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Deciphering Potential Drug Targets in *Clostridium Perfringens* through Metabolic Pathway Analysis

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ABSTRACT

Background: In our day-to-day life, we are facing many dreadful diseases caused by many infectious pathogens. These pathogens invade the living organisms (host) and lethally damaging them. These dreadful pathogens were also be used as bioweapons. Among them, *Clostridium perfringens* is taken for the study. *Clostridium perfringens* is an anaerobic, rod shaped, gram positive bacteria capable of forming spores. It is prevalent in the environment and in the intestine of humans and other animals. It is the causative agent for a wide range of diseases including food borne diseases, gas gangrene and flesh eating disease called necrotizing fasciitis. *C. perfringens* is commonly found on raw meat and poultry that espouse to grow in conditions with very little or no oxygen, and under ideal conditions can multiply very rapidly. These conditions are occasionally lethal due to the substantial number of toxins such as alpha toxin, beta toxin, epsilon toxin and iota toxin produced by *C. perfringens*. It is significantly important to analyze the Drug targets of the pathogen in order to destroy them.

Objective: The present work aims in identifying potential drug targets in *C. perfringens* through metabolic pathway analysis.

Method: Primarily, the metabolic pathways of the host and pathogen are compared to identify unique pathways in the bacteria. Among the enzymes that catalyze unique metabolic pathways, the essential ones for the survival of the pathogen are identified. The druggability of the essential enzymes are predicted through identification of its sub cellular localization and other druggable parameters.

Results: The comparative metabolic pathway analysis result shows that, among the 98 metabolic pathways of *C.perfringens*, 25 pathways were unique that they did not have a counterpart with Human. There were 113 enzymes involved in these unique pathways. The NCBI's protein Blast search against human was done to identify the non-homologous proteins. There were 93 non-homologous proteins. Among the 93 non-homologous proteins, 47 proteins were found to be essential. Based on their sub-cellular localization, 32 proteins were identified as potential drug targets and 15 are probable vaccine candidates.

Conclusion: The present work which started with 25 different pathways with more than a hundred different enzymes, resulted in the identification of 32 putative drug targets against *C.perfringens* infection. All these 32 identified targets did not have any human homolog and are highly essential for the survival of the organism. They were concluded as potential drug targets. Designing of compounds to inhibit these enzymes would be successful for treating the life-threatening infections caused by this pathogen.

Keywords: Gas Gangrene, Necrotizing Fasciitis, Drug Discovery.

I. INTRODUCTION

Human microbes are crucial for human life, having significant impact of human physiology and health [3-5]. A wide range of microbial communities and microbiome exist throughout the human body, playing significant roles in human health and disease [7, 8 and 10]. We are in the peak time to protect ourselves and eradicate the life threatening pathogens. *Clostridium perfringens* is a major food borne pathogen. It is commonly known as cafeteria germ, since the risk places of its outbreak is in cafeterias, prison, and hospitals where large amount of food prepared in advance. This provides ample opportunity to contaminate food. *C.perfringens* is a causative agent of food poisoning, gas gangrene, and necrotizing fasciitis. Other symptomes of this illness include abdominal cramping, fever, vomiting, diahorrea which is often bloody and a distented bowel. It is found in human intestine, raw meat and poultry and in the incompletely food. It is a gram-positive, rod shaped, non-motile, endospore forming, anaerobic bacteria. The spores and vegetative cells are relatively heat-resistance, facilitating its survival in incompletely cooked food. It has a doubling time of less than 10 minutes. The optimal temperature for its growth is 43-45^{0}c. It produces two toxins that active in human gastrointestinal tract. The condition becomes lethal due to substantial production of various toxins such as alpha toxin, beta toxin, epsilon toxin, iota toxin, etc.

These spores and toxins are used as biowarfare agents. The spores are incorporated into weapons which cause traumatic injury which leads to the delivery of spores deep into tissues would results in the development of gas gangrene. *C.perfringens* is one of the leading cause of food poisoning. CDC estimates it causes nearly 1 million cases of foodborne illness each year [6]. Contaminated meat in stews, soups and gravies are usually responsible for outbreak. About 250,000 cases were reported in United States every year. Gas gangrene is a clostridial myonecrosis that produces gas in tissues which leads to death and decomposition of body tissue. About 1000 cases occurs yearly. Nectrotizing fasciitis is a flesh-eating disease results in death of body's soft tissue under the skin and spreads rapidly. Symptoms include red or purple skin in affected area. It occurs in 650-800 people every year. This foodborne necrotic fasciitis still occurs in several regions of Southeast Asia.

