Prauserella muralis sp. nov., from the indoor environment

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A novel Gram-stain-positive, mycelium-forming actinobacterium (05-Be-005^T) isolated from the wall of an indoor environment was studied for its taxonomic position. The isolated strain formed a substrate mycelium that fragmented into rod-shaped cells and showed an aerial mycelium on medium M79. On the basis of 16S rRNA gene sequence similarity studies, strain 05-Be-005^T was shown to belong to the genus *Prauserella*, closely related to *Prauserella rugosa* DSM 43194^T (96.6 % similarity), *Prauserella alba* YIM 90005^T (95.9 %) and *Prauserella halophila* YIM 90001^T (95.4 %). The predominant menaquinone was MK-9(H₄); whole-cell hydrolysates contained *meso*-diaminopimelic acid as the diagnostic diamino acid of the cell wall and arabinose and galactose as the main sugars. Mycolic acids were absent. The polar lipid profile consisted of the lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, phosphatidylserine and an unknown phospholipid. Major fatty acids C_{16:0} iso, C_{16:0}, C_{17:1ω8c} and C_{17:1ω6c} supported the affiliation of strain 05-Be-005^T to the genus *Prauserella*. The results of physiological and biochemical tests allowed clear phenotypic differentiation of strain 05-Be-005^T from the three known *Prauserella* species. Strain 05-Be-005^T represents a novel *Prauserella* species, for which we propose the name *Prauserella muralis* sp. nov., with the type strain 05-Be-005^T (=CCUG 57426^T =NRRL B-24780^T =CCM 7635^T =DSM 45305^T).

The genus *Prauserella* was described by Kim & Goodfellow (1999) to accommodate the single species *Prauserella rugosa*, which had originally been classified in the genus *Amycolatopsis*. *P. rugosa* was found to form a substrate mycelium that undergoes fragmentation. The type strain contained *meso*-diaminopimelic acid (*meso*-A₂pm), arabinose and galactose (chemotype IV *sensu* Lechevalier *et al.*, 1977), as well as a complex mixture of branched-chain and saturated fatty acids, it lacked mycolic acids, had di- and tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologues and contained major amounts of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine and phosphatidylmethylethanolamine. Li *et al.* (2003) described two additional *Prauserella* species, sharing most of these chemotaxonomic features.

Strain 05-Be-005^T was isolated from a wall colonized with moulds. After extraction of a 1 g sample by shaking for 15 min

in 10 ml 0.9 % NaCl solution containing 0.01 % (v/v) Tween 80, aliquots of the suspension were spread on agar plates containing mineral agar according to Gauze *et al.* (1983) (containing l⁻: 20 g soluble starch, 1 g KNO₃, 0.5 g K₂HPO₄, 0.5 g MgSO₄ · 7H₂O, 0.5 g NaCl, 0.01 g FeSO₄ · 7H₂O, 20 g agar). The agar plates were incubated for 2 weeks at 28 °C. The isolated strain was maintained on organic medium M79 [containing l⁻: 10 g glucose, 10 g peptone (Bacto), 2 g casein hydrolysate, 2 g yeast extract, 6 g NaCl, 15 g agar; <http://www.dsmz.de>] and preserved at –80 °C as a 1 : 1 mixture of well-growing cultures in M79 broth and glycerol preservation medium (Chakrabarty & Brown, 1978). The strain formed grey, slightly orange-coloured substrate mycelium and white aerial mycelium on M79 at 28 °C.

Morphological properties, Gram staining and cell morphology were observed microscopically as described by Kämpfer & Kroppenstedt (2004). Isolation of the DNA was performed with a commercial DNA extraction kit (GenElute plant genomic DNA kit; Sigma) after disruption of cells by a 1 min bead-beating step with 1 g of 0.1 mm diameter zirconia beads at maximum speed.

Multiple sequence alignment and analysis of the data were performed using the software package MEGA version 4

Abbreviations: A₂pm, diaminopimelic acid; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 05-Be-005^T is FM956091.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses) showing the position of strain 05-Be-005^T. Multiple alignment, distance calculations (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were performed by using the software package MEGA version 4 (Tamura *et al.*, 2007). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.005 substitutions per nucleotide position.

