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In Silico Putative Drug Target Identification in Enterobacter Cloacae and Homology Modelling of a Candidate Drug Target

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Abstract

Enterobacter cloacae is a clinically significant Gram-negative, facultatively-anaerobic, rod-shaped bacterium belonging to the family of Enterobacteriaceae. It has emerged as a prevalent nosocomial pathogen due to high level resistance to disinfectants and antimicrobial agents. The availability of complete genome sequence of E. cloacae has paved the new way to identify the novel drug targets. In the present work comparative analysis of the metabolic pathways of the pathogen and host was performed to identify the novel drug targets involved in pathogen but absent in Homo sapiens. All enzymes involved in the metabolic pathways of E. cloacae were searched against the proteome of H. sapiens using the BLASTp program. The threshold of percentage identity was set to as < 30%, E-value > 0.001, and the query coverage < 50. Using these parameters approximately 44 unique putative targets were identified. Out of those non-homologous targets, 22 coding genes for putative targets were identified as essential genes from the DEG database. Based on extensive literature search, 8 targets such as UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase, UDP-N-acetylmuramate-L-alanine ligase, UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2, 6-diaminopimelate ligase, UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D-alanine ligase, UDP-N-acetylmuramate-L-alanine ligase (MurC) α-isopropylmalate synthase, UDP-N-Acetylglucosamine Enolpyruvyl Transferase, UDP-N-acetylenolpyruvylglucosamine reductase and Aspartate beta-semialdehyde dehydrogenase were identified as potential drug targets. Among these drug targets, UDP-N-acetylmuramate-L-alanine ligase (MurC) was chosen as potential therapeutic drug target. The homology modeling of MurC was performed using SWISS-MODEL and HHPred servers respectively. Subsequently, all the predicted models were evaluated using the SAVES server. The stereochemical parameters of all 3D models suggest that the best model was predicted by HHPred server. In order the refine the modeled structure, energy minimization was also performed using Deep View tool. The refined 3D structure was further validated by ProSA server. In future, the 3D structure of MurC in E. cloacae might be exploited for the discovery of novel inhibitors that could potentially inhibit this nosocomial pathogen.

Keywords: Enterobacter cloacae, KEGG, Metabolic pathways, Drug targets, Homology modelling.

Introduction

Enterobacter cloacae is a clinically significant Gram-negative, facultatively-anaerobic, rod-shaped bacterium belonging to the family of Enterobacteriaceae[1]. E. cloacae is a nosocomial pathogen that can cause a range of infections such as bacteremia, lower respiratory tract infection, skin and soft tissue infections, urinary tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis and ophthalmic infections[2]. ICU pathogens can cause morbidity and mortality and the management of these bacterial infections is complicated by the organism's multiple antibody resistance. These bacteria contain beta-lactamase, which is undetectable in vitro and is highly resistant to antibiotics such as third generation cephalosporins. E. cloacae can be found

on human skin and tissues as well as fruits, vegetables, and devices such as a hot water treatment tank. Although this organism is mainly a pathogen for human and causes disease. This pathogen has been used as a biological control for plant disease such as the seed-rotting oomycete in Pythium ultimum, and used to control insect pests on mulberry leaves and suppress disease[3]. This bacterium is an opportunistic type of bacteria which attacks (cause disease) in the host system (Homo sapiens) after it has been weakened by some other infections or injury. The E. cloacae is a type of species of Enterobacter, which is prevalent nosocomial pathogen due to high level resistance to disinfectants and antimicrobial agents[4]. The E. cloacae ATCC 13047 strain was first isolated from human cerebrospinal fluid by Edwin Oakes Jordan in 1890 and is the type strain of E. cloacae subsp. cloacae[5]. The aim of present research work was to identify potential therapeutic drug targets in E. cloacae using metabolic pathway using metabolic pathways analysis, and to model the 3D structure of candidate drug target.

