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Leuconostoc gelidum sp. nov. and *Leuconostoc carnosum* sp. nov. from Chill-Stored Meats

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A numerical taxonomic study was performed on 52 *Leuconostoc* spp. strains isolated from chill-stored meats. Three clusters were observed; representative strains from these clusters, together with the type strains of previously described species, were examined by performing a cellular fatty acid analysis and deoxyribonucleic acid (DNA)-DNA hybridization. Cluster II contained seven strains, which were assigned to *Leuconostoc mesenteroides* subsp. *mesenteroides* on the basis of DNA relatedness and biochemical properties. Clusters I (30 strains) and III (15 strains) were shown to represent two new species, for which the names *Leuconostoc gelidum* and *Leuconostoc carnosum*, respectively, are proposed. The type strains of *L. gelidum* and *L. carnosum* are strains NCFB 2775 and NCFB 2776, respectively.

The genus *Leuconostoc* is presently composed of four species, viz., *Leuconostoc mesenteroides* (containing three subspecies, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc mesenteroides* subsp. *dextranicum*, and *Leuconostoc mesenteroides* subsp. *cremoris*), *Leuconostoc paramesenteroides*, *Leuconostoc lactis*, and *Leuconostoc oenos* (8). This classification is based on phenotypic characteristics, including nutritional requirements (3, 5), immunological relationships of dehydrogenases (10, 13), and deoxyribonucleic acid (DNA) homologies (6, 14).

The habitats of all of these species except *Leuconostoc oenos* are reported to be milk and dairy products and plant material; *Leuconostoc oenos* is isolated only from wine and related habitats (8, 21). However, there have been several reports of leuconostocs occurring among the dominant microbial population on meats stored in vacuum packs or under modified gas atmospheres containing carbon dioxide (1, 11, 20, 22), where they may contribute to spoilage. Some isolates from meat have been identified by using an early classification scheme (3) as belonging to groups equivalent to *Leuconostoc mesenteroides* subsp. *mesenteroides* or *Leuconostoc paramesenteroides* (1, 20). However, in our experience many isolates are not identifiable as any of the previously described species.

In this study we used numerical taxonomic techniques to group leuconostocs from stored packaged meats and determined the relationships of the groups to the previously described species. Groups were also studied by performing a cellular fatty acid analysis and DNA homology experiments, which revealed two new species, for which the names *Leuconostoc gelidum* and *Leuconostoc carnosum* are proposed.

MATERIALS AND METHODS

Strains studied. A total of 52 *Leuconostoc* spp. strains from eight samples of raw and cooked vacuum-packed meats were examined (Table 1). The isolates deemed to be leuconostocs were gram-positive, catalase-negative cocci or coccobacilli that produced gas from glucose and formed more than 95% of their lactate as the D-(−) isomer. The type strains of five of the six *Leuconostoc* species and subspecies described in *Bergey's Manual of Systematic Bacteriology*, vol. 2 (8), were included for reference purposes. The type strain of *Leuconostoc oenos* was not included, as this species was known to differ from the meat strains in its requirement for special media with a pH of 4.8 or less (5).

Cultures were maintained at 0 to 1°C in cooked meat medium and were subcultured every 3 months. Except where otherwise stated, inocula for tests were grown in BM broth (28) incubated at 20°C.

Morphological, physiological, and biochemical tests. Gram reaction and morphology were determined after 24 h of incubation of TSY agar (12). Gas production from glucose, ammonia production from arginine, reduction of 0.01% 2,3,5-triphenyltetrazolium chloride, and sugar fermentation were determined as described by Shaw and Harding (23), and acetoin production and hydrolysis of esculin were determined as described by Wilkinson and Jones (28). The isomer of lactate formed from glucose was determined by using the procedure of Shaw and Harding (22), except that D-lactate and L-lactate were determined enzymatically by using reagents obtained from Boehringer GmbH, Mannheim, Federal Republic of Germany. The *o*-nitrophenyl-β-D-galactopyranoside test was performed by using the method of Lowe (18). All other tests were performed as described by Shaw and Harding (22).

