Prauserella halophila sp. nov. and *Prauserella alba* sp. nov., moderately halophilic actinomycetes from saline soil

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The actinomycete strains YIM 90001^T and YIM 90005^T were isolated on starch-casein agar [20% (w/v) NaCl, pH 7.0] from a soil sample collected from Xinjiang Province in the west of China. The two isolates were aerobic and Gram-positive. The substrate mycelium was fragmented and an aerial mycelium was well developed on Czapek medium. The aerial mycelium formed long spore chains with branched short or long spore chains at maturity, which were straight to flexuous, and spores were non-motile. Based on the results of metabolic, chemotaxonomic and molecular analyses, the two isolates were identified as two new species of the genus *Prauserella*, for which the names *Prauserella halophila* for strain YIM 90001^T (= DSM 44617^T = CCTCC AA001015^T) and *Prauserella alba* for strain YIM 90005^T (= DSM 44590^T = CCTCC AA001016^T) are proposed.

Two actinomycete isolates were isolated from a soil sample collected from hypersaline habitats in Xinjiang Province in the west of China, using starch-casein media [20% (w/v) NaCl, pH 7.0] incubated at 28 °C for about 4 weeks. The strains were maintained on ISP medium 2 and Czapek agar slants (Shirling & Gottlieb, 1966) at 4 °C and as glycerol suspensions (20%, v/v) at –20 °C. Biomass for chemotaxonomic analyses was obtained from growth in broth (pH 7.2) containing glucose (4 g l^{–1}), yeast extract (4 g l^{–1}), malt extract (10 g l^{–1}) and NaCl (100 g l^{–1}) at 28 °C. Biomass for molecular systematic studies was obtained by growth in shake flasks (about 150 r.p.m.) using ISP 2 liquid medium (10% NaCl) supplemented with vitamin mixtures of the Humic-vitamin medium described by Hayakawa & Nonomura (1987) at 28 °C for 3 weeks.

Morphological observations

Morphological features were observed on Czapek agar (10% NaCl) and yeast extract-malt extract (ISP 2 medium; containing 10% NaCl) following incubation for 4 weeks at 28 °C (Fig. 1). Colour determinations were made by comparing the pure cultures with colour chips from the ISCC-NBS colour charts (standard samples, no. 2106) (Kelly, 1964). Morphology of spores and mycelia was

examined by scanning electron microscopy with a JEOL model JSM5600LV. The substrate mycelia of the two isolates YIM 90001^T and YIM 90005^T were fragmented, and the aerial mycelia were well developed on Czapek medium. While strain YIM 90001^T developed aerial hyphae on most media tested, only Czapek agar supported the formation of an aerial mycelium of strain YIM 90005^T.

Metabolic properties

Physiological features were observed on media commonly used for characterization of *Streptomyces* species (Shirling & Gottlieb, 1966) prepared with 10% NaCl. Cultural characteristics were determined after 4 weeks at 28 °C by using the International *Streptomyces* Project (ISP) methods (Shirling & Gottlieb, 1966). The media and procedures used for physiological features and carbon source utilization of the isolates were those described by Shirling & Gottlieb (1966) and Locci (1989). The metabolic reactions of strains YIM 90001^T and YIM 90005^T are shown in Table 1. The ranges of carbon and nitrogen utilization of the two strains are wide as they could utilize most carbon and nitrogen sources tested.

Chemotaxonomic properties

Amino acid and sugar analysis of whole-cell hydrolysates followed procedures described by Stanek & Roberts (1974). Isoprenoid quinones were extracted and purified using the small-scale integrated procedure of Minnikin *et al.* (1984). A

The GenBank accession numbers for the 16S rDNA sequences of strains YIM90001^T and YIM90005^T are AF466190 and AF435077, respectively.

