Emended Description of *Paenibacillus amylolyticus* and Description of *Paenibacillus illinoisensis* sp. nov. and *Paenibacillus chibensis* sp. nov.

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The taxonomic position of unidentified group 6 of *Bacillus circulans* as described by Nakamura and Swezey (L. K. Nakamura and J. Swezey, Int. J. Syst. Bacteriol. 33:46–52, 1983) was determined, and the taxonomy of *Paenibacillus amylolyticus* was reexamined. The results of PCR amplification of a 16S rRNA gene fragment with a specific primer and comparative analysis of 16S rRNA gene sequences warranted placing the two taxa in the genus *Paenibacillus*. The levels of DNA reassociation among the strains revealed four groups (designated groups I, II, III, and 6), each with a high level of intragroup relatedness (>72%). Clustering based on phenotypic characteristics correlated well with DNA relatedness grouping. *P. amylolyticus* strains were scattered in groups I, II, and III. Strains labeled the type strain of *P. amylolyticus* from different culture collections appeared in groups I and III. Strains found in group I were identified as *P. amylolyticus* sensu stricto, and the one strain found in group III was identified as *Paenibacillus lautus*. Group 6 encompassed strains formerly assigned to *B. circulans* group 6, and group II contained other strains identified as *P. amylolyticus*. Groups 6 and II were phenotypically and genetically distinct taxa that were distinguishable from the previously described species. These findings showed that groups 6 and II were new species, for which we propose the names *Paenibacillus illinoisensis* and *Paenibacillus chibensis*, respectively.

Nakamura and Swezey (11, 12) observed the taxonomic heterogeneity of *Bacillus circulans* strains and separated these organisms into *B. circulans* sensu stricto and nine groups at the species level. Six of these groups were reclassified as the following new *Bacillus* species: *Bacillus amylolyticus*, *Bacillus lautus*, *Bacillus pabuli*, *Bacillus validus*, *Bacillus alginolyticus*, and *Bacillus chondroitinus* (13, 14). Recently, Ash et al. proposed the transfer of *B. amylolyticus*, *B. pabuli*, and *B. validus* to the genus *Paenibacillus* (2), and Heyndrickx et al. proposed the transfer of *B. lautus* to the genus *Paenibacillus* (8). One of the three remaining groups, the group designated group 6 (12) containing six strains, merited further study.

Also, anomalies noted during preliminary DNA relatedness studies prompted a reexamination of the taxonomy of the genus *Paenibacillus*. To clarify the taxonomic position of the group 6 strains and to reexamine the taxonomy of *Paenibacillus amylolyticus*, we determined the DNA base compositions, levels of DNA relatedness, phenotypic characteristics, chemosystematic properties, 16S rRNA gene sequences, and phylogenetic relationships of these organisms.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Working stocks were cultured on tryptic soy agar (Difco Laboratories, Detroit, Mich.) plates for 24 h at 37°C and stored at room temperature.

Identification of strains at the genus level by 16S rRNA gene fragment amplification. Strains in the genus *Paenibacillus* were identified by PCR amplification of a 16S rRNA gene fragment with specific forward primer PAEN515F (5'-GCTCGGAGAGTGACGGTACCTGAGA-3') and universal reverse primer 1377R (19). The sequence of reverse primer 1377R, PCR amplification of the 16S rRNA gene from chromosomal DNA, and detection of PCR products have been described previously (18).

DNA base composition and DNA relatedness. The procedures used to isolate and purify chromosomal DNA and to estimate DNA base composition were the procedures described by Takagi et al. (20). DNA relatedness values were determined as described by Ezaki et al. (4). Probes for DNA-DNA hybridization were prepared from *Bacillus* sp. strains NRRL NRS-1175 and NRRL NRS-1356^T (T = type strain) and *P. amylolyticus* IFO 13625^T, NRRL B-377^T, NRRL B-142^T, NRRL B-14939, and NRRL NRS-290^T.

Determining and comparing 16S rRNA gene DNA sequences. The methods used for preparation of chromosomal DNA and PCR amplification of the 16S rRNA gene from chromosomal DNA and the primers used for PCR amplification have been described previously (18). Amplified 16S rRNA genes purified with a QIAquick Spin PCR purification kit (QIAGEN GmbH, Hilden, Germany) were used for sequencing templates. Sequencing was carried out as described by Sanger et al. (16) by using a Dye terminator cycle sequencing FS Ready Reaction kit (Perkin-Elmer Co., Foster City, Calif.) and a model ABI 373A automatic DNA sequencer (Perkin-Elmer Co.). The seven sequencing primers were used as described by Fox et al. (6).

