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 (AM49T and AM141T) were found to be members of the genus Paenibacillus, on the basis of the results of phenotypic and phylogenetic analyses. Strains AM49T and AM141T were found to have a cell-wall peptidoglycan based on meso-diaminopimelic acid, MK-7 as their predominant menaquinone and anteiso-C15 : 0as their major fatty acid. The DNA G+C contents of strains AM49T and AM141T 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 AM49T and AM141T was 97 ± 6 %, and 16S rDNA similarity values between strains AM49T and AM141T 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 AM49T and AM141T 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 AM49T and AM141T be placed in the genus Paenibacillus as two distinct novel species, Paenibacillus kribbensis (AM49T = KCTC 0766BPT = JCM 11465T) and Paenibacillus terrae (AM141T = KCCM 41557T = JCM 11466T).

INTRODUCTION

Aerobic or facultatively anaerobic, endospore-forming, rod-shaped bacteria are widely distributed in nature (Slepecky & Hemphill, 1991; Claus & Berkeley, 1986). These microorganisms 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 (AM49T and AM141T) 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.
(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 AM49T and AM141T by using a polyphasic taxonomic approach. On the basis of the data presented here, it is proposed that strains AM49T and AM141T be placed into the genus Paenibacillus as two distinct novel species, Paenibacillus kribbensis and Paenibacillus terrae, respectively.

METHODS

Bacterial strains and culture conditions. Strains AM49T and AM141T 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 KH2PO4, 0·5 g MgSO47H2O, 0·5 g NaNCl, 0·01 g FeSO47H2O, 0·01 g MnSO47H2O, 0·1 g CaCl2, and 15 g agar (pH 7·0). Paenibacillus jumilae DSM 13815T, Paenibacillus polymyxa DSM 361T, Paenibacillus azotoferans DSM 5976T and Paenibacillus peeriae DSM 8320T 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 AM49T and AM141T 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 growth at 3–4 days growth 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 microscope 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) H2O2 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 after incubation in an anaerobic chamber with TSA 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 AM49T were cream-coloured, circular to slightly irregular in shape, flat to low convex and translucent; colonies of strain AM141T were cream-coloured, irregular in shape, thin and translucent after 3–4 days growth on TSA.

Isolation of DNA. Chromosomal DNA was isolated and purified as described previously (Yoon et al., 1996a), 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 AM49T and AM141T had similar micromorphological characteristics. Both strains were Gram-variable. Cells of strains AM49T and AM141T 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 AM49T were cream-coloured, circular to slightly irregular in shape, flat to low convex and translucent; colonies of strain AM141T were cream-coloured, irregular in shape, thin and translucent after 3–4 days growth on TSA.
Cultural and physiological characteristics

Strain AM49 T grew optimally at 30–37 °C, and strain AM141T grew optimally at 30 °C. Strains AM49 T and AM141T 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 AM141T 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 AM141T 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 AM141T did not. Neither strain grew in the presence of 5 % (w/v) NaCl. Strains AM49 T and AM141T 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 AM141T. Nitrate was reduced to nitrite by both strains. The phenotypic characteristics of strains AM49 T and AM141T were compared with those of their closest phylogenetic relatives, namely *P. jamilae*, *P. polymyx*, *P. azotofixans* and *P. peoriae*. As shown in Table 1, strains AM49 T and AM141T 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 AM141T 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. polymyx* DSM 36 T, *P. azotofixans* DSM 5976 T and *P. peoriae* DSM 8320 T. Strains AM49 T and AM141T 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 AM141T) (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. polymyx* 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 AM141T 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 AM141T were different from recognized *Paenibacillus* spp. The chemotaxonomic data for strains AM49 T and AM141T 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 AM141T 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 AM141T 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 AM141T both fell within the radiation of the cluster comprising *Paenibacillus* spp. and, in particular, formed a coherent cluster with *P. jamilae*, *P. polymyx*, *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 AM141T showed levels of 16S rDNA similarity of 98.1–98.7 % and 97.6–98.5 %, respectively, to
the type strains of *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae*. Levels of 16S rDNA similarity between strain AM49<sup>T</sup> and the type strains of other *Paenibacillus* spp. and between strain AM141<sup>T</sup> and the type strains of other *Paenibacillus* spp. were in the ranges 90<sup>–</sup>6<sup>–</sup>95<sup>–</sup>4 % and 90<sup>–</sup>3<sup>–</sup>94<sup>–</sup>7 %, respectively. These data indicate that strains AM49<sup>T</sup> and AM141<sup>T</sup> 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<sup>T</sup> and AM141<sup>T</sup>, and between the two strains and the type strains of the *Paenibacillus* spp. that were phylogenetically related to strains AM49<sup>T</sup> and AM141<sup>T</sup>. Strains AM49<sup>T</sup> and AM141<sup>T</sup> exhibited two independent levels of DNA–DNA similarity, of 14<sup>–</sup>6 and 15<sup>–</sup>3 %. Accordingly, strains AM49<sup>T</sup> and AM141<sup>T</sup> 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<sup>T</sup> and AM141<sup>T</sup> 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<sup>T</sup> and AM141<sup>T</sup> from *P. jamilae*, *P. polymyxa*, *P. azotofixans* and *P. peoriae* (Wayne et al., 1987).

