In silico identification of vaccine and drug targets for *Corynebacterium ulcerans* and the recently described *C. silvaticum*

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Abstract

The availability of complete genomes of pathogens enables *in silico* analyzes that can be used to develop new control methods, reducing the time, cost, and necessity of pathogen cultivation. In this study, we developed a novel pipeline for the prediction of common broad-spectrum drug and vaccine candidates against the diphtheria toxin producing *Corynebacterium ulcerans* and the recently described *C. silvaticum species*, considered as a zoonotic potential. We found four common, non-host homologous, virulent, essential cytoplasmic druggable proteins that belong to metabolic pathways and are involved in the regulation in other essential genes. In addition, the docking analysis showed natural compounds from the ZINC database as drug candidate against the target proteins. We also identified nine vaccine candidates involved in transport and regulation of permeability of substances important to the cell. We hypothesize that these identified therapeutic targets and antimicrobial drugs could be considered for prophylaxis and hence, should be subjected to further experimental validations. We suggest that the pipeline can be used on any pathogen.

1. Introduction

Bacteria of the genus *Corynebacterium* are Gram positive, non-motile and sporeless rods, with strains capable of producing diphtheria toxin (DT) or carrying the gene. *C. ulcerans* is a pathogenic bacterium belonging to the same clade as *C. diphtheria* and *C. tuberculosis*. It is a species of economic and social importance, as it is the main current etiological agent of diphtheria in addition to infecting several mammals. Some strains produce DT, others do not produce the toxin yet cause other human diseases (endocarditis, septic arthritis, osteomyelitis and sepsis) (Hacker et al. 2016). *C. silvaticum* was recently described and is closely related to *C. ulcerans*. This bacterium has pig and deer as reservoirs, but its potential host range is still unknown. It is known so far that it is cytotoxic in humans (Möller et al. 2021). Therefore, the bacterium has a zoonotic potential that leads to a potential economic loss in animal production (Dangel et al. 2020; Viana et al. 2020).

C. ulcerans was selected based on its emergence as a causative agent of diphtheria and the fact that It has a wide range of reservoir species (Hacker et al. 2016; Tiwari et al. 2008), which increases its threat potential. *C. silvaticum* was selected based on its ability to infect production animals and has a, as yet, unknown potential to cause zoonotic infections (Dangel et al. 2020; Viana et al. 2020). The current vaccine for diphtheria uses the toxoid from *C. diphtheriae* (Rappuoli and Malito 2014) and the lack of sequence diversity could lower the efficiency of the current vaccine and antitoxin against *C. ulcerans tox*$^{+}$ strains (Otsuji et al. 2019) and could play a role in diphtheria cases in vaccinated individuals (Vandentorren et al. 2014). Other factors that hinder the control of this disease are that immunization does not last for a lifetime and decreases with time, and also has the possibility of immunized people being colonized and transmitting the disease (Truelove et al. 2020). Additionally, non-toxigenic strains of *C. ulcerans* can cause severe infections such as endocarditis, septic arthritis, osteomyelitis and sepsis (Grosse-Kock et al. 2017; Zasada 2013).

Genomic data can directly assist in the development of control methods. Reverse vaccinology is the prediction of target proteins for vaccine development based on genomic sequencing data, decreasing the time and cost required and the need for cultivation of the pathogen (Rappuoli et al. 2016; Rappuoli 2000). Examples of products developed from this approach are the vaccines *Bexsero* for *Neisseria meningitidis* (Christodoulides and Heckels 2017) and *Engerix-B* for Hepatitis B (Keating and Noble 2003). Recently, vaccine targets have been predicted *in silico* and tested *in vitro* or *in vivo* for *N. meningitidis* (Masignani et al. 2019), *Shigella* (de Alwis et al. 2021; Hajialibeigi et al. 2021), *Leishmania mexicana* (Burgos-Reyes et al. 2021), and *Rhipicephalus bursa* (Couto et al. 2021). Drug targets and their respective drugs can also be predicted by a *in silico* approach (Das et al. 2021).

In this work, we developed a novel pipeline and applied it to the pathogens *Corynebacterium ulcerans* and *C. silvaticum*for the identification of common vaccine and drug targets. While this pipeline is demonstrated with these *Corynebacterium* species, it can be used on any other pathogen.

2. Materials And Method

2.1. Samples

Genome data of 108 strains of were retrieved from GenBank, 72 of *C. ulcerans* and 36 of *C. silvaticum* (Supplementary Table S1). Of the 108 genomes, 76 were available as sequencing reads from the Sequence Read Archive (Supplementary Table S1, SRR accession only). They were assembled in PATRIC (Davis et al. 2020) using Unicycler (Wick et al. 2017). All the genomes were annotated in PATRIC using RASTtk (Brettin et al. 2015). The taxonomy of the samples had previously been verified as *C. ulcerans* or *C. silvaticum* (Viana et al. 2020)

using FastANI (Jain et al. 2018), and were additionally submitted to the Type Strain Genome Server (Meier-Kolthoff and Göker 2019) to confirm their taxonomy.

2.2. Identification of new targets

2.2.1. Identification of core, non-homologous to human host, essential genes, and cell localization

The core proteome were obtained using OrthoFinder v2.3.11 (Emms and Kelly 2015). Of these, proteins not homologous to the host were identified by BLASTp (Camacho et al. 2009) against the human proteome (GenBank accession GRCh38.p13). Furthermore, the dataset of core and non-host homologous proteins were used for essential protein identification by the Pipeline Builder for Identification of Targets (PBIT) (Shende et al. 2017). Finally, the cellular location of the proteins was identified using the SurfG + v1.2.1 (Barinov et al. 2009). Putative Surface Exposed (PSE), secreted and membrane proteins were screened for probable vaccine targets, while cytoplasmatic proteins were screened for probable drug targets.

2.2.2. Characterization of vaccine targets

For secreted PSE, and membrane proteins, the Vaxign2 web-based server (He et al. 2010) was used to identify likely vaccines targets using the following criteria: i) epitope binding to the Major Histocompatibility Complex I and II (MHC I and MHC II) and; ii) probability of being an adhesin greater than 0.51. Cleavage sites, transmembrane domains, functional domains, and virulence factors were predicted using SignalP 5.0 server (Petersen et al. 2011), TMHMM (Sonnhammer et al. 1998), InterProScan (Mitchell et al. 2015) and PBIT, respectively. These genes were reannotated for function using eggNOG-mapper (Huerta-Cepas et al. 2017). Genes in genomic islands (GI), specifically in pathogenic islands (PAIs), were predicted using GIPSy (Soares et al. 2016) with *C. glutamicum* 13032 (CP025533.1) as non-pathogenic reference.

