Ropy slime-producing *Lactobacillus sake* strains possess a strong competitive ability against a commercial biopreservative

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Abstract

Aseptically handled Frankfurters were treated with a commercial *Lactobacillus alimentarius* biopreservate and inoculated with different cell concentrations of four ropy slime-producing *Lactobacillus sake* strains. The packages were vacuum sealed and kept at 6°C for 28 days, after which the production of ropy slime was evaluated. The inoculation test was controlled by sealing the different control packages containing either aseptically manufactured sausages without any bacterial inoculation, packages containing biopreservate only or packages inoculated only with the four different ropy slime-producing strains. Authenticity of the biopreservate strain after the cold storage period was ascertained by performing *Eco*RI restriction endonuclease analysis of 30 randomly selected isolates originating from the biopreservate control packages. All patterns were identical to the pattern of the original *L. alimentarius* biopreservate strain. The biopreservate was found to be quite ineffective against the four ropy slime-producing *L. sake* strains. The strongest slime producers inoculated with approximately 1 CFU/cm^2 could compete efficiently with the *L. alimentarius* having an onset concentration of 10^7 CFU/cm^2 on sausage surfaces. This commercial biopreservate failed to occupy the vital niche of the four ropy slime-producing *L. sake* strains leading to spoilage in almost all packages.

Key words: biopreservation, protective culture, ropy slime, *Lactobacillus sake*, spoilage, vacuum package, inoculation test

1. Introduction

Vacuum packaging is commonly used for increasing the shelf life of cooked meat products. At chill temperatures lactic acid bacteria (LAB) are the dominant spoilage bacteria in these products, as reviewed by Korkeala and Björkroth (1997). Since the growth of LAB is slower, spoilage is retarded compared to spoilage in aerobic conditions, and the products stored at 6°C are expected to retain good sensorial quality for at least three to four weeks. Certain LAB, however, have strong spoilage potential (Reuter, 1970a; 1970b; 1975; Mäkelä et al., 1992a; Dykes et al., 1994a; 1994b) and if a product becomes contaminated, sensorial defects, such as formation of sour odour and taste (Pierson, et al., 1970; Egan and Shay, 1982; Egan, 1983; Korkela et al., 1987; Schillinger and Lücke, 1987) or appearance of ropy polysaccharide compounds (Korkeala, et al., 1988, Korkeala and Björkroth, 1997), may appear before the sell-by day.

LAB associated with a meat processing environment are usually not, with the exception of *Weissella viridescens* (Niven, et al., 1954; 1957; Milbourne, 1983; Borch et al., 1988), thermotolerant and the contamination is mainly a post-heat-treatment issue (Mäkelä et al., 1992a; 1992b; Nerbrink and Borch, 1993). Recent studies of a vacuum-packaged cooked meat product (Björkroth and Korkeala, 1996b; Björkroth and Korkeala, 1997) have shown raw meat to be the main source of spoilage LAB. Manufacturers can deal with LAB contamination by using three different approaches or a combination thereof. Extremely high production hygiene must always be maintained leading the demands towards to clean room technology. Products can be treated also by post-packaging pasteurisation, etc. decontamination treatment. The third approach is biopreservation which means extended storage life and also the enhanced safety of foods using natural microflora, mainly LAB, and/or their antibacterial products as a method of preservation.

There are only few accepted or commercially available biopreservatives, such as nisin (Delves-Broughton, 1990) and the *FloraCarn L2* (Chr. Hansen A/S, Hørsholm, Denmark). Studies of biopreservation have mainly focused on the inhibition of pathogenic micro-organisms, and little is known about the suitability of these products against specific spoilage organisms (SSO) in different types of food. This study set out to evaluate the effect of *FloraCarn L2* (Chr. Hansen), on the production of ropy slime by four previously characterised (Björkroth and Korkeala, 1996a; Björkroth et al., 1996) *Lactobacillus sake* strains. These strains since *L. sake* has been detected as a SSO in vacuum-packaged cooked meat products (Reuter et al. 1970a; 1970b; 1975; Holzapfel and Gerber, 1986; Korkeala et al., 1988; Egan et al., 1989; Mäkelä et al., 1992c; Dykes and von Holy, 1994; Dykes et al., 1994a) and the detection of ropy spoilage in sensorial tests is very accurate compared to the detection of off-odours or off-tastes.

