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Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas-packaged meat

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Abstract

Contamination of cooked meat products with *Listeria monocytogenes* poses a constant threat to the meat industry. The aim of this study was therefore to investigate the use of indigenous lactic acid bacteria (LAB) as protective cultures in cooked meat products. Cooked, sliced, vacuum- or gas-packaged ham and servelat sausage from nine meat factories in Norway were inoculated with 10^3 cfu/g of a mixture of three rifampicin resistant (*rif*-mutant) strains of *L. monocytogenes* and stored at 8°C for four weeks. Growth of *L. monocytogenes* and indigenous lactic acid flora was followed throughout the storage period. LAB were isolated from samples where *L. monocytogenes* failed to grow. Five different strains growing well at 3°C, pH 6.2, with 3% NaCl, and producing moderate amounts of acid were selected for challenge experiments with the *rif*-resistant strains of *L. monocytogenes,* a nalidixic acid/streptomycin sulphate-resistant strain of *Escherichia coli* O157:H7 and a mixture of three *rif*-resistant strains of *Yersinia enterocolitica* O:3. All five LAB strains inhibited growth of both *L. monocytogenes* and *E. coli* O157:H7. No inhibition of *Y. enterocolitica* O:3 was observed. A professional taste panel evaluated cooked, sliced, vacuum-packaged ham inoculated with each of the five test strains after storage for 21 days at 8°C. All samples had acceptable sensory properties. The five LAB strains hybridised to a 23S rRNA oligonucleotide probe specific for *Lactobacillus sakei*. These indigenous LAB may be used as protective cultures to inhibit growth of *L. monocytogenes* and *E. coli* O157:H7 in cooked meat products.

Keywords: Biopreservation; Protective cultures; *Listeria monocytogenes*; *Escherichia coli* O157: H7; *Yersinia enterocolitica* O:3; Lactic acid bacteria; Indigenous flora; Cooked meat

1. Introduction

In 1992, six cases (one fatal) of listeriosis due to *Listeria monocytogenes* serotype 1/2 following consumption of cooked sliced meat products were reported in Norway (Nesbakken, 1995) and 297 cases (63 fatal) in France were caused by *L. monocytogenes* serotype 4b from pork tongue in aspic (Goulet et al., 1993). In Australia, *L. monocytogenes* was found in 46% of 175 samples of vacuum-packaged processed meats purchased from retail stores (Grau and Vanderlinde, 1992). In other coun-

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tries incidences of *L. monocytogenes* ranging from 10 to 50% have been found in ready-to-eat meats (Schmidt and Kaya, 1990). More than 10^5 cfu/g of *L. monocytogenes* has been observed in sliced vacuum-packed frankfurter-type sausage (Schmidt, 1995).

Vacuum or gas-packed cooked sliced meat products in Norway have pH values of approximately 6.2, salt contents from 2.5 to 3% (in water phase) and water activities of 0.97 or higher. Thus these products have little or no inherent stability against the growth of *L. monocytogenes* even under proper refrigeration (ICMSF, 1996, Blom et al., 1997). *L. monocytogenes* is well adapted to survival on equipment and in production facilities, and the occurrence of *L. monocytogenes* in cooked meat products is found to be connected with cross contamination after heat treatment (Nesbakken, 1995). Packages containing low numbers of *L. monocytogenes* may be found to contain high numbers of the organism towards the end of shelf life, particularly in cases of temperature abuse (Nesbakken, 1995). Spoilage may not be evident to the consumer even in the presence of high numbers of pathogens (Beumer et al., 1996). It is therefore essential, but in practice difficult, to ensure well-controlled chill holding and storage systems during production, storage, distribution and sale. A well-implemented HACCP-system reduces possibilities of cross contamination, but is insufficient to completely prevent the incidence of *L. monocytogenes* in the finished products. Further efforts are therefore required to prevent growth during shelf life particularly under conditions of temperature abuse (Nesbakken, 1995).

Effective inhibition of *L. monocytogenes* in vacuum-packaged meat products may be obtained using permitted food additives, e.g., sodium lactate (Unda et al., 1991; Qvist et al., 1994) or a combination of sodium lactate and sodium acetate (Blom et al., 1997). The latter authors found that a combination of 2.5% (w/v) lactate and 0.25% (w/v) acetate prevented growth of *L. monocytogenes* in sliced servelat sausage and cooked ham when stored at 4°C. However, a consumer acceptance trial showed preference for standard servelat and a need for adjustment of the taste of the servelat sausage on addition of the acids. In addition, the authors emphasised the need for strict temperature control during production and storage.