Previously published 16S rRNA gene sequences were obtained from the EMBL, GenBank, and DDBJ databases. Multiple alignment of sequences, calculation of nucleotide substitution rates (K_{muc} values) (9), construction of a neighbor-joining phylogenetic tree (17), and a bootstrap analysis with 1,000 replicates for evaluation of phylogenetic tree topology (5) were carried out with the CLUSTAL W version 1.5 program (21). Alignment gaps and unidentified base positions were not taken into account.

Phenotypic characterization and numerical analysis. Phenotypic characterization of seven strains of *Bacillus* species, eight *P. amylolyticus* strains, and four *Paenibacillus lautus* strains was carried out as described by Gordon et al. (7) and Takagi et al. (20). The phenotypically based relationships among these 19 strains were determined by performing a numerical analysis as described previously (20).

Cellular fatty acid composition and isoprenoid quinone composition. For cellular fatty acid and isoprenoid quinone analyses, the organisms were cultivated overnight in tryptic soy broth (Difco Laboratories) at 37°C. Preparation and determination of cellular fatty acids and isoprenoid quinones were carried out as described by Komagata and Suzuki (10).

Nucleotide sequence accession numbers. The 16S rRNA gene sequences determined in this study have been deposited in the EMBL, GenBank, DDBJ databases under the accession numbers listed in Table 1.

RESULTS

Identification of strains at the genus level by 16S rRNA gene fragment amplification. The results of 16S rRNA gene fragment amplification of the seven *Bacillus* strains and eight

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TABLE 1. Bacterial strains used in this study

Strain ^a	Source ^b	History ^a	Accession no.
<i>Bacillus circulans</i> group 6 strains			
NRRL NRS-1174	1	H. W. Reuszer, Army strain 1324 (= HSCC 486)	
NRRL NRS-1175	1	H. W. Reuszer, Army strain 1329 (= HSCC 318)	
NRRL NRS-1177	1	H. W. Reuszer, Army strain 1376 (= HSCC 401)	
NRRL NRS-1343	1	F. E. Clark, strain 15, isolated from soil (= HSCC 484)	
NRRL NRS-1356 ^T	1	F. E. Clark, strain 14 ^T , isolated from soil (= HSCC 309 ^T = JCM 9907 ^T = IFO 15959 ^T)	D85397
IFO 15379	2	NRRL NRS-1173 from H. W. Reuszer, Army strain 1304 (= HSCC 302)	
<i>Paenibacillus amylolyticus</i> strains			
NRRL NRS-290 ^T	1	Reacquired from the original NRS-290 ^T stock (= HSCC 434 ^T = JCM 9906 ^T = IFO 15957 ^T)	D85396
IFO 13625 ^T	2	ATCC 9995 ^T from NRS-290 ^T from N. R. Smith from K. F. Kellerman (= HSCC 173 ^T)	
ATCC 9995 ^T	3	NRS-290 ^T from N. R. Smith (= HSCC 430 ^T)	
NRRL B-14940 ^T	1	ATCC 9995 ^T from NRS-290 ^T from N. R. Smith, obtained in 1995 (= HSCC 432 ^T)	
NRRL B-142 ^T	1	FDA strain PCI 221 ^T (= HSCC 442 ^T = JCM 9905 ^T = IFO 15958 ^T)	D85395
NRRL B-14939	1	ATCC 9966 from FDA strain PCI 221, obtained in 1995 (= HSCC 433)	
NRRL NRS-359	1	N. R. Smith (= HSCC 497)	
NRRL NRS-1136	1	ATCC 9966 from FDA strain PCI 221 (= HSCC 474)	
NRRL B-377 ^T	1	NRS-290 ^T from N. R. Smith from K. F. Kellerman (= HSCC 374 ^T = IFO 15876 ^T)	D85609
<i>Paenibacillus lautus</i> strains			
NRRL NRS-666 ^T	1	University of Washington, "Bacillus lautus" (= HSCC 493 ^T)	D78473
NRRL NRS-678	1	N. R. Smith, migratory colonies 3, isolated from soil (= HSCC 426)	
NRRL NRS-679	1	N. R. Smith, migratory colonies 4, isolated from soil (= HSCC 402)	
NRRL B-379	1	NRS-676 from N. R. Smith, migratory colonies 1, isolated from soil (= HSCC 424)	

^a HSCC, Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd., Chiba, Japan; JCM, Japan Collection of Microorganisms, Saitama, Japan; FDA, Food and Drug Administration, Washington, D.C.; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Ill.; IFO, Institute for Fermentation, Osaka, Japan; ATCC, American Type Culture Collection, Rockville, Md.

