in diverse niches (Bellanger et al., 2014). These resources are commonly incorporated in GEI prediction markers (Bellanger et al., 2014). Additionally, the GEIs displayed genes implicated

Arsenical-resistance protein, Vancomycin resistance protein VanJ). Bacterial Horizontal Gene Transfer can be greatly advantageous to the bacterium. This can contribute to the generating novel metabolic functions, genomic plasticity and adaptive value. Therefore, it plays a fundamental role in bacterial evolution (Gal-Mor and Finlay, 2006).

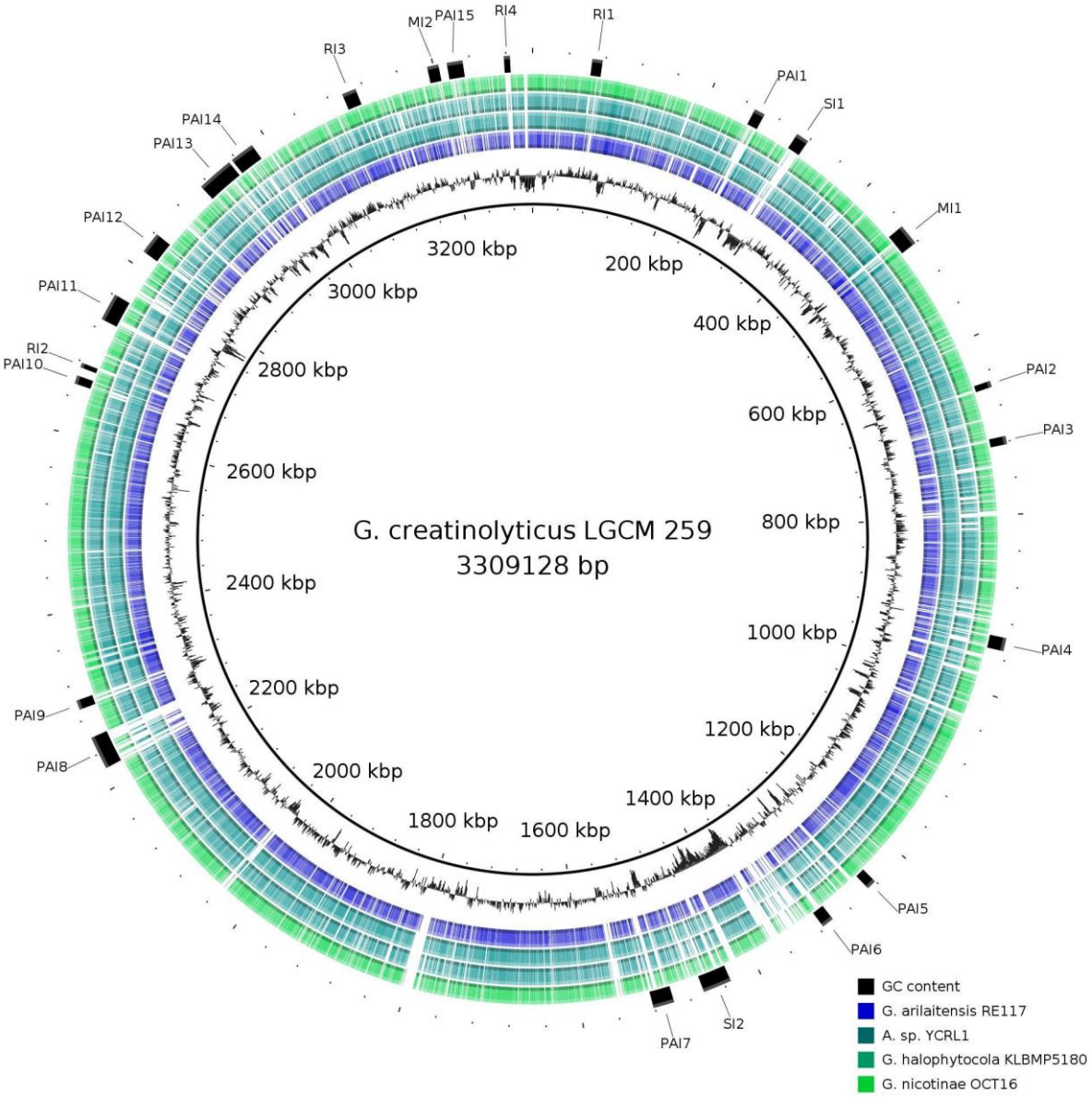

Figure 4: Circular genome map representing all genomic islands found in the *G. creatinolyticus* LGCM 259 genome. Representation, from the inner to the outer circle, is: *G. creatinolyticus* LGCM 259, GC Content, *G. arilaitensis* Re117, *A. sp.* YCRL1, *G.*

hlophytocola KLBM5180 and *G. nicotinae* OTC16. Pathogenicity islands (PAIs); Metabolic islands (MIs), Resistance islands (RIs), and symbiotic islands (SIs).

The genomes of 4 species of the genus *Glutamicibacter* and a genome of the *Arthrobacter* genus were analyzed for the prediction of GEIs. This analysis showed a considerable variation in the number of genomic islands (**Supplementary Table 3**). GEIs were predicted for each genome, with each *Glutamicibacter* genome containing, in average, 25 GEIs. Each species was represented by a strain isolated from a specific niche. Through GEI prediction, we hoped to identify genes in the islands functionally related to the bacterium's specialization to their specific niche.

The comparative analysis between the GEIs of *G. creatinolyticus* LGCM 259 identified 10 specific GEIs for this species (**Supplementary Table 4**) (**Figure 2**). Of these 10 exclusive genomic islands, 5 are PAIs. For PAIs 4 and 5, products were reported (**Table 2**), of which most are involved in sugar transportation, transcriptional regulators, and the products microssistin degradation and copper homeostasis. In addition, the island MI2 was identified, displaying genes involved in the regulation and structure of the bacterium's lipopolysaccharide cover. These genes may be important to the species' classification and identification. Furthermore, 3 RIs, 1 MI and 1 SI were reported, of which SI2 displayed products related to transcriptional regulation, cobalt metabolism and amino acid metabolism (**Table 2**).

Table 2: Description of the products of some exclusive islands of *G. creatinolyticus* LGCM 259.

Strain Name	Exclusive island	Products
***G. creatinolyticus* LGCM 259**	**PAI4**	Cadmium-transporting ATPase, Copper chaperone CopZ, cAMP receptor protein, HAD family hydrolase, Hypothetical protein, Restriction endonuclease, Microcystin degradation protein MlrC

***G. creatinolyticus* LGCM 259**	**PAI5**	Fatty acid desaturase, hypothetical protein, Cold shock protein, IS1380 family transposase, LacI family transcriptional regulator, Alpha-amylase, ABC transporter substrate-binding protein, Maltose ABC transporter permease, Sugar ABC transporter permease, 3,4-dihydroxyphenylacetate 2,3-dioxygenase
***G. creatinolyticus* LGCM 259**	**MI2**	16S ribosomal RNA, 23S ribosomal RNA, 5S ribosomal RNA, Dehydrogenase, Formate dehydrogenase, nitrate-inducible, major subunit precursor, Formate dehydrogenase, nitrate-inducible, iron-sulfur subunit, Polysulfide reductase NrfD, Selenide, water dikinase, Transposase, tRNA-Sec, L-seryl-tRNA(Sec) selenium transferase, Selenocysteine-specific elongation factor, Trehalose 6-phosphate phosphatase
***G. creatinolyticus* LGCM 259**	**SI2**	XRE family transcriptional regulator, Alanine racemase, Aspartate aminotransferase family protein, Recombinase, Amino acid permease, cobT Aerobic cobaltochelatase subunit CobT, IclR family transcriptional regulator, Sulfoacetaldehyde acetyltransferase

In the species *G. arilaitensis* RE117 identified 10 specific GEIs for this species (**Supplementary Table 5; Supplementary figure 4**).We report 5 unique pathogenicity islands, of which PAI3 and PAI12 displayed some proteins, mainly involved in oxidative stress, metal transport, transcriptional regulation, amino acid metabolism, and a toxin-antitoxin system virulence factor (**Table 3**), (Zhang et al., 2017, Monnet et al., 2010) . Genes involved in the environmental adaptation to cheese were not reported in genomic island

content, which may be due to their transmission being chromosomal, not having been acquired from HGT events (Bonham et al., 2017).

Table 3: Description of the products of some exclusive islands of *G. arilaitensis* RE117 and *A. sp*. YRLC1.

Strain Name	Exclusive island	Products
		recombinase family protein,hypothetical protein, single-stranded DNA-binding protein, type VI secretion protein, hypothetical protein, ATP-binding protein, hypothetical protein, ABC transporter ATP-binding protein, Zn-dependent oxidoreductase, membrane protein, class I SAM-dependent methyltransferase, M23 family
***G. arilaitensis* RE117**	**PAI3**	metallopeptidase,chromosome partitioning protein ParB, DUF2637 domain-containing protein, DNA primase, ImmA/IrrE family metallo-endopeptidase, AraC family transcriptional regulator, 6 ABC transporter ATP-binding protein, iron ABC transporter permease, siderophore-interacting protein, helix-turn-helix domain-containing protein, DNA cytosine methyltransferase, conjugal transfer protein, DNA-processing protein DprA, IS3 family transposase, integrase
***G. arilaitensis* RE117**	**PAI12**	IclR family transcriptional regulator, dihydroorotate dehydrogenase, MFS transporter, Zn-dependent hydrolase, dihydroorotate oxidase electron transfer subunit, dihydroorotase, aspartate ammonia-lyase, cyclohexadienyl dehydratase, SDR family oxidoreductase, MFS transporter, transketolase, GntR family transcriptional regulator, transcriptional regulator, type II toxin-antitoxin system HipA family toxin, alkene reductase,SDR family oxidoreductase.

***A. sp*. YRLC1**	**MI3**	cyclic amidohydrolase, ribonuclease BN, hypothetical protein, monooxygenase, glutaminase, aldehyde dehydrogenase, hydrolase, ferredoxin, dehydrogenase, phosphoenolpyruvate synthase, cytochrome, IclR family transcriptional regulator, metal-dependent phosphohydrolase, monooxygenase, formate--tetrahydrofolate ligase
***A. sp*. YRLC1**	**MI6**	glycosyltransferase, hypothetical protein, acetyltransferase, CAAX protease, alkaline phosphatase, sugar ABC transporter permease, sugar ABC transporter substrate-binding protein, fructose-bisphosphate aldolase, hypothetical protein, sugar isomerase
***A. sp*. YRLC1**	**SI1**	hypothetical protein, iron-binding protein, ABC transporter, phosphonate ABC transporter permease, phosphonate ABC transporter, phosphonate ABC transporter substrate-binding protein, oxidoreductase, GntR family transcriptional regulator, HAD family hydrolase, transcriptional regulator

In the species *A. sp.* YRLC1, we report 10 specific GEIs (**Supplementary Table 6; Supplementary figure 5**), them being: 1 pathogenicity, 3 of resistance, and 4 metabolic. Of these, MI3 displays proteins involved in electron transportation, which are important in soil with high mineral content. (**Table 3**) (Zhang et al., 2017). MI6 displayed some products involved in sugar transportation and metabolism, which are involved in nutrient rich soil (**Table 3**). 2 SIs were also found, of which SI1 displayed proteins involved in transmembrane transportation and transcriptional regulation, both of which are important to virulence and soil adaptation. (**Table 3**) (Zhang et al., 2017). For *G. nicotinae* OCT16, the metabolic islands MI1 and SI3 were reported as exclusive genomic islands (**Supplementary Table 7; Supplementary figure 6**), these two islands display genes encoding for proteins involved in sugar transportation, oxidative stress and transcriptional regulation, all of which are important in adaptation to soil environments (**Table 4**). Strains YCRL1 and OTC16 were

isolated from soil, but belonged to different soils, which is why islands were predicted individually. In addition, these 2 strains, as well as strain RE117 (isolated from cheese) had the largest number of shared genomic islands.

The species *G. halophytocola* KLMB5180 had 18 exclusive genomic islands (**Supplementary Table 8; Supplementary figure 7**). Of these 18 GEIs, 2 are symbiotic islands, 5 pathogenic, and 7 metabolic. Islands MI1, MI10 and MI13 display genes involved in transcriptional regulation, sugar transportation, and iron and amino acid metabolism (**Table 4**). Genomic islands functionally involved in niche adaptation were reported, but genes involved in specialization were not found, which could be due to the fact that specialization genes cannot be transferred through HGT (Bonham et al., 2017). In addition, strains isolated from specific niches are likely not niche restricted, having an ample niche adaptation spectrum (Feng et al., 2017).

Table 4: Description of the products of some exclusive islands of *G. nicotinae* OCT16 and *G. halophytocola* KLMB5180.

Species	Exclusive island	Products
***G. nicotinae* OCT16**	**MI1**	amino acid permease, hypothetical protein, TRAP transporter large permease subunit, NAD(P)-dependent oxidoreductase, methyltransferase , IclR family transcriptional regulator, tripartite tricarboxylate transporter TctB family protein, tripartite tricarboxylate transporter permease, tripartite tricarboxylate transporter substrate binding protein, lycoside hydrolase family 3 protein, TRAP transporter large permease, TRAP transporter small permease, LacI family transcriptional regulator, glucuronate isomerase, mannitol dehydrogenase family protein, D-galactonate dehydratase family protein

***G. nicotinae* OCT16**	**SI3**	glycerate kinase, MFS transporter, SDR family oxidoreductase, M20 family peptidase, PLP-dependent aminotransferase family protein, M24 family metallopeptidase, LysR family transcriptional regulator, aspartate aminotransferase family protein, NAD-dependent succinate-semialdehyde dehydrogenase, ald alanine dehydrogenase, ulP family inorganic anion transporter, GGDEF domain-containing protein, EAL domain-containing protein, glycosyltransferase family 2 protein
***G. halophytocola* KLMB5180**	**MI1**	LacI family transcriptional regulator, MFS transporter, mandelate racemase/muconate lactonizing enzyme family protein, sugar ABC transporter permease, carbohydrate ABC transporter permease, gfo/Idh/MocA family oxidoreductase, NAD(P)-dependent oxidoreductase, M24 family metallopeptidase, sugar phosphate isomerase/epimerase, dihydrodipicolinate synthase family protein, carbohydrate ABC transporter substrate-binding protein
***G. halophytocola* KLMB5180**	**MI10**	N-acetyltransferase, AraC family transcriptional regulator, ABC transporter ATP-binding protein, iron-siderophore ABC transporter substrate-binding protein, iron ABC transporter permease, hypothetical protein, ROK family protein, extracellular solute-binding protein, sugar ABC transporter permease, arbohydrate ABC transporter permease, alpha-amylase, FAD-dependent oxidoreductase, APC family permease, TetR/AcrR family transcriptional regulator

***G. halophytocola* KLMB5180**	**MI13**	sugar ABC transporter substrate-binding protein, antibiotic biosynthesis monooxygenase, galE UDP-glucose 4-epimerase, galK galactokinase, galT galactose-1-phosphate uridylyltransferase, DeoR/GlpR transcriptional regulator, alpha-galactosidase, sugar ABC transporter permease, carbohydrate ABC transporter permease, sugar ABC transporter substrate-binding protein

In this experiment 126 GEIs were predicted, of which 67 are homologous between two or more genomes. In the results, there were only 2 GEIs present in all genomes, suggesting that these 2 GEIs may be a characteristic of the genus. The pathogenicity-related islands accounted for 43.65% (55 in total), MIs accounted for 25.39% (32 in total), RIs accounted for 19.04% (24 in total) and SIs accounted for 11.9% (15 in total).

Antibiotic and bioremediation resistance genes in *G. creatinolyticus*

The search of the resistance and virulence repertoire shared between genomes, was facilitated by BLASTX sequence comparison to the MEGARes: An Antimicrobial Database for High-Throughput Sequencing database (Lakin et al., 2017) (percentages of identity with the matching regions are shown in the parenthesis).

The genome of the *G. cretinolyticus* LGCM 259 displayed various antibiotic resistance genes. The rifamycin-resistant beta-subunit of RNA polymerase (rpoB) (77%) gene was identified, a marker that includes amino acid substitutions which disrupt the affinity of rifampin for its binding site (Miller et al., 1994) . Elfamycin EF-Tu_inhibition (75%) was also detected. The *tufA* and *tufB* genes encode the components of EF-Tu, the target of the elfamycins. Mutations in them may cause elfamycin resistance via evasion of the

mechanism of action (Vorstenbosch et al., 2000). Among the other identified genes are Aminocoumarin (100%) resistance genes. Aminocoumarin acts through the inhibition of the DNA-gyrase enzyme involved in bacterial cell division (Flatman et al., 2005), and the ABC transporter (100%) gene, one of the most widely recognized mechanisms of multidrug resistance, which can be considered a hijacking of their normal roles in the transport of xenobiotics, metabolites and signaling molecules across cell membranes (Fletcher et al., 2016). Besides, the genome of strain LGCM 259 presents arsenic resistance genes, as the *arsC* and *arsB* genes. ArsC codes for a small-molecular mass arsenate reductase, and ArsB codes an arsenic efflux pump protein which forms an anion-translocating ATPase (Cai et al., 2009). Moreover, heavy metal resistance genes (related to copper, arsenic, chromium, and cobalt-zinc-cadmium resistance) were identified.

Interestingly, rifampicin resistance genes are shared among all genomes. Other resistances genes antibiotic is present in one or more genomes (**Figure 5**). Only strain LGCM 259 displayed chloramphenicol resistance genes. Elfamycin resistance genes is shared only by strains KLBM5180 and YCRL1. Fluoroquinolone resistance genes was present in nearly all genomes, except for strain KLBM5180. Aminocoumarin resistance genes was present in strains LGCM 259, KLBM5180, and OTC-16. Spiramycin resistance genes could only be detected in the OTC-16 genome. The A. sp. YCRL1genome displayed a gene conferring Multi-drug resistance. The ABC transporter resistance gene was present only in strains LGCM 259 and YCRL1.

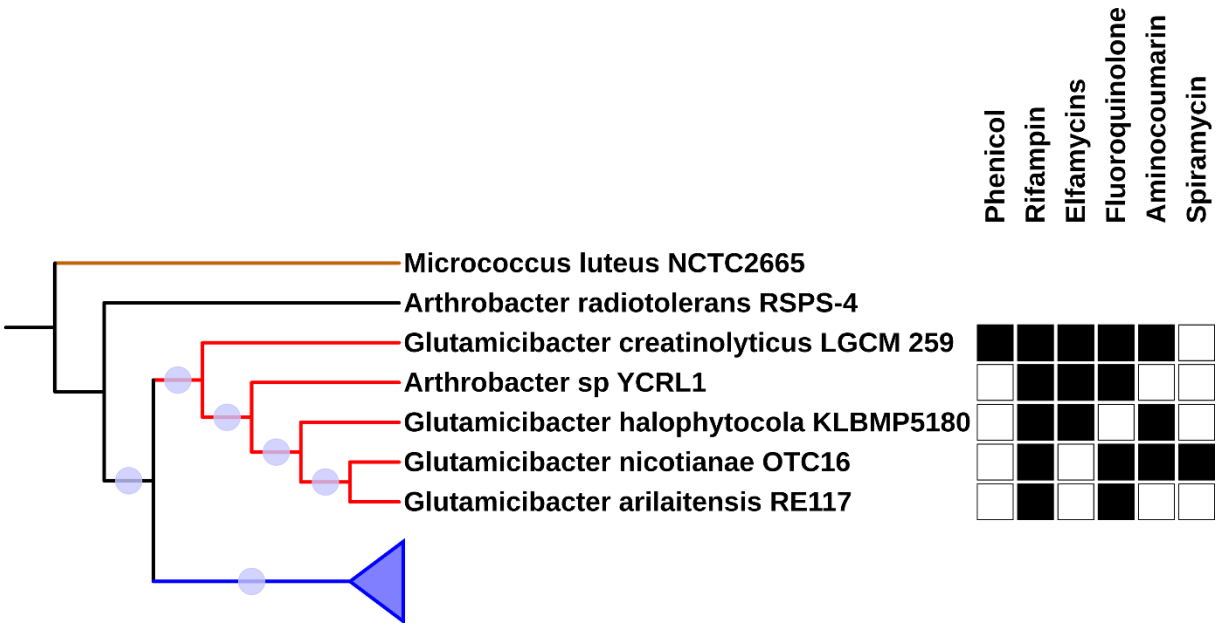

Figure 5: Cluster of the genus *Glutamicibacter*, together with an f the genus *Arthrobacter* species including information on the presence of resistance classes of genes. The cluster is highlighted in red and black squares represent gene presence of the resistance class. Purple balls represent a bootstrap of 100%.

