



ELSEVIER

International Journal of Food Microbiology 80 (2003) 89–97

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Leuconostoc gasicomitatum is the dominating lactic acid bacterium in retail modified-atmosphere-packaged marinated broiler meat strips on sell-by-day

Tuija Susiluoto*, Hannu Korkeala, K. Johanna Björkroth

Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki,
P.O. Box 57, FIN-00014 Helsinki, Finland

Accepted 15 March 2002

Abstract

Lactic acid bacteria (LAB) in retail, modified-atmosphere-packaged (MAP), marinated broiler meat strips on sell-by-day were mainly identified as *Leuconostoc gasicomitatum*. A total of 32 packages, three to five packages of seven differently marinated broiler meat products, were studied at the end of the producer-defined shelf life (at 6 °C, 7–9 days depending on the manufacturer). Prior to the microbiological analyses, appearance and smell of the product was checked and pH measured. Bacteria were cultured on MRS and Tomato Juice Agar (TJA), Rogosa SL agar (SLA), Plate Count Agar (PCA) and Streptomycin Thallium Acetate Agar (STAA) for the enumeration of LAB, lactobacilli, total bacterial count and *Brochothrix thermosphacta*, respectively. The average CFU/g of the 32 packages was 2.3×10^8 on PCA. The highest bacterial average, 3.1×10^8 , was recovered on TJA, the corresponding CFU/g averages on MRS and SLA being 2.3×10^8 and 1.3×10^8 , respectively. Despite the high LAB numbers detected, radical spoilage changes such as unpleasant odor, slime production and formation of gas were not seen. *B. thermosphacta* did not form a significant part of the bacterial population since none of the levels exceeded the spoilage threshold level of 10^5 CFU/g reported in previous studies for this organism. In order to characterize the dominating LAB population, as many as 85, 85 and 88 colonies from MRS, TJA and SLA, respectively, were randomly picked and cultured pure. LAB were identified to species level using a 16 and 23S rDNA *Hind*III RFLP (ribotyping) database. Fifty-six of the 170 isolates picked from the non-selective LAB media (MRS and TJA) were identified as *L. gasicomitatum*, followed by *Carnobacterium divergens* (41 isolates), *Lactobacillus sakei* and *Lactobacillus curvatus* subsp. *melibiosus* (31 isolates) and *L. curvatus* subsp. *curvatus* (20 isolates) species. SLA proved not to be completely selective for lactobacilli because the growth of *Leuconostoc* spp. was not inhibited, *Carnobacterium* spp. were the only species not detected on SLA. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Lactic acid bacteria; *Leuconostoc gasicomitatum*; MAP; Broiler; Spoilage; Ribotyping

1. Introduction

Lactic acid bacteria (LAB) and *Brochothrix thermosphacta* are the main spoilage organisms in cold-stored, vacuum or modified atmosphere packaged

* Corresponding author. Tel.: +358-9-19149701; fax: +358-9-19149718.

E-mail address: tuija.susiluoto@helsinki.fi (T. Susiluoto).

(MAP) meat and meat products (Egan, 1983; Korkeala et al., 1987; von Holy et al., 1991; Borch et al., 1996, a review; Jay, 1996; Taylor, 1996; Hansen and Bautista, 2000; Nychas and Drosinos, 2000). *Lactobacillus* spp., *Leuconostoc* spp. and *Carnobacterium* spp. have mainly been associated with the spoilage of these products (Schillinger and Lücke, 1987; Korkeala et al., 1988; Borch and Agerhem, 1992; Dainty and Mackey, 1992; Borch et al., 1996, a review; Björkroth et al., 1998). Raw, MAP broiler (young fowl) meat products are consumed increasingly in Finland for they are low in fat and easy to cook. In 2000, 13.3 kg of poultry meat were consumed per capita in Finland, which is about 50% more than in 1990. Most of the raw broiler meat is sold MAP and marinated in Finland. Marinating means here mixing the meat with water-oil-based, spiced sauces and these products have a wide diversity varying from carcass cuts to skinned fillet strips. Consumers can use the marinated products as such without further spicing and this results in a very convenient way of making a meal.

Despite the increasing popularity of MAP, marinated broiler meat products, only few studies of the levels and species of LAB (Björkroth et al., 2000) or *B. thermosphacta* in these products have been carried out. Tomato based products, such as many of the marinades, have generally been found susceptible to spoilage by LAB (Juven and Weisslowitch, 1981; Porretta and Vicini, 1993; Björkroth and Korkeala, 1997). A severe LAB spoilage was faced in a tomato-marinated broiler meat product packaged under CO₂ (80%) and N₂ (20%) (Björkroth, et al., 2000). These tomato marinated broiler meat strips were spoiled in 5–6 days at 6 °C by a LAB population which produced unpleasantly smelling compounds, and also formed large amounts of gas. This resulted in severe bulging of the packages and withdrawal of the product from the market. A novel LAB species was found to predominate in these spoiled products and it was named as *Leuconostoc gasicomitatum* (Björkroth et al., 2000). This name was validated in 2001 (Anonymous).

