**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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**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.** 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 48 hours.

**Figure 2.** No differences in OMV biogenesis between control, cold, and heat culture groups.

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**REFERENCES**


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