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Identification and Characterization of *Leuconostoc carnosum*, Associated with Production and Spoilage of Vacuum-Packaged, Sliced, Cooked Ham

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Leuconostoc carnosum **was shown to be the specific spoilage organism in vacuum-packaged, sliced, cooked ham showing spoilage during 3 weeks of shelf life. Identification of the specific spoilage organism was done by use of phenotypic data and *Cla*I, *Eco*RI, and *Hin*dIII reference strain ribopatterns. One hundred *L. carnosum* isolates associated with the production and spoilage of the ham were further characterized by pulsed-field gel electrophoresis (PFGE), together with some meat-associated *Leuconostoc* species: *L. citreum*, *L. gelidum*, *L. mesenteroides* subsp. *dextranicum*, and *L. mesenteroides* subsp. *mesenteroides*. *Apa*I and *Sma*I digests divided the industrial *L. carnosum* strains into 25 different PFGE types, *Apa*I and *Sma*I types being consistent. Only one specific PFGE type was associated with the spoiled packages. This type also was detected in air and raw-meat mass samples. The spoilage strain did not produce bacteriocins. Only seven isolates belonging to three different PFGE types produced bacteriocins. Similarity analysis of the industrial *L. carnosum* strains revealed a homogeneous cluster which could be divided into eight subclusters consisting of strains having at most three-fragment differences. The *L. carnosum* cluster was clearly distinguished from the other meat-associated leuconostoc clusters, with the exception of the *L. carnosum* type strain. Ribotyping can be very helpful in the identification of *L. carnosum*, but its discriminatory power is too weak for strain characterization. PFGE provides good discrimination for studies dealing with the properties of homogeneous *L. carnosum* strains.**

Lactic acid bacteria (LAB) are the major spoilage bacteria in vacuum-packaged, cooked meat products (1, 2, 10, 13, 25, 27, 31, 38, 44, 47, 56). *Lactobacillus* and *Leuconostoc* have been the main genera associated with the spoilage of these products, *Lactobacillus sake* and *Lactobacillus curvatus* being isolated commonly (12, 16, 18, 19, 24, 27, 30, 35, 39, 43–46). Compared to aerobic spoilage bacteria, spoilage LAB produce their typical sensory changes, such as souring, gas formation, and/or slime formation, later, at the stationary phase (29, 44), and a vacuum-packaged product is usually expected to maintain good sensory quality for at least 3 to 4 weeks. However, due to an increased level of LAB contamination or particularly active spoilage strains, spoilage may occur during the shelf-life period, subjecting the producer to recalls (30, 31, 33, 46).

In an LAB contamination study of vacuum-packaged, sliced, cooked ham, 982 LAB isolates from the spoiled product and production line were characterized in order to determine the underlying reasons for fluctuations in product quality (4, 6). Many lots had been showing spoilage changes, i.e., sour odor and taste, before the sell-by date. In that study, ribotyping (21) was used as a tool for contamination analysis. Based on *Eco*RI and *Hin*dIII ribopatterns, two major spoilage LAB types, types G and A, were detected. Contamination with these spoilage LAB was shown to have occurred postcooking, and a probable site of air-mediated contamination from the macerated raw-meat mass to the cooked product was revealed. Because type G showed the typical *Eco*RI and *Hin*dIII ribopatterns of *L. sake* (5), no further identification or characterization studies were warranted. However, the most important specific spoilage organism, type A, was not identified to the species level. Type A had been detected as the dominant type in the macerated raw-meat mass and in the spoiled packages with the strongest changes in sensory characteristics (6). It had also persisted in the plant during the 1-year study period, consisting of two separate large-scale contamination experiments (4, 6).

In this study, we set out to identify type A LAB to the species level and characterize in more detail the 100 isolates possessing the type A *Eco*RI and *Hin*dIII ribopatterns. Since phenotypic characteristics alone are seldom sufficient for species identification of LAB (15), a reference strain library was created by ribotyping and was used with phenotypic data. Pulsed-field gel electrophoresis (PFGE) was applied in order to provide further strain-level characterization. Production of bacteriocins was determined for evaluation of the impact of this characteristic in a population associated with process contamination and product spoilage.

MATERIALS AND METHODS

Bacterial strains. One hundred type A LAB possessing the same *Eco*RI and *Hin*dIII ribopatterns had been isolated during a contamination study of a meat plant (6). All isolates were gram-positive, oval cocci isolated from a macerated raw-meat mass, air in the macerating room, surfaces and air in the cooking room, worker's gloves, surfaces of the ham prior to slicing, and vacuum-packaged, sliced, cooked ham cultured on the sell-by date. Isolates originating from different sources are listed in Table 1.

