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IDENTIFICAÇÃO COMPUTACIONAL DE ALVOS DE DROGAS E CANDIDATOS A VACINAS EM *Mycoplasma genitalium*

Belo Horizonte
2021

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Dissertação apresentada ao Programa Interunidades de Pós-graduação em Bioinformática da Universidade Federal de Minas Gerais como requisito parcial para a obtenção do título de Mestre em Bioinformática.

Orientador: Prof. Dr. Rommel Thiago Jucá Ramos

Co-orientador: Prof. Dr. Vasco Ariston de Carvalho Azevedo

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ATA DA DEFESA DE DISSERTAÇÃO

WYLERSON GUIMARÃES NOGUEIRA

Às nove horas do dia **28 de janeiro de 2021**, reuniu-se, no aplicativo Zoom a Comissão Examinadora de Dissertação, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho intitulado: **"Identificação computacional de alvos de drogas e candidatos a vacinas em Mycoplasma genitalium"**, requisito para obtenção do grau de Mestre em **Bioinformática.** Abrindo a sessão, o Presidente da Comissão, **Dr. Rommel Thiago Juca Ramos**, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra ao candidato, para apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa do candidato. Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	**Instituição**	**Indicação**
Dr. Rommel Thiago Juca Ramos	UFPA	Aprovado
Dr. Vasco Ariston de Carvalho Azevedo	UFMG	Aprovado
Dr. Sandeep Tiwari	UFMG	Aprovado
Dr. Siomar de Castro Soares	UFTM	Aprovado

Pelas indicações, o candidato foi considerado: Aprovado

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

Belo Horizonte, 28 de janeiro de 2021.

Dr. Rommel Thiago Juca Ramos - Orientador

Dr. Vasco Ariston de Carvalho Azevedo - Coorientador

Dr. Sandeep Tiwari

Dr. Siomar de Castro Soares

Documento assinado eletronicamente por **Sandeep Tiwari**, **Usuário Externo**, em 28/01/2021, às 10:47, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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À todas as LGBTQAI+, dentro e fora da ciência. É tudo nosso, manas.

"*There is no such thing as apolitical science*."

(H. Holden Thorp
– editor-in-chief of *Science*, 2020)

RESUMO

Mycoplasma genitalium é um patógeno sexualmente transmissível caracterizado como uma bactéria pleomórfica, em forma de pêra, de crescimento lento e intracelular obrigatório. É um dos patógenos de ISTs (infecções sexualmente transmissíveis) associados à uretrite não gonocócica em homens e a várias síndromes inflamatórias do trato reprodutivo em mulheres, como cervicite, doença inflamatória pélvica (DIP) e infertilidade. Neste trabalho, aplicamos vacinologia reversa e abordagens de genômica subtrativa para a predição *in silico* de potenciais candidatos a vacinas e alvos de drogas utilizando cinco genomas completos de *M. genitalium*. Identificamos 403 genes compartilhados por todas as cinco cepas usando Orthofinder, dos quais 104 proteínas são não-homólogas ao hospedeiro. Destas, 44 são expostas, secretadas ou de membrana, e 60 proteínas são citoplasmáticas, de acordo com as análises do software SurfG+. A essencialidade, funcionalidade e afinidade de ligação estrutural destas proteínas também foram verificadas. No geral, predizemos: 19 alvos de vacinas candidatas usando o software Vaxign – uma permease de transportador ABC; um componente EIICBA de glicose PTS; uma proteína P1 de adesão; uma diacilgliceril transferase; uma proteína M bloqueadora de IgG; e 14 proteínas hipotéticas; e, 7 alvos de fármacos – uma proteína de restrição-modificação do tipo I; Acilhidroperóxido redutase, *Ahp*; fator de ligação ao ribossomo A, *RbfA*; Proteína L32 de 50S ribossomal; Classe Ib ribonucleosídeo difosfato redutase, *NrdI*; uma proteína contendo o domínio DUF3217; e uma proteína hipotética. Além disso, realizamos uma análise de docking molecular usando AutoDock Vina para cada alvo de droga contra um banco de dados de 5008 compostos naturais antimicrobianos, extraído do ZINC Database, selecionando as moléculas ZINC08636510, ZINC04235924, ZINC15709489, ZINC04237087, ZINC04236001 e ZINC35415766 por suas interações favoráveis contra os resíduos do sítio ativo dos respectivos alvos. Em suma, predizemos 14 candidatos a vacinas que ainda não foram descritos. Em relação aos alvos de drogas, este estudo foi o primeiro a revelar tanto a proteína hipotética WP_009885876.1 quanto a proteína contendo o domínio DUF3217 WP_009885939.1 como novos possíveis alvos de drogas contra *M. genitalium*. Por fim, tanto as vacinas candidatas quanto os alvos de drogas aqui identificados podem contribuir para o desenvolvimento futuro de estratégias terapêuticas para controlar a disseminação de *M. genitalium* em todo o mundo.

Palavras-chave: *Mycoplasma genitalium*. Infecções Sexualmente Transmissíveis (ISTs). Vacinologia reversa. Candidatos à vacina. Alvos de drogas.

ABSTRACT

Mycoplasma genitalium is a sexually transmitted pathogen characterized as a pleomorphic, flask-shaped, slow-growing, and obligate intracellular bacterium. It is one of the STI (sexually transmitted infections) pathogens associated with non-gonococcal urethritis in men and several inflammatory reproductive tract syndromes in women, such as cervicitis, pelvic inflammatory disease (PID), and infertility. Here, we applied reverse vaccinology and subtractive genomic approaches for the *in silico* prediction of potential vaccine candidates and drug targets against five strains of *M. genitalium*. We identified 403 genes shared by all five strains using Orthofinder, from which 104 proteins are non-host homologous. From those, 44 exposed, secreted, or membrane, and 60 cytoplasmic proteins, as per SurfG+ analyses. Their essentiality, functionality, and structure-based binding affinity were also checked. Overall, we predicted: 19 candidate vaccine targets using Vaxign software – an ABC transporter permease; a PTS glucose EIICBA component; an adhesion P1 protein; a diacyl glyceryl transferase; an IgG blocking protein M; and 14 hypothetical proteins; and, 7 drug targets – Type I restriction-modification protein; Akylhydroperoxide reductase, *Ahp*; ribosome-binding factor A, *RbfA*; 50S ribosomal protein L32; Class Ibribonucleoside diphosphate reductase, *NrdI*; a DUF3217 domain-containing protein; and a hypothetical protein. Furthermore, we performed a molecular docking analysis using AutoDock Vina for each drug target against a 5008 antimicrobial natural-compounds database retrieved from the ZINC Database, selecting ZINC08636510, ZINC04235924, ZINC15709489, ZINC04237087, ZINC04236001, and ZINC35415766 as promising molecules with favorable interactions with the target's active site residues. Finally, we predicted 14 potential vaccine targets that have not yet been described. Concerning our predicted drug targets, this study was the first to reveal both predicted hypothetical protein (WP_009885876.1) and the DUF3217 domain-containing protein (WP_009885939.1) as novel putative drug targets against *M. genitalium*. Altogether, both vaccine candidates and drug targets identified here may contribute to the future development of therapeutic strategies to control the spread of *M. genitalium* worldwide.

Key-words: *Mycoplasma genitalium*. Sexually transmitted infections (STIs). Reverse vaccinology. Vaccine candidates. Drug targets.

ESTRUTURA DA DISSERTAÇÃO

Essa dissertação está organizada em três seções e dois anexos:

(I). Introdução, Justificativa e Objetivos;

(II). Manuscrito completo em língua inglesa, formatado de acordo com as normas do periódico científico internacional *Genomics*;

(III). Considerações finais e Perspectivas.

O Anexo A está constituído de um artigo publicado na revista *Gene*, desenvolvido durante o mestrado e relacionado à imunobioinformática do patógeno bacteriano zoonótico *Corynebacterium pseudotuberculosis*.

O Anexo B está constituído de um capítulo de livro publicado em e-book junto ao Programa 1000 Futuros Cientistas da Universidade Federal de Minas Gerais, desenvolvido durante o evento I Congresso Nacional de Inovação e Popularização da Ciência e relacionado a relatos de estudantes de pós-graduação durante a pandemia de COVID-19.

E, por fim, Anexo C contém o currículo lattes dos últimos dois anos relatando as atividades realizadas durante o mestrado, tais como participações em eventos, organizações de eventos, monitorias, apresentações de palestras, minicursos ministrados e premiações.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

ONU	Organização das Nações Unidas
OMS	Organização Nacional da Saúde
ISTs	Infecções Sexualmente Transmissíveis
HSV	Herpes Simplex Vírus
HIV	Vírus da Imunodeficiência Humana
HPV	Papilomavírus Humano
AIDS	Síndrome da Imunodeficiência Adquirida
SUS	Sistema Único de Saúde
PeP	Profilaxia Pós-Exposição
PreP	Profilaxia Pré-Exposição
kB	Quilobyte
GC	Razão de Conteúdo Guanina-Citosina
Mgpar	Repetições do Elemento Gênico MgPa
DIP	Doença Inflamatória Pélvica
HSH	Homens que fazem Sexo com Homens
qPCR	Reação em Cadeia de Polimerase Quantitativa
RV	Vacinologia Reversa
MenB	*Neisseria meningitidis* Sorogrupo B
DNA	Ácido Desoxirribonucleico
RNA	Ácido Ribonucleico
ORFs	*Open Reading Frames*
PGRV	Vacinologia Reversa Pangenômica
DEG	Banco de Dados de Genes Essenciais
ACD	*Available Chemicals Database*
NCBI	*National Center for Biotechnology Information*

SUMÁRIO

1. INTRODUÇÃO GERAL

1.1. INFECÇÕES SEXUALMENTE TRANSMISSÍVEIS (ISTs)

A Agenda 2030 para o Desenvolvimento Sustentável da Organização das Nações Unidas (ONU) dedica a sua meta nº 03 para saúde global, a fim de "garantir vidas saudáveis e promover o bem-estar para todos em todas as idades". Conforme descrito pela Organização Mundial da Saúde (OMS), um componente importante para o alcance desses objetivos são os princípios norteadores e as ações prioritárias para o fim da epidemia de Infecções Sexualmente Transmissíveis (ISTs) como problema de saúde pública (UNITED NATIONS, 2018).

O termo IST é usado para se referir a uma condição transmitida de uma pessoa a outra por meio do contato sexual, podendo ser adquiridas por sexo vaginal, anal ou oral desprotegido com alguém que tenha a IST (SUBBARAO; AKHILESH, 2017). As ISTs são a principal causa global de doenças agudas, infertilidade, incapacidade de longo prazo e morte, com graves consequências médicas e psicológicas para milhões de homens, mulheres e crianças (DE WAURE *et al.*, 2015). Além disso, ISTs frequentemente resultam em estigmas, estereótipos, vulnerabilidade e têm sido associadas à violência de gênero (AMIN; MORENO, 2013). A OMS estimou em 2012 que havia 357,4 milhões de novos casos globais de clamídia, gonorreia, sífilis e tricomoníase, quatro das ISTs curáveis mais comuns (NEWMAN *et al.*, 2015).

Apesar da terminologia, existem inúmeras formas de transmissão das ISTs além do contágio por intermédio de relações sexuais. Dependendo da IST específica, as infecções também podem ser transmitidas por meio do compartilhamento de agulhas e da amamentação. Durante uma gestação, também é possível que mulheres grávidas transmitam ISTs ao feto durante a gravidez ou ao recém-nascido durante o parto (WATSON-JONES *et al.*, 2002). Em recém-nascidos, as ISTs podem causar complicações severas como encefalite neonatal, infecções oculares e pneumonia. Em alguns casos, essas infecções podem ser fatais ou demandarem parto por cesariana para reduzir o risco de transmissão durante o momento de nascimento (MULLICK *et al.*, 2005).

Os sintomas mais comuns de ISTs são dor ou desconforto durante o sexo ou urinar; feridas, inchaços ou erupções na pele ou ao redor do pênis ou vagina, testículos, ânus,

nádegas, coxas ou boca; corrimento anormal ou sangramento dos genitais; testículos doloridos ou inchados; e, coceira vaginal ou nos seus entornos (WAGENLEHNER *et al.*, 2016). Os sintomas específicos podem variar, dependendo da IST. As ISTs orais nem sempre são perceptíveis. Quando causam sintomas, geralmente incluem dor de garganta ou feridas ao redor da boca ou garganta (CHOW; FAIRLEY, 2019). Contudo, também é possível contrair uma IST sem desenvolver sintomas e, em muitos casos, as ISTs não causam sintomas perceptíveis (LIMA *et al.*, 2018).

O diagnóstico de ISTs não pode ser realizado apenas com base nos sintomas, sendo necessário o uso de testes de diagnósticos (WI *et al.*, 2019). Diversas ISTs podem ser diagnosticadas através de exames de urina ou sangue, sejam estes testes rápidos ou testes laboratoriais de maior precisão. A coleta de amostras de corrimentos ou feridas em órgãos genitais também pode ser empregada para fins de exame de saúde sexual (PEELING *et al.*, 2006). No caso da confirmação positiva de infecção, é essencial que o tratamento seja obtido o mais rápido possível, haja vista que um quadro instaurado de IST pode aumentar as chances de contrair outras doenças (WARD; RÖNN, 2010).

Os mais diversos tipos de organismos são responsáveis por ISTs, desde bactérias e vírus à pequenos artrópodes e protozoários, sendo estas últimas geralmente tratáveis com medicamentos orais ou tópicos (MARKLE; CONTI; KAD, 2013). Dentre as ISTs bacterianas mais comuns temos: clamídia, causada por *Chlamydia trachomatis*, a mais comumente relatada nos Estados Unidos; sífilis, por *Treponema pallidum*, que costuma passar despercebida em seus estágios iniciais, porém, se não for tratada, em estágio avançado pode levar a perda de visão, perda de audição, perda de memória, doença mental, infecções do cérebro ou da medula espinhal, doenças cardíacas e morte; e, gonorreia, *Neisseria gonorrhoeae*, outra IST bacteriana bastante comum (APEA-KUBI *et al.*, 2004). Todas estas, se diagnosticadas precocemente, são facilmente tratadas com antibióticos. Apesar disto, em *Neisseria gonorrhoeae*, a resistência a antibióticos cresce de modo alarmante, dificultando cada vez as chances de sucesso de seu tratamento (WI *et al.*, 2017). Enquanto que outras bactérias como *Mycoplasma genitalium*, outro agente causador de IST, conseguem evadir o sistema imunológico do hospedeiro e persistem mesmo após o tratamento com macrólidos e tetraciclinas (UNEMO; JENSEN, 2017). Infecções causadas por *M. genitalium* persistentes no organismo do indivíduo podem causar sequelas para toda a vida, tais como danos teciduais no sistema urogenital e infertilidade (SETHI *et al.*, 2012).

Dentre as ISTs virais, as mais comuns são o Herpes Simplex Vírus (HSV ou Herpes), o Vírus da Imunodeficiência Humana (HIV) e o Papilomavírus Humano (HPV) (ALLSWORTH; LEWIS; PEIPERT, 2008). Os quadros clínicos de HSV subdividem-se em herpes genital ou oral, caracterizados pela presença de feridas que se desenvolvem nos órgãos genitais, na boca ou em torno destes. As feridas de herpes geralmente formam crostas, cicatrizam em algumas semanas e causam surtos dolorosos (LOOKER *et al.*, 2015). O HIV pode danificar o sistema imunológico e aumentar o risco de contrair outros vírus ou bactérias e certos tipos de câncer. Se não for tratada, pode levar ao estágio 3 do HIV, conhecido como Síndrome da Imunodeficiência Adquirida (AIDS) (CALDERÓN *et al.*, 2015). O HPV possui muitas cepas diferentes do vírus, algumas são mais perigosas do que outras, e o sintoma mais comum do HPV são verrugas nos genitais, boca ou garganta. Algumas cepas de infecção por HPV podem levar ao câncer, incluindo cancros oral, cervical, vulvar, peniano e retal (GUSTAVSSON *et al.*, 2011).

As ISTs virais não são tratáveis por antibióticos, sendo necessário o uso de medicamentos antivirais, ou antirretrovirais no caso do HIV. Embora a maioria das infecções virais não tenha cura, opções de tratamento estão disponíveis para aliviar os sintomas e reduzir o risco de transmissão (GARCIA; WRAY, 2020). Por exemplo, existem medicamentos disponíveis para reduzir a frequência e a gravidade dos surtos de herpes (WORKOWSKI; BOLAN, 2015). Da mesma forma, o tratamento precoce e eficaz pode ajudar a interromper a progressão do HIV garantindo uma vida saudável ao indivíduo soropositivo. Além disso, o tratamento pode reduzir a níveis indetectáveis a quantidade de HIV no organismo, impedindo a transmissão do HIV a um parceiro sexual (EISINGER; DIEFFENBACH; FAUCI, 2019). Para o HPV, não há tratamento, porém, existe uma vacina disponível para proteger contra algumas das cepas mais perigosas, tais como HPV 16 e HPV 18, maiores responsáveis pelos casos cancerígenos da infecção pelo Papilomavírus Humano (WHEELER *et al.*, 2012).

