J Chem Biol (2010) 3:165–173
DOI 10.1007/s12154-010-0039-1

ORIGINAL ARTICLE

In silico identification of common putative drug targets in *Leptospira interrogans*

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Received: 6 December 2009 / Accepted: 22 April 2010 / Published online: 14 May 2010

Abstract Infectious diseases are the leading causes of death worldwide. Hence, there is a need to develop new antimicrobial agents. Traditional method of drug discovery is time consuming and yields a few drug targets with little intracellular information for guiding target selection. Thus, focus in drug development has been shifted to computational comparative genomics for identifying novel drug targets. Leptospirosis is a worldwide zoonosis of global concern caused by *Leptospira interrogans*. Availability of *L. interrogans* serovars and human genome sequences facilitated to search for novel drug targets using bioinformatics tools. The genome sequence of *L. interrogans* serovar Copenhageni has 5,124 genes while that of serovar Lai has 4,727 genes. Through subtractive genomic approach 218 genes in serovar Copenhageni and 158 genes in serovar Lai have been identified as putative drug targets. Comparative genomic approach had revealed that 88 drug targets were common to both the serovars. Pathway analysis using the Kyoto Encyclopaedia of Genes and Genomes revealed that 66 targets are enzymes and 22 are non-enzymes. Sixty two common drug targets were predicted to be localized in cytoplasm and 16 were surface proteins. The identified potential drug targets form a platform for further investigation in discovery of novel therapeutic compounds against *Leptospira*.

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Keywords Leptospirosis · Subtractive genomics approach · Novel drug targets · KEGG

Introduction

The widespread emergence of bacterial resistance to existing antibiotics is a global health threat and has emphasized the need to develop new antibacterial agents directed towards novel targets [1]. Leptospirosis is a globally widespread infectious zoonosis with more than 500,000 severe cases annually in the world [2]. Human leptospirosis is caused mainly by spirochete pathogen *Leptospira interrogans* [3]. People exposed to recreational activities, farming, and post-flood conditions are at major risk of getting infected through contaminated water, rodents, or pet animals [4, 5]. High prevalence of leptospiral antibodies was observed in rural areas with sanitation workers and sugarcane workers being the most frequent victims [6, 7]. Leptospirosis occurs as either anicteric leptospirosis syndromes in 85-90% of cases or icteric leptospirosis in 5-10% of cases. In anicteric leptospirosis, there are two stages, viz., septicemia stage and the immune stage. Symptoms of infection include fever, chills, headache, and severe myalgia. In 5-15% cases, multiple organ damage is reported and the mortality rate has been shown to be 5-40% [8, 9]. As number of animals act as hosts, the acquiring of infection can have considerable economic implications in developing countries like India.

Prevention of infection by controlling environmental factors is difficult to practice in developing countries. Drugs like oxytetracycline, doxycycline and penicillin have been used in management of infection. A review of clinical trials revealed inconclusive evidence on the benefit or

safety of antibiotics for leptospirosis [10]. More than 200 diverse pathogenic *Leptospira* serovars remain as a challenge to develop an effective and safe leptospirosis vaccine [11]. Vaccines for serovars like *Hardjo*, *Pomona*, *Canicola*, *Grippotyphosa*, and *Icterohaemorrhagiae* have been developed and showed disadvantages like suboptimal protection, requirement of booster doses, and the need of the vaccine for local serovars [5]. Available leptospiral vaccines were relatively unsuccessful in preventing leptospirosis [11], thus underscores the need to search for a new drug target for development of effective drug against the pathogen. Subtractive genomic approach locates essential genes of the pathogen and the non-human homologous proteins which help in preventing the unexpected cross reactivity in the host. The putative common drug targets of *L. interrogans* serovars Copenhageni and Lai were reported in the present study through subtractive genomic approach [12] and metabolic pathway analysis [13]. The drug targets elucidating unique physiology of the versatile spirochetal pathogen were documented. It is anticipated that the identified common drug targets will illuminate understanding of the molecular mechanisms of leptospiral pathogenesis and facilitate the identification of novel drug candidates.