Discussion and conclusion

The bacteria that make up the *Glutamicibacter* genus are aerobic, Gram-positive, and metabolically diverse bacteria that are broadly distributed between soil, human and, in this case, as an occasional pathogen associated to infections (Hou et al., 1998). In general, this study seeks increase our comprehension of the genomic resources of *G. Creatinolyticus* LGCM259 that may be employed in order to resist and survive in a habitat unlike those previously described for the genus.

Bacterial virulence factors allow for a bacterium to replicate and multiply within a host (Cross, 2008). The *G. creatinolyticus* LGCM 259 genome has displayed virulence factors associated with an increase in virulence, probably influencing the bacterium's capacity to infect, and survive within, a host, as well as virulence factors essential to cellular viability and pathogenicity (Muñoz-Elías and McKinney, 2005; Wall et al., 2005b).

Genomes share many common islands, but some of them are unique to each species, suggesting that these unique GEIs may be related to the adaptation of each species, but if these islands are unique for each strain or if it is species related, further studies are needed involving more representatives of each species. The *G. creatinolyticus* LGCM 259 genome is smaller than that of the other four species used in this work, due to the loss of genomic regions. This functional loss is likely tied to the adaptation of *G. creatinolyticus LGCM 259* to its specialized environment, or animal host (mare). Typically, when a bacterium is adapted to infecting a host, there is a reduction of its genome size, since environmental species need genes encoding environmental stress resistance, such as to extreme temperatures, pH, and high salinity (Krasensky and Jonak, 2012; Toft and Andersson, 2010).

The genome displayed a broad repertoire of antimicrobial resistance genes. The emergence of antimicrobial resistant bacteria has become a major public health issue, due

to the lack of effective antimicrobial agents available for the treatment of bacteria-caused diseases (Aslam et al., 2018). We also observed that the 4 genomes were resistant to multiple antibiotic classes, such as Rifampin, Elfamycin and Fluoroquinolone. Multiple drug efflux mechanisms contribute significantly to intrinsic and acquired antimicrobial agent resistance. The following locus tags were found (GcLGCM259_1698, GcLGCM259_0905 and GcLGCM259_1698) in the LGCM 259 genome, which may be involved in multiple drug resistance. Among resistance mechanisms, the multiple drug efflux system, or pump system, deserves special attention, since it allows the bacterium to reduce, or even nullify, its susceptibility to a broad range of antimicrobial agents (Moreira et al., 2004). According to the World Health Organization, antimicrobial resistance is a complex problem that affects all of society and is driven by many interconnected factors. Single isolated interventions have limited impact. Coordinated action is required to minimize the emergence and spread of antimicrobial resistance (World Health Organization, 2018).

According to Prum, 2018, *G. creatinolyticus* has proven to be efficient in the decontamination of arsenic contaminated water, through the interaction between plants and bacteria (Prum et al., 2018). That is because it can tolerate higher levels of arsenic toxicity, as well as its capacity to produce higher levels of indol-3-acetic acid (IAA) and ammonia than *Bacillus subtilis* (Prum et al., 2018). In other works, *G. creatinolyticus* has proven to be a potent producer of extracellular urease for the determination of heavy metal ions (Ramesh et al., 2014) and been deployed as a potentiometric biosensor for the determination of urea content in milk, using mobilized urease (Ramesh et al., 2015). Heavy metals are very useful, due to being used in the manufacture of tools, such as pipes and batteries, but are also among the most well-known toxic substances. However, some bacteria isolated from various sources have demonstrated a capacity to survive in high concentration of toxic heavy metals (Castro-Silva et al., 2003; Pontes et al., 2007).

G. creatinolyticus has displayed genes that may help confer it resistance to heavy metals such as: arsenic, cobalt-zinc-cadmium (*cobT*, *cobS*, *cadA*), and chrome composts (locus tag GcLGCM259_2553 - Chromate efflux transporter ChrA), which are serious environmental contaminants. Species such as *Klebsiella pneumoniae* MS 1.5 and *Mangrovibacter yixingensis* strain MS 2.4 have displayed a high capacity to reduce chrome, and because of it, may be useful for the decontamination of chrome contaminated environments (Sanjay et al., 2018). The genome also displayed copper tolerance genes (*copZ*, *csoR_1*, *cutC*, *aniA*, *pcoC*), copper is an essential element amongst heavy metals,

which plays a major role in the growth and physiology of aerobic organisms, however, this metal can cause cellular death when in excess. In the species *Cupriavidus metallidurans* CH34, *Pseudomonas syringae* pv. *tomato* PT23*, Xanthomonas axonopodis*pv. *Vesicatoria*E3C5 and *Pseudomonas aeruginosa* PAO1, the *cop* genes have been identified (*copA*, *copB*, *copC* e *copD*) which confer copper resistance (Altimira et al., 2012). Some microorganisms are responsible for environmental transformations of the metal, and therefore may be useful as indicators for assays both in polluted and non-polluted environments (Castro-Silva et al., 2003; Trevors et al., 1985). This implies there may be biotechnological applications for metal resistant bacteria in the control of toxic metals in residual water treatment (Sanjay et al., 2018). These discoveries are relevant for the mining industry, as they pertain to bioremediation (Sanjay et al., 2018), however, additional studies are necessary, in order to better understand and explore this bacterium's characteristics and metabolic mechanisms.

In a previous study's regarding the identification of these species, authors report that there are difficulties in identifying these species of clinical samples by conventional biochemical tests because their reliability at the species level is limited (Yamamoto et al., 2017). Infections due to this species may have been underestimated because a correct identification of *G. creatinolyticus* is only possible by applying further identification methods (i.e., 16S rRNA gene sequencing or MALDI-TOF MS) (Funke et al., 1996; Yamamoto et al., 2017).

Finally, our results provide important new information about the genetic background of a strain of *G. creatinolyticus*. Comparative genomic analyses between the genome of *G. creatinolyticus* LGCM 259 and other genomes of *Glutamicibacter* and an *Arthrobacter* revealed the genetic mechanisms of its virulence, as well as genetic reductions, probably responsible for the adaptation to the host. Genomic islands displayed many genes vital to each species adaptation to their specific niche, such as oxidative stress, and transcriptional regulation. The genome also displays various genes which may be related to heavy metal resistance. However, more studies are needed to evaluate the importance and distribution of this species in its various habitats.

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.

Author Contributions

Wrote the manuscript (RS, NS, TC, LG, RH); designed the study (RS, NS, TC, AR, R.C and); experimental work (RS, NS, TC and LG.); conducted in silico analyses and interpreted the results (RS, RP, NS, RH); critically reviewed and revised the manuscript (NS, FR, CV, SJ, MG, AG, AG, RP, RH, and AR); supervised the study (NS, VA.). Funding (BB, VA and NS.). All authors approved this manuscript for publication.

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Additional information

Accession codes *G. creatinolyticus* LGCM 259 genome publicly deposited in GenBank under accession number CP034412.

References

Alikhan, N.-F., Petty, N. K., Ben Zakour, N. L., and Beatson, S. A. (2011). BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics* 12, 402. doi:10.1186/1471-2164-12-402.

Altimira, F., Yáñez, C., Bravo, G., González, M., Rojas, L. A., and Seeger, M. (2012). Characterization of copper-resistant bacteria and bacterial communities from copper-polluted agricultural soils of central Chile. *BMC Microbiol.* 12, 193. doi:10.1186/1471-2180-12-193.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. doi:10.1016/S0022-2836(05)80360-2.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., et al. (2018). Antibiotic resistance: a rundown of a global crisis. *Infect. Drug Resist.* 11, 1645–1658. doi:10.2147/IDR.S173867.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19, 455–77. doi:10.1089/cmb.2012.0021.

Barcellos, F. G., Menna, P., da Silva Batista, J. S., and Hungria, M. (2007). Evidence of horizontal transfer of symbiotic genes from a Bradyrhizobium japonicum inoculant strain to indigenous diazotrophs Sinorhizobium (Ensifer) fredii and Bradyrhizobium elkanii in a Brazilian Savannah soil. *Appl. Environ. Microbiol.* 73, 2635–43. doi:10.1128/AEM.01823-06.

Begg, S. L. (2019). The role of metal ions in the virulence and viability of bacterial pathogens. *Biochem. Soc. Trans.* 47, 77–87. doi:10.1042/BST20180275.

Bellanger, X., Payot, S., Leblond-Bourget, N., and Guédon, G. (2014). Conjugative and mobilizable genomic islands in bacteria: evolution and diversity. *FEMS Microbiol. Rev.* 38, 720–760. doi:10.1111/1574-6976.12058.

Bosi, E., Donati, B., Galardini, M., Brunetti, S., Sagot, M.-F., Lió, P., et al. (2015). MeDuSa: a multi-draft based scaffolder. *Bioinformatics* 31, 2443–2451. doi:10.1093/bioinformatics/btv171.

Busse, H.-J. (2016). Review of the taxonomy of the genus Arthrobacter, emendation of the genus Arthrobacter sensu lato, proposal to reclassify selected species of the genus Arthrobacter in the novel genera Glutamicibacter gen. nov., Paeniglutamicibacter gen. nov., Pseudoglutamicibacter gen. nov., Paenarthrobacter gen. nov. and Pseudarthrobacter gen. nov., and emended description of Arthrobacter roseus. *Int. J. Syst. Evol. Microbiol.* 66, 9–37. doi:10.1099/ijsem.0.000702.

Cai, L., Liu, G., Rensing, C., and Wang, G. (2009). Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. *BMC Microbiol.* 9, 4. doi:10.1186/1471-2180-9-4.

Castro-Silva, M. A., Lima, A. O. de S., Gerchenski, A. V., Jaques, D. B., Rodrigues, A. L., Souza, P. L. de, et al. (2003). Heavy metal resistance of microorganisms isolated from coal mining environments of Santa Catarina. *Brazilian J. Microbiol.* 34, 45–47. doi:10.1590/S1517-83822003000500015.

Chen, L., Yang, J., Yu, J., Yao, Z., Sun, L., Shen, Y., et al. (2004). VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res.* 33, D325–D328. doi:10.1093/nar/gki008.

Cross, A. S. (2008). What is a virulence factor? *Crit. Care* 12, 196. doi:10.1186/cc7127.

Darling, A. C. E., Mau, B., Blattner, F. R., and Perna, N. T. (2004). Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* 14, 1394–403. doi:10.1101/gr.2289704.

Dobrindt, U., and Hacker, J. (2001). Whole genome plasticity in pathogenic bacteria. *Curr. Opin. Microbiol.* 4, 550–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11587932 [Accessed April 24, 2019].

Edwards, A. N., Patterson-Fortin, L. M., Vakulskas, C. A., Mercante, J. W., Potrykus, K., Vinella, D., et al. (2011). Circuitry linking the Csr and stringent response global regulatory systems. *Mol. Microbiol.* 80, 1561–1580. doi:10.1111/j.1365-2958.2011.07663.x.

Egas, C., Barroso, C., Froufe, H. J. C., Pacheco, J., Albuquerque, L., and da Costa, M. S. (2015). Complete genome sequence of the radiation-resistant bacterium Rubrobacter radiotolerans RSPS-4. *Stand. Genomic Sci.* 9, 1062–1075. doi:10.4056/sigs.5661021.

Feng, W.-W., Wang, T.-T., Bai, J.-L., Ding, P., Xing, K., Jiang, J.-H., et al. (2017). Glutamicibacter halophytocola sp. nov., an endophytic actinomycete isolated from the roots of a coastal halophyte, Limonium sinense. *Int. J. Syst. Evol. Microbiol.* 67, 1120–1125. doi:10.1099/ijsem.0.001775.

Fletcher, J. I., Williams, R. T., Henderson, M. J., Norris, M. D., and Haber, M. (2016). ABC transporters as mediators of drug resistance and contributors to cancer cell biology. *Drug Resist. Updat.* 26, 1–9. doi:10.1016/j.drup.2016.03.001.

Funke, G., Hutson, R. A., Bernard, K. A., Pfyffer, G. E., Wauters, G., and Collins, M. D. (1996). Isolation of Arthrobacter spp. from clinical specimens and description of Arthrobacter cumminsii sp. nov. and Arthrobacter woluwensis sp. nov. *J. Clin. Microbiol.* 34, 2356–63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8880479 [Accessed April 24, 2019].

Gal-Mor, O., and Finlay, B. B. (2006). Pathogenicity islands: a molecular toolbox for bacterial virulence. *Cell. Microbiol.* 8, 1707–1719. doi:10.1111/j.1462-5822.2006.00794.x.

Gold, B., Rodriguez, G. M., Marras, S. A., Pentecost, M., and Smith, I. (2001). The Mycobacterium tuberculosis IdeR is a dual functional regulator that controls transcription of genes involved in iron acquisition, iron storage and survival in macrophages. *Mol. Microbiol.* 42, 851–65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11722747 [Accessed February 15, 2019].

Hou, X.-G., Kawamura, Y., Sultana, F., Shu, S., Hirose, K., Goto, K., et al. (1998). Description of Arthrobacter creatinolyticus sp. nov., isolated from human urine. *Int. J. Syst. Bacteriol.* 48, 423–429. doi:10.1099/00207713-48-2-423.

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.

Krasensky, J., and Jonak, C. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.* 63, 1593–608. doi:10.1093/jxb/err460.

Krizova, L., and Nemec, A. (2010). A 63 kb genomic resistance island found in a multidrug-resistant Acinetobacter baumannii isolate of European clone I from 1977. *J. Antimicrob. Chemother.* 65, 1915–1918. doi:10.1093/jac/dkq223.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi:10.1093/molbev/msy096.

Lakin, S. M., Dean, C., Noyes, N. R., Dettenwanger, A., Ross, A. S., Doster, E., et al. (2017). MEGARes: an antimicrobial resistance database for high throughput sequencing. *Nucleic Acids Res.* 45, D574–D580. doi:10.1093/nar/gkw1009.

Letunic, I., and Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* 44, W242-5. doi:10.1093/nar/gkw290.

Li, C.-T., Liao, C.-T., Du, S.-C., Hsiao, Y.-P., Lo, H.-H., and Hsiao, Y.-M. (2014). Functional characterization and transcriptional analysis of galE gene encoding a UDP-galactose 4-epimerase in Xanthomonas campestris pv. campestris. *Microbiol. Res.* 169, 441–452. doi:10.1016/J.MICRES.2013.08.005.

Lindahl, E., and Elofsson, A. (2000). Identification of related proteins on family, superfamily and fold level 1 1Edited by F. C. Cohen. *J. Mol. Biol.* 295, 613–625. doi:10.1006/jmbi.1999.3377.

Maguire, M., Coates, A. R. M., and Henderson, B. (2002). Chaperonin 60 unfolds its secrets of cellular communication. *Cell Stress Chaperones* 7, 317–29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12653476 [Accessed February 15, 2019].

Monnet, C., Loux, V., Gibrat, J.-F., Spinnler, E., Barbe, V., Vacherie, B., et al. (2010). The Arthrobacter arilaitensis Re117 Genome Sequence Reveals Its Genetic Adaptation to the Surface of Cheese. *PLoS One* 5, e15489. doi:10.1371/journal.pone.0015489.

Moreira, M. A. S., Souza, E. C. de, and Moraes, C. A. de (2004). Multidrug efflux systems in Gram-negative bacteria. *Brazilian J. Microbiol.* 35, 19–28. doi:10.1590/S1517-83822004000100003.

Muñoz-Elías, E. J., and McKinney, J. D. (2005). Mycobacterium tuberculosis isocitrate lyases 1 and 2 are jointly required for in vivo growth and virulence. *Nat. Med.* 11, 638–44. doi:10.1038/nm1252.

Pacheco, L. G. C., Pena, R. R., Castro, T. L. P., Dorella, F. A., Bahia, R. C., Carminati, R., et al. (2007). Multiplex PCR assay for identification of Corynebacterium pseudotuberculosis from pure cultures and for rapid detection of this pathogen in clinical samples. *J. Med. Microbiol.* 56, 480–486. doi:10.1099/jmm.0.46997-0.

Pontes, D. S., Lima-Bittencourt, C. I., Chartone-Souza, E., and Amaral Nascimento, A. M. (2007). Molecular approaches: advantages and artifacts in assessing bacterial diversity. *J. Ind. Microbiol. Biotechnol.* 34, 463–473. doi:10.1007/s10295-007-0219-3.

Prum, C., Dolphen, R., and Thiravetyan, P. (2018). Enhancing arsenic removal from arsenic-contaminated water by Echinodorus cordifolius−endophytic Arthrobacter creatinolyticus interactions. *J. Environ. Manage.* 213, 11–19. doi:10.1016/J.JENVMAN.2018.02.060.

Ramesh, R., Aarthy, M., Gowthaman, M. K., Gabrovska, K., Godjevargova, T., and Kamini, N. R. (2014). Screening and production of a potent extracellular *Arthrobacter creatinolyticus* urease for determination of heavy metal ions. *J. Basic Microbiol.* 54, 285–295. doi:10.1002/jobm.201200561.

Ramesh, R., Puhazhendi, P., Kumar, J., Gowthaman, M. K., D'Souza, S. F., and Kamini, N. R. (2015). Potentiometric biosensor for determination of urea in milk using immobilized Arthrobacter creatinolyticus urease. *Mater. Sci. Eng. C* 49, 786–792. doi:10.1016/j.msec.2015.01.048.

Richter, M., Rosselló-Móra, R., Oliver Glöckner, F., and Peplies, J. (2016). JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32, 929–931. doi:10.1093/bioinformatics/btv681.

Sanjay, M. S., Sudarsanam, D., Raj, G. A., and Baskar, K. (2018). Isolation and identification of chromium reducing bacteria from tannery effluent. *J. King Saud Univ. - Sci.* doi:10.1016/J.JKSUS.2018.05.001.

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068–2069. doi:10.1093/bioinformatics/btu153.

Shen, Z., Han, J., Wang, Y., Sahin, O., and Zhang, Q. (2013). The Contribution of ArsB to Arsenic Resistance in Campylobacter jejuni. *PLoS One* 8, e58894. doi:10.1371/journal.pone.0058894.

Soares, S. C., Geyik, H., Ramos, R. T. J., de Sá, P. H. C. G., Barbosa, E. G. V., Baumbach, J., et al. (2016). GIPSy: Genomic island prediction software. *J. Biotechnol.* 232, 2–11. doi:10.1016/j.jbiotec.2015.09.008.

Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig W, and Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int. J. Syst. Evol. Microbiol.* 60, 249–266. doi:10.1099/ijs.0.016949-0.

Toft, C., and Andersson, S. G. E. (2010). Evolutionary microbial genomics: insights into bacterial host adaptation. *Nat. Rev. Genet.* 11, 465–475. doi:10.1038/nrg2798.

Trevors, J. T., Oddie, K. M., and Belliveau, B. H. (1985). Metal resistance in bacteria. *FEMS*

Microbiol. Lett. 32, 39–54. doi:10.1111/j.1574-6968.1985.tb01181.x.

Tumapa, S., Holden, M. T., Vesaratchavest, M., Wuthiekanun, V., Limmathurotsakul, D., Chierakul, W., et al. (2008). Burkholderia pseudomallei genome plasticity associated with genomic island variation. *BMC Genomics* 9, 190. doi:10.1186/1471-2164-9-190.

Unal, C. M., and Steinert, M. (2014). Microbial Peptidyl-Prolyl cis/trans Isomerases (PPIases): Virulence Factors and Potential Alternative Drug Targets. *Microbiol. Mol. Biol. Rev.* 78, 544–571. doi:10.1128/MMBR.00015-14.

