

EFFECT OF SELECTED LAB ON *L. MONOCYTOGENES* DURING PRODUCTION OF TRADITIONALLY FERMENTED SAUSAGES

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ABSTRACT

Within the project "Safety of traditional fermented sausages: Research on protective cultures and bacteriocins" the task of Work Package 5 (WP5) was to produce traditional fermented sausages inoculated with pathogens using selected protective cultures and/or bacteriocins. As a result from previous work packages, three strains of *Lactobacillus sakei* (I-151, I-154 and I-155) showed bacteriocin-based antimicrobial activity against *Listeria monocytogenes* only. The Task 5.1. was production of fermented sausages inoculated with *L. monocytogenes*, together with the three selected protective cultures. Three batches of the indigenous sausages were prepared according to original recipes. For each batch four groups of sausages were produced: a positive control group (sausages inoculated only with *L. monocytogenes*), and three groups of sausages inoculated with *L. monocytogenes* and one of the three protective strains of *Lb. sakei* (I-151, I-154 and I-155). A slight advantage of the strain I-151 over the other two was observed in antilisterial activity, which may suggest that the three investigated strains represented just three isolates of the same bacteria. This hypothesis was supported by results of genetic investigation of bacteriocin determinants of the isolates carried out in WP3, where it was verified that all the Italian strains are subclones of the same population. Having in mind these results, it may be suggested to use only *Lb. sakei* I-151 as a protective starter culture in further experimental investigation.

Key words: *L. monocytogenes*, LAB, bacteriocins, fermented sausages

INTRODUCTION

The task of Work Package 5 (WP5) of the "SAFE-TYSAUSAGE" project was pilot production of fer-

mented sausages inoculated with pathogens using selected protective cultures or bacteriocins. Resulting from the previous work packages, three strains of *Lactobacillus sakei* (I-151, I-154 and I-155) showed desirable bacteriocin production and antilisterial activity was used. These protective strains, as well as the strain of *L. monocytogenes*, were sent by the Italian partner and used for inoculation of sausages.

MATERIALS AND METHODS

1. Preparation of sausages

For the purpose of this task, three batches of the indigenous sausages were prepared. Within each batch, four groups of sausages were produced: a positive control group (sausages inoculated only with *L. monocytogenes*), and three groups of sausages inoculated with *L. monocytogenes* and one of the three protective strains of *Lb. sakei* (I-151, I-154 and I-155). The final number of *L. monocytogenes* in the filling mixture should be 10^4 - 10^6 cells/g.

2. Sampling schedule

Three batches of sausages were used for the experiments carried out within a 3-month period or simultaneously within a month. Samples were taken from each batch at day 0, 3, 7, 14 and 28 of fermentation and ripening process, with day 0 being the day of sausage formulation. Three sausages were taken every sampling day from each batch laboratory and subjected to physicochemical (pH) and microbiological analysis (total viable count, lactic acid bacteria

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count and *L. monocytogenes* count for both enrichment and plating) in laboratory. Sausages were kept at 4°C until analysis.

RESULTS AND DISCUSSION

Results of microbiological analyses of sausage samples are presented in Figure 1.

All three investigated strains of *Lb. sakei* showed a desirable impact on decrease of *L. monocytogenes* in Bosnian sudzuk in all batches. The growth of the pathogen in control sausages was observed up to the Day 3, after which a stationary phase lasted to the Day 7 with mean value log cfu/g 5,52 (Fig. 1). During the rest of fermentation and ripening phase *L. monocytogenes* count was slightly decreasing from 4,59 log cfu/g on Day 14 to 3,83 log cfu/g on Day 28. Growth of the pathogen in sausages inoculated with the three protective strains showed similar trend during the first seven days of fermentation in all batches. Relatively the best effectiveness against *L. monocytogenes* showed the strain I-151 in all batches.

