Proposal of *Sporolactobacillus nakayamae* subsp. *nakayamae* sp. nov., subsp. nov., *Sporolactobacillus nakayamae* subsp. *racemicus* subsp. nov., *Sporolactobacillus terrae* sp. nov., *Sporolactobacillus kofuensis* sp. nov., and *Sporolactobacillus lactosus* sp. nov.

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Catalase-negative spore-forming lactic acid bacteria were isolated from soil samples and fermentation starters for Asian traditional alcoholic beverages. The isolates were characterized by determining morphological, biochemical, physiological, and chemotaxonomic properties and were found to be members of the genus *Sporolactobacillus*. Twelve isolates and some authentic strains belonging to this genus were used in DNA base composition and DNA relatedness studies, and the results revealed that the strains tested could be divided into six groups which correlated with the phenotypic characteristics. One of the groups corresponded to the previously described species *Sporolactobacillus inulinus*. In addition, we propose the following four new species and two new subspecies for the remaining five groups: *Sporolactobacillus nakayamae* subsp. *nakayamae* sp. nov., subsp. nov. (type strain, M-114 [= JCM 3514]), *Sporolactobacillus nakayamae* subsp. *racemicus* subsp. nov. (type strain, M-17 [= JCM 3417]), *Sporolactobacillus terrae* sp. nov. (type strain, M-116 [= JCM 3516]), *Sporolactobacillus kofuensis* sp. nov. (type strain, M-19 [= JCM 3419]), and *Sporolactobacillus lactosus* sp. nov. (type strain, X1-1 [= JCM 9690]).

Originally, the genus *Sporolactobacillus* consisted of catalase-negative, spore-forming, homofermentative lactic acid bacteria and contained a single species, *Sporolactobacillus inulinus*, which was first isolated from chicken feed (13, 14). Later, Nakayama and Yanoshi (18, 19) isolated a number of spore-forming lactic acid bacteria from the soil of plant rhizospheres and proposed four species for them. However, the names proposed by Nakayama and Yanoshi did not appear on the Approved Lists of Bacterial Names (24) and have not been validly published. Previously, we studied the strains isolated by Nakayama and Yanoshi (18, 19), as well as the type strains of *S. inulinus*, *Lactobacillus plantarum*, and *Bacillus coagulans*, by performing morphological, biochemical, and physiological characterization (32) and DNA-DNA hybridization (33) experiments. These studies revealed that the genus *Sporolactobacillus* is phenotypically distinct from the genus *Bacillus*; in particular, it lacks catalase activity and requires carbohydrates for growth. The strains of the genus *Sporolactobacillus* have been divided into several groups. Fox et al. (7) described a distant relationship between *S. inulinus* and *Bacillus* species based on 16S rRNA oligonucleotide cataloging data. Ash et al. (2) classified the genus *Bacillus* into five groups by performing a comparative analysis of 16S rRNA primary sequences (ca. 95% of the total molecule), and *S. inulinus* was phylogenetically separated from all of the *Bacillus* species by the results. Suzuki and Yamasato (27) determined the 16S rRNA gene sequences of 16 strains of spore-forming lactic acid bacteria and found that the isolates of Nakayama and Yanoshi formed one cluster with *S. inulinus* and that this cluster could be further divided into five subclusters, including four catalase-negative subclusters

and one catalase-positive subcluster. Recently, Andersch et al. (1) revived the name *Bacillus laevolacticus* for a group of catalase-positive strains. Although the genus *Sporolactobacillus* has been studied taxonomically frequently, species other than *S. inulinus* have not been established previously. As isolation sources of *Sporolactobacillus* strains were rather limited, we tried to isolate strains of this genus from fermentation starters of alcoholic beverages in Asian countries and from soil samples. In this paper we describe isolation of strains belonging to the genus *Sporolactobacillus* and propose four new species and two new subspecies, *Sporolactobacillus nakayamae* subsp. *nakayamae*, *Sporolactobacillus nakayamae* subsp. *racemicus*, *Sporolactobacillus terrae*, *Sporolactobacillus kofuensis*, and *Sporolactobacillus lactosus*, for our isolates and the strains isolated by Nakayama and Yanoshi (18, 19).

