Outer Membrane Vesicle Production of *Pseudomonas fluorescens* when co-cultured with *Rhodococcus*

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This experiment focuses on *Pseudomonas fluorescens (Pf)* and its ability to create outer membrane vesicles (OMVs) in response to environmental cues. OMVs may contain virulence factors, proteins, and DNA. There is a growing body of research on the regulation of OMVs in bacterial pathogenesis, resistance to environmental stressors, and many other survival mechanisms. Apart from eliciting an antibacterial immune response in plants, there is minimal research published on the role of *Pf'*s OMVs specifically (McMillan et al. 2021). As a preliminary experiment, we sought to analyze the effect on OMV production of *Pf* when co-cultured with *Rhodococcus*, a Gram-positive bacterium that is pathogenic towards plants. Both of these organisms were isolated from samples of periphyton that were growing in Lake Killarney in Federal Way. We hypothesized that introducing *Pf* and *Rhodococcus* into a co-culture would increase *Pf'*s OMV production. Our results were quantified using a fluorescent probe and showed no significant data on the production and regulation of the OMVs from *Pf* when in a co-culture with *Rhodococcus*. We suspect an interaction between these two bacteria that impacts their growth rates. Other methods of co-culturing and quantification of bacterial replication in culture will be required in the continuation of this project. These include differential staining methods, fluorescent microscopy and introducing filters in the co-culture to keep the organisms separated. Continuing this research we used a carboxyfluorescein succinimidyl ester (CFSE) stain on *Pf* and hexidium iodide on *Rhodococcus* to be able to differentiate the bacteria using fluorescence microscopy.