Membrane vesicle and phage interactions in *Clostridioides difficile*

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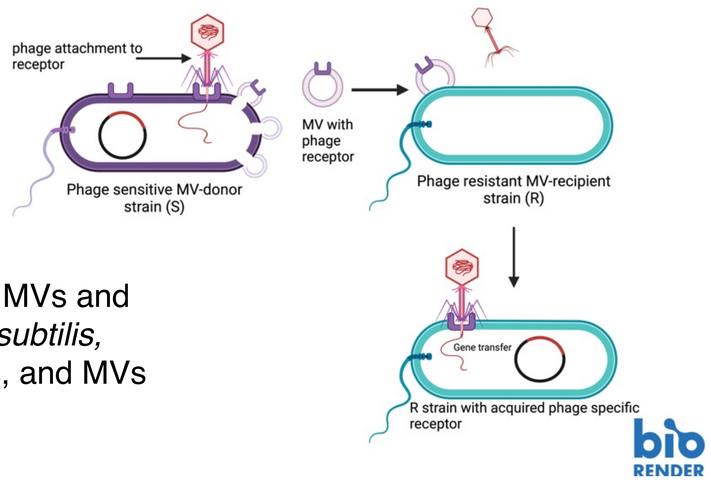
1. Introduction

Membrane vesicles (MVs) are lipid nanoparticles produced by all domains of life. They encapsulate and display genetic material, proteins and lipids, and are involved in many biological processes including horizontal gene transfer. Bacterial viruses (phages) infect and kill bacteria but can also confer exogenous genetic traits to bacterial host cells.

C. difficile has a highly dynamic genome, indicating diverse and active evolution mechanisms at play. E.g. horizontal gene transfer aided by phages and MVs.



There is limited knowledge on *C. difficile* MVs and interactions with phages. But in *Bacillus subtilis,* phages mediated MVs release from cells, and MVs aided phage infection (**Fig. 1**).

We hypothesize that *C. difficile* releases MVs, which transfer phage receptors between *C. difficile* strains. This work aims to:

Figure 1. MVs phage receptor transfer adapted from Tzipilevich et al., 2017.

1. Characterize MVs of R20291 (WT, lysogen of phi027), and NCTC11207 (indicator of phi027).

2. Investigate phage sensitization of CD80 by co-culture with NCTC11207 and phi027, where CD80 is phage-resistant and an MV-recipient (R), while NCTC11207 is phage-sensitive and an MV-donor (S)