The purpose of this study was to determine which LAB species dominate the microflora of MAP, marinated broiler meat strips in retail products which do not yet show clear spoilage changes at the end of the producer-defined shelf life. The microbial quality was

as well evaluated and different media suitable for the determination of LAB levels were compared. *L. gasicomitatum* strains were found to dominate in these products and *Carnobacterium divergens*, *Lactobacillus sakei* subsp. *sakei* and *Lactobacillus curvatus* subsp. *curvatus* were the next common species detected. LAB levels were high on all three selective media used whereas *B. thermosphacta* did not form a significant part of the bacterial population.

2. Materials and methods

2.1. Packages

MAP broiler meat strips were purchased from retail dealers. The upper storage temperature limit for meat kept for sale in retail shops in Finland is 6 °C. After purchase, the packages were immediately brought to our laboratory and, if necessary, stored at 6 °C until the sell-by-day. Products from three different large-scale manufacturers were included. The typical MA for broiler meat containing 80% CO₂ and 20% N₂ had been used by all manufacturers. Altogether 32 packages of seven differently marinated products were studied, three to five packages of each product. The products consisted of skinned breast fillet, which had been mixed with the marinade and packaged under MA conditions. The marinades contained different spices but common ingredients in all were water, vegetable oil, sucrose or glucose, citric or acetic acid, sodium chloride (0.7–1.3%), potassium sorbate, sodium benzoate, xanthan gum and guar gum.

2.2. Sample preparation for the microbial analysis

Prior to the microbiological analysis, the odor, texture and pH of meat was checked and notice was paid to the amount of gas in each package. The purpose of checking of odor and appearance was to assure that none of the packages studied were showing clear signs of spoilage due to leaking etc and were therefore possessing typical late shelf life quality. On the sell-by-day (± 1 day), a sterile 22 g sample was homogenized in 198 ml of 0.1% peptone water using a Stomacher blender (NCFA, 1988). Serial 10-fold dilutions of the homogenized

samples were made from 10^{-2} to 10^{-8} in 0.1% peptone water.

2.3. Aerobic plate count

Aerobic plate count was done on Plate Count Agar (PCA) (Difco Laboratories, Detroit, USA) using the pour plate technique and the medium was incubated for 3 days in aerobic atmosphere in accordance with the NCF (1989) method. The plates were incubated at 25 °C as recommended for psychrotrophic organisms; incubation at 25 °C is adequate for both *Lactobacillus* and *Leuconostoc* genera (Batt, 2000; Lonvaud-Funel, 2000).

2.4. Enumeration of LAB

In order to show culture medium related effects on the enumeration of LAB, CFU/g was determined by parallel plating of the samples on MRS Agar (Oxoid, Basingstoke, England), Tomato Juice Agar (TJA, Oxoid) and Rogosa SL agar (SLA, Difco). MRS Agar supports the growth of all LAB (Reuter, 1985; Baird et al., 1987; Holzapfel, 1992, a review). TJA was chosen in order to show how LAB associated with marinades grow on a tomato-based medium. Many of these marinades contain tomato pure and tomato is known to stimulate the growth of some LAB (Garvie and Mabbitt, 1967). According to the manufacturer, SLA is a selective medium for culturing of lactobacilli. Acetic acid content and low pH in SLA is supposed to prevent the growth of other LAB (Reuter, 1985; Baird et al., 1987; Holzapfel, 1992, a review). The media were inoculated using the spread plate technique and all plates were incubated in an anaerobic CO₂ atmosphere (Anaerogen, Oxoid, 9–13% CO₂ according to the manufacturer) at 25 °C for 5–6 days.

2.5. Detection of *B. thermosphacta*

B. thermosphacta was detected using the Streptomycin Thallium Acetate Agar (STAA) medium (Oxoid). Plates were incubated at 25 °C for 2–3 days aerobically in accordance with the NCF (1991) method. *Pseudomonas* spp. were ruled out by performing the oxidase test (Baird et al., 1987; NCF, 1991) with 1% *N,N*-phenylenediammonium

dichloride-solution (Tamro Laboratories, Vantaa, Finland).

2.6. Selection and management of LAB for species identification

From each sample, one to three randomly picked colonies from the three LAB media (MRS, TJA and SLA) were tested for growth in MRS broth (Difco) at 25 °C for 2 days. Inoculation of all randomly selected colonies into MRS broth resulted in growth. MRS broth and agar were subsequently used for further propagation of the strains. A total of 85, 85 and 88 colonies from MRS, TJA and SLA media, respectively, were cultured pure and altogether 258 LAB isolates were subjected to the species-level identification. For DNA extraction, MRS broth was inoculated and the tubes were grown at 25 °C for 1–2 days depending on the growth rate. After incubation, cells harvested from 1.5 ml of MRS broth were used for DNA extraction. The isolates were stored in MRS broth at –70 °C.