In order to obtain a library for species identification, the following reference strains were ribotyped with *Cla*I, *Eco*RI, and *Hin*dIII: *Leuconostoc carnosum* NCFB (National Collection of Food Bacteria) 2776^T, *Leuconostoc citreum* (*Leuconostoc amelibiosum*) D1 (35), *Leuconostoc fallax* CCUG (Culture Collection of University of Gothenburg) 30061^T, *Leuconostoc gelidum* NCFB 2775^T, *Leuconostoc lactis* CCUG 30064^T, *Leuconostoc mesenteroides* subsp. *mesenteroides* DSM (Deutsche Sammlung von Mikroorganismen) 20343^T, *Leuconostoc mesenteroides* subsp. *cremoris* CCUG 21965^T, *Leuconostoc mesenteroides* subsp. *dextranicum* DSM 20484^T, *Leuconostoc pseudomesenteroides* DSM 20193^T, *Weissella halotolerans* ATCC (American Type Culture Collection) 35410^T, *Weissella viri-*

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TABLE 1. Division of the isolates into different types and certain phenotypic properties

Type[a]	Isolates[b]	Restriction enzyme profile		Production of[c]:	
		*Apa*I	*Sma*I	Slime from sucrose	Bacteriocin
A I-a	M2n, M2h	A1	S1	−	−
A I-b	M3h, M6f, M6h	A2	S2	−	−
A I-c	P31a, M41, M4m	A3	S3	−	−
A I-d	I27e	A4	S4	+	−
A I-e	M5i, M5j	A5	S5	−	−
A I-f	P36b	A6	S6	−	−
A I-g	M6j	A7	S7	+	−
A I-h	V8a–o, V9a–o, V11a–m, V13a–o, I2b, I27a, M5o, M6a	A8	S8	−	−
A I-i	M6o	A9	S9	−	−
A I-j	M2e, M2l, M2o, M3f, M3l	A10	S10	−	+
A I-k	M6g	A11	S11	+	−
A II-a	I1b	A12	S12	−	−
A II-b	I1c, I1f	A13	S13	−	−
A II-c	I26b, I28b	A14	S14	+	−
A II-d	I2a	A15	S15	−	−
A III-a	M2j, M3e, M3m	A16	S16	+	−
A III-b	M1e	A17	S17	+	−
A III-c	M5k	A18	S18	+	−
A IV	M2d	A19	S19	−	+
A V-a	M3o, M6i	A20	S20	+	−
A V-b	M1f	A21	S21	+	+
A VI	M1j	A22	S22	+	−
A VII-a	I27f	A23	S23	+	−
A VII-b	M1i	A24	S24	−	−
A VIII	M1c	A25	S25	−	−

[a] Types sharing the same roman numeral differ by at most three bands in the restriction enzyme profiles.
[b] Sampling was described previously (6). Sources were as follows: M, raw-meat mass; P, surface; I, air; V, spoiled product. Groups of lowercase letters indicate a series of isolates; e.g., a–o indicates 15 isolates from V8a to V8o.
[c] +, production; −, no production.

descens ATCC 12706^T, and *Weissella paramesenteroides* DSM 20288^T. In addition, the previously established (5, 7) *Cla*I, *Eco*RI, and *Hin*dIII *Lactobacillus* ribotypes were compared with the *Leuconostoc* and *Weissella* ribotypes characterized in this study.

The meat-associated reference strains *L. carnosum* NCFB 2776^T, *L. citreum* (*L. amelibiosum*) D1 (35), *L. gelidum* NCFB 2775^T, *L. mesenteroides* subsp. *dextranicum* DSM 20484^T, *L. mesenteroides* subsp. *mesenteroides* DSM 20343^T, *L. pseudomesenteroides* DSM 20193^T, and *W. paramesenteroides* DSM 20288^T were characterized by PFGE along with the industrial isolates.

All strains were maintained in MRS broth (Difco, Detroit, Mich.) at −70°C and cultured with MRS broth or MRS agar (Oxoid, Basingstoke, United Kingdom) as previously described (28).

Phenotypic characterization. The anaerobic growth of all industrial isolates on Rogosa selective *Lactobacillus* agar (Orion Diagnostica, Espoo, Finland) was determined, and the scheme of Villiani et al. (55) was used for the presumptive identification of *Leuconostoc* spp. Gas production from glucose was tested with modified MRS broth in Durham tubes (51). Production of ammonia from arginine was observed by the method of Briggs (14), and dextran formation was studied with 5% sucrose-containing agar (22). Fermentation of carbohydrates was determined by use of the API 50 CH *Lactobacillus* identification system (Biomerieux, Marcy l'Etoile, France) for five randomly selected isolates (I27a, M1f, V8a, M6f, and P31a), which were also tested for the ability to produce different lactic acid isomers by an enzymatic method (57) with D- and L-lactate dehydrogenases (Boehringer GmbH, Mannheim, Federal Republic of Germany). The five randomly selected isolates were also tested for growth in MRS broth at 8, 10, 15, and 37°C.