An alternative method for inhibition of *L. monocytogenes* and other undesired organisms is the use of a protective microflora, usually lactic acid bacteria (LAB) to extend storage life and increase safety of food products. Unlike starter cultures, protective cultures should alter the sensory properties of the product as little as possible (Schillinger and Lücke, 1990). LAB are considered as 'food-grade' organisms being safe to consume (Adams and Marteau, 1995; Sameshima et al., 1998) and have a long history of use in food. During storage, LAB naturally dominate the microflora of many foods including raw meats and fish that are chill stored under vacuum or in an environment with elevated CO_2 concentration (Holzapfel et al., 1995; Stiles, 1996). Food-grade safety, adaptation and suitability for a specific food determine eventual use of such cultures (Holzapfel et al., 1995).

The purpose of this study was therefore to isolate indigenous strains of lactic acid bacteria, which inhibit the growth of *L. monocytogenes* in Norwegian vacuum- or gas-packed sliced meat products during shelf life, and evaluate their suitability as protective cultures. In addition, it was of interest to test the effect of selected strains on growth of *Escherichia coli* O157:H7 and of *Yersinia enterocolitica* O:3 in cooked vacuum-packaged ham. *E. coli* O157:H7 is an emerging food pathogen capable of growth at ≤ 10°C (Buchanan and Doyle, 1997). *Y. enterocolitica* grows at refrigeration temperatures of 0–4°C (Nesbakken and Kapperud, 1985). Cross contamination of vacuum- or gas-packaged meat with either of these bacteria could therefore have serious consequences in these products which have few, if any, inherent hurdles and which may support growth of pathogens under abuse conditions. In order to simulate abuse conditions, the storage temperatures used in the experiments were 8°C for *L. monocytogenes* and *Y. enterocolitica* and 10°C for *E. coli* O157:H7.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Wild-type and rifampicin-resistant mutants (*rif* mutants) of three different strains of *L. monocytogenes* (strain 2230/92 serotype 1, strain 169, serotype 4b, strain 187, serotype 4b) were the same cultures as were used and described by Blom et al.

(1997). Primary stock cultures of both wild type and mutant strains were maintained at −80°C in Bacto Brain Heart Infusion broth (BHI, Difco Laboratories, Detroit, Michigan, USA) containing 20% (v/v) glycerol (Bacto Glycerol, Difco). Working stock cultures were prepared by incubating overnight in BHI at 37°C without shaking. Plate counts of *rif* mutants were performed on blood agar (Bacto Blood Agar Base, Difco) with 5% sterile defibrinated horse blood, with 100 μg/ml rifampicin (R-3501, Sigma Chemical Co, St. Louis, USA). Haemolytic colonies on blood agar were identified as *L. monocytogenes*. Inoculum was prepared by mixing equal volumes of the three strains. *E. coli* O157: H7 NCTC 1200, a non-toxigenic and nalidixic acid streptomycin (NAS) resistant strain was used in challenge experiments. Primary stock cultures were maintained at −80°C in tryptone soya yeast extract (TSY) broth (CM 129, Oxoid Ltd., Basingstoke, Hampshire, UK) with 0.1% yeast extract (L21, Oxoid) containing 20% (v/v) glycerol. Working stock cultures were prepared by incubating overnight in TSY at 37°C without shaking. Plate counts of NAS-resistant mutants were performed on Blood agar with 50 μg/ml nalidixic acid (N-4382, Sigma) and 1000 μg/ml streptomycin sulphate (S-6501, Sigma). *Rif*-resistant mutants of three different strains of *Y. enterocolitica* serotype O:3 were obtained according to Foegeding et al., 1992. Primary stock cultures of both wild type and *rif*-resistant mutant strains were maintained at −80°C in TSY broth with 20% (v/v) glycerol. Working stock cultures were prepared by incubating overnight in TSY at 30°C without shaking. Plate counts of *rif* mutants were performed on blood agar with 100 μg/ml rifampicin. Growth rates of all *rif*-resistant mutants were comparable with those of the wild type strains when examined on the Bioscreen C instrument. Inoculum was prepared by mixing equal volumes of the three strains. MRS (de Man, Rogosa, Sharpe) medium (CM 359, Oxoid) pH 6.2 was used for growth and maintenance of LAB. Colony counts were made on MRS agar. Mesophilic aerobic bacterial counts were made on plate count agar (PCA, Difco) incubated at 30°C. Peptone saline, 0.9% NaCl, 0.1% peptone, (L37, Oxoid) was used as diluent for plate counts. Prior to inoculation, bacterial suspensions were washed twice, centrifuged and resuspended in saline (0.9% NaCl). Detection of antagonistic activity using an agar spot test and a well diffusion assay was done using a modification of the method described by Schillinger and Lücke (1989). MRS agar, which contains 2% glucose (MRS-2 agar), was used for an agar spot test for screening of antagonistic effects. MRS agar containing only 0.2% glucose (MRS-0.2 agar) was used for an agar well diffusion assay for detection of antibacterial substances in the culture medium. A cell-free suspension was obtained by filtering the culture through a 0.2 μm-pore size Millex-GS filter (SLGS 025 BS, Millipore, Bedford, MA 01730, USA). The supernatant was adjusted to pH 6.5. Inhibitory activity from H_2O_2 was prevented by the addition of 5 mg/ml catalase (C-9322, Sigma). The API 50 CH system (BioMérieux, France) was used for identification and discrimination of LAB strains. Total bacterial DNA was isolated from cultures grown overnight in MRS medium and subjected to randomly amplified polymorphic DNA (RAPD) PCR analysis essentially as described by Johansson et al. (1995). The RAPD-PCR profiles obtained were compared with those of validly described species of the genus *Lactobacillus*. Species determination by hybridization of DNA with oligonucleotide probes (15–20 bp) specific for *Lactobacillus sakei* were carried out as described by Nissen and Dainty (1995). LAB strains were stored in MRS broth containing 20% (v/v) glycerol at −80°C.

