***Lactobacillus algidus* sp. nov., a psychrophilic lactic acid bacterium isolated from vacuum-packaged refrigerated beef**

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***Lactobacillus algidus* sp. nov. is described on the basis of 40 strains isolated as one of the predominant bacteria from five specimens of vacuum-packaged beef collected from different meat shops and stored at 2 °C for 3 weeks. These strains were quite uniform in the overall characteristics examined. They are facultatively anaerobic, psychrophilic, Gram-positive, non-spore-forming, non-motile, lactic acid-homofermentative rods. The cells occurred singly and in pairs on agar media and in rather long chains in broth media. They differed in several cultural and biochemical characteristics from the authentic meso-diaminopimelic acid-positive or psychrophilic lactic acid bacteria in the genera *Lactobacillus*, *Carnobacterium* and *Brochothrix*. The SDS-PAGE whole-cell protein pattern was clearly distinctive. DNA–DNA hybridization and phylogenetic analysis of 16S rDNA also failed to associate these strains closely with any of the validly described organisms used. The phylogenetic analysis showed that these strains are rather remotely but most closely related to *Lactobacillus mali* (93% sequence similarity), which belongs to the *Lactobacillus casei*/*Pediococcus* group. Therefore, these strains should be included in the genus *Lactobacillus* and considered to represent a new species, *Lactobacillus algidus* sp. nov. The type strain is M6A9^T (= JCM 10491^T).**

Keywords: *Lactobacillus algidus* sp. nov., lactic acid bacteria, psychrophilic, vacuum-packaged beef

INTRODUCTION

Psychrophilic lactic acid bacteria have invariably been the predominant group of micro-organisms found on vacuum-packaged refrigerated meat and meat products (Schillinger & Lücke, 1987). In earlier studies, most organisms were not identified to the species level and were described as ‘atypical streptobacteria’ (Hitchener *et al.*, 1982). Some of these were later classified as new species, such as *Lactobacillus divergens* and *Leuconostoc gelidum* (Holzapfel & Gerber, 1983; Shaw & Harding, 1984, 1985). To accommodate atypical *Lactobacillus* strains that were

unable to grow on acetate agar (pH 5.6) and produced a major amount of L(+)lactic acid, Collins *et al.* (1987) established a new genus, *Carnobacterium*, into which some common meat bacteria (*L. divergens* and *Lactobacillus carnis*) were transferred. It is now well established that these, together with *Leuconostoc gelidum*, are common organisms on vacuum-packaged refrigerated beef. However, some strains still remain to be identified.

During studies on the microbial flora of vacuum-packaged refrigerated raw beef, we also came across some atypical meso-diaminopimelic acid (mDAP)-positive *Lactobacillus* strains. The strains differed sufficiently in cultural and biochemical properties from other presently known mDAP-positive or psychrophilic lactic acid bacteria and those isolated previously from refrigerated meat. A study was therefore undertaken to determine the systematic position of these strains.

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Abbreviation: mDAP, meso-diaminopimelic acid.

The DDBJ accession number for the 16S rRNA gene sequence of *Lactobacillus algidus* M6A9^T is AB03329.

METHODS

Strains studied. Forty strains of mDAP-positive and psychrophilic lactic acid bacteria were isolated from vacuum-packaged beef stored at 2 °C for up to 3 weeks. The strains were designated M6A1 to M6A40. M6A9^T (= JCM 10491^T), M6A19 (= JCM 10492) and M6A40 (= JCM 10493) have been deposited in the Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (JCM). The vacuum-packaged refrigerated beef was homogenized with PBS and incubated anaerobically on MRS agar (Oxoid) and glucose-blood-liver agar (BL agar; Nissui) at 7 °C for 14 d and aerobically on trypticase soy agar (TS agar; Nissui) at 7 °C for 7 d. The authentic reference strains of mDAP-positive and/or psychrophilic lactic acid bacteria and related organisms (Table 1), some of which are commonly found in meat, were obtained from the JCM and the DSMZ. The strains were stored at -80 °C in TS broth (Nissui) with 10% (w/v) glycerol. Working cultures were grown anaerobically on BL agar at 20 °C and subcultured two or three times before use.

