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

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## Introduction

**Outer Membrane Vesicles (OMVs) are membrane bound, endocytic vesicles that are synthesized during bacterial growth[3].**

OMVs consist of proteins, lipids, and nucleic acids[3]. Transportation and secretion of various cellular components, bacterial pathogenesis against immune defense systems, resistance to stress, as well as biofilm development are among the functions of OMVs[3]. Subsequently, the role of OMVs in the cellular transport of bacterial surface antigens indicates that these vesicular structures have applications in vaccine design and production[1]. The bacterial species of interest for this study were obtained from periphyton samples at Lake Killarney in Federal Way, WA and include *Pseudomonas fragi*, *Pseudomonas fluorescens*, and *Rhodococcus sp*. *P. fragi* is a gram-negative motile bacteria correlated with spoilage and is known for its ability to create antibiotic resistant biofilms as well as its adaptability to temperature stressors from the environment[2,4]. This study aims to further understand OMV production and biofilm formation of Periphyton bacterial species for possible future applications in bioremediation and biotechnology.

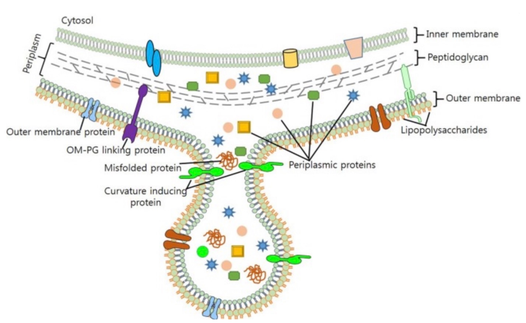



**Figure 1. Bacterial OMV Synthesis[3].**

## Objectives

**[1]** Elucidate the responses of arsenic resistant periphyton bacteria in different environments and address the knowledge gap on how temperature fluctuation affects outer membrane vesicle (OMV) production by *P. fragi*.

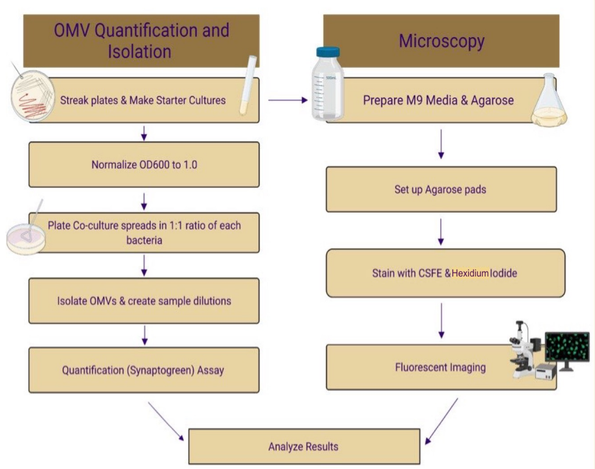
**[2]** Study the biofilm morphologies of monocultures and co-cultures of *P. fragi*, *P. fluorescens,* and *Rhodococcus sp*.

## Hypotheses

**[1]** Exposure to different temperatures such as cold at 4°C and heat at 37°C, will increase the total amount of OMVs produced by *P. fragi*.

**[2]** Co-cultures of the various bacterial species will display a greater symbiotic relationship than the monocultures which may result in enhanced biofilm formation.

## Methods




## OMV Quantification Results

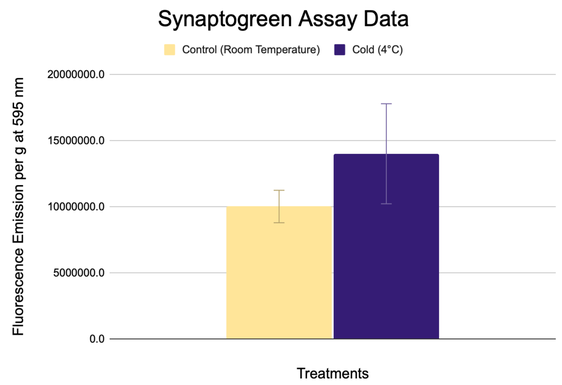



**Figure 2. No difference in OMV biogenesis between control and cold culture groups.** The control group was incubated at room temperature for 48 hours while the cold group was incubated at 4°C for 96 hours. After isolating extracellular polymeric substance (EPS) from plate-grown bacterial biofilms, the Synaptogreen assay was used to detect OMVs. Fluorescence emission per gram at 595 nm is shown for the control group at room temperature (yellow) and the cold group at 4°C (purple). The difference between the two treatment groups is statistically insignificant with a T-test P-value of 0.237. Error bars on graph +/- SEM.