### Table 1. Phenotypic properties useful in distinguishing strains AM49<sup>T</sup> and AM141<sup>T</sup> from some *Paenibacillus* spp.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
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<td>Spore shape</td>
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<td>Oval</td>
<td>Oval</td>
<td>Oval</td>
<td>Ellipsoidal</td>
<td>Ellipsoidal</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>NT</td>
<td>−</td>
<td>NT</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tween 80</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>W</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>NT</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>L- Arabinose</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Citrate</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>W</td>
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<td>Succinate</td>
<td>NT</td>
<td>−</td>
<td>NT</td>
<td>+</td>
<td>W</td>
<td>W</td>
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<tr>
<td>Acid production from:</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adonitol</td>
<td>NT</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L- Arabinose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>D- Arabinose</td>
<td>NT</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Gentiose</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glycerol</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>Trehalose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>Methyl β-D-xyloside</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>W</td>
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<td>Methyl α-D-mannoside</td>
<td>NT</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>W</td>
</tr>
<tr>
<td>2-Keto-D-glucurate</td>
<td>NT</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5-Keto-D-glucurate</td>
<td>NT</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>W</td>
</tr>
<tr>
<td>Growth in presence of 2 % NaCl</td>
<td>+</td>
<td>NT</td>
<td>V</td>
<td>NT</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Optimum growth temperature (°C)</td>
<td>30</td>
<td>30</td>
<td>30–37</td>
<td>30</td>
<td>30–37</td>
<td>30</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>41</td>
<td>43–46</td>
<td>48–53</td>
<td>45–47</td>
<td>48</td>
<td>47</td>
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Table 2. Cellular fatty acid profiles of the type strains of some Paenibacillus spp. and strains AM49T and AM141T

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<td>Saturated fatty acid:</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C11:0 2OH</td>
<td>0.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>C14:0</td>
<td>0.7</td>
<td>5.0</td>
<td>1.0</td>
<td>1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.5</td>
<td>2.2</td>
<td>2.6</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>C16:0</td>
<td>9.1</td>
<td>15.5</td>
<td>6.3</td>
<td>10.2</td>
<td>9.1</td>
</tr>
<tr>
<td>C16:0 N alcohol</td>
<td>ND</td>
<td>0.4</td>
<td>0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C17:0</td>
<td>ND</td>
<td>ND</td>
<td>0.4</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.1</td>
<td>0.3</td>
<td>0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unsaturated fatty acid:</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C16:1 ω11c</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>C17:1 ω6c</td>
<td>0.7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>C18:1 ω5c</td>
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<td>iso-C13:0</td>
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<td>0.4</td>
<td>ND</td>
<td>0.1</td>
<td>ND</td>
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<td>anteiso-C13:0</td>
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<td>1.8</td>
<td>0.3</td>
<td>ND</td>
<td>0.2</td>
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<tr>
<td>iso-C14:0</td>
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<td>4.7</td>
<td>2.2</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>iso-C15:0</td>
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<td>8.7</td>
<td>8.7</td>
<td>10.4</td>
<td>6.5</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>49.9</td>
<td>45.4</td>
<td>56.4</td>
<td>52.4</td>
<td>62.3</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>7.7</td>
<td>5.3</td>
<td>7.4</td>
<td>6.4</td>
<td>4.5</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>7.0</td>
<td>1.1</td>
<td>5.9</td>
<td>6.3</td>
<td>3.8</td>
</tr>
<tr>
<td>iso-C17:1 3OH</td>
<td>ND</td>
<td>2.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>anteiso-C17:1</td>
<td>16.7</td>
<td>2.1</td>
<td>7.9</td>
<td>8.8</td>
<td>8.3</td>
</tr>
<tr>
<td>iso-C18:1 H*</td>
<td>ND</td>
<td>0.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Summed feature 4†</td>
<td>ND</td>
<td>1.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, Not detected.