Morphological, cultural and biochemical tests. All tests were performed at 20 °C unless otherwise stated. Colony morphology, Gram's reaction and cellular morphology were determined on cells grown anaerobically on MRS agar and in MRS broth (Oxoid) incubated for 2 d. Heat resistance was tested by heating the cells (grown on BL agar at 20 °C for 1 week) at 70 °C for 10 min. Gas production from glucose and gluconate, motility, reduction of nitrate, gelatin hydrolysis and growth in 15% (v/v) alcohol were determined according to Barrow & Feltham (1993). Acid production, reduction and curd formation in litmus milk (Difco) were read after 7 d incubation. Acid production from carbohydrates was tested by the method of Mitsuoka (1969). Volatile and non-volatile fatty acids produced from glucose were analysed by the method of Holdeman *et al.* (1977) by using gas chromatography (model GC-7A1; Shimadzu) with the hydrogen flame-ionization detector. Isomers of lactate formed from glucose were determined enzymically with the F kit (Boehringer). Production of acetoin from glucose was tested using VP medium (Eiken Chemicals) supplemented with 0.5% yeast extract (Difco). Production of dextran from sucrose was observed on MRS agar in which glucose was replaced by 5% (w/v) sucrose (Hitchener *et al.*, 1982). Decarboxylation of and ammonia production from arginine were determined using Moeller's broth (Difco) supplemented with 0.5% yeast extract. The effect of temperature on growth was examined in Briggs liver broth (Mitsuoka, 1969) at 0, 5 and 15 °C for 14 d and 25, 30 and 35 °C for 7 d. Growth in the presence of 6.5% (w/v) NaCl was observed in Briggs liver broth for 7 d. Growth on acetate agar (pH 5.4) was tested as described by Hammes *et al.* (1991).

mDAP in cell walls. mDAP in cell walls was analysed by a modification of the method of Becker *et al.* (1964). Cells were grown in MRS broth and collected by centrifugation at 3000 r.p.m. for 20 min. Hydrolysis of the cell walls was carried out at 100 °C for 1–3 h in a sealed glass ampoule, to which 100 µl 6 M HCl had been added. The lysate was spotted on a TLC plate (Funakoshi) and dried. Ascending chromatography was carried out for about 2 h with methanol/pyridine/concentrated HCl/distilled water (160:20:3:35, v/v). Ninhydrin solution (Tokyo Chemical Industry) was sprayed onto the dried plate, followed by heating at 100 °C for 10 min. DL-2,6-Diaminopimelic acid (Sigma) was used as a standard for the discrimination of mDAP from LL-DAP and *Lactobacillus plantarum* JCM 1149^T was used as an mDAP-positive reference strain.

Cellular fatty acids. Strains were grown on BL agar for 2 d. Fatty acid extraction, methyl ester preparation and separation by GC were performed as described previously (Uchida & Mogi, 1972).

DNA base composition and DNA–DNA hybridization. Cells were grown in Briggs liver broth and were harvested in the exponential growth phase. The DNA extraction protocol was based on procedures described previously (Marmur, 1961; Lambert *et al.*, 1998). The G + C content was determined by HPLC (Tamaoka & Komagata, 1984). DNA from calf thymus (Worthington Biochemicals) was included in each set of analyses as a reference standard; the G + C content of the calf thymus DNA was regarded as 42 mol%. DNA–DNA hybridization was performed by using the colorimetric microplate method (Ezaki *et al.*, 1989). DNA was labelled with photobiotin (Vector Laboratories). DNA was hybridized at 45 °C for 4 h in hybridization mixture containing 50% (v/v) formamide and 2.5% (w/v) dextran sulfate to lower the hybridization temperature and to shorten the hybridization time. The hybridized DNAs were coloured with HRP-streptavidin (Zymed Laboratories) and 3,3',5,5'-tetramethylbenzidine (Wako Chemicals) for enzyme assay. The absorbance of the coloured solution was read at 630 nm on a microplate autoreader (Sanko Junyaku).

