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STARTER CULTURE WITH ADDED VALUE FOR PRODUCTION OF FERMENTED, DRY SAUSAGES: Bactoferm™ F-LC

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Background

Application of selected strains of lactic acid bacteria (LAB) as a competitive culture to inhibit undesirable bacteria such as spoilage organisms and pathogens should be considered as an additional biological hurdle to give a higher degree of product safety. LAB possess useful anti-microbial properties among which the most powerful are: production of organic acids, such as lactic acid or acetic acid. Competition for nutrients makes it difficult for the indigenous bacteria to grow. Production of bacteriocins which are heat-stable polypeptides produced by certain LAB, which may have an inhibitory effect on other, mostly closely related, bacteria (Holzapfel, 1995). It is possible to use LAB as bioprotective cultures, as they are considered GRAS-organisms (Generally Recognised As Safe) (Stiles, 1996). Hurdle technology will ensure safe foods through a synergistic effect of a controlled microflora, anti-microbial metabolites together with other inhibiting factors such as packaging, avoid temperature abuse and GMP.

Preservation through fermentation by LAB is a well-known technology which traditionally has been used in the production of a wide range of foods such as yoghurt, kimchi and similar fermented vegetables, and fermented sausages. The reason for fermenting food products is partly the ability of LAB to influence the technological properties and partly the bioprotective effect. Organic acids, mainly lactic acid, are produced from available carbohydrates which result in a lowering of pH. Hereby the growth of many spoilage and pathogenic organisms are inhibited or delayed. Furthermore, the flavour and texture of the food may change and turn the product into a new type of food, with fermented, dry sausages as an example.

Objectives

The aim of this work was to investigate the anti-listerial effect of two bacteriocin producing LAB strains: *Lactobacillus curvatus* MI401 and *Pediococcus acidilactici* PA-2. The strains produce bavaricin A and pediocin PA-1, respectively. The effect was studied in different meat slurries at different temperatures. Furthermore, the properties of the two strains mixed with a staphylococci given a starter culture with added value for the production of safer fermented, dry sausages was investigated.

Methods

The following bacterial strains were added to relevant codes: *Listeria monocytogenes* V80 (Lm) at the level of 10^3-10^4 CFU/g. *Lactobacillus curvatus* MI401 (Lc), *Pediococcus acidilactici* PA-2 (Pa), and a bac$^-$ variant (plasmid cured) of *Pediococcus acidilactici* PA-2 (Pa-bac$^-$) (Christensen and Jørgensen, 1994). The starter culture Bactoferm™ F-LC (F-LC) is a combination of Lc + Pa in the ratio 1:1 combined with *Staphylococcus xylosus*. In the tests the total level of inoculated LAB is 10^7 CFU/g combined in the culture with $5x10^6$ CFU/g staphylococci. The two LAB strains are combined in the culture to obtain a broad inhibitory spectrum.

Two different recipes were used. **A**: recipe for the production of a fatty, fermented, dry sausage (salami) contained approx. 26% pork, 26% beef, and 42.5 % back fat added 2.7% salt, 0.7% maltodextrin SPG 20, 0.5% glucose, 0.05% ascorbate and 100 PPM nitrite and spices. **B**: for the production of Pepperoni approx. 36% pork, 36% beef, and 22% back fat added 3.0% salt, 0.5% glucose, 0.05% ascorbate and 100 PPM nitrite and ColorLife spice blend were used. Tests were conducted both in meat slurries and in salami sausages. The salami slurry was immersed in a thermostat equipped water bath with the temperature adjusted to 24°C and samples were analysed on day 0, 1, 2, 3, 4, and 6. Pepperoni slurry immersed at 40°C was analysed initially and after 12, 24, 36, and 62 hours. The salami sausages were processed in accordance with a three weeks climate chamber programme. A code without starter culture but with Lm added served as control code. The sausages were explored with respect to pH in the sausages, weight loss and bacteriological examinations. Two sausages were analysed on day 0, 1, 2, 3, 6, 13, and 20.

Listeria was enumerated on Palcam with 2.5% egg yolk emulsion (both Oxoid) added, incubated microaerophilic for two days at 37°C. Low levels of Lm were examined semi quantitatively after enrichment (modified method after McClain & Lee, 1988 and Campanini et al, 1993). LAB were detected by pour plating (enumeration) as well as spread plating (visual recognition and microscopically examination) on MRS (Oxoid), anaerobically incubated for three days at 30°C.

Results and discussion

LAB develop as expected to a level of approx. 10^8 CFU/g homogeneously in all samples with inoculated LAB. Whether or not the Lm is added does not influence the increase. In the uninoculated samples the indigenous LAB develop to the same level as the inoculated within two days at 24°C and 12 hours at 40°C. Data not shown.

L. monocytogenes develops in the salami slurry kept at 24°C (figure 1). After 24 hours a stable level of approx. 10^6 CFU/g is reached. The acid production by Pa-bac$^-$ is only sufficient to prevent an increase in Lm. Whereas Lm is suppressed when Pa is added which is probably due to a combined bacteriocin and acid production. Addition of F-LC displays the same picture.

