Geodermatophilus ruber sp. nov., isolated from rhizosphere soil of a medicinal plant

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A novel actinobacterial strain, designated CPCC 201356^T, was isolated from a rhizosphere soil sample of the medicinal plant *Astragalus membranaceus* and subjected to a polyphasic taxonomic analysis. Good growth occurred at 20–32 °C, at pH 7.0–7.5 and with 0–1 % (w/v) NaCl. Colonies on R2A and ISP 2 agar were light red to red, round and lacked aerial mycelium; cells adhered to the agar. The peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid. The predominant menaquinones were MK-9(H_4) and MK-9. Polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine and two unknown phospholipids. The major cellular fatty acids were iso-$C_{16:0}$, iso-$C_{15:0}$ and $C_{17:1}\omega8c$. The G+C content of the genomic DNA was 72.8 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain CPCC 201356^T belonged to the family *Geodermatophilaceae* and consistently formed a distinct sub-branch with *Geodermatophilus obscurus* DSM 43160^T. The organism showed 16S rRNA gene sequence similarity of 97.7 % with *G. obscurus* DSM 43160^T. DNA–DNA hybridization between strain CPCC 201356^T and *G. obscurus* DSM 43160^T was 17.4 %. On the basis of evidence from this polyphasic taxonomic study, a novel species, *Geodermatophilus ruber* sp. nov., is proposed; the type strain is CPCC 201356^T (=DSM 45317^T =CCM 7619^T).

The family *Geodermatophilaceae* was initially proposed by Normand *et al.* (1996), but was only formally described recently (Normand, 2006). The family contains the genera *Geodermatophilus* (type genus), *Blastococcus* and *Modestobacter*. The present investigation was designed to clarify the taxonomic position of a novel strain belonging to the genus *Geodermatophilus*.

During a screening programme for new antibiotics, a bacterial colony was picked and purified on an R2A (DSMZ 830 medium) plate. The strain was isolated using the dilution plating method and incubation at 28 °C for 3 weeks. A rhizosphere soil sample from a medicinal plant, *Astragalus membranaceus*, was collected from Xining (37° 35′ N 101° 49′ E; elevation 2800 m), Qinghai Province, north-west China. The isolate, designated CPCC 201356^T, was maintained on R2A slants at 4 °C and as suspensions of cells in 20 % (v/v) glycerol. Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using R2A without agar or TSB (DSMZ 545 medium) incubated at 28 °C for 5 days. All physiological and biochemical tests were performed at 28 °C using *Geodermatophilus obscurus* DSM 43160^T in parallel experiments. Colony morphology was determined after 3 days at 28 °C on R2A and TSA media. Gram staining was carried out by the standard Gram reaction and observed by light microscopy (Olympus BH-2). Motility of cells was examined on TSB swarming agar (0.3 %, w/v). Cellular morphology was studied using a JEOL JEM-1010 electron microscope with cells from exponentially growing cultures. Oxidase activity was detected using API oxidase reagent according to the manufacturer's instructions. Catalase activity was determined by production of bubbles after the addition of a drop of 3 % H_2O_2. The temperature range and optimum for growth were tested at 4–55 °C on R2A medium. NaCl tolerance was tested and the pH range for growth was investigated between pH 4.0 and 10.0 (at intervals of 0.5 pH units) using the buffer system described by Xu *et al.* (2005). Carbon utilization and acid production were tested using Biolog GEN III MicroPlates and the API 50CH (bioMérieux) system according to the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CPCC 201356^T is EU438905.

manufacturers' instructions. Other physiological tests were examined as described previously (Yuan *et al.*, 2008).

Cells of strain CPCC 201356^T were Gram-reaction-positive, coccoid and motile with periplasmic flagella (Fig. 1). Light-red colonies with a maximum diameter of 1.2 mm were formed on R2A agar or TSA after incubation for 72 h at 28 °C. Colonies were opaque with a moist surface. Strain CPCC 201356^T grew well at 20–32 °C; poor growth was observed at 10 and 40 °C, and no growth occurred at 4 or 45 °C. Growth was observed at initial pH between 6.0 and 8.0 in R2A or TSB medium. The isolate grew optimally at pH 7.0–7.5 and in the presence of 0–1 % NaCl. Detailed physiological and biochemical characteristics of the strain are given in Table 1 and in the species description.

