Strain 211018\textsuperscript{T} was isolated from mangrove *Excocaria agallocha* rhizosphere soil. 16S rRNA gene sequence analysis showed the highest similarity to the type strains of *Micromonospora olivasterospora* DSM 43868\textsuperscript{T} (98.6 %) and *Micromonospora pattaloongensis* TJ2-2\textsuperscript{T} (98.4 %). *gyrB* gene sequence analysis also indicated that strain 211018\textsuperscript{T} should be assigned to the genus *Micromonospora*. The characteristic whole-cell sugars are xylose, mannose and arabinose. The predominant menaquinone is MK-9(H\textsubscript{4}) and the major fatty acids are iso-C\textsubscript{15 : 0} (27.5 %), 10-methyl C\textsubscript{17 : 0} (14.2 %), C\textsubscript{17 : 1\textsubscript{o}}8c (12.8 %), iso-C\textsubscript{16 : 0} (12.6 %), anteiso-C\textsubscript{15 : 0} (6.1 %), iso-C\textsubscript{17 : 0} (4.1 %) and anteiso-C\textsubscript{17 : 0} (4.0 %). The phospholipid profile comprises phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerol and phosphatidylglycerol mannosides. The DNA G+C content is 70.8 mol\%. The chemotaxonomic data of the strain coincided with those of the genus *Micromonospora*. Furthermore, a combination of DNA–DNA hybridization results and some physiological and biochemical properties indicated that the novel strain could be readily distinguished from the closest phylogenetic relatives. On the basis of these phenotypic and genotypic data, strain 211018\textsuperscript{T} represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora rhizosphaerae* sp. nov. is proposed. The type strain is 211018\textsuperscript{T} (=CGMCC 4.5599\textsuperscript{T} = DSM 45431\textsuperscript{T}).

The genus *Micromonospora*, which was first described by Ørskov (1923), is widely distributed in different environments such as soil, root nodules and marine sediment (Maldonado et al., 2005; Trujillo et al., 2005, 2006; Ara & Kudo, 2007). Mangroves grow in the interface between land and sea at tropical and subtropical latitudes in conditions of high salinity, extreme tides, strong winds and high temperature (Kathiresan & Bingham, 2001). The rhizosphere of mangroves has been shown to be a good source for novel actinomycetes (Hatano, 1997; Takeuchi & Hatano, 1998, 1999).

In the course of exploiting mangrove actinomycete resources in Hainan Province, China (Hong et al., 2009), strain 211018\textsuperscript{T} was isolated from a soil sample of the *Excocaria agallocha* rhizosphere. One gram of soil was heated in a hot air oven at 120 °C for 60 min, treated with a solution of 1.0 % chloramine-T for 20 min and diluted to 10\textsuperscript{-2}, then 100 μl of the resultant solution was inoculated on agar plates of humic acid-vitamin medium (HV; Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l\textsuperscript{-1}), nystatin (50 mg l\textsuperscript{-1}) and potassium dichromate (100 mg l\textsuperscript{-1}). After 21 days of aerobic incubation at 28 °C, the isolate, which formed a yellowish-white colony, was transferred and purified on yeast extract-malt extract [International Streptomycetes Project (ISP) 2] agar (Shirling & Gottlieb, 1966) and maintained as working cultures on M8 agar (Castiglione et al., 2008) containing: soluble starch, 20.0 g; glucose, 10.0 g; yeast extract, 2.0 g; hydrolysed casein, 4.0 g; meat extract, 2.0 g; CaCO\textsubscript{3}, 3.0 g; and agar, 15.0 g in 1 l distilled water (pH 7.0).

Morphological characteristics were observed by light microscopy (80i, Nikon) and scanning electron microscopy (FEI QUANTA) using cultures grown on ISP 3 medium (Shirling & Gottlieb, 1966) at 28 °C for 21 days. Cultural characteristics of strain 211018\textsuperscript{T} were tested using 14-day-old cultures grown at 28 °C on various agar media. The ISCC-NBS colour charts were used to determine the names and designations of colony colours (Kelly, 1964).
Phenotypic characteristics of strain 211018\textsuperscript{T} and its phylogenetically close neighbours were examined using several standard methods. Temperature range (4–45 °C), pH (4–11) and NaCl (0–30 %) tolerance for growth were determined on ISP 2 for 14–21 days at 28 °C. Gelatin liquefaction, milk peptonization, nitrate reduction and starch hydrolysis were determined through cultivation on various media as described by Arai (1975) and Williams & Cross (1971). Carbon-source utilization was tested by using ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with 1 % (final concentration) carbon source. The utilization of amino acids as nitrogen source was tested as described by Williams et al. (1983). Production of melanoid pigments was examined using tyrosine agar (ISP 7; Shirling & Gottlieb, 1966).

Freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in M8 broth on a rotary shaker at 28 °C for 4 days. Amino acids and sugars in whole-cell hydrolysates were analysed according to the procedure of Lechevalier & Lechevalier (1980). The acyl group of the muramic acid in the peptidoglycan was determined by the method of Uchida & Aida (1984). Analysis of phospholipids was carried out by TLC according to Minnikin et al. (1984). Fatty acids were extracted by the method of Sassor (1990) and the composition was determined by gas chromatography (Oliver & Colwell, 1973). Menaquinones were extracted from freeze-dried biomass, purified according to Minnikin et al. (1984) and finally analysed by HPLC with an ODS-BP C\textsubscript{18} column (4.6 × 250 mm). The elution solvent was a mixture of methanol and 2-propanol (3:2, v/v).

Genomic DNA was extracted as described by Pospiech & Neumann (1995). The DNA G+C content of strain 211018\textsuperscript{T} was determined using the HPLC method (Pospiech et al., 1989). The level of DNA relatedness between strain 211018\textsuperscript{T} and the reference strain Micromonospora olivasterospora JCM 7348\textsuperscript{T} was determined by total DNA–DNA hybridization. DNA probes were labelled with digoxigenin modified dUTP according to Minnikin et al. (1984). Unlabelled and denatured DNA from freeze-dried biomass, purified according to Minnikin et al. (1984) and finally analysed by HPLC with an ODS-BP C\textsubscript{18} column (4.6 × 250 mm). The elution solvent was a mixture of methanol and 2-propanol (3:2, v/v).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were carried out as described by Nakajima et al. (1999). The 16S rRNA gene sequence of strain 211018\textsuperscript{T} was multiply aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X software. The alignment was manually verified and adjusted prior to the reconstruction of a phylogenetic tree. A phylogenetic tree was reconstructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Kluge & Farris, 1969) in MEGA, version 4.0 (Tamura et al., 2007). Confidence values for the branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Values for sequence similarity among the closest strains were calculated manually after obtaining pair-wise alignments using CLUSTAL X (Thompson et al., 1997). Gaps and ambiguous nucleotides were eliminated from the calculations. Catellatospora citrea IMSNU 22008\textsuperscript{ST} was used as an outgroup. PCR amplification of the gyrB gene and sequencing of the PCR products were carried out as described by Kasai et al. (2000). Phylogenetic analysis was performed as described above.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{phylogenetic_tree.png}
\caption{Neighbour-joining phylogenetic tree (Saitou & Nei, 1987), based on almost-complete 16S rRNA gene sequences, showing the relationships between strain 211018\textsuperscript{T} and phylogenetically related species of the genus Micromonospora. Catellatospora citrea IMSNU 22008\textsuperscript{ST} was used as an outgroup. Asterisks indicate branches that were also found using the maximum-parsimony (Kluge & Farris, 1969) method. Numbers at branch points indicate bootstrap percentages (based on 1000 replicates); only values >50 % are indicated. Bar, 0.005 substitutions per nucleotide position.}
\end{figure}
The morphological and chemical properties of strain 211018T are consistent with its classification as a member of the genus *Micromonospora* (Kawamoto, 1989). Strain 211018T produced well-developed and branched substrate hyphae on ISP 2 medium, but no aerial hyphae. Sparse spores were borne singly from the substrate hyphae having a diameter of approximately 0.9 μm, and the spore surface appeared warty (Supplementary Fig. S1, available in IJSEM Online). Good growth was observed on ISP 2 medium, ATCC 172 agar (http://www.lgcstandards-atcc.org/Attachments/2915.pdf), M8 agar and GYM agar (Wiese et al., 2008); moderate growth was observed on ISP 1 medium and ISP 6 medium (Shirling & Gottlieb, 1966), tap water agar, Czapek’s agar (Wiese et al., 2008) and modified Bennett agar (Jones, 1949); poor growth was observed on ISP 3 medium, ISP 4 medium, ISP 5 medium and ISP 7 medium (Shirling & Gottlieb, 1966). The colour of the substrate hyphae was brilliant orange, spore colour was yellowish-white, and a yellow, soluble pigment was produced on ISP 2, ATCC 172 and M8 agar.

The cell-wall hydrolysates contained glutamic acid, glycine, alanine and dianimonopellic acid (A2pm); the A2pm isomer was meso, indicating that this strain has wall chemotype II (Lechevalier & Lechevalier, 1970) and peptidoglycan type A1γ (Schleifer & Kandler, 1972). The acyl type of the cell-wall muramic acid was glycolyl. Glucose, xylose, mannose, ribose and arabinose were found as whole-cell sugars, but rhamnose was not detected (whole-cell sugar pattern D; Lechevalier & Lechevalier, 1970). Phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides were detected, corresponding to phospholipid type PII (Lechevalier et al., 1977). The major cellular fatty acids were iso-C15:0 (27.5 %), 10-methyl C17:0 (14.2 %), C17:1ω8c (12.8 %), iso-C16:0 (12.6 %), anteiso-C15:0 (6.1 %), iso-C17:0 (4.1 %) and anteiso-C17:0 (4.0 %) (Supplementary Table S1). This pattern corresponds to fatty acid type 3b (Kroppenstedt, 1985). The predominant menaquinones were MK-9(H4) (47.2 %), MK-9(H6) (28.2 %) and MK-9(H2) (15.9 %); small amounts of MK-10(H4) (8.6 %) were also present. The G+C content of the DNA was 70.8 mol%.

