Transfer of *Bacillus mucilaginosus* and *Bacillus edaphicus* to the genus *Paenibacillus* as *Paenibacillus mucilaginosus* comb. nov. and *Paenibacillus edaphicus* comb. nov.

Xiu-Fang Hu, Shi-Xiao Li, Jin-Guang Wu, Jian-Feng Wang, Qiong-Lou Fang and Ji-Shuang Chen

Correspondence
Ji-Shuang Chen
chenjs@zstu.edu.cn

Institute of Bioengineering, Zhejiang Sci-Tech University, Road 2, 310018, Xiasha Hangzhou, PR China

Bacillus mucilaginosus and *Bacillus edaphicus* were reclassified based on their 16S rRNA and *gyrB* gene sequences, DNA–DNA hybridization, fatty acid methyl esters and other taxonomic characteristics. Phylogenetic analysis based on 16S rRNA and *gyrB* gene sequences indicated that strains of *B. mucilaginosus* and *B. edaphicus* were members of the genus *Paenibacillus*, with over 90.4% and 70.3% sequence similarity, respectively. Their DNA G + C contents were 54.5–56.8 mol%. The DNA–DNA relatedness values of *B. edaphicus* VKPM B-7517^T with *B. mucilaginosus* KNP414 and *B. mucilaginosus* CGMCC 1.236 were 89.2% and 88.7%, respectively. The major isoprenoid quinone of *B. mucilaginosus* and *B. edaphicus* was MK-7 (94.1–95.7%). The peptidoglycan type was A1 γ (meso-diaminopimelic acid) and the major polar lipids were phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids were anteiso-C_{15:0}, C_{16:1} ω 11c and C_{16:0}. Phenotypic features and fatty acid profiles supported the similarity of *B. mucilaginosus* and *B. edaphicus* to *Paenibacillus validus* CCTCC 95016^T and confirmed their relationship with members of the genus *Paenibacillus*. Therefore, it is proposed that *Bacillus mucilaginosus* and *Bacillus edaphicus* be transferred to the genus *Paenibacillus* as *Paenibacillus mucilaginosus* comb. nov. (type strain HSCC 1605^T=VKPM B-7519^T=VKM B-1480D^T=CIP 105815^T=KCTC 3870^T) and *Paenibacillus edaphicus* comb. nov. (type strain VKPM B-7517^T=DSM 12974^T=CIP 105814^T), respectively.

Bacillus mucilaginosus, which can release nutritional ions from soils and minerals, is widely used as a biofertilizer in agricultural applications (Glick, 1995; Sheng *et al.*, 2000; Liu *et al.*, 2006). It was first described phenotypically in 1967 (Aleksandrov *et al.*, 1967) and phylogenetically characterized in 1986 (Avakyan *et al.*, 1986). However, the name was not validly published until 1998, which

coincided with the identification of *Bacillus edaphicus*, a novel species with similar functions (Shelobolina *et al.*, 1997). Recently, *B. mucilaginosus* and *B. edaphicus* were found to be more closely related to the genus *Paenibacillus* than to the genus *Bacillus* on the basis of phylogenetic analysis (Hu *et al.*, 2006), but this finding requires further clarification.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the *Bacillus mucilaginosus* strains are DQ898307 (CGMCC 1.2326), DQ898308 (CGMCC 1.231), DQ898309 (CGMCC 1.3714), DQ898310 (CGMCC 1.153) and FJ039528 (VKPM B-7519^T). The accession numbers for the *gyrB* gene sequences for the *B. mucilaginosus* strains are FJ009516 (KNP413), FJ039525 (KNP414), FJ009517 (CGMCC 1.231), FJ009518 (CGMCC 1.2326), EU711070 (CGMCC 1.153), FJ039527 (CGMCC 1.3714) and FJ009520 (VKPM B-7519^T); the accession numbers for the other strains are FJ009519 (*B. edaphicus* VKPM B-7517^T), EU711067 (*B. subtilis* CCTCC 92068^T), FJ009514 (*B. azotoformans* ACCC 10226^T), EU711068 (*B. circulans* ACCC 10228^T), EU711066 (*B. megaterium* ACCC 10245^T), J009515 (*P. validus* CCTCC 95016^T), EU711069 (*P. polymyxa* CCTCC 92076^T), FJ009513 (*P. apiarius* ACCC 10193^T) and FJ039526 (*P. azotofixans* ACCC 10251^T).

