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HTH (HELIX –TURN -HELIX) MOTIF AND 3D STRUCTURE PREDICTION AND VISUALIZATION USING INSILICO TECHNIQUES

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ABSTRACT

The background of the present study is selecting the outer membrane porin D, OprD to identify the multi- drug resistant sites of the motif regions using Insilico protocols. Since a variety of strategies have been developed by bacteria to resist the effects of antibiotics, identifying the mechanism of resistance could aid in discovering and designing novel antimicrobial agents. The amino acid sequence of OprD porin is applied to a Helix-Turn-Helix motif prediction tool in order to identify the motif region and the specific amino acid score. 3D prediction studies are then done using Swiss Model server and the identified 3D structure is viewed using Discovery studio which would play a major role in drug designing. Identifying the potential multi-drug resistant drug binding site is a challenge in the field of pharmacology. Hence, our research is aimed at identifying the potential drug inhibiting sites in the MDR protein.

Keywords: Discovery Studio, Helix-Turn-**Helix,** Multi Drug **Resistant, OprD**, Swiss Model **S**erver.

INTRODUCTION

The widespread use of antibiotics in the clinic, along with a scarcity of novel antibiotic classes, has resulted in a progressive growth of bacterial pathogen resistance to these drugs. Those regulating inflow and efflux are particularly important among the several methods by which bacteria withstand antibiotic treatment, since they restrict the drug's contact with its intracellular targets and, as a result, it harmful effects on the cell (Fernandez L et al., 2012). Antibiotics having a molecular weight more than 1400 Da, such as Vancomycin and Daptomycin, are unable to penetrate through Gram-negative bacteria's outer membrane. This characteristic of the outer membrane is one of the major roadblocks to developing a new antibacterial drug that targets Gram-negative infections (Lee et al., 2013). Extended- spectrum Beta-lactamase (ESBLs) is a major resistance mechanism that obstructs antimicrobial therapy of illnesses caused by Enterobacteriaceae and poses a severe threat to the present antibiotic arsenal. Because bacteria have evolved numerous tactics to combat antibiotic effects, identifying the resistance mechanism might aid in the discovery and development of novel antimicrobial medicines (Shaikh S et al., 2015). Porins are proteins found in the outer membrane of cells that regulate cellular permeability and antibiotic resistance (Choi U & Lee C, 2019). Porins of the outer membrane are transmembrane pore-forming proteins with a barrel structure that creates a water-filled open channel that allows hydrophilic substances to flow through passively. (Pages et al.,2008). The outer membrane acts as a second barrier, preventing the transfer of harmful substances such as bile acid and antibiotics (O'Shea & Moser, 2008).

METHODOLOGY

Sequence Retrieval System: The protein sequence of the potential multi-drug resistant (MDR) bacterial protein OprD (Malaciobacter mytili) was retrieved from KEGG database in FASTA format. **Sequence Analysis:** The selected sequence was analysed using Helix turn Helix (Helix-Turn-Helix Motif Prediction) server to identify the functional motif amino acid regions present in the selected MDR protein. **3D structure prediction:** The amino acid sequence of MDR protein was converted into 3D structure using an advanced automated homology modelling server called SWISS-model. **3D structure visualization**: The modelled structure was visualized using the molecular visualization tool, Discovery Studio Software to view the 3D effect of the modelled MDR protein in different formats.

RESULTS AND DISCUSSION

Porins are proteins found in the outer membrane of cells that govern cellular permeability and antibiotic resistance. Porins are connected to antibiotic resistance in Gram-negative bacteria because they allow medicines to pass through the outer membrane passively. Efflux processes have a big role in antibiotic resistance (Li et al., 2015). Bacterial multidrug resistance can be caused in one of two ways. To begin, these bacteria may acquire many genes, each coding for drug resistance to a single therapy, within a single cell. On resistance (R) plasmids, this form of accumulation is particularly common. Second, increased expression of multidrug efflux pumps, which expel a variety of medicines, can lead to multidrug resistance. Antibiotic resistance has grown more widespread among pathogen strains, and some have acquired multidrug resistance, or resistance to many antibiotics and chemotherapeutic treatments (Hiroshi N., 2009).

