Thermoactinomyces guangxiensis sp. nov., a thermophilic actinomycete isolated from mushroom compost

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A novel thermophilic actinomycete, designated strain CD-1 T, was isolated from mushroom compost in Nanning, Guangxi province, China. The strain grew at 37–55 °C (optimum 45–50 °C), pH 6.0–11.0 (optimum pH 7.0–9.0) and with 0–2.0 % NaCl (optimum 0–1.0 %), formed well-developed white aerial mycelium and pale-yellow vegetative mycelium, and single endospores (0.8–1.0 μm diameter) were borne on long sporophores (2–3 μm length). The endospores were spherical-polyhedron in shape with smooth surface. Based on its phenotypic and phylogenetic characteristics, strain CD-1 T is affiliated to the genus Thermoactinomyces. It contained meso-diaminopimelic acid as the diagnostic diamino acid; the whole-cell sugars were ribose and glucose. Major fatty acids were iso-C 15 : 0, C 16 : 0, anteiso-C 15 : 0 and iso-C 17 : 0. MK-7 was the predominant menaquinone. The polar phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylethanolamine containing hydroxylated fatty acids, ninhydrin-positive glycopolyphospholipid, an unknown phospholipid and glycolipids. The G+C content of the genomic DNA was 48.8 %. 16S rRNA gene sequence analysis showed that the organism was closely related to Lihuaxuella thermophila YIM 77831 T (95.69 % sequence similarity), Thermoactinomyces daqus H-18 T (95.49 %), Laceyella putida KCTC 3666 T (95.05 %), Thermoactinomyces vulgaris KCTC 9076 T (95.01 %) and Thermoactinomyces intermedius JCM 3312 T (94.55 %). Levels of DNA–DNA relatedness between strain CD-1 T and Lihuaxuella thermophila JCM 18059 T, Thermoactinomyces daqus DSM 45914 T, Laceyella putida JCM 8091 T, Thermoactinomyces vulgaris JCM 3162 T and Thermoactinomyces intermedius JCM 3312 T were low (22.8, 33.3, 24.7, 29.4 and 30.0 %, respectively). A battery of phenotypic, genotypic and DNA–DNA relatedness data indicated that strain CD-1 T represented a novel species of the genus Thermoactinomyces, for which the name Thermoactinomyces guangxiensis sp. nov. is proposed. The type strain is CD-1 T (=ATCC BAA-2630 T=CGMCC 4.7156 T).

The genus Thermoactinomyces was established by Tsilinsky (1899) on the basis of the single species Thermoactinomyces vulgaris, and thereafter five species were added to this genus. Yoon et al. (2005) emended the genus into four genera, Thermoactinomyces sensu stricto, Laceyella, Seinonella and Thermostreikomycibium, based on phenotypic, phylogenetic and chemotaxonomic analyses, and the two species Thermoactinomyces vulgaris and Thermoactinomyces intermedius remained in this genus at that time. The family Thermoactinomycetaceae was described by Matsuo et al. (2006) to accommodate the genus Thermoactinomyces and other related genera.

Recently, Thermoactinomyces daqus was described as another novel species of the genus Thermoactinomyces by Yao et al. (2014). Strains in the genus Thermoactinomyces are Gram-stain-positive, non-acid-fast and chemo-organotrophic. Aerial mycelium is abundant and white. Well-developed, branched and septeate substrate mycelium is formed. Endospores are sessile and formed singly on aerial and substrate hyphae or on unbranched sporophores. Members of the genus Thermoactinomyces are thermophilic and grow at 55 °C but not at 30 °C. They have meso-diaminopimelic acid as a cell-wall diamino acid, MK-7 as the predominant menaquinone, the major fatty acid acids are iso-C 15 : 0 and iso-C 17 : 0, the major phospholipids are diphosphatidylglycerol,

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The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain CD-1 T is KJ027602.

Four supplementary figures and two supplementary tables are available with the online Supplementary Material.