Bacteriocin determination. The agar spot test method modified by Schillinger and Lücke (48) was used for screening bacteriocin activity. Based on existing literature, *L. mesenteroides* subsp. *mesenteroides* DSM 20343^T was selected as the indicator bacterium (3, 26, 40, 54, 58).

In vitro isolation of DNA and ribotyping for species identification. Reference strains and the five randomly selected industrial isolates, already known to possess similar *Eco*RI and *Hin*dIII ribotypes, were characterized with *Cla*I, *Eco*RI, and *Hin*dIII (New England BioLabs, Beverly, Mass.). These enzymes were selected because they characterize LAB well (4–6). DNA was isolated by the guanidium thiocyanate method of Pitcher et al. (42) as modified by Björkroth and Korkeala (4) by combined lysozyme and mutanolysin treatments. Restriction endonuclease treatment of 3 μg of DNA was done as specified by the manufacturer (New England BioLabs). Genomic blotting was done by vacuum blotting (Vacugene; Pharmacia, Uppsala, Sweden), and the ribosomal DNA probe for ribotyping was labeled by reverse transcription (avian myeloblastosis virus reverse transcriptase [Promega, Madison, Wis.]; Dig DNA labeling kit [Boehringer]) as previously described by Blumberg et al. (11). Membranes were hybridized at 68°C as described by Björkroth and Korkeala (5). Similarity between all ribopatterns was determined visually.

In situ DNA isolation and PFGE. Cells were harvested from 2 ml of MRS broth cultures grown overnight at 30°C. DNA isolation in situ from agarose blocks was performed as described by Maslow et al. (37) with the modifications described by Björkroth et al. (9). Initially, 11 rare-cutting restriction enzymes, *Apa*I, *Asc*I, *Eag*I, *Mlu*I, *Not*I, *Nru*I, *Rsr*II, *Sac*II, *Sma*I, *Xba*I, and *Xho*I, were tested for the cleavage of DNA of three strains (NCFB 2776^T, M6f, and I27a). *Apa*I and *Sma*I, which produced convenient numbers of fragments with discriminatory patterns, were chosen for the cleavage of all strains. The samples were electrophoresed through a 1.2% (wt/vol) agarose gel (SeaKem Gold; FMC BioProducts, Rockland, Maine) in 0.5× TBE (45 mM Tris, 4.5 mM boric acid [pH 8.3], 1 mM sodium EDTA) at 14°C by use of a Gene Navigator system with the hexagonal electrode (Pharmacia). Interpolation ramping from 0.5 to 15 s for 20 h at 200 V was used for both enzyme digests.

PFGE data management. Photographs of the PFGE banding patterns were scanned with a ScanJet 4c/T scanner (Hewlett-Packard Co., Boise, Idaho). Numerical analysis of macrorestriction patterns was performed with a GelCompar system (version 4.0; Applied Maths, Kortijk, Belgium). The similarity between all pairs was expressed by Dice coefficient correlation, and clustering by the unweighted pair-group method with arithmetic averages was used for the construction of the dendrogram. Types were considered closely related (53) in the presence of at most a three-band difference (one genetic event). This relationship was indicated in the type nomination by a shared roman numeral.

RESULTS

The 100 isolates did not grow on Rogosa selective *Lactobacillus* agar; all produced gas from glucose but did not produce ammonia from arginine. Fifteen isolates (11 different PFGE

