Microbispora hainanensis sp. nov., isolated from rhizosphere soil of Excoecaria agallocha in a mangrove

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Strain 211020T was isolated from rhizosphere soil of Excoecaria agallocha in Hainan, China. The strain produced longitudinal pair spores branching from aerial hyphae. 16S rRNA gene sequence analysis showed that the isolate belonged to the genus Microbispora, exhibiting the highest 16S rRNA gene sequence similarity (98.75%) to Microbispora corallina JCM 10267T with a low DNA–DNA relatedness value (13 ± 0.6%). The isolate contained meso-diaminopimelic acid as the diagnostic diamino acid but madurose was not detected. The predominant menaquinones were MK-9(H4), MK-9(H2) and MK-9(H6), and the major fatty acids were iso-C16:0, iso-C15:0 and C17:0. The phospholipid profile of strain 211020T comprised phosphatidylinositol mannoside, phosphatidylethanolamine, diphosphatidylglycerol and phospholipids of unknown structure containing glucosamine. The DNA G+C content was 70.8 mol%. On the basis of phenotypic and genotypic data, strain 211020T can be distinguished as a novel species of the genus Microbispora, for which the name Microbispora hainanensis sp. nov., is proposed. The type strain is 211020T (=CGMCC 4.5595T=DSM 45428T).

The genus Microbispora belonging to the family Streptosporangiaceae was proposed by Nomomura & Ohara (1957), based on producing longitudinally paired spores on aerial mycelia. M. rosea (Nomomura & Ohara, 1957) is the type species. In the List of Prokaryotic names with Standing in Nomenclature (http://www.bacterio.cict.fr/index.html), 16 species and two subspecies of the genus Microbispora were cited at the time of writing, including Microbispora rosea (Nomomura & Ohara, 1957), Microbispora chromogenes, Microbispora diastatica, Microbispora parva, Microbispora anethystogenes (Nomomura & Ohara, 1960), Microbispora bispora (Lechevalier, 1965), Microbispora thermodiastatica, Microbispora thermorosea (Nomomura & Ohara, 1969), Microbispora echinospora (Nomomura & Ohara, 1971), Microbispora aerata (Cross, 1974), Microbispora viridis (Miyadoh et al., 1985), Microbispora indica, Microbispora karnatakensis (Rao et al., 1987), Microbispora mesophila (Zhang et al., 1998), Microbispora corallina (Nakajima et al., 1999) and Microbispora siamensis (Boondaeng et al., 2009). A number of species have been moved out of, or into the genus Microbispora. In 1990, Kroppenstedt et al. and Miyadoh et al. transferred M. echinospora and M. viridis to the genus Actinomadura as A. echinospora (Kroppenstedt et al. 1990) and A. rugatobispora (Miyadoh et al. 1990), respectively. According to DNA–DNA hybridization experiments, 10 species of the genus Microbispora were suggested to be combined into the type species M. rosea with two subspecies, M. rosea subsp. rosea and M. rosea subsp. aerata (Miyadoh et al. 1990). Wang et al. (1996) proposed to transfer M. bispora to the new genus Thermobispora as Thermobispora bispora based on the analysis of 16S rRNA gene sequence. Thermomonospora mesophila, which produces spores singly borne on aerial hyphae, was reclassified to be a new member of the genus Microbispora as M. mesophila by Zhang et al. (1998). The rhizosphere of
mangroves has proven to be a good source of novel actinobacteria (Wang et al., 2011a, b). During a study of selective isolation of Microbispora cultures from mangrove soil, 19 isolates were obtained and found to belong to the genus Microbispora by their 16S rRNA gene sequence analysis (Xu et al., 2009). Strain 211020T was isolated on humic acid vitamin agar plates from rhizosphere soil of Excoecaria agallocha collected from a mangrove in Hainan, China (19° 37.563′ N, 110° 47.731′ E). On the basis of a taxonomic study using a polyphasic approach, strain 211020T represents a novel species of the genus Microbispora.

Morphological characteristics of strain 211020T were observed by light microscopy (80i; Nikon) and scanning electron microscopy (Quanta; FEI) using cultures grown on ISP 3 medium (Shirling & Gottlieb, 1966) at 28 °C for 14 days. Growth characteristics of strain 211020T were tested using 14 day cultures grown at 28 °C on various agar media. The ISCC-NBS colour charts were used to determine the designations of colony colours (Kelly, 1964). Phenotypic characteristics of strain 211020T were examined using several standard methods: the temperature range (4–55 °C), pH (4–11) and NaCl (0–30 %) tolerance for growth were determined on ISP 2 for 14–21 days at 28 °C, except for the temperature tests; gelatin liquefaction, milk peptonization, nitrate reduction and starch hydrolysis were determined through cultivation on various media as described by Williams & Cross (1971) and Arai (1975); carbon-source utilization was tested by using ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with 1 % (final concentration) carbon sources; the utilization of amino acids as sole nitrogen sources was tested as described by Williams et al. (1983); production of melanoid pigments was examined using tyrosine agar (ISP 7).