2.2.3. Identification of drug targets and docking analysis

For the 84 cytoplasmic proteins, 3D structures were predicted through the MHOLline pipeline (http://www.mholline2.lncc.br/). Genes with predicted structures classified as G2 (e-value $\leq 10e^{-5}$ and Identity ≥ 0.25 and Length Variation Index ≤ 0.7) were selected for the following steps. The Ramachandran plot of the structures was generated PROCHECK (Laskowski et al. 1993) implemented in SAVES server v6.0 (https://saves.mbi.ucla.edu/). Furthermore, the target proteins were prioritized according to function and metabolic pathway using eggNOG-mapper, molecular weight using Uniprot (Wasmuth et al. 2017), virulence using Virulence Factor Database (VFDB) (CHEN *et al.*, 2005) and PBIT, and genomic islands using GIPSy.

The residues with high drug scores were identified using DoGSiteScorer (Volkamer et al. 2012). InterProScan online software was used to identify the domains of each of these proteins (JONES *et al.*, 2014). Moreover, the DoGSiteScorer pocket with the highest drug score and the residues of the protein domain were used for the preparation of targets for docking using AutoDockTools (Morris et al. 2009). To identify drugs for these target proteins, a library of 5,008 drug-like natural compounds were obtained from the ZINC database (http://zinc.docking.org/). The docking analysis was performed using AutoDock Vina (Trott and Olson 2010). The top 10 compounds were analyzed for interactions between their residues interaction and the target proteins using Chimera (Pettersen et al. 2004).

2.3. Workflow

The methods used here and the total number of proteins in each step are summarized in Fig. 1.

3. Results

3.1. Vaccine targets

1,622 proteins were identified as the core proteome, and of these, 325 were characterized as non-host proteins, 168 as essential, 71 as PSE or secreted and 97 as cytoplasmatic proteins. Within the PSE, Vaxign2 predicted nine vaccine targets with adhesin probability > 0.51 and binding epitopes for MHC I and II (Fig. 1, Table 1). Two of these were in pathogenicity islands. An ABC transporter, substrate-binding protein (*mntA*) was found in PAI 3, and a phosphate ABC transporter, substrate-binding protein PstS (TC 3.A.1.7.1) (*pstS*) was found in PAI 6 (Fig. 2, Supplementary Table S2).

Table 1
Vaccine targets candidates for *Corynebacterium ulcerans* and *C. silvaticum.*

Product (Gene)	Adhesin probability	MHC class	Virulence factor / (GI)[a]	PATRIC ID
ABC transporter, substrate-binding protein (cluster 14, Mn/Zn)	0.630	I and II	No	996634.5.peg.167
ABC transporter, substrate-binding protein (cluster 14, Mn/Zn) (*mntA*)	0.602	I and II	Yes (PAI 3)[b]	996634.5.peg.623
Glutamate ABC transporter, permease protein 1 GluC (*gluC*)	0.555	I and II	No	996634.5.peg.835
Integral membrane protein	0.566	I and II	No	996634.5.peg.316
Hypothetical protein	0.512	I and II	No	996634.5.peg.652
Hypothetical protein	0.586	I and II	No	996634.5.peg.823
Hypothetical protein	0.661	I and II	No	996634.5.peg.1276
Phosphate ABC transporter, substrate-binding protein PstS (TC 3.A.1.7.1) (*pstS*)	0.696	I and II	No (PAI 6)	996634.5.peg.1997
Phospholipase / thioesterase	0.571	I and II	No	996634.5.peg.1106

[a] genomic island

[b] pathogenicity island

3.2. Drug targets

For 84 proteins, the sequence alignment with a reference protein had the structure quality classified as G2 (e-value ≤ 10 − 5, identity ≥ 0.25% and Length Variation Index ≤ 0.1). From these, only the four proteins with alignment quality classified as Very High (identity ≥ 0.75% and Length Variation Index ≤ 0.1) were selected for further analysis (Table 2). The Ramachandran plot for 3D structure validation of these final 4 targets is shown in Supplementary Figure S1, and their respective actives residues in Table S3. These four potential targets are essential and only DtxR is a virulence factor according to the PBIT database prediction (Supplementary Table S2). None of the drug targets were in a PAI or GI (Fig. 2).

Table 2
Drug target candidates for *Corynebacterium ulcerans* and *C. silvaticum.*

Product (Gene)	Molecular Function	Biological Process	Mass (Da)	Metabolic Pathways	Virulence Factor	Ramachandran residues in most favoral region	PATRIC ID
Iron-dependent repressor IdeR/DtxR (*dtxR*)	DNA-binding transcription factor activity, protein dimerization activity, transition metal ion binding	Transcription, Transcription regulation	25,203	-	Yes	92.2%	996634.5.peg.1487
Uridine monophosphate kinase UMPK (EC 2.7.4.22) (*pyrH*)	ATP binding, UMP kinase activity	'*de novo*' CTP biosynthetic process	26,168	CTP biosynthesis via de novo pathway and in Pyrimidine metabolism	No	94.2%	996634.5.peg.1586
Fructose-biphosphate aldolase class II (EC 4.1.2.13) (*fba*)	Fructose-bisphosphate aldolase activity, zinc ion binding	Glycolytic process	37,144	Synthesizes D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose	No	94.9%	996634.5.peg.2154
Transcriptional regulator, AcrR family	DNA binding, magnesium ion binding	Regulation of transcription	21,831	-	No	95.5%	996634.5.peg.1300

We have used DoGSiteScorer to select the druggable pockets with score greater than 0.79 (Supplementary Table S3). For the docking analysis, we selected three natural compounds (for each target) based on lower binding energy values and the largest number of hydrogen bonds. The output was the 10 top ranked ligands, based on the best interactions with the proteins actives residues (domain), according to the requisites mentioned. We selected the final 3 out of 10 best compounds for each target protein. The hydrogen bonds, binding energy values, ligands IDs and proteins information are shown in Table 3. For the graphic representation of the docking analysis, we selected the best one compound for each protein (Figs. 3–6).