Computer analysis of phenetic data. A total of 23 characters were identical for all strains and were omitted from the calculation of similarities among strains. The remaining 23 characters were coded in the following states: 1 (positive) and 2 (negative). The level of similarity among strains was estimated by using the simple matching coefficient of Sokal and Michener (25) and clustering by unweighted pair-group average linkage analysis (25).

The 23 tests giving varied results for different strains were repeated with 10% of the strains to determine test reproducibility.

Cellular fatty acids. Cultures were grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 3 g of Lab Lemco (Oxoid Ltd., Basingstoke, United Kingdom) per liter. Cells were harvested by centrifugation (10,000 × g, 10 min) following static incubation of 200 ml of medium in 250-ml Duran bottles for 72 h at 20°C. Then 40 to 60 mg (wet weight) of cells was transferred to a screw-cap tube and saponified with 1 ml of 15% (wt/vol) NaOH in 50% aqueous methanol for 30 min at 100°C with Vortex mixing initially and after 25 min. After cooling, the

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TABLE 1. Strains studied

Cluster	Name as received	No. of strains	Strain designation(s)	Source	Isolated from:
I (*Leuconostoc gelidum*)	*Leuconostoc* spp.	30	SML1 to SML3, SML5 to SML30, SML52	Our isolates	Vacuum-packaged beef
II (*Leuconostoc mesenteroides* subsp. *mesenteroides*)	*Leuconostoc* spp.	7	SML41 to SML45, SML45, SML50	Our isolates	Vacuum-packaged cooked ham
	Leuconostoc mesenteroides subsp. *mesenteroides*	1	NCFB 523^T (= ATCC 8293^T)	NCFB[a]	Fermenting olives
III (*Leuconostoc carnosum*)	*Leuconostoc* spp.	15	SML31 to SML40^T, SML46, SML48, SML49, SML51, SML53	Our isolates	Vacuum-packaged beef, pork, bacon, cooked ham, and luncheon meat
Unclustered	*Leuconostoc mesenteroides* subsp. *dextranicum*	1	NCFB 529^T	NCFB	Unknown
	Leuconostoc mesenteroides subsp. *cremoris*	1	NCFB 543^T	NCFB	Dried starter powder
	Leuconostoc paramesenteroides	1	NCFB 803^T	NCFB	Unknown
	Leuconostoc lactis	1	NCFB 533^T	NCFB	Milk

[a] NCFB, National Collection of Food Bacteria, Agricultural and Food Research Council, Institute of Food Research, Reading Laboratory, Shinfield, Reading, United Kingdom.

released fatty acids were converted to methyl esters by adding 2 ml of 54% (vol/vol) 6 N HCl in methanol, followed by heating at 80°C for 10 min and then rapid cooling in cold running water. The methyl esters were extracted into 1.25 ml of hexane-ether (1:1) by gentle mixing on a specimen tumbler for 10 min. The phases were allowed to separate, and the lower (aqueous) phase was discarded. The sample was then washed with 3 ml of 1.2% (wt/vol) NaOH by mixing on a specimen tumbler for 5 min. Then 2 drops of a saturated NaCl solution was added to assist separation of the phases.