MATERIALS AND METHODS

Isolation procedure. Sixty soil samples were collected mainly from the rhizospheres of plants growing in wet regions in various parts of Japan. Twelve samples of fermentation starters of traditional alcoholic beverages in Asian countries, including luck pang in Thailand, bubod in the Philippines, ragi in Indonesia, and lepar hu in Bhutan, were also used as isolation sources. A 1-g portion of a sample was mixed with 5 ml of GYP medium, which contained 2% glucose, 1% yeast extract, 1% peptone, 1% sodium acetate, and 0.5% (vol/vol) salts solution (pH 6.8). The salts solution contained 4% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2% $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.2% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.2% NaCl. Each sample suspension was heated at 80°C for 10 min and then incubated anaerobically at 30°C to remove non-spore-forming aerobic bacteria. After 4 to 7 days of incubation, 1 drop of the turbid broth was spread onto GYP agar containing 1% CaCO_3 , and the culture was incubated in an anaerobic jar in a 100% CO_2 atmosphere. Acid-producing bacteria were recognized by the appearance of clear zones around colonies. These bacteria were purified by repeated isolation, and the production of lactic acid was confirmed by high-performance liquid chromatography. A Shodex Ionpack KC-811 column was used with 0.1% H_3PO_4 as the eluent (1 ml/min) at 60°C, and lactic acid was detected by refractometry. Some of the strains, those lacking catalase activity, were used in further taxonomic studies. The catalase activity of cells grown on GYP agar slants (32) was determined by adding a 3% hydrogen peroxide solution.

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Strains used and cultivation. *S. inulinus* JCM 6014^T (T = type strain) was obtained from the Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, Japan. The 30 strains whose designations begin with M- were strains isolated by Nakayama and Yanoshi (18, 19). The strains were cultivated on GYP medium at 30°C unless otherwise stated (32).

Morphological, biochemical, and physiological characteristics. Conventional tests were performed by using previously described methods (32). Acid production from carbohydrates was examined by the method of Mitsuoka (17), modified by using liquid media without L-cysteine-HCl. The isomers of lactic acid produced were determined by the enzymatic method described by Okada et al. (21).

Chemotaxonomy. The presence of diaminopimelic acid isomers in cell walls was determined by the method of Stanek and Roberts (25) by using whole-cell hydrolysates. Cellular fatty acid compositions were analyzed by using methyl esters prepared from lyophilized cells with anhydrous 5% methanolic hydrochloric acid (Kokusen Chemical Works, Tokyo, Japan) as described by Ikemoto et al. (10). A model GC-7A gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a Rascot OV-1 capillary column (50 m by 0.25 mm; Nihon Chromato, Tokyo, Japan) was used to determine the composition. Isoprenoid quinones were extracted from lyophilized cells with chloroform-methanol (2:1, vol/vol) and were purified by thin-layer chromatography; the isoprenoid quinone composition was determined by reverse-phase high-performance liquid chromatography (15, 29).

DNA studies. Cells grown on GYP medium at 30°C were collected by centrifugation at the late exponential growth phase. DNA was isolated by the phenol method of Saito and Miura (22) with some modifications, as described previously (33). The DNA base composition was calculated from the melting temperature (T_m) of DNA by using the formula of Marmur and Doty (16). The T_m was determined with a model DU-8 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). The sodium dodecyl sulfate-membrane filter method described previously (26, 33) was used for DNA-DNA hybridization experiments.

RESULTS

Isolation of bacteria. Twenty strains of spore-forming lactic acid bacteria were isolated from soil samples, and 15 strains were isolated from fermentation starters. The proportions of catalase-negative strains among the spore-forming lactic acid bacteria isolated from soil samples and fermentation starters were 39 and 63%, respectively. The strains with catalase activity were not included in this study. Twelve representative strains were selected for further study. Strains MB-1, ME-1', and MH-3 were isolated from soil samples obtained in Yamaguchi Prefecture. Strains F-4, I-3, and SH-3 were isolated from soil samples obtained in Saitama, Shizuoka, and Tokushima Prefectures, respectively. The strains isolated from Asian fermentation starters were strain X1-1^T from luck pang in Thailand, strain X3-1 from bubod in the Philippines, strains X5-5 and X6-1 from ragi in Indonesia, and strains X9-1 and X9-3 from lepar hu in Bhutan.