Vorstenbosch, E. L., Potapov, A. P., de Graaf, J. M., and Kraal, B. (2000). The effect of mutations in EF-Tu on its affinity for tRNA as measured by two novel and independent methods of general applicability. *J. Biochem. Biophys. Methods* 42, 1–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10647810 [Accessed April 25, 2019].

Wall, D. M., Duffy, P. S., Dupont, C., Prescott, J. F., and Meijer, W. G. (2005a). Isocitrate lyase activity is required for virulence of the intracellular pathogen Rhodococcus equi. *Infect. Immun.* 73, 6736–41. doi:10.1128/IAI.73.10.6736-6741.2005.

Wall, D. M., Duffy, P. S., Dupont, C., Prescott, J. F., and Meijer, W. G. (2005b). Isocitrate lyase activity is required for virulence of the intracellular pathogen Rhodococcus equi. *Infect. Immun.* 73, 6736–41. doi:10.1128/IAI.73.10.6736-6741.2005.

Wang, Y., Wang, C., Li, A., and Gao, J. (2015). Biodegradation of pentachloronitrobenzene by *Arthrobacter nicotianae* DH19. *Lett. Appl. Microbiol.* 61, 403–410. doi:10.1111/lam.12476.

Wattam, A. R., Abraham, D., Dalay, O., Disz, T. L., Driscoll, T., Gabbard, J. L., et al. (2014). PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res.* 42, D581-91. doi:10.1093/nar/gkt1099.

Yamamoto, K., Hayakawa, K., Nagamatsu, M., Fujiya, Y., Mawatari, M., Kutsuna, S., et al. (2017). Bacteremia Due to <i>Arthrobacter creatinolyticus</i> in an Elderly Diabetic Man with Acute Cholangitis. *Jpn. J. Infect. Dis.* 70, 201–202. doi:10.7883/yoken.JJID.2016.033.

Yamamoto, K., Hirao, K., Oshima, T., Aiba, H., Utsumi, R., and Ishihama, A. (2005).

Functional characterization in vitro of all two-component signal transduction systems from Escherichia coli. *J. Biol. Chem.* 280, 1448–56. doi:10.1074/jbc.M410104200.

Yang, A.-S., and Honig, B. (2000). An integrated approach to the analysis and modeling of protein sequences and structures. II. On the relationship between sequence and structural similarity for proteins that are not obviously related in sequence 1 1Edited by F. Cohen. *J. Mol. Biol.* 301, 679–689. doi:10.1006/jmbi.2000.3974.

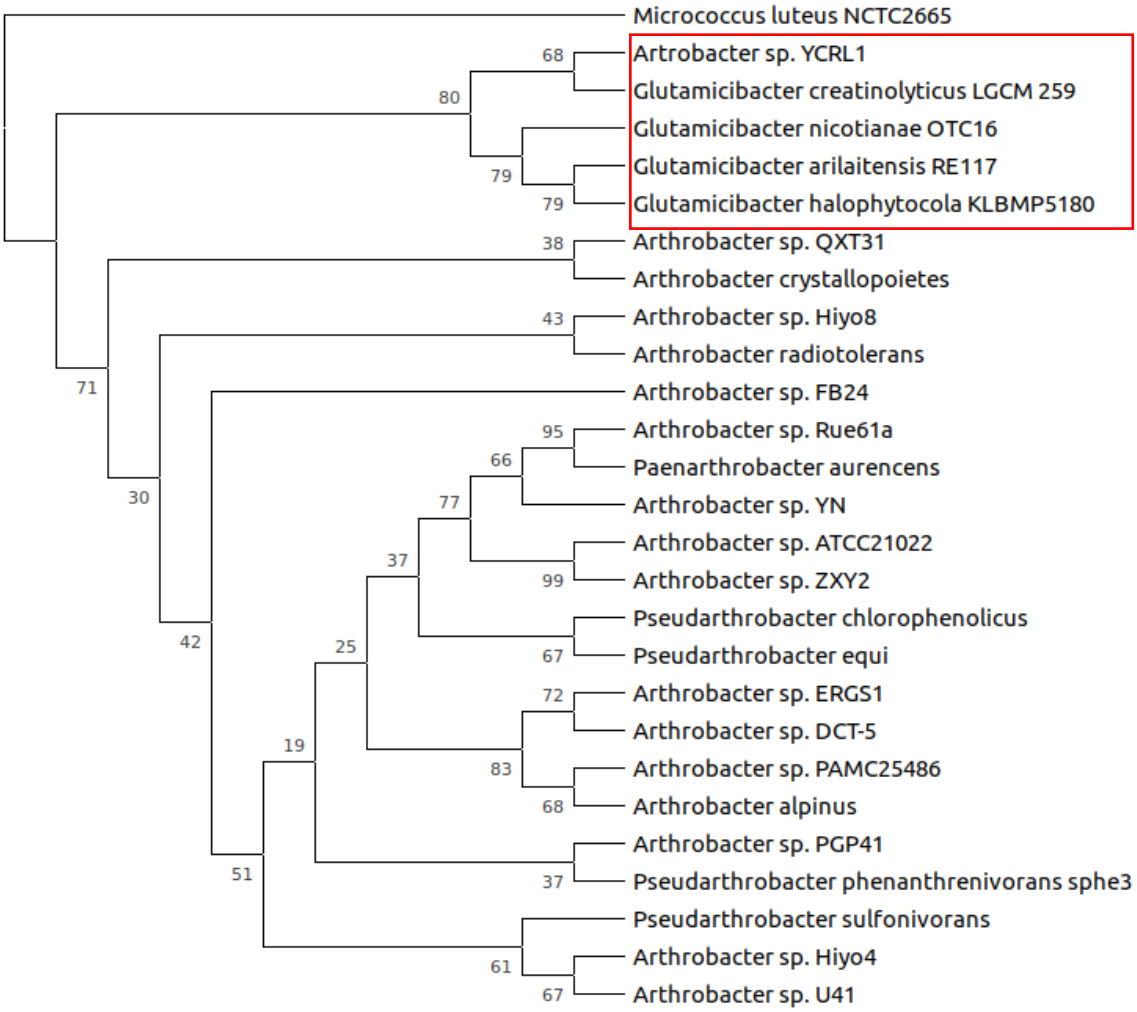
Yao, Y., Tang, H., Su, F., and Xu, P. (2015). Comparative genome analysis reveals the molecular basis of nicotine degradation and survival capacities of Arthrobacter. *Sci. Rep.* 5, 8642. doi:10.1038/SREP08642.

Zhang, H., Wang, R., Chen, S., Qi, G., He, Z., and Zhao, X. (2017). Microbial taxa and functional genes shift in degraded soil with bacterial wilt. *Sci. Rep.* 7, 39911. doi:10.1038/srep39911.

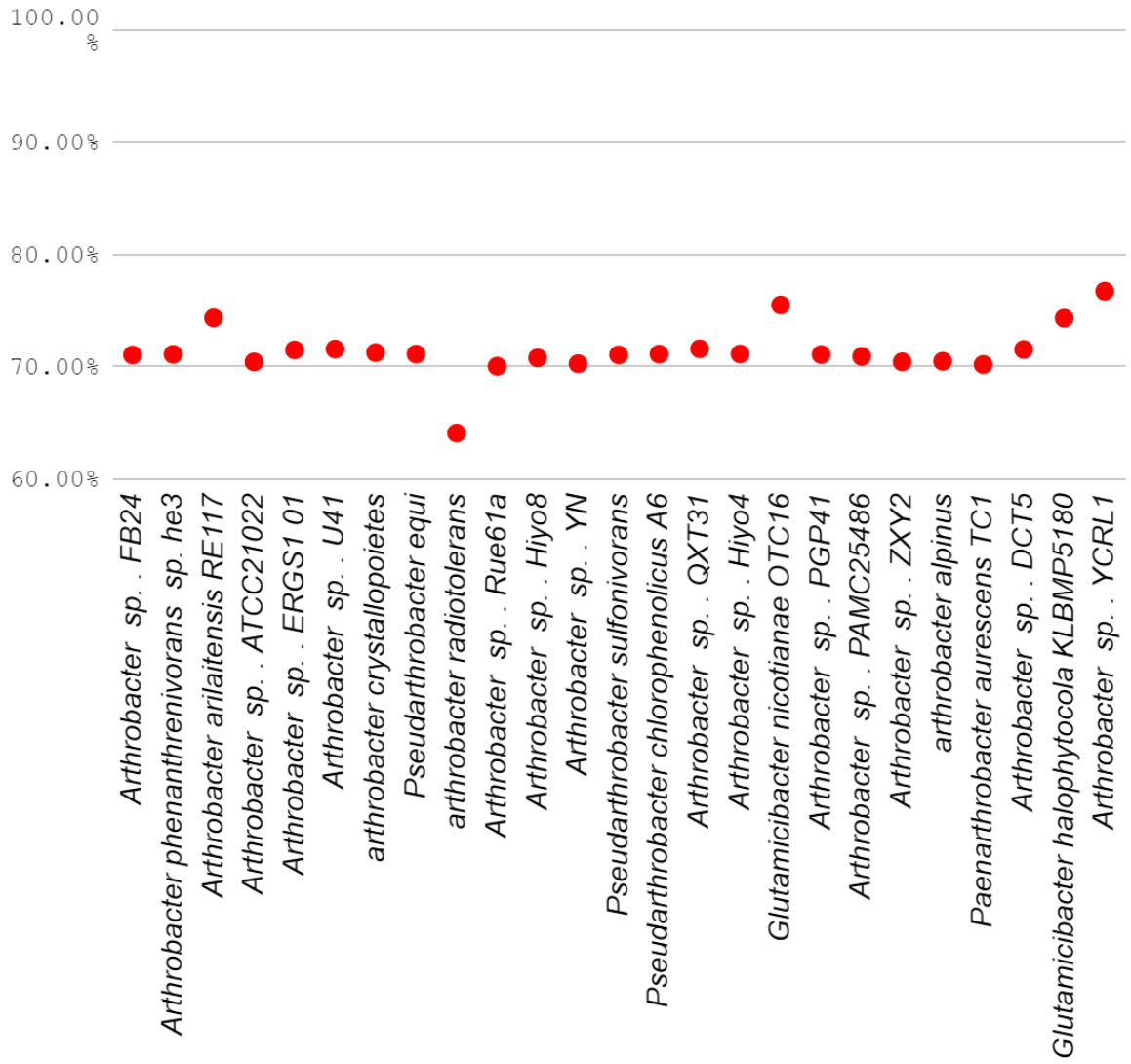
5. MATERIAL SUPLEMENTAR

Supplementary Figures and Tables

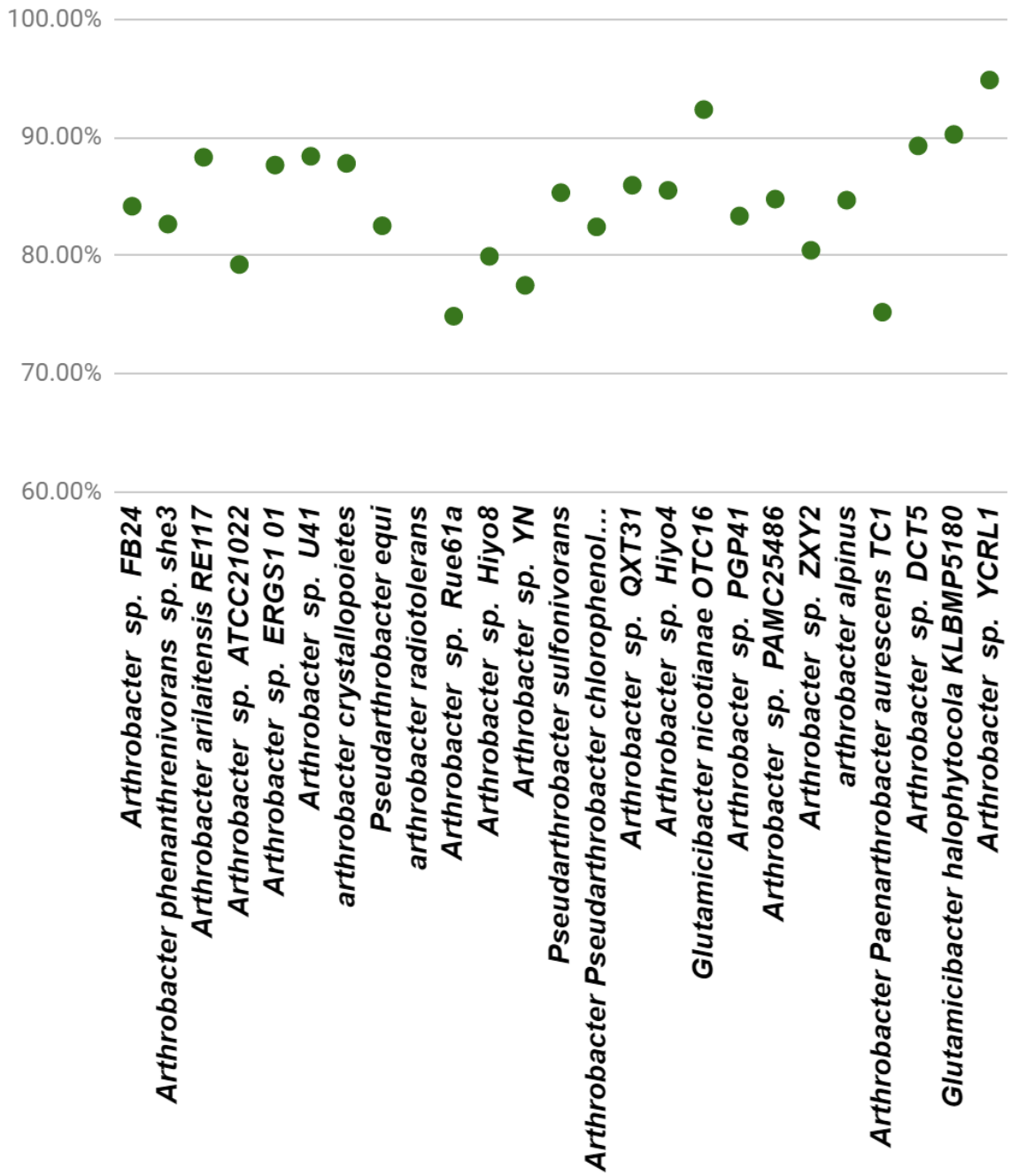
Supplementary Figures



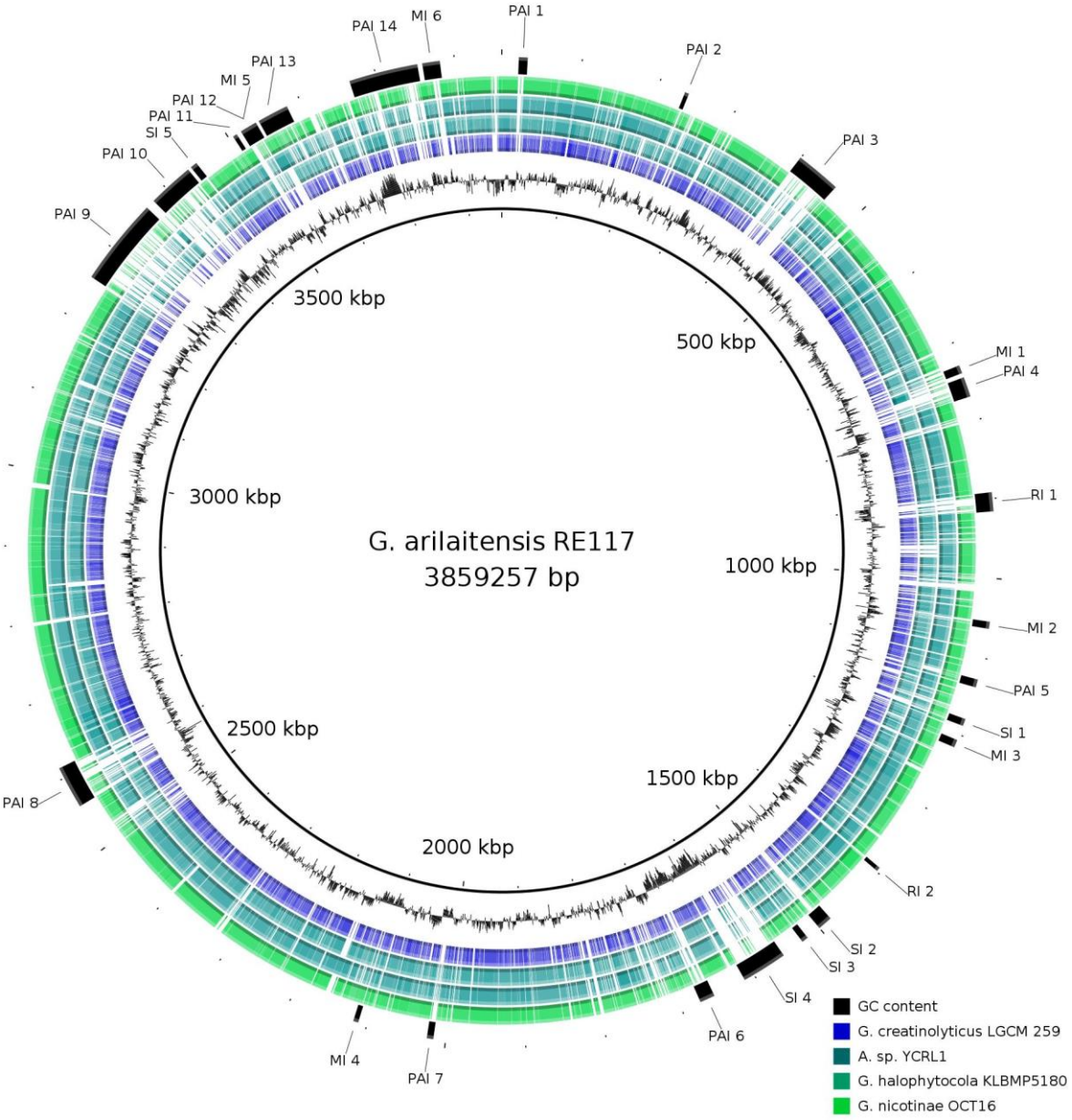
Supplementary Figure 1: The tree based on 16S (1224 nt) sequences was constructed using MEGA X Phylogenetic maximum - likelihood method. The values indicate the percentages of occurrence in 1000 bootstrapped tree. The 16S sequence of *Micrococcus luteus* NCTC 2665 was used as an outgroup. In red, the position of *G. creatinolyticus* LGCM 259, along with the *Glutamicibacter* genus, is highlighted.



