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Paenibacillus telluris sp. nov., a Novel Phosphate-Solubilizing Bacterium Isolated from Soil

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A phosphate-solubilizing bacterial strain designated PS38^T was isolated from farm soil. The isolate was a Gram-positive, motile, endospore-forming, rod-shaped bacterium. It grew optimally at 37°C and pH 7.5. The predominant cellular fatty acids were anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, and iso-$C_{16:0}$. The DNA G+C content was 49.5 mol% and the predominant menaquinone was MK-7. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain PS38^T belonged to the genus *Paenibacillus* and was most closely related to *Paenibacillus chibensis* JCM 9905^T, *P. barengoltzii* SAFN-016^T, *P. timonensis* 2301032^T, and *P. motobuensis* MC10^T with 96.3%, 96.0%, 95.9%, and 95.5% 16S rRNA gene sequence similarity, respectively. On the basis of morphological, chemotaxonomic, physiological, and phylogenetic properties, strain PS38^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus telluris* sp. nov. is proposed. The type strain is PS38^T (=KCTC 13946^T =CGMCC 1.10695^T).

***Keywords*:** *Paenibacillus telluris* sp. nov., taxonomy, Gram-positive

Phosphorus (P) is an essential element for all living cells and one of the major macronutrients for plants. P is applied to soil in the form of phosphatic fertilizers, but a large portion of mineral phosphate is in an insoluble form and so is unavailable to plants (Goldstein, 1986). Free-living phosphate-solubilizing microorganisms (PSM) are always present in soils and the ability of soil microorganisms to convert insoluble forms of P to an accessible form is important for increasing plant yields. The densities of PSM can be very low $<10^2$ CFU/g of soil were reported in a soil in Northern Spain (Peix *et al*., 2001). In this study, we isolated and identified phosphate-solubilizing bacteria (PSB) from a farming field. The genus *Paenibacillus* was originally proposed by Ash *et al*. (1993) (valid publication by Ash *et al*., 1994) its members are facultative anaerobes or strict aerobes, produce spores in swollen sporangia and have DNA G+C contents that range from 45-54 mol%.

Materials and Methods

Collection of microorganisms

During the screening for PSB, strain PS38^T was isolated from an upland farming soil (36°20′08.80″N 127°27′18.89″E) at Daejeon, Korea. For isolation, soil samples were diluted serially with 0.85% NaCl (w/v). Aliquots of each serial dilution were spread on Luria-Bertani agar (LB Difco) containing 0.5% (w/v) tricalcium phosphate (TCP) as the sole P source for selective screening of bacteria having the ability to release inorganic phosphate from tricalcium phosphate (Nautiyal *et al*., 2000). After 1-2 days of incubation at 37°C, PSB produced clear zones around colonies. PS38^T was selected and was routinely grown aerobically on tryptic soy broth (TSB Difco) for 24 h at 37°C, except where indicated otherwise.

Phylogenetic analysis

Amplification and sequencing of the 16S rRNA gene of strain PS38^T was carried out as described by Lee and Yoon (2008). Briefly, a single colony of strain PS38^T grown on TSA was resuspended in 100 μl of 5% (w/v) Chelex-100 solution (Bio-Rad, USA) and boiled for 5 min to prepare crude genomic DNA lysates. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the Eubac 27F and 1492R primers (DeLong, 1992) and the PCR products were purified using the QIAquick PCR Purification kit (QIAGEN, Germany). Sequencing of the purified 16S rRNA gene was performed using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems, USA) as recommended by the manufacturer using five primers (337F, 785F, 1225F, 518R, 1100R). The purified reaction mixtures were electrophoresed automatically using an Applied Biosystems model 3730XL automatic DNA sequencer. The resultant 16S rRNA gene sequence of strain PS38^T was compared with available sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/ BLAST/) to determine an approximate phylogenetic affiliation and was aligned with those of closely related members using the CLUSTAL W program (Thompson *et al*., 1994). Sequence similarity values were computed using the EzTaxon server (http://www.eztaxon.org/Chun *et al.*, 2007). Gaps at the 5′- and 3′- ends of the alignment were omitted for further analyses. Phylogenetic trees were inferred using three tree-making algorithms, the neighbor-joining (NJ), maximum-likelihood (ML), and maximum-parsimony (MP) method available in the PHYLIP software package version 3.65 (Felsenstein, 2005). Evolutionary distance matrices for the NJ method were calculated using the algorithm of the Kimura 2-parameter model (Kimura, 1980) with the DNADIST program. To evaluate the stability of the phylogenetic tree, a bootstrap analysis (1,000 replications) was per-

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formed with SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE programs in the PHYLIP package.

