Paenibacillus prosopidis sp. nov., isolated from the nodules of *Prosopis farcta*

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A bacterial strain, designated PW21^T, was isolated from root nodules of *Prosopis farcta* in Tunisia. Phylogenetic analysis based on 16S rRNA gene sequences placed the isolate into the genus *Paenibacillus*, with its closest relatives being *Paenibacillus glycanilyticus* DS-1^T and *Paenibacillus castaneae* Ch-32^T with identity values of 96.9%. DNA–DNA hybridization measurements showed values of less than 25% with respect to these two species. The isolate was a Gram-variable, motile and sporulating rod. Catalase activity was positive and oxidase activity was weakly positive. Aesculin, CM-cellulose, xylan and starch were hydrolysed but casein and gelatin were not. Acetoin production was weakly positive and nitrate reduction was negative. Urease production was negative. Growth was supported by many carbohydrates and organic acids as carbon sources. MK-7 was the predominant menaquinone and anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{15:0} were the major fatty acids. Major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, a glycolipid, six phospholipids, an unidentified lipid and two unknown aminophosphoglycolipids. *meso*-Diaminopimelic acid was not detected in the peptidoglycan. The DNA G+C content of the isolate was 52.9 mol%. Phylogenetic, chemotaxonomic and phenotypic analyses showed that strain PW21^T should be considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus prosopidis* sp. nov. is proposed. The type strain is PW21^T (=LMG 25259^T =CECT 7506^T =DSM 22405^T).

Prosopis farcta is a legume species widely distributed in North Africa and the Middle East. It is the only species of the genus *Prosopis* that is native to Tunisia and grows naturally in two distinct areas, in the north-east and the south-east, respectively (Pottier-Alapetite, 1979). It is known that this species mainly prefers arid conditions, is well adapted to drought and high temperatures, and exhibits a high degree of salt-tolerance (Dafni & Negbi, 1978). Legumes are

nodulated by several species of rhizobia, and nodules of species of the genus *Prosopis* in Africa are produced by *Mesorhizobium plurifarum* (de Lajudie *et al.*, 1998), *Rhizobium etli* (Odee *et al.*, 2002) *Sinorhizobium saheli* (de Lajudie *et al.*, 1994), *Sinorhizobium kostiense* and *Sinorhizobium arboris* (Nick *et al.*, 1999). However, it is known that other bacteria may be found in legume root nodules (Trujillo *et al.*, 2006a, b; García-Fraile *et al.*, 2008). In the present study, we describe a novel non-nodulating strain belonging to the genus *Paenibacillus*, PW21^T, isolated from root nodules of *Prosopis farcta* growing in Tunisia. Based on genotypic and phenotypic characterization, strain PW21^T should be classified as a novel species of the genus *Paenibacillus*, for which we propose the name *Paenibacillus prosopidis* sp. nov.

Root nodules used for isolation of strain PW21^T were washed several times with sterile distilled water and were

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *P. prosopidis* PW21^T is FJ820995.

Electron micrographs of strain PW21^T, a neighbour-joining tree based on nearly complete 16S rRNA gene sequences and a two-dimensional thin-layer chromatogram of polar lipids of strain PW21^T are available with the online version of this paper.

then surface sterilized in ethanol (95 %, v/v) for 15 s and then in HgCl_2 (2.5 %, w/v) for 2 min. Nodules were rinsed five times with sterile distilled water and then crushed using a sterile glass rod. Homogenized nodule tissue was inoculated on modified yeast extract mannitol agar (YMA; Vincent, 1970; 10 g mannitol l^{-1} , 1 g yeast extract l^{-1} , 0.2 g K_2HPO_4 l^{-1} , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ l^{-1} , 0.5 g NaCl l^{-1} , 20 g agar l^{-1}) and the plates were incubated at 28 °C for 4 days. Cultures used in further phenotypic and molecular studies were purified from a single colony after 2 days of incubation at 28 °C on YMA. After isolation, the strain was grown in TY medium (3 g yeast extract l^{-1} , 4 g tryptone l^{-1} , 0.9 g CaCl_2 l^{-1} , 20 g agar l^{-1}) or TSA (Difco, Becton Dickinson, BBL). On TSA medium, colonies of strain PW21^T were white-cream, round, smooth and convex with approximate diameters of 1–3 mm.