2.2. Growth rates in liquid media

Media were inoculated with $\sim 10^7$ cells/ml. Aliquots (400 μl) were dispensed into microtitre plate wells. Absorbance at 580 nm was measured every 2 h and growth estimated by calculation of the area under the growth curve using a Bioscreen C instrument fitted with Biolink software (Labsystems Co., Helsinki, Finland). Growth rates of *L. monocytogenes* and LAB strains were compared at 3, 5 and 8°C with and without 2.5% NaCl added to the medium. The initial pH of the medium was adjusted to 6.2.

2.3. Isolation and screening of lab strains with inhibitory effect

Freshly made cooked, sliced, vacuum- or gas-packaged (two of the nine factories) ham and servelat sausage (a Norwegian non-fermented cooked meat sausage) produced according to current commercial recipes, were received on ice from 9, geo-

graphically widely distributed, meat plants in Norway. Each package contained three slices of approximately 10 g. The packages were inoculated by injecting 0.1 ml of a cocktail of the three *rif* mutants of *L. monocytogenes,* giving approximately 10^3 cfu/g, through gas probe sealing tape (Toray Engineering Co. Ltd., England) and stored at 8°C for four weeks. A growth of *L. monocytogenes*, the total number of aerobic and lactic acid bacteria and changes in pH were followed throughout the storage period. Samples were analysed after 0, 3, 14 and 28 days. Bacterial counts were determined using two parallel packages for each sampling point.

2.4. Testing five isolated LAB strains against L. monocytogenes, E. coli O157:H7 and Y. enterocolitica O:3

In this experiment, cooked ham was received from only two of the nine factories and was vacuum-packed in the laboratory. The ham, produced according to current recipes, was cooked whole in its casing to a core-temperature of 72°C for 3 min, cooled immediately to 2°C, and stored on ice until used. The casing was removed and the ham was sliced manually under sterile conditions. Slices of ~30 g were vacuum-packed and stored in the refrigerator until the following day. Packages were inoculated with the five LAB test strains (one strain per package) at 10^4 cfu/g and 10^6 cfu/g, respectively and 10^3 cfu/g of the *L. monocytogenes* cocktail as described above. Controls were made with 10^4 and 10^6 cfu of the LAB omitting *L. monocytogenes* and with *L. monocytogenes* omitting LAB. Growth and pH changes were measured after 0, 3, 7, 14 and 28 days as described. As a control, LAB colonies were picked from the MRS plates from the inoculated samples after storage for 28 days at 8°C. The carbohydrate fermentation profiles of the isolated colonies were analysed using the API 50 CH system and compared with the profiles from the original strains.

In a similar experiment, 10^2 –10^3 cfu/g of the *E. coli* O157 NAS mutant strain was inoculated into cooked, sliced and vacuum-packaged ham from one factory and tested against 10^5 cfu/g of each of the

Table 1
Sensory evaluation of cooked ham with different strains of lactic acid bacteria

