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In Silico Putative Drug Target Identification of *Proteus mirabilis* by comparative analysis of Metabolic Pathways

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ABSTRACT

Proteus mirabilis is a significant problem mostly to the vulnerable immune systems individual and cause of 90% Complex Urinary Tract Infections. The treatment is becoming more difficult because 48% *P.mirabilis* strain is resistant to broad-range antibiotics. Development of these drug resistant varieties have led to search for novel drug targets. We have performed an insilico comparative analysis of metabolic pathways of *Homo sapiens* with the pathogen *P.mirabilis* and found 97 unique, nonhomologus, essential proteins that are present only in *P.mirabilis* and absent in humans and presented as list of putative drug target.

Introduction

Urinary tract infections (UTIs) are chronic diseases and can become lethal if the infection spreads to other systems in the body. Although *P. mirabilis* is the major causative agent of urinary tract infections, but not a common pathogen. After Pneumonia, generally the long-term hospital patients get infected by this facultative pathogen [1, 14]. The risk of infection increases to 44% in the individuals with complicated urinary tract problems such as patients suffering with functional or anatomical abnormalities or with chronic instrumentation such as long-term catheterization [2, 3-9].

P. mirabilis is a gram negative bacterium, member of the Enterobacteriaceae family. This rod shaped bacterium can produce high levels of Urease that hydrolyzes the Urea to Ammonia(NH3), Urease makes the urine more alkaline and increases the pH to >7.2 with subsequent precipitation of magnesium ammonium phosphate (struvite) and calcium phosphate (apatite) crystals [10 -13].

These ammonium and calcium crystals block the catheter lumen and cause acute urinary retention. This results in development of bacteriuria and other ascending infections such as pyelonephritis, bacteremia, and in rare case acute cystitis develops due to obstruction of urine flow. Some times *P. mirabilis* can enter into the blood stream, inducing a Systemic Inflammatory Response Syndrome (SIRS) in which mortality rate is approximately 20%-50% [15]. The treatment is becoming more difficult because 48% of *P. mirabilis* strains are resistant to Amoxicillin, Penicillin, Fluoroquinolones, B-Lactams, Aminoglycosides Trimethoprim/Sulfamethoxazole and other broad-range activity antibiotics. Because of the resistant *P.mirabilis* to the currently available drugs, invention of new drugs becomes very essential[23].

Any protein that is necessary for the viability of a pathogenic organism may be a possible drug target, particularly when the protein is nonhomologous (little or no similarity) to host. The differences in the proteins of the host and the pathogen can be effectively used for designing a drug to target the pathogen [16]. A computational approach was used to predict the drug target of *P. mirabilis* by comparing the essential nonhomologous proteins of the pathogen with the host organism i.e. Human by making the use of the metabolic pathway data in KEGG database. This computational comparative strategy has been used to investigate novel drug targets in other pathogenic organisms such as *Pseudomonas aeruginosa, Helicobacter pylori and in Mycobacterium tuberculosis* [17-19].

Materials and Methods

Retrieval of unique pathways of Pathogen from KEGG Database

Metabolic pathway identification numbers of the host *H. sapiens* and the pathogen *P. mirabilis* were extracted from the Kyoto Encyclopedia of Gene and Genome (KEGG) database (http://www.genome.jp/pathways.html) [19]. Pathways which do not appear in the *H. sapiens*, but present in the *P. mirabilis* according to KEGG database annotation, were identified as unique pathways to *P. mirabilis*.

Enzymes in these unique pathways were also involved in other metabolic pathways such as carbohydrate metabolism, amino acid metabolism, lipid metabolism, energy metabolism, vitamin and cofactor biosynthesis and nucleotide metabolism were identified. The corresponding protein sequences were retrieved from the KEGG and Uniprot database.

Nonhomologous Enzymes Searching

The proteins were subjected to BLASTp [22] for searching nonhomologous proteins against the non-redundant database with the e-value inclusion threshold set to 0.005.

The search was restricted to proteins from *H. sapiens* through an option available in the BLAST program, which allows the user to select the organism to which the search should be restricted. In the current context, the objective was to find only nonhomologous targets of pathogen by comparing human and *P.mirabilis* protein.

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Enzymes, which do not have hits below the e-value inclusion threshold of 0.005, were picked out as potential drug targets.

Finding the essential targets of Pathogen

Essential proteins are indispensable for the survival of an organism, since their functions are considered as foundation of life. In this study proteins which are involved in more than one metabolic pathways were considered as Essential proteins.

