Actinoplanes luteus sp. nov., isolated from soil

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A novel filamentous bacterial strain, A-T 5190T, which developed irregular sporangia at the end of sporangiophores on substrate mycelia, was isolated from dry evergreen forest soil collected in Thailand. The 16S rRNA gene sequence and phylogenetic analysis indicated that strain A-T 5190T belonged to the genus Actinoplanes and was related most closely to Actinoplanes palleronii NBRC 14916T (98.88 % similarity) and Actinoplanes rectilineatus NBRC 13941T (98.54 %). DNA–DNA relatedness values between strain A-T 5190T and its closest relatives were below 70 %. The cell-wall peptidoglycan contained meso-diaminopimelic acid. The whole-cell sugars contained rhamnose, ribose, galactose and xylose. The predominant menaquinone was MK-9(H4). The diagnostic phospholipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The predominant cellular fatty acids were unsaturated fatty acid C17 : 1ω7c and branched fatty acids iso-C16 : 0, iso-C15 : 0 and anteiso-C17 : 0. The G+C content of the genomic DNA was 71.9 mol%. Evidence from phenotypic, chemotaxonomic and genotypic studies indicate that strain A-T 5190T represents a novel species of the genus Actinoplanes, for which the name Actinoplanes luteus sp. nov. is proposed. The type strain is A-T 5190T (=BCC 41582T=NBRC 109644T).

The genus Actinoplanes was first described by Couch (1950). It belongs to the family Micromonosporaceae. Actinoplanes philippinensis is the type species. Members of this genus develop spherical, cylindrical, digitate, lobate, bottle-or flask-shaped or irregular sporangia that contain motile sporangiophores with a clump of polar or peritrichous flagella at the tip of sporangiophores on substrate mycelia (Goodfellow et al., 1990). Cells are Gram-positive, non-acid-fast and aerobic. The cell walls of this genus contain meso-diaminopimelic acid, but hydroxyl-diaminopimelic acid is also present. The whole-cell sugars contain xylose as a marker. Menaquinone MK-9(H4) is the major isoprenoid quinone. The phospholipid pattern is of type II and usually includes phosphatidylethanolamine as the diagnostic phospholipid. Predominant cellular fatty acids are often present as iso-/anteiso-branched and monounsaturated components and/or cis-9,10-octadecenoic acid (Tamura & Hatano, 2001). The DNA G+C content is 67–76 mol% (Sazak et al., 2012).

During a study on the diversity of actinomycetes in Thailand, strain A-T 5190T was isolated from dry evergreen forest soil collected in Lamphun Province. The soil sample was taken from the organic layer on the soil surface and kept at 4 °C. The sample was dried at room temperature for 10 days, then heat-dried at 100 °C for 1 h. The dried sample was treated according to the method of Suzuki (2001) and plated on soil extract agar (Suriyachadkun et al., 2009) supplemented with (per litre) 25 mg nalidixic acid, 50 mg cycloheximide and 1 mg terbinafine, then incubated at 28 °C for 21 days. The isolate was purified and maintained on soil extract agar as a working culture. The pure culture was preserved in glycerol (10 %, v/v) at −80 °C and in liquid-dried form for long-term storage.

