

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

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12 Key words

13 Lactic acid bacteria, *Leuconostoc gasicomitatum*, MAP, broiler, spoilage, ribotyping

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18

1 Abstract

2

3 Lactic acid bacteria (LAB) in retail, modified-atmosphere-packaged (MAP), marinated broiler meat  
4 strips on sell-by day were mainly identified as *Leuconostoc gasicomitatum*. A total of 32 packages,  
5 3 to 5 packages of 7 differently marinated broiler meat products, were studied at the end of the  
6 producer-defined shelf life (at 6°C, 7 to 9 days depending on the manufacturer). Prior to the  
7 microbiological analyses, appearance and smell of the product was checked and pH measured.  
8 Bacteria were cultured on MRS and Tomato Juice Agar (TJA), Rogosa SL agar (SLA), Plate Count  
9 Agar (PCA) and Streptomycin Thallium Acetate Agar (STAA) for the enumeration of LAB,  
10 lactobacilli, total bacterial count and *B. thermosphacta*, respectively. The average CFU/g of the 32  
11 packages was  $2.3 \times 10^8$  on PCA. The highest bacterial average,  $3.1 \times 10^8$ , was recovered on TJA,  
12 the corresponding CFU/g averages on MRS and SLA being  $2.3 \times 10^8$  and  $1.3 \times 10^8$ , respectively.  
13 Despite the high LAB numbers, radical spoilage changes such as unpleasant odor, slime production  
14 and formation of gas were not recognized. *B. thermosphacta* did not form a significant part of the  
15 bacterial population since none of the levels exceeded the spoilage threshold level of  $10^5$  CFU/g  
16 reported in previous studies. In order to characterize the dominating LAB population, as many as  
17 85, 85 and 88 colonies from MRS, TJA and SLA, respectively, were randomly picked and cultured  
18 pure. LAB were identified to species level using a 16 and 23S rDNA *Hind*III RFLP (ribotyping)  
19 database. Fifty-six of the 170 isolates picked from the non-selective LAB media (MRS and TJA)  
20 were identified as *Leuconostoc gasicomitatum*, followed by *Carnobacterium divergens* (41  
21 isolates), *Lactobacillus sakei* and *Lactobacillus curvatus* subsp. *melibiosus* (31 isolates) and  
22 *Lactobacillus curvatus* subsp. *curvatus* (20 isolates) species. SLA proved not to be completely  
23 selective for lactobacilli because the growth of *Leuconostoc* spp. was not inhibited, *Carnobacterium*  
24 spp. were the only species not detected on SLA.

25

## 1. Introduction

Lactic acid bacteria (LAB) and *Brochothrix thermosphacta* are the main spoilage organisms in vacuum or modified atmosphere packaged (MAP) meat and meat products (Egan, 1983; Korkeala, 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). 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 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 which 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 *Brochothrix 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 Weisslowich, 1981; Porretta and Vicini, 1993; Björkroth and Korkeala, 1997). A severe LAB spoilage was faced in a tomato-marinated broiler meat product packaged under CO<sub>2</sub> (80%) and N<sub>2</sub> (20%) (Björkroth, et al. 2000). These tomato marinated broiler meat strips were spoiled in 5 to 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*. 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.

1 *sakei* and *Lactobacillus curvatus* subsp. *curvatus* were the next common species detected. LAB  
2 levels were high on all three selective media used whereas *B. thermosphacta* did not form a  
3 significant part of the bacterial population.

## 4 5 2. Materials and methods

### 6 7 2.1 Packages

8 MAP broiler meat strips were purchased from retail dealers. The upper limit for storage temperature  
9 of meat kept for sale in retail shops in Finland is 6°C. After purchase the packages were  
10 immediately brought to our laboratory and, if necessary, stored at 6°C until the sell-by-day.  
11 Products from three different large-scale manufacturers were included. The typical MA for broiler  
12 meat containing 80% CO<sub>2</sub> and 20% N<sub>2</sub> had been used by all manufacturers. Altogether 32 packages  
13 of 7 differently marinated products were studied, 3 to 5 packages of each product. The products  
14 consisted of skinned breast fillet, which had been mixed with the marinade and packaged under MA  
15 conditions. The marinades contained different spices but common ingredients in all were water,  
16 vegetable oil, sucrose or glucose, citric or acetic acid, sodium chloride (0.7 to 1.3%), potassium  
17 sorbate, sodium benzoate, xanthan gum and guar gum.

