Actinokineospora soli sp. nov., a thermotolerant actinomycete isolated from soil, and emended description of the genus Actinokineospora

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A strain of thermotolerant actinomycete, designated YIM 75948T, was isolated from a soil sample in Yunnan province, China. The strain grew at 25–55 °C (optimum 37 °C). The substrate mycelium and aerial mycelium produced on Czapek’s agar were both pale yellow to white. The diagnostic diamino acid of the cell-wall peptidoglycan was meso-diaminopimelic acid, and the whole-cell sugars were mannose, ribose, glucose, galactose and arabinose. The major fatty acids were iso-C15 : 0, iso-C16 : 0 and C16 : 0 and the predominant respiratory quinone was MK-9(H4). The polar lipids consisted of phosphatidylethanolamine, phosphatidylethanolamine with hydroxy fatty acids, diphosphatidylglycerol, phosphatidylinositol and two unidentified phospholipids. The genomic DNA G+C content was 73.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM 75948T belonged in the genus Actinokineospora and that its closest relative among recognized species was Actinokineospora fastidiosa DSM 43855T (97.6 % sequence similarity). The mean level of DNA–DNA relatedness between the novel strain and A. fastidiosa DSM 43855T was, however, only 47.8 %. Based on the phenotypic, chemotaxonomic and phylogenetic data and the results of the DNA–DNA hybridizations, strain YIM 75948T represents a novel species of the genus Actinokineospora for which the name Actinokineospora soli sp. nov. is proposed. The type strain is YIM 75948T (JCM 17695T =DSM 45613T). The description of the genus Actinokineospora is emended to reflect the fact that the genomic DNA G+C contents of A. fastidiosa DSM 43855T and the type strain of Actinokineospora soli sp. nov. recorded in the present study fell above the range given in previous descriptions of this genus.

In 1988, the genus Actinokineospora was proposed by Hasegawa, with Actinokineospora riparia NRRL B-16432T as the type strain of the type species (Hasegawa, 1988a, b). Subsequently, Labeda et al. (2010) proposed not only an emended description of the genus, to accommodate those Actinokineospora species that are unable to produce motile spores, but also the transfer of Amycolatopsis fastidiosa to

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 75948T is JN005785. A supplementary table and three supplementary figures are available with the online version of this paper.
the genus (as Actinokineospora fastidiosa comb. nov.). At the
time of writing, there are 11 species in the genus: Actinoki-
neospora riparia, Actinokineospora inagensis, Actinoki-
neospora globicatena, Actinokineospora terrae, Actinokineospora
diaphysa, Actinokineospora auranticolor, Actinokineospora en-
zanensis, Actinokineospora fastidiosa, Actinokineospora baliensis,
Actinokineospora cibodasensis and Actinokineospora cianjuren-
sis (Hasegawa, 1988a; Tamura et al., 1995; Otoguro et al., 2001;
Labeleda et al., 2010; Lisidiyanti et al., 2010).

During a project to isolate rare actinobacterial strains from
soil samples in the Chinese province of Yunnan, an
actinomycete was isolated that displayed moderate growth
at 50 °C on International Streptomyces Project (ISP)
medium 2 (Shirling & Gottlieb, 1966). The taxonomic
affiliations of this isolate, which was designated strain YIM
75948T, were investigated using a polyphasic approach. In a
phylogenetic analysis based on 16S rRNA gene sequences,
the novel strain was found to be closely related to
Actinokineospora inagensis type strain DSM 43855T. However, these
two strains differed sufficiently in several characteristics for
strain YIM 75948T to be considered a novel species in the
genus Actinokineospora.

Strain YIM 75948T was isolated by plating dilutions from a
soil sample collected in Yunnan province on ISP 2 agar.
The pure culture was preserved in 20 % (v/v) glycerol at
−80 °C. Once it became clear that, in terms of its 16S
rRNA gene sequence, A. fastidiosa DSM 43855T was the
recognized species most closely related to the new strain
(see below), the morphological, physiological, biochemical
and chemotaxonomic characteristics of strain YIM 75948T
were carefully compared with those of A. fastidiosa DSM
43855T. The latter strain was obtained from the Deutsche
Sammlung von Mikroorganismen und Zellkulturen at
Braunschweig, Germany. The two strains were routinely
cultivated on Czapek’s agar or ISP media at 37 °C (Shirling
& Gottlieb, 1966). The colours of the substrate and aerial
mycelia and any soluble pigments produced were determined
by comparison with chips from the ISCC-NBS
colour charts (Kelly, 1964). Unless otherwise indicated, the
phenotypic characteristics were studied using standard
procedures (Smibert & Krieg, 1994; Tang et al., 2011).
Antibiotic susceptibility was examined by using antibiotic
discs on Czapek’s agar, as described by Williams et al.
(1989). The cell morphology of strain YIM 75948T
was observed under a light microscope (model BH2; Olympus)
and in a scanning electron microscope (JSM5600LV; JEOL)
after incubation for 28 days at 37 °C on ISP 2 agar.
Electron microscopy was performed as described by
Nedashkovskaya et al. (2005).

