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Paenibacillus kribbensis sp. nov. and *Paenibacillus terrae* sp. nov., bioflocculants for efficient harvesting of algal cells

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Two strains of Gram-variable, rod-shaped, endospore-forming, peritrichously flagellated, rod-shaped bacteria were isolated from a soil sample collected in Taejon City, Korea. The two strains (AM49^T and AM141^T) were found to be members of the genus *Paenibacillus*, on the basis of the results of phenotypic and phylogenetic analyses. Strains AM49^T and AM141^T were found to have a cell-wall peptidoglycan based on *meso*-diaminopimelic acid, MK-7 as their predominant menaquinone and anteiso-$C_{15:0}$ as their major fatty acid. The DNA G+C contents of strains AM49^T and AM141^T were 48 and 47 mol%, respectively. The two strains formed distinct phylogenetic lineages within the radiation of the cluster comprising *Paenibacillus* spp. and a coherent cluster with *Paenibacillus jamilae*, *Paenibacillus polymyxa*, *Paenibacillus azotofixans* and *Paenibacillus peoriae*. The level of 16S rDNA similarity between strains AM49^T and AM141^T was 97·6 %, and 16S rDNA similarity values between strains AM49^T and AM141^T and the type strains of other *Paenibacillus* spp. ranged from 90·3 to 98·7 %. Levels of DNA–DNA similarity between the two strains and members of the genus *Paenibacillus* indicated that strains AM49^T and AM141^T were distinguishable from each other and from four phylogenetically related *Paenibacillus* spp. Therefore, on the basis of their phenotypic properties, phylogeny and genomic distinctiveness, it is proposed that strains AM49^T and AM141^T be placed in the genus *Paenibacillus* as two distinct novel species, *Paenibacillus kribbensis* (AM49^T =KCTC 0766BP^T =JCM 11465^T) and *Paenibacillus terrae* (AM141^T =KCCM 41557^T =JCM 11466^T).

INTRODUCTION

Aerobic or facultatively anaerobic, endospore-forming, rod-shaped bacteria are widely distributed in nature (Slepecky & Hemphill, 1991; Claus & Berkeley, 1986). These micro-organisms are very important from industrial and economic points of view (Slepecky & Hemphill, 1991; Priest, 1977; Chung *et al.*, 2000; Seo *et al.*, 1999). This group of bacteria includes many strains that are used in the production of various extracellular enzymes, polysaccharides, amino acids, secondary metabolites, etc. In the course of screening microbial flocculants for the recovery of algal cells from culture solution, we have isolated two endospore-forming, rod-shaped bacterial strains that showed higher flocculation efficiency than achieved with chemical methods. These novel strains (AM49^T and AM141^T) were found to be members of the genus *Paenibacillus*, on the basis of 16S rDNA sequence comparisons.

The genus *Paenibacillus* was created with 11 *Bacillus* spp. by Ash *et al.* (1993). Since its creation, continuous transfers of *Bacillus* spp. to the genus and descriptions of novel *Paenibacillus* spp. have increased the number of recognized *Paenibacillus* spp. considerably (Heyndrickx *et al.*, 1996; Shida *et al.*, 1997a, b; Pettersson *et al.*, 1999; Yoon *et al.*, 1998b; Tcherpakov *et al.*, 1999; Van der Maarel *et al.*, 2000; Elo *et al.*, 2001). At the time of writing, there were 28 validly described species belonging to the genus *Paenibacillus*. It has been shown that many recently described *Bacillus* spp. possess the general characteristics of the genus *Paenibacillus*

The GenBank accession numbers for the 16S rDNA sequences of *Paenibacillus kribbensis* AM49^T and *Paenibacillus terrae* AM141^T are AF391123 and AF391124, respectively.

The phylogenetic tree from which Fig. 2 was taken is available as supplementary data in IJSEM Online (http://ijs.sgmjournals.org).

(Shida *et al.*, 1997a). There may also be additional *Bacillus* spp. or strains that possess characteristics of the genus *Paenibacillus*, and rod-shaped endospore formers with the characteristics of the genus *Paenibacillus* may be relatively common in nature. Accordingly, descriptions of novel *Paenibacillus* spp. will contribute to the field of taxonomy and to our understanding of the biological diversity of the genus *Paenibacillus*. The aim of the present study was to unravel the taxonomic positions of strains AM49^T and AM141^T by using a polyphasic taxonomic approach. On the basis of the data presented here, it is proposed that strains AM49^T and AM141^T be placed into the genus *Paenibacillus* as two distinct novel species, *Paenibacillus kribbensis* and *Paenibacillus terrae*, respectively.