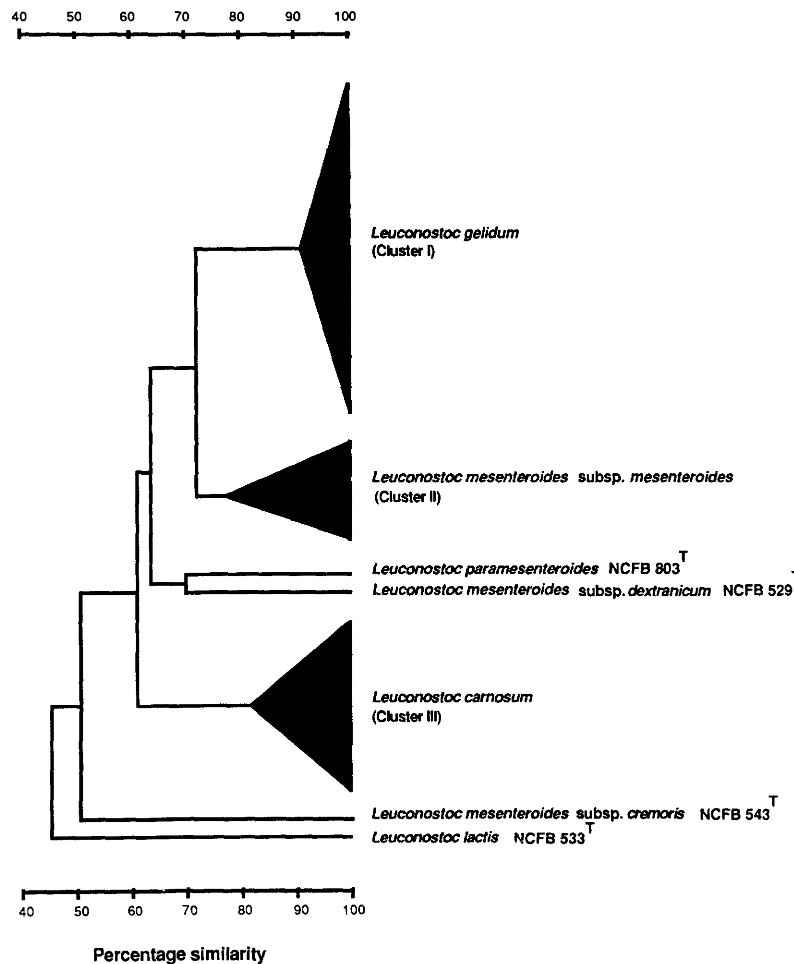

FIG. 1. Simplified dendrogram showing the phenotypic relationships of *Leuconostoc* species as determined by a numerical analysis.

TABLE 2. Properties of the three clusters of leuconostocs detected at a similarity level of 78%[a]

Characteristic	% of strains positive		
	Cluster I (*Leuconostoc gelidum*; *n* = 30)[b]	Cluster II (*Leuconostoc mesenteroides* subsp. *mesenteroides*; *n* = 8)	Cluster III (*Leuconostoc carnosum*; *n* = 15)
Growth at 37°C	3	100	7
Dextran formation	80	100	93
ONPG[c]	100	100	0
Esculin hydrolysis	100	100	66
Hydrolysis of:			
Tween 40	0	0	27
Tween 60	0	88	13
Acid production from:			
Amygdalin	100	13	0
L-Arabinose	100	100	0
Arbutin	100	0	0
Cellobiose	100	13	6
Galactose	0	100	0
Lactose	0	13	0
Maltose	47	100	7
D-Mannose	100	100	80
Melibiose	100	100	13
α-Methyl-D-glucoside	100	25	100
Raffinose	100	88	0
Ribose	63	0	27
Salicin	100	13	7
D-Xylose	100	88	0

[a] All strains produced gas from glucose, produced >95% of their lactate as the D-(−) isomer, grew on acetate agar, grew at 4°C, produced deoxyribonuclease, and produced acid from D-fructose, D-glucose, sucrose, and trehalose. All strains were negative for arginine dihydrolase, acetoin production, reduction of 0.01% tetrazolium, and hydrolysis of Tween 20 and Tween 80, and none produced acid from D-arabinose, dulcitol, erythritol, gluconate, glycerol, inositol, mannitol, D-melezitose, rhamnose, sorbitol, or L-sorbose.

[b] *n* is the number of strains tested.

[c] *o*-Nitrophenyl-β-D-galactopyranoside.

The upper phase was transferred to a gas chromatography autosampler vial and sealed with a crimp cap.