Biomass used for chemotaxonomic analyses was obtained from cultures grown in ISP 2 broth on a rotary shaker (approximately 180 r.p.m.) at 28 °C for 4 days. Amino acids and sugars in whole-cell hydrolysates were analysed according to the procedure of Lechevalier & Lechevalier (1980). Menaquinones were extracted from freeze-dried biomass, purified according to Minnikin et al. (1984) and finally analysed by HPLC with an ODS-BP C18 column (4.6 × 250 mm). The elution solvent was methanol/2-propanol (3:2, v/v). Fatty acids were extracted by the method of Sasser (1990) and the composition was determined by gas chromatography (Oliver & Colwell, 1973). Analysis of the phospholipids was carried out by TLC according to Minnikin et al. (1984).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were carried out using the procedure described by Goodfellow et al. (2007). The 16S rRNA gene sequence of strain 211020T was aligned with selected sequences obtained from the GenBank/EMBL/DDJB databases using CLUSTAL_X software. The multiple sequence alignment was manually verified and adjusted prior to the construction of a phylogenetic tree. The phylogenetic tree was generated via the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms in MEGA software version 4.0 (Tamura et al., 2007). The confidence values for the branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The values for pairwise 16S rRNA gene sequence similarity among the closest strains were determined using the EzTaxon server (http://www.Eztaxon.org) (Chun et al., 2007). Gaps and ambiguous nucleotides were eliminated from the calculations. Chromosomal DNA was extracted as described by Pospiech & Neumann (1995). The DNA G + C content of strain 211020T was determined by the HPLC method (Mesbah et al., 1989). DNA–DNA hybridization values between strain 211020T and M. corallina JCM 10267T were measured on nylon membranes using the method described by Wang et al. (2011a).

Strain 211020T was Gram-stain-positive. A non-fragmenting substrate mycelium was formed. A 14-day-old culture grown on ISP 3 agar media showed longitudinal pairs of spores branching from aerial hyphae (see Fig. S1, available in IJSEM online). Each spore was oval and its surface was smooth. Neither motile spores nor sporangium-like structures were observed. Strain 211020T exhibited good growth on yeast extract-malt extract agar (ISP 2) and oatmeal agar (ISP 3); moderate growth on peptone-yeast extract-iron agar (ISP 6); poor growth on glycerol-asparagine agar (ISP 5) and tyrosine agar (ISP 7); and almost no growth on inorganic salts-starch agar (ISP 4). Strain 211020T developed abundant white aerial hyphae on ISP 2 and ISP 3 media, but not on ISP 1, ISP 5, ISP 6 or ISP 7 media.

Whole-cell hydrolysates of strain 211020T contained meso-diaminopimelic acid. Glucose and galactose were detected, but malachro was not (Fig S2). Phosphatidylinositol mannoside, phosphatidylethanolamine, diphosphatidylglycerol and phospholipids of unknown structure containing glucosamine were detected, corresponding to phospholipid type IV (Fig S3). The cellular fatty acids were iso-C16 : 0 (37.11 %), iso-C15 : 0(16.82 %), C17 : 0 (8.88 %), iso-C17 : 0 (5.85 %), summed feature 3 (contains 2-OH iso-C15 : 0 and/or C16 : 1o7c) (5.58 %), C15 : 0 (5.01 %), C16 : 0 (3.99 %), C17 : 1ω8c (3.99 %), 10-methyl C17 : 0 (2.14 %), iso-C16 : 0 (1.89 %), anteiso-C17 : 0 (1.62 %), C14 : 0 (1.39 %), 10-methyl C16 : 0 (1.31 %), C17 : 1ω8c (1.28 %), iso-C18 : 0 (1.26 %), anteiso-C15 : 0 (1.04 %) and uncertain fatty acids (0.85 %) (Table S1). The predominant menaquinones were MK-9(H4) (24.86 %), MK-9(H2) (23.00 %) and MK-9(H6) (14.46 %); small amounts of MK-10(H4) (2.21 %) were also present. The G + C content of the genomic DNA was 70.8 mol%.

An almost-complete 16S rRNA gene sequence (1480 nt) was obtained for strain 211020T and compared with those available in public databases. A phylogenetic tree that includes members of the genus Microbispora and other selected strains with validly published names from the family...
Streptosporangiaceae is shown in Fig. 1. The 16S rRNA gene similarities between strain 211020<sup>T</sup> and the type strains of recognized species of the genus Microbispora ranged from 98.75 % (M. corallina) to 98.09 % (M. amethystogenes). The highest level of 16S rRNA gene sequence similarity was with M. corallina JCM 10267<sup>T</sup> (98.75 %). The characteristics shown in Table 1 clearly indicate that strain 211020<sup>T</sup> possesses distinct phenotypic and chemotaxonomic profiles that distinguish it from its closest phylogenetic relative, M. corallina JCM 10267<sup>T</sup>. Furthermore, a low level of DNA–DNA relatedness (12.4–13.6 %) was observed between strain 211020<sup>T</sup> and M. corallina JCM 10267<sup>T</sup> (Fig. S4). It is therefore evident from these genotypic and phenotypic data that strain 211020<sup>T</sup> is distinguishable from previously described species of the genus Microbispora, and thus represents a novel species of the genus Microbispora, for which the name Microbispora hainanensis sp. nov. is proposed.

