CHAPTER - 4

Bactericidal mechanism of *p*-coumaric acid, a principal compound of *Termitomyces heimii*: A bioinformatics insight

4.1. Introduction

Beside nutritional and neutraceutical attributes, mushrooms also possess potential bioactive compounds (Ferreira et al. 2009). In recent times, there are growing number of reports on various mushroom species containing large variety of phenolic compounds. Phenolic compounds identified in edible mushrooms are basically belong to either benzoic acid derivatives or cinnamic acid derivatives (Alves et al. 2014). Among them, *p*- coumaric acid (*p*-CA) is a hydroxy-derivative of cinnamic acid. It is one of the important phenolic compounds found in mushrooms. It is found universally either in free or in bound form in variable concentrations. *p*- Coumaric acid is present in esterified or free acid forms in fruits (apples, pears, grapes, oranges, tomatoes and berries), vegetables (beans, potatoes and onions), as well as in cereals (maize, oats and wheat). Biological abundance of free *p*-CA is relatively higher in certain mushroom species than any cereals, pulses, fruits and vegetables amounting a hundred or even thousand times higher concentrations (Pei et al. 2015). *p*-CA is naturallysynthesised through the shikimic acid pathway with tyrosine and phenylalanine as precursors.*p*-CA may be water-soluble or water-insoluble. *p*-CA can be esterified with alcohols, amines, monosaccharides and oligosaccharides or can form glycosides with these sugars to produce water-soluble conjugates. Esterification of *p*-CA with long chain alcohol, polysaccharides and lignin or etherified linked to lignin are producing water-insoluble *p*-CA. It is evident that, in comparison with free *p*-CA, itsconjugates display stronger biological activities (Pei et al. 2015). In purified form *p*-CA is a solid powder and exhibited the properties like melting point-211.5 °C, molecular weight - 164.16 g/mol, count of hydrogen bond donor - 2, count of hydrogen bond acceptor - 3, count of rotatable bond - 2, formal charge - 0, count of heavy atom- 12 (PubChem CID 637542).

In recent past extensive investigations have revealed that *p*-CA possess strong antimicrobial potentials (Kuete et al. 2009; Orhan et al. 2010) along with other bioactivities including antioxidant (Zang et al. 2000), anti-inflammatory (Zhang et al. 2015), antimutagenic (Ferguson et al. 2003), anti-ulcer (De Barros et al. 2008), anti-cancer (Kong et al. 2013) activities, in addition to mitigating atherosclerosis (Shen et al. 2019) and diabetes (Bahadoran et al. 2013).

According to previous results of this thesis, the methanolic extract of *T. heimii* showed significant antibacterial activity against *Staphylococcus aureus* followed by *Shigella flexneri*. Moreover, HPLC analysis of crude methanolic extract of *T. heimii* has confirmed the presence of *p*-CA (section 3.3.3). Earlier, Puttaraju et al. (2006) reported that the amount of free *p*-CA is highest in *T. heimii* (3700 mg/kg DM) than any other mushrooms.

Staphylococcus aureus is a common human pathogen. Approximately 30% of people is colonized with *S. aureus*. It is the major cause of bacteremia and infective endocarditis (IE) in addition to osteoarticular, skin and soft tissue, pleuropulmonary, and device related infections. *S. aureus* causes a variety of soft tissue and skin infections starting from the benign (e.g., uncomplicated cellulitis with impetigo) to life threatening level. *S. aureus* is a major cause of life-threateningpneumonia and has a predominant effect on hospitalized patients causing respiratory infections. Patients having cystic fibrosis are more prone to *S. aureus*.

S. aureus is now the most common cause of infective endocarditis (IE) in the industrialized world (Fowler et al. 2005). *S. aureus* also causesblood poisoning, septicaemia occurs when it enters into a person's bloodstream. Then the bacteria can travel to different organs located deep within body, to produce infections in brain, heart or lungs, bones and muscles, as well as within surgically implanted devices, such as artificial joints and pacemakers. Osteoarticular infections within children are very common. *S. aureus* is the most predominant pathogen in all three classes of osteoarticular infection, commonly called, osteomyelitis (OM) (Sheehy et al. 2010), septic arthritis in joints (Khan et al. 2013), and prosthetic joint infection (PJI) (Rodriguez et al. 2010). Septic arthritis is frequently caused by a staph infection. Knees, shoulders, hips, and fingers or toes are the main target of the bacteria. Joint swelling, severe pain occurred in the infected joint, fever are the symptoms of this disease. Due to its tendency to cause severe human diseases and its growing antibiotic resistance, *S. aureus* is a deadly pathogenic bacterium.

E. coli is a common cause of colitis, bacteremia, diarrhea, infant mortality, peritonitis, and urinary tract infections that kill roughly two million humans in every year (Russo and Johnson. 2003; Kaper et al. 2004). Some strains may also cause cancer (Arthur et al. 2012). In general, Enteropathogenic *E. coli* (EPEC) is a non-invasive organism belongs to a group of pathogenic bacteria capable of causing attaching and effacinglesions on the surface of the intestinal epithelium in host. According to Griffin and Tauxe. 1991; Robinson et al. 2006), *E. coli* O157: H7 is the most common enterohaemorrhagic strainwhich produces a shiga-like toxin. This toxin targets small blood vessels, causing intestinal cell death, bloody diarrhea and severe abdominal pain. Moreover, it causes hemolytic uremic syndrome (HUS), a deadly condition which involve clots in capillaries, hemolytic anemia, thrombocytopenia and renal failure (Griffin et al. 1988; Kaper et al. 2004).

Earlier, Lou et al. (2012) has demonstrated the anti-bacterial activity of *p*-CA against three Gram positive bacteria (*Streptococcus pneumoniae*, *Staphylococcus aureus* and *Bacillus subtilis*) and three Gramnegative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium*). *p*-CA can effectively hinder the growth of different Gram positive and Gram negative bacteria but showing different MIC values ranging from 10 - 80 µg/ml for *S. dysentriae*, *S. aureus* and *E. coli*respectively.

The trans-membrane protein sequences are the primary receptors of different molecules including nutrients, effectors and inhibitors. The actual bactericidal mechanism of *p*-CA was not reported so far. To understand the proper mechanism of action, initially the trans-membrane proteins of *S. aureus* were targeted in the present investigation. In this scenario, an *in silico* study has been designed to understand the mechanism of action of *p*-CA to inhibit *Staphylococcus aerues* at the molecular interaction level. Furthermore, to find out the molecular mechanism of *p*-CA action the results were analyzed through phylogenetic tree construction with *S. aureus* and *E. coli* transmembrane proteins followed by molecular docking.

4.2. Materials and Methods

4.2.1. Study of Minimum Inhibitory Concentration (MIC)of p-CA against two human pathogenic bacteria

The minimum inhibitory concentrations (MIC) of *p*-CA (SIGMA Life Science; ≥98.0% HPLC grade) against *S. aureus* MTCC96 and *Escherichia coli* MTCC118 were measured by broth dilution method (Wiegand et al. 2008).

4.2.2. Sequence retrieval

The transmembrane protein sequences from finished genome of *Staphylococcus aureus* (ID: 04-02981) and *Escherichia coli* (ID: ATCC 25922) were downloaded from IMG-JGI whole genome database (https://img.jgi.doe.gov/).

4.2.3. Sequence selection

From the retrieved transmembrane proteins of *Staphylococcus aureus* and in *Escherichia coli*, unicopy sequences were selectedon the basis of single amino acid change and function of the proteins.

4.2.4. Tertiary structures prediction

Sequences of selected trans-membrane proteins from *Escherichia coli* and *Staphylococcus aureus* were subjected to tertiary structure prediction using Phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2). Phyre2 follows the principles of homology modelling even if the sequential diversity between template and query remain medium or low. This server rely on Hidden Markov Models (HMMs)or profiles and calculates the mutational pattern at the level of non-synonymous SNPs for each amino acid positions and predict the structures based on various statistical models (Kelley et al. 2015).

4.2.5. Quality assessment of predicted structures

Predicted structures were subjected to ProFunc server (https://www.ebi.ac.uk/thornton-srv/databases/profunc/) for quality assessment through Ramachandran plot diagram (Laskowski et al. 2005). ProFunc is an authentic and useful tool for the qualitative and functional analysis of proteins both at sequence and structure level. It is also being used for the identification of hypothetical proteins. It helps to identify the functional motifs as well as close relationships with functionally similar proteins.

4.2.6. Ligand structure retrieval

The 3D structures of *p*-CA were downloaded from PubChem, a chemical information database,(https://pubchem.ncbi.nlm.nih.gov/) in “sdf”’ format (Kim et al. 2016) and that has been converted to “pdb” format from Discovery Studio Visualization-BIOVIA (https://www.3dsbiovia.com/products/datasheets/discovery-studio-visualizer.pdf).

4.2.7. Molecular docking

The 3D molecular structure of *p*-CA was docked with all the selected and predicted tertiary structures using Patchdock server (Schneidman-Duhovny et al. 2005). Patchdock usually perform the docking between protein-protein or protein- small molecules with the use of user provided coordinate files in PDB format. It follows the algorithm built on shape complementarity principles during molecular docking. First, it calculates the RMSD cluster values considering the minimum resolution value of 4 Å, followed by the complementarity-determining regions (CDRs) identification and potential binding site findings.

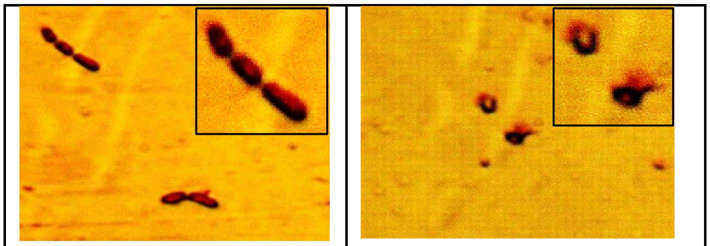
4.2.8. Phylogenetic tree construction

The multiple sequence alignment and phylogenetic tree between *Staphylococcus aureus* and*Escherichia coli* selected trans-membrane proteins were performed using Clustal X2 (Larkin et al. 2007) and PHYLIP 3.69 (Tunimal 1989).

4.3. Results and Discussions

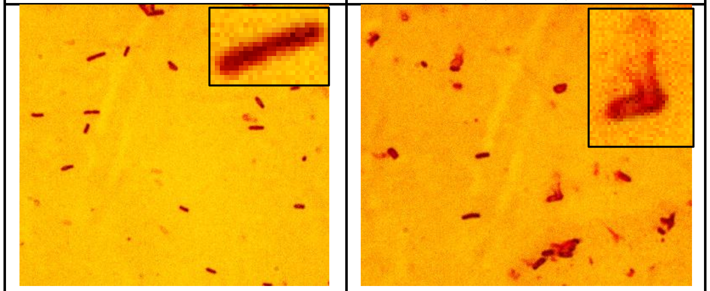
*4.3.1. Minimum Inhibitory Concentration (MIC) of p-*CA

The antibacterial effects of *Termitomycesheimii* extract against *S. aureus* and *E. coli* were previously observed and mentioned in chapter-3 (Table 3.1). Along with some other molecules, abundant and highest amount of *p*-CA was found in HPLC result (Fig-3.3). On that basis, initially in this chapter-4 the antibacterial activity of pure *p*-CA was observed against two human pathogens through MIC value calculation. For *S. aureus* and *E. coli,* the MIC values were found to be 80 µg/ml and 30 µg/ml respectively. The microscopic observations of *E. coli* and *S. aureus* cells before and after *p*-CA treatment were represented in Fig- 4.1.After 3 hours of incubation with respective MIC value concentrations of*p*-CA (80 µg/ml and 30 µg/ml),intact cells of both bacteria were lysed. Antibacterial effect of *p*-CA against six pathogenic bacteria was previously reported by Lou et al. (2012) and the MIC value of*p*-CA against *S. aureus* and *E. coli*measured as20µg/mland80 µg/ml respectively. This MIC value differences may be due to strain specificity of same species. These results indicated that pure form of *p*-CA has aremarkable bactericidal effect against pathogenic bacteria.



S. aureus untreated (1000X)

S. aureus after p-CA treatment (1000X)



E. coli untreated (1000X)

E. coli after p-CA treatment (1000X)

Fig 4.1- Microscopic observation of untreated and *p*-CA treated *S. aureus* and *E. coli* cells

4.3.2. Analysis of retrieved trans-membrane proteins

To study the actual inhibitory mechanism of *p*-CA, initially *Staphylococcus aureus* trans-membrane proteins were retrieved. A total of 642 trans-membrane protein sequences of *Staphylococcus aureus* were retrieved from whole genome (ID: 04-02981) present in IMG JGI microbial whole genome database. Among them, some proteins were found with multi-copy. Finally 330 uni-copy sequences, tabulated in Table 4.1, were selected for further study.

