Description of *Bacillus laevolacticus* (ex Nakayama and Yanoshi 1967) sp. nov., nom. rev.

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The name “*Bacillus laevolacticus*” Nakayama and Yanoshi 1967 was not included on the Approved Lists of Bacterial Names and therefore has no standing in bacteriological nomenclature. In this study 22 catalase-positive, acid-tolerant, facultatively anaerobic, lactic acid-producing *Bacillus* strains were examined taxonomically and compared with a number of strains belonging to phenetically similar *Bacillus* species (*Bacillus coagulans*, *Bacillus smithii*, “*Bacillus vesiculiferous*”) and with *Sporolactobacillus*. The G+C contents (43 to 45 mol%), DNA-DNA homology values (72 to 98%), and results of phenetic similarity analyses revealed that the members of the “*B. laevolacticus*” group were very homogeneous in their phenotypic and genotypic characteristics and clearly distinguishable from other *Bacillus* and *Sporolactobacillus* species. On the basis of these findings, revival of the name *Bacillus laevolacticus* is proposed.

Traditionally, production of lactic acid is observed in microorganisms which are grouped under the term “lactic acid bacteria.” However, a considerable number of lactic acid-producing, aerobic, spore-forming organisms have been described. These bacteria have been isolated from food or in connection with spoilage of preserved food, from milk (12, 29), from tomato purée (4), from the rhizospheres of various plants (25), from a sugar production factory (18), and from the intestines of crayfish (34). It is possible that lactic acid production is much more widely distributed among *Bacillus* species than we realize at this time. The species of the genus *Bacillus* which have been reported to produce lactic acid include two recognized species, *Bacillus coagulans* and *Bacillus smithii*, and three species whose names have not been validly published previously, “*Bacillus laevolacticus*,” “*Bacillus racemilacticus*,” and “*Bacillus vesiculiferous*.”

B. coagulans was first isolated by Hammer in 1915 from spoiled canned milk and was described as a new species. In a number of later studies it was noted that the cell morphology, spore surface morphology, and sporangium morphology varied from strain to strain. This high degree of variability led to the creation of a number of other species names which later were recognized as subjective synonyms, including “*Bacillus thermoacidurans*” (5), “*Bacillus thermoacidificans*” (28), “*Bacillus dextralacticus*” (1), and “*Lactobacillus cereale*” (26). Later, it was observed that clustering of strains was obtained when certain physiological tests were performed (18, 21, 35). Nakamura et al. (24) distinguished DNA relatedness groups, of which DNA group 1 was identified to represent the species *B. coagulans* sensu stricto. DNA group 2 was described as a new species, which was named *B. smithii*.

Other bacterial strains that share some of the characteristics of the genera *Lactobacillus* and *Bacillus* were isolated from chicken feed by Kitahara and Suzuki (17). These strains were similar to members of the genus *Lactobacillus* in their lack of catalase, microaerophilic growth, and lactic acid fermentation characteristics, but production of typical endospores and cell

walls containing diaminopimelic acid were consistent with the description of the genus *Bacillus*. Accordingly, these organisms were placed in a new genus, *Sporolactobacillus*, as *Sporolactobacillus inulinus*. Similar organisms, including “*Sporolactobacillus laevas*,” “*Sporolactobacillus laevas* var. *intermedius*,” and “*Sporolactobacillus racemicus*,” have been isolated from the rhizospheres of wild plants, but these bacteria have not been validly described as new species (36, 37).

