<https://helda.helsinki.fi>

Leuconostoc gelidum and Leuconostoc gasicomitatum strains dominated the lactic acid bacterium population associated with strong slime formation in an acetic-acid herring preserve

Lyhs, Ulrike

Elsevier

2003

International Journal of Food Microbiology. 2003. 90(2): 2207-2218.

<http://hdl.handle.net/1975/517>

[http://dx.doi.org/10.1016/S0168-1605\(03\)00303-9](http://dx.doi.org/10.1016/S0168-1605(03)00303-9)

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

1
2
3 *Leuconostoc gelidum* and *Leuconostoc gasicomitatum* strains dominated the
4 lactic acid bacterium population associated with strong slime formation in
5 an acetic-acid herring preserve
6

7 Ulrike Lyhs¹, Joanna M.K. Koort², Hanna-Saara Lundström² and K. Johanna Björkroth*²

8 ¹*Department of Food Technology/Meat Technology, University of Helsinki, Finland*

9 ²*Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine,*
10 *University of Helsinki, Finland*
11
12
13
14
15
16

17 *Corresponding author: Professor K. Johanna Björkroth
18 Department of Food and Environmental Hygiene
19 Faculty of Veterinary Medicine
20 P.O. Box 57
21 00014 University of Helsinki
22 Finland
23 Phone: +358-9-19149705
24 Fax: +358-9-19149718
25 E-mail: johanna.bjorkroth@helsinki.fi

1 **Abstract**

2 Spoilage characterised by strong slime and gas formation affected some manufacture lots of
3 an acetic-acid Baltic herring (*Culpea haerengus membras*) preserve after few weeks' storage
4 at 0-6°C. The product consisted of herring filets in acetic acid marinade containing sugar,
5 salt, allspice and carrot slices. Microbiological analyses of the spoiled product showed high
6 lactic acid bacterium (LAB) levels ranging from 4.5×10^8 to 2.4×10^9 CFU/g. Yeasts were
7 not detected in any of the herring samples. Since LAB contaminants seldom are associated
8 with fresh fish, LAB populations associated with marinade ingredients (carrots, allspice)
9 were also analysed. The highest LAB levels exceeding 10^7 CFU/g were detected in
10 equilibrium modified atmosphere packaged baby carrots whereas the levels detected in the
11 allspice samples did not exceed 4.3×10^5 . A total of 176 randomly selected LAB isolates
12 originating from herring, carrot and allspice samples were further identified to species level
13 using a 16 and 23S rRNA gene RFLP (ribotyping) database. *Leuconostoc gelidum* and
14 *Leuconostoc gasicomitatum* strains dominated both in the spoiled herring and carrot samples.
15 These species are heterofermentative producing CO₂ from glucose and they also produce
16 dextran from sucrose. Inoculation of some commercial herring products with spoilage-
17 associated *L. gelidum* and *L. gasicomitatum* strains verified that these strains have the
18 capability of producing slime and gas in herring preserves although slime formation was not
19 as strong as in the original samples. Since *L. gelidum* and *L. gasicomitatum* strains were
20 commonly detected in carrots, carrot slices used for the fish marinade were considered to be
21 the probable source of these specific spoilage organisms.

22

1 **1 Introduction**

2 Spoilage of semi-preserved, marinated fish products occurs usually due to the growth of non-
3 putrefactive organisms, such as acetic acid-tolerant lactic acid bacteria (LAB) (Blood, 1975).
4 The marinating process applied, i.e. the treatment of the fish with acetic acid and sodium
5 chloride, is responsible for the microbial selection (Blood, 1975; Sharpe and Pettipher, 1983).
6 Spoilage organisms must tolerate low pH (< 5) together with high NaCl concentrations
7 (usually from 2.5 to 5%). Gaseous spoilage type, manifested by bulging of the lids of the jars
8 after some storage weeks, has been associated with acetic acid fish preserves (Blood, 1975;
9 Sharpe and Pettipher, 1983; Lyhs et al., 2001). *Lactobacillus* spp. (Meyer, 1956b; Kreuzer,
10 1957; Lerche, 1960; Reuter, 1965; Erichsen, 1967; Sharpe and Pettipher, 1983; Lyhs et al.,
11 2001) or yeasts (Somners, 1975) have been the specific spoilage organisms detected in these
12 herring marinades. Limited information exists about the microbial ecology associated with
13 other types of spoilage in marinated fish products. Borgström (1953) reported upon some
14 cases of sliminess occurring in the brine of sugar-salted herring. *Pseudomonas* spp. and less
15 frequently *Leuconostoc* spp. were considered to cause this slime formation.

16 In this study, an unusual spoilage phenomenon affecting an acetic-acid Baltic herring
17 (*Culpea haerengus membras*) preserve is described. During a problematic manufacturing
18 period, some plastic containers started to show bulging due to gas formation after 2 to 3
19 storage weeks. At the same time, extremely strong slime and gas formation was observed in
20 the marinade. The products were manufactured in one processing plant and they were
21 expected to maintain good quality during a shelf-life of 6 months at the recommended storage
22 temperature between 0°C to 6°C. Spoilage had affected only some production lots and
23 occurred from time to time. According to the manufacturer, fresh, good quality raw fish had
24 always been used for the product but the quality of some other ingredients, such as carrot
25 slices and spices used in the marinade, had been called in question. This was considered

1 meaningful, since only certain LAB species have been found in low numbers in the normal
2 microflora of healthy fish (Ringø et al., 1998) and it has been suggested that bacterial strains
3 thriving from other sources may contaminate herring products (Borgström, 1953; Lerche,
4 1960; Krüger, 1973; Lyhs et al., 2001).

5 The aim of this study was to identify the organisms associated with this unusual
6 spoilage case. Microbiological analyses enumerating LAB and yeasts were performed and a
7 16 and 23S rRNA gene RFLP (ribotyping) database was used for the identification of the
8 spoilage LAB. In order to evaluate the contamination risk associated with the non-fish
9 ingredients used for the fish marinade, we identified LAB populations in carrot and allspice
10 samples. Finally, the spoilage potential of the dominating herring strains was verified by
11 inoculation tests in some commercial herring products.