Strain PW21^T was grown in TY agar for 48 h at 22 °C to check for motility by phase-contrast microscopy using the hanging drop method. Gram staining was carried out by the procedure described by Doetsch (1981) after 24 h of incubation at 28 °C. The flagellation type was determined by electron microscopy after 48 h of incubation in TSA at 22 °C as previously described by Rivas *et al.* (2005). For scanning electron microscopy, cells were fixed overnight in phosphate buffer (pH 7.0) containing 2 % paraformaldehyde and 0.2 % glutaraldehyde, dehydrated through a

graded ethanol series, critical-point dried and sputter-coated with gold. Samples were observed under a Zeiss DSM 490 electron microscope. Strain PW21^T was Gram-variable and motile by means of a polar flagellum [Supplementary Fig. S1(a), available in IJSEM Online]. Round, terminal spores were formed in slightly or non-swollen sporangia [Supplementary Fig. S1(b)].

The 16S rRNA gene of strain PW21^T was analysed as described by Rivas *et al.* (2007). The sequence obtained was compared with those from the GenBank database using the BLASTN (Altschul *et al.*, 1990) and EzTaxon (Chun *et al.*, 2007) programs. Sequences were aligned using CLUSTAL X software (Thompson *et al.*, 1997). Distances were calculated according to Kimura's two-parameter method (Kimura, 1980). A phylogenetic tree was inferred by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Felsenstein, 1983) methods. Bootstrap analysis was based on 1000 resamplings. The MEGA4.0 package (Tamura *et al.*, 2007) was used for all analyses. The resulting neighbour-joining tree corresponding to 16S rRNA gene sequences is shown in Fig. 1 (an extended tree is available as Supplementary Fig. S2). Similar results were obtained by using the maximum-parsimony method (data not shown). The results of the phylogenetic analyses indicated that strain PW21^T is related to members of the genus *Paenibacillus*. Strain PW21^T formed a cluster together with *Paenibacillus*

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relatedness among strain PW21^T and representative species of the genus *Paenibacillus*. *Lactobacillus delbrueckii* subsp. *lactis* DSM 20072^T was used as an outgroup. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 2 nt substitutions per 100 nt.

castaneae Ch-32^T, *Paenibacillus glycanilyticus* DS-1^T and *Paenibacillus xinjiangensis* B538^T with 96.9, 96.9 and 96.4 % sequence identities, respectively.

For base composition analysis, DNA was prepared according to Chun & Goodfellow (1995). The mol% G+C content of DNA was determined by using the thermal denaturation method (Mandel & Marmur, 1968). The DNA G+C content of strain PW21^T was 52.9 mol%. DNA–DNA hybridization was performed by using the method of Ezaki *et al.* (1989), following the recommendations of Willems *et al.* (2001). DNA–DNA relatedness values between strain PW21^T and *P. castaneae* Ch-32^T, *P. glycanilyticus* DS-1^T and *P. xinjiangensis* B538^T were lower than 25 %, confirming that the new isolate represents a novel species of the genus *Paenibacillus* according to the current species concept (Wayne *et al.*, 1987).

Chemotaxonomic analyses were carried out by the Identification Service and Dr B. J. Tindall, DSMZ, Braunschweig, Germany. The respiratory quinones and polar lipids were analysed as described by Tindall (1990) and the cellular fatty acids according to the instructions of the Microbial Identification System (MIDI; Microbial ID). For analysis of the peptidoglycan, whole cells of strain PW-21^T were hydrolysed with HCl at 100 °C for 15 h. The hydrolysates were subjected to thin-layer chromatography on cellulose plates using the solvent system of Rhuland *et al.* (1955). Menaquinone 7 (MK-7) was the major respiratory quinone in strain PW21^T and the presence of *meso*-diaminopimelic acid was not detected in its peptidoglycan. The fatty acid profile of strain PW21^T consisted of anteiso-C_{15:0} (48.98 %), iso-C_{16:0} (15.30 %), iso-C_{15:0} (10.09 %), C_{16:0} (4.75 %), anteiso-C_{17:0} (4.59 %), iso-C_{17:0} (5.68 %), iso-C_{14:0} (2.39 %), C_{15:0} (1.96 %), C_{16:1ω11c} (1.78 %), C_{16:1ω7c} alcohol (1.71 %), iso-C_{17:1ω10c} (1.05 %) and other compounds in proportions lower than 1 %. The fatty acid profile of strain PW21^T differs with respect to those of *P. glycanilyticus* DS-1^T and *P. castaneae* Ch-32^T in the proportions of iso-C_{17:0}, iso-C_{16:0}, iso-C_{15:0} and C_{16:0} and with respect to that of *P. xinjiangensis* DSM 16970^T mainly in the proportions of C_{16:0} and iso-C_{16:0} (Supplementary Table S1, available in IJSEM online). The lipid profile of strain PW21^T (Supplementary Fig. S3) consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, a glycolipid, six phospholipids, an unidentified lipid, and two unknown aminophosphoglycolipids. This profile was very different to that reported for *Paenibacillus polymyxa* by Kämpfer *et al.* (2006); nevertheless, as polar lipids have not been analysed in most species of the genus *Paenibacillus*, at this time they are mainly useful for differentiation from adjacent genera such as *Cohnella* (Kämpfer *et al.*, 2006), *Saccharibacillus* (Rivas *et al.*, 2008) and *Fontibacillus* (Saha *et al.*, 2010).