Table 4.1- List of selected trans-membrane protein sequences of *S. aureus*

Sl. No.	A		
1	ABC-2 family transporter protein	2	ABC-2 type transport system permease protein
3	accessory gene regulator B	4	Acetyl esterase lipase
5	acetyl-CoA acyltransferase	6	acetyl-CoA synthetase
7	ACT domain protein	8	acyl-phosphate glycerol-3-phosphate acyltransferase
9	alanine or glycine cation symporter, AGCS family	10	amino acid ABC transporter substrate-binding protein, PAAT family amino acid ABC transporter membrane protein, PAAT family
11	amino acid polyamine organocation transporter, APC superfamily	12	aminobenzoyl-glutamate transport protein
13	ammonium transporter	14	anion transporter
15	arginine ornithine antiporter, APA family	16	arsenite efflux membrane protein ArsB
17	ATP synthase F0 subcomplex B subunit	18	ATP synthase F0 subcomplex C subunit
19	ATP-binding cassette, subfamily B, AbcABmrA	20	ATP-binding cassette, subfamily B, MsbA
21	ATP-binding cassette, subfamily C, CydC	22	ATP-binding cassette, subfamily C, CydD
	B		
23	bacitracin transport system permease protein	24	Bacteriocin (Lactococcin_972)
25	bacteriocin-associated integral membrane (putative immunity) protein	26	Beta - N-acetylglucosaminidase
27	betaine carnitine transporter, BCCT family	28	bile acid Na^+ symporter, BASS family
29	biofilm PGA synthesis N-glycosyltransferase PgaC	30	biotin biosynthesis protein BioX
31	biotin transport system substrate-specific component	32	Biotin-lipoyl like
33	Branched-chain amino acid transport protein	34	branched-chain amino acid cation transporter, LIVCS family
	C		
35	CAAX protease self-immunity	36	camphor resistance protein CrcB
37	Capsular polysaccharide biosynthesis protein	38	capsular polysaccharide synthesis enzyme
39	carbohydrate ABC transporter membrane protein 1, CUT1 family	40	carbohydrate ABC transporter membrane protein 2, CUT1 family
41	carboxyl-terminal processing protease	42	cardiolipin synthetase 2
43	cation diffusion facilitator family transporter	44	cationic antimicrobial peptide transport system permease protein
45	c-di-AMP phosphodiesterase,	46	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase
47	cell division protein DivIC	48	cell division protein FtsL
49	cell division protein FtsQ	50	cell division-specific peptidoglycan biosynthesis regulator FtsW
51	cell elongation-specific peptidoglycan D,D-transpeptidase	52	chloramphenicol-sensitive protein RarD
53	choline carnitine betaine transport	54	citrate-$Mg^{2+}H^+$ or citrate-$Ca^{2+}H^+$ symporter, CitMHS family
55	clumping factor A	56	clumping factor B
57	cobalt transport protein	58	cobalt-zinc-cadmium efflux system protein
59	competence protein ComEA	60	competence protein ComEC
61	competence protein ComGC	62	competence protein ComGD
63	conserved hypothetical integral membrane	64	conserved hypothetical protein TIGR01655

	protein		
65	Cu$^+$-exporting ATPase	66	CubicO group peptidase, beta-lactamase class C family
67	cytochrome aa3 quinol oxidase subunit 1 apoprotein	68	cytochrome aa3 quinol oxidase subunit 2
69	cytochrome aa3 quinol oxidase subunit 3	70	cytochrome aa3 quinol oxidase subunit 4
71	cytochrome bd-I ubiquinol oxidase subunit 1 apoprotein	72	cytochrome bd-I ubiquinol oxidase subunit 2 apoprotein
73	cytochrome c oxidase assembly protein subunit 15	74	cytochrome oxidase maturation protein, cbb3-type
	D		
75	D-alanine transfer protein	76	D-alanyl-D-alanine carboxypeptidase (penicillin-binding protein 56)
77	D-Ala-teichoic acid biosynthesis protein	78	deferrochelataseperoxidaseEfeB
79	diguanylate cyclase (GGDEF) domain-containing protein	80	divalentanionNa$^+$ symporter, DASS family
81	D-methionine transport system permease protein	82	DNA segregation ATPase FtsKSpoIIIE, S-DNA-T family
83	DNA-binding transcriptional regulator, XRE-family HTH domai	84	DoxX protein
85	drug resistance transporter, BcrCflA subfamily	86	drug resistance transporter, EmrBQacA subfamily
87	D-serineD-alanineglycineproton symporter, AAT family		
	E		
88	EamA domain-containing membrane protein RarD	89	energy coupling factor transporter S component ThiW
90	energy-coupling factor transport system permease protein	91	energy-coupling factor transport system substrate-specific component
92	enterotoxin	93	Excalibur calcium-binding domain-containing protein
94	exfoliative toxin AB		
	F		
95	ferrous iron transport protein B	96	formate nitrite transporter
97	F-type H$^+$-transporting ATPase subunit a	98	Fucose 4-O-acetylase
	G		
99	Gas vesicle protein	100	General stress protein CsbA
101	gluconate permease GntP	102	glucose uptake protein
103	Glutamate synthase domain-containing protein 2	104	glutamateNa+ symporter, ESS family
105	glycerol uptake facilitator protein	106	glycerophosphoryl diester phosphodiesterase
107	glycine betaine transporter	108	glycosyl-4,4'-diaponeurosporenoate acyltransferase
109	Glycosyltransferase involved in cell wall bisynthesis		
	H		
110	Helix-turn-helix domain-containing protein	111	heme uptake protein IsdB
112	heme uptake protein IsdC	113	hemin transport system permease protein
114	hemolysin III	115	Hemolysin, contains CBS domains
116	HemX family protein	117	high-affinity iron transporter
118	high-affinity nickel-transport protein	119	holin-like protein
120	holin-like protein LrgB	121	hydrolase, MutTnudix family
122	hydroxyethylthiazole kinase	123	hypothetical protein
	I		

124	immunodominant antigen B	125	immunoglobulin G-binding protein A
126	Inhibitor of apoptosis-promoting Bax1	127	inner membrane protein
128	inorganic phosphate transporter, PiT family	129	integral membrane protein, YkoY family
130	intercellular adhesin biosynthesis polysaccharide N-deacetylase	131	intracellular adhesion protein D
132	iron complex transport system permease protein	133	iron complex transport system substrate-binding protein
134	iron-regulated surface determinant protein A	135	ironzinccopper transport system permease protein
	K		
136	K$^+$-transporting ATPase ATPase A chain	137	K$^+$-transporting ATPase ATPase B chain
138	K$^+$-transporting ATPase ATPase C chain		
	L		
139	lactate permease	140	large conductance mechanosensitive channel
141	leukocidinhemolysin toxin family protein	142	LexA-binding, inner membrane-associated putative hydrolase
143	lipoprotein	144	lipoteichoic acid synthase
145	L-lysine exporter family protein LysEArgO	146	Low temperature requirement protein LtrA
147	L-proline dehydrogenase	148	lysineproton symporter, AAT family
149	LysM domain-containing protein		
	M		
150	magnesium transporter	151	Major Facilitator Superfamily protein
152	manganese transport protein	153	membrane protease FtsH catalytic subuni
154	Membrane protease YdiL, CAAX protease family	155	Membrane protein CcdC involved in cytochrome C biogenesis
156	membrane protein DedA, SNARE-associated domain	157	membrane protein involved in D-alanine export
158	Membrane protein involved in the export of O-antigen and teichoic acid	159	membrane protein YdbS, contains bPH2 (pleckstrin homology) domain
160	Membrane proteinase PrsW, cleaves anti-sigma factor RsiW, M82 family	161	Membrane-associated phospholipid phosphatase
162	Membrane-bound acyltransferase YfiQ, involved in biofilm formation	163	methicillin resistance protein. Metallo peptidase. MEROPS family M56
164	MFS transporter, ACS family, glucarate transporter	165	MFS transporter, DHA1 family, bicyclomycinchloramphenicol resistance protein
166	MFS transporter, DHA1 family, multidrug resistance protein	167	MFS transporter, DHA2 family, multidrug resistance protein
168	MFS transporter, MHS family, prolinebetaine transporter	169	MFS transporter, NNP family, putative nitrate transporter
170	MFS transporter, OPA family, glycerol-3-phosphate transporte	172	MFS transporter, OPA family, hexose phosphate transport protein UhpT
173	Mg^{2+} and Co^{2+} transporter CorA	174	Mg^{2+} and Co^{2+} transporter CorB, contains DUF21, CBS pair, and CorC-HlyC domains
175	micrococcal nuclease	176	Mn^{2+} and Fe^{2+} transporters of the NRAMP family
177	molybdate transport system permease protein	178	monofunctional glycosyltransferase
179	multicomponent Na$^+$H$^+$ antiporter subunit C	180	multidrug efflux pump
181	Multidrug efflux pump subunit AcrB	182	Multidrug Resistance efflux pump
183	multisubunitsodiumproton antiporter, MrpA subunit	184	multisubunitsodiumproton antiporter, MrpB subunit
185	multisubunitsodiumproton antiporter, MrpC subunit	186	multisubunitsodiumproton antiporter, MrpD subunit
187	multisubunitsodiumproton antiporter, MrpE subunit	188	multisubunitsodiumproton antiporter, MrpF subunit
189	multisubunitsodiumproton antiporter, MrpG subunit	190	MutS domain V

	N		
191	N-acetylmuramoyl-L-alanine amidase	192	NAD(P)H-quinone oxidoreductase subunit 5
193	NDP-sugar epimerase, includes UDP-GlcNAc-inverting 4,6-dehydratase FlaA1 and capsular polysaccharide biosynthesis protein EpsC	194	neurotransmitterNa$^+$ symporter, NSS family
195	NitTTauT family transport system permease protein	196	Nuclease-related domain-containing protein
197	Nucleoside recognition	198	nucleoside recognition GATE domain-containing membrane protein YjiH
199	nucleoside transport protein	200	nucleoside transporter
	O		
201	O-antigen biosynthesis protein WbqP	202	O-antigen ligase like membrane protein
203	oligopeptide transport system permease protein	204	osmoprotectant transport system permease protein
205	osmoprotectant transport system substrate-binding protein		
	P		
206	PASPAC sensor signal transduction histidine kinase	207	pathogenicity island protein
208	penicillin-binding protein 1	209	penicillin-binding protein 1A
210	Peptidase family M23	211	Peptidase M50B-like
212	peptide-methionine (S)-S-oxide reductase	213	peptidenickel transport system permease protein
214	peptidoglycan-N-acetylmuramate O-acetyltransferase	215	phage protein
216	phosphate ABC transporter membrane protein 1, PhoT family	217	phosphate ABC transporter membrane protein 2, PhoT family
218	phosphateNa$^+$ symporter	219	phosphatidate cytidylyltransferase
220	phosphatidylglycerol lysyltransferase	221	Phospho-N-acetylmuramoyl-pentapeptide-transferase
222	phosphonate transport system permease protein	223	phosphonate transport system substrate-binding protein
224	Phosphotransferase system, fructose-specific IIC component	225	polysaccharide export protein, MPA1 family
226	potassium uptake protein, TrkH family	227	Predicted arabinose efflux permease, MFS family
228	Predicted PurR-regulated permease PerM	229	Predicted small secreted protein
230	preprotein translocase subunit SecE	231	preprotein translocase subunit SecG
232	preprotein translocase subunit SecY	233	Prolipoproteindiacylglyceryl transferase
234	protein translocase subunit secF protein translocase subunit secD	235	protein translocase subunit secYsec61 alpha
236	protein translocase subunit yajC	237	protoheme IX farnesyltransferase
238	proton glutamate symport protein	239	proton-dependent oligopeptide transporter, POT family
240	pseudouridine-5'-phosphate glycosidase	241	PTS system D-glucose-specific IIA component, Glc family PTS system D-glucose-specific IIB component, Glc family PTS system D-glucose-specific IIC component, Glc family
242	PTS system D-mannitol-specific IIB component, Fru family PTS system D-mannitol-specific IIC component, Fru family	243	PTS system D-mannose-specific IIA component, Fru family PTS system D-mannose-specific IIB component, Fru family PTS system D-mannose-specific IIC component, Fru family
244	PTS system IIB component, Glc family PTS system IIC component, Glc family	245	PTS system IIC component, Gat family
246	PTS system IIC component, L-Asc family	247	PTS system lactose-specific IIB component, Lac

			family PTS system lactose-specific IIC component, Lac family
248	PTS system N-acetylglucosamine-specific IIB component, Glc family PTS system N-acetylglucosamine-specific IIC component, Glc family	249	PTS system sucrose-specific IIB component, Glc family PTS system sucrose-specific IIC component, Glc family
250	PTS system trehalose-specific IIB component, Glc family PTS system trehalose-specific IIC component, Glc family	251	purine nucleoside transport protein
	R		
252	respiratory nitrate reductase gamma subunit	253	Rhodanese-related sulfurtransferase
254	rhomboid protease GluP	255	Riboflavin transporter FmnP
256	ribonucleoside-diphosphate reductase class Ib beta subunit	257	ribonucrease Y
258	rod shape determining protein RodA	259	rod shape-determining protein MreC
260	rod shape-determining protein MreD		
	S		
261	soluteNa^{+} symporter, SSS family	262	sec-independent protein translocase protein TatA
263	sec-independent protein translocase protein TatC	264	Sensor histidine kinase YesM
265	sensor protein	266	Septation ring formation regulator, EzrA
267	serine protease, S1-C subfamily, contains C-terminal PDZ domain	268	serine-aspartate repeat family protein, SdrH
269	serine-aspartate repeat-containing protein CDE	270	serinethreonine exchange transporter, LAT family
271	serinethreonine protein kinase	272	signal peptidase I
273	signal peptidase II Aspartic peptidase. MEROPS family A08	274	site-2 protease. Metallo peptidase. MEROPS family M50B
275	small conductance mechanosensitive channel	276	sodiumproton antiporter, CPA1 family
277	sortase A. Cysteine peptidase. MEROPS family C60A	278	sortase B
279	spermidineputrescine transport system permease protein	280	spermidineputrescine transport system substrate-binding protein
281	succinate dehydrogenase subunit C	282	Sugar phosphate permease
283	Sugar transferase involved in LPS biosynthesis (colanic, teichoic acid)		
	T		
284	tandem five-transmembrane protein	285	teichoic acid transport system ATP-binding protein
286	teichoic acid transport system permease protein	287	Thioldisulfide interchange protein DsbA
288	Threoninehomoserine efflux transporter RhtA	289	ThreonineSerine exporter, ThrE
290	TIGR00659 family protein	291	TM2 domain-containing protein
292	transcriptional attenuator, LytR family	293	transcriptional regulator, MerR family
294	transcriptional regulator, TetR family	295	Transmembrane secretion effector
296	transporter family-2 protein	297	transporter, CPA2 family
298	transporter, NhaC family	299	triacylglycerol lipase
300	trk system potassium uptake protein TrkH	301	Two-component signal transduction system YycFG, regulatory protein YycH
302	Two-component signal transduction system YycFG, regulatory protein YycI	303	two-component system, LytT family, sensor histidine kinase LytS
304	two-component system, NarL family, vancomycin resistance sensor histidine	305	two-component system, OmpR family, phosphate regulon sensor histidine kinase PhoR

	kinase VraS		
306	two-component system, OmpR family, sensor histidine kinase ArlS	307	two-component system, OmpR family, sensor histidine kinase BraSBceS
308	two-component system, OmpR family, sensor histidine kinase GraS	309	two-component system, OmpR family, sensor histidine kinase ResE
310	two-component system, OmpR family, sensor histidine kinase SaeS	311	type 4 prepilin peptidase 1 Aspartic peptidase. MEROPS family A24A
312	type I signal peptidase. Serine peptidase. MEROPS family S26A	313	type II secretion system protein F (GspF)
314	type VII secretion protein EsaA, N-terminal domain-containing protein	315	type VII secretion protein EssA
316	type VII secretion protein EssB		
	U		
317	UDP-GlcNAcundecaprenyl-phosphate GlcNAc-1-phosphate transferase	318	undecaprenol kinase diacylglycerol kinase
319	Undecaprenyl-diphosphatase	320	uracil permease
321	urea transporter		
	V		
322	V8-like Glu-specific endopeptidase	323	Virus attachment protein p12 family protein
	X		
324	xanthine permease		
	Y		
325	YbbR domain-containing protein	326	YggT family protein
327	YhgEPip N-terminal domain-containing protein	328	YibEF-like protein
329	YidCOxa1 family membrane protein insertase		
	Z		
330	zinc transport system permease protein		

Whereas the trans-membrane proteins ofGram negative *E. coli* were also considered for analysis. A total of 614 sequences were retrieved from 1121 sequences of Gram negative *Escherichia coli*, enlisted in Table 4.2.

