Actinokineospora guangxiensis sp. nov., isolated from soil

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A novel actinomycete, designated strain GK-6T, was isolated from a soil sample from Nanning, Guangxi province, PR China. The strain grew at 20–40 °C, pH 6.0–11.0 and with 0–7.0 % NaCl. It formed well-developed aerial and vegetative mycelia. The aerial mycelium was white and the vegetative mycelium was yellow. The long branching aerial mycelia yielded rod-shaped arthrospores, the spores had smooth surfaces and were non-motile. Strain GK-6T contained meso-diaminopimelic acid as the diagnostic diamino acid, the whole-cell sugars were galactose and arabinose. Major fatty acids were iso-C16:0, iso-C18:0 and C17:0. MK-9(H4) was the predominant menaquinone. The polar phospholipids were phosphatidylethanolamine, phosphatidylethanolamine-containing hydroxylated fatty acids, diphosphatidylglycerol, ninyhdrin-positive glycophaspholipid and an unknown phospholipid. The G+C content of the genomic DNA was 73.4 mol%. The 16S rRNA gene sequence analysis indicated that the organism was a member of the genus Actinokineospora and its closest relative among recognized species was Actinokineospora soli JCM 17695T (97.7 % sequence similarity). But the phenotypic characteristics of strain GK-6T were significantly different from those of A. soli JCM 17695T and DNA–DNA hybridization showed low relatedness (22.6–28.3 %) between strain GK-6T and JCM 17695T. On the basis of the phenotypic and phylogenetic data, strain GK-6T represents a novel species of the genus Actinokineospora, and the name Actinokineospora guangxiensis sp. nov. is proposed. The type strain is GK-6T (=DSM 46779T =CGMCC 4.7154T).

The genus Actinokineospora was established by Hasegawa (1988) based on the type strain Actinokineospora riparia and which was described as a motile arthrospore-bearing actinomycete, it was emended by Labeda et al. (2010) to accommodate the species with non-motile spores. Currently, the genus Actinokineospora contains 13 species with validly published names (http://www.bacterio.net/actinokineospora.html), among them, eight species were isolated from soil (Henssen et al., 1987; Tamura et al., 1995; Otoguro et al., 2001; Labeda et al., 2010; Lisdiyanti et al., 2010; Tang et al., 2012; Intra et al., 2013), and five species were isolated from fallen leaves (Hasegawa, 1988; Tamura et al., 1995; Otoguro et al., 2001; Lisdiyanti et al., 2010). Most species of the genus Actinokineospora have arthrospores which can exhibit motility when suspended in sterile distilled water, except for three species (Actinokineospora fastidiosa, Actinokineospora soli and Actinokineospora bangkokensis) with non-motile spores, all of which were isolated from soil. Strains in the genus Actinokineospora have meso-diaminopimelic acid as cell-wall diamino acid, contain arabinose and galactose in their whole-cell hydrolysates, MK-9(H4) as the predominant menaquinone, phospholipid type II, iso-C16:0 fatty acid as the predominant fatty acid and have G+C contents of the DNA of 69.1–74.0 mol%.

During a survey of the biodiversity of rare actinomycetes from Guangxi province, PR China, a mycelium-forming actinomycete strain was isolated from a soil sample collected on 2 March 2013 in Nanning (GPS coordinates 22° 51’ 18.46” N 108° 18’ 19.41” E). The strain is easy to culture at 30 °C and grows well on most of the media tested, even the water agar. The soil samples were collected and isolated by the dilution plate technique, grown on HV medium (Hayakawa & Nonomura, 1987) and incubated at 30 °C in the dark for 5 days under aerobic conditions. Strain GK-6T was maintained on ISP (International Streptomycetes Project) 2 medium (Shirling & Gottlieb, 1966) at 4 °C and as 20 % glycerol suspensions (v/v) at −20 and −80 °C, the on the cultures preserved at −20 °C were restored each year.

Cultural characteristics of strain GK-6T were tested on ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7 media (Shirling &

The GenBank/DDJB/EMBL accession number of the 16S rRNA gene sequence of strain GK-6T is KJ0027603.

