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In silico characterization of putative drug targets in *Staphylococcus saprophyticus*, causing bovine mastitis

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Abstract:
The bovine mastitis caused by coagulase negative staphylococci (CNS) has increased in many herds of urban and rural areas of India. Emergence of multi drug resistant bacteria has further made its management more complex and serious. Therefore, innovation of novel specific drug for the treatment of disease caused by particular organism remained to be a challenge. Hence, in the present study a bacterium was isolated from milk of the cow with bovine mastitis and was identified as *S. saprophyticus*, 44 pathways of *S. saprophyticus* retrieved (KEGG) from web server were found to be non homologous to the host *Bos taurus,* out of which 39 pathways were found to be in cytoplasm, 2 in cell wall and 3 in the cell membrane. The knowledge of the present study could make the drug discovery easier which have high affinity to the target site of the causative organism.

Keywords: Bovine mastitis, *S. saprophyticus*, Phylogenetic tree, KEGG, Metabolic pathway, Drug.

Background:
Mastitis is one of the serious diseases causing huge loss to the dairy industry worldwide. Bacteria involved in bovine mastitis infections are *S. aureus*, Coagulase negative staphylococci (CNS), *E. coli, Serretia marcescens, Bacillus subtilis, Streptococcus* species etc **[1].** Bovine mastitis has remained a major infectious disease which is difficult to manage and control it. Further emergence of multi drug resistant bacteria causing mastitis has made its management more complex. It is more serious in the countries like India which is the highest milk producer in the world which requires an urgent attention and novel methods of intervention to control the disease. In this context that all the drugs selected for the treatment of bovine mastitis against a particular infectious agent has to be screened through the *in silico* study by using the bioinformatics tools and will have to be notified through a database dedicated for this purpose which help in identifying the high prevalence of bovine mastitis. Coagulase negative staphylococcus is one of the most important gram positive bacteria, which have a potential to infect both animals and humans. Coagulase negative Staphylococci (CNS) are increasing as causes of bovine intramammary infection throughout the world in recent years the treatment of staphylococcal mastitis still remains difficult in spite of using of antibiotics with a high *in vitro* efficacy. The *in silico* based approach involves a series of screening of proteins that can be used as potential drug targets and vaccine candidates **[2].** Using such approach, novel targets have been identified successfully for various pathogens **[3-9].** Therefore in the present study we have selected CNS for the *in silico* investigation of drug target pathway in the bacterial cell and characterization of proteins involved in the metabolic pathways and identifying location of pathway in the bacterial cell.

Methodology:
Identification of bacteria
CNS has been isolated from the milk of the cow with bovine mastitis and was identified by staining, colony morphology on a specific media, catalase and coagulase tests.

Molecular identification of the bacteria

Molecular identification of CNS was done by using 16s rRNA primers forward 5¹CAG GCC TAA CAC ATG CAA GTC 3¹and reverse 3¹ GGG CGG AGT GTA CAA GGC 5¹ primers used in the study were designed by using Primer 3, isolation of DNA was carried out by **[10]** and confirmed by 16s rRNA.

Prediction of target for the drug discovery

Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used as a source of metabolic pathway information **[11],** all the metabolic pathways of the *S. saprophyticus* were retrieved from the KEGG. Pathways which do not appear in the host but present in the pathogen have been considered as pathways unique to pathogen according to KEGG database annotation **[12].** The corresponding amino acid sequences of the proteins involved in the pathway were retrieved from the KEGG database. They were subjected to a Basic Local Alignment Search Tool (BLAST) search against the non-redundant database with the e-value inclusion threshold set to 0.005. The search was restricted to proteins from *Bos taurus* through an option available in the BLAST program. This analysis was carried out for the detection of non homologues pathways in the host, which do not have hits below the e-value inclusion threshold of 0.005 and, were picked out as potential drug targets. All the non homologous pathway genes were believed to be essential genes of the pathogen. Essential genes are those indispensable for the survival of an organism, and their functions are therefore, considered a foundation of life **[13].** These crucial genes were absent in the host **[14].** All the essential pathways amino acid sequences were subjected to the PSORT (http://www.psort.org/psortb/index.html) analysis for the prediction the location of the pathway in the cell. This is required to find out the surface membrane proteins which could be probable drug targets. Further these surface membrane pathway protein sequences were characterized by using Peptide Statistic (PEPSTAT) program which is available online via European Molecular Biology Open Software Suite (EMBOSS) web server **[15].**

