BACTERIOCINOGENIC LACTOBACILLI IN THE FERMENTATION OF RAW SAUSAGES

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42nd International Congress of Meat Science and Technology
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Keywords: bacteriocinogenic lactobacilli, *Listeria monocytogenes*, dry fermented sausage

Introduction. There is an increasing consumer demand for food products which are free of chemical additives, reduced in common salt and nitrite, and processed as little as possible. Such products are microbiologically rather instabile, i.e. they cannot be savely produced or stored unless alternative barriers against unwanted microorganisms are introduced. Certain lactic acid bacteria (LAB) have been used since decades as starter cultures for the production of various foods and, because of their natural antagonistic activities against many unwanted microorganisms, they also act as protective cultures. Recently, interest has focussed on bacteriocinogenic LAB. *Listeria monocytogenes (L.m.)*, a pathogen frequently found in meat, has become a typical target for the application of such strains (Abee *et al.*, 1995). *Lactobacillus sake* and *Lactobacillus curvatus* are common starter cultures for fermented meats and both compete well in this environment. Strains excreting bacteriocines have been isolated from various meats and, sakacin P appears to be the most common anti-listerial bacteriocin in *Lactobacillus sake* (Holck *et al.*, 1994; Kröckel & Faulhammer, 1994; Hühne *et al.*, 1996). An almost identical bacteriocin, curvacin 1071, is produced by *L. curvatus* Lb1071(Kröckel & Faulhammer, 1994). Both bacteriocins, sakacin P and curvacin 1071 of *L. sake* Lb674 and *L. curvatus* Lb1071, resp., are *in vitro* highly active against listeria. The following study was undertaken to evaluate the application potential of the strains Lb674 and Lb1071 as starter and protective cultures for fermented sausages.

Experimental Methods. LAB starter cultures included *L. sake* Lb674 (sakacin P), *L. curvatus* Lb1071 (curvacin 1071) and for comparison *L. curvatus* Lc3, a bacteriocin-negative commercial strain. The lipolytic and proteolytic strain *Staphylococcus xylosus* St7 was used as a second starter component to investigate possible effects of the bacteriocins on *Micrococcaceae*. A pool of four different serovars of *L.m.* was used in the challenge experiments. *In vitro*, all of them were inhibited by sakacin 674 and curvacin 1071 (*L.m.* strain /serovar/relative bacteriocin sensitivity: Li20/ 1/2c/ +, Li37/ 4b/ +++, Li52/ 1/2a/ +, Li70/ 1/2b/ ++). For sausage inoculation, liquid cultures of LAB, *S. xylosus* and *L.m* were grown in routine laboratory media. Microbial counts were determined on selective media by standard procedures. Bacteriocin activity (AU, arbitrary units/ml) was estimated using *Listeria ivanovii* Li4 as an indicator organism. To determine bacteriocin activity in the meat, five grams of sausage sample were heated 10 min at 100°C. The resulting fat-water emulsion was centrifuged and aliquots of the aqueous phase containing the bacteriocin activity were applied in an agar diffusion assay. The proteinaceous nature of the inhibitory substance was confirmed by trypsin digestion. - Salami-type sausage batters containing 33% lean pork, 33% lean beef, 33% pork back fat, 2.8% nitrite curing salt, 0.4% saccharose, 0.4% pepper and 0.03% garlic powder were produced in 4 kg batches and stuffed into 60 mm fiber casings (400 g / sausage). LAB inocula ranged from 10^3 - 10^6 /g and staphylococci were applied at 10^6 /g of meat batter. Listeria were inoculated at 10^4 /g. The ripening regime was 2 days at 23°C/ 90 % r.H., then 5 days at 20°C/ 90 % r.H., then 7 days at 18°C/ 85 % r.H., and then 14 days at 15°C/ 80 - 85 % r.H. Smoke was not applied and mould growth was regularly wiped off with paper towels.