### 18 19 2.2 Sample preparation for the microbial analysis

20 Prior to the microbiological analysis, the odor, texture and pH of meat was checked and notice was  
21 paid to the amount of gas in each package. The purpose of checking of odor and appearance was to  
22 assure that none of the packages studied were showing clear signs of spoilage due to leaking etc and  
23 were therefore possessing typical late shelf life quality. On the sell-by day ( $\pm$  1d) a sterile 22g  
24 sample was homogenized in 198g of 0.1% peptone water using a Stomacher blender (NCFA, 1988).  
25 Serial 10-fold dilutions of the homogenized samples were made from 10<sup>-2</sup> to 10<sup>-8</sup> in 0.1% peptone  
26 water.

### 27 28 2.3 Aerobic plate count

29 Aerobic plate count was done on Plate Count Agar (PCA) (Difco laboratories, Detroit, USA) using  
30 the pour plate technique and the medium was incubated for 3d in aerobic atmosphere in accordance  
31 with the NCFA method (1989) with the exception of incubating the plates at 25°C. Incubation at

1 25°C is adequate for both *Lactobacillus* and *Leuconostoc* genera (Batt, 2000; Lonvaud-Funel,  
2 2000).

3

#### 4 *2.4 Enumeration of LAB*

5 In order to show culture medium related effects on the enumeration of LAB, CFU/g was determined  
6 by parallel plating of the samples on MRS Agar (Oxoid, Basingstoke, England), Tomato Juice Agar  
7 (TJA, Oxoid) and Rogosa SL agar (SLA, Difco). MRS Agar supports the growth of all LAB  
8 (Reuter, 1985; Baird et al., 1987; Holzapfel, 1992, a review). TJA was chosen to in order to show  
9 how LAB associated with marinades grow on a tomato-based medium. Many of these marinades  
10 contain tomato pure and tomato is known to stimulate the growth of some LAB (Garvie and  
11 Mabbitt, 1967). According to the manufacturer, SLA is a selective medium for culturing of  
12 lactobacilli. Acetic acid content and low pH in SLA is supposed to prevent the growth of other LAB  
13 (Reuter, 1985; Baird et al., 1987; Holzapfel, 1992, a review). The media were inoculated using the  
14 spread plate technique and all plates were incubated in an anaerobic CO<sub>2</sub> atmosphere (Anaerogen,  
15 Oxoid, 9-13% CO<sub>2</sub> according to the manufacturer) at 25°C for 5 to 6 days.

16

#### 17 *2.5 Detection of B. thermosphacta*

18 *B. thermosphacta* was detected using the Streptomycin Thallium Acetate Agar (STAA) medium  
19 (Oxoid, Basingstoke, England). Plates were incubated at 25°C for 2 to 3 days aerobically in  
20 accordance with the NCFA method (1991). *Pseudomonas* spp. was ruled out by performing the  
21 oxidase test (Baird et al., 1987; NCFA, 1991) with 1% N,N-phenylenediammonium dichloride-  
22 solution (Tamro Lab, Vantaa, Finland).

23

#### 24 *2.6 Selection and management of LAB for species identification*

25 From each sample, 1 to 3 randomly picked colonies from the three LAB media (MRS, TJA and  
26 SLA) were tested for growth in MRS broth (Difco) at 25°C for one to two days. Inoculation of all  
27 randomly selected colonies into MRS broth resulted in growth. MRS broth and agar were  
28 subsequently used for further propagation of the strains. A total of 85, 85 and 88 colonies from  
29 MRS, TJA and SLA media, respectively, were cultured pure. Altogether 258 LAB isolates were  
30 submitted to species level identification. For DNA extraction, MRS broth was inoculated and the  
31 tubes were grown at 25°C for one to two days depending on the growth rate. After incubation, cells  
32 harvested from 1.5 ml of MRS broth were used for DNA extraction. The isolates were stored in  
33 MRS broth at -70°C.