2. Materials and Methods

2.1 *Description of FloraCarn L-2*

FloraCarn L-2 (Chr. Hansen) is a pure culture of *Lactobacillus alimentarius*. The strain does not produce hydrogen peroxide and no production of bacteriocins has been detected. It is supplied in aluminium foil sachets of 50 g containing the freeze-dried strain with glucose as a carrier. The biopreservation culture, prepaired for use by diluting one lyophilised package into 2 litres of water, yields a concentration of approximately 10^9 cells/ml. Sterile water was used in this study to avoid water-mediated contamination. According to the manufacturer, both raw and cooked meat products can either be sprayed with or dipped into the solution. This treatment will result in approximately 10^7 cells/cm^2 on the surface of a product.

2.2 Lactobacillus sake strains used for inoculation

Four representatives, A 210, C1, R51 and R 152, from the main groups of ropy slime-producing *L. sake* previously characterised by carbohydrate patterns, ribotyping, RAPD and macrorestriction patterns (Björkroth and Korkeala, 1996a; Björkroth et al., 1996) were inoculated. Strains were grown overnight in MRS broth (Oxoid, Basingstoke, UK) at 30ºC. The next morning, the cultures were serially diluted 10-fold (-1 to -6) into 0.1% peptone water to provide inocula with different cell concentrations. *L. sake* cells in the four cultures were also enumerated on MRS agar (Difco, Detroit, MI, USA) in order to provide the true inoculation concentrations CFU/cm^2 and CFU/g (Table 1). All plates were incubated anaerobically at 25°C for 5 days using a H_2 and CO_2 gas generating kit (Oxoid) in anaerobic jars.

2.3 Aseptic manufacture of Frankfurters and application of the biopreservation culture

Frankfurter mass, containing 80 ppm sodium nitrite and about 1.8% sodium chloride, and stuffing into natural casings were done in line with normal industrial procedure. The Frankfurters were steam cooked until the core temperature reached 68-70°C. Immediately after, they were transferred aseptically into sterile plastic packages using sterile gloves, and cooled to 4°C before being inoculated. Preparation of the biopreservative was performed as per the manufacturer's instructions (Chr. Hansen A/S, Hørsholm, Denmark). Treatment with the biopreservative was done by pouring a vast amount of the culture into the plastic packages containing the aseptically manufactured sausages. Closed packages were shaken to ensure even distribution of the protective culture. Using sterile gloves, the Frankfurters were aseptically removed from the plastic packages and divided further into smaller sterile plastic packages containing three sausages each. A control batch was left without the biopreservative.

Each package weighed approximately 120 g. The length of one Frankfurter was approximately 14 cm and the radius 1 cm. The superficial content, 88 cm^2, of one Frankfurter was estimated by adding the area of a 12 cm long cylinder to the area of a 1 cm radius ball representing both ends of the Frankfurter. Using this estimation, the total superficial content of three sausages is approximately 264 cm^2. These figures together with the results from enumeration of *L. sake* cultures were used to evaluate the of final inoculation concentrations CFU/g and CFU/cm^2 presented in Table 1.

2.4 Inoculation with Lactobacillus sake and controlling the inoculation test

One hundred µl from four undiluted *L. sake* cultures and each of the dilution were used as inocula. All *L. sake* strains were inoculated into two parallel packages. Three negative control packages were left without any bacterial inoculation, and three biopreservative treated packages were left without *L. sake* inoculation. The ability of four *L. sake* strains to produce ropy slime characteristically (Björkroth et al., 1996) was confirmed by inoculating sterile, non-treated sausages. All inoculations were done aseptically using sterile equipment. The packages were vacuum sealed (-990 mbar) and kept at 6°C for 28 days, which is the normal shelf-life of this Frankfurter type.