Phenotypic characterization

Cell biomass of strain PS38^T was obtained from TSB cultures at 37°C in a rotary shaker at 150 rpm. Cell growth was monitored by measuring the optical density at 600 nm. Requirement for, and tolerance of, NaCl (0-10%) were determined in TSA. To test for growth in the absence of NaCl, the salt was omitted from TSB. Growth was tested at different temperatures (4-60°C) and pH values (5.0-10.0) in TSB. Cell morphology was studied using light microscopy and transmission electron microscopy. Motility was observed at 12 and 36 h in wet mounts using a Nikon E600 light microscope. The flagellum type was examined by transmission electron microscopy using cells from the exponential growth phase. Cells were mounted on Formvar-coated copper grids and negatively stained with 1% potassium phosphotungstate (pH 7.0). Grids were examined in a Phillips 201 transmission electron microscope operating at 80 kV. Endospores were stained as described previously (Smibert and Kreig, 1981). Gram reaction was determined using the bioMérieux Gram Stain kit according to the manufacturer's instructions and a 3% KOH test. Catalase activity was determined by bubble production in 3% (v/v) aqueous hydrogen peroxide. Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine using a Bactident Oxidase strip (Merck). The hydrolysis of compounds was checked on TSA according to the methods described by Cowan and Steel (1965), Lanyi (1987), and Smibert and Krieg (1994). Nitrate reduction and hydrolysis of tyrosine, Tween-20, Tween-80, aesculin, casein, starch, and gelatin were performed as previously described (Lanyi, 1987). Carbon-source utilization tests, acid-production tests and additional physiological tests were performed using API 50 CH (combined with API 50CHB/E), API 20NE and API ZYM galleries according to the instructions of the manufacturer (bioMérieux). Growth under anaerobic conditions was determined in an anaerobic chamber (Mart Microbiology) in an atmosphere of 5% H_2, 10% CO_2, and 85% N_2 after 2 days incubation at 37°C on TSA.

Chemotaxonomy

Fatty acid methyl esters were analyzed by gas chromatography/mass spectrometry according to the instructions of the Sherlock Microbial Identification System (MIDI) (Sasser, 1990) with the TSBA40 database. Biomass for cellular acid analysis was prepared from a 24 h culture grown on TSA at 30°C. Isoprenoid quinones were analyzed as described previously (Komagata and Suzuki, 1987) using a high-performance liquid chromatography (HPLC) apparatus fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM). Methanol/2-propanol (2:1, v/v) was used as the mobile phase and quinone was detected at 270 nm. The peptidoglycan structure was elucidated by the DSMZ identification service. The G+C content (mol %) was determined by reverse-phase HPLC using the method of Tomaoka and Komagata (1984).

The GenBank accession number for the 16S rRNA gene sequence of strain PS38^T is HQ257247.

