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IDENTIFICATION OF POTENTIAL ANTIBIOTIC TARGETS IN THE PROTEOME OF MULTI-DRUG RESISTANT EISENBERGIELLA TAYI

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Abstract:

Eisenbergiella tayi is a pathogen that affects the oral cavity, gastrointestinal tract, skin, and vagina, and shows some resistance to existing antibiotics. Identifying new antibiotic targets through computational methods could expedite the process. At the same time, there are numerous opportunities to develop new antibiotics to address infections caused by this pathogen. In this study, the proteome of *E. tayi* was progressively reduced to pinpoint potential antibiotic targets. The main goals were to identify proteins that are non-redundant, unique to the pathogen, essential, located in the cytoplasm, and associated with virulence and resistance. The druggability of these proteins was assessed using the BLASTp tool from the DrugBank

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database against FDA-approved drugs. The study found that the core proteome of the pathogen consists of 6,044 proteins. Of these, 2,598 were identified as non-homologous to human proteins, and 1,169 were deemed essential to the pathogen. Sub-cellular localization revealed that 594 proteins are cytoplasmic, with 76 being selected as virulent. Metabolic pathway analysis linked 32 proteins to unique pathogen-specific pathways and identified six as druggable. Further analysis highlighted the "argD" protein as both resistant and a promising target for future drug development. These results could lay the groundwork for creating new antibiotics to combat *E. tayi* infections.

Keywords: *Eisenbergiella tayi,* subtractive proteomics, drug targets, metabolic pathways, **argD**

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Introduction:

Eisenbergiella tayi as anaerobic bacteria constitute an intrinsic part of the human microbial biota (Lobo, Jenkins et al. 2013), although culture-independent molecular methods have added more knowledge about the bacterial richness of human associated environments such as the mouth, gut, skin and vagina (Dethlefsen, McFall-Ngai et al. 2007). *Eisenbergiella* gen. nov. was proposed in 2014 to describe an obligate anaerobic, structurally Gram-positive but Gram-stain-negative-appearing bacillus recovered from the blood culture of an elderly Israeli man (Bernard, Burdz et al. 2017) including this a novel strain of a Gram-stain negative, non-motile, non-spore forming rod-shaped, obligate anaerobic bacterium, designated AT11^{T}, was isolated from a stool sample of a morbidly obese woman living in Marseille, France. This bacterium was characterized using biochemical, chemotaxonomic, and phylogenetic methods (Togo, Diop et al. 2018). Characterization of a total of eight isolates which were closest to *E. tayi* took place by using molecular identification and other methods. Two of these strains were described upon receipt as Gram-positive-staining and the remaining 6 isolates as Gram-negative-staining forms (Bernard, Burdz et al. 2017). Phenotypic characterization of the another strain B086562^{T} was carried out using standard methods, as recommended in the Wadsworth manual using trypticase yeast extract haemin (TYH) broth for fermentation reactions (Choi, Kim et al. 2019). The *E. tayi* reference strain NML 110608 proteome data was obtained from the Universal Protein Resource Knowledgebase (UniProtKB)(Boutet, Lieberherr et al. 2016). CD-HIT suite (Huang, Niu et al. 2010) was applied to reveal redundant proteins in the proteome and BLASTp (Lavigne, Seto et al. 2008) against human proteome was performed to remove homologous protein sequences in non-redundant proteins at a threshold expectation value. To obtain essential proteins, Database of Essential Genes (DEG) (Luo, Lin et al. 2021) was used. The final set of non-homologous essential proteins was subjected to PSORTb (Yu, Wagner et al. 2010) and CELLO (Bernstein, Ma et al. 2021). For better understanding of essential cytoplasmic proteins as possible drug targets, their sequences were subjected to BLASTp search of Virulent Factor Database (VFDB)(Liu, Zheng et al. 2022). Virulent proteins were mapped to pathogen metabolic pathways using KEGG Automatic Annotation Server (KAAS)(Moriya, Itoh et al. 2005). The unique metabolic pathway proteins were further unveiled for drugability through DrugBank (Wishart, Feunang et al. 2018). selected druggable

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proteins were evaluated for molecular weight calculation using Expasy ProParam tool (Gasteiger, Hoogland et al. 2005). Antibiotic Resistance Database (ARDB) (McArthur, Waglechner et al. 2013) and Comprehensive Antibiotic Resistant Database (CARD) (McArthur, Waglechner et al. 2013) were used to disclose resistant targets.