Results. In the sausage matrix, both bacteriocin producers grew well, produced detectable bacteriocin activities, and independent from the inoculation level dominated the LAB flora. The salami aroma was influenced by the initial LAB inoculum. At 10^6 LAB/g the sausage pH dropped within one day to 5.2 (Lb674) and 4.8 (Lb1071) and after 28 days the salamis had a sour smell and taste (not shown). Initial inocula of 10^3 /g resulted in a strongly delayed pH drop (Figure 1) and in aromatically more balanced products. After reaching a maximum number of 10^8-10^9/g, viable counts of Lb1071 decreased 1-2 log cycles until day 28. No such decrease was observed for strain Lb674 (Figure 1). Bacteriocin activities in sausages with Lb1071 always were several times higher than with Lb674. They peaked in the late exponential phase of growth and thereafter showed a biphasic 60% decrease until day 28. Lb674 was an unreliable bacteriocin producer in salami and segregated bacteriocin-negative variants. Nevertheless Lb674 always dominated the LAB flora. This was indicated by the biochemical phenotype of the LAB and from the reversion of bacteriocin-negative isolates from MRS plates to the bacteriocin-positive phenotype after 2-3 passages in MRS broth at 25°C. No such segregants were observed for Lb1071. Therefore, Lb1071 was chosen as a protective culture against *L.m.* in challenge studies. In the presence of Lb1071, *L.m.* counts decreased within 28 days 25 times more than in the presence of the bacteriocin-negative strain Lc3 (Figure 2). The major inactivation occured in the first 3 days of ripening. However, a complete elimination of *L.m.* was not observed. To separate effects of pH and a_w from the bacteriocin effect, listerial numbers were plotted against the pH values of the sausage samples of the first two ripening days (Figure 3). During this time period the changes in a_w can be neglected and a decrease in listerial numbers only depends on pH and other antagonistic factors (e.g. bacteriocin). From day 0 to day 2 listeria were reduced 5 times more by Lb1071 than by Lc3. Probably due to the higher acidifying activities of Lb1071 the *Micrococcaceae* were more reduced over a 28 days period. A bacteriocin effect on the *Micrococcaceae* was not apparent when micrococcal numbers were plotted against pH (Figure 3).

Conclusions. The bacteriocin producers *L. sake* Lb674 and *L. curvatus* Lb1071 grow well in salami and both are suitable for the production of dry fermented sausages. At ripening temperatures of 23°C inocula less than 10^6 /g of these strains should be used if mildly acidified products are preferred. Bacteriocin production in salami is more pronounced and reliable with Lb1071. Strain Lb674 tends to segregate bacteriocin-negative variants and therefore is less suitable as a protective culture. Anti-listerial bacteriocin is produced in salami by Lb1071 in sufficient amounts and, once produced the bacteriocin activity is retained over a long time. Curvacin 1071, together with nitrite, pH and a_w, contributes to the reduction of *Listeria monocytogenes* in salami but has no effect on *Micrococcaceae*.