1

## 2 2.7 16 and 23S rDNA HindIII RFLP, Ribotyping

3 DNA was isolated using a modified (Björkroth and Korkeala, 1996) guanidium thiocyanate method  
4 of Pitcher et al. (1989). In this modification, the cell lysis solution contains mutanolysin (250 U/ml,  
5 Sigma, St. Louis, Missouri) in addition to lysozyme (25 mg/ml, Sigma). Restriction endonuclease  
6 treatment of 8µg of DNA was done using *HindIII* restriction enzyme (New England Biolabs,  
7 Beverly, Mass., USA) as specified by the manufacturer. *HindIII* was chosen because it has been  
8 found to provide species-specific patterns for various spoilage LAB (Björkroth and Korkeala,  
9 1996a, 1997; Björkroth et al., 1998, 2000). REA was performed as described before (Björkroth and  
10 Korkeala, 1996). Genomic blots were made using a vacuum device (Vacugene, Pharmacia,  
11 Uppsala, Sweden) and the rDNA probe for ribotyping was labeled by reverse transcription (AMV-  
12 RT, Promega, Madison, Wisc., USA and Dig DNA Labeling Kit, Roche Molecular Biochemicals,  
13 Mannheim, Germany) as previously described by Blumberg et al. (1991). Membranes were  
14 hybridized at 58°C overnight and the detection of the digoxigenin label was performed as  
15 recommended by Roche Molecular Biochemicals.

16

## 17 2.8 Numerical analysis of HindIII ribopatterns

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

29

## 30 3. Results

31

32 In the 32 broiler meat packages, CFU/g of LAB on TJA, MRS and SLA varied between  $10^6$  and  $10^8$   
33 CFU/g (Table 1). *B. thermosphacta* formed only a minor part ( $<10^5$  CFU/g in all samples) of the

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

5  
6 The dominating LAB species in MAP, marinated broiler meat strips was *L. gasicomitatum* and  
7 these strains were detected in the products of all producers and in six of the seven marinades (Table  
8 2). Fifty-six isolates possessed patterns (Ia to Id, Fig. 1) similar to or identical with the ribotype of  
9 *L. gasicomitatum* type strain. *L. gasicomitatum* cluster was homogenous and it could be divided into  
10 four subclusters consisting of strains (ribotypes Ia, Ib, Ic and Id) having a similarity higher than  
11 80%. Most *L. gasicomitatum* strains possessed identical ribopatterns with the *L. gasicomitatum* type  
12 strain LMG 18811 (ribotype Ia). The two almost similar ribotypes Ib and Ic differed from the type  
13 strain Ia by one fragment only. Ribotype Id missed one fragment compared to pattern of the *L.*  
14 *gasicomitatum* type strain (Ia, Fig. 1). *L. gasicomitatum* strains had a similarity of 50% compared to  
15 the *Leuconostoc mesenteroides* cluster which diverged closest to the *L. gasicomitatum* cluster (Fig  
16 1.). Initially, *L. gasicomitatum* strains were recovered more often from TJA (58,9% of *L.*  
17 *gasicomitatum* strains originated from TJA) than from MRS agar (41.1% of *L. gasicomitatum*  
18 strains originated from MRS). *L. gasicomitatum* strains also grew on SLA.

19  
20 *Leuconostocs* branched separately from the other LAB and were well demarked as their own genus  
21 (Fig. 1). Fourteen isolates possessed identical patterns with *Leuconostoc* ribotypes V, VI and VII  
22 (Fig. 1, Table 3). Two strains had identical ribopatterns with *L. mesenteroides* type strain (ribotype  
23 V), 10 strains with *Leuconostoc carnosum* type strain (ribotype VI) and 2 strains with *Leuconostoc*  
24 *gelidum* type strain (ribotype VII). *L. mesenteroides*, *L. carnosum* and *L. gelidum* strains were  
25 found in five of the seven packages and they were also detected on SLA.

