Actinoplanes atraurantiacus sp. nov., isolated from soil

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A Gram-positive-staining bacterium, designated Y16T, was isolated from a soil sample from Yunnan Province, China. The isolate grew optimally at 25–30 °C, grew at pH 6.0–9.0 and could grow with 3 % NaCl. Strain Y16T had cell-wall peptidoglycan based on meso-diaminopimelic acid. The predominant menaquinones were MK-9(H4) and MK-9(H2). The major fatty acid methyl esters were anteiso-C15:0, iso-C15:0, anteiso-C17:0 and C16:0. These chemotaxonomic characteristics suggested that the organism belonged to the genus Actinoplanes. Strain Y16T shared 98.7, 98.3 and 97.9 % 16S rRNA gene sequence similarity with Actinoplanes deccanensis IFO 13994T, A. abujensis A4029T and A. brasiliensis DSM 43805T, respectively. The DNA G+C content of the isolate was 70.8 mol%. DNA–DNA relatedness between the novel isolate and the type strains of A. deccanensis, A. abujensis and A. brasiliensis was 35.2, 32.0 and 22.3 %, respectively. In addition, the pattern of phenotypic properties distinguished strain Y16T from its closest phylogenetic neighbours. It is therefore concluded that strain Y16T (=CGMCC 4.6857T=JCM 17700T) represents a novel species of the genus Actinoplanes, for which the name Actinoplanes atraurantiacus sp. nov. is proposed.

The genus Actinoplanes was proposed by Couch (1950). At the time of writing, the genus comprised 30 species with validly published names. The type species of the genus is Actinoplanes philippinensis. With the development of polyphasic taxonomic techniques, the positions of some species in the classification system have been redefined. Actinoplanes armeniacus (Kalakoutskii & Kusnetsov, 1964), Actinoplanes caeruleus (Horan & Brodsky, 1986) and Actinoplanes minuta-isoropangus (Ruan et al., 1985) were transferred to Streptomycetes armeniacus (Wellington & Williams, 1981), Couchioplanes caeruleus (Tamura et al., 1994) and Cryptosporangium arantiacum (Tamura & Hatano, 2001), respectively, on the basis of their morphology, cell-wall chemotype and phage susceptibility. The phenotypic analysis of the genus Actinoplanes has been given by Goodfellow et al. (1990) and Tamura & Hatano (2001). Organisms placed in the genus are characterized by the presence of spherical, cylindrical, digitate, lobate, bottle- or flask-shaped or very irregular sporangia. Aerial hyphae are scant. The predominant isoprenoid quinone is always MK-9(H4). The major cellular fatty acids are often present as iso-/anteiso-branched and monounsaturated fatty acids and/or cis-9,10-octadecanoic acid (Tamura & Hatano, 2001). Many species of the genus Actinoplanes produce bioactive compounds; for example, teicoplanin, frilimincins and 2-hydroxyethyl-3-methyl-1,4-naphthoquinone are produced by Actinoplanes teichomyceticus, Actinoplanes friulensis and Actinoplanes capillaceus (Wink et al., 2006; Vértesy et al., 2000; Fukami et al., 2000).

A soil sample was collected at a depth of 15 cm in the forest of Yunnan Province, China. Strain Y16T was isolated on glucose-yeast extract-malt extract agar (ISP 2), which had been incubated at 28 °C for 1 week. The isolate was maintained on ISP 2 at 28 °C and stored as a mycelial fragment suspension in 20 % (v/v) glycerol at −20 °C. Biomass for chemotaxonomic studies was obtained by incubation in tryptone soy broth (TSB; Oxoid) with shaking (160 r.p.m.) at 28 °C for 7 days.

The micromorphological and colony characteristics of strain Y16T were studied after incubation for 7, 14 and 21 days at 28 °C on various media described by Shirling & Gottlieb (1966): yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salt-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7), yeast extract-soluble starch medium (YS medium; containing 1−1: 2 g yeast

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Y16T is HQ839788.

A supplementary figure is available with the online version of this paper.
extract, 10 g soluble starch, 15 g agar; pH 7.3) and Bennett agar (containing 0.1 % yeast extract, 0.1 % beef extract, 0.2 % NZ amine type A, 1.0 % maltose monohydrate, 1.5 % agar; pH 7.3; Jones, 1949). The arrangement of hyphae and sporangia of strain Y16"T was observed after incubation for 14 and 21 days, respectively, at 28 °C on ISP 4 by light microscopy (BH 2; Olympus). The morphology of the sporangia and the surface ornamentation of the sporangiospores were observed by examining gold-coated dehydrated specimens with a model FEI QUANTA scanning electron microscope. On ISP 2, colonies were rough, circular to slightly irregular, slightly raised, dark orange and 2.0–3.0 mm in diameter after 7 days at 28 °C. The isolate grew well on ISP 2, 3, 4 and 7, Bennett agar and YS medium, but only moderate growth was observed on ISP 5 and 6. Aerial mycelium was absent on these media. The substrate mycelium was light yellow to dark orange on all media tested. A pink soluble pigment was formed on ISP 7. The surface of the spores was smooth. The morphological characteristics of the isolate are shown in Fig. 1. Fragmentation of the substrate mycelium was not observed.

