Paenibacillus rhizosphaerae sp. nov., isolated from the rhizosphere of Cicer arietinum

Raúl Rivas,1 Carmen Gutiérrez,2 Adriana Abril,3 Pedro F. Mateos,1 Eustoquio Martínez-Molina,1 Antonio Ventosa2 and Encarna Velázquez1

1Departamento de Microbiología y Genética, Lab. 209, Edificio Departamental de Biología, Universidad de Salamanca, Campus M. Unamuno, 37007 Salamanca, Spain
2Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain
3Catedra de Microbiología Agrícola, Facultad de Agronomía, Universidad de Córdoba, Argentina

Two sporulating bacterial strains designated CECAP06T and CECAP16 were isolated from the rhizosphere of the legume Cicer arietinum in Argentina. Almost-complete 16S rRNA gene sequences identified the isolates as a Paenibacillus species. It was most closely related to Paenibacillus cineris LM4 18439T (99-6% sequence similarity), Paenibacillus favisporus LMG 20987T (99-4% sequence similarity) and Paenibacillus azoreducens DSM 13822T (97-7% sequence similarity). The cells of this novel species were motile, sporulating, rod-shaped, Gram-positive and strictly aerobic. The predominant fatty acids were anteiso-C15:0, C16:0 and iso-C16:0. The DNA G+C content of strains CECAP06 and CECAP16 was 51-3 and 50-9 mol%, respectively. Growth was observed from many carbohydrates, but gas production was not observed from glucose. Catalase and oxidase activities were present. The isolates produced β-galactosidase and hydrolysed aesculin. Gelatinase, caseinase and urease were not produced. The results of DNA–DNA hybridization showed that the strains from this study constitute a novel species of the genus Paenibacillus, for which the name Paenibacillus rhizosphaerae sp. nov. is proposed. The type strain is CECAP06T (=LMG 21955T = CECT 5831T).

The genus Paenibacillus (Ash et al., 1994) contains several recently described species isolated from plant rhizospheres (Elo et al., 2001; Berge et al., 2002; Daane et al., 2002; von der Weid et al., 2002). Moreover, many species of this genus, including the type species, Paenibacillus polymyxa, have been reported as plant growth-promoting rhizobacteria (Lebuhn et al., 1997; Timmusk & Wagner, 1999; Timmusk et al., 1999; Helbig, 2001; Beatty & Jensen, 2002; Maes & Baeyen, 2003; von der Weid et al., 2003). Nevertheless, many rhizospheric bacterial species present in many soils remain unknown and their identification may be valuable for future studies of plant growth promotion.

In the present report, we describe the chemotaxonomic, morphological, phylogenetic and physiological characteristics of two bacterial strains, CECAP06T and CECAP16, isolated from the rhizosphere of Cicer arietinum, a legume cultivated in the Chaco Arido (Argentina). The soils from the Chaco Arido are of alluvial origin (Abril et al., 2005) and have been severely and extensively degraded by over-grazing and deforestation. In the dry winter season, the water balance is negative, resulting in a soil moisture deficit. C. arietinum is a legume that is able to grow in semi-arid soils and which may be an alternative crop in sustainable agriculture and soil recovery. On the basis of the data presented in this work, strains CECAP06T and CECAP16 should be placed in a novel species of the genus Paenibacillus, for which the name Paenibacillus rhizosphaerae sp. nov. is proposed.

The isolation was carried out on nutrient agar, as described by Peix et al. (2003), from the rhizospheric soil of two C. arietinum plants, at flowering stage, growing in a soil from the Chaco Arido (Argentina). The colonies of the strains isolated were cream-coloured, opaque, rounded and convex.

The strains were grown on nutrient agar medium for 48 h to check for motility, using phase-contrast microscopy (Axioskop 2; Zeiss). Cells were gently suspended in sterile water, stained with 0-2% (w/v) uranyl acetate and examined at 80 kV with a Zeiss EM 209 transmission electron
microscope (Peix et al., 2003). For scanning electron microscopy, cells and spores were treated as described previously (Valverde et al., 2003) and were observed under a Philips PSEM 500 electron microscope. The Gram behaviour of cells was ascertained by staining (Doetsch, 1981). Cells of CECAP06T and CECAP16 were Gram-positive, rod-shaped, sporulating, motile by means of subpolar flagella and commonly observed as single cells. The spores were subterminal, oval and caused slight swelling of the sporangia (see Supplementary Fig. A available in IJSEM Online). The spore ornamentation resembles that of *Paenibacillus favisporus* (Velázquez et al., 2004).

