Actinophytocola sediminis sp. nov., an actinomycete isolated from a marine sediment

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A novel actinomycete strain, designated YIM M13705T, was isolated from a marine sediment sample of the South China Sea and its characteristics were determined by a polyphasic approach. The slowly growing, Gram-stain-positive, aerobic strain produced branched substrate mycelium and aerial hyphae, and no diffusible pigment was produced on the media tested. At maturity, spore chains were formed on aerial hyphae and substrate mycelium was not fragmented. Whole-cell hydrolysates of the strain contained meso-diaminopimelic acid and galactose, glucose, ribose and rhamnose. The predominant menaquinones were MK-9(H4) and MK-10(H2). The polar lipids detected were diphosphatidylglycerol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylinositol and ninhydrin-positive phosphoglycolipids. The major fatty acid was iso-C16:0. The G+C content of the genomic DNA was 68.2 mol%. On the basis of 16S rRNA gene sequence, the strain was shown to be most closely related to species of the genus Actinophytocola. DNA–DNA hybridization relatedness values (70 %) of the isolate with its closest neighbour Actinophytocola xinjiangensis QAIII60T supported classification of the isolate as a representative of a novel species. On the basis of phylogenetic analysis, and phenotypic and genotypic data, it is concluded that the new isolate belongs to a novel species of the genus Actinophytocola, for which the name Actinophytocola sediminis sp. nov. (type strain YIM M13705T=DSM 45939T=BCRC 16956T) is proposed.

The genus Actinophytocola belongs to the family Pseudonocardiaeae and was proposed by Indananda et al. (2010). Currently, the genus Actinophytocola contains five species with validly published names, which were isolated from different habitats. Actinophytocola oryzae was isolated from the roots of Thai glutinous rice plants (Indananda et al., 2010), while Actinophytocola timorensis (Otoguro et al., 2011), Actinophytocola corallina (Otoguro et al., 2011), Actinophytocola burenkhanensis (Ara et al., 2011) and Actinophytocola xinjiangensis (Guo et al., 2011) were from soils. Members of the genus grow slowly, form non-fragmented substrate mycelium and, on some media, produce aerial mycelium that fragments into spore chains or spore chain-like structures. The cell wall contains meso-diaminopimelic acid and mycolic acids are absent. The major fatty acid, diagnostic menaquinone and polar phospholipid are iso-C16:0, MK-9(H4) and phosphaethylanolamine, respectively (Indananda et al., 2010; Ara et al., 2011; Otoguro et al., 2011; Guo et al., 2011). However, A. xinjiangensis contains more MK-10(H2) (76.5 %) than it does MK-9(H4) (23.5 %), while the other species have only MK-9(H4). On account of characteristics more similar to those of the genus Actinophytocola, A. xinjiangensis QAIII60T

The GenBank/EMBL/DDJ accession number for the 16S rRNA gene sequence of strain YIM M13705T is KJ013500.

Three supplementary figures and two supplementary tables are available with the online version of this paper.
was considered as a member of this genus (Guo et al., 2011). In this study, we report a new member of the genus *Actinophytocola* closely related to *A. xinjiangensis*.

To investigate actinobacterial diversity from marine sediments, strain YIM M13705T was isolated from a sample collected from the South China Sea (112° 30.203’ E 18° 1.654’ N) at a depth of 2439 m, and was obtained by using the serial dilution technique. Sediment sample (1 g) was added to 9 ml sterile distilled water and mixed by vortexing. A 10-fold dilution of this soil suspension was prepared in sterilized distilled water and 0.1 ml was spread on Starch–casein–nitrate agar medium (10.0 g soluble starch; 0.3 g casein; 2 g KNO3; 0.05 g MgSO4 .7 H2O; 35 g NaCl; 2 g K2HPO4; 0.02 g CaCO3; 10 mg FeSO4; 20 g agar; pH 7.2; distilled water 1 litre). The plate was then incubated at 28 °C for 30 days. The strain was cultivated, maintained on ISP (International *Streptomyces* Project) medium 2 (yeast extract-malt extract agar; Shirling & Gottlieb, 1966), and stored as aqueous glycerol suspensions (20 %, v/v) at −80 °C.