The phylogenetic position of strain A-T 5190T was determined by 16S rRNA gene sequence analysis. Genomic DNA was prepared according to the method of Saito & Miura (1963). The 16S rRNA gene was amplified by PCR as described previously (Kawasaki et al., 1993; Yamada et al., 2000; Katsura et al., 2001; Suriyachadkun et al., 2009). The PCR product for the 16S rRNA gene was sequenced by Macrogen using universal primers. A BLAST analysis was used to compare the nearly complete 16S rRNA gene sequence (1438 bp) of strain A-T 5190T with sequences of representatives of the genus Actinoplanes.
retrieved from the GenBank database. 16S rRNA gene sequence similarity was calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Multiple alignments were carried out with CLUSTAL W (Thompson et al., 1994) of the software BioEdit Sequence Alignment Editor (version 7.0.0). Phylogenetic trees were generated by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Felsenstein, 1983) and maximum-likelihood (Felsenstein, 1981) methods using MEGA 5. The robustness of individual branches was estimated by bootstrapping with 1000 replications (Felsenstein, 1985). DNA–DNA hybridization was carried out using a photobiotin-labelling method in microdilution wells as described by Ezaki et al. (1989). DNA–DNA relatedness was determined using the colorimetric method (Verlander, 1992). The G+C content of the genomic DNA was determined using the method of Tamaoka & Komagata (1984) after treatment with P1 nuclease and alkaline phosphatase. HPLC was carried out using a Shimadzu LC-6AD apparatus equipped with a Cosmosil 5C18-AR column (4.6 × 150 mm; Nacalai Tesque).

Pairwise sequence similarities between strain A-T 5190T and reference type strains were calculated based on a 16S rRNA gene sequence of 1438 bases. This strain exhibited highest 16S rRNA gene sequence similarities of 98.88 and 98.54 % with Actinoplanes palleronii NBRC 14916T and Actinoplanes rectilineatus NBRC 13941T, respectively. Phylogenetic trees reconstructed with the neighbour-joining (Fig. 1), maximum-parsimony (Fig. S1a, available in the online Supplementary Material) and maximum-likelihood (Fig. S1b) methods showed a similar topology. The phylogenetic relationship between strain A-T 5190T and members of the genus Actinoplanes revealed that the novel strain formed a cluster with A. palleronii NBRC 14916T. The G+C content of the genomic DNA of strain A-T 5190T was 71.9 mol%. Levels of DNA–DNA relatedness between strain A-T 5190T and A. palleronii NBRC 14916T and A. rectilineatus NBRC 13941T were 44–53 and 32–35 %, significantly below the 70 % cut-off point recommended by Wayne et al. (1987) for the delineation of separate bacterial species. Results of 16S rRNA gene sequence analysis and DNA–DNA relatedness were sufficient to categorize strain A-T 5190T as representing a species distinct from recognized Actinoplanes species.

The morphological characteristics of strain A-T 5190T were observed with a light microscope (model CX 31; Olympus) with a 40 x long-working-distance objective lens (model LUCPLFLN40XR; Olympus) and a scanning electron microscope (model JSM-5410 LV; JEOL) using cultures grown on modified soil extract agar (Suriyachadkun et al., 2009) at 28 °C for 14–21 days. Cultures for scanning electron microscopy were prepared as described by Itoh et al. (1989). The cultural characteristics of strain A-T 5190T as compared with A. palleronii NBRC 14916T and A. rectilineatus NBRC 13941T were determined on various media described by Shirling & Gottlieb (1966), namely ISP2, 3, 4, 5 and 7 and yeast extract-starch agar, following incubation at 28 °C for 14 and 21 days. Colony colour was determined using ISCC-NBS colour charts (Kelly, 1964). Phenotypic characteristics were examined using several standard methods. Temperature, pH and NaCl tolerances were determined using ISP2 as a basal medium. Temperature range for growth was determined at 20, 25, 30, 37 and 45 °C. The pH range for growth was determined at pH 4, 5, 6, 7, 8, 9, 10, 11 and 12 at 30 °C. NaCl tolerance was determined with 0, 1, 2, 3, 4 and 5 % (w/v) NaCl at 30 °C. Utilization of carbohydrates as sole carbon sources was tested using ISP9 as a basal medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % of the carbon source. Hydrolysis of various compounds was examined using the basal medium recommended by Gordon et al. (1974). Tests in the API ZYM (bioMérieux) commercial system were performed according to the manufacturer’s instructions. Gelatin liquefaction, peptonization of milk, nitrate reduction and starch hydrolysis were determined through cultivation on various media as described by Arai (1975) and Williams & Cross (1971). Catalase and oxidase activities were determined using the method of Greenwood & Pickett (1979).

