

Prauserella endophytica sp. nov., an endophytic actinobacterium isolated from *Tamarix taklamakanensis*

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Abstract A novel endophytic actinobacterium, designated strain SP28S-3^T, was isolated from a surface-sterilized stem of *Tamarix taklamakanensis* collected from the southern edge of Taklamakan desert, Xinjiang, China. Strain SP28S-3^T was found to show chemotaxonomic and morphological properties consistent with its classification in the genus *Prauserella*. The polar lipids were found to consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphoglycolipid, phosphatidylcholine, phosphatidylinositol, a glycolipid, an aminolipid and unidentified phospholipids. The major fatty acids (>10 %) were identified as iso-C_{16:0} and C_{16:0}. The genomic DNA G+C content was determined to be 69.7 mol%. Phylogenetic analysis of strain SP28S-

3^T clearly showed that the strain had the highest similarity of 16S rRNA gene sequence with *Prauserella coralliicola* SCSIO 11529^T (99.9 %), followed by *Prauserella marina* DSM 45268^T (97.0 %) and is affiliated with the genus *Prauserella*. The low level (47.8 ± 5.5 %) of DNA–DNA relatedness between strain SP28S-3^T and *P. coralliicola* SCSIO 11529^T combined with other polyphasic taxonomic evidence clearly support the conclusion that strain SP28S-3^T represents a novel *Prauserella* species, for which the name *Prauserella endophytica* sp. nov. is proposed. The type strain is SP28S-3^T (=DSM 46655^T = CGMCC 4.7182^T).

Keywords *Prauserella endophytica* sp. nov. · *Tamarix taklamakanensis* · Endophytic actinobacterium · Taklamakan desert

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Introduction

Since *Amycolatopsis rugosa* (Lechevalier et al. 1986) was reclassified by Kim and Goodfellow (1999), the genus *Prauserella* was established and then emended by Li et al. (2009). Members of this genus are aerobic, Gram-positive, non-motile actinobacteria which form extensively branched substrate mycelia which can fragment into irregular rod-shaped cells. They usually contain *meso*-diaminopimelic acid as the major diamino acid and arabinose and galactose as predominant sugars in the whole-cell hydrolysate; MK-9(H₄) as major menaquinone; either phosphatidylcholine or phosphatidylethanolamine as diagnostic polar lipid; and both branched and saturated fatty acids as major fatty acids (Kim and Goodfellow 2012). The range of DNA G+C contents is from 65.8 to 70.1 mol% (Li et al. 2003, 2009). At the time of writing, the genus comprises 11 validly named species and their 16S rRNA gene sequence similarities are from 95.4 % between *Prauserella halophila* and *Prauserella muralis* to 99.9 % between *Prauserella flava* and *Prauserella salsuginis* (Schäfer et al. 2010; Li et al. 2009). The level of DNA–DNA relatedness between the latter two species is 56.9 % (Kim and Goodfellow 2012). Seven of these 11 species were isolated from Xinjiang, northwest China, including *Prauserella shujinwangii* (Liu et al. 2014); *P. salsuginis*, *P. flava*, *Prauserella aidingensis*, *Prauserella sediminis* (Li et al. 2009); and *P. halophila* and *Prauserella alba* (Li et al. 2003).

During an investigation of the cultivable actinobacterial diversity associated with psammophytes collected from the southern edge of Taklamakan desert, Xinjiang, China, strain SP28S-3^T, the first endophytic actinobacterium affiliated with the genus *Prauserella*, was isolated from a surface-sterilized stem of *Tamarix taklamakanensis*. The present study was aimed to establish the taxonomic status of isolate SP28S-3^T. Based on phylogenetic analysis, the isolate SP28S-3^T showed relatively high levels of 16S rRNA gene sequence similarities to members of the genus *Prauserella*. A polyphasic taxonomic study showed that strain SP28S-3^T can be distinguished from previously described validly named species of the genus *Prauserella* and represents a novel species, for which the name *Prauserella endophytica* sp. nov. is proposed.

