Journal of Computer Science & Systems Biology - Open Access

Research Article
OPEN ACCESS Freely available online
doi:10.4172/jcsb.1000058
JCSB/Vol.3 Issue 3

In silico Analysis Metabolic Pathways for Identification of Putative Drug Targets for *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is one of the most important and studied gram positive bacterial strains, which have a great potential to infect human beings as well as other mammals. The hospital-acquired methicillin-resistant, vancomycin-susceptible gram–positive bacteria strain is responsible for much life threatening diseases like Toxic-shock syndrome, staphylococcal scarlet fever, meningitis, osteomyelitis, etc. This antibiotic resistance strain, lead to development of the new antibiotics or drug molecules which can kill or suppress the growth of *Staphylococcus aureus*. We have performed an insilico comparative analysis of metabolic pathways of the host *Homo sapiens* and the pathogen *S. aureus*. The e-value threshold cut-off was set to 0.005. We have identified total 235 enzyme sequences, which are non homologous to *Homo sapiens* protein sequences and among them 59 enzymes are found to be essential for survival of the *S. aureus* according to the DEG database. Further PA-SUB analysis Results showed that about 52.5% enzymes are found to be in the cytoplasm, 13.5% enzymes are found to be in extracellular, 6.7% enzymes are plasma membrane protein and 27.1% enzymes are given no positive prediction. In this comparative analysis, we have also found 5 unique pathways among 59 essential and 23 non homologous enzymes.

Keywords: *Staphylococcus aureus*; Drug targeting; Pathway analysis; DEG; PA-SUB

Introduction

Staphylococcus aureus a member of *Staphylococcacea* is considered as an opportunistic pathogen for the different mammals including Livestock as well as humans (Lowy, 1998; Projan and Novick, 1997). It has been reported that *Staphylococcus aureus* is resistance against varies antibiotics present in the market (Lowy, 1998; Walsh and Bowe, 2002). This bacterial strain world widely known for causing many of the severe and deadly diseases like osteomyelitis, bacteremia, endocarditis, meningitis, Scalded Skin Syndrome, Toxic Shock Syndrome, food poisoning, etc. (Lowy, 1998; Diekema et al., 2001). It is the primary cause of lower respiratory tract infections and surgical site infections (Richards et al., 1999) and the second leading cause of nosocomial bacteremia (Wisplinghoff et al., 2004), pneumonia, and cardiovascular infections (Richards et al., 1999). Besides these diseases, it also found on the skin of the human beings and causing major problems like pimple, sour throat, hair follicle infection, acne, and sties (a sty is an inflammation of a gland in the eyelid). It also causes boils, which are deeper pus-filled abscesses of the skin and underlying tissue (Freeman-cook and Freeman-cook, 2006; Carleton et al., 2004; King et al., 2006). In common with other facultative aerobes, *S. aureus* can grow in the absence of oxygen either by fermentation or by using an alternative terminal electron acceptor, such as nitrate. Various studies suggest that oxygen plays a role in the pathogenesis of *S. aureus*, in both its capacity to produce virulence factors and its ability to persist and grow in different and often hostile environmental niches (Chan and Foster, 1998; Clements and Foster, 1999; Kass et al., 1987; Ohlsen et al., 1997; Ross and Onderdonk, 2000; Yarwood and Schlievert, 2000). The bacteria contain or can produce a variety virulence factor like adhesion, colonization, exoenzyme and exotoxins, capsule, etc. These virulence factors help the bacteria to attach to the host cells, it can bind to proteins such as fibronectin, laminin, vitronectin, and collagen, which form the extracellular matrix of epithelial and endothelial surfaces (Gillaspy et al., 1998; Freeman-cook and Freeman-cook, 2006). The resistance to antibiotics emerged and spread rapidly among strains of *S. aureus*. About 90% of *S. aureus* strains are currently resistant to penicillin and derivatives. To combat this problem, new derivatives of penicillin were introduced (Lowy, 2003; Freeman-cook and Freeman-cook, 2006). Today, around 50% of all *S. aureus* infections are multi-drug resistant (resistant to penicillin, methicillin, tetracycline, and erythromycin). One antibiotic stood for years as a drug that did not cause resistant bacteria to emerge. It often thought of as a drug of "last resort," the name implies exactly how it has been used. Thus, the battle between humans and bacteria continues (Freeman-cook and Freeman-cook, 2006). The computational approach has been used to investigate novel drug targets in other pathogenic organisms such as Pseudomonas aeruginosa (Sakharkar et al., 2004; Perumal et al., 2007) and in *Helicobacter pylori* (Dutta et al., 2006). As most currently known, antibacterial are essentially inhibitors of certain bacterial enzymes, all enzymes specific to bacteria can be considered as potential drug targets (Michael and Eugene, 1999). In this study, we have adopted a strategy for comparative metabolic pathway analysis to find out some potential targets against *S. aureus*. Only those enzymes which show unique properties than the host were selected as the target.