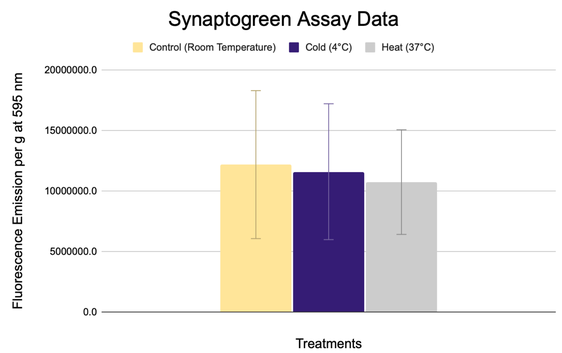



**Figure 3. No differences in OMV biogenesis between control, cold, and heat culture groups.** All plated *P. fragi* were grown at room temperature for 24 hours then the cold and heat groups were incubated at their appropriate temperatures for 48 hours while the control was left at room temperature. After isolating extracellular polymeric substance (EPS) from plate-grown bacterial biofilms, the Synaptogreen assay was used to detect OMVs. Fluorescence emission per gram at 595 nm is shown for the control group at room temperature (yellow), cold group at 4°C (purple), and heat group at 37°C (gray). Differences between the three treatment groups is statistically insignificant with T-test P-values of 0.301 between the control and cold, 0.308 between the control and heat, along with 0.366 between the cold and heat groups. Error bars on graph +/- SEM.

## Microscopy Results

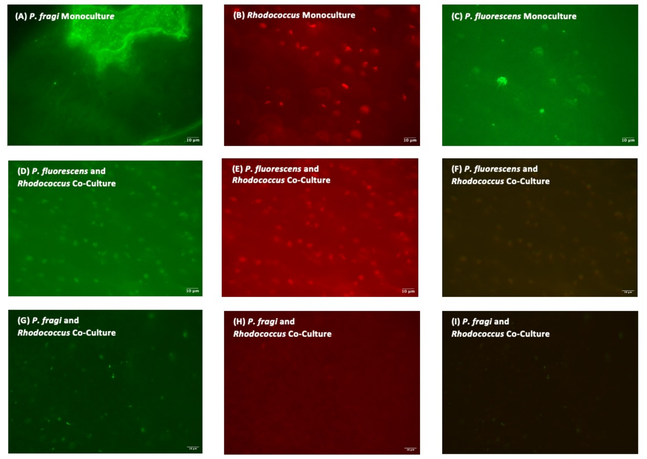



**Figure 4. Fluorescence microscopy biofilm images.** Carboxyfluorescein Succinmidyl Ester (CFSE) stains *Pseudomonas* (gram-negative) species which yields images that emit a green fluorescence signal. Hexidium Iodide (HE) stains *Rhodococcus* (gram-positive) species which yields images that emit a red fluorescence signal. Images **(A)**, **(B)**, **(C)**, **(D)**, & **(E)** were composed using a 1:1 ratio. Images **(G)** and **(H)** were composed using a 1:10 ratio. Images **(F)** and **(I)** are composite images of the two bacterial species in the co-culture. The ratios represent the amount of agarose/M9 solution compared to bacteria that was added in the 12-well plate prior to making the agarose pads for the fluorescence microscopy.

## Conclusions and Future Directions

### OMV Quantification

These results do not support the hypothesis since temperature stressors did not promote OMV biogenesis because multiple replicates in different growth conditions demonstrated statistically insignificant differences in fluorescence emission. Future research may investigate the effects of nutrient availability and exposure to various doses of arsenic to further characterize the bacteria.

### Fluorescence Microscopy

*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.

## References

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