Prauserella marina sp. nov., isolated from ocean sediment of the South China Sea

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A novel actinomycete strain, designated MS498^T, was isolated from an ocean sediment sample collected from the South China Sea. It was subjected to a polyphasic analysis to determine its taxonomic position. The phylogenetic tree based on 16S rRNA gene sequences showed that strain MS498^T had the highest similarity (96.5 %) with members of the genus *Prauserella* and was loosely associated with *Prauserella rugosa* DSM 43194^T and *Saccharomonospora halophila* DSM 44411^T. Based on 16S rRNA gene sequence analysis, phenotypic characteristics and chemotaxonomic data, the new isolate is proposed to represent a novel species of the genus *Prauserella*, named *Prauserella marina* sp. nov. (type strain MS498^T=CCTCC AA 208056^T =DSM 45268^T).

The historical paradigm of the deep ocean as a biological 'desert' has shifted to one of a 'rainforest' owing to the isolation of many novel microbes and their associated bioactive compounds (Demain & Zhang, 2005). Marine microbes can sense, adapt and respond quickly to diverse environments, and can compete for defence and survival by producing unique secondary metabolites (Knight *et al.*, 2003; Zhang *et al.*, 2005). The hidden wealth of this source needs to be explored further. From freshly collected samples of South China Sea sediments, we recovered and isolated many marine fungi by various isolation methods (Demain & Zhang, 2005). A marine microbial extract library has also been established and screened for various biological activities (Bian *et al.*, 2008). We systematically evaluated the quality of the microbial natural products library from the aspects of high diversity and low redundancy in terms of strains, extracts and bioactive compounds (Bian *et al.*, 2009). Identifying beauvericin as an antifungal potentiator was a good example of the use of the library for drug discovery (Zhang *et al.*, 2007; Zhang, 2005; Demain & Zhang, 2005).

Since Kim and Goodfellow established the genus *Prauserella*, eight species have been described, namely *Prauserella rugosa* (Kim & Goodfellow, 1999), *Prauserella halophila*, *Prauserella alba* (Li *et al.*, 2003b), *Prauserella muralis* (Schäfer *et al.*, 2010), *Prauserella salsuginis*, *Prauserella flava*, *Prauserella aidingensis* and *Prauserella sediminis* (Li *et al.*, 2009). Members of this genus are aerobic, Gram-positive, non-acid-fast, and halophilic or halotolerant. Substrate mycelia fragment into irregular rod-shaped elements and aerial mycelia form spores in chains. The hydrolysates of the whole cells contain *meso*-diaminopimelic acid, and major amounts of galactose and arabinose (some strains also ribose). MK-9(H_4) is the predominant menaquinone. The DNA G+C contents are between 65.8 and 69.9 mol% (Kim & Goodfellow, 1999; Li *et al.*, 2003b).

The genus *Saccharomonospora*, described by Nonomura & Ohara (1971), is phylogenetically closely related to the genus *Prauserella*. The genus *Saccharomonospora* is char-

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MS498^T is FJ444996.

A scanning electron micrograph of strain MS498^T and a table detailing cultural characteristics of strain MS498^T and *Prauserella rugosa* are available with the online version of this paper.

acterized by the formation of petiolated spores borne singly or in longitudinal pairs, a DNA G+C content of 66–74 mol%, presence of MK-9(H_4) as the predominant menaquinone, presence of *meso*-diaminopimelic acid in the peptidoglycan, and arabinose and galactose in cell hydrolysates (Nonomura & Ohara, 1971; Hu, 1987; Greiner-Mai *et al.*, 1988; Hu *et al.*, 1988; Jin *et al.*, 1998; Al-Zarban *et al.*, 2002; Li *et al.*, 2003a; Syed *et al.*, 2008).

During an investigation of marine microbial diversity and high-throughput screening of microbial natural products, strain MS498^T was isolated from an ocean sediment sample collected from the South China Sea (GPS coordinates for the sample site are 114° 34′ 58.700″ E 17° 54′ 0.446″ N, at a depth of 3602 m), after 4 weeks of incubation at 22 °C on MOPS-proline agar medium, which contains 1 g MOPS, 1 g proline, 1 g $(NH_4)_2SO_4$, 1 g NaCl, 1 g $CaCl_2$, 1 g K_2HPO_4, 1 g $MgSO_4.7H_2O$ and 20 g agar per litre. The sediment sample was collected by a grab bucket as described by Tian *et al.* (2009). The isolate was maintained on ISP 2 (International *Streptomyces* Project medium 2; Shirling & Gottlieb, 1966) agar slants at 4 °C, and as suspensions of mycelia fragments in glycerol (20 %, v/v). Biomass for chemical and molecular studies was obtained by cultivation in shaking flasks (150 r.p.m.) with ISP 2 (pH 7.0) at 28 °C for one week.

