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Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the Genus *Paenibacillus* and Emended Description of the Genus *Paenibacillus*

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We determined the taxonomic status of six *Bacillus* species (*Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus*) by using the results of 16S rRNA gene sequence and cellular fatty acid composition analyses. Phylogenetic analysis clustered these species closely with the *Paenibacillus* species. Like the *Paenibacillus* species, the six *Bacillus* species contained anteiso-$C_{15:0}$ fatty acid as a major cellular fatty acid. The use of a specific PCR primer designed for differentiating the genus *Paenibacillus* from other members of the *Bacillaceae* showed that the six *Bacillus* species had the same amplified 16S rRNA gene fragment as members of the genus *Paenibacillus*. Based on these observations and other taxonomic characteristics, the six *Bacillus* species were transferred to the genus *Paenibacillus*. In addition, we propose emendation of the genus *Paenibacillus*.

Rod-shaped, aerobic, endospore-forming bacteria have generally been assigned to the genus *Bacillus*, a systematically diverse taxon (5). 16S rRNA gene sequence analyses have identified at least 10 phylogenetic groups in the genus *Bacillus* (2, 3, 7, 23, 26, 31, 33, 34, 36). Five of the groups have been reclassified as the new genera *Alicyclobacillus* (36), *Paenibacillus* (2), *Halobacillus* (33), *Brevibacillus* (31), and *Aneurinibacillus* (31). The genus *Paenibacillus* as proposed by Ash et al. (2) consists of the following 11 species: *Paenibacillus polymyxa*, *Paenibacillus alvei*, *Paenibacillus gordonae*, *Paenibacillus larvae*, *Paenibacillus pulvifaciens*, *Paenibacillus macerans*, *Paenibacillus azotofixans*, *Paenibacillus pabuli*, *Paenibacillus macquariensis*, *Paenibacillus amylolyticus*, and *Paenibacillus validus*. *Clostridium durum* has also been transferred to this genus (6). Recently, Heyndrickx et al. reported that *P. gordonae* was a synonym of *P. validus* (12), that *P. pulvifaciens* was a subspecies of *P. larvae* (11), and that *Bacillus lautus* and *Bacillus peoriae* should be transferred to the genus *Paenibacillus* (13). In addition, Nakamura proposed the new species *Paenibacillus apiarius* (22). Consequently, the genus *Paenibacillus* consists of 13 species and one subspecies.

Members of the genus *Paenibacillus* are facultatively anaerobic organisms that produce spores in definitely swollen sporangia and have G+C contents ranging from 45 to 54 mol%, and some of these organisms excrete diverse assortments of extracellular polysaccharide-hydrolyzing enzymes (5, 25). Interestingly, many recently described *Bacillus* species possess the general characteristics of the genus *Paenibacillus*. The noteworthy ability of these species to hydrolyze complex carbohydrates, including alginate (19), chondroitin (19), chitin (25), curdlan (14), and other polysaccharides (25), suggests that some of them may be related to the genus *Paenibacillus*. To understand the taxonomic position of these *Bacillus* species among the *Bacillaceae*, we determined the sequences of their 16S rRNA genes and compared these sequences with homologous sequences available for other members of the *Bacillaceae*. In addition, a highly specific PCR amplification primer was designed on the basis of the 16S rRNA gene sequence alignments for differentiating the genus *Paenibacillus* from other aerobic, endospore-forming rods.

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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 plates (Difco Laboratories, Detroit, Mich.) for 24 h at 37°C. The strains were stored at room temperature.

Sequencing the 16S rRNA genes. 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 amplifications have been described previously (31). 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. (29) 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 used have been described by Fox et al. (9).

Comparison of 16S rRNA gene sequences. Sequences determined in this study were compared with 16S rRNA gene sequences obtained from the EMBL, GenBank, and DDBJ databases. Multiple alignment of sequences, calculation of nucleotide substitution rates (K_{nuc} values) (15), construction of a neighbor-joining phylogenetic tree (28), and a bootstrap analysis with 1,000 replicates for evaluation of phylogenetic tree topology (8) were carried out with the CLUSTAL W version 1.5 program (35). Alignment gaps and unidentified base positions were not taken into account for the calculations.

Cellular fatty acid compositions. Cells of all of the strains listed in Table 1 except the *P. macquariensis* strains were cultivated overnight in tryptic soy broth (Difco Laboratories) at 37°C. The *P. macquariensis* strains were cultured in tryptic soy broth overnight at 23°C. Preparation and determination of cellular fatty acids were carried out as described by Komagata and Suzuki (16).

Identification of *Paenibacillus* strains by 16S rRNA gene amplification. Strains belonging to the genus *Paenibacillus* were identified by 16S rRNA gene PCR amplification by using specific forward primer PAEN515F (5′-GCTCGGAGA GTGACGGTACCTGAGA-3′) and universal reverse primer 1377R. The sequences of universal forward primer 27FC and reverse primer 1377R and the methods used for PCR amplification of the 16S rRNA gene from chromosomal DNA with a detection primer and detection of PCR products have been described previously (31).

