In silico Identification of Putative Drug Targets in *Pseudomonas aeruginosa* Through Metabolic Pathway Analysis

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Abstract. Comparative genomic analysis between pathogens and the host *Homo sapiens* has led to identification of novel drug targets. Microbial drug target identification and validation has been the latest trend in pharmacoinformatics. In order to identify a suitable drug target for the pathogen *Pseudomonas aeruginosa* an *in silico* comparative analysis of the metabolic pathways between the pathogen and the host *Homo sapiens* was performed. Detection of bacterial genes that are non-homologous to human genes, and are essential for the survival of the pathogen represents a promising means of identifying novel drug targets. Metabolic pathways for the pathogen and *H.sapiens* were obtained from the metabolic pathway database KEGG and were compared to identify unique pathways present only in the pathogen and absent in the host. We identified 361 enzymes from both unique and common pathways between the pathogen and the host of which 50 belong to the 12 unique pathways. Enzymes from both genomes were subject to a BLASTp search and sequences homologous to human were removed as non essential. *P.aeruginosa* targets without human homologs were identified when the e-value threshold was set as 10^{-2}. Of the 214 targets that had no hits only 30 targets belong to unique pathways. These 30 targets were then compared with the list of candidate essential genes identified by mutagenesis. Only 8 targets matched with the essential genes list and these were considered as potential drug targets. We have built homology model for the four target genes lpxC, kdsA, kdsB and waaG using MODELLER software. This approach enables rapid potential drug target identification, thereby greatly facilitating the search for new antibiotics.

Keywords: *Pseudomonas aeruginosa*, *Homo sapiens*, Comparative microbial genomics, KEGG, Homology, MODELLER, kdsA, kdsB, waaG, lpxC, Potential drug targets.

1 Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium and an opportunistic human pathogen as well as an opportunistic pathogen for plants. It mainly target

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immunocompromised patients and typically infects the pulmonary tract, urinary tract and even causes blood infections. *P. aeruginosa* is highly resistant to a wide range of antibiotics and disinfectants [23]. The pathogen has been reported to have lower outer membrane permeability to small molecules [10]. There is also the presence of several multidrug efflux pumps from the major facilitator superfamily (MFS), multidrug and toxic compound extrusion (MATE) families, ATP-binding cassette (ABC) and small multi-drug resistance (SMR) that have increased its intrinsic resistance to many efficient antibiotics. Thus, developing new antibacterial drugs against this pathogen has been a challenging problem over these years.

Over the last decade, complete genome sequences of several pathogenic bacteria have been sequenced and many more such projects are currently under investigation. This global effort has focused primarily on pathogens which encompass the majority of all genome projects, and has generated a large amount of raw material for *in silico* analysis. These data pose a major challenge in the post-genomic era, i. e. to fully exploit this treasure trove for the identification and characterization of virulent factors in these pathogens, and to identify novel putative targets for therapeutic intervention [16].

Genomics can be applied to evaluate the suitability of potential targets using two criteria, i. e. "essentiality" and "selectivity" [19]. The target must be essential for the growth, replication, viability or survival of the microorganism, i. e. encoded by genes critical for pathogenic life-stages. The microbial target for treatment should not have any well-conserved homolog in the host, in order to address cytotoxicity issues. This can help to avoid expensive dead-ends when a lead target is identified and investigated in great detail only to discover at a later stage that all its inhibitors are invariably toxic to the host. Genes that are conserved in different genomes often turn out to be essential [7] [25] [12] [11]. A gene is deemed to be essential if the cell cannot tolerate its inactivation by mutation, and its status is confirmed using conditional lethal mutants.

The complete genome sequence of the pathogen *Pseudomonas aeruginosa* [23] and the host *Homo sapiens* [The Genome International Consortium, 2001] is available. *Pseudomonas aeruginosa* PA01 strain is the largest bacterial genome sequenced with 6.3 million base pairs and with 5,570 predicted open reading frames (ORFs). Comparative analysis between the two genomes has led to know about the pathogenicity of the bacterium and offers to identify new novel antimicrobial drug targets. Galperin and Koonin, 1999 suggested that targets that serve as inhibitors of certain bacterial enzymes and specific to bacteria can be developed as potential drug targets. Comparative metabolic pathway analysis results in the identification of unique pathways and enzymes that are present in the pathogen but absent in the host. Our approach by differential genome analysis identified bacterial genes that are non-homologous to human and thus making them attractive targets for new frontline antibiotics. Our *in silico* approach enabled us to identify suitable targets from the pathogen resulting in homology modeling of these targets and further analysis using molecular docking studies.