26  
27 At the genus level, lactobacilli were the most prevalent and as many as 53 isolates possessed  
28 ribotypes IIa, IIb, IIc, IId, IVa, IVb, IVc and IVd (Fig. 1, Table 3). The patterns of 24 isolates were  
29 identical with *L. sakei* ribotypes IVb, IVc and IVd, 22 were identical with *L. curvatus* subsp.  
30 *curvatus* ribotypes IIa, IIb, IIc and IId and 7 were identical with *L. curvatus* subsp. *melibiosus*  
31 ribotype IVa (Table 3). *L. curvatus* subsp. *melibiosus* ribotype IVa and *L. sakei* subsp. *sakei*  
32 ribotype IVc clustered together with a similarity over 90%, as reported before by Lyhs et al. (1999).  
33 *L. sakei* subsp. *carnosum* ribotype IVb was as well branched close to *L. sakei* subsp. *sakei* and  
34 *L. curvatus* subsp. *melibiosus* type strains. *L. curvatus* subsp. *curvatus* ribotypes IIa, IIb and IIc

1 formed a clearly distinct cluster with a low level of similarity (20%) to the other LAB. Ribotype  
2 IVd was a common finding within the *L. sakei* subspecies. Isolates identical with the ribotype IVd  
3 had a one-band difference to *L. sakei* subsp. *carnosum* type strain (Fig 1.). Bacteria belonging to the  
4 genus *Lactobacillus* were detected in all packages studied (Table 2).

5  
6 Forty-one isolates showed ribotypes IIIa, IIIb and IIIc similar to the ribotype of *C. divergens* type  
7 strain. The pattern of one isolate (ribotype IXb) was similar to the ribotype of *Carnobacterium*  
8 *piscicola* (IXa) (Fig 1, Table 3). Carnobacteria did not grow on SLA and were detected only in  
9 marinated products B1, B2 and B3 (Table 2). The species of four isolates on nonselective media and  
10 seven isolates on SLA remained unidentified. They possessed ribotypes X, XI, XII, XIII (Fig. 1).

#### 11 12 4. Discussion

13  
14 *L. gasicomitatum* was the dominating LAB species in MAP, marinated broiler meat products not  
15 yet showing signs of spoilage at the end of shelf life. LAB levels were relatively high,  $10^6$  to  $10^8$   
16 CFU/g in all samples whereas the levels of *B. thermosphacta* did not exceed  $10^5$  CFU/g previously  
17 reported as the spoilage threshold level (Holley, 2000). Fifty-% carbon dioxide MA inhibits the  
18 growth of *B. thermosphacta* (Holt et al., 1994; Holley, 2000). The initial CO<sub>2</sub> level in these products  
19 was 80% apparently providing unfavorable circumstances for *B. thermosphacta* or the  
20 contamination level with this organism was low in these products. In addition to *L. gasicomitatum*,  
21 *Lactobacillus sakei* and *curvatus* spp. were frequently detected.

22  
23 *L. gasicomitatum* species has been described by Björkroth et al. (2000) and it was assumed to be a  
24 specific spoilage organism in, MAP, tomato-marinated broiler meat strips showing a gaseous  
25 spoilage type. Higher numbers of *L. gasicomitatum* was detected in the spoiled samples (55,8% of  
26 isolates on MRS) than in these late shelf life samples (32,9% of isolates on MRS and TJA).  
27 Therefore, the growth of *L. gasicomitatum* in MAP marinated broiler meat strips is likely to result  
28 in spoilage and *L. gasicomitatum* can be considered as a specific spoilage organism in these  
29 products. *L. carnosum*, *L. mesenteroides* subsp. *mesenteroides* and *L. gelidum* strains were detected  
30 only in small amounts and they do not probably play a major role in the spoilage of marinated  
31 broiler meat products. In addition to the leuconostocs, *L. sakei* and *L. curvatus* strains were  
32 commonly (31.2% of the isolates on nonselective media) detected. These species were also  
33 associated with the spoiled tomato-marinated broiler product (Björkroth et al. 2000), where they

1 formed 40% of the spoilage flora recovered from MRS medium. Carnobacteria were not detected at  
2 all in the spoiled samples (Björkroth et al. 2000) whereas *C. divergens* was a common finding in  
3 these late shelf life samples although it was not found in all packages. According to Barakat et al.  
4 (2000), *C. divergens* belongs also to the microflora of cooked, refrigerated MAP poultry meat.