In 1967, Nakayama and Yanoshi (25) isolated and described catalase-positive, acid-tolerant, facultatively anaerobic, mesophilic *Bacillus* strains that produced lactic acid, which they named “*B. laevolacticus*” and “*B. racemilacticus*.” These organisms could be distinguished from each other only by the ability of “*B. racemilacticus*” to grow in the presence of 3.5% NaCl and to produce DL-lactic acid instead of D-(–)-lactic acid [“*B. laevolacticus*” produced D-(–)-lactic acid]. “*B. laevolacticus*” and “*B. racemilacticus*” were distinguished from *B. coagulans* on the basis of their lower growth temperatures, greater acid tolerance, requirement of carbohydrates for growth, and stereospecificity of the lactate produced [*B. coagulans* produces only L-(+)-lactic acid from glucose]. As early as 1981, Collins and Jones (9) stated that, on the basis of chemotaxonomic data, the acid-tolerant *Bacillus* strains (namely “*B. laevolacticus*,” “*B. racemilacticus*,” “*Bacillus myxolacticus*,” and “*Bacillus dextralacticus*”) and *Sporolactobacillus* strains could be grouped together as members of a separate taxon close to the genus *Bacillus*. Yanagida et al. (36, 37) included a number of “*B. laevolacticus*” and “*B. racemilacticus*” strains in their studies. Certain strains clustered together, but clear separation from *Sporolactobacillus* strains was not possible. On the basis of the results of 16S rRNA sequencing studies performed with a set of these strains, Suzuki and Yamasoto (32) found that most of them clustered more or less around the genus *Sporolactobacillus*, forming several subclusters, while a single strain of “*B. racemilacticus*” formed a separate branch and one strain clustered in *Bacillus* rRNA group 1 (2).

In order to clarify the taxonomic position of 22 catalase-positive, acid-tolerant, facultatively anaerobic, mesophilic *Bacillus* strains with respect to a number of previously recognized *Bacillus* species, we examined the phenotypic and chemosystematic characteristics, DNA base compositions, and levels of DNA relatedness of these organisms.

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TABLE 1. Acid-tolerant *Bacillus* strains examined in this study

Strain	Received as:	Source ^a	Strain history and original designation ^a	Other designation(s)
DSM 442 ^T	M 8 ^T	1	<O. Nakayama, " <i>B. laevolacticus</i> ," rhizosphere of <i>Ranunculus sceleratus</i>	NCIMB 10269 ^T , ATCC 23492 ^T , JCM 2513 ^T
DSM 444 ^b	M 14	1	<O. Nakayama, " <i>B. racemilacticus</i> ," rhizosphere of <i>Lactuca dentata</i>	NCIMB 10274, ATCC 23496, JCM 2517
DSM 2310	NCIMB 10270	2	<NCIMB < O. Nakayama, M 1, " <i>B. laevolacticus</i> ," rhizosphere of <i>Trifolium repens</i>	ATCC 23493, JCM 2514
DSM 2315 ^c	NCIMB 10276	2	<NCIMB < O. Nakayama, M 64, " <i>B. racemilacticus</i> ," rhizosphere	
DSM 2316	NCIMB 10271	2	<NCIMB < O. Nakayama, M 7, " <i>B. laevolacticus</i> ," rhizosphere of <i>Allium japonicum</i>	ATCC 23494, JCM 2515
DSM 2317	NCIMB 10272	2	<NCIMB < O. Nakayama, M 40, " <i>B. laevolacticus</i> ," rhizosphere	ATCC 23495
DSM 2318	NCIMB 10273	2	<NCIMB < O. Nakayama, M 4, " <i>B. laevolacticus</i> ," rhizosphere of <i>Hemerocallis flava</i>	ATCC 23549, JCM 2516
DSM 6474	IAM 12329	3	<IAM < O. Nakayama, M 71, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6475	IAM 12331	3	<IAM < O. Nakayama, M 104, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6476	IAM 12326	3	<IAM < O. Nakayama, M 105, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6477	M 81	1	<O. Nakayama, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6478	IAM 12379	3	<IAM < O. Nakayama, M 75, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6510	M 89	1	<O. Nakayama, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6511	M 121	1	<O. Nakayama, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6547	IAM 12327	3	<IAM < O. Nakayama, M 66, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6548	IAM 12328	3	<IAM < O. Nakayama, M 68, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6549	IAM 12330	3	<IAM < O. Nakayama, M 91, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6763	M 120	1	<O. Nakayama, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6764	M 95	1	<O. Nakayama, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 6771	M 100	1	<O. Nakayama, " <i>B. laevolacticus</i> ," rhizosphere	
DSM 2309	NCIMB 10275	2	<NCIMB < O. Nakayama, M 5, " <i>B. racemilacticus</i> ," rhizosphere of <i>Rumex acetosa</i>	ATCC 23497, JCM 2518
DSM 2314 ^d	NCIMB 10276	2	<NCIMB < O. Nakayama, M 39, " <i>B. racemilacticus</i> ," rhizosphere	ATCC 23498

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^b Designated the type strain of "*B. racemilacticus*" by Gibson and Gordon (10) but identified as a "*B. laevolacticus*" strain by Blumenstock (6).