The fatty acid methyl esters were analyzed by using a model 5890 flame ionization gas chromatograph (Hewlett-Packard Co., Avondale, Pa.) equipped with a fused silica capillary column (25 m by 0.2 mm) coated with a 0.33-μm film of cross-linked 5% phenyl methyl silicone (part numbers 19091B-102; Hewlett-Packard) at a column temperature programmed from 170 to 310°C at 5°C/min. Data were recorded with a model 3392A electronic integrator (Hewlett-Packard), and fatty acid methyl esters were identified by computer (series 300; Hewlett-Packard) comparison of retention times with retention times of authentic standards (part number 19298-60500 Rev. B; Hewlett-Packard).

DNA base composition and DNA-DNA hybridization. To prepare DNA, cells harvested from BM broth incubated for 18 h at 25°C were lysed overnight at 37°C with lysozyme and proteinase K (self digested) in 3% 4-aminosalicylate, as described by Garvie (6). DNA was extracted and purified by the procedure of Owen and Pitcher (19). DNA base composition was estimated by the thermal denaturation method, as described by Garvie (7). DNA to be used in hybridization tests was labeled by nick translation with [*methyl*-^{3}H]thymidine triphosphate (kit N5500; Amersham International plc, Amersham, Buckinghamshire, England). DNA homology values were determined by using the S1 nuclease procedure (2) as described by Owen and Pitcher (19). Hybridizations were performed for 18 h under optimum conditions (25°C below the thermal denaturation temperature).

RESULTS

Clustering of strains based on phenetic data. The relationships of strains revealed by the numerical analysis are shown in the simplified dendrogram in Fig. 1. The average probability of error, which was calculated from the repeated test results by using the formula of Sneath and Johnson (24), was 0.6%, which would not produce a serious distortion of taxonomic structure. The cophenetic correlation value was 0.94, showing that the dendrogram gives a good depiction of the relationships among strains.

Of the 57 strains examined, 53 were contained in three clusters (clusters I, II, and III) at a similarity level of 78%. These clusters contained all 52 strains isolated from meat. Strain NCFB 523^{T} (T = type strain) (*Leuconostoc mesenteroides* subsp. *mesenteroides*) was present in cluster II, but the other four reference strains, strains NCFB 529^{T} (*Leuconostoc mesenteroides* subsp. *dextranicum*), NCFB 543^{T} (*Leuconostoc mesenteroides* subsp. *cremoris*), NCFB 803^{T} (*Leuconostoc paramesenteroides*), and NCFB 533^{T} (*Leuconostoc lactis*), were distinct from clusters I, II, and III.

Physiological and biochemical properties of the clusters. As is typical of leuconostocs, no strain hydrolyzed arginine and all of the strains produced >95% of their lactate as the D-(−) isomer (Table 2). Dextran formation was common in all three clusters. All cluster II strains grew at 37°C, whereas clusters I and III contained high percentages of strains that were incapable of growth at this temperature. Otherwise, differences in the properties of the clusters occurred mainly in the fermentation tests, in which cluster III strains were relatively inactive.

Cellular fatty acid composition. All of the strains had cellular fatty acid profiles consisting of straight-chain saturated, monounsaturated, and cyclopropane ring acids (Table 3). The following fatty acids were detected in all strains: tetradecanoic ($C_{14:0}$), *cis*-9,10-hexadecenoic ($C_{16:1}\Delta 9$), hexadecanoic ($C_{16:0}$), *cis*-11,12-octadecenoic ($C_{18:1}\Delta 11$), and *cis*-11,12-methyleneoctadecanoic ($C_{cyclo\text{-}19}\Delta 11$) acids. Despite this qualitative similarity, two different fatty acid profiles were discerned among the three clusters of strains isolated from meat. Cluster I strains and 4 of the 10 cluster III strains had lower *cis*-11,12-octadecenoic acid contents (7 to 17.5%) and higher *cis*-11,12-methyleneoctadecanoic acid contents (21 to 29%) than the cluster II strains and the other cluster III strains, which had *cis*-11,12-octadecenoic acid contents of 21 to 29% and *cis*-11,12-methyleneoctadecanoic acid contents of 4.5 to 9%. The former group also contained 1.5 to 5.5% of methylenehexadecanoic acid, which was not detected in the other fatty acid profile group.