Materials and Method

Identification of potential drug targets

KEGG (Kanehisa et al., 2002) pathway database was used as a source

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Received May 31, 2010; **Accepted** June 10, 2010; **Published** June 10, 2010

Citation: Morya VK, Dewaker V, Mecarty SD, Singh R (2010) *In silico* Analysis Metabolic Pathways for Identification of Putative Drug Targets for *Staphylococcus aureus*. J Comput Sci Syst Biol 3: 062-069. doi:10.4172/jcsb.1000058

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Sl. No.	Target Enzyme/s	Pathways in which the target is/are involved	length
1	PTS system, glucose-specific enzyme II, A component (EC:2.7.1.69) GI: 15927003	sau00010 Glycolysis / Gluconeogenesis sau00500 Starch and sucrose metabolism sau00520 Amino sugar and nucleotide sugar metabolism sau02060 Phosphotransferase system (PTS)	166 aa
2	fructose specific permease (EC:2.7.1.69) fruA GI: 15926377	sau00051 Fructose and mannose metabolism sau01100 Metabolic pathways sau02060 Phosphotransferase system (PTS)	652 aa
3	mannitol-1-phosphate 5-dehydrogenase (EC:1.1.1.17) GI: 15927740	sau00051 Fructose and mannose metabolism	368 aa
4	galactose-6-phosphate isomerase subunit LacA (EC:5.3.1.26) GI: 15927775	sau00052 Galactose metabolism	142 aa
5	tagatose-6-phosphate kinase (EC:2.7.1.144) lacC GI: 15927773	sau00052 Galactose metabolism	310 aa
6	Phosphotransacetylase (EC:2.3.1.8) eutD GI: 15926266	sau00430 Taurine and hypotaurine metabolism sau00620 Pyruvate metabolism sau00640 Propanoate metabolism	328 aa
7	F0F1 ATP synthase subunit B (EC:3.6.3.14) atpF GI: 15927681	sau00190 Oxidative phosphorylation sau01100 Metabolic pathways	173 aa
8	respiratory nitrate reductase alpha chain (EC:1.7.99.4) narG GI: 15927975	sau00910 Nitrogen metabolism sau02020 Two-component system	1229 aa
9	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC:2.5.1.7) murA GI: 15927674	sau00520 Amino sugar and nucleotide sugar metabolism sau00550 Peptidoglycan biosynthesis sau01100 Metabolic pathways	421 aa
10	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC:2.5.1.7) murZ GI: 15927698	sau00520 Amino sugar and nucleotide sugar metabolism sau00550 Peptidoglycan biosynthesis sau01100 Metabolic pathways	419 aa
11	UDP-N-acetylmuramate--L-alanine ligase (EC:6.3.2.8) murC GI: 15927317	sau00471 D-Glutamine and D-glutamate metabolism sau00550 Peptidoglycan biosynthesis sau01100 Metabolic pathways	437 aa
12	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase (EC:6.3.2.9) murD GI: 15926766	sau00471 D-Glutamine and D-glutamate metabolism sau00550 Peptidoglycan biosynthesis sau01100 Metabolic pathways	449 aa
13	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D-alanyl ligase; (EC:6.3.2.10) murF GI: 15927657	sau00300 Lysine biosynthesis sau00550 Peptidoglycan biosynthesis sau01100 Metabolic pathways	452 aa
14	FmhB protein (EC:2.3.2.-) fmhB GI: 15927842	sau00550 Peptidoglycan biosynthesis sau01100 Metabolic pathways	421 aa
15	factor essential for expression of methicillin resistancefemA; (EC:2.3.2.-) GI: 15926954	sau00550 Peptidoglycan biosynthesis sau01100 Metabolic pathways	420 aa
16	FemB protein (EC:2.3.2.-) femB GI: 15926955	sau00550 Peptidoglycan biosynthesis sau01100 Metabolic pathways	419 aa
17	D-alanyl-alanine synthetase A (EC:6.3.2.4) ddl PDB: 2I87 2I80 2I8C GI: 15927658	sau00473 D-Alanine metabolism sau00550 Peptidoglycan biosynthesis	356 aa
18	phosphoenolpyruvate-protein phosphatase (EC:2.7.3.9) ptsI GI: 15926670	sau02060 Phosphotransferase system (PTS)	572 aa
19	PTS system, N-acetylglucosamine-specific IIABC component (EC:2.7.1.69) ptaA GI: 15927303	sau00520 Amino sugar and nucleotide sugar metabolism sau02060 Phosphotransferase system (PTS)	488 aa
20	PTS system, glucose-specific IIABC component ptsG GI: 15928117	sau00520 Amino sugar and nucleotide sugar metabolism sau02060 Phosphotransferase system (PTS)	688 aa
21	PTS system, mannitol specific IIA component mtlA GI: 15927739	sau00051 Fructose and mannose metabolism sau02060 Phosphotransferase system (PTS)	144 aa
22	preprotein translocase subunit SecY GI: 15927810	sau03060 Protein export sau03070 Bacterial secretion system	430 aa
23	preprotein translocase subunit SecA azi, div GI: 15926430	sau03060 Protein export sau03070 Bacterial secretion system	843 aa
24	preprotein translocase subunit SecA azi, div GI: 15928235	sau03060 Protein export sau03070 Bacterial secretion system	796 aa
25	glutamate racemase (EC:5.1.1.3) murI GI: 15926734	sau00471 D-Glutamine and D-glutamate metabolism sau01100 Metabolic pathways	266 aa
26	DNA-directed RNA polymerase subunit alpha (EC:2.7.7.6) rpoA GI: 15927804	sau00230 Purine metabolism sau00240 Pyrimidine metabolism sau01100 Metabolic pathways sau03020 RNA polymerase	314 aa
27	DNA polymerase III PolC (EC:2.7.7.7) polC GI: 15926847	sau00230 Purine metabolism sau00240 Pyrimidine metabolism sau01100 Metabolic pathways sau03030 DNA replication sau03430 Mismatch repair sau03440 Homologous recombination	1438 aa
28	tryptophan synthase subunit beta (EC:4.2.1.20) trpB GI: 15926952	sau00260 Glycine, serine and threonine metabolism sau00400 Phenylalanine, tyrosine and tryptophan biosynthesis sau01100 Metabolic pathways	404 aa

