TBIOMD495

Abstract

The Effect of Temperature Stressors on Pseudomonas fragi OMV Production

Arianna Koroma, Carla Rezk, Michelle Nguyen, Dr. Sarah Alaei

The bacterium of interest is isolate #24 obtained from periphyton samples from Lake Killarney in Federal Way, WA. *Pseudomonas fragi* is a gram negative motile bacteria involved in meat and dairy spoilage. *P. fragi* is known for its ability to create antibiotic resistant biofilms because of its adaptability to temperature stressors from the environment. OMV production and biofilm formation enable bacterial species survival during stress. The goal of this study is to elucidate this bacterium's response in different environments by addressing the knowledge gap on how temperature fluctuation affects outer membrane vesicle (OMV) production by *P. fragi*. The hypothesis is that exposure to different temperatures such as cold at 4°C and heat at 37°C, would increase the total amount of OMVs produced by *P. fragi*. 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 which uses a synaptogreen dye as a fluorescent probe for lipid bilayers. This allows for the detection of OMVs by labeling the lipid bilayers after theoretically removing all the cells, so the dye will only bind to the lipid bilayers present which is correlated with the amount of OMVs. After growing the control (room temperature) for 48 hours and the cold (4°C) for 96 hours, the culture groups showed no statistically significant difference in fluorescence emission per gram at 595 nm. When growing all the culture groups at room temperature for 24 hours then exposing the appropriate treatment groups to their corresponding temperatures for 48 hours, the control (room temperature), cold (4°C), and heat (37°C) exhibited no statistically significant differences in fluorescence emission per gram at 595 nm. Moreover, the bacteria grew adequately at 4°C possibly indicating that the cold temperature was not a stressor. 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 directions of research involve investigating the effects of various temperatures and other factors such as nutrient availability, on pellicles to explore whether pellicle formation may be enhanced by OMV production as a result of OMVs' signaling and communication roles.