^c Identified as a "*B. racemilacticus*" strain by Nakayama (24a) and as a "*B. laevolacticus*" strain by Blumenstock (6).

^d Identified as a "*B. racemilacticus*" strain by Nakayama and Yanoshi (25) and as a *B. coagulans* strain by Blumenstock (6).

MATERIALS AND METHODS

Bacterial strains. The *Bacillus* strains used in this study are listed in Table 1. Seven strains were obtained from the National Collections of Industrial and Marine Bacteria, Ltd., Aberdeen, Scotland, and seven strains were provided by the Institute of Applied Microbiology, Tokyo, Japan. Eight strains were donated by O. Nakayama, Yamanashi University, Kofu, Japan. The reference strains used were obtained from the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

Phenotypic characterization. Unless indicated otherwise, phenotypic characterization was carried out as described by Gordon et al. (11), as amended by Claus and Berkeley (8). The media used for the minimum and maximum growth temperature, NaCl tolerance, anaerobic growth, lysozyme resistance, gelatin hydrolysis, egg yolk lecithinase reaction, and tyrosine degradation tests were replaced by the glucose medium of

Nakayama and Yanoshi (25). For the nitrate reduction and indole formation tests the medium was supplemented with 1% (final concentration) glucose.

The following tests were performed by previously described methods: flagellum staining (13), Gram staining (3), oxidase activity (19), aminopeptidase activity (with test strips obtained from Merck, Darmstadt, Germany) (7), and pullulanase activity (23).

Chemosystematic characterization. The chemosystematic characterization tests were performed by previously described methods for cell wall analysis (16), analysis of fatty acids (30), and analysis of quinones (27, 33).

DNA isolation, DNA base composition, and DNA relatedness. DNA was isolated by the method of Marmur (22). The G+C content of the DNA and the levels of DNA relatedness were determined as described by Spanka and Fritze (31).

Lactic acid production. The stereospecificity of lactic acid was determined as described by Hohorst (14).

TABLE 2. G+C contents and levels of homology of the DNAs of acid-tolerant *Bacillus* strains

Organism		G+C content (mol%)	% Homology to DSM 6511 ^a
Species	Strain		
<i>"B. laevolacticus"</i>	DSM 442 ^T	43	79
	DSM 444	43	87
	DSM 2310	44	84
	DSM 2315	43	80
	DSM 2316	43	75
	DSM 2317	43	79
	DSM 2318	43	80
	DSM 6476	44	81
	DSM 6547	43	61
	DSM 6548	45	72
	DSM 6474	44	84
	DSM 6549	43	83
	DSM 6475	43	98
	DSM 6478	44	81
	DSM 6477	44	84
	DSM 6510	44	78
	DSM 6764	43	83
	DSM 6771	44	81
	DSM 6763	43	86
	DSM 6511	44	100
<i>"B. racemilacticus"</i>	DSM 2309	37	27

^a Values are the means of three or four determinations.

RESULTS

DNA base composition. On the basis of the results of an analysis of their G+C contents (Table 2), the strains assigned to "*B. laevolacticus*" formed a relatively tight group. The G+C contents of most of these organisms were between 43 and 44 mol%; one strain, DSM 6548, had a slightly higher G+C content (45 mol%). The G+C content of strain DSM 2309, which is assigned to "*B. racemilacticus*," was 37 mol%. Strain DSM 2314 had exactly the same G+C content as the type strain of *B. coagulans* (47 mol%).