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doi:10.4172/jcsb.1000058
JCSB/Vol.3 Issue 3

29	urease subunit gamma (EC:3.5.1.5) ureA GI: 15927868	sau00230 Purine metabolism sau00330 Arginine and proline metabolism sau01100 Metabolic pathways	100 aa
30	urease subunit beta (EC:3.5.1.5) ureB GI: 15927869	sau00230 Purine metabolism sau00330 Arginine and proline metabolism sau01100 Metabolic pathways	136 aa
31	DNA polymerase III, alpha chain (EC:2.7.7.7) dnaE GI: 15927280	sau00230 Purine metabolism sau00240 Pyrimidine metabolism sau01100 Metabolic pathways sau03030 DNA replication sau03430 Mismatch repair sau03440 Homologous recombination	1065 aa
32	replicative DNA helicase (EC:3.6.1.-) dnaC GI: 15925721	sau03030 DNA replication	466 aa
33	DNA primase; (EC:2.7.7.-) dnaG GI: 15927142	sau03030 DNA replication	605 aa
34	excinuclease ABC subunit C uvrC GI: 15926730	sau03420 Nucleotide excision repair	593 aa
35	recombination protein F recF GI: 15925709	sau03440 Homologous recombination	370 aa
36	PriA, primosomal protein (EC:3.6.1.-) priA GI: 15926795	sau03440 Homologous recombination	802 aa
37	potassium-transporting ATPase subunit A (EC:3.6.3.12) kdpA, SCCmec GI: 29165619	sau02020 Two-component system	558 aa
38	chromosomal replication initiation protein dnaA GI: 15925706	sau02020 Two-component system	453 aa
39	two-component sensor histidine kinase vraS GI: 15927459	sau02020 Two-component system	374 aa
40	30S ribosomal protein S17; rpsQ GI: 15927821	sau03010 Ribosome	87 aa
41	50S ribosomal protein L18 rplR GI: 15927814	sau03010 Ribosome	119 aa
42	50S ribosomal protein L30 rpmD GI: 15927812	sau03010 Ribosome	59 aa
43	30S ribosomal protein S4 rpsD GI: 15927296	sau03010 Ribosome	200 aa
44	50S ribosomal protein L10 rplJ GI: 15926217	sau03010 Ribosome	166 aa
45	50S ribosomal protein L35 rpmI GI: 15927258	sau03010 Ribosome	66 aa
46	50S ribosomal protein L31 type B rpmE2 GI: 15927694	sau03010 Ribosome	84 aa
47	50S ribosomal protein L9 rplI GI: 15925720	sau03010 Ribosome	148 aa
48	30S ribosomal protein S6 rpsF GI: 15926066	sau03010 Ribosome	98 aa
49	50S ribosomal protein L28 rpmB GI: 15926807	sau03010 Ribosome	62 aa
50	50S ribosomal protein L21 rplU GI: 15927227	sau03010 Ribosome	102 aa
51	30S ribosomal protein S20 rpsT GI: 15927166	sau03010 Ribosome	83 aa
52	Phosphopentomutase (EC:5.4.2.7) drm GI: 15925843	sau00030 Pentose phosphate pathway sau00230 Purine metabolism	392 aa
53	acetate kinase (EC:2.7.2.1) ackA GI: 15927288	sau00430 Taurine and hypotaurine metabolism sau00620 Pyruvate metabolism sau00640 Propanoate metabolism sau01100 Metabolic pathways	400 aa
54	Undecaprenyl pyrophosphate phosphatase (EC:3.6.1.27) uppP GI: 15926360	sau00550 Peptidoglycan biosynthesis	291 aa
55	PTS system, arbutin-like IIBC component glvC GI: 15927903	sau00010 Glycolysis / Gluconeogenesis sau02060 Phosphotransferase system (PTS)	534 aa
56	preprotein translocase subunit SecY GI: 15928239	sau03060 Protein export sau03070 Bacterial secretion system	403 aa
57	potassium-transporting ATPase subunit A (EC:3.6.3.12) kdpA GI: 15927652	sau02020 Two-component system	558 aa
58	50S ribosomal protein L29 rpmC GI: 15927822	sau03010 Ribosome	69 aa
59	50S ribosomal protein L33 rpmG GI: 15927131	sau03010 Ribosome	49 aa

