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Protective effect of *Lactobacillus sakei* in fermented sausages

Lactobacillus sakei als Schutzkulturen in Rohwürsten

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Summary

Lactobacillus (Lb.) sakei I151, I154 and I155 isolated from Italian fermented sausages were implemented in the production of Croatian traditionally fermented sausages in order to evaluate the protective effect against experimentally inoculated *Listeria (L.) monocytogenes* (4–5 $\log_{10}$ CFU g^{-1}). Three batches of sausages were produced and samples were taken after 0, 3, 7, 14 and 28 days of processing and subjected to microbiological analyses (total viable count, lactic acid bacteria, *L. monocytogenes*) and pH measurement. Using the protective cultures, significantly ($p < 0.05$) lower *L. monocytogenes* counts were observed between the 3rd and 14th day compared to control samples. Sausages produced with *Lb. sakei* I155 were *Listeria* negative already after 14 days, while control sausages and sausages with *Lb. sakei* I151 and I154 after 28 days of ripening.

Keywords: *Lactobacillus sakei, biopreservation, fermented sausages*

Zusammenfassung

Lactobacillus (Lb.) sakei I151, I154 und I155, isoliert aus italienischen Rohwürsten, wurden bei der Herstellung von traditionellen kroatischen Rohwürsten angewendet, um deren Schutzwirkung gegen experimentell inokulierte *Listeria (L.) monocytogenes* zu prüfen. Es wurden drei Chargen von Würsten hergestellt und an Tag 0, 3, 7, 14 und 28 der Reifung wurden Stichproben entnommen zur mikrobiologischen Analyse (Gesamtkeimzahl, Zahl der Milchsäurebakterien, *L. monocytogenes*) und pH-Bestimmung. Die Verwendung von Schutzkulturen verringerte die Keimzahl von *L. monocytogenes* zwischen dem 3. und 14. Tag der Reifung im Vergleich mit den Kontrollwürsten signifikant ($p < 0{,}05$). Die mit *Lb. sakei* I155 hergestellten Rohwürste waren schon am 14. Tag der Reifung *Listeria* negativ, die Kontrollen und die mit *Lb. sakei* I151 u I154 produzierten Rohwürste erst am 28. Tag.

Schlüsselwörter: *Lactobacillus sakei, Biokonservierung, Rohwürste*

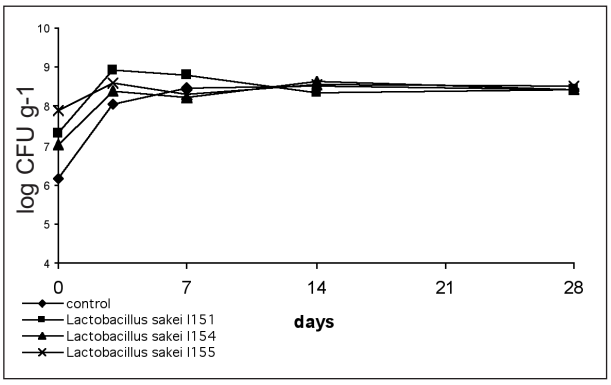

FIGURE 1: *Changes in total viable count* during the ripening of fermented sausages inoculated with L. monocytogenes and protective cultures.*

*Mean of three fermentations.

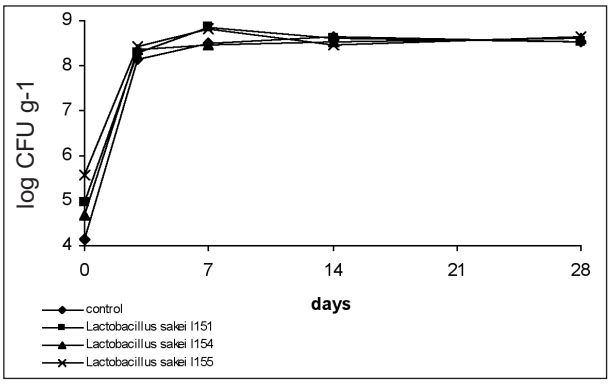

FIGURE 2: *Changes in lactic acid bacteria count* during the ripening of fermented sausages inoculated with L. monocytogenes and protective cultures.*

*Mean of three fermentations.

Introduction

Bacteriocin-producing lactic acid bacteria have been frequently isolated from different sources including meat and meat products (Schillinger and Lücke, 1989; Lewus et al., 1991; Urso et al., 2004). The inhibitory capacity of individual strains towards emerging foodborne pathogens has been investigated intensively in recent years both in vitro and in practical use, i.e. during the production process or storage of many types of food (Hugas et al., 2002; Katla et al., 2002; Hadžiosmanović et al., 2005; Zdolec et al., 2005a). Regarding fermented sausages, the most encouraging results have been found using bacteriocinogenic lactic acid bacteria in the control of *Listeria* spp. (Foegeding et al., 1992; Työppönen et al., 2003; Čaklovica et al., 2005). *Lactobacillus* (*Lb.*) *sakei* is well adapted to meat as substrate and is a normal constituent of the natural lactic acid bacteria (LAB) population in various types of fermented sausages (Hammes, 1990). In addition to technological effects, the protective role of particular *Lb. sakei* strains in fermented sausages has been reported (Schillinger et al., 1991; De Martinis and Franco, 1998; Liserre et al., 2002).