After 28 days, the production of ropy slime was evaluated on a scale ranging from negative (-) to abundant (+++) by two trained judges as previously described by Björkroth et al. (1996). The drip from three negative control packages and three packages treated only with the biopreservative were enumerated for lactic acid bacteria. One hundred µl of the drip and 10-fold dilutions were plated on MRS (Oxoid) agar. The authenticity of isolates in three biopreservate control packages after 28 days was ascertained by performing *Eco*RI restriction endonuclease analysis (REA) of 30 randomly selected isolates. Isolation of DNA, restriction digestion and agarose gel electrophoresis were performed as previously described by

Björkroth and Korkeala (1996a; 1996b). The patterns of the isolates were compared to the pattern of the original *L. alimentarius* strain cultured from the biopreservation solution used.

3. Results and discussion

The four *L. sake* strains produced ropy slime in the control packages without the biopreservative characteristically for each strain (Björkroth et al., 1996). The production of ropy slime by the different inoculations of *Lactobacillus sake* in aseptically packaged Frankfurters treated with the biopreservative is shown in Table 1. Parallel inoculations yielded similar slime production results. No lactic acid bacteria could be isolated from the drip of the three negative control packages. Those packages treated with biopreservate only showed growth of 1.2 to 4.9×10^{10} CFU/ml of drip. The REA (Fig. 1) of 30 isolates from the control packages showed the same pattern as the original *L. alimentarius* strain, thus confirming the vitality of the biopreservative during testing.

FloraCarn L2 has been reported as suppressing the growth of *Listeria monocytogenes*, *Brochotrix thermosphacta*, gram-negative flora and indigenous LAB producing gas or slime (Jelle 1987; 1991; Andersen, 1996). However, in this study the biopreservate did not suppress the growth of the ropy slime-producing *L. sake* strains. LAB cause slimy spoilage of meat products either by accumulating on product surfaces or by producing slimy substances. The *Lactobacillus sake* strains tested here excrete a ropy exopolysaccharide compound (Korkeala et al., 1988) and this form of slimy spoilage may be more difficult to prevent. *L. sake* has also previously been reported as being very competitive in a biopreservation test (Schillinger and Lücke, 1987) and as a very common organism in vacuum-packaged meat products (Reuter et al. 1970a; 1970b; 1975; Shaw and Harding 1984; Mäkelä et al., 1992c; Vogel et al., 1993;

Dykes and von Holy, 1994; Dykes et al., 1994), *L. sake* strains may cause considerable problems in biopreservation.

The cooking of meat products inactivates LAB (Allen and Foster, 1960; Mäkelä and Korkeala, 1987; Mäkelä et al., 1992a) and shortly after packaging LAB populations are under the normal detection limit, <10 CFU/g (Nerbrink and Borch, 1993; Björkroth and Korkeala, 1996b). According to our results (Björkroth and Korkeala, 1996b; Björkroth and Korkeala, 1997; Björkroth, 1997), LAB spoilage problems may occur during shelf life even when the product shows good microbiological quality immediately after packaging. In this study also remarkably low doses (Table 1) of ropy slime-producing *L. sake* were able to generate the spoilage changes. Exception for Type VIII and X lowest inoculation concentration, no biopreservation effect was noticed. When an inoculation study is performed in order to evaluate the effect of a biopreservative on spoilage LAB, very low spoilage strain concentrations must be tested if testing is supposed to simulate a normal production situation.

Since clean room technology demands huge material investments and post-package-heat-treatment has been shown to increase the portion of *Clostridia* and *Bacillus* in the packages (Franz and von Holy, 1996a; 1996b), biopreservation is a promising technique. However, the wide variety of both spoilage and pathogenic microbes really challenges it. Many problematic factors are known to be associated with the food matrix and bacteriocins (Schillinger and Lücke, 1991; Degnan and Luchansky, 1992; Crandall and Montville, 1993; Vandenbergh, 1993; Abee et al., 1995; Hugas et al. 1995; Holzapfel et al., 1995; Leisner et al., 1996; Stiles, 1996), and this experiment showed how difficult it may be for a biopreservative to occupy vital niches in a product. Fears have been expressed towards clean room production lowering the natural contaminant level in the product and leading perhaps to more vulnerable products. Our study showed the differences between two different LAB in a situation of competition indicating that higher LAB contamination level is not necessarily a guarantee to inhibit growth of all harmful microbes.