16S rDNA sequencing. A single colony was treated using InstaGene purification matrix (Bio-Rad) according to the manufacturer's instructions. PCR amplification of 16S rDNA was performed in a total volume of 50 µl containing 2 µl DNA extract, 0.5 units *rTaq* DNA polymerase (Takara Shuzo) and 0.08 µM each of primers 46f (5'-TGGCTCAGATTGAACGCTGGC-3') and 75r (5'-ACTTCTACCTTGTTACGACTT-3'). The reaction involved 30 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min and extension at 72 °C for 2 min. After checking the integrity of the PCR product by agarose gel electrophoresis, the product was purified using the QIAquick-spin PCR purification kit (Qiagen). Sequences were determined directly using the dye terminator cycle sequencing FS ready reaction kit (Perkin Elmer Applied Biosystems) under conditions recommended by the manufacturer. Sequencing reactions were run on an automated DNA sequencer (model 373A; Perkin Elmer) using the manufacturer's recommendations.

To establish the relatives of the strain M6A9^T, preliminary searches in the DDBJ database were performed with the program BLAST. Sequences of close relatives were retrieved from DDBJ and aligned with the newly determined sequences using the program GENETYX-MAC (Software Development).

The following reference sequences were obtained from DBGET (Web/Net): M58803 (*Lactobacillus agilis* DSM 20509^T), M58804 (*Lactobacillus alimentarius* DSM 20249^T), M58808 (*Lactobacillus aviarius* DSM 20655^T), M58809 (*Lactobacillus bifermens* DSM 20003^T), M58810 (*Lactobacillus brevis* ATCC 14869^T), M58811 (*Lactobacillus buchneri* DSM 20057^T), D16551 (*Lactobacillus casei* ATCC 393^T), M58813 (*Lactobacillus coryniformis* DSM 20001^T), U62624 (*Lactobacillus fermentum* KRM), X76330 (*Lactobacillus fructivorans* DSM 20203^T), M58821 (*Lactobacillus hilgardii* DSM 20176^T), AB024300 (*Lactobacillus kefir* NRIC 1693^T), M58827 (*Lactobacillus mali* DSM 20444^T), D79212 (*Lactobacillus paracasei* JCM 8130^T), D79210 (*L. plantarum* JCM 1149^T), L23507 (*Lactobacillus reuteri* DSM 20016^T), D16552 (*Lactobacillus rhamnosus* JCM 1136^T), M58828 (*Lactobacillus ruminis* DSM 20403^T), M58829

Table 1. Characteristics that differentiate between the strains (*L. algidus*) isolated from vacuum-packaged refrigerated beef and authentic reference lactic acid bacteria

Strains: 1, strain M6A9^T (*L. algidus*); 2, 39 further strains of *L. algidus*; 3, *L. agilis* JCM 1187^T; 4, *L. casei* JCM 1134^T; 5, *L. mali* JCM 1116^T; 6, *L. pentosus* JCM 1558; 7, *L. plantarum* JCM 1149^T; 8, *L. ruminis* JCM 1152^T; 9, *L. sakei* JCM 1157^T; 10, *L. sharpeae* JCM 1186^T; 11, *Carnobacterium divergens* JCM 5816^T; 12, *Carnobacterium gallinarum* JCM 4847^T; 13, *Carnobacterium mobile* JCM 5348^T; 14, *Carnobacterium piscicola* JCM 5348^T; 15, *Brochothrix thermosphacta* DSM 20171^T. Characteristics are scored as : +, positive; −, negative; w, weakly positive; D, strain-dependent.