Table 1: Essential enzymes for survival of *S. aureus,* (Identified from DEG analysis)

of metabolic pathway information. Metabolic pathway identification numbers of the host *H. sapiens* and the pathogen *Staphylococcus aureus* was extracted from the KEGG database. Pathways which do not appear in the host but present in the pathogen according to KEGG database annotation have been identified as pathways unique to *Staphylococcus aureus* as in comparison to the host *H. sapiens*. Enzymes in these 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. They were subjected to a BLASTp (Altschul et al., 1997) 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 allows 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 homologues. 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

The targets from the unique pathways were also subjected to a cluster of orthologous groups (COGS) search to identify homologues in other pathogens. After pBLAST analysis the 235 nonhomologous enzymes were further analysed for essentiality to pathogen by DEG database (http://tubic.tju.edu.cn/deg/). Essential genes are those indispensable for the survival of an organism, and their functions are therefore, considered a foundation of life (Zhang et al., 2004). 59 enzymes out of 235 from different pathways were found to be essential. These imperative 59 enzymes were submitted in Drug Bank database against approve drug targets and small molecules legends.

Finding of biological significance of the targets

For biological significance and distribution of these essential targets were analysed by PA-SUB (Proteome Analyst Specialized Sub Cellular Localization) Server v2.5 (Lu et al., 2004). This is required to find out the surface membrane proteins which could be probable vaccine targets.

Results and Discussion

From KEGG server all pathways associated with *S. aureus* have

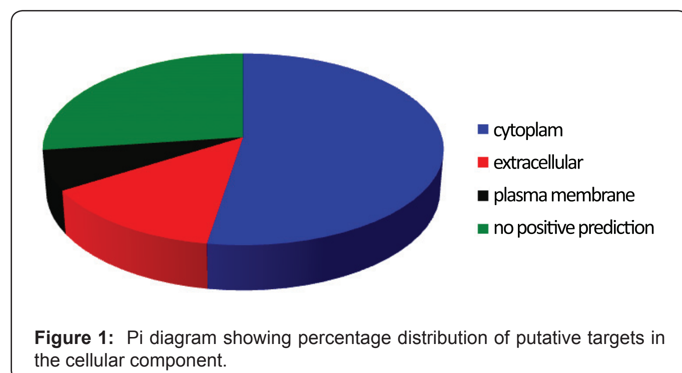

Figure 1: Pi diagram showing percentage distribution of putative targets in the cellular component.

been extensively analyzed and each of enzymes of pathways was compared with proteins from the host *Homo sapiens*, by performing a BLASTp search against the non-redundant database restricted to the *H. sapiens* subset. The e-value threshold cutoff was set to 0.005 to remove homologous sequence. Total 235 enzymes were identified as non-homologous to Human protein sequences. Design and targeting inhibitors against these non-homologous sequences could be the better approach for generation of new drugs. As it has reported in various texts that there are two types of enzymes or pathways are found in any living system i.e. dispensable and indispensable. Any dispensable or non-essential pathway or enzyme can't be a good choice as a drug target. Thus these 235 enzymes were further analysed with DEG server and considered cutoff score was >100 to enhance the specificity of enzyme in *S. aureus*. Total 59 enzymes out of all were found to be essential for *S. aureus* life cycle (Table 1). These targets were found to be potential targets and could be considered for rational drug design. Using metabolic pathway information as the starting point for the identification of potential targets has its advantages as each step in the pathway is validated as the essential function for the survival of the bacterium. The sub cellular localization analysis of all supposed essential and unique enzymes of *Staphylococcus aureus* were evaluated by PA-SUB server. As it was suggested that membrane associated protein could be the better target for developing vaccines. After analysis 31 proteins were found to be located in the cytoplasm, 8 as extra cellular, 4 on plasma membrane while 16 without any positive prediction (Figure 1).

