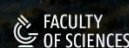
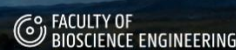


Lysin-based treatment strategies against bovine mastitis-associated *Escherichia coli*: engineering, activity and safety assessment in mammospheres

Julie Bellemans



INVITATION PUBLIC DEFENSE

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23 October 2026

PROMOTORS

Prof. dr. E. Meyer
Faculty of Veterinary Medicine, UGent

Prof. dr. Y. Briers
Faculty of Bioscience Engineering, UGent

Prof. dr. J. Van der Eycken
Faculty of Science, UGent

Prof. dr. S. De Vliegheer
Faculty of Veterinary Medicine, UGent

Curriculum Vitae

Julie Bellemans was born on 13 June 1997 in Hamm, Germany. She obtained her bachelor's degree in Veterinary Medicine from the University of Antwerp in 2018 and her master's degree in Veterinary Medicine from Ghent University in 2021, specializing in livestock with a research option. From 2021 to 2022, she worked as a doctoral assistant in the Biochemistry Research Group under the supervision of Prof. Evelyne Meyer. In 2022, she was awarded a personal PhD Fellowship Strategic Basic Research from the Research Foundation - Flanders (FWO-SB).

Her interdisciplinary doctoral project brought together three research groups with complementary expertise in veterinary medicine, biotechnology, and organic chemistry. By integrating these disciplines, she aimed to develop an innovative antimicrobial therapeutic as an alternative to conventional antibiotics. Her research contributed to the fight against antimicrobial resistance from a One Health perspective, recognizing the close connections between animal, human, and environmental health.

During her doctoral research, Julie supervised two master's thesis students and contributed to the scientific literature through three first-author publications. She presented her work at several national and international conferences, where she received awards for both the best poster and the best pitch presentation. She was also given the opportunity to present her research at the Joint Research Centre of the European Commission in Ispra, Italy.

Alongside her research, Julie was an active board member of the Veterinary PhD Community and serves on the societal advisory committee of Beter voor Dieren. Her professional interests lie at the interface of scientific research and policy, with a particular focus on translating interdisciplinary knowledge into evidence-based and socially relevant One Health solutions.

Where?

The defense will take place on Friday, 23 October 2026, at 5.30 p.m.

Auditorium B

Faculty of Veterinary Medicine
Ghent University, Campus Merelbeke
Salisburylaan 133, Merelbeke

A reception and celebration afterwards will take place at Gusb-Sportbar (Watersportlaan 3, 9000 Gent).

How to attend?

If you would like to attend, please register before 15 October 2026, via the following link: <https://form.jotform.com/262721083466054>

Members of the Jury

Prof. dr. Filip Van Immerseel
Chairman of the Jury
Faculty of Veterinary Medicine, UGent

Prof. dr. Marion Boutinaud
INRAE, France

dr. Roberto Vázquez Fernández
IPLA-CSIC, Spain

dr. Jorick Franceus
Faculty of Bioscience Engineering, UGent

dr. Bruno Toledo Silva
Secretary
Faculty of Veterinary Medicine, UGent

Summary

Antimicrobial resistance has become a major One Health challenge, increasing societal and political pressure to reduce antimicrobial use while preserving effective treatment for humans and animals. In livestock production, this policy shift is reflected in restrictions on prophylactic antimicrobial use and the curative use of antimicrobials that are particularly important for human medicine. At the same time, emphasis is placed on infection prevention, diagnosis and targeted treatment. These developments are directly relevant to the dairy sector, where bovine mastitis remains a major animal-health and production challenge associated with extensive antibiotic use. Mastitis-associated *Escherichia coli* (MAEC) is an important cause of bovine clinical mastitis. Although antibiotics can often be avoided in non-severe cases, rapid and effective intervention remains necessary for severely affected cows. This dissertation investigated bacteriophage-derived lysins as potential intramammary antimicrobials against MAEC. In parallel, physiologically relevant three-dimensional (3D) bovine mammary models were developed for the in vitro safety assessment of these candidate therapeutics.

The first research objective was to improve the biological relevance and reproducibility of the current in vitro bovine mammary epithelial models. Luminal BME-UVI and basal-like MAC-T bovine mammary epithelial cell (bMEC) lines were combined in 3D co-culture systems. A matrix-free ultra-low attachment culture method was selected to generate mammospheres, i.e. 3D spherical aggregates of mammary epithelial cells that reproduce selected in vivo features of mammary tissue organization. These mammospheres showed organized cytoskeletal structures and cell-cell junctions, while casein expression was consistent with lactogenic differentiation. Complementary fluorescence imaging, longitudinal live-cell imaging and flow cytometry provided a reproducible platform for initial cytotoxicity screening in these established mammosphere models.

Lysins are bacteriophage-derived enzymes that kill bacteria by cleaving the peptidoglycan (PG) of the bacterial cell wall. However, when applied externally to Gram-negative bacteria, lysin activity is limited because the bacterial outer membrane (OM) prevents these enzymes from reaching the underlying PG. The second research objective was therefore to improve the accessibility of lysins across this OM. To enable site-specific chemical modification, three natural lysins with activity against Gram-negative bacteria were engineered through the site-specific incorporation of p-azido-L-phenylalanine (AzPhe). This created a bioorthogonal handle for click-chemistry-based modification. LysMK34-AzPhe was selected as the lead lysin variant and used to evaluate a modular approach for attaching a synthetic molecule intended to enhance the applicability of lysins against Gram-negative *E. coli*. The resulting construct was evaluated for enzymatic and antibacterial activity.

Subsequent experiments focused on the context dependence of unconjugated LysMK34-AzPhe. The MAEC reference strain *E. coli* P4 grown in a minimal defined medium was more susceptible to LysMK34-AzPhe than bacteria grown in a nutrient-rich medium. AzPhe incorporation provided no consistent or sustained antibacterial advantage and appeared less stable than the wild-type protein during prolonged incubation. Moreover, standalone activity observed in phosphate-buffered saline was lost in both milk and in bMEC culture media. The antibacterial potential of wild-type LysMK34 was also investigated under additional treatment conditions relevant to bovine mastitis. The evaluated treatments caused no acute cytotoxicity in the mammosphere model under the tested conditions.

Overall, this dissertation contributed to the development of physiologically relevant 3D bovine mammosphere models for initial cytotoxicity screening as well as to the engineering and evaluation of lysins against Gram-negative bacteria, particularly with relevance in the context of bovine coliform mastitis.