As a proof of concept, many of the genes identified by our approach are also reported as essential by experimental methods. Of the 30 distinct targets belonging to the unique pathways of *P.aeruginosa* the experimentally determined candidate essential genes generated by Jacobs *et al.,* 2003 listed out only 8 targets as the most essential ones. By further analyses of these genes with PDB structures only 3 were

selected as the most suitable antibacterial drug targets. Using homology modeling, a target sequence can be modeled with reasonable accuracy with the template sequence based on the sequence similarity between them. Our approach was successful in modeling 4 potential drug targets enabling us further validation and characterization in the laboratory in near future.

2 Materials and Methods

2.1 Identification of Unique Enzymes as Drug Targets

Metabolic pathway information was obtained from the pathway database Kyoto Encyclopedia of Genes and Genomes [9]. Enzyme commission numbers (EC) of the pathogen *P.aeruginosa* and the host *H.sapiens* were extracted from the KEGG database. Pathways unique to *P. aeruginosa* were filtered out. Twelve unique pathways were observed [Table 1]. These are the pathways that do not appear in the host (*H. sapiens*) but are present in the pathogen. We further identified unique enzymes among shared pathways under carbohydrate metabolism, energy metabolism, lipid metabolism, nucleotide metabolism, amino acid metabolism, glycan biosynthesis and metabolism and metabolism of cofactors and vitamins were obtained from the KEGG database. A total of 361 enzymes that are present in *P. aeruginosa* but absent in *H. sapiens* were obtained and their corresponding protein sequences were retrieved from the KEGG database.

The protein sequences for these 361 unique enzymes were retrieved and were subject to BLAST [1] search against human protein sequences database at an expectation E-value cutoff of 10^{-2} to identify non-homologous genes in *P. aeruginosa*. Removing enzymes from the pathogen that share a similarity with the host protein ensures that the targets have nothing in common with the host proteins, thereby, eliminating undesired host protein-drug interactions. The above search resulted in 214 enzymes that had "no hits" in BLAST search. Thirty of these 214 "no hits" belonged to the unique pathways set and the remaining 184 belong to unique enzymes in shared pathways.

Table 1. Pathways unique to *Pseudomonas aeruginosa*

S.No	Pathways and their enzymes	Gene	EC #
1	**Polyketide sugar unit biosynthesis**		
	Glucose 1-phosphate thymidylyltransfease	rmlA	2.7.7.24
	dTDP-D-Glucose 4,6 dehydratase	rmlB	4.2.1.46
	dTDP-4-dehydrorhamnose 3,5 epimerease	rmlC	5.1.3.13
	dTDP-4-dehydrorhamnose reductase	rmlD	1.1.1.133
2	**Biosynthesis of siderophore group nonribosomal peptides**		
	Isochorismate synthase	pchA	5.4.4.2
	Isochorismate pyruvate lyase	pchB	4.1.99.-
3	**Toluene and xylene degradation**		
	catechol 1,2-dioxygenase	catA	1.13.11.1

Table 1. (*continued*)