Strain Y16"T was examined for a range of phenotypic properties. Gram-staining was performed using the method of Smibert & Krieg (1994). Temperature, pH and sodium chloride concentration for growth were determined on ISP 2 for up to 21 days. Carbon source utilization was tested using carbon source utilization medium (ISP 9; Shirling & Gottlieb, 1966) supplemented with the tested carbon sources (final concentration 1 %). Qualitative enzyme tests were performed using the API ZYM system (bioMérieux), according to the manufacturer’s instructions. The following biochemical tests were recorded after 7 days at 28 °C. Hydrolysis of urea was determined using the method described by Goodfellow & Orchard (1974). Gelatin hydrolysis was determined on peptone-gelatin medium (containing 1−1 distilled water: 5 g peptone, 120 g gelatin). Milk coagulation and peptoneization was determined using 20 % (w/v) skimmed milk. Resistance to antibiotics was examined by placing impregnated filter-paper discs on ISP 2 (Goodfellow & Orchard, 1974). The test antibiotics were ciprofloxacin, clindamycin, erythromycin, gentamicin, kanamycin, ofloxacin, streptomycin and tobramycin. The phenotypic properties of strain Y16"T are summarized in the species description and are compared with those of its closest phylogenetic neighbours in the genus Actinoplanes in Table 1.

Isolation of chromosomal DNA from strain Y16"T and PCR amplification of the 16S rRNA gene were carried out according to Rainey et al. (1996). The amplification

Table 1. Comparison of properties of strain Y16"T and its closest phylogenetic neighbours in the genus Actinoplanes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
</tr>
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<tbody>
<tr>
<td>Growth at (°C):</td>
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<td></td>
<td></td>
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<tr>
<td>10</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>37</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>40</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Decomposition of (0.5%, w/v):</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth on:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>w</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>NaCl range for growth (% w/v)</td>
<td>0−3</td>
<td>0−3</td>
<td>0−3</td>
<td>0−5</td>
</tr>
</tbody>
</table>
products were sequenced directly using the method of Lu et al. (2001). Sequence gel electrophoresis was performed and the nucleotide sequences were obtained using an automatic DNA sequencer (model 377; Applied Biosystems). BLAST analysis was used to compare the 16S rRNA gene sequence of strain Y16T with sequences from public databases. A multiple sequence alignment with corresponding sequences from all type strains of members of the genus Actinoplanes was generated using CLUSTAL_X version 2.0 (Larkin et al., 2007). Phylogenetic trees were obtained using the maximum-parsimony and neighbour-joining methods with 1000 bootstrap replications in MEGA version 4.1 (Tamura et al., 2007). Evolutionary distances were calculated using distance options according to Kimura’s two-parameter model (Kimura, 1980). The G+C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962). DNA–DNA relatedness between the isolate and its closest phylogenetic neighbours was studied using thermal denaturation (De Ley et al., 1970; Huß et al., 1983) with a UV-1206 spectrophotometer (Shimadzu) fitted with a TB-85 thermostab and standard software (Jahnke, 1992). The results were expressed as the mean of three determinations. A nearly complete 16S rRNA gene sequence (1402 bp) was obtained from strain Y16T. 16S rRNA gene sequence similarity between strain Y16T and Actinoplanes decannensis IFO 13994T, A. abuensis A4029T and A. brasiliensis DSM 43805T was 98.7, 98.3 and 97.9%, respectively. In the neighbour-joining dendrogram (Fig. 2), the isolate formed a distinct lineage in a cluster containing A. decannensis IFO 13994T, A. abuensis A4029T and A. brasiliensis DSM 43805T. The cluster was recovered in the maximum-parsimony tree.