*The double bond position indicated by a capital letter is unknown.
†Summed feature 4 represents iso-C17:1 I and/or anteiso-C17:1 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 AM49T and AM141T belong to the genus Paenibacillus. The differences in some of their phenotypic characteristics and their phylogenetic and genomic distinctiveness distinguish strains AM49T and AM141T from previously described Paenibacillus spp. On the basis of the data described above, strains AM49T and AM141T 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, KORIBB, where taxonomic studies on this species were performed).

Cells are facultatively anaerobic rods with dimensions of 1–3 × 1–8 μm. Colonies are cream-coloured, circular and 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. 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. Colony characteristics and their phylogenetic and genomic distinctiveness distinguish strains AM49T and AM141T from previously described Paenibacillus spp. On the basis of the data described above, strains AM49T and AM141T should be placed in the genus Paenibacillus as two distinct, novel species, for which we propose the names Paenibacillus kribbensis and Paenibacillus terrae, respectively.

Table 3. Levels of DNA–DNA similarity between strains AM49T and AM141T, and some Paenibacillus spp.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Percentage reassociation with AM49T</th>
<th>Percentage reassociation with AM141T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain AM49T</td>
<td>100</td>
<td>15-3</td>
</tr>
<tr>
<td>Strain AM141T</td>
<td>14-6</td>
<td>100</td>
</tr>
<tr>
<td>P. jamilae DSM 13815T</td>
<td>31-2</td>
<td>28-9</td>
</tr>
<tr>
<td>P. polymyxa DSM 36T</td>
<td>22-9</td>
<td>20-7</td>
</tr>
<tr>
<td>P. azotofixans DSM 5976T</td>
<td>11-4</td>
<td>12-5</td>
</tr>
<tr>
<td>P. peoriae DSM 8320T</td>
<td>15-6</td>
<td>16-8</td>
</tr>
</tbody>
</table>
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, glycerogen 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, xyitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-keto-D-glucuronate or 5-keto-D-glucuronate. Cell-wall peptidoglycan contains meso-diaminopimelic acid. Predominant menaquinone is MK-7. Major fatty acid is anteiso-C15:0. 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 AM141T, which has been deposited in the Korean Collection for Type Cultures as KCTC 0766BP\textsuperscript{T} and the Japan Collection of Microorganisms as JCM 11466\textsuperscript{T}.

**Description of Paenibacillus terrae sp. nov.**


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, glycerogen, gentiobiose and D-turanose; acid is weakly produced from methyl β-D-xyloside and 5-keto-D-glucuronate. Acid is not produced from erythritol, D-arabinose, L-xylose, adonitol, sorbose, rhamnose, dulcitol, sorbitol, melezitose, xyitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-keto-D-glucuronate or 5-keto-D-glucuronate. Cell-wall peptidoglycan contains meso-diaminopimelic acid. Predominant menaquinone is MK-7. Major fatty acid is anteiso-C15:0. 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\textsuperscript{T}, which has been deposited in the Korean Culture Center of Microorganisms as KCCM 41557\textsuperscript{T} and the Japan Collection of Microorganisms as JCM 11466\textsuperscript{T}.

**ACKNOWLEDGEMENTS**

This work was supported by grant HSS0310134 and the NRL research programme (grants M10104000294–01J00012800 and NLW0070111) of the Ministry of Science and Technology (MOST) of the Republic of Korea, and by the research fund of the Probiotic Corporation of Korea.

**REFERENCES**


