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

Angel Valverde,1 Amira Fterich,2 Mosbah Mahdhi,2 Martha-Helena Ramírez-Bahena,3† Miguel A. Caviedes,4 Mohamed Mars,2 Encarna Velázquez3 and Ignacio D. Rodriguez-Llorente4

1 Instituto de Recursos Naturales y Agrobiología de Salamanca, Consejo Superior de Investigaciones Científicas (IRNASA-CSIC), Salamanca, Spain
2 Laboratoire de Biotechnologies Végétales Appliquées a l’Amélioration des cultures, Faculté des Sciences de Gabes, Gabes, Tunisia
3 Departamento de Microbiología y Genética, Universidad de Salamanca, Salamanca, Spain
4 Departamento de Microbiología y Parasitología, Facultad de Farmacia, Sevilla, Spain

Correspondence
Encarna Velázquez
evp@usal.es

A bacterial strain, designated PW21T, 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-1T and *Paenibacillus castaneae* Ch-32T 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-C15 : 0, iso-C16 : 0 and iso-C15 : 0 were the major fatty acids. Major polar lipids were diphosphatidylglycerol, phosphatidylyethanolamine, 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 PW21T 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 PW21T (=LMG 25259T =CECT 7506T =DSM 22405T).

**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 plurifarium* (de Lajudie et al., 1998), *Rhizobium etli* (Odee et al., 2002) *Sinorhizobium saheli* (de Lajudie et al., 1994), *Sinorhizobium kostiensae* 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*, PW21T, isolated from root nodules of *Prosopis farcta* growing in Tunisia. Based on genotypic and phenotypic characterization, strain PW21T 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 PW21T were washed several times with sterile distilled water and were...
then surface sterilized in ethanol (95%, v/v) for 15 s and then in HgCl₂ (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 K₂HPO₄ l⁻¹, 0.2 g MgSO₄·7H₂O 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 CaCl₂ l⁻¹, 20 g agar l⁻¹) or TSA (Difco, Becton Dickinson, BBL). On TSA medium, colonies of strain PW21T were white–cream, round, smooth and convex with approximate diameters of 1–3 mm.

Strain PW21T 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 PW21T 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 PW21T 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 PW21T is related to members of the genus Paenibacillus. Strain PW21T formed a cluster together with Paenibacillus

![Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relatedness among strain PW21T and representative species of the genus Paenibacillus. Lactobacillus delbrueckii subsp. lactis DSM 20072T 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.](http://ijs.sgmjournals.org/2183)
The DNA thermal denaturation method (Mandel & Marmur, 1968).

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 PW21T 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 PW21T and *P. castaneae* Ch-32T, *P. glycanyticus* DS-1T and *P. xinjiangensis* B538T 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 PW21T 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 PW21T and the presence of meso-diaminopimelic acid was not detected in its peptidoglycan. The fatty acid profile of strain PW21T consisted of anteiso-C15:0 (48.98 %), iso-C16:0 (15.30 %), iso-C15:0 (10.09 %), C16:0 (4.75 %), anteiso-C17:0 (4.59 %), iso-C17:0 (5.68 %), iso-C14:0 (2.39 %), C15:0 (1.96 %), C16:0 iso11c (1.78 %), C16:1o7c (1.71 %), iso-C17:1o10c (1.05 %) and other compounds in proportions lower than 1 %. The fatty acid profile of strain PW21T ares with respect to those of *P. glycanyticus* DS-1T and *P. castaneae* Ch-32T in the proportions of iso-C17:0 iso-C16:0 iso-C15:0 and C16:0 and with respect to that of *P. xinjiangensis* DSM 16970T mainly in the proportions of C16:0 and iso-C16:0 (Supplementary Table S1, available in IJSEM online). The lipid profile of strain PW21T (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 NaHPO4/Na2HPO4 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. glycanyticus*, *P. castaneae* and *P. xinjiangensis*, were included in the phenotypic study as references. Results are given in the species description below. Strain PW21T could be differentiated from *P. glycanyticus* 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 PW21T 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. prosopidis name of a plant, and also a botanical genus name; L. gen. n. prosopidis of *Prospis*, isolated from *Prospis 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-C15:0, iso-C16:0, and iso-C15:0. 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...
The type strain, PW21T (the type strain is 52.9 mol% DNA G+C content of the type strain is 52.9 mol%), was isolated from the nodules of *Prosopis farcta* in Tunisia.

### Table 1. Differential phenotypic characteristics of strain PW21T and phylogenetically related species of the genus Paenibacillus

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
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<tr>
<td>Gram stain</td>
<td>v</td>
<td>+</td>
<td>v</td>
<td>+</td>
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<tr>
<td>Colony colour</td>
<td>White–cream</td>
<td>Pinkish yellow</td>
<td>Yellow</td>
<td>White–cream</td>
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<tr>
<td>Oxidase</td>
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<td>+ +†</td>
<td>Yellow</td>
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<tr>
<td>Growth at: pH 5.7</td>
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<td>–</td>
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<tr>
<td>40 °C</td>
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<td>Voges-Proskauer reaction</td>
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<td>Starch</td>
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<td>Assimilation of:</td>
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<td>+</td>
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*Oxidase reported as negative by Yoon *et al.* (2005).
†Data from this study.
‡Negative in flooding with Lugol’s iodine solution.

Acknowledgements

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