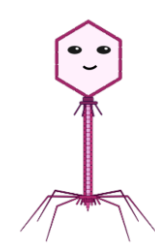


Stability And Lytic Activity Assessment In Milk Of Bacteriophages Targeting *Escherichia Coli* Causing Bovine Mastitis

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Introduction

Bovine mastitis is a major production disease in dairy cattle and the antimicrobial treatments to treat this infection contributes to the emergence and the spread of antimicrobial resistances. Phage therapy could be a promising approach, but the biological and physicochemical properties of milk could affect the phage's ability to control the infection. The objective of this study was to compare the stability and lytic activity of newly isolated phages targeting *Escherichia coli* in raw and heat-treated milk.

Material and methods

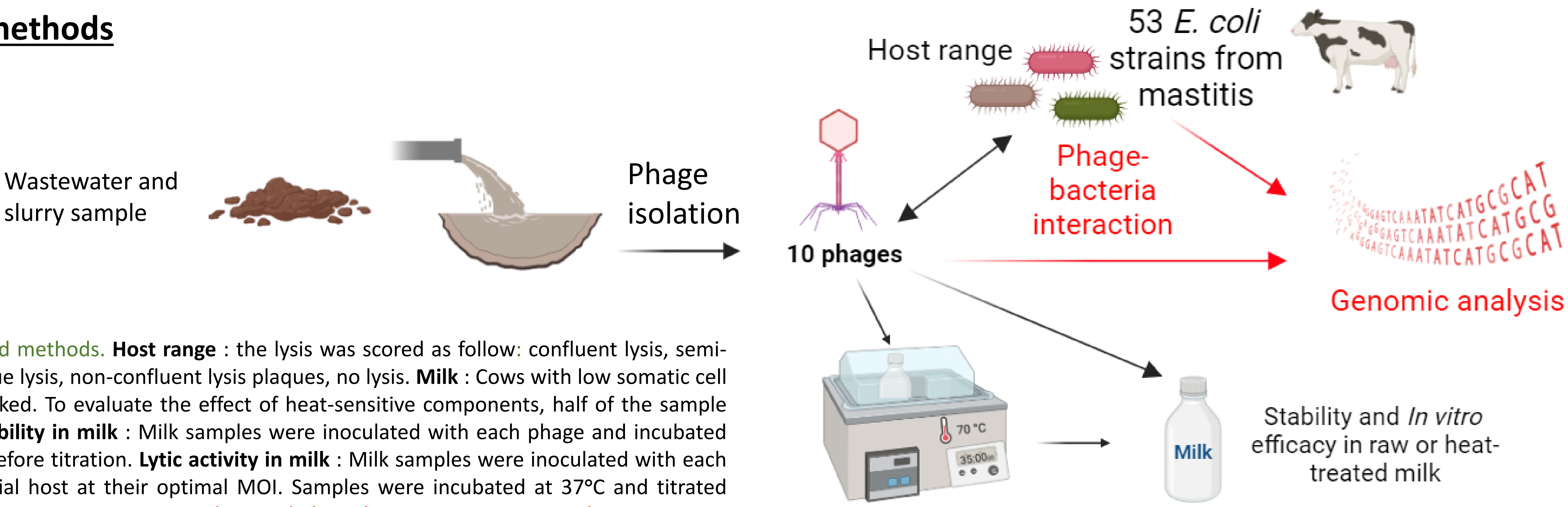


Figure 1. Material and methods. **Host range** : the lysis was scored as follow: confluent lysis, semi-confluent lysis, opaque lysis, non-confluent lysis plaques, no lysis. **Milk** : Cows with low somatic cell count (SCC) were milked. To evaluate the effect of heat-sensitive components, half of the sample was heat-treated **Stability in milk** : Milk samples were inoculated with each phage and incubated for 6 hours at 37°C before titration. **Lytic activity in milk** : Milk samples were inoculated with each phage and its bacterial host at their optimal MOI. Samples were incubated at 37°C and titrated after 1, 3 and 5h. **Perspectives: Genomic analysis and phage-bacteria interaction study**

Results

1. Host range

On average, the phages lysed 9,5% of the bacterial collection. 19/53 Strains weren't lysed by any phage. (Figure 2).