5  
6 Egan (1983) stated that the number of LAB in a sample is usually equal to the number of bacteria  
7 on a nonselective media, such as PCA. In our study, somewhat lower CFU/g counts were obtained  
8 using PCA or MRS instead of TJA. The fact that *L. gasicomitatum* and other leuconostocs were  
9 more frequently recovered from TJA corresponds to the finding that the growth of some  
10 leuconostocs is supported by factors existing in tomato juice (Garvie and Mabbitt, 1967). In case of  
11 this leuconostoc-rich population this may have been the reason for the detection of highest bacterial  
12 CFU levels on TJA. MRS does not apparently support the growth of all *L. gasicomitatum* strains  
13 during the initial recovery even the strains can later be cultivated using this medium. It was also  
14 shown clearly that SLA is not a selective agar suitable for determining the number of *Lactobacillus*  
15 spp. All *Leuconostoc* species detected in this study were also recovered from SLA.

16  
17 According to our study, relatively high levels of LAB are found in the marinated products already  
18 after 7 to 9 day's storage, during which marinated MAP broiler meat strips are to be sold. Vacuum  
19 packaged red meat and poultry products have shown spoilage when LAB levels reach  $10^7$  (Korkeala  
20 et al., 1987; Von Holy et al., 1991; Sawaya et al., 1993). The expected shelf life for red meat  
21 products under refrigerated storage in a combined CO<sub>2</sub> and N<sub>2</sub> atmosphere is over weeks (Borch et  
22 al., 1996, a review). Results concerning the shelf life of un-marinated chicken products give a  
23 reason to suspect that also these products have a longer shelf life than the marinated products (Gill  
24 et al., 1990; Kakouri and Nychas, 1994; Nychas and Tassou, 1997). LAB reached only  $1.8 \times 10^6$   
25 CFU/g in 100% CO<sub>2</sub>-atmosphere when stored at 3°C for 12 days (Nychas and Tassou, 1997). The  
26 chicken carcasses packaged under 100% CO<sub>2</sub> studied by Gill et al. (1990) had approximately the  
27 same total amount of bacteria than in our study. However, these carcasses (Gill et al., 1990) had  
28 been stored for four weeks at 3°C. The carcasses were considered spoiled only in the seventh  
29 storage week, when the bacterial numbers had reached  $6.4 \times 10^9$  CFU/g (Gill et al., 1990). When a  
30 MAP tomato-marinated broiler meat strip product was showing gaseous spoilage, the LAB numbers  
31 had risen up to  $10^{10}$  CFU/g (Björkroth et al., 2000).

32  
33 Because high CFU/g values were obtained from the products possessing both high (in this case pH  
34 5.9) or low (pH 4.9) pH values, no association of the pH with the microbial product quality can be

1 made. The initial acidity of the marinades is high but the meat buffers the pH quite rapidly.  
2 Compared to the spoilage of pork or beef products, the spoilage flora of marinated broiler meat  
3 seems to be quite different. *L. gasicomitatum* strains have yet been detected only in broiler meat  
4 products despite numerous studies of beef and pork products. In marinating, the initial LAB  
5 contamination of broiler meat and the marinade is combined and the ecological niche of the final  
6 product results from this combination. LAB growth and spoilage associated with marinated broiler  
7 meat is therefore a more complex event than in a “plain meat product”. Even sensory panel was not  
8 used during this study we also noticed that it was difficult to evaluate the odour of the different  
9 products. Marinades apparently mask some of the early spoilage effects.

10  
11 Both consumers and producers would benefit from better understanding of the effects the marinades  
12 have on the developing LAB population and shelf life of broiler meat products. Now when the  
13 species distribution in different marinades is known, we are able to study further the interaction  
14 between various LAB and the role of the marinade ingredients in the spoilage. This will be aim of  
15 our further studies.

## 16 17 5. Conclusion

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

## 22 23 24 References

- 25         Anonymous (2001) Validation list no.79. Int. J. System. Evol. Microbiol. 51, 263-265.
- 26         Baird, R. M. , Corry, J. E. L. and Curtis, G. D. W. (Ed.) (1987) Pharmacopoeia of culture  
27 media for food microbiology. De Man, Rogosa and Sharpe (MRS) agar. Int. J. Food Microbiol. 5,  
28 228-229.
- 29         Barakat, R. K., Griffiths M. W. and Harris, L. J. (2000) Isolation and characterization of  
30 Carnobacterium, Lactococcus, and Enterococcus spp. from cooked, modified atmosphere packaged,  
31 refrigerated, poultry meat. Int. J. of Food Microbiol. 62, 83-94.
- 32         Batt, C. A. (2000) *Lactobacillus*, Introduction. In: Robinson, K., Batt, C. A. and Patel, P. D.  
33 (Ed.). Encyclopedia of Food Microbiology 2, Academic press, Bath, pp. 134-136.