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P. amylolyticus strains studied are shown in Fig. 1. When specific forward primer PAEN515F and universal reverse primer 1377R were used, 0.8-kb PCR fragments were amplified in all 15 strains tested. These results suggested that all of the strains examined were members of the genus *Paenibacillus*.

DNA base compositions and DNA relatedness. The G+C contents of the 15 strains mentioned above ranged from 46.3 to 53.2 mol% (Table 2). Low levels of relatedness (range, 2 to 40%) showed that the group 6 reference strain (NRRL NRS-1356) is not closely related to the type strains of previously established *Paenibacillus* species (Table 3). High levels of intragroup DNA relatedness among the *B. circulans* group 6 strains (NRRL NRS-1174, NRRL NRS-1175, NRRL NRS-1177, NRRL NRS-1343, NRRL NRS-1356^T, and IFO 15379) (range, 78 to 100%) demonstrated the genetic homogeneity of this distinct group (Table 2).

Because preliminary studies revealed anomalies in the DNA relatedness of *P. amylolyticus* strains, the relationships of three strains of this species to each other and to the type strains of other species were also studied (Table 3). Low levels of DNA relatedness (range, 0 to 26%) showed that the three *P. amylolyticus* strains were not related to each other. However, a high level of DNA relatedness (86%) was noted between NRRL B-377^T and NRRL NRS-666, the type strain of *P. lautus*.

To determine the cause of the anomalous data obtained with the *P. amylolyticus* strains, the levels of DNA relatedness of strains from several different culture collections were determined. Table 2 shows the levels of relatedness of the *P. amylolyticus* strains from several different sources. These strains were separated into three independent groups based on DNA relatedness values greater than 72%. Group I encompassed the type strains of *P. amylolyticus* acquired from different sources (namely, strains IFO 13625^T, NRRL B-14940^T, ATCC 9995^T, and NRRL NRS-290^T) (Table 1). Four other *P. amylolyticus* strains (NRRL B-142^T, NRRL B-14939, NRRL NRS-359, and

NRRL NRS-1136) made up group II. Type strain NRRL B-377 was the sole constituent of group III.

Phylogenetic relationships. The nucleotide sequences (1,435 to 1,440 nucleotides) of the 16S rRNA gene from strains NRRL NRS-1356^T, NRRL NRS-290^T, NRRL B-142^T, and NRRL B-377^T were determined. The sequence similarity values for strains NRRL NRS-1356^T, NRRL NRS-290^T, and NRRL B-142^T and 19 *Paenibacillus* species were greater than 90.6%; the similarity values for strains NRRL NRS-1356^T, NRRL NRS-290^T, and NRRL B-142^T and the other members of the family *Bacillaceae* were less than 90.1% (data not shown). Strains NRRL NRS-1356^T, NRRL NRS-290^T, and NRRL B-142^T were placed in a robust (bootstrap value, 1,000) monophyletic cluster consisting of the *Paenibacillus* species (Fig. 2). Moreover, NRRL NRS-1356^T (a group 6 strain), *P. amylolyticus*, NRRL B-142^T (a group II strain), *P. lautus*, *Paenibacillus glucanolyticus*, *Paenibacillus macquariensis*, and *Paenibacillus pabuli* appeared to form a subgroup within the genus *Paenibacillus*. The 16S rRNA gene from NRRL B-377^T exhibited a high level of similarity (98.7%) with the 16S rRNA gene from the type strain of *P. lautus* (data not shown).

Phenotypic characterization. Because *B. circulans* group 6, *P. amylolyticus* groups I and II, and *P. lautus* formed a putative phylogenetic subgroup, 98 of their phenotypic characteristics were compared. The 19 strains of these species exhibited 18 differences among the characteristics tested (Table 4).

According to the results of a numerical analysis based on these differential characteristics, the *P. amylolyticus* strains tested could be separated into three clusters at a similarity level of 95%; the group 6 strains also formed a discrete cluster (Fig. 3). These clusters correlated well with the groups circumscribed by DNA relatedness data.