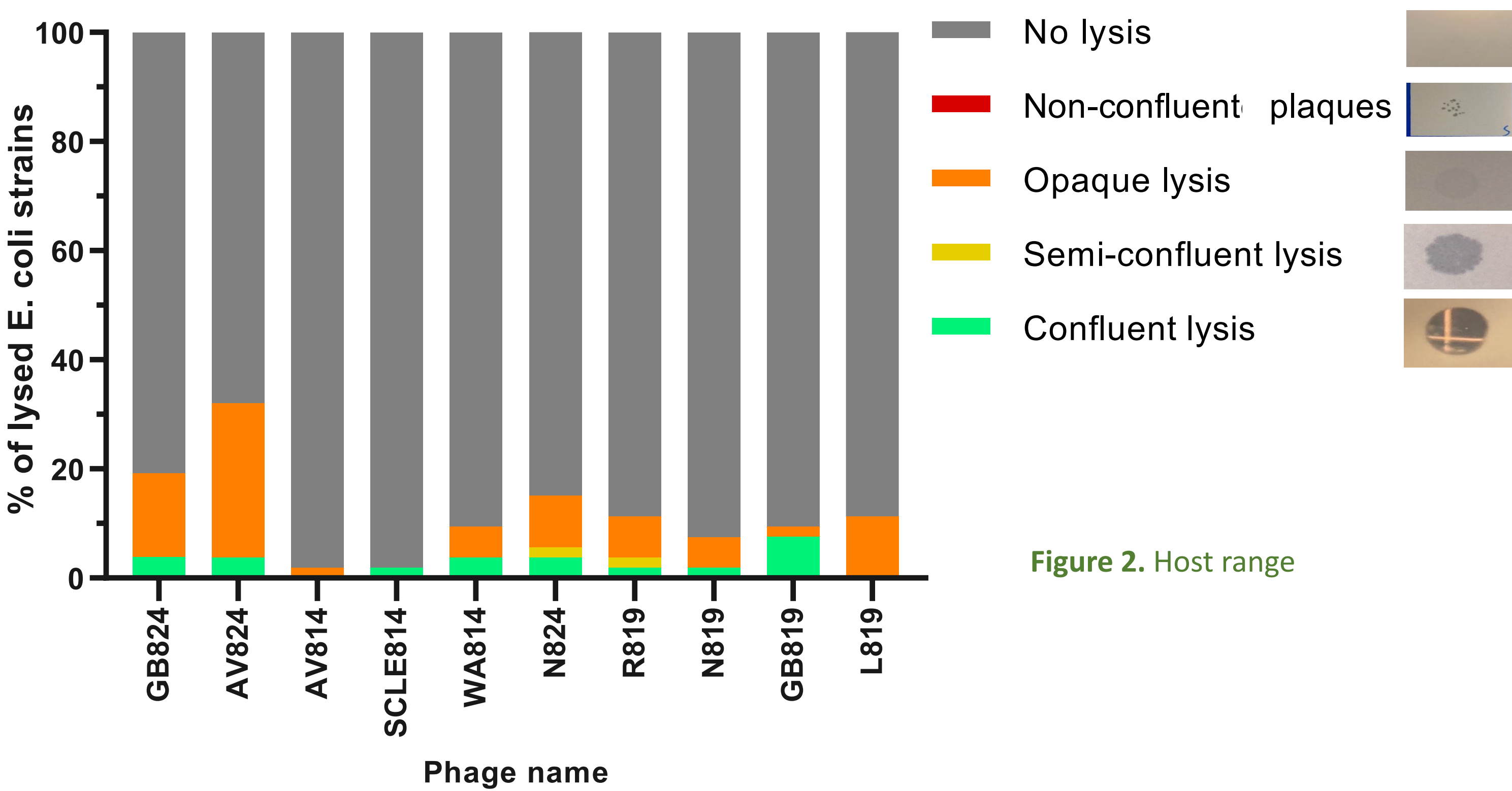


Figure 2. Host range

2. Stability in milk

No significant effect of milk pasteurization on stability was observed. Inoculation of phages in raw or HT milk didn't significantly decrease the phage titer. Multiple comparisons showed a decrease in HT milk for phage N819 (Figure 3).