DNA-DNA hybridization. Table 2 shows that 19 of the 20 strains assigned to "*B. laevolacticus*" exhibited DNA-DNA reassociation values with strain DSM 6511 that were greater than 70%. One strain, DSM 6547, exhibited a lower degree of binding (61%) to strain DSM 6511. The high level of DNA-DNA homology between strains DSM 6547 and DSM 6478

(77%) supported placement of DSM 6547 in the taxon "*B. laevolacticus*." The level of reassociation between strain DSM 6511 DNA and "*B. racemilacticus*" DSM 2309 DNA was 27%. The level of DNA-DNA homology between strain DSM 2314 and the type strain of *B. coagulans* was 82% (data not shown).

Cellular fatty acid composition, quinone system, and cell walls. More than 95% of cellular fatty acids were branched-chain fatty acids; the most common fatty acids were anteiso-C_{15:0} and C_{17:0} fatty acids (each around 35 to 40%). Small amounts of iso-C_{15:0} and C_{16:0} (each approximately 10%) were present. Neither unsaturated fatty acids nor omega-cyclohexane and -heptane fatty acids were found.

The murein of the cell walls of all strains contained *meso*-diaminopimelic acid at the third position of the peptide side chains, which were connected directly without an interpeptide bridge (*meso*-diaminopimelic acid direct type). The quinones were predominantly (>90%) MK-7.

Lactic acid production. All "*B. laevolacticus*" and "*B. racemilacticus*" strains produced D-(-)-lactic acid from glucose in variable amounts; a few strains (especially DSM 2309) also produced L-(+)-lactic acid. In contrast, strain DSM 2314 (*B. coagulans*) produced only L-(+)-lactic acid (data not shown).

Phenotypic characterization. Vegetative cells of "*B. laevolacticus*" were 0.4 to 0.7 µm wide. The widths of "*B. racemilacticus*" and *B. coagulans* cells were similar (0.4 to 0.8 µm). The lengths of the cells of all of these organisms were variable, ranging from 3 to 9 µm. Whereas the morphology of the vegetative cells of the acid-tolerant *Bacillus* strains, including *B. coagulans*, could not be used for differentiation, the shapes and sizes of the spores could be used to distinguish the species. The spores of *B. coagulans* were usually ellipsoid (0.6 to 0.8 µm wide and 1.3 to 1.7 µm long). The "*B. laevolacticus*" strains usually produced somewhat smaller spores which were short ellipsoids or sometimes nearly round; the spores of these organisms were 0.6 to 0.8 µm wide and 0.8 to 1.2 µm long (Fig. 1). The strain representing "*B. racemilacticus*," DSM 2309, produced banana-shaped spores (which were best seen in young stages) that were 0.8 µm wide and 1.2 to 1.3 µm long.

The acid-tolerant strains assigned to "*B. laevolacticus*" yield relatively uniform results in the classical diagnostic tests for the genus *Bacillus*. In contrast to *B. coagulans* and "*B. racemilacticus*," all strains require growth medium supplementation with glucose. Therefore, of the test media the NB or NA basis of most had to be replaced by the glucose medium, and some media had to be at least supplemented with glucose (see

FIG. 1. Sporulating and nonsporulating cells of "*B. laevolacticus*" DSM 442^T (A) and "*B. racemilacticus*" DSM 2309 (B). Bar = 10 µm.

TABLE 3. Differentiation of "*B. laevolacticus*" from other lactic acid-producing *Bacillus* strains and *S. inulinus*

