

## Evaluation of *Lactobacillus sake* Contamination in Vacuum-Packaged Sliced Cooked Meat Products by Ribotyping

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### ABSTRACT

Contamination of sliced cooked meat products with a *Lactobacillus sake* starter strain was suspected to cause spoilage in the products before the end of the expected shelf life. The cooked products were sliced and vacuum packaged in the room in which a fermented product was handled. Since *L. sake* strains are known to be a dominant part of spoilage microflora associated with vacuum-packaged meat products, a contamination study was performed. One hundred and eighteen strains were isolated from six spoiled vacuum-packaged meat products and from the surfaces of the packaging room and adjacent refrigerators. DNA was isolated from these strains and cleaved using *EcoRI* and *HindIII* restriction endonucleases to obtain characteristic ribotypes. Corresponding ribotypes of the *L. sake* starter strain were compared by using *EcoRI* digestions to the 14 different patterns obtained from the strains growing in spoiled products and on surfaces. The *L. sake* starter strain was shown to contaminate the packaging room and it was also isolated from one of the products. However, it was not a dominant strain in this product and it could not be linked to the other products. Our results indicated that handling the fermented product in the refrigerating and packaging rooms together with cooked products was not the major cause of spoilage in these products.

Key words: Contamination, *Lactobacillus sake*, starter strain, vacuum-packaged, meat products (sliced and cooked), ribotyping

Sour off odors and off flavors were increasingly noticed in vacuum-packaged sliced meat products before the end of the expected shelf life, indicating lactic acid bacterial contamination during manufacturing. *Lactobacillus sake* starter strain was suspected of causing the spoilage, because a product fermented with *L. sake* was sliced and packaged in the same room as the cooked meat products. Since *Lactobacillus sake* (5, 13, 21) or bacterial groups resembling this species (8, 16) are known to be very common spoilage organisms in vacuum-packaged meat products, testing for product contamination by the *L. sake* starter strain was considered necessary. It has been shown previously that

fermented sausages can be a source of lactic acid bacterial contamination (9). Ropy slime-producing *L. sake* strains were able to multiply during the fermentation process so that the end product became a possible contamination source of these strains.

The cooking of meat products has been shown to reduce the lactic acid bacteria population to a level at which they could not be detected (3, 6, 11, 17). *Weissella viridescens* (former *L. viridescens*) is the only species reported to survive heating processes generally accepted to be adequate (2, 14, 18). Cooked meat products have been found to become contaminated with lactic acid bacteria during chilling, cold storage, slicing, and packaging (2, 3, 6, 10, 15, 17). Lactic acid bacteria have also been recovered from air and product contact surfaces in the manufacturing environment (3, 6, 10, 12, 17). The air, physical facilities, working surfaces, and workers hands are generally assumed to be the sources of lactic acid bacterial contamination. Our aim was to evaluate the contamination of the *L. sake* starter strain in the packaging room and adjacent refrigerators where the fermented product was handled as well as the cooked meat products.

Ribotyping (4) was chosen for the characterization method because it has been used successfully in typing many bacterial species, including lactic acid bacteria (20). In the present study characteristic banding patterns for the starter *L. sake* strain were obtained. These were compared to corresponding patterns of strains growing in spoiled products to see whether the starter strain formed a dominant part of the lactic acid bacteria in the spoiled products. Spread of the starter strain in the packaging room and adjacent refrigerators was also assessed.

### MATERIALS AND METHODS

#### Sampling

All the meat products studied were packaged in the same room as the fermented product. Typical sensory quality faults and elevated lactic acid bacterial counts had been observed mainly in the whole meat products packaged next to the fermented product line, with the exception of smoked cooked ham. Deteriorations in quality were not usually found in sausages packaged on the other side of the room. Samples studied were selected to contain whole

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meat products and sausages. Two packages were sampled from each set of products (see Table 1) packaged in the same line.

Six samples for lactic acid bacterial analysis were taken from the surfaces of the packaging room and adjacent refrigerators where the fermented product was stored and packaged. Sampling was performed by swabbing approximately 25-cm<sup>2</sup> areas with a sterile cotton wool swab moistened in MRS broth (Difco Laboratories, Detroit, MI, USA). The head of the swab was cut off and dropped into a tube containing MRS broth.