AcrR family protein is a transcriptional regulator that normally acts as a repressor and had better interaction with the compound ZINC04258896 (Fig. 3). Fructose-biphosphate aldolase is an enzyme that participates in the glycolytic pathway and showed the best docking with the compound ZINC04235626 (Fig. 4). The IdeR/DtxR target is an iron-dependent transcriptional regulator, classified as a virulence factor that showed the best interaction with the ligand ZINC03840461 (Fig. 5). Finally, Uridine monophosphate kinase is a converting enzyme in the metabolism of pyrimidines, It had the best docking with the ligand ZINC08300249 (Fig. 6).

Table 3
Docking results of candidate proteins targets and its ligands showing number of hydrogen bonds with its respective residues and binding energy values.

Protein (PATRIC ID)	Ligand ZINC ID	IUPAC name	Binding energy value (Kcal/mol)	H bond / Residues
Transcriptional regulator, AcrR family (996634.5.peg.1300)	ZINC04258896	1-(1,3-benzodioxol-5-yl)-N-methyl-2-[(E)-3-phenylprop-2-enoyl]-1,3,4,9-tetrahydropyrido[3,4-b]indole-3-carboxamide	-7.9	3/Arg21, Arg92
	ZINC04277685	N-[(1R,9S)-11-(naphthalene-2-carbonyl)-6-oxo-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-5-yl]cyclohexanecarboxamide	-8	2/Tyr29, Asp54
	ZINC08300353	N-[(1R,9S)-11-(4-fluorobenzoyl)-6-oxo-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-3-yl]-2H-1,3-benzodioxole-5-carboxamide	-8.2	2/Arg22, Asp94
Fructose-biphosphate aldolase class II (996634.5.peg.2154)	ZINC04235626	(3R,3aS,6S,6aR)-6-(naphthalene-2-sulfonamido)-hexahydrofuro[3,2-b]furan-3-yl N-(4-methoxyphenyl)carbamate	-9.1	3/Thr29, Ser53, Asp276
	ZINC04235829	(3R,3aS,6S,6aR)-6-(naphthalene-2-sulfonamido)-hexahydrofuro[3,2-b]furan-3-yl N-(4-acetylphenyl)carbamate	-9.3	3/Thr29, Ser53, His212
	ZINC08300421	N-[(1R,9S)-11-(1-acetylpiperidin-4-yl)-6-oxo-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-3-yl]-2H-1,3-benzodioxole-5-carboxamide	-9.6	2/Gly213, Thr277
Iron-dependent repressor IdeR/DtxR (996634.5.peg.1487)	ZINC03840461	N-[(11S,13S)-5-(4-chlorophenyl)-2,10-dioxo-1,9-diazatricyclo[9.4.0.0³,⁸]pentadeca-3(8),4,6-trien-13-yl]pyridine-3-carboxamide	-9.8	2/Ser70, Thr159
	ZINC08300249	(1R,9S)-11-(2H-1,3-benzodioxole-5-carbonyl)-5-[3-(trifluoromethyl)phenyl]-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-6-one	-10.2	2/Thr124, Leu135
	ZINC04259070	(1R,9R)-5-{[(3-fluorophenyl)carbamoyl]amino}-N-(4-methoxyphenyl)-6-oxo-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-diene-11-carboxamide	-10.4	2/Thr24, Thr191
Uridine monophosphate kinase (996634.5.peg.1586)	ZINC08300249	(1R,9S)-11-(2H-1,3-benzodioxole-5-carbonyl)-5-[3-(trifluoromethyl)phenyl]-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-6-one	-10.3	2/Gly59, Arg64
	ZINC08300441	3-[(1R,9S)-11-(1-acetylpiperidin-4-yl)-6-oxo-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-3-yl]-1-(3-cyanophenyl)urea	-9.9	3/Gly21, Asp79, Met140
	ZINC08300421	N-[(1R,9S)-11-(1-acetylpiperidin-4-yl)-6-oxo-7,11-diazatricyclo[7.3.1.0²,⁷]trideca-2,4-dien-3-yl]-2H-1,3-benzodioxole-5-carboxamide	-9.9	2/Gly59, Arg64

4. Discussion

C. ulcerans can infect humans and a variety of wild and domestic animals that act as a reservoir. Manifestations can be asymptomatic, or cause damage to the respiratory tract, mastitis and gangrenous dermatitis (Hacker et al. 2016; Tiwari et al. 2008). Non-toxigenic strains can be classified as tox-negative or non-toxigenic but *tox* gene-bearing (NTTB) (Dangel et al. 2019; Fuursted et al. 2015). *C. silvaticum* is a recently described NTTB species close to *C. ulcerans* that causes caseous lymphadenitis in wild animals such as wild boar and roe deer, which are reservoirs, and domestic pigs (Dangel et al. 2020). Its potential host range is unknown, and It could potentially cause zoonotic infections (Viana et al. 2020).

Subtractive genomics, as the name suggests, is an approach based on several steps of filtering proteomic sequences, in order to select and identify final proteins that are indispensable to microorganisms and that are not homologous to the host. This methodology is widely used in reverse vaccinology, a method which includes subtractive genomics, modeling, docking and other in silico techniques in order to predict vaccine and drug targets, without financial expense and in a short time (Hassan et al. 2018; Masignani et al. 2019). The Bexsero

vaccine for Neisseria meningitidis (Christodoulides and Heckels 2017) and Engerix-B vaccine for Hepatitis B (Keating and Noble 2003) were developed using these approaches.

We predicted nine vaccine targets, four drug targets and four probable dug molecules for *C. ulcerans* and *C. silvaticum* using reverse vaccinology and *in silico* drug targeting (Tables 1 and 2). Among nine predicted vaccine targets, four were annotated as subunits of ABC transporters. Two are substrate-binding protein for manganese (Mn) and zinc (Zn), one for phosphate, and one is a permease for glutamate. Both metals are vital for several organisms, required by transcriptional transporters and regulators and oxidative stress response (Mn), and several ribosomal proteins and tRNA synthetases (Zn). The acquisition of both metals is disputed with the host and those ABC transporters are required for colonization (Juttukonda and Skaar 2015; Nies and Grass 2009). One of the transporter's subunits (*mntA*) is in PAI 3 (Fig. 2). Glutamate is an essential metabolite that plays an important role in the metabolism of nitrogen and carbon. The ABC glutamate transporter has already been characterized in other bacterial genera and is a known vaccine target, considering its indispensable role in the survival of the bacterium (Bhatia et al. 2014). Phosphate is essential for energy metabolism and is a component of nucleic acids, phospholipids, and other cell molecules. The Phosphate ABC transporter, substrate-binding protein PstS is part of the complex PstSACB involved in phosphate import (Santos-Beneit 2015). Inactivation of the *pstS* gene increased the susceptibility to penicillin in *Streptococcus pneumoniae* (Soualhine et al. 2005). The transport of these essential nutrients makes those proteins promising vaccine candidates.