13 **2 Materials and methods**

14 **2.1 Acetic-acid herring preserve samples associated with spoilage**

15 A total of 7 containers of marinated herring were studied. Five of the containers showed
16 strong slime and gas formation indicating spoilage (Fig. 1), and two had a normal
17 appearance. The total weight of a container was 2400 g, of which 1420 g were fish, 80 g
18 ingredients used for spicing and decoration, and the rest consisted of water and acetic acid.
19 All containers held herring cut into pieces, onions, carrots, water, sugar, salt (NaCl), acetic
20 acid and allspice. According to the manufacturer, the salt and sugar concentrations of the
21 product were 2.4% and 18% (w/w), respectively. The recommended storage temperature was
22 from 0 to 6°C. At the time of the study, only few weeks less than the 6 months expected
23 shelf-life (at the recommended temperature) were still remaining.

2.2 Carrot and allspice samples

A total of 9 packages of different types of carrots were analyzed for spoilage LAB. Unfortunately the ingredients used for the spoiled lots were not anymore available but the handling of the carrots mimicked the protocols used for the herring manufacture. Equilibrium modified atmosphere (EMA) packaged carrots were also studied because sometimes carrots sliced and packaged elsewhere might be used. Five of the carrot samples consisted of 200 g EMA-packaged baby carrots, 2 were 500 g packages of washed common carrots and the last 2 were 500 g packages of washed and organically-grown carrots. EMA-packaged baby carrots and the peels of the other carrot types were analyzed immediately. After peeling, the common and “organic” carrots were sliced and stored in plastic bags at 4°C up to 4 weeks in order to simulate the circumstances associated with the herring manufacture. During the storage period, the slices were analyzed once a week.

A total of 8 packs of commercial allspice from 5 different Finnish companies were analyzed for spoilage LAB. Five of the packs contained whole and 3 grind allspice.

2.3 Sensory evaluation of the acetic-acid herring preserves and determination of the swell type

Evaluation of color, odor, appearance and texture of the spoiled herring products was performed by five trained judges as described by Korkeala and Lindroth (1987).

CO₂ swell was distinguished from hydrogen or H₂S swell using KOH as described by Jay (2000).

2.4 Microbiological analyses and pH measurement

LAB in all samples were enumerated from 10-fold serial dilutions on MRS agar (Oxoid, Basingstoke, UK) as described by Lyhs et al. (1999). The plates were incubated in an anaerobic jar with a H₂+CO₂ generating kit (Oxoid) at 25°C for 5 days. The allspice samples

1 were also enriched in MRS broth (Difco, Detroit, Michigan, USA) containing 1% sorbic acid
2 as a yeast inhibitor (MRS-S broth) at 25°C for 3-4 days. For enrichment, 1 g of allspice was
3 weighed into 9 ml of MRS-S broth and if growth was detected, a loop-full of the broth was
4 spread onto MRS agar to provide colonies.

5 Yeasts were determined by the method of the Nordic Committee on Food Analysis
6 (1993) using OGYE agar (Oxoid). The plates were incubated aerobically at 25°C for 3 days.

7 The pH was determined from the first sample dilution by a WTW-530 Digital-pH-
8 meter (Wissenschaftliche-Technische Werkstätten, Weilheim, Germany).

9 **2.5 Selection the LAB strains for species identification**

10 Altogether 176 colonies were picked randomly from the MRS plates (Oxoid) and
11 cultured pure using MRS broth (Difco) and MRS agar (Oxoid) as described by Lyhs et al.
12 (2002). A total of 76 colonies were selected from the herring samples, of which 10 colonies
13 originated from a container possessing a normal appearance and 66 were from the ones
14 showing spoilage. From the carrot samples, a total of 63 colonies were picked. These
15 included 19, 10 and 34 colonies from the baby, common, and organically-grown carrots,
16 respectively. From the allspice, 12 colonies were selected from the plates without enrichment
17 and seven strains were cultured pure from the enrichment broths. All 176 isolates were
18 subjected to species identification and they were stored, if needed, in MRS broth (Difco) at
19 -70°C.

20 **2.6 Isolation of DNA, restriction endonuclease analysis (REA) and 16 and 23S rRNA** 21 **RFLP (ribotyping)**

22 Cells harvested from 1 to 2 ml (depending on the growth) of MRS broth (Difco)
23 culture were used for DNA analyses. DNA was isolated by the guanidium thiocyanate
24 method of Pitcher et al. (1989) as modified by Björkroth and Korkeala (1996a) by the

1 combined lysozyme and mutanolysin (Sigma, St. Louis, Missouri) treatment. Restriction
2 endonuclease treatment of 3µg of DNA was done using *Hind*III restriction enzyme as
3 specified by the manufacturer (New England Biolabs, Beverly, Mass.). The rDNA probe was
4 labeled for ribotyping by reverse transcription (AMV-RT, Promega, Madison, Wisconsin and
5 Dig DNA Labeling Kit, Roche Molecular Biochemicals, Mannheim, Germany) as described
6 by Blumberg et al. (1991). REA, genomic blots and hybridization of the membranes were
7 done as described (Björkroth and Korkeala, 1996a). *Hind*III enzyme was chosen because it
8 has been found to provide species-specific patterns for various LAB (Björkroth and Korkeala,
9 1996b; Björkroth and Korkeala, 1997; Björkroth et al., 1998; Björkroth et al., 2000;
10 Björkroth et al., 2001).

