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Peptide-based vesicle isolation method for *P. aeruginosa*

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Introduction

Pseudomonas aeruginosa is an organism that can act as an opportunistic pathogen for humans and animals, where it can cause from mild diseases, such as otitis, to serious illnesses (life-threatening diseases) such as septicemia (1).

In the last decades, new elements have been associated with the pathogenicity of *Pseudomonas* strains (2). This is the case with outer membrane vesicles (OMVs), which are produced when part of the outer membrane (including periplasmic content) generates a bleb. Bacterial vesicles have diverse roles in physiology and virulence (3). However, the difficulties related to vesicle purification (i.e. long protocols or the need of specialized instruments) have become a major obstacle for their characterization. In this context, we decided to analyze the efficacy of a peptide designed in our lab to bind *P. aeruginosa* vesicles and use it to develop a new vesicle purification method.

Results and discussion

We first determine the best conditions to purify vesicles of *P. aeruginosa* PAO1 strain using a commercial kit (ExoBacteria OMV isolation kit) (Figure 1). To that end, we grew the strain in rich medium (KB) or M9 minimal medium. In parallel, we also used KB medium and M9 minimal medium without bacteria as negative control.

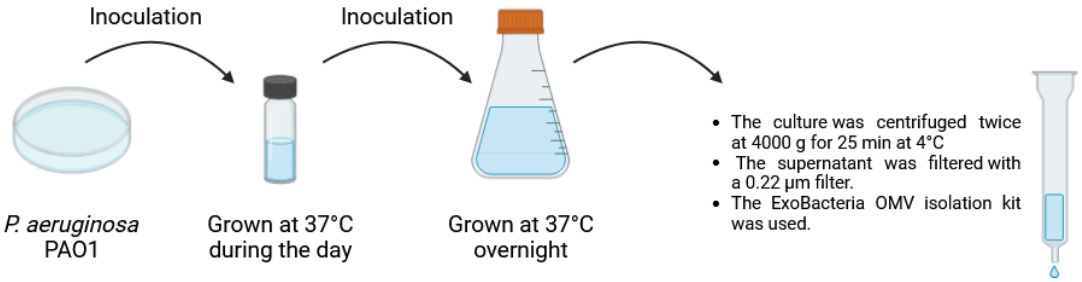

Figure 1. Diagram showing the main steps for vesicle purification. P. aeruginosa *PAO1 was grown in a cetrimide plate and then used to inoculate 2 ml of the corresponding medium. The strain was grown at 37°C during the day, and then used to inoculate 30 of fresh medium. After overnight incubation at 37°C and continuous shaking, the culture was centrifuged twice, filtered (0.25 µm) and processed with the ExoBacteria OMV Isolation according to the manufacturer instructions. Image created with BioRender.com*

We proceeded to characterize all samples—including those from *P. aeruginosa* cultures and negative controls in KB and M9 minimal media—using dynamic light scattering, electron microscopy, and protein quantification assays. Our findings demonstrated that the M9 minimal medium was the most effective for vesicle purification with the ExoBacteria OMV isolation kit. The negative control in M9 minimal medium exhibited a profile consistent with the absence of particle populations, whereas the negative control in KB medium (without bacteria) showed the presence of particle populations, likely due to protein aggregates (figure not shown). Furthermore, our analysis confirmed the presence of *P. aeruginosa* vesicles in the M9 minimal medium (Figure 2). Consequently, we selected these vesicles for subsequent experiments.

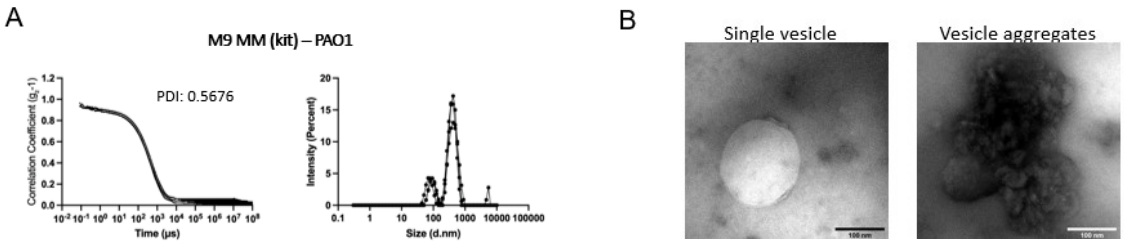

Figure 2. ExoBacteria OMV Isolation Kit was used to purify vesicles/particles from M9 minimal medium inoculated with P. aeruginosa *PAO1 strain. (A) Analysis of growth media for vesicle purification by Dynamic Light Scattering (DLS) using DLS (Zetasizer). The three curves in each graph represent the three readings of the instrument (triplicate). PDI: polydispersity index. (B) Electron microscopy images.*

We later synthesized an amphipathic and cationic 9-mer peptide that was previously described to bind LPS of *Escherichia coli* and *P. aeruginosa* (4) and used it to perform a modified Differential Radial Capillary Action of Ligand Assay (DraCaLa) assay (5). This DraCaLa assay relied on the colorimetric properties of our peptide conjugated to tetramethylrhodamine for visualization of migration on the nitrocellulose membrane (instead of the traditional radiolabeling). Our results suggested that the 9-mer peptide could bind the vesicles of *P. aeruginosa* PAO1. Interestingly, similar results were found for human exosomes (although with lower affinity).

Additional experiments are needed in order to determine the binding affinity between the peptide and the vesicles. In this context, ELISA experiments will be performed.
Altogether, these results contribute to broadening the knowledge on *Pseudomonas* vesicle purification and generate a new alternative for future applications.

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