Characteristic	" <i>B. laevolacticus</i> " ^a	" <i>B. racemilacticus</i> " ^b	<i>B. coagulans</i> ^c	<i>B. smithii</i> ^d	" <i>B. vesiculiferous</i> " ^e	<i>S. inulinus</i> ^f
Cell width (µm)	0.4-0.7	0.6-0.8	0.4-0.8	0.8-1.0	0.8-1.2	0.7-0.8
Spore width (µm)	0.6-0.8	0.8	0.6-0.8	0.6-0.8	0.6-0.8	0.8
Spore length (µm)	0.8-1.2	1.2-1.3	1.3-1.7	1.3-1.5	0.8-1.0	1.0
Sporangia swollen	+	+/-	+/-	+/-	+/-	+
Catalase activity	+	+	+	+	-	-
Oxidase activity	-	-	-	+	ND ^g	ND
Growth in NB at:						
pH 4.5	-	-	+	+	-	-
pH 6.8	-	-	+	-	-	-
Growth in CASO bouillon at:						
pH 4.5	+	+	+	-	+	+
pH 7.7	+	+	+	-	-	+
Maximum temp (°C)	40	45	60	65	40	40
Voges-Proskauer reaction	+	+	+	-	-	ND
pH in Voges-Proskauer broth	3.8-4.0	4.3	4.0-4.4	4.3-4.5	No alkalization	ND
Indole production	-	-	-	-	+	-
Acid produced from:						
Mannitol	+	-	-	+	-	+
Starch	+	-	+	-	-	-
Lactic acid produced	Homofermentative, D-(-) lactic acid ^h	Homofermentative, DL-lactic acid ^h	Homofermentative, L-(+) lactic acid	Homofermentative, L-(+) lactic acid	Heterofermentative, ND	Homofermentative, D-(-) lactic acid
Hydrolysis of DNA	-	-	+	+	ND	ND
G+C content (mol%)	43-45	37	45-47	39-40	39	38-39

^a Data based on 20 strains.^b Data based on one strain.^c Data from Claus and Berkeley (8).^d Data from Nakamura et al. (24).^e Data from Trinkunaitte et al. (34).^f Data from Kitahara and Suzuki (17) and Kandler and Weiss (15).^g ND, not determined.^h Nearly homofermentative according to Nakayama and Yanoshi (25).

Materials and Methods). Positive reactions were observed in the following tests for all strains: catalase activity, growth in CASO bouillon at pH 4.5, growth at 15 and 40°C, growth at pH 5.7, hydrolysis of starch, anaerobic growth, production of acetylmethylcarbinol (Voges-Proskauer test), and acid production from glucose and mannitol. Voges-Proskauer medium was acidified to 3.8 to 4.0. Negative reactions were observed in the following tests for all strains: oxidase activity, growth at pH 4.5 or 6.8 on NA, growth at 5 or 50°C, growth in the presence of 5% NaCl, growth in the presence of 0.2% azide, resistance to lysozyme, acid production from D-xylose and D-arabinose, gas production from glucose, hydrolysis of casein, gelatin, or DNA, egg yolk lecithinase activity, degradation of tyrosine, reduction of nitrate to nitrite, utilization of citrate or propionate, formation of indole, and deamination of phenylalanine.

DISCUSSION

Nakayama and Yanoshi (25) differentiated their catalase-positive, acid-tolerant, facultatively anaerobic, mesophilic, lactic acid-producing strains from *B. coagulans* on the basis of the following characteristics: lower growth temperature, greater acid tolerance, requirement of carbohydrates for growth, and stereospecificity of the lactate produced [DL-lactic acid was produced by "*B. racemilacticus*" and D(-)-lactic acid was produced by "*B. laevolacticus*"].

In our study "*B. racemilacticus*" DSM 2309 was the only strain that produced significant amounts of DL-lactic acid. This strain differed from the other strains by its low G+C content (37 mol%), which was 6 to 8 mol% lower than the G+C contents "*B. laevolacticus*" strains. This finding seemed to be reflected in the remote position of DSM 2309 relative to "*B. laevolacticus*" strains as determined by the analysis of 16S rRNA sequences (32). The results of a DNA-DNA reassociation experiment performed despite the obvious difference between "*B. laevolacticus*" and "*B. racemilacticus*" DNAs (the G+C contents differed by more than 6 mol%) also supported this finding and confirmed the suspected separate species status of the organism (degree of binding, <30%). Revival of the name "*B. racemilacticus*" based on the properties of a single strain, however, is not reasonable, especially since the former type strain of "*B. racemilacticus*," strain DSM 444, clearly is a member of "*B. laevolacticus*."

The G+C content of DSM 2314, which produced L-(+)-lactic acid, was 47 mol%, and this value most closely resembled the G+C content of *B. coagulans* (46 to 47 mol%). As strain DSM 2314 also exhibited a high level of DNA homology with the type strain of *B. coagulans*, strain DSM 2314 was placed in this species.

