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Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products

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Abstract

In this study, 91 strains, originating from meat products, were subjected to a step-by-step screening and characterisation to search for potential protective cultures to be used in the cooked cured meat industry. Strains were first tested on their homofermentative and psychrotrophic character and salt tolerance. Secondly, the antibacterial capacities towards *Listeria monocytogenes*, *Leuconostoc mesenteroides*, *Leuconostoc carnosum* and *Brochotrix thermosphacta* were determined in an agar spot test. In total, 38% of the tested strains were inhibitory towards all indicator strains. However, 91%, 88% and 74% of the strains could inhibit, respectively, *L. monocytogenes*, *B. thermosphacta* and *Leuc. mesenteroides*. Finally, 12 strains, with the highest antibacterial capacities, were evaluated on their competitive nature by comparing their growth rate, acidifying character and lactic acid production at 7 °C under anaerobic conditions in a liquid broth. All 12 strains, except for a bacteriocin producing *Lactobacillus plantarum* strain and the lactocin S producing *Lactobacillus sakei* 148, combined a fast growth rate with a deep and rapid acidification caused by the production of high levels of lactic acid. The 12 selected strains were then further investigated for their growth capacity on a model cooked ham product to establish whether the presence of these cultures on the ham did not negatively influence the sensory properties of the ham. All strains grew in 6 days at 7 °C from a level of 10^5–10^6 to 10^7–10^8 cfu/g and again the bacteriocin producing *L. plantarum* strain was the slowest growing strain. As the glucose level of the model cooked ham product was low ($0.09 \pm 0.03\%$), growth of the putative protective cultures resulted in glucose depletion and a limited lactic acid production and accompanying pH decrease. Cooked ham inoculated with isolates 13E, 10A, 14A (all three identified as *L. sakei* subsp. *carnosus* by SDS-PAGE) and with strains *L. sakei* 148 (LS5) and *L. sakei* subsp. *carnosus* SAGA 777 (LS8) were not rejected by the sensory panel at the 34th day of the vacuum packaged storage at 7 °C. Therefore, these strains could have potential for the use as protective culture in cooked meat products.

Keywords: Biopreservation; Cooked meat products; Lactic acid bacteria; Protective cultures

1. Introduction

Cooked cured meat products are economically important refrigerated products with a high consumption in European countries. Since these products are heated to a temperature of 65–75 °C, most vegetative cells are

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killed and post-heat treatment recontamination determines their shelf life (Borch et al., 1996). Product handling after cooking plus slicing prior to packaging recontaminates these products with about 0.5–2 log cfu/g of total bacteria, mainly lactic acid bacteria (LAB) (Holley, 1997; Samelis et al., 2000). When stored anaerobically and under refrigeration, e.g. through vacuum packaging or modified atmosphere packaging (MAP), LAB will dominate the spoilage process. The metabolic activity of LAB results in spoilage appearing as sour, off-flavours, off-odours, milky exudates, slime production, swelling of the package through gas production and discolouration such as greening (Samelis et al., 2000). The spoilage flora of vacuum or MA-packaged cooked meats consist mainly of *Lactobacillus* spp., predominantly *L. sakei* and *L. curvatus*, followed by *Leuconostoc* spp., *Weisella* spp. and *Carnobacterium* spp. *Brochotrix thermosphacta* may also be a dominant part of the flora depending on the film permeability and the residual oxygen obtained through the vacuum process (Borch et al., 1996; Samelis et al., 2000). Also the psychrotrophic pathogen *Listeria monocytogenes* can be found as a result of post-contamination in cooked meat products (Uyttendaele et al., 1999).

Biopreservation has gained increasing attention as a means of naturally controlling the shelf life and safety of cooked cured meat products. Some LAB, among those commonly associated with meats, demonstrate antagonism towards pathogenic and spoilage organisms. Antagonistic cultures added to meat products to inhibit pathogens and/or prolong the shelf life, while changing the sensory properties as little as possible, are termed protective cultures (Lücke, 2000). Their antagonism refers to inhibition through competition for nutrients and/or production of one or more antimicrobially active metabolites such as organic (lactic and acetic) acids, hydrogen peroxide, antimicrobial enzymes, bacteriocins and reuterin (Holzapfel et al., 1995). Studies on biopreservation in cooked meat products have, until now, mainly focused on inhibition of food pathogens such as *L. monocytogenes* (Andersen, 1995; Hugas et al., 1998; Bredholt et al., 1999, 2001; Amezquita and Brashears, 2002; Budde et al., 2003; Mataragas et al., 2003). However, little is known about the activity of protective cultures against specific spoilage organisms of meat products. Kotzekidou and Bloukas (1996) reported 1 week extension of the shelf life of vacuum packaged sliced cooked ham stored at 4 °C when inoculated with the bioprotective culture *L. alimentarius* FloraCarn L2 and later they reported a shelf life extension of 19 days on vacuum packaged frankfurters at 6–8 °C (Kotzekidou and Bloukas, 1998). However, the same culture failed to prevent growth of ropy slime producing *L. sakei* strains leading to spoilage on frankfurters (Björkroth and Korkeala, 1997). Metaxopoulos et al. (2002) inoculated sliced cooked cured pork shoulder with the bacteriocin producing *Leuconostoc mesenteroides* L124 and *L. curvatus* L422. Results under vacuum packaging showed that in the non-inoculated samples the spoilage microflora grew but in the inoculated ones the number of *B. thermosphacta* and enterococci reduced during storage. Results from this study showed that the bacteriocins, however, did not inhibit the endogenous LAB. To our knowledge, no study has ever investigated the effect of homofermentative LAB towards heterofermentative LAB on cooked meat products. Furthermore, most studies investigate bacteriocin production, while other types of antagonism are less frequently studied. Despite the stream of promising information and laboratory studies (Budde et al., 2003; Mataragas et al., 2003), bacteriocinogenic strains often suffer from a limited effectiveness in foods by a range of factors such as a narrow activity spectrum (not active towards Gram negative bacteria), spontaneous loss of bacteriocinogenicity (genetic instability), limited diffusion in solid matrices, inactivation through proteolytic enzymes or binding to food ingredients such as lipids, poor adaptation of the culture to (refrigerated) food environments, low production level and the emergence of bacteriocin-resistant bacteria (Holzapfel et al., 1995; Rodriguez et al., 2002). An alternative to overcome these disadvantages is the use of non-bacteriocinogenic but nevertheless very competitive cultures, e.g., *L. alimentarius* BJ-33 (Andersen, 1995) and *L. sakei* TH1 isolated by Bredholt et al. (2001). One hypothesis to explain the antagonistic character of such strains is based on the production of lactic acid and the accompanying acidification causing growth arrest of bacteria (Juven et al., 1998). The full explanation is probably more complex and may be due to a combination of effects such as production of antimicrobials and competition for or depletion of specific nutrients (Devlieghere et al., 2004).

In this study, a step-by-step isolation, screening and characterization was performed to search for potential protective cultures to be used in the cooked cured meat industry. Especially LAB that are homofermentative, salt tolerant, psychrotrophic and adapted to meat substrates have a good potential to be used for bioprotection of meat products. The screening was focused on cultures, showing inhibitory activity towards *L. monocytogenes* and spoilage organisms, typically associated with cooked cured meat products as a result of post process contamination. After studying their growth and acidifying character at low temperatures, the characterization of the potential cultures was continued by inoculating them onto a model cooked ham product to find out if they were not negatively influencing the sensory properties of the cooked ham, a prerequisite to use them as protective cultures.

2. Materials and methods

2.1. Collection of relevant strains

Based on a literature study, contacts with commercial suppliers of food starter cultures and earlier research experiments in our lab, 15 lactic acid bacteria were collected for this study. The group of 15 strains consisted out of 7 strains, known to produce bacteriocins, and 8 strains assumed to be bacteriocin negative and known for their very competitive character. In this text, these cultures are further described as 'collected' strains.