Materials and methods

Isolation and maintenance of the organism

The endophytic strain SP28S-3^T was isolated from internal tissue of a surface-sterilized stem sample of *T. taklamakanensis* collected from the southern edge of Taklamakan desert, Xinjiang, China. The sample was processed as described by Qin et al. (2009). After sterilization, the sample was ground into powder by using a micromill and distributed on SP agar media (containing l⁻¹: 1 g sodium propionate, 0.2 g L-asparaginate, 0.9 g KH₂PO₄, 0.6 g K₂HPO₄, 0.1 g MgSO₄·7H₂O, 0.02 g CaCl₂·2H₂O, 20.0 g agar) adjusted to pH 8.0 and supplemented with 1 % (v/v) plant tissue extract. The plates were incubated at 28 °C for 4 weeks. Colonies were transferred onto International *Streptomyces* Projects (ISP) 2 agar slants (Shirling and Gottlieb 1966) at pH 8.0 using the plating technique and were incubated at 28 °C until a pure isolate was obtained. The purified isolate was maintained at 4 °C on ISP 2 agar slants (pH 8.0) and preserved in aqueous glycerol suspensions (20 %, v/v) at –80 °C. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC) as strain CGMCC 4.7182^T and in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) as strain DSM 46655^T.

The reference strains *Prauserella coralliicola* SCSIO 11529^T and *Prauserella marina* DSM 45268^T were obtained from South China Sea Institute of Oceanology, Chinese Academy of Sciences (Guangdong, China) and the DSMZ (Braunschweig, Germany), respectively.

Phenotypic characteristics

Morphological properties of strain SP28S-3^T were observed by light microscopy (BH2; Olympus) and scanning electron microscopy (Quanta 200; FEI) using the coverslip technique described by Zhou et al. (1998) after growth for 14 days on ISP 2 agar plates at 28 °C. Cultural characteristics of strain SP28S-3^T were determined after 2 weeks growth at 28 °C and compared with those of the type strains *P. coralliicola* SCSIO 11529^T and *P. marina* DSM 45268^T on ISP 2, ISP 3, ISP 4, ISP 5, ISP 7 agars (Shirling and Gottlieb 1966), Bennett's agar (Jones 1949), Czapek's agar (Waksman 1961), nutrient agar (Difco), PDA

(Waksman 1961), R2A (Difco) and TSA (Difco). Colours of substrate mycelia, aerial mycelia and soluble pigment were determined by comparison with chips of the ISCC-NBS colour charts (Kelly 1964). The ranges of growth temperature (0, 4, 10, 18, 28, 37, 42 and 50 °C) and tolerance of NaCl concentrations (0, 1, 3, 5, 7, 10, 15 and 20 % w/v) were determined on ISP 2 agar plates for 2 weeks. The pH range for growth was determined in buffered ISP 2 liquid media of pH 4–12 (at intervals of 1 pH unit) for 2 weeks using the buffer system described by Wang et al. (2010) and Luo et al. (2008).

Gram-staining test was performed as described by Magee et al. (1975). Cell motility was studied on ISP 2 swarming agar (0.4 %, w/v). For physiological and biochemical studies, biomasses of strain SP28S-3^T and the two reference type strains were obtained after cultivation on ISP 2 agar plates (pH 8.0) at 28 °C for 7 days. Catalase and oxidase activities were tested in 3 % (v/v) H₂O₂ and 1 % (w/v) tetramethyl-*p*-phenylenediamine, respectively. Hydrolysis of starch, gelatin and Tween-20, Tween-40, Tween-80 was determined as described by Cowan and Steel (1965). Nitrate reduction was determined according to the method of Lányi (1987). Carbon utilization and acid production were determined by Biolog GEN III Microplates and API 50CH system (bioMérieux), respectively. Enzyme activities were examined using the API ZYM kit (bioMérieux) following the manufacturer's instructions. Other physiological and biochemical tests were performed with the methods described by Williams et al. (1983) and Kämpfer et al. (1991).