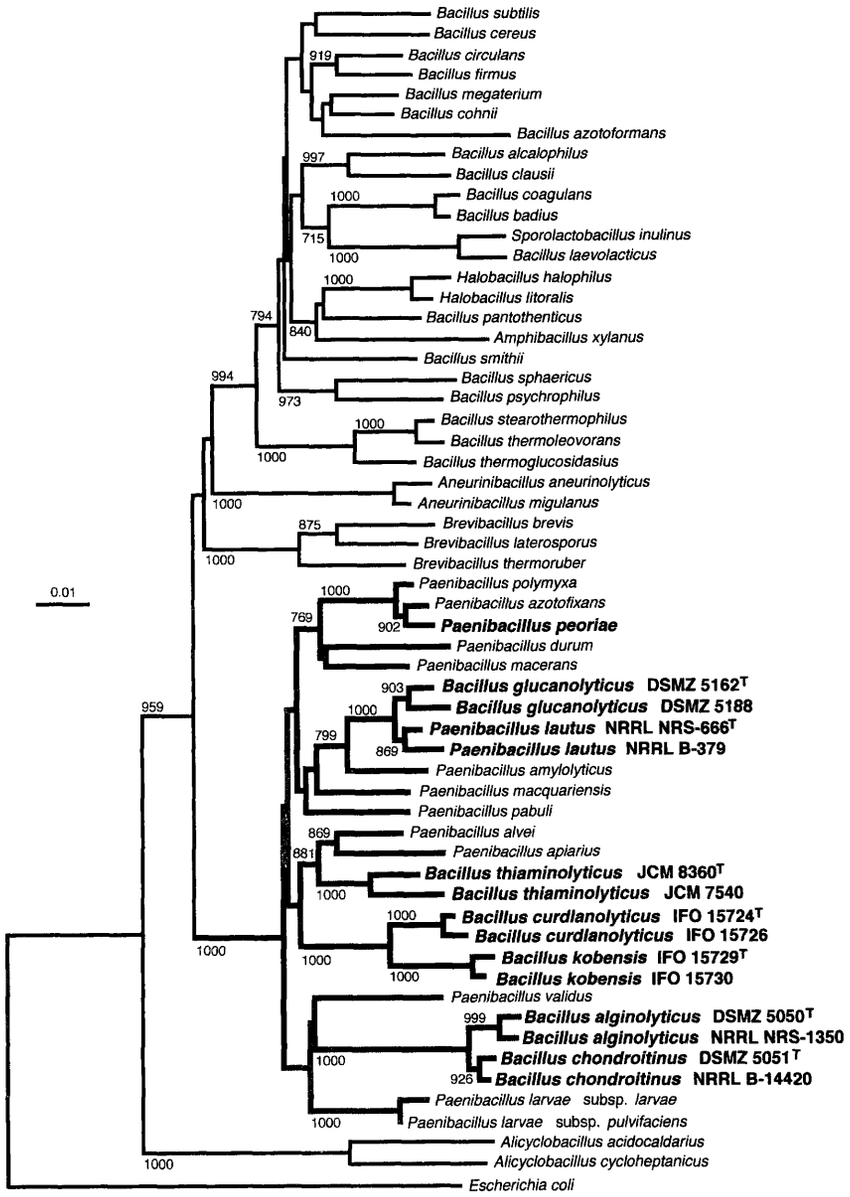

FIG. 1. Phylogenetic relationships of *Paenibacillus* species and some aerobic, rod-shaped, endospore-forming bacteria based on 16S rRNA gene sequences. The branching pattern was generated by the neighbor-joining method. The numbers indicate bootstrap values greater than 700. Boldface lines and boldface type indicate the cluster consisting of *Paenibacillus* species and the species sequenced in this study, respectively. Bar = 0.01 nucleotide substitution per site.

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

RESULTS

Phylogenetic relationship. We determined the nucleotide sequences (1,393 to 1,437 bases) of the 16S rRNA gene from two strains (the type strain and a sensu stricto strain) of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, *Bacillus thiaminolyticus*, and *Paenibacillus lautus* and the type strain of *Paenibacillus peoriae*. The intraspecies similarity values for the six *Bacillus* species and *P. lautus* were greater than 97.1%. 16S

Position		490	500	510	520
PAEN515F	:	GCTCGGAGAGTGACGGTACCTGAGA			
Paenibacillus polymyxa	:	GAGTAACT---NNN-----N---------N---AGAAAGCCCC			
Paenibacillus macerans	:	GAGTAACT--NACAN---------N--------AGAAAGCCCC			
Paenibacillus alvei	:	GAGTNACTN--NNTN-G--N---------N---AGAAAGCCCC			
Paenibacillus pulvifaciens	:	GAGTNACT--C-NTN----------N-T-----AGAAAGCCCC			
Paenibacillus azotofixans	:	GAGTAACT---ACA----------N--------AGAAAGCCCC			
Paenibacillus macquariensis	:	GAGTAACT----TC-AG---------------AGAAAGCCCC			
Paenibacillus pabuli	:	GAGTNACTN---TN-AG--N---------N---AGAAAGCCCC			
Paenibacillus validus	:	GAGTAACT----T---G----------T-----AGAAAGCCCC			
Paenibacillus lautus	:	GAGTAACT---A-------------T-----AGAAAGCCCC			
Paenibacillus peoriae	:	GAGTAACT---ACA----------N--------AGAAAGCCCC			
Paenibacillus amylolyticus	:	GAGTAACT---ATCG---------C--------AGAAAGCCCC			
Paenibacillus apiarius	:	GAGTAACT----TCT-G---------------AGAAAGCCCC			
Paenibacillus durum	:	GAGTAACT---ACA----------------AGAAAGCCCC			
Bacillus alginolyticus	:	GAGTAACT-N--TNT-T---------TAG----AGAAAGCCCC			
Bacillus chondroitinus	:	GAGTAACT--CT-TT-T---------TAG----AGAAAGCCCC			
Bacillus curdlanolyticus	:	GAGTAACT----TTGAGT------N--------AGAAAGCCCC			
Bacillus glucanolyticus	:	GAGTAACT-T-AT-------------T-----AGAAAGCCCC			
Bacillus kobensis	:	GAGTAACT----TC-AG-------N--------AGAAAGCCCC			
Bacillus thiaminolyticus	:	GAGTAACT-T--CATAG-------C--------AGAAAGCCCC			
Bacillus subtilis	:	TCGAATAG-GCG-T-CCT----------A-CCAGAAAGCCAC			
Bacillus circulans	:	GAGTAACT---T-T-CCT----------A-CCAGAAAGCCAC			
Bacillus firmus	:	GAGTAACT--CG-T-CCT------N------CCAGAAAGCCAC			
Bacillus megaterium	:	GAGTAACTN--N-TNCCT----------A-CCAGAAAGCCAC			
Bacillus sphaericus	:	TAGTAACTNGCTNT-CCT----------T-TTAGAAAGCCAC			
Bacillus psychrophilus	:	GAGTNACT--C--TGCCN----------N-TTAGAAAGCCAC			
Bacillus stearothermophilus	:	TCGAAGAG-GCG-NGCG----------C-CGAGAAAGCCCC			
Sporolactobacillus inulinus	:	GAGGAAAT---G-TGCT---------T-C-GCCAGAAAGCCAC			
Halobacillus halophilus	:	ACGAACACAGCG-TACCT----------A-CGAGGAAGCCCC			
Brevibacillus brevis	:	TCGAATAG-GCG-T-CCT----------CGAGAAAGCCAC			
Aneurinibacillus aneurinolyticus	:	CGGGATGAC---CCGGTC----------A-CGAGAAAGCCCC			
Alicyclobacillus acidocaldarius	:	AGTGGAAA--C-CAT-C-A---------GAGTGAGGAAGCCCC			
Amphibacillus xylanus	:	TCGAATAG-G-G-T-CCT----------A-CGAGAAAGCCCC			

FIG. 2. Sequence of the detection primer for members of the *Paenibacillus* cluster (PAEN515F) and alignment of the 16S rRNA gene sequences of *Paenibacillus* species and some related taxa. Dashes indicate nucleotides identical to those of primer PAEN515F.