2.2. Isolation and identification of isolates from meat products

Twenty-seven different types of commercial, cooked and/or fermented, vacuum packaged or MA-packaged, sliced meat products were obtained from different Belgian supermarkets and stored at 7 °C up to the use by date. On the use by date, a 30 g sample of the product was taken aseptically and a decimal dilution serial in Pepton Physiologic Salt solution (PPS; 8.5 g/l NaCl (VWR International, Leuven, Belgium) and 1 g/l peptone (Oxoid, Basingstoke, Hampshire, UK)) was prepared to spreadplate the sample on de Man Rogosa Sharpe agar (MRS, Oxoid), supplemented with 1.4 g/l of sorbic acid (Sigma-Aldrich, Steinheim, Germany) to inhibit yeast growth, and modified CHALMERS medium (Vanos and Cox, 1986) to allow isolation of LAB. The modified CHALMERS medium gives easily distinguishable colonies for LAB due to the characteristic colony type (small pink-red colonies with a light halo) and allows to distinguish the high acid producing colonies among a large population of LAB as the halo around the colony, due to $CaCO_3$ dissolution by lactic acid, is larger for strongly acidifying LAB (Vanos and Cox, 1986). Composition of the modified CHALMERS medium according to Vanos and Cox (1986): 20 g/l lactose (VWR International), 20 g/l D-(+)-glucose (Sigma-Aldrich), 3 g/l soy peptone (VWR International), 3 g/l meat extract (VWR International), 3 g/l yeast extract (Oxoid), 20 g/l $CaCO_3$ (Sigma-Aldrich), 15 g/l agar (Oxoid), 0.5 ml of 1% (w/v) neutral red (Sigma-Aldrich) solution, final pH of 6.0 before sterilization and after sterilization 3 vials/l (32,000 IU/vial) of the antibiotic polymyxin-B (International Medical Products-LabM, Brussels, Belgium) were added to reach a concentration of polymyxin-B-sulfate of 100 IU/ml of medium. After microaerophilic incubation for 72 h at 30 °C of both types of plates, five colonies were picked from both media. Attention was given to choose colonies with different macroscopic morphology and to include at least two colonies having a pronounced and large halo on the modified CHALMERS medium, indicating a high acid production. Isolates were reinoculated in MRS broth, incubated at 30 °C and checked for purity by streaking on MRS agar. Plates with pure cultures were used to test for cell morphology by phase contrast microscopy, Gram reaction by the KOH method and catalase formation by dropping a 3% H_2O_2 (VWR International) solution directly onto each plate. Gram positive and catalase negative strains were further investigated for gas production from glucose and slimy appearance on MRS agar and finally the carbohydrate fermentation profile was determined using the API50CH system (BioMERIEUX, Brussels, Belgium) to select for homofermentative LAB, not demonstrating a slimy or ropy appearance on MRS agar and not identical to each other, as indicated by the fermentation profile.

2.3. Psychrotrophic character and salt tolerance

Both, collected and isolated, strains were tested on their potential to grow in buffered modified BHI broth at low temperatures (4 and 7 °C) combined with salt concentrations occurring in the water phase of cooked meat products (3% or 6% of NaCl). The buffered modified BHI broth consisted of Brain Heart Infusion broth (BHI, Oxoid) (37 g/l) supplemented with 18 g/l D-(+)-glucose (Sigma-Aldrich), 3 g/l yeast extract (Oxoid), 4.6 g/l Na_2HPO_4 (Sigma-Aldrich), 20 mg/l $NaNO_2$ (UCB, Leuven, Belgium) and the pH was adjusted to 6.2. Devlieghere et al. (1998) demonstrated this medium to be suitable as simulation medium for cooked ham. Additional NaCl (VWR International) was added to obtain a level of 3% or 6% of NaCl, being representative NaCl concentrations for the broad spectrum of cooked meat products. All strains were inoculated at a level of 10^6–10^7 cfu/ml in 5 ml of modified BHI broth containing 3% or 6% of NaCl and stored at 4 or 7 °C. Each day, during 5 and 8 weeks at, respectively, 7 and 4 °C, growth was followed by visually examining the turbidity of the broth.

2.4. Antibacterial activity towards L. monocytogenes, Leuc. mesenteroides, Leuc. carnosum and B. thermosphacta

The strains, selected in the previous screening steps, were investigated on their antibacterial properties towards both *L. monocytogenes* and spoilage organisms, typically associated with cooked cured meats. Indicator organisms were a *L. monocytogenes* isolate from cooked ham (L.mono1), *L. monocytogenes* LMG 10470 (L.mono2) and *L. monocytogenes* Scott A (L.mono3), *Leuc. mesenteroides* subsp. *dextranicum* LMG 6908^T (L.mes3), *Leuc. carnosum* LMG 11498 (L.carn1) and a *B. thermosphacta* isolate from cooked ham (B.therm1). Antibacterial activity was assessed by an agar spot test (Juven et al., 1998). The putative protective culture was applied as a single spot of 10 μl on MRS agar and incubated at 30 °C for 24 h in micro-aerophilic conditions. After incubation, plates were covered with 7 ml of semisoft (0.7% agar) BHI or MRS agar inoculated with the indicator strain at a level of 1% (1 ml of an overnight culture in 100 ml of medium). Separate plates containing the test culture were overlaid with each of the six indicator strains and each test was performed in triplicate. After incubation for 24 h at the optimal growth temperature of the indicator strain, lawns were examined for evidence of inhibition. Based on the results of this study and the tests on the potential of the strains to grow at refrigeration temperatures (4 and 7 °C) and 3% and 6% of NaCl, a selection was made of organisms for further study. Strains with the highest antibacterial properties were further subjected to a bacteriocin assay according to Buncic et al. (1997) to find out if the antibacterial properties are the result of bacteriocin production. The putative producer strains were grown in MRS broth for 24 h at 30 °C. Two 10-μl aliquots were spotted on MRS agar containing 0.2% glucose to avoid acid production and these plates were incubated at 30 °C anaerobically to avoid H_2O_2 production. After incubation, 10 μl spots of chymotrypsin (Sigma-Aldrich) and proteinase K (Sigma-Aldrich) (0.05 g/100 ml) were brought next to one of the lactobacilli spots to inactivate any bacteriocin produced. After adding the enzyme spots, plates were held for 2 h at room temperature to allow diffusion of the enzyme before plates were overlaid with 7 ml semisoft (0.7 % agar) BHI agar, inoculated at a level of 1% with indicator strain L.mono1. Plates were incubated for 24 h at 37 °C. Bacteriocin production was indicated by a clear inhibition zone around the untreated spot and a half inhibition zone around the treated spot.