The reason for studying this microbe is, since it causes these life threatening infections it provokes our interest to identify the potential drug targets of C*lostridium perfringens* through *in silico* metabolic pathway analysis.

II. TOOLS AND DATABASES

With the advancements in the field of complete genome sequencing and computational approaches development have lead to metabolic pathway analysis. Various computational biology tools and databases were adopted for the study.

2.1. KEGG database

Kyoto Encyclopedia for Genes and Genomes, KEGG is employed for the representation and analysis of molecular networks involving diseases and drugs [9]. It can be accessed at http://www.genome.jp/kegg/pathway.html. KEGG PATHWAY database is a collection of pathway maps which represents the molecular interactions, reactions and relations. It also contains pathway maps for the molecular systems in both normal and pertubed states.

2.2. UniProt database

Uniprot is an expertly curated protein database, whch supports the biological research by providing a freely accessible, stable, comprehensive, fully classified, richly and accurately annotated protein sequence

knowledgebase, with extensive cross-references and querying interfaces [16]. It can be accessed at http://www.uniprot.org/.

2.3. BlastP

BLAST is an acronym for Basic Local Alignment Search Tool, and is the name given to a suite of tools for identifying imperfect matches between a given query sequence and a database of sequences [1]. It uses the algorithm of Altschul *et al.* to search for similarities between a query sequence and all the sequences in a database. Blast is also a general name for a group of programs. Furthermore, blast can be run for protein searches as well as nucleotide ones. The program input is a biological sequence, it is also called the query sequence and it is compared to every database sequences. Among the different types of BLAST, BLAST-P is used for the study. The protein aminoacids query was searched against all the protein databases. It can be accessed at https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins.

2.4. DEG database

Zhang *et al* (2004) have constructed a Database of Essential Genes (DEG), which contains all the essential genes that are currently available [19]. It can be accessed at http://www.essentialgene.org/. Essential genes are genes that are indispensable to support cellular life. Users can BLAST the query sequences against DEG. If homologous genes are found, it is possible that the queried genes are also essential. Users can search for essential genes by their function or name. Users can also browse and extract all the records in DEG. Essential gene products comprise excellent targets for antibacterial drugs.

2.5. Cello V.2.5 tool

Cello V.2.5 tool is used to study the localization of proteins in the subcellular [18]. CELLO uses 4 types of sequence coding schemes: the amino acid composition, the di-peptide composition, the partitioned amino acid composition and the sequence composition based on the physico-chemical properties of amino acids. It can be accessed at http://cello.life.nctu.edu.tw/.

2.6. Drugbank database

DrugBank is a freely available web-enabled database which can be accessed at https://www.drugbank.ca , that combines detailed drug data with comprehensive drug-target and drug-action information [17]. It was specifically designed to facilitate *in silico* drug-target discovery, drug design, drug-metabolism prediction, drug-interaction prediction, and general pharmaceutical education.