Results in the Pepperoni slurry at 40°C are depicted in figure 2. The development in Lm and the inhibition by Pa-bac- are similar to the above-mentioned tests but the decline in Lm with Pa and F-LC added is more pronounced. After 12 hours <10 CFU Listeria/g is detected. These results indicate that the faster pH drop combined with the production of bacteriocins are efficient to prevent Listeria in Pepperoni produced at high temperatures. In slurries only the capacity of decline in pH and the production of bacteriocins are tested. Whereas it is impossible to predict the impact of other preserving parameters such as smoke and water loss which are part of the production of fermented, dry sausages. Therefore it was chosen to test the efficiency of the bacteriocin producing strains in a salami type fermented at 24°C. The high fat content in this recipe results in a high level of salt-in-water which is expected to stress the added cultures. In figure 3 the development of Lm is illustrated. Listeria is able to survive in the sausages. Also here the acidifying effect of Pa only has a limited effect on Lm. Furthermore, it is obvious that the additional production of bacteriocin by Pa improves the inhibition of Lm and the addition of the combination of the two bacteriocin producing strains in Bactoferm™ F-LC results in an even better safety.

Not only has Bactoferm™ F-LC proved to suppress Listeria added at a level of 10^3 CFU/g to a salami sausage. But the starter culture has also good properties as a semi-fast fermenting culture for the production of dry, fermented sausages. In figure 4 the typical developments of pH and weight loss of salami with F-LC added are shown.

Conclusions

Bactoferm™ F-LC is a starter culture with added value. Besides controlling the acidification, the bioprotective properties enhance the control of Listeria. The production of bacteriocins ensure a better safety of fermented sausages produced with the culture.

Literature

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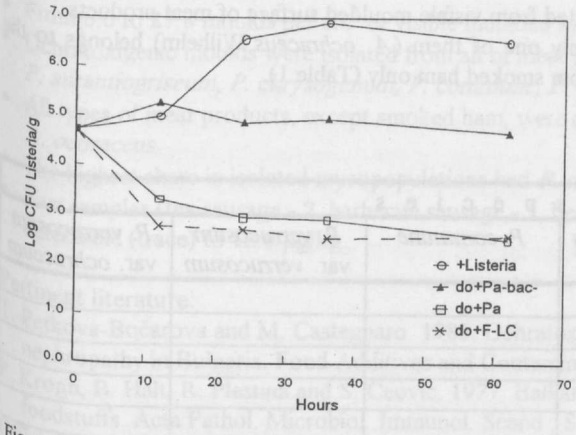

Fig. 1 Development of Listeria in salami mix at 24°C

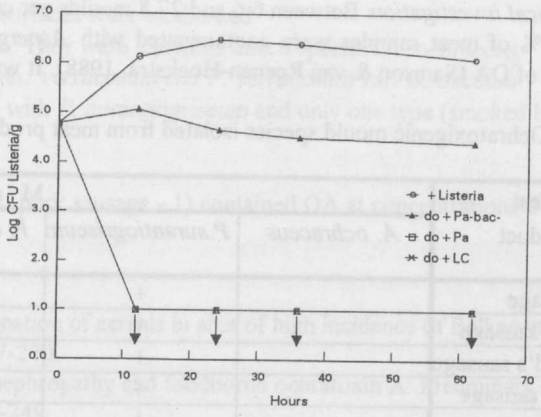

Fig. 2 Development of Listeria in Pepperoni mix at 40°C

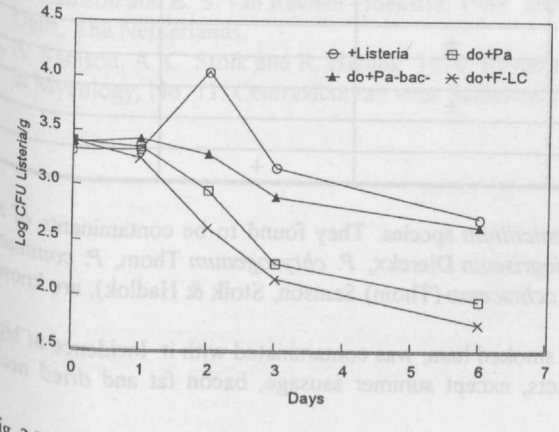

Fig. 3 Development of Listeria in salami at 24°C

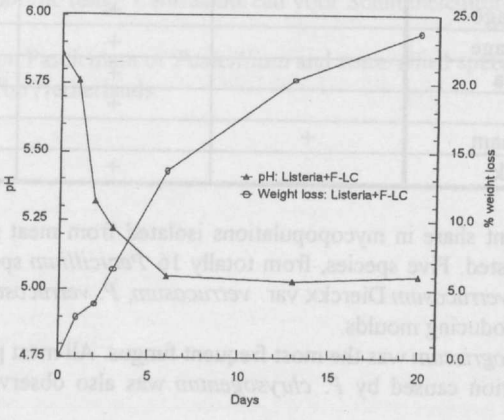

Fig. 4 Properties of F-LC in salami sausages