Table 4.2- List of selected trans-membrane protein sequences of *E. coli*

Sl. No.	**Name of the trans-membrane proteins**		
1	[NiFe]-hydrogenase I apocytochrome b subunit	2	[NiFe]-hydrogenase I apoprotein, small subunit
3	[NiFe]-hydrogenase II apocytochrome b subunit	4	[NiFe]-hydrogenase II apoprotein, small subunit
5	[sulfur carrier protein ThiS] adenylyltransferase	6	1-acyl-sn-glycerol-3-phosphate acyltransferase
7	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase	8	4-amino-4-deoxy-L-arabinose transferase
9	4-azaleucine resistance probable transporter AzlC	10	4-hydroxybenzoate polyprenyltransferase

11	5-hydroxyisourate hydrolase	12	40-residue YVTN family beta-propeller repeat-containing protein
	A		
13	ABC 3 transport family protein	14	ABC-2 type transport system permease protein
15	ABC-type uncharacterized transport system, permease component	16	acyl-CoA dehydrogenase
17	acyl-phosphate glycerol-3-phosphate acyltransferase	18	adhesininvasin
19	adsorption protein B	20	aerobic C4-dicarboxylate transport protein
21	AI-2 transport protein TqsA	22	alanine or glycinecation symporter, AGCS family
23	Alkyl sulfatase BDS1, metallo-beta-lactamase superfamily	24	allantoin permease
25	allose ABC transporter membrane protein	26	allose-binding protein
27	amino acid ABC transporter membrane protein 1, PAAT family	28	amino acid ABC transporter membrane protein 2, PAAT family
29	amino acid adenylation domain-containing protein	30	amino acid exporter, AAE family
31	amino acidpolyamineorganocation transporter, APC superfamily	32	ammonium transporter
33	AmpE protein	34	anaerobic C4-dicarboxylate transporter DcuA
35	anaerobic C4-dicarboxylate transporter DcuB	36	anaerobic dimethyl sulfoxide reductase subunit C
37	anion transporter	38	Antimicrobial peptide resistance and lipid A acylation PagP
39	Apolipoprotein N-acyltransferase	40	arginineagmatine antiporter, APA family
41	aromatic amino acidproton symporter, AAT family	42	arylsulfate sulfotransferase
43	AsmA family protein	44	AsmA protein
45	asparaginase	46	asparagineproton symporter, AAT family
47	ATP-binding cassette, subfamily B, MsbA	48	ATP-binding cassette, subfamily B, multidrug efflux pump
49	ATP-binding cassette, subfamily B, salmochelinenterobactin exporter	50	ATP-binding cassette, subfamily C, CydC
51	ATP-binding cassette, subfamily C, CydD	52	autotransporter secretion inner membrane protein TamB
53	Bacteriophage P21 holin S	54	benzoate membrane transport protein
55	Beta-barrel assembly machine subunit BamE	56	biofilm PGA synthesis N-glycosyltransferase PgaC
57	Branched-chain amino acid transport protein (AzlD)	58	branched-chain amino acidcation transporter, LIVCS family
	C		
59	C4-dicarboxylate transporter, DcuC family	60	Ca^{2+}H^{+} antiporter
61	cadaverinelysine antiporter, APA family	62	camphor resistance protein CrcB
63	capsular polysaccharide transport system permease protein	64	carbohydrate ABC transporter membrane protein 1, CUT1 family (2)
65	carbohydrate ABC transporter membrane protein 1, CUT1 family	66	carbohydrate-specific outer membrane porin
67	Carbon starvation protein CstA (2)	68	carboxylateaminoacidamine transporter
69	cardiolipin synthetase 2	70	cationacetate symporter
71	cationH^{+} antiporter	72	cationic peptide transport system permease protein
73	cd2 zn^{2+} exporting ATPase	74	CDP-diacylglycerol--glycerol-3-phosphate

			3-phosphatidyltransferase
75	Cell division and transport-associated protein TolQ	76	Cell division and transport-associated protein TolR
77	cell division protein FtsB	78	cell division protein FtsL
79	cell division protein FtsQ	80	cell division protein FtsX
81	cell division-specific peptidoglycan biosynthesis regulator FtsW	82	cell elongation-specific peptidoglycan biosynthesis regulator RodA
83	Cell wall-associated hydrolase, NlpC family	84	celllulose biosynthesis operon protein BcsFYhjT
85	cellulose synthase (UDP-forming)	86	cellulose synthase operon protein YhjU
87	cellulose synthase subunit	88	chain length determinant protein (polysaccharide antigen chain regulator)
89	chemotaxis protein MotA	90	Chemotaxis protein MotB
91	chloride channel protein, CIC family	92	cholineglycineproline betaine transport protein
93	citratesuccinate antiporter	94	cobalamin-5'-phosphate synthase
95	colicin V processing peptidase. Cysteine peptidase. MEROPS family C39 alp	96	colicin V secretion protein
97	competence protein ComEC	98	conserved hypothetical integral membrane protein
99	CTP synthase	100	Cu^{+}-exporting ATPase
101	CubicO group peptidase, beta-lactamase class C family	102	curli production assembly transport component CsgF
103	cyd operon protein YbgE	104	cytochrome b561
105	Cytochrome bb6petB	106	cytochrome bd-I ubiquinol oxidase subunit 1 apoprotein
107	cytochrome bd-I ubiquinol oxidase subunit 2 apoprotein	108	cytochrome bd-II ubiquinol oxidase subunit 1 apoprotein
109	cytochrome bd-II ubiquinol oxidase subunit 2 apoprotein	110	cytochrome bo3 quinol oxidase subunit 1 apoprotein
111	cytochrome bo3 quinol oxidase subunit 2	112	cytochrome bo3 quinol oxidase subunit 3
113	cytochrome bo3 quinol oxidase subunit 4	114	cytochrome c biogenesis protein CcmG, thioldisulfide interchange protein DsbE
115	cytochrome c peroxidase	116	cytochrome c-type biogenesis protein CcmE
117	cytochrome c-type biogenesis protein CcmF	118	cytochrome c-type biogenesis protein CcmH
119	cytosine permease		
	D		
120	DamX protein	121	DegQ peptidase. Serine peptidase. MEROPS family S01B
122	DegS peptidase. Serine peptidase. MEROPS family S01B	123	D-fructuronate permease
124	Di- and tricarboxylate transporter	125	diacylglycerol kinase
126	Dicarboxylate transport	127	diguanylate cyclase (GGDEF) domain-containing protein
128	diguanylate cyclasephosphodiesterase with PASPAC sensor(s) signal transduction protein	129	diguanylate cyclasephosphodiesterase
130	dipeptide transport system permease protein	131	disulfide bond formation protein DsbB
132	divalent anionNa^{+} symporter, DASS family	133	D-methionine transport system permease protein
134	DNA translocase FtsK	135	DNA-binding transcriptional regulator, LysR family
136	DnaJ like chaperone protein	137	drug resistance transporter, BcrCflA subfamily
138	D-serineD-alanineglycineproton symporter, AAT		

	family		
	E		
139	EAL domain, c-di-GMP-specific phosphodiesterase class I (or its enzymatically inactive variant)	140	EamA domain-containing membrane protein RarD
141	EamA-like transporter family protein	142	ElaB protein
142	electron transport complex protein RnfA	143	electron transport complex protein RnfB
144	electron transport complex protein RnfD	145	electron transport complex protein RnfE
146	energy-coupling factor transport system permease protein	147	energy-coupling factor transport system substrate-specific component
148	entericidin B		
	F		
149	Fatty acid hydroxylase superfamily protein	150	ferrous transport protein B
151	Fe-S-cluster-containing dehydrogenase component	152	filamentous hemagglutinin
153	fimbrial chaperone protein	154	flagellar biosynthesis protein FlhA
155	flagellar biosynthetic protein FlhB	156	flagellar biosynthetic protein FliQ
157	flagellar biosynthetic protein FliP	158	flagellar biosynthetic protein FliR
159	flagellar FliL protein	160	flagellar M-ring protein FliF
161	flagellar protein FliOFliZ	162	formate dehydrogenase (quinone-dependent) iron-sulfur subunit
163	formate dehydrogenase gamma subunit	164	formatehydrogenlyase subunit 3
165	formatehydrogenlyase subunit 4	166	formate transporter
167	Fucose permease	168	fumarate reductase subunit C
	G		
169	gammaaminobutyrateprotonsymporter AAT family	170	GlpG protein
171	glucitol operon activator protein	172	gluconate permease GntP
173	gluconate permease GntT	174	gluconateH^+ symporter, GntP family
175	glucose ABC transporter membrane protein galactose ABC transporter membrane protein	176	glucose uptake protein
177	glucose-binding protein galactose-binding protein	178	glucuronide carrier protein
179	glutamategamma-aminobutyrate antiporter, GGA family	180	glutamateNa^+ symporter, ESS family
181	glutathione transport system permease protein	182	glutathionylspermidineamidasesynthetase
183	glycerol 3-phosphate ABC transporter membrane protein	184	glycine betaineproline transport system permease protein
185	glycolate permease	186	glycosidepentosidehexuronidecation symporter, GPH family
187	Glycosyl transferases group 1	188	Glycosyltransferase, catalytic subunit of cellulose synthase and poly-beta-1,6-N-acetylglu
189	Gnt-I system low-affinity gluconate transporter		
	H		
190	HdeAHdeB family protein	191	Heat shock protein. Metallo peptidase. MEROPS family M48B
192	heme exporter protein B	193	heme exporter protein C
194	heme exporter protein D	195	hemolysin A secretion protein HlyD(8.A.1.3.1)
196	hemolysin III	197	Hemolysin, contains CBS domains
198	hemolysinA	199	high-affinity iron transporter
200	His Kinase A (phospho-acceptor) domain-containing protein	201	histidine kinase TorS
202	Histidine phosphatase superfamily (branch 1)	203	holin-like protein
204	Hpt sensor hybrid histidine kinase	205	hypothetical protein
	I		
206	Inner membrane protein YlaC	207	inner membrane protein

208	inner membrane transporter RhtA	209	integral membrane protein, TerC family
210	integral membrane protein, YccSYhfK family	211	Integrase core domain-containing protein
212	Intracellular growth attenuator protein IgaA	213	intracellular septation protein
214	iron complex transport system permease protein		
	K		
215	K^+-transporting ATPase ATPase C chain	216	Kef-type potassiumproton antiporter, CPA2 family
	L		
217	L alanine exporter	218	L,D-transpeptidase YbiS
219	L-arabinose ABC transporter membrane protein	220	Large conductance mechanosensitive channel
221	L-arginine ABC transporter membrane protein	222	lauroyl-KDO2-lipid IV(A) myristoyltransferase
223	L-carnitinegamma-butyrobetaine antiporter	224	L-cystine ABC transporter membrane protein Diaminopimelate ABC transporter membrane protein
225	leucine efflux protein	226	L-glutamate ABC transporter membrane protein L-aspartate ABC transporter membrane protein
227	L-glutamine ABC transporter membrane protein	228	L-histidine ABC transp
229	L-histidine-binding protein	230	linoleoyl-CoA desaturase
231	lipid-A kinase	232	lipid-A-disaccharide kinase
233	lipopolysaccharide export system permease protein	234	lipopolysaccharide export system protein LptC
235	lipopolysaccharide exporter	236	lipoprotein-releasing system permease protein
237	L-lactate permease	238	L-leucine ABC transporter membrane protein L-isoleucine ABC transpo
239	L-lysine exporter family protein LysEArgO	240	low-affinity inorganic phosphate transporter
241	L-tartratesuccinate antiporter	242	lysine decarboxylase transcriptional regulator, CadC
243	lysineproton symporter, AAT family	244	lysozyme
	M		
245	macrolide transport system ATP-bindingpermease protein	246	Major Facilitator Superfamily protein
247	major pilin subunit PapA	248	maltooligosaccharide ABC transporter membrane protein
249	manganeseiron transport system permease protein	250	manganeseiron transport system substrate-binding protein
251	MarC family integral membrane protein	252	membrane fusion protein, Cu(I)Ag(I) efflux system
253	membrane glycosyltransferase	254	membrane protease FtsH catalytic subunit
255	membrane protein (2)	256	membrane protein DedA, SNARE-associated domain
257	Membrane protein involved in the export of O-antigen and teichoic acid	258	Membrane protein TerC, possibly involved in tellurium resistance
259	membrane protein YqaA, SNARE-associated domain	260	membrane protein, MarC family
261	membrane protein	262	Membrane-anchored ribosome-binding protein, inhibits growth in stationary phase, ElaBYqjDDUF883
263	metabolite-proton symporter	264	methyl-accepting chemotaxis sensory transducer with PasPac sensor
265	methyl-accepting chemotaxis sensory transducer	266	methylthioribose-1-phosphate isomerase (2)

	with TarH sensor		
267	MFS transporter DAH2 family , multidrug resistance protein	268	MFS transporter, ACS family, D-galactonatetransporter
269	MFS transporter, ACS family, glucarate transporter	270	MFS transporter, ACS family, hexuronate transporter
271	MFS transporter, ACS family, probable galactarate transporter	272	MFS transporter, CP family, cyanate transporter
273	MFS transporter, DHA1 family, 2-module integral membrane pump EmrD	274	MFS transporter, DHA1 family, arabinose polymer transporter
275	MFS transporter, DHA1 family, bicyclomycinchloramphenicol resistance protein	276	MFS transporter, DHA1 family, L-arabinoseisopropyl-beta-D-thiogalactopyranoside export protein
277	MFS transporter, DHA1 family, multidrug resistance protein	278	MFS transporter, DHA1 family, multidrugchloramphenicol efflux transport protein
279	MFS transporter, DHA1 family, purine ribonucleoside efflux pump	280	MFS transporter, DHA2 family, multidrug resistance protein
281	MFS transporter, ENTS family, enterobactin (siderophore) exporter	282	MFS transporter, FHS family, L-fucose permease
283	MFS transporter, FSR family, fosmidomycin resistance protein	284	MFS transporter, MHS family, metaboliteH+ symporter
285	MFS transporter, MHS family, shikimate and dehydroshikimate transport protein	286	MFS transporter, NHS family, nucleoside permease
287	MFS transporter, NNP family, nitratenitrite transporter	288	MFS transporter, OFA family, oxalateformate antiporter
289	MFS transporter, OHS family, lactose permease	290	MFS transporter, OPA family, glycerol-3-phosphate transporter
291	MFS transporter, OPA family, hexose phosphate transport protein UhpT	292	MFS transporter, OPA family, sugar phosphate sensor protein UhpC
293	MFS transporter, PAT family, beta-lactamase induction signal transducer AmpG	294	MFS transporter, putative metabolite transport protein
295	MFS transporter, putative metaboliteH+ symporter	296	MFS transporter, putative signal transducer
297	MFS transporter, SET family, sugar efflux transporter	298	MFS transporter, SHS family, sialic acid transporter
299	MFS transporter, SP family, arabinoseH+ symporter	300	MFS transporter, SP family, galactoseH+ symporte
301	MFS transporter, TsgA protein	302	MFS transporter, UMF2 family, putative MFS family transporter protein
303	MFS transporter, YNFM family, putative membrane transport protein	304	Mg^{2+}-importing ATPase
305	MHS family proline betaine transporter	306	microcin C transport system permease protein
307	microcin C transport system substrate-binding protein	308	miniconductance mechanosensitive channel
309	minor fimbrial subunit	310	modulator of FtsH protease
311	molybdate transport system permease protein	312	monofunctional biosynthetic peptidoglycan transglycosylase
313	monosaccharide ABC transporter membrane protein, CUT2 family	314	monosaccharide ABC transporter substrate-binding protein, CUT2 family
315	Msc C family membrane protein	316	MscS family membrane protein
317	multidrug efflux pump	318	multidrug resistance protein MdtO
319	multidrug resistance protein, MATE family	320	multiple antibiotic resistance protein
321	murein DD-endopeptidase	322	Murein L,D-transpeptidase YafK
	N		