Among the non-transporter vaccine target candidates is a bifunctional protein from the Phospholipase/Thioesterase family, with the gene located in pathogenicity island PAI 6 (Fig. 2). This enzyme is involved in the non-ribosomal synthesis of peptides, including antimicrobial peptides (Santucci et al. 2018; Schneider and Marahiel 1998). The Integral membrane protein belong to the "Putative Actinobacterial Holin-X, holin superfamily III". Holins are produced by double-stranded DNA bacteriophages that use an endolysin-holin strategy to lysogenize their hosts (InterPro entry IPR009937). The other three candidates are hypothetical proteins classified by InterProScan as "Papain-like cysteine peptidase superfamily" (InterPro entry IPR038765) (PATRIC ID 996634.5.peg.652), "Calcium-dependent phosphotriesterase" (SSF63829) (996634.5.peg.1276) and a transmembrane protein (996634.5.peg.823).

Within the drug targets, DtxR was the only predicted virulence factor. It is an iron-dependent repressor in prokaryotes that regulates the expression of genes involved in iron homeostasis and virulence factors, such as diphtheria toxin (*tox*) (WHITE *et al.*, 1998). DtxR regulates genes according to the concentration of Fe_2^+ ions in the environment, allowing to adjust iron uptake. It is considered a virulence factor due to its importance for host colonization (Merchant and Spatafora 2014). Furthermore, DxtR has been considered an attractive drug target due to its role in regulating genes involved in iron homeostasis in bacteria, and such genes are generally virulence factors (Cheng et al. 2018; Parise et al. 2021). Residue SER 70 is hydrogen bonded to the nitrogen of the aromatic ring, THR 159 is hydrogen bonded to the third oxygen of the compound and residues ARG 69 and LEU 135 have hydrophobic interactions with the three aromatic rings.

Uridine Monophosphate Kinase is an important enzyme that acts on the metabolism of pyrimidines in bacteria, specifically on uridine monophosphate (UMP). It converts UMP to UDP that is later used in the nucleoside biosynthesis. UMPK plays an essential role in the production of nucleotides that are constituents of nucleic acids. This enzyme is of interest, as It has been recognized as a potential drug target for tuberculosis (ARVIND et al. 2013) as It also plays a role in regulating the balance of pyrimidine and purine nucleosides. In addition, It is structurally different from the eukaryotic UMP kinases (Rostirolla et al. 2011), which makes It a more attractive candidate. ASP 79 makes two hydrogen bonds with NH (NH-O), GLY 21 makes two hydrogen bonds with the first oxygen of the molecule. The 2D image shows hydrogen bonding of MET 140 to the last nitrogen. MET 83 shows hydrophobic interaction with the last aromatic ring.

The Fructose-1,6-bisphosphate aldolase (FBA) is a glycolytic protein that participates in the glucose metabolic pathway and in the production of organic acid when oxygen is scarce (Altenhoff and Dessimoz 2009; Teramoto et al. 2010). In some genera, It has been shown that reducing FBA leads to cell death. This enzyme is present in the membrane and exposed on the bacterium's cell surface, and its association to virulence is already know as It binds to human plasminogen and mammalian cells (Shams et al. 2014). FBA and other proteins are expressed under conditions of alkaline stress, which indicates that this enzyme is also involved in the physiological breakdown of the bacterium during environmental changes. For these reasons, this protein has previously been recognized as a potential candidate for drug and vaccine targets in various pathogens such as bacteria, fungi and parasites of animals and humans (Pirovich et al. 2021)(Shams et al. 2014). SER 53 is hydrogen bonded to two oxygens (one from the pentose), HIS 212 is hydrogen bonded to an oxygen at the ligand end, and THR 29 is hydrogen bonded to one oxygen (O = S = O). THR 29, THR 33 and GLN 280 have a hydrophobic interaction with naphthalene.

The AcrR family of transcriptional regulators belongs to the one-component system and is associated with several essential cellular mechanisms of bacteria and archaea, such as cell signaling, carbon, nitrogen and lipid metabolism, amino acid metabolism and cofactor

metabolism, production of antibiotics, among other physiological factors. They normally act as repressors, having different regulatory mechanisms in different species of bacteria, which can be positive or negative. In addition, these regulators play a key role in antibiotic resistance (Cuthbertson and Nodwell 2013). For these reasons, proteins from this family have been identified as a broad-spectrum drug target (Deng et al. 2013) and for novel treatments (Gonzáles et al. 2018). ARG 21 has two hydrogen bonds with the oxygen that is attached to the aromatic ring, ARG 92 has a hydrogen bond with an oxygen of the ligand. The two aromatic rings show hydrophobic interactions with VAL 73 and ARG 69.

Conclusion

In this work we presented a first-time approach applied to these bacteria, in which we use common drugs and vaccines for both species. We predicted nine vaccines and four drug target candidates, as well as the respective drug molecules for *C. ulcerans* and *C. silvaticum*. This type of analysis could reduce the cost and time for the development of vaccine and identification of antimicrobial compounds and should be subjected to further experimental validation, as these species can be cultivated. This pipeline is not restricted to *Corynebacterium* but could be used on any prokaryotic or eukaryotic pathogen.

Declarations

CONFLICT OF INTEREST

None declared.

