The Evolution of Antibiotic Resistance Through Protein Mutations

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The purpose of this experiment is to determine if HisKPC-3 mutated beta-lactamase proteins are more antibiotic resistant than HisKPC-2 proteins, and what is occurring at a molecular level that is allowing this protein to achieve such a high level of resistance with only one single amino acid mutation. The development of a three-dimensional model of a beta-lactamase protein helps analyze the molecular interactions that the protein is partaking in. This will allow researchers to predict how inhibitors can be designed to hinder the protein's ability to stabilize and make antibiotics obsolete. These inhibitors can be utilized as drug leads that aid an antibiotic in the treatment process because they can effectively inhibit all beta-lactamase proteins. The approach taken to complete this experiment began with the designing of a DNA primer and developing it into a transferable plasmid. DNA primers underwent thermal cycles in a polymerase chain reaction that would amplify the genetic sequence and provide millions of copies in plasmid form. The plasmid was transformed into a strain of non-pathogenic E.coli that are favorable for protein purification because they can easily initiate their defense mechanism. Next, a favorable solution was developed to grow KPC-3 protein crystals. Through the use of x-ray crystallography, the crystals were shot at with a high-powered x-ray, resulting in an electron diffraction pattern. This pattern was computed and developed into a three-dimensional model of the KPC-3 protein. Also, a minimum inhibitory concentration test was completed to compare the resistance of KPC-2 and KPC-3. This quantitative data will help understand how resistant KPC-3 is and what researchers must do to overcome its resistance to antibiotics