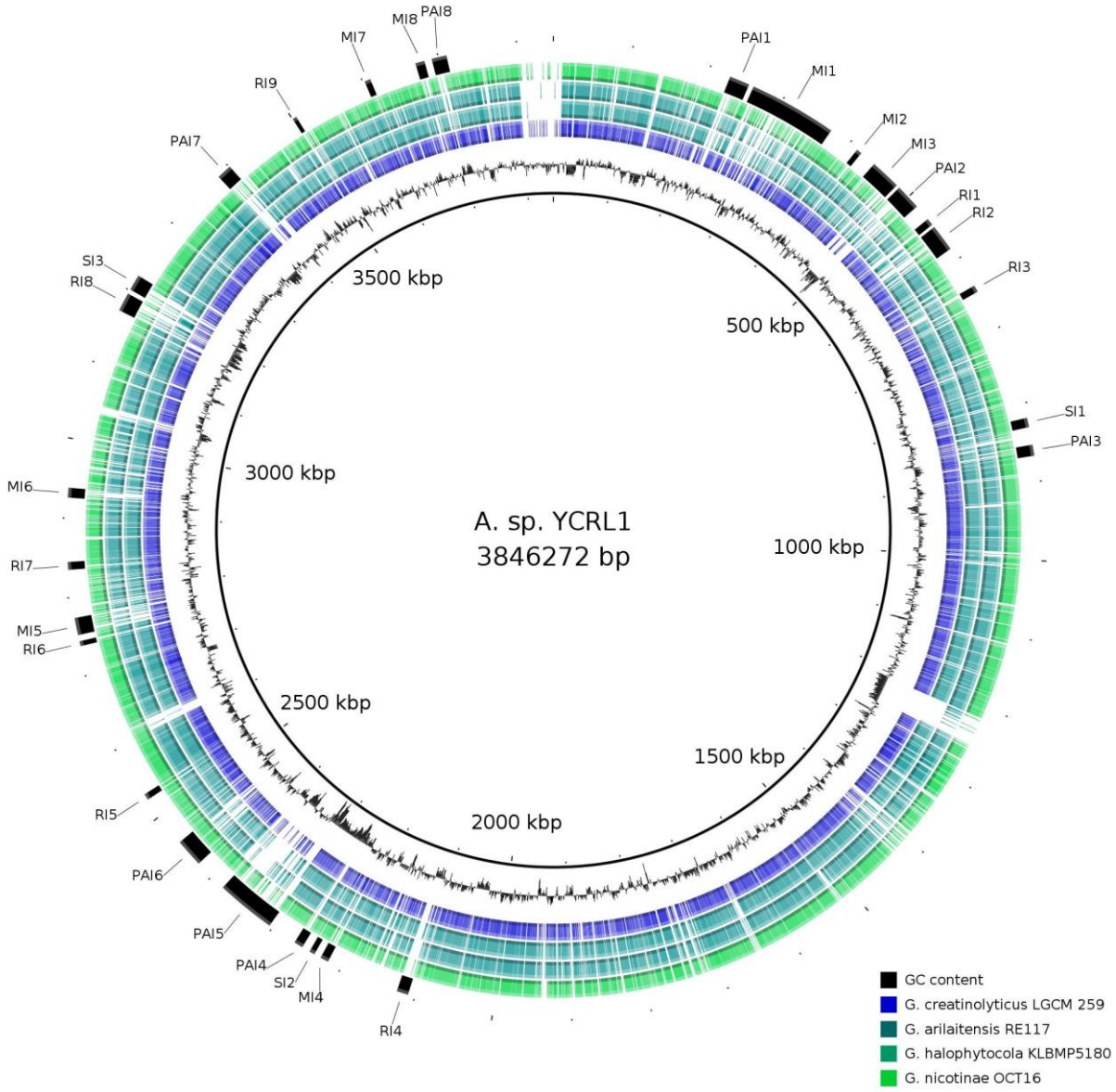
Supplementary figure 2: ANIb (%) of the 25 genomes selected from NCBI with *G. creatinolyticus* LGCM 259 as reference. The threshold frontier to consider two organisms to belong to the same species could be set at >95% identity for ANI. Suggesting that no strain belongs to the same species.



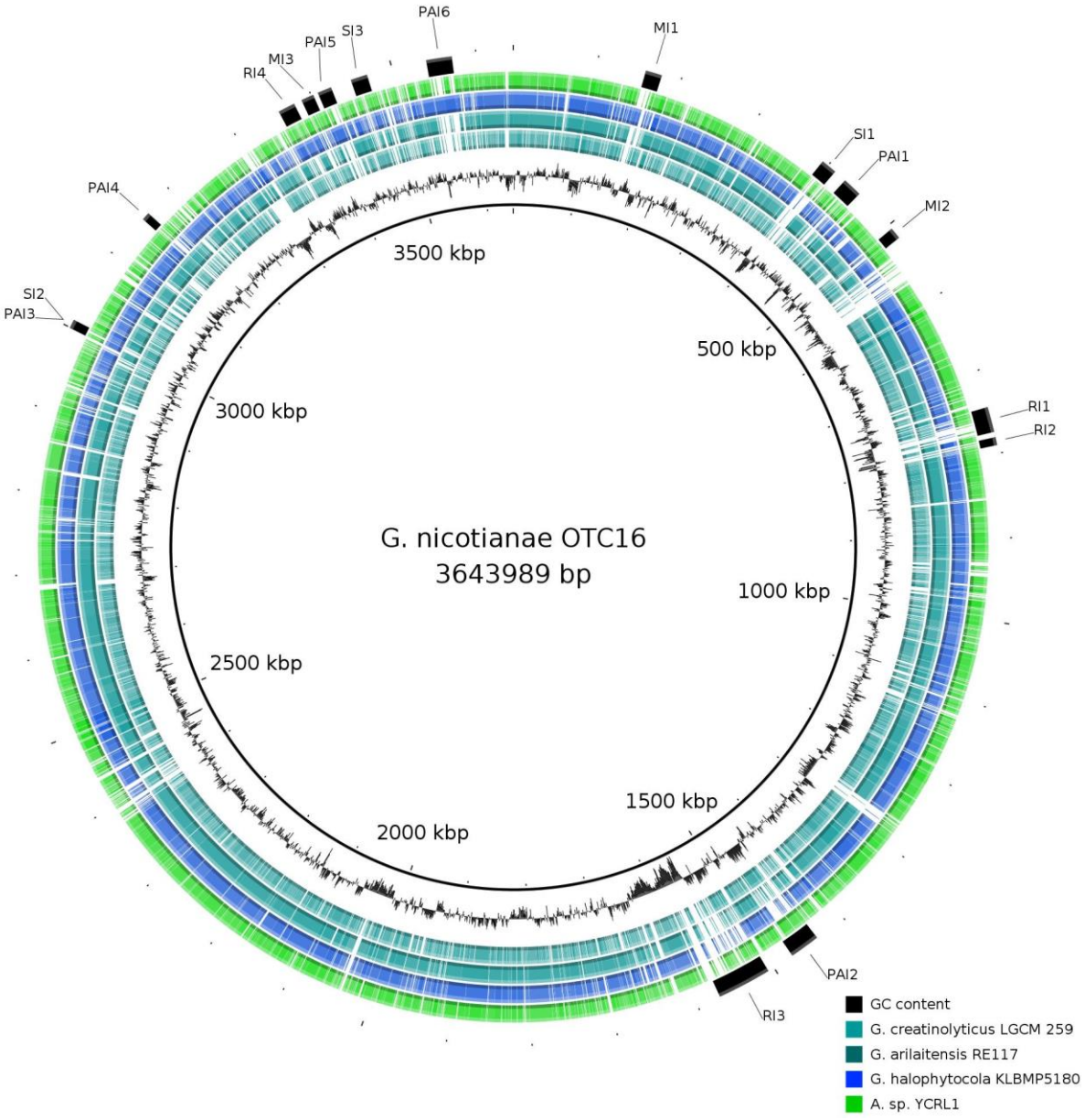
Supplementary figure 3: Tetra nucleotide (%) of the 25 genomes selected from NCBI with *G. creatinolyticus* LGCM 259 as reference. The threshold frontier to consider two organisms to belong to the same species could be set at >99% for Tetra. Suggesting that no strain belongs to the same species.



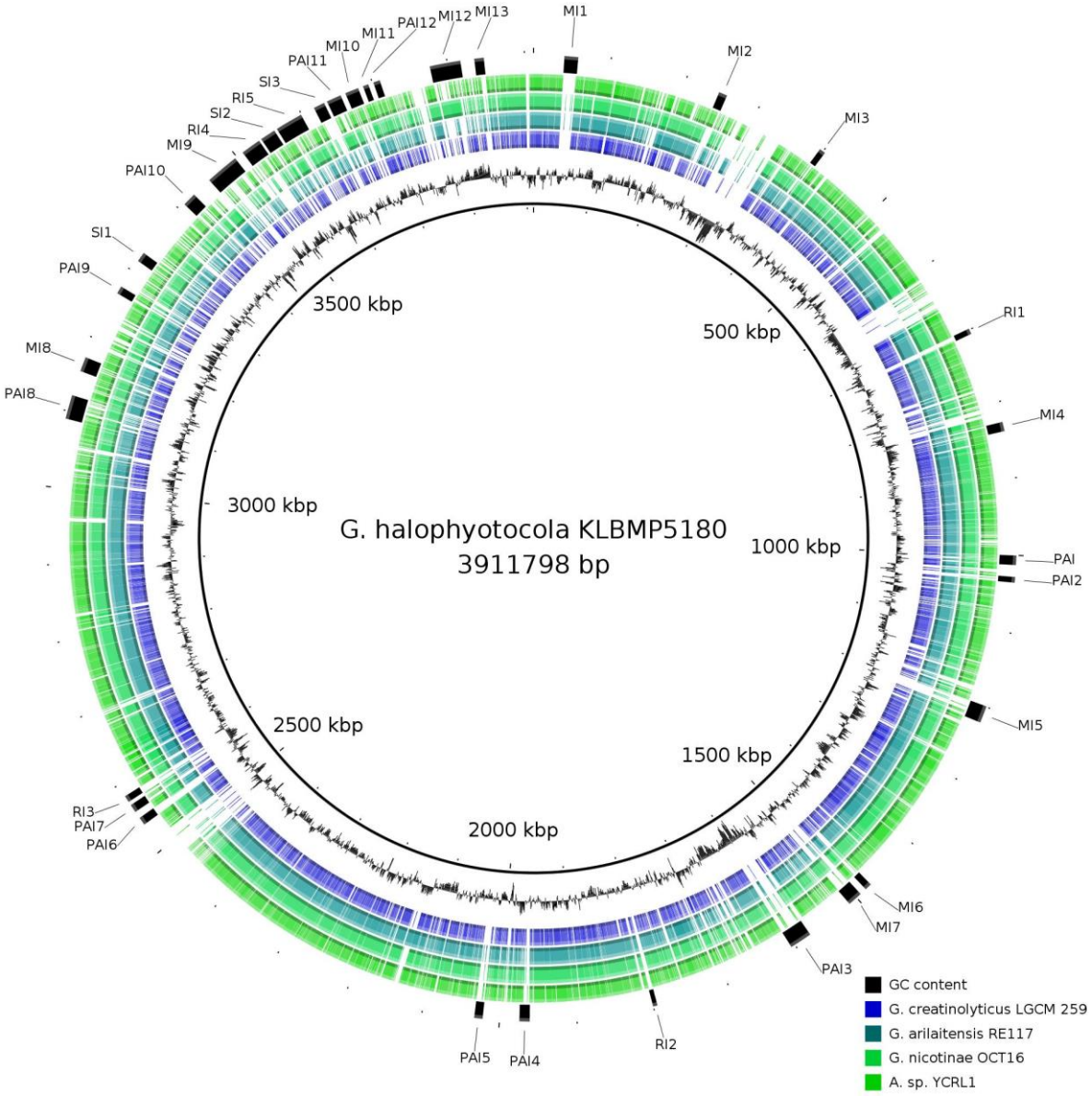
Supplementary figure 4: Circular genome map representing all genomic islands found in the *G. arilaitensis* RE117 genome. Representation, from the inner to the outer circle, consist: *G. arilaitensis* Re117, GC Content, *G. creatinolyticus* LGCM 259, A. *sp.* YCRL1, *G. hlophytocola* KLBM5180 and *G.nicotinae* OTC16. Pathogenicity islands (PAIs); Metabolic islands (MIs), Resistance islands (RIs), and symbiotic islands (SIs).



Supplementary figure 5: Circular genome map representing all genomic islands found in the *A. sp.* YCRL1 genome. Representation, from the inner to the outer circle, consist: *A. sp.* YCRL1, GC Content, *G. creatinolyticus* LGCM 259, *G. arilaitensis*Re117, *G. hlophytocola* KLBM5180 and *G. nicotinae* OTC16. Pathogenicity islands (PAIs); Metabolic islands (MIs), Resistance islands (RIs), and symbiotic islands (SIs).



Supplementary figure 6: Circular genome map representing all genomic islands found in the *G.nicotinae* OTC16 genome. Representation, from the inner to the outer circle, consist: *G.nicotinae* OTC16, GC Content, *G. creatinolyticus* LGCM 259, *G. arilaitensis* Re117, *G. hlophytocola* KLBM5180 and *A. sp.* YCRL1. Pathogenicity islands (PAIs); Metabolic islands (MIs), Resistance islands (RIs), and symbiotic islands (SIs).



Supplementary figure 7: Circular genome map representing all genomic islands found in the *G. hlophytocola* KLBM5180 genome. Representation, from the inner to the outer circle, consist: *G. hlophytocola* KLBM5180, GC Content, G. *creatinolyticus* LGCM 259, *G. arilaitensis* Re117, *G. nicotinae OTC16* and *A. sp. YCRL1*. Pathogenicity islands (PAIs); Metabolic islands (MIs), Resistance islands (RIs), and symbiotic islands (SIs).

Supplementary Tables

Supplementary table 1. Functional characterization of genome sequences of G. creatinolyticus LGCM 259 connected with Kyoto Encyclopedia of Genes and Genomes (KEGG) using BLASTKOALA.

KEGG Category	Percentage (%)
Global and Overview Maps	50
Carbohydrate metabolism	11.7
Protein families: genetic information processing	11.63
Genetic Information Processing	10.83
Protein families: signaling and cellular processes	10.42
Amino acid metabolism	8.94
Environmental Information Processing	6.99
Unclassified: metabolism	5.51
Metabolism of cofactors and vitamins	5.45
Unclassified	5.38
Nucleotide metabolism	4.91
Energy metabolism	4.37
Cellular Processes	2.69
Lipid metabolism	2.62
Protein families: metabolism	2.15
Unclassified: signaling and cellular processes	1.68
Metabolism of other amino acids	1.55
Unclassified: genetic information processing	1.08
Glycan biosynthesis and metabolism	1.08
Metabolism of terpenoids and polyketides	0.87
Xenobiotics biodegradation and metabolism	0.61
Organismal Systems	0.34
Human Diseases	0.27

Supplementary table 2: Virulence factor from *G. creatinolyticus LGCM 259* found in VFDB database.

VFDB prediction	**Query ID**	**Subject ID**	**%ID**	**%Query coverage**
VFG001381(gb|YP_177728) (icl) Isocitrate lyase	GcLGCM259_0286	VFG001381(gb|YP_177728) [Mycobacterium tuberculosis H37Rv]	78.571	96
VFG000079(gb|NP_463763) (clpC) endopeptidase Clp	GcLGCM259_0100	VFG000079(gb|NP_463763) [Listeria monocytogenes EGD-e]	60.843	95
VFG001386(gb|NP_215271) (phoP) Possible two component system response transcriptional	GcLGCM259_0460	VFG001386(gb|NP_215271) [Mycobacterium tuberculosis H37Rv]	60.00	97
VFG001826(gb|NP_217099) (relA) Probable GTP pyrophosphokinase RelA	GcLGCM259_1510	VFG001826(gb|NP_217099) [Mycobacterium tuberculosis H37Rv]	59.754	97
VFG001406(gb|NP_217227) (ideR) Iron-dependent repressor and activator	GcLGCM259_0465	VFG001406(gb|NP_217227) [Mycobacterium tuberculosis H37Rv]	58.291	85

VFG013286(gb|NP_438515) (galE) UDP-glucose 4-epimerase	GcLGCM259_1204	VFG013286(gb|NP_438515) [Haemophilus influenzae Rd KW20]	57.143	91
VFG001855(gb|YP_094724) (htpB) Hsp60, 60K heat shock protein	GcLGCM259_2021	VFG001855(gb|YP_094724) [Legionella pneumophila subsp. pneumophila str. Philadelphia 1]	54.237	99
VFG000925(gb|NP_752606) (fepC) ferrienterobactin ABC transporter	GcLGCM259_0734	VFG000925(gb|NP_752606) [Escherichia coli CFT073]	53.668	73
VFG048830(gb|YP_002920353.1) (gnd) 6-phosphogluconate dehydrogenase [capsule (VF0560)]	GcLGCM259_1336	VFG048830(gb|YP_002920353.1) [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	53.664	96
VFG041304(gb|YP_095978) (lirB) Dot/Icm type IV secretion system effector LirB	GcLGCM259_0015	VFG041304(gb|YP_095978) [Legionella pneumophila subsp. pneumophila str. Philadelphia 1]	52.047	90

Supplementary table 3: General information of the number of genomic islands predicted in the 5 genomes of genus *Glutamicibacter*.

STRAINS	GEIs
Glutamicibacter creatinolyticus LGCM 259	23
Arthrobacter sp. YCRL1	28
Glutamicibacter arilaitensis RE117	27
Glutamicibacter halophytocola KLBMP5180	33
Glutamicibacter nicotianae OTC16	16

Supplementary table 4: Description and comparison of predicted Genomic Island of each GEI of *G. creatinolyticus* LGCM 259. The GEIs of blue color are unique to the genome. The GEIs of black color are islands partially shared with the other genomes. Pident: Percent identity

Reference GEIs	Start	End	Size (bp)	CDS	G + C content (%)	*A. sp. YCRL1 (pident)*	*G. arilaitensis RE117 (pident)*	*G. halophytocola KLBMP5180 (pident)*	*G. nicotianae OTC16 (pident)*
PAI1	251445	263403	11958	11	57.35	-	PAI10 (85.94%)	-	PAI4 (92.15%)
PAI2	651564	658580	7016	6	62.61	-	-	-	-
PAI3	713872	723020	9148	10	63.09	-	-	-	-
PAI4	935666	950270	14604	14	64.89	-	-	-	-
PAI5	1240581	1251390	10809	10	63.02	-	-	-	-
PAI6	1302109	1315487	13378	11	62.78	-	-	-	-
PAI7	1498070	1521457	23387	20	62.99	-	MI3 (100.00%)	-	-
PAI8	2222517	2259175	36658	33	62.08	MI1 (78.82%)	-	-	-
PAI9	2292345	2302836	10491	13	58.11	SI3 (88.98%)	-	-	-

PAI10	2654531	2663734	9203	10	64.48	-	PAI9 (86.72%)	-	-
PAI11	2730539	2760868	30329	34	63.89	-	-	MI8 (82.97%)	RI1 (77.90%)
PAI12	2817223	2840919	23696	23	69.59	-	-	-	-
PAI13	2911553	2952087	40534	35	64.16	RI2 (79.10%)	-	-	PAI1 (92.30%)
PAI14	2955902	2983064	27162	24	61.19	PAI2 (74.71%) RI1 (80.60%)	PAI13 (74.57%)	PAI10 (86.45%)	MI3 (73.66%)
PAI15	3214956	3232451	17495	19	66.38	PAI3 (81.20%)	-	-	-
RI1	65306	76712	11406	12	70.08	-	-	-	-
RI2	2673211	2678370	5159	8	61.67	-	-	-	-
RI3	3277658	3284318	6660	5	58.84	MI1 (82.70%)	PAI10 (85.77%) PAI14 (84.44%)	-	-
RI4	3095953	3111607	15654	25	64.48	-	-	-	-
MI1	3193131	3205763	12632	15	65.71	-	-	-	-
MI2	457021	480448	23427	10	62.57	PAI8 (97.04%)	PAI13 (82.37%)	-	RI3 (92.50%)
SI1	1432230	1463879	31649	27	61.51	MI4 (76.61%)	PAI12 (81.05%) MI5 (81.05%)	-	-
SI2	304738	318982	14244	11	56.94	-	-	-	-

Supplementary table 5: Description and comparison of predicted Genomic Island of each GEI of *G. arilaitensis* RE117. The GEIs of blue color are unique to the genome. The GEIs of black color are islands partially shared with the other genomes. The GEIs of red color are shared among all 5 genomes. Pident: Percent identity

Reference GEIs	Start	End	Size (bp)	CDS	G + C content (%)	*G. creatinolyticus LGCM 259 (pident)*	*A. sp. YCRL1 (pident)*	*G. halophytocola KLBMP5180 (pident)*	*G. nicotianae OTC16 (pident)*
PAI 1	21170	32323	11153	16	59.58	-	-	-	-
PAI 2	233851	238986	5135	6	55.53	-	PAI5 (79.19%) PAI2 (79.19%)	-	-
PAI 3	399421	456750	57329	56	61.18	-	-	-	-
PAI 4	744635	769767	25132	28	58.25	-	RI8 (98.59%)	-	-
PAI 5	1127478	1137567	10089	9	58.91	-	RI7 (82.24%)	-	-
PAI 6	1655915	1674902	18987	17	52.88	-	PAI2 (98.62%) PAI4 (85.12%)	PAI3 (92.94%)	-
PAI 7	2015238	2023496	8258	10	54.91	-	-	-	-
PAI 8	2559811	2613522	53711	14	55.98	-	PAI5 (88.34%) PAI2 (81.87%)	-	PAI4 (98.16%)
PAI 9	3255381	3366084	110703	109	57.34	PAI10 (86.72%)	RI8 (90.67%) PAI2 (89.47%)	-	RI2 (86.42%)
PAI 10	3377834	3435884	58050	64	57.53	RI3 (85.77%)	PAI2 (99.04%) RI8 (84.24%)	-	SI1 (90.36%)
PAI 11	3505349	3510736	5387	8	62.66	-	-	-	-
PAI 12	3517107	3538442	21335	21	56.78	-	-	-	-
PAI 13	3542620	3582146	39526	41	59.55	MI2 (82.37%)	MI1 (80.26%)	SI2 (82.78%)	RI3 (79.97%)

PAI 14	3666297	3753374	87077	67	63.69	RI3 (84.44%)	MI1 (76.68%)	PAI12 (84.05%)	PAI4 (80.67%) MI3 (100.00%)
RI 1	892062	915999	23937	18	53.74	-	PAI2 (98.96%)	-	PAI4 (98.22%)
RI 2	1393740	1398869	5129	8	59.21	-	-	-	-
MI 1	728766	739016	10250	13	63.55	-	SI3 (86.84%)	-	RI1 (90.90%)
MI 2	1053976	1062833	8857	11	62.14	-	-	-	-
MI 3	1207000	1216860	9860	11	64.31	PAI7 (100.00%)	-	-	-
MI 4	2111154	2117530	6376	8	55.32	-	-	-	-
MI 5	3517107	3538442	21335	21	56.78	SI1 (81.05%)	-	-	-
MI 6	3759558	3781727	22169	20	62	-	PAI1 (81.84%)	MI13 (75.11%)	-
SI 1	1179030	1188017	8987	10	56	-	-	-	-
SI 2	1481558	1499903	18345	13	55.79	-	PAI2 (78.13%)	-	MI2 (83.54%)
SI 3	1518902	1527042	8140	8	57.78	-	PAI6 (78.17%)	-	PAI2 (94.50%)
SI 4	1555969	1613291	57322	43	54.23	-	PAI5 (87.23%)	PAI3 (84.27%)	RI3 (89.47%)
SI 5	3440264	3448942	8678	12	57.52	-	-	-	-

Supplementary table 6: Description and comparison of predicted Genomic Island of each GEI of *A. sp.* YCRL1. The GEIs of blue color are unique to the genome. The GEIs of black color are islands partially shared with the other genomes. The GEIs of red color are shared among all 5 genomes. Pident: Percent identity.