Three supplementary figures and three supplementary tables are available with the online Supplementary Material.
Gottlieb, 1966), Czapek solution agar, potato dextrose agar (Waksman, 1961), Gause’s asparagine agar (Gause et al., 1983), Bennett’s agar (Jones, 1949), HV medium (Hayakawa & Nonomura, 1987) and water agar (15.0 g agar, 1000 ml tap water) after incubation for 7 and 14 days at 30 °C. Morphological characteristics were observed by light microscopy (80i; Nikon) and scanning electron microscopy (VEGA3 SBU; Tescan) after incubation for 7 days on ISP2 agar at 30 °C. Motility was observed by light microscopy using cells grown for 7–10 days at 30 °C on ISP 2 agar. The colours of the substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the Inter-Society Color Council-National Bureau of Standards (ISCC–NBS) colour charts (Kelly, 1964). Growth temperature was tested on ISP2 medium at 4, 16, 25, 30, 37, 40, 45 and 50 °C and observed after 28 days. Tolerance to NaCl concentrations between 0 and 15 % (at intervals of 1 %) was determined on ISP2 and read after 7 and 14 days at 30 °C. The pH range and the optimum pH for growth were examined using Bennett’s agar with the pH ranging between pH 4.0 and 11.0 (at intervals of 1.0 pH unit) using the following buffer systems: pH 4.0–6.0, 0.1 M citric acid/0.1 M sodium citrate; pH 7.0–9.0, 0.1M Tris/0.1 M HCl; pH 9.5–10.0, 0.1 M NaHCO3/0.1 M Na2CO3 and pH 11.0, 0.05 M Na2HPO4/0.1 M NaOH. After 7 and 14 days incubation at 30 °C, growth was scored as a positive result. Carbon-source utilization for growth was examined on ISP9 as described by Shirling & Gottlieb (1966). Acid fastness was tested by using the method of Gordon et al. (1974). Oxidase, catalase, esterase, nitrate reduction, degradation of adenine, guanine, hypoxanthine, xanthine, aesculin, L-tyrosine, xylan, casein, gelatin, starch, allantoin and urea were examined as described by Williams et al. (1983).

GK-6T forms well-developed aerial mycelium and vegetative mycelium on HV medium, Bennett’s agar, ISP2, ISP3, ISP4, Czapek’s media and Gause’s asparagine agar, exhibits moderate growth on ISP6, ISP7, potato agar and water agar and poor growth on ISP5, no aerial mycelium on ISP5 and ISP6. The aerial mycelium is white and the vegetative mycelium is yellow. The long branching aerial mycelia yield rod-shaped arthrospores at maturity. The spores’ diameter is 0.3–0.5 μm, they have smooth surfaces and do not exhibit motility when suspended in sterile distilled water (Fig. S1, available in the online supplementary material). The strain does not produce diffusible pigment on any of the media tested. No sporangia or sporangium-like structures on GK-6T were observed. GK-6T grew at 20–40 °C with an optimum temperature range of 25–37 °C and at pH 6.0–11.0 (optimum pH 7.0–9.0) and with 0–7 % (w/v) NaCl (optimum between 0 and 3 %) (Table 1). Other data for physiological and biochemical properties are given in the species description.

Biomass for chemotaxonomic studies was obtained by centrifugation of cultures grown in Bennett’s broth for 3 days at 30 °C and freeze-dried. The cell-wall diamino acid of strain GK-6T was determined from whole-cell hydrolysates as described by Hasegawa et al. (1983). Whole-organism sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). Polar lipids were extracted and examined by two-dimensional TLC and identified according to the method of Minnikin et al. (1984). Menaquinones were extracted and purified using the method of Collins et al. (1987) and separated by HPLC (Kroppenstedt, 1982). Extraction and analysis of mycolic acids followed the procedure described by Minnikin et al. (1980). Cellular fatty acid methyl esters were prepared and analysed by GC according to the instructions of the Microbial Identification System (version 2.11; MIDI), using the AEROBE package including the TSBA (version 3.9), CLIN (version 3.9) and MI17H10 (version 3.8) databases for the identification of fatty acids. The DNA G+C content of genomic DNA was determined by reverse-phase HPLC according to the method of Mesbah et al. (1989). DNA–DNA hybridization values between GK-6T and Actinokineospora soli JCM 17695T (both were labelled for forward and reverse probes) were determined on nylon membranes using the method described by Wang et al. (2011).