Listeria (*L.*) *monocytogenes* is undoubtedly a serious problem in food safety concepts. Its characteristics and ubiquity result in frequent isolation from almost all kinds of foodstuffs and food processing plants (Loncarevic, 1998; Kozačinski and Hadžiosmanović, 2001; Thévenot et al., 2005). Despite hurdles like low a_w, pH or higher salt content, *L. monocytogenes* has been isolated from fermented sausages (Colak et al., 2007). Fortunately, the natural contamination of raw materials and the final number of pathogens in fermented sausages are low (Thévenot et al., 2005). In the present study, high inoculum levels were used (4–5 $\log_{10}$ CFU g^{-1}), unusual in natural conditions, in order to evaluate the effectiveness of the natural ripening process and protective cultures of *Lb. sakei* I151, 154 and I155 in reduction of *L. monocytogenes* populations.

Material and Methods

Strains and preparation of inoculums

Sakacin P producing strains *Lb. sakei* I151, I154, I155 isolated from fermented sausages of the Friuli Venezia Giulia region (Urso et al., 2004) and *L. monocytogenes* NCTC 10527 were received from University of Udine, Food Science Department, Udine, Italy.

Protective strains were grown overnight at +30 °C in MRS broth (BioMerieux, Marcy l`Etoile, France) and streaked on MRS agar. Each strain was subcultured twice, and 500 ml of the active culture was centrifuged at 2000 rpm for 15 minutes. Cells were resuspended in 50 ml sterile saline water. The number of each strain in the thus prepared solutions was enumerated on MRS agar before inoculation of the sausages. *L. monocytogenes* inoculum was prepared in an equivalent procedure in BHI broth, but 10 ml of active culture was centrifuged, and cells were resuspended in 10 ml of sterile saline water. The cell number in this solution was also checked before inoculation.

Sausage production

Three batches of sausages were produced following the Standard Operating Procedure (SOP) as described recently (Kozačinski et al., 2006). Briefly, frozen meat (pork, 60 %; beef, 10 %) and fat (pork back fat, 24 %) were brought to –2 °C before use. After grinding to 12 mm in diameter, other ingredients (salt with 0.5 % $NaNO_2$, 2.5 %; sugar, 0.5 %; spices (ground black pepper, minced red pepper, garlic), 3.0 %) were added separately. Meat and fat were further cut to 2 mm in diameter under continuous mixing and homogenisation of the mixture.

The prepared filling was inoculated with a suspension of *L. monocytogenes* (10^9/ml) during the mixing, reaching a final number of 4–5 $\log_{10}$ CFU g^{-1} in the mixture. After dividing into 4 equal parts, the first one was directly filled into porcine casings (32–34 mm in diameter) under vacuum, representing the control sample, while each of the other three parts was inoculated with one of the protective culture strains (5–6 $\log_{10}$ CFU g^{-1} in final mixture). After stuffing, the sausages were allowed to equilibrate at room temperature (12 hours at +20 °C and 95 % relative humidity (RH)), then cold smoked for 48 hours at the same temperature and 85–90 % RH. Lastly, sausages were kept in a fermentation chamber till day 28. During the ripening process, temperature and relative humidity were gradually reduced to +16–18 °C and 75 % RH, respectively.

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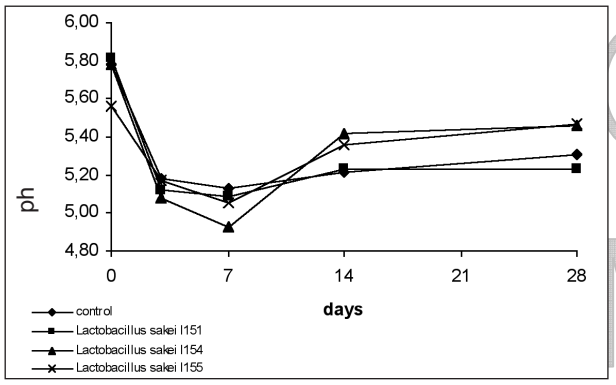

FIGURE 3: *Changes in pH* during the ripening of fermented sausages inoculated with L. monocytogenes and protective cultures.*

*Mean of three fermentations.

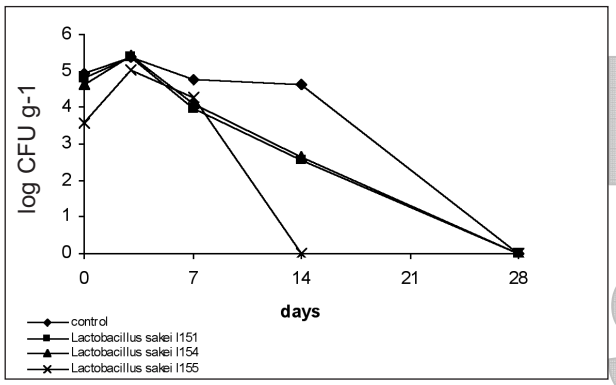

FIGURE 4: *Changes in L. monocytogenes count* during the ripening of fermented sausages inoculated with protective cultures.*

*Mean of three fermentations.