Materials and Methods

Drug target identification

In the present work KEGG pathway database has been used (http://www.genome.jp/kegg/) as a source of metabolic pathway information about E. cloacae strain ATCC 13047. Total 86 different types of metabolic pathways were analyzed, and the enzymes involved in these unique pathways were identified from KEGG database[6]. The protein sequences of all the enzymes involved in different metabolic pathways of E. cloacae were subjected to BLASTp[7] search against the proteome of Homo sapiens. In order to identify the non-homologous encoding genes to the host (human), the threshold values were set to as E-value > 0.001, % identity <30 %, and query coverage < 50%. Using these parameters, 172 targets have been identified as non-homologous to the H. sapiens. Further analysis of the identified putative targets was carried out, and the enzymes present in multiple pathways (duplicates) of the pathogen were removed. Finally, 44 targets were identified as unique putative drug targets in E. cloacae. The genes encoding for the important enzymes have been further searched in the DEG database (http://tubic.tju.edu.cn/deg/)[8] to identify the essentiality and non-essentiality of the genes for the survival of the pathogen. Among 44 drug targets, 22 targets were identified as the essential for the survival of E. cloacae. Out of these 22 targets, 8 drug targets were identified as potential therapeutic drug target from the extensive literature review. Among these targets, MurC was selected as candidate drug target for further study.

Sub-cellular localization of putative drug targets: The sub-cellular localization (SCL) of all 22 essential gene products in E. cloacae was predicted using the PSORTb v3.0.2 program (http://www.psort.org/psortb/). It is the first sub-cellular localization predictor exclusively devised for all prokaryotes, including archaea and bacteria with atypical membrane/cell wall topologies. It handles archaeal sequences as well as Gram-positive and Gram-negative bacterial protein sequences. This program consists of various analytical modules, each of which analyzes one biological feature known to influence or be characteristic of sub-cellular localization. The modules may act as a binary predictor, classifying a protein as either belonging or not belonging to a particular localization site, or they may be multi-category, able to assign a protein to one of several localization sites.

Protein-protein interaction network analysis: In order to analyze the molecular interaction networks of candidate drug target UDP-N-acetylmuramate-L-alanine ligase (MurC), the protein-protein interaction study was carried out from the architecture of E. cloacae interactome using the STITCH v3.1web server (http://stitch.embl.de/). This server explores known and predicted interactions of proteins and chemicals.

Physico-chemical propertics prediction of MurC: The ProtParam computes various physico-chemical properties that can be deduced from a protein sequence. Various parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). These physico-chemical properties of MurC protein in E. cloacae were analyzed using the ProtParam server (http://web.expasy.org/protparam/).

Homology Modeling and evaluation of MurC: The homology modeling was performed to build a three-dimensional model of UDP-N-acetylmuramate-L-alanine ligase (MurC) based on one more related protein of known structure (the template). The protein sequence of MurC was retrieved from the Uniprot database and submitted to Swiss-model and HHPred server respectively. The PDB file of the modeled structure was downloaded and 3D structures of modeled structures were visualized using the Rasmol tool. Subsequently, the Structure Analysis and Verification Servers (SAVES) were used to evaluate the stereo-chemical properties of all the models predicted by various servers. SAVES uses PROCHECK, WHAT_IF, ERRAT, VERIFY 3D, and PROVE molecules to check any anomaly present in the structure. The best predicted model of MurC was of HHpred server. The energy minimization of the modeled structure of MurC was carried out to improve the overall quality of the model using the DeepView tool. The energy minimized modelled structure of MurC was further validated using SAVES and ProSA server respectively.