Fig. 1. Scanning electron micrographs of aerial mycelium of strains YIM 90001^T (a) and YIM 90005^T (b) grown on Czapek medium, supplemented with 10 % NaCl for 28 days at 28 °C. Bars, 1 µm.

dried preparation was dissolved in 200 µl 2-propanol and 1–10 µl amounts were injected onto an HPLC column without further purification. The menaquinones were separated on a Lichrosorb RP-18 column (4 × 250 mm) kept at 40 °C with acetonitrile/2-propanol (65:35, v/v) as

solvent (Kroppenstedt, 1982; Kroppenstedt *et al.*, 1981). Polar lipids were extracted, examined by two-dimensional thin layer chromatography and identified using published procedures (Minnikin *et al.*, 1984). Fatty acid methyl esters were prepared from 40–80 mg wet cells (Miller 1982). The

Table 1. Comparison of morphological, physiological and chemical characteristics of strains YIM 90001^T, YIM 90005^T and *Prauserella rugosa* DSM 43194^T

All strains utilize cellobiose, fructose, glucose, mannitol, rhamnose, ribose, sucrose, xylitol and xylose, and L-alanine and L-proline as nitrogen source. All strains are negative for milk peptonization, starch hydrolysis, cellulose degradation, nitrate reduction, H₂S production and melanin production.

Characteristic	YIM 90001 ^T	YIM 90005 ^T	<i>Prauserella rugosa</i> DSM 43194 ^T
Aerial mycelium	+	+	–
Spore formation	+	+	–
Range of NaCl concentration for growth (%)	5–25	0–25	0–20
Optimal growth concentration of NaCl (%)	10–15	10–15	5–10
Gelatin liquefaction	+	+	–
Milk coagulation	+	+	–
Urease production	+	–	–
Dehalogenase activity	–	+	–
Carbon utilization:			
Arabinose	–	+	+
Galactose	–	+	+
Maltose	–	+	+
Trehalose	ND	–	+
Inositol	+	+	–
Nitrogen utilization:			
L-Arginine	+	+	–
Asparagine	+	+	–
Histamine	+	+	–
Hydroxyproline	+	+	–
L-Serine	+	+	–
L-Threonine	+	+	–
Major menaquinone	9(H ₄)	9(H ₄)	9(H ₂ ,H ₄)
DNA G + C content (mol%)	65·8	66·7	67–68·9

extracts of the methanolysates were analysed by the MIDI microbial identification system as described by Sasser (1990). The results of chemotaxonomic investigations were compared with the data of *Prauserella rugosa* DSM 43194^T (Kim & Goodfellow, 1999). Analyses of whole-cell amino acids and sugars revealed *meso*-diaminopimelic acid and arabinose plus galactose for strains YIM 90001^T and YIM 90005^T, as expected for members of the genus *Prauserella* (Lechevalier *et al.*, 1986). The main menaquinone was MK-9 (H₄) for all three strains. The fatty acid composition of the two isolates is similar to that reported for *Prauserella rugosa* (Mertz & Yao, 1993). Hydroxyphosphatidylethanolamine and hydroxymethylphosphatidylethanolamine, present in *Prauserella rugosa* DSM 43194^T (Yassin *et al.*, 1993), were missing in strains YIM 90001^T and YIM 90005^T.

Molecular analyses

Methods for genomic DNA extraction, PCR of the 16S rRNA genes and the sequencing primers used were as described by Cui *et al.* (2001). For the phylogenetic analyses, reference strains were chosen from BLAST (Altschul *et al.*, 1997) search results. Multiple alignments of the sequences determined in this study, together with reference sequences obtained from databases and calculations of levels of sequence similarity were carried out using CLUSTAL W 1.8 (Thompson *et al.*, 1994). The phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) based upon *K*_{nuc} values of Kimura (1980, 1983). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The accession numbers of the reference strains are listed in Fig. 2.

Chromosomal DNA was prepared following the method of Marmur (1961). The G+C content of the DNA of the test strains was determined using the thermal denaturation method of Marmur & Doty (1962). DNA–DNA hybridization was carried out according to published methods (De Ley *et al.*, 1970; Huss *et al.*, 1983; Jahnke, 1992).

