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In silico analysis of penicillin- binding protein 5 as an inhibitory target of beta-lactam antibiotics in *Enterococcus faecalis*

Maryam Pourhajibagher[1], Mohammad Mahdi Karimi Yazdi[2] and Abbas Bahador[1*]

[1]*Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ABSTARCT

Enterococcus faecalis is an important pathogen that is associated with a range of infections. Beta-lactam antibiotics are a broad class of antibiotics that are used for treatment of E. faecalis infections, but it has intrinsic and acquired resistance to some of them. Penicillin binding protein (PBP) 5 is one of the members of PBPs family that plays as inhibitors of beta-lactam antibiotics. In this study, we used an in silico strategy and focused on PBP5 in E. faecalis and explained its characterization with the help of bioinformatics tools. The results of primary structure prediction showed that PBP5 is a stable protein and belong to hydrolase protein family. The secondary structure was predicted that random coil is predominantly present followed by extended strand and alpha helix. The three-dimensional structure was modeled using Swiss model workspace and the structure was validated that gives valuable understanding for the improvement of helpful rational strategies for experiments. Ramachandran plot analysis showed that 98% and > 99.8% of all residues of PBP5 were found in favored and allowed regions, respectively. Computer simulation studies, including molecular modeling, having knowledge of PBP5 structure and combination of this information may affect the development of new ways of inhibiting PBP5 to can be designed improved drugs.

Key words: Computer simulation, *Enterococcus faecalis*, *In silico*, Penicillin binding proteins

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INTRODUCTION

Enterococcus faecalis is a frequent cause of a wide diversity of infections in humans such as hospital and community-acquired infections, urinary tract infection (UTI), bacteremia, endocarditis, abdomen, biliary tract, endodontic infection, wound infections, intraabdominal and pelvic infections and indwelling foreign devices (like intravascular catheters) [1, 2, 3]. Also, it has isolated from a range of oral conditions including carious lesions, chronic periodontitis, and has been associated with persistent apical periodontitis [4]. *E. faecalis* is one of the most important bacteria that have been frequently found in root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases [5]. Enterococci contain penicillin-binding proteins (PBPs) and tolerate or resist beta-lactam antibiotics [6].

The main mechanism of resistance to beta-lactam antibiotics is due to a change in PBPs. There are evidences to state that the emergence of altered PBPs caused by genetic exchanges [7]. Recombination exchanges and mutation in PBP-coding genes are involved in creating of low-affinity PBP to beta-lactams [8].

Enterococci have both an intrinsic and acquired resistance to antibiotics. They are intrinsically resistant to cephalosporins, penicillinase-resistant penicillins, penicillinase-susceptible penicillin, nalidixic acid, macrolides, aztreonam, clindamycin and aminoglycosides [9]. They also have acquired resistance, which comprises resistance to penicillin by beta-lactamases, tetracyclines, rifampin, fluoroquinolones, chloramphenicol, aminoglycosides and vancomycin [9, 10].

Penicillin resistance can be related to glycopeptide resistance and high-level resistance to gentamicin due to overproduction of PBP5 or synthesis of beta-lactams [11]. However new antibiotics can be proposed with the awareness of the general structure of PBP5 to prevent the emergence of multidrug resistance. New bioinformatics analysis tools are one of the most accurate methods for this goal. We used bioinformatics tools in this study to identify and characterize PBP5 as an inhibitor target of beta-lactam antibiotics in *E. faecalis*.

MATERIALS AND METHODS

Molecular modeling and sequence alignment of Penicillin binding protein-5

PBP5 sequence of *E. faecalis* (Accession No. CAA55190.1) was obtained from NCBI (http://www.ncbi.nlm.nih.gov). Protein-BLAST algorithm was performed in Protein Data Bank (PDB) for the sequence homology search to identify proteins with sequences similar to that of PBP5. PBP5 sequences were aligned in CLUSTAL W (http://www.genome.jp/tools/clustalw).

