A new genus of the family *Micromonosporaceae*, *Polymorphospora* gen. nov., with description of *Polymorphospora rubra* sp. nov.

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Two actinomycete strains were isolated from soil surrounding mangrove roots. The isolates formed short spore chains with spores showing diverse shapes. The isolates contained glutamic acid, glycine, alanine and *meso*-diaminopimelic acid in the cell wall, 3-O-methylmannose, mannose, galactose and glucose as the whole-cell sugars and MK-10(H₆), MK-10(H₄), MK-9(H₆) and MK-9(H₄) as the predominant isoprenoid quinones. The isolates formed a distinct taxon in the phylogenetic tree of the *Micromonosporaceae* based on analysis of 16S rRNA gene sequences and showed chemical and phenotypic properties that were different from members of all of the other genera of this family. Based on these observations, it is proposed that the novel isolates belong to a new genus, *Polymorphospora* gen. nov. The type species of the genus is proposed as *Polymorphospora rubra* sp. nov., with strain TT 97-42^T (=NBRC 101157^T = DSM 44947^T) as the type strain.

The family *Micromonosporaceae* was originally found to possess cell walls of chemotype II/D (Goodfellow *et al.*, 1990) according to the classification of Lechevalier & Lechevalier (1970). However, the genera *Catenuloplanes* (Yokota *et al.*, 1993) and *Couchioplanes* (Tamura *et al.*, 1994), which harboured chemotype VI, were subsequently added to this family based on phylogenetic analysis. The description of this family was later emended by Koch *et al.* (1996) and Stackebrandt *et al.* (1997) on the basis of 16S rRNA gene sequence analysis. This family currently consists of 13 genera, including *Micromonospora* (Orskov, 1923), *Actinoplanes* (Couch, 1950; Stackebrandt & Kroppenstedt, 1987), *Pilimelia* (Kane, 1966), *Dactylosporangium* (Thiemann *et al.*, 1967), *Catellatospora* (Asano & Kawamoto, 1986; Lee & Hah, 2002), *Catenuloplanes* (Kudo *et al.*, 1999; Yokota *et al.*, 1993), *Couchioplanes* (Tamura *et al.*, 1994), *Spirilliplanes* (Tamura *et al.*, 1997), *Verrucospora* (Rheims *et al.*, 1998), *Virgisporangium* (Tamura *et al.*, 2001), *Asanoa* (Lee & Hah, 2002), *Longispora* (Matsumoto *et al.*, 2003) and *Salinispora* (Maldonado *et al.*, 2005).

Mangroves are formed from tropical shrubs and trees belonging to the genera *Rhizophora*, *Bruguiera* and *Sonneratia*. These plants grow in shore mud with tangled roots that are partly aerial. Mangroves spread from the shore into

the mouths of rivers in the tropical and subtropical areas of Africa, America and Asia (Spalding *et al.*, 1977) and they are known to be the habitat of various micro-organisms (Chapman, 1976; Odum & Heald, 1972; Robertson & Duke, 1987). During the course of taxonomic studies on the rare actinomycetes associated with mangroves, two novel strains were isolated from soil samples surrounding mangrove roots in Okinawa, Japan. These strains produced short spore chains and contained *meso*-diaminopimelic acid (A₂pm) and glycine in their cell walls. The novel isolates belonged to the family *Micromonosporaceae*, but formed a lineage distinct from previously recognized genera. The data presented in this paper show that the novel isolates are members of a new genus, *Polymorphospora* gen. nov.

Strains TT 97-42^T and TT 97-44 were isolated from soil samples collected near the roots of *Bruguiera gymnorrhiza* and *Sonneratia alba* at the mouth of the River Shiira on Iriomote Island, Okinawa, Japan. The two strains were isolated using humic acid-vitamin (HV) agar (Hayakawa & Nonomura, 1987) using the yeast extract–SDS method (Hayakawa & Nonomura, 1989). Freeze-dried cells for chemotaxonomic analyses were obtained from cultures grown in yeast extract–glucose broth (10 g yeast extract and 10 g D-glucose in 1 l distilled water, pH adjusted to 7.0) incubated at 28 °C on a rotary shaker for 4 days.