Sensory character	Strain designation					
	37	53	62	71	TH1	Control
Intensity of odour	6.31a[a]	6.40a	5.96ab	6.13a	6.04a	5.23b
Off-odour	2.50ab	2.68a	2.02ab	2.30ab	2.50ab	1.49b
Meat odour	3.17c	2.96c	3.34bc	3.43bc	3.62abc	4.79a
Acidic odour	2.74bc	2.23c	2.87bc	2.73bc	3.46abc	4.62a
Sour odour	4.26a	4.75a	4.11ab	3.76ab	3.10abc	1.36c
Metallic odour	3.82	3.89	4.11	3.43	3.90	3.66
Whiteness	5.74	5.30	5.36	2.52	5.66	5.43
Hue	4.67b	5.43a	5.02ab	4.91ab	4.62b	4.72b
Intensity of colour	3.91b	4.66a	4.44ab	4.24ab	3.99b	4.34ab
Intensity of flavour	6.41a	6.46a	6.26a	6.11ab	6.08ab	5.51b
Off-flavour	2.62	2.67	2.35	2.51	2.51	1.79
Meat flavour	3.77b	3.61b	4.09ab	4.02b	4.19ab	5.17a
Acidic flavour	3.18ab	2.74b	3.21ab	3.36ab	3.52ab	4.69a
Sour flavour	3.52ab	3.97a	3.82a	3.44ab	2.94abc	1.34c
Salty flavour	5.67a	5.69a	5.47a	5.68a	5.64a	5.16a
Metallic flavour	3.59ab	3.96a	3.84a	3.57ab	3.88a	3.19b
Bitter flavour	3.99a	4.23a	3.97a	3.73ab	3.87ab	3.19b
Rancid flavour	1.52	1.62	1.69	1.42	1.61	1.06
Hardness	4.62ab	4.83ab	5.04a	4.93ab	4.79ab	4.52b
Tenderness	5.93	5.73	5.51	5.81	5.96	6.14
Juiciness	5.31	5.42	5.35	5.04	5.33	5.30
Stickiness	1.42	1.48	1.47	1.51	1.46	1.47

[a] Samples with the same letter are not significantly different ($p > 0.05$).

five test LAB strains. The packages were incubated at 10°C for 28 days. Likewise 10^4 cfu/g *rif*-mutant *Yersinia enterocolitica* were tested against each of the five LAB strains at 8°C for 28 days. Microbiological data were converted to logarithms after obtaining the means of two parallel samples. Results were subjected to analysis of variance (ANOVA).

2.4.1. Sensory evaluation of ham inoculated with five test strains LAB

Ham was cooked whole in its casing as described, sliced, and vacuum-packaged. Packages were inoculated with 10^6 cfu/g of the five test lactic acid cultures and stored at 8°C for 21 days. Growth of added LAB strains and indigenous LAB in control packages as well as pH values, were registered after 0, 3, and 21 days. At the end of this period, the inoculated ham was evaluated and compared to control samples without added culture. The analyses were carried out in compliance with ISO 6564-1985, in a sensory laboratory fulfilling international standard requirements with a professional sensory panel of nine persons. Samples were evaluated according to the intensity of the following attributes: odour, colour, flavour and texture (Table 1). The results of the sensory evaluation were registered in a specially constructed system from CSA Compusense, Canada and converted to numerical values from 1.0 = no intensity to 9.0 = strong intensity. Colours were given numerical values from 1.0 = yellowish-red to 9.0 red. These numerical values were subjected to statistical analysis using the data programme STATISTIX 4.0. Significant differences at the 5% level were subjected to 'Tukey's pairwise comparisons of means-test'.

3. Results

3.1. Isolation and screening of antilisterial LAB strains

The level of indigenous LAB on newly prepared cooked, sliced, vacuum- and gas-packaged ham varied from 10^2–10^3 to 10^5–10^6 cfu/g. Numbers on the servelat sausage were from 10^3–10^4 to 10^6–10^8 cfu/g. When *L. monocytogenes* was added to cooked ham, the pathogen failed to grow on hams from six of the nine meat plants. One of these plants used gas-packaging. One example is shown in Fig. 1a. An example of a sample where *L. monocytogenes* had increased to $>10^7$ cfu/g after 28 days at 8°C is given in Fig. 1b. Likewise, on servelat sausage, *L. monocytogenes* failed to grow on samples from three of the factories, of which two used gas-packaging (not shown). Inhibition of growth of *L. monocytogenes* occurred together with rapid growth of the LAB to 10^7–10^8 cfu/g. The mesophilic aerobic