2. Methodology

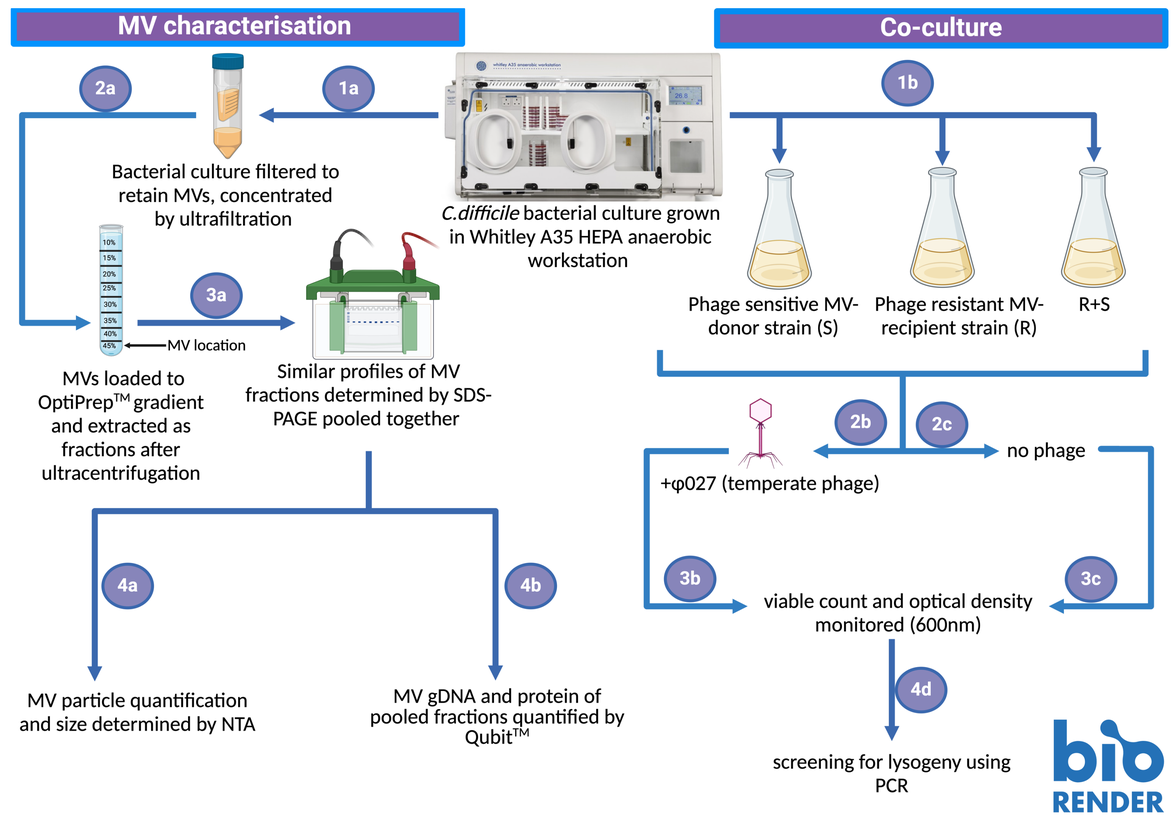


Figure 2. MV characterization. MVs in filtered culture supernatants were concentrated and purified through OptiPrep™ density gradient (1a-2a), retrieved as fractions (2a), and analysed by SDS-PAGE (3a). Similar protein fractions were pooled and assessed for size distribution (4a), particle concentration (4a), genomic DNA and protein (4b). **Co-culture.** S and R bacterial cultures were grown either separately or together (1b), and either infected with phi027 (2b) or left uninfected (2c). Bacterial growth was monitored (3b; 3c), and R colonies derived from mixed culture were screened for phage infection by PCR.

3. Results

- S and WT produced MVs in similar amounts over time. Changes in protein concentration were not significant (**Fig. 3**).
- Protein profiles of crude and purified MVs of S and WT were similar, except WT had a >200 kDa band, which was absent in S (**Fig 4**).

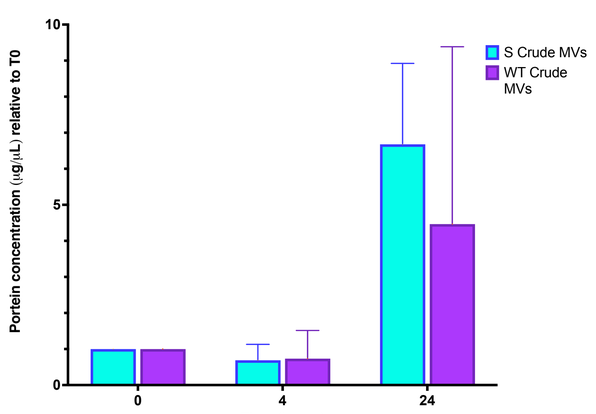


Figure 3. Protein concentration of crude MVs (n = 3; p > 0.05)

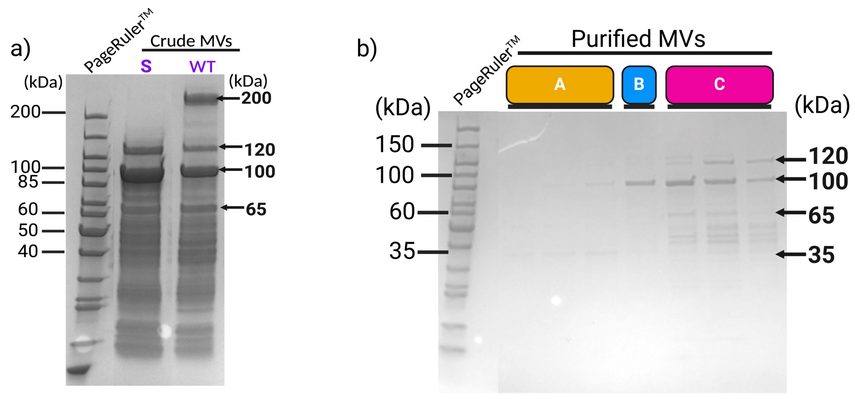


Figure 4. SDS-PAGE of crude MVs (**a**), and purified MV preps from S (**b**) and WT (**c**).