Morphological characteristics were observed by light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 14 days of growth on ISP 2 agar medium. Cultural characteristics were determined after 2 weeks by methods used in the ISP (Shirling & Gottlieb, 1966). The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). For strain MS498^T, substrate mycelia were well developed and fragmented. Spore chains were borne on aerial mycelia and spores were non-motile (Supplementary Fig. S1, available in IJSEM online). The isolate could be distinguished from the type species, *P. rugosa*, by examining a battery of cultural characteristics (Supplementary Table S1).

Growth at different temperatures (4, 10, 15, 20, 28, 37, 40, 45, 55 and 65 °C) was tested on ISP 2. For NaCl tolerance experiments, ISP 2 was used as the basal medium, with NaCl concentrations of 0–20 % (w/v), at intervals of 1 %. The pH range for growth was investigated between 4.0 and 10.0 at intervals of 1 pH unit, using the following buffer system: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0–10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3. Media and procedures used for determination of physiological features and carbon source utilization were those described by Williams *et al.* (1989). The detailed morphological and physiological properties of strain MS498^T are given in the species description. The isolate could be distinguished from the other described species of the genus *Prauserella* by a series of morphological and physiological properties (Table 1).

Isomers of diaminopimelic acid and whole-cell sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983). Polar lipids were extracted and examined by two-dimensional TLC and identified using procedures described by Minnikin *et al.* (1984). Menaquinones were isolated according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). Strain MS498^T, together with the reference type strain, *P. rugosa* DSM 43194^T, contained *meso*-diaminopimelic acid as the diagnostic diamino acid, with galactose and arabinose as the major whole-cell sugars. The phospholipids comprised phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol, together with some unknown phospholipids. The predominant menaquinone of MS498^T was MK-9(H_4), which is different from *P. rugosa* DSM 43194^T that has MK-9(H_2) and MK-9(H_4) as the predominant menaquinones, but the same as *P. halophila* YIM 90001^T, *P. alba* YIM 90005^T, *P. salsuginis* YIM 90625^T, *P. flava* YIM 90630^T, *P. aidingensis* YIM 90636^T and *P. sediminis* YIM 90694^T. However, the minor menaquinone components of our novel isolate, MK-8(H_4), MK-9(H_6) and MK-10(H_4), differed from all other species. For example, the predominant menaquinone of *P. halophila* YIM 90001^T is MK-9(H_4), but the minor components for this species are MK-8(H_4), MK-9 and MK-9(H_2). The chemotaxonomic data for strain MS498^T were consistent with its assignment to the genus *Prauserella* (Lechevalier *et al.*, 1986; Kim & Goodfellow, 1999; Li *et al.*, 2003b).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). Multiple alignments with sequences of all species of the genus *Prauserella* and calculations of levels of sequence similarity were carried out by using the EzTaxon server 2.0 (Chun *et al.*, 2007). Phylogenetic analysis was performed using three tree-making algorithms, namely neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980) using 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. Genomic DNA of four strains for the determination of G+C content was prepared according to the method of Marmur (1961). The G+C content of the DNA of strain MS498^T was determined by reverse-phase HPLC of nucleosides according to Mesbah *et al.* (1989) to be 66.1 mol%.

The length of the 16S rRNA gene sequence of strain MS498^T was 1443 bp. Comparison with sequences from the GenBank database indicated that the new isolate had the highest sequence similarity to members of the genus *Prauserella*. Phylogenetic analysis showed that MS498^T fell into a separate subclade between the genera *Prauserella* and *Saccharomonospora*, and was loosely associated with *P.*

Table 1. Phenotypic characteristics that differentiate strain MS498^T from the other recognized species of the genus *Prauserella*