AGAGACAAGGGACGTTTTCTTTCTTTTTGACGTTAGCGGCGGACGGGTGAGT
AACACGTGGGTAACCTACCTATAAGACTGTGAATCTGCGGGAAACCGGAGC
TAATACCGGATAAATTCCCCCTTCGAATGGTTCG

Figure 1: Sequence of 16s r RNA

Results:

Identification of bacteria

The bacteria used in the present study could be identified as *S. saprophyticus* based on the colony morphology, gram staining and biochemical test. The identification of isolates as *S. saprophyticus* were confirmed by PCR amplification of the gene encoding the 16s rRNA. The sequence of the 16s r RNA gene is shown **(Figure 1).** Identification of species was further confirmed by computational analysis through BLAST.

Identification of potential drug targets

A total of 99 pathways of *S. saprophyticus* were retrieved from KEGG database (http://www.kegg.jp/kegg-bin/search_pathway_text) out of which 44 pathways were non homology to the host *Bos Taurus* **Table 1 (see supplementary material).** As bovine mastitis causative agents are emerging multi drug resistant, the discovery of an alternative treatment for this disease has profound scope and significance. Drug discovery process is time consuming and multistep process which includes test on many models and clinical trials. One of the major reasons for drug failures in bovine mastitis treatment is the indiscriminate use of drug without performing the *in vitro* sensitivity test to the causal organism **[16, 17]**. It's reported that drug resistant in Coagulase negative staphylococcus is also of growing concerned in bovine mastitis, poor drug-affinity and pharmacokinetic properties of lead compounds. Computer aided method is a rapid and significant screening approach because it selects the lead molecules with good pharmacological and druggish properties.

Prediction of targets distribution in the cellular components

ALL the 44 pathways amino acid sequence were Subjected to PSORT analysis (http://www.psort.org/psortb/index.html) for prediction of location of pathway in cell, out of 44 pathways, 2 pathways were found to be in cell wall (4%), 3 pathways were located in cell membrane (7%) and 39 pathways in cytoplasm (89%)**.** Similar studies have been carried out by **[12]** for finding the location of sub cellular components by using Proteome Analyst Specialized Sub Cellular Localization (PA-SUB) Server v2.5 **[18].** This is required to find out the cell wall proteins which could be probable drug targets. Folate biosynthesis pathway and Beta lactamase pathways were found to be present in the cell wall of the bacteria, these both cell wall Proteins were characterized by a PEPSTAT program **[19].** The abundance of each amino acid in the pathways and their molecular percent with respect to dayhoffstat and properties of residues is given in **Table 2 & 3 (see supplementary material).**

Folate biosynthesis pathway

Folic acid is necessary for the biosynthesis of DNA. Bacteria that are sensitive to a drug that inhibits an enzyme necessary for the biosynthesis of folic acid in bacteria are unable to acquire folic acid from their environment. In the presence of folic acid inhibitor they are unable to synthesize the folic acid essential for cell growth and multiplication. Drugs that inhibit the folic acid biosynthesis pathway of pathogenic bacteria do not harm their hosts, however, hosts (*Bos taurus,* human) acquire folic acid from their diet which lack the enzyme necessary for synthesizing folic acid **[20].** Folate biosynthesis pathway comprises a very important hydrolase enzyme responsible for removal of phosphate group from the environment, presence of phosphate group in the environment prevents the passage of organic molecules through the membrane **[21],** and however inhibition of alkaline phosphatase enzyme in the prokaryotic system makes the bacteria unable to take up the organic matter. Hence cause the death of bacteria. Therefore, folate biosynthetic pathway was believed to be the best and safe target for designing the drug against the *S. saprophyticus causing* bovine mastitis.

Beta-lactamase precursor pathway

Nowadays methicillin resistant staphylococcus (MRS) is of serious concern. This is due to the failure of beta – lactam ring to bind to the Penicillin Binding Proteins (PBP). PBP helps to cross link the polymer of repeated units of N-acetylglucosamine (NAG) and N- acetylemuramic acid (NAM), which is the final step in the biosynthesis of cell wall. However, beta-lactam antibiotics are becoming ineffective against pathogenic bacteria.