Molecular description and functional characterization of Penicillin binding protein-5

Overall quality about sequence of PBP5 was carried in out at a glance using UniProt (http://expasy.org) and Expasy ProtParam server (http://www.expasy.org/cgi-bin/protpraram).

Prediction of protein backbone of Penicillin binding protein-5

Homology modeling of PBP5 sequence of *E. faecalis* was used as template to generate a comparative three-dimensional model of PBP5 by SWISS-MODEL server (http://www.swissmodel.expasy.org). Garnier- Osguthorpe-Robson (GOR) IV was used to predict alpha helices, beta sheets, and random coil secondary structures and the three-dimensional structure of PBP5 was obtained from http://www.ncbi.nlm.nih.gov/Structure/VAST.

Analysis of the PBP5 was done by RCSB PDB to check whether the residues are falling in the most favored region in the Ramachandran's Plot or not.

The secondary structure of PBP5 was obtained using the PSIPRED Protein Sequence Analysis Workbench (http://bioinf.cs.ucl.ac.uk/psipred/). The secondary structure of 4CPK_A was derived from psipred protein (http://bioinf.cs.ucl.ac.uk/psipred/). Transmembrane protein topology with a hidden markov model (TMHMM) server (http://www.cbs.dtu.dk/services/TMHMM-2.0) was used for the prediction of transmembrane helices in PBP5.

RESULTS

Primary structure prediction of PBP5 from *E. faecalis* (UniprotKB-Swiss-Prot Accession No. Q47800) has 679 amino acids that its estimated structure weight was 148744.20 dalton. It shows structural similarity with the crystal structure of chain A, crystal structure of Pbp2a double clinical mutant N146k- E150k from Mrsa (PDB ID: 4CPK_A). The score for protein alignment between PBP5 and 4CPK_A was 28.77, with a 37% identity. 4CPK_A was selected as a template on the basis of lowest e-value (2e-98), highest resolution (2.72˚A). Target and template information and alignment of them are shown in Table 1 and figure 1, respectively.

Table 1. Target and template protein properties

Target Name	Template Name	Template PDB ID	Sequence Identity	Query cover	E value
Penicillin Binding Protein-5 (*E. faecalis)*	chain A, crystal structure of Pbp2a double clinical mutant N146k-E150k from Mrsa	4CPK_A	37%	78%	2e-98

Primary structure prediction showed that PBP5 had 81 positively charged residues (Arg + Lys) and 86 negatively charged residues (Asp + Glu). The very high aliphatic index was 80.91 and instability index (31.21) provides the estimate of the stability of protein in a test tube. Its theoretical isoelectric point and extinction coefficient were 5.48 and 66240, respectively. Its grand average of hydropathicity value was -0.438.