Algumas outras ISTs não são causadas por vírus nem bactérias, como é o caso dos piolhos-da-púbis (*Pthirus púbis*), ou Chatos (ORION; MATZ; WOLF, 2004). Eles são pequenos insetos que podem fixar residência em seus pelos púbicos e se alimentam de sangue humano. Os sintomas comuns de Chatos incluem coceira e pequenas saliências rosas ou avermelhadas ao redor dos órgãos genitais ou do ânus, febre baixa, falta de energia e irritabilidade. Se não forem tratados, mordidas arranhadas podem infeccionar e acarretar em novas doenças (ORION *et al.*, 2006). Outra doença é a tricomoníase, causada por um protozoário chamado *Trichomonas vaginalis*, associada à micção frequente e, em mulheres, a

um forte odor relacionado com a secreção vaginal. Se não for tratada, a tricomoníase pode levar a infecções da uretra, doença inflamatória pélvica e infertilidade (BOUCHEMAL; BORIES; LOISEAU, 2017). As infestações de Chatos podem ser administradas com o auxílio de pinças para a remoção dos piolhos púbicos, de tratamentos tópicos sem prescrição e da limpeza de roupas, roupas de cama, toalhas e casa. Enquanto a tricomoníase pode ser tratada com antibióticos (WORKOWSKI; BOLAN, 2015).

Outras ISTs menos comuns incluem o cancroide, o linfogranuloma venéreo, o granuloma inguinale, o molusco contagioso e a sarna (MARKLE; CONTI; KAD, 2013). É importante que os parceiros sexuais também sejam tratados com sucesso para ISTs antes da retomada de atividade sexual. Caso contrário, pode acarretar em quadros de reinfecção ou transmissão para novos indivíduos (WYNN *et al.*, 2019).

Atualmente, existem diversas estratégias de profilaxia disponíveis, sendo a principal e mais eficaz de todas o uso de preservativos, geralmente com o emprego da camisinha masculina (HOLMES; LEVINE; WEAVER, 2004). Outras formas de profilaxia, como barreiras dentais ou a camisinha feminina, também podem ser utilizadas (STEEN *et al.*, 2009). No Brasil, o Sistema Único de Saúde (SUS) também disponibiliza gratuitamente a PeP (profilaxia pós-exposição) e a PreP (profilaxia pré-exposição) como alternativas profiláticas para impedir a contaminação por HIV (BENZAKEN *et al.*, 2019). Para prevenir a acepção de ISTs por recém-nascidos, os médicos encorajam que mulheres grávidas incluam também uma triagem por possíveis ISTs durante o seu acompanhamento pré-natal (MULLICK *et al.*, 2005).

Atualmente, a OMS estima que quase um milhão de pessoas são infectadas todos os dias com qualquer uma das quatro principais ISTs curáveis, clamídia, gonorreia, sífilis e tricomoníase (UNEMO *et al.*, 2017). Apesar de sua alta incidência global, as ISTs continuam sendo uma área de pesquisa negligenciada, o que agrava o combate à emergentes infecções multirresistentes, causando uma crise de saúde pública em todo o mundo (WI *et al.*, 2017). Sendo assim, isso torna essencial os estudos e descobertas em novas medidas profiláticas contra ISTs resistentes à tratamentos, tais como vacinas e novos alvos de drogas, para o cumprimento das metas estabelecidas pela Nações Unidas para a saúde global.

1.2. A ESPÉCIE *Mycoplasma genitalium*

Micoplasmas são um gênero de pequenas bactérias (0,2–0,3 μm) cuja principal característica é a ausência de parede celular (TAYLOR-ROBINSON; JENSEN, 2011). O termo *Mycoplasma* (do grego, *Mykes* = fungo, e *Plasma* = forma da célula) surgiu em 1950 e, nos anos 60, estes foram designados como membros da classe *Mollicutes*, que deriva das palavras em Latim macio (*mollis*) e pele (*cutis*) (MARCONE, 2014; SARAYA, 2017). Os micoplasmas são pleiomórficos, não podem ser corados por corante de Gram e tampouco identificados por microscopia óptica (CAZANAVE; MANHART; BÉBÉAR, 2012). Fazem parte deste gênero inúmeros patógenos humanos importantes, tais como *M. pneumoniae*, uma das espécies associadas com a pneumonia bacteriana; *M. penetrans*, agente infeccioso oportunista de pacientes soropositivos para o vírus HIV e indutor de replicação da AIDS; e, *M. genitalium*, causador de infecções do trato urogenital que podem acarretar em abortos espontâneos e infertilidade (BASEMAN *et al.*, 1995; BLAYLOCK *et al.*, 2004; LO *et al.*, 1991, 1993).

Mycoplasma genitalium é um patógeno sexualmente transmissível caracterizado como uma bactéria pleiomórfica, em forma de pêra, de crescimento lento, intracelular obrigatório e é conhecido como o menor procarioto capaz de auto-replicação, com um genoma de 580 kB de comprimento, 32% de guanina / citosina (GC), contendo em média 485 genes codificadores de proteínas (TAYLOR-ROBINSON; JENSEN, 2011). Esta espécie foi cultivada pela primeira vez a partir de espécimes uretrais obtidos de homens com uretrite não-gonocócica em 1981 e foi um dos primeiros genomas bacterianos a ser totalmente sequenciado em 1995 (FRASER *et al.*, 1995; TULLY *et al.*, 1981).

O genoma de *M. genitalium* é o menor dentre os *Mollicutes* totalmente sequenciados, considerado um exemplo do conceito de "célula mínima" (RAZIN, 1997). Apesar do tamanho mínimo, 4% do genoma é composto por elementos repetidos, chamados Mgpar ou repetições de MgPa, apresentando homologia com o gene *mgpB*, por sua vez, codificando a proteína imunodominante MgPa, a principal proteína de adesão de *M. genitalium* (TAYLOR-ROBINSON; JENSEN, 2011). Muitos estudos demonstram que a habilidade de *M. genitalium* de se aderir e penetrar nas células hospedeiras é a causa deste organismo ser capaz de persistir em indivíduos infectados (BASEMAN *et al.*, 1995; JENSEN; BLOM; LIND, 1994). Tais mecanismos de inserção no interior das células epiteliais do hospedeiro são os principais agentes que possibilitam a persistência de *M. genitalium* no organismo mesmo após

o tratamento com antibióticos, assegurando evasão do sistema imune ao patógeno e, também, dificultando o diagnóstico preliminar da infecção por métodos clássicos de testagem (MCGOWIN; POPOV; PYLES, 2009; MONDEJA *et al.*, 2018).

M. genitalium é um dos patógenos de IST associados com a uretrite não-gonocócica em homens e várias síndromes inflamatórias do trato reprodutivo em mulheres, como cervicite, doença inflamatória pélvica e infertilidade (HORNER *et al.*, 1993; JENSEN *et al.*, 1993). Alguns estudos relataram a infecção de *M. genitalium* como causa de infertilidade e resultados adversos da gravidez, como trabalho de parto prematuro (HITTI *et al.*, 2010). Além disso, existem evidências de uma associação de *M. genitalium* com endometrite e doença inflamatória pélvica (DIP). O trato urogenital é o principal local de infecção por *M. genitalium*. A transmissão está mais frequentemente relacionada a relações vaginais desprotegidas (MANHART; KAY, 2010). Transporte retal assintomático foi relatado em homens que fazem sexo com homens (HSH), mas nenhum transporte faríngeo foi reportado (BRADSHAW *et al.*, 2009).

Em relação aos pacientes HIV-positivos, um estudo serial de prevalência foi realizado em uma população de alto risco para HIV na África, durante 2007-2014 (MAHLANGU *et al.*, 2019). Os resultados mostraram que a incidência de infecções por *M. genitalium* foi maior em mulheres HIV-positivas em comparação com mulheres HIV-negativas, sugerindo comportamento de risco ou uma maior imunossupressão em mulheres HIV-positivas, promovendo a aquisição e persistência deste *Mycoplasma* (COHEN *et al.*, 2007). Mais recentemente, os autores de um estudo entre 527 mulheres australianas relataram que as infecções por *M. genitalium* foram significativamente mais frequentes em mulheres HIV-positivas ($p = 0{,}003$) (LUSK *et al.*, 2011). Além disso, a infecção por *M. genitalium* pode aumentar a transmissibilidade do HIV, aumentando a carga viral nas secreções vaginais (MANHART *et al.*, 2008).

Atualmente, nos Estados Unidos, o tratamento da maioria das infecções por *M. genitalium* ocorre principalmente no contexto do manejo sindrômico de uretrite, cervicite e DIP, devido à falta de disponibilidade de testes diagnósticos (MANHART, 2009). Na Europa, existem testes disponíveis, contudo, a adesão pela prática clínica da triagem por esta IST não é comum nas rotinas laboratoriais (SALADO-RASMUSSEN; JENSEN, 2014). No mundo, a principal ferramenta de diagnóstico da infecção por *M. genitalium* são exames por reação em cadeia de polimerase quantitativa (qPCR) em tempo real, que, apesar de eficaz, é um método

restrito somente a centros de pesquisa e de saúde altamente especializados (CAMPOS *et al.*, 2015; GNANADURAI; FIFER, 2020).

Sendo assim, se faz necessário o desenvolvimento de ensaios comercialmente disponíveis para uso no diagnóstico de infecção por *M. genitalium* a fim orientar as decisões de tratamento na prática clínica do manejo de ISTs (POND *et al.*, 2014). A determinação da relação custo-efetividade no diagnóstico generalizado de *M. genitalium* em indivíduos sintomáticos e assintomáticos de alto risco, assim como garantir acesso global à fármacos e profiláticos vacinais eficientes, é vital para que haja a preparação adequada no enfrentamento deste patógeno emergente (IOANNIDIS *et al.*, 2017).

1.3. VACINOLOGIA REVERSA (RV)

As vacinas representam a estratégia profilática mais eficaz na história da medicina para o controle da propagação de doenças infecciosas, aumentando a expectativa de vida humana (SAUTTO *et al.*, 2019). Os termos “vacina” e “vacinação” surgiram em 1796, com os estudos de Edward Jenner acerca do desenvolvimento de vacinas contra a varíola, evitando a infecção ao se isolar materiais a partir da vaca (RIEDEL, 2005). Desde então, quando se descobriu que os microrganismos são a causa de doenças infecciosas, as primeiras regras de vacinação foram ditadas por Louis Pasteur, marcando o início do desenvolvimento de vacinas (SOLLNER *et al.*, 2008). Desde então, a erradicação da varíola e a redução maciça de outras doenças infecciosas, como a poliomielite, o sarampo e a difteria, foram algumas das principais conquistas em saúde pública do século passado, alcançadas através da vacinação (MELLERSON *et al.*, 2020).

Com base nos princípios de Pasteur, "isolar, inativar e injetar", os métodos tradicionais utilizados em vacinas de primeira e segunda geração concentraram-se em organismos inteiros (DOOLAN; APTE; PROIETTI, 2014). Com a disponibilidade do sequenciamento de genoma completo do final do séc. XX, a predição de antígenos *in silico* assumiu o foco dos estudos para o desenvolvimento de vacinas de terceira geração. Em especial, com o auxílio da bioinformática e o advento da Vacinologia Reversa (“*reverse vaccinology”* – RV) (BAMBINI; RAPPUOLI, 2009). Por exemplo, o estudo pioneiro na aplicação da abordagem de RV (RAPPUOLI, RINO, 2000), contra *Neisseria meningitidis* sorogrupo B (MenB), demorou menos de 18 meses para identificar mais vacinas candidatas em MenB do que foi descoberto durante os últimos 40 anos por métodos convencionais (HE; XIANG; MOBLEY, 2010), o que acelerou drasticamente o processo de desenvolvimento da vacina.

A vacinologia reversa analisa a sequência genômica de um patógeno, que é uma sequência codificada para todos os possíveis genes expressos ao longo do ciclo de vida de um agente patogênico (SANTOS *et al.*, 2011). A abordagem de RV parte do genoma completo ao invés do organismo inteiro, e identifica todo o catálogo de proteínas que tem o potencial de serem expressas a qualquer momento. Esta estratégia tem o benefício adicional de ser aplicável a espécies cultiváveis e não cultiváveis (RAPPUOLI, 2001).

O termo ‘reversa’, em RV, deriva da inversão da lógica clássica descrita pelo Dogma Central da Biologia (DNA → RNA → Proteína). Tradicionalmente, a identificação de antígenos partia da observação fenotípica ou da caracterização experimental de proteínas

expressas pelo patógeno, para então se chegar aos genes correspondentes. Com o advento da genômica, esse percurso foi invertido: passou-se a partir da sequência genômica para predizer genes e proteínas potencialmente antigênicos, cuja expressão e imunogenicidade são posteriormente validadas experimentalmente (STRASSER, 2006). Nesse contexto, a vacinologia reversa aplica esse princípio ao rastrear, no genoma de um organismo, proteínas ou fragmentos proteicos com potencial antigênico, capazes de induzir resposta imune adquirida no hospedeiro (RAPPUOLI, 2005).

De modo geral, em estudos de RV, as *Open Reading frames* (ORFs) derivadas da sequência de um genoma são analisados por um conjunto de softwares, *e.g.* Vaxign, NetMHC 4.0, MED. 1.0, para aferir e predizer atributos desejáveis dos prováveis produtos gênicos das ORFs como candidatos à vacina (ANDREATTA; NIELSEN, 2016; HE; XIANG; MOBLEY, 2010; SANTOS *et al.*, 2013). Em especial, em atenção às proteínas exportadas, pois estas são essenciais nas interações hospedeiro-patógeno, tais como: adesão às células hospedeiras; invasão da célula à qual há conformidade; danos nos tecidos do hospedeiro; resistência ao estresse ambiental da maquinaria de defesa da célula infectada; e mecanismos para subversão da resposta imune do hospedeiro (BHAVSAR; GUTTMAN; FINLAY, 2007; SIBBALD; VAN DIJL, 2008; SIMEONE; BOTTAI; BROSCH, 2009; STAVRINIDES; MCCANN; GUTTMAN, 2008).

Desde a concepção da RV, o progresso na análise genômica, proteômica e transcriptômica surtiu um enorme impacto no modo com que novos antígenos estão sendo identificados (RAPPUOLI; ADEREM, 2011; RINAUDO *et al.*, 2009). Fatores como localização subcelular e o número de domínios transmembranares são frequentemente considerados na filtragem bioinformática para um alvo vacinal, uma vez que proteínas da membrana externa contendo mais de uma hélice transmembrana são, em geral, difíceis de clonar e purificar (PIZZA *et al.*, 2000). Com a disponibilidade de cepas não patogênicas para diversas espécies, elementos exclusivos de genomas de linhagens virulentas também começaram a ser observados (DHANDA *et al.*, 2016). Para evitar a autoimunidade, os alvos de vacina preditos são também analisados quanto à similaridade de sequência com proteínas do hospedeiro, seja humano ou animal (DE GROOT, 2006). Para otimizar vacinas baseadas em epítopos, tornou-se uma tarefa essencial prever epítopos imunogênicos com base na estrutura de antígenos protetores, chamada de "vacinologia estrutural" por algumas referências (DORMITZER; ULMER; RAPPUOLI, 2008; RAPPUOLI *et al.*, 2016).

Além disso, ao invés de fazer a pesquisa de alvos de vacina em uma única cepa de um organismo, é possível realizar a prospecção em inúmeros de genomas coletivamente, explorando possíveis antígenos para toda a espécie (LAPIERRE; GOGARTEN, 2009). A disponibilidade de grande número de genomas nos bancos de dados públicos levou ao surgimento da RV Pangenômica (PGRV), ou PanRV (NAZ *et al.*, 2019). A PGRV aplica os conceitos de genomas *core*, acessório e *unique* das ciências pangenômicas na busca por candidatos vacinais. Do ponto de vista da vacina, os genomas *core* e *unique* são bons candidatos para compor uma vacina que seja adequada para todas as cepas estudadas, sem perder de vista particularidades de genes específicos em cada estirpe (SANTOS *et al.*, 2011).

Desde o seu primeiro estudo de caso aplicado, o conceito de RV também foi aplicado com sucesso a diversos outros agentes de doenças infecciosas. Dentre eles, os vírus Ebola (ULLAH *et al.*, 2020), Zika (SALVADOR *et al.*, 2019) e vaccínia (SANCHEZ-SAMPEDRO *et al.*, 2013), as bactérias *Bacillus anthracis* (ARIEL *et al.*, 2002), *Porphyromonas gingivalis* (ROSS *et al.*, 2001), *Chlamydia pneumoniae* (MONTIGIANI *et al.*, 2002), *Streptococcus pneumoniae* (DOROSTI *et al.*, 2019), *Mycoplasma pneumoniae* (RODRIGUES *et al.*, 2019), *Helicobacter pylori* (MEZA *et al.*, 2017), *Mycobacterium tuberculosis* (BETTS, 2002), *Staphylococcus aureus* (OPREA *et al.*, 2013) e *Acinetobacter baumannii* (SOLANKI *et al.*, 2018), além de outros patógenos de interesse veterinário e zoonótico, como *Corynebacterium pseudotuberculosis* (ARAÚJO *et al.*, 2019), *Brucella* spp. (HISHAM *et al.*, 2018), *Leptospira* spp. (GRASSMANN *et al.*, 2017; ZENG *et al.*, 2017), *Edwardsiella tarda* (LIU *et al.*, 2017) e *Flavobacterium columnare* (MAHENDRAN *et al.*, 2016).

