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Application of nontraditional meat starter cultures in production of Hungarian salami

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Abstract

Listeria monocytogenes and *Escherichia coli* O111 have been implicated in several outbreaks of food-borne disease linked to smallgoods products. Traditional meat starter cultures, containing a mixture of lactic acid bacteria (LAB) and staphylococci, are used to maintain safety and sensory properties of Hungarian salami. The present study investigated if nontraditional meat starter (NTMS) cultures can be used for improving the safety of Hungarian salami. Salami batter was inoculated with *List. monocytogenes* and *E. coli* and subsequently fermented with NTMS cultures and a commercially available meat starter. A total of 15 NTMS cultures were tested. The salami was monitored for levels of pathogen, LAB and pH. When used in conjunction with the commercial meat starter, 9 NTMS cultures reduced the *E. coli* O111 count by more than 2.5 log units, whereas 10 of the NTMS cultures reduced *List. monocytogenes* by more than 2.5 log units. The commercial meat starter alone reduced *E. coli* and *List. monocytogenes* by 1.2 and 1.3 log units, respectively. Some NTMS cultures reduced the pathogen count without affecting pH of the salami batter. All NTMS cultures survived in salami throughout fermentation and maturation. It was concluded that NTMS cultures, including *Lactobacillus acidophilus* LAFTI™ L10, *L. paracasei* LAFTI™ L26, *L. paracasei* 5119, *Lactobacillus* sp. L24 and *Bifidobacterium lactis* LAFTI™ B94, may be used to increase the safety of Hungarian salami because these cultures gave strong inhibition of both *E. coli* O111 and *List. monocytogenes*.

Keywords: Probiotics; Fermentation; Food safety

1. Introduction

Listeria monocytogenes and enterohaemorraghic *Escherichia coli* (EHEC) have been isolated from faeces (Skovgaard and Morgen, 1988; Chapman et al., 1993) and carcasses of cattle at the time of slaughter (Chapman et al., 1993; McNamara, 1995). As a consequence, these pathogens may be incorporated into meat products during manufacture. Human listeriosis has been linked to metwurst (Loncarevic et al., 1997) and other processed meats (Grau, 1996). Outbreaks of severe diarrhoea and haemolytic uremic syndrome (HUS) have been linked to salami infected by *E. coli* O111 (Shay and Souness, 1995).

Traditional salami is produced by fermentation of a sausage batter containing meat, curing agents, salt and spices. The growth of pathogenic microorganisms is suppressed throughout the fermentation. This is due to the inhibitory environment created by a combination

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of mainly low pH and low water activity (Luecke, 1998). However, contamination of raw meat and the inherent ability of microorganisms to adapt to their environment may result in production of unsafe salami (Taplin, 1982; Cowden et al., 1989; Sauer et al., 1997). This has led to implementation of quality assurance programs for increased safety of fermented meat products. In Australia, starter cultures are mandatory in this type of meat product (Shay and Souness, 1995). As reviewed by Luecke (1998), traditional starter cultures include species of lactic acid bacteria, including *Pediococcus* spp. and *Lactobacillus* spp. The use of such cultures ensures a rapid onset of fermentation and discourages growth of undesirable bacteria, due to release of lactate and a reduction of pH.

Several bacteriocins are also produced by *Pediococcus* and *Lactobacillus*, of which some inhibit *List. monocytogenes* (Harris et al., 1989; Rodriguez et al., 1994). Bacteriocinogenic LAB have been successfully used for control of *Listeria* in fermented meat products (Berry et al., 1990; Hugas et al., 1995).

Probiotics are defined as live microorganisms which when applied to animal or man benefit the host by improving the properties of the indigenous microflora (Holzapfel et al., 1998). Hammes and Hertel (1998) suggested the possibility of developing starter cultures that exhibit probiotic properties and which also produce the required technological and sensory functions in the meat matrix. Suitable probiotic cultures may provide two mechanisms of safety. Firstly, they may inhibit pathogenic bacteria in the sausage (Erkkilä et al., 2000) and, secondly, inhibit pathogens within the gastrointestinal tract (Henriksson and Conway, 2001; Hudault et al., 1997).

