The Effect of Pasteurized Milk Extracellular vesicles on Bacterial Growth

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Introduction

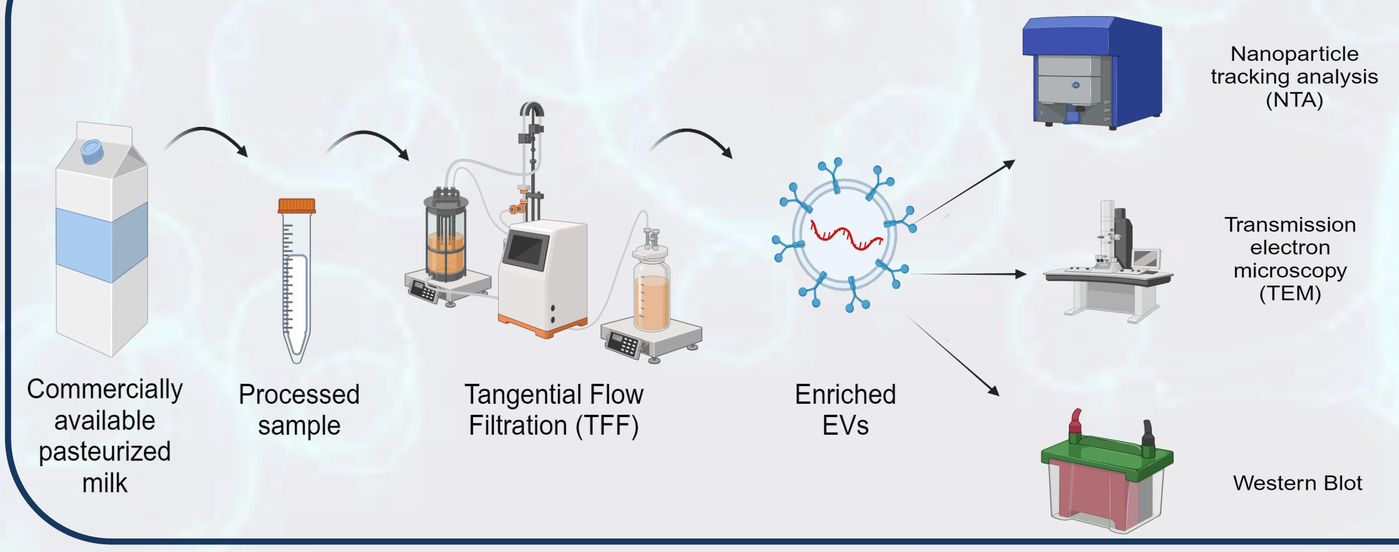
Milk-derived extracellular vesicles (mEVs) have gained attention for their potential biological activities, including antibacterial properties and they can carry bioactive molecules. The mEVs can survive high temperatures and digestion processes while retaining their biological activity. Studies of mammalian EVs are increasingly attracting the interest of researchers; however, there are only a few studies of mEVs' antimicrobial effect.

Hypothesis and Objectives

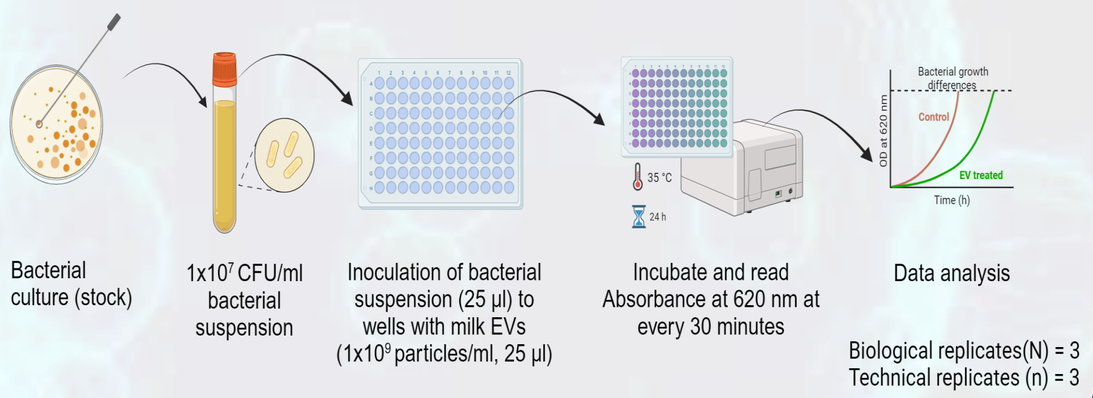
The mEVs may contain bioactive molecules that could exhibit bacterial growth promotion or inhibition and this study investigated the effect of pasteurized cows' milk-derived EVs on the growth of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 53868, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778 and *Pseudomonas aeruginosa* ATCC 27853.

