Novel Drug Targets in *Pseudomonas aeruginosa* : Potential targets for Drug Designing

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Abstract:
Pseudomonas aeruginosa PA01 is one of the most important and studied opportunistic gram negative bacterial strains, which have a great potential to infect human beings as well as other mammals. In the present work unique enzymes were identified from PA01 by comparative metabolic pathway analysis. We have identified nine enzymes are unique to the opportunistic pathogen PA01 and are no significant similarity with *H. sapiens and* will be considered for rational drug design. 437 distinct Potential drug targets were also identified from different metabolic pathways; the study was successful in listing out potential drug targets from the PA01proteome with bioinformatics tools.
Key words: Metabolic pathways; KEGG; Potential drug targets.

1. Introduction

Pseudomonas aeruginosa (*P.aeruginosa*) is the epitome of an opportunistic microorganism that can cause disease in animals, including human beings. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. The symptoms of such infections are generalized inflammation and sepsis. If such colonization's occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal [1]. Because it thrives on most surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics [2]. In case of Human pathology, the role of *P.aeruginosa* is one of the major concerns in intensive care septicemia. Presently the drug resistance strains of *P.aeruginosa* arising mainly by developing multiple mechanisms for its natural and acquired resistance to many of the antimicrobial agents commonly used in clinical practice.

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Outbreaks of multidrug-resistant *P. aeruginosa* colonization or infection have been reported on urology wards, a burn unit, hematology/oncology units, and adult and neonatal critical care units[3-11]. For the last ten years, a notable increase in antibiotic resistance among gram negative *P.aeruginosa* bacteria recovered from hospitalized patients has been reported, especially for critically ill patients[11]. Infections caused by multidrug resistant (MDR) *P. aeruginosa* (MDRPa) have been associated with increased morbidity, mortality and costs [11-13]. To restrict the outbreak of multidrug resistant strains, new drug targets identification is essential. Analysis of complete genomes allows us to compile a list of potential gene products and identify the functions present in the host and absent in the pathogen. This reduces the problem of searching for potential drug targets from a large list to select from a chosen few. As most currently known, antibacterial are essential inhibitors of certain bacterial enzymes, all enzymes specific to bacteria can be considered as potential drug targets[14]. In this study, a strategy of comparative metabolic pathway analysis has been adopted. The enzymes in the metabolic pathways of *P.aeruginosa*, which do not show similarity to any of the host protein (*H.sepians*), represent attractive potential drug targets. The elimination of pseudo drug targets is essential since the cost involved in the investigation of drug targets is prohibitive. Computational tools such as the KEGG, NCBI, and BLAST can be used more efficiently to identify potential targets. Here we find out some potential drug targets against *P.aeruginosa*, only those enzymes which show unique properties based on the results obtained and search in databases than the host. However, every potential target still needs to be experimentally validated but each step in the pathway is validated as an essential function for the survival of the bacterium. In this phase of target identification, *P.aeruginosa* is an especially good

experimental platform, using metabolic pathway information as the starting point for the identification of potential targets.

2. Materials and Method

2.1 Identification of potential drug targets

2.2 Utilization of KEGG for comparative metabolisms

Metabolic pathway information of *P.aeruginosa* was collected from KEGG [15] pathway database. Metabolic pathway identification numbers (E: C**)** of the host *Homo sapiens* and the pathogen *P.aeruginosa* were extracted from the KEGG database. Enzymes which do not appear in the host but present in the pathogen according to KEGG database annotation have been identified as enzymes unique to *P.aeruginosa*. Enzymes in unique pathways as well as enzymes involved in other metabolic pathways under carbohydrate metabolism, amino acid metabolism, lipid metabolism, energy metabolism, vitamin and cofactor biosynthesis and nucleotide metabolism were identified from the KEGG database. The corresponding protein sequences were retrieved from the KEGG database.

2.3 Utilization of BLAST for comparative metabolisms

In this paper I tried to find the unique drug targets in *Pseudomonas aeruginosa*. Here Expect value (E value) is a parameter describes the number of hits one can "expect" to see by chance when searching a database of a particular size. It decreases exponentially as the Score (S) of the match increases. Essentially, the E value describes the random background noise. The lower the E-value, or the closer it is to zero, the more "significant" vice versa the match is. However, keep in mind that virtually identical short alignments have relatively high E values. But some cases the data base search in unable to find e-value, showing there is no significant similarity to that protein. But in case of other essential drug targets E-value threshold is limited to 0.005. They were subjected to a BLASTp[16-17] (http://blast.ncbi.nlm.nih.gov/blast, http://blast.ncbi.nlm.nih .gov/ Blast.cgi? PROG RAM=blastp& PAGE_TYPE=BlastSearch&LINK_LOC=blasthome), search 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 allowed the user to select the organism to which the search should be restricted. In the current context, the objective is to find only those targets, which do not have detectable human significant similarity. Enzymes, which do not have hits below the e-value inclusion threshold of 0.005, were also picked out as potential drug targets. Nine enzymes out of 446 from different pathways were found to be essential and do not have similarity with Human.