To obtain characteristic "fingerprints" of the *L. sake* starter strain, samples of fermented products and the lyophilized starter were also taken.

#### Isolation of lactic acid bacteria

Lactic acid bacterial populations in vacuum-packaged sliced cooked meat products were determined at 30°C on MRS-S (Oxoid, Basingstoke, England) as described by Korkeala and Lindroth (7). One of the pair of packages sampled from each packaging line was cultured immediately and the other on the last day of the expected shelf life (21 days). Until culturing the packages were stored at 5°C. Ten to twenty colonies recovered from these packages were randomly selected for DNA analysis with the exception of sausage type C, which produced only four colonies in the first dilution plated (10<sup>-2</sup>), and the three products which had no bacterial growth.

MRS broths containing cotton-wool swabs were enriched overnight at 30°C and plated on MRS-S agar to produce single colonies. Approximately 10 colonies from each sample site were analyzed further.

*L. sake* starter strain was isolated from the lyophilized ferment by transferring a loopful of the powder into MRS broth (Oxoid) which was incubated at 30°C overnight. A loopful of MRS broth was spread on MRS-S agar to provide single colonies. DNA was isolated from five of the colonies. The use of this particular starter

strain was confirmed by isolating it from the packaged product as well, as described by Korkeala and Lindroth (7).

#### Isolation of chromosomal DNA and restriction enzyme digestion

Cultures were grown overnight in 10 ml of MRS broth at 30°C. Cells were harvested from 1 to 1.5 ml by centrifuging for 2 min at full speed (about 15,000 × g) in a Biofuge A bench centrifuge (Heraeus Sephatec GmbH, Osterode am Kalkberg, FRG) to provide a pellet of approximately 15 mg (wet weight).

Chromosomal DNA was isolated according to the method of Pitcher et al. (19) with slight modifications. Cells were suspended in TE (10 mM Tris-HCl, 1 mM EDTA) solution containing 25 mg/ml lysozyme (Sigma Chemical Company, St. Louis, MO, USA) and 200 U/ml mutanolysin (Sigma). The mixture was incubated at 37°C for 1 to 2 h depending on the resistance of the cell wall.

Initially eleven restriction endonucleases (*AvrII*, *ClaI*, *EcoRI*, *HindIII*, *NarI*, *NheI*, *NotI*, *PvuI*, *RsrII*, *SacII*, *SmaI*; New England Biolabs, Beverly, MA, USA) were tested. *EcoRI* and *HindIII* were chosen for cleaving DNA, since they were found to be most revealing for characterizing *L. sake*. Following the manufacturer's instructions, 2.5 µg of DNA were cleaved with restriction endonucleases. Samples were electrophoresed overnight (25 V) in 0.8% agarose gels (SeaKem IDNA agarose, FMC, Rockland, ME, USA) in a GNA 200 apparatus (Pharmacia, Uppsala, Sweden). Digoxigenin-labeled phage lambda DNA cleaved with *HindIII* (Boehringer Mannheim GmbH, Mannheim, Federal Republic of Germany) was used as a molecular size marker.

#### Preparation of the cDNA probe, Southern transfer, and hybridization

A cDNA probe was prepared from *E. coli* 16S and 23S rRNA (Boehringer Mannheim GmbH) by reverse transcription. The probe was labeled by incorporating digoxigenin-modified dUTP (Boeh-

TABLE 1. Number of strains with different ribotypes detected from products on the last day of the shelf life and from surfaces by *EcoRI* digestions

Source	Ribotype														No. strains	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
<i>L. sake</i> from the ferment	5															5
Fermented product	4															4
Sliced meat product																
Cooked ham type A		20														20
Cooked ham type A							1				12					13
Cooked ham type B			2	8												10
Smoked cooked ham																NG <sup>a</sup>
Sausage type A																NG
Sausage type A																NG
Sausage type B	1				5	4										10
Sausage type C							4									4
Sausage type D							1	11	2	1						15
Surface																
Main refrigerator													10			10
Refrigerator of packaging room												10				10
Pillar between bacon-packaging lines	11										1					12
Pillar between lines 20 m from the bacon line	12									1				1		14
Total	33	20	2	8	5	4	6	11	2	2	13	10	10	1		127

<sup>a</sup> NG, no growth.

ringer Mannheim GmbH) using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI, USA) (1).