Quanto à ISTs, esforços contínuos estão sendo feitos por pesquisadores para identificar novos candidatos a vacinas contra várias infecções sexualmente transmissíveis, como as causadas por Herpes Simplex Virus-1 (SARKAR; ULLAH, 2020), *Neisseria gonorrhoeae*, gonorreia (ZIELKE *et al.*, 2016), *Chlamydia trachomatis*, clamídia (SHIRAGANNAVAR *et al.*, 2020), *Haemophilus ducreyi*, cancroide (FADNAVIS *et al.*, 2018; SAROM *et al.*, 2018), e *Treponema pallidum*, sífilis (JAISWAL *et al.*, 2017).

Apesar desses esforços, vários patógenos de ISTs com infecções multirresistentes e evasivas aos atuais tratamentos, como *M. genitalium*, continuam sem alternativas para o seu combate. Sendo assim, aplicações de abordagens bioinformáticas como a vacinologia reversa, dentre outras, são fundamentais para a geração de novas alternativas de prevenção e tratamento, tais como preparações vacinais, mas, também, novos alvos farmacológicos.

1.4. GENÔMICA SUBTRATIVA E A PROSPECÇÃO DE ALVOS DE DROGAS

No início da década de 1980, a missão de projetar drogas racionalmente usando estruturas de proteínas era um sonho ainda distante dos biólogos estruturais (ANDERSON, 2003), com os primeiros projetos de sucesso sendo publicados apenas no início dos anos 90 (DORSEY *et al.*, 1994; ERICKSON *et al.*, 1990; ROBERTS *et al.*, 1990). Todavia, atualmente, o desenho de medicamentos baseados em estrutura é uma parte vital e integrante da maioria dos programas de descoberta de medicamentos industriais e importante campo da pesquisa acadêmica (MOUNTAIN, 2003).

Atualmente, diversos medicamentos usados para o tratamento de doenças infecciosas causadas por organismos patogênicos apresentam efeitos colaterais de pequeno a grande porte nos pacientes, além de um crescimento alarmante na evolução de cepas multirresistentes aos fármacos de que a medicina dispõe, o que impõe a necessidade de identificar drogas novas e eficazes para combater as doenças (MADABHAVI *et al.*, 2015). Uma abordagem moderna chamada "genômica subtrativa" está amplamente envolvida na identificação de novos e específicos alvos de drogas em organismos patogênicos, como um passo para o reposicionamento ou desenvolvimento de novos fármacos (HOSSAIN *et al.*, 2017).

A genômica subtrativa faz alusão a abordagem matemática para determinar a diferença entre dois valores de uma mesma categoria, onde "subtração" significa literalmente "removido de baixo", ou seja, retirar um pedaço menor de um maior (MADABHAVI *et al.*, 2015). Na genômica subtrativa, geralmente, dois ou mais genomas são utilizados e o conjunto de dados genômicos são subtraídos uns dos outros a fim de revelar os genes específicos de gênero, espécie e fenótipo único (BARH *et al.*, 2011). A identificação de alvos em genômica subtrativa é majoritariamente baseada em genes essenciais e não-homólogos ao hospedeiro (HOSEN *et al.*, 2014).

Genes essenciais são genes necessários para o crescimento, adaptabilidade e sobrevivência de um organismo, sendo letal a deficiência de qualquer um desses genes para o organismo (KAMATH *et al.*, 2003). O Banco de Dados de Genes Essenciais (DEG) é o principal recurso que lista genes essenciais validados experimentalmente em bactérias, fungos, plantas e animais (LUO *et al.*, 2014). O DEG é comumente empregado para a identificação de alvos por abordagens genômicas subtrativas (NAGPAL; USMANI; RAGHAVA, 2018). Um gene essencial não-homólogo de um patógeno, ou seja, não presente no hospedeiro mas presente no patógeno, é considerado o tipo de alvo ideal contra um agente

infeccioso (SAKHARKAR; SAKHARKAR; CHOW, 2008).

Por vias de praxe, um alvo de drogas proteico deve atender a quatro critérios principais: 1- deve ser um gene essencial para a sobrevivência ou patogênese do organismo alvo; 2- "drogabilidade", i.e., tendo características de estrutura de proteína que a tornam passível de se ligar a pequenas moléculas inibidoras; 3- caracterização funcional e estrutural, com ensaios estabelecidos para o rastreio da inibição de pequenas moléculas; e, 4- distinção de alvos de drogas atuais para evitar resistência cruzada (HOLMAN *et al.*, 2009). Além disso, alguns trabalhos em genômica subtrativa também avaliam a localização subcelular de proteínas pré-selecionadas para predizer proteínas de membrana, consideradas alvos putativos de drogas (BARH; MISRA, 2009).

Além disso, somados à genômica subtrativa, estudos *in silico* de *docking* molecular podem levar à descoberta de novos medicamentos para o tratamento de infecções (HOSSAIN *et al.*, 2017). Na triagem virtual, pequenas moléculas ou compostos são encaixados na região de interesse *in silico* e classificados com base nas interações preditas com o sítio-ativo de ligação (ANDERSON, 2003). Existem diversos bancos de dados de biomoléculas para *docking*, e.g., ZINC Database, Available Chemicals Database (ACD) (AZAM; KUMAR; KHAN, 2020; STERLING; IRWIN, 2015). A principal vantagem de realizar o *docking* molecular com compostos de bancos como esses é que os ligantes de sucesso podem ser adquiridos e, finalmente, levados para ensaios *in vitro* e testados por meio de ensaios bioquímicos (SLIWOSKI *et al.*, 2014).

Nos últimos anos, muitos estudos em diversos patógenos empregaram uma abordagem de genômica subtrativa e relataram identificação e reconhecimento bem-sucedidos de novos alvos terapêuticos específicos para suas respectivas espécies de interesse, tais como *Escherichia coli* O157:H7 (MONDAL *et al.*, 2015), *Corynebacterium pseudotuberculosis* (RADUSKY *et al.*, 2015), *Salmonella enterica* (HOSSAIN *et al.*, 2017), *Pseudomonas aeruginosa* (UDDIN; JAMIL, 2018), *Clostridium botulinum* (SUDHA *et al.*, 2019), *Stenotrophomonas maltophilia* (CHAKRABARTY *et al.*, 2020), e *Bartonella bacilliformis* (KHAN *et al.*, 2020). Ainda, alguns desses trabalhos também foram voltados para a genômica subtrativa contra infecções sexualmente transmissíveis, como gonorreia, clamídia, cancroide e sífilis (PRAVEENA *et al.*, 2011; BARH; KUMAR, 2009; JAISWAL *et al.*, 2017; SAROM *et al.*, 2018), demonstrando a importância de contribuições do gênero no enfrentamento das ISTs.

Apesar destes avanços, diversas outras doenças, dentre elas inúmeras ISTs, persistem sem alternativas viáveis de tratamento, sejam por apresentarem infecções resistentes a múltiplas drogas, por ainda não possuírem fármacos eficazes ou por disporem de fatores de virulência que permanecem desconhecidos (YAN; GAO, 2020). Portanto, a genômica subtrativa apresenta-se como grande aliada no combate contra doenças emergentes e vigentes, podendo levar à descoberta de novos medicamentos para o tratamento de infecções atuais e a auxiliar na contenção de grandes surtos futuros.

2. JUSTIFICATIVA

Mycoplasma genitalium é uma bactéria intracelular obrigatória e o patógeno causador de distintas infecções sexualmente transmissíveis (ISTs), incluindo uretrite não gonocócica, endometrite, cervicite e doença inflamatória pélvica (DIP), além de estar relacionada à resultados adversos da gravidez e infertilidade, em ambos os sexos (HITTI *et al.*, 2010). Além disso, a infecção por *M. genitalium* potencializa a transmissibilidade do HIV, aumentando a carga viral nas secreções vaginais (MANHART *et al.*, 2008).

Esse patógeno consegue evadir o sistema imunológico humano e as infecções causadas por *M. genitalium* podem persistir no organismo mesmo após o tratamento com macrólidos e tetraciclinas, causando sequelas por toda a vida (SETHI *et al.*, 2012; UNEMO; JENSEN, 2017). A habilidade de *M. genitalium* de se aderir e penetrar nas células epiteliais hospedeiras também dificultam o diagnóstico preliminar da infecção por métodos clássicos de testagem (MCGOWIN; POPOV; PYLES, 2009; MONDEJA *et al.*, 2018). Atualmente, o combate à *M. genitalium* limita-se ao contexto do manejo sindrômico de infecções do trato urogenital, sendo assim, se faz necessário o desenvolvimento de fármacos e profiláticos vacinais eficientes para o enfrentamento deste patógeno emergente (POND *et al.*, 2014).

Com a disponibilidade do sequenciamento de genoma completo do final do séc. XX, a predição de antígenos *in silico* assumiu o foco dos estudos para o desenvolvimento de candidatos profiláticos com o auxílio da bioinformática (BAMBINI; RAPPUOLI, 2009). Enquanto a vacinologia reversa (RV) tornou-se uma abordagem convencional e popular na era pós-genômica para a seleção de novos candidatos a vacina (BARH *et al.*, 2013), a genômica subtrativa está amplamente envolvida na identificação de alvos de drogas em organismos patogênicos (HOSSAIN *et al.*, 2017). Além disso, somado à triagem virtual, o *docking* molecular pode identificar pequenas moléculas ou compostos já conhecidos como ligantes de sucesso a esses alvos, um passo a mais no desenvolvimento ou reposicionamento de fármacos (SLIWOSKI *et al.*, 2014).

Sendo assim, a aplicação de estratégias de seleção de alvos profiláticos *in silico*, associando a vacinologia reversa à abordagem de genômica subtrativa, proposta por este trabalho, promoverá o estudo e a descoberta de novos candidatos vacinais e alvos de fármacos na investigação de tratamentos para infecções sexualmente transmissíveis persistentes do trato urogenital, visando a prevenção e controle de infecções por *M. genitalium*.

3. OBJETIVOS

3.1. OBJETIVO GERAL

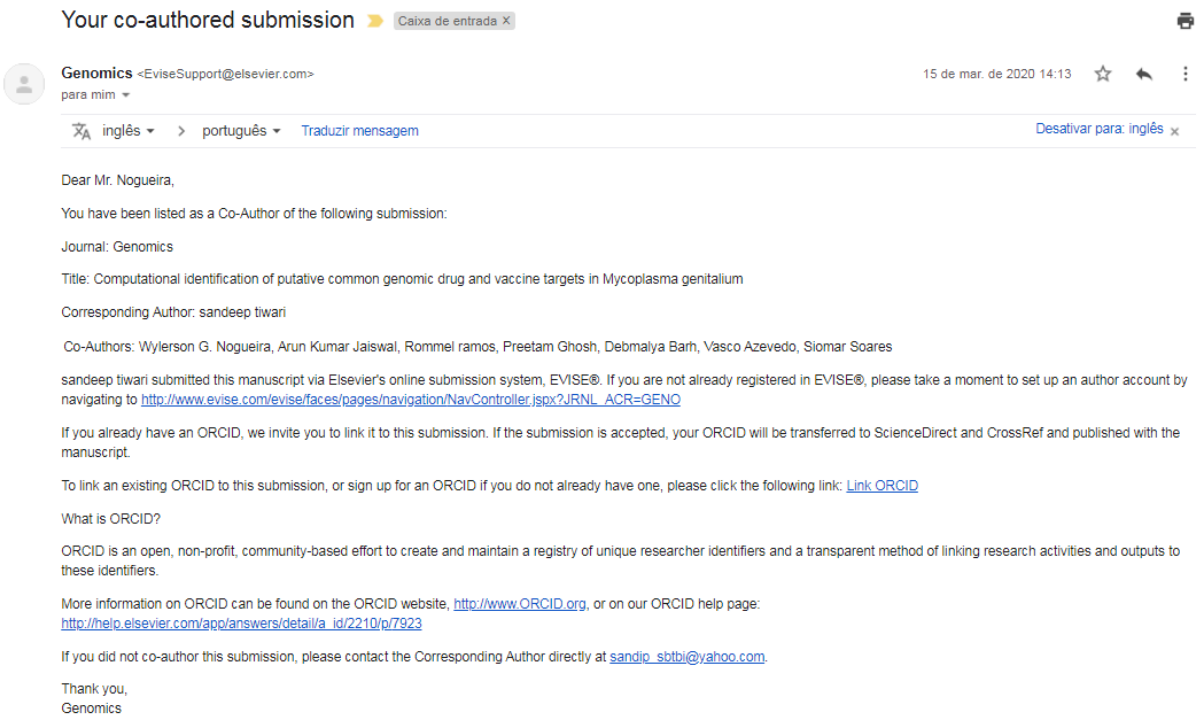
- Realizar a identificação computacional de alvos de drogas e candidatos a vacinas em *Mycoplasma genitalium* por abordagens *in silico*.

3.2. OBJETIVOS ESPECÍFICOS

- Identificar o conjunto de genes compartilhados por todas as linhagens de *M. genitalium*;
- Avaliar o caráter de não-homólogo ao hospedeiro humano, a funcionalidade e a localização subcelular do conjunto de proteínas compartilhadas;
- Realizar a predição de candidatos a vacina por vacinologia reversa;
- Fazer o *screening* virtual de alvos de drogas no conjunto de proteínas citoplasmáticas não-homólogas ao hospedeiro;
- Identificar os compostos naturais com afinidade para com os sítios ativos dos alvos de drogas encontrados por *docking* molecular.

4. MANUSCRITO COMPLETO

O manuscrito completo intitulado “Computational identification of putative common genomic drug and vaccine targets in *Mycoplasma genitalium*”, referente às análises realizadas neste trabalho, foi submetido à revista “Genomics” (ISSN: 08887543) em março de 2020, fator de impacto: 6.205 (2019).



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Computational identification of putative common genomic drug and vaccine targets in *Mycoplasma genitalium*

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Abstract

Mycoplasma genitalium is an obligate intracellular bacterium that is responsible for several sexually transmitted infections, including non-gonococcal urethritis in men and several inflammatory reproductive tract syndromes in women. Here, we applied subtractive genomics and reverse vaccinology approaches for *in silico* prediction of potential vaccine and drug targets against five strains of *M. genitalium*. We identified 403 genes shared by all five strains, from which 104 non-host homologous proteins were selected, comprising of 44 exposed/secreted/membrane proteins and 60 cytoplasmic proteins. Based on the essentiality, functionality, and structure-based binding affinity, we finally predicted 19 (14 novels) putative vaccine and 7 (2 novels) candidate drug targets. The docking analysis showed six molecules from the ZINC database as promising drug candidates against the identified targets. Altogether, both vaccine candidates and drug targets identified here may contribute to the future development of therapeutic strategies to control the spread of *M. genitalium* worldwide.

Keywords: *Mycoplasma genitalium*; sexually transmitted infections (STIs); reverse vaccinology; vaccine candidates; drug targets.

1. Introduction

The 2030 Agenda for Sustainable Development of the United Nations (UN) defines a set of ambitious global health goals. Of particular interest to the proposed strategy was to ensure healthy lives and promote well-being for all ages. This goal builds on the global strategy for the prevention and control of sexually transmitted infections (STIs), promoted by the health sector contributions. As described by the World Health Organization (WHO), an important component towards the achievement of these aims are the guiding principles and priority actions for ending the STI epidemic as a public health problem [1].

Mycoplasma genitalium is a sexually transmitted pathogen characterized as a pleiomorphic, flask-shaped, slow-growing, and obligate intracellular bacterium. It is known as the smallest prokaryote capable of self-replication, with a genome of 580 kb long, 32% of guanine/cytosine (GC) content, and containing 485 protein-coding genes [2]. This species was first cultured by Tully et al. isolated from a male with urethritis (non-gonococcal) in 1981 [3], and it was one of the first bacterial genomes to be totally sequenced in 1995 [4].

M. genitalium is one of the sexually transmitted infection (STI) pathogens associated with non-gonococcal urethritis in men and several inflammatory reproductive tract syndromes in women such as cervicitis, pelvic inflammatory disease, and infertility [5,6]. Some studies have reported the infection of *M. genitalium* as a cause for infertility and adverse pregnancy outcomes such as preterm labor [7]. In addition, there was evidence for an association of *M. genitalium* with endometritis and pelvic inflammatory disease (PID). The urogenital tract is the primary site of *M. genitalium* infection. Transmission is more frequently related to unprotected vaginal intercourse [8]. The asymptomatic rectal carriage has been reported in men who have sex with men (MSM), but no pharyngeal carriage has been reported [9].

Concerning HIV-positive patients, a serial study of prevalence was performed in a population at high risk of HIV in Africa during 2007-2014 [10]. The results showed that the incidence of *M. genitalium* infections was higher in HIV-positive women compared to HIV-negative women, suggesting risk behavior or higher immunosuppression in HIV-positive women promoting the acquisition and persistence of this mycoplasma [11]. More recently, the authors of a study among 527 Australian women reported that *M. genitalium* infections were significantly more frequent in HIV-positive women ($P = 0.003$)

[12]. Furthermore, *M. genitalium* infection may enhance the transmissibility of HIV by increasing the viral load in vaginal secretions [13].