The whole-cell hydrolysates of GK-6T contained meso-diaminopimelic acid as the diagnostic cell-wall peptidoglycan and the whole-cell sugars were galactose, glucose and arabino-bose as diagnostic sugars. No mycolic acids were detected. The polar lipid profile of GK-6T contained phosphatidylethanolamine, phosphatidylethanolamine-

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tr>
<td>Aerial mycelium colour</td>
<td>White</td>
<td>Yellow–white</td>
</tr>
<tr>
<td>Vegetative mycelium colour</td>
<td>Yellow</td>
<td>Yellow–white</td>
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<tr>
<td>pH range</td>
<td>6.0–11.0</td>
<td>7.0–9.0</td>
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<tr>
<td>Temperature range (°C)</td>
<td>20–40</td>
<td>25–55</td>
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<tr>
<td>Tolerance to NaCl (%)</td>
<td>0–7</td>
<td>0–5</td>
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<td>Degradation of:</td>
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<td>Tween 60</td>
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<td>Tween 80</td>
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<td>Hypoxanthine</td>
<td>+</td>
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<td>Nitrate reduction</td>
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<td>Milk coagulation</td>
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<td>Growth on sole carbon sources:</td>
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<tr>
<td>l-arabinose</td>
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<td>Cellobiose</td>
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<td>lactose</td>
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<tr>
<td>Raffinose</td>
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<tr>
<td>Sodium citrate</td>
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<td>Starch</td>
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<td>d-xylene</td>
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<td>–</td>
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<tr>
<td>Xyitol</td>
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Strains: 1, GK-6T; 2, Actinokineospora soli JCM 17695T. +, Positive; –, negative. All data are from this study.
containing hydroxylated fatty acids, diphosphatidylglycerol, phosphatidylglycerol, ninyhydrin-positive glycolipid and two unidentified phospholipids (Fig. S2). The predominant menaquinone of strain GK-6T was MK-9 (H4). The fatty acid profile consisted of major amounts of iso-branched hexadecanoic acids: iso-C16:0 (44.3%), iso-C15:0 (16.9%), and C17:0 (7.3%), and the other components were C17:1ω6c (5.6%), iso-C14:0 (4.7%), C16:0 (3.4%), C17:1ω8c (3.4%), C15:1ω6c (2.7%), iso-C16:1ω7 (2.7%) and C16:1ω7c / C16:1ω6c (2.4%) (Table S1).

The DNA G+C content of strain GK-6T was 73.4 mol%. The chemotaxonomic characteristics of the strain were consistent with those of the members of the genus Actinokineospora, but it could be distinguished from the closely related species Actinokineospora soli JCM 17695T by the lack of mannose and ribose in whole-cell sugars and the different compositions of polar lipids and fatty acids, ninyhydrin-positive glycolipid was the main polar lipid in GK-6T but absent in Actinokineospora soli.

Genomic DNA for PCR amplification was prepared by the method of Li et al. (2007) and the 16S rRNA gene was amplified using primers 27f (5′ GAGTTTGATCCTGCT CAG3′) and 1525r (5′ AGAAAGGAGGTGATCCAGCC3′). The PCR product was purified with a Gel extraction kit (BIOMIGA) and sequenced on an automatic DNA sequencer (model 3730xl; Applied Biosystems). An almost full-length 16S rRNA gene sequence (1421 bp) was aligned and compared with available sequences in the GenBank/EMBL/DDBJ database using BLAST searches (Altschul et al., 1997) and analysed using the EzTaxon server (http://www.ezbiocloud.net/eztaxon/; Kim et al., 2012), revealing that the isolate was a member of the genus Actinokineospora. Multiple alignments with sequences from all species in the genus Actinokineospora with validly published names were carried out using CLUSTAL_X (Thompson et al., 1997). The phylogenetic trees were reconstructed with representative sequences using the methods of neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971). A neighbour-joining tree was reconstructed by using the software package MEGA version 6.0 (Tamura et al., 2013) and calculated by using distances corrected according to Kimura’s two-parameter model (Kimura, 1980). Bootstrap analysis was performed by using 1000 neighbour-joining datasets (Felsenstein, 1985).