4	**1,2 Dichloroethane degradation**		
	Quinoprotein alcohol dehydrogenase	exaA	1.1.99.8
	Probable aldehyde dehydrogenase		1.2.1.3
5	**Type II secretion system**		
	Two-component sensor PilS	pilS	2.7.3.-
	Leader peptidase (prepilin peptidase) / N-methyltransferase	pilD	3.4.23.43
	Methyltransferase PilK	pilK	2.1.1.80
6	**Type III secretion system**		
	Flagellum-specific ATP synthase FliI	fliI	3.6.3.14
7	**Phosphotransfease system (PTS)**		
	phosphotransferase system, fructose-specific IIBC component	fruA	2.7.1.69
	probable phosphotransferase system enzyme I		2.7.3.9
8	**Bacterial Chemotaxis**		
	Methyltransferase PilK	pilK	2.1.1.80
	Two-component sensor PilS	pilS	2.7.3.-
	probable methylesterase		**3.1.1.61
9	**Flagellar Assembly**		
	ATP synthase in type III secretion system		3.6.3.14
10	**D-Alanine metabolism**		
	D-alanine-D-alanine ligase A	ddlA	6.3.2.4
	biosynthetic alanine racemase	alr	5.1.1.1
11	**Lipopolysaccharide Biosynthesis**		
	Probable glucosyltransferases		2.4.-
	3-deoxy-manno-octulosonate cytidylyltransferase	kdsB	**2.7.7.38
	Putative 3-deoxy-D-manno-octulosonate 8-phosphate phosphatase		3.1.3.45
	Tetraacyldisaccharide 4'-kinase	lpxK	**2.7.1.130
	Lipid A-disaccharide synthase	lpxB	**2.4.1.182
	Lipopolysaccharide core biosynthesis protein WaaP	waaP	**2.7.-.-
	Poly(3-hydroxyalkanoic acid) synthase 1	phaC1	2.3.1.-
	UDP-glucose:(heptosyl) LPS alpha 1,3-glucosyltransferase WaaG	waaG	**2.4.1.-
	UDP-2,3-diacylglucosamine hydrolase		**3.6.1.-
	UDP-3-O-acyl-N-acetylglucosamine deacetylase	lpxC	3.5.1.-
	UDP-N-acetylglucosamine acyltransferase	lpxA	2.3.1.129
	ADP-L-glycero-D-mannoheptose 6-epimerase	rfaD	5.1.3.20
	2-dehydro-3-deoxyphosphooctonate aldolase (KDO 8-P synthase)	kdsA	**2.5.1.55
12	**Two component system**		
	Two-component sensor PilS	pilS	2.7.3.-
	Probable 2-(5"-triphosphoribosyl)-3'-dephospho coenzyme-A synthase		2.7.8.25
	Serine protease MucD precursor	mucD	3.4.21.-
	Probable acyl-CoA thiolase		2.3.1.9
	Glutamine synthetase	glnA	6.3.1.2
	Citrate lyase beta chain		4.1.3.6
	Protein-PII uridylyltransferase	glnD	2.7.7.59
	Beta-lactamase precursor	ampC	3.5.2.6
	Anthranilate synthase component II	trpG	4.1.3.27
	Anthranilate phosphoribosyltransferase	trpD	2.4.2.18
	Indole-3-glycerol-phosphate synthase	trpC	4.1.1.48
	Tryptophan synthase alpha chain	tr	4.2.1.20
	Potassium-transporting ATPase	kd	3.6.3.12
	Probable methylesterase		3.1.1.61
	Alkaline phosphatase	phoA	3.1.3.1
	Respiratory nitrate reductase alpha chain	narG	1.7.99.4

** Enzymes that matched with list of candidate essential genes and were considered as potential drug targets.

2.2 Comparison of Unique Enzymes to Essential Gene Data

We further compared the 214 unique enzymes to the list of candidate essential genes of *P.aeruginosa* obtained from transposon mutagenesis studies [8]. It is observed that 83 enzymes in total (8 enzymes from unique pathways and 75 enzymes from shared pathways) are reported as essential [8]. It is noteworthy that 7 of the 8 enzymes from the unique pathways map to a single pathway that of lipopolysaccharide biosynthesis [Table 2]. Literature search revealed that LpxC (UDP-3-O-acyl-N-acetylglucosamine deacetylase) is another enzyme in lipopolysaccharide biosynthesis that is essential but is absent in the transposon mutagenesis data. Our selection of LpxC for further analyses was based on the concept that molecular validation of this enzyme could act as a target for novel antibacterial drugs in *Pseudomonas aeruginosa* [10].

2.3 Comparative Homology Modeling

Annotation screen for the 8 enzymes in unique pathways revealed that one of the enzymes is reported as a conserved hypothetical protein and another one as probable methylesterase. We removed these two proteins for homology modeling. The remaining 6 enzymes from the unique pathways were subject to BLASTp search against PDB. We further removed 3 enzymes that had "no hits" in PDB or had short template sequences and thus modeling is not possible. The final potential drug targets are kdsA (2-dehydro-3-deoxyphosphooctonate aldolase), kdsB (3-deoxy-manno-octulosonate cytidylyl

Table 2. Potential eight drug targets obtained from unique pathways after comparison with the list of candidate essential genes for *P.aeruginosa* [8].The targets which were considered for homology modeling are shaded grey in colour.