Gut microbiota and diet are believed to be associated with the pathogenesis and development of inflammatory bowel disease (IBD). Our study investigated the differences in gut microbiota and dietary factors between Chinses IBD patients and their cohabitating family member controls (Choi, Kim et al. 2019). Over time, individuals with obesity show pathological changes in multiple organs, e.g., liver, muscle, and even the brain. Studies have shown that obesity is closely associated with metabolic disorders, including hyperglycaemia, insulin resistance, dyslipidemia, hypertension (Tian, Wu et al. 2022). A high-fat diet (HFD) induces gut microbiota (GM) disorders, leading to intestinal barrier dysfunction and inflammation and Eisenbergiella Tayi produces major metabolites, eg, butyric acid, acetic acid, lactic acid, and succinic acid (Tian, Geng et al. 2022). Characterization of a previously annotated GUS from *Eisenbergiella tayi* took place and demonstrated that it is, in fact, a GalAse. We determined the crystal structure of this GalAse, identified the key residue that confers GalAse activity, and convert this GalAse into a GUS by mutating a single residue (Liu, Zheng et al. 2022). The protein identified in reference strain NML 110608 is well conserved among other sequenced proteomes of *E. tayi* (DSM26961, NML 120489, NML 150140-1). Only acetylornithine aminotransferase (argD) protein has BLASTp hit against PDB search. Target selection generally implies finding a significant therapeutic agent (Knowles and Gromo 2003). Proper target identification suggests the relationship between drug and disease, which can be further analyzed for possible side effects (Hughes, Rees et al. 2011). ArgD is a member of arg8 protein family. ARG8 encodes acetylornithine aminotransferase, a mitochondrial matrix enzyme that catalyzes the fourth step in the biosynthesis of ornithine (Jauniaux, Urrestarazu et al. 1978), an intermediate in arginine biosynthesis. Arg8p is 68% identical to the acetylornithine aminotransferase from Kluyveromyces lactis, and the K. lactis gene can complement an S. cerevisiae arg8 mutant (Janssen and Chen 1998) . Arg8p is also similar to E. coli ArgD (Heimberg, Boyen et al. 1990). Like other genes encoding arginine biosynthetic

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enzymes, ARG8 is transcriptionally repressed in the presence of arginine and is regulated by general amino acid control (Messenguy 1987).