TABLE 1. Bacterial strains used in this study

Strain[a]	Source[b,c]	History[c]	Accession no.
Bacillus alginolyticus strains			
DSMZ 5050^T	1	NRRL NRS-1347^T from N. R. Smith from F. E. Clark strain 3^T (= HSCC 175^T)	D78465
NRRL NRS-1350	2	N. R. Smith from F. E. Clark strain 8 (= HSCC 609)	D88517
Bacillus chondroitinus strains			
DSMZ 5051^T	1	NRRL NRS-1351^T from N. R. Smith from F. E. Clark strain 12^T (= HSCC 176^T)	D82064
NRRL B-14420	2	L. K. Nakamura, isolated from soil (= HSCC 612)	D88518
Bacillus curdlanolyticus strains			
IFO 15724^T	3	Y. Kanzawa strain YK9^T, isolated from soil (= HSCC 491^T)	D78466
IFO 15726	3	Y. Kanzawa strain YK161, isolated from soil (= HSCC 870)	D88515
Bacillus glucanolyticus strains			
DSMZ 5162^T	1	F. G. Priest strain S93^T from J. R. Norris strain B0030^T (= HSCC 171^T)	D78470
DSMZ 5188	1	F. G. Priest strain E28, isolated from garden soil (= HSCC 872)	D88514
Bacillus kobensis strains			
IFO 15729^T	3	Y. Kanzawa strain YK205^T, isolated from soil (= HSCC 488^T)	D78471
IFO 15730	3	Y. Kanzawa strain YK205, isolated from soil (= HSCC 871)	D88516
Bacillus thiaminolyticus strains			
JCM 8360^T	4	AHU 1393^T (= HSCC 148^T)	D78475
JCM 7540	4	IAM 1034 from K. Arima (= HSCC 197)	D88513
Paenibacillus lautus strains			
NRRL NRS-666^T	2	University of Washington, "*Bacillus lautus*" (= HSCC 493^T)	D78473
NRRL B-379	2	N. R. Smith, migratory colonies 1, isolated from soil (= NRS-676 = HSCC 424)	D85394
Paenibacillus peoriae strains			
IFO 15541^T	3	NRRL B-14750^T from NRRL BD-57^T from B. Delaporte strain 11.B.9^T, isolated from soil (= HSCC 353^T)	D78476
NRRL B-14476	2	L. K. Nakamura, isolated from rotting leaves (= HSCC 452)	
Paenibacillus alvei strains			
IFO 3343^T	3	IMAB B-3-4^T from ATCC 6344^T from N. R. Smith strain 662^T from A. G. Lochhead strain 127^T (= HSCC 146^T)	
NRRL NRS-811	2	J. R. Porter from A. G. Lochhead (= HSCC 897)	
Paenibacillus amylolyticus strains			
NRRL B-377^T	2	N. R. Smith from K. F. Kellerman (= NRS-290^T = HSCC 374^T)	
NRRL B-142	2	FDA strain PCI221 (= HSCC 442)	
Paenibacillus apiarius strains			
NRRL NRS-1438^T	2	H. Katznelson strain BX3^T, isolated from honeybee larvae (= HSCC 603^T)	
NRRL NRS-1578	2	W. C. Haynes (= HSCC 604)	
Paenibacillus azotofixans strains			
NRRL B-14372^T	2	L. Selden strain P3L-S^T, isolated from soil (= HSCC 379^T)	
NRRL B-14359	2	No information available (= HSCC 898)	
Paenibacillus larvae subsp. *pulvifaciens* strains			
IFO 15408^T	3	NRRL B-3685^T from N. R. Smith from J. W. Rouatt from H. Katznelson strain 670^T, isolated from dead honeybee (= HSCC 355^T)	
NRRL B-14152	2	G. J. Bonde (= HSCC 443)	
Paenibacillus macerans strains			
JCM 2500^T	4	CCM 2012^T from R. E. Gordon (= HSCC 179^T)	
IAM 1243	5	IFO 3490 from NRRL B-388 (= HSCC 194)	
Paenibacillus macquariensis strains			
CIP 103269^T	6	DSMZ 2^T from ATCC 23464^T from B. J. Marshall, isolated from soil (= HSCC 358^T)	
NRRL NRS-1534	2	T. Gibson from NCTC 10419 from B. J. Marshall (= HSCC 899)	
Paenibacillus pabuli strains			
NRRL NRS-924^T	2	J. R. Porter from M. Schieblich, "*Bacillus pabuli*" (= HSCC 492^T)	
NRRL BD-537	2	L. K. Nakamura, isolated from Canadian soil (= HSCC 422)	
Paenibacillus polymyxa strains			
JCM 2507^T	4	CCM 1459^T from BUCSAV 162^T (= HSCC 184^T)	
NRRL BD-55	2	B. Delaporte, isolated from soil (= HSCC 416)	
Paenibacillus validus strains			
DSMZ 3037^T	1	NRRL NRS-1000^T from N. R. Smith from J. R. Porter from G. Bredemann, isolated from soil (= HSCC 174^T)	
CIP 103498	6	F. Pichinoty strain Q1, isolated from Spanish soil (= HSCC 357)	
Bacillus subtilis JCM 1465^T	4	IAM 12118^T from ATCC 6051^T from H. J. Cohn strain Marburg^T (= HSCC 182^T)	
Bacillus cereus JCM 2152^T	4	IAM 12605^T from NCIB 9373^T from R. E. Gordon (= HSCC 183^T)	
Bacillus megaterium JCM 2506^T	4	CCM 2007^T from R. E. Gordon (= HSCC 181^T)	
Bacillus circulans IAM 12462^T	5	NCIB 9374^T from R. E. Gordon (= HSCC 161^T)	
Bacillus badius ATCC 14574^T	7	R. E. Gordon from N. R. Smith strain 663^T from B. S. Henry strain 110^T from M. Batchelor (= HSCC 154^T)	
Bacillus sphaericus JCM 2502^T	4	CCM 2120^T from R. E. Gordon (= HSCC 498^T)	

Continued on following page

TABLE 1—*Continued*

Strain[a]	Source[b,c]	History[c]	Accession no.
Bacillus stearothermophilus JCM 2501^T	4	ATCC 12980^T from NCA 26^T (= HSCC 160^T)	
Bacillus coagulans JCM 2257^T	4	IAM 1115^T from ATCC 7050^T from N. R. Smith strain 609^T from J. R. Porter from B. W. Hammer (= HSCC 180^T)	
Sporolactobacillus inulinus NRIC 1133^T	8	K. Kitahara (= HSCC 342^T)	
Brevibacillus brevis JCM 2503^T	4	DSMZ 30^T from ATCC 8246^T from N. R. Smith strain 604^T from J. R. Porter from NCTC 2611^T from W. W. Fors strain 27B^T (= HSCC 186^T)	
Aneurinibacillus aneurinolyticus ATCC 12856^T	7	Y. Ito from R. Kimura (= HSCC 149^T)	

[a] All strains are sensu stricto strains. T = type strain.