2.5. Growth, acidification profile and lactic acid production of 12 selected putative protective cultures

Twelve meat born homofermentative, salt tolerant, psychrotrophic LAB with demonstrated in-vitro antibacterial characteristics, as demonstrated in previous experiments, were selected for this experiment. Aim of this experiment was to compare the 12 putative protective cultures concerning their growth characteristics and acidifying character at 7 °C and under anaerobic conditions at a pH and salt concentration similar to cooked meat products. Therefore, these strains were inoculated at 10^5 cfu/ml in an adapted BHI broth (pH 6.2 and 3% of NaCl). Growth of the cultures was followed during storage under an atmosphere of 100% N_2 at 7 °C to

simulate refrigerated vacuum packaged conditions. The adapted BHI broth consisted of BHI (37 g/l) supplemented with 4 g/l yeast extract (Oxoid), 18 g/l D-(+)-glucose (Sigma-Aldrich), 1 ml/l Tween 80 (Sigma-Aldrich), 0.2 g/l $MgSO_4 \cdot 7H_2O$ (Sigma-Aldrich) and 0.04 g/l $MnSO_4.H_2O$ (Sigma-Aldrich). Additional NaCl was added to obtain a level of 3% of NaCl. At several time intervals during storage, samples of 5 ml were taken by using a sterile needle to determine cell number and pH. Cell numbers were determined by spreadplating on MRS agar by using a Spiral Plater (Spiral Systems, Model D, Led Techno, Eksel, Belgium) and pH measurements were done by using a pH-electrode (Knickn, type 763, Berlin, Germany). At the end of each growth experiment, when the pH was changing no more than 0.01 pH units in 24 h, a sample was taken for the determination of the concentration of lactic acid by using a high-performance liquid chromatograph. The analyses were performed isocratically with the cation exchange column Aminex HPX-87H (Bio-Rad Laboratories, Hercules, CA, USA) at a flow rate of 0.6 ml/min of 5 mM H_2SO_4 (VWR International) at 35 °C and a run time of 25 min. The HPLC equipment consisted of a pump (Gilson, type 307, Villiers Le Bel, France), an injector (Rheodyne 9096, Bensheim, Germany) with a 20-μl loop and a refractive index detector (Gilson, type 132). Growth curves were modelled by the model of Baranyi and Roberts (1994) and pH curves by a modified Gompertz equation used by Linton et al. (1995) for modelling survival curves of *L. monocytogenes* and adjusted for this purpose into:

$$A \times \exp(-\exp((\mathrm{AR} \times 2.718281828 \times (S - t))/A + 1)) - A \times \exp(-\exp((\mathrm{AR} \times 2.718281828 \times S)/A + 1))$$

where A = tail of the sigmoid curve or final pH, t = time (h), S = shoulder of the sigmoid curve (h), AR = acidification rate (h^{-1}) or slope of the linear part of the sigmoid curve.

In this way, estimations for generation time (h), lagphase (h), acidification rate (AR) (h^{-1}), time to acidification starting from 10^6 cfu/ml ($=t_{ac\text{-}6}$) (h) and depth of acidification could be made.

2.6. Behaviour of 12 selected putative protective cultures on a model cooked ham

The major objective of this experiment was to establish if the 12 selected putative protective cultures were not negatively influencing the sensory properties when inoculated onto a real cooked meat product. Furthermore, this experiment allows comparing the 12 LAB in relation to their growth, acidifying capacity and sensory changes on a cooked ham product.

A model cooked ham product was manufactured on a semi-industrial scale at Dera Food Technology (Bornem, Belgium) with following recipe: 80% of pork meat, 20% of water, 18 g/kg nitrited salt (NaCl containing 0.6% of sodium nitrite), 5 g/kg Deraphos C107 (potassium and sodium-di-, tri- and polyphosphates) and 0.5 g/kg Na-ascorbate. After boning and defatting, hams were cut in pieces of ± 10 to 10 to 10 cm. These pieces were homogenised and further minced to 20 mm and finally cuttered in a vacuum bowl cutter (Kilia, Neumünster, Germany) together with the nitrited salt and other ingredients. The cutter mixture was filled in a cook-in-casing to a final diameter of 100 mm and tempered for minimum 2 h at 4 °C before pasteurization occurred at 75 °C to a core temperature of 70 °C in a cooking chamber (Kerres, Sulzbach, Germany) during 2 h and 45 min. After cooling at 4 °C, the cooked ham sausages were sliced with a non-automatic slicer (Omas, S. Vittoria di Gualtieri, Italy) in slices of 2 mm thickness (± 20 g/slice). The product was quick-frozen in a blast freezer (Friginox-Le Froid Professionnel, Frispeed SR-range, Villevallier, France) at − 40 °C to a core temperature of − 10 °C to avoid formation of large ice crystals and finally further stored in a freezing room at − 18 °C. When an experiment started, the necessary amount of product was transferred from the freezer to a cooler at − 3 °C for 48 h and later at 4 °C for 24 h. The chemical composition of the cooked ham was: 24.60 ± 0.63% of dry matter, 2.68 ± 0.02% of NaCl (on aqueous phase), pH of 6.06 ± 0.06 and a water activity of 0.9831 ± 0.001.

The model cooked ham product was inoculated with the 12 strains at 10^5–10^6 cfu/g in three similar experiments. Each experiment consisted of 5 series: 1 blank series of non-inoculated ham and 4 series of ham inoculated with 1 of the 12 strains. Each series was performed in triplicate. The inoculum was sub-

cultured twice (24 h, 30 °C) in 5 ml MRS broth. To reach an inoculation level of 10^5–10^6 cfu/g, 200 μl of a 24 h culture of the test organism was divided over and spread on the surface of 10 slices (125 g/10 slices) of cooked ham. After inoculation, slices were vacuum packaged (10 slices/package) and stored at 7 ± 1 °C in a ventilated refrigerator. Packaging was performed using a Multivac A300/42 (Hagenmüller, Wolfertschwenden, Germany) gas packaging machine in a high barrier film (NX90, Euralpak, Wommelgem, Belgium) of 90 μm thickness with an oxygen transmission rate of 5.2 ml/m^2 24 h atm at 23 °C and 85% of relative humidity.

At days 0, 2, 6, 9, 13, 20, 27 and 34 of the storage period, cooked ham samples were analysed for growth of the inoculated strain, pH and concentration of metabolites. Furthermore, the sensory characteristics were evaluated.

For the microbial analyses, a 15-g sample of ham was taken aseptically and a decimal dilution series in PPS was prepared to plate the appropriate dilutions on MRS agar (aerobic incubation at 22 °C for 3–5 days) and M5 agar (anaerobic incubation at 30 °C for 2 days) to determine the level of LAB. The M5 agar differentiates between homo- and heterofermentative LAB (Zuniga et al., 1993). The blank series was also plated on respectively Plate Count Agar (PCA, Oxoid) (aerobic incubation at 22 °C for 3–5 days), Reinforced Clostridial Agar (RCA, Oxoid) (anaerobic incubation at 37 °C for 3–5 days) and Yeast Glucose Chloramphenicol Agar (YGC, Bio-Rad Laboratories) (aerobic incubation at 22 °C for 3–5 days) to determine respectively total aerobic psychrotrophic count, total anaerobic count and number of yeasts and moulds.

pH measurements and HPLC analyses were performed as described earlier in this study. Before HPLC analysis, meat samples were subjected to an extraction procedure: a 10-g sample was homogenized with 50 ml of distilled water, 5 ml of Carrez I (0.407 M $K_4Fe^{II}(CN)_6$, Sigma-Aldrich) and 5 ml of Carrez II (0.814 M $ZnSO_4$, VWR International) and finally filled up to 100 ml with distilled water. The deproteinized mixture was filtered through a Ø 125 mm filter (Schleicher and Schuell Microscience, Dassel, Germany) and filtered again through a HPLC syringe 0.2 μm filter (Alltech Associates, Lokeren, Belgium) before injection.

Cooked ham samples were evaluated by a nine-member trained sensory panel using a scoring method. Attributes were odour, acid odour, rot odour, taste, acid taste, general appearance, slimy appearance and colour. Attribute scales varied from 1 to 9 with 1 being very good, 5 the limit of acceptability and 9 very bad. A score above 5 indicated the sample being unacceptable. Finally, the panel was asked to evaluate the fitness for human consumption. If five or more of nine persons considered a sample unfit, the sensorial quality was considered as to be rejected.

3. Results and discussion

3.1. Collection of relevant strains

The ‘collected’ LAB, summarised in Table 1, consist of seven strains, known to produce bacteriocins, and eight strains assumed to be bacteriocin negative and known for their very competitive character.