Phenotypic characterization was performed according to the standard methods described by Claus & Berkeley (1986) and Logan & Berkeley (1984) and by using the API 20NE, API20E and API50CH systems (bioMérieux)

according to the manufacturer's instructions. Anaerobic growth was tested in fluid tetrathionate medium (Sigma). Acetoin production, ability to grow in the presence of 2, 5 and 7 % NaCl, nitrate reduction, and phenylalanine deaminase, catalase, gelatinase, caseinase and oxidase activities were analysed as described by Claus & Berkeley (1986). Acid production from glucose, xylose, mannitol and L-arabinose and gas production from glucose were analysed in liquid medium as described by Claus & Berkeley (1986). Amylases, xylanases and cellulases were analysed as described earlier using TSA as basal medium supplemented with the polysaccharides at 7 g l⁻¹ (Rivas *et al.* 2003). Growth was determined at temperatures ranging from 4 to 45 °C in TSA medium. Growth at pH 5.7 and 6.8 was tested as described by Claus & Berkeley (1986); growth at pH 7–8 was tested in TY medium containing 200 mM Na₂HPO₄/NaH₂PO₄ and growth at pH 9 and 10 was tested in the same medium containing 200 mM NaHCO₃/Na₂CO₃. The type strains of the most closely related species, *P. glycanilyticus*, *P. castaneae* and *P. xinjiangensis*, were included in the phenotypic study as references. Results are given in the species description below. Strain PW21^T could be differentiated from *P. glycanilyticus* by colony colour, ability to grow at pH 5.7 and assimilation of L-rhamnose, glycerol and N-acetylglucosamine as carbon source, from *P. castaneae* by colony colour, ability to grow at pH 5.7, gelatin and starch hydrolysis and assimilation of L-rhamnose as carbon source, and from *P. xinjiangensis* by ability to hydrolyse starch and to assimilate L-rhamnose, glycerol and mannitol as carbon source (Table 1).

Strain PW21^T can be genotypically and phenotypically differentiated from previously described species and therefore represents a novel species, which we propose to name *Paenibacillus prosopidis* sp. nov.

Description of *Paenibacillus prosopidis* sp. nov.

Paenibacillus prosopidis (pro.so'pi.dis. L. n. *prosopis* name of a plant, and also a botanical genus name; L. gen. n. *prosopidis* of *Prosopis*, isolated from *Prosopis farcta*).

Cells are Gram-variable and motile by means of a polar flagellum. Round, terminal spores are formed in slightly or non-swollen sporangia. Catalase-positive and weakly oxidase-positive. Colonies on nutrient agar medium are white-cream, round, smooth and convex with approximate diameters of 1–3 mm. The major quinone is MK-7 and *meso*-diaminopimelic acid is not detected in the peptidoglycan. The lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, a glycolipid, six phospholipids, an unidentified lipid, and two unknown aminophosphoglycolipids. The main fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{15:0}. Anaerobic growth is negative. Does not grow at pH 5.7 or at pH 9. Grows between pH 6.5 and 8 (optimal pH 7). Grows in the presence of 2 % NaCl but not 5 % NaCl. Grows at 37 °C but not at 4 °C. Growth at 40 °C is weak. Optimal temperature

Table 1. Differential phenotypic characteristics of strain PW21^T and phylogenetically related species of the genus *Paenibacillus*

Strains: 1, *Paenibacillus prosopidis* sp. nov. PW21^T (data from this study); 2, *Paenibacillus glycanilyticus* DS-1^T (Dasman *et al.*, 2002; Yoon *et al.*, 2005); 3, *Paenibacillus castaneae* Ch-32^T (Valverde *et al.*, 2008); 4, *Paenibacillus xinjiangensis* B538^T (Lim *et al.*, 2006). +, Positive reaction; –, negative reaction; w, weakly positive reaction; v, variable.