2.7. 16 and 23S rDNA HindIII RFLP, ribotyping

DNA was isolated using a modified (Björkroth and Korkeala, 1996a,b) guanidium thiocyanate method of Pitcher et al. (1989). In this modification, the cell lysis solution contains mutanolysin (250 U/ml, Sigma, St. Louis, MO, USA) in addition to lysozyme (25 mg/ml, Sigma). Restriction endonuclease treatment of 8 µg of DNA was done using *Hind*III restriction enzyme (New England Biolabs, Beverly, MA, USA) as specified by the manufacturer. *Hind*III was chosen because it has been found to provide species-specific patterns for various spoilage LAB (Björkroth and Korkeala, 1996a, 1997; Björkroth et al., 1998, 2000). REA was performed as described before (Björkroth and Korkeala, 1996a,b). Genomic blots were made using a vacuum device (Vacugene, Pharmacia, Uppsala, Sweden) and the rDNA probe for ribotyping was labeled by reverse transcription (AMV-RT, Promega, Madison, WI, USA and Dig DNA Labeling Kit, Roche Molecular Biochemicals, Mannheim, Germany) as previously described by Blumberg et al. (1991). Membranes were hybridized at 58 °C overnight and the detection of the digoxigenin label was performed as recommended by Roche Molecular Biochemicals.

2.8. Numerical analysis of HindIII ribopatterns

The membranes were scanned with a Hewlett-Packard ScanJet 4c/T tabletop scanner (Boise, ID, USA). Numerical analysis of the ribopatterns was performed using the Bionumerics 2.0 software package (Applied Maths, Kortrijk, Belgium). Based on internal controls, 1.5% position tolerance and 0.5% optimization was allowed for the bands/patterns. The similarity between all pairs was expressed by Dice coefficient correlation, and the unweighted pair-group method with arithmetic averages (UPGMA) was used for the construction of the dendrogram. The ribopatterns were compared with the corresponding patterns in the LAB database at the Department of Food and Environmental Hygiene, University of Helsinki, Finland. It comprises patterns of all relevant spoilage LAB in the genera of *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, *Enterococcus* and *Weissella* (Björkroth and Korkeala, 1996b, 1997; Björkroth et al., 1998, 2000; Lyhs et al., 1999). Identification of the isolates was made on the basis of locations of the type strains in the clusters.

3. Results

In the 32 broiler meat packages, CFU/g of LAB on TJA, MRS and SLA varied between 10^6 and 10^8 CFU/g (Table 1). *B. thermosphacta* formed only a

minor part ($<10^5$ CFU/g in all samples) of the total flora. Five of the seven products studied showed growth at levels higher or close to 10^8 CFU/g. The pH of the samples was from 4.9 to 5.9, the average pH being 5.6 (Table 1). LAB were best recovered on TJA agar. When the average enumeration levels (CFU/g) of all 32 packages are compared, 57.1%, 25.3% and 24.1% less grew on SLA, PCA and MRS, respectively, than on TJA.

Based on the isolates obtained from the two non-selective media, MRS and TJA, *L. gasicomitatum* was the dominating LAB species in MAP, marinated broiler meat strips. These strains were detected in the products of all producers and in six of the seven marinades (Table 3). Fifty-six isolates possessed patterns (Ia to Id, Fig. 1) similar to or identical with the ribotype of *L. gasicomitatum* type strain. *L. gasicomitatum* cluster was homogenous and it could be divided into four subclusters consisting of strains (ribotypes Ia, Ib, Ic and Id) having a similarity higher than 80%. Most *L. gasicomitatum* strains possessed identical ribopatterns with the *L. gasicomitatum* type strain LMG 18811 (ribotype Ia). The two almost similar ribotypes Ib and Ic differed from the pattern of the type strain by one fragment only. Ribotype Id missed one fragment compared to pattern of the *L. gasicomitatum* type strain (Ia, Fig. 1). *L. gasicomitatum* strains had a similarity of 50% compared to the *Leuconostoc mesenteroides* cluster which diverged closest to the *L. gasicomitatum* cluster (Fig. 1). *L.*

Table 1

The averages of colony forming units (CFU) in each marinated product determined from the CFU/g results of three to five packages of seven differently marinated broiler meat strip products packaged under modified atmosphere