Microbiological analysis and pH measurement

For microbiological analyses and pH measurement, three samples of sausages were taken from each batch at days 0, 3, 7, 14 and 28 of processing. The samples were transported to the laboratory at +4 °C and kept at the same temperature up to analysis.

A 25 g portion of each test sample was diluted in 225 ml of salt peptone water and homogenised for 2 minutes (Stomacher 400 Circulator, Seward, UK). After serial dilution, appropriate dilution samples (1 or 0.1 ml) were poured or spread on agar plates. Total viable count (TVC) was determined on Plate Count Agar (PCA, BioMerieux) at +30 °C for 72 hours; lactic acid bacteria (LAB) count on MRS agar (BioMerieux) overlaid with 5 ml of the same medium at +30 °C for 48–72 hours and *L. monocytogenes* count on Palcam agar at +37 °C for 24–48 hours. Measurements of pH were performed by means of a digital pH-meter (WTW, Germany) in 10 g samples diluted and homogenized in 90 ml of distilled water.

Statistical analysis

Statistical analysis was performed using Statistica 7.1 software (StatSoft Inc., Tulsa, USA). Differences among control sausages and sausages with protective strains regarding microbiological parameters and pH during the ripening phase were analysed by ANOVA ($p < 0.05$).

Results and Discussion

Changes in TVC, LAB, pH and *L. monocytogenes* count in traditionally fermented sausages and sausages inoculated with protective cultures are shown in Figures 1–4.

The total viable count increased till the 14th day in control sausages, while in sausages with *Lb. sakei* cultures an increase in TVC was observed only up to the 3rd day of ripening (Fig. 1). During the fermentation phase TVC was significantly lower in control sausages ($p < 0.05$). In the final products no differences in TVC between sausages existed. Lactic acid bacteria populations rapidly increased towards the 3rd day of fermentation in all groups of sausages (Fig. 2). Significantly higher numbers were found using protective cultures at days 0 and 7. Similarly as in TVC, no differences existed among sausages in the LAB count during the last phase of production ($p < 0.05$). The pH decreased till the 7th day parallel with the rapid increase of the LAB populations (Fig. 3). During the fermentation phase significantly lower pH values were found using *Lb. sakei* I155 (day 0) and I154 (from day 3 to day 7).

The results shown in Figure 4 indicate a higher degree of reduction of *Listeria* untill the 14th day of ripening in sausages with bacteriocinogenic *Lb. sakei* cultures in comparison to the control. Those differences were evident at days 7 and 14, i.e. the number of pathogens was significantly ($p < 0.05$) lower using protective cultures. Sausages with *Lb. sakei* I155 were *L. monocytogenes* negative at the 14th day, while in the other groups of sausages the pathogen was reduced towards the end of the production process.

Results obtained in our study are in accordance with present literature data concerning the antilisterial effectiveness of bacteriocin-producing LAB cultures, including *Lb. sakei* (De Martinis and Franco, 1998; Benkerroum et al., 2005). In addition, the investigation confirmed also the potency of the natural ripening process in suppressing the growth of *Listeria* spp. during fermentation, and in reducing pathogens during the drying phase (Thévenot et al., 2005; Zdolec et al., 2005b). The effectiveness of *Lb. sakei* I151, I154 and I155 against *L. monocytogenes* was recently investigated in traditionally fermented Hungarian salami, Bosnian sudzuk and Serbian sremska sausage (čaklovica et al., 2005; Gasparik-Reichardt et al., 2006). In Hungarian salami, the pathogen was reduced by 2 log cycles during the 28 days ripening process (from initial 5.5 to 3.5 $\log^{10}$ CFU g^{-1}), in the control sausage only by 1.5 log. Bosnian sudzuk produced with protective cultures of *Lb. sakei* was free of *Listeria* spp. at the end of the manufacturing process (5 log cycles reduction). In the control sudzuk samples the pathogen was reduced from initial 4.85 to 3.83 log10 CFU g^{-1}. In addition, similar results were obtained in sremska sausage with 4.0–4.5 log cycles reduction of the pathogen using protective strains, while in control samples *Listeria* spp. survived the ripening process (< 2 log CFU_{10} g^{-1}).

In our study, *L. monocytogenes* populations started to decrease in sausages with protective cultures of *Lb. sakei* between the 3rd and 7th day of fermentation, when the lowest pH values and the highest LAB counts were determined. Furthermore, a stronger reduction of *Listeria* spp. was found between day 7 and day 14 when pH increased and LAB populations stabilised (8.5 $\log_{10}$ CFU

g^{-1}), so it could be assumed that the pathogen decrease was enhanced due to sakacin activity. From our results and the results of others it could be concluded that *Lb. sakei* I151, I154 and I155 are potentially protective cultures in the production of fermented sausages as a part of the hurdle concept (Leistner and Gorris, 1995) in control of *L. monocytogenes*.

Acknowledgements

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