The most common reason is due to the production of beta-lactamase enzyme which catalyzes the hydrolysis of the antibiotics through the formation of carboxyl group degrading beta-lactam ring. Hydrolyzed antibiotics lose its activity or binding affinity towards the PBP hence no effect against bacteria. Resistant bacteria possessing beta lactamase gene hydrolysis beta lactum antibiotic, hence this antibiotic fails to bind PBP **[22].** Beta-lactamse enzyme is an extra cellular enzyme in Gram-positive bacteria and found in periplasmic membrane in Gram-negative bacteria **[23].** More than 200 types of beta-lactamse have been found. The difference among them is only the catalytic efficacy and turns over rate range from 0.004 to 1,200 molecules per second by 1 molecule of enzyme. Beta-lactamase became wide spread via the mechanism of plasmid exchange/insert among the pathogens **[24].** The rapid spread and evolution of these enzymes have seriously threatened the present antimicrobial arsenal. Therefore *In silico* approach in finding out the beta-lactamase inhibitors coupled with beta-lactam antibiotics could be the solution for rapid spread of multi drug resistant bacteria. These enzyme inhibitors would function to inactivate the beta-lactamase in the periplasmic space so that the partner antibiotics can reach its target, penicillin binding protein (PBP), and interrupt the biosynthesis of cell wall causing the death of the bacteria.

It is worth characterizing the physicochemical properties of the protein by using its primary structure or amino acid sequence which aid in finding out whether the protein share any basic physicochemical properties with other proteins that have been studied experimentally. The most important use of this analysis is while qualitatively assessing particular regions of a protein sequence that may have common characteristics with others in alignments with distantly related protein sequences. A typical physicochemical parameter values can be obtained by using this analytical program.

Conclusion:

In the present study attempts were made to find the putative targets of *S. saprophyticus* for the drug discovery with all the sources available in databases by computational methods. *S. saprophyticus* was isolated and confirmed by 16s rRNA gene amplification. A total 44 non homologues pathways amino acid sequence were screened for their location in bacterial cell out of which 39 pathway were found to be in cytoplasm, 2 in cell wall and 3 in the cell membrane. As pathways of cell wall were believed to be the most putative targets for the drug discovery the, proteins involved in the cell wall pathway were further characterized for the determination of physicochemical properties of the protein. Perhaps the knowledge of the present study made the drug discovery easier which have high affinity to the target site. Possible drug discovery to manage bovine mastitis with a help of bioinformatics tool is more significant, specific, reduce the time and complications involved in the clinical trials.

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References:

[1] Kurjogi MM & Kaliwal BB, *Advances in Applied Science Research.* 2011 **2**: 229
[2] John J *et al. Indian journal of biotechnology*. 2011 **10**: 432
[3] Amineni U *et al. J Chem Biol*. 2010 **3**: 165 [PMID: 21572503]
[4] Rathi B *et al. Bioinformation*. 2009 **4**: 143 [PMID: 20198190]
[5] Koteswara Reddy G *et al. Int J Bioinform Res*. 2010 2: 12
[6] Dutta A *et al. In silico Boil*. 2006
[7] Barh D & Kumar A, *In silico Boil*. 2009 **2**: 225
[8] Singh S *et al. J Bioinform Comput Biol*. 2007 **5**: 135 [PMID: 17477495]
[9] Anishetty S *et al. Comput Boil Chem.* 2005 **29**: 368 [PMID: 16213791]
[10] Kurjogi MM *et al. International journal of recent scientific research*. 2012 **3**: 10
[11] Kanehisa M *et al. Nucleic Acids Res*. 2002 **30**: 42 [PMID: 11752249]
[12] Morya V K *et al. J Comput Sci Syst Biol*. 2010 **3**: 062
[13] Zhang R *et al. Nucleic Acids Res*. 2004 **32:** D271
[14] Koonin E V *et al. Curr opin struc Biol*. 1990 **10**: 571
[15] Labarga A *et al. EMBnet News*. 2005 **11**: 18
[16] Sumathi BR *et al. Vet World*. 2008 **8:** 237
[17] Kaliwal BB *et al.Vet World*. 2011 **4**: 158
[18] Lu Z *et al. Bioinformatics*. 2004 **20**: 547 [PMID: 14990451]
[19] Rodrigo Lopez, *Bioinformatics*. 2007
[20] Williams *et al. Baltimore.* 2002
[21] James W *et al. Science.* 1985 **227:** 1338 [PMID: 17793769]
[22] Abraham EP & Chain E, *Nature.* 1940 **146**: 837
[23] Bowden GA & Georgiou G, *J Biol Chem*. 1990
[24] Sykes RB & Richmond MH, *Nature.* 1970 **226**: 952 [PMID: 4910935]