3.2. Isolation and identification of isolates from meat products

From the 27 different meat products, 76 strains were isolated. All isolates, except 2, were Gram positive and catalase negative confirming the selectivity of MRS supplemented with sorbic acid and the Chalmers medium. From the 74 remaining strains, only 3 (4.05%) were heterofermentative and identified as *Leuc. mesenteroides* subsp. *mesenteroides* with the API50CH system while 4 (5.04%) demonstrated a slimy and/or ropy appearance on MRS agar and were not further used. Based on their API50CH fermentation profile, 37 strains were selected for further study. These strains were Gram positive, catalase negative and homofermentative, did not show a slimy appearance on MRS agar and moreover were completely different from each other which means that either they were isolated from a different meat product or they were isolated from the same meat product but showed a different fermentation profile. This severe selection criterion explains why only 37 strains were further investigated. The 37 remaining strains are further described as ‘isolated’ strains. The percentage of these strains isolated out of cooked or fermented meat products per species is

Table 1
Overview of 'collected' lactic acid bacteria implicated in this study

Strain	Code	Origin (reference)	Properties	Obtained from
Lactobacillus plantarum ALC	LP1	Not reported	Pediocin AcH	Danisco
Pediococcus acidilactici PA-2	PA1	Not reported	Pediocin	Chr. Hansen
Lactococcus lactis BB24	LL3	Fermented sausage (Rodriguez et al., 1995)	Nisin	Dr. J.M. Rodriguez[a]
Lactococcus lactis G18	LL4	Fermented sausage (Rodriguez et al., 1995)	Nisin	Dr. J.M. Rodriguez
Lactobacillus sakei 148	LS5	Spanish dry sausage (Sobrino et al., 1991)	Lactocin S	Dr. J.M. Rodriguez
Lactobacillus sakei Lb 706	LS6	Vacuum packaged meat (Schillinger and Lücke, 1989)	Sakacin A	FRC[b] 195
Lactococcus lactis UW1	LL2	Frankfurter sausage	Nisin	LMG[c] 7930
Lactobacillus alimentarius BJ-33 (in 1998, renamed to *L. sakei*)	LS1	MA-packaged fresh meat (Andersen, 1995)	Not reported	Chr. Hansen
Lactococcus lactis subsp. *lactis* L201	LL1	Vacuum packaged cooked sausage (Elsser, 1998)	Not reported	Danisco
Lactobacillus plantarum	LP5	Fermented sausage	Not reported	LFMFP[d] 143
Pediococcus pentosaceus	PP1	Fermented sausage	Not reported	LFMFP 155
Lactobacillus curvatus	LC4	Fermented sausage	Not reported	LFMFP 540
Lactobacillus sakei subsp. *carnosus* GERT17	LS7	Cooked ham (Devlieghere et al., 1998)	Not reported	LFMFP 216
Lactobacillus sakei SAGA 777	LS8	Not reported	Not reported	Quest International
Lactobacillus plantarum	LP3	Not reported	Not reported	LMG 8155

[a] Departamento de Nutricion y Bromatologia III, Universidad Complutense de Madrid (Madrid, Spain).
[b] Federal Research Center for Nutrition, Institut for Hygiene and Toxicology (Karlsruhe, Germany).
[c] LMG, Laboratory Microbiology Gent (Gent, Belgium).
[d] LFMFP, Laboratory of Food Microbiology and Food Preservation (Gent, Belgium).

presented in Table 2. As *L. sakei* is not included in the database of the API50CH system and pure *L. sakei* strains are identified by the API50CH system as *Lactobacillus fermentum*, it was not surprising that the majority of the isolates (32.4% of total isolates and 47.6% of cooked meat isolates) was identified as *L. fermentum*, an organism that is not described as a typical spoilage organism of cooked meat products. Earlier results (not shown) indicate that such *L. fermentum* strains, in most cases, are identified through gelelectroforese (SDS-PAGE) and cluster analysis as members of the *L. sakei*/*curvatus* group (Devlieghere et al., 1998). Difficulties in correctly identifying strains of the *L. sakei*/*curvatus* group have been reported before (Champomier-Vergès et al., 2002). Further, also *Lactococcus lactis* subsp. *lactis* (32.4%) and *L. curvatus* (13.5%) were rather frequently isolated from the meat products. Dominance of the *L. sakei*/*curvatus* group in the spoilage microbial association of cooked meat products has been demonstrated by many authors (Devlieghere et al., 1998; Samelis et al., 2000). *L. lactis* subsp. *lactis* is not typically associated with cooked meat products but, however, has already been isolated from cooked meats by Barakat et al. (2000) and Hamasaki et al. (2003) and from fermented products by Rodriguez et al. (1995). Other species, e.g., *Carnobacterium divergens* were less frequently isolated but nevertheless typical for cooked meat products (Samelis et al.,

Table 2
Percentage distribution of homofermentative LAB isolated out of cooked or fermented meat products per species as identified by the API50CH system

Species	Total	Cooked meat products	Fermented meat products
Number of isolates	37 (100%)	21 (100%)	16 (100%)
Lactobacillus fermentum[a]	12 (32.4%)	10 (47.6%)	2 (12.5%)
Lactococcus lactis subsp. *lactis*	9 (24.3%)	5 (23.8%)	4 (25.0%)
Lactobacillus curvatus	5 (13.5%)	2 (9.5%)	3 (18.8%)
Lactobacillus plantarum	2 (5.4%)	0 (0.0%)	2 (12.5%)
Carnobacterium divergens	3 (8.1%)	3 (14.3%)	0 (0.0%)
Pediococcus pentosaceus	1 (2.7%)	0 (0.0%)	1 (6.25%)
Leuconostoc lactis	1 (2.7%)	0 (0.0%)	1 (6.25%)
Lactobacillus acidophilus	1 (2.7%)	1 (4.76%)	0 (0.0%)
Lactobacillus brevis	1 (2.7%)	0 (0.0%)	1 (6.25%)
Leuconostoc mesenteroides subsp. *mesenteroides*/*dextranicum*	1 (2.7%)	0 (0.0%)	1 (6.25%)

[a] As *L. sakei* is not included in the database of the API50CH system and pure *L. sakei* strains are identified by the API50CH system as *L. fermentum*, these isolates were presumably belonging to the *L. sakei/curvatus group.*

1998). *L. sakei*, *L. curvatus*, *Lactobacillus plantarum* and *Pediococcus pentosaceus*, commonly used as starter cultures (Montel, 1999) were isolated from the fermented meat products.

3.3. Psychrotrophic character and salt tolerance

This test was performed for the 15 collected strains (7 bacteriocinogenic and 8 non-bacteriocinogenic) and 37 isolated strains. Within the group of the bacteriocinogenic strains only lactocin S producing *L. sakei* 148 (LS5) and sakacin A producing *L. sakei* Lb 706 (LS6) were able to grow at both, 4 and 7 °C combined with 3% and 6% of NaCl. It has been described earlier that only a limited number of bacteriocinogenic strains are able to grow at low temperatures (Hugas, 1998). None of the *L. lactis* strains could grow at 4 or 7 °C, while strains LP1 and PA1 could not grow anymore when the low temperature was combined with 6% of salt. From *L. lactis* it is known that they can grow at temperatures as low as 10 °C (Batt, 1999; Hamasaki et al., 2003). However, according to Sobrino et al. (1991), strains LL3 and LL4 are able to grow at 4 °C, but this was not confirmed in this study. Only LS5 was used for further study as not so much is known about the potential of this lactocin S producing strain in real meat applications. Five out of eight strains within the group of the collected non-bacteriocinogenic strains were psychrotrophic and salt tolerant. All these five strains, *L. alimentarius* BJ33 (LS1), a *L. sakei* subsp. *carnosus* isolate from cooked ham (LS7), *L. sakei* SAGA777 (LS8), *L. plantarum* (LP5) and *L. curvatus* (LC4) were used for the next steps of the screening. Again, the *L. lactis* strain (LL1) could not grow at the low temperatures and LP3 and PP1 were sensitive for 6% of salt. Among the isolates, 76% (28/37) was able to grow at low temperatures combined with higher salt concentrations, although all of these cultures were isolated from refrigerated meat products. The group of seven isolates, not able to grow at 4 or 7 °C combined with 3% or 6% NaCl, consisted of three *C. divergens* isolates, one *L. plantarum* isolate, the sole *P. pentosaceus* and *Leuc. lactis* isolates, and one *L. curvatus* isolate. Most strains belonging to the *L. sakei*/*curvatus* group could grow at the low temperatures and high salt concentrations. *L. sakei* is known to be one of the most psychrotrophic species of lactobacilli since some strains grow at 2–4 °C (Champomier-Vergès et al., 2002). In total, 34 strains were useful for further tests, consisting of 6 collected strains and 28 isolated cultures.