323	N-acetylmuramoyl-L-alanine amidase	324	N-acetylneuraminate epimerase
325	NAD(P) transhydrogenase subunit alpha	326	NAD(P) transhydrogenase subunit beta
327	NADH dehydrogenase subunit A	328	NADH dehydrogenase subunit H
329	NADH dehydrogenase subunit J	330	NADH dehydrogenase subunit K
331	NADH dehydrogenase subunit L	332	NADH dehydrogenase subunit M
333	NADH dehydrogenase subunit N	334	nickel transport system permease protein
335	nickelcobalt exporter	336	nicotinamide mononucleotide transporter
337	nitrate transporter NirC	338	Nucleoside recognition
339	nucleoside transporter	340	Nucleoside-specific channel-forming protein, Tsx
	O		
341	O-acetylserinecysteine efflux transporter	342	O-antigen ligase
343	oligogalacturonide transporter	344	oligopeptide transport system permease protein
345	oligopeptide transport system substrate-binding protein	346	oligosaccharide repeat unit polymerase
347	osmoprotectant transport system permease protein	348	Osmotically-inducible protein OsmY, contains BON domain
349	outer membrane autotransporter barrel domain-containing protein	350	Outer membrane murein-binding lipoprotein Lpp
351	Outer membrane protein OmpA	352	outer membrane protein, YaiO family
353	outer membrane transport energization protein ExbB	354	outer membrane usher protein
	P		
355	P pilus assembly protein, chaperone PapD	356	PAP2 superfamily protein
357	paraquat-inducible protein A	358	Paraquat-inducible protein B
359	PASPAC sensor hybrid histidine kinase	360	PASPAC sensor signal transduction histidine kinase
361	penicillin-binding protein 1A	362	penicillin-binding protein 1B
363	peptidebleomycin uptake transporter	364	peptidenickel transport system permease protein
365	peptidoglycan glycosyltransferase cell elongation-specific peptidoglycan D,D-transpeptidase	366	peptidoglycan synthetase FtsI
367	Peptidoglycanxylanchitin deacetylase, PgdACDA1 family (2)	368	peptidyl-prolyl cis-trans isomerase D
369	periplasmic nitrate reductase subunit NapC	370	periplasmic nitrate reductase subunit NapH
371	Permease of the drugmetabolite transporter (DMT) superfamily	372	PgaD-like protein
373	Phage minor tail protein U	374	phage shock protein B
375	phage shock protein C (PspC) family protein	376	phage shock protein G
377	Phage terminase, large subunit GpA	378	Phage-related protein, tail component
379	phenylalanineproton symporter, AAT family	380	phosphateNa$^+$ symporter
381	phosphatidate cytidylyltransferase	382	phosphatidylethanolamineKdo2-lipid A phosphoethanolamine transferase
383	phosphatidylglycerol-membrane-oligosaccharide glycerophosphotransferase	384	phosphatidylglycerophosphatase
385	Phosphatidylglycerophosphate synthase	386	Phosphoethanolamine transferase for glucans (OPG), alkaline phosphatase superfamily
387	phosphoglycerate mutase	388	phospholipidcholesterolgamma-HCH transport system permease protein
389	phospholipidcholesterolgamma-HCH transport system substrate-binding protein	390	Phospho-N-acetylmuramoyl-pentapeptide-transferase
391	phosphonate transport system permease protein	392	p-hydroxybenzoic acid efflux pump subunit

			AaeA
393	p-hydroxybenzoic acid efflux pump subunit AaeB	394	Pilin (type 1 fimbria component protein)
395	pilus assembly protein HofN	396	pilus assembly protein HofO
397	Pilus-assembly fibrillin subunit, chaperone	398	Polyferredoxin
399	potassium efflux system protein	400	potassiumproton antiporter, CPA1 family
401	Predicted arabinose efflux permease, MFS family	402	Predicted exporter
403	Predicted membrane protein (DUF2207)	404	Predicted membrane protein (DUF2238)
405	Predicted protein-tyrosine phosphatase	406	Predicted PurR-regulated permease PerM
407	prepilin peptidase dependent protein D	408	prepilin-type N-terminal cleavagemethylation domain-containing protein
409	preprotein translocase subunit SecD	410	prolineproton symporter, AAT family
411	prophage endopeptidase	412	protease FtsH subunit HflC
413	protease FtsH subunit HflK	414	protease-4
415	protein C Serine peptidase. MEROPS family S49	416	protein HokA
417	protein HokCD	418	protein PsiE
418	protein SanA, affects membrane permeability for vancomycin	419	protein translocase subunit secEsec61 gamma
420	protein translocase subunit secF	421	protein translocase subunit secG
422	protein translocase subunit secYsec61 alpha	423	protein translocase subunit yajC
424	protoheme IX farnesyltransferase	425	proton glutamate symport protein
426	proton-dependent oligopeptide transporter, POT family	427	psiF repeat-containing protein
428	PTS system ascorbate-specific IIC component, L-Asc family	429	PTS system D-fructose-specific IIB component (F1P-formin
430	PTS system D-galactosamine-specific EIIC component, Man family	431	PTS system D-glucose-specific IIB component, Glc family PTS system D-glucose-specific IIC component, Glc family
432	PTS system D-mannitol-speci	433	PTS system D-mannitol-specific IIB component, Fru family PTS system D-mannitol-speci
434	PTS system D-mannose-specific IIC component, Man family	435	PTS system D-mannose-specific IID component, Man family
436	PTS system D-sorbitol-specific IIB component, Gut family PTS system D-sorbitol-speci	437	PTS system D-sorbitol-specific IIC component, Gut family
438	PTS system galactitol-specific EIIC component, Gat family	439	PTS system IIC component, L-Asc family
440	PTS system L-sorbose-specific IIC component, Man family	441	PTS system L-sorbose-specific IID component, Man family
442	PTS system N,N'-diacetylchitobiose-specific IIC component, Lac family	443	PTS system N-acetylgalactosamine-specific EIIC component, Man family
444	PTS system N-acetylgalactosamine-specific EIID component, Man family	445	PTS system trehalose-specific IIB component, Glc family PTS system trehalose-specific
446	PTS system unknown substrate IIC component, Fru family	447	PTS system unknown substrate IIC component, Gat family
448	PTS system, cellobiose-specific IIC component	449 450	Putative 3TM holin, Phage_holin_3
451	putative ABC transport system permease protein	452	putative ABC transport system permease protein
453	putative colanic acid biosysnthesis UDP-glucose lipid carrier transferase	454	putative colanic acid polymerase
455	putative copper resistance protein D	456	putative efflux protein, MATE family
457	putative glucuronide porin	458	Putative inner membrane protein YbhQ

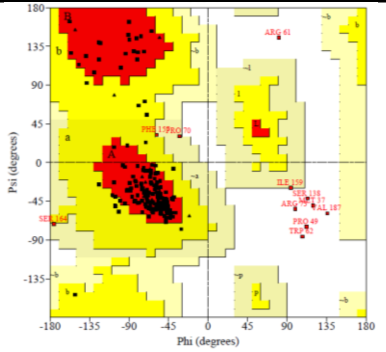
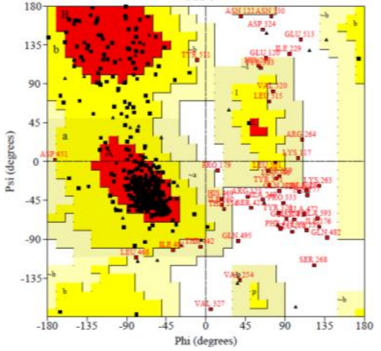
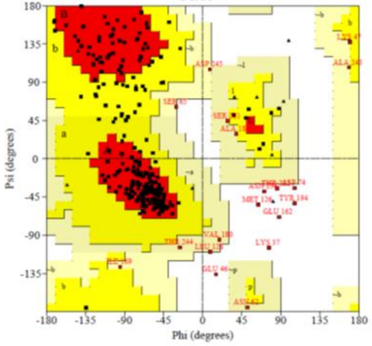
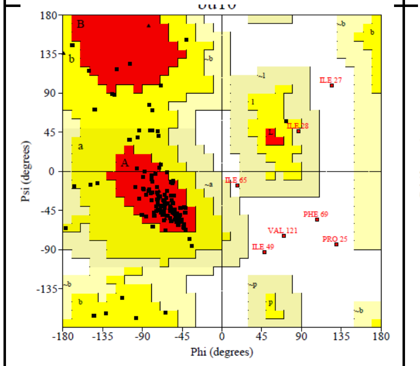
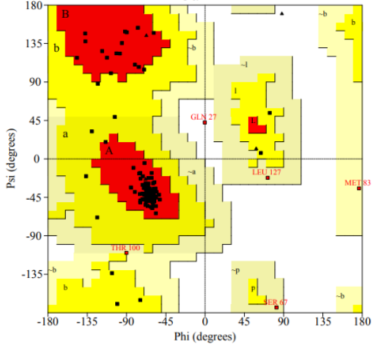
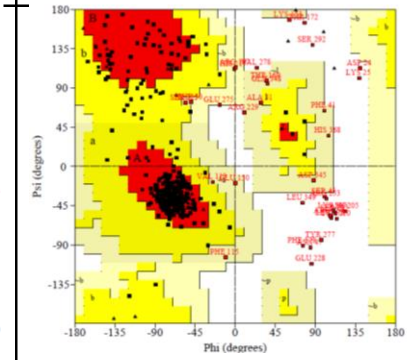
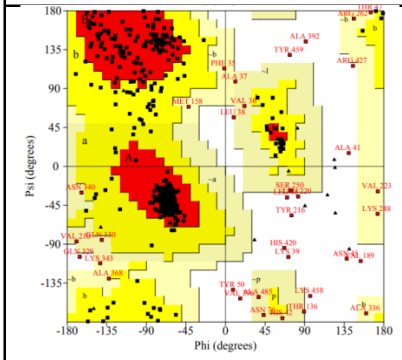
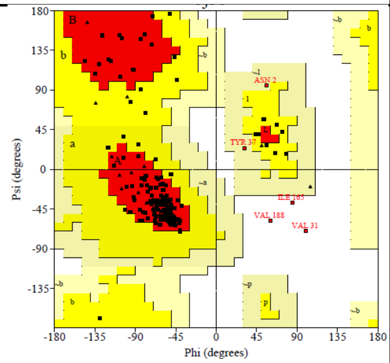
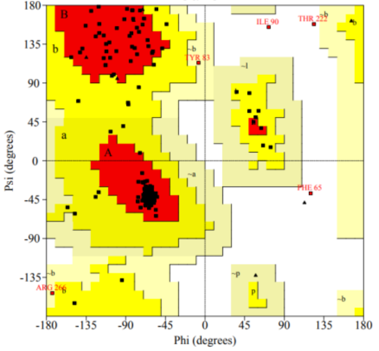
459	Putative inner membrane protein	460	putative membrane protein
461	putative MFS transporter, AGZA family, xanthineuracil permease	462	putative Mg^{2+} transporter-C (MgtC) family protein
463	Putative Mn^{2+} efflux pump MntP	464	putative peptidoglycan lipid II flippase
465	Putative phage holin	466	putative pseudouridine transporter
467	putative pyrimidine permease RutG	468	putative spermidineputrescine transport system permease protein
469	putative thiamine transport system permease protein	470	putative transport protein
471	putrescineornithine antiporter, APA family	472	putrescineproton symporter, AAT family
	Q		
473	quaternary ammonium compound-resistance protein SugE	474	quinoprotein glucose dehydrogenase
	R		
475	regulator RcnB of Ni and Co efflux	476	respiratory nitrate reductase gamma subunit
477	respiratory nitrite reductase specific cytochrome c biogenesis protein NrfE	478	respiratory nitrite reductase specific cytochrome c biogenesis protein NrfF
479	respiratory nitrite reductase specific cytochrome c biogenesis protein NrfG	480	respiratory nitrite reductase specific menaquinol--cytochrome-c reductase complex subunit NrfD
481	Rhodanese-related sulfurtransferase	482	ribosome-dependent ATPase
483	rod shape-determining protein MreC	484	rod shape-determining protein MreD
	S		
485	SanA protein	486	SecD export protein N-terminal TM region
487	Sec-independent protein translocase TatE	488	sensor c-di-GMP phosphodiesterase, contains CSS-motif sensor and EAL domain
499	Septal ring factor EnvC, activator of murein hydrolases AmiA and AmiB	500	serine protease autotransporter
501	serine transporter	502	serinethreonine transporter
503	SH3 domain protein	504	short-chain fatty acids transporter
505	Sigma-70, region 4	506	signal peptidase II Aspartic peptidase. MEROPS family A08
507	site-2 protease. Metallo peptidase. MEROPS family M50B	508	small conductance mechanosensitive channel
509	small multidrug resistance family-3 protein	510	small multidrug resistance pump
511	small toxic polypeptide LdrABCD	512	Small-conductance mechanosensitive channel
513	sodiumpantothenate symporter	514	sodiumproline symporter
515	sodiumproton antiporter, CPA1 family	516	sodiumproton antiporter, NhaA family
517	sodiumproton antiporter, NhaB family	518	soluteNa^{+} symporter, SSS family
519	spermidine export protein MdtI	520	spermidine export protein MdtJ
521	Spore maturation protein SpmB	522	succinate dehydrogenase subunit C
523	succinate dehydrogenase subunit D	524	sulfate permease, SulP family
525	sulfonate transport system permease protein	526	Superinfection exclusion protein B
527	Surface polysaccharide O-acyltransferase, integral membrane enzyme		
	T		
528	Tar ligand binding domain homologue	529	Tat-targeted selenate reductase subunit YnfH
530	taurine transport system permease protein	531	tellurite resistance protein
532	thiamine transport system permease protein	533	Thioldisulfide interchange protein DsbA
534	Thioldisulfide interchange protein DsbB	535	Thioldisulfide interchange protein DsbD
536	thioredoxin reductase (NADPH)	537	thiosulfate reductase cytochrome b subunit
538	threonine transporter	539	TIGR00645 family protein
540	TIGR01666 family membrane protein	541	TIGR02099 family protein