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References

1. Altenhoff AM, Dessimoz C (2009) Jan;5(1):e1000262 Phylogenetic and functional assessment of orthologs inference projects and methods. PLoS Comput. Biol. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19148271
2. de Alwis R, Liang L, Taghavian O, Werner E, The HC, Thu TNH et al (2021 Dec) The identification of novel immunogenic antigens as potential Shigella vaccine components. Genome Med 13(1):8
3. ARVIND A, JAIN V, SARAVANAN P (2013) MOHAN CG. Uridine Monophosphate Kinase as Potential Target for Tuberculosis: From Target to Lead Identification.Interdiscip Sci Comput Life Sci. ;296–311
4. Barinov A, Loux V, Hammani A, Nicolas P, Langella P, Ehrlichh D et al (2009) Prediction of surface exposed proteins in Streptococcus pyogenes, with a potential application to other Gram-positive bacteria. Proteomics 9(1):61–73
5. Bhatia B, Ponia SS, Solanki AK, Dixit A, Garg LC (2014) Identification of glutamate ABC-Transporter component in Clostridium perfringens as a putative drug target. Bioinformation 10(7):401–405
6. Brettin T, Davis JJ, Disz T, Edwards R, Gerdes S, Olsen GJ et al (2015 Feb) RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365
7. Burgos-Reyes MA, Baylón-Pacheco L, Espíritu-Gordillo P, Galindo-Gómez S, Tsutsumi V, Rosales-Encina JL Effect of Prophylactic Vaccination with the Membrane-Bound Acid Phosphatase Gene of Leishmania mexicana in the Murine Model of Localized Cutaneous Leishmaniasis. Laurenti M, editor. J. Immunol. Res. 2021 Apr;2021:6624246
8. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K et al (2009) BLAST+: architecture and applications. BMC Bioinformatics 10:421
9. Cheng Y, Yang R, Lyu M, Wang S, Liu X, Wen Y et al (2018) IdeR, a DtxR Family Iron Response Regulator, Controls Iron Homeostasis, Morphological Differentiation, Secondary Metabolism, and the Oxidative Stress Response in Streptomyces avermitilis.Appl. Environ. Microbiol.; 84(22)
10. Christodoulides M, Heckels J (2017) Novel approaches to Neisseria meningitidis vaccine design. Pathog Dis 75(3):1–16

11. Couto J, Seixas G, Stutzer C, Olivier NA, Maritz-Olivier C, Antunes S et al (2021) Mar Probing the rhipicephalus bursa sialomes in potential anti-tick vaccine candidates: A reverse vaccinology approach.Biomedicines. ; 9(4)
12. Cuthbertson L, Nodwell JR (2013) The TetR Family of Regulators. Microbiol Mol Biol Rev 77(3):440–475
13. Dangel A, Berger A, Konrad R, Sing A (2019) NGS-based phylogeny of diphtheria-related pathogenicity factors in different Corynebacterium spp. implies species-specific virulence transmission. BMC Microbiol BMC Microbiology 19(1):1–16
14. Dangel A, Berger A, Rau J, Eisenberg T, Kämpfer P, Margos G et al (2020) Corynebacterium silvaticum sp. Nov., a unique group of NTTB corynebacteria in wild boar and roe deer. Int J Syst Evol Microbiol 70(6):3614–3624
15. Das P, Sercu T, Wadhawan K, Padhi I, Gehrmann S, Cipcigan F et al (2021) Mar Accelerated antimicrobial discovery via deep generative models and molecular dynamics simulations.Nat. Biomed. Eng.
16. Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler RRM, Butler RRM et al (2020) The PATRIC Bioinformatics Resource Center: Expanding data and analysis capabilities. Nucleic Acids Res, vol 48. Oxford University Press, pp D606–D612. D1
17. Deng W, Li C, Xie J (2013) Jul;25(7):1608–13 The underling mechanism of bacterial TetR/AcrR family transcriptional repressors. Cell. Signal. [Internet]. Elsevier Inc.; Available from: http://dx.doi.org/10.1016/j.cellsig.2013.04.003
18. Emms DM, Kelly S (2015) OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol Genome Biology 16(1):1–14
19. Fuursted K, Søes LM, Crewe BT, Stegger M, Andersen PS, Christensen JJ (2015 Oct) Non-toxigenic tox gene-bearing Corynebacterium ulcerans in a traumatic ulcer from a human case and his asymptomatic dog. Microbes Infect 17(10):717–719
20. Gonzáles A, Fillat MF, Lanas Á (2018) Transcriptional regulators: valuable targets for novel antibacterial strategies. Future Med Chem 10:541–560
21. Grosse-Kock S, Kolodkina V, Schwalbe EC, Blom J, Burkovski A, Hoskisson PA et al (2017) Genomic analysis of endemic clones of toxigenic and non-toxigenic Corynebacterium diphtheriae in Belarus during and after the major epidemic in 1990s. BMC Genomics BMC Genomics 18(1):1–10
22. Hacker E, Antunes CA, Mattos-Guaraldi AL, Burkovski A, Tauch A (2016 Sep) Corynebacterium ulcerans, an emerging human pathogen. Future Microbiol 11(9):1191–1208
23. Hajialibeigi A, Amani J, Gargari SLM (2021 Feb) Identification and evaluation of novel vaccine candidates against Shigella flexneri through reverse vaccinology approach. Appl Microbiol Biotechnol 105(3):1159–1173
24. Hassan SS, Jamal SB, Radusky LG, Tiwari S, Ullah A, Ali J et al (2018) The druggable pocketome of Corynebacterium diphtheriae: A new approach for in silico putative druggable targets. Front Genet 9(FEB):1–9
25. He Y, Xiang Z, Mobley HLT (2010) Vaxign: The First Web-Based Vaccine Design Program for Reverse Vaccinology and Applications for Vaccine Development. J. Biomed. Biotechnol. [Internet]. ;2010:1–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20671958
26. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, Von Mering C et al (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol 34(8):2115–2122
27. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S (2018 Dec) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 9(1):5114
28. Juttukonda LJ, Skaar EP (2015) Manganese homeostasis and utilization in pathogenic bacteria. Mol Microbiol 97(2):216–228
29. Keating GM, Noble S (2003) Recombinant Hepatitis B Vaccine (Engerix-B). Drugs 63(10):1021–1051
30. Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr 26(2):283–291
31. Masignani V, Pizza M, Moxon ER (2019) The development of a vaccine against Meningococcus B using reverse vaccinology. Front Immunol 10(MAR):1–14
32. Meier-Kolthoff JP, Göker M (2019 Dec) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10(1):2182
33. Merchant AT, Spatafora GA (2014) Feb;29(1):1–10 A role for the DtxR family of metalloregulators in gram-positive pathogenesis. Mol. Oral Microbiol. [Internet]. Available from: http://doi.wiley.com/10.1111/omi.12039
34. Mitchell A, Chang H-Y, Daugherty L, Fraser M, Hunter S, Lopez R et al (2015 Jan) The InterPro protein families database: the classification resource after 15 years. Nucleic Acids Res 43(D1):D213–D221