Cellular fatty acid composition and isoprenoid quinone composition. Strains NRRL NRS-1175 and NRRL NRS-1356^T in group 6, strains IFO 13625^T and NRRL NRS-290^T in group

FIG. 1. Amplification of 16S rRNA gene by PCR with detection primer PAEN515F and universal reverse primer 1377R. Lane M contained *Hind*III-digested λ DNA as a molecular weight marker.

I, and strains NRRL B-142^T and NRRL B-14939 in group II were analyzed to determine their cellular fatty acid compositions and isoprenoid quinone compositions. Table 5 shows that all of the strains tested contained anteiso-C_{15:0} acids, which accounted for 36.4 to 57.8% of the total cellular fatty acids. All of the strains tested contained menaquinone 7, which accounted for more than 93% of the total menaquinones.

DISCUSSION

The strains in *B. circulans* group 6 are aerobic rod-shaped organisms that produce ellipsoidal spores in swollen sporangia (Fig. 4B). A PCR amplification analysis of 16S rRNA gene fragments and a comparative analysis of the 16S rRNA gene sequence of NRRL NRS-1356^T revealed that group 6 belongs

TABLE 2. DNA base compositions and levels of DNA relatedness for strains of *B. circulans* group 6 and *P. amylolyticus*

Strain	G+C content (mol%)	% Reassociation with DNA from ^a :						
		NRRL NRS-1175	NRRL NRS-1356 ^T	NRRL NRS-290 ^T	IFO 13625 ^T	NRRL B-142 ^T	NRRL B-14939	NRRL B-377 ^T
NRRL NRS-1174	47.8	80	85	7	13	12	21	10
NRRL NRS-1175	48.0	100	100	21	20	19	25	2
NRRL NRS-1177	48.3	100	78	20	25	22	16	1
NRRL NRS-1343	47.7	100	79	19	22	18	20	17
NRRL NRS-1356 ^T	48.1	100	100	19	33	15	23	16
IFO 15379	47.6	84	95	23	39	25	17	2
NRRL NRS-290 ^T	46.4	23	13	100	100	5	4	0
IFO 13625 ^T	46.3	20	19	72	100	1	3	5
NRRL B-14940 ^T	46.6	19	24	88	100	1	5	16
ATCC 9995 ^T	46.5	21	17	100	100	6	3	3
NRRL B-142 ^T	52.8	34	31	16	14	100	83	5
NRRL B-14939	52.5	28	22	18	25	100	100	15
NRRL NRS-359	53.2	30	26	15	16	100	95	3
NRRL NRS-1136	52.6	33	35	10	3	100	92	0
NRRL B-377 ^T	48.9	35	40	30	23	26	28	100

^a Reassociation values are averages of two determinations; the maximum difference found between two determinations was 9%.

TABLE 3. DNA base compositions and levels of relatedness of selected *B. circulans* group 6 and *P. amylolyticus* strains to the type strains of previously recognized *Paenibacillus* species

Strain	G+C content (mol%)	% Reassociation with DNA from ^a :			
		NRRL NRS-1356 ^T	NRRL NRS-290 ^T	NRRL B-142 ^T	NRRL B-377 ^T
<i>P. amylolyticus</i> NRRL NRS-290 ^T	46.4	13	100	5	0
<i>P. amylolyticus</i> NRRL B-142 ^T	52.8	31	16	100	5
<i>P. amylolyticus</i> NRRL B-377 ^T	48.9	40	0	26	100
<i>P. alginolyticus</i> DSMZ 5050 ^T	45.7	7	18	23	21
<i>P. alvei</i> IFO 3343 ^T	45.6	10	3	13	15
<i>P. apiarius</i> NRRL NRS-1438 ^T	52.3	3	10	21	15
<i>P. azotofixans</i> NRRL B-14372 ^T	46.7	7	28	20	4
<i>P. chondroitinus</i> DSMZ 5051 ^T	46.3	7	11	28	24
<i>P. curdlanolyticus</i> IFO 15724 ^T	53.1	10	23	26	8
<i>P. glucanolyticus</i> DSMZ 5162 ^T	48.3	3	22	10	36
<i>P. kobensis</i> IFO 15729 ^T	51.7	15	28	18	5
<i>P. larvae</i> subsp. <i>pulvificiens</i> IFO 15408 ^T	44.2	4	10	6	9
<i>P. lautus</i> NRRL NRS-666 ^T	50.4	10	26	18	86
<i>P. macerans</i> JCM 2500 ^T	51.8	2	6	25	5
<i>P. macquariensis</i> CIP 103269 ^T	40.2	3	5	8	9
<i>P. pabuli</i> NRRL NRS-924 ^T	45.3	12	33	17	0
<i>P. peoriae</i> IFO 15541 ^T	46.2	8	19	2	0
<i>P. polymyxa</i> JCM 2507 ^T	46.8	1	24	21	13
<i>P. thiaminolyticus</i> JCM 8360 ^T	52.2	2	0	8	0
<i>P. validus</i> DSMZ 3037 ^T	51.7	7	9	11	0