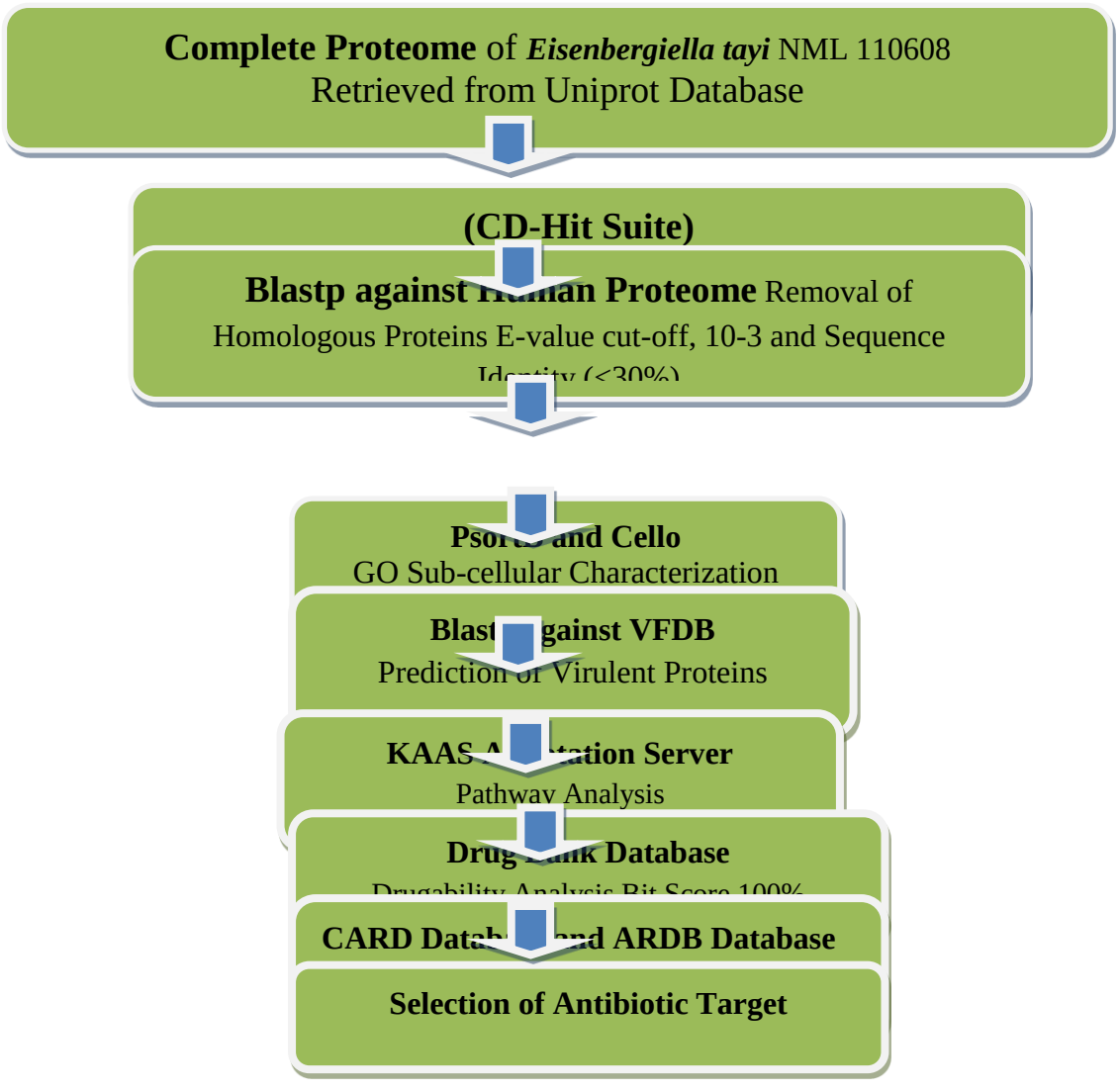

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Fig. 1. Schematic flow for prediction of potential drug targets against *E. tayi*

2. Materials and methods

2.1. Complete proteome retrieval

The *E. tayi* reference strain NML 110608 proteome data was obtained from the Universal Protein Resource Knowledgebase (UniProtKB)(Boutet, Lieberherr et al. 2016). Additionally, the proteomes of three other fully sequenced strains—DSM, CYPM1, and AOUC—were retrieved from the GenBank database at the National Center for Biotechnology Information (NCBI). This expanded dataset enabled a comprehensive comparison and analysis across multiple strains, enhancing the robustness of the study and facilitating the identification of conserved and strain-specific features.

2.2. Eliminating redundant proteins

CD-HIT suite (Huang, Niu et al. 2010) was utilized to identify redundant proteins within the proteome of *E. tayi*. The complete proteome was input into the CD-HIT suite with a sequence identity cutoff set at 80%, while all other parameters were maintained at their default settings. This approach facilitated the removal of redundant sequences, allowing for a more refined and non-redundant dataset for subsequent analyses.

2.3. Removal of homologous proteins

BLASTp (Lavigne, Seto et al. 2008) search against the human proteome was conducted to eliminate homologous protein sequences from the non-redundant proteins. This analysis was performed using a threshold expectation value (E-value) of 10^−3, with sequence identity and bit score cut-offs set at ≤30% and 100, respectively. These criteria ensured the exclusion of proteins with significant similarity to human proteins, focusing on those less likely to induce cross-reactivity or adverse effects in therapeutic contexts.

2.4. Identifying essential proteins

To obtain essential proteins, Database of Essential Genes (DEG) (Luo, Lin et al. 2021) was employed to identify essential proteins among the host non-homologous proteins. For this analysis, the minimum sequence identity threshold was set at ≥30%, and the bit score threshold

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was established at ≥100. These criteria were applied to ensure the selection of proteins with significant similarity and reliability in their essential roles.

2.5. Sub-cellular localization assessment

The final set of non-homologous essential proteins was subjected to PSORTb (Yu, Wagner et al. 2010) , CELLO (Bernstein, Ma et al. 2021) and CELLO2GO (Yu, Cheng et al. 2014) . Proteins consistently predicted as cytoplasmic across all three analytical tools were designated as potential drug targets. This consistent prediction underscores their suitability for targeting, as cytoplasmic proteins are generally more accessible for drug interactions compared to those in other cellular compartments.

2.6. Prediction of virulent proteins

For better understanding of essential cytoplasmic proteins as possible drug targets, their sequences were subjected to BLASTp search of Virulent Factor Database (VFDB)(Liu, Zheng et al. 2022). Proteins exhibiting a sequence identity of ≥30% and a bit score of ≥100 were classified as virulent and selected for further analysis. This threshold ensured the inclusion of proteins with significant similarities to known virulence factors, thus prioritizing those with a higher likelihood of contributing to pathogenicity and offering potential as drug targets (Gupta, Pradhan et al. 2017).

2.7. Metabolic pathways analysis

Virulent proteins were mapped to pathogen metabolic pathways using KEGG Automatic Annotation Server (KAAS)(Moriya, Itoh et al. 2005). Proteins specific to bacterial pathways were categorized as unique, whereas those shared between humans and bacteria were classified as common and thus excluded from further consideration. This distinction ensures that the focus remains on targets that are unique to the pathogen, thereby minimizing the risk of off-target effects and enhancing the potential efficacy of therapeutic interventions (Ahmad, Raza et al. 2017, Gupta, Pradhan et al. 2017).

2.8. Drugability potential of unique proteins

The proteins involved in unique metabolic pathways were further assessed for their druggability by querying the DrugBank database (Wishart, Feunang et al. 2018). This evaluation aimed to identify potential interactions with known drugs and assess the feasibility of targeting these proteins for therapeutic development, focusing on their capacity to bind drug-

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like compounds and their relevance in drug discovery with bit score set to ≥100 (Sanober, Ahmad et al. 2017).