Paenibacillus, a genus separated from the genus *Bacillus*, was first proposed by Ash *et al.* (1993) and later emended by Shida *et al.* (1997). Many species in this genus possess useful properties, such as nitrogen fixation (Achouak *et al.*, 1999) and macromolecule degradation (Ash *et al.*, 1993), and play an economically important role in agriculture. The typical characteristics of species in the genus *Paenibacillus* are as follows (Shida *et al.*, 1997): rod-shaped with a flagellum; producing ellipsoidal spores with swollen sporangia; positive for catalase; negative for H₂S production; variable for oxidase, nitrate reduction and Voges–Proskauer reaction; DNA G + C content ranging between 45% and 54 mol%, with anteiso-C_{15:0} as the major cellular fatty acid, MK-7 as the major isoprenoid quinone,

meso-diaminopimelic acid as the diagnostic diamino acid, and more than 89.6 % intra-genus similarity in 16S rRNA gene sequences. These distinctive characteristics distinguish the genus *Paenibacillus* from other members of the family *Bacillaceae*. In this study, we present phenotypic and phylogenetic data that support the transfer of *B. mucilaginosus* and *B. edaphicus* to the genus *Paenibacillus*.

The following strains were used in this study: *B. edaphicus* VKPM B-7517^T, *B. mucilaginosus* VKPM B-7519^T, CGMCC 1.231, CGMCC 1.2326, CGMCC 1.3714, CGMCC 1.153, KNP413 and KNP414 (Hu *et al.*, 2006), *Bacillus circulans* ACCC 10228^T, *Bacillus megaterium* ACCC 10245^T, *Bacillus subtilis* ACCC 92068^T, *Bacillus azotoformans* ACCC 10226^T, *Paenibacillus apiarius* ACCC 10193^T, *Paenibacillus azotofixans* ACCC 10251^T, *Paenibacillus validus* CCTCC 95016^T and *Paenibacillus polymyxa* CCTCC 92076^T. The *B. mucilaginosus* and *B. edaphicus* strains were cultured on Aleksandrov agar (Avakyan *et al.*, 1986) and the other strains were grown on nutrient agar medium. Aleksandrov medium consisted of (w/v) 1.0 % sucrose, 0.2 % K₂HPO₄·3H₂O, 0.05 % MgSO₄·7H₂O, 0.1 % CaCO₃ and 2.0 % agar, pH 7.5. All strains were incubated for 24–48 h at 30 °C.

Genomic DNA extraction, amplification and sequencing of the 16S rRNA gene were performed as described previously (Hu *et al.*, 2006). The 16S rRNA gene sequences were aligned with representatives from the genera *Bacillus* and *Paenibacillus* that had been deposited in the GenBank, EMBL and DDBJ databases. Phylogenetic trees were constructed with three methods: neighbour-joining (MEGA version 3.1), maximum-parsimony and maximum-likelihood (PHYLIP version 3.65) (Saitou & Nei, 1987). Bootstrap resampling analysis was performed to estimate the confidence of the tree topologies.

The almost-complete 16S rRNA gene sequence (1502 bases) was determined for *B. mucilaginosus* strains CGMCC 1.231, CGMCC 1.2326, CGMCC 1.3714 and CGMCC 1.153. The 16S rRNA gene sequence-based phylogenetic analysis revealed that the intraspecies similarity values for *B. mucilaginosus* were 99.1–99.9 %, with the exception of strain VKM B-1480D^T, which shared only 94.3–96.9 % similarity with the other strains. However, strain HSCC 1605^T, deposited in Japan, presented a high intraspecies similarity of 99.1–99.9 %. Thus, the 16S rRNA gene sequence of strain VKM B-1480D^T, which is similar to that of VKPM B-7519^T was analysed, and it found that the sequence submitted as GenBank accession no. AF006077 might contain various errors. Resequencing of the 16S rRNA gene proved that the sequence of strain VKPM B-7519^T differed significantly from that of strain VKM B-1480D^T. In addition, there was only one base discrepancy between the sequences of strains VKPM B-7519^T (FJ039528) and HSCC 1605^T (AB045091), indicating the similarity of these two strains. Therefore, the strains of *Bacillus mucilaginosus* tested were members of the same species with 99.0–99.9 % intraspecies similarity.