Materials & Methods

Phase 1: Preparation and characterization of mEVs



Phase 2: *In vitro* Microbiology assay



Results

1. Nanoparticle Tracking Analysis (NTA) results

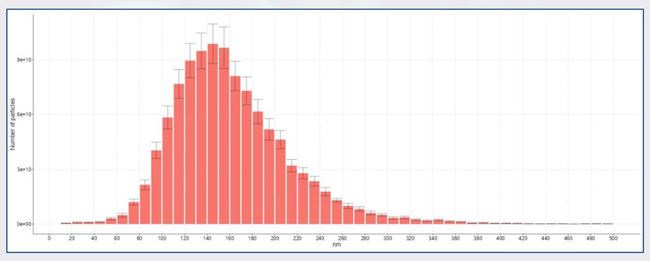


Figure 1: Size distribution of pasteurized mEVs and highest number of particles reported in the study were on the size range of 100-200 nm

2. Transmission Electron Microscopy (TEM) results

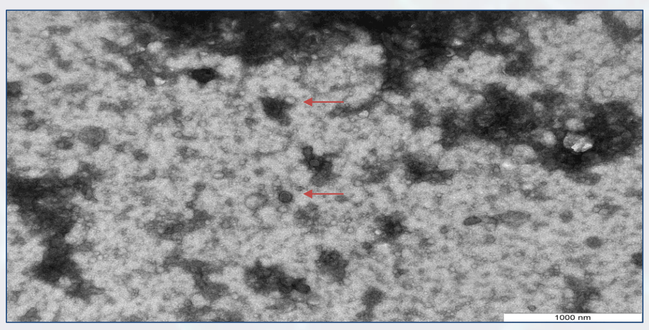
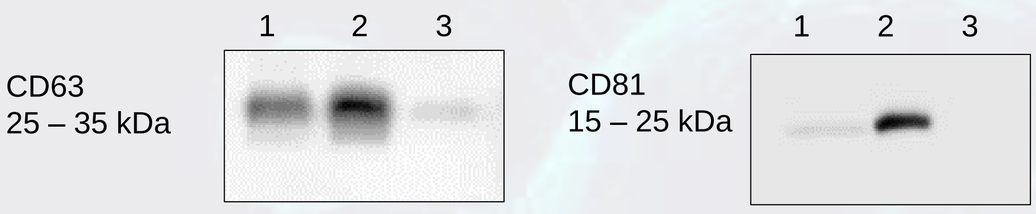


Figure 2: TEM image of pasteurized mEVs

3. Western Blot analysis of EV markers



1 - -80 °C stored TFF milk EV; 2 – Lyophilized milk EVs; 3 – Whey

Figure 3: Western blot analysis of EV markers CD63 and CD81 indicates the presence of EVs in pasteurized milk

