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

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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. *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. 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.

For this work we are using *Rhodobacter sphaeroides*, a facultative alphaproteobacterium with features that make it attractive for analysis. *R. sphaeroides* cultures were grown at 30°C and 200 RPM shaking in Sistrom's minimal medium until the culture reached an OD_{600} of 0.4. Cells were then treated with the antibiotic chloramphenicol. Suspensions of the cells were stained with the membrane dye FM4-64 and deposited onto 200 mesh R2/1 copper Quantifoil grids in 5 μL aliquots, blotted, and plunge frozen in liquid ethane using a Vitrobot Mark IV (ThermoScientific). An additional sample was used for epifluorescence microscopy. Cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) data were collected using a Titan Krios TEM (ThermoScientific) operated at 300 kV, equipped with a Bioquantum energy filter and a K3 direct electron detector (Gatan). Single axis tilt series were acquired using SerialEM [1], with an increment of 2° covering −60° to +60°, or an increment of 3° covering −60° to +60° and a cumulative dose under 45 $e^-/Å^2$ at a defocus range between −4 and −10 μm. Tomograms were reconstructed using IMOD/eTomo [2], and 3D rendering was performed using EMAN2 neural network segmentation training[3].

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 (Figure 1). 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 (Figure 1) 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. sphaeorides* (Figure 2). 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 [4].

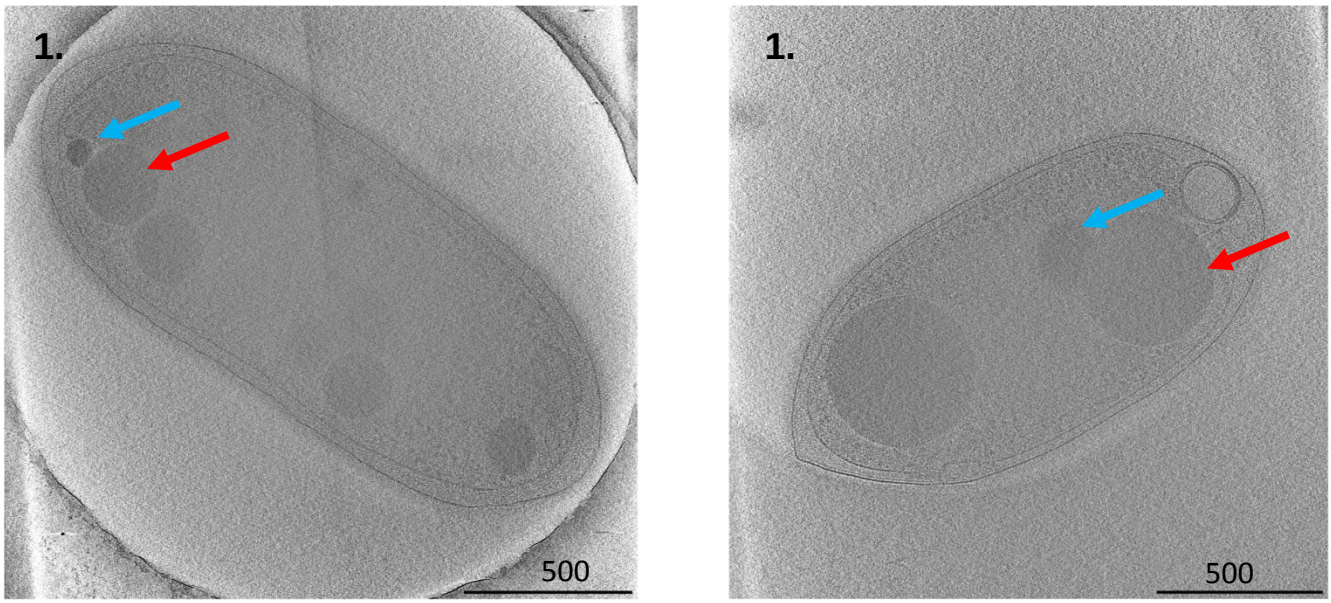

Figure 1. PHB and PP accumulate in granules upon Cm treatment. An *R. sphaeroides* culture was grown in SIS medium and left to grow (A) or treated with 200 µg/mL Cm (B). Scale bars are 500 nm.

