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Stability And Lytic Activity Assessment In Milk Of Bacteriophages Targeting *Escherichia Coli* Causing Bovine Mastitis

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Bovine mastitis is a major production disease in dairy cattle and complementary treatments to antimicrobials are urgently needed. Intramammary phage therapy is a promising approach but characterizing isolated phages in milk is a crucial initial step. This study aimed to compare the stability and lytic activity of 10 phages targeting *E. coli* in milk and assessing their stability at different temperatures and pH.

Ten bacteriophages isolated from wastewater were spotted on 53 *E. coli* strains isolated from bovine mastitis to evaluate their host range. The phage stability was evaluated across different pH (2-12) and T°C (25-60°C). Stability in milk was assessed after 6h of incubation at 37°C. *In vitro* efficacy assays involved inoculating milk with *E. coli* and phages and tracking bacterial titers at different timepoints in raw, heat-treated and UHT milk.

A narrow host spectrum was observed and phage stability was maintained at pH ranging from 4 to 10 and temperatures ranging from 25 to 45°C. At 60°C, only 5/10 phages persisted but with a significant degradation. Stability analysis in milk showed that all phages remained stable in raw and heat-treated milk. Lytic activity assays demonstrated a bacterial decrease with all phages, but for 5/10 phages, bacterial regrowth occurred after 5h of incubation.

In conclusion, milk components are not an obstacle for phage therapy to control bovine mastitis. However, bacterial regrowth suggests the presence of resistances that could be bypassed with the use of phage cocktails. DNA sequencing of the phages will be performed to ensure their safety.

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