11 **2.7 Pattern analysis**

12 The *Hind*III ribopatterns were compared with the corresponding patterns in the
13 previously established LAB database of the Department of Food and Environmental Hygiene,
14 University of Helsinki. This database comprises patterns of all relevant spoilage LAB in the
15 genera of *Carnobacteria*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus* and
16 *Weissella* (Björkroth and Korkeala, 1996b; Björkroth and Korkeala, 1997; Björkroth et al.,
17 1998; Björkroth et al., 2000; Björkroth et al., 2001). For numerical analysis, ribopatterns
18 were scanned using a Hewlet Packard (Boise, Idaho) ScanJet 4c/T scanner and analyzed
19 using the BioNumerics 2.5 software package (Applied Maths, Sint-Martens-Latem,
20 Belgium). The similarity between all pairs was expressed by Dice coefficient correlation and
21 UPGMA (unweighed pair group method using arithmetic averages) clustering was used for
22 the construction of the dendrograms. Based on the use of internal controls, pattern
23 optimization and band position tolerances of 1.0 and 1.5%, respectively, were allowed.
24 Species identification was based on the locations of the type strains in the clusters formed.

2.8 Verification of the spoilage potential by inoculation tests

Four specific spoilage strains originating from the product possessing slimy appearance were used for the inoculation test. The strains were selected based on their ribopatterns and they represented the major spoilage-associated bacterial types: 2 *L. gasicomitatum* strains KSL 3-8 and KSL 3-15 possessing ribotypes IIIId and IIIb and 2 *L. gelidum* strains KSL 3-13 and KSL 3-14 possessing ribotypes IIb and IIa, respectively. Three different strain combinations A, B and C were used for the inoculations. Combination A contained cells from all four strains, combination B the two *L. gasicomitatum* strains and combination C the two *L. gelidum* strains. Four different (Table 4) commercial herring products possessing initial LAB levels <100 CFU/mg and pH values from 4.3 to 4.5 were used as the test material. LAB were enumerated as described in the section 2.4. NaCl content of the products varied from 2.5 to 3.5% (w/w) and the highest sugar contents, 28 to 30% (w/w) were in the tomato and sugar-spice marinated products whereas 19 to 20% (w/w) had been used in acetic acid and acid marinated products. Combinations A, B and C were made using MRS broth culture mixes adjusted according their OD₆₀₀ values to the final cell density of approximately 5×10^5 CFU/ml. One ml of the strain combination was added into 100 g of commercial herring product placed in a low oxygen-permeable plastic pack. This resulted in the initial level of approximately 5000 CFU/g. The packs were vacuum sealed and kept at 6°C for 2 weeks. During the storage, gas and slime formation was observed and finally the packs were opened and the slime and gas formation were judged (3 judges) from none to strong using a score from 0 to 5, respectively. Table 4 shows the product and strain combinations studied.

1 **3 Results**

2 **3.1 Microbiological, pH, sensory and swell type analyses**

3 Table 1 shows the LAB counts on MRS agar and the corresponding pH values
4 obtained from the 7 herring containers. The spoiled samples showed LAB growth up to $2.4 \times$
5 10^9 CFU/g whereas one of the unspoiled samples showed no growth and the other 9.8×10^5
6 CFU/g. Yeasts were detected neither in the unspoiled nor in the spoiled samples. The pH of
7 the spoiled samples ranged from 4.7 to 4.8 whereas pH from 4.3 to 4.4 was detected in the
8 unspoiled samples. Visual examination of the 5 containers showed clear bulging of lids,
9 significant slime formation, and gas bubbles in the marinade (Fig. 1). All judges deemed the
10 contents unfit for human consumption and also detected an off-odor described as buttery,
11 butyrate-like or curd cheese-like. However, the texture of the fish was found normal. No
12 negative remarks were associated with the two normal-looking contents. The swell type
13 detected was a CO₂-swell.

14 Table 2 shows the results from the microbial enumeration on MRS agar and the
15 corresponding pH values in all carrot samples. LAB counts up to 2.3×10^7 CFU/g were
16 observed in the EMA-packaged baby carrots. The peels of carrots showed growth from $7 \times$
17 10^4 to 2.1×10^6 CFU/g. In the slices of the common carrots, the LAB counts increased from
18 10^3 CFU/g of the first storage week up to 6.0×10^5 CFU/g in the fourth week. In the
19 organically-grown carrots, counts ranged from the initial 1.2×10^4 CFU/g to 8.7×10^6 CFU/g
20 at the end of the 4 weeks storage. The pH values in the carrot samples varied between 5.4 and
21 6.6 showing reduction parallel to the increasing LAB levels.

22 In the allspice, levels of LAB between 1.1×10^4 CFU/g and 4.3×10^5 CFU/g and pH
23 values from 5.2 to 5.6 were detected.

3.2 LAB associated with the herring product

Table 3 shows the division of the *Hind*III ribotypes of LAB strains from the spoiled and unspoiled herring samples. The different patterns obtained and a dendrogram based on the pattern similarity are seen in Fig. 2. Three main clusters (cluster I-III) were formed at the similarity level of 56% (Fig. 2). Cluster I contained the type strain of *Leuconostoc mesenteroides* (CCUG 21965), cluster II of *L. gelidum* (NCFB 2775) and cluster III of *L. gasicomitatum* (LMG 18811). The herring isolates in these 3 clusters either shared identical pattern with the type strain or showed high (83-90%) similarity with them (Fig. 2) and were thus identified according the position of the type strain in the cluster. In all spoiled samples, an even distribution of *L. gelidum* and *L. gasicomitatum* strains was observed types IIa, IIb, IIIb and IIIc being the most prevalent. Also in the one sample looking normal but already showing LAB growth of 9.8×10^5 CFU/g these strains were detected.

