Outer Membrane Vesicle Production of *Pseudomonas fluorescens* when Co-Cultured with *Rhodococcus*

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Introduction

Pseudomonas fluorescens is a Gram-negative bacterium that elicits an immune response in plants.

Outer membrane vesicles (OMVs) are produced by the budding of the outer membrane of Gram-negative bacteria. These vesicles contain a variety of different cargo including virulence factors, toxins, proteins, and DNA (2). OMVs contribute to a bacteria's survival mechanisms and pathogenesis, as well as the regulation of microbial interactions (4). Our research focused on *Pseudomonas fluorescens (P. fluorescens)*, a Gram-negative bacterium isolated from Lake Killarney in Federal Way, WA along with a community of other bacteria co-existing in periphyton. Apart from eliciting an antibacterial immune response in plants, there is minimal research published on the role of *P. fluorescens'* OMVs specifically (3). As a preliminary experiment, we sought to analyze the effect on OMV production of *P. fluorescens* when co-cultured with *Rhodococcus*, a Gram-positive bacterium that is pathogenic towards plants and isolated from the same periphyton as *P. fluorescens*. We hypothesized that introducing *P. fluorescens* and *Rhodococcus* into a co-culture would increase *P. fluorescens* OMV production.

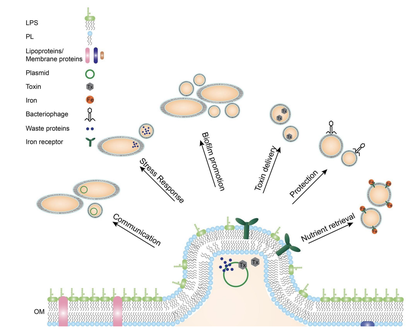
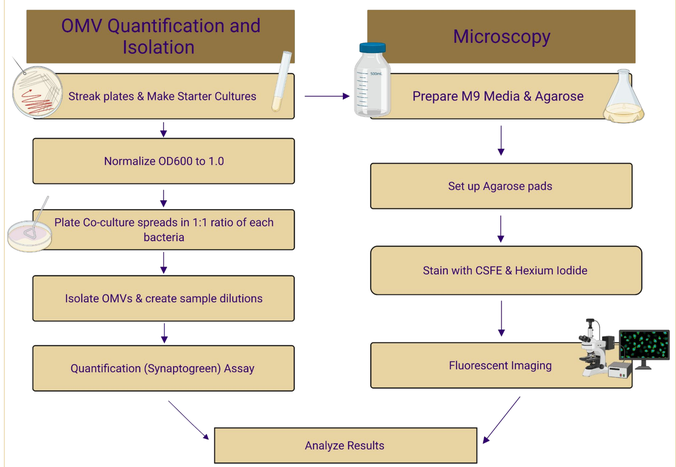


Fig 1: Modified image of the different mechanisms outer membrane vesicles contribute to.[2]

Question:
How is OMV production of *P. fluorescens* affected by co-culturing with *Rhodococcus*?

Hypothesis:
The production of OMVs in *P. fluorescens* will increase when co-cultured with *Rhodococcus* due to increased environmental stimulation for *P. fluorescens.*

Methods



Results

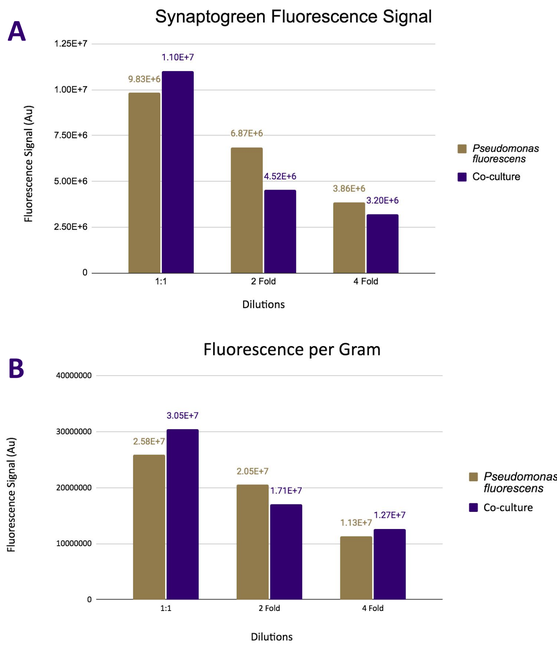
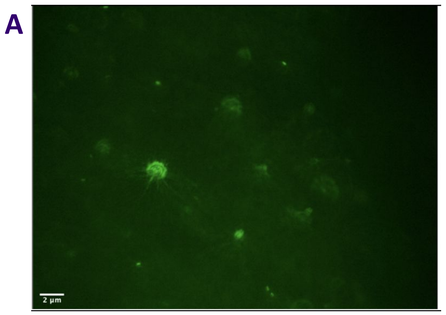


Fig 2: Quantification of *P. fluorescens'* OMVs with a synaptgreen assay. (A) Average fluorescence signal of each condition with their given dilutions. *Rhodococcus* signal was subtracted from the co-culture in order to get relative amount of *P. fluorescens'* OMVs. **(B)** Average fluorescence signal per gram of bacteria with the respective dilutions. *Rhodococcus* signal was subtracted from the co-culture in order to get relative amount of *P. fluorescens'* OMVs.

Conclusion

It is possible that *P. fluorescens'* OMV production is influenced when in a co-culture with *Rhodococcus*. Continued tests and research would need to be done in order to have confidence that the OMV production increase or decreases.

Continued Research - Microscopy



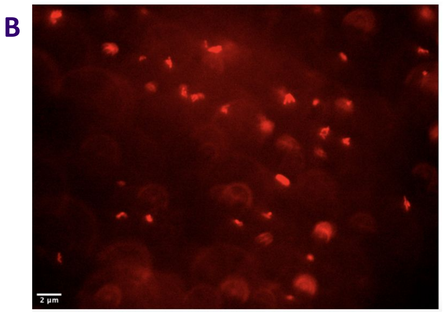


Fig 3: *P. fluorescens* and *Rhodococcus* monoculture biofilm images using fluorescence microscopy. (A) *P. fluorescens* monoculture stained with Carboxyfluorescein Succinmidyl Ester (CFSE) which emits a green fluorescence signal. **(B)** *Rhodococcus* monoculture stained with Hexium Iodide which emits a red fluorescence signal.

References

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4. Schwechheimer C, Kuehn MJ. 2015. Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. Nature Reviews Microbiology. 13(10):605–619.

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