[b] 1, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; 2, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Ill.; 3, Institute for Fermentation, Osaka, Japan; 4, Japan Collection of Microorganisms, Saitama, Japan; 5, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; 6, Collection des Bactéries de l'Institut Pasteur, Paris, France; 7, American Type Culture Collection, Rockville, Md.; 8, NODAI Research Institute, Culture Collection Center, Tokyo University of Agriculture, Tokyo, Japan.

[c] HSCC, Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd., Chiba, Japan; AHU, Department of Agricultural Chemistry, Hokkaido University, Hokkaido, Japan; IMAB, Institute of Microbiology and Agropecurious Industry, Castelar, Argentina; FDA, Food and Drug Administration, Washington, D.C.; CCM, Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic; NCTC, National Collection of Type Cultures, Central Public Health Laboratory Service, London, United Kingdom; BUCSAV, Biologicky Ustav, Czeskslovenska Akademie Ved, Prague, Czech Republic; NCIB, National Collection of Industrial and Marine Bacteria, Ltd., Aberdeen, Scotland, United Kingdom; NCA, National Canners Association, Washington, D.C.; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Ill.; IFO, Institute for Fermentation, Osaka, Japan; DSMZ and DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; ATCC, American Type Culture Collection, Rockville, Md.; IAM, Institute of Applied Microbiology, Tokyo, Japan; JCM, Japan Collection of Microorganisms, Saitama, Japan; CIP, Collection des Bactéries de l'Institut Pasteur, Paris, France; NRIC, NODAI Research Institute, Culture Collection Center, Tokyo University of Agriculture, Tokyo, Japan.

rRNA gene sequence similarity values greater than 89.6% (data not shown) placed the six *Bacillus* species, *P. lautus*, and *P. peoriae* within the realm of the genus *Paenibacillus*. In a phylogenetic tree, the two strains belonging to each of the six *Bacillus* species and the two *Paenibacillus* species were members of a robust monophyletic cluster containing the *Paenibacillus* species (Fig. 1). An inspection of the tree revealed close relationships between *P. peoriae* and *P. azotofixans*, between *B. glucanolyticus* and *P. lautus*, between *B. thiaminolyticus* and the *P. alvei-P. apiarius* complex, between *B. curdlanolyticus* and *B. kobensis*, and between *B. alginolyticus* and *B. chondroitinus*.

Cellular fatty acid compositions. A total of 47 strains of aerobic, endospore-forming rods were analyzed to determine their cellular fatty acid compositions. All of the strains in the *Paenibacillus* cluster contained anteiso-$C_{15:0}$ acid as a major cellular fatty acid, and the level of this fatty acid ranged from 36.9 to 81.0% (Table 2).

Identifying members of the *Paenibacillus* cluster by 16S rRNA gene amplification. Primer PAEN515F designed for detection of the *Paenibacillus* cluster spanned positions 491 to 515 (Fig. 2). PCR amplifications with primers PAEN515F and 1377R produced a 0.8-kb PCR fragment in preparations of the strains of all of the members of the *Paenibacillus* cluster tested (including the six *Bacillus* species), but not in preparations of strains belonging to other clusters (Fig. 3 [only data for the type strains are shown]). A 1.3-kb fragment was amplified in all of the strains tested with primers 27FC and 1377R (data not shown).

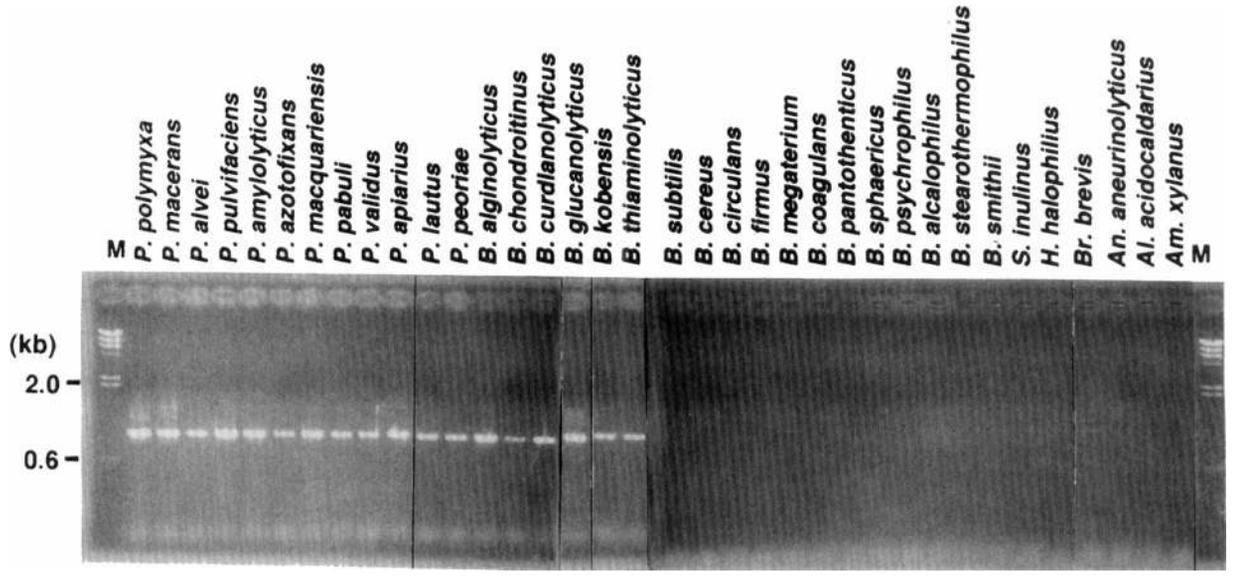

FIG. 3. Amplification of the 16S rRNA gene by PCR with detection primer PAEN515F and universal reverse primer 1377R. Results for only type strains are shown. Lane M contained *Hin*dIII-digested λ DNA as a molecular weight marker. Abbreviations: *P.*, *Paenibacillus*; *B.*, *Bacillus*; *S.*, *Sporolactobacillus*; *H.*, *Halobacillus*; *Br.*, *Brevibacillus*; *An.*, *Aneurinibacillus*; *Al.*, *Alicyclobacillus*; *Am.*, *Amphibacillus*.