Secondry structure prediction of PBP5 in *E. faecalis* by using PSIPRED showed the location and spatial arrangement of each amino acid separately (Fig. 2). The extended strand (16.49%) and alpha helix (33.14%) were the least frequent, whereas the random coil was more frequent at 50.37%.

```
PBP5   MERSNRNKKSSKNPLILGVSALVLIAAAVGGYYAYSQWQAKQELAEAKKTATTFLNVLSKQEFDKLPS
4CPK_A ----------------------------------------------KDKEINNTIDAIEDKNFKQVYK

PBP5   VVQEASLKKNGYDTKSVVEKYQAIYSGIQAEGVKASDVQVKKAKDNQYTFTYKLSMSTPLGEMKDLSY
4CPK_A DSSYISKSDNG--EVEMTERPIKIYNSLGVKDINIQDRKIKKVSKNKKRVDAQYKIKTNYGNIDRN-V

PBP5   QSSIAKKGDTYQIAWKPSLIFPDMSGNDKISIQVDNAKRGEIVDRNGSGLAINKVFDEVGVVPGKLGS
4CPK_A QFNFVKEDGMWKLDWDHSVIIPGMQKDQSIHIEKLKSKRGKILDRNNVELANTGTAYEIGIVPKNVSK

PBP5   GAEKTANIKAFSDKFGVSVDEINQKLSQGWVQADSFVPITVASEPVTELP-----TGAATKDTESRYY
4CPK_A -----KDYKAIAKELSISEDYIKQQMDQNWVQDDTFVPLKTVKKMDEYLSDFAKKFHLTTNETESRNY

PBP5   YYPLGEACAINRVYGTITAEDIEKNPELS---STGVIGKTGLERAFDKELRGQDGGSLVILDDKEN-V
4CPK_A NYPLGKATSHLLGYVGPINSEELKQKEYKGYKDDAVIGKKGLEKLYDKKLQHEDGYRVTIVDDNSNTI

PBP5   KKALQTKEKKDGQTIKLTIDSGVQQQAFAIFDKRPGSAVITDPQKGDLLATVSSPSYDPNKMANGISQ
4CPK_A AHTLIEKKKKDGKDIQLTIDAKVQKSIYNNMKNDYGSGTAIHPQTGELLALVSTPSYDVYPFMYGMSN

PBP5   KEYDAYNNNKDLPFTARFATGYAPGSTFKIITGAIGLDAGTLKPDEELEINGLKWQKDKSWGGYFATR
4CPK_A EEYNKLTEDKKEPLLNKFQITTSPGSTQKILTAMIGLNNKTLDDKTSYKIDGKGWQKDKSWGGYNVTR

PBP5   VKEASPVNLRTALVNSDNIYFAQQTLRMGEDKFRAGLNKFIFGEELDLPIAMTPAQISNEDKFNSEIL
4CPK_A YEVVNGNIDLKQAIESSDNIFFARVALELGSKKFEKGMKKLGVGEDIPSDYPFYNAQISNKNLDNEIL

PBP5   LADTGYGQGQLLISPIQQATMYSVFQNNGTLVYPKLVLDKETKKKDNVISANAANTIATDLLGSVEDP
4CPK_A LADSGYGQGEILINPVQILSIYSALENNGNINAPHLLKDTKNKVWKKNIISKENINLLTDGMQQVVN-

PBP5   SGYVYNMYNPNFSLAAKTGTAEIKDKQDTDGKENSFLLTLDRSNNKFLTMIMVENSGENGSATDISKP
4CPK_A KTHKEDIYRSYANLIGKSGTAELKMKQGETGRQIGWFISYDKDNPNMMMAINVKDVQDKGMASYNAKI

PBP5   LIDYLEATIK----------
4CPK_A SGKVYDELYENGNKKYDIDE
```

Figure 1. Alignment of target and template proteins

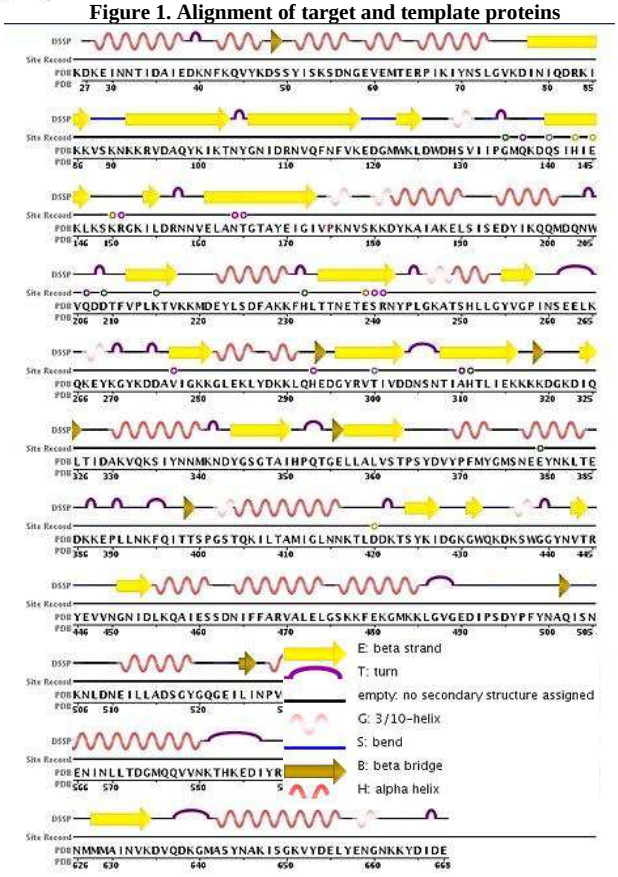

Figure 2. Secondary structure prediction of PBP5 in *E. faecalis* by Psipred

Graphical representation of location of PBP5 in *E. faecalis* showed that all parts of the protein are present outside of the cell (Fig. 3).