The type strains of the three subspecies of *Leuconostoc mesenteroides* (strains NCFB 523^{T}, NCFB 529^{T}, and NCFB 543^{T}) all had fatty acid profiles similar to those of the cluster II strains. The fatty acid profile of *Leuconostoc paramesenteroides* NCFB 803^{T} was notable for a relatively low *cis*-9,10-hexadecenoic acid content (4%) and a high *cis*-11,12-octadecenoic acid content (46.5%). *Leuconostoc lactis* NCFB 533^{T} contained a small amount of octadecanoic acid; this acid was not detected in any other strain.

DNA base composition and DNA-DNA hybridization. Strains in cluster I were 81 to 114% homologous with their reference strain (strain SML9^{T}) and exhibited low levels of DNA base sequence relatedness to reference strains from the other clusters and type strains of previously described

TABLE 3. Cellular fatty acid compositions

Strain	Fatty acid composition (%)[a]							
	$C_{14:0}$	$C_{16:1}\Delta9$	$C_{16:1}$*trans*-9	$C_{16:0}$	$C_{cyclo\text{-}17}$	$C_{18:1}\Delta11$	$C_{18:0}$	$C_{cyclo\text{-}19}\Delta11$
Leuconostoc gelidum (cluster I)								
SML1	11.0	20.5		33.5	2.0	7.5		24.5
SML7	9.5	19.0		32.5	2.0	7.0		29.0
SML9^T	9.5	19.0		31.5	2.5	7.0		29.5
SML10	9.5	19.5		30.5	1.5	10.5		28.5
SML28	10.5	19.0		31.5	1.5	7.5		28.5
Leuconostoc mesenteroides subsp. *mesenteroides* (cluster II)								
NCFB 523^T	8.5	29.0		34.5		18.5		9.0
SML42	7.5	27.5		31.5		28.0		4.5
SML43	6.0	25.0		33.0		30.0		5.5
SML44	7.5	27.5		32.5		27.5		5.5
SML45	7.0	27.0		31.5		29.5		4.5
SML50	7.0	25.5		31.0		29.5		5.5
Leuconostoc carnosum (cluster III)								
SML31	5.5	15.0	V	34.0		37.5		6.5
SML33	5.5	15.0		35.5		37.5		4.5
SML34	5.5	15.5		34.0		37.0		6.0
SML35	5.5	18.0		35.5		35.0		5.0
SML38	6.0	17.5		35.0		34.5		5.5
SML40^T	5.5	18.5		34.5		35.5		4.5
SML48	7.5	16.5		33.5	3.5	17.5		21.0
SML49	7.0	12.0		35.5	4.0	11.0		28.5
SML51	5.5	10.5		38.0	3.5	11.0		27.5
SML53	13.5	16.5	V	31.5	5.5	9.5		20.5
Leuconostoc mesenteroides subsp. *dextranicum* NCFB 529^T	8.0	24.5		33.5		16.0		17.0
Leuconostoc mesenteroides subsp. *cremoris* NCFB 543^T	9.5	31.5		33.5		18.0		8.0
Leuconostoc paramesenteroides NCFB 803^T	2.0	4.0	6.0	25.5		46.5		14.0
Leuconostoc lactis NCFB 533^T	2.0	8.5		33.5		31.5	1.5	21.5

[a] Each value is the mean from three analyses. V, Detected in some analyses at a level of <3%. Abbreviations for fatty acids are illustrated by the following examples: $C_{16:0}$, straight-chain saturated hexadecanoic acid; $C_{18:1}$, monounsaturated octadecenoic acid; $C_{cyclo\text{-}19}\Delta11$, *cis*-11,12-methyleneoctadecanoic acid.

species (Table 4). Cluster II strains all showed >60% relatedness to the type strains of the subspecies of *Leuconostoc mesenteroides* (*Leuconostoc mesenteroides* subsp. *mesenteroides* NCFB 523, *Leuconostoc mesenteroides* subsp. *dextranicum* NCFB 529, and *Leuconostoc mesenteroides* subsp. *cremoris* NCFB 803). All of the cluster III strains showed high levels of DNA relatedness (>70%) to the reference strains (strains SML40^T and SML49) representing the two fatty acid profile types in that cluster, whereas homology values were low (0 to 35%) against reference strains from clusters I and II and the type strains of the previously described species.