Despite of extensive research conducted in the field of biopreservation, many factors affecting niche occupation in different foods are still unknown. In order to search for or develop new biopreservatives, the microbial ecology of different spoilage types should be studied further.

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References

Abee, T., Krockel, L. and Hill, C. (1995) Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int. J. Food. Microbiol. 28, 169-185.

Allen, J.R. and Foster, E.M. (1960) Spoilage of vacuum-packed sliced processed meats during refrigerated storage. Food Res. 25, 19-25.

Andersen, L. (1995) Preservation of meat products with a lactic acid bacteria culture-FolraCarn L-2. abstr. p.303. In: Proceedings of the 41st Annual International Congress of Meat Science and Technology 1995, San Antonio, Texas, USA

Björkroth, J. (1997) DNA-based characterisation methods for contamination analysis of spoilage lactic acid bacteria in food processing. Thesis. University of Helsinki, Helsinki.

Björkroth, J. and Korkeala, H. (1996a) rRNA gene restriction patterns as a characterization tool for *Lactobacillus sake* strains producing ropy slime. Int. J. Food Microbiol. 30, 293-302.

Björkroth, K.J. and Korkeala, H.J. (1996b) Evaluation of *Lactobacillus sake* contamination in vacuum-packaged sliced cooked meat products by ribotyping. J. Food Prot. 59, 398-401.

Björkroth, J., Ridell, J. and Korkeala, H. (1996) Characterization of *Lactobacillus sake* strains associated with production of ropy slime by randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) patterns. Int. J. Food Microbiol. 31, 59-68.

Björkroth, K.J. and Korkeala, H.J. (1997) Use of rRNA gene restriction patterns to evaluate the lactic acid bacterium contamination of vacuum-packaged sliced cooked whole-meat product in a meat processing plant. Appl. Environ. Microbiol. 63, 448-453.

Borch, E., Nerbrink, E. and Svensson, P. (1988) Identification of major contamination sources during processing of emulsion sausage. Int. J. Food Microbiol. 7, 317-330.

Crandall, A.D. and Montville, T.J. (1993) Inhibition of *Clostridium botulinum* growth and toxigenesis in a model gravy system by coinoculation with bacteriocin-producing lactic acid bacteria. J. Food Prot. 56, 485-488.

Degnan, A.J. and Luchansky, J.B. (1992) Infuence of beef tallow and muscle on the antilisterial activity of pediocin AcH and liposome-encapsulated pediocin AcH. J. Food Prot. 7, 552-554.

Delves-Broughton, J. (1990) Nisin and its uses as a food preservative. Food Technol. 44, 100-117.

Dykes, G.A. and von Holy, A. (1994) Taxonomic status of atypical *Lactobacillus sake* and *Lactobacillus curvatus* strains associated with vacuum-packaged meat spoilage. Curr. Microbiol. 28, 197-200.

Dykes, G.A., Britz, T.J. and von Holy, A. (1994a) Numerical taxonomy and identification of lactic acid bacteria from spoiled, vacuum-packaged Vienna sausages. J. Appl. Bacteriol. 76, 246-252.

Dykes, G.A., Cloete, T.E. and von Holy, A. (1994b) Identification of *Leuconostoc* species associated with the spoilage of vacuum-packaged Vienna sausages by DNA-DNA hybridization. Food Microbiol. 11, 271-274.

Egan, A.F. (1983) Lactic acid bacteria of meat and meat products. Antonie van Leeuwenhoek, 49, 327-336.

Egan, A.F. and Shay, B.J. (1982) Significance of lactobacilli and film permeability in the spoilage of vacuum-packaged beef. J. Food Sci. 47, 1119-1126.

Egan, A.F, Shay, B.J. and Rogers, P.J. (1989) Factors affecting the production of hydrogen sulphide by *Lactobacillus sake* L13 growing on vacuum-packaged beef. J. Appl. Bacteriol. 67, 255-262.