^a Reassociation values are averages of two determinations; the maximum difference found between two determinations was 9%.

to the genus *Paenibacillus*. This finding is supported by the DNA base compositions and other chemosystematic characteristics. For example, the G+C content, 48 mol%, falls within the range exhibited by *Paenibacillus* species (45 to 54 mol%) (2, 19). Also, the anteiso-C_{15:0} fatty acid content (56 to 57% of the total fatty acids) and the presence of menaquinone 7 as the principal quinone are characteristic of the genus *Paenibacillus* (2, 19).

Group 6 strains are phenotypically distinguishable from the closely related *Paenibacillus* species, as shown in Table 6. DNA relatedness and phenotypic characterization analyses showed that group 6 is a genetically distinct taxon and that it merits recognition as a new species, for which the name *Paenibacillus illinoisensis* is proposed.

Four strains (NRRL NRS-290^T [= NRRL B-377^T], NRRL NRS-359, NRRL NRS-1136, and NRRL B-142^T) were identified as *P. amylolyticus* strains (formerly assigned to *B. amylolyticus*) on the basis of their DNA reassociation values, the G+C contents of their DNAs, and their phenotypic characteristics (12, 13). DNA relatedness studies and a phenotypic re-examination of the *P. amylolyticus* strains revealed the presence of three separate taxa in this species (Table 2). The group I strains were identified as members of *P. amylolyticus* sensu stricto, and the single group III strain was identified as *P. lautus*. Group I strains NRRL B-14940^T, IFO 13625^T, NRRL NRS-290^T, and ATCC 9995^T and group III strain NRRL B-377^T were purportedly derived from the same strain, strain NRS-290^T. Therefore, the anomalous identification of NRRL B-377^T as *P. lautus* probably resulted from mislabeling in the culture collection. Strains NRRL B-142^T, NRRL B-14939, NRRL NRS-359, and NRRL NRS-1136 formed group II, another genetically and phenotypically distinct taxon. This confusing result was probably due to erroneous identification in previous reports (12, 13).

Taxonomic reevaluation has changed *P. amylolyticus* into a taxon with four strains that have a common provenance, namely, strain NRS-290^T. An emended description of *P. amylolyticus* resulting from our study is given below.

16S rRNA gene analysis revealed that group II strain NRRL

B-142^T was closely related to *Paenibacillus* species (Fig. 2). The DNA relatedness, phenotypic characterization, and phylogenetic analyses revealed that group II is a genetically distinct taxon that represents a new species whose members have G+C

TABLE 4. Distinctive phenotypic characteristics of *B. circulans* group 6 and *P. amylolyticus* group I, II, and III strains

Characteristic	% of positive strains			
	Group 6 (<i>P. illinoisensis</i>) (n = 6) ^a	Group I (<i>P. amylolyticus</i> sensu stricto) (n = 4)	Group II (<i>P. chibensis</i>) (n = 4)	Group III (<i>P. lautus</i>) (n = 5)
Colony color				
Brownish yellow	0	0	100	80
Grayish white	100	100	0	20
Anaerobic growth	100	100	0	100
Nitrate reduction	0	100	100	100
Utilization of:				
Acetate	100	0	0	0
Ammonium	100	0	100	0
Nitrate	100	0	100	100
Hydrolysis of:				
Casein	100 (w) ^b	100 (w)	0	0
Gelatin	100	100	0	100
Starch	100	100	100	100
Tween 20	100	100	0	0
Tween 60	0	100	0	0
Growth at 40°C	100	0	100	100
Acid produced from:				
Glycerol	100	100	0	100
D-Mannose	100	100	0	100
Raffinose	100	0	0	0
Resistance to lysozyme (0.01%)	33	0	100	100
Reaction of litmus in litmus milk	100	0	100	40

^a n is the number of strains examined.

^b w, weakly positive.