Morphological, biochemical, and physiological characteristics. All of the isolates were gram-positive rods and produced endospores that were resistant to heating at 80°C for 10 min. Motility was observed with all of the isolates except strain X1-1^T. Morphological, biochemical, and physiological characteristics of the isolates are shown in Table 1 along with the characteristics of strains described previously (32).

Chemotaxonomic characteristics. All of the isolates had *meso*-diaminopimelic acid in their whole-cell hydrolysates. The predominant isoprenoid quinone of the isolates was a menaquinone with seven isoprene units (MK-7); MK-5 and MK-6 were minor components. Ubiquinone was not detected. The major components of the cellular fatty acids of the isolates were 12-methyl tetradecanoic acid (anteiso-C_{15:0}) and 14-methyl hexadecanoic acid (anteiso-C_{17:0}); 13-methyl tetradecanoic acid (iso-C_{15:0}) and 14-methyl pentadecanoic acid (iso-C_{16:0}) were also present.

DNA base compositions and DNA relatedness. DNA base compositions and levels of DNA relatedness are shown in Table 2. The DNA base compositions of the isolates ranged from 43 to 47 mol% G+C. Strains X3-1, X5-5, X6-1, X9-1,

X9-3, SH-3, MB-1, MH-3, and I-3 exhibited high levels of DNA relatedness to strain X1-1^T and formed a DNA homology group. Although strains ME-1' and F-4 exhibited low levels of relatedness to strain X1-1^T, these isolates were closely related to strain M-114^T, one of the isolates of Nakayama and Yanoshi (19). None of the isolates exhibited a high level of DNA relatedness to the type strain of *S. inulinus*.

DISCUSSION

Catalase-negative spore-forming lactic acid bacteria were isolated from some fermentation starters and soil samples. All of the isolates contained *meso*-diaminopimelic acid in their cell walls, MK-7 was the predominant isoprenoid quinone, and the cellular fatty acids were anteiso- and iso-branched-chain fatty acids. The DNA base compositions ranged from 43 to 50 mol% G+C. These chemotaxonomic properties were consistent with previous descriptions of the genus *Sporolactobacillus* (5, 12–14, 30, 31). The salient characteristics of the six genera of aerobic, endospore-forming rods and cocci are shown in Table 3.

As in the previous studies of *Sporolactobacillus* strains (6, 14, 19), a wide distribution of this genus was found in this study. Furthermore, species diversity was also found in the genus *Sporolactobacillus*. The isolates of Nakayama and Yanoshi (18, 19) were classified into five groups based on DNA relatedness, as shown previously (33). It is interesting that most of our isolates used in the present study did not belong to any of these five groups; the only exceptions were strains ME-1' and F-4, which fell into group 2. The remaining isolates aggregated in a sixth group (Table 2). The six groups based on DNA relatedness data were well-characterized phenotypically and were consistent with the biochemical and physiological properties, as shown in Table 1. Group 1 contains *S. inulinus*. Strain M-86 exhibited a high level of DNA relatedness to the type strain of *S. inulinus*, strain JCM 6014, and most of the phenotypic characteristics of these two strains were similar (the only exceptions were motility, growth at 45°C, and acid production from sorbitol and inulin). The strains belonging to the five other groups are distinct from *S. inulinus* and can be classified in five new taxa based on phenotypic characteristics and DNA relatedness data. However, the levels of DNA relatedness between group 2 and group 4 are slightly higher than the levels of relatedness among the other groups and are within the range of levels of relatedness for different subspecies of the same species, as proposed by Johnson (11). Therefore, below we describe two new subspecies of one new species for the strains belonging to groups 2 and 4. We also describe three new *Sporolactobacillus* species for groups 3, 5, and 6. The taxa of the genus *Sporolactobacillus*, including *S. inulinus*, are distinguished from one another phenotypically by a combination of characteristics (Table 4).