Sub Cellular Localization Analysis of Target proteins

Nonhomologous essential surface membrane proteins of bacteria illustrate their potentiality for becoming the possible vaccine targets. CELLO v2.5 [21] tool was required to find out the surface membrane proteins which could be probable vaccine targets. These tools utilize various protein properties such as amino acids properties, dipeptide composition, physicochemical properties and evolutionary information using PSI BLAST. The results obtained were further validated with PSORTb [20].

Druggable target prioritization

The Drug Bank (http://www.drugbank.ca) database contains detailed drug data (chemical, pharmacological and pharmaceuti cal) with comprehensive drug target information (sequence, structure and pathway). Druggability of the predicted drug targets were further checked using the drug presented in Drug Bank.

Results and Discussion

From KEGG server 22 metabolic pathways were identified as unique to *P. mirabilis* and not present in host *H. sapiens*

1. Secondary bile acid biosynthesis
2. Beta-Lactam resistance
3. Benzoate degradation
4. Phosphonate and phosphinate metabolism
5. D-Alanine metabolism
6. Streptomycin biosynthesis
7. Polyketide sugar unit biosynthesis
8. Lipopolysaccharide biosynthesis
9. Peptidoglycan biosynthesis
10. Chloroalkane and chloroalkene degradation
11. Naphthalene degradation
12. Aminobenzoate degradation

Table 1. List of Unique Essential Nonhomologous target proteins of *P.mirabilis*

Sl No	Entry ID	Gene Name	GI No	Protein Name	Related Metabolic Pathway
1	PMI1408	NA	GI:197285267	conjugated bile acid hydrolase	Secondary bile acid biosynthesis , Metabolic pathways
2	PMI1367	mppA	GI:197285226	periplasmic murein peptide-binding protein	beta-Lactam resistance, ABC transporters
3	PMI0873	nagZ	GI:197284758	beta-hexosaminidase	beta-Lactam resistance, Amino sugar and nucleotide sugar metabolism, Biosynthesis of secondary metabolites
4	PMI0765	ompF	GI:197284658	outer membrane porin	beta-Lactam resistance, Two-component system
5	PMI2349	tolC	GI:197286195	outer membrane channel protein	beta-Lactam resistance, Bacterial secretion system
6	PMI3021	mrcA	GI:197286848	peptidoglycan synthetase	beta-Lactam resistance, Peptidoglycan biosynthesis, Metabolic pathways
7	PMI0426	pbpA	GI:197284326	penicillin-binding protein 2	beta-Lactam resistance, Peptidoglycan biosynthesis
8	PMI2076	ftsI	GI:197285923	penicillin-binding protein	beta-Lactam resistance, Peptidoglycan biosynthesis
9	PMI3079	phnX	GI:197286900	phosphonoacetaldehyde hydrolase	Phosphonate and phosphinate metabolism, Metabolic pathways,Microbial metabolism in diverse environments
10	PMI0085	phnA	GI:197283998	hypothetical protein	Phosphonate and phosphinate metabolism, Microbial metabolism in diverse environments
11	PMI1243	ddlA	GI:197285102	D-alanine--D-alanine ligase	D-Alanine metabolism, Peptidoglycan biosynthesis, Metabolic pathways
12	PMI1508	dadB	GI:197285367	alanine racemase, catabolic	D-Alanine metabolism, Metabolic pathways
13	PMI2273	lpxA	GI:197286119	UDP-N-acetylglucosamine acyltransferase	Lipopolysaccharide biosynthesis, Metabolic pathways
14	PMI2064	lpxC	GI:197285911	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	Lipopolysaccharide biosynthesis, Metabolic pathways
15	PMI2160	lpxH	GI:197286007	UDP-2,3-diacylglucosamine hydrolase	Lipopolysaccharide biosynthesis, Metabolic pathways
16	PMI2272	lpxB	GI:197286118	lipid-A-disaccharide synthase	Lipopolysaccharide biosynthesis, Metabolic pathways
17	PMI0719	lpxK	GI:197284613	tetraacyldisaccharide 4'-kinase	Lipopolysaccharide biosynthesis, Metabolic pathways
18	PMI3167	waaA	GI:197286981	3-deoxy-D-manno-octulosonic-acid transferase	Lipopolysaccharide biosynthesis, Metabolic pathways
19	PMI1686	htrB	GI:197285545	lipid A biosynthesis lauroyl acyltransferase	Lipopolysaccharide biosynthesis, Metabolic pathways
20	PMI0722	kdsB	GI:197284616	3-deoxy-manno-octulosonate cytidylyltransferase	Lipopolysaccharide biosynthesis, Metabolic pathways
21	PMI1090	kdsA	GI:197284961	2-dehydro-3-deoxyphosphooctonate aldolase	Lipopolysaccharide biosynthesis, Metabolic pathways
22	PMI2258	gmhB	GI:197286104	D,D-heptose 1,7-bisphosphate phosphatase	Lipopolysaccharide biosynthesis, Metabolic pathways
23	PMI0349	gmhA	GI:197284250	phosphoheptose isomerase	Lipopolysaccharide biosynthesis, Metabolic pathways