III. METHODS AND MATERIAL

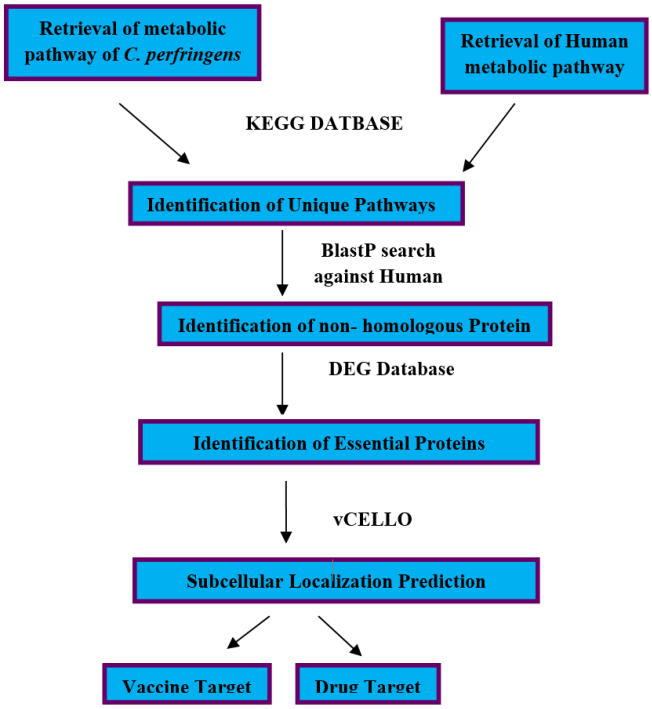

Figure 3.1 Methodology flowchart

3.1. Analysis of host and pathogen metabolic pathways:

The present study focuses on *in silico* based comparative metabolic pathway analysis. Whole genome-wide metabolic pathway analysis of host (*Homo sapiens)* and pathogen (*C.perfringens)* was

performed through KEGG (Kyto Encyclopedia of Genes and Genomes) PATHWAY database. The metabolic pathway information of the host and the pathogen were extracted. The metabolic network pathway consists of molecular interactions, reactions and the enzymes/proteins involved in that pathway.

3.2. Identification of unique metabolic pathways and non-homologous proteins:

Initially, the metabolic pathways of Human and *C.perfringens* were retrieved. A manual sorting and comparison was then performed. The pathways that were not present in the host but present in the pathogen were identified as 'Unique pathways'. The enzymes/proteins involved in these unique pathways were retrieved from UniProt database. They were subjected to NCBI's Protein BLAST search. The BLASTP search was done against the non-redundant protein databases restricted to *Homo sapiens* subset and the e-value threshold cutoff was set to 0.005 to remove homologous sequence. The non-homologous proteins were identified. This provides a way to study the host-pathogen interaction. These proteins were selected for further study.

3.3. Finding Essential genes:

Then, the essentiality of the proteins was checked, where these proteins contribute to the growth and survival of the organism. The essentiality is studied by comparing these non-homologous protein against DEG (Database of Essential Genes) using BlastP search available specifically for prokaryotic organism. This allows us to predict the potential drug targets among large number of non-homologous proteins involved in the unique metabolic pathway.

3.4. Testing the druggability:

Here we present a stradegy to prioritize pathogen proteins based on whether their properties meet criteria is considered as desirable drug target. The most important criteria in determining the therapeutic targets are (i) localization and toxicity (ii) presence or absence of transmembrane. And so, the subcellular location of the essential proteins were found using Cello V.2.5 tool. Cello V.2.5 is a publicly available, web-based system. The proteins which were located in cytoplasm and inner membrane were considered as drug targets and those which were located in surface membrane, periplasmic and extracellular were considered as vaccine candidates.

Additionally, experimentally and computationally solved 3D structures were detected by searching the Protein Data Bank (PDB).

IV. RESULTS AND DISCUSSION

The metabolic pathways of *Clostridium perfringens* and *Homo sapiens* were analysed through KEGG database. The comparative metabolic pathway analysis results shows that, among the 98 metabolic pathways of *C.perfringens*, 25 pathways were unique that they did not have a counterpart with Human. There were 113 enzymes involved in these unique pathways. The NCBI's protein Blast search against human was done to identify the non-homologous proteins. There were 93 non-homologous proteins. They did not show any similarity with the human proteins. The Table-1 shows the enzymes involved in the unique pathways, the protein Blast results shows whether it is a human homolog or not. The DEG results shows whether it is a essential gene or not. Among the 93 non-homologous proteins, 47 proteins were found to be essential. These proteins/enzymes plays a role in pathogenicity which can be concluded as potential targets. The Table-2 shows the subcellular location of essential proteins and its target priotization. Based on their sub-cellular localization, 32 proteins were identified as potential drug targets and 15 are probable vaccine candidates. The drug bank search for the organism, resulted in two proteins namely, pencillin binding protein A and Choloylglycine hydrolase.