3.3 LAB associated with carrots and allspice

In Fig. 3, the different patterns and the clustering based on the similarity of the patterns is shown. Ten main clusters (cluster I-X) were formed at the similarity level of 75%. Seventy-four isolates from the different carrot products and their peels possessed the ribotypes Ic, IIb, IIc, IIIc, IIId, IVb and IVc and were assigned to the genus *Leuconostoc*. Forty isolates were identified as *L. gelidum*, of which 35 isolates possessed type IIc and 5 isolates had patterns identical with the pattern (IIb) of the *L. gelidum* type strain. From the total of 22 isolates, 21 isolates possessed ribotype IIIc being identical with the ribotype of *L. gasicomitatum* (LMG 18811) type strain. The ribotype IIId (one isolate) differed from the pattern of the type strain by one fragment only. In the organic carrots and their peels more *L. gelidum* strains than *L. gasicomitatum* were found (Table 3). Cluster IV contained the different patterns (IVa-IVc) gained from 11 isolates together with the *Leuconostoc citreum* type and reference strains (LMG 11417 and LMG 9824). The reference strains shared an

1 unique pattern, ribotype IVa. The patterns of the five isolates possessing ribotype IVb had
2 one band difference compared to ribotype IVc possessed by six isolates. Four isolates and the
3 type strain of *Enterococcus faecium* (LMG 11423) shared identical patterns (Cluster VI).
4 Patterns in the cluster V were also considered to belong to the genus *Enterococcus* but they
5 did not match any reference strains closely. Cluster VIII, divided into eight subclusters
6 (VIIIa-VIIIh) contained the different ribotypes gained from seven isolates together with the
7 type strains of *Lactococcus lactis* subsp. *hordniae* (LMG 8520), *L. lactis* subsp. *lactis* (LMG
8 6890) and *L. lactis* subsp. *cremoris* (LMG 6897). Due to the adjacent *Lactococcus* cluster,
9 stains in the cluster IX were also considered as lactococci but species-level identification was
10 not obtained. The 4 strains possessing types Xa and Xb were not identified by this database.
11 Table 3 shows the amount of the isolates possessing the different *Hind*III ribotypes and the
12 corresponding LAB species detected in the carrot and allspice samples.

13 **3.4 Verification of spoilage potential by strain inoculation**

14 Table 4 shows the EPS and gas formation in 4 commercial herring products. Gas production
15 was seen in all products but the strongest EPS production was detected in the tomato and
16 sugar-spice marinated herring products. Already after 2 days marinades turned cloudy (could
17 not be observed in the not-transparent tomato marinade) and gas formation was visible in all
18 products in few days. pH of the products remained unchanged or were slightly reduced (by
19 0.1 to 0.2).

20

21 **4 Discussion**

22 *Leuconostoc* spp. were the specific spoilage organisms (SSO) in this acetic-acid
23 herring preserve; 50% of all isolates were identified as *L. gelidum* and 48% as
24 *L. gasicomitatum* species. They are unusual LAB species for this type of a fish preserve.
25 *L. gelidum* species was described by Shaw and Harding (1989) and it has been reported to

1 occur in vacuum or modified atmosphere-packed (MAP) meat products stored at chilled
2 temperatures (Williamson, 1959; Leisner et al., 1995). *L. gasicomitatum* had previously been
3 associated only with MAP poultry products (Björkroth et al., 2000; Susiluoto et al., 2002) and
4 fresh cut produce (Jacxsens et al., 2001). This species was described by Björkroth et al.
5 (2000) and it was then considered to be a SSO in MAP, tomato-marinated broiler meat strips
6 showing gaseous spoilage. This finding was supported recently by Susiluoto et al. (2002)
7 revealing that *L. gasicomitatum* is dominating the developing spoilage LAB population in
8 retail, MAP, marinated broiler meat strips.

9 Slime formation is a rare spoilage type in fish products. It has been reported twice
10 affecting the brine of sugar-salted herring (Borgström, 1953; Magnússon and Möller, 1985).
11 *Pseudomonas* spp., *Leuconostoc* spp. and halophilic Gram-negative, oxidase-positive, aerobic
12 rods were then considered to be responsible for the sliminess. Some LAB are able to produce
13 exopolysaccharides (EPS), which can be secreted into the extra cellular environment (De
14 Vuyst and Degeest, 1999). The formation of EPS can be advantageous to some products
15 serving as viscosifying, stabilizing or gelling agents (Cerning, 1990; van den Berg et al.,
16 1993; Stingele et al., 1996; De Vuyst and Degeest, 1999; Duboc and Mollet, 2001). On the
17 other hand, EPS from *Pedococcus* spp. and heterofermentative lactobacilli may spoil
18 alcoholic beverages such as beers, ciders and wines (Williamson, 1959; Llaubères et al.,
19 1990; Lonraud-Funel et al., 1993; Back, 1994; Duenas et al., 1995; Manca de Nadra and
20 Strasser de Saad, 1995; Fernandez et al., 1996; Satokari et al., 2000). Also in vacuum-
21 packaged, cooked meat products the formation of ropy-slime is a known spoilage sign and in
22 these products it has mainly been associated with *L. sakei* species (Korkeala and Lindroth,
23 1987; Mäkelä et al., 1992b; Korkeala and Björkroth, 1997).

24 The slime in the acetic acid preserve was resisting deformation and had a thick,
25 clumpy texture resembling wall paper paste (Fig. 1). This confers to the viscosity of dextran,

1 a homopolysaccharide produced by the action of dextran-sucrase of *Leuconostoc* spp. on
2 sucrose. *L. carnosum*, *L. gasicomitatum* and *L. mesenteroides* species detected in this acetic
3 acid preserve are all able to produce dextran from sucrose (Björkroth et al., 2000; Garvie,
4 1979; Garvie, 1983; Shaw and Harding, 1989) and also the inoculation test verified the
5 slime-production in two of the herring products tested. *L. gasicomitatum* and *L. carnosum*
6 strains have not been reported to produce slime on ham or broiler products (Björkroth et al.
7 1998, 2000). This is probably due to the lack of sucrose in these meat products. On the
8 contrary to leuconostocs, the ropy-slime producing *L. sakei* strains cause slimy spoilage also
9 in sucrose-devoid meat products (Korkeala et al., 1988). The EPS produced by *L. sakei* are,
10 however, usually very viscous glucose and galactose-containing heteropolysaccharides
11 (Duboc and Mollet, 2001) and their formation is very different from homopolysaccharides
12 like dextran (Monsan et al, 2001).

