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 75948^T, 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-C_{15:0}, iso-C_{16:0} and C_{16:0} 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 75948^T belonged in the genus *Actinokineospora* and that its closest relative among recognized species was *Actinokineospora fastidiosa* DSM 43855^T (97.6 % sequence similarity). The mean level of DNA–DNA relatedness between the novel strain and *A. fastidiosa* DSM 43855^T was, however, only 47.8 %. Based on the phenotypic, chemotaxonomic and phylogenetic data and the results of the DNA–DNA hybridizations, strain YIM 75948^T represents a novel species of the genus *Actinokineospora* for which the name *Actinokineospora soli* sp. nov. is proposed. The type strain is YIM 75948^T (=JCM 17695^T =DSM 45613^T). The description of the genus *Actinokineospora* is emended to reflect the fact that the genomic DNA G + C contents of *A. fastidiosa* DSM 43855^T 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.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 75948^T is JN005785.

A supplementary table and three supplementary figures are available with the online version of this paper.

In 1988, the genus *Actinokineospora* was proposed by Hasegawa, with *Actinokineospora riparia* NRRL B-16432^T 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

the genus (as *Actinokineospora fastidiosa* comb. nov.). At the time of writing, there are 11 species in the genus: *Actinokineospora riparia*, *Actinokineospora inagensis*, *Actinokineospora globicatena*, *Actinokineospora terrae*, *Actinokineospora diospyrosa*, *Actinokineospora auranticolor*, *Actinokineospora enzanensis*, *Actinokineospora fastidiosa*, *Actinokineospora baliensis*, *Actinokineospora cibodasensis* and *Actinokineospora cianjurenensis* (Hasegawa, 1988a; Tamura *et al.*, 1995; Otoguro *et al.*, 2001; Labeda *et al.*, 2010; Lisdiyanti *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 75948^T, 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 fastidiosa* DSM 43855^T. However, these two strains differed sufficiently in several characteristics for strain YIM 75948^T to be considered a novel species in the genus *Actinokineospora*.

Strain YIM 75948^T 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 43855^T was the recognized species most closely related to the new strain (see below), the morphological, physiological, biochemical and chemotaxonomic characteristics of strain YIM 75948^T were carefully compared with those of *A. fastidiosa* DSM 43855^T. 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 75948^T 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 sequences with CLUSTAL_X (Thompson *et al.*, 1997). 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 topologies were evaluated by 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 75948^T and *A. fastidiosa* DSM 43855^T 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 lyophilized 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 75948^T 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 75948^T 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 phenotypic properties that can be used to distinguish strain YIM 75948^T from *A. fastidiosa* DSM 43855^T are shown in Table 1. The main characteristics of strain YIM 75948^T are summarized in the species description.

In phylogenetic analyses based on the almost-complete 16S rRNA gene sequence of strain YIM 75948^T and the corresponding sequences of closely related species, strain

Table 1. Morphological and physiological characteristics that distinguish strain YIM 75948^T from *A. fastidiosa* DSM 43855^T

Strains: 1, YIM 75948^T; 2, *A. fastidiosa* DSM 43855^T. All data from this study. Both strains showed optimum growth at 37 °C and were negative for oxidase and tryptophan deaminase activities but positive for catalase activity. Both hydrolysed gelatin, starch and Tween 20 but not cellulose, gave a positive result in the methyl red test but did not produce H₂S. Both utilized cellobiose and D-glucose as sole carbon sources, but not L-arabinose, dulcitol, glycerol, inositol, lactose, raffinose, L-rhamnose, sodium citrate, sodium oxalate, starch or trehalose. Both utilized L-alanine, L-arginine, glycine, D-histidine, L-hydroxyproline, hypoxanthine, D-phenylalanine, L-lysine, D-proline, L-tyrosine, D-threonine, D-valine and xanthine (but not glutamic acid) as sole nitrogen sources. +, Positive; –, negative; s, sensitive; R, resistant.

Characteristic	1	2
Colony reverse colour	Yellow–white	Pale yellow–yellow
Colour of aerial mycelium	Yellow–white	White–pale pink
Optimum pH	8	7–8
Growth at 55 °C	+	–
Urease	–	+
Nitrate reduction	–	+
Lipase tests		
Tween 40	+	–
Tween 60	–	+
Tween 80	–	+
Carbohydrate utilization		
Mannitol	+	–
D-Mannose	+	–
Maltose	+	–
D-Xylose	–	+
D-Fructose	–	+
D-Galactose	+	–
Sucrose	+	–
Xylitol	+	–
Nitrogen utilization		
L-Serine	–	+
L-Aspartic acid	–	+
Antibiotic susceptibility		
Clindamycin	S	R
Penicillin	S	R
Erythromycin	S	R
Amikacin	R	S
Tetracycline	R	S

YIM 75948^T appeared to belong in the genus *Actinokineospora* and was clustered with *A. fastidiosa* DSM 43855^T 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 75948^T and *A. fastidiosa* DSM 43855^T 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 75948^T and *A. fastidiosa* DSM 43855^T 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 75948^T was 73.8 mol% and its major fatty acids were iso-C_{15:0} (15.9 %), iso-C_{16:0} (37.4 %) and C_{16:0} (12.2 %). Under the same incubation and analysis conditions, the genomic DNA G + C content of *A. fastidiosa* DSM 43855^T was 73.5 mol% and its major fatty acids were iso-C_{15:0} (14.2 %), iso-C_{16:0} (41.1 %), C_{16:0} (10.1 %) and C_{17:1ω6C} (11.3 %). The detailed fatty acid profiles of the novel strain and *A. fastidiosa* DSM 43855^T are displayed in Table S1. The predominant respiratory quinone of strain YIM 75948^T 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 75948^T was similar to that of *A. fastidiosa* DSM 43855^T, consisting of phosphatidylethanolamine, phosphatidylethanolamine with hydroxy fatty acids, diphosphatidylglycerol, phosphatidylinositol and, as minor components, two unidentified phospholipids (Fig. S3). For both strain YIM 75948^T and *A. fastidiosa* DSM 43855^T, 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 arabinose.

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 75948^T 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 75948^T was fairly distinct from that of *A. fastidiosa* DSM 43855^T (Table S1). Based on the results of the morphological, physiological and chemotaxonomical characterizations and DNA–DNA hybridizations, strain YIM 75948^T 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,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain YIM 75948^T in relation to members of the genus *Actinokineospora*. Bootstrap values >50%, based on 1000 replications, are shown at branching points. Bar, 0.01 substitutions per nucleotide position.

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 is susceptible to chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), penicillin (10 µg), netilmicin (30 µg), novobiocin (5 µg), rifampicin (5 µg), tobramycin (10 µg) and vancomycin (30 µg), but resistant to ampicillin (10 µg), amikacin (30 µg), norfloxacin (10 µg), tetracycline (30 µg) and trimethoprim (1.25 µg). Cell hydrolysates contain *meso*-diaminopimelic acid as the diagnostic cell-wall diamino acid, and glycine, glutamic acid, alanine and aspartic acid are the main amino acids in the peptidoglycan. Extracts of whole-cell sugars contain

mannose, ribose, glucose, galactose and arabinose. The polar lipids consist of phosphatidylethanolamine, phosphatidylethanolamine with hydroxy fatty acids, diphosphatidylglycerol, phosphatidylinositol and two unidentified phospholipids. The major fatty acids are iso-C_{15:0}, iso-C_{16:0} and C_{16:0}.

The type strain, YIM 75948^T (=JCM 17695^T=DSM 45613^T), 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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