Genomic DNA was extracted from the isolate as described by Li et al. (2007). The 16S rRNA gene sequence obtained in this study was compared with sequences from EzBioCloud using BLAST (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012). The 16S rRNA gene sequences were aligned with corresponding sequences (retrieved from the GenBank/EMBL/DDBJ database) using CLUSTAL X 1.83 (Thompson et al., 1997). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms by using the software package *MEGA* version 5.0 (Tamura et al., 2011). The topologies of the resultant trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The method of Marmur (1961) was used to prepare genomic DNA of the isolate for the analysis of base composition. The DNA G+C content was determined as described by Mesbah et al. (1989). The quantitative microplate DNA–DNA hybridizations were carried out under optimal conditions as described by Ezaki et al. (1988, 1989). The result was generated according to the method described by Zhang et al. (2013).

Strain YIM M13705T was cultured for 50 days at 28 °C on ISP 2 medium using the covespar technique of Kawato & Shinobu (1959), and morphological properties were observed by using a light microscope (BH-2; Olympus) and a scanning electron microscope (Quanta 200; FEI). Cultural characteristics were observed on various agar media (yeast extract-malt extract agar, oatmeal agar, inorganic salt-starch agar, glycerol-asparagine agar, Czapek’s agar, nutrient agar and trypticase soy agar) at 15 and 30 days at 28 °C, according to the methods described by Shirling & Gottlieb (1966). Colours of the aerial and substrate mycelia were determined by using colour chips from the ISCC-NBS colour charts (standard sample no. 2106; Kelly, 1964). Carbon- and nitrogen-source utilization tests were performed according to the methods described by Shirling & Gottlieb (1966) and Athalye et al. (1985) using the basal medium recommended by Pridham & Gottlieb (1948). Growth at various NaCl concentrations (0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 10.0 %, w/v) and different temperatures (5, 10, 15, 20, 25, 28, 30, 35, 37 and 40 °C) was examined by growing the strain on tryptic soy agar (TSA, Difco) as the basal medium. Growth at pH 4.0–10.0 (at intervals of 1.0 pH unit) was examined on TSA medium using the buffer system described by Xu et al. (2005). Oxidase activity was determined by the oxidation of tetramethyl-p-phenylenediamine. Catalase activity was determined by using 3 % H2O2, and a positive reaction was identified by gas production. Degradation of cellulose was tested using the basal medium recommended by Pridham & Gottlieb (1948), supplemented with 0.5 % CM-cellulose and peptone. Nitrate reduction and degradation of gelatin and urea were determined by using standard procedures as described by Goodfellow (1971) and Athalye et al. (1985).

The biomass used for analyses of cellular fatty acids was obtained from cultures grown in tryptic soy broth (Difco) for 14 days, while that for the study of other chemotaxonomic characteristics was obtained from cultures grown in ISP 2 medium for 28 days. They were all cultured in shaken flasks at 170 r.p.m. at 28 °C, harvested by centrifugation at 2219 g (for 10 min), and washed twice with distilled water. Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the method of Sasser (1990) and the manufacturer’s instructions. Fatty acid methyl esters were then analysed by GC (7890A GC System; Agilent Technologies) by using the Microbial Identification software package (Sherlock Version 6.1; MIDI database TSBA6). The diaminopimelic acid isomer was identified by using TLC as described by Staneck & Roberts (1974). The whole-cell sugars were separated by HPLC after precolumn derivatization with 1-phenyl-3-methyl-5-pyrazoline (Tang et al., 2009). Menaquinones were extracted (Collins et al., 1977) and detected by HPLC (Tamaoka et al., 1983). Polar lipids were determined according to published procedures (Minnikin et al., 1979; Collins & Jones, 1980).

An almost complete 16S rRNA gene sequence (1528 bp) was generated for isolate YIM M13705T. The isolate showed relatively low 16S rRNA gene sequence similarities with the type strains of species of the genus *Actinophytocola*, i.e. 98.7 % similarity with *A. xinjiangensis* QAIII60T, 96.5 % similarity with *A. oryzae* GMKU 367T, 96.2 % similarity with *A. timorensis* NBRC 105524T, 96.0 % similarity with *A. burenkhanensis* MN08-A0203T and 95.8 % similarity with *A. corallina* NBRC 105525T. Further comparative 16S rRNA gene sequence analysis showed that the strain was related phylogenetically to members of the genus *Actinophytocola* (Fig. 1). In the phylogenetic tree reconstructed using the neighbour-joining algorithm, it clustered with the type strain of *A. xinjiangensis* and had 100 % bootstrap support. This relationship was also supported by the other two tree-making methods used in this study with high bootstrap supports of 99 % (Figs S1 and S2, available in the online content).
Supplementary Material). The level of DNA–DNA relatedness of strain YIM M13705<sup>T</sup> with <i>A. xinjiangensis</i> QAIII60<sup>T</sup> was 46.3% (SD 2.3%). The value is well below the 70% cut-off point recommended for the delineation of genomic species (Wayne <i>et al.</i>, 1987). The results of the 16S rRNA gene sequence comparisons and low level of DNA–DNA relatedness demonstrated that strain YIM M13705<sup>T</sup> should represent a novel member of the genus <i>Actinophytocola</i>.