2.9. Molecular weight estimation

The selected druggable proteins were evaluated for their molecular weight using the Expasy ProParam tool (Gasteiger, Hoogland et al. 2005). This analysis is essential for characterizing the proteins and ensuring their suitability for further drug development studies, as molecular weight can impact protein behavior, purification processes, and potential interactions with therapeutic compounds.

2.10. Resistance analysis

The prevalence of antibiotic resistance genes in bacterial genomes has been significantly exacerbated by the widespread use of new drugs. This high distribution of resistance genes contributes to the growing challenge of combating bacterial infections, as these genes facilitate the bacteria's ability to evade the effects of antimicrobial agents, leading to increased treatment failures and the need for novel therapeutic strategies (Lock and Harry 2008). Therefore, Antibiotic Resistance Database (ARDB) (McArthur, Waglechner et al. 2013) and Comprehensive Antibiotic Resistant Database (CARD) (McArthur, Waglechner et al. 2013) were used to disclose resistant targets.

2.11. Structure prediction

The proteins selected as potential antibiotic targets were evaluated for the availability of their experimental three-dimensional (3D) structures. This assessment is crucial for facilitating further computational studies, such as molecular docking and dynamics simulations, which are essential for understanding protein-drug interactions and optimizing drug design. For this purpose, BLASTp was performed against Protein Data Bank (PDB) (Laskowski, Hutchinson et al. 1997). In absence of experimental 3D structure, Phyre 2 (Kelley, Mezulis et al. 2015) was used to model the target protein structure.

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Result

Given the escalating issue of multi-drug resistance in *E. tayi*, the development of new therapeutics has become critically important. This study was designed to identify novel therapeutic targets that could be instrumental in developing effective treatments against *E. tayi*-associated infections. The number of proteins shortlisted at each phase of the study is illustrated in Figure 2, highlighting the rigorous selection process and the identification of promising targets for drug development.

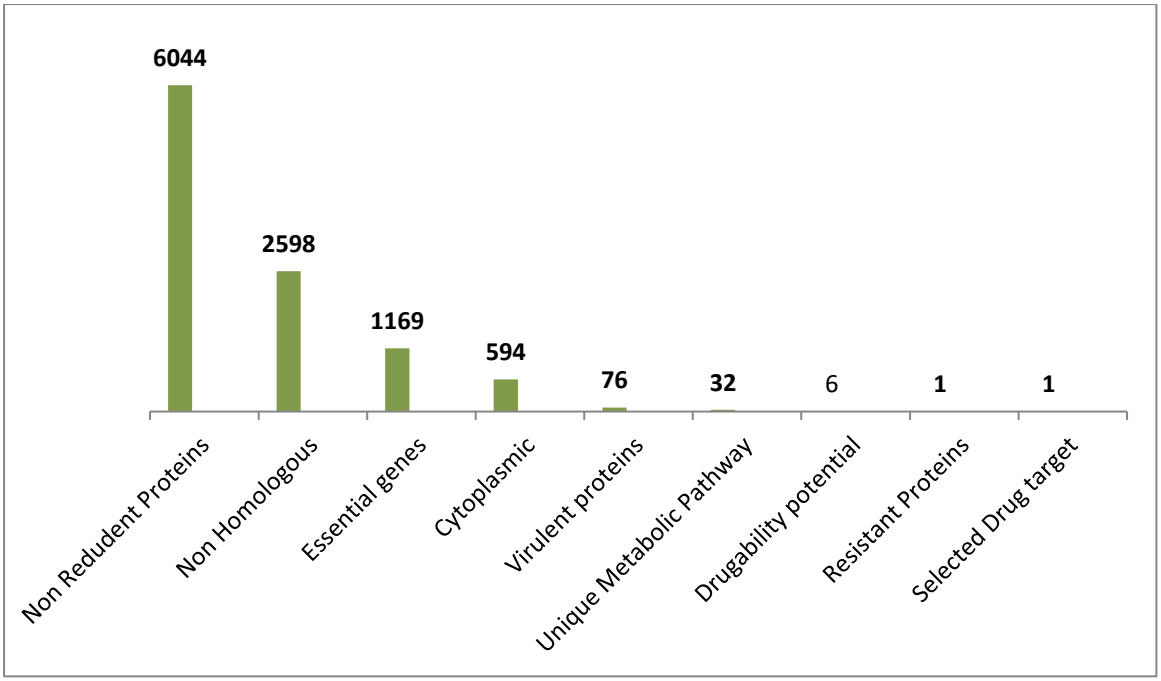

Fig. 2. Summary of the screened proteins obtained at the end of each step of subtractive proteomics

3.1. Eliminating redundant proteins

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CD-HIT analysis identified 6,044 proteins as non-redundant in the proteome of the reference strain HI4320. These proteins were selected for subsequent analyses due to their higher conservation across strains, which enhances the likelihood of developing broad-spectrum antibiotics. In contrast, redundant proteins, which are specific to particular strains, were excluded from further consideration to avoid targeting proteins with limited applicability and to focus on those with broader relevance (Sanober, Ahmad et al. 2017).