Genomic DNA was extracted and prepared for 16S rRNA
gene amplification, the determination of G + C content and
DNA–DNA hybridization tests using the method described by
Marmur (1961). PCR amplification of the 16S rRNA
gene sequence was performed as described by Li et al.
(2007). The 16S rRNA gene sequence was manually aligned
with reference sequences retrieved from the GenBank
database following BLAST searches. Phylogenetic trees were
constructed using version 4.0 of the MEGA software package
(Tamura et al., 2007) after multiple alignments of the se-
quences with CLUSTAL_X (Thompson et al., 1997). Cor-
corrected evolutionary distances were calculated according to
Kimura’s two-parameter model (Kimura, 1980, 1983) and
phylogenetic trees were constructed using the neighbour-
joining and maximum-parsimony algorithms. Tree topol-
ologies were evaluated by the bootstrap analysis with 1000
resamplings (Felsenstein, 1985).

Genomic DNA G + C content was determined by reverse-
phase HPLC according to Mesbah et al. (1989). DNA–DNA
hybridizations were performed by the fluorometric method,
in microdilution wells with photobiotin, as described by
Ezaki et al. (1989). Such hybridizations were performed
between strain YIM 75948T and A. fastidiosa DSM 43855T
with eight replicates. The bacterial biomass used for the
chemotaxonomic analyses (of amino acids, whole-cell
sugars, fatty acids, respiratory quinones and polar lipids)
was produced by incubation in ISP 2 broth at 37 °C for
3 days. Respiratory quinones were extracted from lyophi-
лизed cells and the extracts were purified and analysed by
HPLC, using the procedures reported by Hu et al. (2001).
The procedures used for the identification of whole-cell
sugars were those described by Tang et al. (2009a). The
isomer of diaminopimelic acid in the cell walls was analysed
according to the procedures developed by Hasegawa et al.
(1983). The amino acids in peptidoglycan hydrolysates were
analysed by HPLC after precolumn derivatization with
o-phthalaldehyde (Tang et al., 2009b). Cellular fatty acids
were investigated as described by Sasser (1990), using
version 6.1 of the Sherlock Microbial Identification System
(MIDI) and the TSBA6 database. Polar lipids were extracted,
separated by two-dimensional TLC and identified by
following the procedures of Minnikin et al. (1984).

Strain YIM 75948T showed good growth on Czapek’s agar,
inorganic salts/starch (ISP 4) agar and nutrient agar;
moderate growth was observed on ISP 2, oatmeal (ISP 3),
glycerol/asparagine (ISP 5) and potato dextrose (PD) agars.
Aerial mycelium was produced on Czapek’s, ISP 2 and
nutrient agars but not on PD, ISP 3, ISP 4 or ISP 5 agar.
Motile spores were never observed on any of the media.
Both the aerial and substrate mycelia were white to pale
yellow in colour (or, occasionally, pale pink) and rod-
shaped spores with smooth surfaces were produced (Fig.
S1, available in IJSEM Online). Soluble pigments were not
produced. Strain YIM 75948T grew at 25–55 °C (optimum
37 °C), at pH 7.0–9.0 (optimum pH 8.0) and with 0–5 %
(w/v) NaCl (optimum between 0 % and 2 %). The pheno-
typic properties that can be used to distinguish strain
YIM 75948T from A. fastidiosa DSM 43855T are shown in
Table 1. The main characteristics of strain YIM 75948T are
summarized in the species description.

In phylogenetic analyses based on the almost-complete
16S rRNA gene sequence of strain YIM 75948T and the
 corresponding sequences of closely related species, strain
Table 1. Morphological and physiological characteristics that distinguish strain YIM 75948T from A. fastidiosa DSM 43855T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Colony reverse colour</td>
<td>Yellow–white</td>
<td>Pale yellow–yellow</td>
</tr>
<tr>
<td>Colour of aerial mycelium</td>
<td>Yellow–white</td>
<td>White–pale pink</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>8</td>
<td>7–8</td>
</tr>
<tr>
<td>Growth at 55 °C</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lipase tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 40</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tween 60</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate utilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Mannose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Malrose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Fructose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Xyitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Nitrogen utilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-Serine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>l-Aspartic acid</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Antibiotic susceptibility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Penicillin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>R</td>
<td>S</td>
</tr>
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</table>

YIM 75948T appeared to belong in the genus Actinokineospora and was clustered with A. fastidiosa DSM 43855T in a deep branch of each of the phylogenetic trees (Figs 1 and S2). Since the level of 16S rRNA gene sequence similarity between strain YIM 75948T and A. fastidiosa DSM 43855T was fairly high (97.6 %), DNA–DNA hybridizations between these two strains were indicated (Stackebrandt & Goebel, 1994). The mean level of DNA–DNA relatedness between strain YIM 75948T and A. fastidiosa DSM 43855T was, however, only 47.8 ± 4.2 %, a value that falls well below the threshold value, of 70 %, that, when exceeded, is taken to indicate that the hybridized strains probably belong to the same species (Stackebrandt & Goebel, 1994).

