

## **Title: TEMPERATURE STABILITY OF BACTERIOCINS PRODUCED BY RUMINAL BACTERIA**

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### **Abstract:**

Bacteriocins, antimicrobial peptides produced by bacteria, have been widely isolated and characterized. The bacteriocin-producing trait can contribute to survival of the producing cells by killing other bacteria that might compete for nutrients in the same environment. Bacteriocins are produced by many bacteria isolated from rumen and they can show broad spectrum of activity and thermal stability, properties useful for biotechnological application. The aim of this study was to investigate the temperature stability of the bacteriocins produced by ruminal isolates. 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. Bacterial cells were grown in BHI broth, at 39°C, in anaerobiosis. The bacteriocin extracts were obtained from stationary phase cells, using successive centrifugation steps and acidic NaCl solution (100mM, pH 2); after 2h at room temperature the cells were centrifuged, the supernatant was collected, lyophilized and named crude bacteriocin extract. The bacteriocin activity was evaluated by agar well diffusion assay, using *Listeria monocytogenes*, *Streptococcus macedonicus* or *Enterococcus faecalis* as indicator microorganisms. Antimicrobial activity was expressed as arbitrary units per mL (AU/mL). Temperature sensitivity was assessed by heating samples of crude bacteriocin extract to 45°C and 70°C for 30 min and 60 min, and also 121°C for 15 min; additionally, the samples were stored at -80°C and -4°C for 21 days. Heat-treated samples were evaluated regarding the antimicrobial activity by the agar well diffusion assay. The bacteriocin produced by the isolate C6I9 was highly stable, maintaining its full antibacterial activity (1600 AU/mL) after storage for 21 days at -80°C and -4°C, and also after heating at 45°C, 70°C and 121°C (*L. monocytogenes* inhibition growth halo of 22 mm of diameter). The bacteriocin produced by C6I8 was also stable at the majority of the temperatures tested (133 AU/mL), but lost the antimicrobial activity upon autoclaving. The activity of the others crude bacteriocin extracts was not detected after thermal treatment and storage. The bacteriocins produced by the ruminal isolates C6I9 and C6I8 showed important heat resistance and storage stability, features that have been considered for industrial application.

**Keywords:** bacteriocin, *Listeria monocytogenes*, inhibition, rumen

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