METHODS

Bacterial strains and culture conditions. Strains AM49^T and AM141^T were isolated from a soil sample collected in Taejon City, Korea, by the dilution plating technique on a solid medium containing (l^{-1}) 30 g glucose, 2 g yeast extract, 2 g KH_2PO_4, 0·5 g $MgSO_4.7H_2O$, 0·5 g NaCl, 0·01 g $FeSO_4.7H_2O$, 0·01 g $MnSO_4.H_2O$, 0·1 g $CaCO_3$ and 15 g agar (pH 7·0). *Paenibacillus jamilae* DSM 13815^T, *Paenibacillus polymyxa* DSM 36^T, *Paenibacillus azotofixans* DSM 5976^T and *Paenibacillus peoriae* DSM 8320^T were used as reference strains and were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). For the investigation of their morphological and physiological characteristics, strains AM49^T and AM141^T were, in most cases, cultivated on trypticase soy agar (TSA; BBL) or in trypticase soy broth (TSB; BBL) at 30 °C. Cell biomass for the analyses of cell wall and menaquinone, and for DNA extraction was obtained from TSB cultures grown at 30 °C. All strains were cultivated on a horizontal shaker at 150 r.p.m.; the broth cultures were checked for purity by microscopic examination before being harvested by centrifugation. For fatty acid methyl ester (FAME) analysis, cell masses of strains AM49^T and AM141^T and the reference strains were obtained from agar plates after growth for 3 days at 30 °C on TSA.

Morphological characterization. Colony and cell morphologies were examined by using colonies and cells grown on TSA. Observation of cell micromorphology was performed using light microscopy and transmission electron microscopy (TEM). Flagellum type was examined by TEM using cells from exponentially growing cultures. For TEM observations, cells were negatively stained with 1 % (w/v) phosphotungstic acid and, after air-drying, the grids were examined by using a model CM-20 transmission electron microscope (Philips).

Physiological characterization. Oxidase activity was determined by oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined by bubble formation in a 3 % (v/v) H_2O_2 solution. Hydrolysis of aesculin and nitrate reduction were determined as described previously (Lanyi, 1987). Hydrolyses of casein, gelatin, hypoxanthine, starch, Tween 80, tyrosine and xanthine, and urease activity were determined as described by Cowan & Steel (1965). Acid production from carbohydrates was determined by using the API 50CH system (bioMérieux). Utilization of various substrates as sole carbon and energy sources was determined as described by Shirling & Gottlieb (1966). Growth under anaerobic conditions was determined after incubation in an anaerobic chamber with TSA that was prepared anaerobically. Tolerance to NaCl was measured in TSB containing 1–6 % (w/v) NaCl. Growth at various temperatures was measured on TSA at 4–50 °C.

Isolation of DNA. Chromosomal DNA was isolated and purified as described previously (Yoon *et al.*, 1996), with the exception that ribonuclease T1 was used together with ribonuclease A.

Chemotaxonomic characterization. The isomer type of the diaminopimelic acid of the peptidoglycan layer was analysed by the method of Komagata & Suzuki (1987). Menaquinones were analysed as described previously (Komagata & Suzuki, 1987) using reversed-phase HPLC. For quantitative analysis of cellular fatty acid compositions, a loop of cell mass was harvested and FAMEs were prepared and identified following the instructions of the Microbial Identification System (MIDI).

DNA G+C content. This was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

16S rDNA sequencing and phylogenetic analysis. 16S rDNA was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998a). The PCR product was purified with a QIAquick PCR purification kit (Qiagen). Sequencing of the purified 16S rDNA was performed using an ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) as recommended by the manufacturer. The purified sequencing reaction mixtures were electrophoresed automatically using an Applied Biosystems model 377 automated DNA sequencer. Alignment of sequences was carried out using the CLUSTAL W software (Thompson *et al.*, 1994). Gaps at the 5′ and 3′ ends of the alignment were omitted from further analyses. Phylogenetic trees were inferred by using three tree-making algorithms, i.e. the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods contained within the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated by the algorithm of Jukes & Cantor (1969) using DNADIST. The stability of relationships was assessed by a bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

DNA–DNA hybridization. This was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. Of the values obtained, the highest and lowest values in each sample were excluded and the remaining three values were used for the calculation of similarity values. Hence, DNA–DNA similarity values are expressed as the mean of three values.