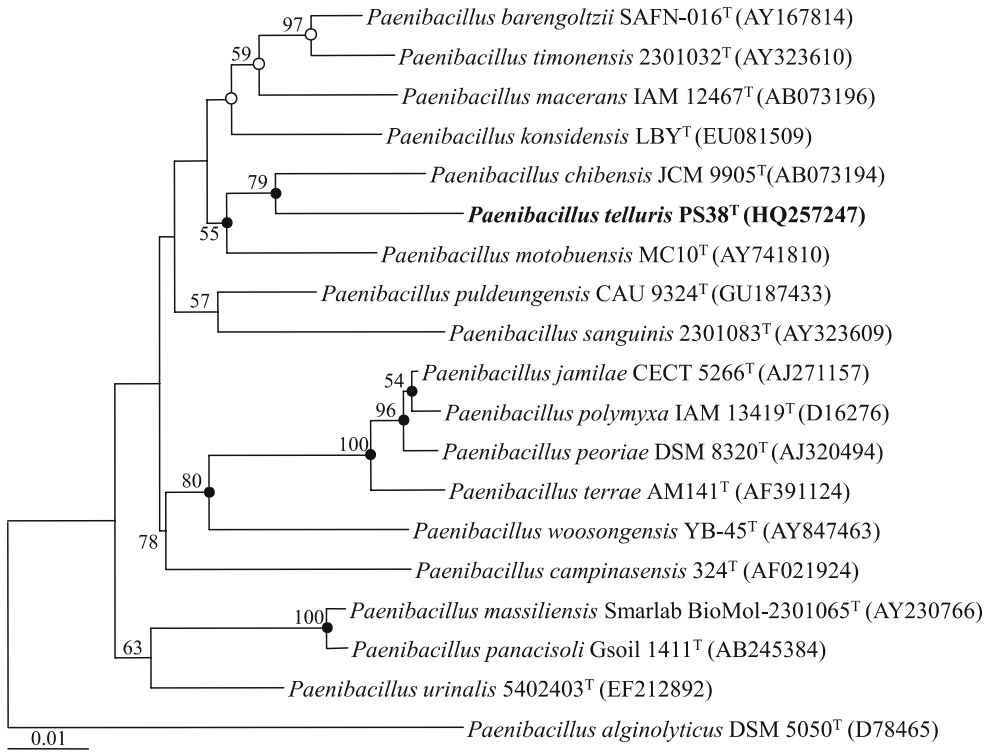

Fig. 1. NJ tree based on 16S rRNA gene sequences, showing phylogenetic relationship of strain PS38^T and related taxa. Bootstrap values (percentages of 1,000 replications) are shown where >50%. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum parsimony algorithms. Open circles denote node recovery with one of the algorithms. *P. alginolyticus* DSM 5050^T was used as an outgroup. The scale bar equals 0.01 substitutions per nucleotide position.

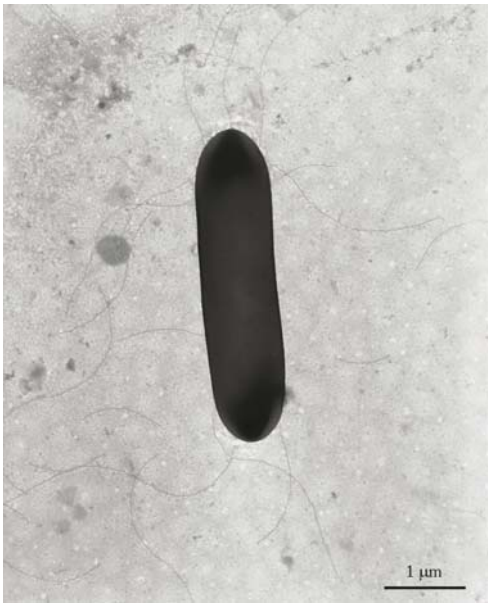

Fig. 2. Transmission electron micrograph showing general morphology of negatively stained cells of strain PS38^T after growth for 24 h at 37°C on TSA.

Results and Discussion

Phylogenetic analysis

The almost complete 16S rRNA gene sequence (1,484 nt) of strain PS38^T was obtained and the 16S rRNA gene sequence search using BLAST revealed that strain PS38^T exhibited low levels of similarity with respect to the recognized species of *Paenibacillus*, which was sufficient to indicate that strain PS38^T represented a novel member of the genus (Stackebrandt and Goebel, 1994). The 16S rRNA gene sequence similarities of strain PS38^T to *P. chibensis* JCM 9905^T, *P. barengoltzi* SAFN-016^T, *P. timonensis* 2301032^T, *P. motobuensis* MC10^T, and *P. chibens* LBY^T were 96.3%, 96.0%, 95.9%, 95.5%, and 95.5%, respectively. The phylogenetic tree (Fig. 1) constructed by NJ analysis showed that strain PS38^T was affiliated to the genus *Paenibacillus* and formed a distinct phylogenetic line diverging from closely related species *P. chibensis* JCM 9905^T and *P. motobuensis* MC10^T with a 79% and 55% bootstrap value, respectively. The topologies of the phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms were similar to that of the NJ tree (Fig. 1).

Phenotypic characteristics

Strain PS38^T isolated on LB showed a high phosphate-solubilizing activity and formed colonies on TSA that were 1-1.5 mm in diameter, light yellow, low convex, and circular with slightly irregular edges, translucent and glossy after cultivation at 37°C within 24 h. The strain was a facultative anaerobe and cells of the isolates were motile rods (0.7-1.0 μm wide and 4.0-5.0 μm long) (Fig. 2). Growth was observed at temperatures ranging from 15-45°C; optimum growth occurred at 37°C and pH 7.0. The cells were Gram-positive, catalase-positive, oxidase-positive, and reduced nitrate to nitrite. The isolate produced an ellipsoidal terminal endospore in a swollen sporangium. Strain PS38^T was different from the phylogenetically related species in a 3% KOH test. Other physiological and biochemical characteristics are provided in Table 1 and species description.

Chemotaxonomy

The cellular fatty acid profile of the strain was characterized by the presence of saturated fatty acids, mainly anteiso-$C_{15:0}$ (33.5%), $C_{16:0}$ (20.0%), anteiso-$C_{17:0}$ (16.8%), and iso-$C_{16:0}$ (11.9%), following growth on TSA. This pattern was similar to those of related species of the genus *Paenibacillus*, but the value of anteiso-$C_{17:0}$ and $C_{16:0}$ was higher than the other close species of *Paenibacillus* (Table 2). The predominant isoprenoid quinone was MK-7. The DNA G+C content of strain PS38^T was 49.5 mol%. The major fatty acid profile, isoprenoid qui-