3.4. Antibacterial activity towards *L. monocytogenes*, *Leuc. mesenteroides*, *Leuc. carnosum* and *B. thermosphacta*

The aim of this experiment was to examine the 34 selected LAB for their antibacterial capacity towards *L. monocytogenes* and representative spoilage organisms. It should be noticed that a positive result, this is the detection of an inhibition zone, may result from lactic acid, bacteriocin or hydrogen peroxide production. However, this test was meant to select for strains with the highest antibacterial activity, as it was not possible to continue working with all 34 strains. This test was not yet meant to reveal the mechanism of inhibition. All strains, except two showed antibacterial activity towards at least one of the indicator strains. Only 13/34 (38.2%) strains were active towards all 6 indicator strains. Towards L.mono1, L.mono2 and L.mono3, respectively, 27/34 (79.4%) 31/34 (91.2%) and 26/34 (76.5%) strains were demonstrating antibacterial activity. Towards L.mes3, L.carn1 and B.therm1, respectively, 25/34 (73.5%), 17/34 (50%) and 30/34 (88.2%) strains were demonstrating antibacterial activity. The largest inhibition zones were observed towards L.mono2 and B.therm1 indicating that especially L.mono2 and B.therm1 were inhibited by the test strains, while L.carn1 was only slightly inhibited. Within the group of collected strains, LP5 showed the highest and LC4 the lowest antibacterial activity towards the six indicator strains, while the antibacterial activity of LS1, LS5, LS7 and LS8 was intermediate. LS5 did not show a larger inhibition zone in comparison to the other strains although this strain is known to produce lactocin S (Sobrino et al., 1991). Inhibition zones can be the result of different antimicrobial compounds, e.g., for LS1, lactic acid production has been indicated as the causative agent for the antagonistic character of the strain (Juven et al., 1998). Within the group of isolated strains, isolates 9A (vleeskoek, a typical Belgian cooked meat product), 20C (rolled pork), 10A (cooked turkey fillet), 13E (boulogne), 14A (fermented sausage) and 16G (chorizo) showed the

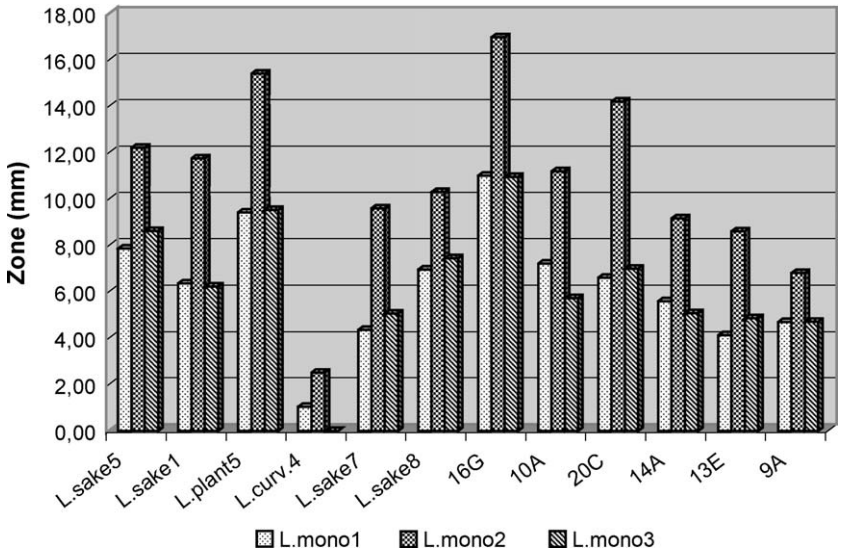

Fig. 1. Antibacterial properties of the 12 selected lactic acid bacteria towards three different *L. monocytogenes* strains; zone (mm) = radius of inhibition zone minus radius of the spot.

highest antibacterial activities towards the indicator strains and were selected for further studies. Especially strain 16G showed large inhibition zones. In Figs. 1 and 2, the antibacterial activity of the six collected LAB and six selected isolates towards, respectively, the three *L. monocytogenes* indicator strains and the three spoilage indicator strains is shown.

The 12 selected strains were subjected to a bacteriocin assay, confirming that LS5 produced a bacteriocin and revealing that LP5, previously assumed as bacteriocin negative, is also producing a bacteriocin. All other strains did not show inhibitory zones towards L.mono1 in repeating bacteriocin assays. To absolutely prove that they do not produce bacterio-

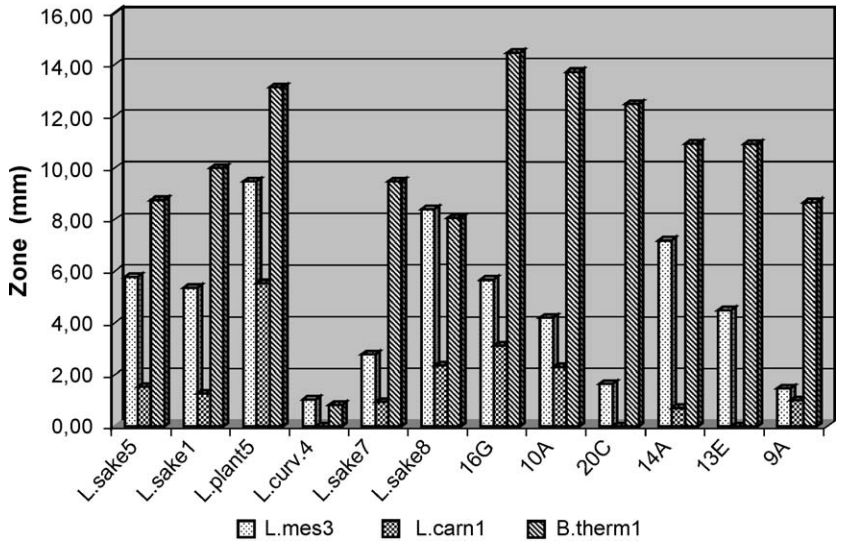

Fig. 2. Antibacterial properties of the 12 selected lactic acid bacteria towards the spoilage organisms *L. mesenteroides*, *L. carnosum* and *B. thermosphacta*; zone (mm) = radius of inhibition zone minus radius of the spot.

cins, specialized genetic techniques need to be used but this was beyond the scope of this study. Therefore, the 10 other strains are further assumed to be non-bacteriocinogenic. Sobrino et al. (1991) investigated the inhibitory spectrum of lactocin S producing *L. sakei* 148 (LS5). Activity was mainly observed towards indicator strains *L. curvatus*, *Leuc. mesenteroides* and *L. monocytogenes*.

The 6 selected isolates were further identified through SDS-PAGE. Based on the API50CH system strains 9A, 20C and 10A were identified as *L. fermentum*, strains 13E and 14A as *Leuc. mesenteroides* subsp. *mesenteroides*/*dextranicum* and 16G as *L. curvatus*. However, through SDS-PAGE, isolates 9A, 20C, 10A, 13E and 14A were identified as *L. sakei* subsp. *carnosus* and 16G was confirmed to be a *L. curvatus* strain.