The aim of this study was to investigate the effect of probiotic and other NTMS cultures on the survival of food borne pathogens in Hungarian salami, and if these NTMS cultures survive during manufacture of salami.

2. Material and methods

2.1. Microbial cultures

A commercial meat starter (FloraCarn FF1-200), consisting of a mixture of *Pediococcus pentosaceus* and *Staphylococcus xylosus*, was kindly provided by Chr.Hansen Australia (Bayswater, Australia). Fifteen strains of LAB and bifidobacteria of dairy product origin and human faecal origin were obtained. The origin and source of these strains is given in Table 1. *E. coli* O111 (UNSW 097700) and *List. monocytogenes* (UNSW 030800) was obtained from the University of New South Wales (Sydney, Australia). Cultures were stored at −80 °C in 30% (w/v) glycerol. Lactobacilli and pediococci were maintained in MRS broth (CM 359). Bifidobacteria were maintained in reinforced costridal medium (RCM, CM 149). Cultures of lactobacilli, pediococci and bifidobacteria were incubated in anaerobic jars for 48 h at 37 °C. Anaerobic conditions were achieved using AnaeroGen™ kits (Oxoid, Melbourne, Australia). Pathogens were maintained in tryptone soya broth (TSB, CM 129) and incubated in aerobic conditions at 37 °C for 24 h. The inoculum size used for all broth cultures was 1% of the final culture volume. All cultures were incubated at static conditions. Media used in this study was purchased from Oxoid.

2.2. Preparation of salami batter and fermented sausages

Fermented sausages were prepared following the recipe for Hungarian salami (Don Smallgoods, Victoria, Australia). The meat formulation consisted of pork back fat, sow hind quarter, beef chuck and pork shoulder. Frozen meat was minced and mixed with NaCl, spices, dextrose, milk solids, wine, $NaNO_2$ and $NaNO_3$. Salami batter was inoculated with either (1) an NTMS culture, (2) a pathogen and FloraCarn FF1-200 (FloraCarn), or (3) an NTMS culture, FloraCarn and a pathogen. Natural fermentation was monitored in salami batter prepared without added cultures. FloraCarn was added to the sausage batter according to instructions by the manufacturer ($>10^6$ cfu/g). NTMS cultures and pathogens used for inoculation of salami batter were prepared as described above. NTMS cultures were added where appropriate to achieve initial levels of 10^6 cfu/g. Pathogens were added to yield populations of 10^5–10^6 cfu/g sausage batter. All added cultures had reached stationary phase of growth. For monitoring survival of pathogenic bacteria and pH, aliquots of salami batter (25 g) were incubated in specimen jars at 25 °C for 7 days. Natural fermentation

Table 1
Log reduction of pathogenic bacteria and pH after fermentation of sausage batter for 7 days

Species name	Strain designation	Origin	pH	*E. coli*	*List. monocytogenes*
FloraCarn[a] only	NA	NA	4.71 ± 0.36	1.2 ± 0.4	1.3 ± 0.5
P. pentosaceus	L22*	human intestine	4.61 ± 0.31	2.6 ± 0.3	>2.5 ± 0.2
P. pentosaceus	L27*	human intestine	4.66 ± 0.80	2.2 ± 0.2	>2.5 ± 0.3
Lactobacillus sp.	L24*	human intestine	4.46 ± 0.13	3.3 ± 0.2	>2.5 ± 0.1
L. paracasei	LAFTI™ L25*	human intestine	4.61 ± 0.51	1.4 ± 0.5	1.1 ± 0.3
L. paracasei	LAFTI™ L26*	human intestine	4.47 ± 0.09	2.6 ± 0.1	>2.5 ± 0.1
L. paracasei	CSCC 5277***	dairy	4.75 ± 0.40	0.8 ± 0.1	1.0 ± 0.3
L. paracasei	CSCC 5119***	dairy	4.71 ± 0.45	3.2 ± 0.4	>2.5 ± 0.5
L. paracasei	CSCC 5120***	dairy	4.85 ± 0.40	0.3 ± 0.3	0.2 ± 0.3
L. casei imunitas	CSCC 5281***	dairy	5.07 ± 0.46	1.2 ± 0.3	0.5 ± 0.2
L. reuteri	CSCC 5310***	dairy	4.40 ± 0.14	3.0 ± 0.5	>2.5 ± 0.1
L. acidophilus	LAFTI™ L10**	dairy	4.51 ± 0.07	3.2 ± 0.6	>2.5 ± 0.1
L. acidophilus	L21*	human intestine	4.87 ± 0.35	1.7 ± 0.1	1.2 ± 0.1
Bifidobacterium sp.	LAFTI™ B74*	human intestine	4.40 ± 0.24	4.2 ± 0.3	>2.5 ± 0.2
B. longum	LAFTI™ B22*	human intestine	4.25 ± 0.20	2.5 ± 0.1	>2.5 ± 0.1
B. lactis	LAFTI™ B94*	human intestine	4.38 ± 0.16	3.7 ± 0.4	>2.5 ± 0.1