Chemotaxonomic characteristics

For analysis of menaquinones, polar lipids and cell wall components, strain SP28S-3^T and the two reference type strains were cultured in ISP 2 liquid medium at 28 °C for 7 days on a rotary shaker (180 r. p. m). Menaquinones were extracted as described by Collins et al. (1977), separated by HPLC (Groth et al. 1997) and then confirmed on a single quadrupole mass spectrometer LCMS-2020 (Shimadzu) as described by Guo et al. (2015). Polar lipids were extracted and examined by two-dimensional TLC on silica gel 60 F₂₅₄ plates (Merck) using methods described by Minnikin et al. (1984). Isomers of diaminopimelic acid and whole-cell sugar composition were identified

by TLC on cellulose plates using procedures described previously (Staneck and Roberts 1974; Schleifer and Kandler 1972, respectively). For analysis of fatty acids, biomass of strain SP28S-3^T and the two reference strains were harvested from ISP 2 agar plates at 28 °C, when the bacterial communities reached the late-exponential stage of growth according to the four quadrants steak method (Sasser 1990). The whole-cell fatty acids were saponified, methylated and extracted according to the method described by Kuykendall et al. (1988), and analyzed on an Agilent 7890A gas chromatograph coupled with an Agilent 5975C, and a single quadrupole mass spectrometer equipped with Nist08 Library software database. A capillary column HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Technologies) was used for separation of fatty acid methyl esters. The initial temperature was maintained at 90 °C for 1 min, raised to 180 °C at the rate of 10 °C/min, then to 210 °C at the rate of 2 °C/min, finally to 270 °C at the rate of 20 °C/min and kept at that temperature for 2 min. Helium was used as carrier gas with a flow rate of 1.0 ml/min. Injection (2 μl) was made in the splitless mode at an injector temperature of 270 °C. Mass spectra were obtained using electron impact (EI, 70 eV).

Phylogenetic analyses

The 16S rRNA gene was amplified from extracted genomic DNA as described by Li et al. (2007). The PCR amplicon was cloned using a pEASY-T1 cloning kit (TransGen Biotech) and sequenced using an ABI PRISMTM 3730XL DNA Analyzer. The similarity analysis of the 16S rRNA gene sequence was carried out using the EzTaxon server (<http://eztaxon-e.ezbiocloud.net/>; Kim et al. 2012). The 16S rRNA gene sequence of strain SP28S-3^T was aligned with corresponding sequences (obtained from the GenBank/EMBL/DDBJ database) using BioEdit (Hall 1999). Phylogenetic trees were generated with the maximum-parsimony (Fitch 1971), maximum-likelihood (Felsenstein 1981) and neighbour-joining (Saitou and Nei 1987) methods by using MEGA version 5 (Tamura et al. 2011). The stability of the clades in the trees was appraised by bootstrap analysis with 1000 replications (Felsenstein 1985). A distance matrix was generated using the Kimura two-parameter model (Kimura 1983).

DNA base composition and DNA–DNA relatedness

To calculate the G+C content, genomic DNA was prepared by the method described by Marmur (1961). The G+C content was determined by reverse-phase HPLC analysis and calculated from the ratio of nucleosides according to Mesbah et al. (1989).

DNA–DNA relatedness between strain SP28S-3^T and *P. coralliicola* SCSIO 11529^T was carried out in triplicate according to the thermal renaturation method (DeLey et al. 1970) by using a PharmaSpec UV-2550 UV–VIS Spectrophotometer (Shimadzu) equipped with a Peltier-thermostatted cell holder and a temperature controller (Model S-1700, Shimadzu) with an in situ temperature probe. The DNA concentration was adjusted spectrophotometrically to approximately OD₂₆₀ = 1 using 0.1 × saline sodium citrate (SSC). The renaturation rates of sheared DNA were determined at 81 °C.

Results and discussion

Molecular characteristics

Comparative analyses of the 16S rRNA gene sequence (1484 bp, GenBank/EMBL/DDJB accession number KM 670438) showed that strain SP28S-3^T is affiliated with the genus *Prauserella*. The highest similarity was found between strain SP28S-3^T and *P. coralliicola* SCSIO 11529^T, *P. marina* DSM 45268^T (99.9 and 97.0 %, respectively) and the similarities between strain SP28S-3^T and other *Prauserella* species were all less than 97.0 %, ranging from 96.4 % (*Prauserella rugosa*) to 95.4 % (*P. sediminis*). In the phylogenetic tree based on the neighbor-joining method, strain SP28S-3^T and *P. coralliicola* SCSIO 11529^T fell into a distinct subclade with *P. marina* DSM 45268^T. This subclade was associated with *P. rugosa* DSM 43194^T and *P. muralis* 05-Be-005^T. This phylogenetic relationship was also found and supported in trees constructed with maximum-likelihood and maximum-parsimony methods as shown in Fig. 1. Strain SP28S-3^T showed low DNA–DNA relatedness value of 47.8 ± 5.5 % to *P. coralliicola* SCSIO 11529^T, indicating that the whole genomic DNA relatedness value is well below the delineating 70 % cut-off point for bacterial species identification (Wayne et al. 1987).