Metabolic pathway analysis of the essential proteins in *Staphylococcus aureus* done by KEGG Automatic Annotation Server (KAAS). A result of comparative analysis of the metabolic pathways of the host and pathogen by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database reveals some pathways that are unique to *Staphylococcus aureus* and are not present in the humans like D-Alanine metabolism, Peptidoglycan biosynthesis, Phosphotransferase system (PTS), Bacterial secretion system and Two-component system (Table 4). In addition to these pathways other pathways like streptomycin biosynthesis, novobiocin biosynthesis, C5-Branched dibasic acid metabolism and many other pathways are not present in humans, and some enzymes we found non homologous to human protein sequence, but they are not essential for survival of the *S. aureus*. Non homologous sequences from these pathways and other pathways are present in the 235 enzyme table as well as in the essential enzyme table. We again examined these unique pathways' enzymes' gene in the COGs (http://www.ncbi.nlm.nih.gov/COG/) to identify homologues in other pathogens. This COG id also can be shown from KEGG database (Table 4). Note that we are not considering the hypothetical or putative protein sequences (Table 2 and Table 3). This small group of proteins is required to be further verified for their role in *Staphylococcus aureus* survival and virulence by mutagenesis study. Further we analyze our essential enzymes of DEG database result against the drug bank database, and we identified about 8 approved drug target and 24 small molecule drug target (Table 2 and Table 3). The pathways and their enzyme and contribution were discussed bellow.

Unique pathways : peptidoglycan biosynthesis

All the bacterial species almost share a common feature of the cell wall which is helping them to maintain their structure as well as helping the bacterium to withstand tremendous internal pressure (up to 350 lbs/cm^2) to keep the bacterial cell from exploding. The cell wall is composed of peptidoglycan, teichoic acids, and proteins (Schleifer, 1983). Chemical analysis of the cell wall indicates that more than 70% of the weight of the cell wall is peptidoglycan and that the teichoic acid is covalently bound to the peptidoglycan through a phosphodiester bond (Coley et al., 1978). Peptidoglycan is made of glycan chains of alternating N-acetylglucosamine and N-acetylmuramic acid cross-linked by short stem peptides attached to the N-acetylmuramic acid (Ghuysen, 1968, Schleifer and Kandler, 1972). However, the cells also must grow. For this to occur, the cell wall must enlarge. Various enzymes are required for this process. Enzymes called autolysis break the cross-links in the webbing of the cell wall, and enzymes called transpeptidases enlarge the cell wall and reseal it. Cell wall biosynthesis can be separated into two phases—the six intracellular enzymatic steps and the three steps that occur outside of the plasma membrane. Amongst the cytoplasmics steps involved in the biosynthesis of peptidoglycan, four ADP forming ligases (MurC, MurD, MurE and MurF) catalyze the assembly of its peptide moiety by successive additions of l-alanine, d-glutamate, a

s.no.	Enzyme	Accession no	Drug bank target	score
1	mannitol-1-phosphate 5-dehydrogenase	gi|15927740|	4476 Mannitol dehydrogenase (DB00742)	65
2	respiratory nitrate reductase alpha chain	gi|15927975|	4344 Dimethyl sulfoxide/trimethylamine N-oxide reductase (DB01093)	53
3	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	gi|15927674|	520 UDP-N-acetylglucosamine 1-carboxyvinyltransferase (DB00828)	362
4	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	gi|15927698|	520 UDP-N-acetylglucosamine 1-carboxyvinyltransferase (DB00828)	278
5	D-alanyl-alanine synthetase A	gi|15927658|	D-alanine--D-alanine ligase A (DB00260)	298
6	DNA-directed RNA polymerase subunit alpha	gi|15927804|	DNA-directed RNA polymerase alpha chain (DB00615)	242
7	30S ribosomal protein S4	gi|15927296|	30S ribosomal protein S4 (DB00254; DB00256; DB00453; DB00560; DB00595; DB00618; DB00759; DB01017)	171
8	50S ribosomal protein L10	gi|15926217|	50S ribosomal protein L10 (DB00254; DB00314; DB00446; DB00778; DB00931; DB01190; DB01211; DB01321; DB01627)	102

Table 2: Approved drug target identified by the drug bank server.

