Effects of Chloramphenicol Treatment on Cellular Storage Granules and Membrane Structures in *Rhodobacter sphaeroides*

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Project Goals: This project seeks to define the physiological responses of bacterial cells to antibiotic treatments. This information will be used to improve our understanding of bacterial stress responses, including the induced accumulation of cellular structures that may be beneficial for bio-industrial and bioenergy purposes. Bacterial storage compartments, such as polyhydroxybutyrate (PHB) granules and polyphosphate (PP) granules are cellular structures highly enriched in specific chemical compounds. Bacterial membranes are a source for a variety of lipids. All of these structures may serve as important sources of primary material for a number of pharmaceutical, industrial, and biofuel applications. In this work, we use the photoheterotrophic bacterium *Rhodobacter sphaeroides* to study the accumulation of storage granules and membranes using cryo-electron tomography (cryo-ET), fluorescence microscopy, and biochemical purifications. Addressing these goals will provide a significant benefit to the development of renewable biofuels and bioproducts.

Rhodobacter sphaeroides is an excellent model organism for the studies of cellular structures and bioenergy. It is a facultative photoheterotrophic bacterium that generates diverse cellular structures, including various storage granules and intracytoplasmic membranes, depending on its growth conditions. In this study, we observed the behavior of three cellular structures in response to the translation blocking antibiotic, chloramphenicol (Cm). The storage granules we studied were: PHB, a subcellular compartment where butyrate monomers are polymerized and are stored under nutrient rich conditions; PP, a chain of inorganic phosphate residues linked together to serve as storage for reducing potential; and internal and external cell membrane derived structures. An *R. sphaeroides* culture, grown in Sistrom's medium, was treated with 200 µg/mL Cm and samples were collected for cryo-ET, fluorescence microscopy, and gas chromatography mass spectrometry (GCMS). Upon Cm treatment, the occurrence of PHB granules inside of individual cells changed. Using cryo-ET, it was observed that the average radius of individual PHB granules increased nearly 7-fold, and the corresponding volume of PHB per cell increased approximately 5-fold, despite this volume being comprised of fewer granules per cell. The accumulation of PHB in the cell was further verified by GCMS analysis. In addition to PHB granules, *R. sphaeroides* cells accumulate PP in granules that are smaller, more electron dense, and less labile to electron dose than PHB. These observations will be presented. The presence of PP granules can be controlled by growth in a medium lacking excess phosphate, further corroborating their identity as PP granules. Accumulation of PP is also increased by Cm treatment. In addition to the observation of changes to these nutrient storage granules, it was also noted in cryo-ET volumes that aggregations of cellular membranes were present in the wild-type strain of *R. sphaerorides*. Highly irregular membrane aggregations in the periplasmic space formed both at the pole and along the cell body. These membrane aggregations were apparent by fluorescence microscopy using membrane staining. Progress toward analyzing the changes in fatty acid and lipid profiles following Cm treatment will be presented.

Our observations lead to an understanding of the cellular responses to antibiotic treatment. Since chloramphenicol blocks translation, thereby inhibiting cellular processes, it is possible that accumulation of nutrient and energy stores may occur as a method of mitigating and preparing for Cm induced stress. Accumulation of irregular membrane structures demonstrates that perhaps misregulation of intracellular membrane production occurs. Our findings lead to a better understanding of mechanisms bacteria may use to respond to, and possibly cope with, antibiotic stress, thus having the potential to impact studies of antibiotic resistance. Additionally, these analyses demonstrate conditions under which *R. sphaeroides* can be encouraged to produce stores of carbohydrates, lipids, and reducing power – all of which may have utility toward creating better chemicals for industrial purposes and biofuels.

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