The other 20 strains examined in this study, which previously were designated "*B. laevolacticus*" strains (18 strains) or "*B. racemilacticus*" strains (2 strains), produced D(-)-lactic acid. Overall, these strains were phenotypically homogeneous and exhibited high levels of DNA-DNA relatedness (more than 70%). Only one strain, DSM 6547, exhibited a lower degree of binding (61%) to strain DSM 6511. Because of its overall high levels of similarity to the other strains in the group, strain DSM 6547 is considered a member of "*B. laevolacticus*"; this placement is supported by the high level of DNA-DNA homology (77%) between strains DSM 6547 and DSM 6478. Four of the strains included in this study (DSM 442^T [T = type strain], DSM 444, DSM 2310, and DSM 6476) were included in the rRNA sequence analysis study of Suzuki and Yamasato (32). Interestingly, Suzuki and Yamasato found that these strains form a tight cluster together with a number of strains allocated to *Sporolactobacillus* and *Bacillus* species whose names have

not been validly published which appear to be relatively distantly related to the type strain of *S. inulinus*.

Table 3 shows a number of phenotypic characteristics that clearly differentiate "*B. laevolacticus*" from other lactic acid-producing *Bacillus* and *Sporolactobacillus* species, including *B. coagulans*, *B. smithii*, "*B. racemilacticus*," "*B. vesiculiferous*," and *S. inulinus*.

The following key characteristics easily distinguish "*B. laevolacticus*" from other catalase-positive, facultatively anaerobic, Voges-Proskauer-positive *Bacillus* species having similar morphology (the cells of all strains are less than 1 µm in diameter): *Bacillus alvei* grows well on NA, forms dihydroxyacetone and indole, hydrolyzes casein and gelatin, and is resistant to lysozyme, and the G+C content of the type strain of *B. alvei* is 45 mol%; *Bacillus azotofixans* does not hydrolyze starch and produces gas from glucose, the final pH values of *B. azotofixans* cultures in Voges-Proskauer medium are 4.5 to 5.1, and the G+C content of the type strain of *B. azotofixans* is 52 mol%; *Bacillus licheniformis* grows well on NA, grows at 50°C, reduces nitrate to nitrite, grows in the presence of 5% NaCl, produces acid from xylose, and hydrolyzes casein and gelatin, and the G+C content of the type strain of *B. licheniformis* is 46 mol%; and *Bacillus polymyxa* grows well on NA, reduces nitrate to nitrite, produces acid from xylose, produces gas from glucose, and hydrolyzes casein and gelatin, and the G+C content of the type strain of *B. polymyxa* is 44 mol%.

On the basis of our results, we consider the strains of "*B. laevolacticus*" genetically and phenotypically distinct from other lactic acid-producing, aerobic, spore-forming organisms. Therefore, we propose that the name *Bacillus laevolacticus* should be revived and assigned to the same taxon to which it was originally applied, in accordance with Rules 27, 28a, 33a, and 33c of the *International Code of Nomenclature of Bacteria* (20). A description of the species is given below.

Description of *Bacillus laevolacticus* sp. nov., nom. rev. *Bacillus laevolacticus* (ex Nakayama and Yanoshi 1967) [lae. vo.lac'ti.cus. M. L. adj. *laevolacticus*, referring to D(-)-lactic acid, the only lactic acid produced by the organisms]. Young cells are gram positive. The cell width is 0.4 to 0.7 µm. Motile by means of a few polarly and laterally inserted flagella. The spores are ellipsoid (0.6 to 0.8 µm wide and 0.8 to 1.2 µm long) and swell the sporangium. On glucose-CaCO₃ agar the colonies of most strains are typical white pinpoint colonies that are about 2 mm in diameter; each colony has a clear halo around it which is produced by acid production.

The peptidoglycan side chains are directly linked via *m*-diaminopimelic acid. MK-7 is the main (>90%) menaquinone. Ubiquinones are not present. The G+C content of the DNA is 43 to 45 mol% (as determined by the thermal denaturation method). The predominant cellular fatty acids are branched-chain anteiso-C_{15:0} and C_{17:0} fatty acids.