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Supplementary material:

Table 1: Information about Non- homologues pathways and their locations in *S. saprophyticus* searched against *Bos Taurus*

Sl no	Gene id	Pathway	Location	No of Amino acids
1	SSP1071	Citrate synthase	Cytoplasm	372
2	SSP0629	Galactokinase	Cytoplasm	386
3	SSP0068	UDP-glucose 6-dehydrogenase	Cytoplasm	334
4	SSP0324	3-hydroxy-3-methylglutaryl-coa synthase	Cytoplasm	389
5	SSP0146	Choloylglycine hydrolase	Cytoplasm	331
6	SSP2380	NADH dehydrogenase subunit	Cell membrane	509
7	SSP1268	L-asparaginase	Cytoplasm	324
8	SSP1439	Aspartate kinase	Cytoplasm	455
9	SSP2163	Branched-chain amino acid aminotransferase	Cytoplasm	358
10	SSP2423	Acetyl-coa acetyltransferase	Cytoplasm	394
11	SSP0817	Threonine dehydratase	Cytoplasm	422
12	SSP1325	2-oxoglutarate dehydrogenase E1	Cytoplasm	933
13	SSP2186	Beta-lactamase precursor	Cell wall	290
14	SSP1531	Monooxygenase	Cytoplasm	432
15	SSP1325	2-oxoglutarate dehydrogenase	Cytoplasm	933
16	SSP0933	Aspartate alpha-decarboxylase	Cytoplasm	077
17	SSP2168	Alkylphosphonate uptake protein	Cytoplasm	177
18	SSP1645	Glutamate racemase	Cytoplasm	269
19	SSP1013	D-alanine aminotransferase	Cytoplasm	281
20	SSP1351	Alanine racemase	Cytoplasm	357
21	SSP0105	Beta-D-galactosidase	Cytoplasm	992
22	SSP1272	NAD(P)H-dependent glycerol-3-phosphate dehydrogenase	Cytoplasm	332
23	SSP1455	Glutathione peroxidase	Cytoplasm	157
24	SSP2389	Acetate-coa ligase	Cytoplasm	523
25	SSP1531	Monooxygenase	Cytoplasm	432
26	SSP0824	Acetolactate synthase large subunit	Cytoplasm	596
27	SSP1313	Dihydrofolate reductase	Cytoplasm	161
28	SSP1432	Catalase	Cytoplasm	495
29	SSP0824	Acetolactate synthase large subunit	Cytoplasm	596
30	SSP1288	Biotin-acetyl-coa carboxylase ligase	Cytoplasm	323
31	SSP1850	Lipoyl synthase	Cytoplasm	313
32	SSP0592	Folate biosynthesis	Cell wall	477
33	SSP0762	Aldehyde dehydrogenase	Cytoplasm	475
34	SSP1218	Glycine cleavage system aminomethyltransferase T	Cytoplasm	363
35	SSP2401	Adenylylsulfate kinase	Cytoplasm	204
36	SSP1530	Fabg	Cytoplasm	250
37	SSP1707	Phosphoenolpyruvate-protein phosphotransferase	Cytoplasm	571
38	SSP1270	Ribosomal protein S1	Cytoplasm	393
39	SSP0689	DNA-directed RNA polymerase subunit alpha	Cytoplasm	314
40	SSP0897	Exonuclease	Cytoplasm	184
41	SSP0683	Preprotein translocase subunit secy	Cell membrane	430
42	SSP1122	Bifunctional preprotein translocase subunit secd/secf	Cell membrane	758
43	SSP2253	Transcription-repair coupling factor	Cytoplasm	1170
44	SSP1465	DNA mismatch repair protein muts	Cytoplasm	887