1 Björkroth, J. K.; Geisen, R.; Schillinger, U.; Weiss, N.; de Vos, P.; Holzapfel, W. H.;  
2 Korkeala, H. J. and Vandamme, P. (2000) Characterisation of *Leuconostoc gasicomitatum* sp. nov.  
3 associated with spoiled raw tomato-marinated broiler meat strips packaged under modified  
4 atmosphere conditions. *Appl. Environ. Microbiol.* 66, 3764-3772.

5 Björkroth, J. K.; Vandamme, P. and Korkeala, H. J. (1998) Identification and  
6 Characterization of *Leuconostoc carnosum*, Associated with Production and Spoilage of Vacuum-  
7 Packaged, Sliced, Cooked Ham. *Appl. Environ. Microbiol.* 64, 3313-3319.

8 Björkroth, J. K. and Korkeala, H. K. (1997) *Lactobacillus fructivorans* spoilage of tomato  
9 ketchup. *J. Food Prot.* 60, 505-509.

10 Borch, E. and Agerhem, H. (1992) Chemical, microbial and sensory changes during the  
11 anaerobic cold storage of beef inoculated with a homofermentative *Lactobacillus* sp. or a  
12 *Leuconostoc* sp. *Int. J. Food Microbiol.* 15, 99-108.

13 Borch, E., Berg, H. and Holst, O. (1991) Heterolactic fermentation by a homofermentative  
14 *Lactobacillus* sp. during glucose limitation in anaerobic continuous culture with complete cell  
15 recycle. *J. Appl. Bacteriol.* 71, 265-269.

16 Borch, E., Kant-Muermans, M-L. and Blixt, Y. (1996) Bacterial spoilage of meat and cured  
17 meat products. A review. *Int. J. Food Microbiol.* 33, 103-120.

18 Dainty, R. H. and Mackey, B. M. (1992) The relationship between the phenotypic properties  
19 of bacteria from chill- stored meat and spoilage processes. *J. Appl. Bacteriol.* 73, 103S-114S.

20 Egan, A. F. (1983) Lactic acid bacteria of meat and meat products. *Antonie van*  
21 *Leeuwenhoek* 49, 327-336.

22 Garvie, E. and Mabbitt, L. A. (1967) Stimulation of the Growth of *Leuconostoc oenos* by  
23 tomato juice. *Archiv für Mikrobiologie* 55, 398-407.

24 Gill, C. O. , Harrison, J. C. L. and Penney, N. (1990) The storage life of chicken carcasses  
25 packaged under carbon dioxide. *Int. J. Food microbiol.* 11, 151-158.

26 Hansen, K. M. J. and Bautista, D. A. (2000). Spoilage problems, problems caused by  
27 bacteria. In: Robinson, K. , Batt, C. A. and Patel, P. D. (Ed.). *Encyclopedia of Food Microbiology*  
28 3, Academic press, Bath, 2051-2056.

29 Holley, R. A. *Brochothrix*. (2000) In: Robinson, K. , Batt, C. A. and Patel, P. D. (Ed.).  
30 *Encyclopedia of Food Microbiology*, vol 1, Academic press, Bath, 314-318.

31 Holzapfel, W. H. (1992) Culture media for non- sporulating Gram- positive food spoilage  
32 bacteria. A review. *Int. J. Food Microbiol.* 17, 113- 133.

33 Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (1994) *Bergeys*  
34 *Manual of Determinative Bacteriology*, 9th edn., Williams & Wilkins, Baltimore, pp. 787.