Type of product (no. of packages)	CFU/g (\log_{10}) average value				
	pH	MRS ^a	TJA ^b	PCA ^c	RA ^d
A1 (5)	5.2	8.8 ± 1.0	8.7 ± 1.0 ^c	8.7 ± 0.9	8.2 ± 1.0
A2 (5)	5.9	8.7 ± 0.5	8.9 ± 0.2	8.8 ± 0.1	8.7 ± 0.5
A3 (4)	5.2	6.3 ± 0.5	6.2 ± 0.5	6.3 ± 0.5	5.9 ± 0.5
B1 (5)	5.1	7.8 ± 0.2	8.1 ± 0.2	7.8 ± 0.2	6.5 ± 1.0
B2 (3)	5.8	8.2 ± 0.2	8.8 ± 0.2	7.9 ± 0.2	7.8 ± 0.4
B3 (5)	5.7	8.1 ± 0.6	8.4 ± 0.4	8.3 ± 0.3	7.8 ± 0.8
C1 (5)	5.5	6.2 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	6.0 ± 0.1
Total (32)	5.6	8.4 ± 1.1	8.5 ± 1.2	8.4 ± 1.1	8.1 ± 1.2

^a MRS Agar (Oxoid).

^b Tomato Juice Agar (Oxoid).

^c Plate Count Agar (Difco Laboratories).

^d Rogosa SL Agar (Difco).

^e The average of four samples.

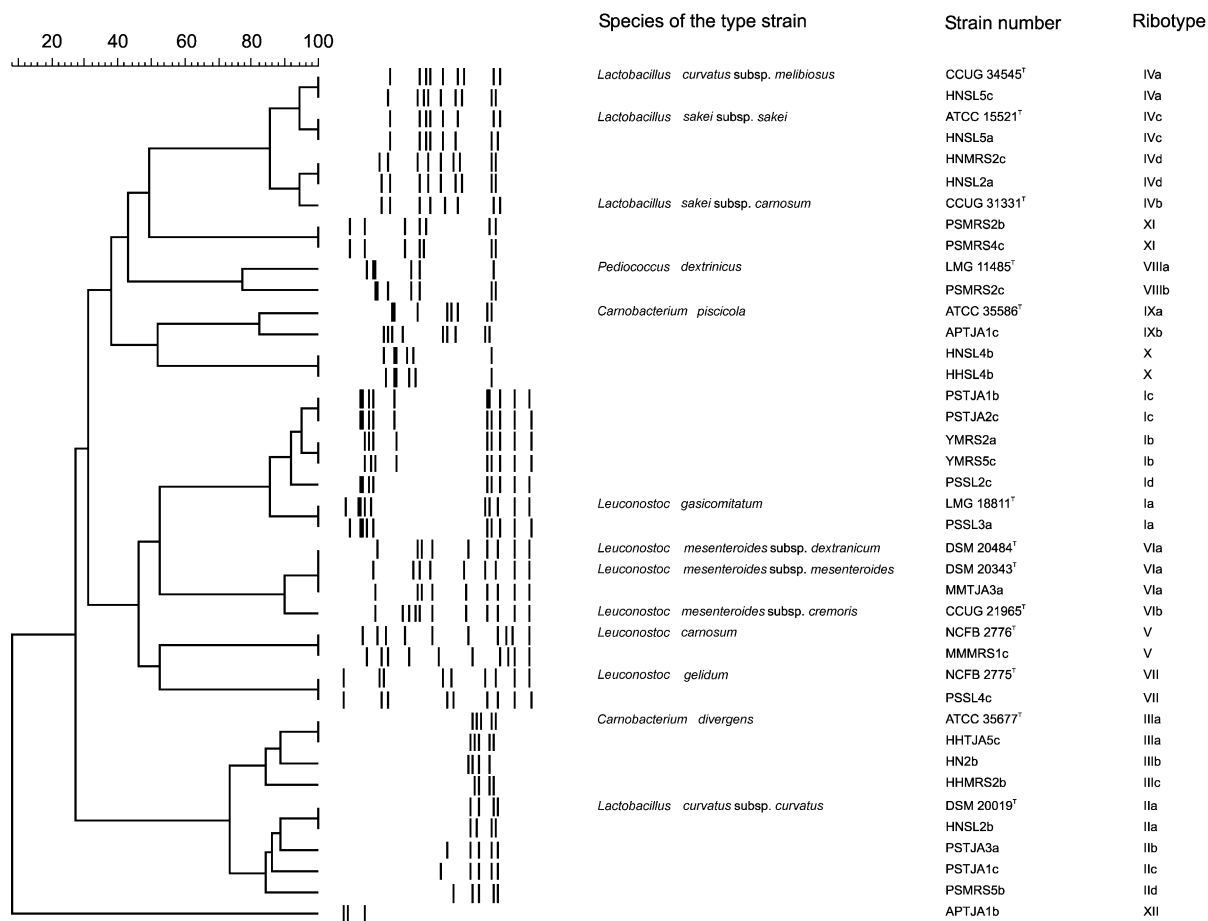


Fig. 1. Schematic patterns of *Hind*III ribopatterns and the numerical analysis of the patterns presented as a dendrogram. Left side of the banding patterns, high molecular masses <23 kbp and right the low molecular mass >1000 bp.

gasicomitatum strains were recovered more often from TJA (58.9% of *L. gasicomitatum* strains originated from TJA) than from MRS agar (41.1% of *L. gasicomitatum* strains originated from MRS) and they also grew on SLA (Table 2).