Freeze-dried cells used for chemotaxonomic analysis were obtained from a culture growing in glucose-yeast extract broth (Tamura et al., 1994) on a rotary shaker at 30 °C for 7 days. The isomer of diaminopimelic acid in the cell wall was determined using the method of Staneck & Roberts (1974). The acyl type of the peptidoglycan was determined using the method of Uchida & Aida (1984). Whole-cell sugars were analysed using TLC on cellulose according to the method of Komagata & Suzuki (1987). Polar lipids in whole cells were extracted and analysed according to the method of Minnikin et al. (1984). Cellular fatty acid methyl esters were prepared and analysed following the instructions for the ACTINO method of the Microbial Identification System (MIDI, Sherlock Version 4.0B) (Sasser, 1990; Kampfer & Kroppenstedt, 1996). Isopenoid quinones were extracted and purified using the method of Collins et al. (1977) and identified using reversed-phase HPLC (Cosmosil 5C18 column (4.6 × 150 mm); Nacalai Tesque) with a mixture of methanol and 2-propanol (2 : 1, v/v) as elution solvent (Wu et al., 1989).

Strain A-T 5190T had characteristics typical of the genus Actinoplanes. It developed small irregular sporangia (5–10 μm) containing motile sporangiospores at the tip of sporangiophores on substrate mycelia (Fig. 2). The spores were motile, as observed by light microscopy after incubating sporangia in 0.1 M potassium phosphate buffer (pH 7) at 28 °C for 30–60 min. Fragmentation of substrate mycelium was not observed. Phenotypic characteristics of strain A-T 5190T compared with A. palleronii NBRC 14916T and A. rectilineatus NBRC 13941T are indicated in detail in Table 1 and in the species description. Strain A-T 5190T grew well on ISP2, 4 and 7 and yeast extract-starch agar. Moderate growth was observed on
Fig. 1. Phylogenetic relationships, derived from 16S rRNA gene sequences, between strain A-T 5190T and the type strains of other Actinoplanes species. The phylogenetic tree was reconstructed by using the neighbour-joining method. Dactylosporangium aurantiacum DSM 43157T was used as an out-group. Asterisks represent clades that were also recovered with the maximum-likelihood and maximum-parsimony algorithms. Numerals at nodes indicate bootstrap percentages derived from 1000 replications (only values greater than 50 % are indicated). Bar, 0.005 substitutions per nucleotide position.
ISP5. Substrate mycelium was light orange–yellow to strong orange–yellow. Soluble pigment was not observed on the media used.

Cell-wall amino acids of strain A-T 5190T contained meso-diaminopimelic acid. The acyl type of the cell-wall muramic acid was glycolylmuramic acid. This strain contained rhamnose, ribose, galactose, glucose and xylose as whole-cell sugars (whole-cell sugar pattern D of Lechevalier & Lechevalier, 1970). Menaquinones were MK-9(H2) (1%), MK-9(H4) (72%), MK-9(H6) (3%) and MK-10(H4) (23%). Phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unidentified glycolipids and three unidentified phospholipids were detected, while phosphatidylcholine was not detected (Fig. S2). The unsaturated fatty acid C17:1 (19.9%) and branched fatty acids iso-C16:0 (15.3%), iso-C15:0 (13.9%) and anteiso-C17:0 (11.5%) were major components (>10% each) and unsaturated fatty acid C18:1 (7.2%) and branched fatty acid anteiso-C15:0 (6.7%) were found in moderate amounts. Mycolic acids were absent.