Ash et al. (2) performed a phylogenetic analysis based on 16S ribosomal DNA sequences and found a great deal of diversity in the genus *Bacillus*. *S. inulinus* was placed on an independent branch within the large *Bacillus* group. Furthermore, Suzuki and Yamasato (27) found that 12 representative strains of spore-forming lactic acid bacteria isolated by Nakayama and Yanoshi (18, 19) and *S. inulinus* formed one cluster with very similar 16S rDNA sequences (levels of similarity, more than 97.2%). In addition, these authors mentioned that this cluster contains some catalase-positive strains and that the definition of the genus *Sporolactobacillus* should be amended to accommodate both catalase-negative and catalase-positive strains (27). Apart from this problem, it is evident that the

TABLE 1. Biochemical and physiological characteristics of spore-forming lactic acid bacteria

DNA homology group ^a	Strain	Spore formation	Growth at:						Growth in the presence of:					Litmus milk		Acid production from carbohydrates																										
			Motility	Catalase	15°C	25°C	30°C	40°C	45°C	50°C	3% NaCl	4% NaCl	5% NaCl	7% NaCl	10% NaCl	Acidification	Reduction	Coagulation	Liquefaction	Arabinose	Ribose	Xylose	Glucose	Fructose	Galactose	Mannose	Rhamnose	Cellobiose	Lactose	Maltose	Melibiose	Sucrose	Raffinose	Salicin	Trehalose	Mannitol	Sorbitol	Starch	Methyl-D-glucoside	Inulin	Control	Lactic acid isomer
1	JCM 6014 ^{Tb}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D
	M-86 ^c	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D
2	M-45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D
	M-46	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D
	M-47	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D
	M-50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-51	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-54	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-56	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D		
	M-59	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D		
	M-84	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-87	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-88	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-101	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-103	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-113	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-114 ^T	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	ME-1'	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	F-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
3	M-16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	
	M-33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D		
	M-61	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D		
	M-85	N	+	+	+	+	+	+	+	+	+	+	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D		
	M-99	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D		
	M-115	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D		
	M-116 ^T	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																			

^a Data for groups 1 to 5 are from reference 33.
^b Type strain of *S. inulinus*.
^c Data for the strains whose designations begin with M- are from reference 32.
^d +, positive; -, negative; N, not determined.

catalase-negative strains of Nakayama and Yanoshi are members of the genus *Sporolactobacillus*. Additional studies that include catalase-positive strains would make the taxonomic relationship between the genera *Sporolactobacillus* and *Bacillus* clearer.

Descriptions of the four new species and two new subspecies are given below. The following characteristics are common features of these species and subspecies of the genus *Sporolactobacillus*: gram-positive spore-forming rods that are generally 0.4 to 1.0 by 2.0 to 4.0 µm in 7-day-old cultures on GYP

TABLE 2. Levels of DNA relatedness for isolates and some catalase-negative spore-forming lactic acid bacteria

DNA homology group ^a	Source of DNA	G+C content (mol%)	% DNA-DNA reassociation with DNA from:					
			JCM 6014 ^T	M-114 ^T	M-17 ^T	M-116 ^T	M-19 ^T	X1-1 ^T
1	JCM 6014 ^T	47	100 (100) ^b	25 (22)	24	22	22	12
	M-86	50	88	35	27		19	
2	M-45	45	32	75	47	38		
	M-46	46	41	76				
	M-50	45	41	81				
	M-51	43	34	70				
	M-54	45	42	72				
	M-56	45	38	73				
	M-59	45	33		64	51		
	M-84	46	47	101	60	52		
	M-87	45	33	80			23	
	M-88	47	34 (32)	(77)	61	35		22
	M-101	47	41		67	48		
	M-103	47	33	78			21	
	M-113	45	43	86				
	M-114 ^T	45	37 (42)	100 (100)	61	46		28
	ME-1'	47	(30)	(82)				18
	F-4	47	(34)	(73)				22
4	M-13	46	22		91	28		
	M-17 ^T	45	(36)	(65)	100	37		37
	M-96	43	32	58				
	M-98	45	26	57	84	41		
3	M-16	43	25		40	55		
	M-32	46	25		43	64		
	M-33	46	17		30	64		
	M-61	45	25		46	72		
	M-85	46	40	54	38	91		
	M-99	45	35	48	41	63		
	M-115	46	39		38	119		
	M-116 ^T	44	42	53	34	100		
5	M-18	43	24 (26)	(24)	29	19	101	32
	M-19 ^T	43	24	28	25	20	100	
6	X1-1 ^T	46	(28)	(30)				100
	X3-1	45	(25)	(27)				105
	X5-5	45	(25)	(29)				104
	X6-1	46	(25)	(32)				103
	X9-1	46	(25)	(25)				98
	X9-3	43	(23)	(23)				86
	SH-3	46	(25)	(33)				94
	MB-1	45	(30)	(31)				76
	MH-3	44	(23)	(24)				69
	I-3	46	(22)	(23)				65
	Calf thymus	43	0 (4)	1 (4)	4	5	1	2

