Mutagenesis and Expression of BF4112 in E. Coli and Bacteroides Fragilis

Summer 2017 Research Summary

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Previous work has been done by the Benson lab to characterize a cysteine tyrosine crosslink in the active site of the protein BF4112. While characterization had been done with wild type protein, some specific point mutants had been created in order to further characterize the cross link and its mechanism. Previous mutants that had been made were Y52F, Y4F, Y4F/Y109F, and C98S.

My work in the first part of the summer consisted of creating more point mutants of BF4112 using Quikchange mutagenesis. The point mutants C98S/Y4F, C98S/Y109F, C98S/Y4F/Y109F, E16K, and E16Q were all successfully created. The mutants that change cysteine and tyrosine to serine and phenylalanine, respectively, are aimed at further characterizing the cysteine tyrosine crosslink and the mutants that change the glutamic acid to lysine and glutamine strive to fix solubility issues. Once the point mutations were confirmed by sequencing, the plasmid was transformed into two different strains of E. coli, TOP10 and BL21. The TOP10 strain is engineered to generate plasmid and the BL21 strain to make protein. The colonies from the transformation plates were grown up in liquid cultures from which either the plasmid or protein was harvested. The protein was then purified using an affinity column and a size exclusion column. Other members of my team then performed experiments to further characterize the cysteine tyrosine crosslink in the mutated forms of BF4112.

My work in the second part of the summer consisted of expressing BF4112 in *Bacteroides fragilis*. *Bacteroides fragilis* is an anaerobic gut bacteria where BF4112 is naturally expressed. In order to express BF4112 in *Bacteroides fragilis*, we used a plasmid, pER 153, which originally contained BS2, a gene for a fluorescent protein. With the use of restriction sites, we cut BS2 out of the plasmid and ligated the BF4112 gene into the plasmid. This plasmid was transformed into *Bacteroides fragilis* by first making the *Bacteroides fragilis* cells electrocompetent then performing electroporation to get the plasmid into the cells. The bacteria was grown up in liquid cultures and harvested with the goal of purifying BF4112 in order to answer questions concerning the naturally expressed form of BF4112. So far we have successfully electroporated the cells, grown them up into 1-liter cultures, and harvested the cells. The next step will be to purify the protein using the affinity column and size exclusion column and finally to perform experiments to answer questions concerning certain aspects of this naturally expressed form of BF4112.

This summer's research has been greatly beneficial to me. After Calvin, I want to attend graduate school to further my study of molecular biology. I have taken labs at Calvin, but this summer has truly allowed me to learn so much more than I would in the structured lab setting. Through summer research, I have not only learned and refined lab techniques, learned about new instruments and methods, and had the opportunity to deeply learn about a specific area of research, but I have also learned what it means to problem solve in the lab. This skill cannot truly be taught in the structured lab setting. In the research lab setting, one must be able to think on their feet, learn to design their experiments with their own goals in mind, both prep and execute the experiment, then figure out the next step that must be taken based on the results of the previous experiment. Overall, this summer has been such an amazing learning experience for me.