3.5. Growth, acidification profile and lactic acid production of 12 selected putative protective cultures

For the six collected strains LS5, LS1, LS7, LS8, LP5 and LC4, and six isolates 9A, 20C, 10A, 13E, 14A and 16G, growth curves and pH curves at 7 °C were obtained. By using the model of Baranyi and Roberts (1994), an estimation for the growth rate (h^{-1}) and lagphase (h) was obtained. The pH model resulted in a shoulder *S* (h), an acidification rate AR (h^{-1}) and a tail A or final pH, used to calculate the acidification depth, being the difference between the initial pH (6.2) and the estimated final pH. To obtain a parameter indicating the time before acidification in the medium starts, that is independent from the initial cell number (varying between 10^5–10^6 cfu/ml), the shoulder obtained from the pH model was corrected for the time necessary to reach 10^6 cfu/ml starting from the initial count. In this way, the time to acidification starting from 10^6 cfu/ml ($=t_{ac\text{-}6}$) was calculated. The most important model parameters and the amount of lactic acid produced during growth are summarised in Table 3. The majority of the strains (LS8, LS7, LS1, 10A, 13E, 9A, 20C and 14A) showed a similar growth and pH evolution with short lagphases and short generation times. Within this group, all strains were *L. sakei* strains. Acidification depth for this dominant group ranged from 1.92 to 2.19 pH units. The lactocin S producing LS5 demonstrated a different pattern: a longer generation time and accompanying longer $t_{ac\text{-}6}$ was observed together with a limited acidification of 1.58 pH units, which is significantly less than for all other strains. Strains LC4 and 16G, both *L. curvatus*, had a comparable evolution with longer generation times and longer acidification rates than the *L. sakei* group. The sole *L. plantarum* LP5 strain grew and acidified the medium very slowly, but resulted in a deep acidification. Most strains produced levels of lactic acid around 130 mM, although LS7 and 14A produced a higher level of

Table 3
Model parameters of the growth and acidification experiment for the 12 selected lactic acid bacteria in adapted BHI broth at 7 °C under anaerobic conditions

Strain code	Lagphase (h)	Generation time (h)	Time to acidification $t_{ac\text{-}6}$ (h)[a]	Acidification rate (h^{-1})	Depth of acidification[b]	Produced level of lactic acid (mM)
LS8	46.19 ± 4.40	3.88 ± 0.56	14.37 ± 2.00	0.047 ± 0.001	2.19 ± 0.01	131.98 ± 0.26
LS7	39.13 ± 2.20	3.00 ± 0.25	13.32 ± 3.31	0.030 ± 0.001	2.24 ± 0.01	149.86 ± 0.54
LS1	45.93 ± 5.29	3.78 ± 0.52	29.48 ± 8.50	0.039 ± 0.000	2.21 ± 0.01	139.76 ± 0.25
LS5	56.63 ± 6.08	7.07 ± 0.60	74.47 ± 6.72	0.018 ± 0.000	1.58 ± 0.01	82.28 ± 1.34
LP5	178.15 ± 1.96	10.39 ± 0.86	118.48 ± 4.13	0.015 ± 0.000	2.39 ± 0.01	121.91 ± 1.09
LC4	60.88 ± 4.66	4.50 ± 0.48	33.18 ± 0.72	0.040 ± 0.002	2.22 ± 0.00	128.25 ± 0.29
9A	91.17 ± 4.76	5.33 ± 0.42	23.70 ± 1.91	0.032 ± 0.001	2.17 ± 0.01	128.31 ± 0.31
10A	25.09 ± 0.74	3.67 ± 0.14	14.48 ± 1.24	0.023 ± 0.001	1.92 ± 0.01	128.37 ± 1.50
13E	54.17 ± 3.37	4.13 ± 0.40	27.17 ± 0.41	0.037 ± 0.001	2.13 ± 0.02	129.52 ± 0.65
14A	26.96 ± 7.31	4.37 ± 0.97	14.92 ± 1.34	0.033 ± 0.001	2.21 ± 0.01	153.59 ± 1.44
16G	17.61 ± 5.15	7.67 ± 0.54	32.32 ± 0.79	0.017 ± 0.000	1.86 ± 0.00	130.05 ± 2.09
20C	33.93 ± 2.97	3.10 ± 0.25	15.81 ± 1.62	0.045 ± 0.000	2.15 ± 0.01	128.49 ± 0.82

[a] starting from 10^6 cfu/ml.
[b] Difference between initial pH (6.2) and estimated final pH.

± 150 mM. Lactocin S producing strain LS5 produced significantly less (82 mM) lactic acid in comparison to the other strains.

Especially strains with fast growth rates at low temperatures have potential as protective cultures as fast growth rates are an indication for greater competitiveness for nutrients and give the LAB a selective advantage over slower growing competitors (Bredholt et al., 1999). However, fast growth rates are for LAB most often accompanied by a high acidification rate and acidification depth. On real cooked meats, this could result in a rapid and large pH decrease, creating undesired sensory deviations. Therefore, strains were further investigated to see to what extent they are influencing organoleptic properties of cooked meat products.

3.6. Behaviour of 12 selected putative protective cultures on a model cooked ham

The non-inoculated cooked ham had an initial contamination with LAB, enumerated on MRS agar, of 1.3 $\log_{10}$ cfu/g. Aerobic count and LAB count were of the same magnitude and no yeasts or moulds were detected. Near the end of the storage period (day 34), the level of endogenous LAB increased up to 10^4–10^6 cfu/g. The initial microbial load was very low compared to the obtained inoculation level of 5×10^5–5×10^6 cfu/g. Furthermore, dominance of inoculated strains over background flora was confirmed by identical API profiles of inoculum and isolates (results not shown). In Fig. 3, the growth of the tested strains, as enumerated on M5 agar, on the model cooked ham, is presented. At day 6, the majority of the strains reached a cell concentration of about 10^8 cfu/g. Similar to the growth experiment in the adapted BHI broth, strain LP5 grew slower than the other strains and reached a lower maximal population level, although the inoculation level was by accident slightly higher than the mean inoculation level of the other LAB. The initial level of glucose and lactic acid of the cooked ham product was, respectively, 0.09 ± 0.03% and 0.70 ± 0.12%. The latter level corresponds to the level of 0.76% lactate, mentioned by Stekelenburg and Kant-Muermans (2001), being the level naturally present in meat. The pH of the model cooked ham was about 6.09 ± 0.05 at the start of the experiment. Near the end of the storage period, the pH of the inoculated ham decreased significantly while the pH of the non-inoculated series did not decrease (Fig. 4). The pH decrease at day 27 varied between 0.2 and 0.3 pH units, depending on the type of strain. In the cooked ham series that were still sensory acceptable at day 34 (strains 13E, 10A, 14A, LS8 and LS5), the pH decreased to a value of about 5.80–5.75 on day 34. Significant differences in pH values between inoculated and related non-inoculated series were observed from day 9 on for LS7, 16G, LC4, 10A and 14A, from

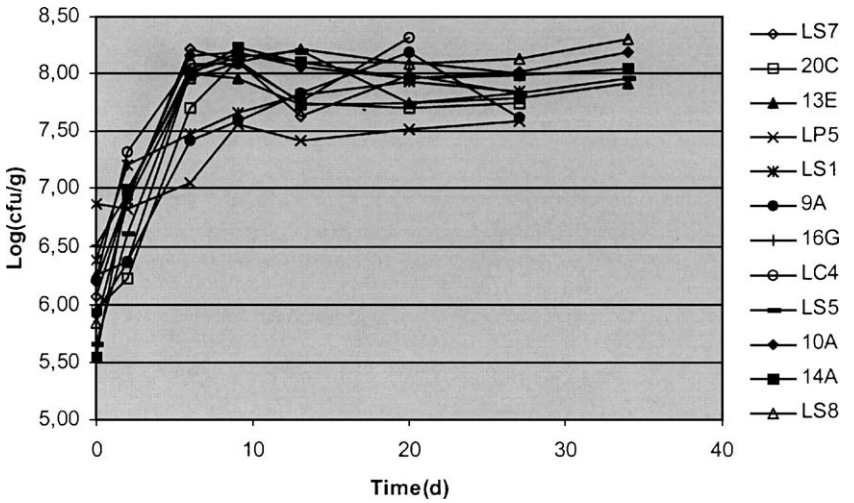

Fig. 3. Growth of the 12 selected lactic acid bacteria on the vacuum packaged model cooked ham product during storage at 7 °C.