Thus, strain SP28S-3^T should be considered as representing a new species of the genus *Prauserella*. We did not perform DNA–DNA hybridizations between strain SP28S-3^T and other type strains in the genus *Prauserella* because of the low 16S rRNA gene sequence similarities (≤97.0 %). The G+C base composition of strain SP28S-3^T was determined to be 69.7 mol%, which is in the range for the members of the genus *Prauserella* (Li et al. 2003, 2009).

Phenotypic characteristics

Strain SP28S-3^T was found to grow well on most of the tested media. Good growth was observed on ISP 2, ISP 3, ISP 4 and ISP 7 agars, Bennett's agar, Czapek's agar, nutrient agar, R2A and TSA; poor growth was observed on ISP 5 agar; and no growth was observed on PDA. The colour of the substrate mycelia ranged from yellowish white to strong orange. The mycelia were found to fragment into rod-shaped cells with 0.4 µm in width and 0.8–2.2 µm in length (Supplementary Fig. S1). White aerial mycelia could be observed after growth for 2 weeks on most of the tested media. No soluble pigments were observed. The detailed cultural characteristics of strain SP28S-3^T and the two reference type strains are shown in Supplementary Table S1. Growth of strain SP28S-3^T was observed at 18–42 °C, 0–7 % NaCl (w/v) and pH 6.0–11.0, with optimum growth at 28–37 °C, 0–5 % NaCl (w/v) and pH 8.0. The cells were found to be Gram-positive and non-motile. The detailed physiological and biochemical properties of strain SP28S-3^T and the two reference type strains are shown in Table 1 and in the species description.

Chemotaxonomic characteristics

The predominant menaquinone was identified as MK-9(H₄) (90.6 %) and the minor components as MK-8(H₄) (7.0 %) and MK-10(H₄) (2.4 %). The polar lipids were found to be comprised of diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylcholine, phosphatidylinositol, a glycolipid, an aminolipid and some unidentified phospholipids (Fig. 2). *Meso*-diaminopimelic acid was identified as the diamino acid; arabinose and galactose were also found in the whole-cell hydrolysate of the strain SP28S-3^T. The major fatty

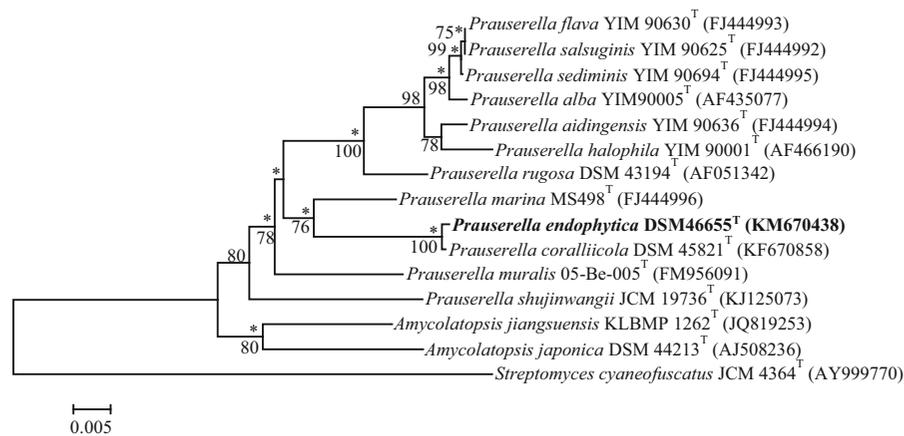


Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequences of strain SP28S-3^T and related strains in the genus *Prauserella*. Bootstrap values >50 % are shown at nodes. Bar,

acids were identified as iso-C_{16:0} (30.6 %) and C_{16:0} (28.7 %), along with lower amounts of C_{16:1}ω9c (7.5 %), C_{16:0} 2OH (7.3 %), C_{17:0} 10-methyl (7.0 %), anteiso-C_{17:0} (4.8 %), iso-C_{15:0} (4.5 %), C_{17:0} (4.0 %), C_{16:1}ω7c (3.9 %) and C_{14:0} (1.7 %). The comparison between the fatty acids of strain SP28S-3^T and the two reference type strains is shown in Table 2.