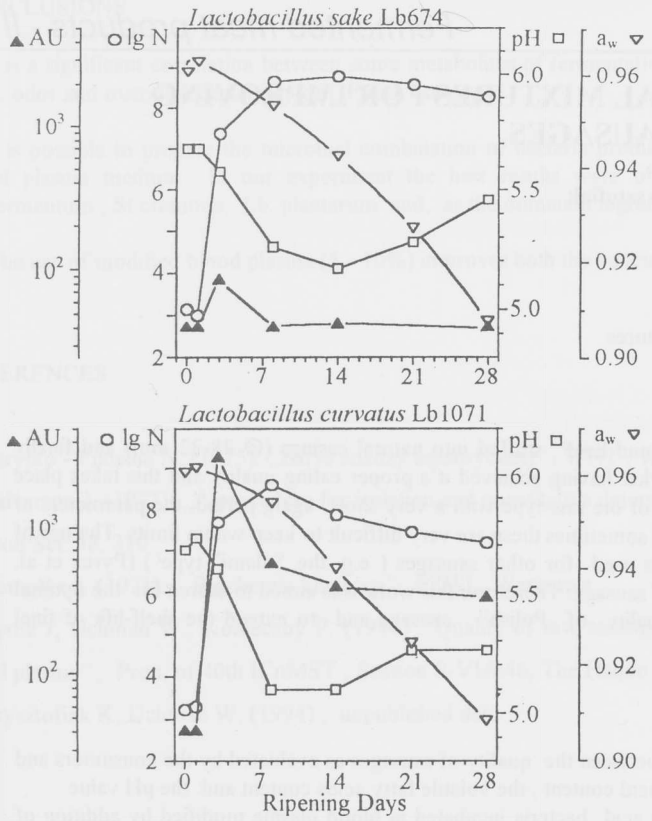

Figure 1. Development of LAB numbers (lg N), bacteriocin activity (AU; AU = 40 means zero activity), pH and a_w in the sausages during the production of salami with *L. sake* Lb674 (sakacin P) and *L. curvatus* Lb1071 (curvacin 1071).

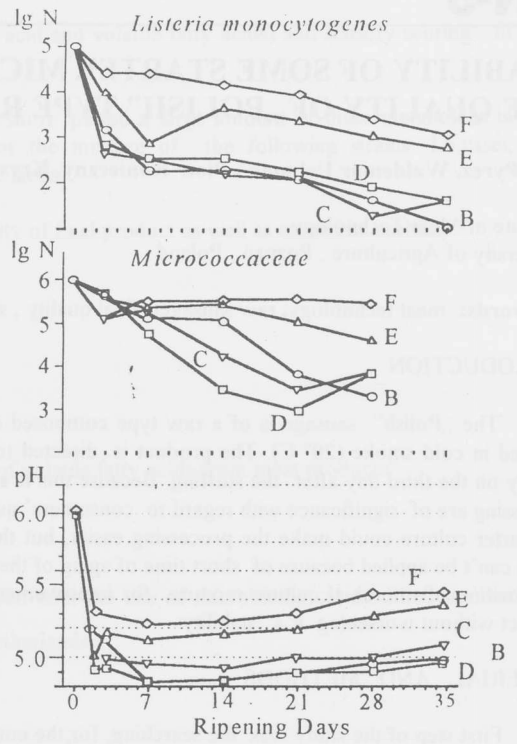

Figure 2: Development of listerial and micrococcal numbers (lg N), and of pH in dry fermented sausages inoculated with different levels of *L. curvatus* Lb1071 (B: 10^4/g, C: 10^5 - 10^6/g, D: 10^6 - 10^7/g) and *L. curvatus* Lc3 (E: 10^4 - 10^5/g, F: 10^6 - 10^7/g)

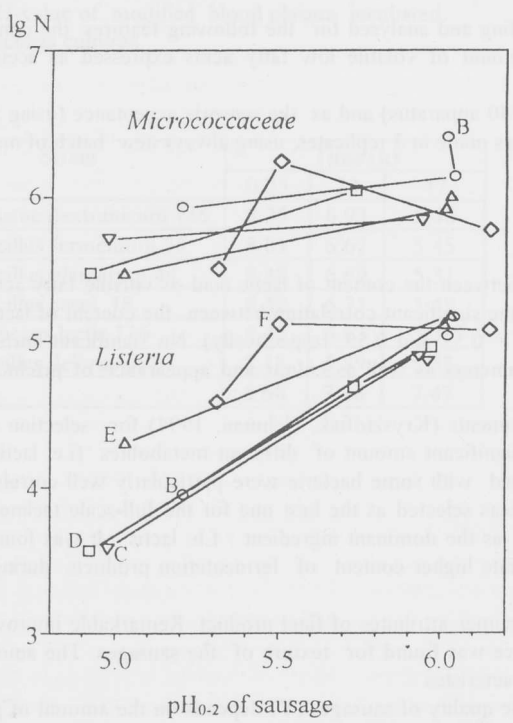

Figure 3. Correlation between sausage pH and the corresponding numbers (lg N) of *L. monocytogenes* and *Micrococcaceae* during the first two days of salami ripening in the presence of the *L. curvatus* strains Lb1071 (bacteriocin-positive) and Lc3 (bacteriocin-negative). Compare Figure 2 for symbols.

References

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