Sl No	Entry ID	Gene Name	GI No	Protein Name	Related Metabolic Pathway
24	PMI3168	waaQ	GI:197286982	lipopolysaccharide core biosynthesis glycosyl transferase	Lipopolysaccharide biosynthesis, Metabolic pathways
25	PMI3174	waaC	GI:197286988	ADP-heptose--LPS heptosyltransferase	Lipopolysaccharide biosynthesis, Metabolic pathways
26	PMI3175	waaF	NCBI-GI:197286989	ADP-heptose--LPS heptosyltransferase	Lipopolysaccharide biosynthesis, Metabolic pathways
27	PMI3169	wabG	GI:197286983	lipopolysaccharide core biosynthesis glycosyl transferase	Lipopolysaccharide biosynthesis, Metabolic pathways
28	PMI3163	rfaL	GI:197286977	O-antigen ligase	Lipopolysaccharide biosynthesis, Metabolic pathways
29	PMI3661	murA	GI:197287464	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Amino sugar and nucleotide sugar metabolism, Peptidoglycan biosynthesis, Metabolic pathways
30	PMI3248	murB	GI:197287061	UDP-N-acetylenolpyruvoylglucosamine reductase	Amino sugar and nucleotide sugar metabolism, Peptidoglycan biosynthesis, Metabolic pathways
31	PMI2069	murC	GI:197285916	UDP-N-acetylmuramate--L-alanine ligase	D-Glutamine and D-glutamate metabolism, Peptidoglycan biosynthesis, Metabolic pathways
32	PMI2072	murD	GI:197285919	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	D-Glutamine and D-glutamate metabolism, Peptidoglycan biosynthesis, Metabolic pathways
33	PMI2074	murF	GI:197285921	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	Lysine biosynthesis, Peptidoglycan biosynthesis, Metabolic pathways
34	PMI2073	mraY	GI:197285920	phospho-N-acetylmuramoyl-pentapeptide-transferase	Peptidoglycan biosynthesis, Metabolic pathways
35	PMI2070	murG	GI:197285917	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	Peptidoglycan biosynthesis, Metabolic pathways
36	PMI2075	murE	GI:197285922	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase	Lysine biosynthesis, Peptidoglycan biosynthesis
37	PMI0423	dacA	GI:197284323	D-alanyl-D-alanine carboxypeptidase	Peptidoglycan biosynthesis, Metabolic pathways
38	PMI0729	aphA	GI:197284623	acid phosphatase/phosphotransferase	Aminobenzoate degradation, Riboflavin metabolism, Microbial metabolism in diverse environments
39	PMI1059	budA	GI:197284937	alpha-acetolactate decarboxylase	Butanoate metabolism,C5-Branched dibasic acid metabolism
40	PMI1771	ackA	GI:197285629	acetate kinase	Taurine and hypotaurine metabolism, Pyruvate metabolism, Propanoate metabolism, Methane metabolism, Metabolic pathways, Microbial metabolism in diverse environments, Carbon metabolism
41	PMI1772	Pta	GI:197285630	phosphate acetyltransferase	Taurine and hypotaurine metabolism, Pyruvate metabolism, Propanoate metabolism, Methane metabolism, Metabolic pathways, Microbial metabolism in diverse environments, Carbon metabolism
42	PMI1524	glpX	GI:197285383	fructose 1,6-bisphosphatase II	Glycolysis / Gluconeogenesis,Pentose phosphate pathway,Fructose and mannose metabolism,Methane metabolism,Metabolic pathways,Biosynthesis of secondary metabolites,Microbial metabolism in diverse environment, Carbon metabolism
43	PMI0243	fbaA	GI:197284152	fructose-bisphosphate aldolase	Glycolysis / Gluconeogenesis,Pentose phosphate pathway, Fructose and mannose metabolism, Methane metabolism, Metabolic pathways, Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments, Carbon metabolism, Biosynthesis of amino acids
44	PMI3227	Ppc	GI:197287040	phosphoenolpyruvate carboxylase	Pyruvate metabolism, Methane metabolism, Metabolic pathways, Microbial metabolism in diverse environments, Carbon metabolism
45	PMI1421	ppsA	GI:197285280	phosphoenolpyruvate synthase	Pyruvate metabolism, Methane metabolism, Metabolic pathways, Microbial metabolism in diverse environments, Carbon metabolism
46	PMI1043	arnB	GI:197284921	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase	Amino sugar and nucleotide sugar metabolism, Biosynthesis of secondary metabolites, Two-component system
47	PMI3571	narG	GI:197287375	respiratory nitrate reductase 1 subunit alpha	Nitrogen metabolism,Microbial metabolism in diverse environments,Two-component system