Taxonomic conclusion

Strain SP28S-3^T can be distinguished from the reference type strain *P. marina* DSM 45268^T not only by its low 16S rRNA gene sequence similarity (97.0 %) but also by differences in chemotaxonomic characteristics. Strain SP28S-3^T contained aminolipid and glycolipid as polar lipids, which were absent in *P. marina* DSM 45268^T (Fig. 2). Meanwhile, strain *P. marina* DSM 45268^T contained larger amounts of C_{14:0} and iso-C_{15:0} compared to strain SP28S-3^T (Table 2). Although strain SP28S-3^T has relatively high 16S rRNA gene sequence similarity (99.9 %) with the reference type strain *P. coralliicola* SCSIO 11529^T, they exhibit notable differences in chemotaxonomic characteristics. For example, the polar lipids were more varied in strain SP28S-3^T compared to strain *P. coralliicola* SCSIO 11529^T, as aminolipid, phosphoglycolipid, glycolipid and phosphatidylinositol were only found in strain SP28S-3^T; C_{16:1}ω9c was one of the major fatty acids in strain *P. coralliicola* SCSIO 11529^T but was a minor fatty acid in strain SP28S-3^T; and C_{16:0} 2OH was found exclusively in strain SP28S-3^T and not in strain *P. coralliicola* SCSIO 11529^T. The

0.005 changes per nucleotide position. Asterisks indicate branches that are also found using the maximum-likelihood and maximum-parsimony tree-making methods

predominant menaquinone in the three strains was found to be MK-9(H₄) but the minor menaquinone components were different (Table 1). Furthermore, differences were found in oxidase activity, milk coagulation and the hydrolysis of starch, Tween 20 and Tween 80. The assimilation of sole carbon sources, acid production and activities of *N*-acetyl-β-glucosaminidase, β-glucosidase, lipase (C₁₄) and α-mannosidase were also different (Table 1). In conclusion, the observed molecular, chemotaxonomic and phenotypic differences, especially the low DNA–DNA relatedness between isolate SP28S-3^T and the closest phylogenetic relative *P. coralliicola* SCSIO 11529^T, clearly support the conclusion that strain SP28S-3^T represents a new species of the genus *Prauserella* for which the name *Prauserella endophytica* sp. nov. is proposed.

Description of *Prauserella endophytica* sp. nov

Prauserella endophytica (en.do.phy'ti.ca. Gr. pref. *endo*, within; Gr. *phyton* plant; Gr. n. *phyton*, plant; L. fem. suff. *-ica*, adjectival suffix used with the sense of belonging to; N.L. fem. adj. *endophytica*, within plant, endophytic, pertaining to the original isolation from plant tissues).

An aerobic, Gram-positive, non-motile endophytic actinobacterium that forms mycelium-like filaments which can fragment into rod-shaped cells 0.4 μm in width and 0.8–2.2 μm in length. The colour of the substrate mycelia ranges from strong orange (on ISP 2 medium) to yellowish-white (on ISP 3 and ISP 4

Table 1 Phenotypic characteristics of strain SP28S-3^T and two reference type strains