13 Since fish barely contains carbohydrates, the dominating *Leuconostoc* spp. in the
14 present case must have used the sucrose added by the manufacturer as crystal sugar and
15 carrots in the marinade. Also in our inoculation test, the strongest EPS production was
16 observed in the two herring products containing approximately 30% (w/w) sugar. When
17 Magnússon and Möller (1985) studied the ability of the slime-producing bacteria associated
18 with the brine of sugar-salted herring, they did not detect ropiness in brine where sucrose had
19 been substituted by glucose. In order to avoid sliminess Magnússon and Möller (1985)
20 recommended the use of glucose instead of sucrose. However, obligatory heterofermentative
21 LAB produce gas during glucose fermentation and substituting sucrose with glucose may
22 therefore lead to gaseous spoilage type. The butter-like off-odor detected in the spoiled
23 product was likely associated with diacetyl formation. Because the fish muscle is not rich in
24 citrate, another precursor producing pyruvate may have triggered diacetyl formation.

25

1 The slime formation in the acetic acid preserve was accompanied with bulging of the
2 containers and a slight increase in the pH. Meyer (1956a) was the first to report this type of
3 LAB spoilage and he called it as “protein swell”. It has been suggested that the acetic acid
4 provides an environment suitable for the action of proteolytic enzymes present in the fish
5 muscle. The products of this proteolysis, i.e. amino acids, provide an energy source for the
6 acetic acid-tolerant LAB (Meyer, 1962b; Stamner, 1976). Usually in the case of LAB
7 spoilage, product pH falls due to the formation of lactic acid but in “protein swell” pH is
8 elevated. This has been attributed to the production of ammonia by deamination of amino
9 acids. In “protein swell”, CO₂ production in the product may be due to the decarboxylation of
10 amino acids being independent from the heterofermentative glucose utilization. This type of
11 LAB spoilage has been caused by *Lactobacillus* spp. in marinated herring products (Meyer,
12 1956b; Kreuzer, 1957; Lerche, 1960; Meyer, 1962a; Reuter, 1965; Erichsen, 1967; Sharpe
13 and Pettipher, 1983; Lyhs et al., 2001). It has also been associated with anchovy-stuffed
14 olives (Harmon et al., 1987) and it was suspected to cause gaseous spoilage in MAP, raw,
15 tomato-marinated broiler meat strips (Björkroth et al., 2000). *Leuconostocs* are, however,
16 obligatory heterofermentative LAB and produce gas (CO₂) also during the fermentation of
17 glucose. This was probably the reason for gas formation in the inoculated herring products.
18 Since the pH of these products did not rise, we were not able to repeat the decarboxylation
19 reaction considered to have happened in the spontaneously spoiled acetic acid herring
20 preserve. These results emphasize how the type of the spoilage reaction varies between the
21 different products. Future studies are warranted in order to clarify the metabolism associated
22 with these bacterial strains in herring products.

23 During this study, EMA-packaged baby carrots and carrots stored up to four weeks at
24 4°C showed higher numbers of LAB (Table 2) than the levels of 10³ to 10⁴ CFU/g reported
25 previously (Carlin et al., 1989; Garg et al., 1990; Liao and Fett, 2001). An increase of LAB

counts up to 10^5 CFU/g was observed during the 4-weeks cold storage of carrot slices. LAB have also previously been associated with cold-stored carrot products (Kakiomenour et al., 1995; Barry-Ryan and O'Beirne, 2000). In our study, the majority of the strains isolated from EMA-packaged baby and organically-grown carrots and their peels were identified as *L. gelidum* and *L. gasicomitatum*. In the common carrots and their peels, mostly *L. citreum* strains were found. The occurrence of leuconostocs in vegetable products has also been reported previously (Garg et al., 1990; Garcia-Gimeno and Zurera-Cosano, 1997) but the species were not identified.

We considered the carrots as a risk for *L. gelidum* and *L. gasicomitatum* contamination even there were some differences between the ribotypes associated with the spoiled herring preserve and carrot samples. In the allspice samples, mostly strains of *E. faecium* and *L. lactis* subsp. *hordniae* were identified and these species did not play any apparent role in the spoilage process. After this study, the manufacturer started to pay closer attention to the carrot quality and storage times for sliced carrots. It is now already a year since the last spoiled lot was detected. These results emphasize the fact that all ingredients, even used only as small amounts for decoration and spicing, play an important role in the hygiene of food manufacture.

5 Conclusion

Leuconostoc spp. were the specific spoilage organisms (SSO) in an acetic-acid herring preserve showing slimy spoilage type. Fifty-% of all isolates were identified as *L. gelidum* and 48% as *L. gasicomitatum* species. These same species were also commonly detected in carrot samples and during cold storage their levels were increased. Cold-stored carrots must therefore be considered as a risk for *L. gelidum* and *L. gasicomitatum* contamination.

6 Acknowledgments

The financial support from the Academy of Finland (Project 100479) is gratefully acknowledged. The authors wish to thank Ms. Henna Niinivirta for her excellent technical assistance. Ms. Maria Stark is acknowledged for the photograph of a spoiled herring container.

7 References

- Barry-Ryan, C., O'Beirne, D., 2000. Effects of peeling methods on the quality of ready- to-use carrot slices. *Int. J. Sci. Technol.* 35, 243-250.
- Björkroth, J., Korkeala, H., 1996a. Evaluation of *Lactobacillus sake* contamination in vacuum-packaged sliced cooked meat products by ribotyping. *J. Food Prot.* 59, 398-401.
- Björkroth, 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, J., Korkeala, H., 1997. *Lactobacillus fructivorans* spoilage of tomato in 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. Characterization 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.

1 Björkroth, K.J., Geisen, R., Schillinger, U., Weiss, N., Hoste, B., Holzapfel, W.H., Korkeala,
2 H.J., Vandamme, P., 2002. Taxonomic study of *Weissella confusa* and description of
3 *Weissella cibaria* sp. nov., a novel species in food and clinical samples. Int. J. Syst.
4 Evol. Microbiol. 52, 141-148.

5 Blood, R.M. 1975. Lactic acid bacteria in beverages and food. In: Proc. 4th Long Ashton
6 Symposium 1973. Academic Press, London, p. 195.