Results and Discussion

Drug target identification

The availability of complete genome sequence has paved the new way to identify the novel potential drug targets in E. cloacae. Earlier several potential drug targets have been identified in numerous pathogens using the metabolic pathways analysis[9-11]. In the present work, metabolic pathways of E. cloacae were analyzed, and total 86 metabolic pathways were present in the KEGG pathways databases. All the enzymes involved in the metabolic pathways of E. cloacae were searched against the proteome of H. sapiens using the BLASTp program. The threshold of percentage identity was set to as < 30%, E-value > 0.001, and the query coverage < 50 %. Total 172 putative drug targets were identified. Out of 172 drug targets, there was found some targets or genes which were involved in more than one metabolic pathway. All the duplicates targets were removed from the list, and total 44 unique putative drug targets have been found. The genes encoded for 44 unique putative drug targets were again searched

against the DEG (Database of Essential Genes) database to identify the essentiality of the genes for the survival of E. cloacae. Total 22 encoding genes have been identified as essential for the survival of this pathogen (Table 1).

Table 1: List of Essential genes for Enterobacter cloacae

S.No	Gene ID	Gene Name	DEG ID	TARGETS	Pathways
1.	ECL_00191	dlgD	DEG10050373	2,3-diketo-L-gulonate reductase [EC:1.1.1.130]	Pentose and glucuronate interconversions
					Ascorbate and aldarate metabolism
2.	ECL_05074	rhaD	DEG10040567	rhamnulose-1-phosphate aldolase [EC:4.1.2.19]	Pentose and glucuronate interconversions
					Fructose and mannose metabolism
3.	ECL_00002	dnaN	DEG10040692	DNA polymerase III subunit beta [EC:2.7.7.7]	Purine metabolism
					Pyrimidine metabolism
4.	ECL_03056	dnaN	DEG10110044	DNA polymerase III subunit delta [EC:2.7.7.7]	Purine metabolism
					Pyrimidine metabolism
5.	ECL_04389	ureC	DEG10100307	urease subunit alpha [EC:3.5.1.5]	Purine metabolism
					Arginine and proline metabolism
6.	ECL_04795	asd	DEG10040525	aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]	Glycine, serine and threonine metabolism
					Cysteine and methionine metabolism
					Lysine biosynthesis
7.	ECL_02168	asd	DEG10130082	aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]	Glycine, serine and threonine metabolism
					Cysteine and methionine metabolism
					Lysine biosynthesis
8.	ECL_04130	tdcG	DEG10040468	L-serine dehydratase [EC:4.3.1.17]	Glycine, serine and threonine metabolism
					Cysteine and methionine metabolism
9.	ECL_02018	metE	N/A*	5-methyltetrahydropteroyl triglutamate--homocysteine methyltransferase [EC:2.1.1.14]	Cysteine and methionine metabolism
					Selenocompound metabolism
10.	ECL_04966	metE	DEG10100167	5-methyltetrahydropteroyl triglutamate--homocysteine methyltransferase [EC:2.1.1.14]	Cysteine and methionine metabolism
					Selenocompound metabolism
11.	ECL_04024	leuA	DEG10100584	2-isopropylmalate synthase [EC:2.3.3.13]	Valine, leucine and isoleucine biosynthesis
					Glycerophospholipid metabolism
12.	ECL_00882	murE	DEG10040016	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase [EC:6.3.2.13]	Lysine biosynthesis
					Peptidoglycan biosynthesis
13.	ECL_00883	murF	DEG10040617	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase [EC:6.3.2.10]	Lysine biosynthesis
					Peptidoglycan biosynthesis

On the basis of extensive literature review, out of 22 Essential drug targets, 8 putative drug targets were reported as potential therapeutic drug targets in various pathogens. These targets were also involved in more than one metabolic pathways of E. cloacae. Those potential drug targets are listed below:

i. UDP-N-acetylmuramoylalanyl-D-glutamate-2, 6-diaminopimelate ligase
ii. UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase
iii. UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase
iv. UDP-N-acetylmuramate-L-alanine ligase
v. α-Isopropylmalate synthase
vi. UDP-N-Acetylglucosamine Enolpyruvyl Transferase
vii. UDP-N-acetylenolpyruvylglucosamine reductase
viii. Aspartate-beta-semialdehyde dehydrogenase

Sub-cellular localization of putative drug targets in *E.cloacae*

In order to predict the sub-cellular localization (SCL) of all 22 gene products (putative drug targets) in E. cloacae, the PSORTb v3.0.2 program was used. In this program several modules were used for the prediction of sub-cellular localization of bacterial proteins. All 22 unique drug targets crucial for the survival of E. cloacae were subjected for the prediction of sub-cellular localization. It was found that all the targets were localized in cytoplasmic region (Appendix Table I), which might be easily accessible in bacterial cell being targeted for inhibitor designing.