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OPEN ACCESS Freely available online
doi:10.4172/jcsb.1000058
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Sl. No.	Enzyme name	Accession no	Drug bank target	score
1	fructose specific permease	gi|15926377|	Nitrogen regulatory protein (DB03131)	64
2	mannitol-1-phosphate5-dehydrogenase	gi|15927740|	Mannitol dehydrogenase (DB00742; DB01907)	65
3	galactose-6-phosphate isomerase subunit LacA	gi|15927775|	Sugar-phosphate isomerase (DB03661; DB03684)	77
			Ribose-5-phosphate isomerase B (DB03108; DB04496)	61
4	phosphotransacetylase	gi|15926266|	Phosphate acetyltransferase (DB02897)	417
5	F0F1 ATP synthase subunit B	gi|15927681|	ATP synthase B chain (DB03091)	59
6	respiratory nitrate reductase alpha chain	gi|15927975|	Dimethyl sulfoxide reductase (DB02153; DB02379)	59
			Dimethyl sulfoxide/trimethylamine N-oxide reductase (DB01093)	53
7	UDP-N-acetylglucosamine1-carboxyvinyltransferase	gi|15927674|	UDP-N-acetylglucosamine1-carboxyvinyltransferase (DB01867; DB01879; DB02435; DB02995; DB03089; DB04174; DB04474)	369
			2306 UDP-N-acetylglucosamine1-carboxyvinyltransferase (DB03397)	362
			UDP-N-acetylglucosamine1-carboxyvinyltransferase (DB00828)	362
8	UDP-N-acetylglucosamine1-carboxyvinyltransferase	gi|15927698|	UDP-N-acetylglucosamine1-carboxyvinyltransferase (DB01867; DB01879; DB02435; DB02995; DB03089; DB04174; DB04474)	280
			UDP-N-acetylglucosamine1-carboxyvinyltransferase (DB03397)	278
			UDP-N-acetylglucosamine1-carboxyvinyltransferase (DB00828)	278
9	UDP-N-acetylmuramate--L-alanine ligase	gi|15927317|	UDP-N-acetylmuramate--L-alanine ligase(DB01673; DB03909; DB04395)	152
10	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	gi|15926766|	UDP-N-acetylmuramoylalanine--D-glutamate ligase (DB01673; DB02314; DB03431; DB03801)	179
11	UDP-N-acetylmuramoylalanyl-D-glutamyl-2, 6-diaminopimelate-D-alanyl-D-alanyl ligase	gi|15927657|	UDP-N-acetylmuramoylalanine--D-glutamate ligase (DB01673; DB02314; DB03431; DB03801)	50
12	D-alanyl-alanine synthetase A	gi|15927658|	D-alanine--D-alanine ligase A (DB00260)	298
			D-alanine--D-alanine ligase (DB03431	210
			D-alanine--D-alanine ligase B (DB03431)	187
13	phosphoenolpyruvate-protein phosphatase	gi|15926670|	Pyruvate, phosphate dikinase (DB02522)	115
			Pyruvate, phosphate dikinase, chloroplast(DB01819)	112
14	preprotein translocase subunit SecA	gi|15926430|	Preprotein translocase subunit secA (DB03431)	1036
			Preprotein translocase secA 1 subunit(DB03431)	723
15	preprotein translocase subunit SecA	gi|15928235|	Preprotein translocase subunit secA (DB03431)	512
			Preprotein translocase secA 1 subunit(DB03431)	440
16	glutamate racemase	gi|15926734|	Glutamate racemase (DB02174)	164
17	DNA-directed RNA polymerase subunit alpha	gi|15927804|	DNA-directed RNA polymerase alpha chain(DB00615)	242
18	DNA polymerase III PolC	gi|15926847|	DNA polymerase III subunit epsilon (DB01643; DB01867)	69
19	DNA primase	gi|15927142|	DNA primase (DB03166)	244
20	chromosomal replication initiation protein	gi|15925706|	Chromosomal replication initiator protein dnaA(DB03431)	213
21	30S ribosomal protein S4	gi|15927296|	30S ribosomal protein S4 (DB00254; DB00256; DB00453; DB00560; DB00595; DB00618; DB00759; DB01017)	171
22	50S ribosomal protein L10	gi|15926217|	50S ribosomal protein L10 (DB00254; DB00314; DB00446; DB00778; DB00931; DB01190; DB01211; DB01321; DB01627)	102
23	30S ribosomal protein S6	gi|15926066|	30S ribosomal protein S6 (DB01867)	53
24	acetate kinase	gi|15927288|	Acetate kinase (DB02423; DB03166; DB03431)	374

Table 3: Small molecule drug target evaluated by the drug bank server.

diamino acid (usually diaminopimelate or l-lysine) and d-alanine–d-alanine to UDP N-acetylmuramic acid (Rogers et al., 1980; Van Heijenoort, 1994) among the enzyme involved in the peptidoglycan biosynthesis we found the total 9 enzyme (KAAS RESULT) in which murA, muC, murD, murF which are non homologous to *Homo sapiens* and homologous to other prokaryotes are essential for survival of *S. aureus* and ddl, uppP, femX, femA, femB so considering these gene/gene product protein for inhibitor screening may also result from a good drug which can help in curing the disease caused by this microorganism.