Sl No	Entry ID	Gene Name	GI No	Protein Name	Related Metabolic Pathway
48	PMI3572	narH	GI:197287376	respiratory nitrate reductase 1 subunit beta	Nitrogen metabolism,Microbial metabolism in diverse environments,Two-component system
49	PMI3573	narJ	GI:197287377	respiratory nitrate reductase 1 subunit delta	Nitrogen metabolism,Microbial metabolism in diverse environments,Two-component system
50	PMI3574	narI	GI:197287378	respiratory nitrate reductase 1 subunit gamma	Nitrogen metabolism,Microbial metabolism in diverse environments,Two-component system
51	PMI3586	frdC	GI:197287389	fumarate reductase subunit C	Oxidative phosphorylation, Pyruvate metabolism, Metabolic pathways, Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments, Carbon metabolism, Two-component system
52	PMI3585	frdD	GI:197287388	fumarate reductase subunit D	Oxidative phosphorylation, Pyruvate metabolism, Metabolic pathways, Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments, Carbon metabolism, Two-component system
53	PMI0574	cydB	GI:197284471	cytochrome D ubiquinol oxidase subunit II	Oxidative phosphorylation, Metabolic pathways,Two-component system
54	PMI0573	cydA	GI:197284470	cytochrome D ubiquinol oxidase subunit I (	Oxidative phosphorylation,Metabolic pathways,Two-component system
55	PMI0435	gltK	GI:197284335	glutamate/aspartate ABC transporter permease	ABC transporters,Two-component system
56	PMI0436	gltJ		glutamate/aspartate ABC transporter permease	ABC transporters,Two-component system
57	PMI1681	ttrA	GI:197285540	tetrathionate reductase subunit A	Sulfur metabolism, Microbial metabolism in diverse environments, Two-component system
58	PMI1683	ttrB	GI:197285540	tetrathionate reductase subunit B	Sulfur metabolism, Microbial metabolism in diverse environments, Two-component system
59	PMI1682	ttrC	GI:197285541	tetrathionate reductase subunit C	Sulfur metabolism, Microbial metabolism in diverse environments, Two-component system
60	PMI1672	flhD	GI:197285531	transcriptional activator FlhD	Two-component system, Flagellar assembly
61	PMI1671	flhC	GI:197285530	transcriptional activator FlhC	Two-component system, Flagellar assembly
62	PMI1665	NA		methyl-accepting chemotaxis protein	Two-component system, Bacterial chemotaxis
63	PMI1667	Chew	GI:197285526	purine-binding chemotaxis protein	Two-component system, Bacterial chemotaxis
64	PMI1664	cheR	GI:197285523	chemotaxis methyltransferase CheR	Two-component system, Bacterial chemotaxis
65	PMI1663	cheB	GI:197285522	chemotaxis response regulator protein-glutamate methylesterase	Two-component system, Bacterial chemotaxis
66	PMI1631	fliG	GI:197285490	flagellar motor switch protein G	Bacterial chemotaxis, Flagellar assembly
67	PMI1637	fliM	GI:197285496	flagellar motor switch protein FliM	Bacterial chemotaxis, Flagellar assembly
68	PMI1638	fliN	GI:197285497	flagellar motor switch protein FliN	Bacterial chemotaxis, Flagellar assembly
69	PMI1670	motA	GI:197285529	flagellar motor protein MotA	Two-component system, Bacterial chemotaxis, Flagellar assembly
70	PMI1669	motB	GI:197285528		Bacterial chemotaxis, Flagellar assembly
71	PMI0090	rbsB	GI:197284002	D-ribose transporter subunit RbsB	ABC transporters, Bacterial chemotaxis
72	PMI1378	sapA	GI:197285237	peptide transport periplasmic protein	ABC transporters, Bacterial chemotaxis
73	PMI1666	NA	GI:197285525	methyl-accepting chemotaxis protein	Two-component system, Bacterial chemotaxis
74	PMI1619	fliC2	GI:197285478	flagellin 2	Two-component system, Flagellar assembly
75	PMI1830	crr	GI:197285685	PTS system glucose-specific transporter	Glycolysis / Gluconeogenesis,Starch and sucrose metabolism, Amino sugar and nucleotide sugar metabolism, Phosphotransferase system (PTS)
76	PMI2292	ptsG	GI:197286138	PTS system glucose-specific transporter subunit IIBC	Glycolysis / Gluconeogenesis, Amino sugar and nucleotide sugar metabolism, Phosphotransferase system (PTS)
77	PMI0455	nagE	GI:197284355	PTS system N-acetylgalactosamine-specific transporter subunit EIICBA	Amino sugar and nucleotide sugar metabolism, Phosphotransferase system (PTS)
78	PMI2982	NA	GI:197286811	PTS system transporter subunit EIIBC	Glycolysis / Gluconeogenesis, Starch and sucrose metabolism, Phosphotransferase system (PTS)