The diagnostic isomer of diaminopimelic acid in whole-cell hydrolysates (4 M HCl, 100 °C, 15 h) of strain CPCC 201356^T was identified by TLC on cellulose plates using the solvent system of Schleifer & Kandler (1972). Sugar analysis of whole-cell hydrolysates was carried out as described by Staneck & Roberts (1974). Polar lipids were extracted and examined by two-dimensional TLC and identified using previously described procedures (Minnikin *et al.*, 1984). Menaquinones were isolated using the method of Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). Analysis of the whole-cell fatty acid pattern followed described methods using the MIDI system (Microbial ID) (Kroppenstedt, 1985; Meier *et al.*, 1993).

The peptidoglycan type of strain CPCC 201356^T was A1γ (*meso*-diaminopimelic acid direct). No characteristic sugars were detected in whole-cell hydrolysates. The polar lipid profile contained significant amounts of phosphatidylethanolamine and diphosphatidylglycerol and small amounts of phosphatidylinositol; two unknown phospholipids were also detected. The predominant menaquinones were MK-9(H_4) (62.9 %) and MK-9 (37.1 %). The fatty acid profile was characterized by large amounts of iso-$C_{16:0}$ (24.8 %), iso-$C_{15:0}$ (13.0 %) and $C_{17:1}\omega8c$ (24.7 %) and smaller amounts of anteiso-$C_{17:0}$ (9.3 %), $C_{16:0}$ (7.8 %), anteiso-$C_{15:0}$ (5.1 %), $C_{18:1}\omega9c$ (4.0 %), $C_{17:0}$ (3.0 %), iso-$C_{16:1}$ H (3.0 %), $C_{16:1}\omega6c$ (2.2 %), iso-$C_{17:0}$ (1.3 %) and iso-$C_{14:0}$ (1.3 %).

Table 1. Differential phenotypic characteristics of strain CPCC 201356^T and *G. obscurus* DSM 43160^T

Data were obtained in this study.

Characteristic	CPCC 201356^T	*G. obscurus* DSM 43160^T
Colony colour on R2A	Light-red, red	Black
Colony surface on R2A	Moist	Dry
Nitrate reduction	+	−
Degradation of:		
Starch	−	+
Gelatin	−	+
Utilization as sole carbon source of:		
D-Arabitol	−	+
D-Mannose	+	−
Trehalose	−	+
D-Sorbitol	−	+
Raffinose	−	+
Rhamnose	−	+
Oxidase activity	−	+
Acid production from:		
D-Fructose	+	−
D-Arabinose	−	+
D-Glucose	−	+
Predominant menaquinone(s)	MK-9(H_4), MK-9	MK-9(H_4)
Major fatty acids (>10 %)*	i-$C_{16:0}$, i-$C_{15:0}$, $C_{17:1}\omega8c$	i-$C_{16:0}$, i-$C_{15:0}$, $C_{17:1}\omega8c$, $C_{18:1}\omega8c$

*i, iso-branched.

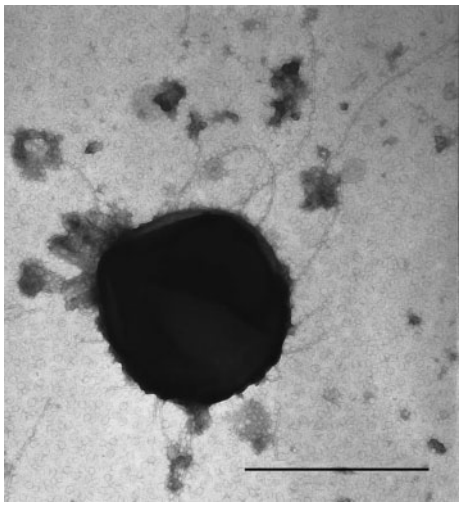

Fig. 1. Scanning electron micrograph of a cell of strain CPCC 201356^T grown on R2A medium for 4 days at 28 °C. Bar, 1 μm.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were conducted as described by Li *et al.* (2007). Multiple alignments with sequences of closely related strains were carried out using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983) and MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The G+C content of the genomic DNA was determined as 72.8 mol% by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989). DNA–DNA hybridization was carried out according to the thermal renaturation method (De Ley *et al.*, 1970) using a

UV-1700 spectrophotometer (Shimadzu) equipped with a DCW-2008 water bath. The hybridization temperature was 86 °C.