Strain YIM M13705<sup>T</sup> grew well but slowly on ISP 2 medium and formed black colonies. Greyish-black pigment was produced after 30 days. Investigations of a 50-day-old culture revealed that the characteristics of the mycelium on a coverslip varied with the distance from the agar medium. Abundant substrate mycelium was formed near to the agar medium while scant aerial mycelium formed with no spores or spore chains (Fig. 2a). But spore chains with irregular round spores were observed far away from the agar medium (Fig. 2c). Meanwhile, spore-chain-like formations were formed on the aerial mycelium in the middle (Fig. 2b). It is probable that the strain needs oligotrophic conditions to produce aerial mycelium and spore chains. Strain YIM M13705<sup>T</sup> could grow on all the media tested and no obvious pigment was produced on any media within 30 days. Aerial mycelium was produced on ISP 2 and ISP 4 (inorganic salts-starch agar) media, and the colour of the aerial mycelium and substrate mycelium depended on the medium tested. Detailed cultural characteristics are shown in Table S1.

Strain YIM M13705<sup>T</sup> could grow at pH 6.0–9.0 (optimum pH 8.0), 10–30 °C (optimum 25 °C) and with 0–6.0% (w/v) NaCl (optimum 0–2.0%). The results of other physiological and biochemical analyses are summarized in Table 1 and the species descriptions below. The range of carbon source utilization of strain YIM M13705<sup>T</sup> could not be determined in this study because of negative reactions caused by extremely poor growth in basal media, but that of strain <i>A. xinjiangensis</i> QAIII60<sup>T</sup> was determined to be consistent with the description by Guo <i>et al.</i> (2011).

Strain YIM M13705<sup>T</sup> exhibited chemical characteristics consistent with the other five species of genus <i>Actinophytocola</i>, although there were some minor differences. It contained meso-diaminopimelic acid as the cell-wall diamino acid; galactose, glucose, rhamnose and ribose as whole cell sugars; diphasphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, hydroxypidophosphatidylethanolamine.
and ninhydrin-positive phosphoglycolipids as major polar lipids (Fig. S3); MK-9(H₄) (12.8 %) and MK-10(H₂) (87.2 %) as the predominant menaquinones; and iso-C₁₆ : ₀ as the major fatty acid (Table S2). The genomic DNA G + C content of strain YIM M13705ᵀ was 68.2 mol%. Strain YIM M13705ᵀ had minor amounts of phosphatidylinositol, which was not reported in existing species of the genus Actinophytocola. In addition, MK-10(H₂) as the predominant menaquinone was only found in A. xinjiangensis QAIII60ᵀ (Guo et al., 2011). These properties easily separated strain YIM M13705ᵀ from current species with validly published names.

On the basis of phenotypic, chemotaxonomic and phylogenetic analysis, together with the low level of DNA–DNA relatedness and 16S rRNA gene similarities, strain YIM M13705ᵀ could be distinguished from known members of the genus Actinophytocola. More characteristics that differentiate strain YIM M13705ᵀ from its closest neighbour, Actinophytocola xinjiangensis QAIII60ᵀ, and other species of genus Actinophytocola are summarized in Tables 1 and S2. Thus, it is considered that strain YIM M13705ᵀ is representative of a novel species of the genus Actinophytocola, for which the name Actinophytocola sediminis sp. nov. is proposed.

**Description of Actinophytocola sediminis sp. nov.**

Actinophytocola sediminis (se.di′ni.nis. L. gen. n. sediminis of a sediment).