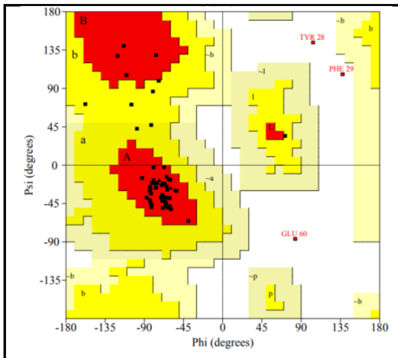
542	TIR domain-containing protein	543	trimethylamine-N-oxide reductase (cytochrome c), cytochrome c-type subunit TorY
544	trimethylamine-N-oxide reductase (cytochrome c), cytochrome c-type subunit TorC	546	tRNA A37 threonylcarbamoyladenosine dehydratase
547	tryptophan-specific transport protein	548	two-component system, CitB family, cit operon sensor histidine kinase CitA
549	two-component system, CitB family, sensor histidine kinase DcuS	550	two-component system, NarL family, capsular synthesis sensor histidine kinase RcsC
551	two-component system, NarL family, nitratenitrite sensor histidine kinase NarX	552	two-component system, NarL family, sensor histidine kinase RcsD
553	two-component system, NarL family, sensor histidine kinase UhpB	554	two-component system, NtrC family, C4-dicarboxylate transport sensor histidine kinase DctB
555	two-component system, NtrC family, sensor histidine kinase HydH	556	two-component system, OmpR family, heavy metal sensor histidine kinase CusS
557	two-component system, OmpR family, osmolarity sensor histidine kinase EnvZ	558	two-component system, OmpR family, sensor histidine kinase BaeS
559	two-component system, OmpR family, sensor histidine kinase CreC	560	two-component system, OmpR family, sensor histidine kinase KdpD
561	two-component system, OmpR family, sensor histidine kinase PhoQ	562	two-component system, OmpR family, sensor histidine kinase QseC
563	two-component system, OmpR family, sensor histidine kinase RstB	564	two-component system, OmpR family, sensor kinase
565	Two-component-system connector protein	566	type 4 prepilin peptidase 1 Aspartic peptidase. MEROPS family A24A
567	type II secration system protein I (GspI)	568	type II secration system protein J (GspJ)
569	type II secretion system protein A	570	type II secretion system protein B
571	type II secretion system protein C (GspC)	572	type II secretion system protein G (GspG)
573	type II secretion system protein H (GspH)	574	type II secretion system protein K (GspK)
575	type II secretion system protein M (GspM)	576	type II secretion system protein
577	type VI secretion system protein ImpK	578	TypeIIsecration system protein F (GspF)
579	tyrosine-protein kinase EtkWzc	580	tyrosine-specific transport protein
	U		
581	UDP-N-acetylmuramoylalanine--D-glutamate ligase	582	Uncharacterised protein family protein
583	Uncharacterized membrane protein YdjX, TVP38TMEM64 family, SNARE-associated domain	584	Uncharacterized membrane protein YeiH
585	Uncharacterized membrane protein HdeD, DUF308 family	586	Uncharacterized membrane protein YgdD, TMEM256DUF423 family
587	Uncharacterized membrane protein YhaH, DUF805 family	588	Uncharacterized membrane protein YhdT
589	Uncharacterized membrane protein YhhN	590	Uncharacterized membrane protein YkgB
591	Uncharacterized membrane protein YqaE, homolog of Blt101, UPF0057 family	592	Uncharacterized membrane protein YqiK, contains Band7PHBSPFH domain
593	uncharacterized protein	594	undecaprenyl phosphate-alpha-L-ara4N flippase subunit ArnE
595	undecaprenyl phosphate-alpha-L-ara4N flippase subunit ArnF	596	Undecaprenyl-diphosphatase
597	undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase	598	universal stress protein B

599	UPF0716 protein FxsA	600	UPF0755 protein
	V		
601	vitamin B12 transport system permease protein	602	voltage-gated potassium channel
	X		
603	xanthine permease XanP	604	xanthine permease XanQ
605	xanthine permease	606	xylose ABC transporter membrane protein
	Y		
607	YebO-like protein	608	YggT family protein
609	YniB-like protein	610	YobH-like protein
611	YqjK-like protein		
	Z		
612	zinc resistance-associated protein	613	zinc transport system permease protein
614	zinc transporter, ZIP family		

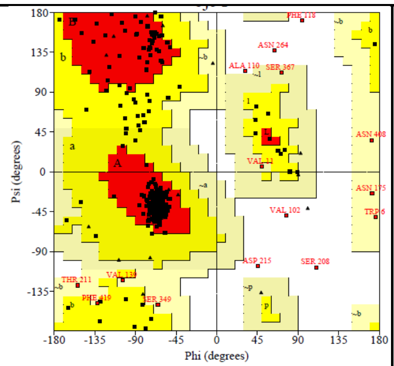
4.3.3. Tertiarystructure prediction and quality assessment

To perform the molecular docking study tertiary protein structures were needed. Selected 330 trans-membrane protein sequences were subjected to homology modelling against available multiple X-ray crystallographic structures present in the structure database. All the predicted structures were subjected to structural quality assessment through Ramachandran plot analysis. Among 330 proteins, 13.13% structures showed amino acids in between 90-100% of allowed regions, 68.68% structures showed amino acids in between 80-89% and 18.18% structures showed amino acids in between 70-79% of allowed regions. Most structures in 80-89% allowed region group were found with 90% accuracy value when added with their respective additionally allowed region values. 23 good quality structures were represented through Ramachandran plot (Fig- 4.2), where the range of added percent value of residues in most favoured regions (A,B,L) and residues in additional allowed regions (a,b,l,p) was 91.8-99%. Two structures namely Alanine or glycinecation symporter AGCS family and YibEF-like protein showed 89.8% and 89.9% amino acids within most favoured and additional allowed regions respectively. Good quality PDB among all the predicted structures were selected for molecular docking study with the inhibitor *p*-CA.

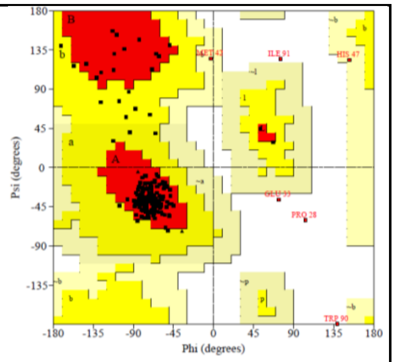
		
1. ABC-2 family transporter protein (88.3 %+ 6.9% = 95.2%)	3. Alanine or glycinecation symporter AGCS family (76.6 %+ 13.2% = 89.8%)	9. Beta- N-acetylglucosaminidase (73.9 %+ 18.8% = 92.7%
		
10. Biotin biosynthesis protein BioX (74.8%+20.9%= 95.7%)	13. CAAX protease self-immunity (90.4 %+ 6.6% = 97%)	14. Capsular polysaccharide biosynthesis protein (80.8%+11.1%= 91.9%)
		
16. Cationic antimicrobial peptide transport system permease protein (79.7%+14.4% = 94.1%)	17. CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase (84.5% +12.5% = 97%)	19. Cobalt transport protein (87.9%+10.1% = 99%)



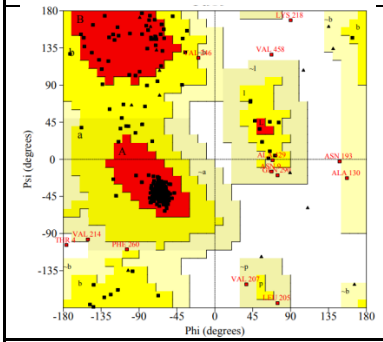
29. General stress protein CsbA (82.5%+12.3% = 94.8%)



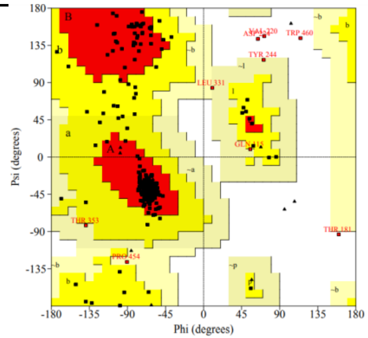
30. Gluconate permease GntP (84.5% +11.6% = 96.1%)



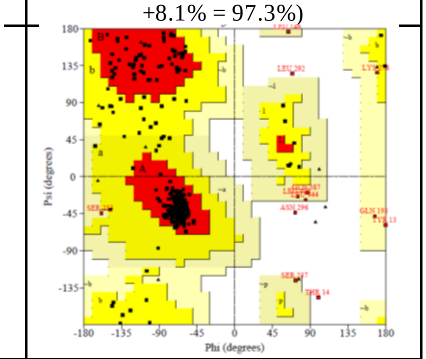
40. Membrane protein DedA, SNARE-associated domain (89.2% +8.1% = 97.3%)



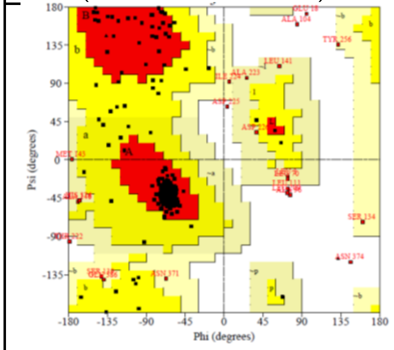
47. MFS transporter, DHA2 family, multidrug resistance protein (86.6%+10.1% = 96.7%)



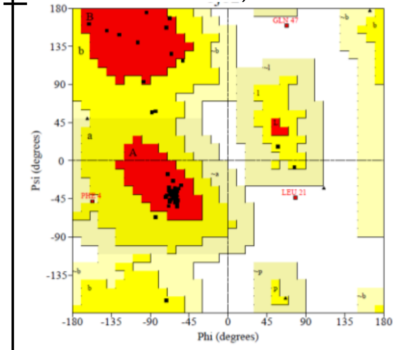
48. MFS transporter, MHS family, prolinebetaine transporter (88%+10% = 98%)



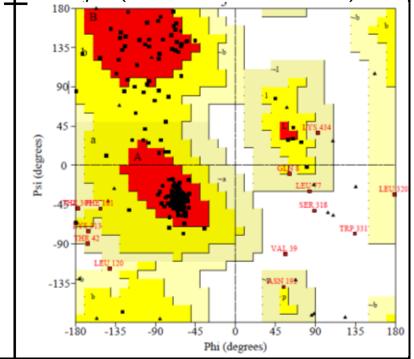
57. Nucleoside recognition GATE domain-containing membrane protein YjiH (85.3% + 11.7% = 97%)



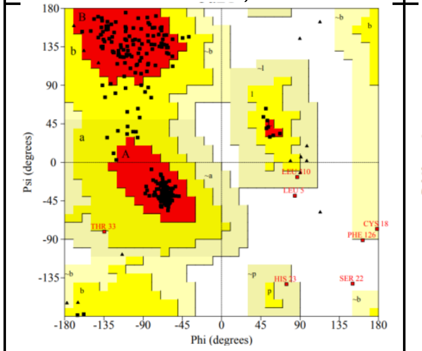
69. Predicted arabinose efflux permease, MFS family (85.0%+8.6% = 93.6%)



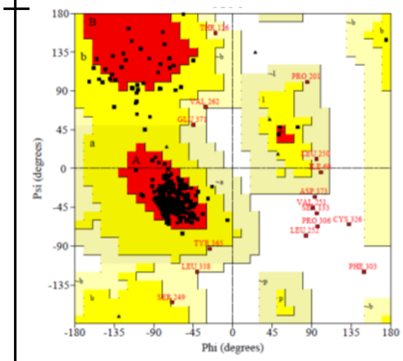
71. Preprotein translocase subunit SecG (85.1% +10.4 % = 95.5 %)



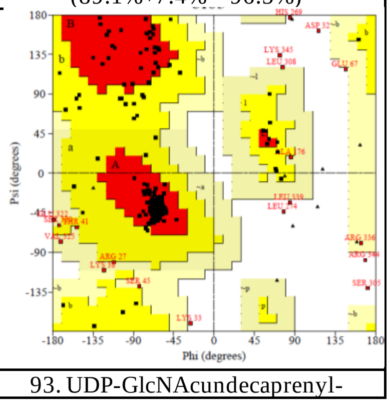
84. Serine threonine exchange transporter, LAT family (89.1%+7.4% = 96.5%)



86. Spermidineputrescine transport



88. Thioldisulfide interchange protein



93. UDP-GlcNAcundecaprenyl-

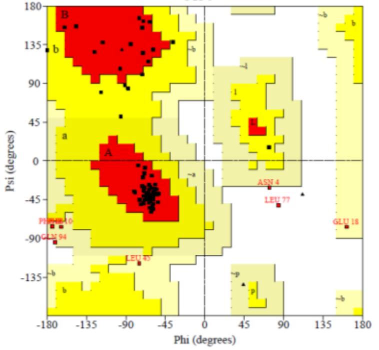
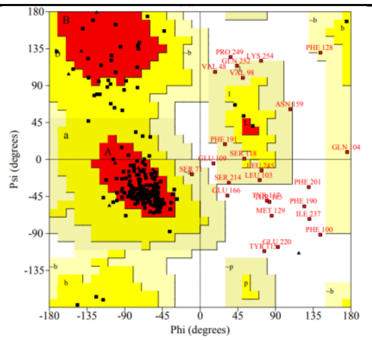
system substrate-binding protein (89.3%+ 8.5% = 97.8%)	DsbA (87.2%+8.6% = 95.8%)	phosphate GlcNAc-1-phosphate transferase (83.8%+10.0% = 93.8%)
		
97. YggT family protein (82.4%+9.4%=91.8)	99. YibEF-like protein (74.8%+15.1% = 89.9%)	

Fig4.2- Ramachandran plot analysis of some good quality structures [Residues in most favoured regions (A,B,L)% + Residues in additional allowed regions (a,b,l,p)%]

4.3.4. Molecular docking analysis with p-CA

To understand the actual inhibition pattern of *p*-CA, predicted good quality structures were individually used as receptor against the 3D ligand structure of *p*-CA. Molecular docking analysis revealed that *p*-CA have multidimentional inhibition properties (Table 4.3). Based on Atomic Contact Energy (ACE) value calculated through Patchdock, *p*-CA showed higher affinity towards 99 trans-membrane protein structures of *S. aureus* listed in Table 4.3. Among them 62 proteins were transport proteins, 24 proteins were involved with different functional responses, 11 proteins were found as different molecule synthesising proteins and 2 were unknown proteins.