EC no	Protein name	Gene
3.6.1.-	conserved hypothetical protein	ybbF
2.7.7.38	3-deoxy-manno-octulosonate cytidylyltransferase	kdsB
2.7.1.130	tetraacyldisaccharide 4*-kinase	lpxK
2.5.1.55	2-dehydro-3-deoxyphosphooctonate aldolase	kdsA
2.4.1.182	lipid A-disaccharide synthase	lpxB
3.1.1.61**	probable methylesterase	
2.7.-.-	LPS biosynthesis protein RfaE	rfaE
2.4.1.-	"UDP-glucose:(heptosyl) LPS alpha 1,3-glucosyltransferase WaaG"	waaG

** Enzyme belonging to bacterial chemotaxis pathway. All other remaining enzymes belong to the lipopolysaccharide biosynthesis pathway.

transferase), waaG ("UDP-glucose: (heptosyl) LPS alpha 1,3- glucosyltransferase WaaG") and lpxC (UDP-3-O-acyl-N-acetylglucosamine deacetylase).

A homology 3D model was built for the four potential drug targets kdsA, kdsB, waaG and lpxC [Figure 1] using MODELLER program [20]. The structural homologues from PDB were used as templates for building the 3D models for the four potential targets. All the selected templates had identity of more than 35% with the target protein and had a resolution of <3.0Å. The structural homologue used as template for kdsA is 2-dehydro-3-deoxyphosphooctonate aldolase (68% identity) from *Escherichia coli* with PDB identifier 1G7U [2], kdsB is 3-deoxy-manno-octulosonate cytidylyltransferase (53% identity) from *Haemophilus influenzae* with PDB identifier 1VIC [3], waaG is a synthetic construct with PDB identifier 2CMU[18] with 41% identity and lpxC is UDP-3-O-acyl-N-acetylglucosamine deacetylase from *Aquifex aeolicus* with PDB identifier 1P42 [26] with 36% sequence identity. The stereo chemical quality of the modeled protein structures was assessed

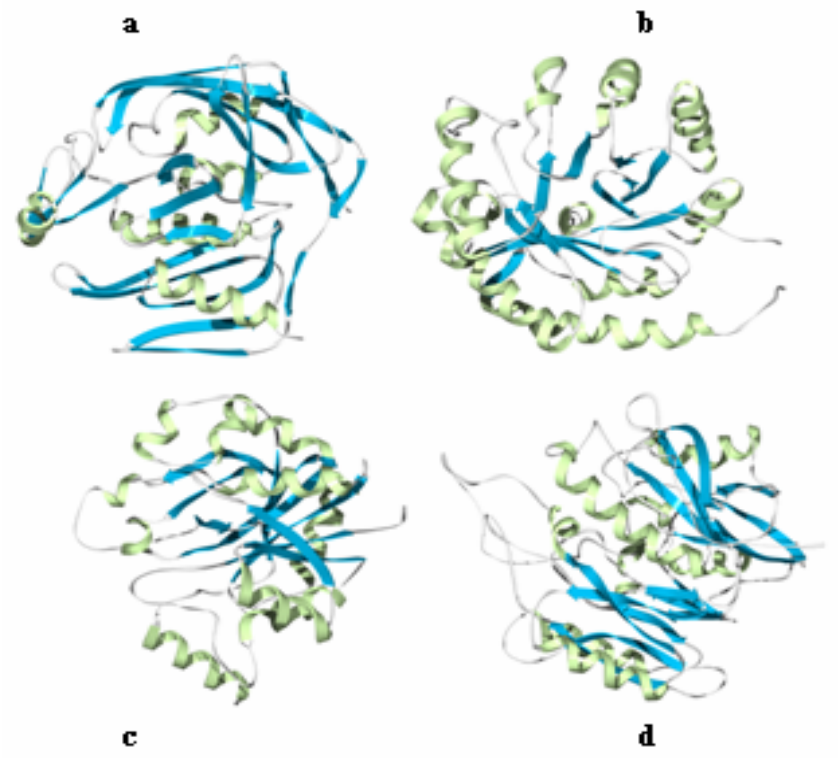

Fig. 1. Models generated by DeepView. Ribbon representation of the following 3D models a) LpxC, b) KdsA, c) KdsB and d) WaaG.