Currently, treatment of most *M. genitalium* infections occurs mainly in the context of syndromic management for urethritis, cervicitis, and PID, owing to the lack of diagnostic test availability in the United States [14]. Testing is readily available in Europe, but it is not clear how commonly it is used [15]. Hence, further development of commercially available assays for use in diagnosing *M. genitalium* infection and guiding treatment decisions is critical [16]. Determining the cost-effectiveness of widespread diagnosis of *M. genitalium* in symptomatic and asymptomatic high-risk individuals is urgently needed to drive this agenda forward, besides the need for more commercially available assays.

In addition, characterization of the surface-exposed antigenic epitopes, important for antibody-mediated killing, should be a priority for the future development of effective drugs and vaccines against bacterial pathogens [17]. Finally, the use of well-defined animal models, the continued exploration of enzymes required for recombination, and the regulation of recombination and other significant processes in this unique bacterium are critical to our understanding of the pathobiology of this emerging pathogen and its associated disease processes [18].

With the advent of new high-throughput sequencing technologies and the rise of genomic data, scientists can use computational methods (i.e., subtractive genomics) to identify new targets, which are more time and cost-effective than classical approaches. Reverse vaccinology (RV) is a conventional and popular approach in the post-genomic era for the prompt identification of novel vaccine targets [19,20]. Approaches adopting RV methods are being widely utilized for targets identification in a myriad of human pathogens, including *Mycobacterium tuberculosis* [21], *Helicobacter pylori* [22], *Burkholderia pseudomallei* [23], *Pseudomonas aeruginosa* [24], *Salmonella typhi* [25], *Neisseria gonorrhoeae* [26], *Haemophilus ducreyi* [27] and *Treponema pallidum* [28].

The availability of pathogen and host genomes makes bioinformatics approaches more attractive and feasible [29,30]. Comparative and subtractive genomic approaches associated with an analysis of metabolic pathways efficiently contribute to the identification of non-host homologous essential proteins of the pathogen [31,32]. When identified, these so-called putative targets are a starting point for drug and vaccine development studies. In the case of a vaccine, this target also needs to be able to elicit a proper and accurate adaptive immune response [26]. Alternatively, in the case of drugs, this target must also be

selected for structure-based selective inhibitor development as a measure of suitable druggability [33,34].

In this study, we mainly focus on the *in silico* identification of putative vaccine and drug targets against *M. genitalium* using reverse vaccinology and subtractive genomics. Furthermore, we also identify natural new lead antimicrobial compounds and possible drug molecules that show favorable interactions, lowered energy values, and high complementarity with the predicted targets.

2. Materials and Methods

The workflow used in this work for the prediction of putative vaccine and drug candidates against *M. genitalium* is detailed in Fig. 1.

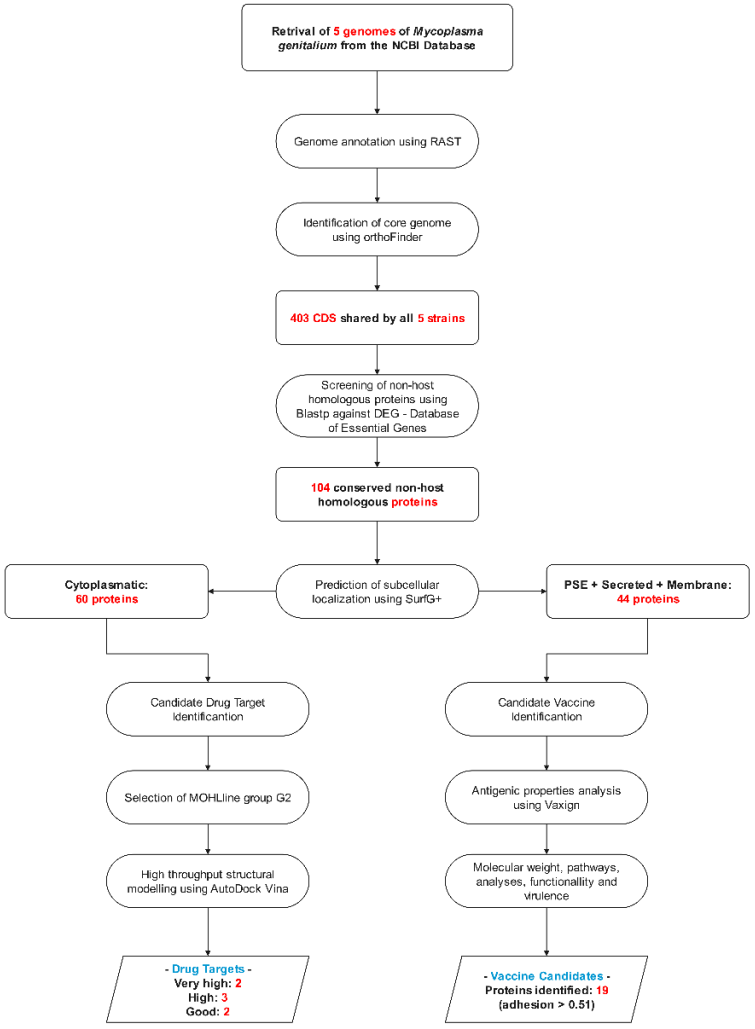

Fig. 1. Designed workflow with the methodologies used for the prediction of vaccine targets and drug candidates and the total number of proteins identified in each step. Categories of drug targets are defined accordingly to MHOLline's quality group parameters [35] (CDS: coding DNA sequence; PSE: putative surface exposed).

2.1. *Collection of Genome Data*

The genome sequences of five *M. genitalium* strains (Table 1) were retrieved from the GenBank database available at the National Center for Biotechnology Information (NCBI) [36]. For homogeneity in the genome annotations, all genomes were annotated using the RAST server (Rapid Annotations using Subsystems Technology) [37]. Furthermore, these annotated genome sequences were used for analysis.

Table 1. General information about the complete genome of the five *M. genitalium* strains used in this work.

Organism/Name	Strain	Assembly	Size (Mb)	GC%	Genes	Proteins	Level
Mycoplasma genitalium G37	G-37	GCA_000027325.1	0.580076	31.7	563	509	Complete Genome
Mycoplasma genitalium M2321	M2321	GCA_000292405.1	0.579977	31.7	564	480	Complete Genome
Mycoplasma genitalium M6320	M6320	GCA_000292485.1	0.579796	31.7	563	474	Complete Genome
Mycoplasma genitalium M2288	M2288	GCA_000292505.1	0.579558	31.7	566	491	Complete Genome
Mycoplasma genitalium M6282	M6282	GCA_000292445.1	0.579504	31.7	567	449	Complete Genome

2.2. *Identification of Intra-Species Conserved Non-Host Homologous Proteins*

The orthologous genes were clustered to obtain a framework to provide insightful information from multiple genomes, highlighting the conservation and divergence of gene families and biological processes. For pathogenic bacteria, clustering orthologs can facilitate the screening for drug and vaccine candidates [28]. We compared five strains of *Mycoplasma genitalium* using orthoFinder software [38] with an E-value of 1×10^{-50}. CDSs shared by all strains were considered the core genome. The possible candidates for drugs and vaccines should be non-homologous to human proteins; hence, autoimmunity is circumvented, and precise a immune response is elicited against the targeted pathogen.

Given that, these core genes were subjected to a BLASTp [39] against the human genome for the identification of non-host homolog targets using an *in house* script.

2.3. *Assessment of Essential Genes*

A subtractive genomics approach was applied to identify conserved targets that were essential to this pathogen [26]. The Database of Essential Genes (DEG) contains experimentally validated data from a series of organisms, including bacteria, archaea, and eukaryotes, that encompass currently reported essential genomic elements, plus protein-coding genes that are indispensable to support cellular life [40]. We prioritized proteins considered essential according to the DEG Database for further analysis since if the target interferes with some vital metabolic pathway of the bacteria, the response to the protein target as a possible drug or vaccine candidate is more likely to be effective [41]. The amino acid sequences from the set of core-conserved proteins of *M. genitalium* was subjected to BLASTp search against the DEG database for homology analyses. The cut-off values used for BLASTp were: E-value ≤ 0.0001, bit score ≥ 100, and identity ≥ 25% [20,22,42].

2.4. *Reverse Vaccinology Approach for Prediction of Putative M. genitalium Vaccine Targets*

The non-host homologous conserved proteome of *M. genitalium* was screened using SurfG+ software [43] to identify secreted proteins, membrane proteins, and putative surface exposed proteins. The presence of signal peptides and the number of transmembrane helixes in a protein candidate are significant criteria for vaccine development, as they are relevant in the process to clone, express, and purify proteins [44]. Hence, we searched for cleavage sites and transmembrane helices in the proteins, using SignalP [45] and TMHMM (Transmembrane Helix prediction server, based on a hidden Markov model), respectively [46]. Also, we predicted the presence of functional domains for the proteins with InterProScan, which uses several databases for domain prediction [47]. The dataset was screened by Vaxign [42], using default parameters, searching for proteins with the following features: major histocompatibility complex (MHC II and I) binding properties; adhesion probability greater than 0.51; with no similarity to host proteins.

2.5. *Ligand Libraries, Virtual Screening and Molecular Docking Analyses*

The ligand library of ZINC drug-like molecules (Natural Product and its derivatives) was downloaded from the ZINC database [48,49]. The 5008 ligands were acquired in .SDF format and then converted into .PDB by using the OpenBabel (v. 2.4.1) tool [50]. Also, for the docking analysis, the 3D structures of the identified final drug targets were assessed and converted to the .PDBQT format using the AutoDockTools MGL tool (v. 1.5.4) [51]. The active site residues of the target proteins were identified by using DoGSiteScorer, a web-based automated pocket detection and analysis tool for calculating the druggability of protein cavities [52]. For each detected cavity, the tool returns the pocket residues and a druggability score ranging from 0 to 1, which indicates highly druggable protein cavities that are most likely to bind ligands with high affinity [53]. Exported protein data bank files (.PDB) for each protein target were imported into the DoGSiteScorer web-tool for subsequent computational binding-site prediction and druggability assessment, determining the grid box parameters needed to perform the docking. Using AutoDock Vina [54], we performed the virtual screening of the ligands based on the grid box parameters with a drug score greater than 0.8 for each target putative druggable pocket. Virtual screening was performed using the bash script vina_screen_local.sh, and the Python script topmolecule.py identified the five top-ranked ligand molecules. The 3D poses of docked molecules were examined in Chimera [55], and 2D poses were displayed in PoseView [56].

3. Results

3.1. *Identification of intra-species conserved non-host homologous proteins*

We compared five genomes of *M. genitalium* strains (Table 1) using the software orthoFinder [38]. Coding DNA sequences (CDSs) shared by all strains of a species is considered the core genome, which corresponds to 403 CDSs, according to our analyses for *M. genitalium*. Considering the human genome as the host organism, using an *in house* script to perform BLASTp against the DEG database, we submitted the protein sequences of the set of genes shared by all strains to select non-homologous sequences (≤ 35%) in higher organisms, and we found that 104 are non-host homologous proteins according to the percentage of identity.

3.2. *Predicted vaccine targets through Reverse Vaccinology*

Regarding the screening for vaccine targets, we considered only secreted, putative surface exposed (PSE) and membrane proteins because they are more likely to be exposed to the immune cells of the host [43]. To test the adhesion and binding capacity to major histocompatibility complex (MHC) class I and class II, all membrane, secreted, and PSE

protein targets of *M. genitalium* were submitted to the Vaxign software [42], a method based on genomic features for predicting vaccine targets in the reverse vaccinology platform. Sequences of the 44 PSE, secreted or membrane proteins were submitted to this web-based platform in the .FASTA format for analysis of antigenic properties. Moreover, screened proteins with adhesion capacity greater than 0.51 were also considered immunogenic and selected for further analysis [57] (Table 2). Subsequently, to create a final list of putative targets, the same dataset of 44 proteins was used to predict the MHC I and MHC II binding potential, adhesion probability, and the number of transmembrane helices (TMHs) (Table 2).

Table 2. Putative vaccine candidates shared by all analyzed strains of *M. genitalium*.

Protein ID	Gene Name	Subcellular Localization	SignalP (Cleavage Site)	TMHMM	InterProScan	Gene Product	MHC I & II Binding	Adhesion Probability
WP_009885633.1	MG389	Membrane	YES (b/w 23-24)	TMH= 1	NO	Hypothetical Protein	yes	0.551
WP_009885924.1	-----	Membrane	YES (b/w 26-27)	TMH= 2	NO	Hypothetical Protein	yes	0.558
WP_014894034.1	-----	Membrane	NO	TMH= 4	NO	Hypothetical Protein	yes	0.580
WP_014894048.1	-----	Membrane	YES (b/w 34-35)	TMH= 2	NO	Hypothetical Protein	yes	0.526
WP_009885577.1	MG456	PSE	YES (b/w 34-35)	TMH= 2	NO	Hypothetical Protein	yes	0.515
WP_009885652.1	MG095	PSE	YES (b/w 34-35)	TMH= 0	NO	Hypothetical Protein	yes	0.865
WP_010869319.1	MG074	PSE	YES (b/w 34-35)	TMH= 1	Protein of unknown function DUF5426 (IPR035339)	Hypothetical Protein	yes	0.586
WP_010869403.1	MG277	PSE	YES (b/w 31-32)	TMH= 12	NO	Hypothetical Protein	yes	0.709
WP_010869397.1	MG256	PSE	NO	TMH= 3	NO	Hypothetical Protein	yes	0.526
WP_009885703.1	MG040	PSE	YES (b/w 32-33)	TMH= 0	ABC transporter substrate-binding protein PnrA-like Domain IPR003760	Hypothetical Protein	yes	0.761

WP_009885565.1	MG468	PSE	YES (b/w 33-34)	TMH= 8	NO	ABC transporter permease	yes	0.668
WP_009885925.1	ptsG MG069	PSE	YES (b/w 36-37)	TMH= 10	Glucose permease domain IIB IPR036878 Duplicated hybrid motif IPR011055	PTS glucose EIICBA component	yes	0.541
WP_009885795.1	MG260	PSE	YES (b/w 28-29)	TMH= 1	Mycoplasma lipoprotein, central domain Domain IPR004984	Hypothetical Protein	yes	0.769
WP_010869472.1	MG412	PSE	YES (b/w 32-33)	TMH= 0	PBP domain Domain IPR024370	Hypothetical Protein	yes	0.600
WP_010869366.1	mgpA MG191	PSE	YES (b/w 30-31)	TMH= 2	Adhesin P1 Family IPR022400 Adhesin P1 domain Domain IPR004940	Adhesin P1	yes	0.803
WP_009885643.1	lgt MG086	PSE	NO	TMH= 7	Phosphatidyl glycerol—prolipo protein diacylglyceryl transferase *lgt* (IPR001640)	Diacylglyceryl transferase	yes	0.675
WP_010869408.1	MG286	Secreted	YES (b/w 30-31)	TMH= 1	Protein of unknown function DUF5452 IPR035219	Hypothetical Protein	yes	0.501
WP_010869364.1	MG186	Secreted	YES (b/w 26-27)	TMH= 0	SNase-like, OB-fold superfamily IPR035437	Hypothetical Protein	yes	0.588
WP_010869405.1	MG281	Secreted	YES (b/w 33-34)	TMH= 1	IgG-blocking virulence domain Domain IPR030942	IgG-blocking protein M	yes	0.683

In this approach, the 19 final vaccine candidates were selected, and their respective protein products were investigated – an ABC transporter permease; a PTS glucose EIICBA

component; an adhesion P1 protein; a diacylglyceryl transferase; an IgG blocking protein M; and 14 hypothetical proteins.

The ABC transporter is a large family mainly comprising of high-affinity branched-chain amino acid transporter proteins, with proteins from the galactose, ribose, and fructose transport system permeases also being found within this family [58–61]. Protein members of the ABC transporter family containing this domain have been described in several biological roles, such as cell division in Gram-negative and Gram-positive bacteria as a component of the septal ring, where it may insert division proteins into the cytoplasmic membrane [62,63]; involved as part of the tripartite efflux system MacAB-TolC [64]; as part of an ATP-dependent Lipoprotein-releasing system transmembrane protein known as LolCDE [65]; acting in bacitracin export through the ABC transporter complex BceAB [66]; playing a role in acetoin utilization during stationary phase and sporulation [67]; and, involved in peptide resistance mechanisms [68]. The Gene Ontology (GO) terms associated with this protein family are transmembrane transport (GO:0055085), transmembrane transport activity (GO:0022857), and integral component of membrane (GO:0016021), for biological process, molecular function, and cellular component, respectively.

The phosphoenolpyruvate-dependent sugar phosphotransferase system (sugar PTS), a major carbohydrate active transport system, catalyzes the phosphorylation of incoming sugar substrates concomitantly with their translocation across the cell membrane. This system is involved in glucose transport [69]. The PTS glucose EIICBA complex involves three main domains, EIIA, EIIB, and EIIC, which are responsible for transferring a phosphoryl group on to the next domain, which is then transferred to the sugar substrate simultaneously with the sugar uptake, and by forming the translocation channel which contains the specific substrate-binding site, respectively [70]. A series of studies have worked on the modification of PTS glucose EIICBA components, and overall glucose transportation systems and central metabolic pathways, for biotechnological purposes, such as the improvement of the production of amino sugars and amino acids of interest, for instance, N-acetylglucosamine (GlcNAc) [71], L-tryptophan [72] and L-threonine [73].