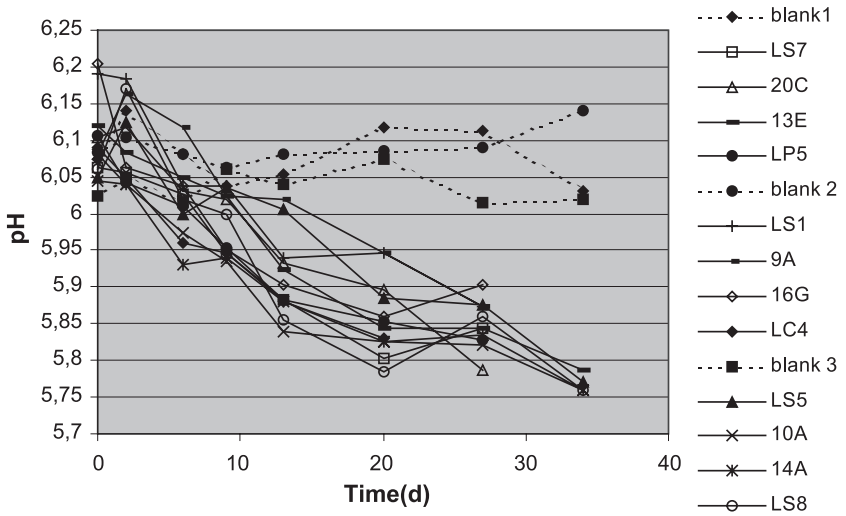

Fig. 4. pH evolution of the vacuum packaged model cooked ham product, non inoculated (- - -) and inoculated (—) with the 12 selected lactic acid bacteria during storage at 7 °C.

day 13 on for LP5, LS1 and LS8 and from day 20 on for 9A and LS5. This indicates that strains 10A, 14A, LC4, LS7 and 16G are more rapidly acidifying the cooked ham, while strains 9A and LS5 are less rapidly acidifying the product. LS5 had also a low acidification rate in the previous experiment when growing in the adapted BHI broth. However, the limited acidification of the broth by LS5 was not confirmed in the cooked ham experiment, as the pH of the cooked ham, inoculated with LS5, decreased to 5.80 ± 0.05. In general, the pH decrease is very limited but this can easily be understood taking into account the small level of glucose ($0.09 \pm 0.03\%$), initially present in the cooked ham. Since LAB can form maximum 2 mol of lactic acid by conversion of 1 mol of glucose, the maximum expected amount of lactic acid produced out of $\pm 0.1\%$ glucose is about 0.1% of lactic acid, depending on the heterofermentative character of the strain. This level is very low compared to the initial lactic acid level of $0.70 \pm 0.12\%$ and explains why no significant lactic acid production was found for none of the cultures. Korkeala et al. (1990) concluded that a level of lactic acid above 0.4% is an indication of spoilage, while here levels of lactic acid varied between 0.4% and 1.2% during storage and high levels were not correlated with rejection of samples by our sensory panel. However, the study of Korkeala et al. (1990) was performed on cooked ring sausages, while cooked ham was the product used here. Juven et al. (1998) found a significant lactic acid production of 50 mM or 0.45% (from 90 mM or 0.81% lactic acid initially to 142 mM or 1.28% lactic acid after 9 weeks at 4 °C) on ground beef, inoculated with *L. alimentarius* FloraCarn L-2 (LS1 in this study). Presumably, the glucose content of the ground beef was higher than the glucose content of the model cooked product used here. However, Juven et al. (1998) did not evaluate the sensory properties of this product after these 9 weeks.

Near the end of storage, the glucose level of the cooked ham decreased significantly and finally no glucose was detected anymore from day 13 on for LS1, 16G, LC4, LS8 and 14A, from day 20 on for LS7, 13E and 10A and from day 27 on for LP5, 9A, LS5 and 20C. The glucose concentration at the last day of the storage period of the non-inoculated product did not differ significantly from the initial glucose level.

Sensory analysis indicated that cooked ham inoculated with strains 13E, 10A, 14A, LS5 and LS8 was not rejected by the sensory panel even on the 34th day of the storage period, although the pH, at that moment, was already decreased to 5.75–5.80. It seems that this pH is not sufficiently low to result in a sensory deviation on the model cooked ham. Statistical analysis (*t*-tests) confirmed that there were no

significant differences for all sensory attributes on day 34 between blank 1 and 13E and between blank 3 and 14A and blank 3 and LS5. Although the ham inoculated with 10A and LS8 was not rejected on day 34, cooked ham inoculated with 10A and LS8 had a significant higher score for respectively taste and acid taste and acid taste only than the non-inoculated ham. However, the scores were still lower then 5, the limit of acceptability. Bredholt et al. (1999) also compared vacuum packaged reference cooked ham and cooked ham, inoculated with several homofermentative LAB and found all of the inoculated packages after 21 days of storage at 8 °C to be acceptable, although some strains resulted in a slight more sour taste and smell. However, in the study of Bredholt et al. (1999), sensory properties were not followed as a function of time. Table 4 gives the day of rejection and the mean score of the sensory panel for the different sensory attributes at the day of rejection or in case of no rejection at day 34. The table reveals that cooked ham inoculated with strains 20C, LP5 and LS7 is rejected on the basis of a deviating taste but not on the basis of an acid taste, while cooked ham inoculated with strains 16G, 9A, LS1 and LC4 was rejected on the basis of a deviating odour but not on the basis of an acid odour. Furthermore, it is important to notice that scores for taste and odour on the day of rejection, in case of the rejected series, are never higher than 6.5 indicating that the deviating odour and taste are not very pronounced. Scores for general appearance, colour and slime production never reached a value higher than respectively 3.5–4 and 2.5, indicating that inoculation with the LAB did not influence the colour and general appearance of the cooked ham in a negative way and that the strains did not result in slime formation on the surface of the product.

Table 5 summarizes the number of LAB, pH and concentration of glucose and metabolites of the cooked ham at the day of rejection or in case of no rejection at the last day of storage. The judges considered the rejected samples unfit for human consumption after LAB reached levels of 7.4–8.2 $\log_{10}$ cfu/g. This was expected and corresponds to the findings of Korkeala et al. (1987) on cooked ring sausages. However, samples still acceptable at day 34 did also reach these levels of LAB without causing sensory deviations in agreement with the findings of Bredholt et al. (1999). At the moment of rejection, pH values varied between 5.79 and 5.96. This corresponds to the results of Korkeala et al. (1990), finding cooked ring sausages unfit when the pH decreases below 5.8–5.9. However, cooked ham samples, still sensory acceptable at day 34, demonstrated a similar pH decrease without being judged unfit for consumption. Differences between our study and the study of Korkeala et al. (1990) might be explained by our primary selection towards homofermentative LAB,

Table 4
Scores for the different sensory attributes at the day of rejection or in case of no rejection at the last day of the storage experiment