**Description of Microbispora hainanensis sp. nov.**

*Microbispora hainanensis* (hai.nan.en’sis N.L. fem. adj. hainanensis of or pertaining to Hainan, a tropical province of China, from which the mangrove soil sample was collected).

Aerobic, Gram-positive, non-motile actinobacterium that forms extensively branched aerial hyphae and substrate hyphae. Forms longitudinal paired spore chains on ISP 2 and ISP 3 media. Hydrolyses starch, reduces nitrate, and degrades Tween 20 but not Tween 80. Does not produce melanin, urease or H<sub>2</sub>S, neither liquefies gelatin nor coagulates or peptonizes milk. Grows at 20 and 50 °C, but not at 10 or 55 °C. Grows at pH 5–8, and in the presence of 0–1 % (w/v) NaCl. Utilizes (+)-cellubiose, D-galactose, D-glucose, D-mannitol, D-mannose, (+)-melezitose, D-sorbitol, dulcitol, (+)-l-rhamnose, (+)-melibiose, soluble starch, meso-erythritol, dextrin, maltose and hippuric acid, but does not utilize adonitol, D-fructose, lactose, D-ribose, (+)-trehalose, (+)-D-xylene, glycerol, L-arabinose, L-sorbitose, inositol, sucrose, α-ketogluutaric acid, malic acid, lactic acid, succinic acid malonic acid or pyruvic acid as sole carbon sources. Utilizes L-histidine, L-4-hydroxyproline, L-serine and L-valine, but does not utilize L-arginine, L-methionine, L-phenylalanine or L-cysteine as sole nitrogen sources. Contains meso-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan, but madurose is not detected. The phospholipid profile consists of phosphatidylglycerol and phosphatidylethanolamine, diphosphatidylglycerol and phospholipids of unknown structure containing glucosamine. The cellular fatty acids are iso-C<sub>15</sub>:0, iso-C<sub>17</sub>:0, summed feature 3 (contains 2-OH iso-C<sub>15</sub>:0 and/or C<sub>16</sub>:1ω7c), C<sub>15</sub>:0, C<sub>16</sub>:1ω8c, 10-methyl C<sub>17</sub>:0, iso-C<sub>16</sub>:0, anteiso-C<sub>17</sub>:0, C<sub>14</sub>:0, 10-methyl C<sub>16</sub>:0, C<sub>17</sub>:1ω9c, iso-C<sub>18</sub>:0, anteiso-C<sub>15</sub>:0 and uncertain fatty acids. The predominant menaquinones are MK-9(H<sub>4</sub>), MK-9(H<sub>2</sub>) and MK-9(H<sub>0</sub>). The DNA–DNA hybridization related value between the type strain 211020<sup>T</sup> and M. corallina JCM 10267<sup>T</sup> is 13 ± 0.6 %.

The type strain, 211020<sup>T</sup> (=CGMCC 4.5595<sup>T</sup> = DSM 45428<sup>T</sup>), was isolated from rhizosphere soil of *Excoecaria agallocha* in a mangrove in Hainan Province, China. The G+C content of the type strain is 70.8 mol%.

![Neighbour-joining phylogenetic tree (Saitou & Nei, 1987), based on almost-complete 16S rRNA gene sequences (1400 nt), showing the relationships between strain 211020<sup>T</sup>, recognised species of the genus Microbispora, and type species of other genera in the family Streptosporangiaceae. Streptosporangium ambosfaciens ATCC 23877<sup>T</sup> 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, 0.01 substitutions per nucleotide position.](image-url)
Table 1. Diagnostic characteristics differentiating strain 211020T from *M. corallina* JCM 10267T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>211020T</th>
<th><em>M. corallina</em> JCM 10267T</th>
</tr>
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<tbody>
<tr>
<td>Colony colour on ISP 2 media</td>
<td>Brown</td>
<td>Pink</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
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<tr>
<td>D-Fructose/ lactose/ D-ribose/ D-xylene/ glycerol/ L-arabinose/ L-sorbose/ hippuric acid/ α-ketoglutaric acid/ 2-keto-D-glucuronic acid/ malic acid</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Methionine/ L-phenylalanine</td>
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<td>Tween 80 degradation</td>
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<tr>
<td>Gelatin liquefaction</td>
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<tr>
<td>DNA G + C content (mol%)</td>
<td>70.8</td>
<td>71.5*</td>
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</tbody>
</table>

*Data from Nakajima et al., 1999.

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**References**


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