DISCUSSION

Complete agreement was obtained between the classifications of the leuconostocs isolated from meat as determined by numerical analysis of phenotypic properties and by DNA-DNA hybridization. Both techniques revealed the presence of three groups, represented by clusters I, II, and III in the numerical study. The subdivision of cluster III into two groups indicated by the cellular fatty acid composition study was not supported by the DNA hybridization results, which showed that this cluster formed a single homology group. This is reconcilable because the differences were only in the relative amounts of the C_{19} cyclopropane ring acid (*cis*-11,12-methyleneoctadecanoate) and its precursor (*cis*-11,12-octadecenoic acid) and in the presence or absence of the C_{17} cyclopropane ring acid, whose precursor (*cis*-9,10-hexadecenoic acid) was present in both types. These represent very minor enzymatic differences (17) and would not be expected to affect genomic relatedness (26).

All three groups of leuconostocs from meat had intragroup DNA homology values of >70%, showing that each group was composed of a single species (27). The DNA-DNA hybridization results demonstrated that strains in cluster II could be assigned to *Leuconostoc mesenteroides*; high homology values (>60%) were obtained with the type strains of all three subspecies (*Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc mesenteroides* subsp. *dextranicum*, and *Leuconostoc mesenteroides* subsp. *cremoris*). The high levels of DNA relatedness among the subspecies of *Leuconostoc mesenteroides* are in agreement with data from previous studies (6, 14). Strains in cluster II were identified as *Leuconostoc mesenteroides* subsp. *mesenteroides* on the basis of phenotypic similarity, as exemplified by the presence of the type strain (strain NCFB 523) in that cluster in the numerical phenetic analysis. The DNA-DNA hybridization results demonstrated that cluster I and III strains were unrelated to previously described species in the genus *Leuconostoc*. *Lactobacillus confusus* and *Lactobacillus viridescens* are difficult to separate from leuconostocs (8) but were not included in this study. However, there is evidence demonstrating that cluster I and III strains do not belong to these species. Reference strains from each cluster (strains SML9^T and SML40^T) exhibited low (8%) DNA base sequence relatedness to the type strain of *Lactobacillus confusus* (J. A. E. Farrow, personal communication). The guanine-plus-cytosine (G+C) contents of the DNAs of representative strains from clusters I and III were 37 and 39 mol%, respectively, which is outside the range (41 to 44 mol%) for *Lactobacillus viridescens* (16). In contrast to cluster I and III strains, *Lactobacillus viridescens* produces