The digested lactic acid bacterial DNA was transferred from gels to MSI Magnagraph membranes (MSI, Westboro, MA, USA) using a VacuGene XL blotting system (Pharmacia) according to the protocol described by the manufacturer. The DNA was fixed using UV irradiation in optimal crosslink mode in a Spectrolinker XL 1000 (Spectronics Corporation, New York, NY, USA). The membranes were hybridized in a Techne Hybridizer (Techne, Cambridge, United Kingdom) at 68°C. Solutions for hybridization, washes, and development of the digoxigenin label were performed according to the instructions for DIG DNA Labelling and Detection Kit (Boehringer Mannheim GmbH).

## RESULTS

No lactic acid bacteria were found in the products immediately after packaging. From products which had sour off flavors and off odors,  $1.8 \times 10^7$  to  $6.0 \times 10^7$  colony-forming units (CFU)/g were recovered on MRS-S agar on the last day of the shelf life, with the exception of type C sausage, from which only 400 CFU/g was recovered. No lactic acid bacteria were found in type A sausages or smoked cooked ham which also had good sensory qualities. These products had been judged to be good in routine quality control analyses as well.

Table 1 shows the division of different ribotypes obtained from different sampling sites by the *EcoRI* digestions which were most distinctive. Ribotyping distinguished 11 different patterns from six different vacuum-packaged

meat products. One to three strains with different ribotypes were found in each of the packages. With the exception of sausage type B, one major strain type was found among the isolates. The ribotype of *L. sake* starter strain was found only once in sausage type B, and in the other products it could not be detected. Six samples were taken from surfaces in the production premises, but bacterial growth was observed only in 4 of the samples. From the surface sampling sites located in the packaging room, ribotypes of *L. sake* starter strain were detected on a pillar adjacent to the bacon-packaging line and a pillar between the lines, 20 m from the bacon line. *L. sake* starter strain was the most common strain detected in these samples, but strains isolated from adjacent refrigerators had different ribotypes. The diversity of the different ribotypes obtained by *EcoRI* digestions are shown in Fig. 1.

## DISCUSSION

The *L. sake* starter strain was detected in one of the spoiled products, showing that there was contamination of the product from the surroundings. However, it was not a dominant strain type in this package and it was not isolated from the other packages. Thus, contamination of the products with the *L. sake* starter strain cannot be the major cause for quality deterioration during shelf life. The variation of strain types isolated from the packages suggests that contamination takes place during many different stages of production.