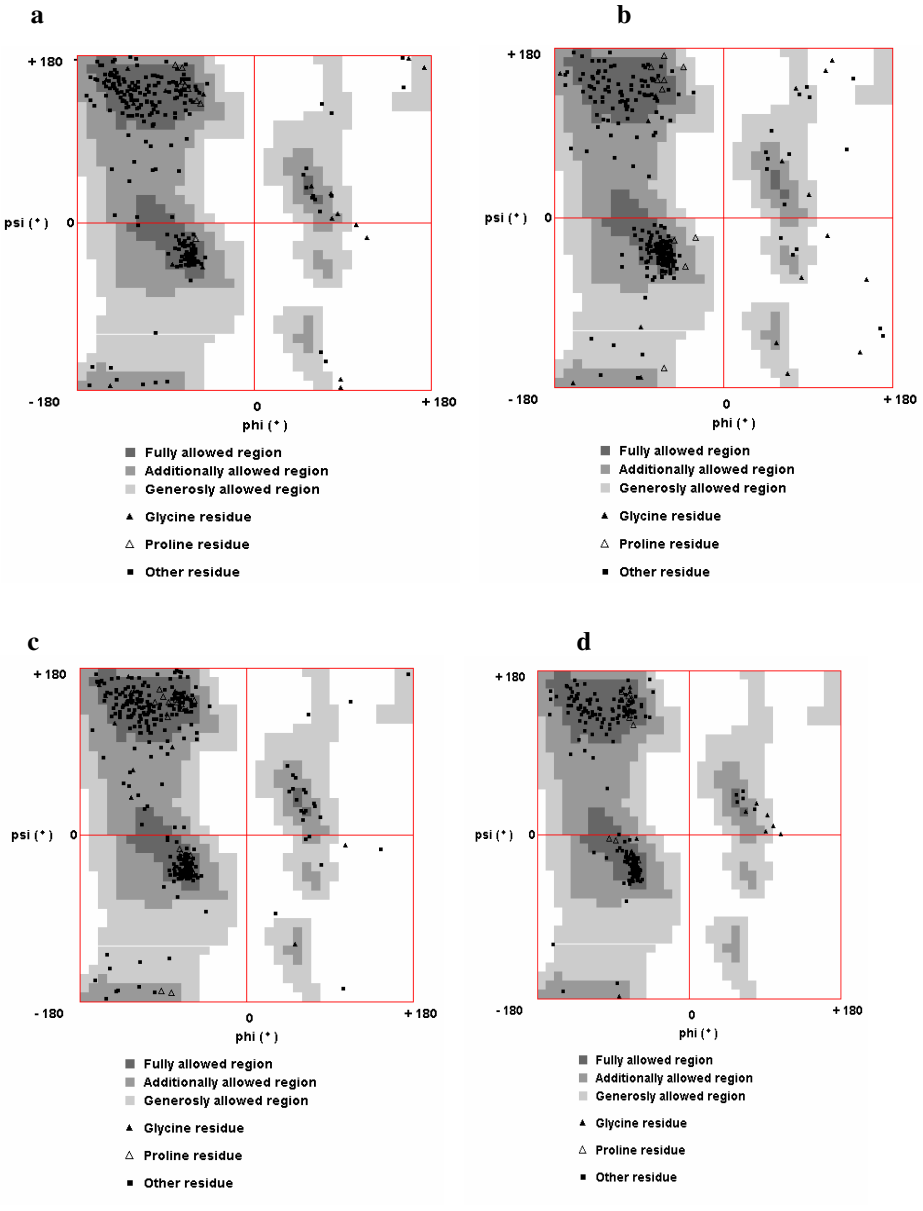

Fig. 2. Ramachandran plot for the following models a) LpxC, b) KdsA, c) KdsB and d) WaaG

by Ramachandran plot (φ vs ψ) for all the 4 models generated using the Ramachandran plot server [22] [Figure 2]. The quality of the model was validated

with the PROCHECK program [13] and the main chain parameters for the model were tabulated [Table 3].

a) Ramachandran plot statistics for the LpxC model

Fully Allowed Region (244 residues)	:	81.06 %
Additionally Allowed Region (42 residues)	:	13.95 %
Generously Allowed Region (6 residues)	:	1.99 %
Outside region (9 residues)	:	2.99 %

Total		100.00 %
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b) Ramachandran plot statistics for the KdsA model

Fully Allowed Region (201 residues)	:	72.04 %
Additionally Allowed Region (49 residues)	:	17.56 %
Generously Allowed Region (20 residues)	:	7.17 %
Outside region (9 residues)	:	3.23 %

Total		100.00 %
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c) Ramachandran plot statistics for the KdsB model