Franz, C.M.A.P. and von Holy, A. (1996a) Thermotolerance of meat spoilage lactic acid bacteria and their inactivation in vacuum-packaged vienna sausages. Int. J. Food Microbiol. 29, 59-73.

Franz, C.M.A.P. and von Holy, A. (1996b) Bacterial populations associated with vacuum-packaged vienna sausages. Food Microbiol. 13, 165-174.

Holzapfel, W. H. and Gerber, E.S. (1986) Predominance of *Lactobacillus curvatus* and *Lactobacillus sake* in the spoilage association of vacuum-packaged meat products, abstr., p. 26. In: Abstracts of the 32nd European Meeting of Meat Research Workers 1986, Ghent.

Holzapfel, W.H., Geisen, R. and Schillinger, U. (1995) Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. Food Microbiol.24, 343-326.

Hugas, M., Garriga, M., Aymerich, M.T. and Monfort, J.M (1995) Inhibition of *Listeria* in dry fermented sausages by the bacteriocinogenic *Lactobacillus sake* CTC494. J. Appl. Bacteriol. 79, 322-330.

Jelle, B. (1987) The preserving effect of lactobacilli on vacuum-packaged beef. Master thesis. Royal Veterinary & Agricultural University, Copenhagen.

Jelle B. (1991) Biopreservation of sliced meat products. Chr. Hansen, Hørsholm, Denmark.

Korkeala, H.J. and Björkroth, K.J. (1997) Spoilage and contamination of vacuum-packaged cooked sausages: A review. J. Food Prot. 60, 724-731.

Korkeala, H., Lindroth, S., Ahvenainen, R. and Alanko, T. (1987) Interrelationship between different parameters in the spoilage of vacuum-packed cooked ring sausages. Int. J. Food Microbiol. 5, 311-321.

Korkeala, H., Suortti, T. and Mäkelä, P. (1988) Ropy slime formation in vacuum-packed cooked meat products caused by homofermentative lactobacilli and a *Leuconostoc* species. Int. J. Food Microbiol. 7, 339-347.

Leisner, J.J., Greer, G.G. and Stiles, M.E. (1996) Control of beef spoilage by sulfide producing *Lactobacillus sake* strain with bacteriocinogenic *Leuconostoc gelidum* UAL187 during anaerobic storage at 2°C. Appl. Environ. Microbiol. 62, 2610-2614.

Mäkelä, P. and Korkeala, H. (1987) *Lactobacillus* contamination of cooked ring sausages at sausage processing plants. Int. J. Food Microbiol. 5, 323-330.

Mäkelä, P.M., Korkeala, H.J. and Laine, J.J. (1992a) Survival of ropy slime-producing lactic acid bacteria in heat processes used in the meat industry. Meat Sci. 31, 463-471.

Mäkelä, P.M., Korkeala, H.J. and Laine. J.J. (1992b) Ropy slime-producing lactic acid bacteria contamination at meat processing plants. Int. J. Food Microbiol. 17, 27-35.

Mäkelä, P., Schillinger, U., Korkeala, H. and Holzapfel, W.H. (1992c) Classification of ropy slime-producing lactic acid bacteria based on DNA-DNA homology, and identification of *Lactobacillus sake* and *Leuconostoc amelibiosum* as dominant spoilage organisms in meat products. Int. J. Food Microbiol. 16, 167-172.

Milbourne, K. (1983) Thermal tolerance of *Lactobacillus viridescens* in ham. Meat Sci. 9, 113-119.

Nerbrink, E. and Borch, E. (1993) Evaluation of bacterial contamination at separate processing stages in emulsion sausage production. Int. J. Food Microbiol. 20, 37-44.

Niven, C.F.Jr. and Evans, J.B. (1957) *Lactobacillus viridescens* nov. spec., a heterofermentative species that produces a green discoloration of cured meat pigments. J. Bacteriol. 73, 758-759.

Niven, C.F.Jr., Buettner, L.G. and Evans, J.B. (1954) Thermal tolerance studies on the heterofermentative lactobacilli that cause greening of cured meat products. Appl. Microbiol. 2, 26-29.