Cultures were grown on HV agar and yeast extract–starch agar (2 g yeast extract and 10 g soluble starch in 1 l distilled water, pH 7.0) by incubating at 28 °C for 3–14 days. Cultures were observed under a light microscope and under a

Abbreviations: A₂pm, diaminopimelic acid; ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains TT 97-42^T and TT 97-44 are AB223089 and AB223090, respectively.

scanning electron microscope (model JSM-5400; JEOL) by using previously described methods (Tamura *et al.*, 1994). Scanning electron micrographs of 14-day-old cultures of strain TT 97-42^T grown on HV agar are shown in Fig. 1. The strain formed short spore chains. At the periphery of the colonies, the shapes of the spores were diverse, but they showed oval to short rod morphology at the centre of the colonies. The spore sizes were 0.5–0.9 µm long × 0.8–1.3 µm wide. The cells were non-motile.

Cultural characteristics were recorded during a 2-week incubation period at 28 °C, according to the scheme specified in the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). Nutrient agar (Difco) was used as the medium. Physiological characteristics were analysed as described previously (Gordon *et al.*, 1974; Yokota *et al.*, 1993). The novel isolates formed strong red to strong vivid reddish-orange colonies on yeast extract-malt extract agar (ISP medium 2), oatmeal agar (ISP medium 3) and inorganic salts-starch agar (ISP medium 4). Soluble pigment was not produced in any of the media tested. Aerial mycelia were rarely observed to develop on HV or water agar. The novel isolates showed good to moderately good growth on ISP media 2, 3 and 4, but no growth, or very poor growth, on glycerol-asparagine agar (ISP medium 5),

peptone-yeast extract iron agar (ISP medium 6) and tyrosine agar (ISP medium 7). The novel isolates utilized D-mannitol, D-melibiose, maltose, L-rhamnose, methyl α-D-glucoside, D-galactose, D-mannose and D-glucose. Only one of the isolates, strain TT 97-42^T, utilized D-raffinose. The strains were positive in tests for starch hydrolysis and NO₃ reduction. However, the strains were negative in tests for aesculin hydrolysis, urea decomposition, growth in Sabouraud dextrose broth, growth on MacConkey agar, utilization of sodium succinate, sodium oxalate, sodium malate and sodium citrate and resistance to 4 % NaCl, methyl violet and pyronine B.

Whole-cell sugars, cell-wall amino acids, isoprenoid quinones, cellular fatty acids, phospholipids, diaminopimelic acid isomers, acyl type of muramic acids, mycolic acids and DNA G+C content were analysed as described previously (Tamura *et al.*, 1994). The predominant menaquinones found in the isolates were MK-10(H₆) (34–35 %), MK-10(H₄) (20–28 %), MK-9(H₆) (20–27 %) and MK-9(H₄) (17–19 %). The novel isolates contained glucose, mannose, galactose and 3-O-methylmannose as whole-cell sugars with varying amounts of xylose, but not arabinose. The cell-wall amino acids were *meso*-A₂pm, alanine, glycine and glutamic acid (at a molar ratio of about 0.6:1.0:1.1:1.2). These amino acids corresponded to murein type A1γ as described by Schleifer & Kandler (1972). Mycolic acids were not detected. The acyl moiety of murein contained glycolyl residues. Phosphatidylethanolamine was detected as a diagnostic phospholipid, but phosphatidylglycerol-, phosphatidylcholine- and glucosamine-containing phospholipids were not detected. The cellular fatty acids consisted of iso-branched, anteiso-branched, saturated and unsaturated fatty acids and corresponded to fatty acid pattern 2a as described by Kroppenstedt (1985). The major cellular fatty acids were iso-C_{16:0} (40–53 %), C_{17:1} (18–19 %), anteiso-C_{17:0} (11 %), anteiso-C_{15:0} (6–8 %) and C_{17:0} (5–8 %). The DNA G+C contents of strains TT 97-42^T and TT 97-44 were 70.2 and 70.7 mol%, respectively.