TABLE 2. Cellular fatty acid compositions of several species in the genera *Bacillus* and *Paenibacillus* and related organisms

Strain	% of total cellular fatty acids														
	Saturated acids												Unsaturated acids		
	Straight chain				iso branched					anteiso branched					
	$C_{13:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{13:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{17:0}$	$C_{13:0}$	$C_{15:0}$	$C_{17:0}$	$C_{16:1}$ ω 9	$C_{16:1}$ ω 11	iso-$C_{17:1}$ ω 10
Bacillus alginolyticus strains															
DSMZ 5050^T	ND[a]	0.3	1.4	2.6	ND	1.2	4.7	10.4	1.7	ND	69.6	6.2	ND	ND	ND
NRRL NRS-1350	ND	0.2	1.1	3.1	ND	0.5	3.4	11.0	1.5	ND	73.0	5.4	ND	ND	ND
Bacillus chondroitinus strains															
DSMZ 5051^T	ND	0.9	2.0	6.4	ND	1.7	2.4	10.4	0.7	ND	69.7	3.2	ND	ND	ND
NRRL B-14420	ND	0.5	0.9	5.0	ND	1.1	3.2	10.3	2.6	ND	67.4	5.3	ND	ND	ND
Bacillus curdlanolyticus strains															
IFO 15724^T	ND	0.5	0.6	6.7	ND	1.6	1.7	24.4	0.8	ND	56.3	3.2	ND	ND	ND
IFO 15726	ND	0.4	0.9	5.0	ND	3.5	1.7	27.1	0.7	ND	56.7	3.0	ND	ND	ND
Bacillus glucanolyticus strains															
DSMZ 5162^T	ND	0.8	0.7	11.2	ND	1.4	3.2	13.9	2.0	ND	56.5	8.2	ND	0.5	0.2
DSMZ 5188	ND	0.4	0.5	10.1	ND	0.7	1.0	12.1	1.6	ND	60.1	12.8	ND	ND	0.2
Bacillus kobensis strains															
IFO 15729^T	ND	1.0	2.8	12.2	ND	0.5	2.0	8.8	0.5	ND	65.7	3.2	ND	ND	ND
IFO 15730	ND	0.7	2.7	8.3	ND	1.1	1.7	16.2	0.7	ND	61.1	4.1	ND	ND	ND
Bacillus thiaminolyticus strains															
JCM 8630^T	ND	0.9	0.6	10.5	ND	0.6	10.5	5.8	6.5	ND	42.4	11.6	ND	5.2	1.7
JCM 7540	ND	0.5	0.5	8.5	ND	0.6	10.7	6.3	7.1	ND	45.3	16.4	ND	1.3	1.0
Paenibacillus alvei strains															
IFO 3343^T	ND	2.3	1.6	15.3	ND	0.3	12.3	2.3	3.3	ND	53.9	3.3	ND	1.3	0.5
NRRL NRS-811	ND	1.8	2.0	12.2	ND	0.2	9.2	2.5	2.0	ND	60.3	4.0	ND	0.8	0.2
Paenibacillus amylolyticus strains															
NRRL B-377^T	ND	0.6	0.5	20.1	ND	0.2	2.9	4.1	5.8	ND	36.9	11.5	ND	0.5	1.0
NRRL B-142	ND	0.3	0.6	5.3	ND	0.8	4.0	12.2	3.2	ND	57.8	14.3	ND	ND	ND
Paenibacillus apiarius strains															
NRRL NRS-1438^T	ND	0.6	2.2	4.8	ND	0.2	8.3	4.2	5.5	ND	60.5	16.3	ND	0.8	0.2
NRRL NRS-1578	ND	0.5	1.4	4.6	ND	0.4	6.1	5.3	6.0	ND	59.3	16.3	ND	0.2	0.3
Paenibacillus azotofixans strains															
NRRL B-14372^T	ND	1.5	0.3	17.6	ND	0.7	1.7	6.7	1.1	ND	62.2	5.0	ND	0.2	ND
NRRL B-14359	ND	0.9	0.2	15.2	ND	0.8	2.0	5.1	0.8	ND	60.8	3.5	ND	0.3	ND
Paenibacillus larvae subsp. *pulvifaciens* strains															
IFO 15408^T	ND	0.6	1.3	6.4	ND	0.2	9.5	5.3	4.5	ND	48.8	20.7	ND	0.8	ND
NRRL B-14152	ND	0.2	1.6	4.7	ND	0.2	8.0	1.3	7.2	ND	40.8	30.2	ND	1.0	ND
Paenibacillus lautus strains															
NRRL NRS-666^T	ND	1.1	0.3	15.6	ND	0.8	1.5	7.4	1.2	ND	57.3	9.7	ND	2.0	0.2
NRRL B-379	ND	0.5	0.3	18.3	ND	0.4	2.6	3.6	6.0	ND	34.8	10.2	ND	0.3	0.4
Paenibacillus macerans strains															
JCM 2500^T	ND	3.7	0.5	17.9	ND	7.9	2.6	16.4	0.6	ND	36.1	12.2	ND	0.1	ND
IAM 1243	ND	1.9	3.1	17.4	ND	1.8	2.6	17.1	4.1	ND	34.5	16.1	ND	0.1	ND
Paenibacillus macquariensis strains															
CIP 103269^T	ND	0.7	1.2	2.9	ND	0.8	5.2	2.6	0.4	ND	81.0	0.7	ND	0.6	0.2
NRRL NRS-1534	ND	0.4	1.5	1.5	ND	1.0	4.1	4.1	0.2	ND	78.2	1.0	ND	0.3	0.3
Paenibacillus pabuli strains															
NRRL NRS-924^T	ND	0.7	0.1	10.1	ND	0.7	2.2	4.8	1.3	ND	73.7	4.1	ND	ND	ND
NRRL BD-537	ND	0.8	0.4	11.0	ND	3.9	1.7	7.7	0.8	ND	69.8	3.0	ND	ND	ND
Paenibacillus peoriae strains															
IFO 15541^T	ND	0.7	0.5	10.6	ND	1.4	7.9	6.7	5.0	ND	54.7	10.5	ND	0.5	0.3
NRRL B-14476	ND	0.8	0.3	9.3	ND	0.9	3.5	6.7	6.0	ND	60.9	10.9	ND	ND	ND
Paenibacillus polymyxa strains															
JCM 2507^T	ND	0.4	0.3	9.3	ND	0.5	1.0	5.6	1.6	ND	62.9	16.9	ND	ND	ND
NRRL BD-55	ND	0.5	0.3	11.0	ND	0.4	0.2	4.5	1.1	ND	51.0	28.8	ND	ND	ND
Paenibacillus validus strains															
DSMZ 3037^T	ND	0.7	1.4	10.8	ND	1.4	4.1	11.7	3.4	ND	57.3	7.5	ND	0.4	0.1
CIP 103498	ND	0.9	0.7	10.2	ND	1.6	6.8	9.1	3.4	ND	58.7	4.9	ND	0.5	0.2
Bacillus subtilis JCM 1465^T	ND	0.3	2.3	1.3	ND	0.4	28.4	2.0	6.1	ND	44.5	6.8	3.3	ND	0.5
Bacillus cereus JCM 2152^T	0.8	3.1	4.9	2.4	7.8	2.4	48.7	2.7	6.2	0.6	3.8	0.7	1.1	4.4	2.8
Bacillus megaterium JCM 2506^T	ND	1.7	1.8	1.6	ND	8.2	32.6	1.1	0.4	ND	45.2	1.1	ND	ND	ND
Bacillus circulans IAM 12462^T	ND	4.3	2.2	3.7	ND	3.9	10.0	3.5	ND	ND	57.3	3.4	ND	ND	ND
Bacillus badius ATCC 14574^T	ND	2.1	9.6	2.4	ND	1.6	60.0	3.2	1.7	ND	7.6	1.8	ND	ND	ND
Bacillus sphaericus JCM 2502^T	ND	1.4	6.8	1.3	ND	6.0	53.4	6.9	2.1	ND	8.8	0.9	ND	ND	ND
Bacillus stearothermophilus JCM 2501^T	ND	ND	ND	3.4	ND	ND	27.5	5.6	17.2	ND	7.8	38.6	ND	ND	ND
Bacillus coagulans JCM 2257^T	ND	0.5	5.3	0.7	ND	ND	2.2	0.4	ND	ND	65.8	22.6	ND	ND	ND
Sporolactobacillus inulinus NRIC 1133^T	ND	ND	5.1	0.8	ND	ND	1.7	0.8	ND	ND	52.9	35.2	ND	ND	ND
Brevibacillus brevis JCM 2503^T	ND	0.4	0.7	2.7	ND	0.6	17.6	3.5	5.3	ND	54.4	9.6	ND	ND	ND
Aneurinibacillus aneurinolyticus ATCC 12856^T	ND	0.8	1.0	5.9	ND	0.5	44.7	13.9	6.3	ND	4.4	1.1	ND	4.1	7.8