(Tamura *et al.*, 2007). Genetic distance calculations (distance options according to Kimura's two-parameter model; Kimura, 1980) and clustering with the neighbour-joining method (Fig. 1) were performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain 05-Be-005^T was a continuous stretch of 1412 bp. Sequence similarity calculations after a neighbour-joining analysis indicated that the closest relatives of strain 05-Be-005^T were *P. rugosa* DSM 43194^T (96.6 % similarity), *Prauserella alba* YIM 90005^T (95.9 %) and *Prauserella halophila* YIM 90001^T (95.4 %).

Standard paper chromatography, HPLC and TLC procedures were used to determine the quinone system (Collins *et al.*, 1977; Groth *et al.*, 1996), whole-organism sugars (Becker *et al.*, 1965) and polar lipids (Minnikin *et al.*, 1979). Analysis of the amino acids in peptidoglycan hydrolysates was carried out according to Schleifer & Kandler (1972) and as described by Groth *et al.* (1996). Menaquinones were extracted and analysed as described by Collins *et al.* (1977). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC as described by Collins *et al.* (1980). The occurrence of mycolic acids was determined by TLC as described by Minnikin *et al.* (1975). For all chemotaxonomic analyses except for fatty acid analyses, cells were grown for 24–48 h in shake flasks in liquid organic medium M79 at 180 r.p.m. at 28 °C; for fatty acid analyses, cells were grown on tryptic soy agar. Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996).

Whole-organism hydrolysates of strain 05-Be-005^T contained *meso*-A₂pm as the diagnostic diamino acid of the peptidoglycan and the sugars arabinose, galactose and glucose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), typical of members of the genus *Prauserella*. The predominant menaquinone was MK-9(H₄) (79 %); minor amounts of MK-8(H₄) (5 %) and MK-8 (6 %) and traces of other menaquinones (<3 %) were also detected.

The phospholipids were composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine,

phosphatidylmethylethanolamine and phosphatidylinositol; these phospholipids were also present in the three described *Prauserella* species. Additionally, phosphatidylserine and an unknown phospholipid were detected. Mycolic acids were absent.

The fatty acid profile of strain 05-Be-005^T was very similar to those of the type strains of the closely related species *P. rugosa*, *P. alba* and *P. halophila* and was congruent with the fatty acid profiles reported by Li *et al.* (2003), although some quantitative and also qualitative differences could be observed (Table 1).

Table 1. Major fatty acids of type strains of species of the genus *Prauserella*

Strains: 1, 05-Be-005^T; 2, *P. alba* DSM 44590^T; 3, *P. rugosa* DSM 43194^T; 4, *P. halophila* DSM 44617^T. Data were obtained in this study; values are percentages of total fatty acids. –, Not detected.

Fatty acid	1	2	3	4
C _{14:0} iso	0.7	0.9	–	1.2
C _{14:0}	0.6	0.6	–	–
C _{15:0} iso	2.0	1.8	1.1	1.8
C _{15:1ω6c}	2.2	–	1.0	2.2
C _{15:0}	6.0	1.4	1.3	2.2
C _{16:1} iso H	4.0	5.2	14.4	4.1
C _{16:0} iso	36.5	27.7	34.9	27.0
Summed feature 3*	6.2	11.4	6.4	11.0
C _{16:0}	9.5	17.8	4.6	8.0
C _{16:0} 10-methyl	4.1	–	2.3	–
C _{17:0} iso	2.0	1.9	2.7	1.6
C _{17:0} anteiso	2.9	5.4	3.4	10.6
C _{17:1ω8c}	5.5	4.9	4.3	8.7
C _{17:1ω6c}	8.7	8.8	15.6	11.0
C _{17:0}	7.2	4.7	2.9	3.7
C _{17:0} 10-methyl	1.5	–	–	–
C _{18:1} iso	–	1.3	1.0	3.7
C _{18:1ω9c}	–	1.9	1.9	2.2
C _{18:0}	–	4.1	2.1	–

*Summed feature 3 contains C_{16:1ω7c} and/or C_{15:0} iso 2-OH.