3.2. Removal of homologous proteins

To eliminate host-homologous proteins from the non-redundant proteome, a BLASTp search was conducted against the human proteome, resulting in the identification of 2,598 proteins as host non-homologous. Proteins classified as host non-homologous are less likely to elicit autoimmune reactions, reducing the risk of adverse effects in therapeutic applications. This refinement step is crucial for ensuring the specificity and safety of potential drug targets by minimizing cross-reactivity with host proteins (Naz, Awan et al. 2015)

3.3. Identification of essential proteins

Essential proteins are vital for sustaining cellular life and comprise a minimal set of proteins necessary for life (Zhang, Ou et al. 2004). A BLASTp search against the Database of Essential Genes identified 1,169 proteins as essential and 1,429 proteins as non-essential. Given that essential proteins are pivotal in regulating critical mechanisms such as nutrient acquisition, virulence, and pathogenicity, they present as highly promising candidates for drug development. Targeting these essential proteins could effectively disrupt key processes vital for the pathogen's survival and disease-causing capabilities, making them attractive targets for therapeutic intervention (Sanober, Ahmad et al. 2017) (Naz, Awan et al. 2015)

3.4. Sub-cellular localization assessment

Non-homologous essential proteins were subjected to further analysis based on their subcellular localization, a critical determinant for assessing their suitability as drug targets. At this stage, only those essential proteins identified as cytoplasmic were considered for further evaluation. A comprehensive analysis using the CELLO server predicted a total of 594 proteins to be cytoplasmic. To ensure the accuracy of these predictions, results were cross-verified with the PSORTb tool, which confirmed that all 594 proteins are indeed localized in the cytoplasm. The precise prediction of cellular localization is pivotal for elucidating protein functions,

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understanding their roles in pathogenic processes, and developing targeted therapeutic strategies. Cytoplasmic proteins are generally more amenable to drug accessibility compared to membrane-bound counterparts, thus rendering them more advantageous as targets for novel drug development (Sanober, Ahmad et al. 2017). Membrane proteins are frequently implicated in energy-driven efflux systems and are known to pump a wide range of drugs. These systems utilize cellular energy to actively expel a variety of compounds, including antimicrobial agents, thereby contributing to multidrug resistance. The broad substrate specificity of these efflux pumps often complicates drug treatment regimens, as they can effectively reduce the intracellular concentrations of therapeutic agents, diminishing their efficacy (Ahmad, Raza et al. 2018) . Additionally, membrane proteins have lower permeation rate thus blocking access of the drug for the target protein.

3.5. Virulence analysis

Virulence analysis was performed using cytoplasmic proteins (Asad, Ahmad et al. 2018). Drugs designed to target the virulent mechanisms of a pathogen aim to address critical factors such as infection establishment, immune evasion, nutrient acquisition, and survival under hostile conditions. A BLASTp search against the Virulence Factor Database (VFDB) identified 76 virulent proteins from an initial set of 594 cytoplasmic proteins. These shortlisted proteins represent optimal candidates for the development of anti-virulent compounds. Unlike traditional antibiotics, which kill or inhibit the growth of bacteria, anti-virulent compounds specifically disarm bacterial pathogens of their ability to cause disease, thereby reducing their virulence without necessarily affecting their viability (Knowles and Gromo 2003).

3.6. Unique and common metabolic pathway analysis

The combination of subtractive proteomics with metabolic pathway analysis proves to be a highly effective methodology for identifying proteins essential to the survival of a pathogen and exclusive to it. This integrative approach ensures the selection of targets that are not only critical for the pathogen’s viability but also specific to the pathogen, thereby minimizing potential off-target effects in therapeutic development (Uddin, Saeed et al. 2015). The essential cytoplasmic and virulent proteins were subjected to metabolic pathway analysis using the

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KAAS (KEGG Automatic Annotation Server) tool. This analysis facilitated the identification and characterization of the specific metabolic pathways associated with these proteins, aiding in the understanding of their roles and relevance in the pathogen's physiology (Moriya, Itoh et al. 2007). Among the 76 proteins analyzed, 32 were identified as being involved in unique metabolic pathways specific to the pathogen. Of these, the majority—92%—are associated with more than one pathogen-specific metabolic pathway. Detailed information about these unique metabolic pathways is presented in Table 1. The remaining proteins were found to be part of common pathways shared with the host (human) and the pathogen. Proteins linked to unique pathways are considered the most promising drug targets due to their absence in the host's pathways, thereby reducing the likelihood of adverse side effects, as illustrated in Figure 1.

