ANTI-LISTERIA POTENTIAL OF LACTIC ACID BACTERIA ISOLATED FROM REGIONAL CHEESES FROM THE TATRA MOUNTAINS REGION

SUMMARY

Listeria monocytogenes is one of the most dangerous foodborne pathogen. These bacteria are resistant to multiple physical-chemical factors and are characterized by an enormous adaptive plasticity, thus are found in various food products. Cheeses produced from unpasteurized milk, manufactured in domestic conditions are particularly exposed to Listeria contamination. Regional cheeses manufactured in the Carpathian Mountains region, such as oscypek, golka, bundz and bryndza, belong to this group of foods. These food products are very popular in our country. Although the probability of Listeria occurrence in these cheeses is high, the available data indicate that this group of foods is well protected against its growth. This fact may be associated with the occurrence of some microorganisms active against Listeria in these foods. Therefore, in this work the ultimate aim was to investigate the indigenous microbiota of the Tatra Mountains regional cheeses with respect to its anti-Listeria activity. Owing to a remarkably high anti-Listeria activity of bacteriocins produced by LAB, especially those belonging to class IIa, this work was focused on this particular group of compounds.

The preliminary research comprised investigation of the regional cheeses and the production environment with respect to Listeria occurrence. Results of the preliminary research allowed determination of the contamination sources and the transmission paths of Listeria. Based on the obtained data is was concluded, that shepards’ houses called bacowka, as well as the equipment used in the manufacturing process were an important source of cheeses contamination with Listeria, including pathogenic strains L. monocytogenes i L. ivanovii. From amongst of the analyzed surfaces, the filtration cloth caused the greatest hazard of Listeria contamination. It was also found that manufactured cheeses were contaminated with Listeria at many stages of the manufacturing process. For all of the analyzed cheeses, the most hazardous stage of the manufacturing process, with respect to Listeria contamination, was milk filtration. However, Listeria was eliminated from the analyzed cheeses during ripening. After 14 days of ripening, no living Listeria cells were detected in all of the analyzed cheeses. This fact clearly indicated that the analyzed cheeses contained anti-Listeria agents.

Since bacteriocinogenic LAB may constitute anti-Listeria agents, in the next stage of this work it was determined whether the analyzed cheeses are a source of such strains. To this end, a metagenomic analysis was performed. The meta-DNA of the regional cheeses microbiota was analyzed with respect to existence of genes encoding anti-Listeria bacteriocins class IIa and sequences characteristic for selected, potentially bacteriocinogenic, LAB genera. The analysis confirmed that some potential bacteriocin class IIa producers were present in all of the analyzed cheeses. Moreover, the genera composition of LAB contained in each particular of the analyzed cheeses was revealed.

The cheeses containing LAB with anti-Listeria potential were subjected to further work. As a result, 807 LAB strains were isolated from all of the analyzed cheeses. Subsequently, these strains were species identified via PCR-RPLP technique and sequencing. It was revealed
that the isolated strains belong to the following species: En. durans, En. faecium, En. hirae, Lc. lactis, Lc. garvieae Lb. casei, Lb. plantarum, Lb. pracasei, Ln. mesenteroides, Ln. citreum, P. acidilactici and P. pentosaceus. Oscypek and golka cheeses were the source of most the listed LAB species. Only 2 to 5 LAB species were isolated from the remaining cheeses. Lb. casei was the common species for all of the analyzed cheeses. The identified strains were subsequently analyzed for their ability to synthesize anti-Listeria bacteriocins, especially of the IIa class. This trait was determined both on the phenotype and the genotype level. The results indicated that more than 50% of the isolated LAB species had the genes encoding bacteriocins class IIa, but only 62% of these strains produced active bacteriocins. Moreover, some strains lacking the genes encoding bacteriocins class IIa were able to synthesize bacteriocins. Thus, it can be concluded, that the indigenous microbiota of the analyzed cheeses produced also bacteriocins of some other than IIa class. In this work, the anti-Listeria potential of a particular LAB species was determined, as well. Moreover, comparison of the analyzed cheeses with respect to antagonistic activity of their indigenous microbiota was carried out. It was determined that oscypek was the richest source of LAB able to produce bacteriocins active against Listeria. Bacteriocins produced by these species were characterized by the broadest activity spectra and exerted the strongest antagonistic activity. LAB with anti-Listeria potential constituted also an important component of the indigenous microbiota of the remaining cheeses. Simultaneously, these strains were the crucial element of the natural barrier of the analyzed cheeses against Listeria contamination.

In the last stage of this work, bacteriocins with the strongest anti-Listeria activity were characterized. Bacteriocins Lc. garvieae AS 6/4/4 and Lb casei AS 4/4/23 were selected for this experiment. After analyses of their biochemical properties, mechanism of action, molecular mass and primary structure it was concluded that these bacteriocins are a new members of bacteriocin IIa class. In model studies the applicability of these bacteriocins in both the prevention of and overcoming L. monocytogenes contamination was confirmed.

As a result of the presented research a unique collection of LAB with anti-Listeria potential was created. This collection may serve as a basis for development o new, attractive starter cultures, protective cultures and biopreparations designed for combating Listeria contaminations.

**Key words:** anti-Listeria bacteriocins, microbiota of Polish regional cheese, LAB

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