In this study, we identified the OprD gene which is responsible for MDR. The protein sequence of the potential multi-drug

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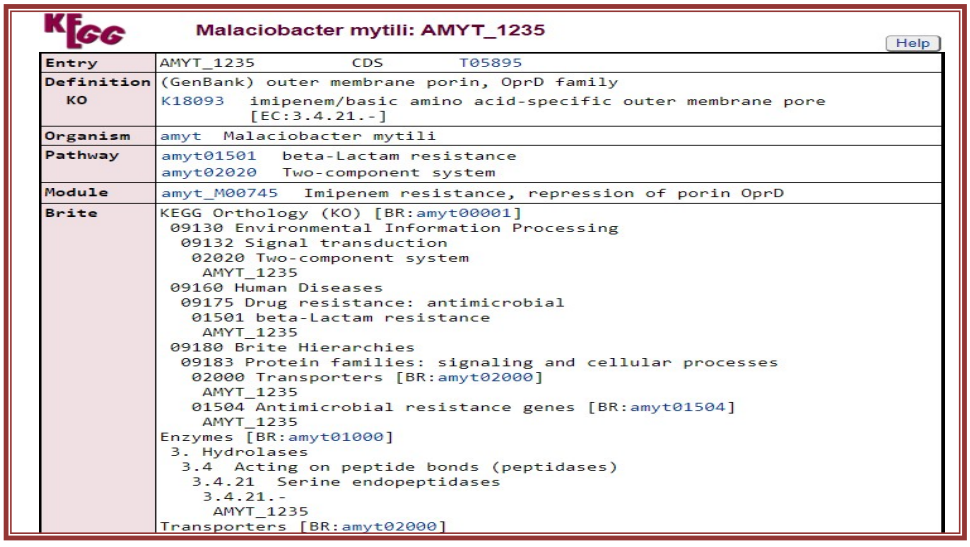
resistant (MDR) bacterial protein OprD (Malaciobacter mytili) was retrieved from KEGG (Kyoto Encyclopedia of Genes and Genomics) database in FASTA format as seen in Figure 1. Figure 2 further elucidates the information of Malaciobacter mytili using KEGG pathway. The selected sequence was analysed using Helix turn Helix (Helix-Turn-Helix Motif Prediction) server to identify the functional motif amino acid regions (The amino acids position starts at :226 and end at 247: TYLEAAYNFLNYSISTQYYNSK) present in the selected MDR protein as shown in figure 3. The score of the 22nd amino acid sequence positioned at 226 is 0.07. Segments with the lowest scores (AAC values) i.e., 0.80 or less can be regarded as a strong candidate for a helix-turn-helix motif (G et al., 1989).

Our goal is to use an automated modelling server to model the OprD. SWISS-model server was used to conduct protein modelling experiments. To conduct drug docking experiments, the OprD-gene- coded amino acid sequence was translated into a 3D structure. Discovery studio software, a molecular visualisation tool, was used to explore the anticipated structure. The 3D structure of the OprD sequence was shown using the molecular visualisation tool, Discovery studio software, as shown in Figure 4 and Figure 5 illustrating the 3D structure of the protein sequence OprD of Malaciobacter mytili showing hydrophobic region.

```
>amyt:AMYT_1235 K18093 imipenem/basic amino acid-specific
outermembrane pore [EC:3.4.21.-] | (GenBank) outer membrane
porin, OprD family (A)
MKKNYLSLTLALMSFLTVSNASTLQDTLSNGKISGTLQAYYFARDKNSGTDNDILTLGLD
ISYESAEYNGFGFKTTFQSASSPWVDEDGKAGRKSNMWGSGAQLSEAFISYTYIKTSAQI
GRMYFSSPLLSGSGSRVNKEAFQGFVITNSNIPDTVVTLAYMNKFQSRTDGKGNIGEFTK
NFKTAAAPWSFKLDDGAYTVSIVNKSLENLTLTAAYVDAIDAFKATYLEAAYNFLNYSIS
TQYYNSKEEGKESGNLFGLQGTASFGPLNFTASYTTTGDDADVLPGLGNGADLAYTWSEA
```

Figure 1. Protein sequence of outer membrane porin D, OprD of Malaciobacter mytili

