Innovative Strategies Utilizing Extracellular Vesicles from Whey and Algae Spent Media to Combat Biofilms of Bovine Mastitis Pathogens

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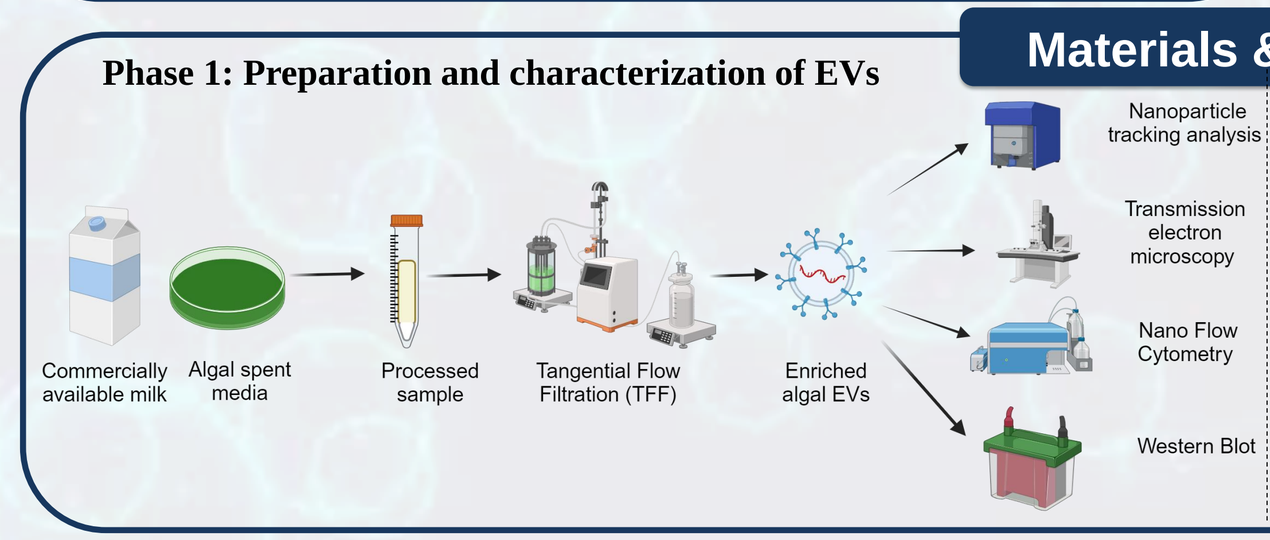
Introduction

- Contamination of surfaces by bacteria is a significant issue in the dairy industry.
- Due to zoonotic potential, it worsens the risk of cattle mastitis.
- Mastitis-causing pathogens like *Staphylococcus aureus*, *Streptococcus agalactiae* (GBS), and *Escherichia coli* are known to form biofilms.
- Extracellular vesicles (EVs) have demonstrated antibacterial properties.

Hypothesis and Objectives

- Whey and algae spent media-derived extracellular vesicles (EVs) exhibit antibiofilm effects on pathogens known to cause mastitis in cattle
- To evaluate the effectiveness of whey (mEV) and algae spent (aEV) media-derived EVs against mastitis-causing pathogens, *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* ATCC BAA 116, and *Escherichia coli* ATCC 53868

Materials & Methods



Results

1. Nanoparticle Tracking Analysis (NTA)

The highest number of particles reported in the mEV and aEV were in the size range of 100-200 nm and 200-255 nm respectively

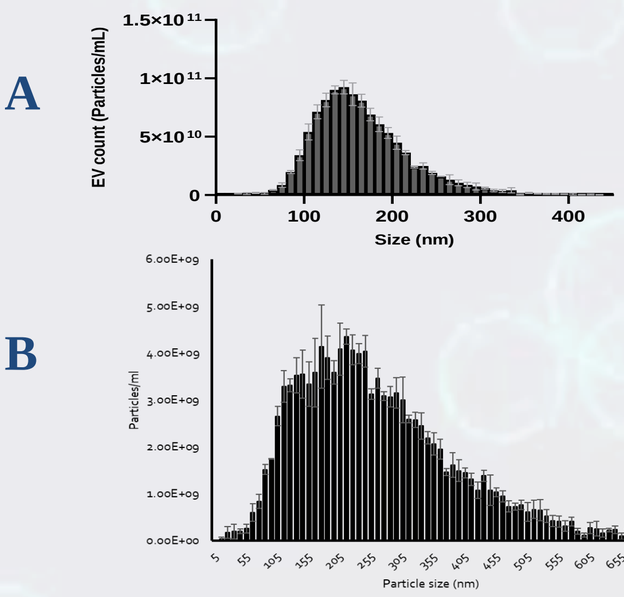


Figure 1: Size distribution of mEVs (A) aEVs (B).