FIG. 2. Phylogenetic relationships of *Paenibacillus* species and some aerobic, rod-shaped, endospore-forming bacteria based on 16S rRNA gene sequences. The branching pattern was generated by the neighbor-joining method. The numbers indicate bootstrap values greater than 700. The boldface lines and boldface type indicate the cluster consisting of *Paenibacillus* species and the strains sequenced in this study, respectively. Bar = 0.01 nucleotide substitution per site.

contents ranging from 52.5 to 53.2 mol%. Group II encompasses three strains (NRRL B-142^T, NRRL B-14939, and NRRL NRS-1136) which have a common origin (PCI 221) and strain NRRL NRS-359. Although group II is represented by only two strains, frequent use of these organisms in antibiotic assays encourages recognition of them as a new species, whose proposed name is *Paenibacillus chibensis*.

Previously, we described a simple method for identifying *Brevibacillus* and *Aneurinibacillus* strains by PCR amplification of 16S rRNA gene fragments with a specific detection primer (18). The usefulness of this method for identification of *Paenibacillus* strains was confirmed in another study (19) and in this study. The detection primer, primer PAEN515F, was highly specific for identification of the genus *Paenibacillus*.

Emended description of *Paenibacillus amylolyticus* (Nakamura 1984) Ash, Priest, and Collins 1993. Cells are rods that are 0.7 to 0.9 by 3.0 to 5.0 μm . Gram positive. Motile with peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia (Fig. 4A). Colonies are flat, smooth, circular, and entire. No soluble pigment is produced on nutrient agar.

Facultatively anaerobic.

Catalase positive and oxidase negative.

Acetylmethylcarbinol is not produced (as determined by the Voges-Proskauer reaction). The pH in Voges-Proskauer broth is 5.2.

Hydrogen sulfide, indole, dihydroxyacetone, and lecithinase (as determined by the egg yolk reaction) are not produced.

Nitrate is reduced to nitrite.

FIG. 3. Phenotypic relationships among strains of *B. circulans* group 6 and *P. amylolyticus* group I, II, and III strains. The dendrogram was produced by using the simple matching coefficient and the unweight pair group arithmetic average algorithm.

TABLE 5. Cellular fatty acid compositions of *B. circulans* group 6 and *P. amylolyticus* group I, II, and III strains

Strain	% of total cellular fatty acids										
	Saturated acids									Unsaturated acids	
	C _{14:0}	C _{15:0}	C _{16:0}	iso-C _{14:0}	iso-C _{15:0}	iso-C _{16:0}	iso-C _{17:0}	anteiso-C _{15:0}	anteiso-C _{17:0}	C _{16:1} ω11	iso-C _{17:1} ω10
Group 6 (<i>P. illinoisensis</i>) strains											
NRRL NRS-1175	1.6	0.3	24.3	1.5	1.6	6.6	1.2	55.9	5.7	ND ^a	ND
NRRL NRS-1356 ^T	2.0	1.0	24.7	1.8	1.5	6.3	1.4	56.6	4.9	ND	ND
Group I (<i>P. amylolyticus</i> sensu stricto) strains											
NRRL NRS-290 ^T	2.9	0.5	12.8	2.3	2.0	9.0	3.5	46.2	2.2	0.1	ND
IFO 13625 ^T	2.4	0.8	13.7	2.6	1.7	8.6	2.3	43.0	2.0	0.2	1.2
Group II (<i>P. chibensis</i>) strains											
NRRL B-142 ^{Tb}	0.3	0.6	5.3	0.8	4.0	12.2	3.2	57.8	14.3	ND	ND
NRRL B-14939	0.4	0.6	9.8	0.5	2.9	10.7	3.6	53.7	15.2	ND	0.2
Group III (<i>P. lautus</i>) strain NRRL B-377 ^{Tb}	0.6	0.5	20.1	0.2	2.9	4.1	5.8	36.9	11.5	0.5	1.0

^a ND, not detected.^b Data from reference 19.

Casein, gelatin, starch, Tween 20, and Tween 60 are hydrolyzed (hydrolysis of casein is weakly positive). DNA, Tween 80, urea, and hippurate are not hydrolyzed.

Tyrosine is not decomposed.

Phenylalanine is not deaminated.

Citrate, propionate, acetate, fumarate, L-malate, lactate, succinate, L-glutamate, L-asparatate, alginate, gluconate, α-ketoglutarate, malonate, and tartrate are not utilized.

Nitrate and ammonium are utilized.

No change occurs in litmus milk.