Characteristic	Strain														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acid production from:															
L-Arabinose	+	+	+	−	+	+	+	−	+	−	+	−	−	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	−	+	−	+	−
Gluconate	−	−	−	+	−	+	+	−	+	−	+	+	−	+	w
Glycerol	−	−	−	−	−	+	+	−	−	−	+	+	+	+	+
Glycogen	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+
Mannitol	−	−	+	+	+	+	+	−	−	−	−	−	−	+	+
Melezitose	−	−	+	+	−	+	+	−	−	−	+	+	−	+	−
α-Methyl D-glucoside	−	−	−	−	+	w	+	−	−	−	−	+	−	+	w
α-Methyl D-mannoside	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−
Rhamnose	−	−	−	−	+	+	+	−	−	−	+	+	−	+	+
Salicin	+	+	+	+	+	+	+	+	w	+	−	+	+	+	+
Sorbitol	−	−	D	+	−	+	+	−	−	−	−	−	−	−	+
Starch	−	−	−	−	−	+	+	D	−	−	−	+	−	+	+
D-Tagatose	−	−	−	+	+	−	−	−	−	−	−	+	−	−	+
Trehalose	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+
Gas production from:															
D-Glucose	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−
Gluconate	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−
Nitrate reduction	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−
Arginine decarboxylase	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−
NH ₃ from arginine	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−
Motility	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−
Acetoin production	−	−	+	+	−	−	−	−	+	−	+	+	+	+	−
H ₂ S production	−	−	−	−	−	−	−	−	+	−	+	+	+	+	−
Growth at:															
0 °C	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
15 °C	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+
30 °C	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−
Lactate isomer*	L	L	L	L	L	DL	D+DL	L	DL	L	L	L	L	L	L
mDAP	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+
Catalase	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
DNA G+C content (mol %) [†]	36.5	36.7 [‡]	43–44	45–47	32–34	46–47	44–46	44–47	42–44	53	33–36	34–36	36–37	34–36	36
DNA–DNA hybridization [§]	100	73–105	10	8	20	13	6	7	11	8	3	10	4	0	15

* Based on the content of L(+)-lactic acid: D, 0–20%; D+DL, 20–40%; DL, 40–60%; L+DL, 60–80%; L, 80–100%.

[†] Data from Talon *et al.* (1988), Kaneuchi *et al.* (1988), Wood & Holzapfel (1995) and this study.

[‡] Mean G+C content for three strains, M6A9^T, M6A19 and M6A40.

[§] Hybridization with photobiotin-labelled DNA from M6A9^T (%). Values are means from two determinations.

(*Lactobacillus sakei* DSM 20017^T), AF089108 (*Lactobacillus salivarius* ATCC 11741^T), X76327 (*Lactobacillus sanfranciscensis* ATCC 27651^T), M58831 (*Lactobacillus sharpeae* DSM 20505^T), X61139 (*Lactobacillus vaginalis* NCTC

12197^T), M58833 (*Pediococcus acidilactici* DSM 20284^T), D87678 (*Pediococcus damnosus* JCM 5886^T), D87679 (*Pediococcus dextrinicus* JCM 5887^T) and D88528 (*Pediococcus parvulus* JCM 5889^T).

Whole-cell protein analysis. Strains were grown anaerobically on BL agar at 20 °C for 2 d. The cells were harvested using a sterile wire loop. Preparation of cellular protein extracts and SDS-PAGE were performed as described previously (Pot *et al.*, 1994). The cells were suspended in 1 ml 0.15 M Tris/HCl buffer (Wako Chemicals). A portion of this suspension (100 µl) was centrifuged at 5000 r.p.m. for 5 min and 100 µl 10 mg lysozyme ml⁻¹ (Wako Chemicals) was added to the pellet and incubated for 90 min. To solubilize the proteins, 100 µl SDS sample buffer [0.125 M Tris/HCl, 4% (w/v) SDS, 20% (w/v) glycerol, 0.002% bromophenol blue, 10% (v/v) 2-mercaptoethanol; Wako Chemicals] was added and heated at 100 °C for 5 min. The proteins were separated by using a 10% (w/v) acrylamide (Wako Chemicals) separating gel [the monomer solution contained 30% (w/v) total acrylamide with 2.67% (w/v) cross-linking bisacrylamide in 0.375 M Tris/HCl (pH 8.8) and 0.1% SDS] and a 3% (w/v) stacking gel [the monomer solution again contained 30% (w/v) total acrylamide with 2.67% (w/v) cross-linking bisacrylamide in 0.125 M Tris/HCl (pH 6.8) and 0.1% SDS]. The gels were run at a constant current of 20 mA per gel in a vertical slab apparatus (Atto). Fixing of the gels was done with 25% (v/v) methanol and 10% (v/v) acetic acid. This was followed by staining with 0.25% Coomassie brilliant blue R250 (Nacalai Tesque) in 25% (v/v) methanol and 10% (v/v) acetic acid. Destaining was done with 25% (v/v) ethanol and 8% (v/v) acetic acid. The protein band patterns were compared visually to those of authentic reference strains.

