The Impact of Extracellular Vesicles from commercially available milk on *Staphylococcus aureus* growth

Deep Bhattacharya[1], Dulmini Nanayakkara Sapugahawatte[1], Getnet Balcha Midekessa[1,2] Suranga Kodithuwakku[1,3], Gayandi Ekanayake[1], Mati Roasto[4] and Alireza Fazeli[1,2,5,*]

1. Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51006 Tartu, Estonia
2. Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Ravila 14b, 50411 Tartu, Estonia
3. Department of Animal Sciences, Faculty of Agriculture, University of Peradeniya, 20400 Peradeniya, Sri Lanka
4. Chair of Veterinary Biomedicine and Food Hygiene, Estonian University of Life Sciences, Kreutzwaldi 52, 51006 Tartu, Estonia
5. Division of Clinical Medicine, School of Medicine & Population Health, University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK

*Email: alireza.fazeli@emu.ee

Introduction

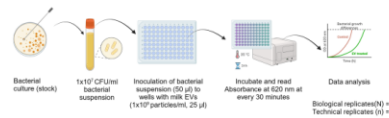
- Antibiotic resistance in *Staphylococcus aureus* is a growing concern for human and animal health.
- Milk, with its strong antibacterial properties, is a promising solution.
- Extracellular vesicles (EVs) play a role in intercellular communication and could be used for targeted antimicrobial delivery and understanding host-pathogen interactions.

Objectives

- Isolation of EVs from pasteurized and unpasteurized whey using TFF
- Investigate the impact of milk-derived EVs on the growth of *S. aureus* ATCC 25923.
- Measure absorbance at 620 nm over 24 hours at 37°C to monitor bacterial growth.

Methods

- TFF system utilized to enrich EVs from different fractions of industrial whey.
- Milk EV (mEV) samples diluted to $1x10^9$ particles/mL.
- Bacterial co-cultures set up with mEVs at concentrations of $1x10^7$, $1x10^6$, and $1x10^5$ CFU/mL.
- Negative control: Phosphate buffer solution (PBS) with bacteria.



Results

1. Nanoparticle Tracking Analysis (NTA)

- The mEVs were successfully purified and enriched from raw samples using TFF and analyzed using NTA
- The size distribution of pasteurized and unpasteurized mEVs and the highest number of particles reported in the study were in the size range of 120-180 nm.

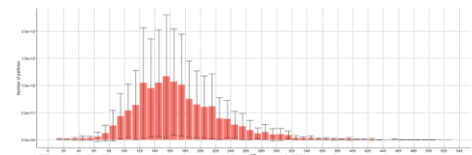


Figure 1: The particle size distribution of EVs

2. In Vitro Microbiology Assay

- Enriched mEVs inhibited the growth of *S. aureus* ATCC 25923 at a lower concentration of $1x10^5$ CFU/mL.
- The percentage of relative bacterial growth inhibition indicates the highest inhibition by pasteurized mEVs (17.07% ± 3.1) followed by concentrated pasteurized mEVs (15.10% ± 9.7), and unpasteurized (8.36% ± 10) after 6h, 7h, and 6h of incubation respectively.

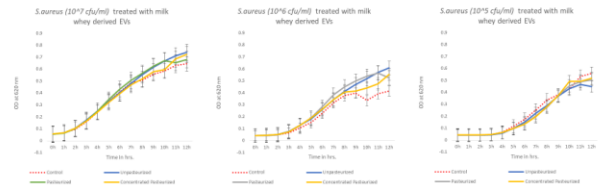


Figure 2: Effect of mEVs from different whey fractions on *S.aureus* growth

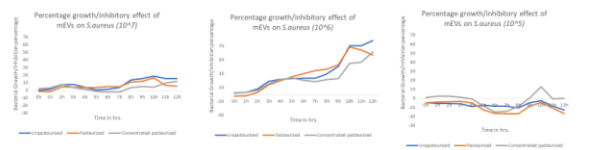


Figure 3: Relative bacterial growth/inhibition percentage

Conclusion

The study successfully isolated extracellular vesicles (EVs) from pasteurized and unpasteurized milk whey using the TFF system. These mEVs exhibited capability to impede the growth of *Staphylococcus aureus* ATCC 25923 at lower concentrations amongst which pasteurized whey derived mEVs showed the most promise. Although the observed inhibition was statistically insignificant (P=0.05, Student's t-test), further confirmation through a bacterial growth inhibition assay is essential. Investigating milk-derived EVs remains crucial in addressing antibiotic resistance.

Acknowledgement

This research was partially supported by the Estonian Research Council grant PRG 1441.

Funded by the European Union