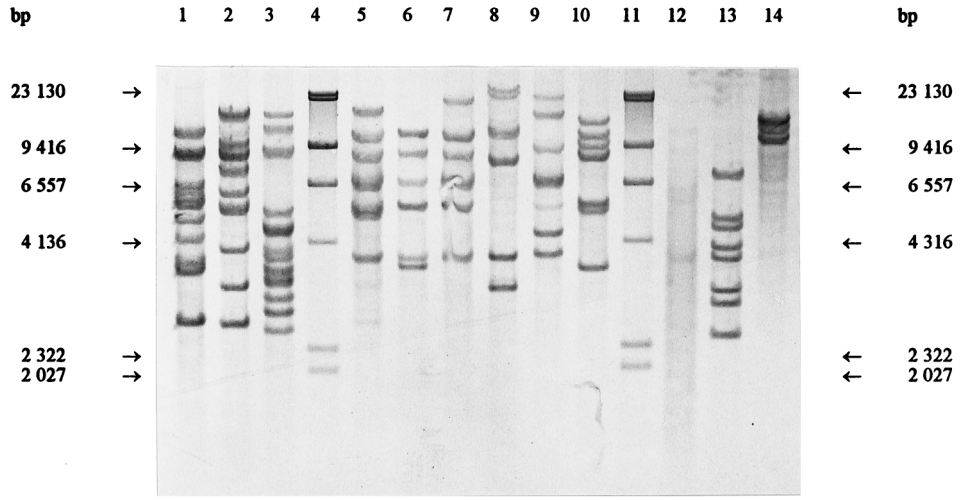

FIG. 1. *Cla*I ribopatterns. Lanes 4 and 11, phage lambda DNA cleaved with *Hin*dIII as a fragment size marker; lane 1, *Weissella viridescens* ATCC 12706^T; lane 2, *Weissella halotolerans* ATCC 35410^T; lane 3, *Weissella paramesenteroides* DSM 20288^T; lane 5, *Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343^T; lane 6, *Leuconostoc mesenteroides* subsp. *cremoris* CCUG 21965^T; lane 7, *Leuconostoc mesenteroides* subsp. *dextranicum* DSM 20484^T; lane 8, *Leuconostoc pseudomesenteroides* DSM 20193^T; lane 9, *Leuconostoc carnosum* NCFB 2776^T; lane 10, *Leuconostoc gelidum* NCFB 2775^T; lane 12, *Leuconostoc lactis* CCUG 30064^T; lane 13, *Leuconostoc fallax* CCUG 30061^T; lane 14, *Leuconostoc citreum* (*Leuconostoc amelibiosum*) D1.

types) produced slime from sucrose, and bacteriocins were produced by 7 isolates (Table 1). The five isolates tested produced only D-lactic acid and had similar fermentation patterns for the utilization of ribose, D-glucose, D-fructose, α-methyl-D-glucoside, *N*-acetylglucosamine, cellobiose, saccharose, trehalose, β-gentiobiose, D-turanose, and gluconate. Growth occurred at 8, 10, and 15°C but not at 37°C.

Previously determined oval cell morphology and the phenotypic characteristics typical of leuconostocs led to the comparison with the *Leuconostoc* and *Weissella* type strains. Figures 1, 2, and 3 show that the *Cla*I, *Eco*RI, and *Hin*dIII ribotypes, respectively, of the reference strains differed clearly from the *Lactobacillus* ribotypes obtained previously (5, 7). The *Cla*I, *Eco*RI, and *Hin*dIII ribopatterns of the industrial isolates were found to be identical to those of *L. carnosum* NCFB 2776^T (Fig. 1, 2, and 3, lanes 10). All of the other type strains were distinct from *L. carnosum* NCFB 2776^T. Based on the phenotypic data and the identical ribopatterns, the industrial isolates were classified as *L. carnosum*. *Hin*dIII and *Eco*RI generated the least distinguishing ribotypes for the *Leuconostoc* and *Weissella* species. *Cla*I was the only enzyme distinguishing *L. mesenteroides* subsp. *mesenteroides* from *L. mesenteroides* subsp. *dextranicum* (Fig. 1, lanes 5 and 7).

Both *Apa*I and *Sma*I generated 25 different patterns for the meat plant isolates when one-band differences are noted. The *Apa*I types were consistent with the *Sma*I types (Table 1). All meat-associated reference strains, with the exception of *L. carnosum* NCFB 2776^T, were clearly distinguished from the industrial isolates (Fig. 4 and 5). Both *Apa*I and *Sma*I resulted in convenient numbers of fragments for macrorestriction anal-