ACE value comparison among all the interaction patterns indicated that *p*-CA more rigidly and frequently interacts mostly with the maximum ion exchange channel proteins like citrate-$Mg^{2+}H^+$ or citrate-$Ca^{2+}H^+$ symporter, CitMHS family, F-type H^+-transporting ATPase subunit A, iron complex transport system permease protein, multisubunitsodiumproton antiporter, MrpA subunit, Mn^{2+} and Fe^{2+} transporters of the NRAMP familyetc.Ion exchange channels are responsible for flagellar locomotionand maintaining osmotic pressure of the cellular compartment (Martinac et al. 2008). Ions are also required for some enzymatic activities. Inhibition of those symporter and antiporter proteins in the cell membrane leads to accumulation of osmotic stress on the treated celland may cease the enzymatic and locomotoryactivities also.

Moreover, *p*-CA also showed affinity towards different amino acids, peptides andcarbohydrate permease proteins like ABC-2 family transporter protein, alanine or glycine cation symporter, AGCS family, Branched-chain amino acid transport protein, PTS system lactose-specific IIB component, Lac family PTS system lactose-specific IIC component, Lac family, PTS system sucrose-specific IIB component, Glc family PTS system sucrose-specific IIC component, Glc family, PTS system trehalose-specific IIB component, Glc family PTS system trehalose-specific IIC component, Glc family. Interaction and inhibition of those proteins may lead to nutrition depletion from the cells. Some other interactions also found which indicated that *p*-CA may inhibit the peptidoglycan systhesis of bacteria. Some important docking results have been represented in Fig- 4.3.

Table 4.3- Selected proteins on the basis of ACE value in molecular docking using Patchdock (T=Transport, R=Response, S=Synthesis)

Sl. No.	Name of the protein	Lowest ACE value
1T	ABC-2 family transporter protein	**-205**
2T	ABC-2 type transport system permease protein	**-151**
3T	alanine or glycine cation symporter, AGCS family	**-241**
4T	amino acid ABC transporter substrate-binding protein, PAAT family amino acid ABC transporter membrane protein, PAAT family	**-188**
5T	aminobenzoyl-glutamate transport protein	**-147**
6T	ammonium transporter	**-159**
7R	arsenite efflux membrane protein ArsB	**-169**
8S	ATP synthase F0 subcomplex B subunit	**-155**
	B	
9S	Beta- N-acetylglucosaminidase	**-205**
10S	biotin biosynthesis protein BioX	**-233**
11T	Branched-chain amino acid transport protein	**-173**
12T	branched-chain amino acid cation transporter, LIVCS family	**-191**
	C	
13R	CAAX protease self-immunity	**-210**
14S	Capsular polysaccharide biosynthesis protein	**-204**
15S	capsular polysaccharide synthesis enzyme	**-186**
16T	cationic antimicrobial peptide transport system permease protein	**-213**

17T	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	**-249**
18T	citrate-Mg^{2+}H^{+} or citrate-Ca^{2+}H^{+} symporter, CitMHS family	**-192**
19T	cobalt transport protein	**-241**
20R	competence protein ComEA	**-169**
21R	conserved hypothetical integral membrane protein	**-160**
22R	cytochrome bd-I ubiquinol oxidase subunit 2 apoprotein	**-185**
	D	
23T	divalent anionNa^{+} symporter, DASS family	**-160**
24T	D-methionine transport system permease protein	**-146**
	E	
25T	energy coupling factor transporter S component ThiW	**-189**
26R	exfoliative toxin AB	**-176**
	F	
27T	F-type H^{+}-transporting ATPase subunit a	**-174**
28T	Fucose 4-O-acetylase	**-188**
	G	
29R	General stress protein CsbA	**-211**
30T	gluconate permease GntP	**-221**
	I	
31R	Inhibitor of apoptosis-promoting Bax1	**-179**
32R	inner membrane protein	**-182**
33R	integral membrane protein, YkoY family	**-181**
34T	iron complex transport system permease protein	**-152**
35T	ironzinccopper transport system permease protein	**-162**
	L	
36T	lactate permease	**-174**
37R	LexA-binding, inner membrane-associated putative hydrolase	**-186**
38T	lysineproton symporter, AAT family	**-162**
	M	
39S	Membrane protein CcdC involved in cytochrome C biogenesis	**-194**
40S	membrane protein DedA, SNARE-associated domain	**-212**
41T	membrane protein involved in D-alanine export	**-199**
42R	membrane protein YdbS, contains bPH2 (pleckstrin homology) domain	**-171**
43R	Membrane proteinase PrsW, cleaves anti-sigma factor RsiW, M82 family	**-195**
44S	Membrane-bound acyltransferase YfiQ, involved in biofilm formation	**-168**
45T	MFS transporter, DHA1 family, bicyclomycinchloramphenicol resistance protein	**-166**
46T	MFS transporter, DHA1 family, multidrug resistance protein	**-197**
47T	MFS transporter, DHA2 family, multidrug resistance protein	**-215**
48T	MFS transporter, MHS family, prolinebetaine transporter	**-209**
49T	MFS transporter, NNP family, putative nitrate transporter	**-197**
50T	MFS transporter, OPA family, glycerol-3-phosphate transporte	**-179**
51T	Mn^{2+} and Fe^{2+} transporters of the NRAMP family	**-191**
52T	multisubunitsodiumproton antiporter, MrpA subunit	**-173**
53T	multisubunitsodiumproton antiporter, MrpB subunit	**-157**
54R	MutS domain V	**-175**
	N	
55S	NAD(P)H-quinone oxidoreductase subunit 5	**-151**
56R	Nucleoside recognition	**-164**
57R	nucleoside recognition GATE domain-containing membrane protein YjiH	**-202**
	O	
58T	oligopeptide transport system permease protein	**-171**
	P	
59R	Peptidase M50B-like	**-188**
60T	peptidenickel transport system permease protein	**-187**
61R	phage protein	**-164**
62T	phosphate ABC transporter membrane protein 1, PhoT family	**-136**
63T	phosphate ABC transporter membrane protein 2, PhoT family	**-181**
64T	phosphatidylglycerol lysyltransferase	**-177**
65T	Phospho-N-acetylmuramoyl-pentapeptide-transferase	**-171**

66T	Phosphotransferase system, fructose-specific IIC component	**-181**
67T	polysaccharide export protein, MPA1 family	**-149**
68T	potassium uptake protein, TrkH family	**-180**
69T	Predicted arabinose efflux permease, MFS family	**-206**
70T	preprotein translocase subunit SecE	**-169**
71T	preprotein translocase subunit SecG	**-224**
72T	preprotein translocase subunit SecY	**-187**
73T	preprotein translocase subunit SecF	**-188**
74T	preprotein translocase subunit SecY	**-165**
75T	protein translocase subunit yajC	**-190**
76T	proton glutamate symport protein	**-156**
77T	proton-dependent oligopeptide transporter, POT family	**-161**
78T	PTS system lactose-specific IIB component, Lac family PTS system lactose-specific IIC component, Lac family	**-159**
79S	PTS system N-acetylglucosamine-specific IIB component, Glc family PTS system N-acetylglucosamine-specific IIC component, Glc family	**-179**
80T	PTS system sucrose-specific IIB component, Glc family PTS system sucrose-specific IIC component, Glc family	**-163**
81T	PTS system trehalose-specific IIB component, Glc family PTS system trehalose-specific IIC component, Glc family	**-171**
	R	
82R	rhomboid protease GluP	**-168**
83T	Riboflavin transporter FmnP	**-187**
	S	
84T	Serine threonine exchange transporter, LAT family	-220
85R	sortase B	**-155**
86T	spermidineputrescine transport system substrate-binding protein	**-201**
87S	Sugar transferase involved in LPS biosynthesis (colanic, teichoic acid)	**-165**
	T	
88R	Thioldisulfide interchange protein DsbA	**-208**
89T	ThreonineSerine exporter, ThrE	**-145**
90R	TM2 domain-containing protein	**-187**
91T	transporter family-2 protein	**-172**
92R	two-component system, LytT family, sensor histidine kinase LytS	**-138**
	U	
93T	UDP-GlcNAcundecaprenyl-phosphate GlcNAc-1-phosphate transferase	**-205**
94T	uracil permease	**-173**
95T	urea transporter	**-174**
	X	
96T	xanthine permease	**-188**
	Y	
97R	YggT family protein	**-217**
98	YhgEPip N-terminal domain-containing protein	**-189**
99	YibEF-like protein	**-214**

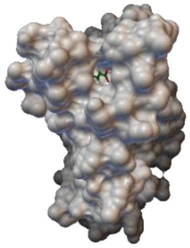
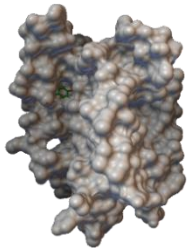
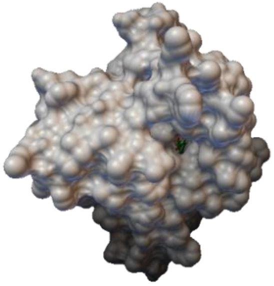
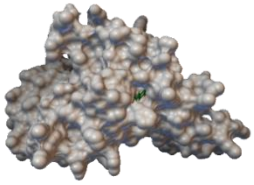
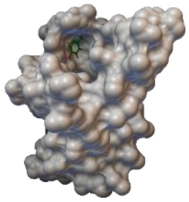
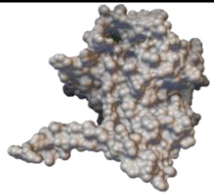
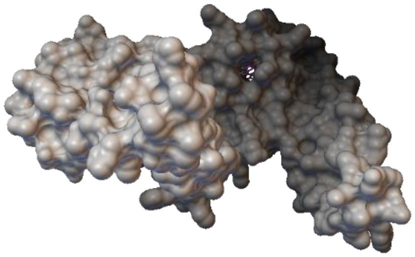
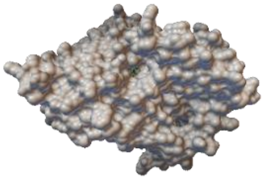
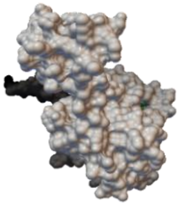
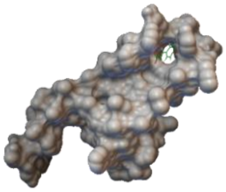
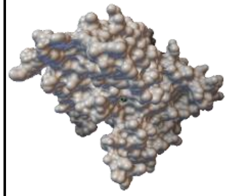
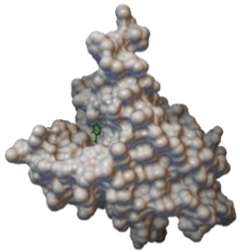
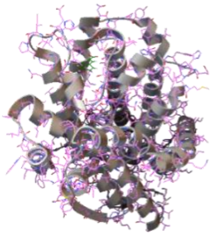
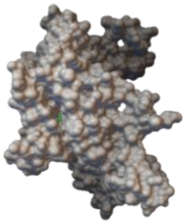
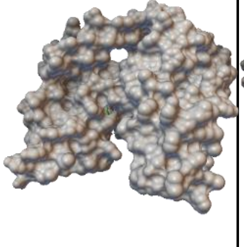
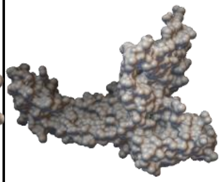
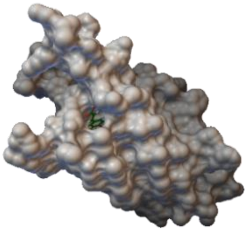
				
ABC-2 family transporter protein	**ABC-2 type transport system permease protein**	**Alanine or glycine cation symporter, AGCS family**	**Beta- N-acetylglucosaminidase**	**Biotin biosynthesis protein BioX**
				
Capsular polysaccharide biosynthesis protein	**Cobalt transport protein**		**Divalent anionNa+ symporter, DASS family**	**Fucose 4-O-acetylase**
				
General stress protein CsbA	**Gluconate permease GntP**	**Ironzinccopper transport system permease protein**	**Internal blocking with Mn2+ and Fe2+ transporters of the NRAMP family**	**Phosphate ABC transporter membrane protein 2, PhoT family**
				
Phosphotransferase system, fructose-specific IIC component	**Predicted arabinose efflux permease, MFS family**	**PTS system lactose-specific IIB component**	**Riboflavin transporter FmnP**	**Internal blocking with Serine threonine exchange transporter, LAT family**

Fig4.3 -Some predicted membrane protein structures of *S. aureus* and their docking with *p*-CA