V. CONCLUSION

With the advancements in the field of computational approaches, the metabolic pathways of *Clostridium perfringens* and *Homo sapiens* were analysed and the potential drug targets of *C.perfringens* were identified. The present work which started with 25 different pathways with more than a hundred different enzymes, resulted in the identification of 32 putative drug targets against *C.perfringens* infection. All these 32 identified targets did not have any human homolog and are highly essential for the survival of the organism. They were concluded as potential drug targets. Designing of compounds to inhibit these enzymes would be successful for treating the life threatening infections caused by this pathogen. Further, the three dimensional structure for the identified drug targets are not available in Protein Databank, experimental procedures to identify the structure of the targets will be of prime importance to treat infections caused by *C.perfringens.*

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Table 1: Proteins Involved in Unique Pathways of *Clostridium perfringens*

S. No	Gene Name	Uniprot ID	Protein Name	Human Homolog	Essentiality
3. Bacterial chemotaxis(cpe02030)					
28.	RbsB	Q8XJX5	Probable ribose ABC transporter	NO	NO
29.	MglB	Q8XKQ3	Probable galactoside ABC transporter	NO	YES
4. Quorum sensing(cpe02024)					
30.	LuxS	Q9XDU6	S-ribosylhomocysteine lyase	NO	NO
31.	Hfq	Q8XL84	RNA-binding protein	NO	NO
32.	GadC	Q8XIQ4	Probable glutamate gamma-aminobutyrate antiporter	NO	NO
33.	GadA/B	Q8XIQ6	Glutamate decarboxylase	YES	-
34.	CcfA	Q8XH28	Membrane protein insertase	NO	YES
35.	FsrD	Q8XM19	Putative AgrB-like protein 1	NO	NO
36.	transporter	Q8XKX3	Probable spermidine/putrescine-binding protein 1	NO	NO
37.	ToxE	Q8XMX2	Riboflavin biosynthesis protein	NO	YES
38.	ToxF	Q8XH76	Uncharacterized protein	NO	NO

5. Two-component system(cpe02020)					
39.	PhoR	Q8XJJ9	Two-component sensor histidine kinase	NO	YES
40.	PhoP	Q8XMP6	Two-component response regulator	NO	YES
41.	PtsS	Q8XMQ2	Probable phosphate ABC transporter	NO	YES
42.	HtrA	Q8XI91	Probable serine proteinase Do	YES	-
43.	DnaA	Q8XPG2	Chromosomal replication initiator protein	NO	YES
44.	GlnA	Q8XHB6	Probable glutamine synthetase	NO	YES
45.	AtoB	Q8XIC6	Acetyl-CoA acetyltransferase	YES	-
46.	RPoN	Q8XKT7	RNA polymerase sigma factor sigma54	NO	YES
47.	PilA	Q8XI37	Uncharacterized protein	NO	NO
48.	MprF	Q8XKZ5	Phosphatidylglycerol lysyltransferase (Also involved in Cationic antimicrobial peptide resistance(cpe01503) pathway)	NO	YES
49.	VanY	Q8XKL5	Probable D-alanyl-D-alanine carboxypeptidase (Also involved in Vancomycin resistance(cpe01502))	NO	NO
50.	Spo0A	Q8XJE7	Phosphorylation-activated transcription factor	NO	NO
51.	cit C	Q8XL96	[Citrate [pro-3S]-lyase] ligase	NO	NO
52.	cit D	Q8XL95	Citrate lyase acyl carrier protein	NO	NO
53.	cit E	Q8XL94	Citrate lyase beta subunit	NO	YES
54.	cit F	Q8XL93	Citrate lyase alpha chain	NO	YES
55.	cit X	Q8XL92	Probable CitG protein	NO	NO
56.	cit G	Q8XL97	Probable 2-(5''-triphosphoribosyl)-3'-dephosphocoenzyme-A synthase	NO	NO
57.	MaeA	Q8XL91	Probable malate oxidoreductase	YES	-
58.	AgrB/B1	Q8XM19	Putative AgrB-like protein 1	NO	NO
59.	YesM	Q8XMW4	Histidine kinase	NO	NO
60.	YesN	Q8XIM8	Two-component response regulator	NO	NO
6. Cationic antimicrobial peptide resistance(cpe01503)					
61.	amiA/C	Q8XP50	Probable N-acetylmuramoyl-L-alanine amidase	NO	NO
62.	degP	Q8XI91	Probable serine proteinase Do	YES	-
7. Vancomycin resistance(cpe01502)					
63.	Ddl	Q8XM71	D-alanine--D-alanine ligase B (Also	NO	YES