^a All of the homology groups except group 6 correspond to the groups described by Yanagida et al. (33).^b The values in parentheses are from reference 33.

agar; generally motile; catalase and cytochromes are absent; facultatively anaerobic; good growth occurs on media containing glucose, and poor or no growth occurs in nutrient broth; D- or DL-lactic acid is produced homofermentatively; acid is produced from glucose, fructose, and mannose; the cellular fatty acid profile includes branched-chain acids, and anteiso-C_{15:0} and anteiso-C_{17:0} are the predominant fatty acids; the cell wall contains *meso*-diaminopimelic acid; and the major isoprenoid quinone is MK-7. The type strains of the taxa described below have been deposited in the Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, Japan.

Description of *Sporolactobacillus nakayamae* subsp. *nakayamae* sp. nov., subsp. nov. *Sporolactobacillus nakayamae* subsp. *nakayamae* (na.ka.ya'mae. M.L. gen. n. *nakayamae*, of Nakayama, named for Ooki Nakayama, a Japanese microbiologist who isolated a number of *Sporolactobacillus* strains). Most strains grow at 15°C. Acid is produced from maltose, but not from arabinose, ribose, xylose, rhamnose, and lactose. Most strains produce acid from galactose, sucrose, and trehalose. Most strains do not produce acid from cellobiose, melibiose, salicin, and starch. The DNA G+C content ranges from 43 to 47 mol% (as determined by the *T_m* method). The habitat is soil. The type strain is strain M-114 (= JCM 3514). The

TABLE 3. Salient characteristics of the genera of aerobic, endospore-forming bacteria

Genus	Rod-shaped cells	Spore shape	Anaerobic growth	Growth in nutrient broth	Catalase activity	Production of lactic acid	Major isoprenoid quinone	Major cellular fatty acids	G+C content (mol%)
<i>Sporolactobacillus</i> ^a	+	Oval	+	—	—	+	MK-7 ^c	anteiso-C _{15:0} , anteiso-C _{17:0}	43–50
<i>Amphibacillus</i> ^d	+	Oval	+	—	—	+	None	anteiso-C _{15:0} , C _{16:0} , iso-C _{16:0} , iso-C _{15:0}	36–38
<i>Bacillus</i> ^e	+	Oval or spherical	V	+	+	V	MK-7	V	32–69
<i>Brevibacillus</i> ^f	+	Oval	V	+	+	NT	MK-7	anteiso-C _{15:0} and iso-C _{15:0} or iso-C _{15:0}	46–57
<i>Paenibacillus</i> ^g	+	Oval	+	+	+	NT	MK-7	anteiso-C _{15:0}	40–54
<i>Sporosarcina</i> ^h	—	Round	—	+ or —	+	NT	MK-7		40–42

^a Data from reference 12 and this study.^b +, positive; —, negative; V, variable; NT, not tested.^c MK-7, menaquinone 7.^d Data from reference 20.^e Data from reference 3.^f Data from references 23 and 28.^g Data from references 8 and 9.^h Data from reference 4.

other strains examined are strains M-45, M-46, M-47, M-50, M-51, M-54, M-56, M-59, M-84, M-87, M-88, M-101, M-103, M-113, ME-1', and F-4.