Table 1. Unique Metabolic Pathway of *E. tayi*

Protein ID	Gene Name	Protein Name	Paathway ID	Pathways
A0A1E3A378	**glyA**	**Serine hydroxymethyltransferase**	K00600	Glycine, serine and threonine metabolism/Cyanoamino acid metabolism/Biosynthesis of secondary metabolites/Antifolate resistance
A0A1E3A3Y3	**carB_1**	**Carbamoyl phosphate synthase large chain**	K01948	Metabolic pathway/Alanine, aspartate and glutamate metabolism/ Microbial metabolism in

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				diverse environments
A0A1E3A5Z9	**groL**	**Chaperonin GroEL**	K04077	RNA degradation/Longevity regulating pathway – worm/Type I diabetes mellitus/Legionellosis
A0A1E3A9E6	**ftsH_2**	**ATP-dependent zinc metalloprotease FtsH**	K08956	Spinocerebellar ataxia
A0A1E3A9G0	**pyrG**	**CTP synthase**	K01937	Pyrimidine metabolism/Metabolic pathways/Nucleotide metabolism/Biosynthesis of cofactors
A0A1E3AJD3	**pfkA_1**	**ATP-dependent 6-phosphofructokinase**	K00850	Glycolysis / Gluconeogenesis/Pentose phosphate pathway/Fructose and mannose metabolism/ Galactose metabolism
A0A1E3AJL8	**adk**	**Adenylate kinase**	K00939	Purine metabolism/Thiamine

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				metabolism/Metabolic pathways/Biosynthesis of secondary metabolites
A0A1E2ZZI6	**prmC**	**Release factor glutamine methyltransferase**	K02493	Brite Hierarchies/Protein families: genetic information processing
A0A1E2ZZZ3	**purM_2**	**Phosphoribosylformylglycinamidine cyclo-ligase**	K11787	Purine metabolism/Metabolic pathways/ Biosynthesis of secondary metabolites
A0A1E3A092	**rnc**	**Ribonuclease 3**	K03685	Ribosome biogenesis in eukaryotes/ Proteoglycans in cancer
A0A1E3A130	**proC_2**	**Pyrroline-5-carboxylate reductase**	K00286	Arginine and proline metabolism/Metabolic pathways/Biosynthesis of amino acids
A0A1E3A1I3	**glpK_3**	**Glycerol kinase**	K00864	Glycerolipid metabolism/Metabolic

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				pathways/PPAR signaling pathway
A0A1E3A1K1	**metG_2**	**Methionine--tRNA ligase**	K01874	Selenocompound metabolism/Aminoacyl-tRNA biosynthesis/ Metabolic pathways
A0A1E3A1K5	**mro**	**Aldose 1-epimerase**	K01785	Glycolysis / Gluconeogenesis/ Galactose metabolism/Microbial metabolism in diverse environments/ Metabolic pathways
A0A1E3A1T3	**nrdB**	**Ribonucleoside-diphosphate reductase subunit beta**	K10808	Purine metabolism/Pyrimidine metabolism/ Glutathione metabolism/p53 signaling pathway
A0A1E3A269	**pgk**	**Phosphoglycerate kinase**	K00927	Purine metabolism/Pyrimidine metabolism/Glutathione metabolism/Metabolic pathways

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A0A1E3A2D8	**ppnK**	**NAD kinase**	K00858	Nicotinate and nicotinamide metabolism/Metabolic pathways/Biosynthesis of cofactors
A0A1E3A2H1	**nifS**	**Cysteine desulfurase IscS**	K04487	Thiamine metabolism/Metabolic pathways/Sulfur relay system
A0A1E3A2H7	**smc_2**	**Chromosome partition protein Smc**	K06674	Cell cycle - yeast
A0A1E3A2I1	**tpiA_2**	**Triosephosphate isomerase**	K01803	Glycolysis / Gluconeogenesis/Fructose and mannose metabolism/Inositol phosphate metabolism/Metabolic pathways
A0A1E3A2L9	**ffh**	**Signal recognition particle protein**	K03106	Quorum sensing/Protein export/Bacterial secretion system
A0A1E3A2Q8	**mutS2_2**	**Endonuclease MutS2**	K08740	Premature ovarian failure

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A0A1E3A2Q9	**gpsA**	**Glycerol-3-phosphate dehydrogenase [NAD(P)+]**	K00006	Glycerophospholipid metabolism/Biosynthesis of secondary metabolites/MAPK signaling pathway - yeast
A0A1E3A2T2	**gpmA_2**	**2,3-bisphosphoglycerate-dependent phosphoglycerate mutase**	K01834	Glycolysis / Gluconeogenesis/ Glycine, serine and threonine metabolism/Metabolic pathways
A0A1E3A2V0	**rpe**	**Ribulose-phosphate 3-epimerase**	K01783	Pentose phosphate pathway/Pentose and glucuronate interconversions/ Metabolic pathways
A0A1E3A2X2	**glyQS_2**	**Glycine--tRNA ligase**	K01880	Aminoacyl-tRNA biosynthesis
A0A1E3A2X3	**gap**	**Glyceraldehyde-3-phosphate dehydrogenase**	K10705	Glycolysis / Gluconeogenesis/ Metabolic pathways/Biosynthesis of secondary metabolites
A0A1E3A331	**argG**	**Argininosuccinate synthase**	K01940	Arginine biosynthesis/