			involved in Peptidoglycan biosynthesis(cpe00550) pathway)		
64.	Alr	Q8XM22	Alanine racemase	NO	YES
65.	MurF	Q8XJA0	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase (Also involved in Peptidoglycan biosynthesis(cpe00550) pathway)	NO	YES
66.	MarY	Q8XJA1	Phospho-N-acetylmuramoyl-pentapeptide-transferase (Also involved in Peptidoglycan biosynthesis(cpe00550) pathway)	NO	YES
67.	MurG	Q8XIQ1	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase (Also involved in Peptidoglycan biosynthesis(cpe00550) pathway)	NO	YES
8. Beta-lactum resistance(cpe01501)					
68.	NagZ	Q8XP12	Probable beta-hexosamidase A	NO	NO
69.	BlaZ	Q8XL58	Probable beta-lactamase	NO	NO
70.	PBP 1a/2	Q8XJ01	Penicillin-binding protein 1A (also involved in Peptidoglycan biosynthesis(cpe00550) pathway)	NO	YES
71.	PBP 2	Q8XIH9	Probable penicillin-binding protein 2	NO	YES
9. Degeradation of Aromatic aminoacids(cpe01220)					
72.	1.1.1.1	Q8XN89	Alcohol dehydrogenase (Also involved in Chloroalkane and chloroalkane degradation(cpe00625) pathway and Napthalene degradation(cpe00626) pathway)	NO	YES
10.Methane metabolism(cpe00680)					
73.	2.7.1.11	Q8XNH2	ATP-dependent 6-phosphofructokinase 1	YES	-
74.	2.7.1.29	Q8XP65	Dihydroxyacetone kinase	YES	-
75.	4.1.2.13	Q8XP79	Fructose-1,6-bisphosphate aldolase	NO	YES
76.	1.1.1.95	Q8XPB1	D-3-phosphoglycerate dehydrogenase	YES	-
77.	5.4.2.12	Q8XP82	Probable phosphoglycerate mutase	NO	NO
78.	2.1.2.1	Q8XJ32	Serine hydroxymethyltransferase	YES	-
79.	4.2.1.11	Q8XKU4	Enolase	YES	-

80.	2.3.1.8	Q8XJN1	Phosphate acetyltransferas	NO	YES
81.	2.7.2.1	Q8XNW5	Acetate kinase 1	NO	YES
82.	1.2.7.4	I1SBA8	Nitrate reductase electron transfer subunit	NO	NO
83.	3.1.3.71	Q8XHC9	Probable 2-phosphosulfolactate phosphatase	NO	NO
11.Secondary bile acid(cpe00121)					
84.	3.5.1.24	P54965	Choloylglycine hydrolase	NO	NO
12. Monobactam biosynthesis(cpe00261)					
85.	2.7.2.4	Q8XJS6	Aspartokinase	NO	YES
86.	1.2.1.11	Q8XJ57	Aspartate-semialdehyde dehydrogenase	NO	YES
87.	4.3.3.7	Q8XJ56	4-hydroxy-tetrahydrodipicolinate synthase	YES	-
88.	1.17.1.8	Q8XJ55	4-hydroxy-tetrahydrodipicolinate reductase	NO	YES
13.Carbapenem biosynthesis(cpe00332)					
89.	2.7.2.11	Q8XHA6	Glutamate 5-kinase	YES	-
90.	1.2.1.41	Q8XHA7	Gamma-glutamyl phosphate reductase	YES	-
14.Benzoate degradation(cpe00460)					
91.	1.1.1.157	Q8XI27	Beta-hydroxybutyryl-CoA dehydrogenase NAD-dependent	YES	-
92.	2.3.1.9	Q8XIC6	Acetyl-CoA acetyltransferase	YES	-
15.Cyanoaminoacid metabolism(cpe00460)					
93.	2.1.2.1	Q8XJ32	Serine hydroxymethyltransferase	YES	-
94.	6.3.1.1	Q8XIY5	Aspartate--ammonia ligase	NO	YES
95.	3.5.1.1	Q8XJK4	L-asparaginase	NO	YES
16.Streptomycin biosynthesis(cpe00521)					
96.	2.7.1.2	Q8XP84	Probable glucose kinase	YES	-
97.	5.4.2.2	Q8XJ88	Probable phosphomannomutase	YES	-
98.	2.7.7.24	Q8XMS2	Glucose-1-phosphate thymidylyltransferase (Also involved in Acarbose and validamycin biosynthesis(cpe00525) pathway and Polyketide sugarunit biosynthesis(cpe00523) pathway)	NO	YES
99.	4.2.1.46	Q8XMR9	dTDP-glucose 4,6-dehydratase (Also involved in Polyketide sugarunit biosynthesis(cpe00523) pathway and Acarbose and validamycin biosynthesis(cpe00525) pathway)	YES	-

