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Insilico Analysis of Multidrug Efflux Pump Protein from *Klebsiella pneumoniae* and its Active Inhibition by Reserpine

S. Amala[1], S. Meenakshi[2] and S. Hemalatha[3]

[1]Lecturer, Dhanalakshmi Srinivasan College of Arts & Science for Women, Perambalur, India
[2&3]Lecturer, Karpagam College of Engineering, Coimbatore, India
E-mail: amala.santhanam@gmail.com

Abstract

Multi drug resistance exhibited by pathogenic strains of bacteria remains always a threat for existing treatment regimens. A pathogenic bacterial strain *Klebsiella pneumoniae* exhibiting multidrug resistance through expressing outer membrane protein efflux pumps was isolated and characterized having an over expressed outer membrane efflux pump protein of approximately molecular weight of 56.1KDa. Reserpine, a potent inhibitor was analyzed for its effectiveness in inhibiting efflux pump in insilico. The protein structure of the predicted membrane protein was constructed by Bioinformatics tools and the efficiency of Reserpine to bind the pump protein was evaluated by docking using Hex programme. Reserpine found efficient in inhibiting the predicted structures, which offers multiple applications in drug transporters as they are supposed to share a considerable homology among themselves.

Keywords: Klebsiella, Reserpine, Multidrug, Efflux pump.

Introduction

Efflux as a mechanism of antibiotic resistance was first reported linearly 1980's for tetracycline Efflux pumps in bacteria contribute to intrinsic resistance to a wide range of antibiotics and often have a broad substrate range. Typically the over expression of multidrug efflux pump confers resistance to antibiotic including fluoroquinolones,

some dyes (eg: Ethidiumbromide), detergents (eg, SDS), and disinfectants (eg: cetrimide) (Piddock LJ *et al.*, 2006). Multidrug resistance efflux pumps). The over expression of multi drug efflux pumps can lead to low level multidrug resistance, which possess a clinical problem

The efflux of a broad range structurally unrelated toxic compounds can be the primary physiological function of multidrug transporters or it can be merely a fortuitous side effect of the transport of an unidentified specific physiological substrate. (Neyfakh, *et al.*, 1991). The latter role of MDRs would be consistent with the observation that multiding transporters belong to four distinct families which are frequently more homologous to substrate specific transporters than to each other.

Klebsiella pneumoniae (and some related species) is an opportunistic Gram-negative rod pathogen involved in the outbreaks of nosocomical infections (in intensive care units), lower respiratory, urinary tract and burn wound infections. In fact nosocomial infections associated with *Klebsiella spp.*, have shown an increase in most part of the world recently (Li *et al*., 2004).

In *Klebsiella pneumoniae*, unusual classes of multiple drug resistance (MDR) mutants exhibiting simultaneous to the structurally unrelated antibiotics were isolated by selection of resistance to beta-lactam antibiotics or to fluoroquinolones (eg. Ofloxacin) (Borsch *et al.*, 1993). The drug-resistant organisms will continually evolve and escape drug treatment, thus providing a threat to health. The challenge is to rapidly identify such organisms as they emerge, assess their potential impact on health, measure their prevalence in the hospital and community, and devise policies to minimize their spread. (Cohen *et al.*, 1992)

Reserpine is a naturally occurring alkaloids produced by several members of the genus Rauwolfia, a climbing shrub indigenous in Southern and South East Asia. There is no known commercial production of synthetic Reserpine. The chemical is extracted from the roots of *Rauwolfia serpentina* with alcohol or aqueous acids and then purified. Reserpine occurs as white or slightly yellow crystals or powder. It is very sparingly soluble in water, slightly soluble in acetone, methanol, ethanol, diethyl ether. Reserpine is used to lower blood pressure.

Alternatively multidrug transporters may have evolved to protect cells form diverse environmental toxins (Schmitz, *et al.*, 1998) and induced in response to cellular damage and stress conditions. Furthermore the substrate spectra of several multidrug transporters include detergents (Putman *et. al.*, 2000) bile salts, organic solvents suggesting that multidrug transporters play a role in protection of the membrane integrity or energy state of the cell.

Materials and methods

To analyze the protein & assign its functional and structural roles various tools & soft wares were used. The primary sequence of the protein was obtained form Genbank at

NCBI. The sequence was then compared for detecting the homologues sequence found in database using BLASTP. Protparam is the tool which was used for the calculation of the physicochemical properties. The secondary structure of the protein was predicted by tools like GOR. The conserved domains in the proteins were detected from the BLAST analysis. Since only the primary sequence information was available from NCBI, no structure in the X-ray crystallographic data was available from PDB. Hence the modeling of the protein has to be done to deduce the 3D structure of protein. Here Homology modeling was done by using Swiss model & the structure was validated. Then the 3D co-ordinate file was visualized in the Rasmol. The transmembrane helices of the efflux protein was predicted by the tool TMpred. The Drug Reserpine structure was retrieved from database and it was allowed to dock with the protein using HEX.

