

**STUDIES ON BACTERIOCHLOROPHYLL *e* BIOSYNTHESIS IN BROWN-COLORED GREEN SULFUR BACTERIA AND IMPLICATIONS FOR UNDERSTANDING THE LOWER LIGHT LIMITS OF PHOTOTROPHY.** J. L. Thweatt<sup>1</sup> and D. A. Bryant<sup>1,2</sup>, <sup>1</sup>Department of Biochemistry and Molecular Biology Pennsylvania State University, University Park, PA 16802, <sup>2</sup>Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717.

**Abstract:** Green sulfur bacteria (GSB) are strictly anaerobic photolithoautotrophs that live in anoxic-sulfidic, or euxinic, environments. There is evidence of large-scale euxinia in the oceans of Earth's geological past [1]. Today, however, GSB are limited to the anoxic-sulfidic zones of stratified lakes, sulfidic springs, and stratified sediments. In addition to their ecophysiological relevance as analogs to past euxinic events on Earth and exoplanetary euxinic environments, GSB live at the lowest light intensities of any known chlorophototrophic organism, defining the lower limit of light intensity for phototrophy on Earth [2]. In order to live in low-light intensities, GSB produce highly efficient light-harvesting antenna complexes called chlorosomes, which consist of up to 250,000 specialized, self-aggregating bacteriochlorophylls (BChl) enclosed in a protein-lipid monolayer envelope [3].

Despite the name, GSB actually come in both green- and brown-colored varieties depending on the type of BChl they have in their chlorosomes. The chlorosomes of green-colored GSB contain either BChl *c* or BChl *d*, while the chlorosomes of brown-colored GSB contain BChl *e*. Brown-colored GSB are typically found lower down in stratified environments and in lower light intensities than their green-colored counterparts. For example, the brown-colored strain *Prosthecochloris* sp. BS-1 grows about 100 m beneath the surface of the Black Sea at a light intensity of  $\sim 3 \text{ nmol photons m}^{-2} \text{ s}^{-1}$ , approximately  $10^{-6}$  fold lower than at the surface. This translates to 1-10 photons per BChl per day, an extremely light-limited environment [4].

Studying brown-colored GSB is important to understanding the lower limits of phototrophy on Earth. In the past 5 years a genetic system was developed in a brown-colored GSB, opening up the possibility of detailed physiological studies of mutants in these organisms [5]. In this study we use an insertional inactivation mutant to confirm that a gene designated *bciD* is necessary for production of BChl *e* in a brown-colored strain of GSB, *Chlorobaculum* (*Cba.*) *limnaeum*. Mutants of *bciD* in *Cba. limnaeum* produce BChl *c* instead of BChl *e*. In addition, *in vitro* characterization of the enzyme encoded by *bciD* showed that it is sufficient to convert

bacteriochlorophyllide (BChlide) *c* into BChlide *e* (the direct biosynthetic precursor of BChl *e*) [6]. Ongoing work comparing the growth physiology and energy transfer characteristics of the green-colored *bciD* inactivated strain of *Cba. limnaeum* to the brown-colored wild-type strain will allow us to better understand the effect of pigment composition on the low-light physiology of these important chlorophototrophs.

**References:** [1] Meyer K. M. and Kump L. R. (2008) *Annu. Rev. Earth Planet. Sci.*, 36, 251–288. [2] Overmann, J. and Garcia-Pichel F. (2013) *The Prokaryotes: Prokaryotic Communities and Ecophysiology*, 203–257. [3] Montaño G. A. et al. (2003) *Biophys. J.*, 85, 2560–2565. [4] Manske A. K. et al. (2005) *Appl. Environ. Microbiol.* 71, 8049–8060. [5] Vogl K. et al. (2012) *Front. Microbiol.* 3, 298. [6] Thweatt J. L. et al. (2017) *J. Biol. Chem.* 292, 1361–1373.