Sl No	Entry ID	Gene Name	GI No	Protein Name	Related Metabolic Pathway
79	PMI3515	NA	GI:197287320	PTS system transporter subunit EIIBC	Starch and sucrose metabolism, Phosphotransferase system (PTS)
80	PMI0291	treB	GI:197284198	PTS system trehalose(maltose)-specific transporter subunit IIBC	Starch and sucrose metabolism, Phosphotransferase system (PTS)
81	PMI2137	agaW	GI:197285984	PTS system N-acetylgalactosamine-specific transporter subunit EIIC	Galactose metabolism,Phosphotransferase system (PTS)
82	PMI2135	agaF	GI:197285982	PTS system N-acetylgalactosamine-specific transporter subunit EIIA	Galactose metabolism,Phosphotransferase system (PTS)
83	PMI2136	agaD	GI:197285983	PTS system N-acetylgalactosamine-specific transporter subunit EIID	Galactose metabolism,Phosphotransferase system (PTS)
84	PMI2138	agaV	GI:197285985	PTS system N-acetylgalactosamine-specific transporter subunit EIIB	Galactose metabolism, Phosphotransferase system (PTS)
85	PMI1775	ulaA	GI:197285633	PTS system ascorbate-specific transporter subunit IIC	Ascorbate and aldarate metabolism, Metabolic pathways, Microbial metabolism in diverse environments,Phosphotransferase system (PTS)
86	PMI1777	NA	GI:197285635	PTS system transporter subunit EIIA	Ascorbate and aldarate metabolism, Metabolic pathways,Microbial metabolism in diverse environments, Phosphotransferase system (PTS)
87	PMI3540	tatB	GI:197287345	Sec-independent protein translocase	Protein export, Bacterial secretion system
88	PMI3541	tatC	GI:197287346	twin-arginine protein translocation system subunit TatC	Protein export,Bacterial secretion system
89	PMI0078	secD	GI:197283991	preprotein translocase subunit SecD	Protein export ,Bacterial secretion system
90	PMI2791	secE	GI:197286620	preprotein translocase subunit SecE	Protein export, Bacterial secretion system
91	PMI3415	secG	GI:197287227	protein-export membrane protein	Protein export,Bacterial secretion system
92	PMI3275	secY	GI:197287088	preprotein translocase subunit SecY	Protein export,Bacterial secretion system
93	PMI0077	yajC	GI:197283990	preprotein translocase subunit YajC	Protein export, Bacterial secretion system
94	PMI3128	oxaA	GI:197286946	inner membrane protein translocase component YidC	Protein export , Bacterial secretion system
95	PMI2061	secA	GI:197285908	preprotein translocase subunit SecA	Protein export , Bacterial secretion system
96	PMI2062	secM	GI:197285909	SecA regulator SecM	Protein export, Bacterial secretion system
97	PMI3183	secB	GI:197286997	preprotein translocase subunit SecB	Protein export , Bacterial secretion system

Table 2. List of common potential drug targets which are highly similar to the target proteins in Drug Bank