The almost complete 16S rRNA gene sequences of strains YIM 90001^T and YIM 90005^T were first analysed by a BLAST search and secondly by a more detailed similarity search with sequences of the strains shown in Fig. 2. The neighbour-joining tree indicated that strains YIM 90001^T and YIM 90005^T were highly related (99.0% sequence

similarity) and both strains were close to the type strain *Prauserella rugosa* DSM 43194^T (97.57 and 97.78% sequence similarity, respectively), forming a distinct branch within the family *Pseudonocardiaceae* (Stackebrandt *et al.*, 1997). Strains YIM 90001^T and YIM 90005^T have no more than 96% sequence similarity to members of this family.

The G+C contents of the genomic DNAs from strains YIM 90001^T and YIM 90005^T were 65.8 and 66.7 mol%, respectively.

DNA–DNA reassociation experiments indicated a low value of 35.4% similarity between strains YIM 90001^T and YIM 90005^T, while the similarity values for these two strains and *Prauserella rugosa* DSM 43194^T were 5 and 35.4% respectively.

Taxonomic conclusions

Based on phylogenetic analyses and chemotaxonomic properties the two isolates YIM 90001^T and YIM 90005^T should be considered members of the genus *Prauserella*. Both strains YIM 90001^T and YIM 90005^T differ in morphology from *P. rugosa* DSM 43194^T (Kim & Goodfellow, 1999). The substrate mycelium is fragmented and the aerial mycelium is well developed. The aerial mycelium forms long spore chains with branched short or long spore chains at maturity, which are straight to flexuous, and spores are non-motile. DNA–DNA relatedness provided important data for determining the taxonomic position of the new isolates. DNA–DNA hybridization among the two new isolates and *P. rugosa* DSM 43194^T revealed significantly lower than 70% similarity values, indicating that strains YIM 90001^T and YIM 90005^T represent two new species in accordance with the recommendations of the committee on reconciliation of approaches to bacterial systematics (Wayne *et al.*, 1987). As the genomic distinctness is also expressed by differences in some metabolic properties (Table 1), we propose the names *Prauserella halophila* sp. nov. and *Prauserella alba* sp. nov. for strains YIM 90001^T and YIM 90005^T, respectively.

Emendation of the genus *Prauserella* Kim & Goodfellow 1999

The genus description (Kim & Goodfellow, 1999) is emended with respect to morphology and the chemical composition of cell constituents.

Fig. 2. Phylogenetic tree obtained by the neighbour-joining method showing the positions of strains YIM 90001^T and YIM 90005^T among phylogenetically related members of the family *Pseudonocardiaceae*. Numerals on branches are bootstrap values (1000 replicates, only values >50% are indicated). Bar, 2 nt substitutions per 100 nt 16S rDNA sequence. The sequence of *Streptosporangium roseum* was used as outgroup.

Aerobic, Gram-positive, non-acid-alcohol-fast, non-motile actinomycetes that form an extensively branched substrate mycelium (0.6–0.8 µm in diameter) which fragments into irregular rods within 24–48 h on rich medium. Aerial hyphae with straight to flexuous spore chains may be formed which are short and branched, or, at maturity, are long. Spores are non-motile. Brownish, soluble pigment may be produced on some media. Some strains grow optimally at 10% or between 10 and 15% NaCl at 28 °C and pH 7.0. Optimal growth between pH 6.8 and 7.2. The temperature range for growth is 10–45 °C, with the optimum temperature at 28 or 34 °C. Mycolic acid absent. Contains *meso*-diaminopimelic acid as the diamino acid, an acetylated peptidoglycan (only one species tested), major amounts of arabinose and galactose (some strains also ribose), di- and tetrahydrogenated menaquinones with nine isoprenoid units or tetrahydrogenated menaquinones with nine isoprenoid units as the predominant isoprenologue. Diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine are diagnostic polar lipids; some strains also contain phosphatidylinositol and methylphosphatidylethanolamine. Fatty acid profile is rich in branched-chain and saturated components. The G+C content of DNA ranges between 65.8 and 69.9 mol% (T_m method). The genus belongs to the family *Pseudonocardiaceae*. The type species is *Prauserella rugosa*.

Description of *Prauserella halophila* sp. nov.