Appendix Table I

S.No	Gene ID	Gene Name	DEG ID	TARGETS	Pathways
1.	ECL_02394	ARO4	DEG10040290	3-deoxy-7-phosphoheptulonate synthase [EC:2.5.1.54]	Tryptophan metabolism
					Phenylalanine, tyrosine and tryptophan biosynthesis
2.	ECL_02983	aroG-2	DEG10040290	3-deoxy-7-phosphoheptulonate synthase [EC:2.5.1.54]	Tryptophan metabolism
					Phenylalanine, tyrosine and tryptophan biosynthesis
3.	ECL_03678	aroC	DEG10050092	chorismate synthase [EC:4.2.3.5]	Tryptophan metabolism
					Phenylalanine, tyrosine and tryptophan biosynthesis
4.	ECL_00885	murD	DEG10110007	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase [EC:6.3.2.9]	D-Glutamine and D-glutamate metabolism
					Peptidoglycan biosynthesis
5.	ECL_00888	murC	DEG10040020	UDP-N-acetylmuramate—L-alanine ligase [EC:6.3.2.8]	D-Glutamine and D-glutamate metabolism
					Peptidoglycan biosynthesis
6.	ECL_00944	panC	N/A*	pantoate--beta-alanine ligase [EC:6.3.2.1]	beta-Alanine metabolism
					Pantothenate and CoA biosynthesis
7.	ECL_04101	cysD	DEG10130159	sulfate adenylyltransferase subunit 2 [EC:2.7.7.4]	Selenocompound metabolism
					Sulfur metabolism
8.	ECL_04571	murA	DEG10040662	UDP-N-acetylglucosamine 1-carboxyvinyltransferase [EC:2.5.1.7]	Amino sugar & Nt. sugar metabolism
					Peptidoglycan biosynthesis
9.	ECL_04946	murB	DEG10110210	UDP-N-acetylenolpyruvylglucosamine reductase [EC:1.3.1.98]	Amino sugar & Nt. sugar metabolism
					Peptidoglycan biosynthesis

Since the UDP-N-acetylmuramate-L-alanine ligase (MurC) was involved in multiple pathways, and it is also essential for the survival of E. cloacae, it was selected as a candidate drug target for further analysis.

Protein-protein interaction network analysis

The protein-protein interactions (PPIs) are crucial in almost all biological processes occur in any organism. Using global protein interaction network (interactome) analysis, the relationships between genes/proteins can be understood in an effective way. The sub-network analysis and hub prioritization of drug target MurC in E. cloacae interactome was carried out using the STITCH 3.1 server.

After analyzing the protein-protein interaction sub-network of MurC in E. cloacae interactome, it was observed that this target is a crucial hub which is highly connected with murD, murB, Ent638_0638, murG, UDP-N-acet.ani, murF, murE, Ent638_0635, mraY, and Ent638_0639 proteins (Figure1). The protein-protein interaction of FBA was predicted with > 0.9 confidence score (Approximate probability), which shows strong association with these proteins (Table 2).