SL NO	Entry Number	Protein Number	Similar Target Present in Database	Drugs Name	Status
1	PMI1408	conjugated bile acid hydrolase	Penicillin acylase	Penicillin V	Approved
2	PMI1367	periplasmic murein peptide-binding protein	Periplasmic oligopeptide-binding protein	NAPHTHALEN-2-YL-3-ALANINE	Experimental
3	PMI0873	beta-hexosaminidase	Beta-hexosaminidase	[[(3R,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(pentanoylamino)oxan-2-ylidene]amino] N-phenylcarbamate [[(3R,4R,5S,6R)-3-(butanoylamino)-4,5-dihydroxy-6-(hydroxymethyl)oxan-2-ylidene]amino] N-phenylcarbamate	Experimental
4	PMI0765	outer membrane porin	Outer membrane protein C	Dodecane	Experimental
5	PMI2349	outer membrane channel protein	Outer membrane protein TolC	Cobalt Hexammine Ion	Experimental
6	PMI3021	peptidoglycan synthetase	Penicillin-binding protein 1A	Cefmetazole, Ertapenem,Cefpiramide, Ceftazidime, Cefazolin, Cefonicid, Cefoperazone, Cefoxitin, Ceftizoxime,Cefradine	Approved

7	PMI0426	penicillin-binding protein 2	Penicillin-binding protein 2	Ertapenem, Ceftazidime, Mezlocillin, Amdinocillin, Cefazolin, Cefonicid, Cefoperazone, Cefepime,Ceftibuten,Imipenem	Approved
8	PMI2076	penicillin-binding protein	Peptidoglycan synthase FtsI	Cefmetazole,Ertapenem,Cefpiramide,Ceftazidime ,Cefazolin,Cefonicid,Cefoperazone,Cefoxitin,Ceftizoxime Cefmenoxime	Approved
9	PMI3079	phosphonoacetaldehyde hydrolase	Phosphonoacetaldehyde hydrolase	Phosphonoacetaldehyde,Ethanesulfonic Acid,Vinylsulphonic Acid	Experimental
10	PMI1243	D-alanine--D-alanine ligase	D-alanine--D-alanine ligase A	Cycloserine	Approved
11	PMI1508	alanine racemase, catabolic	Alanine racemase	Cycloserine	Approved
12	PMI2273	UDP-N-acetylglucosamine acyltransferase	Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase	D-tartaric acid,2-HYDROXYMETHYL-6-OCTYLSULFANYL-TETRAHYDRO-PYRAN-3,4,5-TRIOL	Experimental
13	PMI2064	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	(2R)-N-hydroxy-3-naphthalen-2-yl-2-[(naphthalen-2-ylsulfonyl)amino]propanamide	Experimental
14	PMI0722	3-deoxy-manno-octulosonate cytidylyltransferase	3-deoxy-manno-octulosonate cytidylyltransferase	Cmp-2-Keto-3-Deoxy-Octulosonic Acid	Experimental
15	PMI1090	2-dehydro-3-deoxyphosphooctonate aldolase	2-dehydro-3-deoxyphosphooctonate aldolase	Phosphoenolpyruvate, {[(2,2-Dihydroxy-Ethyl)-(2,3,4,5-Tetrahydroxy-6-Phosphonooxy-Hexyl)-Amino]-Methyl}-Phosphonic Acid,3-Fluoro-2-(Phosphonooxy)Propanoic Acid,1-Deoxy-Ribofuranose-5'-Phosphate	Experimental
16	PMI0349	phosphoheptose isomerase	Phosphoheptose isomerase	D-Glycero-D-Mannopyranose-7-Phosphate	Experimental
17	PMI3661	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Fosfomycin	Approved
18	PMI3248	UDP-N-acetylenolpyruvoylglucosamine reductase	UDP-N-acetylenolpyruvoylglucosamine reductase	Flavin adenine dinucleotide	Approved
19	PMI2069	UDP-N-acetylmuramate--L-alanine ligase	UDP-N-acetylmuramate--L-alanine ligase	Uridine-5'-Diphosphate-N-Acetylmuramoyl-L-Alanine,Adenosine-5'-[Beta, Gamma-Methylene]Triphosphate,Phosphoaminophosphonic Acid-Adenylate Ester	Experimental
20	PMI2072	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	UDP-N-acetylmuramoylalanine--D-glutamate ligase	Uridine-5'-Diphosphate-N-Acetylmuramoyl-L-Alanine ,Uridine-5'-Diphosphate-N-Acetylmuramoyl-L-Alanine-D-Glutamate Lysine Nz-Carboxylic Acid N-[(6-BUTOXYNAPHTHALEN-2-YL)SULFONYL]-L-GLUTAMIC ACID N-[(6-BUTOXYNAPHTHALEN-2-YL)SULFONYL]-D-GLUTAMIC ACID N-{[6-(PENTYLOXY)NAPHTHALEN-2-YL]SULFONYL}-D-GLUTAMIC ACID N-({6-[(4-CYANOBENZYL)OXY]NAPHTHALEN-2-YL}SULFONYL)-D-GLUTAMIC ACID N-({6-[(4-CYANO-2-FLUOROBENZYL)OXY]NAPHTHALEN-2-YL}SULFONYL)-D-GLUTAMIC ACID	Experimental
21	PMI2074	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	2-CHLORO-N-(3-CYANO-5,6-DIHYDRO-4H-CYCLOPENTA[B]THIOPHEN-2-YL)-5-DIETHYLSULFAMOYL-BENZAMIDE	Experimental
22	PMI2070	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine	Uridine-Diphosphate-N-Acetylgalactosamine	Experimental