Phosphotransferase system (PTS)

The phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) is a major mechanism used by bacteria for uptake of carbohydrates, particularly hexoses, hexitol, and disaccharides, where the source of energy is from PEP. The PTS consists of two general components, enzyme, I (EI) and histidine phosphocarrier protein (HPr), and of membrane-bound sugar specific permeases (enzymes II). Each enzyme II (EII) complex consists of one or two hydrophobic integral membrane domains (domains C and D) and two hydrophilic domains (domains A and B). EII complexes may exist as distinct proteins or as a single multidomain protein. The PTS catalyzes the uptake of carbohydrates and their conversion into their respective phosphoesters during transport. There are four successive phosphoryl transfers in the PTS. Initial autophosphorylation of EI, using PEP as a substrate, is followed by transfer of the phosphoryl group from EI to HPr. EIIA catalyzes the self-phosphoryl transfer from HPr after which the phosphoryl group is transferred to histidine or cysteine residues of EIIB. The sugar is transported through the membrane-bound EIIC and is phosphorylated by the appropriate sugar-specific EIIB.The phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) is a major mechanism used by bacteria for uptake of carbohydrates, particularly hexoses, hexitols, and disaccharides, where the source of energy is from PEP. The PTS

Accession no.	Gene	Description	GI NUMBER	COG ID
sau00473 D-Alanine metabolism				
SA1887	ddl	D-alanyl-alanine synthetase A	GI: 15927658	COG1181
sau00550 Peptidoglycan biosynthesis				
SA1902	murA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	GI: 15927674	**COG0766**
SA1561	murC	UDP-N-acetylmuramate--L-alanine ligase	GI: 15927317	**COG0773**
SA1026	murD	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	GI: 15926766	**COG0771**
SA1886	murF	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D-alanyl ligase	GI: 15927657	**COG0770**
SA1887	ddl	D-alanyl-alanine synthetase A	GI: 15927658	COG1181
SA0638	uppP	undecaprenyl pyrophosphate phosphatase	GI: 15926360	COG: COG1968
SA2057	fmhB	FmhB protein	GI: 15927842	COG: COG2348
SA1206	femA	factor essential for expression of methicillin resistance	GI: 15926954	COG2348
SA1207	femB	FemB protein	GI: 15926955	COG: COG2348
sau02060 Phosphotransferase system (PTS)				
SA0935	ptsI	phosphoenolpyruvate-protein phosphatase	GI: 15926670	**COG1080**
SA1255	Crr	PTS system, glucose-specific enzyme II, A component	GI: 15927003	COG: COG2190
SA0655	fruA	fructose specific permease	GI: 15926377	COG1445
SA1547	ptaA	PTS system, N-acetylglucosamine-specific IIABC component	GI: 15927303	COG: COG2190
SA2326	ptsG	PTS system, glucose-specific IIABC component	GI: 15928117	COG: COG2190
SA1962	mtlA	PTS system, mannitol specific IIA component	GI: 15927739	COG: COG1762
SA2114	glvC	PTS system, arbutin-like IIBC component	GI: 15927903	COG: COG1263
sau03070 Bacterial secretion system				
SA2028	SecY	preprotein translocase subunit	GI: 15927810	**COG0201**
SA2446	SecY	preprotein translocase subunit	GI: 15928239	**COG0201**
SA0708	secA , azi, div	preprotein translocase subunit SecA	GI: 15926430	COG: COG0653
SA2442	azi, div	preprotein translocase subunit SecA azi, div	GI: 15928235	COG: COG0653
sau02020 Two-component system				
SA2185	narG	respiratory nitrate reductase alpha chain	GI: 15927975	**COG0243**
SA0068	kdpA, SCCmec	potassium-transporting ATPase subunit A	GI: 29165619	**COG2060**
SA0001	dnaA	chromosomal replication initiation protein	GI: 15925706	**COG0593**
SA1701	vraS	two-component sensor histidine kinase	GI: 15927459	COG: COG4585

Table 4: Information about Non-homologous enzymes from S. aureus searched against Homo sapience.

consists of two general components, enzyme, I (EI) and histidine phosphocarrier protein (HPr), and of membrane-bound sugar specific permeases (enzymes II). Each enzyme II (EII) complex consists of one or two hydrophobic integral membrane domains (domains C and D) and two hydrophilic domains (domains A and B). EII complexes may exist as distinct proteins or as a single multidomain protein. The PTS catalyzes the uptake of carbohydrates and their conversion into their respective phosphoesters during transport. There are four successive phosphoryl transfers in the PTS. Initial autophosphorylation of EI, using PEP as a substrate, is followed by transfer of the phosphoryl group from EI to HPr. EIIA catalyzes the self-phosphoryl transfer from HPr after which the phosphoryl group is transferred to histidine or cysteine residues of EIIB. The sugar is transported through the membrane-bound EIIC and is phosphorylated by the appropriate sugar-specific EIIB. KAAS results showed that 7 enzymes were involving, which can be used as a potential drug target namely PTS system, glucose-specific enzyme II, A component, fructose specific permease, phosphoenolpyruvate-protein phosphatase, PTS system, N-acetylglucosamine-specific IIABC component, PTS system, glucose-specific IIABC component. PTS system, mannitol specific IIA component mtlA. PTS system, arbutin-like IIBC component glvC.