Chemoorganotrophic. Does not grow in NB or NA. Glucose or other carbohydrates are required for growth. Facultatively anaerobic. Catalase positive. Oxidase negative. Mesophilic. Maximum temperature for growth, 40°C. Acid tolerant; growth occurs at pH 4.5. Voges-Proskauer positive; the pH in Voges-Proskauer medium is 3.8 to 4.0. No growth occurs in the presence of lysozyme or 5% NaCl. Hydrolyzes starch and pullulan. Citrate and propionate are not utilized. Gelatin, DNA, tyrosine, and casein are not hydrolyzed. Indole is not produced. Nitrate is not reduced to nitrite. Egg yolk lecithinase negative. Does not deaminate phenylalanine. Acid is produced from glucose and mannitol but not from arabinose or xylose. No gas is produced from glucose. Predominantly D(-)-lactic acid is produced from glucose.

Habitat: rhizospheres of plants.

The type strain is strain M 8 (= ATCC 23492 = DSM 442 = IAM 12321 = NCIB 10269).

The DNA base composition of the type strain is 43 mol%. The type strain has all the characteristics given above for the species and was isolated from the rhizosphere of ditch crowfoot (*Ranunculus sceleratus*).

REFERENCES

- Anderson, A. A., and C. H. Werkman. 1944. Description of a dextro-lactic acid forming organism of the genus *Bacillus*. Iowa State Coll. J. Sci. **14**:187–194.
- Ash, C., J. A. E. Farrow, S. Wallbanks, and M. D. Collins. 1991. Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small-subunit-ribosomal RNA sequences. Lett. Appl. Microbiol. **13**:202–206.
- Bartholomew, J. W. 1962. Variables influencing results in the precise definition of steps in Gram staining as a means of standardizing the results obtained. Stain Technol. **37**:139–155.
- Becker, E. M., and C. S. Pederson. 1950. The physiological characters of *Bacillus coagulans* (*Bacillus thermoacidurans*). J. Bacteriol. **59**:717–725.
- Berry, R. N. 1933. Some new heat resistant acid tolerant organisms causing spoilage in tomato juice. J. Bacteriol. **25**:72–73.
- Blumenstock, I. 1984. *Bacillus coagulans* HAMMER 1915 und andere thermophile oder mesophile, säuretolerante Bacillus-Arten—eine taxonomische Untersuchung. Ph.D. dissertation. Universität Göttingen, Göttingen, Germany.
- Cerny, C. 1978. Studies on the aminopeptidase test for the distinction of gram negative from gram positive bacteria. Eur. J. Appl. Microbiol. Biotechnol. **5**:113–122.
- Claus, D., and R. C. W. Berkeley. 1986. Genus *Bacillus* Cohn 1882, p. 1105–1139. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
- Collins, M. D., and D. Jones. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. Microbiol. Rev. **45**:316–354.
- Gibson, T., and R. E. Gordon. 1974. Genus *Bacillus* Cohn 1872, p. 529–575. In R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. Williams and Wilkins, Baltimore.
- Gordon, R. E., W. C. Haynes, and C. H.-N. Pang. 1973. The genus *Bacillus*. United States Department of Agriculture, Washington, D.C.
- Hammer, B. W. 1915. Bacteriological studies on the coagulation of evaporated milk. Iowa Agric. Exp. Stn. Res. Bull. **19**:119–131.
- Heimbrook, M. E., W. L. L. Wang, and G. Campbell. 1989. Staining bacterial flagella easily. J. Clin. Microbiol. **27**:2612–2625.
- Hohorst, H. J. 1966. L-Lactatbestimmung, p. 266–270. In H. U. Bergmeyer (ed.), Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim, Germany.
- Kandler, O., and N. Weiss. 1986. Genus *Sporolactobacillus* (Kitahara and Suzuki 1963), p. 1139–1141. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
- Kandler, O., and N. Weiss. 1986. Genus *Lactobacillus* (Beijerinck 1901), p. 1209–1219. In P. H. A. Sneath, M. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
