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

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Introduction

Bovine mastitis is a major production disease in dairy cattle (Hogeveen et al., 2019) which is mostly caused by bacterial species, including *Escherichia coli* (*E. coli*) (Krishnamoorthy et al., 2021). Treatment include the use of antimicrobials (Suojala et al., 2013), which contribute to the development and spread of antibiotic resistances. The use of bacteriophages is of growing interest as alternative to antimicrobials. These viruses capable of killing their bacterial hosts present several advantages as their specificity to prokaryotic cells, their capacity to multiply at the site of infection and to co-evolve with their host (Kortright et al., 2019). However, the use of phages to control mastitis entails a detailed characterization to select candidates that could overcome the infection in a safe way. Indeed, intramammarily administrated phages will be in direct contact with milk components, but also with the mammary epithelium and the immune system which have antiviral activity (Drobni et al., 2004; Korhonen et al., 2000; Seifu et al., 2005). This study aimed to assess the stability and the lytic activity in raw and heat-treated (HT) milk of 10 phages targeting *E. coli* to assess if they are suitable for the intramammary treatment of bovine mastitis.

Materials And Methods

Bacterial And Phage Collection

Bacteria were isolated from bovine mastitis. Three bacterial strains were put in contact with 6 different filtered (0,22µm) wastewater and 1 slurry sample. After incubation, samples with lysis were purified to obtain a pure clone of the phage and they were further amplified.

Host Range

Four µl of each phage were spotted on LB agar with a bacterial overlay (Optical density = 0,3). The plates were incubated overnight at 37°C and the lysis plaques were scored: confluent lysis, semi-confluent lysis, opaque lysis, countable lysis plaques, no lysis.

Milk Samples

Raw milk was sampled from healthy cows (Somatic Cell Count <2e5 cells/ml) and half of the sample was HT at 70°C during 35 minutes. A sterility control was performed. Ultra heated and homogenized milk (UHT) was bought from the local supermarket.

Stability Of Phages In Milk

The phage incocula were titrated and 0,5ml of each phage were added to 5ml of fresh raw or HT milk. A second titration was performed after 6h of incubation at 37°C.

Lytic Activity Of Phages In Milk

Phage and bacteria were incubated at their optimal Multiplicity Of Infection (MOI) in raw, HT and UHT milk. The bacterial inoculum was titrated and titration of the samples were

performed after 1, 3 and 5h of incubation at 37°C. The control consisted in bacteria incubated in milk.

Results And Discussion

Bacterial And Phage Collection

A collection of 53 strains isolated from bovine mastitis was established. Six strains were Extended-Spectrum-Beta-Lactamase (ESBL). Ten bacteriophages were isolated.

Host Range

On average, the phages lysed 9,5% of the bacterial collection. 19/53 Strains weren't lysed by any phage. The narrow host range could be linked to the serotype. Indeed, the phage adsorption requires a reversible attachment to the O-antigen of the lipopolysaccharides.

Stability Of Phages In Milk

No significant effect of milk pasteurization on stability was observed. Inoculation of phages in raw or HT didn't significantly decreased the phage titer, except for one phage in HT milk.

In Vitro Efficacy Of Phages In Milk:

All samples demonstrated a bacterial reduction in comparison to the control group. However, 5/10 samples presented a bacterial regrowth after 5 hours incubation, which was still lower than in the control group. This observation could be linked to resistances.

Conclusions

Milk components are not an obstacle for the use of phages in the control of *E. coli* 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 and sequencing of the bacterial collection will allow us to determine their serotypes, and to link them with the phages host range.

Acknowledgements

This program benefited from a financial support of Wallonia in the frame of a BioWin's Health Cluster and Wagralim's Agri-food Innovation Cluster program (Vetphage). The authors acknowledge the Care-FEPEX (Liège, Belgium) and the AIDE (Liège, Belgium).

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Interpretive Summary

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Bovine mastitis is a major production disease in dairy cattle and complementary treatments to antimicrobials are urgently needed to fight antibiotic resistances. 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 newly isolated phages targeting *E. coli* in milk. Our study indicates that milk components are not an obstacle to phage therapy in the context of *E. coli* mastitis. However, bacterial regrowth during the *in vitro* efficacy assessment suggests the presence of resistances that could be bypassed with the use of phage cocktails.

Acknowledgements:
This program benefited from a financial support of Wallonia in the frame of a BioWin's Health Cluster and Wagralim's Agri-food Innovation Cluster program (Vetphage). The authors acknowledge the "Agence Régionale de Santé et d'Identification Animale" (ARSIA, Ciney, Belgium), the Care-FEPEX (ULiège, Liège, Belgium) and the "Association intercommunale pour le démergement et l'épuration des communes de la province de Liège" (AIDE, Liège, Belgium).