Title: IDENTIFICATION AND GENE PROSPECTION OF BACTERIOCINOGENIC BACTERIA ISOLATED FROM RUMEN

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Abstract:

Bacteriocins are an abundant and diverse group of ribosomally synthesized antimicrobial peptides produced by bacteria. The bacteriocins produced by Gram-positive bacteria can be divided into four classes: Class I, represented by lanthionine-containing post-translationally modified bacteriocins, referred to as lantibiotics; Class II, diverse group of non-modified peptides, divided into sub-classes; Class III, large and heat-labile peptides; class IV, complex bacteriocins containing lipid or carbohydrate moieties. Two types of modification systems have been found in lantibiotic gene clusters: the LanB/LanC modification apparatus (responsible for the dehydration reactions and lanthionine formation, respectively), and LanM, a single protein that carries out all modification reactions. The aim of this study was to identify bacteriocinogenic bacteria isolated from rumen and also to analyze the occurrence of genes involved in lantibiotic biosynthesis. In previous studies, six promising bacteriocin-producing strains, named C6I8, C6I9, C7I2, ISO7, ISO37 and AS1.5, were selected from ruminal Gram-positive isolates belonging to the Laboratory of Rumen Microbiology, Embrapa Dairy Cattle. Chromosomal DNA extraction was performed using phenol-chloroform method and the samples were quantified in NanoDrop. The 16S rDNA was amplified by PCR, using the universal primers; the fragments of interest were purified using the kit EasyPrep Gel/PCR purification. Samples were sequenced in automated DNA sequencer and the consensus sequences obtained were compared to databases. The presence of putative genes related to lantibiotic biosynthesis was assessed by PCR, using the degenerate primers for the genes lanB, lanC and lanM. Positive results were determined by the presence of amplification products with 400-500 bp for LanB, 200-300 bp for LanC and LanM. According to the sequencing, three isolates were identified as *Streptococcus equinus*, one as *Streptococcus macedonicus*, one as *Streptococcus lutetiensis* and one as *Streptococcus gallolyticus*. *S. equinus* e *S. gallolyticus* showed positive results for LanB; *S. macedonicus*, *S. equinus* and *S. gallolyticus* for LanC; *S. equinus* and *S. gallolyticus* for LanM. The results obtained suggest that most of the selected bacteria are lantibiotic producers; however, other studies are needed to evaluate the expression of the detected genes and also to analyze the presence of genes related to the biosynthesis of other classes of bacteriocins.

Keywords: rumen, lantibiotics, gene prospection

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