Results

The similarity search for the sequence was carried out with the help of BLAST tool(Fig 1).The Physicochemical properties of the hypothetical protein showed that the number of amino acids are 471 with a Molecular weight of 50694.4 & Isoelectric point as 10.10.The maximum number of the amino acid present in the sequence was found to be Leucine (15.9%) & the least was found to be Glutamine (0.4%).Total number of positively charged residues were 59 and negatively charged residues were 67.The instability index of the protein was computed to be 40.51 which classified the protein as unstable. The grand hydropathicity was calculated to be 0.778.The secondary structural analysis of protein revealed that the random coil (42.46%) was found to be the most frequent followed by alpha helix(35.88%).Extended strand was found to be least frequent (21.66%) (Fig 2).The structure of the hypothetical protein was deduced by homology modeling. The homology modeling was performed using Swiss model workspace (Fig.3).The transmembrane helices prediction showed that 12 helices are found from Inside to outside & 22 helices are found from Outside to inside (Fig 4).The docking of Reserpine revealed that found efficient in inhibiting the predicted Efflux protein structure. (Fig 5). It showed that Reserpine can act as a drug for the multi drug resistant organisms

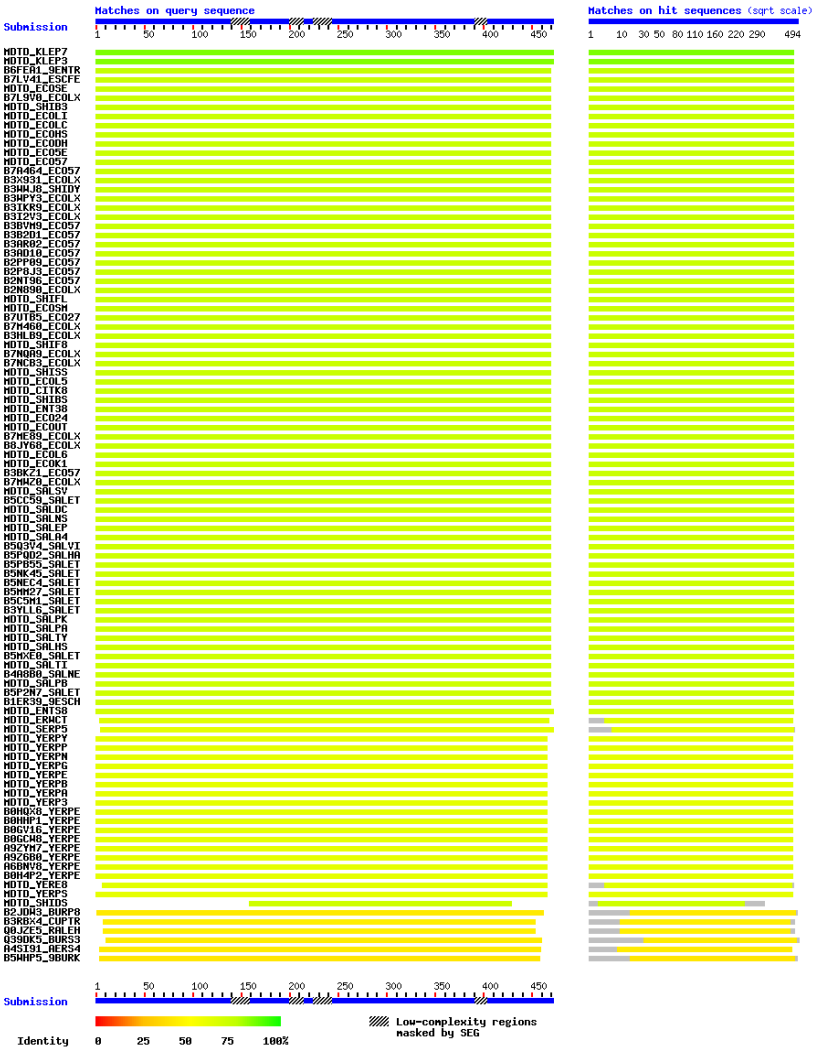

Figure 1: Blast Result.

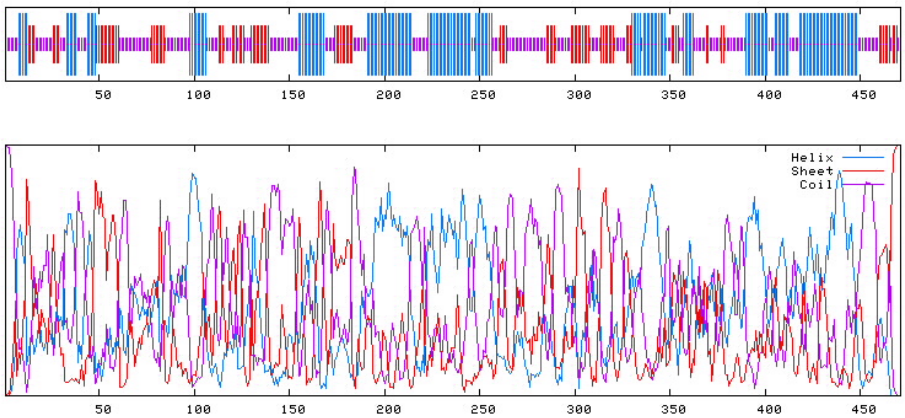

Figure 2: GOR Result.