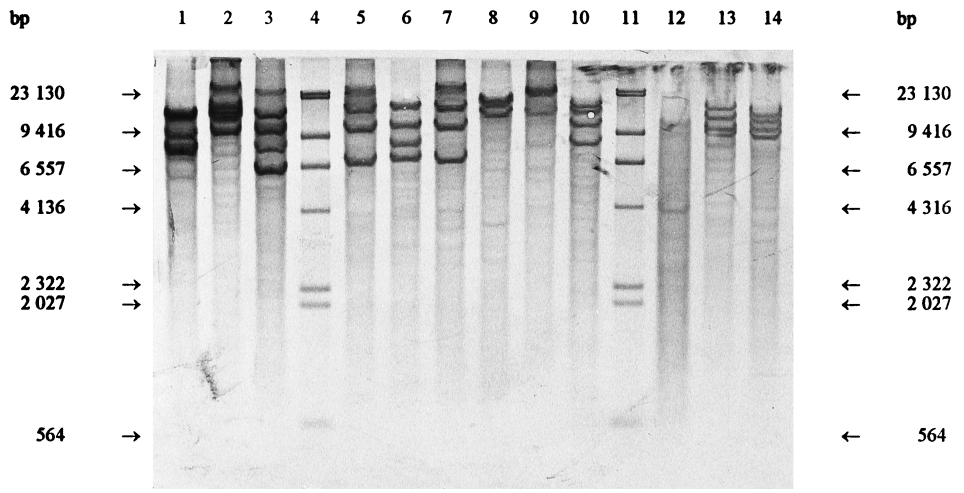

FIG. 2. *Eco*RI ribopatterns. Lanes 4 and 11, phage lambda DNA cleaved with *Hin*dIII as a fragment size marker; lane 1, *Weissella viridescens* ATCC 12706^T; lane 2, *Weissella halotolerans* ATCC 35410^T; lane 3, *Weissella paramesenteroides* DSM 20288^T; lane 5, *Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343^T; lane 6, *Leuconostoc mesenteroides* subsp. *cremoris* CCUG 21965^T; lane 7, *Leuconostoc mesenteroides* subsp. *dextranicum* DSM 20484^T; lane 8, *Leuconostoc pseudomesenteroides* DSM 20193^T; lane 9, *Leuconostoc carnosum* NCFB 2776^T; lane 10, *Leuconostoc gelidum* NCFB 2775^T; lane 12, *Leuconostoc lactis* CCUG 30064^T; lane 13, *Leuconostoc fallax* CCUG 30061^T; lane 14, *Leuconostoc citreum* (*Leuconostoc amelibiosum*) D1.

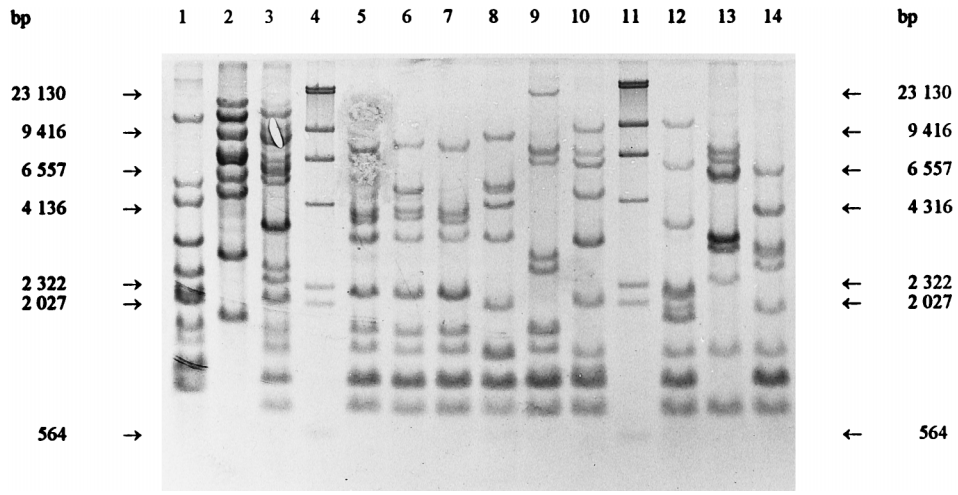

FIG. 3. *Hin*dIII ribopatterns. Lanes 4 and 11, phage lambda DNA cleaved with *Hin*dIII as a fragment size marker; lane 1, *Weissella viridescens* ATCC 12706^T; lane 2, *Weissella halotolerans* ATCC 35410^T; lane 3, *Weissella paramesenteroides* DSM 20288^T; lane 5, *Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343^T; lane 6, *Leuconostoc mesenteroides* subsp. *cremoris* CCUG 21965^T; lane 7, *Leuconostoc mesenteroides* subsp. *dextranicum* DSM 20484^T; lane 8, *Leuconostoc pseudomesenteroides* DSM 20193^T; lane 9, *Leuconostoc carnosum* NCFB 2776^T; lane 10, *Leuconostoc gelidum* NCFB 2775^T; lane 12, *Leuconostoc lactis* CCUG 30064^T; lane 13, *Leuconostoc fallax* CCUG 30061^T; lane 14, *Leuconostoc citreum* (*Leuconostoc amelibiosum*) D1.

ysis (Fig. 4). However, *Sma*I cleaved the DNA efficiently, whereas some partial digestion was occasionally noted with *Apa*I. Because of the better reproducibility, *Sma*I patterns were chosen for the numerical analysis.