- Particle sizes ranged from 10 - 290 nm in S, and 30 - 210 nm in WT (**Fig. 5**)
- Double and single membrane spherical MVs were observed by transmission electron microscopy (TEM) in purified fractions ranging from 50 – 400 nm (**Fig. 6**).
- Stationary growth phase MV-encapsulated DNA concentration was highly variable for WT (**Fig. 7).**

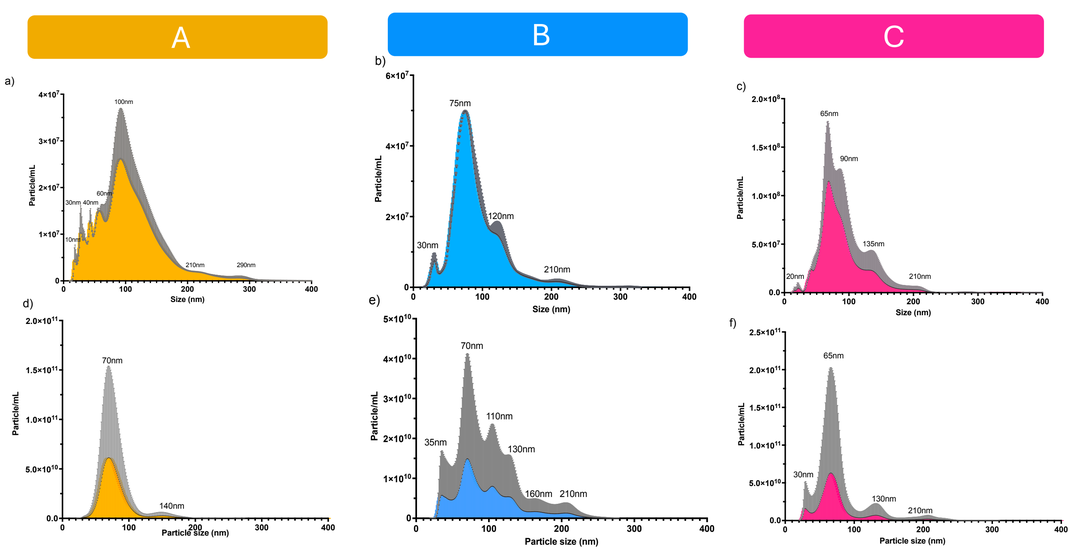


Figure 5. Particle size distribution of S (**top row)** and WT (**bottom row**) purified MV fractions A, B, C. Grey curves= SD (n = 2 or 3)

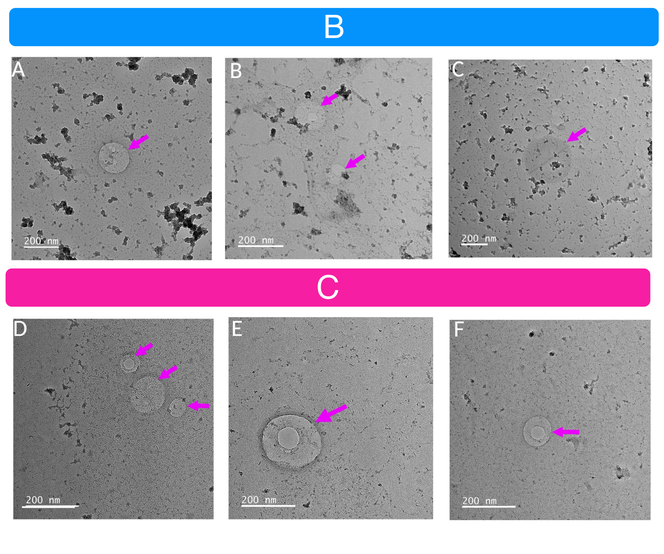


Figure 6. TEM of UranyLess-stained purified MV fractions B (**A-C**) and C (**D-F**) of WT.

