



SCIENTIFIC SYMPOSIUM

“ANTIMICROBIAL RESISTANCE: A CHALLENGE FOR PUBLIC HEALTH, ANIMAL HEALTH AND THE ENVIRONMENT”

June 25th, 2024, 9 am – 12 am

TEMPLATE FOR ABSTRACT SUBMISSION

Submitting author

Name + Surname	Diderich Jacob
Organisation	Veterinary Bacteriology, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, Belgium
Full address	Bât. B43 Quartier Vallée 2 avenue de Cureghem 6 4000 Liège 1 Belgique
E-mail	jacob.diderich@uliege.be

- Only oral presentation
- Only poster presentation
- Poster presentation if oral is not accepted

Stability and lytic activity assessment in milk of bacteriophages targeting *Escherichia coli* causing bovine mastitis

J. Diderich¹, Marc Saulmont², J-N. Duprez¹, D. Thiry¹

¹ *Laboratory of Bacteriology, Department of Infectious and Parasitic Diseases, FARA, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium*

² *Regional Animal Health and Identification Association (ARSIA), Ciney, Belgium*

Introduction

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.

Materials and methods

Ten bacteriophages isolated from wastewater were spotted on 53 *E. coli* strains isolated from bovine mastitis to evaluate their host range. The stability of the phages 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. The optimal multiplicity of infection (MOI) was determined for each phages and used in subsequent lytic activity assays. These assays involved inoculating milk with *E. coli* and phages at the determined MOI and tracking bacterial titers at different timepoints in raw, heat-treated and UHT milk. Illumina sequencing of the phages was performed.

Results and conclusion

A narrow host spectrum was observed for the phages and 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. Regarding the genomic characterization, 2/10 phages presented lysogeny-associated genes.

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. Eight phages demonstrated lytic-associated genes, which are required to ensure the treatment safety. Sequencing the bacterial collection will enable us to determine their serotype and associate them with the phage host range, which are linked.

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 Regional Agency of Animal Health and Identification (Ciney, Belgium), the Care-FEPEX (Liège, Belgium) and the "Association intercommunale pour le démergement et l'épuration des communes de la province de Liège" (AIDE, Liège, Belgium).