The results of analysis of a Croatian traditional sausage show slight differences. The growth of *L. monocytogenes* in control sausages increased from 4,94 on Day 0 up to 5,37 on Day 3 and was 4,76 log cfu/g on the Day 7 (Fig. 1). In the rest of fermentation and ripening phase the pathogen count decreased from 4,63 to 0,00 log cfu/g on Day 14 and 28, respectively. Absence of growth of the pathogen on Day 28 is characteristic only for the Croatian sausage comparing with other three indigenous sausages. Growth of *L. monocytogenes* showed similar trend during first 7 days of fermentation in sausages inoculated with the three protective strains.

In control batch, mean values of *L. monocytogenes* decreased from 3,59 log cfu/g on Day 7, 3,44 log cfu/g on Day 14 to finally 1,7 log cfu/g on Day 28 for Serbian sausage. In the batches with protective cultures, in all three fermentations on the Day 28, the pathogen could not be found even with enrichment procedure. All the three strains of *Lb. sakei* had a positive influence on decrease of *L. monocytogenes* count. *Lb. sakei* I-151 showed a slight advantage over the other two strains with 1,83 log reduction from Day 0 to Day 14, comparing to strain I-154 with

1,33 log reduction in the same period and 1,72 log reduction for I-155. (Fig. 1).

In the sausage produced in Hungary with the used protective starter cultures and combinations of different antimicrobial treatments (salt, nitrite, pH, aw) the count of inoculated *L. monocytogenes* was reduced by about 2 log cycles. The best protective starter culture was I-151 with log reduction of 2,39 from Day 0 to Day 28. The other two protective strains had almost the same results of log reduction in the same period (1,97 and 2,03 for strains I-154 and I-155, respectively; Fig. 1). The traditional ripening process reduced listerial growth only by 1,5 log cycle unit in sausages.