4.3.5. Sequential comparison between trans-membrane proteins of S. aureus and E. coli through phylogenetic tree

The selected 614 sequences of *E. coli* were individually aligned with 330 selected trans-membrane protein sequences of *S. aureus*. According to phylogenetic trees, 72 sequences were found to share sequential similarities (Fig- 4.4). Among them 59 sequences showed functional similarities whereas 13 sequences showed sequential similarities but functional dissimilarities (Table 4.4).12 sequences from functionally similar group, such as, ammonium transporter, CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase, conserved hypothetical integral membrane protein, D-methionine transport system permease protein, gluconate permease GntP, MFS transporter, DHA2 family, multidrug resistance protein, MFS transporter, OPA family, glycerol-3-phosphate transporter, Phospho-N-acetylmuramoyl-pentapeptide-transferase, protein translocase subunit secG, proton glutamate symport protein, proton-dependent oligopeptide transporter, POT family and transporter family-2 protein showed higher affinity of docking with *p*-CA. To understand the structural similarity, these 12 protein structures werealigned with each other (Fig- 4.5) where6 sequences namelyammonium transporter protein, D-methionine transport system permease protein, MFS transporter, OPA family, glycerol-3-phosphate transporter, Phospho-N-acetylmuramoyl-pentapeptide-transferase, proton glutamate symport protein and transporter family-2 protein were found to have very high structural similarity and clearly presented in Table 4.5.The affinity of *p*-CA with those selected protein molecules were found to be higher than others (Fig- 4.6). As per the ACE value (ranged from -205 to -249), the proper channel blocking ofsome transporter proteins by *p*-CA was best observed for CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase (-249) followed by biotin biosynthesis protein BioX (-233), preprotein translocase subunit SecG (-224), gluconate permease GntP (-221), Serine threonine exchange transporter, LAT family (-220), MFS transporter, DHA2 family, multidrug resistance protein (-215), MFS transporter, MHS family, proline betaine transporter (-209) and amino acid ABC transporter substrate-binding protein, PAAT family (-205).

p-CA was found to interact with CDP-diacylglyceral-glycerol-3-phosphate 3-phosphatidyle transferase enzyme of both the selected pathogens (*S. aureus* and *E. coli*). This protein is composed of six α-helix chains, which formed a barrel like structure through transmembrane. According toHirabayashi et al. (1976), CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase is the key enzyme to regulate thesynthesis of phosphatidyl glycerol (PG) which is a very important integral membrane protein tightly associated with the cytoplasmic cell membrane of bacteria. In *S. aureus*, lipoteichoic acid (LTA) contains repeating units of *sn*-glycerol phosphate groups which are the derivative of PG. During the transformation from PG to *sn*-glycerol phosphate, 1,2-diacylglyerol (DAG) is produced as byproduct (Hirabayashi et al. 1976, Jerga et al. 2007, Peleg et al.2012 and Taron et al. 1983).Over accumulation of DAG has a lethal effect on bacterial membrane (Jergaet al. 2007).To avoid this, DAG is being converted back to PG by a four step reaction involving the first enzyme as diacylglycerol kinase, second phosphatidate cytidylyltransferase, third CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase and last one phosphatidylglycerophosphatase.On the other hand, in Gram negative *E. coli* ,CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferasealso plays an important role in regulation of DAG during lipid biosynthesis, lipid metabolism, phospholipid biosynthesis and phospholipid metabolism(Dowhan1992). In this regard, inhibition of a cell membrane bound enzyme, CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase by *p*-CA will influence the accumulation of lethal DAG in bacterial cell and gradual lysis of bacterial cell membrane will occur. This may be the membrane lysis pathway of *p*-CA treatment. According to the Atomic Contact Energy (ACE) value during molecular docking analysis,*p*-CA was found to interact with the LEU 90 and SER 46 of CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase in*S. aureus* (Fig- 4.7). This position resided within transport channel. Whereas, in case of *E. coli*, *p*-CA was found to docked with ALA 43 and THR 132 of CDP -diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase (Fig 4.8). The present findings could able to explain the actual molecular mechanism of membrane damage in *S. aureus* as well as in *E. coli* and this discovery may leads to the better understanding about target specific drug delivery.

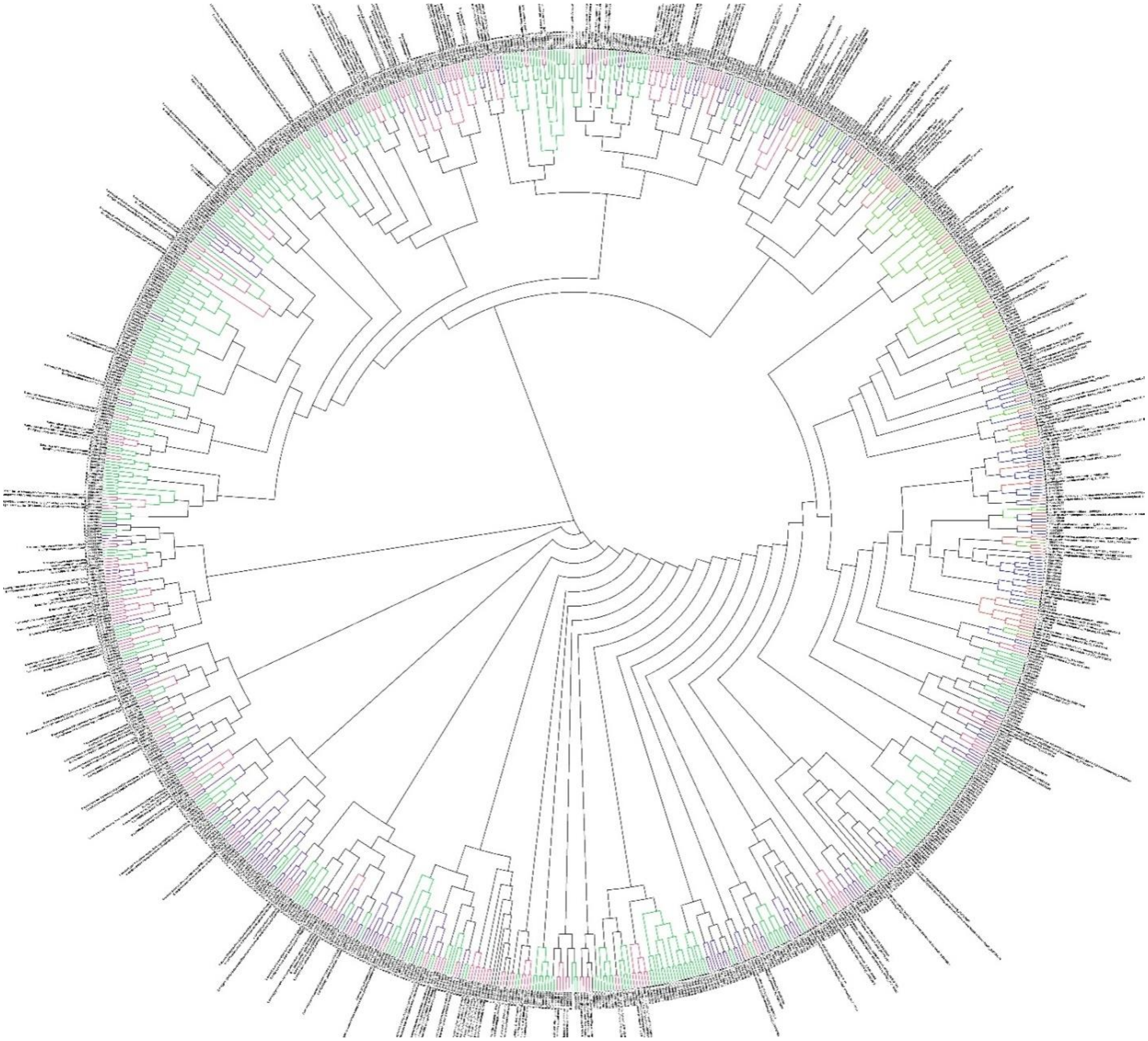
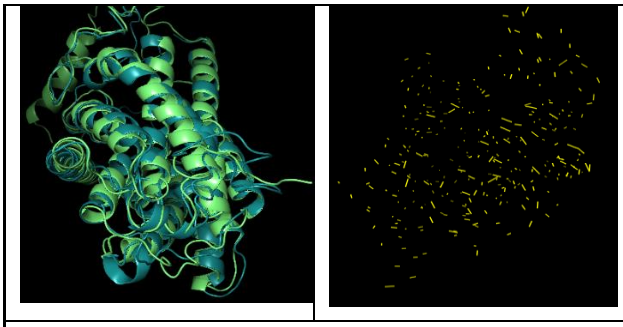


Fig4.4-Phylogenetic tree of all the trans-membrane proteins from *S. aureus* and *E. coli*. The matched sequences were presented elaborately in the figure (Red=match sequences between *S. aureus* and *E. coli;* Blue=only from *S. aureus*; Green=only from *E. coli*)

Table 4.4- Some common or phylogenetically related membrane protein for *S.aureus* and *E. coli.* The bold and star (*) sequences showed similarity with 99 trans-membrane proteins of *S. aureus*, with which *p*-CA was docked rigidly

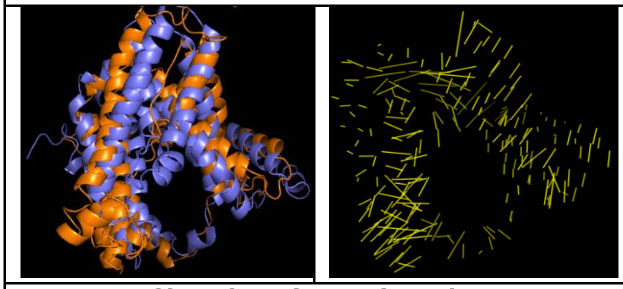
Staphylococcus aureus* and *Escherichia coli	
	Functionally Similar sequences
1	ABC-2 type transport system permease protein
2	acyl-phosphate glycerol-3-phosphate acyltransferase
3	alanine or glycinecation symporter, AGCS family
4	amino acid ABC transporter membrane protein
5	***ammonium transporter**
6	anion transporter
7	4-azaleucine resistance probable transporter AzlC
8	Branched-chain amino acid transport protein
9	branched-chain amino acidcation transporter, LIVCS family
10	Capsular polysaccharide
11	Cationic peptide transport system permease protein
12	***CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase**
13	citratesuccinate antiporter
14	competence protein Com
15	***conserved hypothetical integral membrane protein**
16	divalent anionNa+ symporter, DASS family
17	***D-methionine transport system permease protein**
18	D-serineD-alanineglycineprotonsymporter, AAT family
19	Energy coupling factor transport
20	ferrous iron transport protein B
21	Fucose
22	***gluconate permease GntP**
23	glutamateNa^+ symporter, ESS family
24	hemolysin III
25	inner membrane protein
26	integral membrane protein
27	iron complex transport system permease protein
28	Lactate permease
29	lysineproton symporter, AAT family
30	manganeseiron transport system permease protein
31	membrane protein DedA, SNARE-associated domain
32	MFS transporter, DHA1 family, bicyclomycinchloramphenicol resistance protein
33	MFS transporter, DHA1 family, multidrugchloramphenicol efflux transport protein
34	***MFS transporter, DHA2 family, multidrug resistance protein**
35	***MFS transporter, OPA family, glycerol-3-phosphate transporter**
36	MFS transporter, OPA family, hexose phosphate transport protein UhpT
37	Nucleoside recognition
38	oligopeptide transport system permease protein
39	peptidenickel transport system permease protein
40	phage protein
41	Phosphatidylglycerol
42	***Phospho-N-acetylmuramoyl-pentapeptide-transferase**
43	Predicted arabinose efflux permease, MFS family
44	***protein translocase subunit secG**
45	***proton glutamate symport protein**
46	***proton-dependent oligopeptide transporter, POT family**
47	PTS system N acetyl

48	PTS system trehalose-specific IIB component, Glc family PTS system trehalose-specific IIC component, Glc family	
49	putative MFS transporter, AGZA family, xanthineuracil permease	
50	respiratory nitrate reductase gamma subunit	
51	serinethreonine transporter	
52	Thioldisulfide interchange protein DsbA	
53	*transporter family-2 protein	
54	two-component system, LytT family, sensor kinase	
55	UDP-N-acetylmuramoylalanine--D-glutamate ligase	
56	Uncharacterized membrane protein YgdD, TMEM256DUF423 family	
57	Undecaprenyl-diphosphatase	
58	xanthine permease	
59	YggT family protein	
	Staphylococcus aureus	***Escherichia coli***
	Sequentially Similar but Functionally Dissimilar sequences	
1	*CAAX protease self-immunity	*oligogalacturonide transporter (2598529651)
2	*cytochrome bd-I ubiquinol oxidase subunit 2 apoprotein	*intracellular septation protein(2598528459)
3	*energy coupling factor transporter S component ThiW	*allantoin permease (2598529381)
4	*integral membrane protein, YkoY family	*Membrane protein TerC, possibly involved in tellurium resistance (2598527937)
5	*LexA-binding, inner membrane-associated putative hydrolase	*YniB-like protein (2598528023)
6	*membrane protein involved in D-alanine export	*L-alanine exporter (2598526862)
7	*Mn^{2+} and Fe^{2+} transporters of the NRAMP family	*cation:H^+ antiporter (2598526181)
8	*NAD(P)H-quinone oxidoreductase subunit 5	*inner membrane protein (2598526281)
9	*Nucleoside recognition	*Spore maturation protein SpmB (2598527342)
10	*Phosphotransferase system, fructose-specific IIC component	*modulator of FtsH protease (2598528977)
11	*rhomboid protease GluP	*GlpG protein (2598525958)
12	*uracil permease	*uraA uracil-xanthine permease (650107291)
13	*YggT family protein	*putative transport protein (2598525654)

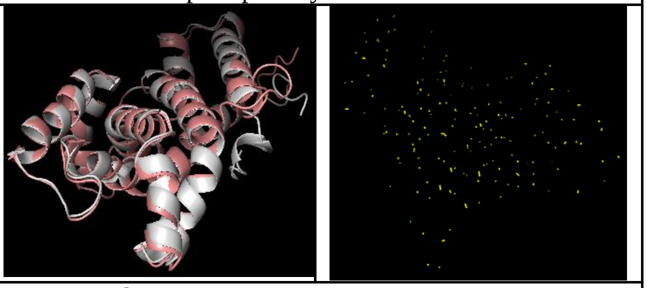


Ammonium transporter protein

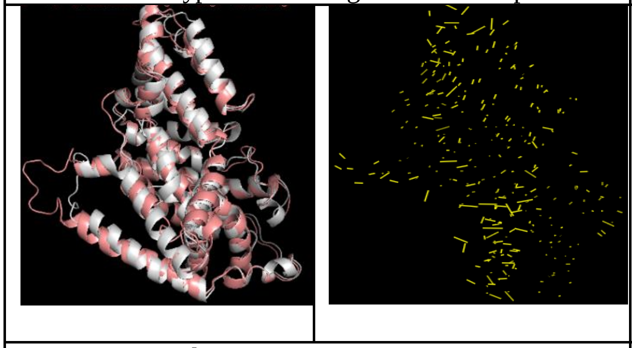
CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase



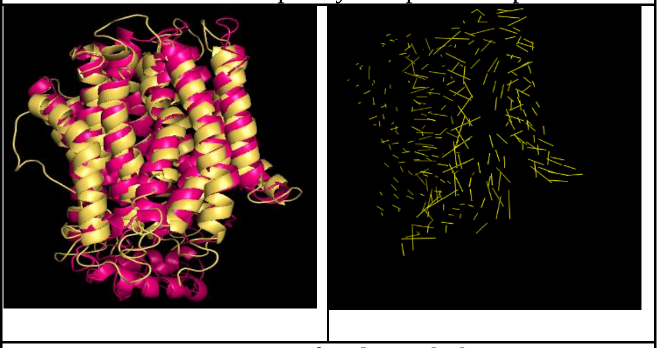
conserved hypothetical integral membrane protein



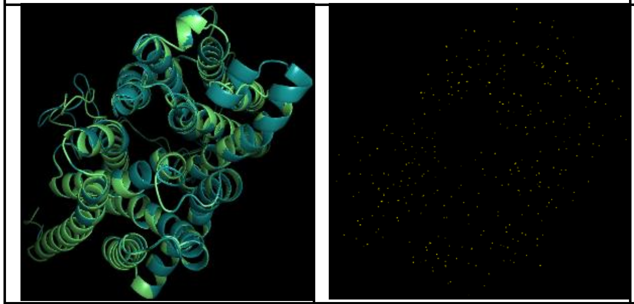
D-methionine transport system permease protein



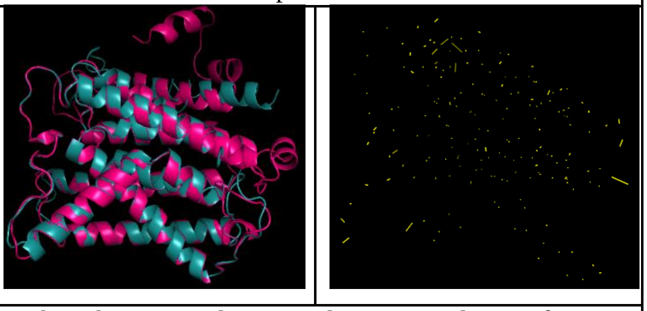
gluconate permease GntP



MFS transporter, DHA2 family, multidrug resistance protein



MFS transporter, OPA family, glycerol-3-phosphate transporter



Phospho-N-acetylmuramoyl-pentapeptide-transferase

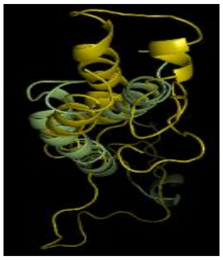
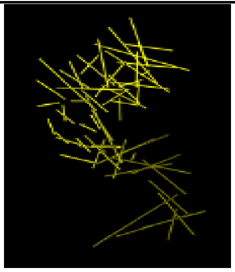
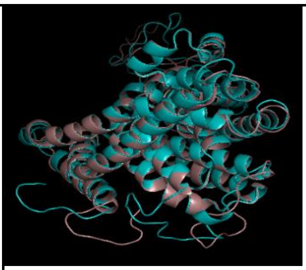
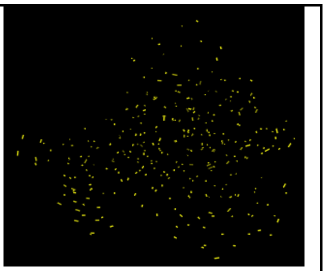
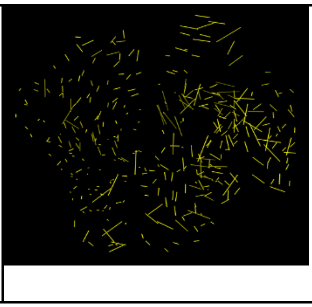
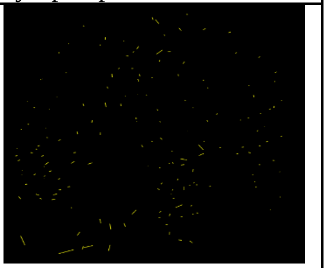
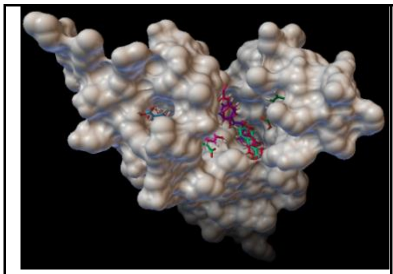
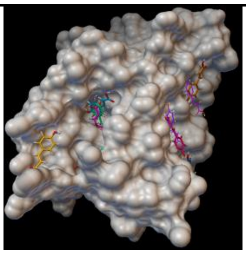
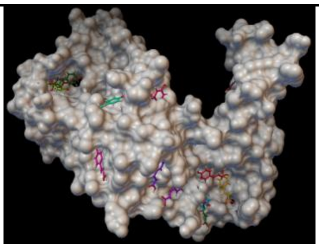
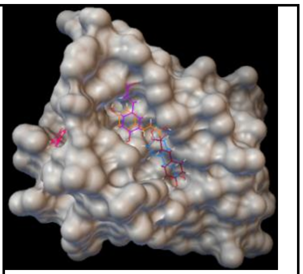
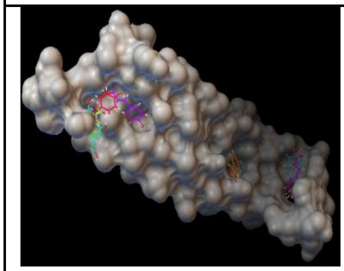
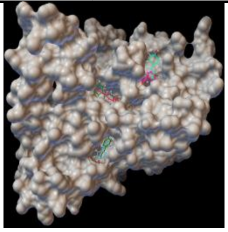
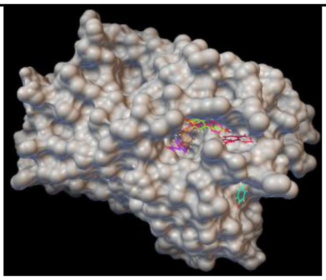
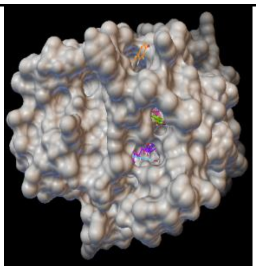
			
protein translocase subunit secG		proton glutamate symport protein	
			
proton-dependent oligopeptide transporter, POT family		transporter family-2 protein	

Fig4.5- Some structurally similar protein present in *S. aureus* and *E. coli*

			
CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase_12 (-249)	alanine or glycine cation symporter, AGCS family (-241)	cobalt transport protein (-241)	biotin biosynthesis protein BioX (-233)
			
preprotein translocase subunit SecG (-224)	gluconate permease GntP (-221)	Serinethreonine exchange transporter, LAT family (-220)	MFS transporter, DHA2 family, multidrug resistance protein (-215)

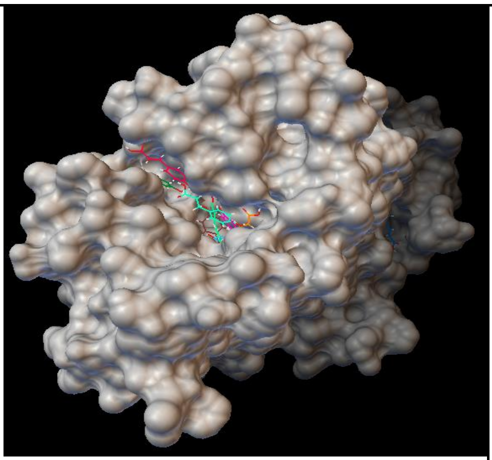
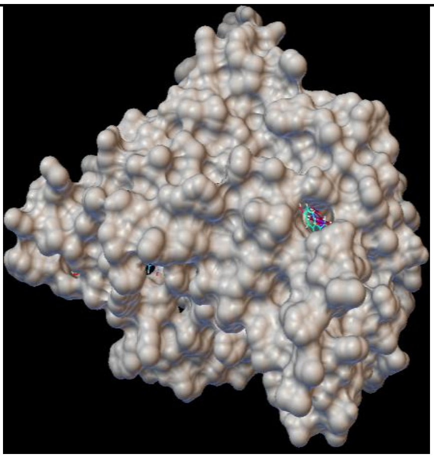
	
MFS transporter, MHS family, prolinebetaine transporter (-209)	amino acid ABC transporter substrate-binding protein, PAAT family (-205)

Fig 4.6- Molecular docking results of *p*-CA with different proteins showing high Atomic Contact Energy(ACE) value

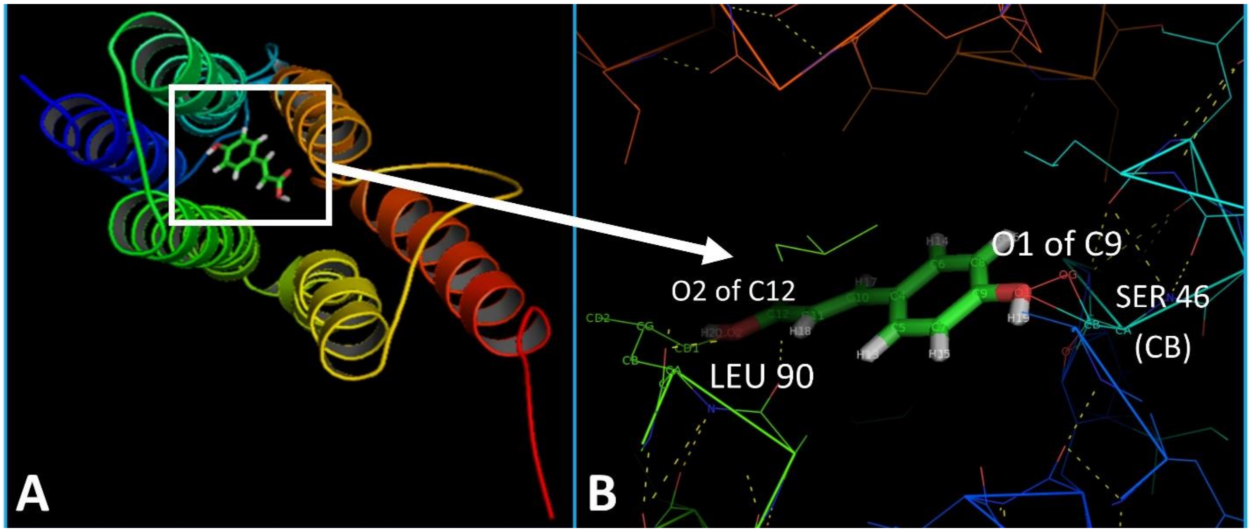


Fig4.7- Docking site (A) and rigid bond pattern (B) of *p*-CA within CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase of *S. aureus*

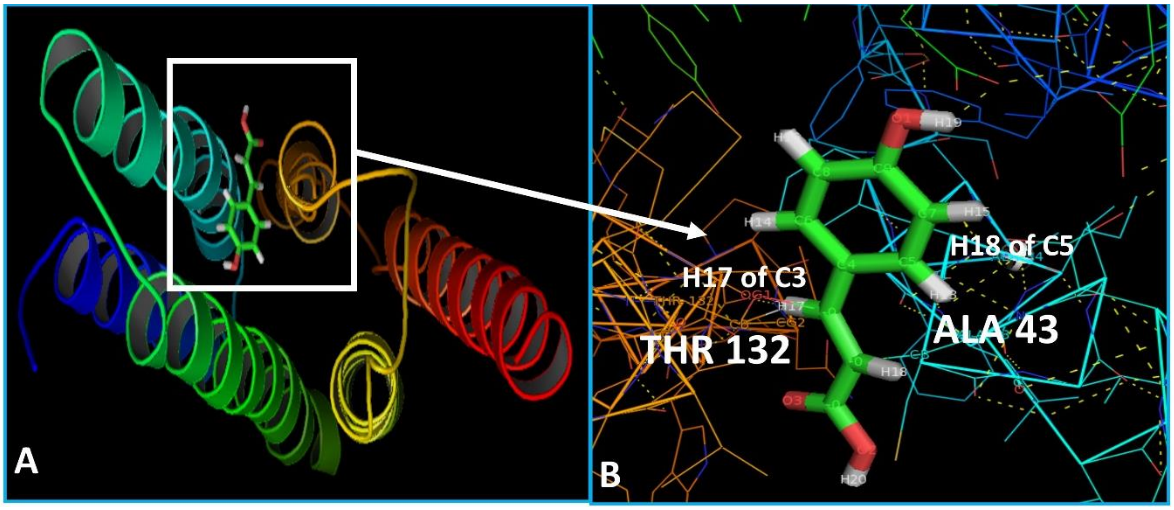


Fig4.8 - Docking site (A) and rigid bond pattern (B) of *p*-CA within CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase of *E. coli*

4.4. Conclusion

p-CA kills Gram positive *S. aureus* as well as Gram negative *E. coli* by multidimensional way. Through molecular docking study 25 common functional proteins were detected from both bacteria which are interactive with *p*-CA. The target proteins were belonging to different ion-exchange channels, nutrient symporter channels, multidrug resistance and efflux proteins and phospholipid biosynthetic proteins. The present study revealed that inactivation of those membrane proteins will lead to osmotic imbalance within the bacterial cell resulting various enzymatic nonfunctioning, nutritional depletion, multidrug susceptibility as well as inhibition of phospholipid biosynthesis. It was found that *p*-CA completely blocked the transport channel of a common membrane protein namely CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyl transferase which converts 1,2-diacylglycerol (DAG) to phosphatidylglycerol (PG). Hence, nonfunctioning of CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyl transferase leads to over accumulation of lethal DAG in the cell causing gradual membrane lysis. The overall in silico study of *p*-CA activity against both Gram positive and Gram negative bacteria could able to explain one of the exact bactericidal mechanism at molecular level and established its broad spectrum antibacterial potentials. Since the wild edible mushroom ***Termitomyces heimii*** has very high *p*-CA content, so it has an immense role in controlling pathogenic bacterial population in the intestine. It is further stated that consumption of *T. heimii* with diet is very much helpful for healthy gut both in microbial and nutritional point of view.