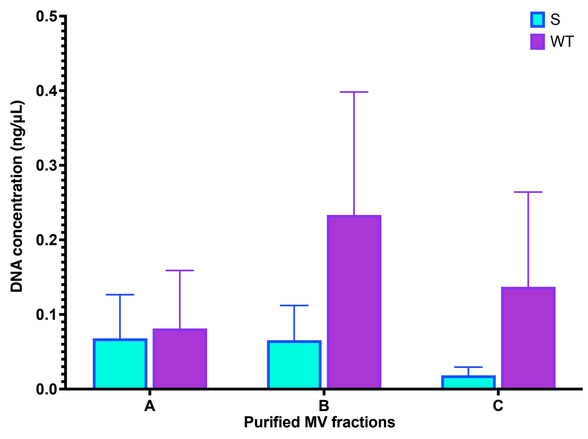


Figure 7. Concentration of DNA of S and WT purified MV fractions (A-C), n = 3; p > 0.05.

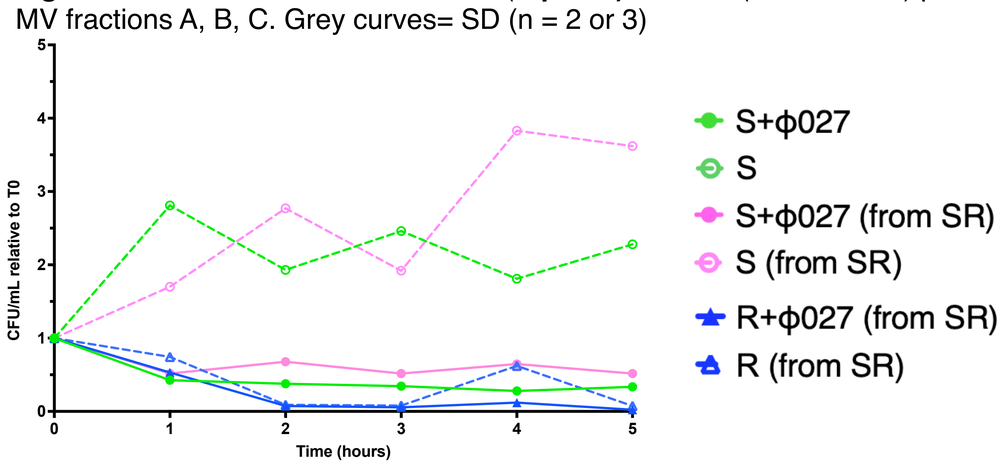


Figure 8. Average viable count of S and R grown separately or together, and either infected with ϕ027 or left uninfected. Data was standardised to t=0h; n=2.

- S infected with ϕ027 did not result in complete cell lysis because ϕ027 is lysogenic (**Fig. 8**, **green curves**)
- As expected, viability of S infected with ϕ027 separately or with R reduced 5-fold over time, while viability of uninfected cultures increased (**Fig. 8, green** and **pink curves**).
- In the presence of S, viability of R decreased over time regardless of ϕ027, suggesting it was out-competed by S rather than lysed by phage (**Fig. 8, blue curves**).
- R lysogens (1.7% of 60) were detected by PCR with phage-specific primers only in the presence of S, indicating ϕ027 infection of R was dependent on S. However no stable lysogens were recovered.
- More S lysogens (4.5% of 110) were detected since S is naturally susceptible to ϕ027.

4. Conclusions

- Like other Clostridia species (e.g., *C. perfringens , C. botulinum*) *C. difficile* MVs are spherical, heterogeneous in size, contain protein and DNA, and could be purified in an iodixanol density gradient (Kobayashi et al., 2022; Jiang et al., 2014; Obana et al., 2017).
- Cell lysates and MVs of S and WT contained major proteins of similar molecular weights. Identification of contents are underway.
- Double and single MVs were observed, while only single membrane MVs were found in another study (Nicholas et al., 2017).
- Detection of R lysogen only in the presence of S suggests that phage infection is mediated by S strain MVs, as reported in *Bacillus subtilis* (Tzipilevich et al., 2017*).* However, failure to isolate stable lysogens suggests phage did not integrate into R genome, possibly due to lack of a ϕ027 phage attachment site.

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References

Jiang, Y. *et al.* (2014) , *International Journal of Medical Microbiology*, 304(3–4), pp. 431–443; **Kobayashi, N.** *et al.* (2022) *Frontiers in Microbiology*, 13; **Nicholas, A.** *et al.* (2017), *Microbial Pathogenesis*, 107, pp. 6–11; **Obana, N.** *et al.* (2017) *Infection and Immunity*, 85(5); **Tzipilevich, E.** *et al.* (2017), *Cell*, 168(1–2).