The adhesion P1 protein is a 170-kDa protein present on the attachment organelle surface that plays a critical role in the binding and gliding process of mycoplasmal pathogenic species, such as *Mycoplasma pneumoniae* [74]. The P1 protein is transmembrane, and serves as the primary host receptor binding protein in *M. pneumoniae*, and is directly responsible for the bacterial cytoadherence [75]. Structure model based on

small-angle X-ray scattering (SAXS) results on recombinant P1 adhesin from *M. pneumoniae* fitted well with the equivalent structure obtained with cryo-electron tomography from our related species of interest, *M. genitalium* [76].

Diacylglyceryl transferases LGT are involved in the pathways for lipoprotein biosynthesis, where it catalyzes the diacylglyceryl transfer of a prolipoprotein, which is the first step in the protein modification towards the formation of mature lipoproteins [77]. Usually, it holds several lipid molecules that are bound within the central cavity of the transferase [78]. The roles and implications of diacylglyceryl transferase LGT presence/absence in the virulence and pathogenicity to the host by several pneumococcal serotypes have been described by many studies on pathogens, such as *Streptococcus mutans* [79], *Streptococcus uberis* [80], *Enterococcus faecalis* [81] and *Streptococcus pneumoniae* [82], even suggesting its association as an inner membrane protein [83].

M-related proteins (Mrp) are group A streptococcal (GAS) receptors for immunoglobulins, being known as Protein M based on its impact on virulence, as it broadly blocks antibody-antigen union and its binding contributes to the ability of GAS to resist phagocytosis into the human host [84]. Studies show that Mrp preferentially binds human IgG and that this binding appears to function by a mechanism of inhibition by anchoring to conserved regions of the antibody light chains in a form to block entrance to macromolecular antigens [85]. Also, recent findings provide evidence of a human IgG bound to the IgG blocking protein M that increases virulence and neutralizes the beneficial effects of IgG opsonization [86].

3.3. *Drug targets identification*

In this subsection, we analyzed the cytoplasmic (SurfG+) non-host homologous conserved proteins present in the core genome of *M. genitalium*. We submitted the 60 non-host homologous amino acid sequences of the cytoplasmic proteins to the MHOLline tool [35], an online web tool that uses the HMMTOP, BLAST, BATS, MODELLER, and PROCHECK software for the prediction of three-dimensional protein modeling. For this, only the first three distinct quality G2 model groups were taken into consideration in this study, namely: 1 – very high-quality model sequences (identity ≥ 75%) (LVI ≤ 0.1), 2 – high-quality model sequences (identity ≥ 50% and < 75%) (LVI ≤ 0.1), and 3 – good quality model sequences (identity ≥ 50%) (LVI > 0.1 and≤ 0.3) [35]. Therefore, we found

seven proteins (2 very high, 3 high, and 2 good) in the first 3 distinct quality G2 model groups (Table 3).

Table 3. Putative drug targets of *Mycoplasma genitalium* identified using Mholline.

Mholline G2 Categories (Very High/High/Good)		
WP_009885596.1	Very High	type I restriction modification protein
WP_009885605.1	Very High	hydroperoxide reductase
WP_009885829.1	High	ribosome-binding factor A
WP_009885876.1	High	hypothetical protein
WP_009885939.1	High	DUF3217 domain-containing protein
WP_009885820.1	Good	50S ribosomal protein L32
WP_010869386.1	Good	class Ib ribonucleoside-diphosphate reductase assembly flavoprotein NrdI

3.4. *Virtual screening and molecular docking*

In molecular docking, the lower energy scores signify better protein-ligand binding when compared to high-energy values [87]. In this work, for each drug target protein, a natural drug-like compounds library downloaded from ZINC Database (5.008 compounds) was used for docking analysis. All the compounds from this library were used one at a time for the screening of the top molecules that presented favorable binding with the target. The name and ID Code of the targets, AutoDock Vina binding affinity for the identified molecules, DoGSiteScore's features, and the number of predicted hydrogen bonds with interacting residues from the best ligands are shown for each target in Table 4. The predicted configuration of the best-docked molecules is shown for each protein target in figures 2–8.

Concerning our predicted drug targets, we refined our candidates to a final list of 7 drug targets – Type I restriction-modification protein; Akylhydroperoxide reductase (Ahp); ribosome-binding factor A (RbfA); 50S ribosomal protein L32; class Ibribonucleoside diphosphate reductase (NrdI); a DUF3217 domain-containing protein; and a hypothetical protein. As for the ligands, we performed a molecular docking analysis for each drug target against compounds from the ZINC Natural Product library. The compounds ZINC08636510, ZINC04235924, ZINC15709489, ZINC04237087, ZINC04236001, and ZINC35415766 were selected as promising molecules with favorable interactions against the active site residues from each target.

Type I restriction enzymes (REases) are large pentameric proteins with distinct subunits for restriction (R), methylation (M), and DNA sequence-recognition (S), involved with the restriction-modification system (RM system) [88]. RM systems are found in

bacteria and some other prokaryotic organisms, where it offers protection against foreign DNA, such as that indwelled by bacteriophages [89]. Type I was the first REases to be discovered and purified, yet they have been hard to characterize. As genome analysis reveals their genes and methylome studies reveal their recognition sequences, we are beginning to grasp a better understanding of those proteins [90]. The docking analysis was performed to identify the maximum number of hydrogen bond interactions and the residues interacting with the compounds from the ZINC Natural Product library. The interaction of compound ZINC08636510 is shown in figure 2.

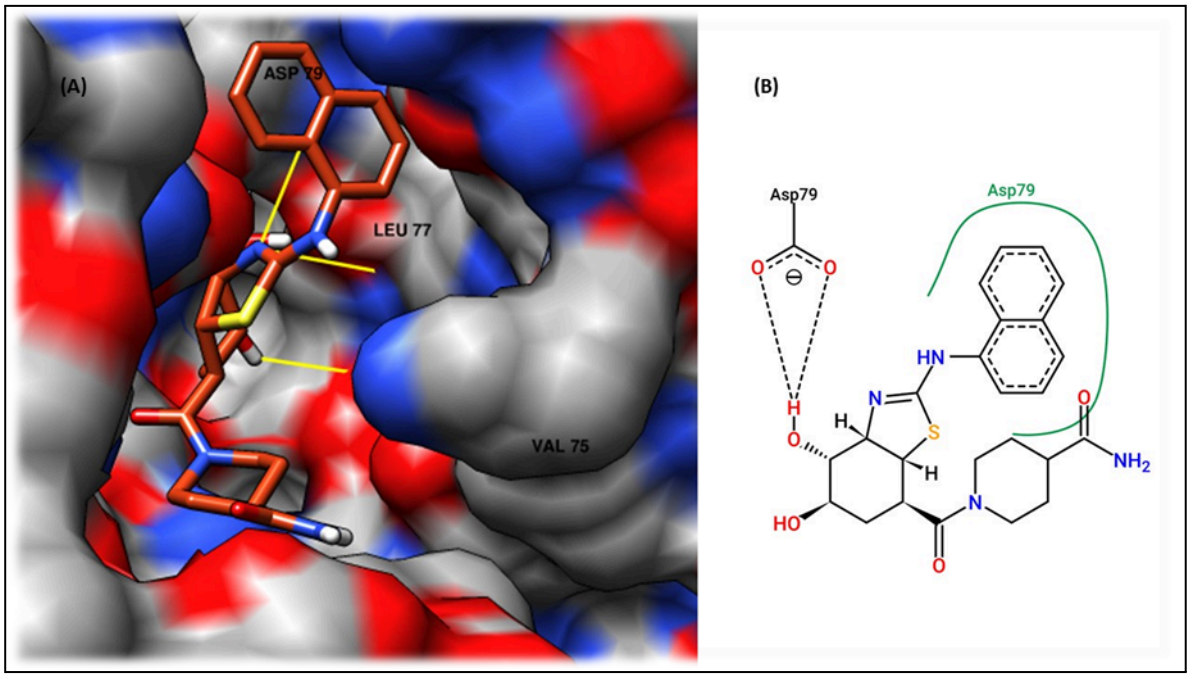

Fig. 2. (a) Three-dimensional surface representation of docking analysis for the structure of WP_009885596.1 (Type I restriction-modification protein) with compound ZINC08636510, and (b) two-dimensional representation of Type I restriction-modification protein with compound ZINC08636510.

Alkyl hydroperoxide reductase (Ahp) is a thiol-specific peroxidase that catalyzes the reduction of hydrogen peroxide and organic hydroperoxides to water and alcohols, respectively [91]. Hydrogen peroxide is generated during aerobic metabolism and can inflict critical damage to biomolecules; therefore, Ahp plays a vital role in cell protection against oxidative stress by detoxifying peroxides and averting its damage on bacteria [92]. The interaction of compound ZINC04235924 is shown in figure 3.

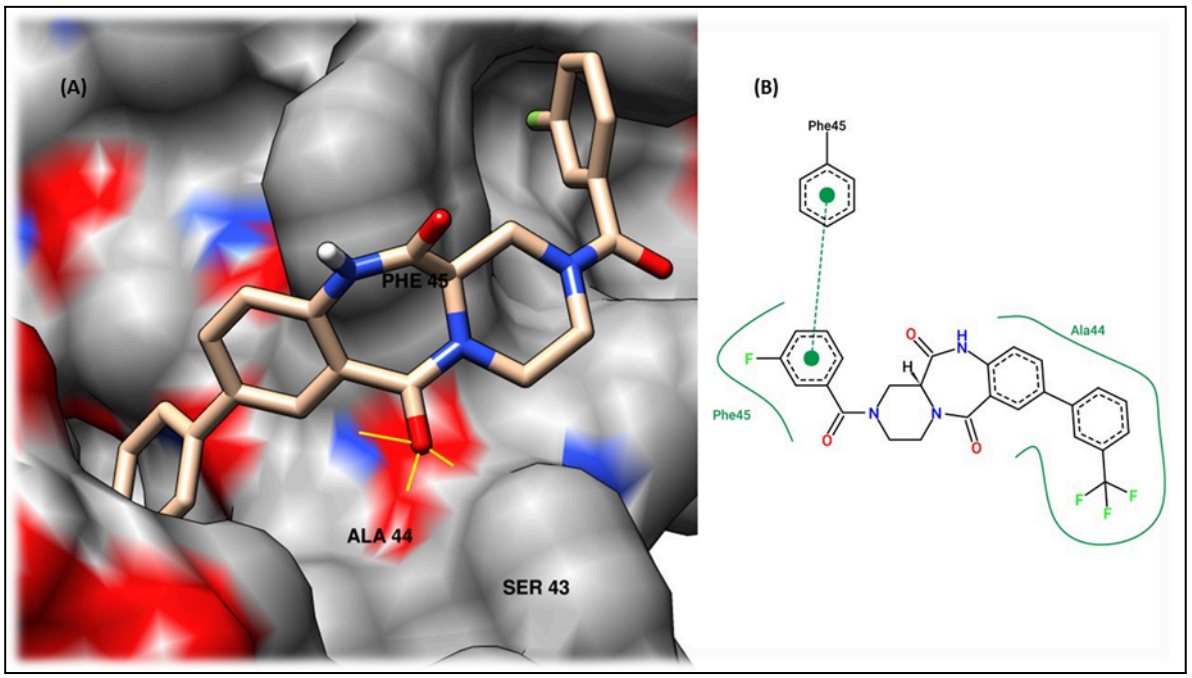

Fig. 3. (a) Three-dimensional flat ribbon representation of docking analysis for the structure of WP_009885605.1 (Akylhydroperoxide reductase – Ahp) with compound ZINC04235924, and (b) two-dimensional representation of Ahp with compound ZINC04235924.

Ribosome-binding factor A (RbfA) is one of the main proteins to assist in the late maturation of the functional core of the 30S subunit, and it serves as a cold-shock adaptation protein in *Escherichia coli* [93,94]. RbfA is crucial for the efficient processing of pre-16S rRNA, and it is a member of a large family of small proteins found in most bacterial organisms, making it a key target for structural proteomics [95]. The docking analysis revealed the interaction of Rbfa to the compound ZINC15709489, a sodium-dependent proline transporter ligand. This compound is known to terminate the action of proline by its high-affinity sodium-dependent reuptake into presynaptic terminals, which belongs to the neurotransmitter sodium symporter (nss) family [96]. Lexicon Pharmaceuticals currently commercialize this drug-like compound, but only its potential as a novel therapeutic target for cognition improvement has been reported [97], which opens the doors for the repurposing of this compound against mycoplasmal bacteria, such as *M. genitalium*. The compound ZINC15709489's interaction with this *M. genitalium* drug target is shown in figure 4.

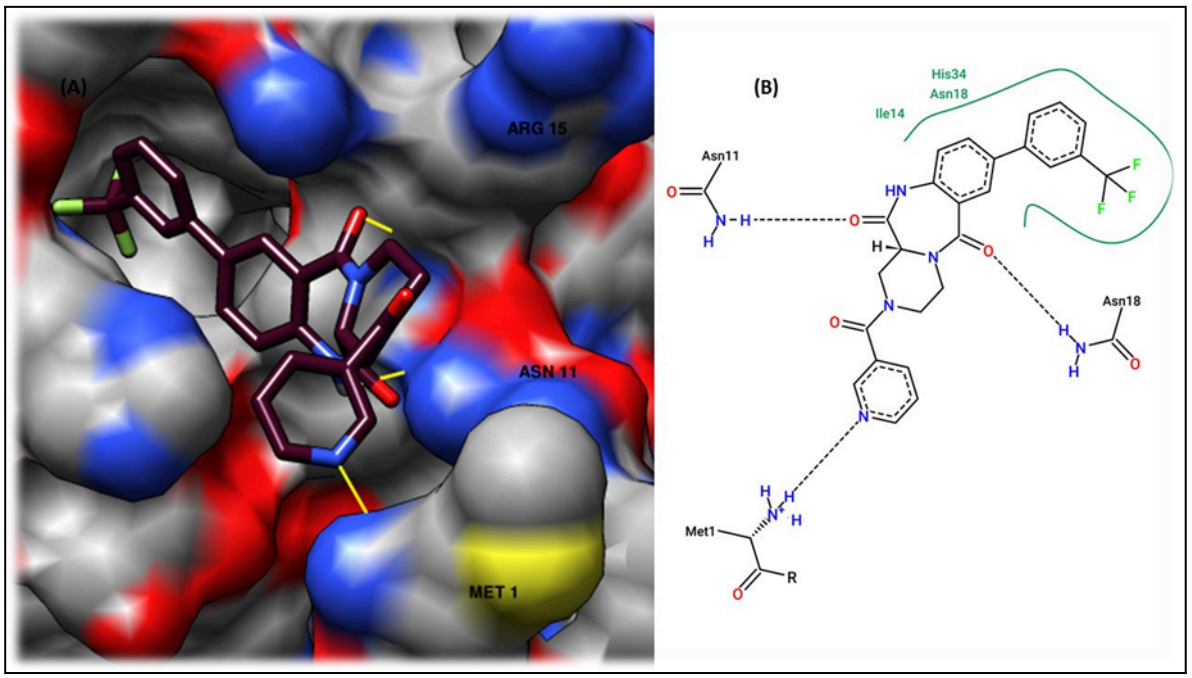

Fig. 4. (a) Three-dimensional flat ribbon representation of docking analysis for the structure of WP_009885829.1 (Ribosome-binding factor A – RbfA) with compound ZINC15709489, and (b) two-dimensional representation of RbfA with compound ZINC15709489.

Hypothetical proteins are elements predicted by gene identification tools during the genome analysis, but for which there is no experimental evidence that it is expressed *in vivo* [98]. Presently, the bioinformatics tools are used to identify new genes during the annotation steps of a genome assembly, and, when it finds the open reading frames (ORFs) in the genome to show less identity than required to known, identified protein in the database, the sequence is labeled as "putative" or "hypothetical protein." These proteins with uncertain functions and existence represent an opportunity for the screening and discovery of new drug targets that may be used in further studies against countless diseases [99]. The docking analysis revealed the interaction of our predicted hypothetical protein to the compound ZINC04237087, a neuronal acetylcholine receptor subunit alpha-4 ligand. Neuronal acetylcholine receptor subunit alpha-4, encoded in humans by the CHRNA4 gene, is a protein subunit of some nicotinic acetylcholine receptors (nAChR) and a member of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses [100]. This protein seems to render in the addictive response to nicotine [101]. The compound ZINC04237087 is known for binding acetylcholine to the AChR receptor, which responds by an extensive change in conformation that affects all subunits and leads to the opening of an ion-conducting channel across the plasma membrane permeable to sodium ions [102]. The interaction of compound ZINC04237087 is shown in figure 5.

