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Abstract

oxidoreductases probably causes resistance in gram negative bacteria

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Abstract:
Treatment of infections are caused by Gram-negative pathogens due to the limited use of antibiotics and increased resistance to drugs and their tendency to develop recurrent infections. Hence, there is increasingly urgent need to identify new goals and thereby combinations to improve anti-bacterial agents. Studying the effects of these factors on pathogenesis and antibiotic resistance is important to identify and design new effective antibacterial agents. Among these essential factors in the pathogenicity and antibiotic resistance some oxidoreductase can be mentioned(1). monooxygenases are a group of oxidoreductase enzymes. They are able to metabolize drugs and some antibiotics, such as imipenem. These enzymes probably due to the ability of oxidation of heterocyclic compounds, including some antibiotics, have a role in biotransformation, making them resistant and enduring infections. In this study, monooxygenase was studied in silico. However, practically the gene from Pseudomonas was amplified by PCR, cloned in a pET-22b and expressed heterologously in E.coli BL21 (DE3). The recombinant proteins were analysed by SDS-PAGE method, purified by Ni-NTA purification system and identified by western blotting. Then, the function of the enzyme in the oxidation of ampicillin was demonstrated in the presence of FAD and NADPH as cofactor and coenzyme. Thus, the ability of the recombinant enzyme to affect ampicillin by oxidative activity was confirmed. Enzyme activity of Fmo5p was assayed photometrically. A total of 100 µl of reactions was used to test the susceptibility of BL21 cells to ampicillin metabolized by disk diffusion method, and the growth inhibition zone was evaluated as bacterial susceptibility. By measuring the growth inhibition zone after 16 hours of culture of susceptible bacteria on agar plate, a diameter of 30 mm was observed for the control plate and 10 mm for the test plate, which was a confirmation of the oxidative activity of Fmo5p on ampicillin. The enzyme could also be used as a target to design new drugs to fight antibiotic resistance.

Biography:
Malihe Mohamadkhani is a graduate of Microbial biotechnology with a master's degree from Semnan university, Iran. She holds a BSc in Cellular and Molecular Biology with a focus on

Biotechnology for 4 years at the University of Isfahan / Iran. She worked on cloning, protein expression and determining the function of a bacterial enzyme to detoxify certain xenobiotic and carcinogenic compounds, and was able to defend her thesis to an excellent degree. She has been a brilliant student in both undergraduate and graduate schools. She is a member of the Iranian Association of Young Mathematicians and Biologists. She has presented an abstract on Reviewing the Safety as well as Biological and Ethical Aspects of CRISPR-Cas Gene-Editing Technology in 2nd International Congress and 10th National Conference of Biotechnology, Islamic Republic of Iran, Tehran, August 2017. She presented another abstract on In Silico Study of Dimethylaniline Monooxygenase Structure from Pseudomonas Aeruginosa in the 3rd International Congress and 11th National Conference of Biotechnology, Islamic Republic of Iran, Tehran, August 2019. She is preparing the main research article extracted from her thesis. Maliheh grew up in Isfahan city and she is interested in cancer topics and drug design.

Publication of speakers:

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