Reference GEIs	**Start**	**End**	**Size (bp)**	**CDS**	**G + C content (%)**	***G. creatinolyticus LGCM 259 (pident)***	***G. arilaitensis RE117 (pident)***	***G. halophytocola KLBMP5180 (pident)***	***G. nicotianae OTC16 (pident)***

PAI1	227567	253411	25844	21	64.65	-	MI6 (81.84%)	-	-
PAI2	483678	516145	32467	27	60.38	PAI14 (77.59%)	PAI10 (99.04%) PAI9 (79.69%)	-	PAI4 (81.43%)
PAI3	853362	867444	14082	15	66.66	PAI15 (81.20%)	-	-	-
PAI4	2254934	2266425	11491	10	50.4	-	PAI6 (85.04%)	-	-
PAI5	2304363	2380169	75806	46	58.66	-	PAI8 (88.29%)	PAI3 (81.01%)	RI3 (78.76%)
PAI6	2422886	2458879	35993	42	66.77	-	SI3 (78.19%)	-	PAI2 (81.31%)
PAI7	3381911	3401053	19142	27	62.04	-	-	-	-
PAI8	3691482	3710573	19091	10	69.11	MI2 (97.04%) PAI14 (95.58%)	-	-	RI3 (86.55%)
RI1	537016	546087	9071	10	61.09	-	-	PAI10 (83.74%)	-
RI2	549929	584224	34295	33	65.96	PAI13 (79.09%)	-	PAI9 (74.81%)	-
RI3	639292	648104	8812	12	64.95	-	-	-	-
RI4	2108898	2123618	14720	12	57.74	-	PAI9 (88.68%)	-	RI2 (87.58%)
RI5	2525750	2533567	7817	8	70.35	-	-	-	-
RI6	2737457	2743759	6302	10	63.91	-	-	MI7 (72.72%)	-
RI7	2834713	2844645	9932	10	62.67	-	PAI5 (82.27%)	MI9 (77.11%)	-
RI8	3171829	3195574	23745	20	55.38	-	PAI9 (99.55%)	-	-
RI9	3500395	3505529	5134	9	57.9	-	-	-	-

MI1	259597	371874	112277	102	65.15	PAI8 (78.84%) RI3 (83.06%)	PAI13 (80.26%)	RI5 (79.83%)	RI4 (79.19%)
MI2	412466	419219	6753	9	66.51	-	-	-	-
MI3	440369	479523	39154	38	63.67	-	-	-	-
MI4	2217913	2228456	10543	10	66.85	SI1 (76.61%)	-	-	-
MI5	2751778	2773695	21917	22	67.14	-	-	-	-
MI6	2925759	2938259	12500	15	66.08	-	-	-	-
MI7	3602535	3610196	7661	10	65.06	-	-	MI3 (79.63%)	-
MI8	3670348	3681973	11625	13	64.97	-	-	MI2 (79.71%)	-
SI1	818826	830877	12051	13	66.46	-	-	-	-
SI2	2237300	2244377	7077	9	61.42	-	-	-	-
SI3	3201194	3221552	20358	18	59.25	PAI9 (88.98%)	MI1 (98.98%)	-	RI1 (82.08%)

Supplementary table 7: Description and comparison of predicted Genomic Island of each GEI of *G. nicotinae* OTC16. The GEIs of blue color are unique to the genome. The GEIs of black color are islands partially shared with the other genomes. The GEIs of red color are shared among all 5 genomes. Pident: Percent identity.

Reference GEIs	**Start**	**End**	**Size (bp)**	**CDS**	**G + C content (%)**	***G. creatinolyticus LGCM 259 (pident)***	***A. sp. YCRL1 (pident)***	***G. arilaitensis RE117 (pident)***	***G. halophytocola KLBMP5180 (pident)***
PAI1	426454	449613	23159	25	63.56	PAI13 (92.30%)	-	-	-
PAI2	1438425	1474298	35873	46	67.44	-	PAI6 (81.99%)	SI3 (94.50%)	-
PAI3	2999355	3009881	10526	11	65.6	-	-	-	PAI8 (82.85%)

PAI4	3153270	3162764	9494	11	57.46	PAI1 (92.06%)	PAI2 (81.37%)	RI1 (98.22%) PAI8 (98.16%)	-
PAI5	3408191	3425059	16868	17	59.64	-	-	-	PAI11 (83.38%)
PAI6	3541160	3571713	30553	29	61.69	-	-	-	MI12 (86.75%)
RI1	744367	774495	30128	36	59.71	PAI11 (77.84%)	SI3 (81.96%)	MI1 (90.90%) PAI9 (92.19%)	MI8 (77.94%)
RI2	780727	790348	9621	12	54.94	-	RI4 (87.58%)	PAI9 (86.42%)	PAI1 (77.41%)
RI3	1507563	1571962	64399	42	60.61	MI2 (92.50%)	PAI5 (78.78%)	SI4 (89.47%)	PAI3 (84.02%)
RI4	3357488	3377739	20251	19	65.62	-	MI1 (79.51%)	-	RI5 (85.03%)
MI1	157504	177052	19548	19	62.83	-	-	-	-
MI2	505228	519029	13801	13	63.98	-	-	SI2 (83.54%) PAI8 (82.85%)	-
MI3	3387250	3402360	15110	15	64.77	PAI14 (73.66%)	-	PAI14 (92.45%)	-
SI1	392155	411072	18917	16	56.58	-	-	PAI10 (90.36%)	-
SI2	2999355	3009881	10526	11	65.6	-	-	-	PAI8 (82.85%)
SI3	3449788	3469786	19998	20	61.73	-	-	-	-

Supplementary table 8: Description and comparison of predicted Genomic Island of each GEI of *G.halophytocola* KLBM5180. The GEIs of blue color are unique to the genome. The GEIs of black color are islands partially shared with the other genomes. ident: Percent identity.

Reference GEIs	Start	End	Size (bp)	CDS	G + C content (%)	*G. creatinolyticus LGCM 259 (pident)*	*A. sp. YCRL1 (pident)*	*G. arilaitensis RE117 (pident)*	*G. nicotianae OTC16 (pident)*
SI 1	3314498	3327157	12659	13	62.55	-	-	-	-
SI 2	3538934	3559111	20177	22	62.35	-	-	PAI13 (82.76%) PAI14 (76.68%)	-
SI3	3617938	3632462	14524	9	59.25	-	-	-	-
PAI 1	1000139	1012495	12356	15	63.93	-	-	-	RI2 (77.35%)
PAI 2	1027889	1035221	7332	8	54.22	-	-	-	-
PAI 3	1576248	1605336	29088	16	50.9	-	PAI5 (80.98%)	SI4 (84.27%) PAI6 (93.46%)	RI3 (84.02%)
PAI 4	1960853	1974182	13329	13	61.4	-	-	-	-
PAI 5	2021782	2032672	10890	14	57.75	-	-	-	-
PAI 6	2539109	2549637	10528	11	54.77	-	-	-	-
PAI 7	2559083	2569774	10691	10	56.99	-	-	-	-
PAI 8	3089899	3121103	31204	30	63.1	-	-	-	SI2 (82.85%) PAI3 (82.85%)
PAI 9	3265041	3275069	10028	12	63.65	-	RI2 (74.81%)	-	-
PAI 10	3408970	3426576	17606	17	60.27	PAI14 (86.45%)	RI1 (83.74%)	-	-
PAI 11	3636638	3655926	19288	24	63.03	-	-	-	PAI5 (83.38%)
PAI 12	3701183	3709978	8795	11	63.24	-	-	PAI14 (84.07%)	-
MI 1	39921	57851	17930	16	57.36	-	-	-	-

MI 2	245545	256541	10996	14	59.03	-	MI8 (79.70%)	-	-
MI 3	395168	403094	7926	10	58.42	-	MI7 (79.59%)	MI6 (75.12%)	-
MI 4	826353	838552	12199	14	65.11	-	-	-	-
MI 5	1199222	1221430	22208	19	60.7	-	-	-	-
MI 6	1474995	1483950	8955	9	61.74	-	-	-	-
MI 7	1492922	1511983	19061	11	57.15	-	RI6 (72.72%)	-	-
MI 8	3153927	3172972	19045	21	63.41	PAI11 (82.979%)	-	-	RI1 (77.98%)
MI 9	3453534	3497205	43671	44	61.27	-	RI7 (77.10%)	-	-
MI 10	3662534	3680322	17788	18	59.18	-	-	-	-
MI 11	3687034	3694257	7223	8	64.3	-	-	-	-
MI 12	3776808	3816925	40117	33	63.05	-	-	-	PAI6 (86.75%)
MI 13	3835049	3847491	12442	12	61.05	-	-	-	-
RI 1	698528	705960	7432	9	53.87	-	-	-	-
RI 2	1794411	1800180	5769	8	54.78	-	-	-	-
RI 3	2574637	2583267	8630	8	53.26	-	-	-	-
RI 4	3509425	3535488	26063	24	63.32	-	-	-	-
RI 5	3562975	3599092	36117	32	60.55	-	MI1 (79.89%)	-	RI4 (85.02%)

6. CONCLUSÃO

A linhagem LGCM 259, isolada de uma égua na Itália, foi identificada como *G. creatinolyticus* através do MALDI Biotyper. A montagem do genoma do isolado LGCM 259 apresenta um repertório de genes de resistência a antibióticos, fatores de virulência essenciais para sua viabilidade celular, virulência e patogenicidade. Análises genômicas e comparativas realizadas neste trabalho evidenciaram a grande plasticidade existente entre os genomas do gênero *Glutamicibacter*, o que provavelmente está relacionado à capacidade de adaptação aos diferentes ambientes.

As análises de sintenia demonstraram que os genomas das linhagens estudadas quando comparadas ao genoma de *G. creatinolyticus* LGCM 259 apresentaram variações no tamanho do genoma. Entretanto, estudos mais aprofundados dessas linhagens devem ser realizados para entender o impacto dessa diferença no conteúdo gênico e na adaptação a determinado nicho.

O pequeno número de linhagens disponíveis para a maioria das espécies do gênero *Glutamicibacter* não permite o estabelecimento de um perfil definitivo de suas propriedades genômicas, principalmente em relação a espécie de *G. creatinolyticus* que até o momento é o primeiro genoma sequenciado e depositado em banco de dados públicos. Os resultados obtidos neste trabalho auxiliam no entendimento de algumas características da linhagem, entretanto, novos estudos são necessários para melhor compreender a biologia desses microrganismos.

7. PERSPECTIVAS

Como perspectivas futuras, pretende-se:

- Realizar uma análise das vias metabólicas dos genes resistentes a metais pesados (cromo, cobre, arsênio, dentre outros).
- Analisar os genes ausentes na linhagem LGCM 259 em relação aos genomas do gênero *Glutamicibacter*.
- Realizar análises genômicas com a outra amostra isolada do abcesso da mesma égua identificada como *Dietzia* spp**.**

8. REFERÊNCIAS BIBLIOGRÁFICAS

ALTIMIRA, F. et al. Characterization of copper-resistant bacteria and bacterial communities from copper-polluted agricultural soils of central Chile. **BMC Microbiology**, v. 12, n. 1, p. 193, 5 set. 2012.

ALVAREZ, R. F. et al. Trimethoprim-sulfamethoxazole versus norfloxacin in the prophylaxis of spontaneous bacterial peritonitis in cirrhosis. **Arquivos de Gastroenterologia**, v. 42, n. 4, p. 256–262, dez. 2005.

BAPTISTA, M. G. DE F. M. **Mecanismos de Resistência aos Antibióticos - Maria Galvão Baptista (20071294)**. [s.l: s.n.]. Disponível em: <http://recil.grupolusofona.pt/bitstream/handle/10437/3264/Mecanismos de Resistência aos Antibióticos Maria Galvão Ba.pdf?sequence=1>. Acesso em: 18 maio. 2019.

BARBOSA, E. et al. On the limits of computational functional genomics for bacterial lifestyle prediction. **Briefings in Functional Genomics**, v. 13, n. 5, p. 398–408, 1 set. 2014.

BARCELLOS, F. G. et al. Evidence of horizontal transfer of symbiotic genes from a Bradyrhizobium japonicum inoculant strain to indigenous diazotrophs Sinorhizobium (Ensifer) fredii and Bradyrhizobium elkanii in a Brazilian Savannah soil. **Applied and environmental microbiology**, v. 73, n. 8, p. 2635–43, 15 abr. 2007.

BERGLUND, E. C.; KIIALAINEN, A.; SYVÄNEN, A.-C. Next-generation sequencing technologies and applications for human genetic history and forensics. **Investigative Genetics**, v. 2, n. 1, p. 23, 2011.

BRUINS, M. R.; KAPIL, S.; OEHME, F. W. Microbial Resistance to Metals in the Environment. **Ecotoxicology and Environmental Safety**, v. 45, n. 3, p. 198–207, 1 mar. 2000.

BUERMANS, H. P. J.; DEN DUNNEN, J. T. Next generation sequencing technology: Advances and applications. **Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease**, v. 1842, n. 10, p. 1932–1941, 1 out. 2014.

BUSSE, H.-J. Review of the taxonomy of the genus Arthrobacter, emendation of the genus Arthrobacter sensu lato, proposal to reclassify selected species of the genus Arthrobacter in the novel genera Glutamicibacter gen. nov., Paeniglutamicibacter gen. nov., Pseudoglutamicibacter gen. nov., Paenarthrobacter gen. nov. and Pseudarthrobacter gen.

nov., and emended description of Arthrobacter roseus. **International Journal of Systematic and Evolutionary Microbiology**, v. 66, n. 1, p. 9–37, 1 jan. 2016.

CHAN, C. X.; RAGAN, M. A. Next-generation phylogenomics. p. 1–6, 2013.

CHOPRA, I.; ROBERTS, M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. **Microbiology and molecular biology reviews : MMBR**, v. 65, n. 2, p. 232– 60 ; second page, table of contents, jun. 2001.

CHOUDHURI, S. Phylogenetic Analysis. In: **Bioinformatics for Beginners**. [s.l.] Elsevier, 2014. p. 209–218.

COLLINS, M. D.; DORSCH, M.; STACKEBRANDT, E. Transfer of Pimelobacter tumescens to Terrabacter gen. nov. as Terrabacter tumescens comb. nov. and of Pimelobacter jensenii to Nocardioides as Nocardioides jensenii comb. nov. **International Journal of Systematic Bacteriology**, v. 39, n. 1, p. 1–6, 1 jan. 1989.

CONN, H. J.; DIMMICK, I. Soil Bacteria Similar in Morphology to Mycobacterium and Corynebacterium. **Journal of bacteriology**, v. 54, n. 3, p. 291–303, set. 1947.

COSTA, D. A. **GENÔMICA COMPARATIVA DE LINHAGENS DE Saccharomyces E Kluyveromyces DE INTERESSE BIOTECNOLÓGICO**. [s.l: s.n.]. Disponível em: <http://www.locus.ufv.br/bitstream/handle/123456789/8473/texto completo.pdf?sequence=1&isAllowed=y>. Acesso em: 15 maio. 2019.

DING, L.; HIROSE, T.; YOKOTA, A. Four novel Arthrobacter species isolated from filtration substrate. **INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY**, v. 59, n. 4, p. 856–862, 1 abr. 2009.

DOBRINDT, U. et al. Toxin genes on pathogenicity islands: impact for microbial evolution. **International Journal of Medical Microbiology**, v. 290, n. 4–5, p. 307–311, 1 out. 2000.

EISEN, J. A. Phylogenomics: improving functional predictions for uncharacterized genes by evolutionary analysis. **Genome research**, v. 8, n. 3, p. 163–7, 1 mar. 1998.

FENG, W.-W. et al. Glutamicibacter halophytocola sp. nov., an endophytic actinomycete isolated from the roots of a coastal halophyte, Limonium sinense. **International Journal of Systematic and Evolutionary Microbiology**, v. 67, n. 5, p. 1120–1125, 1 maio 2017.

FLEISCHMANN, R. D. et al. Whole-genome random sequencing and assembly of

Haemophilus influenzae Rd. **Science (New York, N.Y.)**, v. 269, n. 5223, p. 496–512, 28 jul. 1995.

FRIERI, M.; KUMAR, K.; BOUTIN, A. Antibiotic resistance. **Journal of Infection and Public Health**, v. 10, n. 4, p. 369–378, 1 jul. 2017.

GANZERT, L. et al. Arthrobacter livingstonensis sp. nov. and Arthrobacter cryotolerans sp. nov., salt-tolerant and psychrotolerant species from Antarctic soil. **INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY**, v. 61, n. 4, p. 979–984, 1 abr. 2011.

HOU, X.-G. et al. Description of Arthrobacter creatinolyticus sp. nov., isolated from human urine. **International Journal of Systematic Bacteriology**, v. 48, n. 2, p. 423–429, 1 abr. 1998.

KARDOS, N.; DEMAIN, A. L. Penicillin: the medicine with the greatest impact on therapeutic outcomes. **Applied Microbiology and Biotechnology**, v. 92, n. 4, p. 677–687, 2 nov. 2011.

KONG, Z.; GLICK, B. R. The Role of Bacteria in Phytoremediation. In: **Applied Bioengineering**. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 2017. p. 327–353.

KRIZOVA, L.; NEMEC, A. A 63 kb genomic resistance island found in a multidrug-resistant Acinetobacter baumannii isolate of European clone I from 1977. **Journal of Antimicrobial Chemotherapy**, v. 65, n. 9, p. 1915–1918, 1 set. 2010.

LAND, M. et al. Insights from 20 years of bacterial genome sequencing. **Functional & Integrative Genomics**, v. 15, n. 2, p. 141–161, 2015.