Strains of *B. mucilaginosus* and *B. edaphicus* strain VKPM B-7517^T showed 90.4–96.4 % and 90.3–95.1 % similarity to the representatives of the *Paenibacillus* cluster, respectively. However, both species shared less than 88.5 % similarity with representatives of the other clusters of the family *Bacillaceae*. The >90.4 % 16S rRNA gene sequence similarity to strains of the genus *Paenibacillus* places *B. mucilaginosus* and *B. edaphicus* within the realm of the genus *Paenibacillus* rather than in that of the genus *Bacillus* (Shida *et al.*, 1997). The phylogenetic trees constructed with all three treeing methods indicated that the *B. mucilaginosus* and *B. edaphicus* clustered with the genus *Paenibacillus* (Fig. 1). Within this cluster, *B. mucilaginosus* strains and *B. edaphicus* VKPM B-7517^T were found to be closely related to each other and to the species *P. validus*, which was consistent with an earlier result (Shelobolina *et al.*, 1997).

The *gyrB* gene, a single-copy gene encoding the B-subunit protein of DNA gyrase, is increasingly used as a phylogenetic marker for bacterial systematics and for bacterial taxonomic and phylogenetic studies (Yamada *et al.*, 1999). The *gyrB* gene was used in this study to confirm the taxonomic and phylogenetic placements of the species tested. The amplification of the *gyrB* gene was performed with universal primers (UP-1, CAYGCNGGNGGNAA-RTTYG; UP-2r, CCRTCACRTCTCNGTCNGTCAT; Yamamoto & Harayama, 1995) and the sequencing and phylogenetic analysis were carried out as described above.

The 1210 bp *gyrB* genes of the strains examined were sequenced and their GenBank accession numbers are given in Fig. 2. The intraspecies similarity values for the strains of *B. mucilaginosus* were 98.2–99.9 %. All *B. mucilaginosus* strains showed 70.4–78.0 % gene sequence similarity to the representatives of *Paenibacillus* cluster and to *B. edaphicus* VKPM B-7517^T. *B. edaphicus* VKPM B-7517^T shared 70.3–79.2 % gene sequence similarity with strains of the genus *Paenibacillus*. However, *B. mucilaginosus* and *B. edaphicus* shared only 61.2–68.9 % similarity with representatives of the other clusters of the family *Bacillaceae*. It would appear that there are threshold values of 98 % for a species delineation and 70 % for a genus delineation for the *gyrB* gene, which require further substantiation. The high *gyrB* gene sequence similarity between *B. mucilaginosus* and *B. edaphicus* and members of the genus *Paenibacillus* suggested that these species should be placed within the genus *Paenibacillus* rather than the genus *Bacillus*.

The phylogenetic trees constructed with the three treeing methods (Fig. 2) also indicated that *B. mucilaginosus* and *B. edaphicus* clustered within the genus *Paenibacillus*, and showed that the two species were closely related to each other and to *P. validus*. However, *B. mucilaginosus* strains were divided into two groups, Group I (KNP413, CGMCC 1.3714 and CGMCC 1.2326) and Group II (VKPM B-7519, CGMCC 1.231, CGMCC 1.153 and KNP414). Strains within each group shared 99.6–99.9 % gene sequence

Fig. 1. Phylogenetic tree showing the relationship between *B. edaphicus*, *B. mucilaginosus* and closely related strains. The tree was based on an alignment of 16S rRNA gene sequences and was constructed using the neighbour-joining method (MEGA version 3.1). The stability of the tree was assessed by 1000 bootstrap replications with Felsenstein confidence limits. The sequence of *Escherichia coli* ATCC 11775^T was used as an outgroup. Strains for which the sequences were determined in this study are printed in bold type. Bar, evolutionary distance of 20 substitutions per position.

similarities for the *gyrB* gene, but strains in the different groups showed only 98.2–98.6% sequence similarity. Strains in Group II, which possessed plasmids (about 7 kb) and large capsules (data not shown), differed at 17 positions when compared with those in Group I. Although differences existed between the two groups, which are of

interest for further study, such differences were not significant enough to prevent the two groups from clustering into the same species. The results based on the *gyrB* gene sequence coincided closely with those of the 16S rRNA gene sequence comparisons and further confirmed the powerful discriminative ability of the *gyrB* gene.

Fig. 2. Phylogenetic tree based on an alignment of *gyrB* gene sequences showing the relationship between *B. edaphicus*, *B. mucilaginosus* and closely related strains. For further details of tree construction, see Fig. 1. Bar, evolutionary distance of 50 substitutions per position.

