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Determination of Optimal Conditions for *BCHF* Enzymatic Activity

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Poster Presentation P21

**DETERMINATION OF OPTIMAL CONDITIONS FOR
BCHF ENZYMATIC ACTIVITY**

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Photosynthetic organisms possess the unique ability to capture solar energy and utilize it in the formation of carbon compounds from carbon dioxide and water. Without photosynthesis, all life on Earth would be unable to acquire energy in a usable form. Because this energy conversion evolves oxygen, photosynthesis also sustains life by providing it with the atmospheric conditions needed to breathe. The first step in the photosynthetic process involves the absorption of light energy by pigments. While chlorophylls, found in higher plants and algae, are often the most familiar photosynthetic pigments, photosynthetic bacteria utilize a related pigment called bacteriochlorophyll. Study of bacteriochlorophyll and the mechanism of this pigment's biosynthesis can provide further insight into the crucial process of photosynthesis, as well as its evolutionary history. The previously-identified genetic locus *bchF* encodes an enzyme that catalyzes a certain step in the bacteriochlorophyll *a* biosynthesis pathway. Specifically, water adds to a vinyl group at position three of chlorophyllide *a* to form 3-hydroxyethylchlorophyllide. The steps of bacteriochlorophyll biosynthesis have been well-defined genetically, but the specific requirements for *bchF* enzymatic function have not been explored, such as its optimum pH and temperature. The study of *bchF* is significant because an *in-vitro* assay of this enzyme has never been produced; demonstrating that the enzyme is functional *in-vitro* would be an important step forward in understanding the biosynthesis of bacteriochlorophyll *a*. After inserting the gene into three different expression vectors, substantial levels of protein expression could not be obtained. After exhausting most other options, an optimized, artificial *bchF* gene within an expression vector was purchased. Once expression of the artificial gene is confirmed, the next step is to extract pigments from *bchF* mutants. This will allow for enzyme activity testing using the isolated pigment as a substrate. The ultimate objective is to characterize the optimal conditions for *bchF* enzymatic activity and to determine if the *bchF* gene alone is sufficient to catalyze this step in bacteriochlorophyll biosynthesis.