Strain A-T 5190T developed irregular sporangia, whereas A. palleronii and A. rectilineatus developed globose and cylindrical sporangia. Strain A-T 5190T was able to reduce nitrate and grew at 37°C, while A. palleronii and A. rectilineatus could not (Table 1). Strain A-T 5190T utilized cellobiose, melibiose and salicin, while its closest relatives could not. Low DNA–DNA relatedness values were observed between strain A-T 5190T and the type strains of the closest related species. These phenotypic (Table 1) and genotypic data show that strain A-T 5190T can be

Table 1. Characteristics differentiating strain A-T 5190T from its closest relatives in the genus Actinoplanes

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Colonial characteristics:</td>
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<tr>
<td>Substrate mycelium on ISP2</td>
<td>Light orange–yellow</td>
<td>Deep yellow</td>
<td>Moderate yellow</td>
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<tr>
<td>Substrate mycelium on ISP4</td>
<td>Strong orange–yellow</td>
<td>Moderate orange–yellow</td>
<td>Light yellow</td>
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<tr>
<td>Substrate mycelium on ISP5</td>
<td>Strong orange–yellow</td>
<td>Pale yellow</td>
<td>Vivid yellow</td>
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<tr>
<td>Shape of sporangia</td>
<td>Irregular</td>
<td>Globe</td>
<td>Cylindrical</td>
</tr>
<tr>
<td>Menaquinones</td>
<td>MK-9(H2), MK-9(H4), MK-9(H6), MK-10(H4)</td>
<td>MK-9(H4), MK-9(H6)</td>
<td>ND</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth at 37 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Utilization of:</td>
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<tr>
<td>Cellobiose</td>
<td>+</td>
<td>–</td>
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<td>Glycerol</td>
<td>+</td>
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<td>Lactose</td>
<td>+</td>
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<td>+</td>
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<td>Melibiose</td>
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<td>Salicin</td>
<td>+</td>
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<td>Sucrose</td>
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<tr>
<td>Esterase (C4)</td>
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<td>+</td>
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<td>Esterase Lipase (C8)</td>
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<td>–</td>
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<tr>
<td>Leucine arylamidase</td>
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<td>+</td>
<td>–</td>
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<tr>
<td>Valine arylamidase</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Trypsin</td>
<td>+</td>
<td>+</td>
<td>–</td>
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Fig. 2. Scanning electron micrograph of strain A-T 5190T grown on soil extract agar at 28 °C for 3 weeks. Bar, 1 μm.
distincted from previously described species in the genus *Actinoplanes*. Strain A-T 5190^T^ should therefore be classified as representing a novel species of the genus *Actinoplanes*, for which the name *Actinoplanes luteus* sp. nov. is proposed.

**Description of *Actinoplanes luteus* sp. nov.**

*Actinoplanes luteus* (lu’t’e.us. L. masc. adj. luteus orange–yellow, referring to colony colour on ISP2).

Aerobic, Gram-stain-positive, non-acid-fast actinomycete that forms light orange–yellow substrate mycelia on ISP2 and strong orange–yellow substrate mycelia on ISP4 and ISP5. Irregular sporangia containing motile sporangiospores are formed at the tip of sporangiophores on substrate mycelia. Optimal growth is at 25–30 °C and pH 8–9, but growth occurs at 20–37 °C and pH 6–11. The maximum NaCl concentration for growth is 1 % (w/v). Utilizes cellobiose, D-glucose, glycerol, D-fructose, inositol, lactose, melibiose, L-rhamnose, raffinose, salicin, sucrose, trehalose and D-xyllose, but not L-arabinose, D-arabitol, mannose, sorbose, D-sorbitol or D-xylitol. Hydrolysis of starch, nitrate and peptonization of milk are positive. Positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and catalase. Cell-wall amino acids contain meso-diaminopimelic acid. The acyl type of the cell-wall muramic acid is glycolylmuramic acid. Characteristic whole-cell sugars are rhamnose, ribose, galactose, glucose and xylose. The predominant menaquinone is MK-9(H4). The diagnostic phospholipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The major fatty acids are C_{17}:1, iso-C_{16}:0, iso-C_{15}:0 and anteiso-C_{17}:0. The type strain is A-T 5190^T^ (=BCC 41582^T^=NBRC 109644^T^), which was isolated from dry evergreen forest soil. The G+C content of the DNA of the type strain is 71.9 mol%.

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**References**