7 Blumberg, H.M., Kielbauch, J.A., Wachsmuth, K., 1991. Molecular epidemiology of *Yersinia*
8 *enterocolitica* O:3 infection: use of chromosomal DNA restriction fragment length
9 polymorphism of rRNA gene. J. Clin. Microbiol. 20, 2368-2374.

10 Borgström, G., 1953. Herring delicatessen and marinated products. In: Jul, M. and Kondrup,
11 M. (Eds), The technology of herring utilization. Boktrykkeri Griegs, Bergen, pp. 243-
12 256.

13 Carlin, F., Nguyen-The, C., Cudennec, P., Reich, M., 1989. Microbiology spoilage of fresh,
14 ready-to-use grated carrots. Sci Aliment. 9, 371-386.

15 Cerning, J., 1990. Exocellular polysaccharides produced by LAB bacteria. FEMS Microbiol.
16 Rev. 87, 113-130.

17 De Vuyst, L., Degeest, B., 1999. Heteropolysaccharides from lactic acid bacteria. FEMS
18 Microbiol. Rev. 23, 153-177.

19 Duboc, P., Mollet, B., 2001. Applications of exopolysaccharides in the dairy industry. Int.
20 Dairy J. 11, 759-768.

21 Duenas, M., Irastorza, A., Fernandez, K., Bilbao, A., 1995. Heterofermentative lactobacilli
22 causing ropiness in Basque Country ciders. J. Food Prot. 58, 76-80.

23 Erichsen, I., 1967. The microflora of semi-preserved fish products III. Principal groups of
24 bacteria occurring in tibits. Antonie van Leeuwenhoek 33, 107-112.

- 1 Fernandez, K., Duenas, M., Irastorza, A., Bilbao, A., Del Campo, G., 1996. Characterisation
2 and DNA plasmid analysis of ropy *Pedicoccus* strains isolated from Basque Country
3 ciders. J. Food Prot. 59, 35-40.
- 4 Garcia-Gimeno, R.M., Zurera-Cosano, G., 1997. Determination of ready-to-eat vegetable
5 salad shelf-life. Int. J. Food Microbiol. 36, 31-38.
- 6 Garg, N., Churey, J.J., Splitstoesser, D.F., 1990. Effect of processing conditions on the
7 microflora of fresh-cut vegetables. J. Food Prot. 53, 701-703.
- 8 Garvie, E. I., 1979. Proposal of neotype strains for *Leuconostoc mesenteroides* (Tsenkovskii)
9 van Tieghem, *Leuconostoc dextranicum* (Beijernick) Hucker and Pederson and
10 *Leuconostoc cremoris* (Knudsen and Sørensen) Garvie. Int. J. Syst. Bacteriol. 29, 149-
11 152.
- 12 Garvie, E. I., 1983. *Leuconostoc mesenteroides* subsp. *cremoris* (Knudsen and Sørensen)
13 comb. nov. and *Leuconostoc mesenteroides* subsp. *dextranicum* (Beijernick) comb. nov.
14 Int. J. Syst. Bacteriol. 33, 118-119.
- 15 Harmon, S.M., Kautter, D.A., McKee, C., 1987. Spoilage of anchovy-stuffed olives by
16 heterofermentative lactobacilli. J. Food Safety 8, 205-210.
- 17 Jacxsens, L., Devlieghere, F., Van der Steen, C., Debevere, J., 2001. Effect of high oxygen
18 modified-atmosphere packaging on microbial growth and sensorial qualities of fresh-cut
19 produce. Int. J. Food Microbiol. 71, 197-201
- 20 Jay, J.M. 2001. Modern Food Microbiology. Aspen publishers, Inc. Gaithersburg, Maryland,
21 USA. 679 pp.
- 22 Kakiomenour, N., Kakouri, A. Tassou, C.C., Nychas, G.J.E., 1995. Storage of shredded
23 carrots with modified atmospheres: possible role of microbial metabolites as indicator of
24 spoilage. Int. Biodeterioration and Biodegradation 36, 466.

- 1 Korkeala H., Lindroth, S., 1987. Differences in microbial growth in the surface layer and
2 center of vacuum-packaged cooked ring-sausage. *Int. J. Food Microbiol.* 4, 105-110
- 3 Korkeala, H., Suortti, T., Mäkelä, P., 1988. Ropy slime formation in vacuum-packed cooked
4 meat products caused by homofermentative lactobacilli and a *Leuconostoc* species. *Int.*
5 *J. Food Microbiol.* 7, 339-347.
- 6 Korkeala, H., Björkroth, J., 1997. Microbiological spoilage and contamination of vacuum-
7 packaged cooked sausages: a review. *J. Food Prot.* 60, 724-731.
- 8 Kreuzer, R., 1957. Untersuchungen über den biologisch bedingten Verderb von Fischwaren
9 und seine Verhinderung. I. Kaltmarinaden-Organismen und Milieu. *Arch. Fisch Wiss.*
10 8, 104 -139.
- 11 Krüger, K.-E., 1973. Hygienische Probleme bei der Fischwarenherstellung. *Feinkostwirtsch.*
12 10, 186-190.
- 13 Leisner, J.J., Greer, G.G., Dilts, B.D., Stiles, M.E., 1995. Effect of growth of selected lactic
14 acid bacteria on storage life of beef stored under vacuum and air. *Int. J. Food Microbiol.*
15 26, 231-243
- 16 Lerche, M., 1960. Bombage-Ursache in Fischpräserven. *Berl. Münch. Tierärztl. Wochenschr.*
17 75, 12-14.
- 18 Liao, C.H., Fett, W.F., 2001. Analysis of native microflora and selection of strains
19 antagonistic to human pathogens on fresh produce. *J. Food Prot.* 64, 1110-1115.
- 20 Llaubères, R.M., Richard, B., Lonvaud, A., Dubordieu, D., 1990. Structure of an exocellular
21 β -D-glucan from *Pedococcus* sp., a wine lactic acid bacteria. *Carbohydr. Res.* 203, 103-
22 107.
- 23 Lonraud-Funel, A., Guillox, Y., Joyeux, A., 1993. Isolation of a DNA probe for
24 identification of glucan-producing *Pedococcus damnosus* in wines. *J. Appl. Bacteriol.*
25 74, 41-47.