The DNA base ratio was determined from the thermal denaturation temperature, as described by Marmur & Doty (1962). The DNA G+C contents of strains of *B. mucilaginosus* and *B. edaphicus* ranged from 54.5 to 56.8 mol% (Table 1). These values are similar to those previously determined by Shelobolina *et al.* (1997); however, they exceeded the DNA G+C content level expected for members of the genus *Paenibacillus* (Shida *et al.*, 1997). Most strains of the genus *Paenibacillus* have a DNA G+C content of 45–54 mol% (Shida *et al.*, 1997), with the exception of strains of *Paenibacillus dendritiformis* (Tcherpakov *et al.*, 1999) and *Paenibacillus koleovorans* (Takeda *et al.*, 2002), which have a higher DNA G+C content of 54–55 mol%. Thus, *B. mucilaginosus* and *B. edaphicus* provide further evidence that the DNA G+C content of the genus *Paenibacillus* can exceed 54 %.

DNA–DNA hybridization was performed using a thermal denaturation technique (De Ley *et al.*, 1970). The DNA–DNA hybridization values confirmed that *B. mucilaginosus* strains VKPM B-7519^T, KNP414 and CGMCC 1.2326 were members of the same species. Strain VKPM B-7519^T exhibited 89.2 % and 88.7 % relatedness values with strains KNP414 and CGMCC 1.2326, respectively. Organisms that have <97.0 % 16S rRNA gene sequence similarity will not recombine with DNA relatedness values >60 %, regardless of the hybridization method employed (Stackebrandt & Goebel, 1994). We did not determine the DNA–DNA relatedness values between *B. mucilaginosus* and other strains of the genus *Paenibacillus* because the 16S rRNA gene sequence similarities between them were <96.4 %, which demonstrated that *B. mucilaginosus* and *B. edaphicus* are not related to any of the previously described *Paenibacillus* taxa at the species level.

Table 1. Distinctive phenotypic characteristics of *B. edaphicus*, *B. mucilaginosus* and closely related strains

Strains: 1, *B. edaphicus* VKPMB-7517^T; 2, *B. mucilaginosus* VKPMB-75179^T; 3, CGMCC 1.153; 4, CGMCC 1.231; 5, CGMCC 1.2326; 6, CGMCC 1.3714; 7, KNP414; 8, KNP413; 9, *P. validus* CCTCC 95016^T; 10, *B. subtilis* ACCC 92068. All of the strains possessed rod-shaped cells and ellipsoidal spores and were negative in tests for the production of H₂S. All strains were positive in tests for catalase and for acid production from sucrose and glucose. +, Positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10
Swollen sporangia	+	+	+	+	+	+	+	+	+	–
Motility	–	–	–	–	–	–	–	–	+	+
Capsule	+	+	+	+	+	+	+	+	–	–
Gram stain	–	–	–	–	–	–	–	–	+	+
Voges–Proskauer reaction	–	–	–	–	–	–	+	–	–	+
Oxidase	–	–	+	–	+	+	–	–	–	+
Nitrate reduction	+	–	+	+	+	+	+	+	+	+
Gelatin hydrolysis	–	–	–	–	–	–	–	–	–	+
Growth at 50 °C	–	–	–	–	–	–	–	–	+	+
Growth in NaCl (%):										
1.0	+	+	+	+	–	+	+	+	+	+
1.5	+	+	+	+	–	–	–	+	+	+
2.0	+	+	+	+	–	–	–	–	+	+
2.5	–	–	–	–	–	–	–	–	–	+
DNA G + C content (mol%)	54.7	55.7	55.3	54.9	55.1	54.5	55.9	56.8	51.3	43.5

Cell-wall peptidoglycan was purified and the cell-wall amino acids and the peptides in the cell-wall hydrolysates were analysed by two dimensional ascending TLC on cellulose plates using the solvent systems of Schleifer & Kandler (1972). The isomer of diaminopimelic acid was identified using the method of Rhuland *et al.* (1955). Analyses of polar lipids and menaquinones were performed according to published procedures (Groth *et al.*, 1999). The peptidoglycan type of the two species was A1 γ , based on *meso*-diaminopimelic acid. The major polar lipids were phosphatidylglycerol and diphosphatidylglycerol, while the major isoprenoid quinone was MK-7 (94.1–95.7 %). These data were identical to and consistent with the chemotaxonomic properties characteristic of the genus *Paenibacillus* (Shida *et al.*, 1997; Kämpfer *et al.*, 2006; Chou *et al.*, 2009).