Growth occurs at temperatures between 10 and 40°C and at pH 4.5 to 9.0. Optimum growth occurs at 37°C and at pH 7.0. Growth does not occur at temperatures lower than 10°C or greater than 40°C.

Growth occurs in the presence of 2% NaCl. Growth is inhibited by 5% NaCl, 0.02% sodium azide, and 0.001% lysozyme.

Acid but no gas is produced from D-glucose, L-arabinose, D-fructose, D-galactose, maltose, sucrose, D-xylose, trehalose, glycerol, D-mannitol, D-cellobiose, D-ribose, salicin, D-mannose, melibiose, inositol, inulin, and starch. Neither acid nor gas is produced from lactose, D-sorbitol, L-sorbose, L-rhamnose, raffinose, and adonitol.

The major fatty acids are anteiso-C_{15:0} and C_{16:0}. The major quinone is menaquinone 7.

The G+C content ranges from 46.3 to 46.6 mol%.

The type strain is NRRL NRS-290, which was isolated by K. F. Kellerman. The type strain has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd., Chiba, Japan as strain HSCC 434 (= JCM 9906 = IFO 15957).

Description of *Paenibacillus illinoisensis* sp. nov. *Paenibacillus illinoisensis* (il. li. nois. en'sis. M.L. adj. *illinoisensis*, referring to Illinois, the state where Microbial Properties Research, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, is located). Cells are rods that are 0.5 to 0.8 by 3.0 to 5.0 μm. Gram positive. Motile with peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia (Fig. 4B). Colonies are flat, smooth, circular, entire, and yellowish gray. No soluble pigment is produced on nutrient agar.

Facultatively anaerobic.

Catalase positive and oxidase negative.

Acetylmethylcarbinol is not produced (as determined by the Voges-Proskauer reaction). The pH in Voges-Proskauer broth is 5.0 to 5.2.

Hydrogen sulfide, indole, dihydroxyacetone, and lecithinase (as determined by the egg yolk reaction) are not produced.

Nitrate is not reduced to nitrite.

Casein, gelatin, starch, and Tween 20 are hydrolyzed (hydrolysis of casein is weakly positive). DNA, Tween 60, Tween 80, urea, and hippurate are not hydrolyzed.

FIG. 4. Phase-contrast micrographs of sporulating cells of three species. (A) *P. amylolyticus* NRRL NRS-290^T. (B) *P. illinoisensis* NRRL NRS-1356^T. (C) *P. chibensis* NRRL B-142^T. Cells were cultured on tryptic soy agar plates for 2 days at 37°C.

TABLE 6. Differentiation of *P. illinoisensis*, *P. amylobycticus*, *P. chibensis*, and allied *Paenibacillus* species

Characteristic	<i>P. illinoisensis</i> (group 6) ^e	<i>P. amylobycticus</i> (group 1) ^e	<i>P. pabuli</i> ^b	<i>P. chibensis</i> (group II) ^e	<i>P. lautus</i> (group III) ^e	<i>P. glucanolyticus</i> ^c	<i>P. macquariensis</i> ^d
Gram reaction	+	+	+	+	+	-	-
Anaerobic growth	+	+	+	-	+	+	+
Oxidase	-	-	-	-	-	+	-
Nitrate reduction	-	+	-	+	+	v	-
Utilization of:							
Citrate	-	-	-	-	-	v	-
Acetate	+	-	+	-	-	v	-
Hydrolysis of:							
Casein	w	w	v	-	-	v	-
Gelatin	+	+	NR	-	+	v	-
Growth in the presence of:							
5% NaCl	-	-	-	-	-	+	-
0.001% Lysozyme	v	-	-	+	+	NR	-
Growth at:							
5°C	-	-	+	-	-	-	+
40°C	+	-	+	+	+	v	-
pH 5.7	+	+	+	+	+	+	-
Acid produced from:							
L-Arabinose	+	+	+	+	+	v	-
Glycerol	+	+	NR	-	+	v	-
Lactose	-	-	+	-	-	+	+
D-Mannose	+	+	+	-	+	+	NR
L-Rhamnose	-	-	+	-	-	NR	NR
Raffinose	+	-	NR	-	-	+	NR
Major cellular fatty acids	anteiso-C _{15:0} ^a C _{16:0}	anteiso-C _{15:0} ^a C _{16:0}	anteiso-C _{15:0} ^a C _{16:0}	anteiso-C _{15:0} ^a anteiso-C _{17:0} iso-C _{16:0}	anteiso-C _{15:0} ^a anteiso-C _{17:0} C _{16:0}	anteiso-C _{15:0} ^a iso-C _{15:0} ^f	anteiso-C _{15:0} ^f
G+C content (mol%) ^a	48	46-47	45	53	49-50	48	40

^a Data obtained in this study.^b Data from reference 13.^c Data from references 1 and 15.^d Data from references 3 and 15.^e +, positive reaction; -, negative reaction; v, variable reaction; w, weakly positive; NR, not reported.^f Data from reference 19.