Amplification and sequencing of the 16S rRNA gene was performed as described by Rivas et al. (2003a). The sequences obtained were compared with sequences from the GenBank database, using the MegaBLAST program (Ma et al., 2002). Sequences were aligned using CLUSTAL W software (Thompson et al., 1997). The distances were calculated according to the method of Kimura (1980). Phylogenetic trees were inferred using neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1993) and maximum-parsimony methods (Felsenstein, 1983). Bootstrap analysis was based on 1000 resamplings. The MEGA 2.1.0 package (Kumar et al., 2001) was used for all analyses.

The almost-complete (1547 nt) 16S rRNA gene sequences of isolates CECAP06T and CECAP16 were compared with those held in the GenBank database; we show, here, that the strains from this study are phylogenetically related to species of the genus *Paenibacillus*. Fig. 1 shows the phylogenetic tree obtained with the neighbour-joining method (taken from Supplementary Fig. B in IJSEM Online). The same results were obtained when maximum-likelihood and maximum-parsimony methods were used (data not shown); Fig. 1 shows bootstrap values only for those branches that were found in all three analyses. The results obtained showed that the novel species is closely related to *Paenibacillus cineris*, *P. favisporus* and *Paenibacillus azoreducens*. Percentage differences based on pair-wise sequence comparisons were determined using complete 16S rRNA gene sequences. The data obtained showed 99·6·% similarity with *P. cineris* LMG 18439T, 99·4·% similarity with *P. favisporus* LMG 20987T and 97·7·% similarity with *P. azoreducens* DSM 13822T.

DNA–DNA hybridization analyses and determination of DNA G+C contents were performed as described by Arahl et al. (2001). DNA–DNA hybridization studies were carried out according to the competition procedure of the membrane method described by Johnson (1994). The hybridization temperature was 51 °C, which is within the limit of validity for the filter method (De Ley & Tijtgat, 1970), and the percentage of hybridization was calculated according to Johnson (1994). Three independent determinations were carried out for each experiment and the results reported are mean values. The DNA G+C content of strains CECAP06T and CECAP16 was 51·3 and 50·9 mol%, respectively. These values are similar to those obtained for the phylogenetically closest species of the genus *Paenibacillus* (Meehan et al., 2001; Logan et al., 2004; Velázquez et al., 2004). The results of DNA–DNA hybridization showed 79·% hybridization between strains CECAP06T and CECAP16. The values for DNA hybridization of strain CECAP06T with *P. favisporus* LMG 20987T, *P. cineris* LMG 18439T and *P. azoreducens* DSM 13822T were 42, 45 and 18·%, respectively. The values for DNA hybridization of strain CECAP16 with the aforementioned *Paenibacillus* species were 46, 43 and <10·%, respectively. In terms of DNA–DNA hybridization, the threshold value for the definition of a species is considered to be 70·% (Wayne et al., 1987); consequently, our results indicate that the strains isolated in this study do not belong to any of the known species of *Paenibacillus*.

The fatty acid composition was analysed by GLC as described by Rivas et al. (2003b) and the results are shown in