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PHB granules (red arrows), and Polyphosphate granules (Blue arrows) are indicated. Upon Cm treatment PHB granules are increased significantly in size compared PHB granules in untreated cells. Scale bars are 500 nm.

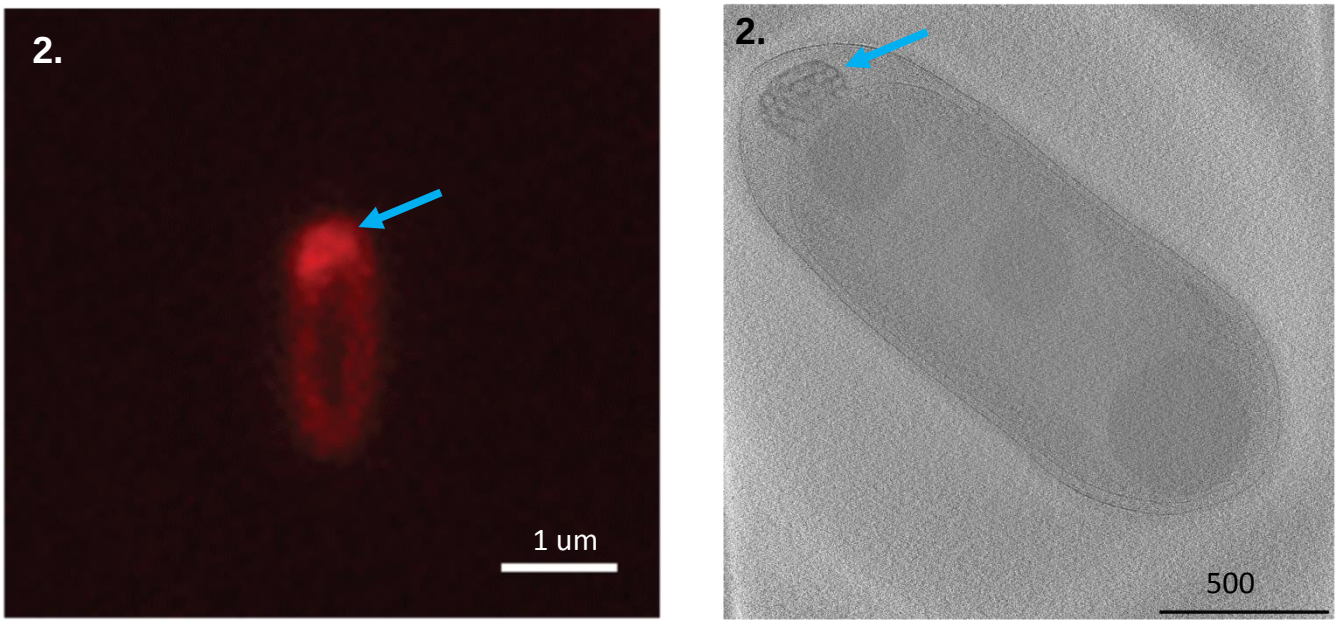

Figure 2. Membrane aggregations are present in response to Cm treatment. An *R. sphaeroides* culture was grown in sis medium and treated with 200 μg/mL Cm. The culture was stained with the membrane stain FM4-64 (red fluorescence) and images were collected by epifluorescence microscopy. Aggregations of cell membranes were apparent as bright regions of staining (blue arrows) Scale bar is 1 μm. B. Membrane aggregations (blue arrows) appear as amorphous, tubular structures in the periplasm in the central slice of a cryo-tomogram. Scale bar is 500 nm.

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