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Cloning the *bchZ* Gene from *Chloroflexus Aurantiacus*

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Poster Presentation 4

CLONING THE *bchZ* GENE FROM *CHLOROFLEXUS AURANTIACUS*

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Chloroflexus aurantiacus is a filamentous photosynthetic bacterium that was first isolated from Japanese hot springs and moves by gliding without the aide of flagella or cilia. Characterized as a green non-sulfur bacterium, *C. aurantiacus* has an interesting evolutionary position in that it shares similarities with both green sulfur and purple non-sulfur bacteria, two different classifications containing photosynthetic bacteria. Therefore, *C. aurantiacus* may be a key organism to study the early processes of photosynthesis and its evolution.

This bacterium has in its chemical composition various photosynthetic pigments used for capturing light energy. The main pigments include bacteriochlorophylls *a* and *c*, which are synthesized depending on light and oxygen conditions of the environment. The bacteriochlorophyll biosynthetic pathway in photosynthetic bacteria is catalyzed by various enzymes encoded by genes organized in a region of the genome called the photosynthesis gene cluster. One group of genes in the cluster is *bchX*, *bchY*, and *bchZ*. These genes code for components of a chlorin reductase which catalyzes the reaction converting chlorophyllide *a* to 2-desacetyl-2-vinyl bacteriochlorophyllide.

The objective of the research was to clone the *bchZ* gene from *C. aurantiacus* by molecular genetic techniques for further study. In general, a gene can be cloned by inserting it into another organism, like that of *Escherichia coli*, such that the gene will be replicated every time the cell divides, thereby creating multiple copies. Because the *bchZ* sequence in *C. aurantiacus* is unknown, polymerase chain reaction (PCR) primers consisting of short oligonucleotides were synthesized based on consensus sequences of the *bchZ* gene from green sulfur and purple non-sulfur bacteria. The PCR primers were used to amplify a small region of the *bchZ* gene which was then ligated into a plasmid vector. The ligated plasmid was used to transform competent *E. coli*. Blue and white screening of colonies and restriction digests indicated whether insert of the amplified portion of *bchZ* actually occurred. DNA sequencing was then used to determine if the cloned insert was indeed *bchZ*.