3. Results and Discussion

Targeting of metabolic pathways has several advantages on its own. Each step in the metabolic pathway is already well validated which marks it as an essential function for bacterial growth. If target enzymes are discarded from the pathogen that shares a similarity with the host proteins, it ensures that the targets have nothing in common with the host proteins, thereby, eliminating undesired host protein–drug interactions.

3.1 Unique enzymes of *P.aeuriginosa* when compared to the *H.sapiens*

The step wise identification and analysis of the potential drug targets has been carryout with comparative metabolic pathway analysis and unique pathways. The nine enzymes addressed in this study do not show similarity to any of the host proteins (listed in Table 1). These enzymes involved in varies metabolic pathways including glyoxalate, dicarboxylate, inositol phosphate, propionate, starch, fructose, mannose, glycolosis, gluconeogenisis, aminosugar, nucleotide sugar (purine), sucrose, nitrogen, riboflavin, fatty acid, tryptophan, Phenylalanine, tyrosine and tryptophan Metabolisms. All the targets in these pathways found by this approach are also under various stages of progress at the Microbial Genome Database**.** Here, the approach is in confirmation with the experimental findings. Most of the targets may have homologues in other pathogenic bacteria.

3.2 Other essential targets

The other drug targets of the PA include lipid metabolism, carbohydrate metabolism, amino acid metabolism, energy metabolism, vitamin-cofactor biosynthetic pathways and nucleotide metabolisms. Several proteins from these pathways were found as potential drug targets which are showing high threshold e-value to the host proteins.

These were also observed and the same are listed in supplementary data table. While some of them are known to be associated with virulence or important for persistence or vital for *P.aeruginosa* metabolism, others should further be investigated for their potential to be drug targets.

Table.1. The enzymes of *P.aeruginosa* do not show significant similarity to any of the host proteins from Blast-P analysis.

S. No	Accec.No	Gene	Name of enzyme	Similarity with Human	Metabolic pathway involved
1	PA4810	fdnI	Nitrate-inducible formate dehydrogenase Gamma subunit	No significant Similarity	Glyoxalate and dicarboxylate metabolism
2	PA0130		Aldehyde dehydrogenase	No significant similarity	Inositol phosphate metabolism
3	PA0796	prpB	2- methylisocitrate lyase	No significant similarity	Proponate metabolism
4	PA5322	algC	Phosphomannomutase	No significant similarity	Starch fructose,mannose , glycolosis, gluconeogenisis amino sugar, nucleotide sugar (purine) and sucrose metabolism
5	PA0519	nirS	Pitrite reductase precursor	No significant similarity	Nitrogen metabolism
6	PA4053	ribE	6,7-dimethyl-8-ribityllumazine synthase	No significant similarity	Riboflavin metabolism
7	PA0651	trpC	Iindole-3-glycerol-phosphate synthase	No significant similarity	Phenylalanine, tyrosine and tryptophan biosynthesis
8	PA1525	alkB2	Alkane-1-monoxygenase-2	No Significant Similarity	Fatty acid metabolism
9	PA2579	kynA	L-tryptophan:oxygen	No significant	Tryptophan metabolism

To establish that it can indeed be a drug target, more insight into the activity and the essential nature of the target in the viability of the pathogen in the host should be gathered from literature where possible. Some of the targets may have been identified by earlier studies. We have included them in our list since our approach has been successful in identifying these targets. As shown in supplementary data table, there are 29 targets from lipid metabolism, 147 from carbohydrate metabolism, 14 targets from energy metabolism, 39 from nucleotide metabolism, 155 from amino acid metabolism, 27 from nitrogen metabolism and 26 from vitamin - cofactor biosynthetic pathways. Some of these targets are linked to more than one pathway. Amongst these, included virulence factors, lipases, esterases and enzymes from energy cycles, glyoxylate cycle and respiratory proteins are important for bacterial persistence.