Description of *Sporolactobacillus nakayamae* subsp. *racemicus* subsp. nov. *Sporolactobacillus nakayamae* subsp. *racemicus* (ra.ce.mi'cus. L. n. *racemus*, rotatory of lactic acid racemi, [rotatory]-lactic acid). DL-Lactic acid is produced. Litmus milk is acidified. Acid is produced from galactose, maltose, melibiose, sucrose, raffinose, trehalose, and inulin, but not from arabinose, ribose, and xylose. The DNA G+C content ranges from 43 to 46 mol% (as determined by the *T_m* method). The habitat is soil. The type strain is strain M-17 (= JCM 3417). The other strains examined are strains M-13, M-96, and M-98.

Description of *Sporolactobacillus terrae* sp. nov. *Sporolactobacillus terrae* (ter'rae. M.L. gen. n. *terrae*, of the earth). Most strains grow at 15°C. Acid is produced from trehalose and inulin, but not from ribose, xylose, rhamnose, lactose, and sorbitol. Most strains produce acid from galactose and sucrose. Most strains do not produce acid from arabinose, melibiose, and starch. The DNA G+C content ranges from 43 to 46 mol% (as determined by the *T_m* method). The habitat is soil. The type strain is strain M-116 (= JCM 3516). The other

strains examined are strains M-16, M-32, M-33, M-61, M-85, M-99, and M-115.

Description of *Sporolactobacillus kofuensis* sp. nov. *Sporolactobacillus kofuensis* (ko.fu.en'sis. M.L. adj. *kofuensis*, belonging to Kofu, Yamanashi, Japan, the source of the soil from which the organism was isolated). Acid is produced from galactose, maltose, sucrose, raffinose, trehalose, mannitol, and inulin, but not from arabinose, ribose, xylose, rhamnose, cellobiose, lactose, melibiose, salicin, and sorbitol. The DNA G+C content is 43 mol% (as determined by the *T_m* method). The habitat is soil. The type strain is strain M-19 (= JCM 3419). The other strain examined is strain M-18.

Description of *Sporolactobacillus lactosus* sp. nov. *Sporolactobacillus lactosus* (lac.to'sus. M.L. adj. *lactosus*, of lactose, pertaining to lactose). Litmus milk is acidified and then reduced by some strains. Acid is produced from galactose, lactose, melibiose, and trehalose. Most strains produce acid from maltose, sucrose, raffinose, and inulin. The DNA G+C content ranges from 43 to 46 mol% (as determined by the *T_m* method). The habitat is soil in Japan and fermentation starters. The type strain is strain X1-1 (= JCM 9690). The other strains examined are strains X3-1, X5-5, X6-1, X9-1, X9-3, SH-3, MB-1, MH-3, and I-3.

TABLE 4. Differential characteristics of *Sporolactobacillus* species and subspecies

Species or subspecies	DNA homology group	Growth at 15°C	Litmus milk		Acid production from carbohydrates								Lactic acid isomer
			Acidification	Reduction	Arabinose	Xylose	Galactose	Cellobiose	Lactose	Melibiose	Starch	Inulin	
<i>S. inulinus</i>	1	— ^a	—	—	—	—	—	—	—	—	+	d	D
<i>S. nakayamae</i> subsp. <i>nakayamae</i>	2	(+)	d	(—)	—	—	(+)	(—)	—	(—)	(—)	(—)	D
<i>S. nakayamae</i> subsp. <i>racemicus</i>	4	+	+	d	—	—	+	d	d	+	d	+	DL
<i>S. terrae</i>	3	(+)	(—)	—	(—)	—	(+)	d	—	(—)	(—)	+	D
<i>S. kofuensis</i>	5	—	—	—	—	—	+	—	—	—	d	+	D
<i>S. lactosus</i>	6	(+)	(+)	(+)	d	d	+	d	+	+	d	(+)	D

^a —, all strains are negative; +, all strains are positive; (—), at least 80% of the strains are negative; (+), at least 80% of the strains are positive; d, 20 to 80% of the strains are positive.

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