[a] ND, not detected.

TABLE 3. Distinctive phenotypic characteristics of *B. alginolyticus*, *B. chondroitinus*, *B. curdlanolyticus*, *B. glucanolyticus*, *B. kobensis*, and species and subspecies in the genus *Paenibacillus*

Characteristic	*B. alginolyticus*[a]	*B. chondroitinus*[a]	*B. curdlanolyticus*[b]	*B. glucanolyticus*[c]	*B. kobensis*[b]	*B. thiaminolyticus*[d]	*P. polymyxa*[e]	*P. azotofixans*[f]	*P. peoriae*[g]	*P. macerans*[h]	*P. durum*[i]	*P. lautus*[j]	*P. amylolyticus*[j]	*P. macquariensis*[k]	*P. pabuli*[j]	*P. alvei*[h]	*P. apiarius*[l]	*P. validus*[m]	*P. larvae* subsp. *larvae*[n]	*P. larvae* subsp. *pulvifaciens*[n]
Spore shape	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval
Swollen sporangia	+[o]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growth	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	NT	+	+	+	+	+	+	+	−	−
Oxidase	−	−	−	NT	−	+	−	−	−	NT	NT	−	−	NT	−	NT	−	−	NT	NT
Nitrate reduction	−	−	+	+	+	+	+	−	+	+	−	+	+	−	−	−	+	v	v	v
Production of:																				
Acetylmethylcarbinol	−	−	−	−	−	−	+	+	+	−	+	−	−	−	−	+	−	v	v	v
Indole	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	+	+
Dihydroxyacetone	NT	NT	NT	NT	NT	−	+	−	−	−	NT	NT	NT	−	NT	+	−	NT	+	+
pH in Voges-Proskauer broth	<5.5	<5.5	5.3–5.4	<5.5	5.3–5.4	4.9–5.5	4.5–6.8	4.5–5.1	5.5–6.5	4.5–5.0	NT	<5.5	<5.5	<6.0	<5.5	4.6–5.2	4.4–5.4	NT	NT	NT
Decomposition of:																				
Tyrosine	−	−	NT	−	NT	+	−	−	−	−	NT	−	−	−	−	v	+	NT	−	−
Thiamine	NT	NT	NT	NT	NT	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Hydrolysis of:																				
Casein	−	−	−	+	−	+	+	−	+	−	NT	−	−	−	v	+	+	NT	+	+
Starch	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	NT	−	−
Urea	+	+	+	−	+	−	−	NT	−	NT	NT	+	v	−	−	NT	+	−	−	−
Alginate	+	+	NT	NT	NT	NT	−	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Tween 80	+	+	NT	NT	NT	−	NT	NT	NT	NT	−	−	−	NT	−	NT	−	−	NT	NT
Egg yolk lecithin	−	−	NT	NT	NT	−	−	NT	−	NT	−	−	−	NT	−	NT	−	NT	NT	NT
Degragation of:																				
Carboxymethyl cellulose[b]	+	+	−	+	−	NT	NT	NT	NT	NT	NT	+	−	NT	+	NT	NT	+	NT	NT
Chitin[p]	NT	NT	NT	−	NT	+	−	NT	−	+	NT	NT	NT	−	−	+	−	NT	NT	NT
Curdlan[b]	−	−	+	+	+	NT	NT	NT	NT	NT	NT	+	+	NT	+	NT	NT	−	NT	NT
Pectin[e,b]	NT	NT	NT	−	NT	−	+	−	+	−	NT	NT	NT	−	+	−	−	NT	NT	NT
Pullulan[b,p]	NT	NT	−	+	−	+	+	NT	+	v	NT	NT	NT	+	−	+	+	NT	NT	NT
Xylan[n]	NT	NT	NT	−	NT	NT	+	NT	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
β-1,2-Glucan[b]	+	−	−	+	−	NT	NT	NT	NT	NT	NT	+	−	NT	−	NT	NT	+	NT	NT
β-1,6-Glucan[b,p]	−	−	+	+	+	−	−	NT	−	−	NT	+	+	−	−	−	−	−	NT	NT
Nitrogen fixation	NT	NT	NT	NT	NT	NT	+	+	NT	v	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Utilization of:																				
Citrate	+	−	−	−	−	+	−	−	+	v	NT	−	−	−	−	−	+	−	−	−
Succinate	+	−	−	−	−	+	−	NT	+	NT	NT	−	−	NT	−	NT	NT	NT	NT	NT
Acetate	+	v	−	NT	−	+	NT	NT	NT	NT	NT	+	v	NT	+	NT	NT	NT	NT	NT
Fumarate	+	−	−	NT	−	+	NT	NT	NT	NT	NT	−	−	NT	−	NT	NT	NT	NT	NT
L-Malate	+	v	−	NT	−	+	NT	NT	NT	NT	NT	−	−	NT	−	NT	NT	NT	NT	NT
Litmus milk	NC	NC	NT	NT	NT	NC	NT	NT	NT	A	NT	A	A	NT	A	A	NT	NT	NT	NT
Optimum growth temp (°C)	28–30	28–30	30	30	30	28	30	30–37	30	30	30	28–30	28–30	20	28–30	28	28	28–35	35–37	28–30
Growth at:																				
pH 5.6	+	+	NT	+	NT	−	+	+	+	+	+	−	+	−	+	−	+	NT	−	−
50°C	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−
Growth in the presence of:																				
0.001% lysozyme	+	−	+	NT	+	−	+	−	v	NT	NT	+	−	−	−	+	+	NT	+	+
5% NaCl	−	−	−	+	−	v	−	−	−	−	NT	+	−	−	v	v	+	NT	−	−
Fermentation of:																				
D-Glucose	+	+	+	+	+	+	++	++	++	++	NT	+	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	v	+	v	++	−	++	++	−	+	+	−	+	−	−	+	−	−
Mannitol	+	+	−	v	−	v	++	++	++	++	−	+	+	+	+	−	−	+	−	+
D-Xylose	+	+	+	+	+	−	++	−	++	++	−	+	+	+	+	−	−	+	−	−
Glycerol	NT	NT	+	NT	−	NT	++	−	−	NT	NT	NT	NT	NT	NT	NT	NT	+	+	+
Raffinose	NT	NT	+	+	−	NT	NT	++	NT	NT	+	NT	NT	NT	NT	NT	NT	v	−	−
G+C content (mol%)	47–49	47–48	50–52	48	50–52	52–54	43–46	48–53	45–47	52–53	50	50–52	53	39	48–50	45–47	52–54	50–52	42–43	42–43