Characteristic	SP28S-3 ^T	<i>P. coralliicola</i> SCSIO 11529 ^T	<i>P. marina</i> DSM 45268 ^T
Aerial mycelia (ISP 2 agar)	+	–	+
Optimum temperature (°C)	28–37	28–45	28–37
Optimum NaCl (%)	0–5	0–3	0–3
Optimum pH	8	7	7
Oxidase activity	+	–	–
Catalase activity	+	+	+
Milk coagulation	–	+	+
Hydrolysis of			
Gelatin	+	+	+
Starch	+	–	–
Tween 20	–	+	–
Tween 40	+	+	(+)
Tween 80	–	+	–
Carbon utilization			
D-Fructose	–	+	–
D-Galactose	+	–	+
<i>myo</i> -Inositol	+	–	–
Maltose	+	–	+
D-Mannose	–	–	+
Raffinose	+	+	–
L-Rhamnose	+	–	+
Acid production from			
D-Arabinose	–	+	–
L-Arabinose	–	–	+
D-Cellobiose	+	–	+
L-Fucose	–	+	–
Mannose	+	(+)	–
L-Rhamnose	–	+	–
D-Ribose	+	–	+
D-Xylose	–	+	+
Enzyme activity			
N-acetyl-β-glucosaminidase	+	–	+
β-Glucosidase	+	–	–
Lipase (C14)	–	–	(+)
α-Mannosidase	+	–	–
Naphthol-AS-BI-phosphohydrolase	+	(+)	+
Trypsin	–	+	–
Valine arylamidase	+	(+)	+
Menaquinones	MK-9(H ₄); MK-8(H ₄); MK-10(H ₄)	MK-9(H ₄); MK-8(H ₄)	MK-9(H ₄); MK-8(H ₄); MK-10(H ₄); MK-9(H ₆)
Predominant fatty acids	iso-C _{16:0} ; C _{16:0}	iso-C _{16:0} ; C _{16:0} ; C _{16:1} ω _{9c}	C _{16:0} ; iso-C _{16:0} ; iso-C _{15:0}

Table 1 continued

Characteristic	SP28S-3 ^T	<i>P. coralliicola</i> SCSIO 11529 ^T	<i>P. marina</i> DSM 45268 ^T
DNA G+C content (mol%)	69.7	70.2 ^a	67.2

Data for all strains were obtained in this study except where indicated. All strains are negative for H₂S production, indole production, cellulose degradation, urease production and nitrate reduction. All strains are negative for utilization of D-cellobiose and lactose. All strains are positive for utilization of D-sorbitol and trehalose. All strains are negative for acidification of D-fucose, D-lactose, D-raffinose, sorbose and L-xylose. All strains are positive for acidification of D-fructose, D-galactose, D-glucose, D-maltose and D-trehalose. All strains are positive for acid phosphatase, alkaline phosphatase, cysteine arylamidase, esterase (C4), esterase lipase (C8), α -glucosidase and leucine arylamidase. All strains are negative for α -chymotrypsin, α -galactosidase, α -fucosidase, β -galactosidase and β -glucuronidase

+ positive, – negative, (+) weakly positive

^a Data was taken from Wu et al. (2014)

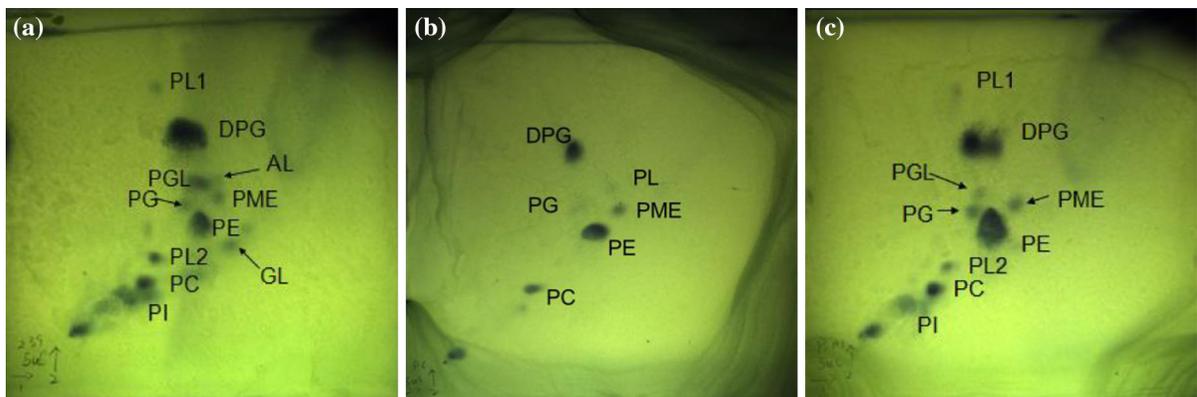


Fig. 2 Polar lipids of strain SP28S-3^T (a), *Prauserella coralliicola* SCSIO 11529^T (b) and *Prauserella marina* DSM 45268^T (c) were separated by two-dimensional TLC, detected by spraying with molybdatophosphoric acid reagent. DPG diphosphatidylglycerol,