1 Lyhs, U., Björkroth, J., Korkeala, H., 1999. Characterization of LAB bacteria from spoiled,
2 vacuum-packaged, cold-smoked rainbow trout using ribotyping. Int. J. Food Microbiol.
3 52, 77-84.

4 Lyhs, U., Korkeala, H., Björkroth, J., 2001. *Lactobacillus alimentarius* – a specific spoilage
5 organism in marinated herring. Int. J. Food Microbiol. 64, 355-360.

6 Lyhs, U., Korkeala, H., Björkroth, J., 2002. Characterization of lactic acid bacteria from
7 spoiled, vacuum-packaged ‘gravad’ rainbow trout using ribotyping. Int. J. Food
8 Microbiol. 72, 147-153.

9 Magnússon, H., Möller, A., 1985. Ropiness in the brine of sugar-salted herring. Int. J. Food
10 Microbiol. 1, 253-261.

11 Mäkelä, P.M., Korkeala, H.J., Laine, J.J., 1992a. Survival of ropy slime-producing lactic acid
12 bacteria in heat processes used in meat industry. Meat Sci. 31, 463-471.

13 Mäkelä, P.M., Korkeala, H.J., Laine, J.J., 1992b. Ropy slime-producing lactic acid bacteria
14 contamination at meat processing plants. Int. J. Food Microbiol. 17, 27-35.

15 Mäkelä, P.M., Schillinger, U., Korkeala, H., Holzappel, W.H., 1992c. Classification of ropy
16 slime-producing lactic acid bacteria based on DNA-DNA homology, and identification
17 of *Lactobacillus sake* and *Leuconostoc amelibiosum* as dominant spoilage organisms in
18 meat products. Int. J. Food Microbiol. 16, 167-172

19 Manca de Nadra, M.C.M., Strasser de Saad, A.M., 1995. Polysaccharide production from
20 *Pedicoccus pentosaceus* from wine. Int. J. Food Microbiol. 27, 101-106.

21 Meyer, V., 1956a. Die Bestimmung der Bombage-Arten bei Fischkonserven. Fischwirtsch. 8,
22 212-224.

23 Meyer, V. 1956b. Probleme des Verderbens von Fischkonserven in Dosen. II.
24 Aminosäuredecarboxylase durch Organismen der *Betabacterium-Buchneri*-Gruppe als
25 Ursache bombierter Marinaden. Veröff. Inst. Meeresforsch. Bremerhaven 4:1-16.

- 1 Meyer, V., 1962a. Über Milchsäurebakterien in Fischmarinaden. Zentrbl. Bakt. Parastikde. 1,
2 Orig. 184, 296-302.
- 3 Meyer, V., 1962b. Probleme des Verderbens von Fischkonserven in Dosen. VII.
4 Untersuchung über die Entstehung von Aminosäuren beim Marinieren von Heringen.
5 Veröff. Inst. Meeresforsch. Bremerhaven 8, 21.
- 6 Monsan, P., Bozonnet, S., Albenne C., Joucla, G., Willemot, R.-M., Remaud-Siméon, M.
7 2001. Homopolysaccharides from lactic acid bacteria. Int. J. Dairy Sci. 11, 675-685.
- 8 Nordic Committee on Food Analysis, 1993. Yeasts. Detection in foods. NCFA method no.
9 149, Espoo, Finland.
- 10 Pitcher, D.G., Saunders, N.A., Owen, R.J., 1989. Rapid extraction of bacteria genomic DNA.
11 Lett. Appl. Microbiol. 8, 151-156.
- 12 Reuter, G., 1965. Das Vorkommen von Laktobazillen in Lebensmitteln und ihr Verhalten im
13 menschlichen Intestinaltrakt. Zentrbl. Bakt. Parastikde. 1, Orig. 197, 468.
- 14 Ringø, E., Gatesoupe, F.-J., 1998. Lactic acid bacteria in fish: a review. Aquaculture 160,
15 177-203.
- 16 Satokari, R., Mattila-Sandholm, T., Suihko, M.-L., 2000. Identification of pedicocci by
17 ribotyping. J. Appl. Microbiol. 88, 260-265.
- 18 Sharpe, M.E., Pettipher, G.L., 1983. Food spoilage by lactic acid bacteria. Econ. Microbiol.
19 8, 199-223.
- 20 Shaw, B.G., Harding, C.D., 1989. *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum*
21 sp. nov. from chill-stored meats. Int. J. System. Bacteriol. 39, 217-223.
- 22 Somers, J.M., 1975. Herring marinades. Food-Progress 2, 2-4.
- 23 Stamner, J.R., 1976. Lactic acid bacteria. In: DeFigueiredo, M.P., Splittstoesser, D.F. (Eds.),
24 Food Microbiology: Public health and spoilage aspects. AVI Publishing, Westport, CT,
25 p. 413.

1 Stingle, F., Neeser, J.-R., Mollet, B., 1996. Identification and characterization of the *eps*
2 (Exopolysachharide) gene cluster from *Streptococcus thermophilus* Sfi6. J. Bacteriol.
3 178, 1680-1690.

4 Susiluoto, T., Korkeala, H., Björkroth, J., 2002. *Leuconostoc gasicomitatum* is the
5 dominating lactic acid bacterium in retail modified-atmosphere-packaged marinated
6 broiler strips on sell-by day. Int. J. Food Microbiol. 80, 89-97.

7 van den Berg, D.J.C., Robijn, G.W., Janssen, A.C., Giuseppin, M.L.F., Vreeker, R.,
8 Kamerling, J.P., Vliegenhart, J.F.G., Ledeboer, A.M., Verrips, C.T., 1993. Production of
9 a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of
10 the polysaccharide. Appl Environm Microbiol. 61, 2840-2844.