[a] Data from reference 19.
[b] Data from reference 14.
[c] Data from reference 1.
[d] Data from reference 21.
[e] Data from reference 20.
[f] Data from reference 30.
[g] Data from references 17 and 20.
[h] Data from reference 10.
[i] Data from reference 4.
[j] Data from reference 18.
[k] Data from reference 5.
[l] Data from reference 22.
[m] Data from reference 12.
[n] Data from reference 11.
[o] +, positive reaction; −, negative reaction; NT, not tested; v, variable reaction; NC, no change; A, acid reaction; ++, acid and gas produced.
[p] Data from reference 25.

DISCUSSION

A phylogenetic analysis based on 16S rRNA gene sequences placed *B. alginolyticus*, *B. chondroitinus*, *B. curdlanolyticus*, *B. glucanolyticus*, *B. kobensis*, *B. thiaminolyticus*, *P. lautus*, and *P. peoriae* in a robust (bootstrap value, 1,000) cluster consisting of the *Paenibacillus* species. Sequence comparisons performed with members of this cluster revealed intracluster similarity values greater than 89.6%. The results of the phylogenetic analysis of *B. thiaminolyticus*, *P. lautus*, and *P. peoriae* correlated well with the results of polyphasic taxonomy reported by Heyndrickx et al. (13). Ash et al. (2) reported that *P. lautus* (formerly *B. lautus*) was a member of rRNA group 1 based on the 16S rRNA sequence of the type strain. The different position of this species may have been due to an erroneous gene sequence used in the study of Ash et al. (2).

Table 3 shows phenotypic characteristics of the members of the *Paenibacillus* cluster. Interestingly, species that formed groups in the *Paenibacillus* cluster exhibited similar polysaccharidase or physiological activities or originated from common habitats. For example, *P. peoriae*, *P. azotofixans*, and *P. polymyxa* are members of a group (Fig. 1) that hydrolyze pectin and xylan and fix nitrogen. The *B. glucanolyticus-P. lautus*, *B. curdlanolyticus-B. kobensis*, and *B. alginolyticus-B. chondroitinus* pairs are closely related organisms that share the ability to hydrolyze various β-glucans, curdlan, and alginate, respectively. *B. thiaminolyticus*, *P. alvei*, and *P. apiarius* appear to be members of a group (Fig. 1) that consists of species which are frequently isolated from honeybee environs.

The general similarity of the distribution of cellular fatty acids in the organisms also indicates the cohesiveness of the *Paenibacillus* cluster (Fig. 3). Furthermore, like many previously described *Paenibacillus* species, the new species exhibit diverse polysaccharidase activities (1, 14, 19, 25).

Detection primer PAEN515F was designed on the basis of a signature DNA sequence found in the 16S rRNA gene of *Paenibacillus* species. Ash et al. (2) showed that slot blot hybridization is useful for identification of the genus *Paenibacillus* when an amplified 16S rRNA gene from chromosomal DNA and a specific probe are used. Shida et al. (31) described a rapid, simple, and efficient method for identifying the genera *Brevibacillus* and *Aneurinibacillus* by PCR amplification of 16S rRNA gene fragments with genus-specific detection primers. The genus *Paenibacillus* was successfully differentiated from the other taxa belonging to the *Bacillaceae* by using PAEN515F as the detection primer. Concomitantly, the six *Bacillus* species placed in the *Paenibacillus* cluster were identified as members of the genus *Paenibacillus*. At this time, the phenotypic characteristics available for differentiating and identifying the genera belonging to the *Bacillaceae* are inadequate (Table 4). The use of probes and specific detection primers should be very useful for identifying and differentiating members of the *Bacillaceae*.

Based on the observations described above, we propose that *B. alginolyticus*, *B. chondroitinus*, *B. curdlanolyticus*, *B. glucanolyticus*, *B. kobensis*, and *B. thiaminolyticus* should be transferred to the genus *Paenibacillus* as *Paenibacillus alginolyticus*, *Paenibacillus chondroitinus*, *Paenibacillus curdlanolyticus*, *Paenibacillus glucanolyticus*, *Paenibacillus kobensis*, and *Paenibacillus thiaminolyticus*, respectively.

Description of *Paenibacillus alginolyticus* (Nakamura 1987) comb. nov. The description of *Paenibacillus alginolyticus* comb. nov. is identical to the description of *B. alginolyticus* given by Nakamura (19). The major cellular fatty acids are anteiso-$C_{15:0}$ and iso-$C_{16:0}$ acids (this study). Type strain DSMZ 5050 (= NRRL NRS-1347 = ATCC 51185 = CIP 103122 = IFO

TABLE 4. Salient characteristics of the genera of aerobic, endospore-forming rods