The traditional meat starter culture was used alone (FloraCarn) or in combination with a nontraditional meat starter culture of dairy or human intestinal origin. Presented results are averages ± standard deviations of five individual experiments.
NA = not applicable.
[a] A mixture of *S. xylosus* and *P. pentosaceus*.
* Culture obtained from CRC for Food Industry Innovation culture collection, UNSW.
** Culture obtained from DSM Food Specialties, Australia.
*** Culture obtained from CSIRO culture collection, Highett, Australia.

and survival of NTMS cultures were monitored in salami batter filled into fibrous casings (75 mm in diameter). Individual sausages were formed by tying the casing at approximately 90 mm intervals. Sausages were fermented at 25 °C for 72 h at a relative humidity (RH) of 85%, followed by maturation at 15 °C and 70% RH for 42 days.

2.3. Microbiology and pH of salami batter

The sausage batter was monitored for changes in population levels of lactobacilli, pediococci, bifidobacteria and pathogens. Samples (10 g) of the sausage batter were homogenised with 90 ml of 0.1% bacteriological peptone. Serial 10-fold dilutions were made and aliquots of 0.1 ml were plated in triplicate on the following agar plates: Rogosa agar (CM 627) for enumeration of lactobacilli and pediococci; reinforced clostridal agar (RCA, CM 151) supplemented with propionate (1.5 g/l) for enumeration of bifidobacteria; PALCAM agar (CM 877) containing selective supplement (CM 469) for enumeration of *List. monocytogenes* and Sorbitol MacConkey agar (CM 813) for enumeration of *E. coli*. Rogosa agar was incubated under anaerobic conditions for 24 h at 37 °C. RCA was incubated anaerobically at 37 °C for 48 h. All other agar plates were incubated at aerobic conditions for 24 h at 37 °C. Salami batter was sampled every second day during fermentation and maturation until pathogens could not be detected. Population levels of NTMS cultures were monitored for 42 days. The pH was measured after a 7-day fermentation of the salami batter. Measurement of pH was conducted after maceration of the salami batter in distilled water.

3. Results

3.1. Inhibition of food borne pathogens using NTMS cultures

Improved reduction of pathogens was observed when NTMS cultures were used in conjunction with FloraCarn. Increased inhibition of *E. coli* and *List. monocytogenes* was achieved by 12 and 10 NTMS cultures, respectively (Table 1).

When used in conjunction with FloraCarn, 9 NTMS cultures reduced the *E. coli* count by more than 2.5 log units. The greatest reduction of *E. coli* was observed in salami batter inoculated with FloraCarn and either *Lactobacillus* sp. L24, *Lactobacillus paracasei* 5119, *L. acidophilus* LAFTI™ L10 (L10), *Bifidobacterium* LAFTI™ B74 (B74) or *Bifidobacterium lactis* LAFTI™ B94 (B94) (Table 1). The reductions of *E. coli* were approximately 2 log units greater when these five strains were used in conjunction with FloraCarn, compared to FloraCarn alone (Table 1). Some reductions in levels of this pathogen were noted during the first 3 days of fermentation; however, the most significant reduction was observed over the subsequent 3 days. Between days 6 and 7, a declined rate of reduction and an increase in numbers of *E. coli* was detected in salami batter inoculated with LAFTI™ B74 (B74) and *Lactobacillus* sp. L24, respectively (Fig. 1).