RESULTS AND DISCUSSION

Morphology

Strains AM49^T and AM141^T had similar micromorphological characteristics. Both strains were Gram-variable. Cells of strains AM49^T and AM141^T were rods that measured approximately 1·3–1·8 × 4·0–7·0 μm in 3-day-old cultures that had been grown on TSB at 30 °C (Fig. 1). The cells were motile by means of peritrichous flagella. Terminal or central ellipsoidal spores were observed in swollen sporangia. Colonies of strain AM49^T were cream-coloured, circular to slightly irregular in shape, flat to low convex and translucent; colonies of strain AM141^T were cream-coloured, irregular in shape, thin and translucent after 3–4 days growth on TSA.

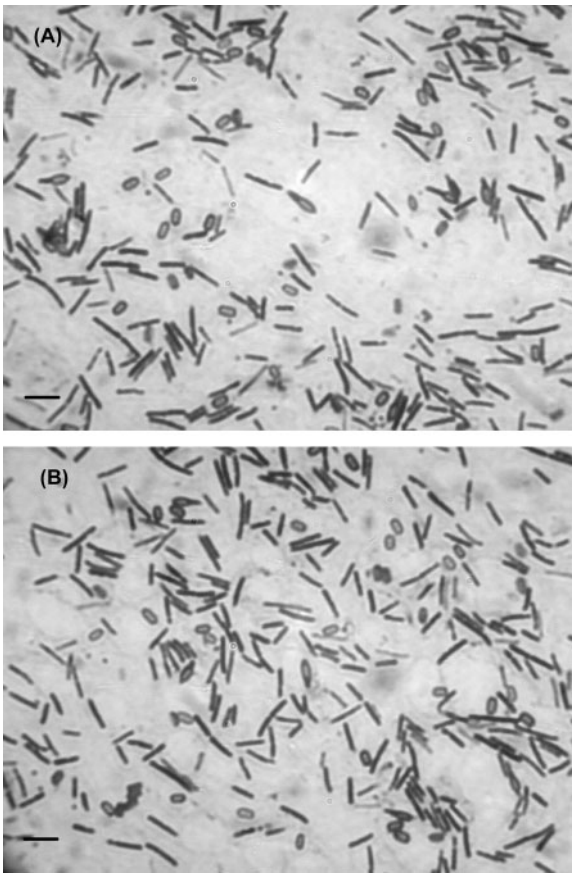

Fig. 1. Light micrographs of strains AM49^T (A) and AM141^T (B) from 3-day-old cultures grown on TSA. Bar, 5 μm.

Cultural and physiological characteristics

Strain AM49^T grew optimally at 30–37 °C, and strain AM141^T grew optimally at 30 °C. Strains AM49^T and AM141^T grew optimally between pH 6·5 and 8·0; no growth of either strain was observed at pH values below 4·0. The two strains grew optimally in the presence of 0–2 % (w/v) NaCl. However, there were differences between strains AM49^T and AM141^T with respect to their maximum growth temperatures and tolerance to NaCl. Strain AM49^T grew at 10 and 44 °C, but not at 4 °C or temperatures above 45 °C. Strain AM141^T grew at 10 and 40 °C, but not at 4 °C or temperatures above 41 °C. Strain AM49^T grew in the presence of 4 % (w/v) NaCl, but strain AM141^T did not. Neither strain grew in the presence of 5 % (w/v) NaCl. Strains AM49^T and AM141^T grew under anaerobic conditions on TSA. Both strains showed catalase activity, but neither showed oxidase nor urease activities. Aesculin, casein, gelatin and starch were hydrolysed by the two strains, but no hydrolysis of hypoxanthine, tyrosine or xanthine was observed for them. Tween 80 was hydrolysed by strain AM49^T, but it was only weakly hydrolysed by strain AM141^T. Nitrate was reduced to nitrite by both strains. The phenotypic characteristics of strains AM49^T and AM141^T were compared with those of their closest phylogenetic relatives, namely *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae*. As shown in Table 1, strains AM49^T and AM141^T were found to have physiological properties that allowed their distinction from the four recognized *Paenibacillus* spp.