Materials and methods

Identification of putative common drug targets

Complete genome sequences of both the serovars of *L. interrogans* were retrieved from the National Centre for Biotechnology Information (ftp://ftp.ncbi.nlm.nih.gov/genome/bacteria) [14, 15]. The Database of Essential Genes (DEG) was accessed at http://tubic.tju.edu.cn/deg/ [16]. The DEG search engine parameters were set to an expectation value (*E* value) cut-off of 10^{-10} and minimum bit score cut off of 100 for screening essential genes from both *Leptospira* serovar genomes. These genes were searched [17] against human proteome in the National Center for Biotechnology Information (NCBI) server. The homologs were excluded and the list of non-human homologs (drug targets) was compiled. The protein products of final list of genes were obtained from Uniprot (http://www.uniprot.org/).

Metabolic pathway analysis

The involvements of drug targets in metabolic pathways were analyzed at the Kyoto Encyclopedia of Genes and Genome (KEGG) [18]. Comparative analysis of the metabolic pathways of the host and pathogen was performed to trace out drug targets involved in pathogen specific metabolic pathways.

Subcellular localization prediction

Computational prediction of the subcellular localization of proteins is a valuable tool for genome analysis and annotation in bacterial pathogens, since the prediction of proteins on the cell surface is of particular interest due to the potential of such proteins to be primary drug or vaccine targets. Proteome analyst specialized subcellular localization server v2.5 [19] was used to predict the surface membrane proteins which could be highly useful as probable vaccine targets. The predicted membrane proteins were further analyzed in psortb v3.0 [20] to confirm if the drug targets are identified as membrane proteins irrespective of the subcellular localization prediction methods.

Results and discussion

Infectious diseases are the second leading cause of death worldwide [21]. Though there is an increasing demand for new antimicrobial agents, their development is hampered due to requirement of huge investment, less market, short-term usage in same patient, and high level of competition with newly developed agents [22]. Developments in Bioinformatics have brought the algorithms, tools, and facilitated the automation of microbial genome sequencing, development of integrated databases over the internet, comparison of genomes, identification of gene product function, and paved the way for development of antimicrobial agents, vaccines, and rational drug design [23].

The *L. interrogans* genome consists of a 4.33-Mb chromosome I and a 350-kb chromosome II. The genome is highly conserved between the serovars Copenhageni and Lai, exhibiting 95% identity at the nucleotide level. The genome sequence of Copenhageni has 4,669 and 455 genes on chromosome I and chromosome II while that of Lai has 4,360 and 367 genes, respectively [14, 15]. In silico drug target identification mainly relies on the principle "a good drug target is a gene essential for bacterial survival yet cannot be found in host" [24]. The principle was well implemented using DEG and the NCBI non-redundant database to develop subtractive genomic approach, which had been widely used for fast screening of potential drug targets from the sequenced genomic information of emerging infectious pathogens [12]. Metabolic pathway analysis is another approach for drug target identification which focuses on enzymes of pathways unique and vital to bacteria [13]. Subtractive genomic approach was practiced to find *L. interrogans* common drug target followed by assessment of these targets for its vitality in pathogens' metabolic pathway.

Sakharkar et al. [25] identified 306 essential genes in *Pseudomonas aeruginosa*; Dutta et al. [26] reported 178

essential genes in *Helicobacter pylori*, and Chan-Eng Chong et al. [27] found 312 essential genes in *Burkholderia pseudomallei* by using the same approach. In the present study, screening of Copenhageni whole genome has resulted 576 essential genes in chromosome I and 32 essential genes in chromosome II, while Lai has 403 and 39 essential genes in chromosome I and II, respectively. About 218 essential genes of Copenhageni and 158 essential genes of Lai were identified as human non-homologs. Each gene product can be considered as drug target to the respective pathogen serovars. Eighty eight drug targets were found common to both the serovars (Tables 1 and 2). Sixty-six common drug targets were enzymes and 22 were non-enzymes. This result also revealed the presence of 11 and 10 putative uncharacterized proteins in Copenhageni and Lai, respectively, as potential drug targets but none of them were common to both (Table 1).