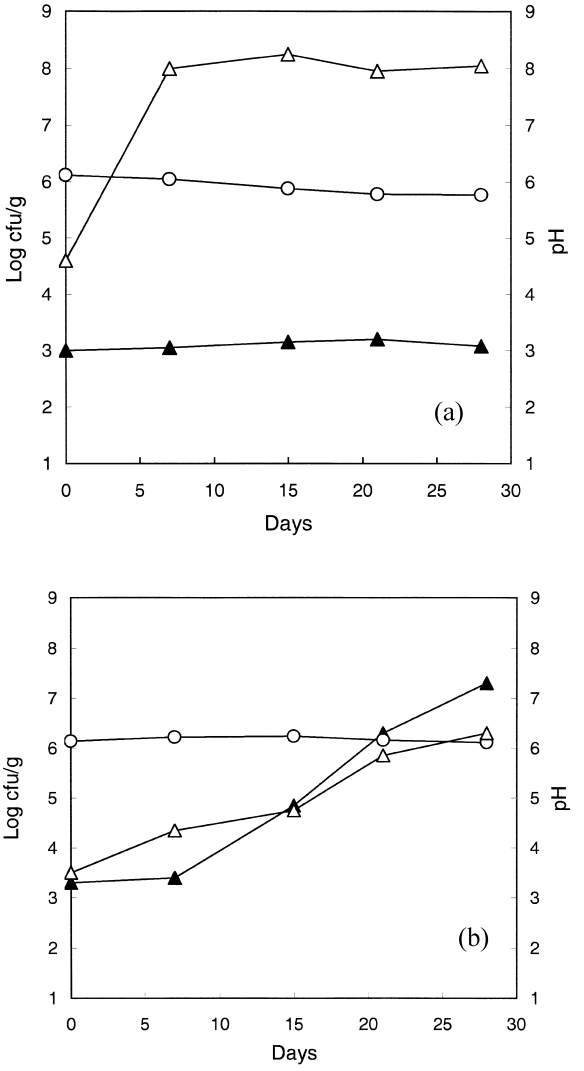

Fig. 1. (a) Inhibition of *L. monocytogenes* by indigenous LAB in cooked, sliced, vacuum-packaged ham at 8°C. ▲ = *L. monocytogenes*, △ = LAB, ○ = pH; (b) growth of *L. monocytogenes* on cooked, sliced, vacuum-packaged ham at 8°C. ▲ = *L. monocytogenes*, △ = LAB, ○ = pH.

counts were of the same order as the number of LAB. Colonies on PCA were mainly LAB. After 4 weeks, the pH of the ham varied from 6.3–5.8 and from 6.2–4.7 in the servelat sausage.

Seventy Gram-positive, catalase-negative strains, selected from MRS agar from samples where *L. monocytogenes* failed to grow after 28 days at 8°C, were examined for production of gas from glucose, and lowering of pH in MRS broth. Non-gas-producing strains were examined for their ability to grow at 3°C, at pH 6.2 and pH 5.8 and with 3% NaCl added to the medium. Five strains were selected for closer examination. The five strains could be distinguished by differences in their API fermentation patterns. Comparison of RAPD-PCR gel profiles showed slight differences between strains, but largely similar to those of known strains of *L. sakei*. The strains also hybridised with *L. sakei* specific nucleotides.