Strain	Day of rejection	Odour	Acid odour	Rot odour	Taste	Acid taste	Slime	Colour	General appearance	% yes[a]
Blank 1	>34	3.38	2.50	2.50	3.13	3.13	1.50	2.00	2.00	100.0
20C	13	3.00	4.00	3.50	5.00	4.38	1.75	2.63	2.63	37.5
13E	>34	3.25	2.63	2.25	3.50	3.38	1.86	2.00	1.88	75.0
LP5	13	3.63	4.50	3.38	5.25	4.38	2.50	2.13	2.13	25.0
LS7	13	3.63	4.73	3.63	5.63	4.50	2.00	2.25	2.63	12.5
Blank 2	>27	3.71	2.50	2.50	3.13	2.29	1.00	1.71	1.57	85.7
16G	9	5.29	4.00	3.5	5.00	5.29	2.00	3.57	3.71	28.6
9A	20	5.25	4.25	4.38	5.5	5.13	1.25	2.00	2.00	42.9
LS1	9	5.14	4.50	3.38	5.25	4.29	2.14	3.43	3.57	28.6
LC4	6	6.14	4.14	3.14	5.57	4.00	1.43	1.86	2.57	28.6
Blank 3	>34	2.80	2.40	2.00	2.00	1.80	1.00	1.20	1.40	100.0
10A	>34	4.00	3.80	3.20	3.20	3.20	1.60	1.40	1.60	100.0
14A	>34	2.40	2.40	2.20	2.20	2.00	1.40	1.20	1.60	100.0
LS5	>34	3.60	2.40	3.00	3.00	2.80	1.00	1.40	1.40	100.0
LS8	>34	3.20	3.00	3.40	3.40	3.00	1.40	1.20	1.20	80.0

[a] % of the panel members willing to consume the product.

Table 5
Summary of the LAB number (log_{10} cfu/g) on M5 agar, pH and concentration (g/kg of ham) of glucose and metabolites of the cooked ham at the day of rejection or in case of no rejection at the last day of the storage experiment

Strain	Day of rejection	Cell number	pH	Glucose	Lactic acid	Acetic acid	Propionic acid	Ethanol
Blank 1	>34	4.2 ± 0.7	6.04 ± 0.00	0.96 ± 0.38	6.98 ± 0.70	0.08 ± 0.21	0.00 ± 0.00	0.00 ± 0.00
20C	13	7.8 ± 0.2	5.93 ± 0.02	0.72 ± 0.09	6.76 ± 0.78	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
13E	>34	7.9 ± 0.1	5.79 ± 0.01	0.00 ± 0.00	10.13 ± 0.98	0.27 ± 0.17	0.00 ± 0.00	0.96 ± 0.83
LP5	13	7.4 ± 0.3	5.88 ± 0.02	0.65 ± 0.23	9.91 ± 1.81	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LS7	13	7.6 ± 0.1	5.88 ± 0.02	0.15 ± 0.26	9.68 ± 1.90	0.08 ± 0.14	0.00 ± 0.00	0.00 ± 0.00
Blank 2	>27	5.2 ± 0.8	6.09 ± 0.07	1.15 ± 0.22	8.18 ± 0.37	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16G	9	8.2 ± 0.4	5.95 ± 0.03	0.13 ± 0.23	5.88 ± 0.96	0.02 ± 0.04	0.04 ± 0.07	0.00 ± 0.00
9A	20	8.2 ± 0.2	5.95 ± 0.02	0.26 ± 0.17	4.87 ± 0.98	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LS1	9	7.7 ± 0.3	6.04 ± 0.02	0.05 ± 0.04	3.89 ± 0.39	0.05 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
LC4	6	8.1 ± 0.2	5.96 ± 0.03	0.38 ± 0.18	6.28 ± 0.55	0.03 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
Blank 3	>34	6.3 ± 0.5	6.02 ± 0.04	0.90 ± 0.52	6.38 ± 3.72	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10A	>34	8.2 ± 0.0	5.76 ± 0.01	0.00 ± 0.00	12.05 ± 5.24	0.00 ± 0.00	0.13 ± 0.00	0.10 ± 0.14
14A	>34	8.1 ± 0.0	5.76 ± 0.00	0.00 ± 0.00	7.84 ± 0.97	0.19 ± 0.02	0.97 ± 1.38	1.75 ± 2.47
LS5	>34	8.0 ± 0.0	5.77 ± 0.01	0.00 ± 0.00	11.81 ± 3.17	0.17 ± 0.24	0.00 ± 0.00	0.00 ± 0.00
LS8	>34	8.3 ± 0.1	5.76 ± 0.01	0.00 ± 0.00	6.35 ± 4.38	0.10 ± 0.15	0.26 ± 0.37	0.39 ± 0.55

while Korkeala et al. (1990) studied the behaviour of the natural spoilage flora.

Although the strains were previously selected on their homofermentative character, small levels of acetic acid (varying between 0.002% and 0.290%), ethanol (varying between 0.005% and 0.750%) or propionic acid (varying between 0.003% and 0.142%) were detected in all inoculated series and even in the non inoculated series near the end of storage (Table 5). This is in agreement with the results from Borch et al. (1991), demonstrating a metabolic switch from homo- to heterolactic fermentation during anaerobic continuous growth of a homofermentative *Lactobacillus* due to glucose depletion. Production of acetate may be induced when the supply of glucose to each individual bacterial cell is insufficient to support a homofermentative metabolism, e.g., on a meat surface during storage (Borch and Agerhem, 1992). The study of Borch and Agerhem (1992) demonstrated that in the presence of the homofermentative *Lactobacillus* spp. on beef packaged in 5% CO_2 and 95% N_2, acetate reached a level of about 6 mmol/kg or 0.036% after 4 weeks at 4 °C. Comparable concentrations were found in this study, but on a cooked ham product. However, for none of the inoculated series, a persistent production of one of these metabolites from a certain moment on could be observed and 95% confidence intervals on the concentration values of these metabolites were rather large. There was also no relation between formation of one of these metabolites and rejection by the sensory panel. Even in the cooked ham samples inoculated with 13E, 10A, 14A, LS5 and LS8 and not rejected at day 34 of the storage experiment, on some days, a small amount of ethanol and/or acetic acid was detected, indicating that these metabolites could not be the reason for rejection. In the experiment of Borch et al. (1991), anaerobic continuous growth of a homofermentative *Lactobacillus* during glucose depletion was accompanied by extensive utilization of amino acids and also sulphide was produced. During this experiment, no typical sulphide odours were detected in the rejected product, although absence or presence of sulphide was not confirmed by chemical analysis. Amino acid catabolism produces a number of compounds including ammonia, amines, aldehydes, phenols, indole and alcohols, all being volatile flavours. Dainty (1996) confirmed that when glucose becomes depleted, other substrates begin to be metabolised. These include lactate, amino acids and creatine under aerobic storage and lactate and arginine during storage in vacuum or MAP.

4. Conclusion

Especially lactic acid bacteria that are homofermentative, salt tolerant, psychrotrophic and adapted to

meat substrates have a good potential to be used for the biopreservation of cooked meat products. Therefore, primary screening tests of 91 meat born LAB resulted in the selection of 12 putative protective cultures, known for their psychrotrophic character, salt tolerance and antibacterial properties towards *L. monocytogenes* and spoilage organisms associated with cooked cured meat products. From these 12 strains, only LC4 can be excluded for further research because this strain has a very limited antibacterial activity and when inoculated on cooked ham, the product was rejected on day 6 of vacuum packaged storage at 7 °C. Strain LP5 is also less suitable for further investigations as this strain grows rather slow on cooked ham in comparison to the other LAB. Furthermore, all strains resulting in rejection of the cooked ham by the sensory panel have less potential for use as protective culture. The remaining four non-bacteriocinogenic (13E, 10A, 14A and LS8, all four identified as *L. sakei* subsp. *carnosus*) and one bacteriocinogenic (LS5 or lactocin S producing *L. sakei* 148) LAB could be the subject of further research aiming at confirming whether these strains do have a shelf-life prolonging effect on cooked meat products due to an inhibitory effect towards heterofermentative LAB, *B. thermosphacta* and *L. monocytogenes*.

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