2. Transmission Electron Microscopy (TEM)

EVs via TEM revealed diameters within the range of 100 to 200 nm. The observed morphology appeared imperfectly spherical cup-shaped

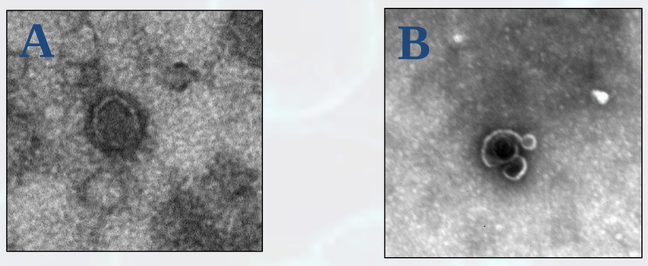
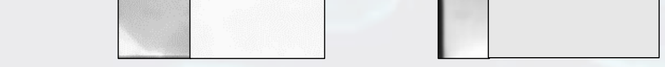


Figure 2: TEM images of mEV (A) and aEV (B)

3. Western Blot analysis of mEV 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. Nano Flow cytometry (NFC) analysis of aEVs

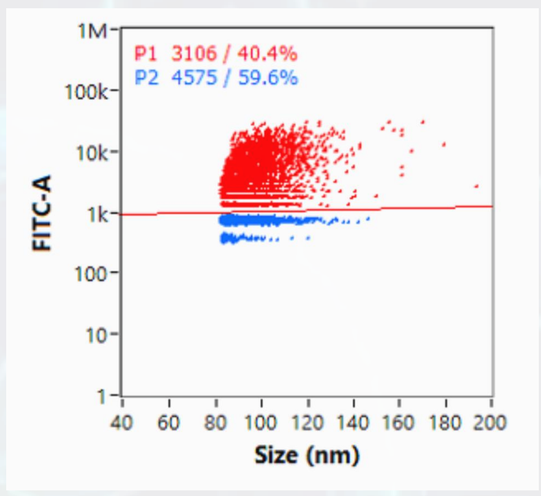


Figure 4: Size profile of CMG-labelled particles with a mean size of 73.11 nm

5. *In vitro* Microbiology Assay

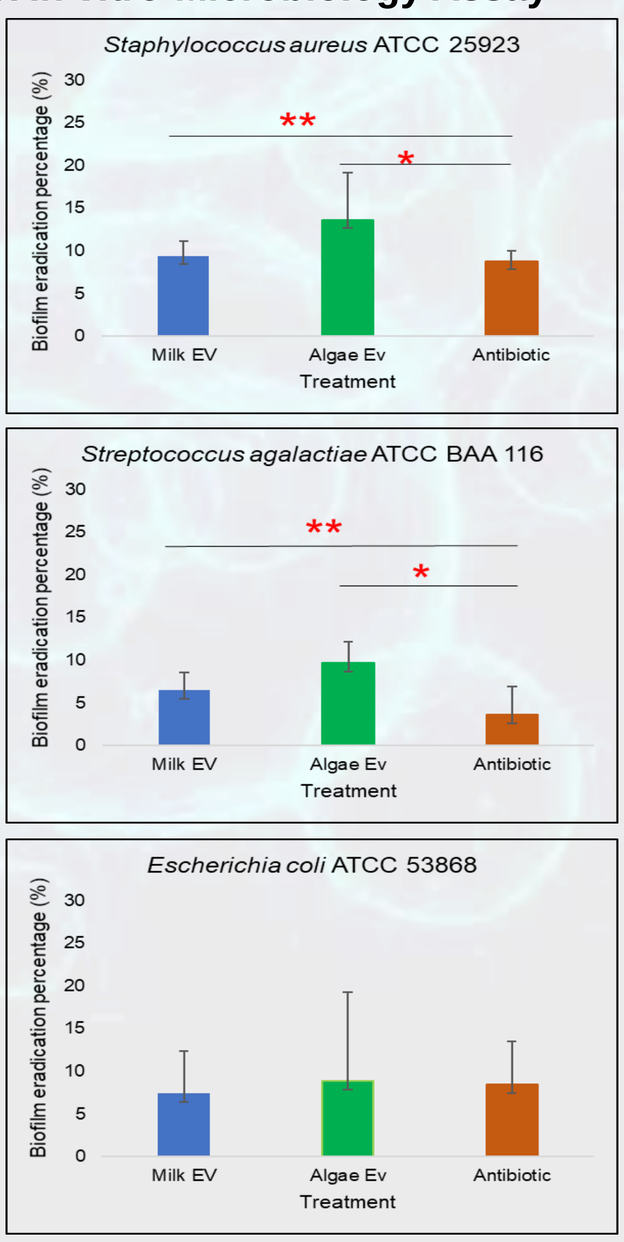


Figure 5: Biofilm eradication effect of mEV and aEV on biofilms of *S. aureus* (A), *S. agalactiae* (B) and *E. coli* (C) revealed that both mEV and aEV significantly inhibit ($p < 0.05$) the biofilms by gram positive bacteria

Conclusion

This study demonstrates the efficacy of mEVs and aEVs in effectively inhibiting the continued growth of established biofilms formed by *S. aureus* ATCC 25923 and *S. agalactiae* ATCC BAA 116. These findings present natural and innovative strategies for combating biofilm formation by pathogens prevalent in the dairy industry.

Acknowledgement

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