Chemotaxonomic characteristics and DNA base content

Strains AM49^T and AM141^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid in their cell-wall peptidoglycan. Unsaturated menaquinone with seven isoprene units (MK-7) was the predominant isoprenoid quinone found in both strains. The cellular fatty acid profiles of the two strains are shown in Table 2, together with those of *P. polymyxa* DSM 36^T, *P. azotofixans* DSM 5976^T and *P. peoriae* DSM 8320^T. Strains AM49^T and AM141^T had cellular fatty acid profiles containing major amounts of branched-saturated fatty acids and anteiso-$C_{15:0}$ as the major fatty acid (approx. 52 % for strain AM49^T and 62 % for strain AM141^T) (Table 2). The fatty acid profiles of the two strains are similar to those of the type strains of the *Paenibacillus* spp. used in this study, but there are differences in the proportions of some fatty acids (Table 2). The fatty acid profiles of *P. polymyxa* DSM 36^T, *P. azotofixans* DSM 5976^T and *P. peoriae* DSM 8320^T obtained in this study were similar to those of the three strains found in a study by Elo *et al.* (2001). The DNA G+C contents of strains AM49^T and AM141^T were 48 and 47 mol%, respectively. The results obtained from the chemotaxonomic analyses were consistent with the results of the phylogenetic analysis, based on 16S rDNA sequences, and the micromorphology of the strains, indicating that strains AM49^T and AM141^T were different from recognized *Paenibacillus* spp. The chemotaxonomic data for strains AM49^T and AM141^T were found to be most similar to the chemotaxonomic properties characteristic of the genus *Paenibacillus* (Shida *et al.*, 1997a; Wainø *et al.*, 1999).

Phylogenetic analysis

The almost-complete 16S rDNA sequences of strains AM49^T and AM141^T were determined directly, following PCR amplification, and comprised 1511 and 1513 nt, respectively, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. The 16S rDNA sequences of strains AM49^T and AM141^T had 36 bp sequence differences (approx. 2·4 % difference) in the region compared. A phylogenetic tree, generated using the neighbour-joining algorithm, showed that strains AM49^T and AM141^T both fell within the radiation of the cluster comprising *Paenibacillus* spp. and, in particular, formed a coherent cluster with *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae* (Fig. 2). This coherent cluster was also found in the tree generated using the maximum-parsimony algorithm (data not shown). Strains AM49^T and AM141^T showed levels of 16S rDNA similarity of 98·1–98·7 % and 97·6–98·5 %, respectively, to

Table 1. Phenotypic properties useful in distinguishing strains AM49^T and AM141^T from some *Paenibacillus* spp.

Species: 1, *P. jamilae*; 2, *P. polymyxa*; 3, *P. azotofixans*; 4, *P. peoriae*; 5, strain AM49^T; 6, strain AM141^T. NT, Not tested; +, positive reaction; −, negative reaction; W, weak reaction; V, variable reaction. All species were positive for anaerobic growth, catalase, acid production from D-fructose, D-glucose, maltose, D-mannitol and salicin, and growth at pH 5·6. All species were negative for oxidase, and growth at 50 °C and in the presence of 5 % NaCl. All species formed swollen sporangia. Data for *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae* are from Aguilera *et al.* (2001), Nakamura (1987), Heyndrickx *et al.* (1995, 1996), Shida *et al.* (1997a) and Pettersson *et al.* (1999).