Table 2. Physiological characteristics of the type strains of *Prauserella* species

Strains: 1, 05-Be-005^T; 2, *P. alba* DSM 44590^T; 3, *P. rugosa* DSM 43194^T; 4, *P. halophila* DSM 44617^T. +, Positive; –, negative; (+), weakly positive. Data were obtained in this study under identical conditions. All strains were negative for hydrolysis of aesculin, *o*-nitrophenyl β-D-galactopyranoside, *p*-nitrophenyl (pNP) β-D-glucuronide, *bis*-pNP phosphate, pNP phenylphosphonate, pNP phosphorylcholine, L-alanine *p*-nitroanilide (pNA), L-glutamate γ-3-carboxy-pNA and L-proline pNA and utilization of *N*-acetyl-D-galactosamine, α-melibiose, *p*-arbutin†, salicin, inositol†, maltitol, mesaconate, putrescine, β-alanine, L-tryptophan and 3-hydroxybenzoate. All strains were positive for utilization of cellobiose*†, D-fructose*†, D-glucose*†, D-galactose†, L-rhamnose*†, ribose*†, D-xylose*†, adonitol, D-mannitol*†, acetate, propionate†, fumarate, 3-hydroxy-DL-butyrate, L-malate, pyruvate†, suberate, L-alanine*†, L-histidine, L-phenylalanine and L-proline*†.

Test	1	2	3	4
Hydrolysis of:				
pNP α-D-glucopyranoside	+	+	+	–
pNP β-D-glucopyranoside	–	–	+	–
pNP β-D-xylopyranoside	+	–	–	–
2-Deoxythymidine-5'-pNP phosphate	–	–	+	–
Assimilation of:				
<i>N</i> -Acetyl-D-glucosamine	+	+	–	–
L-Arabinose	+	+	+	–
D-Mannose	+	+	–	–
Maltose	+	(+)*	+	–
Trehalose	+	+	+	–
D-Sorbitol	+	–	–	+
<i>cis</i> -Aconitate	+	+	+	–
<i>trans</i> -Aconitate	+	+	+	–
Adipate	–	+	+	+
4-Aminobutyrate	+	–	+	–
Azelate	+	–	+	+
Citrate	–	+	+	–
Glutarate	–	–	+	–
Itaconate	+	–	–	–
DL-Lactate	+	+	+	–
Oxoglutarate	+	+	–	–
L-Aspartate	+	–	–	+
L-Leucine	+	+	+	–
L-Ornithine	+	–	–	–
L-Serine	+	+	–	–
4-Hydroxybenzoate	–	–	+	–
Phenylacetate	+	+	+	–

*Data congruent with results for *P. rugosa*, *P. alba* and *P. halophila* given by Li *et al.* (2003).

†Data congruent with results for *P. rugosa* given by Kim & Goodfellow (1999).

Results of the comparative physiological characterization, using identical test conditions in all cases, are given in Table 2 and the species description, with methods described previously (Kämpfer *et al.*, 1991). Several test

results were obtained which enable the differentiation of strain 05-Be-005^T from the other *Prauserella* type strains. We did not perform DNA–DNA hybridizations because of the low 16S rRNA gene sequence similarities (<97%) to all other type strains of *Prauserella*. The isolate forms an aerial mycelium and contains only minor amounts (<1.5%) of MK-9(H₂), characteristics which are in agreement with the description of the genus *Prauserella* after its emendation by Li *et al.* (2003). Kim & Goodfellow (1999) already described the high phenotypic similarity of *P. rugosa* to *Saccharomonospora*. In fact, the two genera can only be separated clearly on the basis of the 16S rRNA gene sequence dissimilarity, and strain 05-Be-005^T clustered clearly with the other known *Prauserella* species. The observed genotypic and phenotypic differences (Tables 1 and 2) warrant the creation of a novel species of the genus *Prauserella*.

Description of *Prauserella muralis* sp. nov.

Prauserella muralis (mu.ra'lis. L. fem. adj. *muralis* pertaining or belonging to walls).

Forms mycelium-like filaments, about 1.3 μm wide. Substrate mycelium on M79 agar is grey to slightly orange. Aerial mycelium on agar is white and becomes fragmented. Gram-stain-positive, oxidase-positive (weak reaction), showing an oxidative metabolism. Good growth occurs after 3 days of incubation on tryptone soy agar and nutrient agar at 25–30 °C. The quinone system is composed of the predominant compound menaquinone MK-9(H₄). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, phosphatidylserine and an unknown phospholipid (phospholipid pattern II *sensu* Lechevalier *et al.*, 1977). Major fatty acids are C_{16:0} iso, C_{16:0}, C_{17:1ω8c} and C_{17:1ω6c}. Carbon source utilization (including differentiating characters using identical conditions in all cases) is indicated in Table 2.

The type strain, 05-Be-005^T (=CCUG 57426^T =NRRL B-24780^T =CCM 7635^T=DSM 45305^T), was isolated in Berlin, Germany, by Dr C. Trautmann, sampled from the wall of a house colonized with moulds.

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