Figure 2: The above figure shows the KEGG pathway interface



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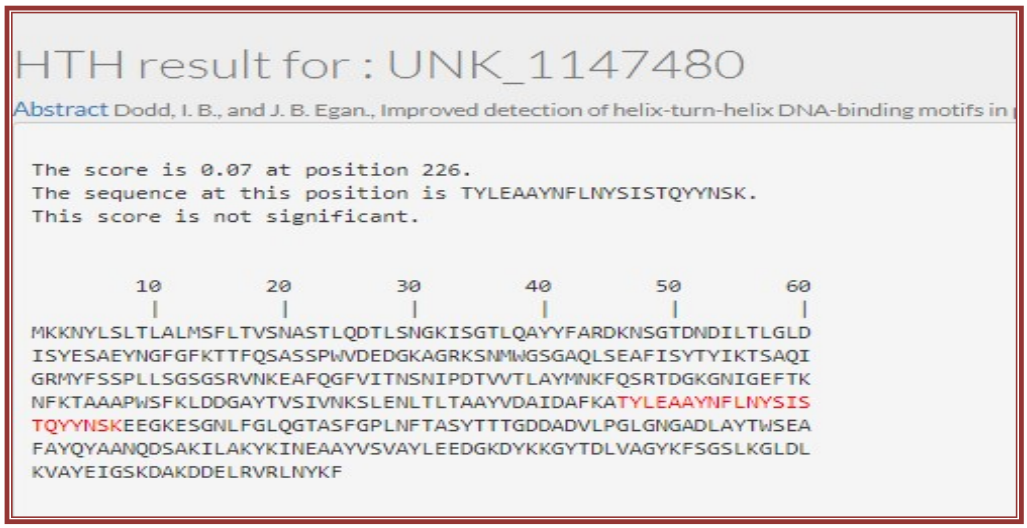


Figure 3: Protein sequence of OprD (Malaciobacter mytili) Shown in Helix-Turn-Helix motif prediction server.

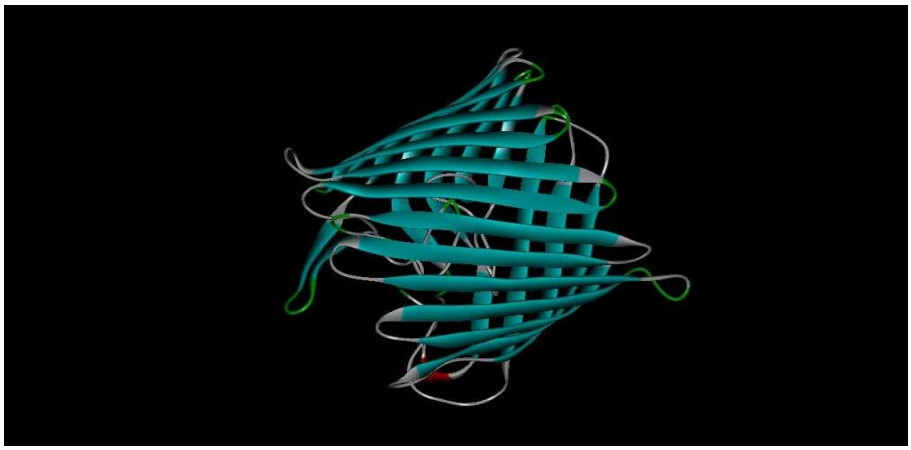


Figure 4: 3D structure of the protein sequence of OprD of Malaciobacter mytili viewed under Discovery studio.

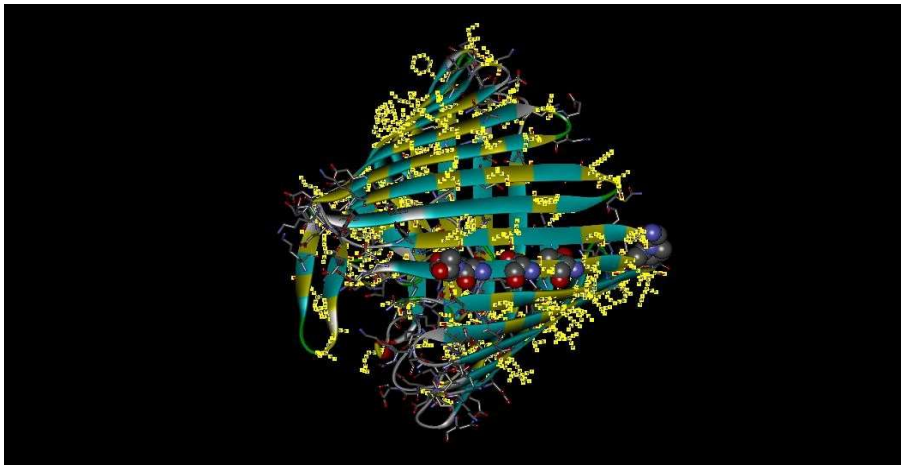
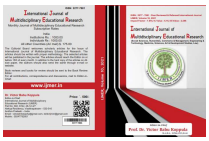


Figure 5: 3D structure of the protein sequence of OprD of Malaciobacter mytili showing hydrophobicregion viewed under Discovery studio.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CONCLUSION

In this research work, we discovered the potential marker HTH (helix-turn-helix) motif sequences of the amino acids of **Malaciobacter mytili**. This finding plays a vital role in the drug binding sites of the multi-drug resistant protein sequence. In future, this drug can be validated using wet lab studies andcan be used as a potential therapeutic agent for diseases caused by resistant bacteria.

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