Table 2: Predicted functional partners of murC protein in the proteome of E. Cloacae

S. No.	Protein/chemical code	Predicted associated protein/chemical in the network	Score (Probability score)
1.	murD	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	0.999
2.	murB	UDP-N-acetylenolpyruvoylglucosamine reductase	0.997
3.	Ent638_0638	D-alanine--D-alanine ligase	0.996
4.	murG	undecaprenyldiphospho-muramoylpentapeptide beta-N- acetylglucosaminyl-transferase	0.993
5.	UDP-N-acet.ani	UDP-N-acetylmuramoyl-L-alanine	0.992
6.	murF	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D- alanine ligase	0.989
7.	murE	UDP-N-acetylmuramoylalanyl-D-glutamate--2, 6-diaminopimelate ligase	0.988
8.	Ent638_0635	cell division protein FtsW	0.984
9.	mraY	phospho-N-acetylmuramoyl-pentapeptide- transferase	0.981
10.	Ent638_0639	cell division protein FtsQ	0.981

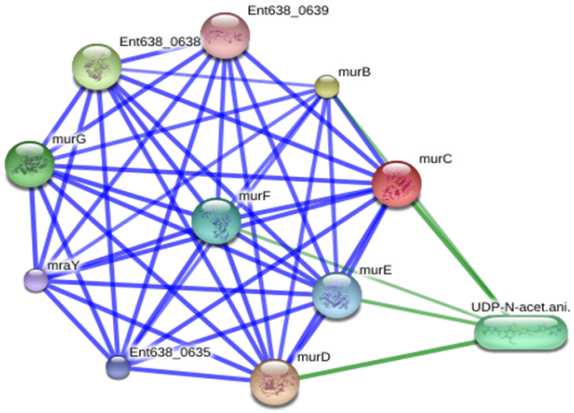


Figure 1: Protein interaction network of UDP-N-acetylmuramate--L-alanine ligase (MurC) in E. cloacae (Protein-protein interactions are shown in blue, chemical-protein interactions in green and interactions between chemicals in red. Stronger associations are represented by thicker lines)

Physico-chemical propertics prediction of MurC

Various physico-chemical parameters such as the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY), were used to analyze the MurC protein of E. cloacae by ProtParam server[12]. (Table II) (Appendix) depicts that total 491 were present in the protein, in which the percentage of Alanine was the highest (11.4) and the percentage of Tryptophan was the lowest (0.2). It also showed that the MurC is a stable protein with an instability index of 30.73. According to this program, a protein whose instability index is smaller than 40 is predicted as stable; on the other hand, a value above 40 predicts that the protein maybe unstable. Above value suggest that this protein is stable and catalytically active under room temperature. The MurC protein has a theoretical pI value of 5.61, and its molecular weight was found to be 53382.5, which suggest moderate size of protein.

Appendix Table II: Evaluation of 3D structure of MurC after Energy minimization

Number of times energy minimization	Procheck result (Ramachandran plot)	Verify 3D result (% of the residues had an average 3D-1D score>0.2	Errat (overall quality factor)	Bad Contacts
1	90.8% core 7.5% Allowed 0.9% gener 0.7% disallowed	92.68%	89.149	5
2	Allowed 0.9% gener 0.7% disallowed 90.8% core 7.5%	92.68%	90.426	3
3	89.4% core 8.9% allowed 1.2% gener 0.5% disallowed	92.68%	90.638	1

Homology Modeling and evaluation of MurC

The homology modeling was performed to build a three-dimensions (3D) structure of candidate drug target UDP-N-acetylmuramate-L-alanine ligase (MurC) based on one more related protein of known structure (the template). The protein sequence of MurC was retrieved from the Uniprot database and submitted to Swiss-model and HHPred server respectively. The 3D structure of target was predicted using the template MurC from Yersinia pestis (PDB ID: 4hv4), which was having 85% sequence identity and 5.3e-90 E-value. The higher percentage of sequence identity and lower E-value suggest that the template is closely related to the target protein, and better quality 3D structure can be predicted using the homology modeling method. The PDB files[13/14] of the modeled structure was downloaded and visualized using the Rasmol tool. Subsequent to structure prediction, Structure Analysis and Verification Server (SAVES)[15-17] was used to evaluate the stereo-chemical properties of all the models predicted by various servers. SAVES uses PROCHECK, WHAT_IF, ERRAT, VERIFY 3D, and PROVE molecules to check any anomaly present in the structure. The best predicted model of MurC was of HHpred server. The energy minimization of the modeled structure of MurC was done to improve the quality of the model using SPDBV tool[18]. The energy minimised of modelled structure of MurC was further validated using SAVES and ProSA server respectively.