Characteristic	1	2	3	4	5	6
Spore shape	Ellipsoidal	Oval	Oval	Oval	Ellipsoidal	Ellipsoidal
Nitrate reduction	+	+	−	+	+	+
Hydrolysis of:						
Casein	+	+	−	+	+	+
Gelatin	+	+	−	+	+	+
Starch	+	+	−	+	+	+
Urea	NT	−	NT	−	−	−
Tween 80	NT	NT	NT	NT	+	W
Tyrosine	NT	−	−	−	−	−
Utilization of:						
L-Arabinose	NT	NT	NT	NT	+	−
D-Fructose	NT	NT	NT	NT	+	−
D-Ribose	NT	NT	NT	NT	+	−
D-Xylose	NT	NT	NT	NT	+	−
Citrate	−	−	−	+	W	W
Succinate	NT	−	NT	+	W	W
Acid production from:						
Adonitol	NT	−	−	−	−	−
L-Arabinose	+	+	−	+	+	+
D-Arabinose	NT	−	−	−	−	−
Gentiobiose	NT	+	+	+	+	+
Glycerol	+	+	−	V	W	+
D-Ribose	+	+	−	+	+	+
Trehalose	+	+	+	−	+	+
D-Xylose	+	+	−	+	+	+
Methyl β-D-xyloside	−	+	−	+	+	W
Methyl α-D-mannoside	NT	−	−	+	W	+
2-Keto-D-gluconate	NT	−	+	−	−	−
5-Keto-D-gluconate	NT	−	+	−	−	W
Growth in presence of 2 % NaCl	+	NT	V	NT	+	+
Optimum growth temperature (°C)	30	30	30–37	30	30–37	30
DNA G+C content (mol%)	41	43–46	48–53	45–47	48	47

the type strains of *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae*. Levels of 16S rDNA similarity between strain AM49^T and the type strains of other *Paenibacillus* spp. and between strain AM141^T and the type strains of other *Paenibacillus* spp. were in the ranges 90·6–95·4 % and 90·3–94·7 %, respectively. These data indicate that strains AM49^T and AM141^T are species that are clearly separate from other *Paenibacillus* spp., with the exceptions of *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae* (Stackebrandt & Goebel, 1994).

DNA–DNA similarity

DNA–DNA hybridization was performed between strains AM49^T and AM141^T, and between the two strains and the type strains of the *Paenibacillus* spp. that were phylogenetically related to strains AM49^T and AM141^T. Strains AM49^T and AM141^T exhibited two independent levels of DNA–DNA similarity, of 14·6 and 15·3 %. Accordingly, strains AM49^T and AM141^T should be considered as members of different species, considering the criterion of DNA similarity for defining a species in current bacterial systematics (Wayne *et al.*, 1987). Levels of DNA–DNA similarity between strains AM49^T and AM141^T and the type strains of *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae* are shown in Table 3. The levels of DNA–DNA similarity observed support the genomic distinction of strains AM49^T and AM141^T from *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae* (Wayne *et al.*, 1987).

Table 2. Cellular fatty acid profiles of the type strains of some *Paenibacillus* spp. and strains AM49^T and AM141^T

Species: 1, *P. polymyxa* DSM 36^T; 2, *P. azotofixans* DSM 5976^T; 3, *P. peoriae* DSM 8320^T; 4, strain AM49^T; 5, strain AM141^T.

Fatty acid	1	2	3	4	5
Saturated fatty acid:					
$C_{11:0}$ 2OH	0·4	ND	ND	ND	ND
$C_{14:0}$	0·7	5·0	1·1	1·9	1·3
$C_{15:0}$	0·5	2·2	2·6	1·7	2·2
$C_{16:0}$	9·1	15·5	6·3	10·2	9·1
$C_{16:0}$ *N* alcohol	ND	0·4	0·1	ND	ND
$C_{17:0}$	ND	ND	0·4	0·5	0·3
$C_{18:0}$	1·1	0·3	0·1	ND	ND
Unsaturated fatty acid:					
$C_{16:1}$ ω11*c*	ND	ND	ND	0·3	0·3
$C_{17:1}$ ω6*c*	0·7	ND	ND	ND	ND
$C_{18:1}$ ω5*c*	ND	1·8	ND	ND	ND
Branched fatty acid:					
iso-$C_{13:0}$	ND	1·3	0·4	ND	0·1
anteiso-$C_{13:0}$	ND	1·8	0·3	ND	0·2
iso-$C_{14:0}$	0·6	4·7	2·2	1·2	1·1
iso-$C_{15:0}$	5·5	8·7	8·7	10·4	6·5
anteiso-$C_{15:0}$	49·9	45·4	56·4	52·4	62·3
iso-$C_{16:0}$	7·7	5·3	7·4	6·4	4·5
iso-$C_{17:0}$	7·0	1·1	5·9	6·3	3·8
iso-$C_{17:0}$ 3OH	ND	2·9	ND	ND	ND
anteiso-$C_{17:0}$	16·7	2·1	7·9	8·8	8·3
iso-$C_{18:1}$ H*	ND	0·8	ND	ND	ND
Summed feature 4†	ND	1·0	ND	ND	ND

ND, Not detected.