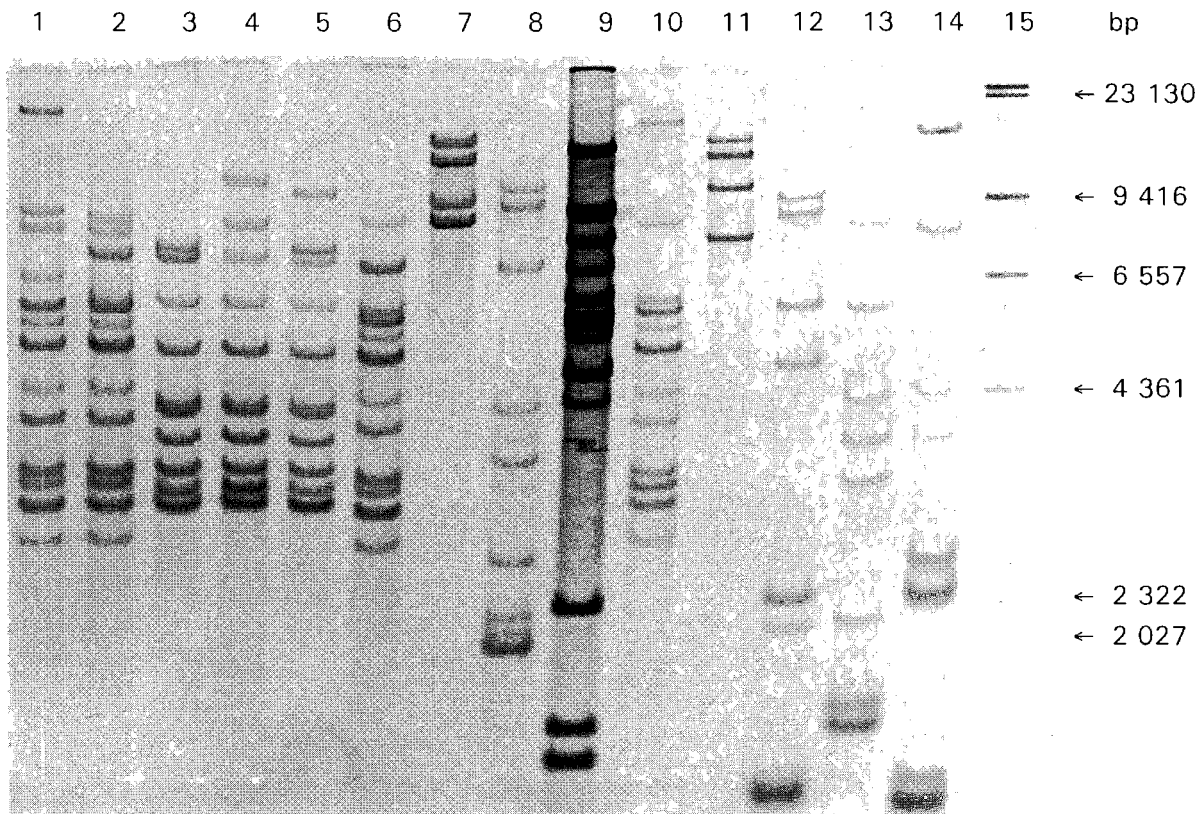


FIGURE 1. Different ribotypes obtained by *EcoRI* digestions of the strains isolated from spoiled products and surfaces. Lanes 1–14, ribotypes numbered as in Table 1; lane 15, phage lambda DNA cleaved with *HindIII* as a fragment size marker.

Analysis of the surface flora of the packaging room showed clearly that contamination of a large area with the *L. sake* starter strain had occurred. If starter strains used in meat fermentation are capable of growing in vacuum-packaged cooked meat products, handling of fermented products in the same packaging room as cooked products may cause spoilage problems. Since lactic acid bacteria are known to be important spoilage organisms in vacuum-packaged cooked meat products, it is advisable to avoid packaging fermented products and cooked products in the same packaging room if the spoilage potentials of starter strains are unknown. Previously it has been reported that fermented products can be a source of *L. sake* strains causing slime formation in vacuum-packaged cooked meat products (9) but to the authors' knowledge starter strains have not been reported to cause spoilage in cooked meat.

Refrigerators have been reported to be one of the major sites of lactic acid bacterial contamination (17). In this study we could not show spreading of the *L. sake* starter strain in the two refrigerators where the fermented bacon was stored before and after packaging. Strains we obtained from refrigerators could not be linked to strains isolated from the products. However, surface samples of lactic acid bacterial populations were enriched because of the small number of cells on the surfaces. During enrichment, some of the species capable of growing in vacuum packages may be lost due to overgrowth of more competitive strains. For the comprehensive assessment of the effect of cold storage, more samples should be analyzed.

Quantitative methods have not proved to be very useful tools for lactic acid bacterial contamination analyses. The products usually contain so few lactic acid bacterial strains after packaging that the spoilage rate during the shelf life cannot be predicted by traditional enumeration procedures (17). The "clean areas" after cooking also harbor so few lactic acid bacteria that detection of contamination sites based on enumeration of microorganisms is usually impossible (3, 6, 12, 17). Ribotyping as a method seems to be a useful tool for qualitative lactic acid bacteria contamination analyses. With this method we can distinguish between different strains and recognize probable clones. By "fingerprinting" the strains, we can answer specific questions concerning contamination sites. When ribotyping is combined with techniques suitable for isolating small numbers of lactic acid bacteria (10), the effect of surface flora in various production areas on product contamination could also be analyzed reliably.

Since the starter strain could not be recognized as a major contaminant causing this spoilage problem, a comprehensive analysis of other contamination sites is needed in order to reduce the risk of contamination and ensure that the quality of the products is maintained during the expected shelf life.

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