The almost-complete 16S rRNA gene sequence (1449 bp) of strain CPCC 201356^T was determined and subjected to comparative analyses. BLAST results showed that the closest relatives of strain CPCC 201356^T were members of the family *Geodermatophilaceae*, with highest 16S rRNA gene sequence similarity of 97.7 % with *G. obscurus* DSM 43160^T. The phylogenetic tree (Fig. 2) of the family *Geodermatophilaceae* based on 16S rRNA gene sequences showed that the novel isolate formed a stable distinct lineage with *G. obscurus* DSM 43160^T among members of the family *Geodermatophilaceae*, with a high bootstrap value of 98 %.

The chemotaxonomic characteristics of the novel strain readily distinguished it from *G. obscurus* DSM 43160^T (Table 1). Additionally, the DNA–DNA hybridization value between CPCC 201356^T and *G. obscurus* DSM 43160^T was 17.4 %, which was much lower than 70 %, the threshold value considered for the delineation of genomic species (Wayne *et al.*, 1987).

Based on the phenotypic (Table 1) and genotypic data presented above, it is proposed that strain CPCC 201356^T represents a novel species of the genus *Geodermatophilus*, with the name *Geodermatophilus ruber* sp. nov.

Description of *Geodermatophilus ruber* sp. nov.

Geodermatophilus ruber (ru′ber. L. masc. adj. *ruber* red).

Cells stain Gram-positive. Catalase-positive and oxidase-negative. Coccoid cells are motile with periplasmic flagella. Colonies are adherent. Newly formed colonies are light red, becoming red after growth for 4 days on R2A, TSA or ISP 2 agar. No diffusible pigments are produced on any medium tested. Utilizes acetic acid, D-fructose, D-glucose, D-mannose, inosine, L-malic acid, quinic acid and succinic acid as sole carbon sources for energy and growth, but not dextrin, D-arabitol, cellobiose, D-fucose, D-galactose, lactose, maltose, D-mannitol, D-rhamnose, D-sorbitol, trehalose, raffinose or sucrose. Acid is produced from D-fructose. L-Cysteine and L-proline can be used as sole nitrogen sources, but not adenine, glycine, hypoxanthine, L-alanine, L-arginine, L-asparagine, L-cystine, L-glutamic acid, L-histidine, L-lysine, L-phenylalanine, L-serine, L-threonine, L-tyrosine, L-valine or xanthine. Positive for reduction of nitrate, but negative for methyl red and Voges–Proskauer tests, cellulose and gelatin hydrolysis, casein and starch degradation, milk coagulation and peptonization and H_2S production. Tests for acid phosphatase, alkaline phosphatase, cystine arylamidase, leucine arylamidase, lipase, naphthol phosphohydrolase, trypsin, valine arylamidase, α-chymotrypsin, α-glucosidase and β-glucosidase are positive; tests negative for *N*-acetyl-β-glucosaminidase, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase and β-glucuronidase. NaCl tolerance range is 0–1 % (w/v). Growth occurs at pH 6.0–8.0 and 10–40 °C. Good growth occurs at 20–32 °C and pH 7.0–7.5. The cell wall contains *meso*-diaminopimelic acid as the diamino acid in the peptidoglycan, without the presence of a diagnostic sugar. The predominant menaquinones are MK-9(H_4) and MK-9. Polar lipids consist mainly of diphosphatidylglycerol, phosphatidylethanolamine and two unknown phospholipids. The major cellular fatty acids are iso-$C_{16:0}$, iso-$C_{15:0}$ and $C_{17:1}\omega 8c$. The genomic DNA G+C content of the type strain is 72.8 mol%.

The type strain is CPCC 201356^T (=DSM 45317^T =CCM 7619^T), isolated from a rhizosphere soil sample of the plant *Astragalus membranaceus*, collected from Xining, Qinghai Province, north-west China.

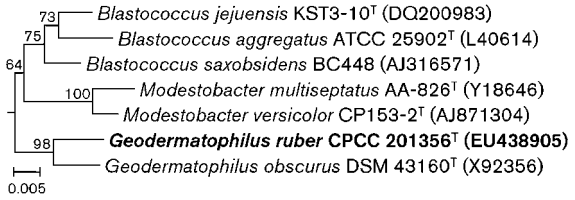

Fig. 2. Neighbour-joining tree derived from aligned 16S rRNA gene sequences showing the position of strain CPCC 201356^T among its phylogenetically nearest neighbours. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 0.005 substitutions per nucleotide position.

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