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Author: Zeynab Zare

Title of thesis: Production of recombinant endolysin KP27 protein antibiotic and investigation of its activity on *E.coli* and *Staphylococcus aureus* bacteria

Antibiotic resistance occurs when bacteria develop mechanisms to resist antibiotics, often by acquiring resistance genes through plasmids or mutations. These changes can include production of antibiotic-degrading enzymes, changes in antibiotic targets, or activation of efflux pumps. Endolysins, enzymes of bacteriophages, break bacterial cell walls and cause lysis. Endolysin KP27, from Klebsiella pneumoniae bacteriophage, shows its potential against antibiotic resistant strains. Klebsiella pneumoniae, found in hospitals and natural environments, has pathogenic factors such as seroresistance and capsular polysaccharides. The high antibiotic resistance of K. pneumoniae highlights the need to study bacteriophages such as Myoviridae phage vB_KpnM_KP27 (KP27). Endolysin KP27 binds zinc ions in its N-terminal monoamine, similar to carboxypeptidases and bacterial peptidases, and cleaves between L-Ala and D-Glu residues in peptidoglycan. This endolysin has 140 amino acids, weighs 15.8 kDa, and shows peptidase activity. Since the phenomenon of drug resistance and pathogenicity and its complications is a global problem and pathogenic bacteria such as E. coli and S. aureus have pathogenic factors such as proteins, toxins and enzymes that cause skin infections, food poisoning and infections It becomes severe like bacteremia and endocarditis.

The KP27 gene in the pET-28a vector was transformed into E. coli BL21 cells and they were cultured on a plate containing kanamycin and selected. After confirmation by PCR and gene sequencing, protein expression was induced by lactose and then purified by column chromatography. After its purification and confirmation by SDS-PAGE gel, its activity was checked on two bacteria S.aureus and E.coli. According to the obtained results, the minimum MIC on S.aureus and E.coli bacteria has an inhibitory effect of 0.004, 0.008 mg/l, respectively, and the minimum lethality (MBC) of 0.1 mg/1 has lethal properties on both has a bacterial species, which causes 99.99% lethality at a concentration of 0.1 mg/l and 50% lethality at a concentration of 0.05 mg/l within 90 minutes. The evaluation of the effect of temperature on the performance of endolysin in the temperature range of 37-70C in a period of 30 minutes, its inhibitory effect was similar to the untreated group. In evaluating the effect of pH on the performance of endolysin, it was observed that it maintains its activity in the pH range of 6-11. The non-toxic effect of endolysin KP27 on red blood cells can be used as a potential of this endolysin as an antimicrobial agent against bacteria, especially antibiotic resistant strains.

Keywords: " endolysin KP27, bacteriophage, cloning, enzyme antibiotic"