LASAT, M. M. Phytoextraction of toxic metals: a review of biological mechanisms. **Journal of environmental quality**, v. 31, n. 1, p. 109–20, 2002.

LI, B.; WEBSTER, T. J. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. **Journal of orthopaedic research : official publication of the Orthopaedic Research Society**, v. 36, n. 1, p. 22–32, 2018.

LISTER, P. D.; WOLTER, D. J.; HANSON, N. D. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. **Clinical microbiology reviews**, v. 22, n. 4, p. 582–610, out. 2009.

LOMAN, N. J.; PALLEN, M. J. **Twenty years of bacterial genome sequencingNature Reviews Microbiology**Nature Publishing Group, , dez. 2015.

MARDIS, E. R. Next-Generation Sequencing Platforms. **Annual Review of Analytical Chemistry**, v. 6, n. 1, p. 287–303, 12 jun. 2013.

METZKER, M. L. Sequencing technologies — the next generation. **Nature Reviews Genetics**, v. 11, n. 1, p. 31–46, 8 jan. 2010.

MONNET, C. et al. The Arthrobacter arilaitensis Re117 Genome Sequence Reveals Its Genetic Adaptation to the Surface of Cheese. **PLoS ONE**, v. 5, n. 11, p. e15489, 24 nov. 2010.

MORADALI, M. F.; GHODS, S.; REHM, B. H. A. Pseudomonas aeruginosa Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. **Frontiers in cellular and infection microbiology**, v. 7, p. 39, 2017.

OCHMAN, H.; DAVALOS, L. M. The nature and dynamics of bacterial genomes. **Science (New York, N.Y.)**, v. 311, n. 5768, p. 1730–3, 24 mar. 2006.

PAN, K.; WANG, W.-X. Trace metal contamination in estuarine and coastal environments in China. **Science of The Total Environment**, v. 421–422, p. 3–16, 1 abr. 2012.

PATEL, S. Infection , Genetics and Evolution Drivers of bacterial genomes plasticity and roles they play in pathogen virulence , persistence and drug resistance. **MEEGID**, v. 45, p. 151–164, 2016.

PETERSON, J. W. **Bacterial Pathogenesis**. [s.l.] University of Texas Medical Branch at Galveston, 1996.

PRUM, C.; DOLPHEN, R.; THIRAVETYAN, P. Enhancing arsenic removal from arsenic-contaminated water by Echinodorus cordifolius−endophytic Arthrobacter creatinolyticus interactions. **Journal of Environmental Management**, v. 213, p. 11–19, 1 maio 2018.

PURANIK, R. et al. A pipeline for completing bacterial genomes using in silico and wet lab approaches. **BMC genomics**, v. 16 Suppl 3, n. Suppl 3, p. S7, 2015.

RAMESH, R. et al. Screening and production of a potent extracellular *Arthrobacter creatinolyticus* urease for determination of heavy metal ions. **Journal of Basic Microbiology**, v. 54, n. 4, p. 285–295, abr. 2014.

RAMESH, R. et al. Potentiometric biosensor for determination of urea in milk using immobilized Arthrobacter creatinolyticus urease. **Materials Science and Engineering: C**, v. 49, p. 786–792, abr. 2015.

SANJAY, M. S. et al. Isolation and identification of chromium reducing bacteria from tannery effluent. **Journal of King Saud University - Science**, 4 maio 2018.

SINGHAL, N. et al. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. **Frontiers in microbiology**, v. 6, p. 791, 5 ago. 2015.

SKERMAN,', V. B. D.; MCGOWAN, V.; SNEATH', P. H. A. **Approved Lists of Bacterial Names**. [s.l: s.n.]. Disponível em: <www.microbiologyresearch.org>. Acesso em: 17 maio. 2019.

SOARES, S. C. et al. Genome sequence of Corynebacterium pseudotuberculosis biovar equi strain 258 and prediction of antigenic targets to improve biotechnological vaccine production. **Journal of Biotechnology**, v. 167, n. 2, p. 135–141, 2013.

TOFT, C.; ANDERSSON, S. G. E. Evolutionary microbial genomics: insights into bacterial host adaptation. **Nature Reviews Genetics**, v. 11, n. 7, p. 465–475, jul. 2010.

TUMAPA, S. et al. Burkholderia pseudomallei genome plasticity associated with genomic island variation. **BMC Genomics**, v. 9, n. 1, p. 190, 25 abr. 2008.

VERLI, H. Bioinformática : da biologia à flexibilidade molecular. 2014.

VILA, J. et al. Identification of Clinically Relevant Corynebacterium spp., Arcanobacterium haemolyticum, and Rhodococcus equi by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. **Journal of Clinical Microbiology**, v. 50, n. 5, p. 1745, maio 2012.

WANG, Y. et al. Biodegradation of pentachloronitrobenzene by *Arthrobacter nicotianae* DH19. **Letters in Applied Microbiology**, v. 61, n. 4, p. 403–410, out. 2015.

YAMAMOTO, K. et al. Bacteremia Due to <i>Arthrobacter creatinolyticus</i> in an Elderly Diabetic Man with Acute Cholangitis. **Japanese Journal of Infectious Diseases**, v. 70, n. 2, p. 201–202, 24 mar. 2017.

YANCEY, R. J. et al. Ceftiofur sodium, a broad-spectrum cephalosporin: evaluation in vitro and in vivo in mice. **American journal of veterinary research**, v. 48, n. 7, p. 1050–3, jul.

1987.

YASSIN, A. F. et al. Auritidibacter ignavus gen. nov., sp. nov., of the family Micrococcaceae isolated from an ear swab of a man with otitis externa, transfer of the members of the family

Yaniellaceae Li et al. 2008 to the family Micrococcaceae and emended description of the suborder Micrococcineae. **INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY**, v. 61, n. 2, p. 223–230, 1 fev. 2011.

Rifici Claudia, et al. 2019 CASE REPORT: BACTERIAL GRANULOMATOUS MYOSITIS IN A HORSE**. BMC Veterinary Research.**

9. ANEXO

9.1.1. CASE REPORT submetido a revista BMC Veterinary Research relacionado a este trabalho.

Neste artigo, realizei o cultivo da amostra, enviada pela Itália, extração do DNA genômico para o sequenciamento, PCR multiplex, preparo da amostra para a técnica de MALDI – TOF e contribuí com a redação da parte metodológica dessas técnicas, bem como com a revisão de todo o artigo.

CASE REPORT: BACTERIAL GRANULOMATOUS MYOSITIS IN A HORSE

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Authors' contribution

CR, GM, SC and SS contributed to concept and design the study.

CR, GM, PO and DeBD performed histopathological and molecular analysis.
ARA performed the bacteriological analysis.
CR, ARA and GM drafted the manuscript and all authors read and approved the final manuscript.
CV contributed to interpret the data, to read and approve the final manuscript.
RGS, NS, TLPC, and HCPF performed PCR quadruplex and MALDI Biotyper.
SSJ, VA read and approved the final manuscript
All authors contributed to acquisition, analysis and interpretation of data.

ABSTRACT

Background: Granulomatous myositis in horses are the less common form caused by infectious agents, such as bacteria, viruses, protozoa and parasites. Bacterial myositis include infections mainly due to *Streptococcus zooepidemicus*, *Actinobacillus equuli*, *Fusobacterium* spp. and *Corynebacterium pseudotuberculosis* that involve the muscle tissue through haematogenous spreading and/or lesions of the skin. The presented case report details the first case of bacterial granulomatous myositis in a horse reared in Italy due to *Corynebacterium pseudotuberculosis*, *Corynebacterium amycolatum*, subsequently identified as *Arthrobacter creatinolyticus* and bacteria of the genus *Dietzia* spp. This case is extremely rare and interesting for the etiologic agents identified, and the peculiar pathological features.

Case Presentation: A 12-yo Quarter Horse mare was evaluated for the presence of diffuse subcutaneous nodules and masses ranged from 2x3 to 5x20 cm in size, adherent to sub-cutis and muscles. They were strongly vascularized and diagnosed as pyogranulomas, cytologically. Following the therapy and an initial disappearance of most of the lesions and a general improvement of the mare, clinical condition worsened along with the appearance of new lesions. Further histological, molecular, bacteriological and biochemical investigations were performed. All the data obtained allowed to a severe and diffuse multibacterial granulomatous myositis diagnosis by *Corynebacterium pseudotuberculosis* and *Corynebacterium amycolatum*. By matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) high-resolution mass spectrometer the sequencing peptides of the two microorganisms were for *Arthrobacter creatinolyticus* and bacteria of the genus *Dietzia* spp.

Conclusion: *Corynebacterium pseudotuberculosis* diagnosed by histopathological analysis in a horse in Italy could represent the first report on this agent to date, as well as *Arthrobacter creatinolyticus* and *Dietzia maris* infection is the first case found in horses.

Keywords: Bacterial myositis, Horses, *Corynebacterium pseudotuberculosis*, *Arthrobacter creatinolyticus, Dietzia* spp.

BACKGROUND

Myopathies are disorders affecting skeletal muscle and, in adult horses, include nutritional myopathies, infectious diseases, immune-mediated disorders, toxic myopathy, inherited myopathies, endocrinopathies, pasture-associated rhabdomyolysis, malignant hyperthermia, and exertional rhabdomyolysis [1]. Granulomatous myositis are less common form caused by infectious agents, such as bacteria, viruses, protozoa and parasites. Bacterial myositis include infections mainly due to *Streptococcus zooepidemicus*, *Actinobacillus equuli*, *Fusobacterium* spp. and *Corynebacterium pseudotuberculosis* that involve the muscle tissue through haematogenous spreading and/or lesions of the skin [2]. *Corynebacterium pseudotuberculosis* is an important animal pathogen. It is a Gram-positive, pleomorphic, facultative intracellular pathogenic bacterium belonging to the *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Rhodococcus* (CMNR) group of the Actinobacteria Phylum. It is the etiological agent of a disease that is commonly called Caseous Lymphadenitis (CLA) or cheesy gland [3]. This disease is found in all the world's major sheep and goat production areas, causing significant economic losses by affecting wool, meat, and milk production [4-8]. It has also been isolated from other animal species such as cattle, camels, swine, buffaloes, horses, in which it causes Ulcerative Lymphangitis and Pigeon Fever [4,9-11]. *C. pseudotuberculosis* could also affect humans, causing distinct kinds of lymphadenitis. Contamination occurs through contact with infected animals and consumption of infected food [3,9,12].

Case Presentation

A 12-yo Quarter Horse mare was evaluated for the presence of diffuse subcutaneous nodules and masses (**fig.1a**) diagnosed as pyogranulomas, cytologically, and then treated with penicillin (6000000 UI die) and dihydrostreptomycin (7.5 g/die) intramuscularly. After three months of therapy, general clinical condition deteriorated and another antibiotic therapy with cephalosporines of third generation (Ceftiofur: 2.2 mg/kg b.w. once-a-day for 10 consecutive days) and tetracyclines (2.5g/die intravenously) was administered. After an initial disappearance of most of the lesions and a general improvement of the mare, clinical condition worsened again and along with the appearance of new lesions (**fig. 1b**). This evolution let the owner to request further investigations. Three masses were surgically removed and examined, macroscopically; the excised masses ranged from 2x3 to 5x20 cm in size and were adherent to sub-cutis and muscles and strongly vascularized, on cut section, they

consisted in a fibrotic tissue surrounding a caseous and necrotic material with dispersed small multiple purulent-like foci (**Fig. 1c**).

An aliquot of excised tissues was sampled and fixed in formalin (10%) and paraffin was embedded for histological diagnosis. Tissue sections of 3-4 μm were stained with Hematoxylin-Eosin (HE), Masson Trichrome, Periodic acid – Schiff (PAS), Grocott and Gram and examined microscopically.

Histological examination revealed a diffuse mixed inflammatory infiltrate invading the endomysium (**Fig. 2A**) constituted by granulocytes neutrophils and mainly eosinophils, macrophages, lymphocytes, plasma cells, epithelioid and multinucleated giant cells (**Fig. 2B**), often arranged between collagenolytic degeneration areas or around foci of necrosis associated with calcification (**Fig. 2C**). PAS and Grocott stains revealed no fungal presence. Gram stain allowed to detect small aggregates of pleomorphic, blue Gram-positive microorganisms free or inside macrophages (**Fig. 2D**).

On the basis of these results, a diagnosis of granulomatous myositis was issued.

Hence, in order to reveal the etiologic agent, cause of the pathology, an aliquot of fixed tissues was sent to the Experimental Zooprophylactic Institute (IZS) of Sicily, Palermo, Italy.

The DNA was quantized and employed in PCR test targeted to the ribosomal region and sequences were amplified by primers annealing at 16S of ribosomal genes, specific primers to amplify a species-specific DNA region of *Corynebacterium* spp. [13]. Amplification of the 16S rRNA region was performed by using pA 5'- AGAGTTTGATCCTGGCTCAG and pH 5'- AAGGAGGTGATCCAGCCGCA universal primers [14]. The full 16S rRNA region was amplified by PCR in a final reaction volume of 50 μl. Each reaction mixture contained approximately 10 ng of template DNA; 0.4 pmol (each) forward (pA) and reverse (pH) primers; 10 μM (each) dATP, dCTP, dGTP, and dTTP; 10X reaction buffer containing 1.5 mM MgCl2 (AB) and 2 U of Taq Gold (AB). The amplification was performed in a 9700-thermal cycler (Applied Biosystems Inc., Foster City, California, 94404, USA). An initial denaturation step (94°C for 5 min) was followed by 35 cycles (with each cycle consisting of DNA denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, and elongation at 72°C for 1 min) and a final extension step at 72°C for 7 min. A non-template negative control was included in each PCR run. The PCR products were purified with a Spin PCR purification kit (QIAGEN, Hilden, 40724, Germany). The DNA was eluted in 25 μl of double-distilled H_2O. The purified PCR products were sequenced with the pA and pH primers in two different reactions using BigDye Terminator cycle sequencing 1.1 kit (Applied Biosystems Inc., Foster City, California, 94404, USA). Sequencing on an ABI Prism 310 DNA sequencer (Applied Biosystems

Inc., Foster City, California, 94404, USA) was performed. The obtained data were checked on the electropherograms and were analyzed by Wu Blast 2 sequence alignment software, considering 97% identity as the stringent parameter for strain identification (P<0.005).

PCR sequence was aligned in GeneBank with the corresponding tract of *Corynebacterium pseudotuberculosis* 16S rRNA gene (strain NCTC 3450: GenBank: X84255.1).

In order to isolate *Corynebacterium pseudotuberculosis* and identify the biovar, two frozen tissue aliquots were sent to the Laboratory of Medical Microbiology and Infectious diseases of School of Biosciences and Veterinary medicine, University of Camerino, Italy. Bacteriological culture was performed onto Columbia Agar (sheep blood 5%) (Liofilchem®, Italy), Columbia C.N.A.M. Agar (sheep blood 5%) (Liofilchem®, Italy), followed by incubation at 37°C for 48-72 hours in 5-10% of CO_2 atmosphere (CampyGen®, ThermoFisher Scientific, Italy). Bacterial identification was achieved using biochemical tests, including catalase, glucose, sucrose, urea, nitrate reduction, casein, and gelatin hydrolysis [15]. Phenotypic identification was performed by commercial gallery RapID™ CB Plus (Remel, Thermo Fisher Scientific, Italy) according to the manufacturer's instructions. The reading was performed by the open REMEL software (http://www.remel.com/ERIC/IdentificationSingle.aspx).

Antibiotic susceptibility testing by the Kirby-Bauer disk diffusion test for Kanamycin (30 µg), Amikacin (30 µg), Ampicillin (10 µg), Penicillin G (1 IU), Streptomycin (300 µg), Cefazolin (30 µg), Cefquinome (30 µg), Colistin sulphate (25 µg), Enrofloxacin (5 µg), Erythromycin (15 µg), Gentamicin (30 µg), Doxycycline (30 µg), Tylosin (30 µg), Rifampin (5 µg), Cotrimazole (23.75 plus 1.25 µg), Azithromycin (15 µg), Cloramphenicol (30 µg), Furazolidone (50 µg), Oxytetracicline (30 µg), Imipenem (10 µg), Fosfomycin (50 µg), was performed. The zone diameters were interpreted according to guidelines set by the European Committee on Antimicrobial Susceptibility Testing (CLSI, 2016).

By the culture agar media, different bacteria were grown. White cream to yellow, circular, aerobic and dry colonies (strain 1) were recovered together with deep orange color, circular colonies (strain 2). They consisted of Gram-positive, rod-shaped and catalase positive bacteria. The biochemical identification showed atypical urease test (-), catalase (+), glucose and sucrose (+), gelatin and casein hydrolysis (-), and nitrate reduction (-) for the first microorganism (**Figure 3**). The second one showed urease test (-), catalase (+), glucose (+), sucrose (-), atypical MAL (-) and LEU (+). By the Remel software reading, the strains resulted *C. pseudotuberculosis* with one test (urease) atypical (bioscore 1/852) and *C. amycolatum* with atypical MAL and LEU (bioscore 1/272). In relation to the reduction of nitrate to nitrite, biovar Ovis was estimated. Antimicrobial drug–

susceptibility testing showed that *C. pseudotuberculosis* strain was susceptible to Ampicillin, Penicillin G, Streptomycin, Cefazolin, Cefquinome, Enrofloxacin, Rifampicin, Cotrimazole.

Subsequently, an aliquot of each sample was frozen in Cryobank® (Mast Group, Germany), stored at -80°C, and sent to the Laboratory of Cellular and Molecular Genetics in Brazil for confirmation through Quadruplex PCR and MALDI Biotyper methods.

http://www.remel.com/ERIC/IdentificationSingle.aspx

For the Quadruplex PCR identification, the extraction of bacterial genomic DNA was performed in accordance with a previously described protocol [16]. Quadruplex PCR reactions were performed with a final volume of 50µL, containing 20ng of genomic DNA, 0.25mM dNTPs, 0.1 unit of Taq DNA polymerase (Life Technologies, Carlsbad, CA, USA), 2mM $MgCl_2$, enzyme buffer (Life Technologies, Carlsbad, CA, USA), 0.5 µM of each primer oligonucleotide, and water to a final volume of 50µL. The primers used in this study anneal to the *16s rRNA*, *rpoB*, *narG*, and *pld* gene sequences of *C. pseudotuberculosis,* in accordance with Almeida et al. [17]. The amplified products were resolved through electrophoresis on 1.0% (w/v) agarose gels and stained using ethidium bromide.

As expected, the positive control using *C. pseudotuberculosis* biovar Equi genomic DNA yielded PCR amplicons corresponding to the 16S rRNA, *rpoB, narG*, and *pld* genes, with 816, 446, ~600, and 203 bp-sized fragments, respectively (**Figure 4**). In turn, the positive control using *C. pseudotuberculosis* biovar Ovis genomic DNA yielded PCR amplicons corresponding only to the 16S rRNA, *rpoB,* and *pld* genes (**Figure 4**). As expected, the *pld* gene product was not detected in any of the two strains **(Figure 4**). None amplification was detected using water as a template (negative control). The amplification profiles seen for the equine strains 1 and 2 clearly differed from the *C. pseudotuberculosis* standard amplification profiles.

Later, a single colony of each bacterial strain was spotted onto a MALDI Biotyper target steel plate using a sterile toothpick. Subsequently, each sample was overlaid with 1µl matrix consisting of a saturated solution of α-cyano-4-hydroxycinnamic acid (HCCA) (Bruker Daltonics, Bremen, Germany). The preparation was allowed to dry at room temperature. Spectra using the FlexControl MicroFlex LT mass spectrometer (Bruker Daltonics) were acquired in accordance with Assis et al. [18]. Previous calibration of the mass spectrometer was performed using a bacterial standard test [19]. As recommended by the manufacturer, scores ≥2.000 indicated species-level identification, while scores ≥1.700 and <2.000 indicated genus-level identification and scores <1.700 indicated no reliable identification.

The strain 1 was identified as *Arthrobacter creatinolyticus* (score of 2.387), while the strain 2 was identified as *Dietzia maris* (score of 1.791). As the score for strain 2 was not above the 2.000

threshold, MALDI Biotyper (Bruker Daltonics, Bremen, Germany) indicated reliable identification only at the genus level.