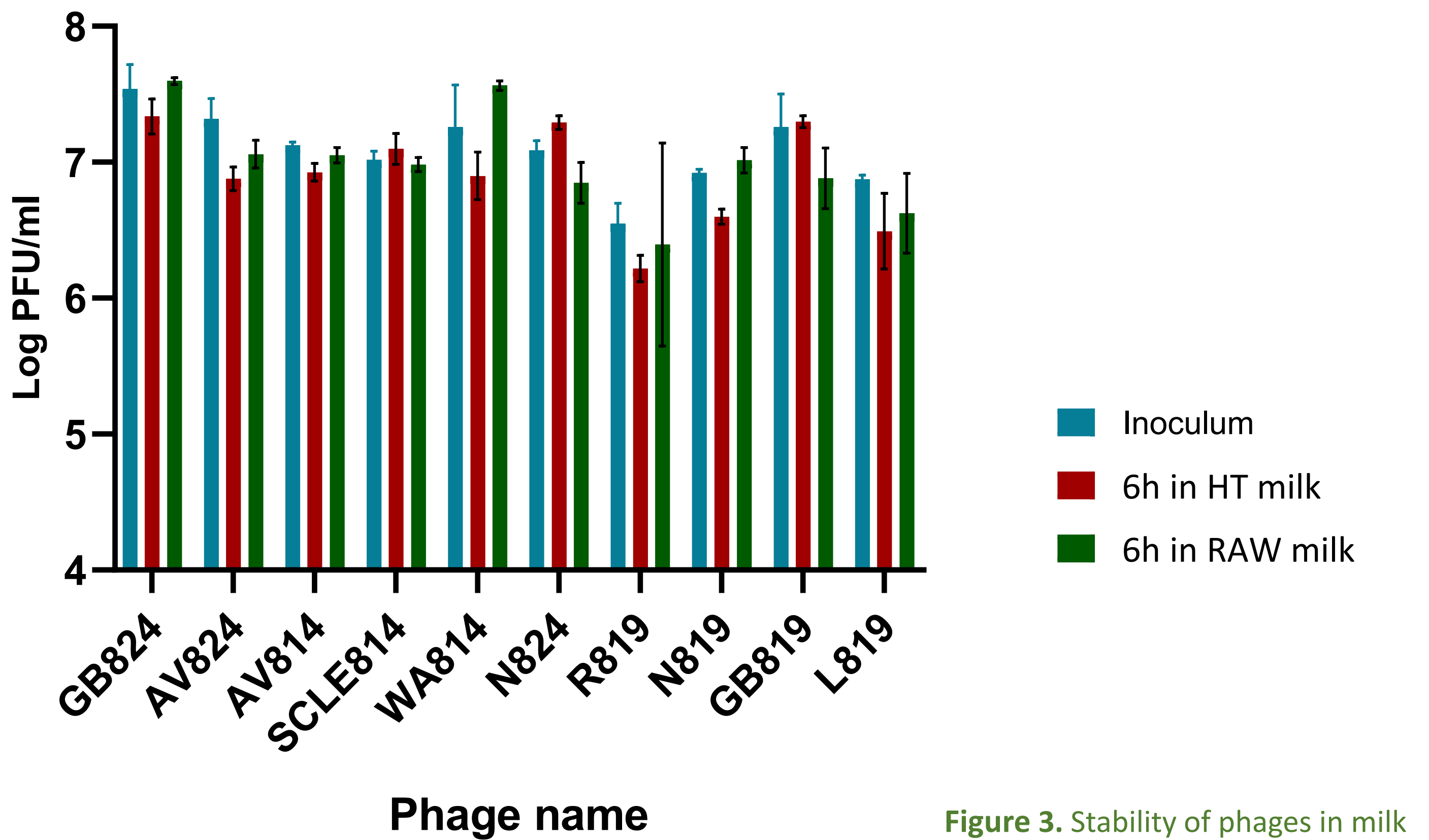


Figure 3. Stability of phages in milk

3. Lytic activity 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 with a titer which was lower than in the control group (Figure 4).

