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The Effect of Temperature stressors on Pseudomonas fragi OMV production

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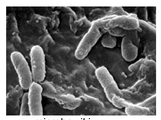
INTRODUCTION

Pseudomonas Fragi is a gram negative bacteria involved in meat and dairy spoilage, it is known for its ability to create antibiotic resistant biofilms because of its adaptability. This project aims to understand how *P. fragi* OMV (outer membrane vesicle) biogenesis is impacted at various temperatures and observe how the bacteria responds to different environmental cues.

Hypothesis: 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*.



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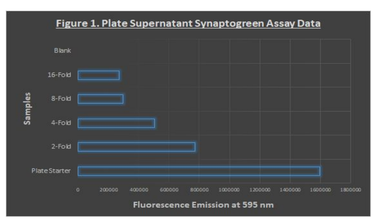
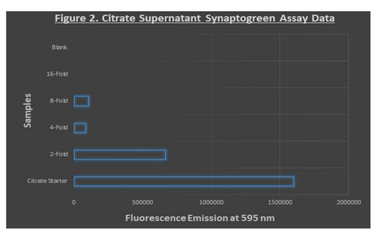
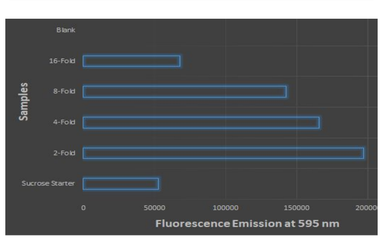
Sample obtained from Periphyton samples from Lake Killarney in Federal Way, WA.

Plate, citrate broth, and sucrose broth supernatant synaptogreen assay data







METHODS AND MATERIALS

Part I: Plate Grown Biofilms Preparation

- Isolate a colony from a sample frozen stock through streak for up to 24 hours on LB agar plates.
- Grow the bacteria in test tube on the shaker for 24 hours.
- Isolate and grow bacteria on different LB agar plates with different temperature conditions

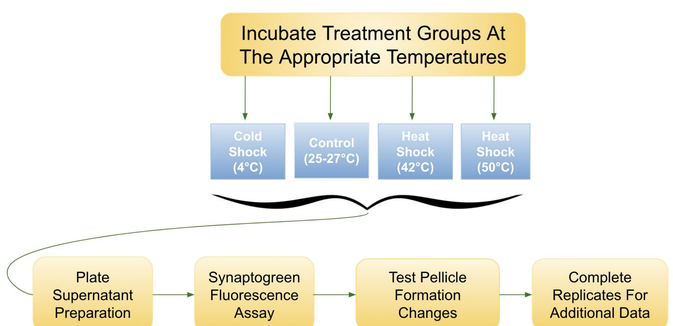
Part II: Extract Cellular Polymeric from Plate-Grown Bacterial Biofilms (Supernatant Preparation)

- The bacteria from different temperature conditions was pipetted into an OMV (outer-membrane vesicles) prep buffer.
- The solution went through a series of vortexes to mix the solution evenly in preparation for centrifuge.
- The mixed pellets were centrifuged and the supernatant containing the OMV released from the bacteria was collected.
- The filtered supernatant was gathered in test tubes separated by different temperature conditions diluted in 1:2 ratio 3 times.

Part III: Synaptogreen Assay

- The supernatant was then transferred to a 96-well plate mixed with Synaptogreen dye (fluorescence dye) with three replicates per sample

Protocol Overview



RESULTS

Figure 1. Synaptogreen Assay Data

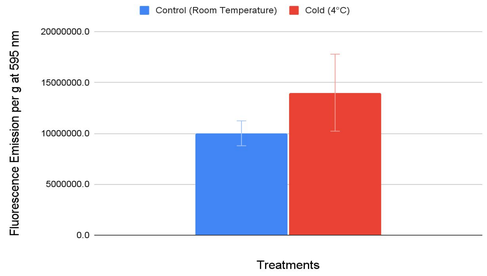


Figure 1. No difference in OMV biogenesis between control and cold culture groups. Control group was incubated at room temperature for 48 hours while the cold group was incubated at 4°C for 96 hours. After isolating extracellular polymeric substance (EPS) from plate-grown bacterial biofilms, the synaptogreen assay was used to detect OMVs. Fluorescence emission per gram at 595 nm is shown for the control group at room temperature (blue) and the cold group at 4°C (red). 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 2. Synaptogreen Assay Data

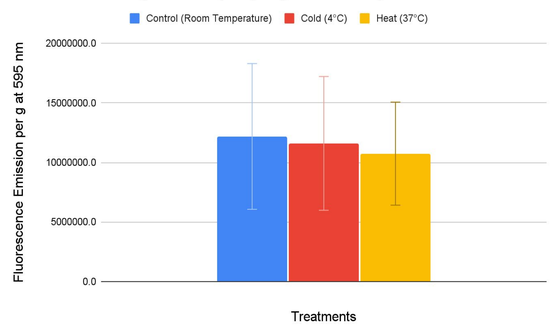


Figure 2. No differences in OMV biogenesis between control, cold, and heat culture groups. All plated *P. fragi* were grown at room temperature for 24 hours then the cold and heat groups were incubated at their appropriate temperatures for 48 hours while the control was left at room temperature. After 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 (blue), cold group at 4°C (red), and heat group at 37°C (yellow). 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.

Conclusion/Analysis

P. fragi exhibited adequate growth for:
- Control at room temperature.
- Cold at 4°C.

→ Indicates cold temperature was not a stressor.

P. fragi exhibited:
- Slow growth at 37°C
- Inadequate growth at 42°C.
- No growth at 50°C.

→ Indicates heat was a stressor.

Temperature stressors have no statistically significant effect on OMV biogenesis according to fluorescence emission data. Fluorescence emission per gram at 595 nm difference were statistically insignificant for all groups in both trials.

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