Expression and Purification of a Putative Regulator involved in Bacterial Fatty Acid Biosynthesis

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The regulation of fatty acid (FA) metabolism in bacteria is a tightly coordinated process that confers the membrane its characteristic permeability properties and prevents the accumulation of free FAs which can be toxic to the organism. Several marine bacteria produce eicosapentaenoic (EPA) and docohexanoic (DHA) acids, both omega-3 FA, in high proportions as part of their normal fatty acid metabolism. These two FAs (EPA and DHA) are considered essential in humans and are important for brain development and cardiac health. Preliminary data show that current sources of EPA and DHA for human consumption comes from oily fish, whose global numbers are being rapidly depleted by overfishing. Thus, it is important to seek new ways of making these fatty acids in cellular systems that are both cost-effective and renewable. Since the microorganisms that make these fatty acids naturally are notoriously difficult to propagate and grow under standard laboratory conditions, there is an active search for ways to transfer entire gene clusters for their production in more industrially amenable organisms, such as *E. coli* or *S. cerevisiae*. The EPA gene cluster contains all the genes that are involved in the biosynthesis of EPA in Shewanella species. The *pfaR* gene in the EPA cluster is positioned between other genes and its predicted secondary structure suggests that it may be a regulator of this pathway. Our hypothesis is that *pfaR* produces a protein and acts as a regulator by binding to other genes in the EPA gene cluster. We will determine whether a PfaR-DNA interaction takes place by electrophoretic mobility shift assays (EMSAs) which requires pure fragments of DNA and pure recombinant PfaR. We will PCR-amplify DNA sequences located directly upstream from the main EPA biosynthesis genes, especially *pfaR* from Shewanella sp. Recombinant PfaR will be made by standard molecular cloning methods and expressed in *E. coli*. Finally, both DNA fragments and recombinant PfaR will be incubated together and resolved on a native polyacrylamide gel and visualized on an Odyssey IR Imaging System. With this work we expect to define the function of PfaR as a possible regulator of fatty acid biosynthesis. The information gained in this project could help to drive the production of polyunsaturated fatty acids in *E. coli,* possibly pushing the production yields closer to commercial scale.