4. *In vitro* Microbiology Assay

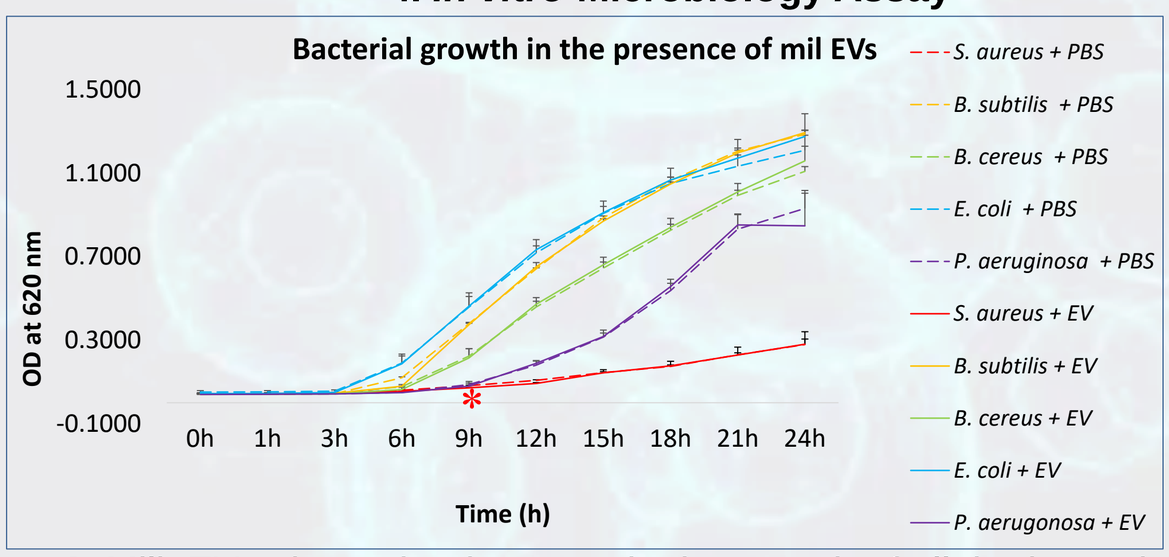


Figure 4: Milk EVs showed a decrease in the growth of all the bacteria except *E. coli*

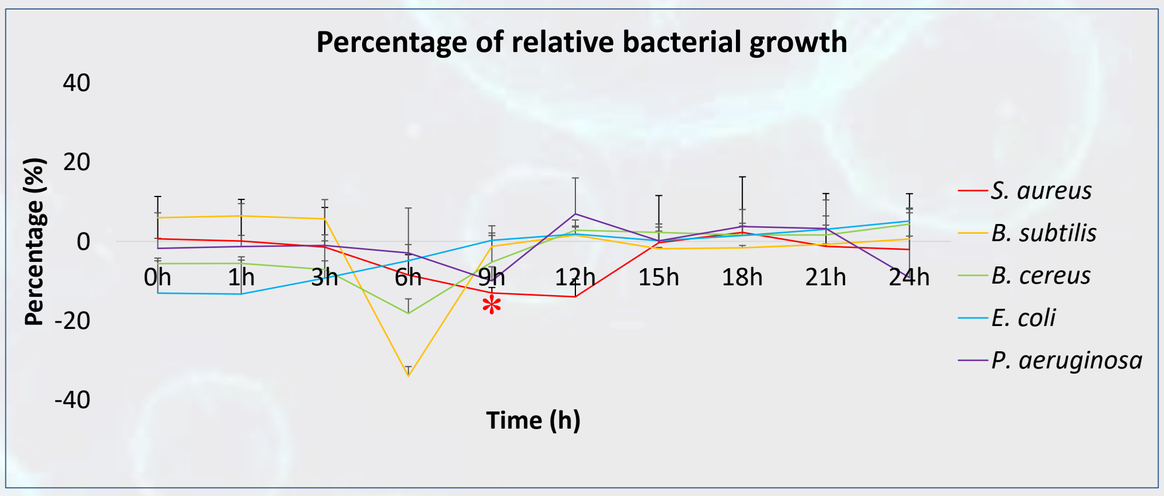


Figure 5: The percentage of relative bacterial growth inhibition indicates the highest growth inhibition by *B. subtilis* (33.9% ± 2.4) followed by *B. cereus* (18.1% ± 3.7), *S. aureus* (12.9% ± 1.3) and *P. aeruginosa (*9.9% ± 3.5) after 6h, 6h, 9h and 9h of incubation respectively

Only the *S. aureus* growth inhibition was statistically significant (**P*=0.022*)*

Conclusion

The mEVs can be absorbed by bacteria and has a selective inhibitory activity on bacteria. The application of pasteurised mEVs for antibacterial activity holds promise as a natural and potentially effective approach to combat bacterial infections. More research is required to understand and harness their potential benefits for therapeutic purposes.

Acknowledgement

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