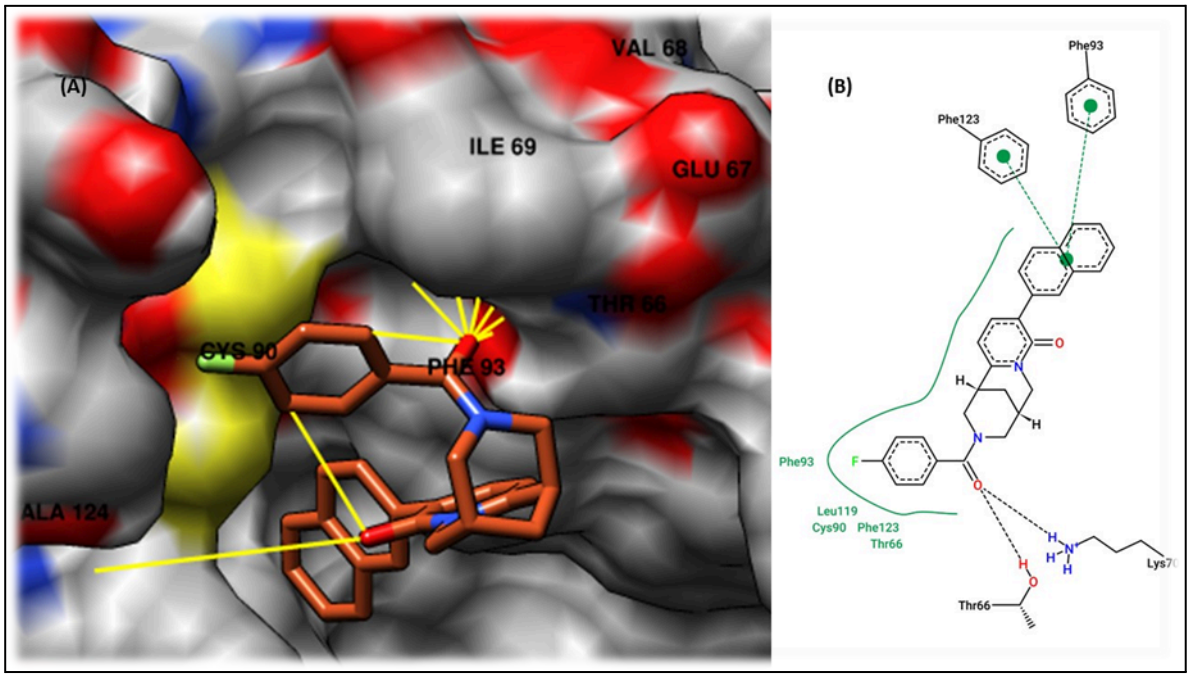

Fig. 5. (a) Three-dimensional flat ribbon representation of docking analysis for the structure of WP_009885876.1 (hypothetical protein) with compound ZINC04237087, and (b) two-dimensional representation of hypothetical protein with compound ZINC04237087.

Domains of unknown function (DUFs) are a massive set of uncharacterized families of proteins that are compiled in the Pfam database, and, up to the present time, DUFs corresponds to 20% of all known protein families [103]. The Pfam database is a collection of protein families and domains that have been widely employed for annotating sequenced genomes and assemblies [104]. The DUF3217 (PF11506) is a domain present and apparently restricted to *Mycoplasma* proteins. Currently, the Pfam database determines that all of the proteins containing this domain appear to belong to *Mycoplasma*. While some members in this family of proteins might be annotated as MG376, this cannot yet be confirmed. The interaction of compound ZINC04236001 is displayed in figure 6.

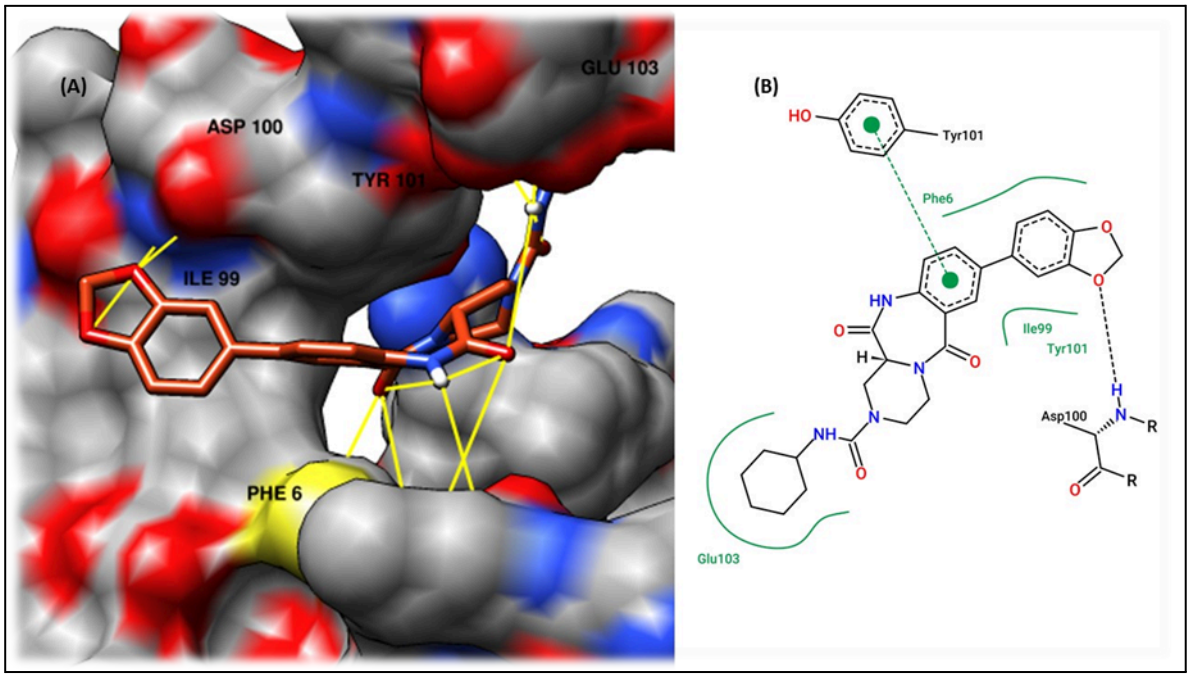

Fig. 6. (a) Three-dimensional flat ribbon representation of docking analysis for the structure of WP_009885939.1 (DUF3217 domain-containing protein) with compound ZINC04236001, and (b) two-dimensional representation of DUF3217 domain-containing protein with compound ZINC04236001.

The L32 protein is a component of the 50S ribosomal subunit that forms a cluster with L17 and L22, holding together all the domains of the 23S rRNA [105]. The 50S ribosomal protein L32 belongs to the bacterial ribosomal protein bL32 family and can be found on the solvent side of the large subunit [106]. It has been described as a drug target using the antibiotic troleandomycin against the eubacteria *Deinococcus radiodurans*, as L32 interacts with the antibiotic, which blocks the peptide exit tunnel [107]. For this one, the docking analysis recognized the same best interacting compound ZINC04236001 from the ZINC Natural Product library as for the previous drug target. The interaction of compound ZINC04236001 is shown in figure 7.

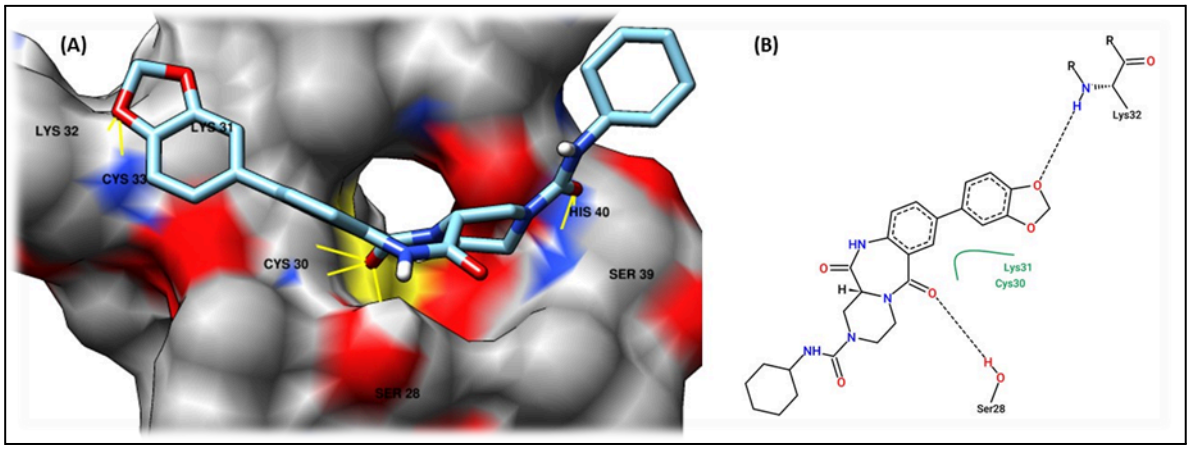

Fig. 7. (a) Three-dimensional flat ribbon representation of docking analysis for the structure of WP_009885820.1 (50S ribosomal protein L32) with compound ZINC04236001, and (b) two-dimensional representation of 50S ribosomal protein L32 with compound ZINC04236001.

Ribonucleotide reductases (RNRs) are responsible for the *de novo* conversion of ribonucleoside diphosphates into deoxyribonucleoside diphosphates, which are essential for DNA synthesis and DNA repair [108]. Three classes of RNRs (I, II, and III) have been characterized to date according to their different mechanisms, their quaternary structural differences, and differing in their cofactors, while Class I RNRs are further subdivided into class Ia and class Ib [109]. Class Ib enzymes contain a dimanganese-tyrosyl radical, involved in the generation of a transient thiyl (sulfanyl) radical on a cysteine residue. In addition, NrdH with NrdEF suffers a stimulatory effect on their ribonucleotide reductase activities due to the presence of NrdI in Salmonella [110]. The interaction of compound ZINC35415766 is shown in figure 8.

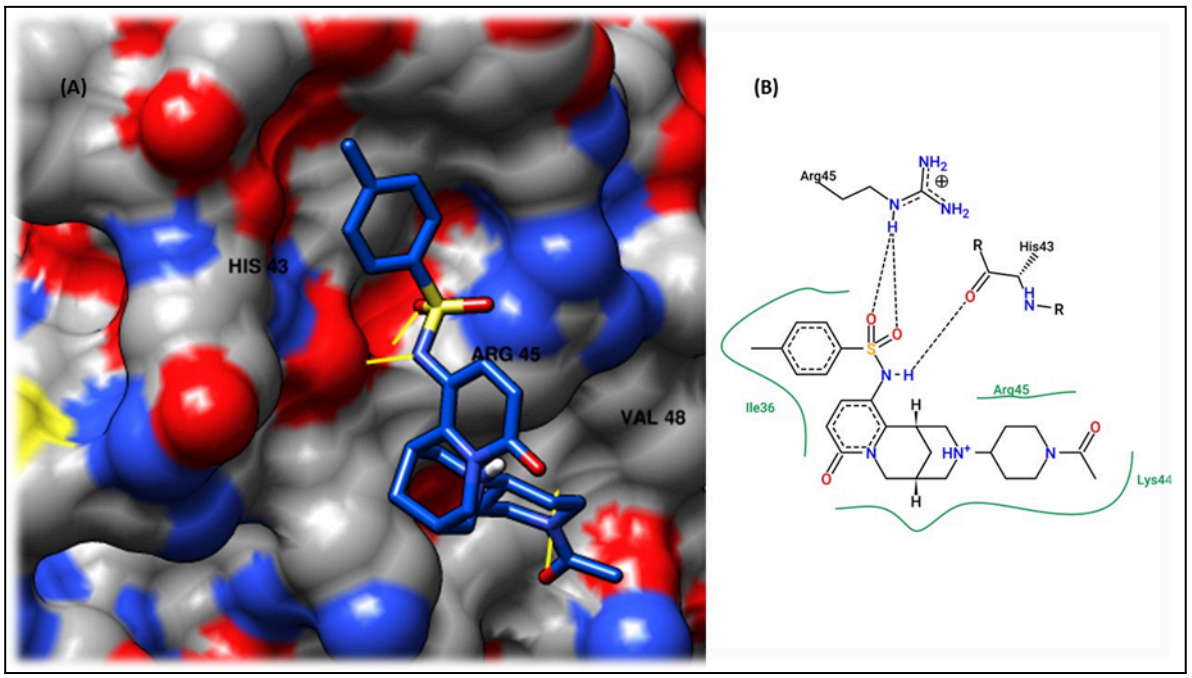

Fig. 8. (a) Three-dimensional flat ribbon representation of docking analysis for the structure of WP_010869386.1 (Class Ibribonucleoside diphosphate reductase NrdI) with compound ZINC35415766, and (b) two-dimensional representation of Class IbNrdI with compound ZINC35415766.

Table 4. DoGSiteScorer's features, AutoDock Vina binding affinity on ZINC Natural Products, and predicted hydrogen bonds for the selected top molecules against each protein as a drug target.

Drug targets		**DoGSiteScorer's results**				**ZINC Natural Products**	**AutoDock Vina**		
Name	Code	Volume A^3	Surface A^2	Drug Score	Residues	Top Compounds	Binding Affinity	H-Bond	Residues
type I restriction modification protein	WP_009885596.1	472.97	616.18	0.82	ARG_171 / ARG_174 / ASP_175 / TYR_177 / ALA_178 / HIS_179 / LEU_181 / PHE_182 / PHE_188 / TRP_192 / LEU_194 / ARG_265 / GLU_268 / PHE_291 / TYR_293 / CYS_294 / ALA_295 / LYS_297 / TYR_298 / ALA_337 / GLY_338 / ILE_340 / VAL_341 / PHE_342 / LEU_344 / ASP_345 / GLN_346 / LEU_348 / ASP_349 / LYS_352	ZINC08636510	-8.0	7	ASP_79 / LEU_77 / VAL_75
hydroperoxide reductase	WP_009885605.1	470.98	719.0	0.78	LEU_46 / LEU_49 / ALA_50 / GLU_53 / LEU_54 / ILE_73 / ASN_74 / ILE_75 / LEU_93 / ILE_95 / HIS_96 / TRP_97 / ILE_112 / ASP_113 / VAL_115 / SER_116 / LYS_117 / ALA_121 / HIS_122 / ASN_123 / LEU_125 / HIS_126 / THR_128 / SER_129 / PHE_131 / LYS_132 / ILE_133 / ASN_134 / ILE_135	ZINC04235924	-9.1	3	PHE_45 / ALA_44 / SER_43
ribosome-binding factor A	WP_009885829.1	538.37	814.88	0.83	ILE_9 / GLU_10 / ASP_12 / ILE_13 / ILE_14 / LEU_16 / ILE_17 / ASN_18 / ILE_21 / VAL_30 / LYS_31 / LEU_32 / GLY_33 / HIS_34 / VAL_35 / VAL_38 / LEU_40 / SER_41 / ASP_43 / PHE_44 / PHE_45 / HIS_46 / ALA_47 / VAL_49 / LEU_51 / PHE_66 / PHE_73 / MET_76 / LEU_77 / LYS_88 / LEU_89	ZINC15709489	-8.8	9	ARG_15 / ASN_11 / MET_1
Hypothetical protein	WP_009885876.1	578.11	991.91	0.89	PHE_62 / THR_66 / ILE_69 / LYS_70 / ASP_73 / TYR_74 / SER_85 / ASP_86 / CYS_89 / CYS_90 / PHE_93 / TYR_94 / LEU_97 / PHE_100 / ILE_101 / LEU_104 / ILE_110 / ARG_114 / PHE_115 / ARG_118 / LEU_119 / ARG_122 / PHE_123 / ILE_125	ZINC04237087	-9.9	19	VAL_68 / GLU_67 / ILE_69 / THR_66 / PHE_93 / CYS_90 / ALA_124
DUF3217 domain-containing protein	WP_009885939.1	501.82	631.05	0.85	ASN_3 / VAL_5 / LEU_7 / GLU_8 / GLY_9 / VAL_25 / ILE_27 / PHE_41 / PHE_42 / VAL_43 / PHE_44 / ILE_64 / SER_65 / ILE_66 / LEU_70 / ARG_71 / THR_72 / TYR_73 / LEU_74 / GLU_75 / LYS_82 / THR_83 / THR_84 / ILE_85	ZINC04236001	-7.9	15	GLU_103 / ASP_100 / TYR_101 / ILE_99 / PHE_6
50S ribosomal protein L32	WP_009885820.1	688.77	1490.79	0.79	SER_28 / VAL_29 / CYS_30 / LYS_31 / LYS_32 / CYS_33 / LYS_35 / LYS_36 / LYS_37 / LEU_38 / SER_39 / HIS_40 / ARG_41 / VAL_42 / CYS_43 / SER_44 / CYS_45 / GLY_46 / MET_47 / TYR_48 / GLY_49 / GLU_50	ZINC04236001	-6.4	10	LYS_32 / LYS_31 / CYS_33 / CYS_30 / SER_28 / HIS_40 / SER_39
class Ib ribonucleoside-diphosphate reductase assembly flavoprotein NrdI	WP_010869386.1	315.33	641.94	0.61	MET_1 / HIS_2 / PRO_20 / PHE_21 / ILE_22 / LEU_39 / PHE_41 / GLN_42 / HIS_43 / GLU_59 / TYR_60 / VAL_61 / ARG_96 / ILE_99 / LEU_126 / GLN_142 / ILE_144 / ILE_145 / PHE_148 / PHE_149 / SER_152	ZINC35415766	-8.1	8	HIS_43 / ARG_45 / VAL_48

4. Discussion

Although it was first identified in the 1980s, *Mycoplasma genitalium* is still a little-known sexually transmitted infection (STI) [111]. *M. genitalium* can evade the human immune system, and infections caused by *M. genitalium* can persist in the body even after treatment with macrolides and tetracyclines, causing lifelong sequelae [112]. Currently, the fight against *M. genitalium* is limited to the context of the syndromic management of urogenital tract infections. Therefore, the development of efficient vaccines, drugs, and prophylactics to face this emerging pathogen is of utmost importance [113].