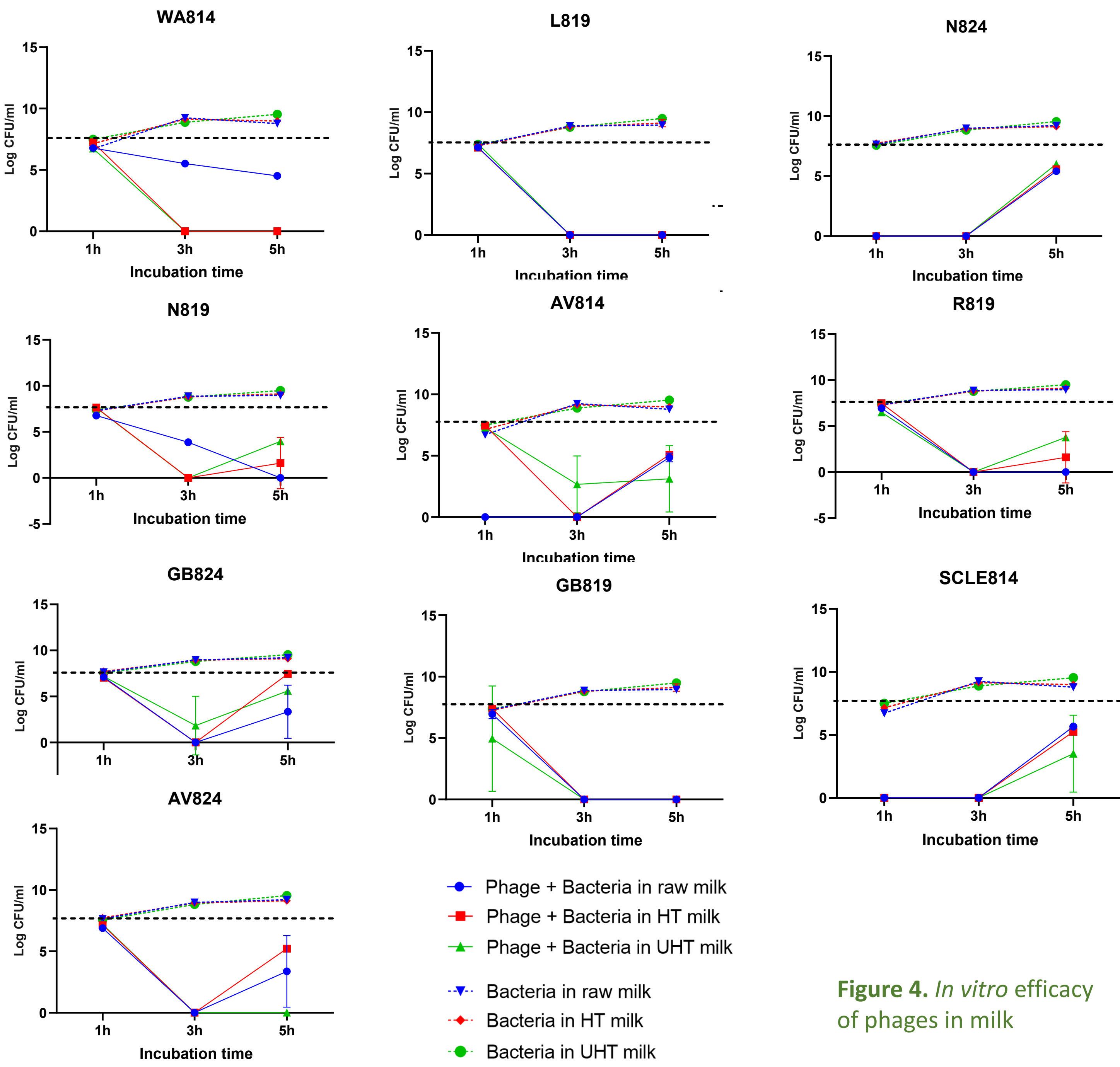


Figure 4. In vitro efficacy of phages in milk

Bacteriophage ID card

- Name:** L819
- Taxonomy:** ND
- Propagation strain:** *E. coli* 819
- Origin:** Slurry
- Host range:** Narrow
- pH and T°C stability:** pH 4-10, T°C 25-45°C
- Stability in milk:** Yes ☒ No ☐
- Lytic activity in milk:** Yes

Optimal MOI: 0.01

Cycle

☒ Lytic ☐ Lysogenic

Delivered by FARAH - ULiège

Conclusion

Milk components are not an obstacle for the use of phages in the control of bovine mastitis caused by *E. coli* (Figure 5). However, bacterial regrowth after 5h of incubation suggests the presence of resistances that could be bypassed with the use of phage cocktails. To ensure their safety, DNA sequencing of the phages will be conducted. Given the narrow host range, sequencing of the bacterial collection will be carried out, allowing an in-depth study of the phage-bacterial interactions.

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