PG phosphatidylglycerol, PGL phosphoglycolipid, GL glycolipids, PE phosphatidylethanolamine, PME phosphatidylmethylethanolamine, PC phosphatidylcholine, PI phosphatidylinositol, AL aminolipid, PL unidentified phospholipids

media) and aerial mycelia are white on most media. Temperature, pH and NaCl tolerance ranges are 18–42 °C, pH 6.0–11.0 and 0–7 % (w/v), respectively. Optimum growth occurs on ISP 2 agar supplemented with 0–5 % NaCl at 28–37 °C and pH 8.0. D-Galactose, *myo*-inositol, maltose, D-mannitol, raffinose, L-rhamnose, D-sorbitol and trehalose can be utilised as carbon sources, but D-fructose, D-mannose, D-cellobiose and lactose cannot be utilised. Acid can be produced from D-cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, mannose, D-ribose and D-trehalose. Oxidase activity, catalase activity, gelatin liquefaction, hydrolysis of starch and Tween 40 are positive but hydrolysis of Tween 20, Tween 80, milk coagulation, production of H₂S and indole, cellulose degradation,

urease activity and nitrate reduction are negative. The cell wall contains *meso*-diaminopimelic acid, galactose and arabinose. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphoglycolipid, phosphatidylcholine, phosphatidylinositol, a glycolipid, an aminolipid and other unidentified phospholipids. The predominant menaquinone is MK-9(H₄); MK-8(H₄) and MK-10(H₄) are also present as minor components. The major fatty acids are iso-C_{16:0} and C_{16:0}. The genomic DNA G+C content of the type strain is 69.7 mol%.

The type strain SP28S-3^T (=DSM 46655^T = CGMCC 4.7182^T) was isolated from surface-sterilized stem of *T. taklamakanensis* collected from the southern edge of

Table 2 Fatty acid composition (%) of strain SP28S-3^T and two reference type strains

Fatty Acids	SP28S-3 ^T	<i>P. corallicola</i> SCSIO 11529 ^T	<i>P. marina</i> DSM 45268 ^T
Saturated			
iso-C _{14:0}	–	–	2.7
C _{14:0}	1.7	–	6.8
iso-C _{15:0}	4.5	1.7	10.3
anteiso-C _{15:0}	–	–	2.7
C _{15:0}	–	–	1.0
iso-C _{16:0}	30.6	34.8	25.4
C _{16:0}	28.7	28.8	28.5
C _{17:0} 10-methyl	7.0	–	4.2
iso-C _{17:0}	–	1.9	–
anteiso-C _{17:0}	4.8	6.6	4.3
C _{17:0}	4.0	2.4	1.9
C _{18:0}	–	1.4	–
Unsaturated			
C _{16:1} ω7c	3.9	3.5	2.0
C _{16:1} ω9c	7.5	14.3	6.5
C _{17:1} ω9c	–	2.7	–
C _{18:1} ω9c	–	1.9	–
Hydroxy			
C _{16:0} 2OH	7.3	–	3.7

All strains were cultured on ISP 2 agar plates at 28 °C for 7 days. All data are from this study

– Not detected in this study

Taklamakan desert, Xinjiang, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SP28S-3^T is KM 670438.

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References

- Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 100:221–230
- Cowan ST, Steel KJ (1965) Manual for the identification of medical bacteria. Cambridge University Press, London
- DeLey J, Caltoir H, Reeynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rate. *Eur J Biochem* 12:133–142
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17:368–376
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20:406–416
- Groth I, Schumann P, Rainey FA, Martin K, Schuetze B, Augsten K (1997) *Demetria terrigena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* 47:1129–1133
- Guo L, Tuo L, Habden X, Zhang Y, Liu J, Jiang Z, Liu S, Dilbar T, Sun C (2015) *Allosalinactinospora lopnorenensis* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae* isolated from soil. *Int J Syst Evol Microbiol* 65:206–213
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Jones KL (1949) Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* 57:141–145
- Kämpfer P, Kroppenstedt RM, Dott W (1991) A numerical classification of the genera *Streptomyces* and *Streptovermicillium* using miniaturized physiological tests. *J Gen Microbiol* 137:1831–1891
- Kelly KL (1964) Inter-Society Color Council–National Bureau of Standards Color Name Charts Illustrated with Centroid Colors. US Government Printing Office, Washington, DC
- Kim SB, Goodfellow M (1999) Reclassification of *Amycolatopsis rugosa* Lechevalier et al. 1986 as *Prauserella rugosa* gen. nov., comb. nov. *Int J Syst Bacteriol* 49:507–512
- Kim SB, Goodfellow M (2012) Genus XII. *Prauserella*. In: Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki KI, Ludwig W, Whitman WB, Parte AC (eds) *Bergey's*