35. Möller J, Busch A, Berens C, Hotzel H, Burkovski A (2021) Newly Isolated Animal Pathogen Corynebacterium silvaticum Is Cytotoxic to Human Epithelial Cells.
36. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS et al (2009 Dec) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 30(16):2785–2791
37. Nies DH, Grass G (2009) Transition Metal Homeostasis. Stewart V, editor. EcoSal Plus [Internet]. Aug 13;3(2):ecosalplus.5.4.4.3. Available from: https://journals.asm.org/doi/10.1128/ecosalplus.5.4.4.3
38. Otsuji K, Fukuda K, Ogawa M, Saito M (2019) Mutation and diversity of diphtheria toxin in Corynebacterium ulcerans. Emerg Infect Dis 25(11):2122–2123
39. Parise D, Teixeira M, Parise D, Cybelle A, Gomide P, Kato RB et al (2021)The Transcriptional Regulatory Network of Corynebacterium pseudotuberculosis.
40. Petersen TN, Brunak S, von Heijne G, Nielsen H (2011 Sep) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8(10):785–786
41. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC et al (2004) UCSF Chimera - A visualization system for exploratory research and analysis. J Comput Chem 25(13):1605–1612
42. Pirovich DB, Da AA, Skelly PJ (2021) Multifunctional Fructose 1, 6-Bisphosphate Aldolase as a Therapeutic Target. 8:1–19August
43. Rappuoli R (2000) Reverse vaccinology. Curr Opin Microbiol 3(5):445–450
44. Rappuoli R, Bottomley MJ, D'Oro U, Finco O, De Gregorio E (2016) Reverse vaccinology 2.0: Human immunology instructs vaccine antigen design. J Exp Med 213(4):469–481
45. Rappuoli R, Malito E (2014) History of Diphtheria Vaccine Development. In: Burkovski A (ed) Corynebacterium diphtheriae Relat. Toxigenic Species. Springer Netherlands, Dordrecht, pp 225–238
46. Rostirolla DC, Breda A, Rosado LA, Palma MS, Basso LA, Santos DS (2011) UMP kinase from Mycobacterium tuberculosis: Mode of action and allosteric interactions, and their likely role in pyrimidine metabolism regulation. Arch. Biochem. Biophys. [Internet]. Elsevier Inc.; ;505(2):202–12. Available from: http://dx.doi.org/10.1016/j.abb.2010.10.019
47. Santos-Beneit F (2015) The Pho regulon: a huge regulatory network in bacteria.Front. Microbiol.Apr;6.
48. Santucci P, Point V, Poncin I, Guy A, Crauste C, Serveau-Avesque C et al (2018) LipG a bifunctional phospholipase/thioesterase involved in mycobacterial envelope remodeling. Biosci Rep 38(6):1–21
49. Schneider A, Marahiel MA (1998) Genetic evidence for a role of thioesterase domains, integrated in or associated with peptide synthetases, in non-ribosomal peptide biosynthesis in Bacillus subtilis. Arch Microbiol 169(5):404–410
50. Shams F, Oldfield NJ, Wooldridge KG, Turner DPJ (2014) Fructose-1,6-bisphosphate aldolase (FBA)-A conserved glycolytic enzyme with virulence functions in bacteria: "Ill met by moonlight. Biochem Soc Trans 42(6):1792–1795
51. Shende G, Haldankar H, Barai RS, Bharmal MH, Shetty V, Idicula-Thomas S et al (2017 Dec) PBIT: Pipeline builder for identification of drug targets for infectious diseases. Bioinformatics 33(6):929–931
52. Soares SC, Geyik H, Ramos RTJ, de Sá PHCG, Barbosa EGV, Baumbach J et al (2016 Aug) GIPSy: Genomic island prediction software. J Biotechnol 232:2–11
53. Sonnhammer EL, von Heijne G, Krogh A (1998) A hidden Markov model for predicting transmembrane helices in protein sequences. Proceedings. Int. Conf. Intell. Syst. Mol. Biol. ;6:175–82
54. Soualhine H, Brochu V, Ménard F, Papadopoulou B, Weiss K, Bergeron MG et al (2005 Dec) A proteomic analysis of penicillin resistance in Streptococcus pneumoniae reveals a novel role for PstS, a subunit of the phosphate ABC transporter. Mol Microbiol 58(5):1430–1440
55. Teramoto H, Inui M, Yukawa H (2010) Regulation of genes involved in sugar uptake, glycolysis and lactate production in Corynebacterium glutamicum. Future Microbiol 5(10):1475–1481
56. Tiwari TSP, Golaz A, Yu DT, Ehresmann KR, Jones TF, Hill HE et al Investigations of 2 cases of diphtheria-like illness due to toxigenic Corynebacterium ulcerans.Clin. Infect. Dis. 2008Feb; 46(3):395–401
57. Trott O, Olson AJ AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. [Internet]. 2010 Jan 30;31(2):455–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19499576
58. Truelove SA, Keegan LT, Moss WJ, Chaisson LH, Macher E, Azman AS et al (2020) Clinical and epidemiological aspects of diphtheria: A systematic review and pooled analysis.Clin. Infect. Dis. p.89–97