The cellular fatty acid compositions (FAME) of *B. edaphicus* VKPM B-7517^T and the *B. mucilaginosus* strains were analysed with the Sherlock Microbial Identification System (MIS Inc.), with *P. validus* strain CCTCC 95016^T as the control. All aspects of the procedure were conducted according to the instructions for the MIDI System (Microbial ID; Sasser, 1990). The resulting FAME profiles were compared with those of known species in the MIDI database, TSBA version 4.10. The major cellular fatty acids of *B. edaphicus* and the *B. mucilaginosus* strains were anteiso-C_{15:0} (43.19–48.45 %), C_{16:1} ω 11c (10.78–14.20 %) and C_{16:0} (8.72–19.83 %). The complete cellular fatty acid profiles are presented in Table 2. Based on the maximum similarity of 1.2–20.2 %, the MIDI identification program indicated that *B. edaphicus* and *B. mucilaginosus* were related to *P. validus*. The profiles were similar to the previously determined profile of *B. mucilaginosus* VKPM B-7519^T, but different from that of *B. edaphicus* VKPM

B-7517^T as determined by Shelobolina *et al.* (1997). The FAME profile difference observed for strain VKPM B-7517^T could be attributed to the different methods utilized in the determination of the FAME profiles. The standard method was used in this study, but Shelobolina *et al.* (1997) developed their own method. The differences between the two methods may be a result of the media utilized; TSBA medium containing nitrogen was used in the standard method instead of Aleksandrov medium. It has already been reported that *B. edaphicus* strains can be significantly influenced by the composition of the medium, especially by the presence of nitrogen (Shelobolina *et al.*, 1997). We also observed that colonies of strain VKPM B-7517^T grown on TSBA medium were small and opaque and were clearly different from those cultured on Aleksandrov medium. Therefore, an observed difference in the FAME profiles of the cultures grown on the two different media is understandable. The predominant anteiso-C_{15:0} fatty acids observed in the profiles of the *B. edaphicus* and *B. mucilaginosus* strains confirmed their association with the genus *Paenibacillus*.

Other characteristics, including Gram stain, Voges–Proskauer reaction, nitrate reduction, gelatin liquefaction, enzymic activities (catalase, oxidase) and NaCl tolerance, were determined according to the methods of Claus & Berkeley (1986). All strains of *B. mucilaginosus* and *B. edaphicus* tested (Table 2) shared similar morphologies with *P. validus* CCTCC 95016^T, except for the presence of a flagellum. Although the presence of a flagellum is a characteristic shared by most species in the genus *Paenibacillus*, there are some species without flagella (Smerda *et al.*, 2005; Sanchez *et al.*, 2005). *B. mucilaginosus* and *B. edaphicus* were variable in tests for oxidase and

Table 2. Fatty acid contents (%) of strains of *B. edaphicus* and *B. mucilaginosus*

Strains: 1, *B. edaphicus* VKPM B-7517^T; 2, *B. mucilaginosus* VKPM B-7519^T; 3, CGMCC 1.153; 4, CGMCC 1.231; 5, CGMCC 1.2326; 6, CGMCC 1.3714; 7, KNP414; 8, KNP413; 9, *P. validus* CCTCC 95016^T. ND, Not detected.

Fatty acid	1	2	3	4	5	6	7	8	9
C _{14:0} iso	1.72	2.97	1.53	1.65	1.00	2.25	3.86	1.84	3.53
C _{14:1} ω5c	ND	2.29	0.45	0.45	0.98	1.55	1.22	0.81	ND
C _{14:0}	1.48	2.64	2.20	2.32	2.37	3.68	4.70	2.86	2.02
C _{15:0} iso	7.41	4.35	3.94	5.29	4.31	4.64	1.36	5.29	10.94
C _{15:0} anteiso	47.59	48.45	43.66	44.97	47.21	48.09	43.19	46.98	50.85
C _{15:0}	3.50	1.99	0.76	0.97	1.34	0.86	1.62	ND	0.93
C _{16:0} iso	4.95	3.09	3.07	2.94	2.00	2.81	3.87	2.63	7.41
C _{16:1} ω11c	12.04	11.10	11.92	10.78	11.85	13.95	14.20	11.32	7.95
C _{16:0}	8.72	10.00	16.19	16.08	19.83	15.74	13.82	15.97	7.36
C _{17:0} anteiso	4.48	2.77	6.38	4.32	4.13	2.82	1.70	3.46	5.52
C _{17:0} iso	3.05	1.86	2.82	2.69	1.83	ND	ND	1.80	3.15
C _{16:1} ω7c/C _{15:0} iso 2-OH	3.65	2.09	3.27	3.82	3.67	5.17	7.01	7.05	2.31