An almost-complete 16S rRNA gene sequence (1481 nt) was obtained for strain 211018T and compared with those deposited in public databases. The highest levels of similarity were with *M. olivasterospora* DSM 43868T (98.6 %) and *Micromonospora pattaloongensis* TJ2-2T (98.4 %). A subset of the closest phylogenetic relatives of strain 211018T based on the neighbour-joining method are presented in Fig. 1. A phylogenetic tree that includes all species of the genus *Micromonospora* with validly published names is available as Supplementary Fig. S2. The gyrB gene sequence analysis also indicated that strain 211018T should be assigned to the genus *Micromonospora*. Similarity values between strain 211018T and *M. olivasterospora* DSM 43868T and *M. pattaloongensis* TJ2-2T were 92.3 % and 88.5 %, respectively. Gyrase B sequence similarities between strain 211018T and other available species of the genus *Micromonospora* ranged from 88.9 % to 94.7 %.

The characteristics shown in Table 1 clearly indicate that strain 211018T possesses some distinct phenotypic and chemotaxonomic profiles that distinguish it from its closest phylogenetic relatives, *M. olivasterospora* JCM 7348T and *M. pattaloongensis* JCM 12833T. Furthermore, a low level of DNA–DNA relatedness (18.2–20.9 %) was observed between strain 211018T and other available species of the genus *Micromonospora* ranged from 88.9 % to 94.7 %. The phylogenetic analysis shown in Fig. 2 included all available sequences for type strains representing species of the genus *Micromonospora* and other members of the family *Micromonosporaceae*.

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![Fig. 2. Neighbour-joining phylogenetic tree based on the gyrB gene sequences of strain 211018T, recognized species of the genus *Micromonospora* and members of the family *Micromonosporaceae*. Asterisks indicate branches that were also found using the maximum-parsimony (Kluge & Farris, 1969) method. Numbers at branch points indicate bootstrap percentages (based on 1000 replicates); only values >50 % are indicated. Bar, 0.02 substitutions per nucleotide position.](image-url)
from previously described species of the genus *Micromonospora*. Therefore, strain 211018T represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora rhizosphaerae* sp. nov. is proposed.

**Description of Micromonospora rhizosphaerae sp. nov.**

*Micromonospora rhizosphaerae* (rhi.zo.spha.e’r.e. Gr. n. rhiza root; L. n. sphaera a ball, sphere; N.L. n. rhizofera rhizophere; N.L. gen. n. rhizoferae of the rhizosphere, pertaining to the soil from which the type strain was isolated).

Aerobic, Gram-positive, mesophilic actinomycete that forms well-developed and branched substrate hyphae. Colonies are brilliant orange to yellowish-white and, on all tested media, raised colonies become white with increased age. Single spores are formed on substrate hyphae. Aerial hyphae are absent. A yellow soluble pigment is produced on ISP 2, M8 and ATCC 172 agar. Nitrate is reduced to nitrite. Utilizes D-glucose and D-fructose and weakly utilizes D-galactose and D-xylose, but does not utilize maltose, sucrose, D-ribose, raffinose, L-rhamnose, D-arabinose, lactose, α-melibiose, melezitose, dulcitol, myo-inositol, salicin, glycerol or mannitol. D-Alanine, L-histidine, L-hydroxyproline and L-serine are used as nitrogen source. Positive for milk peptonization, but negative for starch hydrolysis, gelatin liquefaction, melanin formation and H2S production. The optimal temperature for growth is 28–40 °C; no growth occurs below 10 °C. The pH range for growth is 5–9, with an optimum at 7. The maximum NaCl concentration for growth is 2 %. The acyl type of the cell-wall muramic acid is glycolyl. The cell wall contains meso-diaminopimelic acid. The predominant menaquinone is MK-9(H4). The characteristic whole-cell sugars are xylose, mannose and arabinose. The phospholipid profile comprises phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The major fatty acids of the type strain are iso-C15 : 0, 10-methyl C17 : 0, C17 : 1ω8c (12.8 %), iso-C16 : 0 (27.5 %), 10-methyl C17 : 0 (14.2 %), C17 : 1ω8c (12.8 %) and anteiso-C15 : 0 (13.1 %). The DNA G + C content of the type strain is 70.8 mol%.

The type strain, 211018T (=CGMCC 4.5599T =DSM 45431T), was isolated from mangrove *Exocaria agallocha* rhizophere soil in Hainan Province, China.

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**References**