59. Vandentorren S, Guiso N, Badell E, Boisrenoult P, Micaelo M, Troché G et al (2014 Sep) Toxigenic Corynebacterium ulcerans in a fatal human case and her feline contacts, France, March 2014, vol 19. Euro Surveill. European Centre for Disease Control and Prevention (ECDC), p 20910. 38
60. Viana MVC, Profeta R, da Silva AL, Hurtado R, Cerqueira JC, Ribeiro BFS et al Taxonomic classification of strain PO100/5 shows a broader geographic distribution and genetic markers of the recently described Corynebacterium silvaticum. Kuo C-H, editor. PLoS One [Internet]. 2020 Dec 21;15(12):e0244210. Available from: https://dx.plos.org/10.1371/journal.pone.0244210
61. Volkamer A, Kuhn D, Rippmann F, Rarey M (2012 Aug) DoGSiteScorer: a web server for automatic binding site prediction, analysis and druggability assessment. Bioinformatics 28(15):2074–2075
62. Wasmuth EV, Lima CD, Bateman A, Martin MJ, O'Donovan C, Magrane M et al (2017) UniProt: The universal protein knowledgebase. Nucleic Acids Res. Oxford University Press; Jan;45(D1):D158–69
63. Wick RR, Judd LM, Gorrie CL, Holt KE, Unicycler (2017) : Resolving bacterial genome assemblies from short and long sequencing reads. Phillippy AM, editor. PLOS Comput. Biol. Jun;13(6):e1005595
64. Zasada AA (2013) Nontoxigenic highly pathogenic clone of Corynebacterium diphtheriae, Poland, 2004–2012. Emerg Infect Dis 19(11):1870–1872

Figures

Figure 1

Workflow of the methodologies and the number of genes identified and selected at each step.

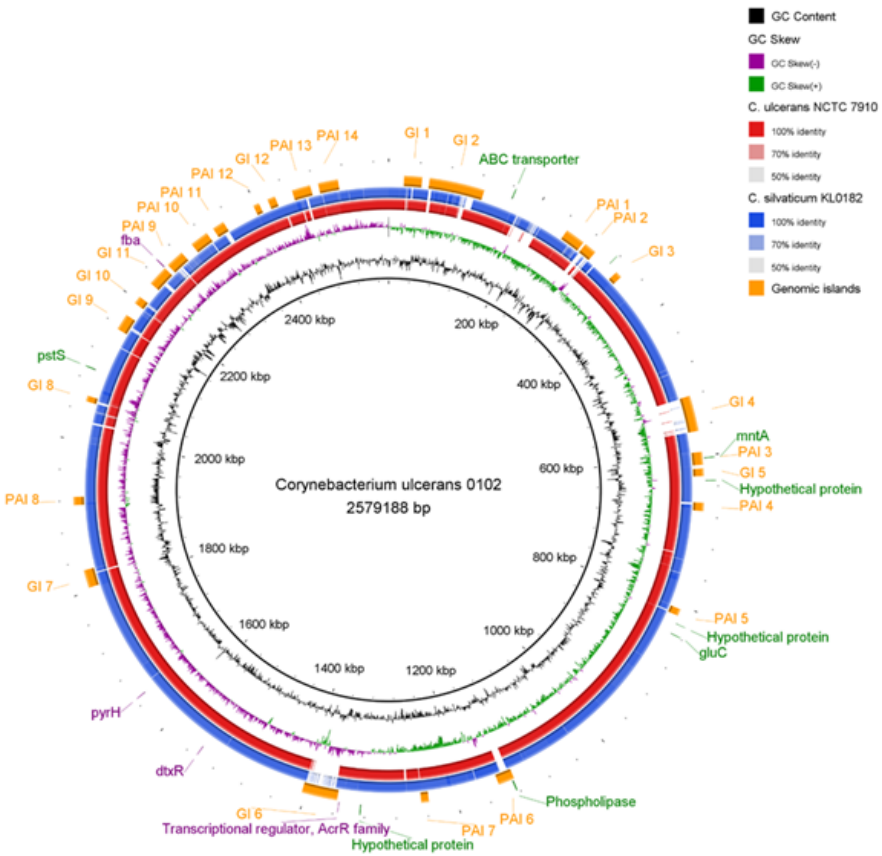
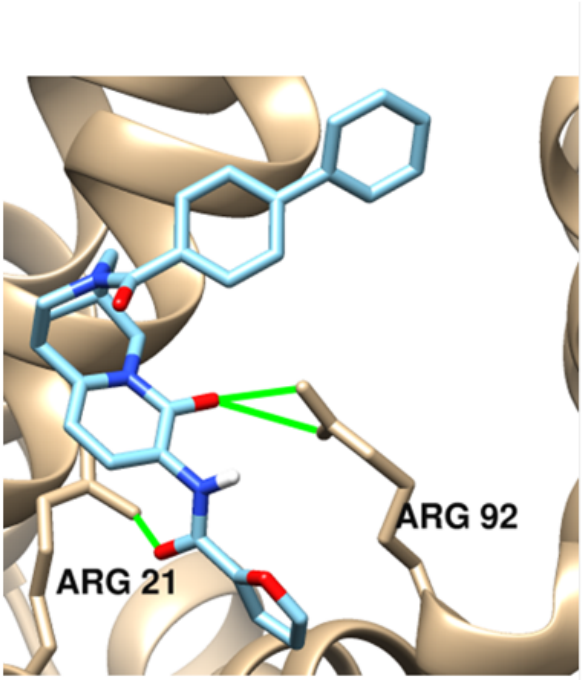


Figure 2

Circular map of *Corynebacterium ulcerans* 0102 showing genomic islands and the position of vaccine (green) and drug targets (purple). GI – genomic island, Pai – pathogenicity island.



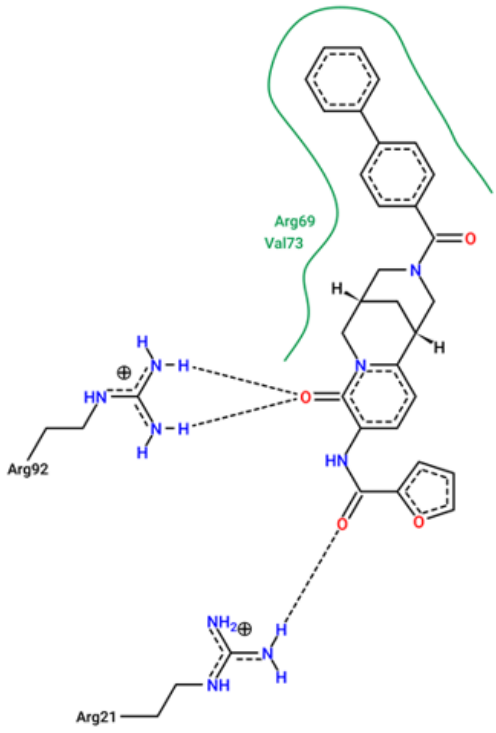


Figure 3

3D and 2D graphic representation of the docking analysis for the most druggable protein cavity of drug target AcrR family protein with ZINC04258896.