The genomic DNA G + C content of strain YIM 75948T was 73.8 mol% and its major fatty acids were iso-C15:0 (15.9 %), iso-C16:0 (37.4 %) and C16:0 (12.2 %). Under the same incubation and analysis conditions, the genomic DNA G + C content of A. fastidiosa DSM 43855T was 73.5 mol% and its major fatty acids were iso-C15:0 (14.2 %), iso-C16:0 (41.1 %), C16:0 (10.1 %) and C17:0 cyclo (11.3 %). The detailed fatty acid profiles of the novel strain and A. fastidiosa DSM 43855T are displayed in Table S1. The predominant respiratory quinone of strain YIM 75948T was MK-9(H4) (91.7 %) but MK-9(H6) (4.0 %), MK-9 (2.2 %) and MK-9(H8) (2.1 %) were detected as minor components. The polar lipid profile of strain YIM 75948T was similar to that of A. fastidiosa DSM 43855T, consisting of phosphatidyl ethanolamine, phosphatidylethanolamine with hydroxy fatty acids, diphasophatidyglycerol, phosphatidylinositol and, as minor components, two unidentified phospholipids (Fig. S3). For both strain YIM 75948T and A. fastidiosa DSM 43855T, meso-diaminopimelic acid was identified as the cell-wall diamino acid, glycine, glutamic acid, alanine and aspartic acid were found to be the main amino acids of the peptidoglycan, and the whole-cell sugars were identified as mannose, ribose, glucose, galactose and arabinoise.

Although the phylogenetic analysis and chemotaxonomic characteristics (major fatty acids, polar lipids, cell-wall diamino acid and respiratory quinones) supported the assignment of strain YIM 75948T to the genus Actinokineospora, certain physiological and biochemical characteristics of the novel strain (growth at 55 °C, nitrate reduction, urease and lipase activities and utilization of particular carbohydrates and nitrogen sources) distinguished it from all recognized species of the genus Actinokineospora. Furthermore, the fatty acid content of strain YIM 75948T was fairly distinct from that of A. fastidiosa DSM 43855T (Table S1). Based on the results of the morphological, physiological and chemotaxonomical characterizations and DNA–DNA hybridizations, strain YIM 75948T represents a novel species of the genus Actinokineospora for which the name Actinokineospora soli sp. nov. is proposed.

Emended description of the genus Actinokineospora

The description is as given for the genus Actinokineospora by Labeda et al. (2010) with the following addition. The range of genomic DNA G+C contents, as determined by HPLC, is 69–74 mol%.

Description of Actinokineospora soli sp. nov.

Actinokineospora soli (so’li. L. neut. gen. n. soli of soil, the source of the type strain).

Cells are Gram-staining-positive, aerobic and thermo-tolerant. The mycelium, which is white to pale yellow (or,
occasionally, pale pink), produces rod-shaped, smooth, non-motile spores. Soluble pigments are not produced. Growth occurs at 25–55 °C (optimum 37 °C), at pH 7.0–9.0 (optimum pH 8.0) and with 0–5 % (w/v) NaCl (optimum between 0 % and 2 %). Positive for catalase activity but negative for oxidase, urease and tryptophan deaminase activities. Hydrolyses starch, gelatin and Tweens 20 and 40, but not cellulose or Tweens 60 or 80. Nitrate is not reduced to nitrite. Negative result in the Voges-Proskauer test but positive result in the methyl red test. H₂S is not produced. As sole carbon sources, cellobiose, D-galactose, D-glucose, mannitol, D-mannose, maltose, sucrose and xylitol are utilized, but not L-arabinose, dulcitol, glycerol, inositol, lactose, raffinose, L-rhamnose, sodium citrate, sodium oxalate, starch, trehalose or D-xylose. As sole nitrogen sources, L-alanine, L-arginine, glycine, D-histidine, L-hydroxyproline, hypoxanthine, D-phenylalanine, L-lysine, D-proline, L-tyrosine, D-threonine, D-valine and xanthine are utilized, but not glutamic acid, L-serine or L-aspartic acid. Acid is produced from cellobiose, dulcitol, D-galactose, D-glucose, mannitol, D-mannose, sucrose and xylitol, but not from L-arabinose, D-fructose, glycerol, inositol, lactose, maltose, raffinose, L-rhamnose, sodium citrate, sodium oxalate, trehalose, D-xylose or starch. The type strain, YIM 75948T (=JCM 17695T =DSM 45613T), was isolated from a soil sample collected in Yunnan province, China. The genomic DNA G+C content of the type strain is 73.8 mol%.

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References


