Outer Membrane Vesicle (OMV) Biogenesis and Biofilm Characterization of Periphyton Bacterial Organisms

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Outer membrane vesicles (OMVs) are endocytic vesicular structures that transport and secrete cellular cargo contributing to bacterial pathogenesis, stress resistance, signaling, and communication. *Pseudomonas fragi* is a gram-negative motile bacteria known for its ability to create antibiotic resistant biofilms as well as its adaptability to temperature stressors from the environment. The bacterial species of interest were obtained from periphyton samples from Lake Killarney in Federal Way, WA. The goal of this study is to elucidate the responses of arsenic resistant periphyton bacteria in different environments by addressing the knowledge gap on how temperature fluctuation affects OMV production by *P. fragi*. This work also aimed to study the biofilm morphologies of monocultures and co-cultures of *P. fragi*, *Pseudomonas fluorescens,* and *Rhodococcus sp*. Exposure to different temperatures such as cold at 4°C and heat at 37°C, is predicted to increase the total amount of OMVs produced by *P. fragi*. Also, it is hypothesized that co-cultures of the various bacterial species will display a greater symbiotic relationship than the monocultures which is associated with enhanced biofilm formation. Fluorescence microscopy was completed after staining the bacteria with Carboxyfluorescein Succinmidyl Ester (CFSE) and Hexidium Iodide (HE) then adding the samples to agarose pads with M9 media. The methodology for OMV quantitation involves isolating extracellular polymeric substance (EPS) from *P. fragi* plate-grown biofilms in the form of filtered supernatant then conducting a Synaptogreen assay. At various growth temperatures (4°C, 20°C, and 37°C) and exposure times (24, 48, and 96 hours), no statistically significant difference in fluorescence emission per gram at 595 nm was detected among the culture groups. These results do not support the hypothesis since temperature stressors did not promote OMV biogenesis as multiple replicates in different growth conditions demonstrated statistically insignificant differences in fluorescence emission. The bacteria grew adequately at 4°C possibly indicating that the cold temperature was not a stressor. *P. fragi*, *P. fluorescens,* and *Rhodococcus sp.* monoculture and co-culture images were yielded using fluorescence microscopy; however, imaging issues resulted from a lack of adhesion of the bacteria as these organisms tended to form pellicles or air-surface level aggregates. Future work can more clearly establish the relationship between the bacterial species in terms of their ability to form biofilms by possibly finding a more precise method to yield thinner and smoother agarose pad slices. Further characterization of the bacteria in terms of OMV synthesis along with biofilm formation may also include investigating the effects of nutrient availability and arsenic exposure.