11 Williamson, D.H., 1959. Studies on lactobacilli causing ropiness in beer. J. Appl Bacteriol. 2,
12 392-402.

13 Yost, C.K., Nattress, F.M., 2002. Molecular typing techniques to characterize the
14 development of a lactic acid bacteria community on vacuum-packaged beef. Int. J. Food
15 Microbiol. 72, 97-105.

1 Table 1. Growth on MRS agar and pH values in an acetic-acid herring preserve possessing
 2 either normal (samples 1 and 2) appearance or slimy spoilage type

Sample no.	Bacterial counts (CFU/g)	pH
1 ^a	< 100	4.3
2 ^a	9.8×10^5	4.4
3	6.8×10^8	4.8
4	9.9×10^8	4.7
5	4.5×10^8	4.7
6	2.4×10^9	4.7
7	1.5×10^9	4.7

3 ^a Sample of normal appearance.

4

1 Table 2. Growth on MRS agar and pH values detected in equilibrium modified atmosphere-packaged baby carrots,
 2 sliced common and organic carrots stored at +4°C up to 4 weeks and their peels at the beginning of the storage

Product	Time of analysis	Package No.	Bacterial counts (CFU/g)	pH
Common carrots, slices	Week 1	1	1.6×10^4	6.4
		2	10^3	6.4
	Week 2	1	3.0×10^4	6.6
		2	10^4	6.5
	Week 3	1	-	6.3
		2	3.6×10^4	6.5
	Week 4	1	6.0×10^5	6.5
		2	1.2×10^5	6.4
Organic carrots, slices	Week 1	1	1.2×10^4	6.5
		2	1.4×10^4	6.4
	Week 2	1	3.6×10^5	6.4
		2	2.3×10^5	6.3
	Week 3	1	2.0×10^6	6.2
		2	4.5×10^5	6.4
	Week 4	1	8.7×10^6	5.4
		2	1.1×10^6	5.5

1 Table 2 continues.

Product	Time of analysis	Package No.	Bacterial counts (CFU/g)	pH
Peels of common carrots		1	8.6×10^5	6.3
		2	2.1×10^6	6.2
Peels of organic carrots		1	7.0×10^4	6.4
		2	1.5×10^5	6.3
Baby carrots		1	1.9×10^6	5.7
		2	2.3×10^7	5.4
		3	4.2×10^6	5.7
		4	4.3×10^6	5.4
		5	5.0×10^6	5.4

1 Table 3. Lactic acid bacterium species distribution, ribotypes and numbers of the strains detected in the herring,, carrot and allspice samples

Species	Ribotype	Number of strains in sources							
		Herring product with normal appearance	Herring product with slimy spoilage	Baby carrots	Organic carrots	Peels of organic carrots	Common carrots	Peels of common carrots	Allspice
<i>Leuconostoc</i>	Ia		1						
<i>mesenteroides</i> subsp. <i>mesenteroides</i>	Ic							1	
<i>Leuconostoc</i>	IIa	7	17						
<i>gelidum</i>	IIb		15	1	2	2			
	IIc			8	21	6			
<i>Leuconostoc</i>	IIIa		2						
<i>gasicomitatum</i>	IIIb	2	5						
	IIIc			9	10	2	1		
	IIId	1	26						
<i>Leuconostoc</i>	IVb						1	4	
<i>citreum</i>	IVc			1			2	3	
<i>Enterococcus</i> sp.	Va								5
	Vb								1

1 Table 3 continues.

Species	Ribotype	Number of strains in sources							
		Herring product with normal appearance	Herring product with slimy spoilage	Baby carrots	Organic carrots	Peels of organic carrots	Common carrots	Peels of common carrots	Allspice
<i>Enterococcus faecium</i>	VI								4
<i>Lactobacillus curvatus</i> subsp. <i>curvatus</i>	VII				1				
<i>Lactococcus lactis</i> subsp. <i>hordniae</i>	VIIIa								2
	VIIIc								1
	VIIId								2
	VIIIe						2		
<i>Lactococcus</i> sp.	IXa								1
	IXb								1
	IXc								1
	IXd								1
Not identified	Xa						3		
	Xb						1		
Total		10	66	19	34	10	10	8	19

1 Table 4. Exopolysaccharide (EPS) and gas formation in 4 commercial herring products after 2
2 weeks storage at 6°C. Inoculations were made using 2 *L. gasicomitatum* strains and 2 *L.*
3 *gelidum* strains as 3 combinations A, B and C containing all four strains, the two *L.*
4 *gasicomitatum* strains or the two *L. gelidum* strains, respectively. Inoculum cell density of $5 \times$
5 10^5 CFU/ml into 100g of herring was used in all tests and the severities of slime and gas
6 formation were judged by scoring from none (0) to severe (5).

LAB strain combination	Herring filets in acetic acid		Herring in acid marinade		Herring in tomato marinade		Herring in sugar and spice marinade	
	Gas	EPS	Gas	EPS	Gas	EPS	Gas	EPS
A	5	1	3	0	4	4	4	3
B	5	1	3	0	5	4	5	2
C	5	1	3	0	ND ^a	ND	ND	ND

7 ^aND, not determined

LEGENDS TO THE FIGURES

Fig. 1. Strong slime formation in an acetic acid herring preserve after 2 to 3 weeks storage at 0 to 6°C.

Fig. 2. *HindIII* 16 and 23S RFLP patterns and numerical analysis of the patterns presented as a dendrogram. Patterns obtained from lactic acid bacterium strains detected in an acetic acid herring preserve showing slimy spoilage. Left side of the banding patterns possesses high molecular weights, < 23 kbp, and right side >1000 bp.

Fig. 3. *HindIII* 16 and 23S RFLP patterns and numerical analysis of the patterns presented as a dendrogram. Patterns obtained from lactic acid bacterium strains detected in carrot and allspice samples. Left side of the banding patterns possesses high molecular weights, < 23 kbp, and right side >1000 bp.

Fig. 1 Lyhs et al.

Fig. 2. Lyhs et al.

Fig. 3. Lyhs et al.