The application of prophylactic targeting strategies *in silico* has become a conventional and popular approach in the post-genomic era with the aid of bioinformatics [114]. Several studies have adopted the combination of reverse vaccinology (RV) strategy to screen for new vaccine candidates in association with subtractive genomics to identify drug targets in pathogenic organisms [115–120], many of which target sexually transmitted causative agents [121–125]. In this study, we used five complete genomes of the species to explore the pangenome for putative therapeutic candidates against the pathogen.

Previous studies already used a single genome of *M. genitalium*, strain G-37, to identify potential drug and vaccine targets, adopting different approaches. For instance, *in silico* analyses and manual mining were performed and 67 proteins were identified as non-homologous essential proteins that could serve as potential drug and vaccine targets [126]. Yang and colleagues carried out the functional characterization of hypothetical proteins (HPs) and described the functional assignments of 61 HPs amongst different functional groups, such as DNA-binding proteins, helicases, and transporters. Among those proteins, a group of 20 proteins was predicted to be virulence factors, indicating pathogenic features of *M. genitalium* [127].

At this point, two genes, *ptsG* (ORF: MG069) and *mgpA* (ORF: MG191) were predicted in previous studies [126,127]. Besides these genes, another seventeen were found by the reverse vaccinology approach adopted in this work. It is important to remind that the RV methods applied in those works, as well as on this one, took into consideration similar factors as essentiality and non-host homology. However, each of the previous studies based its final selection of candidates on different criteria. While the first one stopped at the subcellular localization of the proteins as the final step of selection [126], the most recent work screened for virulence factors [127]. Although the use of the basic steps of RV, neither of these works assessed the immunological features of their datasets. In that sense, including other tools, such

as Vaxign, that predicts the binding capacity to MCH-I and MCH-II complexes [42], to the analysis of the antigenic properties of candidates fits as an alternative to rank potential targets in other RV studies.

The several genomes of the species currently available on public databases allow the execution of studies related to the pangenome, which is composed of three parts: core genome, accessory genome, and strain-specific genes [128]. Targeting the core genes of bacterial species is a promising strategy, as the core genes remain conserved throughout a species, making eventual findings more relatable to diverse infectious strains [129]. In this work, we searched in the pan-exoproteome, i.e. the conserved secreted and potentially surface exposed (PSE) proteins, for the vaccine target identification. Since the proteins characterized as membrane, PSE, and secreted are more favorable to be exposed to the immune cells of the host, it makes them more suitable to the RV analysis [99]. In addition, we performed the virtual screening and molecular docking for the cytoplasmatic portion of the pangenome to identify small molecules or compounds that can act as successful binders to the putative drug targets. This procedure is one step further into the development or repositioning of drugs [130].

Through reverse vaccinology and subtractive genomics approaches, we predicted 19 potential vaccine targets, out of which 14 have not been described on their antigenic properties or biological functions, which may be used in further studies as novel vaccine strategies against the pathogen. In our study, we considered proteins containing any number of transmembrane helixes (TMHs), whereas proteins with two or fewer TMHs are preferable for further expression analysis [44]. Therefore, the WP_014894034.1, WP_010869403.1, WP_010869397.1, WP_009885565.1, WP_009885925.1, and WP_009885643.1 should not be considered for the use of whole protein vaccine formulations. However, all of those should still be considered for future studies on the engineering of multi-epitope vaccine constructs.

Concerning our druggable candidates, we refined our candidates to a final list of 7 putative drug targets. On some of our predicted targets, Type I restriction-modification, hydroperoxide reductase, and Class Ib NrdI proteins have also been predicted by other studies as potential drug targets [131–133]. Zavilgelsky and Rastorguev described proteins *ArdA* and *Ocr* as effective anti-restriction inhibitors of Type I REases [134]. Andrade and Reed included in a review Ahp and other thioredoxin reductases (TrxRs) as drug targets of interest against protozoan parasites [135]. Since human cells lack the Ahp subunit C (AhpC), the distinctive N-terminal epitope of mycobacterial AhpC has also been pointed out as an ideal drug target for further investigations [136]. Likewise, humans do not possess class Ib RNRs and thus

have no NrdI protein. Recent studies show that the NrdI-dependent manganese-containing form of NrdF is required during virulence in the pathogenic species *S. sanguinis* [137]. Hence, Lofstad *et al.* highlighted the NrdI / NrdI reductase as appealing targets for the design of novel, highly selective antibiotics against pathogens that depend on Class Ib RNRs [138].

So far, only the 50S ribosomal protein L32 has been described and certified as a pharmacological target for the approved antibiotic drug Troleandomycin (DB13179) [139]. More recent work also succeeded in predicting this particular protein as a putative drug target on mycoplasmal bacteria [140]. Concerning the RbfA, it is not a new concept that we could have drugs to be directly designed targeting the bacterial ribosome [141]; although, thus far, RbfA has not yet been employed in any antibiotic strategy. Moreover, no matches on the topic of DUF3217 domain-containing proteins could be found in the literature regarding antibiotic activity, strategical binding sites for compounds, or signed as drug targets. Hence, our study was the first to reveal both the predicted hypothetical protein (WP_009885876.1) and DUF3217 domain-containing protein (WP_009885939.1) as novel putative drug targets against *Mycoplasma genitalium*.

As for the ligands, we selected 6 compounds from the ZINC Natural Product library as the top promising ligands against the predicted drug targets. The compound ZINC15709489, a sodium-dependent proline transporter ligand was revealed as the top ligand to the drug target Rbfa. This drug-like compound is currently commercialized by Lexicon Pharmaceuticals as a novel therapeutic target for cognition improvement [97], and its commercial availability enables future *in vitro* and *in vivo* studies. The repurposing of this compound against *M. genitalium* infections could enhance the possibilities of a quicker come through this drug agent into the labor of clinical practice. Another promising molecule is ZINC04236001, predicted the best interacting compound for both the 50S ribosomal protein L32 and the DUF3217 domain-containing protein, revealing its potential as a prophylactic compound since it has naturally at least two essential targets against this Mycoplasmal bacteria.

Prevention against the silent trait of *M. genitalium* infection is known to be challenging [142] and its ability to adhere and penetrate host epithelial cells also hinders the preliminary diagnosis of infection by classical testing methods [143]. Beyond that, *M. genitalium* is one of the sexually transmitted pathogens that cause significant morbidity in the host [127]. Hence, the development of effective therapeutic alternatives, such as the candidates described here emphasized, is urgently needed to counter the multi-drug resistant challenges imposed by this pathogen.

5. Conclusions

Until now, pelvic inflammatory disease (PID) and several inflammatory reproductive tract syndromes caused by the sexually transmitted infection of *Mycoplasma genitalium* are a major cause of infertility, endometritis, and preterm labor. Yet, the only available method that can accurately detect *M. genitalium*'s infection is currently employed only for research purposes with restricted access for patients, and there are no FDA-approved diagnostic tests available in the United States, neither are CDC guideline recommendations available for the screening of asymptomatic patients for this infection. For that reason, we explored an *in silico* workflow to prospect novel proteins to be used as vaccine candidates and drug targets against the bacterium from a common set of genes obtained from five complete genomes through reverse vaccinology and subtractive genomics. According to the non-host homologous proteins, 104 among the 403 core set of sequences were pre-selected for subtractive genomics and RV analysis, which included the prediction of subcellular location, number of transmembrane helices, molecular weight, and antigenicity potential, and molecular docking analysis.

Overall, we predicted 19 candidate vaccine targets and 7 drug targets. Noteworthy, so far, only the 50S ribosomal protein L32 has been described and certified as a pharmacological target against an existing approved antibiotic drug. On some of the others, Type I restriction-modification, alkyl hydroperoxide reductase, and Class IbNrdI proteins have also been successfully predicted by other studies as potential drug targets. In addition, the Ribosome-binding factor A is a remarkably promising protein for targeting the bacterial ribosome that has not yet been employed in any antibiotic strategy.

In conclusion, we predicted 14 potential vaccine targets that have not yet been described as antigenic candidates and may be used in further studies as novel vaccine strategies against the pathogen. Concerning our predicted drug targets, our work was the first to reveal both predicted hypothetical protein (WP_009885876.1) and DUF3217 domain-containing protein (WP_009885939.1) as novel putative drug targets against *M. genitalium*. Altogether, both vaccine candidates and drug targets identified here may contribute to the future development of therapeutic strategies to control the spread of this pathogen worldwide.

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5. CONSIDERAÇÕES FINAIS

Neste trabalho, foram identificados 403 genes codificantes para proteínas em comum para todas as cinco linhagens sequenciadas de *M. genitalium* disponíveis no NCBI. Destas, apenas 103 foram selecionadas como proteínas não-homólogas ao hospedeiro. Após a predição de localização subcelular para todos estes elementos, as 59 proteínas expostas ou secretadas pela bactéria tiveram seu potencial antigênico avaliado, e as 44 proteínas citoplasmáticas sofreram uma triagem de modelagem estrutural e posterior análise por *docking* molecular.

Ao final, previmos 19 alvos vacinais e 7 alvos de drogas, sendo destes 14 e 2 candidatos, respectivamente, inéditos quanto a este tipo de descrição na literatura. Dentre os alvos de droga, a proteína de modificação de restrição Tipo I, a alquil hidroperóxido redutase e a proteína da classe Ib *NrdI* foram preditas com sucesso como potenciais alvos de drogas por outros estudos, e somente a proteína L2 ribossomal de 50S já havia sido descrita e certificada como um alvo farmacológico contra um antibiótico comercialmente aprovado. Além destes, o *Ribosome-binding factor A* é um proteico alvo de drogas promissor, possibilitando direcionar-se diretamente ao ribossomo bacteriano, o que é uma alternativa que ainda não foi explorada em nenhuma estratégia antibiótica.

Em suma, tanto as moléculas de candidatos a vacinas quanto os alvos de drogas identificados por este trabalho podem contribuir no desenvolvimento futuro de estratégias profiláticas e terapêuticas, assim, auxiliando no combate mundial da disseminação desse emergente patógeno sexualmente transmissível.

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6. PERSPECTIVAS

Como perspectivas para este trabalho, pretende-se:

- Desempenhar a construção de uma vacina multi-epítopo para o combate da infecção por *Mycoplasma genitalium* com base nos alvos vacinais identificados neste estudo;
- Realizar os testes para validação dos ligantes e alvos de drogas revelados por este trabalho por soroterapia. Em especial, do ligante ZINC15709489, comercializado pela empresa Lexicon Pharmaceuticals, com o alvo *Ribosome-binding factor A* (*RbfA*);
- Avaliar como os achados deste trabalho se relacionam com os mecanismos patogênicos de adesão celular e evasão ao sistema imune do hospedeiro desempenhados por *M. genitalium*.

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8. APÊNDICE

Nesta seção estão os dados suplementares coletados ao longo do período de coleta e planejamento amostral deste estudo. Segue como:

- APÊNDICE A – Artigo Publicado – Computational identification of putative common genomic drug and vaccine targets in *Mycoplasma genitalium*.

APÊNDICE A – Artigo Publicado – Computational identification of putative common genomic drug and vaccine targets in *Mycoplasma genitalium*

Genomics
Volume 113, Issue 4, July 2021, Pages 2730-2743

Original Article

Computational identification of putative common genomic drug and vaccine targets in *Mycoplasma genitalium*

Wylerson G. Nogueira [a 1], Arun Kumar Jaiswal [a c 1], Sandeep Tiwari [a], Rommel T.J. Ramos [b], Preetam Ghosh [d], Debmalya Barh [e], Vasco Azevedo [a], Siomar C. Soares [c]

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Artigo final oriundo da dissertação, aceito para a publicação no periódico científico *Genomics* e disponível no link de acesso (https://doi.org/10.1016/j.ygeno.2021.06.011).

9. ANEXOS

Nesta seção estão descritos os demais artigos e atividades nos quais eu participei como colaborador, incluindo participações em eventos, organizações de eventos, monitorias, apresentações de palestras, minicursos ministrados e premiações. Seguem como:

- ANEXO A – Artigo Publicado: Prediction of new vaccine targets in the core genome of *Corynebacterium pseudotuberculosis* through omics approaches and reverse vaccinology;

- ANEXO B – Capítulo de Livro Publicado: Relatos de estudantes de pós-graduação em Ciências da Vida no Brasil durante a pandemia de COVID-19;

- ANEXO C – Currículo Lattes: atividades presentes de 2018 a 01/2021.

ANEXO A – Artigo Publicado – Prediction of new vaccine targets in the core genome of *Corynebacterium pseudotuberculosis* through omics approaches and reverse vaccinology

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Research paper

Prediction of new vaccine targets in the core genome of *Corynebacterium pseudotuberculosis* through omics approaches and reverse vaccinology

Carlos Leonardo Araújo[a], Jorianne Alves[a], Wylerson Nogueira[a], Lino César Pereira[a], Anne Cybelle Gomide[b], Rommel Ramos[a], Vasco Azevedo[a], Artur Silva[a], Adriana Folador[a,*]

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ARTICLE INFO

Keywords:
Corynebacterium pseudotuberculosis
Core genome
Omics approaches
Reverse vaccinology

ABSTRACT

Corynebacterium pseudotuberculosis is the etiologic agent of veterinary relevance diseases, such as caseous lymphadenitis, affecting different animal species causing damage to the global agribusiness. So far, there are no completely effective treatment methods to overcome the impacts caused by this pathogen. Several genomes of the species are deposited on public databases, allowing the execution of studies related to the pan-genomic approach. In this study, we used an integrated *in silico* workflow to prospect novel putative targets using the core genome, a set of shared genes among 65 *C. pseudotuberculosis* strains. Subsequently, through RNA-Seq data of the same abiotic stresses in two strains, we selected only induced genes to compose the reverse vaccinology workflow based in two different strategies. Our results predicted six probable antigens in both analysis, which indicates that they have a strong potential to be used in further studies as vaccine targets against this bacterium.

Neste artigo (https://doi.org/10.1016/j.gene.2019.03.049), eu participei na definição de escopo metodológico, análise formal, investigação, redação do rascunho original, revisão e edição, e na visualização dos resultados alcançados.

Dentre os destaques desse artigo, relatamos que: o *core* genoma do patógeno zoonótico *Corynebacterium pseudotuberculosis* é composto por 768 genes; de acordo com os dados de RNA-Seq, 230 genes são induzidos em pelo menos uma condição de estresse; seis genes foram preditos *in silico* como candidatos em diferentes abordagens de vacinologia reversa; esses genes podem contribuir em novos estudos para o desenvolvimento de novas vacinas.

ANEXO B – Capítulo de Livro Publicado – Relatos de estudantes de pós-graduação em Ciências da Vida no Brasil durante a pandemia de COVID-19

Anais do I CNIPC – Resumo
Congresso Nacional de Inovação e Popularização da Ciência (CNIPC)
Ações durante a Covid-19
Evento online – 07, 08 e 09 de outubro de 2020

RELATOS DE ESTUDANTES DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA VIDA NO BRASIL DURANTE A PANDEMIA DE COVID-19

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Resumo: O conhecimento científico é crucial para o desenvolvimento de uma nação e um dos maiores desafios atuais enfrentados pela sociedade é a pandemia de Covid-19. Este trabalho teve como objetivo relatar as experiências de pós-graduandos de diferentes áreas das ciências da vida no Brasil. Estes relatos mostram o impacto da crise global impulsionada pelo vírus refletido diretamente na vida acadêmica dos estudantes de pós-graduação, afetando seus planos e perspectivas dentro do atual cenário social.

Palavras-chave: Ciências da Vida; Pós-graduação; Pandemia

Neste capítulo de livro (ISBN: 978-65-89362-00-5), eu participei na elaboração da proposta de publicação, investigação, redação do rascunho original, revisão e edição dos relatos obtidos. Este e-book é fruto de uma ação de extensão universitária vinculada ao Programa 1000 Futuros Cientistas da Universidade Federal de Minas Gerais, oriundo das discussões e trabalhos apresentados em ambiente virtual no I Congresso Nacional de Inovação e Popularização da Ciência, no decorrer dos dias 07 a 09 de outubro de 2020.

ANEXO C – Currículo Lattes – 2018 a 01/2021

Currículo Lattes

CNPq

Imprimir currículo

Wylerson Guimarães Nogueira

Endereço para acessar este CV: **http://lattes.cnpq.br/7773763584173970**

Última atualização do currículo em 19/01/2021

Resumo informado pelo autor

Mestrando do Programa Interunidades de Pós-Graduação em Bioinformática (CAPES 7) na Universidade Federal de Minas Gerais (UFMG). Bacharel em Biotecnologia pela Universidade Federal do Pará - UFPA. Trabalhou como voluntário na organização sem fins lucrativos AIESEC em Belém do 2º semestre de 2013 ao 2º semestre de 2015. Desenvolveu estudos em imunobioinformática no Laboratório de Engenharia Biológica, hospedado no Parque de Ciência e Tecnologia do Guamá - PCT Guamá / UFPA em 2018. Atualmente, desenvolve pesquisas em vacinologia reversa e One Health em parceria com a RECOM - Rede de Ciências Ômicas.
(Texto informado pelo autor)

Nome civil

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Formação acadêmica/titulação

2018 Mestrado em Bioinformática.
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil
Orientador: Rommel Thiago Jucá Ramos

2011 - 2018 Graduação em Biotecnologia.
Universidade Federal do Pará, UFPA, Belem, Brasil
Título: Predição in silico de alvos antigênicos em pan-exoproteoma de Corynebacterium pseudotuberculosis
Orientador: Rommel Thiago Jucá Ramos
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Formação complementar

2012 - 2012 Extensão universitária em II Curso de Verão em Bioinformática Estrutural da UFMG. (Carga horária: 40h).
Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil

Atuação profissional

1. Universidade Federal do Pará - UFPA

Vínculo institucional

2017 - 2017 Vínculo: Estágio Obrigatório II , Enquadramento funcional: Estágio , Carga horária: 200, Regime: Integral
Outras informações:
Desenvolvimento de projeto de pesquisa na área de Enzimologia e Produção Enzimática no Laboratório de Biotecnologia de Enzimas e Biotransformações (LABEB), no Instituto de Ciências Biológicas (ICB/UFPA), intitulado "ESTUDO METABÓLICO E COMPARATIVO DE LINHAGENS COMERCIAIS DE Saccharomyces sp. DE PÃO E DE VINHO", a fins de cumprimento do Estágio Curricular II para obtenção do título de Bacharel em Biotecnologia.