*Leuconostoc*s branched separately from the other LAB and were well demarked as their own genus (Fig. 1). Fourteen isolates from MRS and TJA possessed identical patterns with *Leuconostoc* ribotypes V, VI and VII (Fig. 1, Table 2). Two strains had identical ribopatterns with *L. mesenteroides* type strain (ribotype V), 10 strains with *Leuconostoc carnosum* type strain (ribotype VI) and 2 strains with *Leuconostoc gelidum* type strain (ribotype VII). *L. mesenteroides*, *L. carnosum* and *L. gelidum* strains

were found in five of the seven packages and they were also detected on SLA.

Fifty-three isolates from MRS and TJA possessed ribotypes IIa, IIb, IIc, IId, IVa, IVb, IVc and IVd (Fig. 1, Table 2) and were assigned to the genus *Lactobacillus*. Twenty-four isolates possessed typical *L. sakei* ribotypes IVb, IVc and IVd (Fig. 1) and 22 were assigned to *L. curvatus* species (Fig. 1) (1998, 2000;). Seven isolates shared identical pattern with *L. curvatus* subsp. *melibiosus* ribotype IVa. *L. curvatus* subsp. *melibiosus* ribotype IVa and *L. sakei* subsp. *sakei* ribotype IVc clustered together with a similarity over 90%, as reported before by Lyhs et al. (1999) who considered this species as misclassified. *L. sakei* subsp. *carosum* ribotype IVb was as well branched

Table 2
Numbers of LAB ribotypes obtained from different media

Ribotype	Number of isolates on media		
	MRS ^a	TJA ^b	RA ^c
Ia	17	29	11
Ib	4	1	–
Ic	2	3	–
Id	–	–	1
IIa	12	7	17
IIb	–	1	–
IIc	–	1	–
IId	1	–	–
IIIa	16	23	–
IIIb	–	1	–
IIIc	1	–	–
IVa	3	4	12
IVb	3	–	9
IVc	5	2	4
IVd	10	4	15
V	2	–	2
VI	4	6	9
VII	1	1	1
VIII	1	–	–
IX	–	1	–
X	–	–	5
XI	2	–	2
XII	–	1	–
XIII	1	–	–
Total	85	85	88

^a MRS Agar (Oxoid).

^b Tomato Juice Agar (Oxoid).

^c Rogosa SL Agar (Difco).

close to *L. sakei* subsp. *sakei* and *L. curvatus* subsp. *melibiosus* type strains. *L. curvatus* ribotypes IIa, IIb and IIc formed a clearly distinct cluster with a low level of similarity (20%) to the other LAB. Ribotype IVd was a common finding within the *L. sakei* subspecies. Isolates identical with the ribotype IVd had a one-band difference from *L. sakei* subsp. *carnosum* type strain (Fig. 1). Bacteria belonging to the genus *Lactobacillus* were detected in all packages studied (Table 3).

Forty-one isolates showed ribotypes IIIa, IIIb and IIIc similar to the ribotype of *C. divergens* type strain. The pattern of one isolate (ribotype IXb) was similar to the ribotype of *Carnobacterium piscicola* (IXa) (Fig. 1). Carnobacteria did not grow on SLA and were detected only in marinated products B1, B2 and B3 (Table 3). The species of four isolates on nonselective media and seven isolates on SLA remained unidentified. They possessed ribotypes X, XI, XII, XIII (Fig. 1).

4. Discussion

L. gasicomitatum was the dominating LAB species in MAP, marinated broiler meat products not yet showing signs of spoilage at the end of shelf life. LAB levels were relatively high, 10^6 – 10^8 CFU/g in all samples whereas the levels of *B. thermosphacta* did not exceed 10^5 CFU/g previously reported as the spoilage threshold limit (Holley, 2000). In previous

Table 3

LAB species division of 258 isolates originating from three to five packages of seven differently marinated broiler meat strip products packaged under modified atmosphere

Pack (no. of packs)	<i>Leuconostoc</i>			Other ^a			<i>Lactobacillus curvatus</i> and <i>Lactobacillus sakei</i>			<i>Carnobacterium</i> <i>divergens</i>			Other LAB ^b			Total				
	<i>gasicomitatum</i>			leuconostocs ^a									MRS TJA SLA			MRS TJA SLA				
	MRS	TJA	SLA	MRS	TJA	SLA	MRS	TJA	SLA	MRS	TJA	SLA	MRS	TJA	SLA	MRS	TJA	SLA		
A1	(5)	6	5	2	4	4	7	1	3	3	–	–	–	–	–	–	–	11	12	12
A2	(5)	9	13	3	–	2	2	6	–	10	–	–	–	–	–	–	–	15	15	15
A3	(4)	4	9	2	–	–	–	4	–	7	–	–	–	–	–	–	–	8	9	9
B1	(5)	–	2	3	2	–	2	–	–	7	12	12	–	–	–	1	14	14	13	13
B2	(3)	–	–	–	–	1	–	8	1	9	–	3	–	–	2	–	8	7	9	9
B3	(5)	1	1	–	–	–	–	9	5	11	5	9	–	–	–	4	15	15	15	15
C1	(5)	3	3	2	1	–	1	6	10	10	–	–	–	4	–	2	14	13	15	15
Total	(32)	23	33	12	7	7	12	34	19	57	17	24	–	4	2	7	85	85	88	88