Table 2: Characterization of alkaline phosphates

PEPSTATS of ssp:SSP0592 from 1 to 477
Molecular weight = 51985.09 Residues = 477
Average Residue Weight = 108.983 Charge = -10.5
Isoelectric Point = 4.9759
A280 Molar Extinction Coefficient = 35700
A280 Extinction Coefficient 1mg/ml = 0.69
Improbability of expression in inclusion bodies = 0.626

Residue	Number	Mole%	DayhoffStat
A = Ala	43	9.015	1.048

B = Asx	0	0.000	0.000
C = Cys	0	0.000	0.000
D = Asp	50	10.482	1.906
E = Glu	20	4.193	0.699
F = Phe	18	3.774	1.048
G = Gly	42	8.805	1.048
H = His	9	1.887	0.943
I = Ile	20	4.193	0.932
J = --	0	0.000	0.000
K = Lys	50	10.482	1.588
L = Leu	23	4.822	0.652
M = Met	12	2.516	1.480
N = Asn	37	7.757	1.804
O = ---	0	0.000	0.000
P = Pro	15	3.145	0.605
Q = Gln	25	5.241	1.344
R = Arg	5	1.048	0.214
S = Ser	31	6.499	0.928
T = Thr	30	6.289	1.031
U = ---	0	0.000	0.000
V = Val	26	5.451	0.826
W = Trp	2	0.419	0.323
X = Xaa	0	0.000	0.000
Y = Tyr	19	3.983	1.172
Z = Glx	0	0.000	0.000
M = Met	12	2.516	1.480
Property	**Residues**	**Number**	**Mole%**
Tiny	(A+C+G+S+T)	146	30.608
Small	(A+B+C+D+G+N+P+S+T+V)	274	57.442
Aliphatic	(A+I+L+V)	69	14.465
Aromatic	(F+H+W+Y)	48	10.063
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	220	46.122
Polar	(D+E+H+K+N+Q+R+S+T+Z)	257	53.878
Charged	(B+D+E+H+K+R+Z)	134	28.092
Basic	(H+K+R)	64	13.417
Acidic	(B+D+E+Z)	70	14.675

Table 3: Characterization for Beta-lactamase precursor

PEPSTATS of ssp: SSP2186 from 1 to 290
Molecular weight = 32144.37 Residues = 290
Average Residue Weight = 110.843 Charge = -0.5
Isoelectric Point = 6.3029
A280 Molar Extinction Coefficient = 21050
A280 Extinction Coefficient 1mg/ml = 0.65
Improbability of expression in inclusion bodies = 0.746

Residue	**Number**	**Mole%**	**DayhoffStat**
A = Ala	23	7.931	0.922
B = Asx	0	0.000	0.000
C = Cys	1	0.345	0.119
D = Asp	15	5.172	0.940
E = Glu	19	6.552	1.092
F = Phe	7	2.414	0.670
G = Gly	15	5.172	0.616
H = His	3	1.034	0.517
I = Ile	24	8.276	1.839
J = --	0	0.000	0.000
K = Lys	27	9.310	1.411
L = Leu	25	8.621	1.165
M = Met	8	2.759	1.623
N = Asn	25	8.621	2.005
O = ---	0	0.000	0.000

P = Pro	10	3.448	0.663
Q = Gln	14	4.828	1.238
R = Arg	5	1.724	0.352
S = Ser	23	7.931	1.133
T = Thr	20	6.897	1.131
U = ---	0	0.000	0.000
V = Val	13	4.483	0.679
W = Trp	1	0.345	0.265
X = Xaa	0	0.000	0.000
Y = Tyr	12	4.138	1.217
Z = Glx	0	0.000	0.000
Property	**Residues**	**Number**	**Mole%**
Tiny	(A+C+G+S+T)	82	28.276
Small	(A+B+C+D+G+N+P+S+T+V)	145	50.000
Aliphatic	(A+I+L+V)	62	21.379
Aromatic	(F+H+W+Y)	23	7.931
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	139	47.931
Polar	(D+E+H+K+N+Q+R+S+T+Z)	151	52.069
Charged	(B+D+E+H+K+R+Z)	69	23.793
Basic	(H+K+R)	35	12.069
Acidic	(B+D+E+Z)	34	11.724