The greatest reductions of *List. monocytogenes* were observed when FloraCarn was used in conjunction with either *Lactobacillus* sp. L24, *L. paracasei* LAFTI™ L26 (L26), *L. reuteri* 5310, *L. acidophilus* LAFTI™ L10 (L10), *Bifidobacterium* sp. B74, *B. longum* LAFTI™ B22 (B22), *P. pentosaceus* L22 and L27, *L. paracasei* 5119 and *B. lactis* B94 (Table 1). In contrast to *E. coli*, the viability of *List. monocytogenes* was reduced by up to 1.5 log units during the first 3 days of fermentation (Fig. 2). By day 7, the reductions of *List. monocytogenes*, using the combination of FloraCarn and these NTMS strains, were more than 1.2 log units greater compared to reductions induced by FloraCarn alone.

3.2. Survival of NTMS cultures in salami

The population of LAB was monitored during fermentation and subsequent maturation up to day 42 (Table 2). Overall, the population of NTMS increased throughout fermentation and maturation and maintained populations greater than 10^6 cfu/g up to day 42 (Table 2). Population levels of 10^8 cfu/g were reached in salami inoculated with *Lactobacillus* sp. L21 and *P. pentosaceus* L22. In naturally fermented salami, indigenous LAB increased in numbers progressively throughout fermentation approaching 10^6 cfu/g by day 21 (Table 2).

3.3. Effect of NTMS cultures on pH

The initial pH of the nonfermented sausage batter was approximately 5.9. By day 7, the FloraCarn

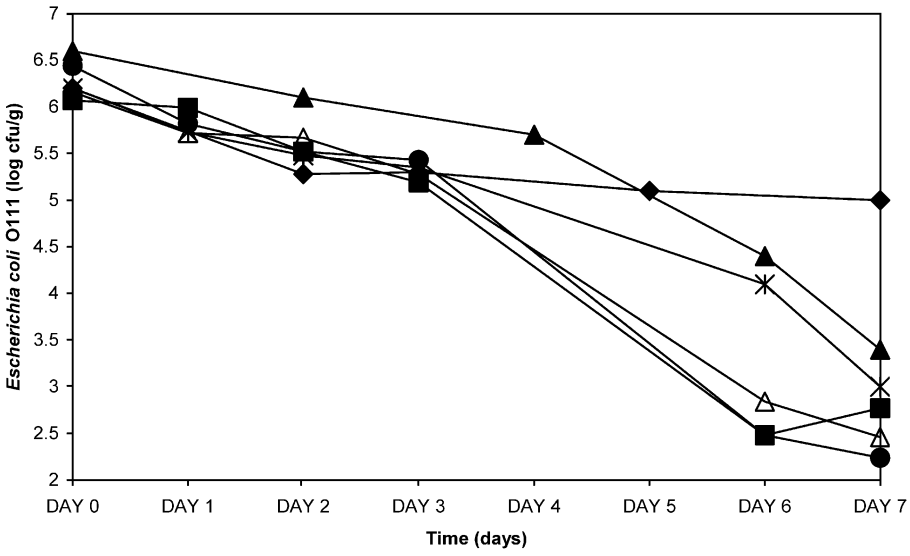

Fig. 1. Inhibition of *E. coli* O111 during fermentation of sausage batter with FloraCarn (♦) or a combination of FloraCarn and L24 (■), 5119 (▲), L10 (✳), B74 (●) and B94 (△). Presented results are typical of three individual experiments.