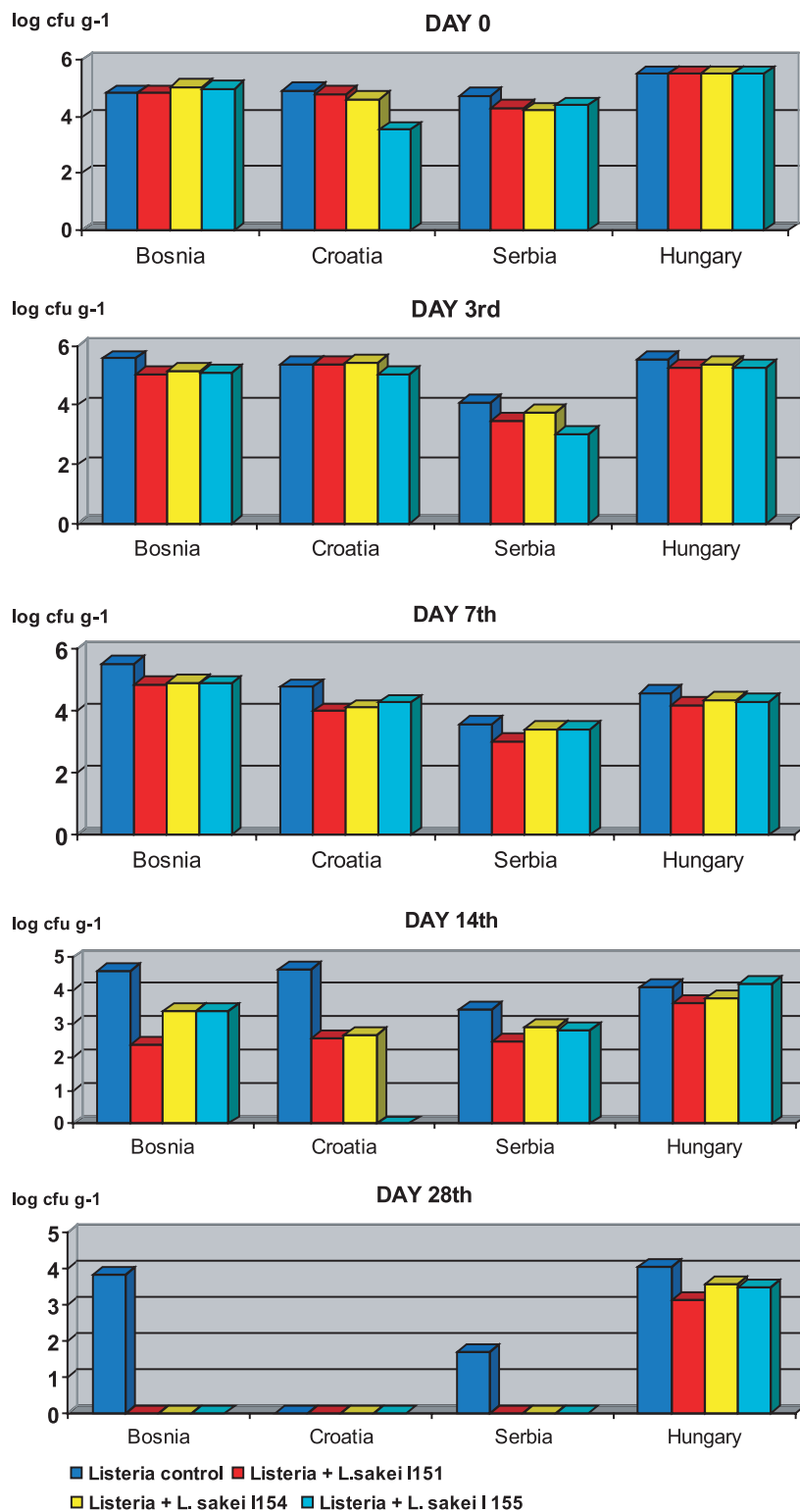
Several authors reported on listerial growth and survival in dry fermented sausages (Johnson et al., 1988; Trussel and Jemmi, 1989; Campanini et al., 1993). Protective effect of *Lb. sakei* strains against *L. monocytogenes* was observed by other authors. Schillinger et al. (1991) conducted a study that resulted in an effective inhibition of *L. monocytogenes* by *Lb. sakei* in meat. That a strain of *Lb. sakei* was able not only to suppress the growth of listeria in dry fermented sausages, but also to diminish their number by 1,25 log compared to the non-bacteriocinogenic control strain reported Hugas et al. (1995).

CONCLUSIONS

1. All the three investigated protective strains were identified as *Lb. sakei* and isolated from Italian sausages;
2. No significant differences in antilisterial activity were observed among the strains;
3. A slight advantage of the strain I-151 over the other two was observed in antilisterial activity, which may suggest that the three investigated strains represented just three isolates of the same bacteria. This hypothesis was supported by results of genetic investigation of bacteriocin determinants of the isolates carried out in previous work, where it was verified that all the Italian strains are subclones of the same population. Having in mind these results, it may be suggested to use only *Lb. sakei* I-151 as a protective starter culture in further experimental investigation.

▼ **Figure 1.** Growth of *L. monocytogenes* in four traditionally fermented sausages (sausage formulation).

▼ **Slika 1.** Rast *L. monocytogenes* u 4 tradicionalne fermentirane kobasice tijekom zrenja



PROŠIRENI SAŽETAK UTJECAJ BAKTERIJA MLIJEČNE KISELINE NA *L. MONOCYTOGENES* TIJEKOM PROIZVODNJE TRADICIONALNO FERMENTIRANI KOBASICA

UVOD

Unutar projekta "Safety of traditional fermented sausages: Research on protective cultures and bacteriocins" zadatak WP5 bio je proizvodnja tradicionalnih trajnih kobasica korištenjem selekcioniranih protektivnih kultura i/ili bakteriocina inokuliranih s patogenim bakterijama. U tu svrhu korištena su tri soja *Lactobacillus sakei* (I-151, I-154 and I-155) za koje je ranije utvrđeno da pokazuju antimikrobnu aktivnost u smislu sprečavanja rasta i razmnožavanja *Listeria monocytogenes*.