RESULTS

Morphology

The 40 strains isolated were facultatively anaerobic, non-motile, non-spore-forming, Gram-positive rods with rounded ends. Cells occurred singly and in pairs and were 0.3–0.7 × 1.1–1.9 µm when grown on MRS agar (Fig. 1a). Some cells were filamentous when grown on BL agar. Cells grown in MRS broth formed rather long chains of coccobacilli and short rods (Fig. 1b). After anaerobic incubation on MRS agar at 20 °C for 2 d, the colonies were 1.0–1.5 mm in diameter, circular, smooth, convex and white to grey in colour.

Cultural and biochemical characteristics

The optimum temperature for growth was 15–25 °C. No growth occurred at 30 °C. Growth was observed even at temperatures as low as –2 °C. Growth on BL agar was enhanced by the addition of 5% (v/v) horse blood. Anaerobic incubation on BL and MRS agars also yielded larger colonies. No strains resisted heating at 70 °C for 10 min. The biochemical characteristics for the strains are given in Table 2. The strains were quite similar to one another with respect to cultural and biochemical characteristics, enough to warrant their placement within a taxon. No gas was produced in Briggs liver broth based on glucose or gluconate, thus showing a homofermentative mechanism. The configuration of lactic acid was mostly L(+) with a small percentage (2–15%) of D(–). mDAP was detected in the cell walls. Lactic acid was the sole end product of glucose fermentation.

Fig. 1. Gram-stained cells of strain M6A9^T (*L. algidus*) grown on MRS agar (a) and in MRS broth (b) at 20 °C for 36 h. Bar, 10 µm.

Cellular fatty acids

A major constituent of the cellular fatty acids detected from strain M6A9^T was oleic acid, a normal unsaturated acid of 18 carbon atoms with a double bond at the ninth carbon [C18:1(Δ9)] constituting 63.3% of all the fatty acids detected. Minor fatty acids detected were C16:0, C16:1(Δ9) and C14:0, in amounts of 11.8, 7.3 and 7.2%, respectively. The remaining acids detected were in amounts of less than 2.0%. No cyclopropane acid was detected.

Whole-cell protein analysis

All 40 strains studied showed similar SDS-PAGE protein band patterns characterized by a single densely staining band of about 38 kDa, with many other variably staining faint bands. As shown in Fig. 2, the SDS-PAGE protein band patterns of strains M6A9^T and M6A38 were more similar to each other than to those of the reference organisms used.

Table 2. Biochemical characteristics for which there were differences among the 40 strains isolated

The following tests gave positive results for all 40 strains: production of acid from *N*-acetyl glucosamine, L-arabinose, arbutin, D-fructose, galactose, D-glucose, D-mannose, ribose, salicin and trehalose; growth at 0, 5, 15 and 25 °C; hydrolysis of aesculin; presence of mDAP in the cell wall; and production of a predominant amount of L(+)lactic acid. The following tests gave negative results for all 40 strains: production of acid from adonitol, D-arabinose, D-arabitol, dulcitol, erythritol, D-fucose, gluconate, glycerol, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, D-lyxose, mannitol, melezitose, α -methyl D-glucoside, α -methyl D-mannoside, α -methyl xyloside, rhamnose, sorbitol, L-sorbose, starch, D-tagatose, D-turanose, xylitol and L-xylose; gas production from glucose and gluconate; growth in 6.5% NaCl and in 15% ethanol; growth at 30 °C; production of acetoin, dextran and arginine decarboxylase and of ammonia from arginine; hydrolysis of gelatin; growth on acetate agar; heat resistance at 70 °C; H₂S production; motility; nitrate reduction; and reaction in litmus milk.

Production of acid from:	Positive (n)	Weakly positive (n)	Negative (n)	Strain M6A9 ^T
Aesculin	33	3	4	+
Amygdalin	4	0	36	—
Cellobiose	18	0	22	+
Lactose	21	0	19	+
Maltose	27	0	13	—
Melibiose	30	0	10	—
D-Raffinose	9	7	24	—
Sucrose	8	1	31	—
D-Xylose	16	0	24	—