The 16S rRNA gene sequence of strain GK-6T showed higher similarity to those of members of genus Actinokineospora (94.6–97.7 %), displayed the highest similarity to Actinokineospora soli JCM 17695T (97.7 %) and to the other strains examined the similarities were all below 96.8 %. In the neighbour-joining tree (Fig. 1) based on the 16S rRNA gene sequences of all species of the genus

**Fig. 1.** A phylogenetic tree showing the relationship between strain GK-6T and all species of the genus Actinokineospora with validly published names reconstructed using the neighbour-joining method based on 16S rRNA sequences. Asterisks indicate that the corresponding branches are supported by both maximum likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values based on 1000 resamplings; only values above 50 % are shown. Bar, 0.01 substitutions per nucleotide position.
Actinokineospora with validly published names, showing the evolutionary divergence between strain GK-6^T and the type strains of species of the genus Actinokineospora, strain GK-6^T formed a monophyletic clade with the closely related species Actinokineospora soli JCM 17695^T with a high bootstrap value of 100 % and supported by all the treeing algorithms applied. The maximum-parsimony tree showed a similar result (Fig. S3). But the mean DNA–DNA relatedness value between GK-6^T and JCM 17695^T was 22.6 % to 28.3 % (Table S2), a value well below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne et al., 1987), showing that GK-6^T represents a distinct genomic species.

The phylogenetic analysis, chemotaxonomic characteristics and morphological characteristic of strain GK-6^T indicate that it belongs to the genus Actinokineospora. The fatty acid content of strain GK-6^T was fairly distinct from that of Actinokineospora soli JCM 17695^T (Table S1). GK-6^T can also be distinctly distinguished from Actinokineospora soli JCM 17695^T on the basis of biochemical and physiological properties (Table 1). The cultural characteristics of strain GK-6^T and Actinokineospora soli JCM 17695^T were compared in detail and they were quite different (Table S3). Therefore, the results of the polyphasic taxonomic study presented here allow us to assign the isolate to a novel species, for which we propose the name Actinokineospora guangxiensis sp. nov.

**Description of Actinokineospora guangxiensis sp. nov.**

Actinokineospora guangxiensis (guang.xi.en‘sis. N.L. fem. adj. guangxiensis pertaining to Guangxi Zhuang Autonomous Region, south-western China, where the location of the type strain was isolated).

Aerobic, Gram-stain-positive, non-acid-fast, non-motile actinomycete. Forms well-developed aerial mycelium and vegetative mycelium on HV medium, Bennett’s agar, ISP2, ISP3, ISP4, Czapek’s media and Gause’s asparagine agar, exhibits moderate growth on ISP6, ISP7, potato agar and water agar and poor growth on ISP5, no aerial mycelium on ISP5 and ISP6. The aerial mycelium is white and the vegetative mycelium is yellow. The long branching aerial mycelia yield rod-shaped arthropores at maturity. The spores’ diameter is 0.3–0.5 µm, they have smooth surfaces and do not exhibit motility when suspended in sterile distilled water. Sporangia are not detected. No soluble pigment is observed in any of the media tested. Growth is observed at 20–40 °C with an optimum temperature range of 25–37 °C and at pH 6.0–11.0 (optimum pH 7.0–10.0) and with 0–7 % (w/v) NaCl (optimum 0–3 %). Hydrolyses L-tyrosine, starch, hypoxanthine, gelatin, allantoin and urea but not casein, guanine, aesculin, adenine, xylan and xanthine. Positive for catalase, nitrate reduction, milk coagulation and peptonization. Negative for oxidase and H₂S production. Twin 20, 40, 60 and 80 are hydrolysed. Utilizes L-arabinose, L-rhamnose, lactose, D-galactose, D-xylose, sucrose, D-fructose, D-mannose, inulin, dextrin, D-sorbitol, maltose, glucose, raffinose, sodium acetate, sodium propionate and sodium citrate as sole carbon sources, but not inositol, mannitol, trehalose, ribose, salicin, xylitol, cellobiose and sodium oxalate are not. As sole nitrogen sources, L-alanine, glycine, D-phenylalanine, L-hydroxyproline, hypoxanthine, L-tyrosine, xanthine, D-valine, L-aspartic acid and methionine are utilized, but not L-arginine, D-threonine, D-histidine, L-lysine, D-proline, L-serine or glutamic acid. Cell hydrolysates contain meso-diaminopimelic acid and the whole-cell sugars are galactose, glucose and arabinose. The polar lipids consist of phosphatidylethanolamine, phosphatidylethanolamine-containing hydroxylated fatty acids, diphasphatidylglycerol, phosphatidylinositol and ninyhydrin-positive glycoprophospholipid. The predominant menaquinone is MK-9(H₄). The major fatty acids are iso-C₁₆ : 0, iso-C₁₅ : 0 and C₁₇ : 0.

The type strain, GK-6^T (=DSM 46779^T=CGMCC 4.7154^T), was isolated from soil in Nanning, Guangxi Zhuang Autonomous Region, south-western China. The DNA G+C content of the type strain is 73.4 mol%.

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### References