- Kitahara, K., and J. Suzuki. 1963. *Sporolactobacillus* nov. subgen. J. Gen. Appl. Microbiol. **9**:59–71.
- Klaushofer, H., and F. Hollaus. 1970. Zur Taxonomie der hochthermophilen, in Zuckerfabriksäften vorkommenden aeroben Sporenbildner. Z. Zuckerind. **20**:465–470.
- Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by oxidase reaction. Nature (London) **178**:703.
- Lapage, S. P., P. H. A. Sneath, E. F. Lessel, V. B. D. Skerman, H. P. R. Seeliger, and W. A. Clark (ed.). 1990. International code of nomenclature of bacteria. 1990 Revision. American Society for Microbiology, Washington, D.C.
- Logan, N. A., and R. C. W. Berkeley. 1981. Identification of *Bacillus* strains using the API system. J. Gen. Microbiol. **130**:1871–1882.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**:208–218.
- Morgan, F. J., K. R. Adams, and F. G. Priest. 1979. A cultural method for the detection of pullulan-degrading enzymes in bacteria and its application to the genus *Bacillus*. J. Appl. Bacteriol. **46**:291–294.
- Nakamura, L. K., I. Blumenstock, and D. Claus. 1988. Taxonomic study of *Bacillus coagulans* Hammer 1915 with a proposal for *Bacillus smithii* sp. nov. Int. J. Syst. Bacteriol. **38**:63–73.
- Nakayama, O. Personal communication.
- Nakayama, O., and M. Yanoshi. 1967. Spore-bearing lactic acid bacteria isolated from rhizosphere. I. Taxonomic studies on *Bacillus laevolacticus* nov. sp. and *Bacillus racemilacticus* nov. sp. J. Gen. Appl. Microbiol. **13**:139–153.
- Olsen, E. 1944. En sporedannende mælkesyrebakterie *Lactobacillus cereale* (nov. sp.). Kem. Maandesbl. Nord. Handelsbl. Kem. Ind. **25**:125–130.
- Readfearn, E. R. 1967. Isolation and distribution of ubiquinones. Methods Enzymol. **10**:381–384.
- Renco, P. 1942. Recherche sur un fermento lattico sporigo (*Bacillus thermoacidificans*). Ann. Microbiol. (Paris) **2**:109–114.
- Sarles, W. B., and B. W. Hammer. 1932. Observations on *Bacillus coagulans*. J. Bacteriol. **23**:301–314.
- Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. U.S. Fed. Culture Collections Newsl. **20**:1–5.
- Spanka, R., and D. Fritze. 1993. *Bacillus cohnii* sp. nov., a new, obligately alkaliphilic, oval-spore-forming *Bacillus* species with ornithine and aspartic acid instead of diaminopimelic acid in the cell wall. Int. J. Syst. Bacteriol. **43**:150–156.
- Suzuki, T., and K. Yamasato. 1994. Phylogeny of spore-forming lactic acid bacteria based on 16S rRNA gene sequences. FEMS Microbiol. Lett. **115**:13–18.
- Tindall, B. J., K. O. Stetter, and M. D. Collins. 1989. A novel, fully saturated menaquinone from the thermophilic, sulphate-reducing archaebacterium *Archaeoglobus fulgidus*. J. Gen. Microbiol. **135**:693–696.
- Trinkunaite, L. L., V. I. Duda, L. L. Mityushina, L. M. Mitskenene, A. V. Lebedinskii, and V. V. Krivenko. 1987. A new spore-forming bacterium, *Bacillus vesiculiferous* sp. nov., forming gas balloons on cells. Mikrobiologiya **56**:108–113.
- Wolf, J., and A. N. Barker. 1968. The genus *Bacillus*: aids to the identification of its species, p. 93–109. In B. M. Gibbs and F. A. Skinner (ed.), Identification methods for microbiologists, part B. Academic Press, Inc., New York.
- Yanagida, F., K.-I. Suzuki, T. Kaneko, M. Kozaki, and K. Komagata. 1987. Morphological, biochemical, and physiological characteristics of spore-forming lactic acid bacteria. J. Gen. Appl. Microbiol. **33**:33–45.
- Yanagida, F., K.-I. Suzuki, T. Kaneko, M. Kozaki, and K. Komagata. 1987. Deoxyribonucleic acid relatedness among some spore-forming lactic acid bacteria. J. Gen. Appl. Microbiol. **33**:47–55.