- 1 Jay, J. M. (1996) Modern Food Microbiology. 5th edn., Chapman & Hall, New York, pp.  
2 661.
- 3 Jones, M. V. (1989) Modified atmospheres. In: Gould, G. W. (Ed.). Mechanisms of Action  
4 of Food Preservation Procedures. Elsevier Applied Science, Essex, 247-277.
- 5 Juven, B. and Weisslowitch, H. (1981) Chemical changes in tomato juices caused by lactic  
6 acid bacteria. J. Food Sci. 46, 1543-1545.
- 7 Kakouri, A. and Nychas, G. J. E. (1994) Storage of poultry meat under modified  
8 atmospheres or vacuum-packs. Possible role of microbial metabolites as indicator of spoilage. J.  
9 Appl. Bacteriol. 76, 163-172.
- 10 Korkeala, H. , Ahvenainen, R. and Alanko, T. (1987) Interrelationship between microbial  
11 numbers and other parameters in the spoilage of vacuum-packed cooked sausages. Int. J. Food  
12 Microbiol. 5, 311-321.
- 13 Korkeala, H., Suortti, T. and Mäkelä, P. (1988) Ropy slime formation in vacuum-packed  
14 cooked meat products caused by homofermentative lactobacilli and *Leuconostoc* species. Int. J.  
15 Food Microbiol. 7, 339-347.
- 16 Lonvaud-Funel, A. (2000) *Leuconostoc*. In: Robinson, K. , Batt, C. A. and Patel, P. D. (Ed.).  
17 Encyclopedia of Food Microbiology 2, Academic Press, Bath, 1183-1194.
- 18 Lyhs, U., Björkroth, J. and Korkeala, H. (1999) Characterisation of lactic acid bacteria from  
19 spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping. Int. J. Food Microbiol. 52,  
20 77-84.
- 21 NCFA, Nordic Committee on Food Analysis. Pretreatment of foods for microbiological  
22 examination. Method no 91, 1988.
- 23 NCFA, Nordic Committee on Food Analysis. Psychrotrophic microorganisms.  
24 Determination by the plate count method. Proposed method no 74, 2, 1989.
- 25 NCFA, Nordic Committee on Food Analysis. *Brochothrix thermosphacta*, determination in  
26 meat and meat products. Method no 141, 1991.
- 27 Nychas, G. J. E. and Drosinos, E. H. (2000) Spoilage of meat. In: Robinson, K. , Batt, C. A.  
28 and Patel, P. D. (Ed.). Encyclopedia of food microbiology 2, Academic Press, Bath, 1253-1260.
- 29 Nychas, G. J. E. and Tassou, C. C. (1997) Spoilage processes and proteolysis in chicken as  
30 detected by HPCL. J. Sci. Food Agric. 74, 199-208.
- 31 Porretta, S. and Vicini, E. (1993) Changes in tomato pulp quality caused by lactic acid  
32 bacteria. Int. J. Food, Sci. and Technol. 28, 611-616.
- 33 Pitcher, D.G., Saunders, N.A., Owen, R.J., 1989. Rapid extraction of bacterial genomic  
34 DNA with guanidium thiocyanate. Lett. Appl. Microbiol. 8, 151-156.

- 1 Reuter, G. (1985) Elective and selective media for lactic acid bacteria. Int. J. Food  
2 Microbiol. 2, 55-68.
- 3 Samelis, J., Kakouri A. and Rementzis, J (2000) The spoilage microflora of cured, cooked  
4 turkey breasts prepared commercially with or without smoking. Int. J. of Food Microbiol. 56, 133-  
5 43.
- 6 Sawaya, W. N. , Abu-Ruwaida, A. S. , Hussain, A. J. , Khalafawi, M. S. and Dashti, B. H.  
7 (1993) Shelf-life of vacuum-packaged eviscerated broiler carcasses under stimulated market  
8 conditions. J. Food Safety 13, 305-321.
- 9 Schillinger, U. and Lücke, F. K. (1987) Lactic acid bacteria on vacuum-packaged meat and  
10 their influence on shelf life. Fleischwirtsch. 67, 1244-1248.
- 11 Taylor, S. A. (1996) Modified atmosphere packing of meat. In: Meat quality and meat  
12 packaging. Taylor, S. A., Raimundo, A. , Severini M. and Smulders, J. M. ECCEAMST, Utrecht,  
13 301-308.
- 14 Tseng, C-P. and Montville, T. J. (1990) Enzyme activities affecting end product distribution  
15 by *Lactobacillus plantarum* in response to changes in pH and O<sub>2</sub> . Appl. Environ. Microbiol. 56,  
16 2761-2763.
- 17 von Holy, A. , Cloete, T. E. and Holzapfel, W. H. (1991) Quantification and characterisation  
18 of microbial populations associated with spoiled, vacuum-packed vienna sausages. Food Microbiol.  
19 8, 95-104.