Comparative analysis of the metabolic pathways of the host and pathogen by using the KEGG pathway database had revealed that 20 pathways are unique to both pathogenic *Leptospira* serovars (Table 3). The results of pathogen specific pathways are in agreement with the findings by Anisetty et al. [13] and Barh and Kumar [28] in *Mycobacterium tuberculosis* and *Neisseria gonorrhoeae*, respectively. Seven unique pathways such as D-alanine metabolism, lipopolysaccharide biosynthesis, peptidoglycan biosynthesis, two-component system, bacterial chemotaxis, phosphotransferase system, and bacterial secretion system were observed to have 20 common drug targets (Tables 1, 2, and 3). The remaining 13 pathogen-specific pathways, namely, geraniol degradation, gamma-hexachlorocyclohexane degradation, novobiocin biosynthesis, streptomycin biosynthesis, polyketide sugar unit biosynthesis, 1- and 2-methylnaphthalene degradation, 1,2-dichloroethane degradation, benzoate degradation via CoA ligation, 3-chloroacrylic acid degradation, styrene degradation, C5-branched dibasic acid metabolism, caprolactam degradation, and flagellar assembly did not show involvement of any leptospiral putative drug target. Barh and Kumar [28] also reported four pathogen specific pathways namely; C5-branched dibasic acid metabolism, streptomycin biosynthesis, polyketide sugar unit biosynthesis and novobiocin biosynthesis did not involve any drug targets in *N. gonorrhoeae*. Thus, out of 66 common enzymes identified as drug target, 46 enzymes were from pathways common to host and pathogen. But the enzymes were non-homologous to human.

Considering all 88 common drug targets reported in the present study, 72 targets are cytoplasmic and 16 are membrane proteins (Tables 1 and 2). The 16 membrane proteins have the potential to act as common efficacious vaccine candidate against different strains of *Leptospira*. However, the membrane proteins from protein export, bacterial secretion system (SecD, SecF, and secY), ABC transporters (CysT, CysW), cell division proteins (FtsW, FtsZ), heavy metal efflux pump (CzcA), sensor protein, and Na+/H+antiporter would be highly useful based on the avalable literature evidences [25–28]. We also proved CzcA as a T cell-driven subunit vaccine cadidate through computational pan-genome reverse vaccinology approach [29].

As has been mentioned, 20 enzymes are identified from the seven pathogen specific pathways. This small group of drug targets would have high impact as drug targets, as absence of these pathways in host eradicate any potential risk factors exerted by the drugs targeting these pathways. Lipopolysaccharides (LPS) and peptidoglycans biosynthesis pathways are two major pathogen-specific pathways. LPS and peptidoglycans, the main constituents of the outer cell wall of Gram-negative bacteria, play an important role in pathogenesis and antibiotic sensitivity. Eight enzymes of lipopolysaccharide pathway (LpxB, LpxC, LpxD, KdsA, KdsB1, GmhA, KdtA, and RfaE2) and seven enzymes of peptidoglycan biosynthesis pathway (MurA, MurC, MurD,

Table 1 Results of the computational analyses of *Leptospira interrogans* serovars Copenhageni and Lai genome

Genes/proteins	Copenhageni	Lai	Common drug targets
Number of genes	5,124	4,727	-
Predicted essential genes product	608	442	-
Predicted non-human homologs (putative drug targets)	218	158	88
Putative uncharacterized drug targets	11	10	NIL
Predicted common drug targets	-	-	88
Number of enzymes as common drug target	-	-	66
Common drug target enzymes present in pathways of both host and pathogen	-	-	46
Common drug target enzymes specific to unique pathways of pathogen	-	-	20
Non-enzymes	-	-	22
Cytoplasmic proteins	-	-	72
Membrane protein (potential vaccine candidate)	-	-	16

Table 2 Common drug targets of *Leptospira interrogans* serovars Copenhageni and Lai