MATERIJAL I METODE

Proizvedene su tri proizvodne serije kobasica, i to: pozitivna kontrola (kobasica inokulirana samo s *L. monocytogenes*) i tri kobasice inokulirane s *L. monocytogenes* i po jednim od sojeva *L. sakei* (I-151, I-154 i I-155). Po tri uzorka kobasica uzimana su iz svake serije 0., 3., 7., 14. i 28. dan zrenja radi fizikalno-kemijske (pH) i mikrobiološke pretrage (ukupni broj bakterija, ukupni broj bakterija mliječne kiseline, broj *L. monocytogenes*).

REZULTATI I DISKUSIJA

Podaci u literaturi ukazuju na mogućnost rasta i preživljavanja bakterije *L. monocytogenes* u trajnim kobasicama (Johnson et al., 1988; Trussel and Jemmi, 1989; Campanini et al., 1993). Drugi autori ističu učinak nekih sojeva *Lb. sakei* na sprečavanje rasta *L. monocyto-*

genes (Schillinger et al., 1991; Hugas et al., 1995).

Rezultati su prikazani po danima uzorkovanja kobasica tijekom zrenja (slika 1). Sva tri soja *Lb. sakei* pokazala su značajan utjecaj na smanjenje broja *L. monocytogenes* u uzorcima bosanskog sudžuka, u kojem je uočen pad broja bakterije od 14. dana zrenja sa 4,59 log cfu/g na 3,83 log cfu/g 28. dana. Relativno najbolji učinak na sprečavanje rasta *L. monocytogenes* imao je soj I-151 u svim proizvodnim serijama. U kontrolnim uzorcima kobasica proizvedenih u Hrvatskoj rast *L. monocytogenes* smanjivao se tijekom zrenja (4,94 log cfu/g 0. dana, 4,76 log cfu/g 7. dana, 4,63 log cfu/g 14 dana), a posljednjeg dana zrenja patogen nije utvrđen. Smanjenje rasta *L. monocytogenes* utvrđeno je i u uzorcima sa zaštitnim sojevima, da bi 28. dana kobasice bile slobodne od patogena. U uzorcima srpske kobasice srednja vrijednost broja *L. monocytogenes* u kontrolnoj kobasici padala je od 3,59 log cfu/g 7. dana do 1,7 log cfu/g 28. dana zrenja. U serijama sa zaštitnim kulturama 28. dana zrenja patogen nije bio utvrđen i sva su tri soja laktobacila pokazala pozitivan utjecaj na smanjenje broja listerija. *Lb. sakei* I-151 pokazao se učinkovitijim od ostalih sojeva u istraživanju (redukcija broja *L. monocytogenes* za 1,83 log od 0. - 14. dana) u usporedbi sa sojem I-154 (redukcija za 1,33 log u istom vremenskom razdoblju) te sojem I-155 (smanjenje broja za 1,72 log). U mađarskim kobasicama broj *L. monocytogenes* bio je smanjen za oko 2 log. Najboljom zaštitnom kulturom pokazao se soj I-151 (smanjenje od 2,39 do 28. dana zrenja). U kontrolnom uzorku rast listerije smanjen je za 1,5 log.

ZAKLJUČCI

Uočena je prednost soja I-151 u odnosu na druga dva soja *L. sakei* korištena u pokusu iz čega je moguće zaključiti da tri ispitivana soja predstavljaju tri izolata iste bakterije. Ta je hipoteza potvrđena i rezultatima genetičkog istraživanja tvorbe bakteriocina učinjenog unutar zadatka istog projekta. Imajući to na umu možemo zaključiti kako je moguće preporučiti samo soj I-151 kao protektivnu starter kulturu u daljnjim eksperimentalnim proizvodnjama kobasica.

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