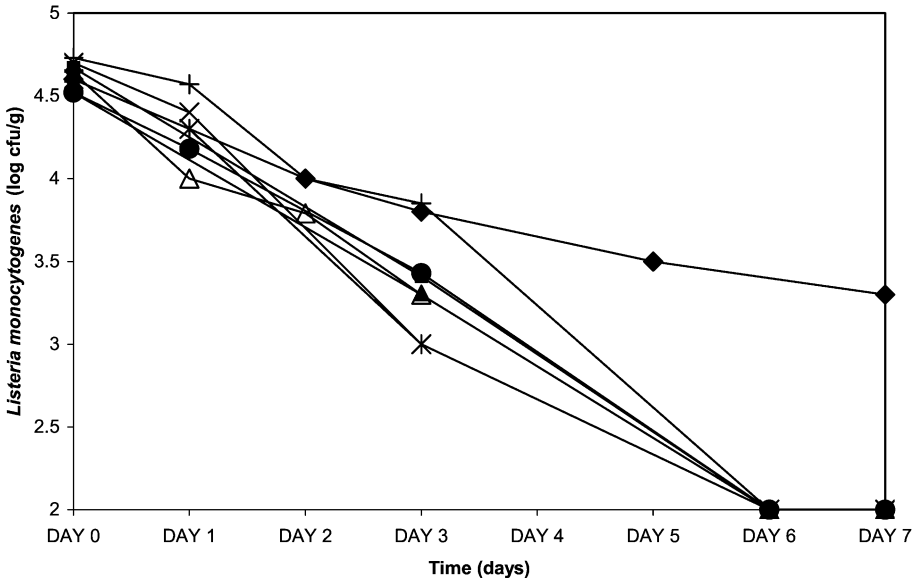

Fig. 2. Inhibition of *List. monocytogenes* during fermentation of sausage batter with FloraCarn (♦) or a combination of FloraCarn and L24 (■), L26 (▲), 5310 (×), L10 (✱), B74 (●), B22 (+) and B94 (△). Presented results are typical of three individual experiments.

reduced the pH to 4.71. The pH of salami fermented with a combination of FloraCarn and the NTMS culture ranged from 4.25 to 5.07 (Table 1).

Table 2
Populations of lactic acid bacteria (log CFU/g) in Hungarian salami during the fermentation, drying and maturation of Hungarian salami inoculated with nontraditional meat starter (NTMS) culture

NTMS	Day 0	Fermentation (day 3)	Maturation	
			Day 21	Day 42
L21	7.5	8.4	8.0	8.1
L22	7.4	8.1	8.0	8.0
L24	6.3	7.2	7.0	7.4
LAFTI™ L25	6.4	7.1	8.5	7.7
LAFTI™ L26	6.5	7.3	8.4	7.5
L27	5.2	7.2	7.9	7.8
CSCC 5277	7.4	7.6	8.3	7.5
CSCC 5119	6.8	7.3	8.1	6.2
CSCC 5281	7.1	7.3	7.8	7.0
CSCC 5310	5.9	7.2	6.6	6.9
LAFTI™ L10	6.9	7.4	7.3	6.0
CSCC 5120	6.6	7.3	7.8	6.6
LAFTI™ B74	7.3	8.2	8.0	7.9
LAFTI™ B22	6.9	8.5	8.2	7.8
LAFTI™ B94	7.2	8.4	7.6	7.1
Natural fermentation	0	5.2	5.9	5.2

4. Discussion

The present study investigated the effect of a collection of lactobacilli, bifidobacteria and pediococci of dairy product origin and human intestinal origin, on levels of *E. coli* and *List. monocytogenes* during fermentation of salami batter. When used in conjunction with FloraCarn, some of these cultures improved the reduction of pathogens, compared to FloraCarn alone. The greatest decline in numbers of *E. coli* was observed during fermentation for up to day 6. However, this was followed by a reduced rate of decline and an increase of the pathogen in salami batter inoculated with *Bifidobacterium* sp. B74 and *Lactobacillus* sp. L24, respectively. *E. coli* may adapt to acidic conditions and develop resistance to bacteriocins (Leyer et al., 1995; Ganzle et al., 1999). This may explain why the effect of B74 and L24 was reduced during prolonged fermentation of salami batter.