Gram-stain-positive, aerobic actinomycete. Growth occurs on ISP 2, ISP 3, ISP 4, ISP 5, TSA, Czapek’s agar and nutrient agar with no production of pigments within 30 days. Forms scant white aerial mycelium on ISP 2 and ISP 3 and the colour of substrate mycelium depends on the medium. Substrate hyphae and aerial mycelium are well developed on ISP 2 at 50 days. At maturity, spore chains with irregular round spores are borne on aerial hyphae. Growth occurs at pH 6.0–9.0 (optimum pH 8.0), 10–30 °C (optimum 25 °C) and with 0–6.0 % (w/v) NaCl (optimum

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**Fig. 2.** Scanning electron micrographs of strain YIM M13705ᵀ. The strain was grown on ISP 2 medium for 50 days: (a) was taken near to the agar medium showing abundant substrate mycelium, scant aerial mycelium and no spores or spore chains; (b) was taken a little further from the agar medium showing spore-chain-like formation; (c) was taken far away from the agar medium showing spore chains with irregular round spores. All were taken from the same coverslip. Bar, 10 μm.
Table 1. Differential phenotypic and chemotaxonomic characteristics of strain YIM M13705T and other members in the genus *Actinophytocola*

Strains: 1. YIM M13705T (data from this study); 2. *A. xinjiangensis* QAIII60T (this study); 3. *A. burenkhanensis* NBRC 105883T (Ara et al., 2011; Guo et al., 2011); 4. *A. coralina* NBRC 105525T (Guo et al., 2011; Otoguro et al., 2011); 5. *A. oryzae* NBRC 105245T (Guo et al., 2011; Otoguro et al., 2011); 6. *A. timorensis* NBRC 105524T (Guo et al., 2011; Otoguro et al., 2011). All strains contain meso-diaminopimelic acid. DPG, diphosphatidylglycerol; NPG, ninhydrin-positive phosphoglycolipid; OH-PE, hydroxyphosphatidylethanolamine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; +, positive; −, negative; w, weakly positive; ND, no data available.

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Spore morphology</td>
<td>Irregular round</td>
<td>Cylindrical</td>
<td>ND</td>
<td>Rod-shaped</td>
<td>Cylindrical</td>
<td>Rod-shaped</td>
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<td>Isolation source</td>
<td>Marine sediment</td>
<td>Forest soil</td>
<td>Soil</td>
<td>Soil</td>
<td>Plant roots</td>
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<td>Colony colour on ISP 2 medium</td>
<td>Olive black</td>
<td>Light yellow</td>
<td>Pale yellow</td>
<td>Melon yellow</td>
<td>Orange-red</td>
<td>White-yellow</td>
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<td>NaCl for growth (% w/v)</td>
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<td>≤ 4</td>
<td>≤ 3</td>
<td>≤ 7</td>
<td>≤ 2</td>
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<td>−</td>
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<td>−</td>
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<td>ND</td>
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<td>Oxidase</td>
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<td>+</td>
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<td>Hydrolysis of:</td>
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<td>+</td>
<td>−</td>
<td>W</td>
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<td>Whole-cell sugars</td>
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<td>Gal, Glu, Rha, Rib</td>
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<td>Ara, Gal, Rha</td>
<td>Ara, Gal, Rha</td>
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<tr>
<td>Predominant menaquinone(s)</td>
<td>MK-9(H₄), MK-10(H₂)</td>
<td>MK-9(H₄), MK-10(H₂)</td>
<td>MK-9(H₄)</td>
<td>MK-9(H₄)</td>
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<td>Major phospholipids</td>
<td>DPG, PE, OH-PE, NPG, PI</td>
<td>DPG, PE, OH-PE, NPG</td>
<td>DPG, PE, OH-PE, NPG</td>
<td>DPG, PE, NPG</td>
<td>DPG, PE, NPG</td>
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<td>DNA G+C content (mol%)</td>
<td>68.2</td>
<td>72.5</td>
<td>70.6</td>
<td>71.2</td>
<td>71.1</td>
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0–2.0 %). Positive in tests for production of oxidase and cellulase. Negative in tests for nitrate reductase, hydrolysis of gelatin and production of H₂S, melanin, urease, amylase and catalase. Utilizes DL-α-alanine, DL-aspartic amide, L-cysteine, hypoxanthine, L-alanine, L-histidine, L-methionine, L-phenylalanine, L-serine and L-threonine, but not glycine, L-glutamate, L-lysine, L-tyrosine or L-tryptophan. Whole-cell sugars contain galactose, glucose, rhamnose and ribose. Predominant menaquinones are MK-9(H₄) and MK-10(H₂). The polar lipids consist of diphosphatidylglycerol, phosphatidylglycinol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, ninhydrin-positive phosphoglycolipids, several unknown phospholipids and unknown polar lipids. The major fatty acid component is iso-C₁₆:0.

The type strain is YIM M13705T (=DSM 45939T=BCRC 16956T), isolated from a sample collected from the South China Sea (112° 30.203’ E 18° 1.654’ N) at a depth of 2439 m. The G+C content of the genomic DNA of the type strain is 68.2 mol%.

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References