	Uniprot ID	Gene product name (Copenhageni)	Gene name	Uniprot ID	Gene product name (Lai)
Enzymes					
Lipopolysaccharide biosynthesis					
1	Q72P96	Lipid-a-disaccharide synthase protein	lpxB	Q8F752	Lipid-A-disaccharide synthase
2	Q72RV5	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	lpxC	Q8F3U4	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase
3	Q72LT1	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	lpxD	Q8F8P2	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase
4	P61656	2-dehydro-3-deoxyphosphooctonate aldolase	kdsA	Q8F3J4	2-dehydro-3-deoxyphosphooctonate aldolase
5	Q72QF5[a]	3-deoxy-manno-octulosonate cytidylyltransferase	kdsB1	Q8F5P2[a]	3-deoxy-manno-octulosonate cytidylyltransferase
6	Q72RC1[a]	Phosphoheptose isomerase	gmhA	Q8F4E9[a]	Phosphoheptose isomerase
7	Q72Q34	3-deoxy-d-manno-octulosonic acid transferase	kdtA	Q8F636	3-deoxy-D-manno-octulosonic-acid transferase
8	Q72S48	ADP-heptose synthase	rfaE2	Q8F3J1	ADP-heptose synthase
Peptidoglycan biosynthesis					
9	Q72MD7	Udp-n-acetylglucosamine 1-carboxyvinyltransferase	murA	Q8EYY3	Udp-n-acetylglucosamine 1-carboxyvinyltransferase
10	Q72R85	UDP-N-acetylmuramate-alanine ligase	murC	Q8F4J0	UDP-N-acetylmuramate-alanine ligase
11	Q72NP4[a]	UDP-N-acetylmuramoylalanine-D-glutamate ligase	murD	Q8F7V4[a]	UDP-N-acetylmuramoylalanine-D-glutamate ligase
12	Q72R81	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase	murE	Q8F4J4	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase
13	Q72Q23	UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D-alanine ligase	murF	Q8F648	UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D-alanine ligase
14	Q72R84[b]	UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase	murG	Q8F4J1[b]	UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase
15	Q72R93	D-alanine-D-alanine ligase	ddl	Q8F4I2	D-alanine-D-alanine ligase
DNA Replication					
16	Q72VS4	DNA polymerase III alpha subunit	dnaE	Q8F9D7	DNA polymerase III, subunit alpha
17	Q72RP3	DNA primase	dnaG	Q8F416	DNA primase
18	Q72WD5	DNA polymerase III beta subunit	dnaN	Q8FA33	DNA polymerase III beta subunit
19	Q72WD1	DNA gyrase subunit A	gyrA	Q8CM44	DNA gyrase subunit A
20	Q72S35	Primosomal protein N'	priA	Q8F3K4	Primosomal protein N'
21	Q72WD4	DNA replication and repair protein recF	recF	Q8FA32	DNA replication and repair protein recF
Histidine metabolism					
22	P61660	Imidazoleglycerol-phosphate dehydratase	hisB	Q8F9R6	Imidazoleglycerol-phosphate dehydratase
23	Q72PG3	Histidinol-phosphate aminotransferase	hisC	Q8F6W9	Histidinol-phosphate aminotransferase
24	P62382	ATP phosphoribosyltransferase	hisG	Q8F6P1	ATP phosphoribosyltransferase
25	P61780	Imidazole glycerol phosphate synthase subunit hisH	hisH	P59118	Imidazole glycerol phosphate synthase subunit hisH
Phenylalanine, tyrosine, and tryptophan biosynthesis					
26	Q72W01[a]	Chorismate synthase	aroC	Q8F9N4[a]	Chorismate synthase
27	Q72NP6	Indole-3-glycerol phosphate synthase	trpC	Q8F7V2	Indole-3-glycerol phosphate synthase
28	Q72PD0	Anthranilate phosphoribosyltransferase	trpD	Q8F708	Anthranilate phosphoribosyltransferase
29	Q72RH2	N-(5'-phosphoribosyl)anthranilate isomerase	trpF	Q8F495	N-(5'-phosphoribosyl)anthranilate isomerase
Protein export, Bacterial secretion system					
30	Q72PD3[b]	SecD	secD	Q8F706[b]	Preprotein translocase subunit SecD
31	Q72PD4[c]	SecF	SecF	Q8F705[c]	Preprotein translocase subunit SecF

Table 2 (continued)