The 16S rRNA gene was amplified by using PCR and the resulting PCR products were directly sequenced as described by Tamura & Hatano (1998) by using a DNA sequencer (ABI PRISM 3100 Genetic Analyzer; PE Applied Biosystems) according to the manufacturer's protocol. The 16S rRNA gene sequences of the novel strains and reference organisms were aligned with those of *Streptomyces ambofaciens* (Pernodet *et al.*, 1989). The CLUSTAL_X 1.83 software package (Thompson *et al.*, 1997) was used to generate evolutionary distances (K_{nuc} value; Kimura, 1980), similarity values and a neighbour-joining phylogenetic tree (Saitou & Nei, 1987) based on the K_{nuc} and bootstrap values (Felsenstein, 1985) for 1000 replications. PAUP 4.0 (Swofford, 2002) was used to generate a maximum-parsimony tree (Swofford & Berlocher, 1987). NJPLOT (Perrière & Gouy, 1996) was used to plot the phylogenetic trees. DNA–DNA relatedness was determined using the microplate hybridization method developed by Ezaki

Fig. 1. Scanning electron micrographs of cells of strain TT 97-42^T grown on HV agar for 2 weeks at 28 °C. Bars, 5 µm (a) and 1 µm (b).

et al. (1988, 1989) with minor modifications (Tamura *et al.*, 1999). Phylogenetic analysis of the 16S rRNA gene sequences revealed that the novel isolates belonged to a cluster of the family *Micromonosporaceae* and represented a new phyletic line that was different from each of the 13 genera in this family with validly published names (Fig. 2). This finding was supported by the topology of the maximum-parsimony tree and by the bootstrap value of 100 % obtained in the neighbour-joining analysis. Comparison of the 16S rRNA gene sequences of the novel isolates and their closest neighbours, members of the genera *Dactylosporangium* and *Micromonospora*, revealed similarity values of between 95.8 and 98.1 %. The gene sequence similarity between isolates TT 97-42^T and TT 97-44 was 100 %. The DNA–DNA relatedness value between the two novel isolates was 72.7–72.9 %.

The novel isolates contained *meso*-A₂pm and glycine in their peptidoglycan. Arabinose was not detected as a whole-cell sugar. Further, arabinose was not found among the members of the genera *Catenuloplanes* and *Spirilliplanes* (Tamura *et al.*, 1997; Yokota *et al.*, 1993). The novel isolates also contained 3-*O*-methylmannose as a whole-cell sugar and this sugar was also detected in members of the genera *Spirilliplanes* and *Virgisorangium* (Tamura *et al.*, 1997, 2001). Furthermore, the presence of *N*-glycolyl muramic acid in the cell-wall peptidoglycan and the absence of mycolic acid are characteristics of the family *Micromonosporaceae*. Therefore, the novel isolates were assigned to the family *Micromonosporaceae* based on chemotaxonomic characteristics.

The novel isolates formed spore chains and differed in this respect from the genera *Dactylosporangium* and *Micromonospora*, the closest phylogenetic neighbours. Thus, the

chemotaxonomic and morphological characteristics of the novel isolates distinguish them from any of the previously recognized members of the family *Micromonosporaceae* (Table 1).

The genotypic and phenotypic data described above suggest that strains TT 97-42^T and TT 97-44 form an independent monophyletic clade within the family *Micromonosporaceae*. Based on their high DNA–DNA relatedness, the two novel strains are concluded to represent the same species. The novel isolates are assigned to a new genus, *Polymorphospora* gen. nov., with the name *Polymorphospora rubra* sp. nov. proposed for the type species of the genus. Strain TT 97-42^T (=NBRC 101157^T=DSM 44947^T) is proposed as the type strain of the type species.

Description of *Polymorphospora* gen. nov.

Polymorphospora (Po.ly.mor.pho.spo'ra. Gr. adj. *polymorphos* multiform; N.L. fem. n. *spora* a spore; N.L. fem. n. *Polymorphospora* polymorphic spore).

Gram-positive, non-acid-fast, aerobic actinomycetes that show extensive branching and non-fragmenting substrate hyphae. Short spore chains develop on short sporophores on the substrate mycelium. Immature spores are oval or of various shapes and short rods are formed (0.6–0.9 µm long × 0.8–1.5 µm wide) on maturation. Spores are non-motile. Strictly aerobic. Optimum temperature for growth generally ranges between 20 and 30 °C. The cell walls contain glutamic acid, glycine, alanine and *meso*-diaminopimelic acid. The chemotype is II according to Lechevalier & Lechevalier (1970) and the peptidoglycan type is presumed to be A1γ according to Schleifer & Kandler (1972). Mannose, 3-*O*-methylmannose, glucose and galactose are detected

Fig. 2. A neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of isolates TT 97-42^T and TT 97-44 and the family *Micromonosporaceae*. *Streptomyces ambofaciens* ATCC 23877^T (GenBank accession number M27245) was used as an outgroup (not shown). Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. Bar, 0.01 *K*_{nuc}.