Strains: 1. MS498^T, 2. *P. rugosa* DSM 43194^T, 3. *P. salsuginis* YIM 90625^T, 4. *P. flava* YIM 90630^T, 5. *P. aidingensis* YIM 90636^T, 6. *P. sediminis* YIM 90694^T, 7. *P. halophila* YIM 90001^T, 8. *P. alba* YIM 90005^T. Data for MS498^T are from this study; data for strain 2 are from Lechevalier *et al.* (1986), Kim & Goodfellow (1999) and Li *et al.* (2003b); data for strains 3–6 are from Li *et al.* (2009); data for strains 7 and 8 are from Li *et al.* (2003b) and Li *et al.* (2009). All strains utilize glucose as a sole carbon source, and L-alanine and L-proline as nitrogen sources. All strains are negative for starch hydrolysis, nitrate reduction, and H_2S production. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Aerial mycelium	+	–	+	–	+	+	+	+
Spore formation	+	–	+	–	+	+	+	+
NaCl range (%)	0–10	0–20	5–15	5–15	5–15	5–20	5–25	0–25
Optimum NaCl concentration (%)	0–5	5–10	8–10	8–10	8–10	10	10–15	10–15
Degradation of:								
Gelatin	+	–	+	+	+	+	+	+
Urea	–	+	–	–	–	–	+	–
Carbon utilization								
L-Arabinose	+	+	+	–	–	–	–	+
Cellobiose	–	+	+	–	–	–	+	+
D-Fructose	–	+	+	+	+	–	+	+
D-Galactose	+	+	+	–	+	–	–	+
myo-Inositol	–	–	+	+	–	–	+	+
Lactose	–	+	+	+	+	+	ND	ND
Maltose	+	+	–	–	–	–	–	+
D-Mannitol	+	+	+	–	–	–	+	+
D-Mannose	+	+	+	–	–	–	ND	ND
Raffinose	–	+	–	–	–	–	ND	ND
L-Rhamnose	+	+	+	–	+	+	+	+
D-Ribose	+	+	+	+	+	–	+	+
Trehalose	+	+	+	–	+	–	ND	–
D-Xylitol	–	+	+	–	–	–	+	+
D-Xylose	+	+	+	+	+	–	+	+
Nitrogen utilization								
Adenine	–	–	–	–	–	–	ND	ND
L-Arginine	+	–	–	–	–	–	+	+
L-Glycine	ND	+	–	–	–	–	ND	ND
L-Histidine	+	ND	+	+	+	+	ND	ND
L-Hydroxyproline	+	–	+	+	+	+	+	+
L-Lysine	–	ND	+	+	–	+	ND	ND
DL-Methionine	–	ND	–	–	–	–	ND	ND
L-Phenylalanine	+	ND	+	+	+	+	ND	ND
L-Serine	+	–	–	–	–	–	+	+
L-Threonine	+	–	+	+	+	+	+	+
DL-Tryptophan	+	ND	+	+	+	+	ND	ND
L-Tyrosine	+	+	+	+	+	+	ND	ND
L-Valine	+	ND	+	+	+	+	ND	ND
Xanthine	+	ND	+	+	+	+	ND	ND
Predominant menaquinone(s)	MK-9(H_4)	MK-9($H_{2,4}$)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)
Predominant fatty acids	i-$C_{16:0}$ (19.9 %)	i-$C_{16:0}$ (33.8 %)	i-$C_{16:0}$ (36.4 %)	i-$C_{16:0}$ (38.6 %)	i-$C_{16:0}$ (36.9 %)	i-$C_{16:0}$ (33.8 %)	i-$C_{16:0}$ (27.0 %)	i-$C_{16:0}$ (42.4 %)
	$C_{16:0}$ (17.4 %)	i-$C_{16:0}$ 2-OH (13.7 %)	ai-$C_{17:1}$ (9.4 %)	ai-$C_{17:1}$ (10.0 %)	$C_{16:1}\omega 9c$ (10.6 %)	i-$C_{16:0}$ 2-OH (9.2 %)	$C_{16:1}\omega 9c$ (11.0 %)	i-$C_{16:0}$ 2-OH (11.5 %)
	i-$C_{15:0}$ (10.0 %)	$C_{16:1}\omega 9c$ (9.2 %)	i-$C_{16:0}$ 2-OH (9.1 %)	i-$C_{16:0}$2-OH (9.0 %)	i-$C_{16:0}$ 2-OH (9.6 %)	ai-$C_{17:1}$ (8.0 %)	ai-$C_{17:0}$ (10.6 %)	ai-$C_{17:0}$ (7.6 %)
							$C_{17:1}\omega 9c$ (8.7 %)	
							$C_{16:0}$ (8.0 %)	
DNA G+C content (mol%)	66.1	67.0–68.9	69.1	69.9	70.1	69.1	65.8	66.7