TABLE 4. Levels of DNA homology for leuconostocs

Species or subspecies	Strain	Mol% G+C	% Homology with [^{3}H]DNA from:							
			Leuconostoc gelidum SML9^T	*Leuconostoc carnosum* SML40^T	*Leuconostoc carnosum* SML49	*Leuconostoc mesenteroides* subsp. *mesenteroides* NCFB 523^T	*Leuconostoc mesenteroides* subsp. *dextranicum* NCFB 529^T	*Leuconostoc mesenteroides* subsp. *cremoris* NCFB 803^T	*Leuconostoc paramesenteroides* NCB 543^T	*Leuconostoc lactis* NCFB 533^T
Leuconostoc gelidum (cluster I)	SML1	37	81	7	21	31	3	8	0	10
	SML3		85	9	5	18	9	0	1	0
	SML7		95	4	15	15	0	23	0	0
	SML9^T	37	100	14	12	27	13	0	0	0
	SML28		114	3	10	9	6	2	3	1
Leuconostoc mesenteroides subsp. *mesenteroides* (cluster II)	NCFB 523^T	38[a]	16	13	1	100	92	75	0	0
	SML42		4	10	13	77	67	69	6	0
	SML43		2	9	10	96	94	106	2	0
	SML44		32	12	14	95	60	94	5	2
	SML45		12	6	15	91	69	97	2	7
	SML50	39	17	17	11	101	98	100	2	17
Leuconostoc carnosum (cluster III)	SML31		0	81	78	32	6	26	3	0
	SML33		31	94	96	31	7	8	2	0
	SML34		14	93	79	19	7	9	4	0
	SML35		3	97	82	29	10	20	4	10
	SML38		16	116	97	29	24	19	6	25
	SML40^T	39	18	100	110	23	8	10	3	19
	SML48		0	97	90	26	22	29	0	24
	SML49	39	20	97	100	25	10	12	4	10
	SML51		2	82	112	25	19	2	0	5
	SML53		12	76	81	26	11	17	0	0
Leuconostoc mesenteroides subsp. *dextranicum*	NCFB 529^T	38[a]	14	8	5	92	100	90	5	6
Leuconostoc mesenteroides subsp. *cremoris*	NCFB 543^T	39[a]	9	10	4	109	81	100	5	5
Leuconostoc paramesenteroides	NCFB 803^T	38[a]	0	3	0	15	10	6	100	0
Leuconostoc lactis	NCFB 533^T	42[a]	0	22	7	25	14	0	0	100

[a] Data from reference 9, calculated the thermal denaturation point relative to reference DNA from *Escherichia coli* K-12 (G+C content, 51.2 mol%) by using the method of Owen and Pitcher (19).

more than 5% of its lactate as the L-(+) isomer (16) and is capable of growth at 37°C (15). Therefore, cluster I and III strains warrant separate species status; we propose the names *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum* sp. nov. for clusters I and III, respectively. These species qualify for membership in the genus *Leuconostoc* on the basis of the following characteristics: gram-positive, catalase-negative cocci or coccobacilli that produce gas from glucose, form more than 95% of their lactate as the D-(−) isomer, and do not hydrolyze arginine. Characteristics use-

TABLE 5. Diagnostic characteristics of *Leuconostoc mesenteroides*, *Leuconostoc paramesenteroides*, *Leuconostoc lactis*, *Leuconostoc gelidum*, *Leuconostoc carnosum*, *Lactobacillus confusus*, and *Lactobacillus viridescens*[a]

Characteristic	*Leuconostoc mesenteroides* subsp. *mesenteroides*	*Leuconostoc mesenteroides* subsp. *dextranicum*	*Leuconostoc mesenteroides* subsp. *cremoris*	*Leuconostoc paramesenteroides*	*Leuconostoc lactis*	*Leuconostoc gelidum*	*Leuconostoc carnosum*	*Lactobacillus confusus*	*Lactobacillus viridescens*
Acid produced from:									
L-Arabinose	+	−	−	d	−	+	−	−	−
Arbutin	d	−	−	−	−	+	−	NT	NT
Fructose	+	+	−	+	+	+	+	+	+
Galactose	+	d	d	+	+	−	−	+	−
Maltose	+	+	d	+	+	d	−	+	+
Salicin	d	d	−	−	d	+	−	+	−
Sucrose	+	+	−	+	+	+	+	+	d
Trehalose	+	+	−	+	−	+	+	−	d
Xylose	d	d	−	d	−	+	−	+	−
Dextran formation	+	+	−	−	−	d	+	+	NT
Growth at 37°C	d	+	−	d	+	−	−	+	+

[a] +, 90% or more of the strains positive; −, 90% or more of the strains negative; d, 11 to 89% of the strains positive; NT, not tested. Data for *Leuconostoc mesenteroides*, *Leuconostoc paramesenteroides*, and *Leuconostoc lactis* are from reference 8. Data for *Lactobacillus confusus* and *Lactobacillus viridescens* are from references 15 and 16.

ful for differentiating these species from previously described taxa are shown in Table 5.