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				Alanine, aspartate and glutamate metabolism/Metabolic pathways
A0A1E3A342	**argD**	**Acetylornithine aminotransferase**	K00819	Arginine and proline metabolism/Metabolic pathways/Biosynthesis of secondary metabolites
A0A1E3A3E9	**purB**	**Adenylosuccinate lyase**	K01756	Purine metabolism/ Alanine, aspartate and glutamate metabolism/ Metabolic pathways
A0A1E3UI56	**adhE_2**	**Aldehyde dehydrogenase EutE**	K00129	Glycolysis / Gluconeogenesis/ Histidine metabolism/ Tyrosine metabolism/ Metabolic pathways
A0A1E3A7W3	BEI61_05298	**Putative multidrug export ATP-binding/permease protein**	K05661	Hereditary stomatocytosis/Microphthalmia/ Familial

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pseudohyperkalemia/Dyschromatosis universalis hereditaria

3.7. Drugability potential

The potential of a protein to bind to drug-like compounds, known as its "druggability potential," was assessed through alignment with DrugBank databases, which include FDA-approved drugs, experimental small molecules, nutraceuticals, and biotech drugs. To evaluate this potential, each drug target was compared to DrugBank entries by sequence similarity. For proteins involved in pathogen-specific pathways of *E. tayi*, druggability potential was determined based on this alignment. Notably, nine target proteins exhibited significant hits in regular sequence searches within the DrugBank database (Wishart, Feunang et al. 2018), The remaining 23 proteins were excluded at this stage. From the initial nine proteins that yielded hits, six were further shortlisted based on a bit score threshold exceeding 100. These six proteins were classified into drug groups, including FDA-approved, investigational, and experimental small molecule compounds, as detailed in Table 2. Additionally, the molecular weight of each potential drug target was analyzed using the Expasy Proparam tool to ensure comprehensive characterization (Gasteiger, Hoogland et al. 2005). A critical factor for selecting proteins in this study is that they should ideally have a molecular weight of less than 110 kDa, which facilitates ease of purification and is preferred for experimental procedures. The identified druggable proteins fall within a molecular weight range of 11-63 kDa, indicating that these filtered proteins are well-suited for further experimentation in drug development studies.

Table 2. Drugability potential of the six drugable targets.

Gene Name	Protein ID	Drug Bank ID	Drugbank Targets	Drug Group	Molecular Weight (kDa)
carB_1	A0A1E3A3Y3	DB06775	Carglumic acid	Approved	11.67

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BEI61_05298	A0A1E3A7W3	DB00997	Doxirubicin	Approved	63.08
argD	A0A1E3A342	DB11638	Artenimol	Approved, Experimental	44.60
rpe	A0A1E3A2V0	DB00153	Ergocalciferol	Approved	23.88
groL	A0A1E3A5Z9	DB09130	Copper	Approved	57.30
purM_2	A0A1E2ZZZ3	DB00642	Pemetrexed	Approved,Investigational	36.34

3.8. Resistance analysis

Druggable proteins were further prioritized based on their resistance profiles. An extensive literature review revealed that *E. tayi* exhibits resistance to various antibiotics, including ornithine, antenimol, pyridoxal phosphate, gabaculine, and canaline. A resistance assessment was performed for six candidate proteins. Among these, only acetylornithine aminotransferase (argD) demonstrated significant resistance to artenimol, with a sequence identity of 41% and a resistance score of 214. In contrast, the remaining proteins lack appropriate structural templates for accurate prediction, as detailed in the accompanying table.

(Table 3), therefore, discouraged in the study.

Table 3. Template search analysis for six potential drug targets.