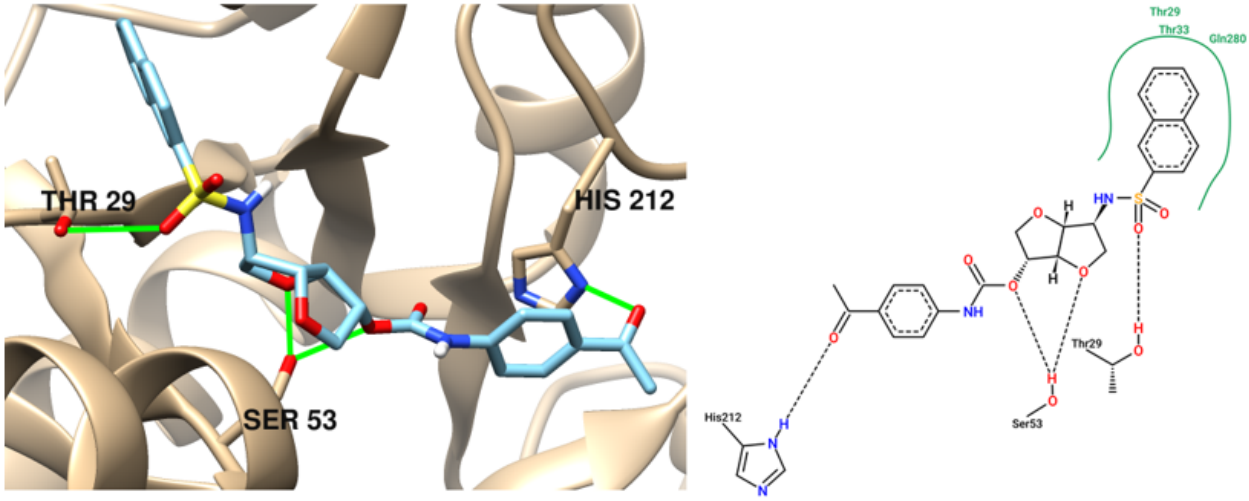


Figure 4

3D and 2D graphic representation of the docking analysis for the most druggable protein cavity of drug target Fructose-bisphosphate aldolase class II with ZINC04235829.

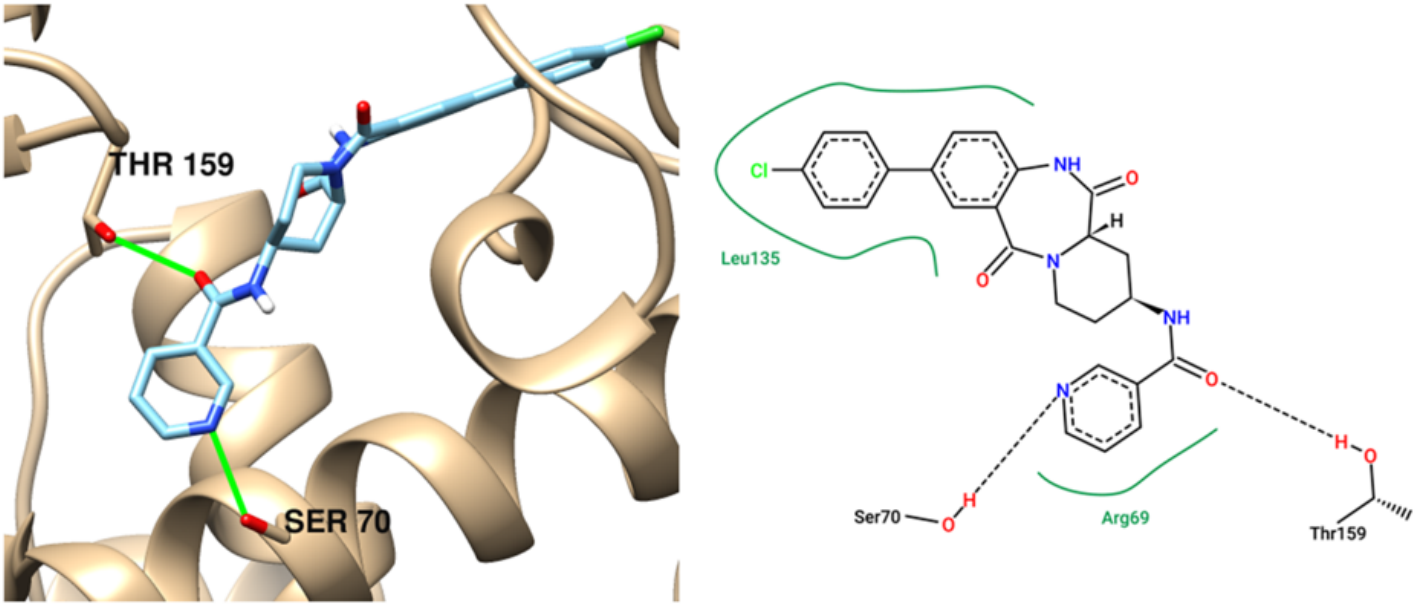


Figure 5

3D and 2D graphic representation of the docking analysis for the most druggable protein cavity of drug target Iron-dependent repressor IdeR/DtxR with ZINC03840461.

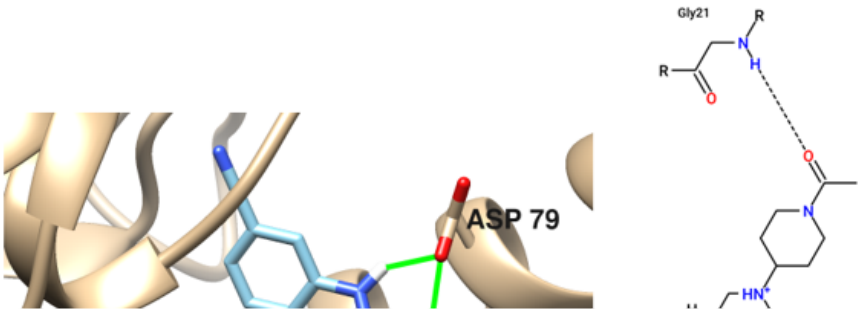


Figure 6

3D and 2D graphic representation of the docking analysis for the most druggable protein cavity of drug target Uridine monophosphate kinase UMPK with ZINC08300441.

Supplementary Files

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- SupplementaryMaterial.zip