Description of *Leuconostoc gelidum* sp. nov. *Leuconostoc gelidum* (ge'li.dum. L.neut.adj. *gelidum*, cold, (referring to the ability to grow on chill-stored meat). Gram-positive, nonmotile, nonsporeforming, spherical but sometimes lenticellular cells usually occur in pairs and chains. Colonies are small, smooth, round, and greyish white. Growth occurs at 1°C, and most strains do not grow at 37°C. Heterofermentative, producing gas from glucose. More than 95% of the lactate is produced as the D-(−) isomer. Catalase negative. Arginine is not hydrolyzed. All strains hydrolyze esculin and are β-galactosidase positive. Voges-Proskauer negative. Deoxyribonuclease positive. Does not hydrolyze Tween 20, Tween 40, Tween 60, or Tween 80. Most strains produce dextran from sucrose. Acid is produced from amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, D-glucose, D-mannose, melibiose, α-methyl-D-glucoside, raffinose, salicin, sucrose, trehalose, D-xylose. Acid is not produced from D-arabinose, dulcitol, erythritol, galactose, gluconate, glycerol, inositol, lactose, mannitol, D-melezitose, rhamnose, sorbitol, or L-sorbose. Some strains produce acid from maltose and ribose.

The cellular fatty acids are of the straight-chain saturated, monounsaturated, and cyclopropane ring types, with tetradecanoic, hexadecanoic, *cis*-9,10-hexadecenoic, *cis*-11,12-octadecenoic, and *cis*-11,12-methyleneoctadecanoic acids predominating. Small quantities of methylenehexadecanoic acid are also present. The G+C content of the DNA of the type strain is 37 mol% (as determined by the thermal denaturation method). Isolated from vacuum-packaged meat stored at low temperatures.

The type strain is strain NCFB 2775 (= SML9). The description of the type strain corresponds to that of the species, except that no growth occurs at 37°C, dextran is produced from sucrose, acid is produced from ribose, and acid is not produced from maltose.

Description of *Leuconostoc carnosum* sp. nov. *Leuconostoc carnosum* (car.no'sum. L.neut.adj *carnosum*, pertaining to flesh). Gram-positive, nonmotile, nonsporeforming, spherical but sometimes lenticellular cells usually occur in pairs and chains. Colonies are small, smooth, round, and greyish white. Growth occurs at 1°C, and most strains do not grow at 37°C. Heterofermentative, producing gas from glucose. More than 95% of the lactate is produced as the D-(−) isomer. Catalase negative. Arginine is not hydrolyzed. Some strains hydrolyze esculin. All strains are β-galactosidase negative. Voges-Proskauer negative. Deoxyribonuclease positive. Does not hydrolyze Tween 20 and Tween 80, but some strains hydrolyze Tween 40 and Tween 60. Most strains produce dextran from sucrose. Acid is produced from D-fructose, D-glucose, α-methyl-D-glucoside, sucrose, and trehalose. Acid is not produced from amygdalin, D-arabinose, L-arabinose, arbutin, dulcitol, erythritol, galactose, gluconate, glycerol, lactose, mannitol, D-melezitose, raffinose, rhamnose, sorbitol, L-sorbose, or D-xylose. Some strains produce acid from cellobiose, maltose, D-mannose, melibiose, ribose, and salicin.

The cellular fatty acids are of the straight-chain saturated, monounsaturated, and cyclopropane ring types, with tetradecanoic, hexadecanoic, *cis*-9,10-hexadecenoic, *cis*-11,12-octadecenoic, and *cis*-11,12-methyleneoctadecanoic acids predominating. Some strains produce methylenehexadecanoic acid. The G+C content of the DNA of the type strain is 39 mol% (as determined by the thermal denaturation method). Isolated from vacuum-packaged meat stored at low temperatures.

The type strain is strain NCFB 2776 (= SML40). The description of the type strain corresponds to that of the species, except that no growth occurs at 37°C, dextran is produced from sucrose, esculin is not hydrolyzed, Tween 40 and Tween 60 are not hydrolyzed, acid is produced from D-mannose and ribose, and acid is not produced from cellobiose, maltose, melibiose, or salicin.

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