D-Alanine metabolism

We find ddl D-alanyl-alanine synthetase. An enzyme and its pdb file is also available and is one of the essential enzymes, which is involved in both D-alanine metabolism as well as in peptidoglycan synthesis, d-Alanine is a necessary precursor in the bacterial peptidoglycan biosynthetic pathway. Thus targeting this enzyme will act on both the pathway.

Bacterial secretion system

Genomic analysis has revealed many novel potential targets for antimicrobial drugs and many of them are in essential and conserved metabolic pathways or cell-cell communication systems (Marraffini et al., 2006, Brown and Wright, 2005; Bassler and Losick, 2006). Such a potential mechanism which is necessary for the virulence of microbes is the bacterial secretion system. Pathogenic bacteria need virulence factors in order to infect their hosts and to survive the immune response. (Coombes et al., 2004; Finlay and McFadden, 2006) The secretion systems used for this purpose are in many cases very important or essential for bacterial virulence, and they are grouped into five classes according to their protein composition, amino acid similarities and mechanism.

Gram+ve bacteria secrete a wide range of proteins whose functions include biogenesis of organelles, such as pilli and flagella, nutrient acquisition, virulence, and efflux of drugs and other toxins. Six distinct secretion systems have been shown to mediate the protein export through the inner and outer membranes of Gram +ve bacteria. These pathways are highly conserved throughout the Gram-negative bacteria species.

In Gram-positive bacteria, secreted proteins are commonly

translocated across the single membrane by the Sec pathway or the two-arginine (Tat) pathway. Type II secretion pathway supports the translocation of proteins associated with the virulence factors, across the outer membrane (Sandkvist, 2001). In our study, we identified 2 genes see, specific can be targeted for drug designing based on their role in the protein export and bacterial secretion system.

Two-component system

Two-component signal transduction systems, comprised of histidine kinases and their response regulator substrates, is the predominant means by which bacteria sense and respond to extracellular signals. These systems allow cells to adapt to prevailing conditions by modifying cellular physiology, including initiating programs of gene expression, catalyzing reactions, or modifying protein–protein interactions. These signaling pathways have also been demonstrated to play a role in coordinating bacterial cell cycle progression and development (Jeffrey et al., 2005). 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 virulence (Barrett and Hoch, 1998; Cai et al., 2005). In the prototypical two-component pathway, the sensor HK phosphorylates its own conserved. HIS residue in response to a signal(s) in the environment. Subsequently, the phosphoryl group of HK is transferred onto a specific Asp residue on the RR. The activated RR can then effect changes in cellular physiology, often by regulating gene expression. Two-component pathways thus eventually enable cells to sense and respond to stimuli by inducing changes in transcription. As they appear to be absent from metazoans, including humans, this class of molecules has been suggested as a major new target for antibacterial and antifungal drug development (Barrett and Hoch, 1998; Stephenson and Hoch, 2002). In this pathway, we found 4 enzymes which we can be used as a drug target namely respiratory nitrate reductase alpha chain narG (also involved in nitrogen metabolism), potassium-transporting ATPase subunit A (kdpA, SCCmec), chromosomal replication initiation protein dnaA and two-component sensor histidine kinase vraS.

Conclusion

Staphylococcus aureus has a circular chromosome and plasmid namely p with the number of nucleotides 2839469, 2614 protein genes as well as 79 RNA genes. In NCBI when we search *Staphylococcus aureus* in protein it shows that this subspecies contains about 6177 proteins. All the protein *Staphylococcus aureus* of that is non-homologous to the human proteome could not be taken directly as targets as these also include a large number of proteins, which are not imperative for the viability of the organism. Therefore, these 236 non homologous sequences were subjected to BLASTP against DEG and 59 enzymes were identified essential with the total score cut off 100 or greater than 100. In the list of DEG result enzymes are involved in Peptidoglycan biosynthesis, Phosphotransferase system (PTS), DNA replication, ribosome, mismatch repair, protein export and other many pathways. These identified putative targets may be exploiting further for developing drugs against *S. aureus.* It is quite obvious that increase of drug resistance properties requires more potential targets and by this Insilco approaches reduces the effort of wet lab and also increases the probability of success. By this present study we have tried to evaluate the targets could be better target fore rational drug designing.

Acknowledgement

Authors are thankful to Head, department of Molecular and Cellular Engineering, Sam Higginbotom Institute of Agriculture, Technology & Sciences, Allahabad, for providing all kind of support.

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