	Uniprot ID	Gene product name (Copenhageni)	Gene name	Uniprot ID	Gene product name (Lai)
32	Q72NI1[b]	Preprotein translocase secY subunit	secY	Q9XD16[b]	Preprotein translocase secY subunit
ABC transporters					
33	Q72PE3[b]	Sulfate ABC transport system permease protein	cysT	Q8F6Z3[b]	Sulfate transport system permease protein cysT
34	Q72PE4[b]	Sulfate ABC transport system permease protein	cysW	Q8F6Z2[b]	Sulfate transport system permease protein cysW
Sulfur metabolism					
35	Q72W82	Serine acetyltransferase	cysE	Q8F9X5	Serine acetyltransferase
36	Q72R95	Homoserine O-acetyltransferase	metX	Q8F4I0	Homoserine O-acetyltransferase
Lysine degradation					
37	Q72SS2[a]	L-lysine 2,3-aminomutase		Q8EXB5	L-lysine 2,3-aminomutase
38	Q72UX3[b]	Penicillin-binding protein1	pbp1	Q8F6T0[b]	Penicillin-binding protein 1A
RNA Polymerase					
39	Q72NI8	DNA-directed RNA polymerase subunit alpha	rpoA	Q9XD09	DNA-directed RNA polymerase subunit alpha
40	Q72S41	RNA polymerase sigma-54 factor	rpoN	Q8F3J8	Transcription initiation factor sigma 54
Enzymes of other pathways					
41	Q72W86	7,8-dihydropteroate synthase protein (Folate biosynthesis)	folP	Q8F9Y0	Dihydropteroate synthase
42	Q72W63	Diaminopimelate epimerase (lysine biosynthesis)	dapF	Q8F9V5	Diaminopimelate epimerase
43	Q72VI0	Ribose 5-phosphate isomerase B (pentose phosphate pathway)	rpiB	Q8F928	Ribose 5-phosphate isomerase B
44	Q72VB8	2,3-bisphosphoglycerate-independent hosphoglycerate mutase (glycolysis/gluconeogenesis)	gpmI	P59173	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate mutase
45	P61724	6,7-dimethyl-8-ribityllumazine synthase (riboflavin metabolism)	ribH	Q8F8T9	6,7-dimethyl-8-ribityllumazine synthase
46	Q72S57	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (terpenoid backbone biosynthesis)	ispH	Q8F3I3	4-hydroxy-3-methylbut-2-enyl diphosphate reductase
47	Q72RV6[a]	Phosphotransferase system enzyme I (phosphotransferase system (PTS))	ptsI	Q8F3U3	Phosphoenolpyruvate-protein phosphotransferase
48	Q72RJ9	N-acetyl-gamma-glutamyl-phosphate reductase (arginine and proline metabolism)	argC	P59307	N-acetyl-gamma-glutamyl-phosphate reductase
49	Q72QN9	Aspartate 1-decarboxylase (beta-Alanine metabolism @@@Pantothenate and CoA biosynthesis)		Q8CVE9	Aspartate 1-decarboxylase
50	Q72Q13[b]	Phosphatidylserine synthase (Glycerophospholipid metabolism)	pssA	Q8F660[b]	Phosphatidylglycerophosphate synthase
51	Q72PZ2[a]	Type III beta-ketoacyl synthase-like protein (Fatty acid biosynthesis)	fabH	Q8F686[a]	3-oxoacyl-[acyl-carrier-protein] synthase III
52	Q72NU6	Methylglyoxal synthase (pyruvate metabolism)	mgsA	Q8F7N5	Methylglyoxal synthase
53	Q72NA2	Mannose-6-phosphate isomerase (amino sugar and nucleotide sugar metabolism)	manA	Q8F8A2	Mannose-6-phosphate isomerase
54	Q72M22	Uroporphyrinogen-III C-methyltransferase (porphyrin and chlorophyll metabolism)	cobA	Q8EXQ3	Cob(I)alamin adenosyltransferase
55	Q72Q21	Phosphoribosylaminoimidazole carboxylase ATPase subunit (purine metabolism)	purK	Q8F650	Phosphoribosylaminoimidazole carboxylase ATPase subunit
56	Q72R77	Chemotaxis protein methyltransferase (two-component system, Bacterial chemotaxis)	cheR	Q8F4J9	Methylase of chemotaxis methyl-accepting proteins
57	Q72RD4	Glutamate-cysteine ligase (Glutathione metabolism)	gshA	Q8F4D5	Glutamate-cysteine ligase
58	Q72W44	Leucyl/phenylalanyl-tRNA-protein transferase	aat	Q8F9T2	Leucyl/phenylalanyl-tRNA-protein transferase
59	Q72VM0[b]	Prenyltransferase		Q8F977[b]	UbiA prenyltransferase family protein

Table 2 (continued)