Species identification performed using a 16S and 23S rRNA *Hind*III RFLP (ribotyping) database.

^a Includes *L. carnosum*, *L. mesenteroides* and *L. gelidum* strains.

^b Includes one isolate of *C. piscicola*, one isolate of *Pediococcus dextrinicus* and 11 isolates of unknown species.

studies, 50% CO₂ MA has inhibited the growth of *B. thermosphacta* (Holt et al., 1994; Holley, 2000). The initial CO₂ level in these products was 80% apparently creating unfavorable circumstances for *B. thermosphacta* or the contamination level with this organism was low at the first place. In addition to *L. gasicomitatum*, the typical meat spoiling lactobacilli, *L. sakei* and *L. curvatus* were frequently detected.

L. gasicomitatum species has been described by Björkroth et al. (2000) and it was assumed to be a specific spoilage organism in, MAP, tomato-marinated broiler meat strips showing a gaseous spoilage type. Higher numbers of *L. gasicomitatum* was detected in the spoiled samples (55.8% of isolates on MRS) than in these late shelf life samples (32.9% of isolates on MRS and TJA). Therefore, the growth of *L. gasicomitatum* in MAP marinated broiler meat strips is likely to result in spoilage and *L. gasicomitatum* can be considered as a specific spoilage organism in these products. *L. carnosum*, *L. mesenteroides* subsp. *mesenteroides* and *L. gelidum* strains were detected only in small amounts and they do not probably play a major role in the spoilage of marinated broiler meat products. In addition to the leuconostocs, *L. sakei* and *L. curvatus* strains were commonly (31.2% of the isolates on nonselective media) detected. These species were also associated with the spoiled tomato-marinated broiler product (Björkroth et al., 2000), where they formed 40% of the spoilage flora recovered from the MRS medium. Carnobacteria were not detected at all in the spoiled samples (Björkroth et al., 2000) whereas *C. divergens* was a common finding in these late shelf life samples although it was not found in all packages. According to Barakat et al. (2000), *C. divergens* belongs also to the microflora of cooked, refrigerated MAP poultry meat.

Egan (1983) stated that the number of LAB in a sample is usually equal to the number of bacteria on a nonselective media, such as PCA. In our study, somewhat lower CFU/g counts were obtained using PCA or MRS instead of TJA. The fact that *L. gasicomitatum* and other leuconostocs were more frequently recovered from TJA corresponds to the finding that the growth of some leuconostocs is supported by factors existing in tomato juice (Garvie and Mabbitt, 1967). In case of this leuconostoc-rich population, this may have been the reason for the detection of highest bacterial CFU levels on TJA. MRS does not appa-

rently support the growth of *L. gasicomitatum* strains as well as TJA during the initial recovery even the strains can later be cultured using this medium. It was also shown clearly that SLA is not a selective agar suitable for determining the number of *Lactobacillus* spp. All *Leuconostoc* species detected in this study were also recovered from SLA.

According to our study, relatively high levels of LAB are found in the marinated products already after 7–9 day's storage at 6 °C. Vacuum-packaged red meat and poultry products have shown spoilage when LAB levels reach 10⁷ (Korkeala et al., 1987; Von Holy et al., 1991; Sawaya et al., 1993). The expected shelf life for red meat products under refrigerated storage in a combined CO₂ and N₂ atmosphere is over weeks (Borch et al., 1996, a review). Results concerning the shelf life of un-marinated chicken products give a reason to suspect that also these products may have a longer shelf life than the marinated products (Gill et al., 1990; Kakouri and Nychas, 1994; Nychas and Tassou, 1997). LAB reached 1.8 × 10⁶ CFU/g in 100% CO₂-atmosphere when stored at 3 °C for 12 days (Nychas and Tassou, 1997). The chicken carcasses packaged under 100% CO₂ studied by Gill et al. (1990) had approximately the same total amount of bacteria than in our study. However, these carcasses (Gill et al., 1990) had been stored for four weeks at 3 °C. The carcasses were considered spoiled only in the seventh storage week, when the bacterial numbers had reached 6.4 × 10⁹ CFU/g (Gill et al., 1990). When a MAP tomato-marinated broiler meat strip product was showing gaseous spoilage, the LAB numbers had risen up to 10¹⁰ CFU/g (Björkroth et al., 2000).