Discussion and conclusions

This report shows the clinical-pathological history of a mare, lasting not less than two years, characterized by the presence of several subcutaneous/muscle lesions treated with different antibiotic therapies that resulted in a decrease in number but never in the total disappearance.

All data resulted in a diffuse granulomatous myositis due to multiple bacteria represented by *Corynebacterium pseudotuberculosis*, *Corynebacterium amycolatum*, subsequently identified as *Arthrobacter creatinoltycus* and bacteria of the genus *Dietzia*, respectively.

Corynebacterium pseudotuberculosis is a microorganism facultative intracellular pathogen that exhibits pleomorphic forms, such as coccoids and rods, non-sporulating, non-capsulated, non-motile bacterium and facultative anaerobe that causes granulomatous and necrotic lesions. In horses, the infection occurs through contact with contaminated soil via skin abrasions or mucous membranes and recent studies support the role of insect vectors, and the incubation period may vary from weeks to months [20]. *C. pseudotuberculosis* can be identified through PCR assay with 16S RNA and classified into two biovars based on their ability to reduce nitrate to nitrite [21-22]. Nitrate-negative strains are grouped into the biovar Ovis and are responsible for Caseous Lymphadenitis (CL), in small ruminants [23] and mastitis in dairy cattle [24]; while the nitrate-positive strains are grouped into the biovar Equi and is responsible of ulcerative lymphangitis and abscesses in internal organs of equines [8,25] and oedematous skin disease in buffalos [10]. Furthermore, *C. pseudotuberculosis* bv. equi infection in horses is commonly known as "Pigeon Fever" because it leads to the formation of external abscesses on the pectoral muscle region, making it expand, similar to a pigeon breast. However, external abscesses may occur in a variety of locations including deep intramuscular, axillary, inguinal, and mammary [26]. Nonetheless, in *C. pseudotuberculosis*, the role of nitrate respiration is not known [27].

Pigeon Fever has been described for the first time in California, since then it has also spread enzootic in some regions of the western United States, which have a low annual precipitation, such as Arizona and Western Texas [28-29]. In our case, on one side, growth site, size, and macroscopic appearance seemed overlapping the typical lesions of Pigeon Fever, although the characterization of the biovar Ovis, together with the absence of a real abscess with purulent material did not allowed the certain diagnosis by *C. pseudotuberculosis* in horses. Furthermore, lesion's gross appearance was characterized by a necrotic caseous material surrounded by a fibrotic pseudo-capsule with small

purulent-like foci attributed to a chronic form of ulcerative lymphangitis, even if there were not a lymphatic system involvement, swelling of the limbs and ulcerative lesions.

This atypical presentation for *C. pseudotuberculosis* bacterial infection is probably due to the finally identification of other etiologic agents: a bacterium of the genus *Arthrobacter creatinolyticus* and *Dietzia* spp. In some individuals, the recurrence or persistence of infection for greater than 1 year can occur [30], and it cannot be excluded a concomitant infection by opportunistic pathogens that could be favored by the immunological reduction induced by *C. pseudotuberculosis* [31-32].

Dietzia spp. represents a group of Gram-positive non-spore-forming cocci growing occasionally as short rods, with aerobic metabolism [33]; the genus *Dietzia* spp. was previously classified as *Rhodococcus* and is closely related to the *Actinomycetes*, among which *Corynebacterium* spp, and easily and rapidly characterized by using MALDI spiral-TOF MS thanks to the presence of mycolic acids in the cell wall, that they act similarly to the outer membranes of Gram-negative bacteria [34].

Arthrobacter species, which belong to the heterogeneous group of coryneform bacteria, consist in Gram positive, catalase-positive, aerobic, non-spore-forming bacteria and, presently, the genus contains two groups of species referred to as the *Arthrobacter globiformis/ Arthrobacter citreus* group and *Arthrobacter nicotianae* group that differs in their peptidoglycan structure [35]. Infection due to *Arthrobacter* species might have been underestimated because a correct identification of *A. creatinolyticus* is only possible by applying further identification methods (i.e., 16S rRNA gene sequencing or MALDI-TOF MS. Vargha et al. [36] reported that sixteen *Arthrobacter* species could be identified by MALDI-TOF MS. Infection by *Arthrobacter creatinolyticus* is a zoonosis. Reports regarding the isolation of *Arthrobacter* species from human clinical specimens (i.e., wound swab, urine, or blood) have increased since the late 1990s [37-38]. In Veterinary literature few species have been isolated between this genus: *Arthrobacter rhombi* isolated from fish [39], *Arthrobacter nasiphocae* isolated from the nasal cavities of a phoca vitulina [40], *Arthrobacter gandavensis* isolated from cattle [41], and *Arthrobacter equi* isolated from genital swabs of a horse [35].

The presented case is extremely rare and interesting for both the etiologic agents found and the peculiar pathological features. *C. pseudotuberculosis* isolated in a horse in Italy could represent the first report on this agent to date, as well as *A. creatinolyticus* and *Dietzia maris* are the first bacterial infections revealed in horses. Both the histopathological and bacteriological diagnosis allowed to the use of a targeted therapy with Trimethoprim and sulfametoxazole (10 g/100 kg orally the first day, and then 5 g/100 kg for 10 days) resulting in an extensive reduction in number and size of the detected lesions. At one year away, the follow-up marked improvement in the general conditions and the pregnancy status of the mare.

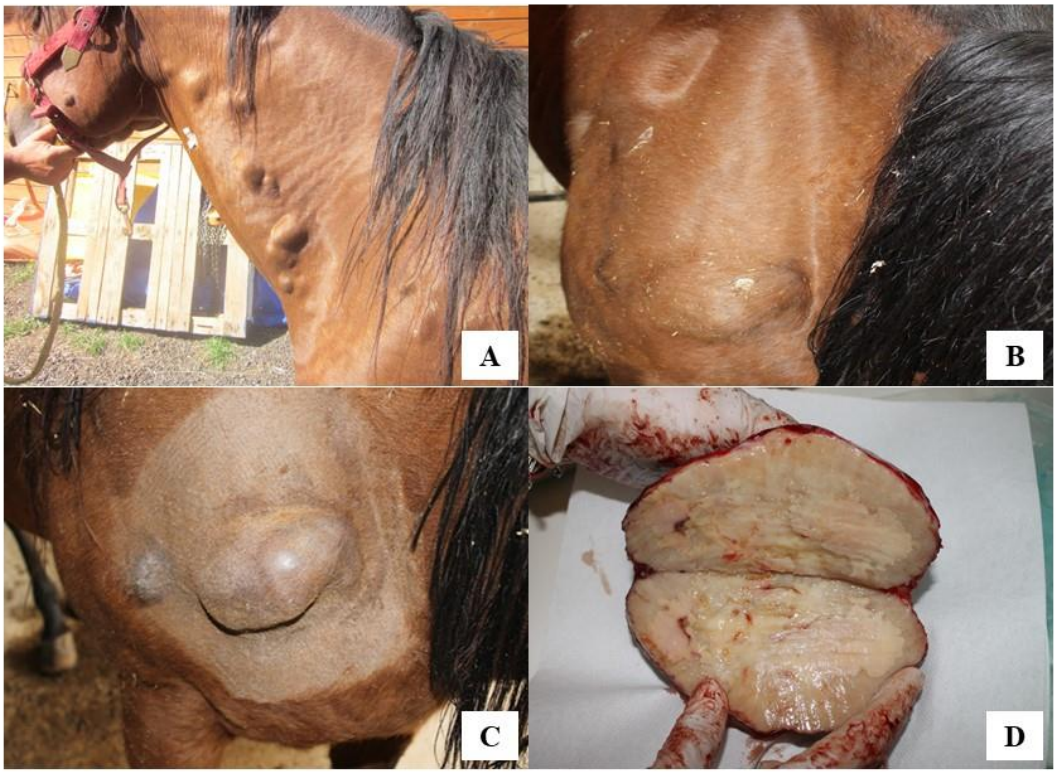

Figure 1: A) Several single or multiple subcutaneous nodules and masses, 3 to more 20 cm in size, B) mainly localized at shoulder regions. C) adherent to muscles, painless and firm in consistency. D) On cut section, evidence of necrotic caseous material surrounded by a fibrotic pseudo capsule with small purulent-like foci externally to the necrotic tissue.

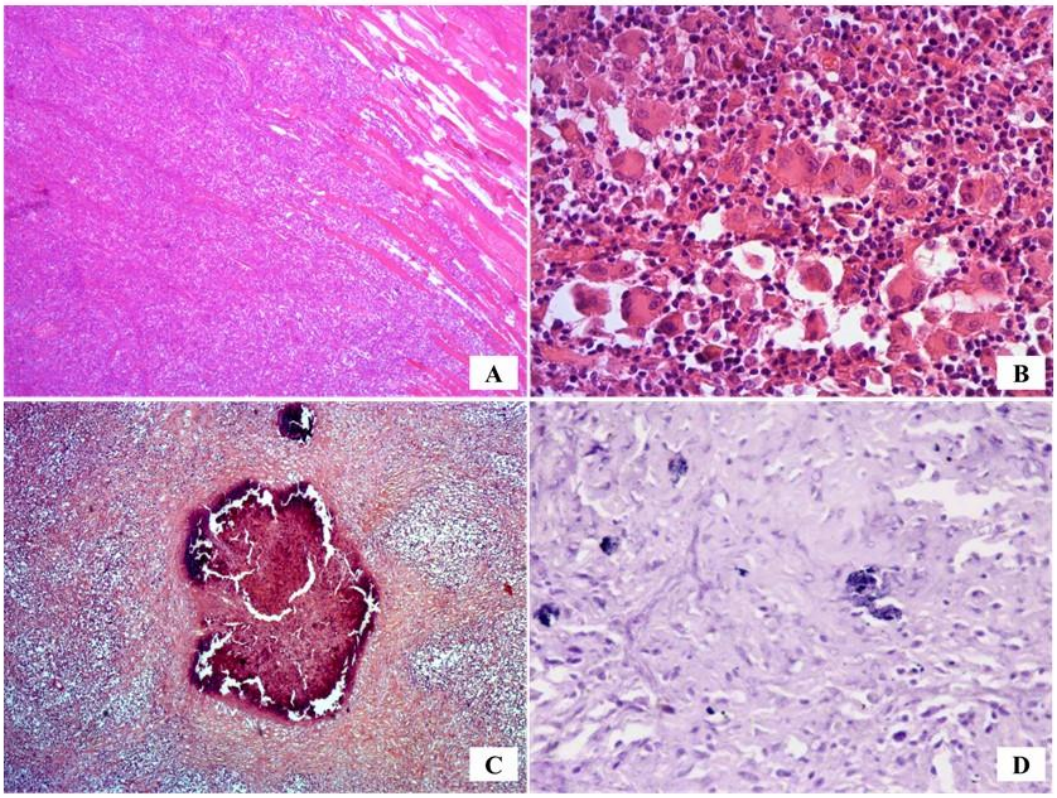

Figure 2: Histological examination**:** A) Widespread inflammatory infiltrate invading the endomysium (EE, 10x); B) Mixed inflammatory infiltrate constituted by granulocytes neutrophils and mainly eosinophils, macrophages, lymphocytes, plasma cells, epithelioid and multinucleated giant cells (EE, 40x); C) Collagenolytic degeneration areas and foci of necrosis associated with calcification (EE, 10x); D) Gram stain allowed to detect small aggregates of pleomorphic, blue Gram-positive microorganisms free or inside macrophages (Gram , 40x).

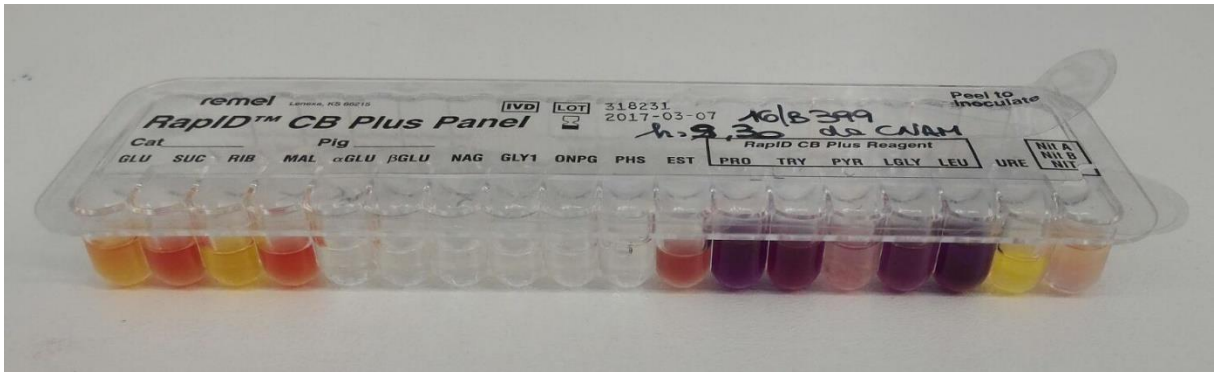

Figure 3: The biochemical identification showed atypical urease test (-), catalase (+), glucose and sucrose (+), gelatin and casein hydrolysis (-), and nitrate reduction (-) for the first microorganism.

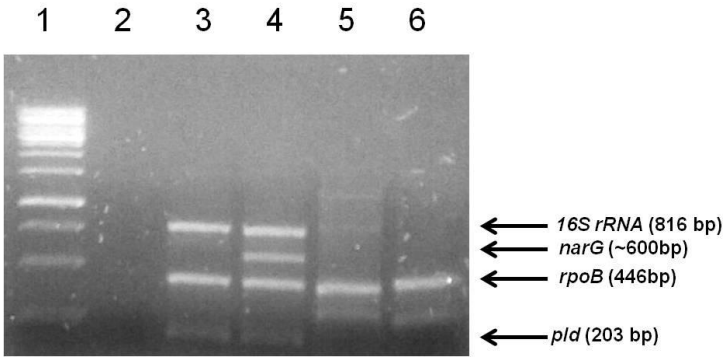

Figure 4. Agarose gel electrophoresis of the quadruplex PCR products for the identification of the two strains isolated from a horse. Lane 1- 1kb Gene Ruler DNA Ladder (Thermo Scientific); Lane 2- Negative Control; Lane 3- *C. pseudotuberculosis* Ovis DNA (strain 1002); 4- *C. pseudotuberculosis* 258 Equ;i 5- DNA from strain 1, isolated from a sick equine; 6- DNA from strain 2, isolated from a sick equine. The arrows indicate the target genes and fragment sizes expected for the *C. pseudotuberculosis* amplification profiles.

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References

1. Le Roux K, Streichenberger N, Vial C, Petiot P, Feasson L, Bouhour F, et al. Granulomatous myositis: a clinical study of thirteen cases. Muscle Nerve. 2007; 35:171-7.
2. Valentine BA. Pathologic findings in equine muscle (excluding polysaccharide storage): a necropsy study. J Vet Diagn Invest. 2008;20(5):572-9.
3. Dorella Alves F, Pacheco Carvalho LG, Oliveira Costa S, Miyoshi A, Azevedo V. *Corynebacterium pseudotuberculosis*: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. Vet. Res. 2006;37: 201–218.
4. Williamson LH. Caseous lymphadenitis in small ruminants, Vet. Clin. North Am. Food Anim. Pract. 2001; 17:359–71.
5. Paton MW, Walker SB, Rose IR, Watt GF. Prevalence of caseous lymphadenitis and usage of caseous lymphadenitis vaccines in sheep flocks, Aust. Vet. J. 2003; 81:91–5.
6. Foley JE, Spier SJ, Mihalyi J, Drazenovich N, Leutenegger CM. Molecular epidemiologic features of *Corynebacterium pseudotuberculosis* isolated from horses. Am J Vet Res. 2004;65:1734–7.

7. Seyffert N, Guimarães AS, Pacheco LG, Portela RW, Bastos BL, Dorella FA, et al. High seroprevalence of caseous lymphadenitis in Brazilian goat herds revealed by *Corynebacterium pseudotuberculosis* secreted proteins-based ELISA. Res Vet Sci. 2010; 88:50–5.
8. Kumar J, Singh F, Tripathi BN, Kumar R, Dixit SK, Sonawane GG. Epidemiological, bacteriological and molecular studies on caseous lymphadenitis in Sirohi goats of Rajasthan, India. Trop Anim Health Prod. 2012; 44:1319–22.
9. Peel MM, Palmer GG, Stacpoole AM, Kerr TG. Human lymphadenitis due to *Corynebacterium pseudotuberculosis*: report of ten cases from Australia and review. Clin Infect Dis. 1997;24(2):185–91.
10. Selim AS. Oedematous skin disease of buffalo in Egypt, J. Vet. Med. B Infect. Dis. Vet. Public Health. 2001; 48:241–58.
11. Yeruham I, Friedman S, Perl S, Elad D, Berkovich Y, Kalgard Y. A herd level analysis of a *Corynebacterium pseudotuberculosis* outbreak in a dairy cattle herd. Vet. Dermatol. 2004; 15:315–20.
12. Trost E, Ott L, Schneider J, Schröder J, Jaenicke S, Goesmann A, et al. The complete genome sequence of *Corynebacterium pseudotuberculosis* FRC41 isolated from a 12-year-old girl with necrotizing lymphadenitis reveals insights into gene-regulatory networks contributing to virulence. BMC Genomics. 2010;11(1):728.
13. Pascual C, Lawson PA, Farrow JA, Gimenez MN, Collins MD. Phylogenetic analysis of the genus Corynebacterium based on 16S rRNA gene sequences. Int J Syst Bacteriol. 1995;45(4):724-8.
14. Hutson RA, Thompson DE, Collins MD. Genetic interrelationships of saccharolytic Clostridium botulinum types B, E and F and related clostridia as revealed by small subunit rRNA gene sequences. FEMS Microbiol Lett. 1993;108(1):103-10.
15. Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical Veterinary Microbiology. 2nd ed. Philadelphia: Mosby Ltd; 2013.
16. Pacheco LGC, Pena RR, Castro TLP, Dorella FA, Bahia RC, Carminati R, et al. Multiplex PCR assay for identification of Corynebacterium pseudotuberculosis from pure cultures and for rapid detection of this pathogen in clinical samples. J. Med. Microbiol. 2007; 56:480–86.
17. Almeida S, Dorneles E M S, Diniz C, Abreu V, Sousa C, Alves J, et al. Quadruplex PCR assay for identification of *Corynebacterium pseudotuberculosis* differentiating biovar Ovis and Equi. BMC Veterinary Research. 2017; 13:290.

18. Assis GBN, Pereira FL, Zegarra AU, Tavares GC, Leal CA, Figueiredo HCP. Use of MALDI-TOF Mass Spectrometry for the Fast Identification of Gram-Positive Fish Pathogens. Front. Microbiol.2017;8:1492. doi: 10.3389/fmicb.2017.01492
19. Seibold E, Maier T, Kostrzewa M, Zeman E, Splettstoesser W. Identification of *Francisella tularensis* by whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry: fast, reliable, robust, and cost-effective differentiation on species and subspecies levels. J. Clin. Microbiol. 2010; doi: 10.1128/JCM.01953-09.
20. Spier SJ, Leutenegger CM, Carroll SP, Loye JE, Pusterla JB, Carpenter TE, et al. Use of a real-time polymerase chain reaction-based fluorogenic5′ nuclease assay to evaluate insect vectors of *Corynebacterium pseudotuberculosis* infections in horses. Am J Vet Res. 2004; 65:829–34.
21. Biberstein EL, Knight HD, Jang S. Two biotypes of *Corynebacterium pseudotuberculosis*. Vet. Rec. 1971; 89:691–92.
22. Muckle CA, Gyles CL. Characterization of strains of *Corynebacterium pseudotuberculosis*, Can. J. Comp. Med. 1982;46:206–8.
23. Baird GJ, Fontaine MC. *Corynebacterium pseudotuberculosis* and its role in ovine caseous lymphadenitis. J Comp Pathol. 2007; 137:179–210.
24. Shpigel NY, Elad D, Yeruham I, Winkler M, Saran A. An outbreak of *Corynebacterium pseudotuberculosis* infection in an Israeli dairy herd. Vet Rec. 1993; 133:89–94.
25. Britz E, Spier SJ, Kass PH, Edman JM, Foley JE. The relationship between *Corynebacterium pseudotuberculosis* biovar *equi* phenotype with location and extent of lesions in horses. Vet J. 2004; 200:282–6.
26. Nogradi N, Spier SJ, Toth B, Vaughan B. Musculoskeletal *Corynebacterium pseudotuberculosis* infection in horses: 35 cases (1999–2009). J Am Vet Med Assoc. 2012; 241:771–7.
27. Gomide AC, Ibraim IC, Alves JTC, Gomesde SP, Oliveira Silva YR, Santana MP, et al. Transcriptome analysis of *Corynebacterium pseudotuberculosis* biovar Equi in two conditions of the environmental stress. Gene. 2018;677:349-60.
28. Szonyi B, Swinford A, Clavijo A, Ivanek R. Re-emergence of Pigeon Fever (*Corynebacterium pseudotuberculosis*). Infection in Texas Horses: Epidemiologic Investigation of Laboratory-Diagnosed Cases. J Equine Vet Sci. 2014;34(2):281-7.