			transferase		
23	PMI2075	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase	Uridine-5'-Diphosphate-N-Acetylmuramoyl-L-Alanine-D-Glutamate, 2,6-Diaminopimelic Acid, Lysine Nz-Carboxylic Acid	Experimental
24	PMI0423	D-alanyl-D-alanine carboxypeptidase	D-alanyl-D-alanine carboxypeptidase DacA	Cefmetazole,Cefoperazone,Cefoxitin	Approved
25	PMI0729	acid phosphatase/phosphotransferase	Class B acid phosphatase	2'-Deoxycytidine	Experimental
26	PMI1771	acetate kinase	Probable butyrate kinase 2	Formic Acid, Adenosine-5'-[Beta, Gamma-Methylene]Triphosphate	Experimental
27	PMI1772	phosphate acetyltransferase	Phosphate acetyltransferase	Acetylphosphate	Experimental
28	PMI0243	fructose-bisphosphate aldolase	Fructose-bisphosphate aldolase class 2	Phosphoglycolohydroxamic Acid	Experimental
29	PMI3227	phosphoenolpyruvate carboxylase	Phosphoenolpyruvate carboxylase	3,3-Dichloro-2-Phosphonomethyl-Acrylic Acid	Experimental
30	PMI1421	phosphoenolpyruvate synthase	Pyruvate, phosphate dikinase	Phosphonopyruvate	Experimental
31	PMI1043	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase	Pyridoxamine-5'-Phosphate,Pyridoxyl-N,O-Cycloserylamide-5-Monophosphate	Experimental
32	PMI3571	respiratory nitrate reductase 1 subunit alpha	Respiratory nitrate reductase 1 alpha chain	N-Formylmethionine, (1S)-2-{[{[(2S)-2,3-DIHYDROXYPROPYL]OXY}(HYDROXY)PHOSPHORYL]OXY}-1-[(PENTANOYLOXY)METHYL]ETHYL OCTANOATE	Experimental
33	PMI3572	respiratory nitrate reductase 1 subunit beta	Respiratory nitrate reductase 1 beta chain	N-Formylmethionine ,(1S)-2-{[{[(2S)-2,3-DIHYDROXYPROPYL]OXY}(HYDROXY)PHOSPHORYL]OXY}-1-[(PENTANOYLOXY)METHYL]ETHYL OCTANOATE	Experimental
34	PMI3574	respiratory nitrate reductase 1 subunit gamma	Respiratory nitrate reductase 1 gamma chain	N-Formylmethionine ,(1S)-2-{[{[(2S)-2,3-DIHYDROXYPROPYL]OXY}(HYDROXY)PHOSPHORYL]OXY}-1-[(PENTANOYLOXY)METHYL]ETHYL OCTANOATE	Experimental
35	PMI3586	fumarate reductase subunit C	Fumarate reductase subunit C	2-[1-(4-CHLORO-PHENYL)-ETHYL]-4,6-DINITRO-PHENOL ,2-HEPTYL-4-HYDROXY QUINOLINE N-OXIDE	Experimental
36	PMI3585	fumarate reductase subunit D	Fumarate reductase subunit D	2-[1-(4-CHLORO-PHENYL)-ETHYL]-4,6-DINITRO-PHENOL,2-HEPTYL-4-HYDROXY QUINOLINE N-OXIDE	Experimental
37	PMI1681	tetrathionate reductase subunit A	Thiosulfate reductase	UBIQUINONE-1	Experimental
38	PMI1683	tetrathionate reductase subunit B	NrfC protein	UBIQUINONE-1	Experimental
39	PMI1665	methyl-accepting chemotaxis protein	Methyl-accepting chemotaxis protein II	1,10-Phenanthroline	Experimental
40	PMI1664	chemotaxis methyltransferase CheR	Chemotaxis protein methyltransferase	S-Adenosyl-L-Homocysteine	Experimental
41	PMI0090	D-ribose transporter subunit RbsB	D-ribose-binding periplasmic protein	beta-D-Ribopyranose	Experimental
42	PMI1378	peptide transport periplasmic protein	Nickel-binding periplasmic protein	3,5-Diiodotyrosine	Experimental
43	PMI1666	methyl-accepting chemotaxis protein	Methyl-accepting chemotaxis protein II	1,10-Phenanthroline	Experimental