It has been proposed by that the enzymes of the glyoxylate cycle are activated during adaptation to the low oxygen environment of the granuloma[19]. The glyoxylate bypass allows the bacterium to synthesize carbohydrates from fatty acids. Succinate and glyoxylate produced by this cycle are supplied to the TCA cycle and gluconeogenesis. Disrupting this pathway by targeting these enzymes has a potential in the treatment of latent infections. Among all the identified targets, PA4810 (nitrate-inducible formate dehydrogenase) has not yet been targeted. It has also been suggested that the *P.aeruginosa* undergoes a metabolic downshift in the hostile O_2 limiting environment of the granuloma and switches to anaerobic nitrate respiration [18-19]. This aids in the persistence of the *P.aeruginosa* under anaerobic conditions. nirS is a gene cluster that encodes for membrane bound nitrate reductase do not have human homologues. AnsA, napA, spuI, antA from nitrogen metabolism have very limited human homologues, which are involved in anaerobic phosphorylative electron transport chain. Since the bacterium has to survive under oxygen limiting conditions, these represent attractive targets. Amongst the new targets from the energy metabolism important ones are PA2691, PA4031, and PA5560. Other important targets from amino acid metabolism, algC, purC, pnP, and relR from nucleotide metabolism, ribE-6, 7-dimethyl-8-ribityllumazinesynthase and bio D-dithiobiotin synthetase from vitamin - cofactor biosynthetic pathways. Most of the antimicrobials target, steps in the later stage of cell wall biosynthesis. The early steps in cell wall biosynthesis have not been targeted. To date, only fosfomycin, which targets MurA (NAG enolpyruvate transferase), has been developed as an antibacterial agent [20]. Along with these, the other unique metabolic pathways peptidoglycan biosynthesis and D-alanine metabolism is common to all bacterial species, and cell wall biosynthetic pathways have long been targeted for antimicrobial discovery. The iron acquisition systems of many pathogenic and saprophytic bacteria relay on the production of small molecules called siderophores. Therefore, here the metabolic pathways are investigated. The enzymes involved in the peptidoglycan biosynthetic pathway, MurA, MurB, MurC, MurD, MurE, MurF, MurX, MurG and ddlA do not have human homologues. The details are presented in Table 2. Targets from the peptidoglycan biosynthesis pathway are under investigation, but the role of peptidoglycon in *p.aeruginosa* is limited because it is gram negative organism.

Table.2: Targets from unique pathways and Homology identity with Humans in BLAST

Accession No	Gene	Description	Uniport id	Identity With Human(%)	e-Value
Peptidoglycon biosynthesis: KEGG pathway ID pae00550					
PA2977	MurB	UDP-N-acetylenolpyruvoylglucosamine reductase	**Q9HZM7**/MURB_PSEAE	29	0.71
PA4411	MurC	UDP-N-acetylmuramate--L-alanine ligase	**Q9HW02**/MURC_PSEAE	25	0.28
PA4414	MurD	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	**Q9HVZ9**/MURD_PSEAE	33	5.2
PA4417	MurE	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase	**Q59650**/MURE_PSEAE	47	4.2
PA4201	ddlA	D-alanine-D-alanine ligase A	**Q9HWI0**/DDLA_PSEAE	26	0.34
PA4415	mraY	phospho-N-acetylmuramoyl-pentapeptide-Transferase	**Q9HVZ8**/MRAY_PSEAE	33	0.24
PA4412	MurG	Undecaprenyldiphospho-muramoyl pentapeptide beta-N-acetylglucosaminyltransferase	**Q9HW01**/MURG_PSEAE	28	3.1
D-alanine metabolism: KEGG pathway ID Pae00473					
PA4930	alr	Alanine racemase	**Q9HUN4** /ALR1_PSEAE	32	0.46
PA4201	ddlA	D-alanine-D-alanine ligase	**Q9HWI0**/DDLA_PSEAE	26	0.34
Thiamine metabolism: KEGG pathway ID pae00730					
PA3976	thiE	Thiamine-phosphate pyrophosphorylase	**Q9HX40** (THIE_PSEAE)	53	6.33
PA3975	thi D	Phosphomethylpyrimidine kinase	**Q9HX41** (Q9HX41_PSEAE)	33	1.5
Streptomycin biosynthesis: KEGG pathway ID pae00521					
PA3193	glk	Glucokinase	**Q9HZ46** (GLK_PSEAE)	40	2e-05
PA5322	algc	Phosphomannomutase AlgC	**P26276** (ALGC_PSEAE)		
PA4069		Hypothetical protein	**Q9HWV9**/Q9HWV9_PSEAE	33	2.0
Pa3818		Extragenic suppressor protein SuhB	**Q9HXI4** (SUHB_PSEAE)	37	7e-39

4. Conclusion

In contempt of availability of effective broad spectrum antibiotics, Pseudomonas remains a leading infectious killer in the world. Many factors, such as pneumonia, co-infection, drug resistance, lack of patient compliance with chemotherapy, variable efficacy of drugs, and various other factors contribute to the rate of mortality due to septicemia. So there is a need to develop new anti-bacterial drugs. The search for new antibacterial agents directed towards novel targets has become highly imperative. We performed an in-depth analysis of *P. aeruginosa* drug targets implemented in BLAST and KEGG and identified that they tend to have high deviations when compared with human being.

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