nitrate reactions, positive for catalase activity, and negative for the Gram and Voges–Proskauer reactions, for gelatin liquefaction and for the production of H₂S. They could not grow at 50 °C or in media containing 2.5 % (w/v) NaCl. From these observations, it is obvious that *B. mucilaginosus* and *B. edaphicus* have phenotypic characteristics in common with strains of the genus *Paenibacillus* (Shida *et al.*, 1997).

The phylogenetic and phenotypic results demonstrate that *B. edaphicus* VKPM B-7517^T and *B. mucilaginosus*, as represented by strain VKPM B-7519^T, are phylogenetically closer to the genus *Paenibacillus* than to the genus *Bacillus*. However, they are independent of any recognized species of the genus *Paenibacillus*. Therefore, it is proposed that *B. edaphicus* and *B. mucilaginosus* be transferred to the genus *Paenibacillus* as *Paenibacillus mucilaginosus* comb. nov. and *Paenibacillus edaphicus* comb. nov., respectively.

Description of *Paenibacillus mucilaginosus* comb. nov.

Paenibacillus mucilaginosus (mu.ci.la.gi.no'sus. L. masc. adj. *mucilaginosus* slimy, sticky).

Basonym: *Bacillus mucilaginosus* Avakyan *et al.* 1998.

The description is identical to that given by Shelobolina *et al.* (1997), with the exception that the Gram stain is negative. The major isoprenoid quinone is MK-7 (94.1–95.7 %). The peptidoglycan type is A1γ (*meso*-diaminopimelic acid) and the major polar lipids are phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are anteiso-C_{15:0}, C_{16:1}ω11c and C_{16:0}. The type strain is HSCC 1605^T (=VKPM B-7519^T=VKM B-1480D^T=CIP 105815^T=KCTC 3870^T).

Description of *Paenibacillus edaphicus* comb. nov.

Paenibacillus edaphicus (e.da.phi'cus. Gr. n. *edaphos* soil; N.L. masc. adj. *edaphicus* of soil).

Basonym: *Bacillus edaphicus* Shelobolina *et al.* 1998.

The description is similar to that given by Shelobolina *et al.* (1997), except that Gram stain is negative and the major fatty acids are anteiso-C_{15:0}, C_{16:1}ω11c and C_{16:0}. The major isoprenoid quinone is MK-7 (94.7 %). The peptidoglycan type is A1γ (*meso*-diaminopimelic acid) and the major polar lipids are phosphatidylglycerol and diphosphatidylglycerol. The type strain is VKPM B-7517^T (=DSM 12974^T=CIP 105814^T).

Acknowledgements

The authors would like to thank Dr Yuri Rybakov (Institute of Genetics of Industrial Microorganisms, Russia) for kindly donating *B. mucilaginosus* VKPM B-7519^T and *B. edaphicus* VKPM B-7517^T. This research was financially supported by the National High Technology Research and Development Program of China (Project No. 2006AA10Z428).

References

- Achouak, W., Normand, P. & Heulin, T. (1999). Comparative phylogeny of *rrs* and *nifH* genes in the *Bacillaceae*. *Int J Syst Bacteriol* **49**, 961–967.
- Aleksandrov, V. G., Blagodyr, R. N. & Ilev, I. P. (1967). Liberation of phosphoric acid from apatite by silicate bacteria. *Mikrobiol Z (Kiev)* **29**, 111–114.
- Ash, C., Priest, F. G. & Collins, M. D. (1993). Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks, and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek* **64**, 253–260.
- Avakyan, Z. A., Pivovarova, T. A. & Karavaiko, G. I. (1986). Characteristics of a new *Bacillus mucilaginosus* species. *Mikrobiologiya* **55**, 477–482 (in Russian).
- Avakyan, Z. A., Pivovarova, T. A. & Karavaiko, G. I. (1988). *Bacillus mucilaginosus* sp. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 66. *Int J Syst Bacteriol* **48**, 631–632.
- Chou, J. H., Lee, J. H., Lin, M. C., Chang, P. S., Arun, A. B., Young, C. C. & Chen, W. M. (2009). *Paenibacillus contaminans* sp. nov.,

isolated from a contaminated laboratory plate. *Int J Syst Evol Microbiol* **59**, 125–129.

Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1140. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. *Can J Microbiol* **41**, 109–117.

Groth, I., Schumann, P., Martin, K., Schuetze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (1999). *Ornithinococcus hortensis* gen. nov., sp. nov., a soil actinomycete which contains L-ornithine. *Int J Syst Bacteriol* **49**, 1717–1724.

Hu, X., Chen, J. & Guo, J. (2006). Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World J Microbiol Biotechnol* **22**, 983–990.

Kämpfer, P., Rosselló-Mora, R., Falsen, E., Busse, H. J. & Tindall, B. J. (2006). *Cohnella thermotolerans* gen. nov., sp. nov., and classification of '*Paenibacillus hongkongensis*' as *Cohnella hongkongensis* sp. nov. *Int J Syst Evol Microbiol* **56**, 781–786.

Liu, W. X., Xu, X. S., Wu, X. H., Yang, Q. Y., Luo, Y. M. & Christie, P. (2006). Decomposition of silicate minerals by *Bacillus mucilaginosus* in liquid culture. *Environ Geochem Health* **28**, 133–140.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955). The behavior of the isomers of α,ϵ -diaminopimelic acid on paper chromatograms. *J Am Chem Soc* **77**, 4844–4846.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sanchez, M. M., Fritze, D., Blanco, A., Sproer, C., Tindall, B. J., Schumann, P., Kroppenstedt, R. M., Diaz, P. & Pastor, F. I. J. (2005). *Paenibacillus barcinonensis* sp. nov., a xylanase producing bacterium isolated from a rice field in the Ebro River delta. *Int J Syst Evol Microbiol* **55**, 935–939.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Shelobolina, E. S., Avakyan, Z. A., Boulygina, E. E., Turova, T. P., Lysenko, A. M., Osipov, G. A. & Karavaiko, G. I. (1997). Description of a new species of mucilaginosus bacteria, *Bacillus edaphicus* sp. nov. and confirmation of the taxonomic status of *Bacillus mucilaginosus* Avakyan *et al.* 1986 based on data from phenotypic and genotypic analysis. *Microbiology* (English translation of *Mikrobiologiya*) **66**, 681–689.

Shelobolina, E. S., Avakyan, Z. A., Boulygina, E. E., Turova, T. P., Lysenko, A. M., Osipov, G. A. & Karavaiko, G. I. (1998). *Bacillus edaphicus* sp. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 66. *Int J Syst Bacteriol* **48**, 631–632.

Sheng, X. F., Huang, W. Y. & Yin, Y. X. (2000). Effects of application of silicate bacteria fertilizer and its potassium release. *J Nanjing Agric Univ* **23**, 43–46 (in Chinese).

Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). 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*. *Int J Syst Bacteriol* **47**, 289–298.

Smerda, J., Sedlacek, I., Pacova, Z., Durnova, E., Smiskova, A. & Havel, L. (2005). *Paenibacillus mendelii* sp. nov., from surface sterilized seeds of *Pisum sativum* L. *Int J Syst Evol Microbiol* **55**, 2351–2354.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Takeda, M., Kamagata, Y., Shinmaru, S., Nishiyama, T. & Koizumi, J. (2002). *Paenibacillus koleovorans* sp. nov., able to grow on the sheath of *Sphaerotilus natans*. *Int J Syst Evol Microbiol* **52**, 1597–1601.

Tcherpakov, M., Ben-Jacob, E. & Gutnick, D. L. (1999). *Paenibacillus dendritiformis* sp. nov., proposal for a new pattern-forming species and its localization within a phylogenetic cluster. *Int J Syst Bacteriol* **49**, 239–246.

Yamada, S., Ohashi, E., Agata, N. & Venkateswaran, K. (1999). Cloning and nucleotide sequence analysis of *gyrB* of *Bacillus cereus*, *B. thuringiensis*, *B. mycoides*, and *B. anthracis* and their application to the detection of *B. cereus* in rice. *Appl Environ Microbiol* **65**, 1483–1490.

Yamamoto, S. & Harayama, S. (1995). PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol* **61**, 1104–1109.