*The double bond position indicated by a capital letter is unknown.

†Summed feature 4 represents iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B, which could not be separated by GLC using the MIDI system.

Conclusion

In view of the combined morphological, physiological, chemotaxonomic and phylogenetic data discussed here, it is evident that strains AM49^T and AM141^T belong to the genus *Paenibacillus*. The differences in some of their phenotypic characteristics and their phylogenetic and genomic distinctiveness distinguish strains AM49^T and AM141^T from previously described *Paenibacillus* spp. On the basis of the data described above, strains AM49^T and AM141^T should be placed in the genus *Paenibacillus* as two distinct, novel species, for which we propose the names *Paenibacillus kribbensis* and *Paenibacillus terrae*, respectively.

Description of *Paenibacillus kribbensis* sp. nov.

Paenibacillus kribbensis (krib.ben′sis. N.L. adj. *kribbensis* arbitrary name formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB, where taxonomic studies on this species were performed).

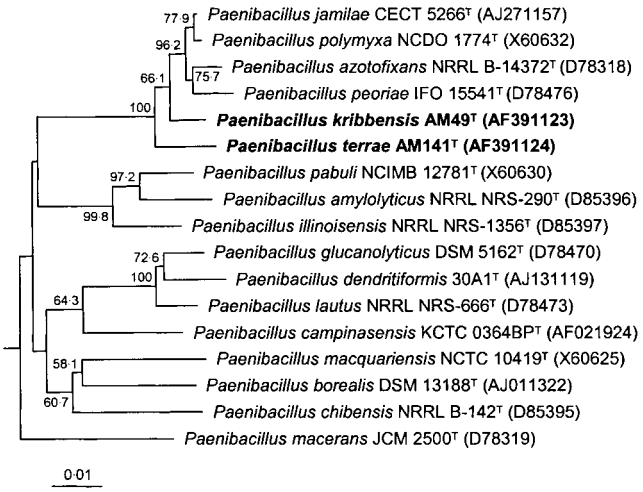

Fig. 2. Neighbour-joining tree, based on 16S rDNA sequences, showing the phylogenetic positions of strains AM49^T and AM141^T, the type strains of some *Paenibacillus* spp. and representatives of some other taxa. Bar, 0·01 substitutions per nucleotide position. Only bootstrap values (expressed as percentages of 1000 replications) of greater than 50 % are shown at the branch points. The tree from which Fig. 2 was taken is available as supplementary data in IJSEM Online (http://ijs.sgmjournals.org/).

Cells are facultatively anaerobic rods with dimensions of 1·3–1·8 × 4·0–7·0 μm on TSA. Gram-variable. Ellipsoidal spores are formed in swollen sporangia. Motile by means of peritrichous flagella. Colonies are cream-coloured, circular to slightly irregular in shape, flat to low convex and translucent on TSA. Optimal growth temperature is between 30 and 37 °C; growth occurs at 10 and 44 °C, but not at 4 or 45 °C. Optimal pH for growth is between pH 6·5 and 8·0; growth is inhibited below pH 4·0. Grows optimally in the presence of 0–2 % (w/v) NaCl; growth occurs in the presence of 4 % (w/v) NaCl, but not in the presence of 5 % (w/v) NaCl. Catalase-positive. Oxidase- and urease-negative. Aesculin, casein, gelatin, starch and Tween 80 are hydrolysed. Hypoxanthine, tyrosine and xanthine are not hydrolysed. Nitrate is reduced to nitrite. L-Arabinose,

Table 3. Levels of DNA–DNA similarity between strains AM49^T and AM141^T, and some *Paenibacillus* spp.