Fig. 2. Whole-cell protein patterns of strains M6A9^T and M6A38 (*L. algidus*) isolated from vacuum-packaged refrigerated beef and mDAP-positive reference strains. Lanes: 1 and 12, molecular mass markers (from top to bottom) myosin (205 kDa), β -galactosidase (116 kDa), phosphorylase B (97.4 kDa), bovine plasma albumin (66 kDa), egg albumin (45 kDa) and carbonic anhydrase (29 kDa); 2, strain M6A9^T; 3, strain M6A38; 4, *L. plantarum* JCM 1149^T; 5, *L. pentosus* JCM 1158; 6, *L. sharpeae* JCM 1187^T; 7, *L. mali* JCM 1116^T; 8, *L. agilis* JCM 1187^T; 9, *L. ruminis* JCM 1152; 10, *B. thermosphacta* DSM 20171^T; and 11, *C. divergens* JCM 5816^T. **Fig. 3.** Phylogenetic tree showing the relationship of strain M6A9^T (*L. algidus*) to some members of the *L. casei*/*Pediococcus* group of *Lactobacillus*. The tree was constructed by the UPGMA method. Bar, 0.01 nucleotide substitutions per site.

DNA studies

The G+C contents of DNA extracted from strains M6A9^T, M6A19 and M6A40 were 36.5, 37.1 and 36.4 mol %, respectively. In DNA–DNA hybridization experiments, photobiotin-labelled DNA from strains M6A9^T and M6A40 reassociated to high levels (73–105%) with unlabelled DNA from other strains (M6A1, M6A3, M6A9^T and M6A19), but at low levels (0–23%) with unlabelled DNA from all the reference strains used (Table 1). Photobiotin-labelled DNA from *L. casei* JCM 1134^T, *L. mali* JCM 1116^T, *L. plantarum* JCM 1149^T, *Carnobacterium divergens* JCM 5816^T, *Carnobacterium piscicola* JCM 5348^T and *Brochothrix thermosphacta* DSM 20171^T reassociated at low levels (8–25%) with unlabelled DNA from five strains (M6A1, M6A3, M6A9^T, M6A19 and M6A40).

Phylogenetic analysis of strain M6A9^T

Approximately 1500 bases of the 16S rDNA gene sequence of strain M6A9^T were determined by direct sequencing of PCR-amplified product. A phylogenetic tree obtained by the unweighted pair group method

with averages (UPGMA) with Kimura's correction (Kimura, 1980) is shown in Fig. 3. Comparisons were made by the analysis of 1505 bases of the 16S rDNA of strain M6A9^T. Among the members of the *L. casei*/*Pediococcus* group of *Lactobacillus*, strain M6A9^T was most closely related to *L. mali* (sequence similarity of 93.0%).

DISCUSSION

The strains studied are facultatively anaerobic, non-motile, non-spore-forming, Gram-positive, rod-shaped bacteria that produce lactic acid homo-fermentatively from glucose as the sole short-chain fatty acid. Table 1 shows the characteristics most useful in distinguishing the strains studied from some members of the genera *Lactobacillus*, *Carnobacterium* and *Brochothrix*. They were clearly differentiated from *Carnobacterium* species including *C. divergens* that have been isolated frequently from vacuum-packaged refrigerated meat (Collins *et al.*, 1987) by growth at 30 °C, carbohydrate fermentation patterns, nitrate reduction and production of arginine decarboxylase, ammonia from arginine, acetoin and hydrogen sulfide. They were distinctly differentiated from *Brochothrix* species, which were identified as one of the classical meat organisms (Sneath & Jones, 1976), by carbohydrate fermentation patterns and catalase production. Furthermore, the strains studied could be distinguished from mDAP-positive or psychrophilic *Lactobacillus* species in many characteristics such as growth at 0, 15 and 30 °C, motility, catalase production, gas production from glucose and gluconate, types of lactic acid isomers, carbohydrate fermentation patterns and/or G + C content of DNA.

Three mDAP-positive *Lactobacillus* species, *Lactobacillus suebicus*, *Lactobacillus vaccinostrercus* and *Lactobacillus vitulinus*, were not included in this study but could be distinguished from the strains studied by many characteristics such as those listed above. More specifically, they differ in growing at temperatures over 30 °C while failing to grow at temperatures below 10 °C. *L. vaccinostrercus* differs in producing mainly DL-lactic acid, whereas the strains studied produced the L(+) isomer. *L. vitulinus* produces D-lactic acid. Both *L. vaccinostrercus* and *L. suebicus* are obligately heterofermentative, whereas the strains studied are homofermentative. The strains studied produced acid from aesculin and trehalose, whereas neither *L. suebicus* nor *L. vaccinostrercus* produces acid from these sugars (Okada *et al.*, 1979; Kleynmans *et al.*, 1989).