Protein ID	Protein Name	Number of Amino Acids	Experimental Structure Availability	Protein Data Bank Hit	Template	identity	Query Length
A0A1E3A3Y3	Carbamoyl phosphate	1064	×	×	×	×	×

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	synthase large chain						
A0A1E3A7W3	Putative multidrug export ATP-binding/permease protein	576	×	×	×	×	×
A0A1E3A342	Acetylornithine aminotransferase	406	×	✓	✓	41%	406
A0A1E3A2V0	Ribulose-phosphate 3-epimerase	222	×	×	×	×	×
A0A1E3A5Z9	Chaperonin GroEL	540	×	×	×	×	×
A0A1E2ZZZ3	Phosphoribosylformylglycinamidine cyclo-ligase	341	×	×	×	×	×

3.9. argD structure modelling

The structural conformation of acetylornithine aminotransferase has been elucidated in various bacterial species and was subsequently utilized as a template for modeling the argD protein via

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Phyre2 (Kelley, Mezulis et al. 2015). The three-dimensional conformation of Acetylornithine aminotransferase (argD) is illustrated in Figure 3. The protein, as characterized in the reference strain NML 110608, demonstrates a high degree of conservation across other sequenced proteomes of *E. tayi* (DSM26961, NML 120489, NML 150140-1). Of particular note, Acetylornithine aminotransferase (argD) uniquely produces a positive BLASTp match in searches against the Protein Data Bank (PDB). The process of target selection inherently involves the identification of a significant therapeutic entity, thereby accentuating the potential of argD as a pivotal candidate for targeted drug development (Knowles and Gromo 2003). Accurate target identification necessitates a comprehensive understanding of the interplay between a pharmacological agent and its disease context, thereby facilitating an in-depth analysis of potential off-target effects and adverse reactions (Hughes, Rees et al. 2011). ArgD belongs to the arg8 protein family, wherein ARG8 encodes acetylornithine aminotransferase. This enzyme, located in the mitochondrial matrix, facilitates the fourth step in the biosynthetic pathway of ornithine (Jauniaux, Urrestarazu et al. 1978), an intermediate enzyme in the arginine biosynthetic pathway. Arg8p exhibits 68% amino acid sequence identity with the acetylornithine aminotransferase from *Kluyveromyces lactis*. Notably, the *K. lactis**gene is capable of functionally complementing an *S. cerevisiae* arg8 mutant, underscoring the conservation and functional interchangeability of this enzyme across species. (Janssen and Chen 1998) . Arg8p is also similar to E. coli ArgD (Heimberg, Boyen et al. 1990). Similar to other genes involved in arginine biosynthesis, ARG8 is subject to transcriptional repression in the presence of arginine. Additionally, its expression is modulated by general amino acid control mechanisms, reflecting the integrated regulatory network governing amino acid metabolism (Lillywhite et al., 2013; Messenguy 1987).

Argenine-responsive transcription factors—namely Arg80p, Arg81p, Arg82p, and Mcm1p—along with their corresponding upstream activating sequences within the ARG8 gene, have been identified. The ARG8 sequence has been adapted to the mitochondrial genetic code, facilitating its use as a marker for mitochondrial transformation. A construct wherein the recoded ARG8 gene substitutes the COX3 coding sequence effectively complements a deletion of the nuclear ARG8 gene. This engineered construct necessitates the presence of COX3

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mRNA-specific translational activators for its expression, highlighting the intricate regulatory interplay required for functional restoration (Steele, Butler et al. 1996).

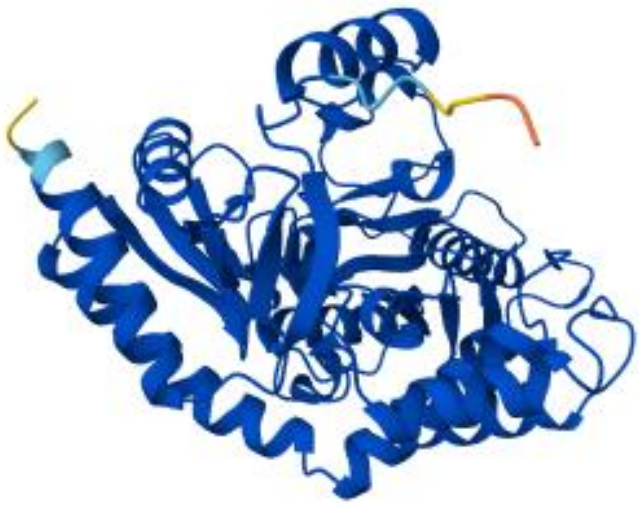

Fig. 3 3D structure of argD.

4. Conclusions

The present investigation employs subtractive proteomics to elucidate prospective antibiotic targets within the proteome of *E. tayi*. This analytical approach identified a mere six proteins of interest: Carbamoyl phosphate synthase large chain, Putative multidrug export ATP-binding/permease protein, Acetylornithine aminotransferase, Ribulose-phosphate 3-epimerase, Chaperonin GroEL, and Phosphoribosylformylglycinamidine cyclo-ligase. Among these, Acetylornithine aminotransferase (argD) was selected as the most viable candidate for drug targeting, primarily due to the availability of an experimental structural template. This protein is thus proposed for further investigation through molecular docking and molecular dynamics simulations to identify and characterize potential therapeutic agents.

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NA

Conflict of Interest

The authors declare no conflict of Interest.

Authors Contributions

I.M. experimented, wrote and reviewed the original draft, also reviewed and assisted in computational tools and conceived the original idea.

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