Strain	Percentage reassociation with	
	Strain AM49^T	Strain AM141^T
Strain AM49^T	100	15·3
Strain AM141^T	14·6	100
P. jamilae DSM 13815^T	31·2	28·9
P. polymyxa DSM 36^T	22·9	20·7
P. azotofixans DSM 5976^T	11·4	12·5
P. peoriae DSM 8320^T	15·6	16·8

D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, melibiose, D-raffinose, L-rhamnose, D-ribose, stachyose, sucrose, D-trehalose, D-xylose, *myo*-inositol, D-mannitol and sodium gluconate are utilized; disodium succinate and trisodium citrate are weakly utilized as sole carbon and energy sources. D-Melezitose, adonitol, D-sorbitol, sodium acetate and sodium benzoate are not utilized as sole carbon and energy sources. In the API 50CH system, when API CHB suspension medium is used, acid is produced from L-arabinose, ribose, D-xylose, methyl β-D-xyloside, galactose, glucose, fructose, mannose, inositol, mannitol, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, raffinose, starch, glycogen and gentiobiose; acid is weakly produced from glycerol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine and gluconate. Acid is not produced from erythritol, D-arabinose, L-xylose, adonitol, sorbose, rhamnose, dulcitol, sorbitol, melezitose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-keto-D-gluconate or 5-keto-D-gluconate. Cell-wall peptidoglycan contains *meso*-diaminopimelic acid. Predominant menaquinone is MK-7. Major fatty acid is anteiso-$C_{15:0}$. DNA G+C content is 48 mol% (as determined by HPLC). Isolated from a soil sample from Taejon City, Korea. The type strain is strain AM49^T, which has been deposited in the Korean Collection for Type Cultures as KCTC 0766BP^T and the Japan Collection of Microorganisms as JCM 11465^T.

Description of *Paenibacillus terrae* sp. nov.

Paenibacillus terrae (ter′rae. L. gen. n. *terrae* of the earth).

Cells are facultatively anaerobic rods with dimensions of 1·3–1·8 × 4·0–7·0 μm on TSA. Gram-variable. Ellipsoidal spores are formed in swollen sporangia. Motile by means of peritrichous flagella. Colonies are cream-coloured, irregular in shape, thin and translucent on TSA. Optimal growth temperature is 30 °C; growth occurs at 10 and 40 °C, but not at 4 or 41 °C. Optimal pH for growth is between pH 6·5 and 8·0; growth is inhibited below pH 4·0. Grows optimally in the presence of 0–2 % (w/v) NaCl; growth occurs in the presence of 3 % (w/v) NaCl, but not in the presence of 4 % (w/v) NaCl. Catalase-positive. Oxidase- and urease-negative. Aesculin, casein, gelatin and starch are hydrolysed; Tween 80 is weakly hydrolysed. Hypoxanthine, tyrosine and xanthine are not hydrolysed. Nitrate is reduced to nitrite. D-Cellobiose, D-galactose, D-glucose, lactose, maltose, D-mannose, melibiose, D-raffinose, L-rhamnose, stachyose, sucrose, D-trehalose, *myo*-inositol, D-mannitol and sodium gluconate are utilized; disodium succinate and trisodium citrate are weakly utilized as sole carbon and energy sources. L-Arabinose, D-fructose, D-melezitose, D-ribose, D-xylose, adonitol, D-sorbitol, sodium acetate and sodium benzoate are not utilized as sole carbon and energy sources. In the API 50CH system, when API CHB suspension medium is used, acid is produced from glycerol, L-arabinose, ribose, D-xylose, galactose, glucose, fructose, mannose, inositol, mannitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, raffinose, starch, glycogen, gentiobiose and D-turanose; acid is weakly produced from methyl β-D-xyloside and 5-keto-D-gluconate. Acid is not produced from erythritol, D-arabinose, L-xylose, adonitol, sorbose, rhamnose, dulcitol, sorbitol, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate or 2-keto-D-gluconate. Cell-wall peptidoglycan contains *meso*-diaminopimelic acid. Predominant menaquinone is MK-7. Major fatty acid is anteiso-$C_{15:0}$. DNA G+C content is 47 mol% (as determined by HPLC). Isolated from a soil sample from Taejon City, Korea. The type strain is strain AM141^T, which has been deposited in the Korean Culture Center of Microorganisms as KCCM 41557^T and the Japan Collection of Microorganisms as JCM 11466^T.

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