Because high CFU/g values were obtained from the products possessing both high (in this case pH 5.9) or low (pH 4.9) pH values, no association of the pH with the microbial product quality can be made. The initial acidity of the marinades is high (about pH 4) but when combined together, meat buffers the low pH of the marinade quite rapidly. Compared to the spoilage of pork or beef products, the spoilage flora of marinated broiler meat seems to be quite different. *L. gasicomitatum* strains have yet been detected only in broiler meat products despite numerous studies of beef and pork products. When the product is marinated, the initial LAB contamination of broiler meat and the marinade is combined and the ecological

niche of the final product results from this combination. LAB growth and spoilage associated with marinated broiler meat is therefore a more complex event than in a “plain meat product”. Judging the spoilage changes in these products is also difficult. Even official sensory panel was not used in this study we noticed that it was difficult to evaluate the odour of the different products. Marinades apparently mask the early spoilage effects.

Both consumers and producers would benefit from better understanding of the effects the marinades have on the developing LAB population and shelf life of broiler meat products. Now when the species distribution in different marinated products is known, we are able to study further the interaction between various LAB and the role of the marinade ingredients in the spoilage. This will be aim of our further studies.

5. Conclusion

L. gasicomitatum was found to be the dominating LAB in retail, MAP, marinated broiler meat strips on sell-by day. In addition, *C. divergens*, *L. sakei* subsp. *sakei* and *L. curvatus* subsp. *curvatus* were the next common species detected. LAB levels were high whereas *B. thermosphacta* did not form a significant part of the bacterial population.

Acknowledgements

We would like to thank Ms. Henna Niinivirta for her excellent technical assistance.

References

- Anonymous, 2001. Validation list no. 79. *Int. J. Syst. Evol. Microbiol.* 51, 263–265.
- Baird, R.M., Corry, J.E.L., Curtis, G.D.W. (Eds.), 1987. *Pharmacopoeia of Culture Media for Food Microbiology*. De Man, Rogosa and Sharpe (MRS) Agar. *Int. J. Food Microbiol.*, vol. 5, pp. 228–229.
- Barakat, R.K., Griffiths, M.W., Harris, L.J., 2000. Isolation and characterization of *Carnobacterium*, *Lactococcus*, and *Enterococcus* spp. from cooked, modified atmosphere packaged, refrigerated, poultry meat. *Int. J. Food Microbiol.* 62, 83–94.
- Batt, C.A., 2000. *Lactobacillus*, introduction. In: Robinson, K., Batt, C.A., Patel, P.D. (Eds.), *Encyclopedia of Food Microbiology*, vol. 2. Academic Press, Bath, pp. 134–136.
- Björkroth, K.J., Korkeala, H.J., 1996a. Evaluation of *Lactobacillus sakei* contamination in vacuum-packaged sliced cooked meat products by ribotyping. *J. Food Prot.* 59, 398–401.
- Björkroth, K.J., Korkeala, H., 1996b. rRNA gene restriction patterns as a characterization tool for *Lactobacillus sakei* strains producing ropy slime. *Int. J. Food Microbiol.* 30, 293–302.
- Björkroth, K.J., Korkeala, H.K., 1997. *Lactobacillus fructivorans* spoilage of tomato ketchup. *J. Food Prot.* 60, 505–509.
- Björkroth, K.J., Vandamme, P., Korkeala, H.J., 1998. Identification and characterization of *Leuconostoc carnosum*, associated with production and spoilage of vacuum-packaged, sliced, cooked ham. *Appl. Environ. Microbiol.* 64, 3313–3319.
- Björkroth, K.J., Geisen, R., Schillinger, U., Weiss, N., de Vos, P., Holzapfel, W.H., Korkeala, H.J., Vandamme, P., 2000. Characterisation of *Leuconostoc gasicomitatum* sp. nov. associated with spoiled raw tomato-marinated broiler meat strips packaged under modified atmosphere conditions. *Appl. Environ. Microbiol.* 66, 3764–3772.
- Borch, E., Agerhem, H., 1992. Chemical, microbial and sensory changes during the anaerobic cold storage of beef inoculated with a homofermentative *Lactobacillus* sp. or a *Leuconostoc* sp. *Int. J. Food Microbiol.* 15, 99–108.
- Borch, E., Kant-Muermans, M.-L., Blixt, Y., 1996. Bacterial spoilage of meat and cured meat products. A review. *Int. J. Food Microbiol.* 33, 103–120.
- Dainty, R.H., Mackey, B.M., 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *J. Appl. Bacteriol.* 73, 103S–114S.
- Egan, A.F., 1983. Lactic acid bacteria of meat and meat products. *Antonie van Leeuwenhoek* 49, 327–336.
- Garvie, E., Mabbitt, L.A., 1967. Stimulation of the growth of *Leuconostoc oenos* by tomato juice. *Arch. Mikrobiol.* 55, 398–407.
- Gill, C.O., Harrison, J.C.L., Penney, N., 1990. The storage life of chicken carcasses packaged under carbon dioxide. *Int. J. Food Microbiol.* 11, 151–158.
- Hansen, K.M.J., Bautista, D.A., 2000. Spoilage problems, problems caused by bacteria. In: Robinson, K., Batt, C.A., Patel, P.D. (Eds.), *Encyclopedia of Food Microbiology*, vol. 3. Academic Press, Bath, pp. 2051–2056.
- Holley, R.A., 2000. *Brochothrix*. In: Robinson, K., Batt, C.A., Patel, P.D. (Eds.), *Encyclopedia of Food Microbiology*, vol. 1. Academic Press, Bath, pp. 314–318.
- Holzapfel, W.H., 1992. Culture media for non-sporulating gram-positive food spoilage bacteria. A review. *Int. J. Food Microbiol.* 17, 113–133.
- Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., 1994. *Bergeys Manual of Determinative Bacteriology*, 9th edn. Williams and Wilkins, Baltimore, p. 787.
- Jay, J.M., 1996. *Modern Food Microbiology*, 5th edn. Chapman & Hall, New York, p. 661.
- Juven, B., Weisslowitch, H., 1981. Chemical changes in tomato juices caused by lactic acid bacteria. *J. Food Sci.* 46, 1543–1545.
- Kakouri, A., Nychas, G.J.E., 1994. Storage of poultry meat under modified atmospheres or vacuum-packs. Possible role of micro-