Strain M6A9^T, a representative of the strains studied, was found to possess oleic acid as a major cellular fatty acid. The presence of oleic acid as a major cellular fatty acid was considered to be distinctive of *Carnobacterium* and to be regarded as differential from *Lactobacillus* (Collins *et al.*, 1987). However, Decallonne *et al.* (1991) and Dykes *et al.* (1995) showed that possession of oleic acid is not always a reliable

characteristic because some *Lactobacillus* species also contain significant amounts of this acid.

Concerning the SDS-PAGE protein pattern analysis, we could not do any computer analysis due to the unavailability of the computer program and so could not construct any relationship. However, visual comparison of the strains studied and reference organisms revealed that the strains studied were more similar to one another than to the mDAP-positive psychrophilic reference strains used, enough to warrant their placement in a taxon at the species level.

Our failure to associate the strains studied closely with the phenotypically and phylogenetically similar organisms listed in Table 1 in DNA-DNA hybridization experiments also suggests that the strains studied represent a new species.

The phylogenetic analysis of 16S rDNA gene revealed relatively close similarity (93.0%) of strain M6A9^T to *L. mali*, which is included in the *L. casei*/*Pediococcus* group of the genus *Lactobacillus* (Collins *et al.*, 1991). However, the similarity of strain M6A9^T to *L. mali* and other members of the *L. casei*/*Pediococcus* group was not sufficiently great to identify strain M6A9^T as any one of these organisms (Stackebrandt & Goebel, 1994). The phylogenetic analysis, together with phenotypic characteristics, indicate that the strains studied represent a new species in the genus *Lactobacillus*, for which we propose the name *Lactobacillus algidus* sp. nov.

Description of *Lactobacillus algidus* sp. nov.

Lactobacillus algidus (al'gi.dus. L. adj. *algidus* cold, referring to the ability to grow at low temperature).

Cells are facultatively anaerobic, non-spore-forming, Gram-positive, non-motile rods. Colonies are circular, convex, smooth, cream-white and 1.0–1.5 mm in diameter after 48 h incubation at 20 °C on MRS agar. The cellular morphology depends on growth medium: coccobacilli or short rods in MRS broth and rods (0.3–0.7 × 1.1–1.9 µm) on MRS agar. Growth occurs at 0–25 °C. No growth at 30 °C. Catalase and oxidase are not produced. Aesculin is hydrolysed. Hydrogen sulfide is not produced. Nitrate is not reduced. Gelatin is not liquefied. No dextran is produced from sucrose. Acid is produced from *N*-acetyl D-glucosamine, L-arabinose, arbutin, D-fructose, galactose, D-glucose, D-mannose, ribose, salicin and trehalose. Acid is not produced from adonitol, D-arabinose, glycerol, glycogen, inulin, mannitol, melezitose, α-methyl D-glycoside, α-methyl D-mannoside, rhamnose, sorbitol, L-sorbose, starch, D-tagatose, D-turanose, xylitol or L-xylose. Most strains produce acid from aesculin and melibiose. Some strains produce acid from cellobiose, lactose, maltose and D-xylose. Most strains do not produce acid from amygdalin, D-raffinose or sucrose (Table 2). The major cellular fatty acids produced by strain M6A9^T are the straight-chain mono-unsaturated oleic acid [C18:1(Δ9)] and straight-chain satu-

rated palmitic acid (C16:0). G + C contents of DNA of strains M6A9^T (= JCM 10491^T), M6A19 (= JCM 10492) and M6A40 (= JCM 10493) are 36.5, 37.1 and 36.4 mol %, respectively, as determined by the HPLC method.

The type strain is M6A9^T (= JCM 10491^T). The description of the type strain corresponds to that of the species except that acid is produced from cellobiose, aesculin, lactose and D-xylose, whereas no acid is produced from amygdalin, maltose, melibiose, raffinose or sucrose. The peptidoglycan type is mDAP. Habitat: vacuum-packaged refrigerated beef stored at low temperatures.

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