	Uniprot ID	Gene product name (Copenhageni)	Gene name	Uniprot ID	Gene product name (Lai)
60	Q72QC5	Perosamine synthetase		Q8F5S2	Perosamine synthetase
61	Q72RB6	NADPH-dependent 7-cyano-7-deazaguanine reductase	queF	Q8F4F6	NADPH-dependent 7-cyano-7-deazaguanine reductase
62	Q72RG8	Biotin synthase	bioB	Q8F498	Biotin synthase
63	Q72RL4	FKBP-type peptidyl-prolyl cis-trans isomerase	slyD	Q8F453	FKBP-type peptidyl-prolyl cis-trans isomerase
64	Q72S40	HPr kinase/phosphorylase	hprK	Q8F3J9	HPr kinase/phosphorylase
65	Q72SA5	Tyrosine recombinase xerD	xerD	Q7ZAM7	Tyrosine recombinase xerD
66	P61611	LexA repressor	lexA	Q8F663	LexA repressor
Non-enzymes					
Ribosome proteins					
1	Q72NH6	50 S ribosomal protein L6	rplF	Q9XD21	50 S ribosomal protein L6
2	Q72NH7[a]	50 S ribosomal proteinL18	rplR	Q9XD20[a]	50 S ribosomal protein L18
3	Q72S27	50 S ribosomal proteinL19	rplS	Q8F3L4	50 S ribosomal protein L19
Cell Division proteins					
4	Q72N67	FtsA	ftsA	Q8F8E6	Cell division protein ftsA
5	Q72R83[b]	Cell division protein	ftsW	Q8F4J2[c]	Cell division protein FtsW
6	Q72N68[b]	Cell division protein ftsZ	ftsZ	Q8F8E5[b]	Cell division protein ftsZ
Vitamin B6 metabolism proteins					
7	Q72NL4[a]	Pyridoxal phosphate biosynthesis protein	pdxA	Q8F7Z2[a]	Pyridoxal phosphate biosynthetic protein pdxA
8	Q3V896	Pyridoxal phosphate biosynthetic protein	pdxJ	Q8F5Z9	Pyridoxal phosphate biosynthetic protein pdxJ
Translation intitiation factors					
9	P61690[a]	Translation initiation factor IF-1	infA[a]	Q9XD14	Translation initiation factor IF-1
10	Q72PK8	Translation initiation factor IF-3	infC	Q8F6Q9	Translation initiation factor IF-3
Other proteins					
11	Q72RW5[e]	Outer membrane protein		Q8F3T2[e]	Predicted outer membrane protein
12	Q72RN3	Magnesium transporter	mgtE	Q8F430	Magnesium transporter
13	Q72RI9	UvrABC system protein C/ Excinuclease ABC subunit C	uvrC	Q8F479	UvrABC system protein C
14	Q72R15[c]	Heavy metal efflux pump	czcA	Q8F5X3[b]	Cation efflux system membrane protein czcA
15	Q72QP4[a]	Cytoplasmic membrane protein	mviN	Q8F5E7[a]	Virulence factor mviN
16	Q72QQ3[b]	Sensor protein		Q8F425[b] Q8EXH1	Sensor protein
17	Q72Q47[b]	Na+/H+antiporter		Q8F621[c]	Na+/H+antiporter
18	Q72PM6	Fatty acid/phospholipid synthesis protein plsX	plsX	Q8F6N9	Fatty acid/phospholipid synthesis protein plsX
19	Q72N21[b]	Acriflavine resistance		Q8F3G5[b]	Acriflavine resistance protein-like protein
20	Q72LR5	ParB	parB	Q8EY30	ParB protein
21	Q72RK4	TldD	tldD	Q8F463 Q8F462	TldD protein
22	Q72WD6	Chromosomal replication initiator protein dnaA	dnaA	Q8FA34	Chromosomal replication initiator protein dnaA

The subcellular localization of drug targets were identified from the consensus results through predictions made by PA-SUB and psortb

[a] Drug targets with no hits in human

[b] Predicted inner membrane proteins

[c] Predicted inner membrane proteins with no hits in human

[d] Predicted outer membrane protein

[e] Predicted outer membrane protein with no hits in human

Table 3 Unique metabolic pathway of *Leptospira interrogans* with respect to human

Sl. no.	Pathway ID (Copenhageni)	Pathway ID (Lai)	Pathway name	No. of common targets
1	lil00281	lic00281	Geraniol degradation	-
2	lil00361	lic00361	gamma-Hexachlorocyclohexane degradation	-
3	lil00401	lic00401	Novobiocin biosynthesis	-
4	lil00473	lic00473	D-alanine metabolism	1[a]
5	lil00521	lic00521	Streptomycin biosynthesis	-
6	lil00523	lic00523	Polyketide sugar unit biosynthesis	-
7	lil00540	lic00540	Lipopolysaccharide biosynthesis	8
8	lil00550	lic00550	Peptidoglycan biosynthesis	7
9	lil00624	lic00624	1- and 2-Methylnaphthalene degradation	-
10	lil00631	lic00631	1,2-Dichloroethane degradation	-
11	lil00632	lic00632	Benzoate degradation via CoA ligation	-
12	lil00641	lic00641	3-Chloroacrylic acid degradation	-
13	lil00643	lic00643	Styrene degradation	-
14	lil00660	lic00660	C5-Branched dibasic acid metabolism	-
15	lil00930	lic00930	Caprolactam degradation	-
16	lil02020	lic02020	Two-component system	1[b]
17	lil02030	lic02030	Bacterial chemotaxis	1[b]
18	lil02040	lic02040	Flagellar assembly	-
19	lil02060	lic02060	Phosphotransferase system (PTS)	1
20	lil03070	lic03070	Bacterial secretion system	3