2011 - 2013 Vínculo: Bolsista , Enquadramento funcional: Bolsista PIBIC/FAPESPA , Carga horária: 20, Regime: Dedicação exclusiva
Outras informações:
Bolsista de Iniciação Científica com o plano de trabalho "Bioprospecção de lipases de interesse [illegible]" "Investigação Sistemática Microbiana com potencial de produção de lipases", sob orientação da Profa. Dra. Luciana Pereira Xavier.

2. Parque de Ciência e Tecnologia - PCT/Guamá - PCT-GUAMÁ

Vínculo institucional

2017 - 2017 Vínculo: Estágio Obrigatório I , Enquadramento funcional: Estágio , Carga horária: 200, Regime: Dedicação exclusiva
Outras informações:
Desenvolvimento de projeto técnico-científico na área de Tecnologia da Panificação no Centro de Valorização de Compostos Bioativos da Amazônia (CVACBA), no PCT-Guamá, Espaço Inovação, a fins de cumprimento do Estágio Curricular I para obtenção do título de Bacharel em Biotecnologia.

3. AIESEC em Belém - @BL

Vínculo institucional

2015 - 2015 Vínculo: Voluntário , Enquadramento funcional: Diretor de Intercâmbios Sociais p Organizaçõe , Carga horária: 20, Regime: Parcial
Outras informações:
VP ICX GCDP - Diretor de Intercâmbios Sociais para Organizações do Terceiro Setor. Líder e gestor de área operacional de entrega de intercâmbios sociais voluntários. Responsável final pelo desenvolvimento de liderança na membresia da maior área operacional da AIESEC em Belém, 2015. Promoveu intercâmbios voluntários, realização de projetos dentro do Terceiro Setor e desenvolvimento de liderança de membresia.

2014 - 2015 Vínculo: Voluntário , Enquadramento funcional: Gerente de Projetos - Smarketing , Carga horária: 20, Regime: Parcial
Outras informações:
Gerente de Projetos, com foco na realização e desenvolvimento do Projeto Smarketing. Gestor e Líder de uma equipe composta por 5 membros. Responsável pela entrega final do Projeto Smarketing, experiência de intercâmbio dos Trainees Voluntários e desenvolvimento de liderança da membresia voluntária do projeto.

2014 - 2014 Vínculo: Voluntário , Enquadramento funcional: Membro de Vendas do Time de iGCDP , Carga horária: 20, Regime: Parcial
Outras informações:
Durante este período de experiência na AIESEC em Belém, organização sem fins lucrativos, atuou em diversas funções, dando suporte aos diversos stakeholders da Organização e Time de iGCDP - Programa de Cidadão Global para Organizações. Dentre stakeholders, Organizações Parceiras, famílias parceiras ao Programa de Host Family e Intercambistas estrangeiros, participantes voluntários do programa de Cidadão Global a fim de desenvolver projetos de impacto no Terceiro Setor em Belém.

4. III Curso de Verão em Bioinformática - UFMG - CVBIOINFO/UFMG

Vínculo institucional

2019 - 2019 Vínculo: Monitoria , Enquadramento funcional: Monitor , Carga horária: 30, Regime: Parcial
Outras informações:
Monitoria no III Curso de Verão em Bioinformática - UFMG.

Prêmios e títulos

2019 Biotecnologias para Remediação e Monitoramento Ambiental: Menção Honrosa., Centro Brasileiro-Argentino de Biotecnologia - CBAB

2006 Olimpíada Brasileira de Astronomia e Astronáutica, Medalha de Bronze, Agência Espacial Brasileira (AEB)

Producão

Produção bibliográfica

Artigos completos publicados em periódicos

1. doi ARAÚJO, CARLOS LEONARDO; ALVES, JORIANNE; **NOGUEIRA, WYLERSON**; PEREIRA, LINO CÉSAR; GOMIDE, ANNE CYBELLE; RAMOS, ROMMEL; AZEVEDO, VASCO; SILVA, ARTUR; FOLADOR, ADRIANA
Prediction of new vaccine targets in the core genome of Corynebacterium pseudotuberculosis through omics approaches and reverse vaccinology. GENE. JCR v.702, p.36 - 45, 2019.

Capítulos de livros publicados

1. **NOGUEIRA, W. G.**; BARATA, L. M.; MESCOUTO, V. A.
RELATOS DE ESTUDANTES DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA VIDA NO BRASIL DURANTE A PANDEMIA DE COVID-19 In: I Congresso Nacional de Inovação e Popularização da Ciência - Ações durante a COVID-19.1ª ed.Belo Horizonte - MG: Instituto de Ciências Exatas, 2020, v.1, p. 285-286.

Trabalhos publicados em anais de eventos (resumo)

1. JAISWAL, A. K.; NOGUEIRA, W. G.; TIWARI, S.; RAMOS, R. T. J.; AZEVEDO, V. A. C.; SOARES, S. C.
An In Silico approach for the identification of vaccine and drug targets against Mycoplasma genitalium, causative agent of sexually transmitted pelvic inflammatory disease (PID) In: X-MEETING 2019 - 15th International Conference of the Brazilian Association of Bioinformatics and Computational Biology (AB3C), 2019, Campos do Jordão.
Anais do X-MEETING 2019 - 15th International Conference of the Brazilian Association of Bioinformatics and Computational Biology (AB3C). , 2019.

2. NOGUEIRA, W. G.; TOSTA, S. F. O.; CAVALCANTE, A. L. Q.; PINHEIRO, K. C.; AZEVEDO, V. A. C.; SILVA, A. L. C.; RAMOS, R. T. J.
IN SILICO CONSTRUCTION OF A MULTI-EPITOPE VACCINE BASED ON PAN-EXOPROTEOME OF NEISSERIA GONORRHOEAE THROUGH AN SUBTRACTIVE GENOMICS AND REVERSE VACCINOLOGY APPROACH In: 30° Congresso Brasileiro de Microbiologia, 2019, Maceió.
Anais do 30º Congresso Brasileiro de Microbiologia. , 2019.

3. NOGUEIRA, W. G.; PINHEIRO, K. C.; CAVALCANTE, A. L. Q.; TOSTA, S. F. O.; AZEVEDO, V. A. C.; SILVA, A. L. C.; RAMOS, R. T. J.
In silico prediction of antigenic targets in pan-exoproteome of Neisseria gonorrhoeae through subtractive genomics and reverse vaccinology In: 55° Congresso da SBMT | CHAGASLEISH | 26° Congresso Brasileiro de Parasitologia, 2019, Belo Horizonte.
Anais do 55º Congresso da SBMT | CHAGASLEISH | 26º Congresso Brasileiro de Parasitologia. , 2019.

4. NOGUEIRA, W. G.; PINHEIRO, K. C.; SILVA, A. L. C.; AZEVEDO, V. A. C.; RAMOS, R. T. J.
In silico prediction of antigenic targets in pan-exoproteome of Corynebacterium pseudotuberculosis In: 14th X-Meeting - International Conference of the Brazilian Association for Bioinformatics and Computational Biology, 2018, São Pedro - SP.
Abstract Book of 14th X-Meeting 2018. , 2018.

5. NOGUEIRA, W. G.; PINHEIRO, K. C.; SILVA, A. L. C.; AZEVEDO, V. A. C.; RAMOS, R. T. J.
In silico prediction of antigenic targets of Staphylococcus lugdunensis: a reverse vaccinology approach In: 14th X-Meeting - International Conference of the Brazilian Association for Bioinformatics and Computational Biology, 2018, São Pedro - SP.
Abstract Book of 14th X-Meeting 2018. , 2018.

Apresentação de trabalho e palestra

1. **NOGUEIRA, W. G.**
Bioinformática_além_do_que_voce_conhece.fasta, 2020. (Conferência ou palestra,Apresentação de Trabalho)

2. **NOGUEIRA, W. G.**
Drug targets and vaccine candidates against Mycoplasma genitalium, 2020. (Seminário,Apresentação de Trabalho)

3. GOIS, B. V. A.; PINHEIRO, K. C.; **NOGUEIRA, W. G.**; ARAGÃO, A. O.; CAVALCANTE, A. L. Q.; FOLADOR, A. C.; RAMOS, R. T. J.
First-ever described Virome of the Amazonian Lake Bolonha: contributions to the understanding of water-related public health concerns, 2020. (Congresso,Apresentação de Trabalho)

4. JAISWAL, A. K.; NOGUEIRA, W. G.; TIWARI, S.; RAMOS, R. T. J.; AZEVEDO, V. A. C.; SOARES, S. C.
An In Silico approach for the identification of vaccine and drug targets against Mycoplasma genitalium, causative agent of sexually transmitted pelvic inflammatory disease (PID), 2019. (Congresso,Apresentação de Trabalho)

5. NOGUEIRA, W. G.; TOSTA, S. F. O.; CAVALCANTE, A. L. Q.; PINHEIRO, K. C.; AZEVEDO, V. A. C.; SILVA, A. L. C.; RAMOS, R. T. J.
IN SILICO CONSTRUCTION OF A MULTI-EPITOPE VACCINE BASED ON PAN-EXOPROTEOME OF NEISSERIA GONORRHOEAE THROUGH AN SUBTRACTIVE GENOMICS AND REVERSE VACCINOLOGY APPROACH, 2019. (Congresso,Apresentação de Trabalho)

6. NOGUEIRA, W. G.; PINHEIRO, K. C.; CAVALCANTE, A. L. Q.; TOSTA, S. F. O.; AZEVEDO, V. A. C.; SILVA, A. L. C.; RAMOS, R. T. J.
In silico prediction of antigenic targets in pan-exoproteome of Neisseria gonorrhoeae through subtractive genomics and reverse vaccinology, 2019. (Congresso,Apresentação de Trabalho)

7. ARAUJO, C. L. A.; ALVES, J. T. C.; NOGUEIRA, W. G.; PEREIRA, L. C.; GOMIDE, A. C.; RAMOS, R. T. J.; AZEVEDO, V. A. C.; SILVA, A. L. C.; FOLADOR, A. C.
Prediction of new vaccine targets in the core genome of Corynebacterium pseudotuberculosis through omics approaches and reverse vaccinology, 2019. (Congresso,Apresentação de Trabalho)

8. NOGUEIRA, W. G.; PINHEIRO, K. C.; SILVA, A. L. C.; AZEVEDO, V. A. C.; RAMOS, R. T. J.
In silico prediction of antigenic targets in pan-exoproteome of Corynebacterium pseudotuberculosis, 2018. (Congresso,Apresentação de Trabalho)

9. NOGUEIRA, W. G.; PINHEIRO, K. C.; SILVA, A. L. C.; AZEVEDO, V. A. C.; RAMOS, R. T. J.
In silico prediction of antigenic targets in pan-exoproteome of Corynebacterium pseudotuberculosis, 2018. (Simpósio,Apresentação de Trabalho)

10. NOGUEIRA, W. G.; PINHEIRO, K. C.; SILVA, A. L. C.; AZEVEDO, V. A. C.; RAMOS, R. T. J.
In silico prediction of antigenic targets of Staphylococcus lugdunensis: a reverse vaccinology approach, 2018. (Congresso,Apresentação de Trabalho)

Eventos

Eventos

Participação em eventos

1. Apresentação de Poster / Painel no(a) **I Congresso Nacional de Inovação e Popularização da Ciência - Ações durante a COVID-19**, 2020. (Congresso)
RELATOS DE ESTUDANTES DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA VIDA NO BRASIL DURANTE A PANDEMIA DE COVID-19.

2. **Reunião Magna Virtual da ABC 2020: O Mundo a Partir da COVID-19.**, 2020. (Encontro)

3. **SIG: Drug-Design 2020**, 2020. (Simpósio)

4. Simposista no(a) **Simpósio Online Horizontes da Biotecnologia**, 2020. (Simpósio)
Bioinformática_além_do_que_voce_conhece.fasta.

5. Apresentação de Poster / Painel no(a) **X-meeting eXperience 2020**, 2020. (Congresso)
First-ever described Virome of the Amazonian Lake Bolonha: contributions to the understanding of water-related public health concerns.

6. **4th Brazilian Student Council Symposium: Women in Bioinformatics**, 2019. (Simpósio)

7. Apresentação de Poster / Painel no(a) **55º Congresso da SBMT | CHAGASLEISH | 26º Congresso Brasileiro de Parasitologia**, 2019. (Congresso)
In silico prediction of antigenic targets in pan-exoproteome of Neisseria gonorrhoeae through subtractive genomics and reverse vaccinology.

8. **ANIMAL SEX DETERMINATION BY GENES, CHROMOSOMES AND THE ENVIRONMENT**, 2019. (Seminário)
ANIMAL SEX DETERMINATION BY GENES, CHROMOSOMES AND THE ENVIRONMENT.

9. **CENTRO BRASILEIRO-ARGENTINO DE BIOTECNOLOGIA (CBAB) - Curso BRA-12: "Biotecnologias para Remediação e Monitoramento Ambiental"**, 2019. (Outra)
Biomimetic biosensor for analysis of endocrine disruptors in samples of clinical, environmental and industrial interest.

10. **Curso OPAS: Tecnologia de sequenciamento genético baseada em nanoporos para investigação temporal e epidemiológica de surto de Dengue: capacitação, pesquisa, vigilância e divulgação científica**, 2019. (Oficina)
ORGANIZAÇÃO PAN-AMERICANA DE SAÚDE (OPAS) & SECRETARIA DE VIGILÂNCIA EM SAÚDE DO MINISTÉRIO DA SAÚDE (SVS).

11. **DOE's Joint Genome Institute (JGI) - Workshop: "Mining Microbial and Viral Genomes and Metagenomes for Biotechnological Applications using the JGI-IMG system"**, 2019. (Oficina)
Workshop: "Mining Microbial and Viral Genomes and Metagenomes for Biotechnological Applications using the JGI-IMG system".

12. **I LIGA BRASILEIRA DE BIOINFORMÁTICA - LBB**, 2019. (Olimpíada)
Primeira e Segunda Fase da I Liga Brasileira de Bioinformática - LBB.

13. **Simpósio e Diplomação dos Membros Afiliados da ABC | Regional MG & CO 2018-2022 / 2019-2023**, 2019. (Simpósio)
.

14. **X-MEETING 2019 - 15th International Conference of the Brazilian Association of Bioinformatics and Computational Biology (AB3C)**, 2019. (Congresso)
An In Silico approach for the identification of vaccine and drug targets against Mycoplasma genitalium, causative agent of sexually transmitted pelvic inflammatory disease (PID).

15. Apresentação de Poster / Painel no(a) **14th X-Meeting - International Conference of the Brazilian Association for Bioinformatics and Computational Biology**, 2018. (Congresso)
In silico prediction of antigenic targets in pan-exoproteome of Corynebacterium pseudotuberculosis.

16. Apresentação de Poster / Painel no(a) **3rd Brazilian Student Council Symposium**, 2018. (Simpósio)
In silico prediction of antigenic targets in pan-exoproteome of Corynebacterium pseudotuberculosis.

Organização de evento

1. **NOGUEIRA, W. G.**
I Workshop Online de Bioinformática, 2020. (Outro, Organização de evento)

2. **NOGUEIRA, W. G.**
IV Curso de Verão em Bioinformática - UFMG, 2020. (Outro, Organização de evento)

3. **NOGUEIRA, W. G.**
55º Congresso da SBMT | CHAGASLEISH | 26º Congresso Brasileiro de Parasitologia, 2019. (Congresso, Organização de evento)

Totais de produção

Produção bibliográfica

Artigos completos publicados em periódico	1
Capítulos de livros publicados	1
Trabalhos publicados em anais de eventos	6
Apresentações de trabalhos (Conferência ou palestra)	1
Apresentações de trabalhos (Congresso)	8
Apresentações de trabalhos (Seminário)	3
Apresentações de trabalhos (Simpósio)	1

Produção técnica

Curso de curta duração ministrado (extensão)	1

Eventos

Participações em eventos (congresso)	7
Participações em eventos (seminário)	3
Participações em eventos (simpósio)	5
Participações em eventos (oficina)	2
Participações em eventos (encontro)	2
Participações em eventos (outra)	3
Organização de evento (congresso)	3
Organização de evento (outro)	2

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