In the present and other similar studies (Hinkens et al., 1996; Faith et al., 1998; Erkkilä et al., 2000), survival of pathogenic bacteria was monitored by direct plating on selective media. It should be noted that severely injured cells might not recover on selective media, and that resuscitation of injured cells in

a nonselective broth before plating may result in an improved recovery (Clavero and Beuchat, 1996; Nakagawa et al., 2000). The significance of results presented here lies in the differences of pathogen survival caused by different starter cultures. These differences are expected on both selective and nonselective agar.

The pH endpoints of most salami batters containing NTMS cultures (9/13) were above 4.5. Similar or greater reductions in pH have previously been observed in other sausage formulations fermented by intestinal lactobacilli (Sameshima et al., 1998). It is likely that a significant reduction of pH would reduce the pathogens' ability to survive in the salami batter. Consequently, the pathogen inhibition resulting from use of *B. longum* B22 and some other NTMS cultures may be due to the considerable pH reduction induced by these strains. In contrast, the use of *L. paracasei* 5119, *P. pentosaceus* L22 and L27 resulted in a pH similar to that of salami fermented by FloraCarn alone. Yet, these strains induced considerable reductions in levels of *List. monocytogenes* and *E. coli*, which indicate that inhibitory compounds other than acids are involved. Bacteriocin producing strains, with activity against *Listeria*, have been described and used in production of fermented meat products (Hugas et al., 1995; Berry et al., 1990). Campanini et al. (1993) found that the addition of starters prevented growth, but not always the survival of *Listeria*. As has been suggested, the application of bacteriocinogenic NTMS strains may provide an additional tool for preventing growth and survival of potentially pathogenic bacteria, and contribute to sensory qualities of fermented sausages. The present study demonstrates that an improved inhibition of pathogens may be achieved by several strains of lactobacilli, bifidobacteria and pediococci of human gastrointestinal origin. Future studies will investigate the mechanisms whereby the NTMS cultures inhibit these pathogens.

Previous studies have demonstrated that the incidence and severity of salmonellae infections is reduced in mice dosed with *B. longum* B22 (Henriksson and Conway, 2001), *L. paracasei* LAFTI™ L25 and L26 (Welin and Henriksson, unpublished observations). Hence, these three strains have a potential for use as probiotics in humans. Probiotic strains may be used in fermentation of salami and thereby contribute to protection against infection by pathogens that may be present on consumption of the product. It has been suggested that for a probiotic strain to be active in the GI tract, it should be consumed in a viable state (Fuller, 1989). Therefore, the survival of probiotic and other NTMS cultures in sausage batter was monitored throughout fermentation and maturation. In the present study, levels of NTMS strains were found to increase during fermentation, and to remain constant throughout maturation. By day 42, the majority of the strains remained at levels of 10^6 cfu/g or more. These observations are in line with those of Sameshima et al. (1998), who reported that a range of lactobacillus of intestinal origin survived during fermentation of sausage batter. Results presented here indicate that the fermented meat product may serve as a vehicle for delivery of probiotics to the GI tract in humans.

Cultures other than traditional meat starter cultures could have a negative impact on the sensory properties of the product. The sensory properties and acceptability of sausages fermented with *L. rhamnosus* are dependent on the particular strain used in the fermentation process (Erkkilä et al., 2001). In a sensory trial conducted at Don Smallgoods, *L. acidophilus* L10, *L. paracasei* L26 and *B. lactis* B94 were applied to salami in conjunction with the traditional meat starter culture. It was concluded that none of the strains impacted negatively on the sensory properties of commercially produced salami (Graeme Kentish, personal communication).

Traditional starter cultures are necessary to achieve the desired fermentation parameters specific for the product type; however, the implementation of other cultures with more significant pathogen inhibitory activities would contribute to increased product safety. In conclusion, this study reports that a range of LAB cultures of dairy product origin and human GI origin may be added to the formulation of fermented sausages, leading to a potentially safer product with no adverse effect on quality. Future studies will investigate if probiotic NTMS cultures provide protection against infection in an animal model, where the animals are fed Hungarian salami contaminated with these pathogens.

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