Figure 5 shows the dendrogram of the industrial isolates and the reference strains. *L. carnosum* formed a homogeneous cluster, within which eight subclusters consisted of strains having at most three-band differences. Reference strains, with the exception of the *L. carnosum* type strain, clustered separately from the industrial isolates. Isolates associated with the sensorially spoiled products all showed the type A I-h pattern (Fig. 4, lanes 8 and 17) and belonged to the largest cluster, consisting of A I types (Fig. 5 and Table 1). Type A I-h was also detected in two raw-meat mass samples and two air samples, one from the macerating room and one from the postcooking form removal area.

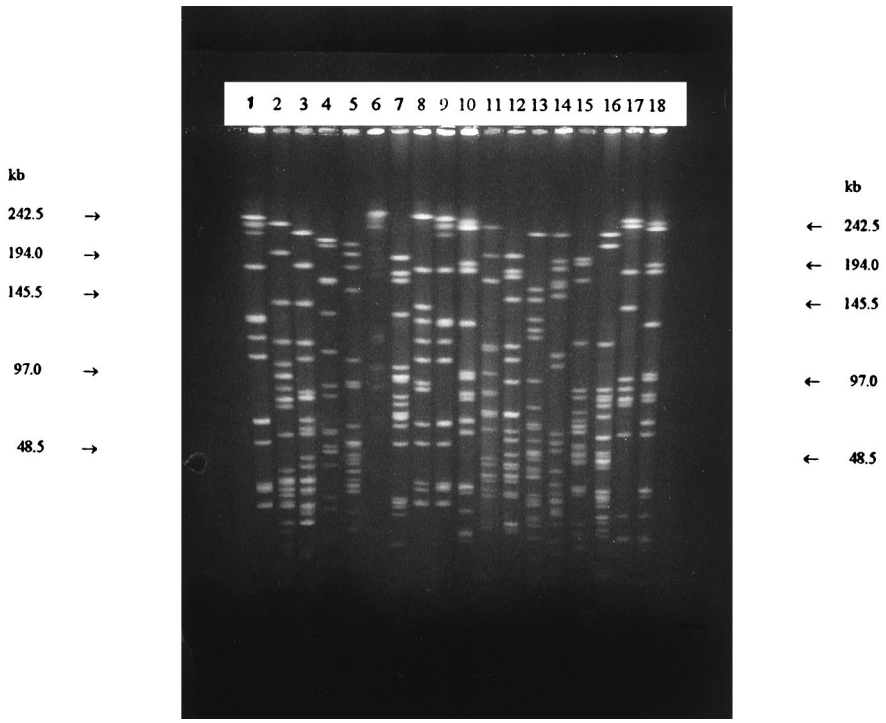

FIG. 4. *Sma*I (lanes 1 to 9) and *Apa*I (lanes 10 to 18) ribopatterns. Lanes 1, 9, 10, and 18, *Leuconostoc carnosum* NCFB 2776^T; lanes 2 and 11, *Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343^T; lanes 3 and 12, *Leuconostoc mesenteroides* subsp. *dextranicum* DSM 20484^T; lanes 4 and 13, *Leuconostoc pseudomesenteroides* DSM 20193^T; lanes 5 and 14, *Weissella paramesenteroides* DSM 20288^T; lanes 6 and 15, *Leuconostoc gelidum* NCFB 2775^T; lanes 7 and 16, *Leuconostoc citreum* (*Leuconostoc amelibiosum*) D1; lanes 8 and 17, *Leuconostoc carnosum* V-8a.