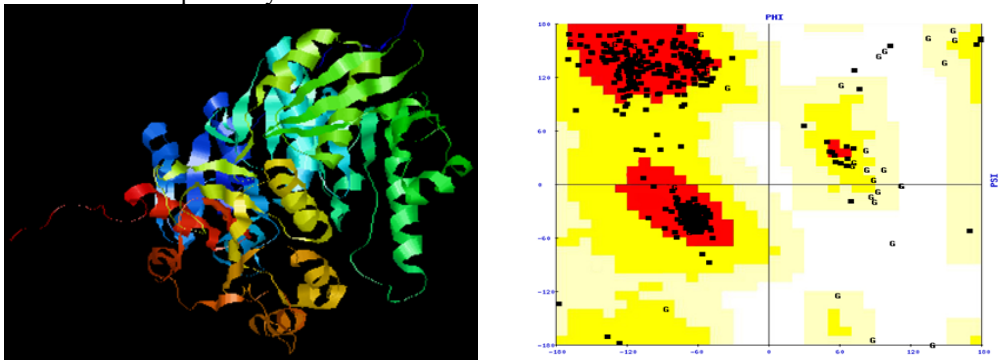
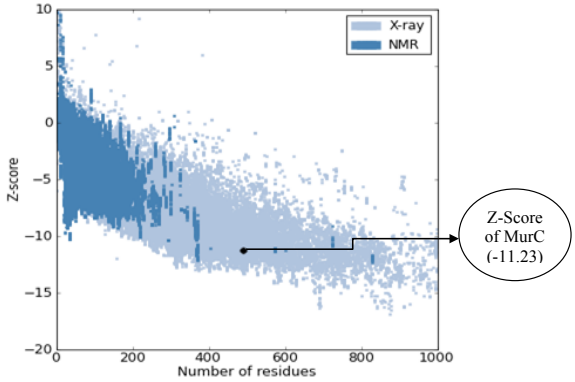


Figure 2: 3D structure and Ramachandran plot of MurC

ProSA (Protein structure analysis)

The modeled 3D structure of UDP-N-acetylmuramate-L-alanine ligase (MurC) was revalidated by the ProSA server[19]. (Figure 3) shows the Z-Score, which indicates the overall quality of the modeled structure.



The Z-Score of MurC protein was found to be -11.23, which indicates good quality of protein. The dark blue colour indicates NMR whereas light blue indicates X-ray technique region. Here the Z-Score lies in the X-ray plot region which signifies that MurC is within the range of scores typically found for native proteins of similar size.

The validation results show that the predicted 3D structure of UDP-N-acetylmuramate-L-alanine ligase (MurC) is of better quality. A good quality model would be expected to have more than 90% amino acid residues in the most favoured (core) regions, and the results suggest that the predicted model is of good quality. The modeled structure of MurC can be exploited as potential therapeutic drug target using structure-based drug designing strategy against the E. cloacae.

Conclusion

The metabolic pathways in the genome of E. cloacae were analyzed, and 8 putataive targets were identified as potential drug targets. Among these targets, UDP-N-acetylmuramate-L-alanine ligase (MurC) was chosen as potential therapeutic drug target. The protein-protein interaction sub-network of MurC suggest that this target is a crucial hub, which is highly connected with several essential proteins. The 3D structure of MurC protein was predicted using the homology modeling method. After stereo-chemical evaluation and further refinement, it was found that predicted model is of good quality. In future, the 3D structure of MurC in E. cloacae might be exploited for the discovery of novel inhibitors that could potentially inhibit the pathogen.

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