100.	5.1.3.13	Q8XMS1	dTDP-4-dehydrorhamnose 3,5-epimerase (Also involved in Polyketide sugarunit biosynthesis(cpe00523)pathway)	NO	YES
101.	1.1.1.18	Q8XP75	Probable dehydrogenase	NO	NO
102.	1.1.1.133	Q8XMS0	dTDP-4-dehydrorhamnose reductase (Also involved in Polyketide sugarunit biosynthesis(cpe00523) pathway)	NO	NO
17.Acarbose and validamycin biosynthesis(cpe00525)					
18.Peptidoglycan biosynthesis(cpe00550)					
103.	2.5.1.7	Q8XID7	UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1	NO	YES
104.	1.3.1.98	Q8XNI0	UDP-N-acetylenolpyruvoylglucosamine reductase	NO	YES
105.	6.3.2.8	Q8XHJ0	UDP-N-acetylmuramate--L-alanine ligase	NO	YES
106.	6.3.2.9	Q8XHM4	UDP-N-acetylmuramoylalanine--D-glutamate ligase	NO	YES
107.	6.3.2.13	Q8XJ99	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase	NO	YES
108.	3.6.1.27	Q8XM93	Uncharacterized protein	NO	NO
109.	LMWPBE	Q8XJF8	D-alanyl-D-alanine carboxypeptidase	NO	NO
19.Chloroalkane and chloroalkane degradation(cpe00625)					
110.	1.2.1.3	Q8XI14	Aldehyde dehydrogenase	YES	-
111.	3.8.1.2	Q8XMC7	Uncharacterized protein	YES	-
20.Napthalene degradation(cpe00626)					
21.Polyketide sugarunit biosynthesis(cpe00523)					
22.Penicillin and cephalosporin biosynthesis(cpe00311)					
112.	3.5.2.6	Q8XL58	Probable beta-lactamase	NO	NO
113.	3.1.1.41	Q8XK07	Probable acetylxylan esterase	NO	NO

TABLE – 2 : Target Prioritization based on Sub-cellular Localization

S. No	Gene name	Uniprot ID	Protein name	Subcellular Location	Trans membrane	Candidate
1.	Sec D/F	Q8XJ20	Protein-export membrane protein	Membrane	Transmembrane	Vaccine Target
2.	Sec E	Q8XHR2	Protein translocase subunit	Cytoplasm	-	Drug Target
3.	Sec Y	Q8XHU3	Protein translocase subunit	Membrane	Transmembrane	Vaccine Target
4.	YidC	Q8XH28	Membrane protein insertase	Membrane	Transmembrane	Vaccine Target
5.	Sec A	Q8XIF0	Protein translocase subunit	Cytoplasm	-	Drug Target
6.	pts I	Q8XHW9	Phosphoenolpyruvate-protein phosphotransferase	Cytoplasm	-	Drug Target
7.	FruA/B	Q8XMV4	PTS system	Membrane	Transmembrane	Vaccine Target
8.	Pts G	Q8XIG3	Probable PTS system	Membrane	Transmembrane	Vaccine Target
9.	Nag E	Q8XP89	Probable PTS system enzyme	Membrane	Transmembrane	Vaccine Target
10.	MalT	Q8XNB9	Probable PTS system	Membrane	Transmembrane	Vaccine Target
11.	GlyC/B	Q8XNY6	PTS arbutin-like enzyme IIBC component	Membrane	Transmembrane	Vaccine Target
12.	UlaA	Q8XN15	Uncharacterized protein	Membrane	Transmembrane	Vaccine Target
13.	MglB	Q8XKQ3	Probable galactoside ABC transporter	Membrane	Transmembrane	Vaccine Target
14.	CcfA	Q8XH28	Membrane protein insertase	Membrane	Transmembrane	Vaccine Target
15.	ToxE	Q8XMX2	Riboflavin biosynthesis protein	Cytoplasm	-	Drug Target
16.	PhoR	Q8XJJ9	Two-component sensor histidine kinase	Cytoplasm	-	Drug Target
17.	PhoP	Q8XMP6	Two-component response regulator	Cytoplasm	-	Drug Target
18.	PtsS	Q8XMQ2	Probable phosphate ABC transporter	Membrane	Extracellular	Vaccine Target
19.	DnaA	Q8XPG2	Chromosomal replication initiator protein	Cytoplasm	-	Drug Target
20.	GlnA	Q8XHB6	Probable glutamine synthetase	Cytoplasm	-	Drug Target