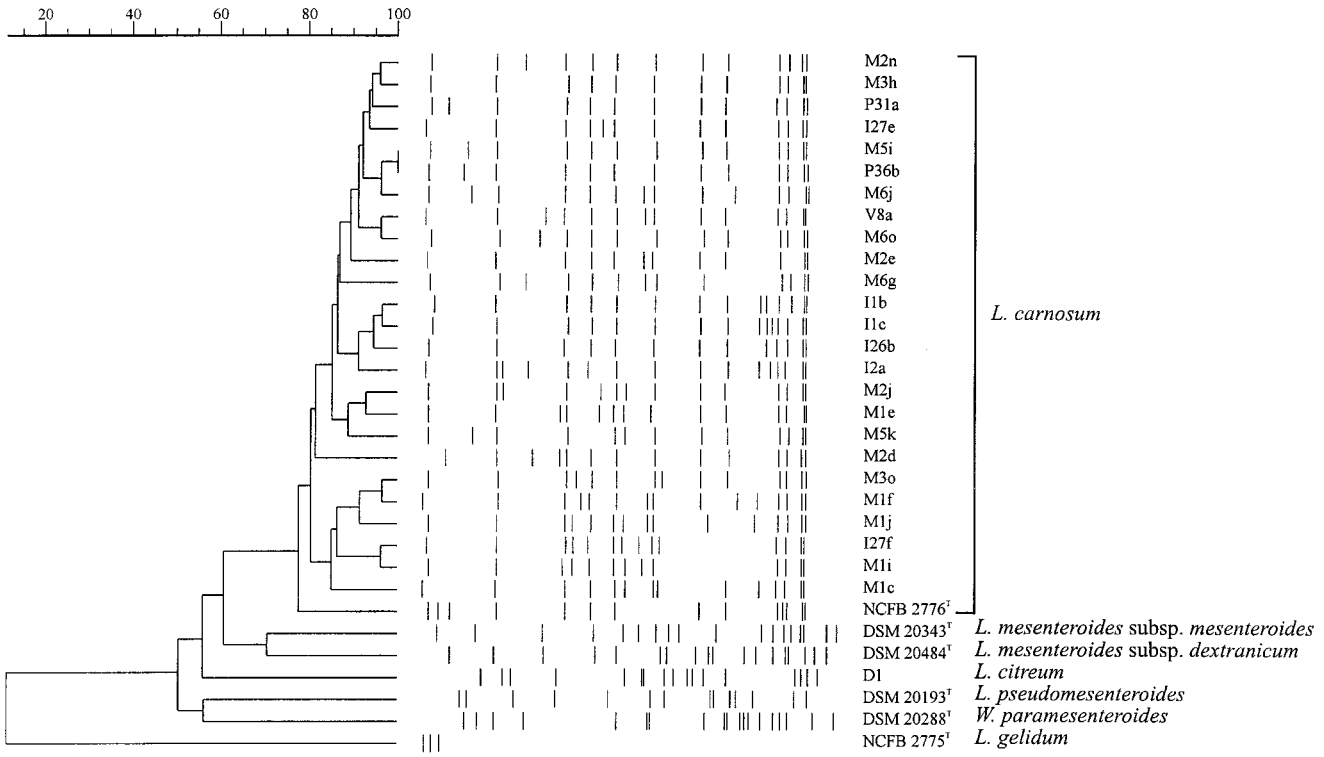

FIG. 5. Dendrogram based on *Sma*I ribopatterns. The similarity between all pairs was expressed by Dice coefficient correlation, and the unweighted pair-group method with arithmetic averages was used for the construction of the dendrogram.

DISCUSSION

L. carnosum was identified as the specific spoilage organism in the vacuum-packaged, cooked ham studied here. This species was described along with *L. gelidum* by Shaw and Harding in 1989 (50). It belongs to the main *Leuconostoc* cluster designated *Leuconostoc* sensu stricto and shares 97 to 99% rRNA homology with the other sensu stricto species: *L. citreum*, *L. gelidum*, *L. lactis*, *L. mesenteroides*, and *L. pseudomesenteroides* (15). Characterization studies of *L. carnosum* have been sparse and have been done with a limited number of strains (50, 58). Studies associated with *L. carnosum* have mainly focused on the production and purification of bacteriocins produced by this species (3, 20, 23, 26, 40, 41, 49, 52, 54).

L. carnosum seems to be strongly associated with ham products. In an earlier meat production plant contamination study (6), type A was found to dominate in the microflora of the raw-pork mass macerated overnight. In this plant, we noted that *L. carnosum* contamination occurred mainly in ham, whereas *L. sake* and *L. curvatus* have been detected in a variety of products (4, 6). Approximately 36% of the spoilage flora in Vienna sausages has been reported to consist of leuconostocs (17). When these *Leuconostoc* species were identified (18), the absence of *L. carnosum* was emphasized. In another characterization study of the LAB causing spoilage in vacuum-packaged, processed meats, a high prevalence of bacteriocin-producing psychrotropic leuconostocs was revealed (58). In that study, nine isolates were identified as *L. carnosum*; eight of these nine originated from different types of ham and one originated from sliced turkey. The strains forming the *L. carnosum* cluster (III) in the work describing this species (50) were from cold-stored, vacuum-packaged beef, pork, bacon, cooked ham, and luncheon meat. Compared with ham and other whole-meat products, emulsion sausages have more variable raw materials, such as different meat mixtures, pork skin emulsion, and spices, and undergo a different type of processing. The process and ingredients used for ham manufacturing may favor the survival and/or growth of *L. carnosum*. However, an adequate cooking process, considered to be the most important factor destroying LAB on products prior to packaging (1, 33, 34, 36), and the use of nitrite are similar in the production of emulsion sausages and whole-meat products.

