

Title: PARTIAL CHARACTERIZATION OF BACTERIOCINS PRODUCED BY RUMINAL ISOLATES

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Abstract:

A wide variety of organisms produce antimicrobial peptides as part of their first line of defense. Bacteria and Archaea members produce ribosomally synthesized peptides known as bacteriocins, which exhibit bactericidal or bacteriostatic activity against target microorganisms. The production of bacteriocins is considered a competitive advantage, allowing the bacteriocin-producing cells to compete with other microorganisms in their natural environments. Bacteriocin-producing cells have been isolated from various environments, including the bovine rumen. The present study aimed to evaluate the bacteriocin production by thirty Gram-positive isolates from rumen fluid and belonging to the Laboratory of Rumen Microbiology, from Embrapa Dairy Cattle. Initially, the production of antagonistic substances by the selected isolates was evaluated by the spot-on-the-lawn method, using *Streptococcus macedonicus*, *Streptococcus equinus*, and *Streptococcus* sp. as indicator microorganisms; the presence of clear zones (> 6 mm of diameter) around the colonies of the selected isolates indicated antagonism. The presence of bacteriophages, acid production and the chloroform action were evaluated in order to exclude possible interfering action on the antagonist activity observed. The proteinaceous nature of antagonistic substances was evaluated upon treatment with the proteolytic enzyme proteinase K (30 U/mg), while the spectrum of activity was determined against both Gram-positive and Gram-negative bacteria (n=13). In the initial screening, six isolates (20.0%) demonstrated antimicrobial activity against at least one of the three indicator bacteria, while the isolates referred as C6I8 BOV, C6I9 BOV, AS1.5 and ISO37 (13.3%) inhibited the growth of two indicator microorganisms. The antagonistic activity observed was not related to the presence of bacteriophages, acid production or chloroform activity. All substances were inactivated after proteinase K treatment, and therefore they were defined as bacteriocins. None of the isolates inhibited the growth of Gram-negative bacteria. The isolates C6I8 BOV, C6I9 BOV and ISO37 were able to inhibit the growth of pathogenic microorganisms, such as *Listeria monocytogenes* and *Enterococcus faecalis*. The results showed the production of bacteriocins by ruminal isolates and further work is required to define the best extraction procedure and also to purify these antimicrobial peptides.

Keywords: bacteriocins, rumen, *Streptococcus*, *Listeria monocytogenes*, *Enterococcus faecalis*.

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