13. C5-Branched dibasic acid metabolism
14. Methane metabolism
15. Limonene and pinene degradation
16. Biosynthesis of siderophore group nonribosomal peptides
17. Bacterial secretion system
18. Phosphotransferase system
19. Flagellar assembly
20. Bacterial chemotaxis
21. Biosynthesis of siderophore group nonribosomal peptides
22. Two-component system

From these metabolic pathways 97 nonhomologous and essential enzymes were identified.

These proteins may act as drug targets, as these pathways are absent in host and eradicate any potential risk factors exerted by the drug targeting these pathways.As example Peptidoglycan biosynthesis pathway is an important pathogen-specific pathway. Peptidoglycan is one of the main constituents of the outer cell wall of Gram -negative bacteria and play an important role in pathogenesis and antibiotic sensitivity. Peptidoglycan synthetase(mrcA), penicillin-binding protein 2(pbpA), penicillin-binding protein(ftsI), D-alanine--D-alanine ligase (ddlA), UDP-N-acetylglucosamine 1-carboxyvinyltransferase(murA), UDP-N-acetylenolpyruvoylglucosamine reductase (murB), UDP-N-acetylmuramate--L-alanine ligase(murC), UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase(murD), UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase(murF), Phospho-N-acetylmuramoylpentapeptidetransferase(mraY), Undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase (murG), UDP-Nacetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase(murE), D-alanyl-D-alanine carboxypeptidase(dacA) were identified as potential common drug targets in the present study. D-alanine--D-alanine ligase (ddlA) also participates in D-alanine metabolism which is essential for the precursor of peptidoglycan backbone and metabolic pathway thus could be considered as good drug target.

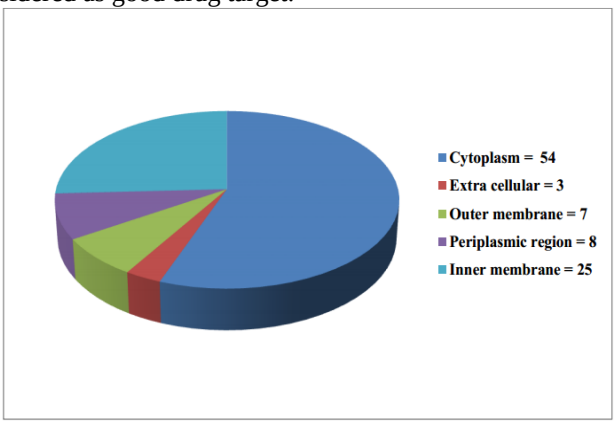

Figure 1. The Sub cellular localization analysis of all supposed essential and unique enzymes of *Proteus mirabilis* by CELLO v2.5 server.

Fumarate reductase subunit C, fumarate reductase subunit D, cytochrome D ubiquinol oxidase subunit II, cytochrome D ubiquinol oxidase subunit I can become good targets as they participate in Two component systems, essential for the growth and survival in adverse environmental conditions.

The sub cellular localization analysis of all supposed essential and unique enzymes of *Proteus mirabilis* were evaluated by using CELLO v2.5 server. After analysis of 97 proteins , 54 were found to be located in the cytoplasm, 3 as extra cellular, 7 in outer membrane , 8 in periplasmic region, and 25 in inner membrane (Fig. 1). The results obtained were further validated with PSORTb. Forty three common potential drug targets were found to be highly similar to the target proteins in Drug Bank (Table 2).

Nine out of 43 drug targets were having approved drugs and the remaining 34 drug targets were having experimental drugs. Further, the drug targets can be used to explore the structure based drug designing (SBDD), to propose novel inhibitor molecules against Proteus infections in human.

Conclusion

In the present study, we have used metabolic pathway analysis method for the identification of drug targets against *P.mirabilis*. Ninety seven common putative drug targets were listed as the drug targets against the pathogen. As the inhibitors will be specific to the pathogen and non homologous to human, therefore not toxic to the host. The present study can be carried forward to design inhibitors to block these targets. The microorganisms are gaining resistance to already existing drugs very rapidly, so for producing better and effective drugs in future these protein targets will be very helpful.

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