21.	RPoN	Q8XKT7	RNA polymerase sigma factor sigma54	Cytoplasm	-	Drug Target
22.	MprF	Q8XKZ5	Phosphatidylglycerol lysyltransferase	Membrane	Transmembrane	Vaccine Target
23.	cit E	Q8XL94	Citrate lyase beta subunit	Cytoplasm	-	Drug Target
24.	cit F	Q8XL93	Citrate lyase alpha chain	Cytoplasm	-	Drug Target
25.	Ddl	Q8XM71	D-alanine--D-alanine ligase B	Cytoplasm	-	Drug Target
26.	Alr	Q8XM22	Alanine racemase	Cytoplasm	-	Drug Target
27.	MurF	Q8XJA0	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	Cytoplasm	-	Drug Target
28.	MarY	Q8XJA1	Phospho-N-acetylmuramoyl-pentapeptide-transferase	Membrane	Transmembrane	Vaccine Target
29.	MurG	Q8XIQ1	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase	Cytoplasm	-	Drug Target
30.	PBP1a/2	Q8XJ01	Penicillin-binding protein 1A	Membrane	Transmembrane	Vaccine Target
31.	PBP 2	Q8XIH9	Probable penicillin-binding protein 2	Extracellular	-	Drug Target
32.	1.1.1.1	Q8XN89	Alcohol dehydrogenase	Cytoplasm	-	Drug Target
33.	4.1.2.13	Q8XP79	Fructose-1,6-bisphosphate aldolase	Cytoplasm	-	Drug Target
34.	2.3.1.8	Q8XJN1	Phosphate acetyltransferase	Cytoplasm	-	Drug Target
35.	2.7.2.1	Q8XNW5	Acetate kinase 1	Cytoplasm	-	Drug Target
36.	2.7.2.4	Q8XJS6	Aspartokinase	Cytoplasm	-	Drug Target
37.	1.2.1.11	Q8XJ57	Aspartate-semialdehyde dehydrogenase	Cytoplasm	-	Drug Target
38.	1.17.1.8	Q8XJ55	4-hydroxy-tetrahydrodipicolinate reductase	Cytoplasm	-	Drug Target
39.	6.3.1.1	Q8XIY5	Aspartate--ammonia ligase	Cytoplasm	-	Drug Target
40.	2.7.7.24	Q8XMS2	Glucose-1-phosphate thymidylyltransferase	Cytoplasm	-	Drug Target

41.	5.1.3.13	Q8XMS1	dTDP-4-dehydrorhamnose 3,5-epimerase	Cytoplasm	-	Drug Target
42.	2.5.1.7	Q8XID7	UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1	Cytoplasm	-	Drug Target
43.	1.3.1.98	Q8XNI0	UDP-N-acetylenolpyruvoyl glucosamine reductase	Cytoplasm	-	Drug Target
44.	6.3.2.8	Q8XHJ0	UDP-N-acetylmuramate--L-alanine ligase	Cytoplasm	-	Drug Target
45.	6.3.2.9	Q8XHM4	UDP-N-acetylmuramoylalanine--D-glutamate ligase	Cytoplasm	-	Drug Target
46.	6.3.2.13	Q8XJ99	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase	Cytoplasm	-	Drug Target
47.	3.5.1.1	Q8XJK4	L-asparaginase	Cytoplasm	-	Drug Target