Fully Allowed Region (222 residues)	:	88.10 %
Additionally Allowed Region (22 residues)	:	8.73 %
Generously Allowed Region (6 residues)	:	2.38 %
Outside region (2 residues)	:	0.79 %

Total		100.00 %
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d) Ramachandran plot statistics for the WaaG model

Fully Allowed Region (300 residues)	:	80.06 %
Additionally Allowed Region (55 residues)	:	14.82 %
Generously Allowed Region (10 residues)	:	2.70 %
Outside region (6 residues)	:	1.62 %

Total		100.00 %

Table 3. Main Chain Parameters

a. LpxC

No	Stereochemical parameter	No.of data pts	Parameter value	Comparison Typical value	Values Band width	No. of band widths from mean	
a.	%-tage residues in A,B,L	268	86.2	88.2	10	-0.2	Inside
b.	Omega angle st dev	302	3.5	6	3	-0.8	Inside
c.	Bad contacts/ 100 residues	9	3	1	10	-0.2	Inside
d.	Zeta angle st dev	280	1.2	3.1	1.6	-1.2	Better
e.	H-bond energy st dev	173	0.8	0.7	0.2	0.3	Inside
f.	Overall G-factor	303	-0.1	-0.2	0.3	0.2	Inside

b. KdsA

No	Stereochemical parameter	No.of data pts	Parameter value	Comparison Typical value	Values Band width	No. of band widths from mean	
a.	%-tage residues in A,B,L	238	84.9	88.2	10	-0.3	Inside
b.	Omega angle st dev	269	4.9	6	3	-0.4	Inside
c.	Bad contacts/ 100 residues	6	2.2	1	10	0.1	Inside
d.	Zeta angle st dev	255	1.6	3.1	1.6	-1	Inside
e.	H-bond energy st dev	164	0.8	0.7	0.2	0.5	Inside
f.	Overall G-factor	274	-0.3	-0.2	0.3	-0.3	Inside

c. KdsB

No	Stereochemical parameter	No.of data pts	Parameter value	Comparison Typical value	Values Band width	No. of band widths from mean	
a.	%-tage residues in A,B,L	220	93.6	88.2	10	0.5	Inside
b.	Omega angle st dev	252	3.8	6	3	-0.7	Inside
c.	Bad contacts/ 100 residues	6	2.4	1	10	0.1	Inside
d.	Zeta angle st dev	238	1.4	3.1	1.6	-1	Better
e.	H-bond energy st dev	150	0.7	0.7	0.2	0.2	Inside
f.	Overall G-factor	254	0	-0.2	0.3	0.5	Inside

d. WaaG

No	Stereochemical parameter	No.of data pts	Parameter value	Comparison Typical value	Values Band width	No. of band widths from mean	
a.	%-tage residues in A,B,L	325	83.4	88.2	10	-0.5	Inside
b.	Omega angle st dev	372	4	6	3	-0.7	Inside
c.	Bad contacts/ 100 residues	17	4.6	1	10	0.4	Inside
d.	Zeta angle st dev	348	1.7	3.1	1.6	-0.9	Inside
e.	H-bond energy st dev	186	0.8	0.7	0.2	0.6	Inside
f.	Overall G-factor	373	-0.2	-0.2	0.3	-0.1	Inside

Main- chain parameters for four models generated by PROCHECK. A- Fully Allowed Region, B- Additionally Allowed Region, L-Generously Allowed Region.

3 Results and Discussion

The impact of microbial genomics on drug discovery has led to the identification of new novel antibacterial drugs. Enzymes mediate the synthesis of many complex molecules from simpler ones in a series of chemical reactions. Targeting enzymes present in the pathogen but absent in the host will make sure the elimination of pseudo drug targets in the pathways. It is therefore essential to identify unique pathways and target only those unique enzymes and thus narrowing down to few potential drug targets.

3.1 Pathways and Enzymes Unique to *P.aeruginosa* When Compared to *H.sapiens*

Metabolic pathways belonging to the pathogen and the host were compared and pathways that are present in the pathogen but not in the host are considered to be unique pathways whose enzymes are suitable antibacterial drug targets. Comparative metabolic pathway analysis resulted in 12 unique pathways: Polyketide sugar unit biosynthesis, biosynthesis of siderophore group non-ribosomal peptides, toluene and xylene degradation, D-alanine metabolism, type II secretion system, type III secretion system, phosphotransferase system, bacterial chemotaxis, flagellar assembly, lipopolysaccharide biosynthesis, 2-component system and 1,2 dichloroethane degradation [Table 1]. Enzymes of these pathways are specific and hence they can be explored by finding suitable inhibitors against them.