Table 1. Phenotypic characteristics that differentiate strain PS38^T from related members of the genus *Paenibacillus*
Species: 1, strain PS38^T; 2, *P. chibensis* JCM 9905^T; 3, *P. motobuensis* MC10^T. All species formed swollen sporangia and were positive for anaerobic growth, catalase positive, acid production from arbutin, D-cellobiose, D-fructose, D-glucose, glycogen, maltose, D-mannose, D-mannitol, esculin, amygdalin, and salicin, and growth at pH 6.0. All species were negative for indole production and growth in the presence of 8% NaCl. All data are from this study. +, positive; −, negative

Characteristic	1	2	3
3% KOH test	−	+	+
Oxidase	+	+	−
Growth at 10°C	−	+	−
Growth in presence of 5% NaCl	+	−	+
ONPG test	−	+	+
H_2S production	−	−	+
Utilization of:			
Glucose	+	−	+
Arabinose	+	−	−
Mannose	+	−	−
Mannitol	+	−	−
N-Acetylglucosamine	+	−	+
Maltose	+	−	+
Acid production from:			
Glycerol	+	−	+
Methyl-β-D-xylopyranoside	−	+	+
L-Rhamnose	−	+	−
Methyl-α D-mannopyranoside	−	+	+
D-Trehalose	−	+	+
D-Melezitose	+	−	−
D-Raffinose	+	−	+
Xylitol	−	−	+
Gluconate	−	+	+
Enzyme activity:			
Alkaline phosphatase	+	−	−
Leucine arylamidase	−	+	+
Acid phosphatase	+	+	−
α-Galactosidase	+	+	−
β-Glucosidase	−	+	+
α-Fucosidase	−	+	−
DNA G+C content (mol%)	49.5	52.8	47.0

Table 2. Cellular fatty acid composition (%) of strains PS38^T and other related *Paenibacillus* type strains grown on TSA for 24 h at 30°C Strains: 1, PS38^T; 2, *P. chibensis* JCM 9905^T; 3, *P. motobuensis* MC10^T. All data from this study. Data are expressed as percentages of total fatty acids. Fatty acids representing <0.5% in all strains are not included.

Fatty acid	1	2	3
Saturated:			
$C_{14:0}$	1.3	1.4	2.6
$C_{16:0}$	20.0	10.9	17.5
Branched-chain:			
iso-$C_{14:0}$	1.7	1.8	2.1
iso-$C_{15:0}$	5.8	8.9	13.2
iso-$C_{16:0}$	11.9	14.2	9.9
iso-$C_{17:0}$	7.7	6.3	13.0
anteiso-$C_{15:0}$	33.5	43.7	29.5
anteiso-$C_{17:0}$	16.8	11.4	9.1

none and the DNA G+C content were typical of the group classically defined as the genus *Paenibacillus* (Ash *et al*., 1993).

In conclusion, the physiological, biochemical and phylogenetic properties of strain PS38^T support its description as a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus telluris* sp. nov. is proposed.

Description of *Paenibacillus telluris* sp. nov.

Paenibacillus telluris (L. gen. n. *telluris*, of the soil or earth). Cells are Gram-positive, endospore-forming (ellipsoidal, terminal) rods (0.7-1.0 μm wide and 4.0-5.0 μm long) that are motile by means of peritrichous flagella. It is an aerobic or facultatively anaerobic phosphate-solubilizing bacterium. Colonies on TSA medium are circular with slightly irregular edges, low convex, light yellow, translucent glossy and usually 1-1.5 mm in diameter within 24 h at 37°C. Growth occurs at 15-45°C (optimum, 37°C) and from pH 6.0-9.0 (optimum, pH 7.0). Grow occurs at salinities of 0-5% (w/v) NaCl (optimum, 3% NaCl). No growth occurs in the presence of more than 8% NaCl. Oxidase-positive and catalase-positive. No hydrogen sulfide and indole are produced, and the Voges-Proskauer and ONPG tests are negative. Nitrate is reduced to nitrite. Aesculin is hydrolysed; gelatin and urea are not hydrolysed. Glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, and gluconate are assimilated, but malate and citrate are not. Acid is produced from L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, methyl-α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, D-melezitose, D-raffinose, starch, glycogen, gentiobiose, and D-turanose. Acid is not produced from erythritol, D-arabinose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, inulin, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-D-gluconate, and 5-keto-D-gluconate. Alkaline phosphatase, esterase, esterase lipase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, and α-glucosidase are produced. Lipase, leucine arylamidase, valine arylamidase, crystine arylamidase, trypsin, α-chymotrypsin, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosamininase, α-mannosidase, and α-fucosidase are not produced. The predominant menaquinone is MK-7. The major fatty acid is anteiso-$C_{15:0}$. The G+C content of the type strain is 49.5 mol% (by HPLC).

The type strain is PS38^T (=KCTC 13946^T =CGMCC 1.10695^T), isolated from a soil at Daejeon in Korea.

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