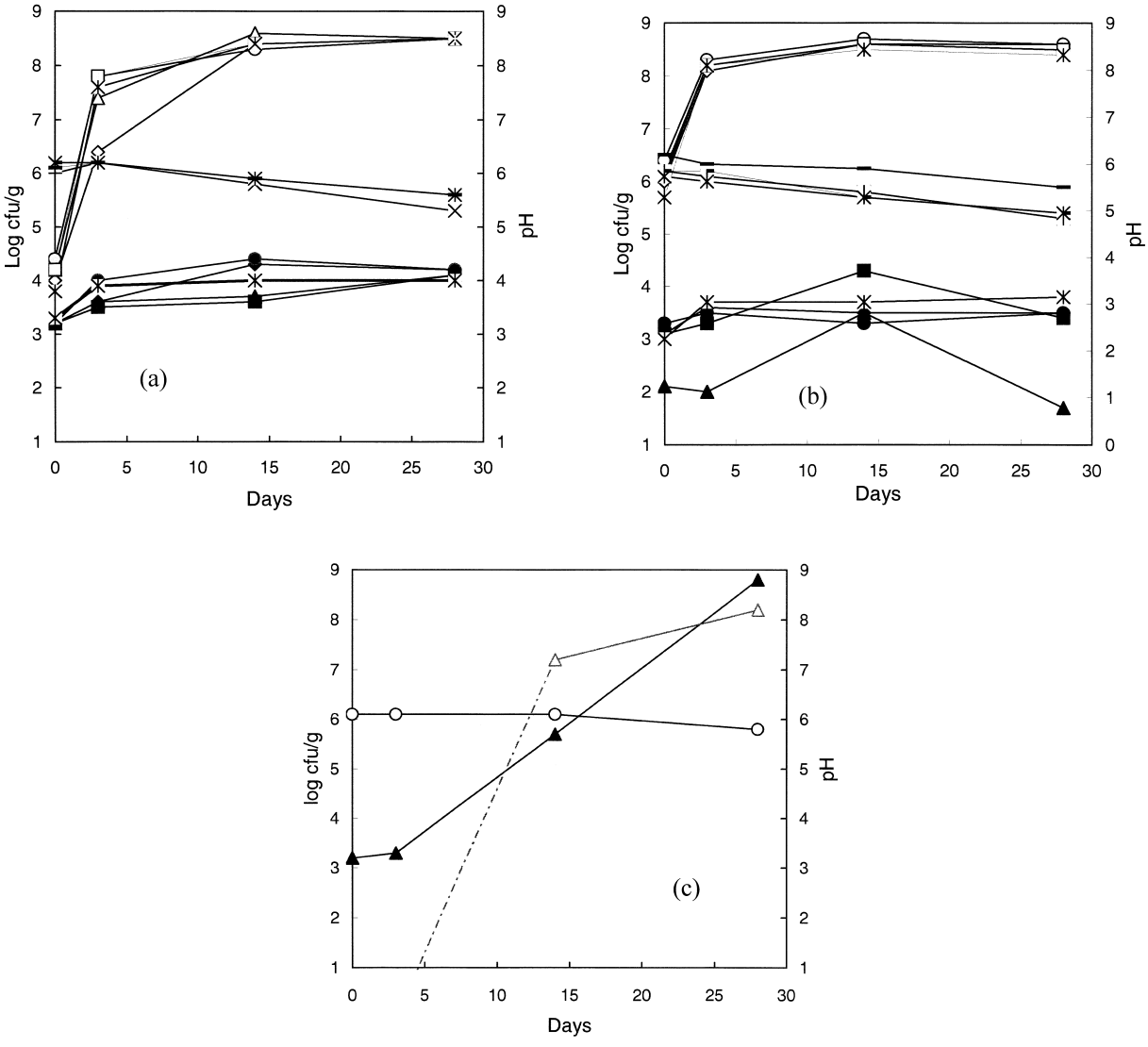

Fig. 2. (a) Inhibition of *L. monocytogenes* on cooked, sliced, vacuum-packaged ham at 8°C by addition of 10^4 cfu/g of five selected LAB strains. Filled symbols = *L. monocytogenes*. Open symbols = LAB strains. pH profiles are shown; (b) Inhibition of *L. monocytogenes* on cooked, sliced, vacuum-packaged ham at 8°C by addition of 10^6 cfu/g of five selected LAB strains. Filled symbols = *L. monocytogenes*, open symbols = LAB strains. pH profiles are shown; (c) Growth of *L. monocytogenes* on cooked, sliced, vacuum-packaged ham at 8°C without added LAB. ▲ = *L. monocytogenes*, △ = LAB, ○ = pH.

3.2. Inhibition of l. monocytogenes by lab strains

Cooked, sliced, vacuum-packaged ham inoculated with *L. monocytogenes,* and each of the 10^4cfu/g or 10^6 cfu/g of the five selected LAB strains was stored for 28 days at 8°C. After three days, the LAB had increased in number from 10^4 cfu/g to 10^8–10^9 cfu/g (Fig. 2a) and from 10^6 cfu/g to 10^8–10^9 cfu/g (Fig. 2b). The difference in added numbers of LAB at the start had little or no influence on the inhibitory effect of the five strains. In the control packages with no added LAB, *L. monocytogenes* grew to 10^8–10^9 cfu/g within 28 days (Fig. 2c); the numbers of indigenous LAB bacteria were initially below the detection limit (<100 cfu/g), but increased to 10^7–10^8 cfu/g after two weeks and to 10^8–10^9 cfu/g after four weeks. Growth of the LAB added to control packages without *L. monocytogenes* was comparable to growth in the samples with *L. monocytogenes* (not shown).

