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Bacteriocin production by lactic acid bacteria (LAB) isolated from traditional cheese

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Abstract. Lactic Acid Bacteria (LAB) are a group of bacteria that are found as natural microbiota in various ecosystems. They can produce a number of antimicrobial metabolites, including organic acids and other organic components, hydrogen peroxide and bacteriocins. The aim of this study was the evaluation of antibacterial activity of LAB isolated during production and maturation of traditional Rugova cheese. Samples for analysis were collected from different points of Rugova region and were transported to the laboratory under constant cooling conditions. The bacterial isolation was performed using standard methods and the isolates of LAB were identified down to the species level using a Biotyper Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Out of 140 tested isolates 105 had the ability to produce bacteriocins. The ability of bacteriocin production by LAB isolated from Rugova cheese can be taken as a measure of quality and safety of this traditional product.

Keywords: Lactic acid bacteria, bacteriocin, cheese, MALDI-TOF MS

Introduction

Lactic Acid Bacteria (LAB) are a group of bacteria that are found as natural microbiota in various ecosystems. They are used to produce a variety of fermented foods, they are important constituents in pharmaceutical formulations and as probiotics in functional foods. The LAB can produce a number of antimicrobial metabolites, including organic acids and other organic components, hydrogen peroxide and bacteriocins [1]. Bacteriocins are ribosomally synthesized peptides with antimicrobial activity. They are secreted to the extracellular medium [2] and can be classified in three classes [3]. Class I are short peptides (19–50 amino acids) that are often post-translationally modified resulting in the non-standard amino acids, such as lanthionine and others. A prominent member of this class is Nisin A. Class II are thermostabile peptides like Pediocin PA-1, lactococcin G, enterocine As-48, ect. Class III comprises large and heat labile proteins. Best known bacteriocins of class III are Colicin, produced by Escherichia coli or various helveticins produced by Lactobacillus helveticus [4-5]. LA bacteria are used to produce a large variety of fermented foods, they occur in pharmaceutical formulations and as probiotics in functional foods [6]. Traditional cheeses are known for a high diversity of microbial communities, dominated by LAB, which are important for developing organoleptic characteristics of cheese [7-8]. Kosovo produces a variety of traditional cheeses by traditional
methods, however little information is available about microbial activities in this products [9]. The aim of this study was the evaluation of antibacterial activity of LAB isolated during production and maturation of traditional Rugova cheese.

Materials and methods

All samples analyzed in this study were collected from different points of Rugova region (Fig. 1), and were transported to the laboratory under constant cooling conditions. Traditional cheese produced by farmers in Rugova region, were collected in different stage of production and maturation.

For bacterial isolation, 1 ml of appropriate dilutions was plated on M17 and MRS agar medium respectively. The plates were incubated at 30°C and 37°C for 48h under aerobic and anaerobic conditions. Gram positive and catalase negative bacteria were selected randomly from M17 and MRS agar. Pour plating technique was used for bacterial purification, by repeated plating in the same agar until pure cultures were obtained [9-11]. The isolates of LAB were identified down to the species level using a Biotyper Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Agar overlay techniques were used to demonstrate the antibacterial activity of natural LAB isolated from traditional Rugova cheese, against 5 indicator strains [12].

Results and Discussion

After identification of our isolates by MALDI TOF-MS, 140 were selected to investigate their bacteriocine production ability in traditional Rugova cheese. Out of 140 tested isolates 105 had the ability to produce bacteriocins against 5 indicator bacteria (Fig. 2).
Forty-seven isolates of Lactococcus lactis showed antibacterial activity against at least one indicator bacteria. Other authors have found little or lack of activity of bacteriocin production of lactococci against Listeria spp. [13]. Forty-three isolates of Enterococcus faecalis showed bacteriocin production against 1 to 5 indicator bacteria. Only one isolate (FA25) had the ability to inhibit the growth of Listeria monocytogenes with a clearly visible inhibition zone. The ability of huge number of isolates of genus Enterococcus for bacteriocin production is in harmony with the findings of other authors [14-16]. Leuconostoc mesenteroides is present with 5 isolates in Rugova cheese that had ability to produce bacteriocin against 3 indicator bacteria. Leuconostoc mesenteroides are used in Swiss type cheese because of metabolite secreted by this bacteria, which also are acceptable for other foods [17-18]. Others bacterial species (Enterococcus durans, Lactobacillus paracaseae, Lactococcus grvrieae and Enterococcus gilvus), had low antibacterial activity against indicator bacteria.

**Conclusions**

The large number of bacteriocin producers demonstrates the great assertiveness of the natural LAB microbiota over potentially existing pathogens. Thus, the ability of bacteriocin production by LAB isolated from Rugova cheese can be taken as a measure of quality and safety of this traditional product. Further investigation is needed to characterize these bacteriocins and elucidate their antibacterial mechanisms.

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