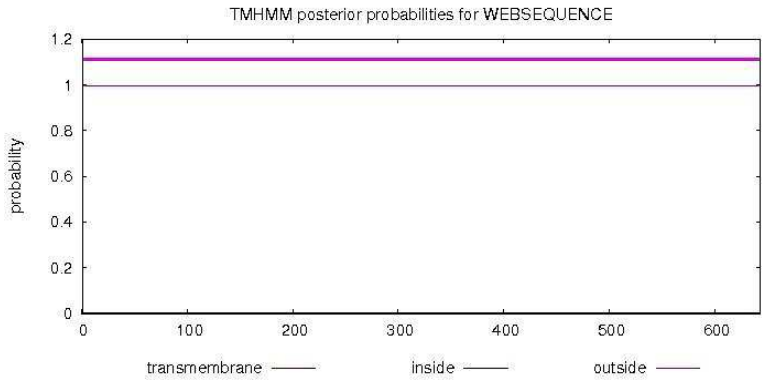

Figure 3. Graphical representation of location of PBP5 in *E. faecalis*

Studying sequence information with three- dimensional structures of proteins gives valuable understanding for the improvement of helpful rational strategies for experiments such as the structure based design of specific inhibitors. No experimental structural information is accessible for the majority of protein sequences thus theoretical methods for proteins structure calculation intending to association this structure knowledge gap have acquired much interest in recent years. Because the three-dimensional structure of PBP5 of *E. faecalis* was not available in PDB, BLAST-p similarity search was performed that obtained 4CPK_A, with complete query coverage that are shown in figure 4.

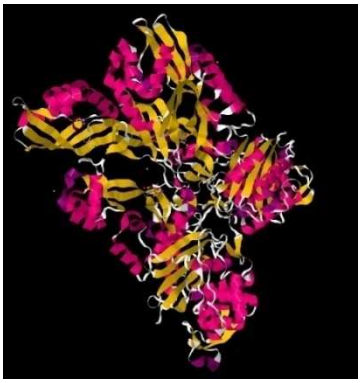

Figure 4. Modeled spatial configuration of PBP5 of *E. faecalis*

Modeled spatial configuration of PBP5 showed that it consists of the following components: 2 cell surface protein A-B, 11 cadmium ion (Molecular Formula: Cd^{+2} and Molecular Weight: 112.411 g/mol), 4 Chloride ion (Molecular Formula: Cl^{-} and Molecular Weight: 35.453 g/mol), and 2 Muramic acid (Molecular Formula: $C_9H_{17}NO_7$ and Molecular Weight: 251.23378 g/mol).

Ramachandran plot analysis of the modeled protein showed that 96.4% (1219/1265) of all residues were found in favored (98%) regions and among the 1265 residues 1258 residues found in allowed (> 99.8%) regions (Fig. 5). All (7) Ramachandran outliers (phi, psi) are presented in Table 2.

Table 2. All (7) Ramachandran outliers

Mol	Chain	Res	Type
1	B	265	LYS
1	B	273	LYS
1	B	504	SER
1	B	267	LYS
1	B	506	LYS
1	A	28	LYS
1	B	274	ASP