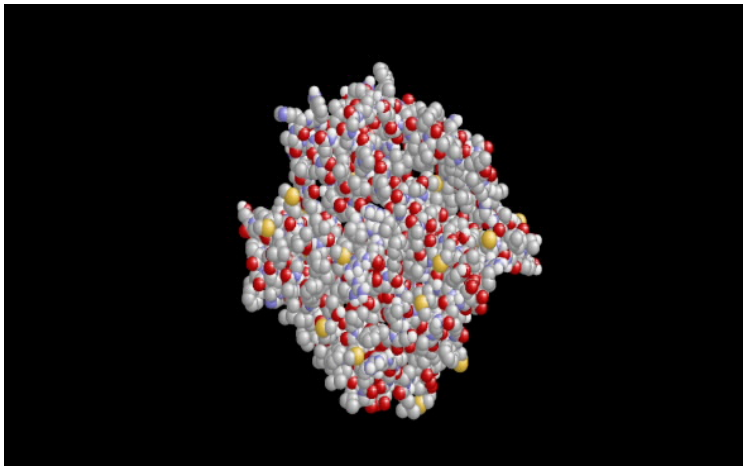

Figure 3: Modelled Efflux protein.

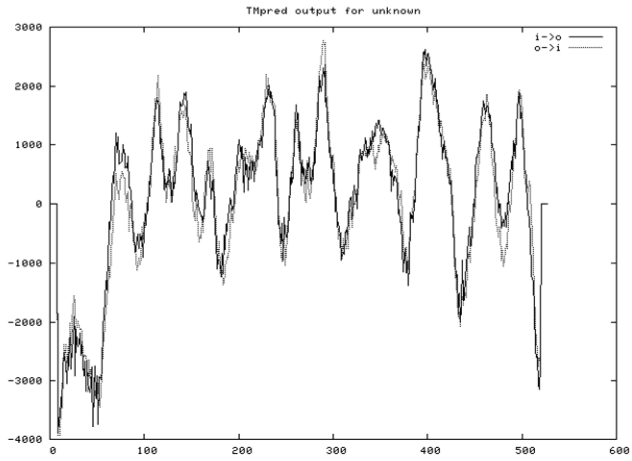

Figure 4: TMPred Result.

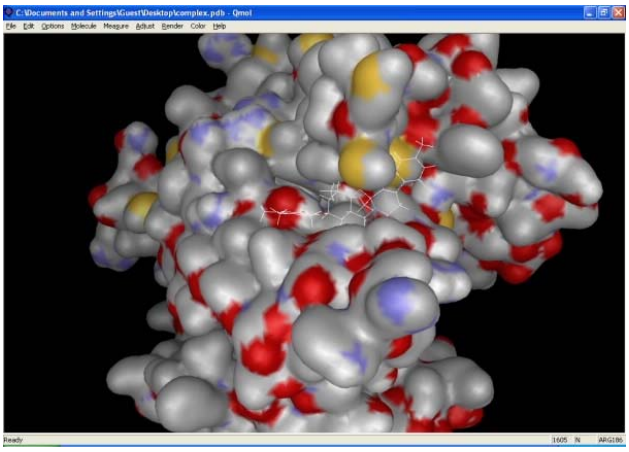

Figure 5: Reserpine docked into the efflux protein.

Conclusion

The current study put forward many suggestions to combat drug resistance mediated by efflux pumps. The molecular characterization by sequencing bioinformatics tools gives opportunity to design ligand (chemical antagonists) by docking and theoretical calculations. Multi drug resistance exhibited by pathogenic strains of bacteria remain always a threat for existing treatment regimens. A pathogenic bacterial strain Klebsiella pneumoniae exhibiting multidrug resistance via expressing outer membrane protein efflux pumps was isolated and characterized having an over expressed outer membrane efflux pump protein of approximately molecular weight of 56.1KDa. Reserpine, a potent inhibitor was analysed for its effectiveness in inhibiting efflux pump in insilico. The protein structure of the predicted membrane protein was constructed through Bioinformatics tools and the efficiency of Reserpine to bind the pump protein was evaluated by docking using Hex programme. Reserpine found efficient in inhibiting the predicted structures, which offers multiple applications in drug transporters as they are supposed to share a considerable homology among themselves. The effect of Reserpine alone was determined to nullify the suggested toxic effects of Reserpine. The results suggest potent application of Reserpine as a combinatorial drug in successful treatment of wide spectrum of multi drug resistant organisms using routinely used antibiotics.

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