Characteristics	1	2	3	4
Gram stain	v	+	v	+
Colony colour	White–cream	Pinkish yellow	Yellow	White–cream
Oxidase	w	+*†	+	+*†
Growth at:				
pH 5.7	–	+	+	–
40 °C	w	+	–	–†
Voges-Proskauer reaction	w	–	–	+†
Hydrolysis of:				
Gelatin	–	–	+	–†
Starch	+	+	–‡	–
Assimilation of:				
D-Mannose	w	+	+	+
L-Rhamnose	–	+	+	+
Fructose	w	+	–	+
Glycerol	–	+	–	+
Mannitol	+	+	+	–
N-acetylglucosamine	–	+	–	w†

*Oxidase reported as negative by Yoon *et al.* (2005).

†Data from this study.

‡Negative in flooding with Lugol's iodine solution.

for growth is 30 °C. Nitrate is not reduced to nitrite. Produces β -galactosidase but does not produce indole, phenylalanine deaminase, urease, arginine dehydrolase, ornithine or lysine decarboxylase. Acetoin production is weak. Hydrolyses aesculin but not gelatin in API20NE and API20E, respectively. Hydrolyses cellulose, starch and xylan but not casein. In the API 20NE system, assimilation of glucose, L-arabinose, mannitol, maltose, gluconate and malate is positive and that of N-acetylglucosamine, caprate, citrate, adipate, L-malate and phenylacetate is negative. Assimilation of mannose is weakly positive. Acid, but not gas, is produced from glucose. In API 50CH, acid production from L-arabinose, D-ribose, D-xylose, methyl α -D-xyloside, galactose, glucose, dulcitol, sorbitol, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, raffinose and amygdalin is positive. Acid production from glycerol, erythritol, D-arabinose, L-xylose, adonitol, L-sorbose, L-rhamnose, inositol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, inulin, melezitose, xylitol, gentiobiose, turanose, L-lyxose, tagatose, D-fucose, L-fucose, D-arabinitol and L-arabinitol is negative. Acid production from mannose, fructose and glycogen is weakly positive. Hydrolysis of arbutin and aesculin is positive. The DNA G + C content of the type strain is 52.9 mol%.

The type strain, PW21^T (=LMG 25259^T =CECT 7506^T =DSM 22405^T), was isolated from the nodules of *Prosopis farcta* in Tunisia.

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References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872, 174^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Dafni, A. & Negbi, M. (1978). Variability in *Prosopis farcta* in Israel: seed germination as affected by temperature and salinity. *Isr J Bot* **27**, 147–159.
- Dasman, Kajiyama, S., Kawasaki, H., Yagi, M., Seki, T., Fukusaki, E. & Kobayashi, A. (2002). *Paenibacillus glycanilyticus* sp. nov., a novel species that degrades heteropolysaccharide produced by the

cyanobacterium *Nostoc commune*. *Int J Syst Evol Microbiol* 52, 1669–1674.

de Lajudie, P., Willems, A., Pot, B., Dewettinck, D., Maestrojuan, G., Neyra, M., Collins, M. D., Dreyfus, B., Kersters, K. & Gillis, M. (1994). Polyphasic taxonomy of rhizobia: emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov. and *Sinorhizobium teranga* sp. nov. *Int J Syst Bacteriol* 48, 715–733.

de Lajudie, P., Willems, A., Nick, G., Moreira, F., Molouba, F., Hoste, B., Torck, U., Neyra, M., Collins, M. D. & other authors (1998). Characterization of tropical tree rhizobia and description of *Mesorhizobium plurifarium* sp. nov. *Int J Syst Bacteriol* 48, 369–382.

Doetsch, R. N. (1981). Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*, pp. 21–33. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.

Felsenstein, J. (1983). Parsimony in systematics: biological and statistical issues. *Annu Rev Ecol Syst* 14, 313–333.

García-Fraile, P., Velázquez, E., Mateos, P. F., Martínez-Molina, E. & Rivas, R. (2008). *Cohnella phaseoli* sp. nov., isolated from root nodules of *Phaseolus coccineus* in Spain, and emended description of the genus *Cohnella*. *Int J Syst Evol Microbiol* 58, 1855–1859.

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.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16, 111–120.

Lim, J.-M., Jeon, C. O., Park, D.-J., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2006). *Paenibacillus xinjiangensis* sp. nov., isolated from Xinjiang province in China. *Int J Syst Evol Microbiol* 56, 2579–2582.

Logan, N. A. & Berkeley, R. C. W. (1984). Identification of *Bacillus* strains using the API system. *J Gen Microbiol* 130, 1871–1882.

Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* 12B, 195–206.

Nick, G., de Lajudie, P., Eardly, B. D., Suomalainen, S., Paulin, L., Zhang, X., Gillis, M. & Lindström, K. (1999). *Sinorhizobium arboris* sp. nov. and *Sinorhizobium kostense* sp. nov., isolated from leguminous trees in Sudan and Kenya. *Int J Syst Bacteriol* 49, 1359–1368.

Odee, D. W., Haukka, K., McInroy, S. G., Sprent, J. I., Sutherland, J. M. & Young, J. P. W. (2002). Genetic and symbiotic characterization of rhizobia isolated from tree and herbaceous legumes grown in soils from ecologically diverse sites in Kenya. *Soil Biol Biochem* 34, 801–811.

Pottier-Alapetite, G. (1979). *Flore de la Tunisie: Angiospermes-Dicotylédones, Apétales Dialypétales*, vol. 1. Tunis: Publications Scientifiques Tunisiennes. Ministère de l'Enseignement Supérieur et de la Recherche Scientifique et le Ministère de l'Agriculture.

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.

Rivas, R., Sánchez, M., Trujillo, M. E., Zurdo-Piñero, J. L., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2003). *Xylanimonas cellulosilytica* gen. nov., sp. nov., a xylanolytic bacterium isolated from a decayed tree (*Ulmus nigra*). *Int J Syst Evol Microbiol* 53, 99–103.

Rivas, R., Gutiérrez, C., Abril, A., Mateos, P. F., Martínez-Molina, E., Ventosa, A. & Velázquez, E. (2005). *Paenibacillus rhizosphaerae* sp. nov., isolated from the rhizosphere of *Cicer arietinum*. *Int J Syst Evol Microbiol* 55, 1305–1309.

Rivas, R., García-Fraile, P., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2007). Characterization of xylanolytic bacteria present in the bract phyllosphere of the date palm *Phoenix dactylifera*. *Lett Appl Microbiol* 44, 181–187.

Rivas, R., García-Fraile, P., Zurdo-Piñero, J. L., Mateos, P. F., Martínez-Molina, E., Bedmar, E. J., Sánchez-Raya, J. & Velázquez, E. (2008). *Saccharibacillus sacchari* gen. nov., sp. nov., isolated from sugar cane. *Int J Syst Evol Microbiol* 58, 1850–1854.

Saha, P., Krishnamurthi, S., Bhattacharya, A., Sharma, R. & Chakrabarti, T. (2010). *Fontibacillus aquaticus* gen. nov., sp. nov., isolated from a warm spring. *Int J Syst Evol Microbiol* 60, 422–428.

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

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24, 1596–1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25, 4876–4882.

Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 66, 199–202.

Trujillo, M. E., Kroppenstedt, R. M., Schumann, P., Carro, L. & Martínez-Molina, E. (2006a). *Micromonospora coriariae* sp. nov., isolated from root nodules of *Coriaria myrtifolia*. *Int J Syst Evol Microbiol* 56, 2381–2385.

Trujillo, M. E., Kroppenstedt, R. M., Schumann, P. & Martínez-Molina, E. (2006b). *Kribbella lupini* sp. nov., isolated from the roots of *Lupinus angustifolius*. *Int J Syst Evol Microbiol* 56, 407–411.

Valverde, A., Peix, A., Rivas, R., Velázquez, E., Salazar, S., Santa-Regina, I., Rodríguez-Barrueco, C. & Igual, J. M. (2008). *Paenibacillus castaneae* sp. nov., isolated from the phyllosphere of *Castanea sativa* Miller. *Int J Syst Evol Microbiol* 58, 2560–2564.

Vincent, J. M. (1970). The cultivation, isolation and maintenance of rhizobia. In *A Manual for the Practical Study of Root-Nodule Bacteria*, pp. 1–13. Edited by J. M. Vincent. Oxford: Blackwell Scientific.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37, 463–464.

Willems, A., Doignon-Bourcier, F., Goris, J., Coopman, R., de Lajudie, P., De Vos, P. & Gillis, M. (2001). DNA-DNA hybridization study of *Bradyrhizobium* strains. *Int J Syst Evol Microbiol* 51, 1315–1322.

Yoon, J.-H., Kang, S.-J., Yeo, S.-H. & Oh, T.-K. (2005). *Paenibacillus alkaliterrae* sp. nov., isolated from an alkaline soil in Korea. *Int J Syst Evol Microbiol* 55, 2339–2344.