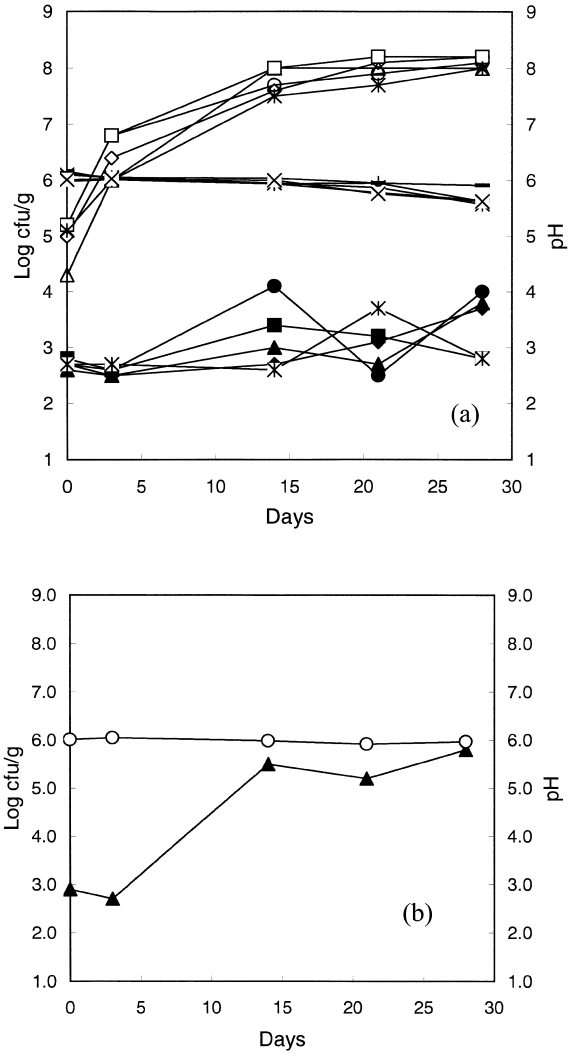

Fig. 3. (a) Inhibition of *E. coli* O157 on cooked, sliced, vacuum-packaged ham at 10°C by addition of 10^4-10^5 cfu/g of selected LAB strains. Filled symbols = *E. coli* O157:H7, open symbols = LAB strains. pH profiles are shown; (b) Growth of *E. coli* O157 on cooked, sliced, vacuum-packaged ham at 10°C without added LAB. ▲ = *E. coli* O157:H7, ○ = pH.

3.3. Inhibition of E. coli O157 H:7 by LAB strains

Addition of 10^4–10^5 cfu/g LAB also had an inhibitory effect on the growth of 10^3 cfu/g *E. coli* O157 H:7 in cooked ham stored at 10°C for four weeks. After 14 days statistically significant differences ($p < 0.05$) were observed between the levels of *E. coli* O157 H:7 in the packages to which LAB were added (Fig. 3a), compared to control packages without LAB (Fig. 3b). No significant growth of *E. coli* O157:H7 was observed upon prolonged storage after 14 days at 10°C in the samples with added LAB. In the absence of added LAB, *E. coli* O157 increased in numbers to 10^{5-6} cfu/g. The numbers of indigenous LAB remained below the detection level (<100/g) in the *E. coli* O157:H7 control samples throughout the experiment.

3.4. Growth of Y. enterocolitica O:3 in cooked ham

Addition of 10^4–10^5 cfu/g of the five LAB strains did not inhibit growth of *Y. enterocolitica* on cooked sliced vacuum-packaged ham stored for 28 days at 8°C (Fig. 4). *Y. enterocolitica* increased in numbers from 10^4 cfu/g at start to 10^{6-8} cfu/g at the end of the storage period. All five LAB strains increased to 10^8 cfu/g during the storage period. In the control samples, the numbers of indigenous LAB were 10^2 cfu/g initially and 10^5 cfu/g at the end of storage.

3.5. Sensory evaluation of ham inoculated with five selected strains LAB

The results of the sensory evaluation are given in Table 1. On comparison with the control packages, all of the inoculated packages after storage for 21 days at 8°C were found to be acceptable and the statistical differences were small. A principal com-

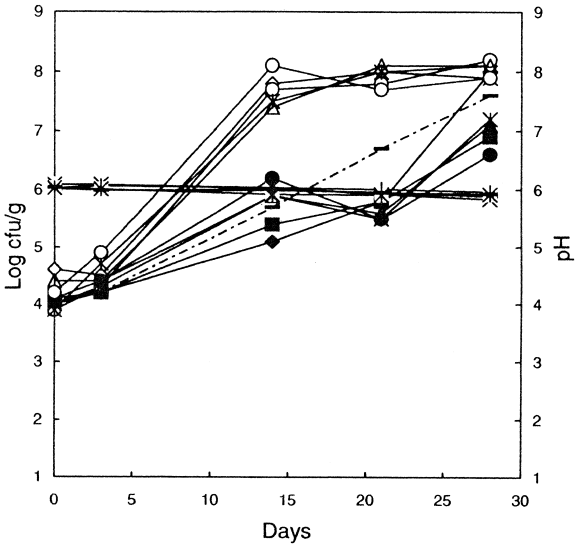

Fig. 4. Growth of *Y. enterocolitica* on cooked, sliced, vacuum-packaged ham at 8°C in the presence of added LAB. Filled symbols = *Y. enterocolitica,* open symbols = LAB strains. pH profiles are shown. The dotted line shows growth of *Y. enterocolitica* in cooked ham without added LAB.

ponent analysis (PCA) showed the characters 'sour' and 'acidic' to be the most distinguishable characters for aroma and taste but the intensities of these characters were all < 5.0. Ham inoculated with strains 53, 62, 71 and TH1 had a slightly more sour taste than the control. Ham inoculated with strains 37, 53, 62 and 71 had a slightly more sour smell than the control. The control was slightly more acidic than the ham with the test strains. Ham samples inoculated with strains 37, 53 and 62 were slightly more bitter than the control.