Table 1. Characteristics that differentiate the genus *Polymorphospora* from other genera of the family *Micromonosporaceae*

Taxa: 1, *Polymorphospora*; 2, *Actinoplanes*; 3, *Asanoa*; 4, *Catellatospora*; 5, *Catenuloplanes*; 6, *Couchioplanes*; 7, *Dactylosporangium*; 8, *Longispora*; 9, *Micromonospora*; 10, *Pilimelia*; 11, *Salinispora*; 12, *Spirilliplanes*; 13, *Verrucosipora*; 14, *Virgisporangium*. Data are taken from Kroppenstedt (1985), Asano & Kawamoto (1986, 1988), Asano *et al.* (1989), Goodfellow (1989), Yokota *et al.* (1993), Tamura *et al.* (1994, 1995, 1997, 2001), Rheims *et al.* (1998), Kudo *et al.* (1999), Lee *et al.* (2000), Lee & Hah (2002), Matsumoto *et al.* (2003) and Maldonado *et al.* (2005). Cell-wall chemotype is based on the classification of Lechevalier & Lechevalier (1970). Fatty acid type is according to the classification of Kroppenstedt (1985) and phospholipid type is assigned according to Lechevalier *et al.* (1977). +, Positive; –, negative; ND, not determined; Ara, arabinose; Gal, galactose; Man, mannose; Rha, rhamnose; Xyl, xylose.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Spore motility	–	+	–	–	+	+	+	–	–	+	–	+	–	+
Spore vesicles	–	+	–	–	–	–	+	–	–	+	–	–	–	+
Cell-wall chemotype	II	II	II	II	VI	VI	II	II	II	II	II	II	II	II
Major whole organism sugars	Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal and Xyl or only Xyl	Xyl	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Xyl	Ara, Xyl	Ara, Gal, Xyl	Gal, Xyl	Man, Xyl	Ara, Gal, Man, Rha, Xyl
Fatty acid type	2a	2c	2d	3b	2c	2c	2d	2d	3b	2b	3a	2d	2b	2d
Major menaquinones (MK-)	10(H ₆ , H ₄), 9(H ₆ , H ₄)	9(H ₄), 10(H ₄)	10(H ₆ , H ₈)	9(H ₄ , H ₆) or 10(H ₄)	10(H ₄), 11(H ₄)	9(H ₄)	9(H ₄ , H ₆ , H ₈)	10(H ₄ , H ₆)	9(H ₄ , H ₆), 10(H ₄ , H ₆)	9(H ₂ , H ₄)	9(H ₄)	10(H ₄)	9(H ₄)	10(H ₄ , H ₆ , H ₈)
Phospholipid type	II	II	II	II	III	II	II	II	II	II	II	II	II	II
DNA G+C content (mol%)	71	72–73	71–72	70–72	70–73	70–72	72–73	70	71–73	ND	70–73	69	70	71

as whole-cell sugars. The predominant cellular fatty acid is iso-C_{16:0}. The major menaquinones are MK-10(H₆), MK-10(H₄), MK-9(H₆) and MK-9(H₄). Phosphatidylethanolamine is present as the diagnostic phospholipid (phospholipid pattern type PII). The acyl type of the cell-wall polysaccharides is glycolyl. Mycolic acid is not detected. The G + C content of DNA is approximately 70–71 mol%. Unique nucleotide signatures are present at positions 1244 (U) of the 16S rRNA gene. This genus belongs to the family *Micromonosporaceae*. The type species is *Polymorphospora rubra*.

Description of *Polymorphospora rubra* sp. nov.

Polymorphospora rubra (ru'bra. L. fem. adj. *rubra* red).

In addition to the morphological, chemotaxonomic and general characteristics described for the genus, the species has the following characteristics. Colonies that develop on ISP media 2, 3 and 4 are red to reddish-orange in colour. Utilizes D-mannitol, D-melibiose, maltose, L-rhamnose, methyl α -D-glucoside, D-galactose, D-mannose and D-glucose. Positive in tests for starch hydrolysis and urea decomposition. No growth in the presence of 4% NaCl. Aesculin is not hydrolysed. The major cellular fatty acids are iso-C_{16:0} and C_{16:1}. The G + C content of DNA is 70 mol%.

The type strain TT 97-42^T (=NBRC 101157^T=DSM 44947^T), was isolated from soil surrounding mangrove roots.

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