Tyrosine is not decomposed.

Phenylalanine is not deaminated.

Acetate is utilized, and citrate, propionate, fumarate, L-malate, lactate, succinate, L-glutamate, L-asparatate, alginate, gluconate, α -ketoglutarate, malonate, and tartrate are not utilized.

Nitrate and ammonium are utilized.

Litmus is reduced in litmus milk.

Growth occurs at temperatures between 10 and 50°C and at pH 4.5 to 9.0. Optimum growth occurs at 37°C and at pH 7.0.

Growth occurs in the presence of 2% NaCl. Growth is inhibited by 5% NaCl and by 0.02% sodium azide. Some strains can grow in the presence of 0.001% lysozyme.

Acid but no gas is produced from D-glucose, L-arabinose, D-fructose, D-galactose, maltose, sucrose, D-xylose, trehalose, glycerol, D-mannitol, D-cellobiose, D-ribose, salicin, D-mannose, melibiose, raffinose, inositol, inulin, and starch. Neither acid nor gas is produced from lactose, D-sorbitol, L-sorbose, L-rhamnose, and adonitol.

The major fatty acids are anteiso-C_{15:0} and C_{16:0}. The major quinone is menaquinone 7.

The G+C content ranges from 47.6 to 48.3 mol%. The G+C content of the type strain is 48.1 mol%.

The type strain is NRRL NRS-1356, which was isolated from soil by F. E. Clark. The type strain has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd., Chiba, Japan as strain HSCC 309 (= JCM 9907 = IFO 15959).

Description of *Paenibacillus chibensis* sp. nov. *Paenibacillus chibensis* (chi.ben'sis. M.L. adj. *chibensis*, referring to Chiba, a Japanese prefecture where the Research Laboratory of Higeta Shoyu Co., Ltd., is located). Cells are rods that are 0.5 to 0.8 by 3.0 to 5.0 μ m. Gram positive. Motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia (Fig. 4C). Colonies are flat, smooth, circular, entire, and brownish yellow. No soluble pigment is produced on nutrient agar.

Strictly aerobic.

Catalase positive and oxidase negative.

Acetylmethylcarbinol is not produced (as determined by the Voges-Proskauer reaction). The pH in Voges-Proskauer broth ranges from 4.6 to 4.7.

Hydrogen sulfide, indole, dihydroxyacetone, and lecithinase (as determined by the egg yolk reaction) are not produced.

Nitrate is reduced to nitrite.

Starch is hydrolyzed. Casein, gelatin, DNA, Tween 20, Tween 60, Tween 80, urea, and hippurate are not hydrolyzed.

Tyrosine is not decomposed. Phenylalanine is not deaminated.

Citrate, propionate, acetate, fumarate, L-malate, lactate, succinate, L-glutamate, L-asparatate, alginate, gluconate, α -ketoglutarate, malonate, and tartrate are not utilized.

Nitrate and ammonium are utilized.

No change occurs in litmus milk.

Growth occurs at temperatures between 10 and 50°C and at pH 4.5 to 9.0. Optimum growth occurs at 37°C and at pH 7.0.

Growth occurs in the presence of 2% NaCl and 0.001% lysozyme. Growth is inhibited by 5% NaCl and by 0.02% sodium azide.

Acid but no gas is produced from D-glucose, L-arabinose, D-fructose, D-galactose, maltose, sucrose, D-xylose, trehalose, D-mannitol, D-cellobiose, D-ribose, salicin, melibiose, inositol, inulin, and starch. Neither acid nor gas is produced from lactose, glycerol, D-sorbitol, L-sorbose, D-mannose, L-rhamnose, and adonitol.

The major fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0}, and iso-C_{16:0}. The major quinone is menaquinone 7.

The G+C content ranges from 52.5 to 53.2 mol%. The G+C content of the type strain is 52.8 mol%.

The type strain is NRRL B-142, which has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd., Chiba, Japan as strain HSCC 442 (= JCM 9905 = IFO 15958).

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