The isomers of diaminopimelic acid and whole-cell sugars were identified according to the procedures developed by Hasegawa et al. (1983) and Lechevalier & Lechevalier (1980). Polar lipids were extracted and identified according to the method of Minnikin et al. (1984). Isoprenoid quinones were extracted and purified according to Collins et al. (1987). Purified menaquinones were determined by reversed-phase HPLC (Wu et al., 1989). Fatty acids were extracted, purified, methylated and quantified by GC using the standard Microbial Identification System (TSBA40; MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The whole-cell hydrolysate of the isolate contained meso-diaminopimelic acid and xylose and arabinose (wall chemotype II; Lechevalier & Lechevalier, 1970). The phospholipids were phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol (Fig. S1, available in IJSEM Online). The characteristic menaquinones were MK-9(H4) and MK-9(H2). Mycolic acids were absent. The cellular fatty acids are anteiso-C15:0 (26.2%), iso-C15:0 (20.8%), anteiso-C17:0 (13.8%), C16:0 (10.1%), C18:0 (7.0%), iso-C16:0 (5.3%), C18:1ω9c (4.6%), iso-C17:0 (4.4%), summed feature 3 (C16:1ω7c and/or iso-C15:0 2-CH2; 2.8%), C17:0 13:0 (1.3%), C14:0 (1.2%), C17:1ω8c (0.7%), iso-C14:0 (0.6%), C16:1ω7c (0.6%) and iso-C17:1ω9c (0.4%). These data supported the classification of the isolate as a member of the genus Actinoplanes (Tamura & Hatano, 2001). In addition, the DNA G+C content of strain Y16T was 70.8 mol%, which is within the range for the genus Actinoplanes (Goodfellow et al., 1990).

Mean DNA–DNA relatedness values between the isolate and the type strains of A. decannensis IFO 13994T, A. abuensis DSM 45518T and A. brasiliensis DSM 43805T were 35.2, 32.0 and 22.3%, respectively, which are values well below the 70% cut-off point recommended by Wayne et al. (1987) for the delineation of genomic species. The isolate could also be distinguished from its closest phylogenetic neighbours by a combination of phenotypic properties (Table 1). These results supported the findings of the 16S rRNA gene sequence analysis.

The physiological and chemotaxonomic characteristics indicated that strain Y16T belonged to the genus Actinoplanes; however, the 16S rRNA gene sequence analysis and DNA–DNA relatedness indicated that it represented a novel species. It is therefore proposed that the isolate is classified in the genus Actinoplanes as Actinoplanes atraurantiacus sp. nov.

Description of Actinoplanes atraurantiacus sp. nov.

Actinoplanes atraurantiacus (a.trau.ran.ti.cus. L. adj. ater -tra -trum black, dark; N.L. adj. aurantiacus -a -um orange; N.L. masc. adj. atraurantiacus dark orange).

Cells are aerobic and Gram-stain-positive. Globose to oval sporangia are formed. The substrate mycelium is light yellow to dark orange on ISP 2, 3, 4, 5, 6 and 7, Bennett agar and YS medium. A pink soluble pigment is formed on ISP 7. Optimal growth is at 25–30 ℃, but no growth occurs at 40 ℃. Grows at pH 6.0–9.0 and with up to 3 % NaCl. Gelatin, starch and urea are hydrolysed. H2S is not produced. Nitrate is reduced. Casein is decomposed, but not L-tyrosine or cellulose. Milk coagulation and peptone-ization is negative. As sole carbon and energy sources, utilizes cellobiose, D-galactose, D-glucose, glycogen, lactose, maltose, D-mannose, D-mannitol (weakly), L-rhamnose, salicin, sucrose and D-xylose, but not L-arabinose, D-arabitol, inositol, melezitose, melibiose, raffinose, D-ribose, D-sorbitol, L-sorbosé or D-tagatose. With API ZYM, positive for β-galactosidase, N-acetyl-β-glucosaminidase, β-glucosidase, alkaline phosphatase, x-chymotrypsin, naphthol-AS-BI-phosphohydrolase, leucine arylamidase and x-glucosidase; weakly positive for acid phosphatase, esterase lipase (C8) and esterase (C4); and negative for cystine arylamidase, x-fucosidase, valine arylamidase, x-mannosidase, x-galactosidase, β-glucuronidase, lipase (C14) and trypsin. Sensitive to ciprofloxacin, erythromycin, gentamicin, kanamycin, ofloxacin, streptomycin and tobramycin, but resistant to clindamycin. The cell-wall peptidoglycan contains meso-diaminopimelic acid. The diagnostic sugars are xylose and arabinose. Mycolic acids are absent. The major menaquinones are MK-9(H2) and MK-9(H2). The major fatty acids (>10%) are anteiso-C15:0.
iso-C₁₅:₀, anteiso-C₁₇:₀ and C₁₆:₀; moderate amounts (5–10 %) of C₁₈:₀ and iso-C₁₆:₀ are also present. The phospholipids are phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol.

The type strain is Y₁₆ᵀ (= CGMCC 4.6857ᵀ = JCM 17700ᵀ), isolated from forest soil in Yunnan Province, China. The G+C content of the DNA of the type strain is 70.8 mol%.

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sp. nov., novel actinomycetes from soil.

A new species of the genus

Actinoplanes – A. armeniacus and some peculiarities of its mode of spore formation. Microbiology (English translation of Microbiologia) 33, 613.


