

Attempting to Solve the Crystal Structure of β -Lactamase, TEM-60

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Antibiotics disrupt the synthesis of harmful bacteria's cell wall such as *Mycobacterium Tuberculosis*. However, β -Lactamases are enzymes which are encoded in the chromosomes of bacterial cells that degrade antibiotics. I am studying a Class A β -Lactamase, TEM-60. By studying the atomic structure of β -lactamases, we can gain a greater understanding of how the structure of the enzyme works to catalyze antibiotics.

In this study, molecular cloning techniques of Gibson Assembly and Pipe Cloning were conducted to create a plasmid with the genetic information to produce TEM-60. Multiple different primer designs and ratios of vector (pET28a) to insert were combined and cloned in 10G *E.Coli* cells to achieve a properly sequenced plasmid. Kanamycin was used throughout as a selective marker for our exogenous plasmid. Recombinant protein expression methods using BL21 *E. Coli* cells produced large amounts of TEM-60 once induced with 0.5mM IPTG. Cells were lysed through sonication, and an affinity chromatography matrix purified the recombinant protein using a nickel resin to bind the His-tagged protein while other proteins flowed through. However, SDS-page gels confirmed that the protein is currently insoluble, and therefore prior protocol will be modified. Although this portion of the research project has not yet been conducted, once TEM-60 is soluble it will be further purified through affinity chromatography and size-exclusion methods. Then it will be concentrated until crystallization experiments are conducted for the optimization of protein crystals using sitting drop vapor diffusion method. These protein crystals will be sent to a synchrotron, and x-ray diffraction pattern data will be collected to generate a structural model of TEM-60.

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