29. Kilcoyne I, Spier SJ, Carter CN, Smith JL, Swinford AK, Cohen ND. Frequency of *Corynebacterium pseudotuberculosis* infection in horses across the United States during a 10-year period. J Am Vet Med Assoc. 2014; 245:309–14.
30. Aleman M, Spier SJ, Wilson WD, Doherr M. *Corynebacterium pseudotuberculosis* infection in horses: 538 cases (1982–1993) J Am Vet Med Assoc. 1996;209:804–9.
31. Bemer-Melchior P, Haloun A, Riegel P, Drugeon HB. Bacteremia due to *Dietzia maris* in an immunocompromised patient. Clin. Infect. Dis. 1999; 29:1338–40.
32. Kowalski K, Szewczyk R, Druszczyńska M. Mycolic acids-potential biomarkers of opportunistic infections caused by bacteria of the suborder Corynebacterineae. Postepy Hig Med Dosw (Online). 2012; 29:66:461-8.
33. Rainey FA, Klatte S, Kroppenstedt RM, Stackebrandt E. Dietzia, a new genus including *Dietzia maris* comb. nov. formerly *Rhodococcus maris*. Int J Syst Bacteriol. 1995; 45:32–6.
34. Kanae T, Tomohiko T, Satoshi H, Takafumi S, Hiroko K, Ken-ichiro S, Hiroaki S. Simple and rapid characterization of mycolic acids from *Dietzia* strains by using MALDI spiral-TOFMS with ultra-high mass-resolving power. The Journal of Antibiotics. 2013; 66:713-17.
35. Yassin AF, Spröer C, Siering C, Hupfer H, Schumann P. *Arthrobacter equi* sp. nov., isolated from veterinary clinical material. Int J Syst Evol Microbiol. 2011;61(9):2089-94.
36. Vargha M, Takaá ts Z, Konopka A, et al. Optimization of MALDI-TOF MS for strain level differentiation of *Arthrobacter* isolates. J Microbiol Methods. 2006; 66:399-409.
37. Mages IS1, Frodl R, Bernard KA, Funke G. Identities of *Arthrobacter* spp. and *Arthrobacter*-like bacteria encountered in human clinical specimens. J Clin Microbiol. 2008;46(9):2980-6.
38. Yamamoto K, Hayakawa K, Nagamatsu M, Fujiya Y, Mawatari M, Kutsuna S, et al. Bacteremia Due to *Arthrobacter creatinolyticus* in an Elderly Diabetic Man with Acute Cholangitis. Jpn J Infect Dis. 2017;70(2):201-2.
39. Osorio CR, Barja JL, Hutson RA, Collins MD. *Arthrobacter rhombi* sp. nov., isolated from Greenland halibut (Reinhardtius hippoglossoides). Int J Syst Bacteriol. 1999; 49:1217–20.
40. Collins MD, Hoyles L, Foster G, Falsen E, Weiss N. *Arthrobacter nasiphocae* sp. nov., from the common seal (*Phoca vitulina*). Int J Syst Evol Microbiol. 2002; 52:569–71.
41. Storms V, Devriese LA, Coopman R, Schumann P, Vyncke F, Gillis M. *Arthrobacter gandavensis* sp. nov., for strains of veterinary origin. Int J Syst Evol Microbiol. 2003; 53:1881-4.

10. APÊNDICE

No apêndix contém trabalhos desenvolvidos durante o mestrado e um resumo de cada análise que realizei nos devidos trabalhos, e por fim o currículo lattes dos últimos dois anos relatando as atividades que realizei durante o mestrado (como certificado de cursos, eventos, apresentação de pôster, monitoria de cursos de verão).

Artigos em que tive participação durante o mestrado.

Artigo publicado

ORIGINAL RESEARCH
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Mutation of the Surface Layer Protein SlpB Has Pleiotropic Effects in the Probiotic *Propionibacterium freudenreichii* CIRM-BIA 129

Fillipe L. R. do Carmo [1,2,3], Wanderson M. Silva [4,5], Guilherme C. Tavares [6], Izabela C. Ibraim [1], Barbara F. Cordeiro [1], Emiliano R. Oliveira [1], Houem Rabah [2,3], Chantal Cauty [2], Sara H. da Silva [1], Marcus V. Canário Viana [1], Ana C. B. Caetano [1], Roselane G. dos Santos [1], Rodrigo D. de Oliveira Carvalho [7], Julien Jardin [2], Felipe L. Pereira [6], Edson L. Folador [8], Yves Le Loir [2,3], Henrique C. P. Figueiredo [6], Gwénaël Jan [2,3]† and Vasco Azevedo [1]†*

OPEN ACCESS

Neste artigo, minha participação foi relacionada a montagem, anotação e deposito do genoma completo no NCBI. Esta bactéria apresentava uma mutação, inserindo um plasmídeo no gene *slpB*. Assim, verifiquei se esse gene mutado estava fazendo parte de um operon. Foi realizado também a geração das imagens do mapa genômico comparativo usando o BRIG e o Mapa circular do genoma utilizando o CGview.

Artigo submetido na Scientific Reports

Detailed Status Information

Manuscript #	SREP-19-21085
Current Revision #	0
Submission Date	20th May 19
Current Stage	Quality Check Started
Title	Whole-genome analyses with a novel multidrug resistant Klebsiella pneumoniae strain from Brazil
Manuscript Type	Original Research
Collection	N/A
Corresponding Author	Dr. Thiago Luiz de Paula Castro (castrotlp@gmail.com) (Universidade Federal da Bahia)
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Authorship	Yes
Abstract	Klebsiella pneumoniae is one of the most important human pathogens, emerging as an agent of severe community infections. A comprehensive analysis of the genome of this microorganism could provide a better understanding of the molecular basis of its virulence and pathogenesis, contributing to new forms of disease control. In this study, we sequenced the genome of a multidrug resistant K. pneumoniae strain, herein named B31, isolated from a patient in Brazil. The genome sequencing of B31 resulted in a 5.27-Mb sized chromosome, one multireplicon plasmid (IncFIIk/ IncFIBk), and an IncI1 replicon likely to belong to another plasmid. The characterization of its genome confirmed that B31 is a multi-drug resistant (MDR) strain and belongs to the sequence type ST15, reported in Brazil for the first time in the present work. Comparative genomic analyses were conducted including B31 and 172 K. pneumoniae genomes from different parts of the world. We provide relevant information on the antibiotic resistance profiles and distribution of virulence-associated genetic elements in B31 and other strains.
Techniques	Life sciences techniques [Genomic analysis]; Life sciences techniques, Genomic analysis [DNA sequencing];
Subject Terms	Biological sciences/Biological techniques/Genomic analysis/Comparative genomics Biological sciences/Microbiology/Antimicrobials/Antimicrobial resistance
Competing Interests Policy	There is **NO** Competing Interest.
Applicable Funding Source	No Applicable Funding

Neste artigo ajudei com o trabalho experimental, e escrita do manuscrito.

Currículo lattes dos últimos dois anos relatando as atividades que realizei durante o mestrado (como certificado de cursos, eventos, apresentação de pôster, monitoria de cursos de verão).

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2017 Mestrado em Bioinformática.
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil
Orientador: Vasco Ariston de Carvalho Azevedo

2012 - 2016 Graduação em Ciências biológicas.
Faculdades Integradas Pitágoras, FIP/MOC, Montes Claros, Brasil
Título: Biotyper para identificação e diferenciação de biovares de *Corynebacterium pseudotuberculosis*
Orientador: Juliana Yporti

2010 - 2011 Ensino Profissional de nível técnico em Técnico em Farmácia.
Instituto Superior de Ciências da Saúde, INCISA, Belo Horizonte, Brasil

Formação complementar

2019 - 2019 Curso de curta duração em Detecção de Variantes, (Carga horária: 4h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil

2014 - 2014 Curso de curta duração em Treinamento na base Integrity Biomarkers. (Carga horária: 3h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil
Palavras-chave: Bioinformática, Proteoma

2013 - 2013 Curso de curta duração em Metabolismo, nutrição e atividade física. (Carga horária: 4h).
Centro Universitário Metodista Izabela Hendrix, IMIH, Belo Horizonte, Brasil
Palavras-chave: metabolismo, nutrição, atividade fisica

2013 - 2013 Curso de curta duração em Genética Forense. (Carga horária: 4h).
Centro Universitário Metodista Izabela Hendrix, IMIH, Belo Horizonte, Brasil

2004 - 2004 Curso de curta duração em Programa como vender mais e melhor. (Carga horária:

20h).

Sebrae, SEBRAE- MG, Brasil

Atuação profissional

1. Universidade Federal de Minas Gerais - UFMG

Vínculo institucional

2017 - Atual Vínculo: Bolsista, Enquadramento funcional: Estudante de mestrado, Regime: Dedicação exclusiva

Outras informações:

Desenvolve atividades de pesquisa no laboratório de Genética Celular e Molecular da Universidade Federal de Minas Gerais

2015 - 2016 Vínculo: Bolsista, Enquadramento funcional: Iniciação Científica, Carga horária: 30, Regime: Parcial

Outras informações:

Laboratório de Genética Celular e Molecular - Universidade Federal de Minas Gerais Supervisor: Prof Vasco Azevedo Projeto: Biotyper para a identificação e diferenciação de biovares de Corynebacterium pseudotuberculosis

2014 - 2015 Vínculo: Estágio voluntário, Enquadramento funcional: Iniciação Científica, Carga horária: 20, Regime: Parcial

Outras informações:

Laboratório de Genética Celular e Molecular - Universidade Federal de Minas Gerais Supervisor: Prof Vasco Azevedo Projeto:Analysis and characterization of functional genes in metabolic pathways of carbohydrate utilization in strains of Corynebacterium pseudotuberculosis 15 using computational system

2. Linhagen Biotecnologia - LINHAGEN

Vínculo institucional

2013 - 2014 Vínculo: Bolsista, Enquadramento funcional: Estagiário, Carga horária: 30, Regime: Parcial

Outras informações:

Atuando na extração DNA, Reação em cadeia da Polimerase (PCR), Preparo de soluções, Rotulagem de amostras (crina, sangue, sêmem)

3. Instituto Materno e Infantil Vila da Serra - HVS

Vínculo institucional

2010 - 2013 Vínculo: CLT, Enquadramento funcional: Auxiliar/ Técnico Famácia , Carga horária: 44, Regime: Integral

Outras informações:

Atuando na dispensa de medicamentos nas unidades do bloco cirúrgico, obstétrico e UTI (Unidade de Tratamento Intensivo), análises de receitas, inventários de medicamentos

4. Prudente casa de lanches LTDA-EPP - PCL

Vínculo institucional

2006 - 2009 Vínculo: CLT, Enquadramento funcional: Atendente e Confeiteira, Carga horária: 40, Regime: Dedicação exclusiva

Projetos

Projetos de pesquisa

2013 - 2014 Caracterização das vias de utilização de fontes de carbono e nitrogênio de 15 linhagens *de Corynebacterium pseudotuberculosis*

Situação: Concluído Natureza: Projetos de pesquisa

Alunos envolvidos: Graduação (1); Mestrado acadêmico (1);

Integrantes: Roselane Gonçalves dos Santos; Carlos Augusto Almeida Diniz; Vasco Azevedo (Responsável); Sintia Almeida

Financiador(es): Fundação de Amparo à Pesquisa do Estado de Minas Gerais-FAPEMIG

Áreas de atuação

1. Genética Molecular e de Microorganismos

Idiomas

Inglês Compreende razoavelmente, Fala Pouco, Escreve razoavelmente, Lê razoavelmente

Português Compreende Bem, Fala Bem, Escreve Bem, Lê Bem

Prêmios e títulos

2017 3º Lugar Melhor Trabalho Apresentado no III Encontro de Ciência, Cultura e Arte ICB- UFMG, UFMG

Produção

Produção bibliográfica

Artigos completos publicados em periódicos

1. DO CARMO, FILLIPE L. R.; SILVA, WANDERSON M.; TAVARES, GUILHERME C.; IBRAIM, IZABELA C.; CORDEIRO, BARBARA F.; OLIVEIRA, EMILIANO R.; RABAH, HOUEM; CAUTY, CHANTAL; DA SILVA, SARA H.; CANÁRIO VIANA, MARCUS V.; CAETANO, ANA C. B.; **Dos Santos, Roselane G.**; DE OLIVEIRA CARVALHO, RODRIGO D.; JARDIN, JULIEN; PEREIRA, FELIPE L.; FOLADOR, EDSON L.; LE LOIR, YVES; FIGUEIREDO, HENRIQUE C. P.; JAN, GWÉNAËL; AZEVEDO, VASCO

Mutation of the Surface Layer Protein SlpB Has Pleiotropic Effects in the Probiotic Propionibacterium freudenreichii CIRM-BIA 129. Frontiers in Microbiology. , v.9, p.1 - 22, 2018.

Referências adicionais : Português. Meio de divulgação: Meio digital. Home page: [doi:10.3389/fmicb.2018.01807]

Apresentação de trabalho e palestra

1. **GONÇALVES-SANTOS, R.**

Genomic characterization of Glutamicibacter creatinolyticus LGCM259: a comparative approach, 2019. (Seminário,Apresentação de Trabalho)

Referências adicionais : Brasil/Português. Meio de divulgação: Outro; Local: ICB-UFMG; Cidade: Belo Horizonte; Evento: Programa de Pós graduação em Bioinformática; Inst.promotora/financiadora: Universidade Federal de Minas Geris

2. RIBEIRO, B. S.; CAETANO, A. C. B.; SILVA, A. L.; **GONÇALVES-SANTOS, R.**; VIANA M.V.C; Profeta R; SEYFFERT, N.; LE-LOIR, Y.; BRENIG, B.; Azevedo, V; CASTRO, T. L. P.

Complete genome sequence of Staphylococcus aureus strain O17, 2018. (Congresso,Apresentação de Trabalho)

Referências adicionais : Brasil/Inglês. Meio de divulgação: Impresso; Local: São Paulo; Cidade: São Pedro; Evento: X-meeting 2018; Inst.promotora/financiadora: 14th International Conference of the AB3C São Pedro

3. **GONÇALVES-SANTOS, R.**

Descubra novas estratégias contra patógenos,, 2018. (Outra,Apresentação de Trabalho)

Referências adicionais : Brasil/Português. Meio de divulgação: Outro; Evento: #Explora UFMG Jovem; Inst.promotora/financiadora: Universidade Federal de Minas Gerais

4. CAETANO, A. C. B.; **GONÇALVES-SANTOS, R.**; PARISE, D.; VIANA M.V.C; Profeta R; TIWARE, S.; Garcia A G; SEYFFERT, N.; Azevedo, V; CASTRO, T. L. P.

Identification of core, accessory and exclusive genes of Staphylococcus aureus isolated from ovine mastitis, 2018. (Conferência ou palestra,Apresentação de Trabalho)

Referências adicionais : Brasil/Português. Meio de divulgação: Impresso; Cidade: Belo Horizonte; Evento: Gene Time Conference; Inst.promotora/financiadora: Universidade Federal de Minas Geris

5. LUZ;, B. S. R.; SEYFFERT, N.; TIWARE, S.; SANTANA, K. T. O; CASTRO, T. L. P.; **GONÇALVES-SANTOS, R.**; CAETANO, A. C. B.; Azevedo, V

CONSTRUÇÃO DE LINHAGENS MUTANTES DE CORYNEBACTERIUM PSEUDOTUBERCULOSIS PARA GLICOCONJUGADOS DA PAREDE CELULAR, 2017. (Outra,Apresentação de Trabalho)

Referências adicionais : Brasil/Português; Cidade: Belo Horizonte; Evento: XXVI SEMANA DE INICIAÇÃO CIENTÍFICA; Inst.promotora/financiadora: Universidade Federal de Minas Gerais

Eventos

Eventos

Participação em eventos

1. **III Curso de Verão em Bioinformática da UFMG**, 2019. (Outra)

.

2. **Darwin day**, 2018. (Simpósio)

.

3. **Doenças Neurodegenerativas no Século XXI**, 2018. (Outra)

.

4. **Explora UFMG Jovem**, 2018. (Encontro)
Descubra novas estratégias contra patógenos.

5. Apresentação de Poster / Painel no(a) **Gene Time Conference**, 2018. (Outra)
Identification of core, accessory and exclusive genes of Staphylococcus aureus isolated from ovine mastitis.

6. **Simpósio de genômica do câncer – Oncologia de Precisão e Personalizada,** 2018. (Simpósio)

.

7. **Técnicas de NGS e principais aplicações e tendências**, 2018. (Outra)

.

8. **Uso de tecnologias de sequenciamento de nova geração na vigilância genômica de Flavivírus emergentes e identificação de novos vírus circulantes**, 2018. (Outra)

.

9. Apresentação (Outras Formas) no(a)**III Mostra de Pós-graduação do 3o Encontro de Ciência, Cultura e Arte do ICB, na Semana do Conhecimento, da UFMG**, 2017. (Exposição)
A bioinformática que você não vê, mas crê.

Organização de evento

1. **GONÇALVES, R. R.**
III Curso de Verão em Bioinformática da UFMG, 2019. (Outro, Organização de evento)
Referências adicionais : Brasil/Português. Meio de divulgação: Outro

2. **GONÇALVES, R. R.**
II Curso de Verão em Bioinformática, 2018. (Outro, Organização de evento)
Referências adicionais : Brasil/Português. Meio de divulgação: Outro

3. **GONÇALVES, R. R.**
Uso de tecnologias de sequenciamento de nova geração na vigilância genômica de

Flavivírus emergentes e identificação de novos vírus circulantes, 2018. (Outro, Organização de evento)

Referências adicionais : Brasil/Português. Meio de divulgação: Outro

Bancas

Bancas

Participação em banca de comissões julgadoras

Outra

1. **“Estudos tecnológicos no tratamento de urina por destilação solar visando a extração de estruvita para utilização como fertilizante natural”**, 2018

Universidade Federal de Minas Gerais

Referências adicionais : Brasil/Português.

2. **“Lanterna guia: Uma proposta para aumentar a autonomia de portadores de deficiência visual”**, 2018

Universidade Federal de Minas Gerais

Referências adicionais : Brasil/Português.

Totais de produção

Produção bibliográfica

Artigos completos publicados em periódico..... 1

Trabalhos publicados em anais de eventos..... 2

Apresentações de trabalhos (Conferência ou palestra) 2

Apresentações de trabalhos (Congresso)..... 3

Apresentações de trabalhos (Seminário)..... 1

Apresentações de trabalhos

(Simpósio)..... 7

Apresentações de trabalhos

(Outra)..... 5

Eventos

Participações em eventos

(congresso)..... 2

Participações em eventos

(seminário)..... 1

Participações em eventos

(simpósio)..... 7

Participações em eventos

(encontro)..... 2

Participações em eventos

(outra)..... 14

Organização de evento

(outro)..... 3

Participação em banca de comissões julgadoras

(outra)..... 2