4. Discussion

L. monocytogenes has a long generation time at refrigeration temperatures, however significant growth can occur during the shelf life of vacuum-packaged products, even when significantly outnumbered by the background flora (Juven et al., 1998; Grau and Vanderlinde, 1992; Schmidt, 1995). During experiments with additives, Blom et al. (1997) observed that *L. monocytogenes* failed to grow in some inoculated additive-free control samples of cooked, sliced, vacuum-packaged servelat sausage and ham. They tentatively suggested that the background flora of LAB was responsible for inhibition of *L. monocytogenes* in these samples. Schmidt (1995) made similar observations in sliced vacuum-packaged frankfurter-type sausage inoculated with both *L. monocytogenes* and a mixed LAB flora taken from a coarse mettwurst. However, after isolation from the frankfurter, the two predominating LAB strains did not, either separately or in combination, show the inhibitory effects of the total flora. Thus, there is considerable variation in the observed inhibitory effect of both the total and individual members of the natural background LAB. Potential protective cultures should therefore be carefully examined before use.

Inhibition of undesired or pathogenic microorganisms by LAB may be due to the effect of one, or synergism between, several mechanisms. Competition for nutrients, lowering of pH, production of lactic acid, acetic acid, hydrogen peroxide or other antimicrobial substances such as bacteriocins, are examples of such mechanisms (Schillinger and Lücke, 1990; Skyttä et al., 1991; Daeschel, 1992; Vandenbergh, 1993). The inhibitory influence of different LAB strains and of LAB mixed cultures isolated from various meat products has been investigated in liquid culture and in agar diffusion tests (Schillinger and Lücke, 1989, 1990). In addition, investigations have been carried out on various meat products (Andersen, 1995; Juven et al., 1998; Schillinger and Lücke, 1989, 1990; Schillinger et al., 1991, Schmidt, 1995; Skyttä et al., 1991). In our experiments, the one bacteriocin-producing strain (no. 53) did not differ in inhibitory effect from the other four strains. The observed inhibition of *L. monocytogenes* does not therefore appear to be due to this bacteriocin. The reason for the observed inhibition of *L. monocytogenes* remains, as yet, to be determined. However, the LAB strains used were selected specifically for their fast growth rate in the products at temperatures ≤ 8°C. Their numbers increased rapidly within the first three days. Faster growth rates and greater competitiveness for nutrients give the LAB a selective advantage over slower growing competitors. The lowering of pH due to the metabolic activity of LAB inhibits most pathogenic bacteria (Schillinger and Lücke, 1990). Homolactic fermentation results in almost exclusively lactic acid under normal conditions and undissociated lactic acid has a bacteriostatic action (Schillinger and Lücke, 1990).

An increase in the concentration of undissociated lactic acid was suggested to be the cause of the reduction in numbers of *L. monocytogenes* in vacuum-packaged ground beef after addition of *L. alimentarius* FloraCarn L2 (Juven et al., 1998).

An inoculum of 10^4–10^5 cfu/g of the five LAB strains had an inhibitory effect on 10^3 cfu/g *E. coli* O157:H7. The storage temperature and inoculum were selected to favour growth of *E. coli* O157:H7 and it is therefore unlikely that the inoculum would be able to grow at 4°C in the presence of 10^4–10^5 cfu/g of the LAB strains. We did not, however, observe any reduction in the numbers of *E. coli* O157:H7 in the presence of the LAB. Taking into account the low infectious dose (2–2000 cells) of this pathogen (Buchanan and Doyle, 1997), it is particularly important to eliminate any risk of cross-contamination in cooked meat products, which are consumed without further heat treatment.

Some results indicate that the ability of *Y. enterocolitica* to compete with other psychrotrophic organisms normally present in foods may be poor (Schiemann, 1989; Stern et al., 1980). However, our results indicate that 10^4 cfu/g of *Y. enterocolitica* is able to grow well at 8°C in vacuum-packaged cooked ham and servelat sausage in the presence of 10^{4-5} cfu/g LAB. It is therefore important to avoid cross-contamination with this bacterium.

The sensory qualities of the product with and without added LAB were evaluated after storage at abuse temperature (8°C) for 21 days. Although rated as statistically significant, the differences in the characteristics ‘acidic’, ‘sour’ and ‘bitter’ were small and the professional sensory panel did not find any deviation from the normal acceptable quality of the product in any of the samples despite extended abnormal storage conditions. Cooked meat products should be held at a temperature of ≤ 4°C during storage and distribution. These products are normally consumed within 11–15 days. Thus there were no important sensory objections to employing these LAB strains as protective cultures in commercial cooked ham and servelat sausage.

5. Conclusions

The strains, identified as *L. sakei,* were isolated from commercial products and inhibited growth of a mixture of three strains of *L. monocytogenes* used in our experiments. These LAB strains should therefore be well adapted to growth in the products and to survival in the production facilities. With their inhibitory effect on *L. monocytogenes* and *E. coli* O157:H7, together with their almost negligible effect on the sensory quality of the products after storage at abuse temperature, the five strains are well suited for application as protective cultures in cooked vacuum-packaged ham and servelat sausage.

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