- bial metabolites as indicator of spoilage. *J. Appl. Bacteriol.* 76, 163–172.
- Korkeala, H., Ahvenainen, R., Alanko, T., 1987. Interrelationship between microbial numbers and other parameters in the spoilage of vacuum-packed cooked sausages. *Int. J. Food Microbiol.* 5, 311–321.
- Korkeala, H., Suortti, T., Mäkelä, P., 1988. Ropy slime formation in vacuum-packed cooked meat products caused by homofermentative lactobacilli and *Leuconostoc* species. *Int. J. Food Microbiol.* 7, 339–347.
- Lonvaud-Funel, A., 2000. *Leuconostoc*. In: Robinson, K., Batt, C.A., Patel, P.D. (Eds.), *Encyclopedia of Food Microbiology*, vol. 2. Academic Press, Bath, pp. 1183–1194.
- Lyhs, U., Björkroth, J., Korkeala, H., 1999. Characterisation of lactic acid bacteria from spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping. *Int. J. Food Microbiol.* 52, 77–84.
- NCFA, 1988. Nordic Committee on Food Analysis. Pretreatment of foods for microbiological examination. Method no. 91.
- NCFA, 1989. Nordic Committee on Food Analysis. Psychrotrophic microorganisms. Determination by the plate count method. Proposed method no. 74. 2.
- NCFA, 1991. Nordic Committee on Food Analysis. *Brochothrix thermosphacta*, determination in meat and meat products. Method no. 141.
- Nychas, G.J.E., Drosinos, E.H., 2000. Spoilage of meat. In: Robinson, K., Batt, C.A., Patel, P.D. (Eds.), *Encyclopedia of Food Microbiology*, vol. 2. Academic Press, Bath, pp. 1253–1260.
- Nychas, G.J.E., Tassou, C.C., 1997. Spoilage processes and proteolysis in chicken as detected by HPCL. *J. Sci. Food Agric.* 74, 199–208.
- Pitcher, D.G., Saunders, N.A., Owen, R.J., 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol.* 8, 151–156.
- Porretta, S., Vicini, E., 1993. Changes in tomato pulp quality caused by lactic acid bacteria. *Int. J. Food Sci. Technol.* 28, 611–616.
- Reuter, G., 1985. Elective and selective media for lactic acid bacteria. *Int. J. Food Microbiol.* 2, 55–68.
- Sawaya, W.N., Abu-Ruwaida, A.S., Hussain, A.J., Khalafawi, M.S., Dashti, B.H., 1993. Shelf-life of vacuum-packaged eviscerated broiler carcasses under stimulated market conditions. *J. Food Saf.* 13, 305–321.
- Schillinger, U., Lücke, F.K., 1987. Lactic acid bacteria on vacuum-packaged meat and their influence on shelf life. *Fleischwirtsch* 67, 1244–1248.
- Taylor, S.A., 1996. Modified atmosphere packing of meat. In: Taylor, S.A., Raimundo, A., Severini, M., Smulders, J.M. (Eds.), *Meat Quality and Meat Packaging*. J. M. ECCEAMST, Utrecht, pp. 301–308.
- von Holy, A., Cloete, T.E., Holzapfel, W.H., 1991. Quantification and characterisation of microbial populations associated with spoiled, vacuum-packed vienna sausages. *Food Microbiol.* 8, 95–104.