- manual of systematic bacteriology, vol 2. Springer, New York, pp 1384–1390
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62:716–721
- Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, London
- Kuykendall LD, Roy MA, O'Neill JJ, Devine TE (1988) Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradorhizobium japonicum*. *Int J Syst Bacteriol* 38:358–361
- Lányi B (1987) Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 19:1–67
- Lechevalier MP, Prause H, Labeda DP, Ruan J-S (1986) Two new genera of nocardioform actinomycetes: *amycolata* gen. nov. and *Amycolatopsis* gen. nov. *Int J Syst Bacteriol* 36:29–37
- Li W-J, Xu P, Tang S-K, Xu L-H, Kroppenstedt RM, Stackebrandt E, Jiang C-L (2003) *Prauserella halophila* sp. nov. and *Prauserella alba* sp. nov., moderately halophilic actinomycetes from saline soil. *Int J Syst Microbiol* 53:1545–1549
- Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R, Xu LH, Stackebrandt E, Jiang CL (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China) and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* 57:1424–1428
- Li Y, Tang S-K, Chen Y-G, Wu J-Y, Zhi X-Y, Zhang Y-Q, Li W-J (2009) *Prauserella salsuginis* sp. nov., *Prauserella flava* sp. nov., *Prauserella aidingensis* sp. nov. and *Prauserella sediminis* sp. nov., isolated from a salt lake. *Int J Syst Microbiol* 59:2923–2928
- Liu M, Zhang L, Ren B, Yang N, Yu X, Wang J, Ding L, Liu X, Liu Z, Goodfellow M, Zhang L (2014) *Prauserella shujinwangii* sp. nov., a new member of the genus *Prauserella* isolated from Xinjiang region, China. *Int J Syst Evol Microbiol* 64:3833–3837
- Luo H-Y, Miao L-H, Fang C, Yang P-L, Wang Y-R, Shi P-J, Yao B, Fan Y-L (2008) *Nesterenkonia flava* sp. nov., isolated from paper mill effluent. *Int J Syst Evol Microbiol* 58:1927–1930
- Magee CM, Rodeheaver G, Edgerton MT, Edlich RF (1975) A more reliable gram staining technic for diagnosis of surgical infections. *Am J Surg* 130:341–346
- Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* 3:208–218
- Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by highperformance liquid chromatography. *Int J Syst Bacteriol* 39:159–167
- Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 2:233–241
- Qin S, Li J, Chen H-H, Zhao G-Z, Zhu W-Y, Jiang C-L, Xu L-H, Li W-J (2009) Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. *Appl Environ Microbiol* 75:6176–6186
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI technical note 101. MIDI inc, Newark
- Schäfer J, Martin K, Kämpfer P (2010) *Prauserella muralis* sp. nov., from the indoor environment. *Int J Syst Microbiol* 60:287–290
- Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* 36:407–477
- Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16:313–340
- Staneck JL, Roberts GD (1974) Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 28:226–231
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 10: 2731–2739
- Waksman SA (1961) The actinomycetes, V2: classification, identification and description of genera and species. Williams & Wilkins, Baltimore
- Wang J, Li Y, Bian J, Tang S-K, Ren B, Chen M, Li W-J, Zhang L-X (2010) *Prauserella marina* sp. nov., isolated from ocean sediment of the South China Sea. *Int J Syst Microbiol* 60:985–989
- Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE et al (1987) International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematic. *Int J Syst Bacteriol* 37:463–464
- Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ (1983) Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* 129:1743–1813
- Wu J-F, Li J, You Z-Q, Zhang S (2014) *Prauserella coralliicola* sp. nov., isolated from the coral *Galaxea fascicularis*. *Int J Syst Evol Microbiol* 64:3341–3345
- Zhou Z-H, Liu Z-H, Qian Y-D, Kim SB, Goodfellow M (1998) *Saccharopolyspora spinosporotrichia* sp. nov., a novel actinomycete from soil. *Int J Syst Bacteriol* 48:53–58