Among these pathways, lipopolysaccharide biosynthesis, polyketide sugar unit biosynthesis, biosynthesis of siderophore group non-ribosomal peptides, phosphotransferase system (membrane transporter) and D- alanine metabolism are considered to be essential pathways whose enzymes can be targeted for novel antimicrobial drugs [10] [6]. We therefore elaborate on the unique enzymes in these pathways and investigate the lipopolysaccharide pathway in detail.

Polyketides are secondary metabolites playing an important role in defence and intercellular communication. Polymerization of acetyl and propionyl subunits results in the formation of polyketides. Polyketides have important biological activities and pharmacological properties and hence targeting enzymes of these pathways would be an ideal one for drug discovery. Enzymes of this pathway RmlA (EC 2.7.7.24), RmlB (EC 4.2.1.46), RmlC (EC 5.1.3.13) and RmlD (EC 1.1.1.133) synthesize deoxy-thymidine di-phosphate (dTDP)-L-rhamnose from dTTP and glucose-1-phosphate and are important targets for the development of new antimicrobial drugs [14]. Cell wall is necessary for viability and hence they are attractive targets with new drugs being developed for inhibition of cell wall synthesis.

Iron chelating compounds secreted by microorganisms are called Siderophores. These are nonribosomal peptides and they help in dissolving Fe3+ ions as soluble Fe3+ complexes that can be taken up by active transport mechanism. *Pseudomonas* B 10 produces a siderophore namely pseudobactin. Nonribosomal peptides are synthesized by specialized nonribosomal peptide-synthetase (NRPS) enzymes and this biosynthesis is in similar with that of polyketide and fatty acid biosynthesis. Enzymes pchB (Isochorismate pyruvate-lyase EC 4.1.99.-) and pchA (Isochorismate

synthase EC 5.4.4.2) are the two target enzymes of this pathway catalyzing the salicylate biosynthesis [4] [21].

Recent studies reveal that pathogens depend on their hosts for nutrients and hence transport of substrates and other products becomes essential thus making bacterial transport proteins as potential drug targets [6]. PTS or Phosphotransferase system is involved in transporting many sugars into pathogen and involves enzymes of the plasma membrane and the cytoplasm making it a multicomponent system. Protein-N (pi)-phosphohistidine--sugar phosphotransferase (EC 2.7.1.69) belongs to enzyme II of phosphotransferase system with a phosphocarrier protein substrate of low molecular mass (9.5 kDa). The other enzyme that has no human homologue is Phosphoenol-pyruvate—protein phosphotransferase (EC 2.7.3.9) which can also serve as a suitable drug target [5]. The presence of many multidrug efflux pumps increases antibiotic resistance and hence prevents the antibiotic action. It therefore becomes necessary to inhibit these efflux pumps enabling the transport of antibiotic molecules [17].

Enzymes D-alanine-D-alanine ligase A ('ddlA' EC 6.3.2.4) and alanine racemase ('alr' EC 5.1.1.1) catalyze the alanine biosynthesis. Alanine is a non-essential α-amino acid and exists as two enantiomers L-form and D-form. D-alanine mainly exists in bacterial cell walls and serves as a precursor for peptidoglycan biosynthesis. The catalytic action of enzymes alanine racemases makes L-form get racemized to its D-form. These two enzymes are rarely present in eukaryotes and hence they can be developed as suitable drug targets [24].

3.1.1 Lipopolysaccharide Biosynthesis

P.aeruginosa is a gram-negative bacterium producing lipopolysaccharide, a major constituent of the outer cell membrane. Lipopolysaccharide (LPS) serves as selectively permeable membrane for organic molecules and also increases the negative charge of the cell wall and stabilizes the overall membrane structure. LPS consist of a polysaccharide chain covalently linked to a lipid moiety, known as lipid A. Lipopolysaccharide has an important role in the structural integrity of the bacteria and its defense against the host and hence the pathways of these enzymes are attractive drug targets. The enzymes of this pathway had no human homologues and hence they served as potential targets. A total of thirteen enzymes formed this pathway of which seven enzymes matched with the list of candidate essential genes obtained by transposon mutagenesis study and four of them lpxC, kdsA, kdsB and waaG were selected for homology modeling LpxC, KdsA, KdsB and WaaG [Figure 1].