Thus, overall 20 enzymes reported here as drug target are from pathways unique to bacteria

[a] The target (ddl) is one of the seven drug targets reported from peptidoglycan biosynthesis pathway

[b] The same drug target (cheR) participates in Two-component system and Bacterial chemotaxis pathway

MurE, MurF, MurG, and Ddl) were identified as potential common drug target in the present study. Both the pathways being unique to Gram-negative bacteria, designing potential inhibitors targeting the common drug targets of the pathways would be highly useful for controlling diseases rendered by Gram-negative bacterial pathogens by making it susceptible to osmotic lysis. The efficiency of the drug targets from the two pathways were well evident from previous studies reporting enzymes of these two pathways as drug target in various bacterial pathogens to identify novel inhibitors [30–35]. Thus, the unique leptospiral drug targets identified from these two pathways would be highly useful for further potential inhibitor design against leptospirosis.

Many of the common drug target code for the basic survival mechanisms of the bacterium. The list of potential drug targets encoded in microbial genomes includes genes involved in translation, transcription, DNA replication, repair, outer membrane proteins, permeases, enzymes of intermediary metabolism, host interaction factors, and many more. Four genes *aroC*, *trpC*, *trpD*, and *trpF* reported as drug targets found to be essential components of phenylalanine, tyrosine, and tryptophan biosynthesis pathway. Therefore, targeting of these enzymes may disrupt pathways essential for *Leptospira* survival and virulence and therefore might be a potential antibacterial therapeutic strategy.

Ddl that participates in D-alanine metabolism is essential for the precursor of peptidoglycan backbone and metabolic pathway thus could be considered as good drug target. Phosphoenolpyruvate-protein phosphotransferase is found to be an ideal target to block the phosphotransferase system and is unique to the pathogen. Type III secretion system is a contact-dependent pathway that plays a role in host-pathogen interaction [36]. Type II secretion pathway supports the translocation of proteins associated with the virulence factors, across the outer membrane [37]. Two component systems, essential for the growth and survival in adverse environmental conditions, are ubiquitous in bacteria, and have been reported to be involved in virulent pathogen [38, 39]. Thus, SecD, SecF, SecY, and chemotaxis protein methyltransferase would be effective drug targets against *Leptospira*.

The computational genomics approach [13, 25–28] stated herein, is likely to speed up drug discovery process by removing hindrances like dead-ends or toxicity that are encountered in classical approaches. Further, homology modeling of these targets will help in identifying the best possible sites that can be targeted for novel drug design.

Conclusion

Drug design and discovery in the post genomic era is shattering old paradigm and routinely reconstructing the drug discovery protocol by including the eons of information encoded in our genome. Subtractive genomic approach identifies putative drug targets thus presenting an opportunity to deal with a manageable number of efficient data through further pathway studies and experimental design. The identification of 88 novel drug targets in the present study provide a basis for computer-aided drug design against *L. interrogans* to overcome the challenges of severe leptospirosis. However, these targets should be experimentally validated for their role in bacterial survival and virulence. Qualitative tertiary structure prediction and identification of functionally important residues of these drug targets would be useful for screening novel inhibitors from millions of compounds from ligand databases. Such a strategy will enable to locate critical pathways and steps in pathogenesis; to target these steps by designing new drugs; and to inhibit the infectious agent of interest with new antimicrobial agents. The drug targets from the unique pathways would also be extended as common targets for designing inhibitors against Gram-negative bacterial pathogens.

Acknowledgments The study was supported by grants from DBT, Ministry of Science and Technology, Government of India, New Delhi. We are grateful to Dr. B. Vengamma, Director for providing constant support and encouragement for research at SVIMS Bioinformatics Centre, where this work has been performed. We thank Prof. K. Venkateswarlu (SK University, Anantapur) for critically reading the manuscript.

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