PFGE characterization of *L. carnosum* confirmed the assumption that the raw-meat mass was the major source of contamination. The type of LAB contamination in a product has been considered to reflect the type of contamination in the processing facility (25, 38). Various LAB types were shown to contaminate the environment associated with the ham processing line studied here (4, 6). The greatest diversity in the different types of LAB was found in the environmental surface samples (4, 6). However, the majority of these LAB types have never been isolated from packaged products (4, 6). Only type A I-h isolates associated with the spoiled packages (V isolates), raw material (M5o and M6a isolates), and air of the macerating room (isolate I2b) and postcooking form removal area (isolate I27a) contaminated the products before they were transferred to the slicing-packaging department. The products were contaminated with a spoilage organism from the raw-meat mass before they entered the slicing line. In this case, the slicing line and the slicing room were not the main site and source of contamination, as is so often thought (25, 38). This route of contamination may be more common than is generally considered, also explaining the link between raw-meat mass and cooked ham.

Identification of species of the genus *Leuconostoc* is difficult

(15, 55), which apparently is the main reason for the sparse population characterizations published. *Leuconostoc* spp. are phenotypically related to *Weissella* spp., heterofermentative lactobacilli, and pediococci and form a natural phylogenetic group with *Weissella confusa*, *W. halotolerans*, *Weissella kandleri*, *Weissella minor*, and *W. viridescens* (15). Due to the variable results obtained, sugar fermentation patterns are of little value in the species identification and could lead to misclassification (15). For presumptive identification, the scheme proposed by Villiani et al. (55) was found practical. However, in this scheme *L. carnosum* is supposed to form dextran. Only 15 of the 100 isolates tested here (11 of the established 25 PFGE types) formed slime from sucrose, lessening the value of this characteristic in *L. carnosum* identification.

It has been stated that reliable differentiation between *L. carnosum* and *L. gelidum* is impossible without DNA-DNA hybridization (15). Our results indicate that ribotyping can be used to distinguish *L. carnosum* from the other phenotypically related leuconostocs. However, care must be taken when enzymes are selected for species identification by ribotyping. Using *Hin*dIII-based ribopatterns, Villiani et al. (55) could not distinguish *L. mesenteroides* subsp. *mesenteroides* from *L. mesenteroides* subsp. *dextranicum* and *L. lactis*. We found *Hin*dIII and *Eco*RI to be the least distinguishing enzymes and *Cla*I to be the only enzyme generating a clear one-band shift in the patterns of these two subspecies (Fig. 1, lanes 5 and 7). *Cla*I may thus provide better results for the discrimination of *L. mesenteroides* subspecies. However, the *Hin*dIII pattern of the *L. lactis* type strain was clearly distinguished from the patterns of the *L. mesenteroides* subspecies (Fig. 3, lanes 5, 7, and 12). Despite its value in species identification and LAB contamination studies dealing with a diversity of species, ribotyping cannot be used for strain characterization when such a homogeneous population, such as the population of *L. carnosum* isolated from the meat production plant studied here, is assessed.

Only one type, A I-h (Fig. 4, lanes 8 and 17), from the largest lineage, was associated with the sensorially spoiled packages; however, even the production environment was not overwhelmingly contaminated by this specific organism. Strains of this type may possess characteristics that aid in growth niche occupation. Specific spoilage organisms have been considered to have better competitive ability, enabling them to prevail in the microflora present (8, 13, 32). Differences in the generation time, production of bacteriocins, strong ability to produce slime or volatile compounds causing sensorial spoilage, and better resistance to different stress factors, such as cold, heat, and disinfectants, are factors considered to be associated with specific spoilage organisms. For the *L. carnosum* population studied here, the production of bacteriocins was not found to be a common characteristic, as reported by Yang and Ray (58). The nine *L. carnosum* isolates studied by Yang and Ray (58) all inhibited *L. mesenteroides*. The true general impact of bacteriocin production in the development of spoilage flora is still not clear. Studies of bacteriocin production have mainly focused on the use of bacteriocins or cultures producing bacteriocins as biopreservatives. Biopreservatives are inoculated at a high initial concentration or a dense population in a freshly prepared product. This situation differs clearly from the situation in which some or one species in a contaminating flora gradually occupies a niche in a package and, finally, when reaching the stationary phase, spoils the product.

Molecular typing methods also provide valuable information for applied microbiology. They can contribute to knowledge of different bacterial populations associated with food processing and enable future research to be focused accurately on specific spoilage organisms and their specific characteristics. Such work will rely mainly on the reliable species identification and good strain characterization of specific spoilage organisms.

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