Characteristic	*Paenibacillus*[a,b]	*Bacillus*[c,d]	*Sporolactobacillus*[c]	*Amphibacillus*[e]	*Halobacillus*[f]	*Brevibacillus*[g]	*Aneurinibacillus*[g]	*Alicyclobacillus*[h]
No. of species	20		1	1	3	10	2	3
Cell shape	Rod	Rod	Rod	Rod	Rod or spherical to oval			
Spore shape	Oval	Oval or spherical	Oval	Oval	Oval	Oval	Oval	Oval
Sporangia	Swollen	Swollen or not swollen	Swollen	Swollen	Swollen	Swollen	Swollen	Swollen or not swollen
Anaerobic growth	v[i]	v	+	+	−	−	−	−
Catalase	v	+	−	−	+	+	+	+
Hydrolysis of thiamine	NT	NT	NT	NT	NT	−	+	NT
Production of:								
Acetylmethylcarbinol	v	v	NT	NT	−	−	−	v
Lactic acid	NT	v	+	+	NT	NT	NT	NT
pH in Voges-Proskauer broth	<6.0	v	NT	NT	NT	>7.0	>7.0	NT
Growth in the presence of 10% NaCl	−	v	−	−	+	−	−	−
Optimum growth conditions								
pH	7.0	v (7.0 to 9.5)	7.0	9.0	7.5	7.0	7.0	3.0
Temp (°C)	23 to 37	v (15 to 55)	30	37	35	30 to 48	37	65
Major isoprenoid quinone	MK-7	MK-7	MK-7	None	ND	MK-7	MK-7	MK-7
Major cellular fatty acid(s)	anteiso-$C_{15:0}$	v	anteiso-$C_{15:0}$, iso-$C_{15:0}$, $C_{14:0}$	anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$	ND	anteiso-$C_{15:0}$ and iso-$C_{15:0}$ or iso-$C_{15:0}$	iso-$C_{15:0}$, $C_{16:0}$, iso-$C_{16:0}$	ω-Alicyclic acids
Peptidoglycan type	*meso*-DAP[j]	*meso*-DAP or L-Lys or Orn-D-Asp	*meso*-DAP	*meso*-DAP	Orn-D-Asp	ND	ND	ND
Intragenus similarity value (%) for 16S rRNA gene sequence	>89.6	NT	100	100	97.9	>93.2	98.6	>92.7
PCR 16S rRNA gene amplification with the following specific primers:								
PAEN515F[b]	+	−	−	−	NT	−	−	−
BREV174F[g]	−	−	−	−	NT	+	−	−
ANEU506F[g]	−	−	−	−	NT	−	+	−
Cross-reaction with antisera against S-layer protein from:								
Brevibacillus choshinensis[g]	−	−	−	NT	NT	+	−	−
Aneurinibacillus migulanus[g]	−	−	−	NT	NT	−	+	−
G+C content (mol%)	39–54	32–69	39	36–38	40–43	46–57	42–43	52–60

[a] Data from references 2, 6, 11–14, 19, and 22.
[b] Data from this study.
[c] Data from reference 5.
[d] Data from reference 32.
[e] Data from reference 24.
[f] Data from reference 33.
[g] Data from reference 31.
[h] Data from reference 36.
[i] v, variable reaction; −, negative reaction; +, positive reaction; NT, not tested; ND, not determined.
[j] *meso*-DAP, *meso*-diaminopimelic acid.

15375 = JCM 9068 = NCIMB 12517) has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd. as strain HSCC 175.

Description of *Paenibacillus chondroitinus* (Nakamura 1987) comb. nov. The description of *Paenibacillus chondroitinus* comb. nov. is identical to the description of *B. chondroitinus* given by Nakamura (19). The major cellular fatty acids are anteiso-$C_{15:0}$ and iso-$C_{16:0}$ acids (this study). Type strain DSMZ 5051 (= NRRL NRS-1351 = ATCC 51184 = IFO 15376 = JCM 9072) has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd. as strain HSCC 176.

Description of *Paenibacillus curdlanolyticus* (Kanzawa et al. 1995) comb. nov. The description of *Paenibacillus curdlanolyticus* comb. nov. is identical to the description of *B. curlanolyticus* given by Kanzawa et al. (14). Type strain IFO 15724 (= ATCC 51898 = CIP 104575) has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd. as strain HSCC 491.

Description of *Paenibacillus glucanolyticus* (Alexander and Priest 1989) comb. nov. The description of *Paenibacillus glucanolyticus* comb. nov. is identical to the description of *B. glucanolyticus* given by Alexander and Priest (1). The major cellular fatty acids are anteiso-$C_{15:0}$ and iso-$C_{15:0}$ acids (this study). Type strain DSMZ 5162 (= IFO 15330 = NCIMB 12809) has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd. as strain HSCC 171.

Description of *Paenibacillus kobensis* (Kanzawa et al. 1995) comb. nov. The description of *Paenibacillus kobensis* comb. nov. is identical to the description of *B. kobensis* given by Kanzawa et al. (14). Type strain IFO 15729 (= ATCC 51900 = CIP 104576) has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd. as strain HSCC 488.

Description of *Paenibacillus thiaminolyticus* (Nakamura 1990) comb. nov. The description of *Paenibacillus thiaminolyticus* comb. nov. is identical to the description of *B. thiaminolyticus* given by Nakamura (21). The major cellular fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, $C_{16:0}$, and iso-$C_{15:0}$ acids (this study). Type strain JCM 8360 (= AHU 1393 = NRRL B-4156 = IFO 15656) has been deposited in the Culture Collection of the Research Laboratory of Higeta Shoyu Co., Ltd. as strain HSCC 148.

Emendation of the genus *Paenibacillus*. The observations made in this study revealed that the genus *Paenibacillus* consists of 19 species and one subspecies. Some characteristics in the original description of this genus as proposed by Ash et al. (2) were not found in several *Paenibacillus* species. Therefore, the description of this genus should be emended, as described below.

Emended description of the genus *Paenibacillus* Ash, Priest, and Collins 1993. Cells are rod shaped. Gram positive, gram negative, or gram variable. Motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. No soluble pigment is produced on nutrient agar.

Facultatively anaerobic or strictly aerobic.

Almost all of the species are positive for catalase. *Paenibacillus larvae* subsp. *larvae* and *Paenibacillus larvae* subsp. *pulvifaciens* are negative for catalase. Oxidase activity is variable.

The Voges-Proskauer reaction (production of acetylmethylcarbinol) is variable, and the pH in Voges-Proskauer broth is less than 6.0.

Hydrogen sulfide is not produced. Indole is produced by some species.

Nitrate reduction to nitrite is variable.

Hydrolysis of casein, hydrolysis of starch, and hydrolysis of urea are variable.

Decomposition of tyrosine is variable.

Growth at pH 5.6 and growth at 50°C are variable. Optimum growth occurs at pH 7.0. The optimum growth temperature of 19 species (all species except *P. macquariensis*) is 28 to 30°C. The optimum growth temperature of *P. macquariensis* is 20 to 23°C. Growth is inhibited by 10% NaCl. Some species do not grow in medium containing 0.001% lysozyme.

Acid is produced from various sugars. *P. polymyxa*, *P. peoriae*, *P. azotofixans*, and *P. macerans* produce gas from various sugars.

Some species decompose polysaccharides.

The major cellular fatty acid is anteiso-$C_{15:0}$ acid.

The G+C contents range from 45 to 54 mol%.

The levels of 16S rRNA gene sequence similarity are more than 89.6% for the members of this genus. A 16S rRNA gene fragment is amplified by PCR with primers PAEN515F and 1377R.

The type species is *P. polymyxa*.

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