3.2 Targets from Pathways Common to Both *P.aeruginosa* and *H.sapiens*

Unique enzymes in common pathways between *H.sapiens* and *P.aeruginosa* present another source for exploration of drug targets. These targets may be responsible for the pathogenicity and other important biological functions of *P.aeruginosa.* Though our approach concentrated mainly on those enzymes present in the unique pathways we also investigated to find potential targets from the common pathways. There are about 84 targets in carbohydrate metabolism, 30 targets in energy metabolism, 14 targets in lipid metabolism, 31 targets in nucleotide metabolism, 122 targets in amino acid metabolism, 65 targets from metabolism of cofactors and vitamins and 10 targets from secondary metabolite biosynthesis [Figure 3]. It must be noted that several targets were functional in more than one pathway.

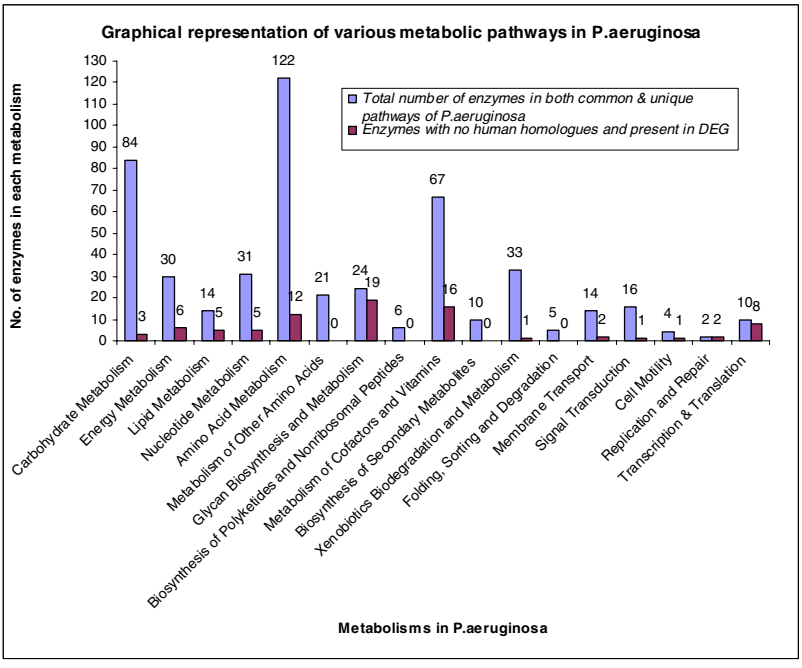

Fig. 3. Metabolic pathways in *P.aeruginosa*

Amino acid metabolism consists of maximum number of targets since amino acids, as precursors of proteins, are essential to all organisms [15]. Many of the enzymes are involved in the biosynthesis of glutamate, lysine, arginine and many other amino acid biosynthesis. Lipid metabolism consists of enzymes that function for lipid biosynthesis as well as for lipid degradation. Many virulence factors including phospholipases C, toxins, lipases and proteases are secreted by *P.aeruginosa.* Outer membrane proteins and membrane transporters are important drug targets in this pathogen due to their involvement in transport of antibiotics. Targets responsible for adhesion and motility are also of great interest in drug targeting.

Many multi-drug efflux systems are present in *P.aeruginosa* thus preventing the action of effective antibiotics. Thus targeting those genes responsible for inhibition of action of antibiotics would prevent the drug resistance property of this pathogen. This example thus illustrates the use of this approach to identify essential genes in pathogens that may be considered as drug targets with more confidence.

4 Conclusion

Our *in silico* approach of comparative metabolic pathway analysis resulted in the identification of potential drug targets. For the first time, the availability of complete genome sequences of many bacterial species is facilitating many computational approaches. The complete definition of all gene products by gene identification tools exemplified here is just the first step. The data presented here demonstrates that

stepwise prioritization of genome open reading frames using simple biological criteria can be an effective way of rapidly reducing the number of genes of interest to an experimentally manageable number. This process is an efficient way for enriching potential target genes, and for identifying those that are critical for normal cell function. The generation of a comprehensive essential gene list will allow an accelerated genetic dissection of traits such as metabolic flexibility and inherent drug resistance that render *P. aeruginosa* such a tenacious pathogen. Such a strategy will enable us 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.

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