

# Isolation and characterization of lytic bacteriophages for mastitis treatment

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Bovine mastitis is one of the primary diseases in dairy farming, and consumes the most antibiotics in milk production. The indiscriminate use of antibiotics promotes the development of resistance, also elevated monetary costs related to the disposal of contaminated milk in dairies. An alternative to the treatment of bacterial infections is phage therapy, which consists of the use of lytic bacteriophages for infection control. The work aimed to isolate and characterize bacteriophages to control *Staphylococcus aureus*, the main bacteria related to the disposal of animals that present mastitis. To make the isolation of the bacteriophages samples were collected in the sewage system of Viçosa-MG and from animal farming of the Federal University of Viçosa. The samples were enriched in 10 mL of 2X LB medium containing 100 µL of the bacterial strain in logarithmic growth phase. The mixture was plated by double-layer method. The resulting lysis plates propagated independently. Seven phages were isolated: Φ046, Φ4081, Φ1334, Φ3212 (urban municipal sewage system), ΦCAP046, ΦCAP 3906 (UFV goat farming), ΦBOV 574 (UFV cattle farming). After isolation, all phages were subjected to host spectrum, the phages Φ3112, Φ046, and ΦCAP 046 became components of Cocktail1 (the first *S. aureus* cocktail). The phages were subjected to transmission electron microscopy and growth curves assay. In the growth curve, the Cocktail1 and the individual phages were evaluated for their activity against 6 different *Staphylococcus aureus* isolates confirmed by polymerase chain reaction. Results regarding the host spectrum varying from 1/12 of Φ BOV 574 to 11/12 of Φ 046. Under microscopy, phages Φ3212, ΦCAP046 and Φ046 showed respectively the following total sizes and morphotypes: 65 nm-podovirus, 61 nm-podovirus, 314-nm myovirus. The Cocktail1 showed good activity against the bacteria in the growth curves, with LB medium for 15 hours starting the experiment with the bacteria in OD<sub>600</sub> 0,1 and resulting in this OD<sub>600</sub> from the controls to the treatment: *S. aureus* 046: from 0,4 to 0,15, *S. aureus* 3212 from 0,5 to 0,15, *S. aureus* 1334 from 0,5 to 0,12, *S. aureus* 222 from 0,4 to 0,15, *S. aureus* 3059 from 0,5 to 0,15 and against *S. aureus* ATCC 33591 from 0,35 to 0,12. The phages individually were also effective against the most part of the bacteria. Although in-vivo studies need to be performed, the phages isolated in this study shown promising results.

**Key words:** Mastitis; Bacteriophages; biocontrol

## Isolamento e caracterização de bacteriófagos para o tratamento de mastite bovina.

Mastite é a doença bacteriana de maior impacto na pecuária leiteira. Este trabalho teve como objetivo isolamento e caracterização de bacteriófagos para biocontrole de *Staphylococcus aureus*, uma das bactérias mais importantes associadas a mastite. Foram isolados 7 fagos e 3 deles se tornaram componentes do Cocktail1, que se mostrou efetivo em curvas de crescimento contra 6 diferentes isolados de *Staphylococcus aureus*. Foram realizadas microscopias dos fagos revelando que apresentavam respectivamente morfotipos e tamanhos: Φ046: miovírus, 314nm, ΦCap046: podovírus, 61nm e Φ3212: podovírus, 65nm. Os fagos apresentaram resultados promissores, porém, testes in-vivo serão necessários para confirmar a eficiência.

**Palavras-chave:** Mastite; bacteriófagos; biocontrole.

Acknowledge: This work was developed having support from institution PBIC/FAPEMIG.