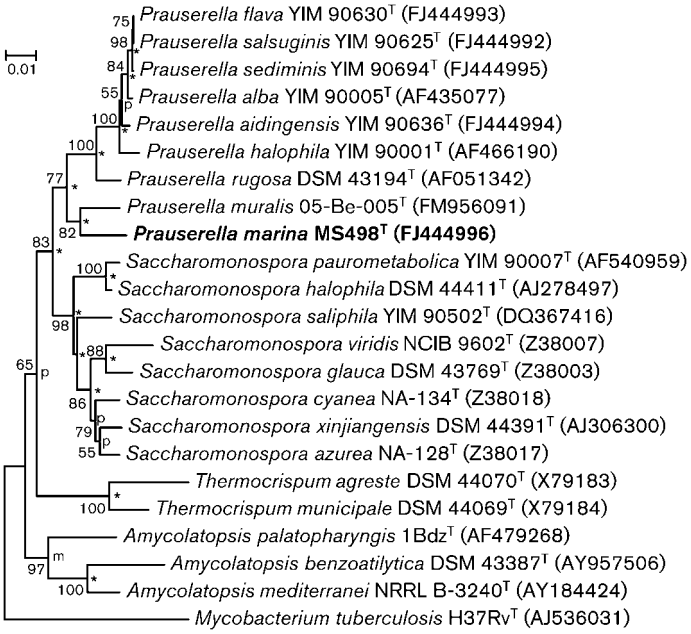

Fig. 1. Phylogenetic dendrogram, obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain MS498^T among its phylogenetic neighbours. Numbers at branch nodes are bootstrap values (expressed as a percentage of 1000 replicates, only values above 50 % are shown) based on the neighbour-joining (Saitou & Nei, 1987) algorithm. 'm' and 'p' indicate branches that were also recovered by using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) treeing algorithms, respectively; asterisks indicate branches recovered with all three methods. The sequence of *Mycobacterium tuberculosis* H37Rv^T (AJ536031) was used as an outgroup. Bar 1 % sequence divergence.

rugosa DSM 43194^T and *Saccharomonospora halophila* DSM 44411^T (Fig. 1). However, strain MS498^T showed the highest 16S rRNA gene sequence similarity (96.5 %) to *P. rugosa* DSM 43194^T and its morphology and chemotaxonomic characteristics were similar to those of members of the genus *Prauserella*. Thus, strain MS498^T should be assigned to the genus *Prauserella*. Strain MS498^T exhibited many phenotypic differences from its closest phylogenetic neighbour *P. rugosa* DSM 43194^T (Table 1). Therefore, we consider that this new isolate represents a novel species of the genus *Prauserella*, for which we propose the name *Prauserella marina* sp. nov.

Description of *Prauserella marina* sp. nov.

Prauserella marina (ma.ri′na. L. fem. adj. *marina* of the sea).

Aerobic, Gram-stain-positive and non-motile. Aerial mycelium is white on most media tested. Substrate mycelium is fragmented, the colour ranging from light grey–white (ISP 5) to pale pink (ISP 3 and potato agar) to moderate reddish-brown (ISP 2). Optimum growth occurs on ISP 2 medium prepared without NaCl at 28–37 °C and pH 7.0. Grows at 15 to 45 °C, pH 6.0–9.0 and 0–10 % (w/v) NaCl. L-Arabinose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, L-rhamnose, D-ribose, trehalose and D-xylose can be utilized as carbon sources, but cellobiose, lactose, D-fructose, *myo*-inositol, raffinose and D-xylitol cannot be utilized. L-Alanine, L-arginine, L-histidine, L-hydroxyproline, hypoxanthine, L-phenylalanine, L-proline, L-serine, L-threonine, DL-tryptophan, L-tyrosine, L-valine and xanthine can be utilized as nitrogen sources, but adenine, L-lysine and DL-methionine cannot be utilized as nitrogen sources. Milk coagulation, gelatin liquefaction and catalase are positive, and hydrogen sulfide, starch hydrolysis, urease activity, nitrate reduction and oxidase are negative. Cell walls contain *meso*-diaminopimelic acid and ribose, arabinose and galactose. Polar lipids are phosphatidylcholine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylmethylethanolamine. The predominant menaquinone is MK-9(H_4) (80.9 %), and MK-8(H_4) (10.1 %), MK-9(H_6) (6.9 %) and MK-10(H_4) (2.1 %) are also present. The cellular fatty acids are iso-$C_{16:0}$ (19.9 %), $C_{16:0}$ (17.4 %), iso-$C_{15:0}$ (10.0 %), iso-$C_{16:1}$ H (6.4 %) and $C_{17:0}$ (2.7 %). The DNA G+C content of the type strain is 66.1 mol%.

The type strain, MS498^T (=CCTCC AA 208056^T =DSM 45268^T), was isolated from an ocean sediment sample from the South China Sea.

Note added in proof

Since this article was accepted for publication, an additional novel species of the genus *Prauserella*, *Prauserella muralis* (Schäfer *et al.*, 2010) has been described.

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