Prauserella halophila (ha.lo'phi.la. Gr. n. *halo* salt; Gr. adj. *phila* loving; N.L. gen. adj. *halophila* salt-loving, referring to the ability to grow at high NaCl concentrations).

Gram-positive and aerobic. The branched substrate mycelium is fragmented, the colour ranging from light grey-white (ISP 5), deep grey-white (ISP 3), light yellow (ISP 2 and 4), deep yellow (potato agar), or light orange-brown (Czapek's agar), all media being prepared with 10% NaCl. A white to yellow aerial mycelium is well developed on most media tested (except for ISP 2), forming branched short or, at maturity, long spore chains, which are straight to flexuous. Spores non-motile. No diffusible pigment produced. Optimum growth occurs on Czapek media supplemented with NaCl at a concentration of 10–15% at 28 °C and pH 7.0. The range of carbon and nitrogen utilization of strain YIM90001^T is wide (Table 1). Galactose, arabinose and ribose are found in whole-cell hydrolysates. The main menaquinone is MK-9(H₄) (83%) and small amounts of MK-8(H₄) (4%), MK-9 (5%) and MK-9(H₂) (8%) are also found. Polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and methylphosphatidylethanolamine. The major cellular fatty acids are iso/anteiso-branched fatty acids: iC16:0 (27%), *cis*-11C17:1 (11%), *cis*-9C16:1 (11.0%), aiC17:0 (10.6%), *cis*-9C17:1 (8.7%), C16:0 (8%), iC16:1 (4.1%), C17:0 (3.9%), *cis*-x18:1 (3.7%), C15:0 (2.2%), C15:1 (2.2%), *cis*-9C18:1 (2.2%), iC17:0 (1.6%), iC15:0 (1.8%), aiC15:0 (1.3%) and iC14:0 (1.2%). The G+C content of the DNA is 65.8 mol%.

Isolated from soil in hypersaline habitats, Xinjiang Province in the west of China. Type strain is YIM 90001^T, deposited in the Chinese Centre of Type Culture Collection as strain CCTCC AA001015^T (=DSM 44617^T).

Description of *Prauserella alba* sp. nov.

Prauserella alba (al'ba. L. adj. *alba* white, referring to the white aerial mycelium).

The branched substrate mycelium is fragmented, the colour ranging from yellow-white and light yellow (nutrient agar, ISP 4, ISP 5), light orange-yellow (Czapek's agar, potato agar), orange-yellow (ISP 2) and grey-white (ISP 3), all media being prepared with 10% NaCl. A white aerial mycelium developed on Czapek medium, forming branched short or, at maturity, long spore chains, which were straight to flexuous; spores non-motile. No diffusible pigment. Optimum growth occurs in Czapek medium supplemented with NaCl at a concentration of 10% at 28 °C and pH 7.0. It can utilize a broad range of carbon and nitrogen sources (Table 1). Galactose, arabinose and ribose are found in whole-cell hydrolysates. Main menaquinone is MK-9(H₄) (90%); MK-9(H₂) occurs in smaller amounts (8%). Traces of MK-8(H₄) and MK-9 are found in addition. Polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and methylphosphatidylethanolamine. The major cellular fatty acids are iso/anteiso-branched fatty acids. Traces of 10-methyl-branched fatty acids are also found. The principal fatty acids are: iC16:0 (29%), *cis*-11C17:1 (8.7%), *cis*-9C16:1 (8.1.0%), aiC17:0 (10.5%), *cis*-9C17:1 (5.3%), C16:0 (8.7%), iC16:1 (6.8%), C17:0 (2.09%), *cis*-x18:1 (7.0%), C15:1 (2.2%), *cis*-9C18:1 (4.2%), iC15:0 (2.2%) and iC17:0 (3.0%). The G+C content of the DNA is 66.7 mol%. Isolated from soil in hypersaline habitats, Xinjiang Province in the west of China. Type strain is YIM 90005^T, deposited in the Chinese Centre of Type Culture Collection as strain CCTCC AA001016^T (=DSM 44590^T).

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