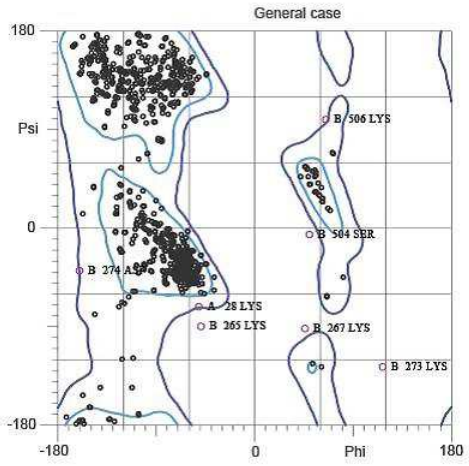

Figure 5. Molprobity Ramachandran analysis

DISCUSSION

Like other bacteria involved in nosocomial infections [12- 16], *E. faecalis* is also commonly considered by its resistance. As reported before, the number of infections associated with antibiotic-resistant bacteria is continuously increasing. *E. faecalis* is among the most antibiotic resistant bacteria known [9]. In trying to prevent further antibiotic resistant *E. faecalis*, drug susceptibility testing is forcefully recommended. New and more specific antibiotics are being developed [17].

Penicillin is one of the antibiotics that have some activity against *E. faecalis*. PBPs are a set of proteins that are able to bind to penicillin by their affinity. The low-affinity PBPs are involved in penicillin resistance in some of gram-negative bacteria, such as *Neisseria gonorrhoeae* and gram-positive bacteria, such as staphylococci and enterococci [18]. The PBPs are usually widely classified into high-molecular-weight (HMW) and low-molecular-weight (LMW) categories. They are chemically similar to the molecular that form the peptidoglycan and can bind to β-lactam antibiotics.

PBP5 is a contributing factor in intrinsic-lactam resistance of enterococci and it is completely differs from other PBPs [19]. PBP5 is a therapeutic target in *E. faecalis*. So, it is important to be able to select an appropriate antibiotic that can bind to PBP5 and inhibits bacterial activity. For this purpose, we must know the overall structure of this protein.

To address the problem of resistance, it will be necessary to change the protocols of use of antimicrobials. Bioinformatics tools, homology modeling algorithms, and *in silico* analysis help in estimating the structural and functional information of a protein molecule that is useful for recommend the appropriate antibiotics [20]. The present study characterized the PBP5 by using various computational tools.

Structure and domain diagrams of PBP5 showed that it comprised 679 amino acid residues, and the total number of negatively charged residues in it was approximately equal to the total number of positively charged residues. Moreover, it had very high aliphatic index, indicating that it was stable at a wide range of temperatures. Comparative analysis of the predicted secondary structure of PBP5 showed that the random coil was present predominantly, followed by an extended strand and an alpha helix. TMHMM is a membrane protein topology prediction method based on a hidden Markov model. It predicts transmembrane helices and discriminate between soluble and membrane proteins with high degree of accuracy. Moreover, further studies have shown that PBP5 is present outside of the cell.

Based on the findings of earlier studies [17, 19], the crystal structure of PBP5 reveals that there were strong inter-residue hydrogen-bonding interactions around the active site, which comprising the signature motifs (SXXK, SXN, and KTG) lay in the cleft between the large α helical cluster of domain I and the five-stranded anti-parallel β-sheet structure.

Now days, nanoparticles are considered a viable alternative to antibiotics and seem to have a high potential for controlling bacterial infections [21- 24]. The possibility of the emergence of resistance against these nanoparticles is rare due to their effects on different sites such as membrane, protein synthesis, inhibiting the replication [25].

Nanoparticles can also be designed by use of computer simulation. Studies have shown that nanosilver is widely used for this purpose and have always been used against various diseases. It acts against *E. faecalis* by alteration of cell wall and cytoplasm [26]. NanoTiO2 is one of the nanoparticles that gives self-cleaning to coated surfaces to it in the presence of ulteraviolet (UV) light. In addition, it is attracting much interest because of its potent antibacterial activity [27, 28].

CONCLUSION

The information on the characterization of the PBP5 in *E. faecalis* obtained from these studies alone is not sufficient. Nonetheless, computer simulation suggests that *in silico* studies, including molecular modeling and having knowledge of PBPs structure is allowed us to discuss new ways of inhibiting PBPs to can be designed improved drugs to combat infections by *E. faecalis* and related Gram-positive bacteria.

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