Pierson, M.D., Collins-Thompson, D.L. and Ordal, Z.J. (1970) Microbiological, sensory and pigment changes of aerobically and anaerobically packaged beef. Food Technol. 24, 1171-1175.

Reuter, G. (1970a) Laktobazillen und eng verwandte Mikroorganismen in Fleisch und Fleischerzeugnissen. 2. Mitteilung: Die Charakterisierung der isolierten Laktobazillenstämme. Fleischwirtsch. 50, 954-962.

Reuter, G. (1970b) Laktobazillen und eng verwandte Mikroorganismen in Fleisch und Fleischwaren. 4. Mitteilung: Die Ökologie von Laktobazillen, Leuconostoc-Species und Pediokokken. Fleischwirtsch. 50, 1397-1399.

Reuter, G. (1975) Classification problems, ecology and some biochemical activities of lactobacilli of meat products. In: Carr, J.G., Cutting, C.V. and Whiting, G.C. (eds.) Lactic acid bacteria in beverages and food. Academic Press, London and New York, pp. 221-229.

Schillinger, U. and Lücke, F-K. (1987) Lactic acid bacteria on vacuum-packaged meat and their influence on shelf life. Fleiscwirtsh. 67, 1244-1248.

Schillinger, U., Kaya, M. and Lücke, F-K. (1991) Behaviour of *Listeria monocytogenes* In meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. 70, 473-478.

Shaw, B.G. and Harding, C.D. (1984) A numerical taxonomic study of lactic acid bacteria from vacuum-packaged beef, pork, lamb and bacon. J. Appl. Bacteriol. 56, 25-40.

Stiles, M.E. (1996) Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek 70, 331-335.

Vogel, R.F., Lohmann, M., Nguyen, M., Weller, A.N. and Hammes, W.P. (1993) Molecular characterization of *Lactobacillus curvatus* and *Lactobacillus sake* isolated from sauerkraut and their application in sausage fermentation. J. Appl. Bacteriol. 74, 295-300.

Vandenbergh, P.A. (1993) Lactic acid bacteria their metabolic products and interference with microbial growth. FEMS Microbiol. Rev. 12, 221-238.

Table 1.

Production of ropy slime by the different inoculations of *Lactobacillus sake* in aseptically packaged Frankfurters treated with a commercial[a] protective culture.

Strain	Genotypic characterisation[b]	Characteristic[bc] production of ropy slime	Inoculation level		Production of ropy slime in biopreservation[c]
			CFU/g	CFU/cm^2	
A 210	Group 1, Type I	+++	1.7	0.8	+++
			1.7×10^2	0.8×10^2	+++
			1.7×10^4	0.8×10^4	+++
			1.7×10^5	0.8×10^5	+++
C 1	Group 2, Type VIII	+++	1.5	0.7	++
			1.5×10^2	0.7×10^2	+++
			1.5×10^4	0.7×10^4	+++
			1.5×10^5	0.7×10^5	+++
R 51	Group 3, Type IX	+	0.7	0.3	+
			0.7×10^2	0.3×10^2	+
			0.7×10^4	0.3×10^4	+
			0.7×10^5	0.3×10^5	+
R 152	Group 4, Type X	++	0.3	0.2	-
			0.3×10^2	0.2×10^2	-
			0.3×10^4	0.2×10^4	+
			0.3×10^5	0.2×10^5	++

[a]*FloraCarn L2* (Chr. Hansen A/S, Hørsholm, Denmark) used in a concentration of approximately 10^7 CFU/cm^2 as recommended by the manufacturer.

[b]Division of different types of *L. sake* based on previous genotypic characterisation using macrorestriction patterns together with characteristic slime-producing ability (Björkroth et al., 1996).

[c]Production of ropy slime categorised from negative (-), slight (+), moderate (++) to abundant (+++) (Björkroth and Korkeala, 1996).

Fig. 1. *Eco*RI digests of chromosomal DNA. Lanes 2 to 16 randomly collected isolates from the biopreservate control packages, lane 17 original *Lactobacillus alimentarius* strain cultured from the biopreservatiom solution. Lanes 1 and 18 phage lambda DNA cleaved with *Hind*III as a fragment size marker.