![Fig. 1. Phylogenetic analysis of 16S rRNA gene sequences from *P. rhizosphaerae* CECAP06T and *P. rhizosphaerae* CECAP16 and those of type strains of closely related *Paenibacillus* species, using the neighbour-joining method. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Only those bootstrap values for the branches that are found with all three analyses used in this study are shown. Bar, 5 nt substitutions per 1000 nt.](image-url)
Table 1. The predominant fatty acids in the two strains from this study were anteiso-C15:0 and C16:0. Other fatty acids detected were iso-C16:0, iso-C15:0, anteiso-C17:0 and small amounts of iso-C17:0, C10:0, C14:0 and C15:0. According to these results, the fatty acid composition of strains CECAP06\textsuperscript{T} and CECAP16 is similar to those reported for P. azoreducens, P. cineris and P. favisporus (Meehan et al., 2001; Logan et al., 2004; Velázquez et al., 2004). The phenotypic characterization of the two novel isolates was performed according to the standard methods described by Claus & Berkeley (1986) and with the API 20E system (bioMérieux) (Logan & Berkeley, 1984), using strains P. favisporus DSM 13822\textsuperscript{T}, P. cineris DSM 18439\textsuperscript{T} and P. azoreducens DSM 13822\textsuperscript{T} as references. The API 20NE system was also used to characterize the isolates (according to the manufacturer’s instructions). Table 2 shows the distinctive phenotypic features of the novel species as well as those of closely related species belonging to the genus Paenibacillus. Strains CECAP06\textsuperscript{T} and CECAP16 differ only in terms of mannose assimilation. The main difference between the novel species and the closely related species is the inability of the former to grow under anaerobic conditions. This characteristic is quite rare among Paenibacillus species, but the species Paenibacillus chibensis, Paenibacillus validus, Paenibacillus cardianalyticus and Paenibacillus kobensis are also unable to grow under anaerobic conditions (Shida et al., 1997). Moreover, the novel species differs from P. cineris with respect to growth in presence of 5 % (w/v) NaCl, from P. favisporus with respect to the Voges–Proskauer reaction, growth at 50°C and acid production from L-arabinose, and from P. azoreducens with respect to the oxidase test, the production of H\textsubscript{2}S, growth in presence of 5 % (w/v) NaCl and acid production from L-arabinose. Therefore, the phenotypic differences between our isolates and phylogenetically related Paenibacillus species are in agreement with the DNA–DNA hybridization studies that support the placement of these isolates within a novel species. Therefore, on the basis of these polyphasic taxonomic data, we propose that the isolates from this study should be classified as members of a novel Paenibacillus species, for which the name Paenibacillus rhizosphaerae sp. nov. is proposed.

Description of Paenibacillus rhizosphaerae sp. nov.

Paenibacillus rhizosphaerae [rhi.zo.spha.‘rae. Gr. fem. n. rhiza root; L. fem. n. sphaera -ae (from Gr. fem. n. sphaira -as) ball, any globe, sphere; N.L. gen. fem. n. rhizosphaerae of the rhizosphere].

Cells are rod-shaped, 3–0–3·1×0·9–1·0 μm and motile by means of peritrichous flagella. Spores are in a subterminal position in the cells and cause slight swelling of the sporangia. Colonies grown on nutrient agar (for 48 h at 28°C) are circular, convex, cream-coloured, opaque and usually 1–3 mm in size. Strictly aerobic and Gram-positive. Growth occurs at 10–37°C and at pH 5–9. The optimum growth temperature is 28°C and the optimum pH is 7. Grows without NaCl and with up to 5·0 % (w/v) NaCl. Oxidase- and catalase-positive. Gas is not produced from glucose. The following phenotypic and biochemical characteristics were obtained by using the bioMérieux API 20E...
and API 20NE systems. Acid is produced from D-glucose, L-arabinose, sucrose, rhamnose, melibiose, xylose, amygdalin and mannitol. N-Acetylglucosamine, maltose and gentio-bose are used as carbon sources. Mannose is assimilated by strain CECAP16, but not by strain CECAP06T. Inositol, sorbitol, citrate, propionate, caprate, adipate, maleate and phenylacetate are not used as sole sources of carbon. Produces β-galactosidase but not gelatinase, urease, caseinase, phenylalanine deaminase, lysine decarboxylase, arginine dehydrodase, ornithine decarboxylase, tryptophan deaminase, tyrosinase, indole, dihydroxyacetone or hydrogen sulfide. Produces acetoin (in Voges–Proskauer medium) and reduces nitrate to nitrite. The predominant fatty acids are anteiso-C₁₅:₀, C₁₆:₀ and iso-C₁₆:₀.

The type strain, CECAP06T (=LMG 21955T = CECT 5831T), was isolated from the rhizosphere of the legume *Cicer arietinum* in Argentina. The G+C content of its DNA is 50–9 mol%.

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**References**


