A Journey To The Crystallized Structure Of KPC-2; β-lactamase Derived From *Klebsiella pneumoniae*

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Klebsiella pneumoniae, a class B bacterium that produces the beta-lactamase protein KPC-2, can be easily controlled under laboratory conditions. Klebsiella organisms are often resistant to multiple types of antibiotics, including the one used in this laboratory, aminoglycosides (kanamycin). Current scientific evidence show that plasmids are the primary source of the resistance genes.

The purpose of this research conducted was to work on the beta-lactamase protein, KPC-2, a gram-negative bacterial enzyme from Klebsiella pneumoniae. The experiment involves molecular cloning of the DNA encoding the KPC-2 sequence into a circular DNA plasmid, recombinant protein expression in E. coli, and protein purification. The structure of KPC-2 has already been solved, but we would like to perform further structural studies of the enzyme by crystallizing the specific enzyme, with hopes to obtain diffraction patterns for crystallographic data analysis by using a bright X-ray light source, such as a third-generation synchrotron. The experiment began with the PCR of various vectors and inserts to create an overlap, and once the DNA that codes for KPC-2 beta-lactamase, came in, a PCR was run in order to produce more copies of the desired DNA. We set up a temperature gradient in order to see which temperature would work the best at producing a readable band. After the PCR was done, a gel was run to determine the best temperature (68.3 degrees Celsius). We then ran a DPN1 digest, a Gibson Assembly, transformed the DNA into DH5α, conducted a colony PCR, conducted a mini prep, and sent the DNA off for sequencing. The protein was successfully cloned on the fourth attempt using the Gibson Assembly process, and protein purification using the expression strain is underway. The protein will be purified by affinity chromatography using a nickel resin and size exclusion chromatography. If successful and time remains, the crystallization process will be attempted to allow more structural studies.

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