1 TABLE 1. The averages of colony forming units (CFU) determined from the CFU/g results of 3 to  
 2 5 packages of 7 differently marinated broiler meat strip products packaged under modified  
 3 atmosphere.

Type of product		CFU/g (log <sub>10</sub> ) average value					
(no. of packages)	pH						
		MRS <sup>a</sup>	TJA <sup>b</sup>	PCA <sup>c</sup>	RA <sup>d</sup>		
A1	(5)	5.2	8.8 ± 1.0	8.7 <sup>e</sup> ± 1.0	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	

19 <sup>a</sup>MRS Agar (Oxoid, Basingstoke, England)

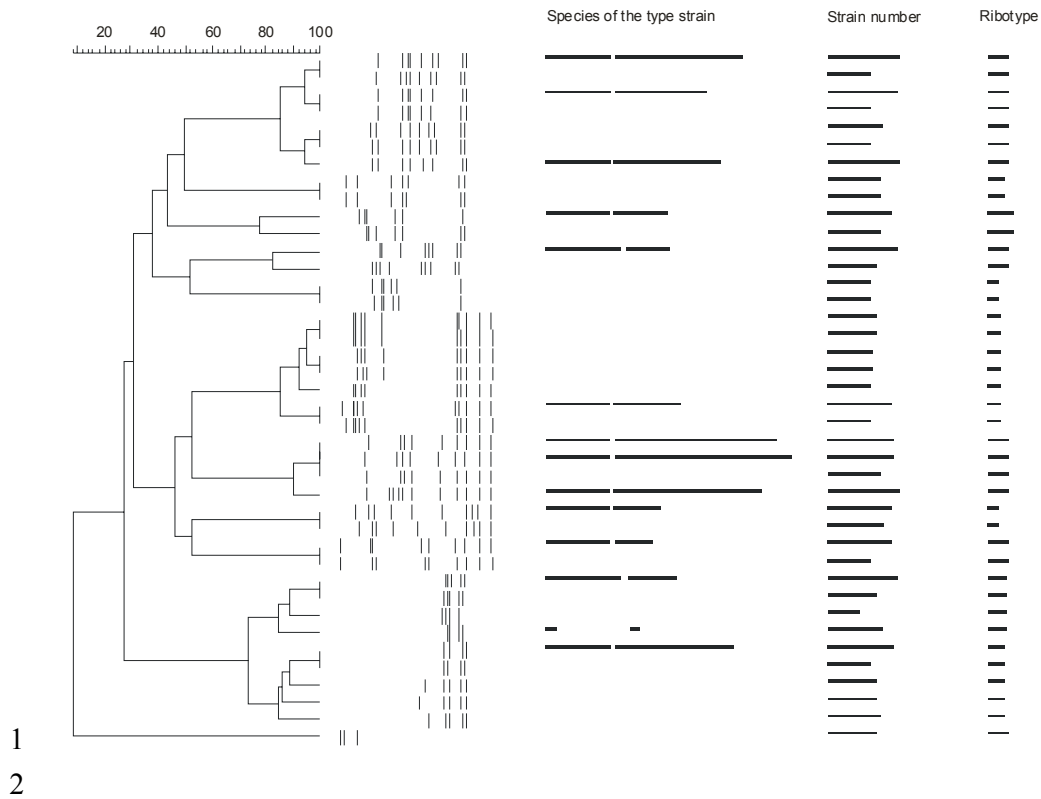
20 <sup>b</sup>Tomato Juice Agar (Oxoid)

21 <sup>c</sup>Plate Count Agar (Difco laboratories, Detroit, USA),

22 <sup>d</sup>Rogosa SL Agar (Difco)

23 <sup>e</sup>The average of four samples

24



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