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Thermoactinomyces species of the genus actinomycetes in mushroom compost, strain CD-1T was isolated by the dilution plate technique, grown on HV medium (Hayakawa & Nonomura, 1987) and incubated at 50 °C in the dark for 3 days under aerobic conditions. Strain CD-1T was maintained on ISP (International Streptomycetes Project) 4 medium (Shirling & Gottlieb, 1966) at 4 °C and as 20 % glycerol suspensions at −20 °C and −80 °C.

Cultural characteristics of strain CD-1T were tested on ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7 media (Shirling & 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 and water agar (15.0 g agar, 1000 ml tap water) after incubation for 2 and 5 days at 50 °C. Morphological characteristics were observed under light microscopy (80×; Nikon) and scanning electron microscopy (VEGA3 SBU; Tescan) after incubation for 2 days on ISP4 medium at 50 °C. 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). Temperature for growth was tested on Bennett’s agar at 4, 16, 25, 30, 37, 40, 45, 50, 55, 60 and 65 °C and observed after 14 days. Tolerance to NaCl between 0 and 15 % (at intervals of 1 %) was tested on Bennett’s agar and observed after 7 and 14 days at 50 °C. The pH range and the optimum pH for growth were examined on Bennett’s agar with the pH range between pH 4.0 and 11.0 (at intervals of 1.0 pH unit) using the following buffer system: pH 4.0–5.0, 0.1 M citric acid/0.1 M NaOH; pH 5.0–6.0, 0.1 M KH2PO4/0.1 M NaOH; pH 6.0–7.0, 0.1 M KH2PO4/0.1 M NaOH; pH 7.0–8.0, 0.1 M KH2PO4/0.1 M NaOH; pH 8.0–9.0, 0.1 M Na2HPO4/0.1 M NaOH; and pH 9.0–10.0, 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 of incubation at 50 °C, growth was scored as a positive result. Carbon-source utilization for growth was examined on ISP9 as described by Shirling & Gottlieb (1966). Media and procedures used for determination of physiological features were conducted as described by Williams et al. (1983).

Strain CD-1T grew well on HV medium, Sauton’s agar (Mordarska et al., 1972), Bennett’s agar, ISP3 and ISP4 (Fig. S1, available in the online Supplementary Material), exhibited moderate growth on Gause’s asparagine agar and ISP7, and poor growth on water agar and potato agar, but no growth on ISP2, ISP5, ISP6 or Czapek’s media. No diffusible pigment could be observed on any of the media. The aerial mycelium was well developed and white, vegetative mycelium was pale-yellow, single endospores (0.8–1.0 μm diameter) were borne on long sporophores (2–3 μm length). The endospores were spherical polyhedral in shape with smooth surface (Fig. S2). No sporangia or naked sporangium-like structures on CD-1T were observed. Strain CD-1T grew at 37–55 °C with an optimum temperature range of 45–50 °C, at pH 6.0–11.0 (optimum pH 7.0–9.0) and with 0–2 % (w/v) NaCl (optimum between 0 and 1 %). Other data for physiological and biochemical properties are given in the species description.

Biomass for chemotaxonomic studies was obtained by centrifugation and freeze-drying of cultures in Bennett’s broth for 2 days at 50 °C. The cell-wall diamino acid of strain CD-1T 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 MI7H10 (version 3.8) databases for the identification of fatty acids. DNA–DNA hybridization values between strain CD-1T and Lixuaxella thermophila JCM 18059T (Yu et al., 2012), Thermoactinomyces daquis DSM 45914T, Laceyputida JCM 8091T (Lacey & Cross, 1989; Yoon et al., 2005), Thermoactinomyces vulgaris JCM 3162T and Thermaactinomyces intermedium JCM 3312T were determined on nylon membranes using the method described by Wang et al. (2011). Genomic DNA G+C content was determined by reverse phase HPLC according to the method of Mesbah et al. (1989).

The whole-cell hydrolysates of strain CD-1T contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan and the whole-cell sugars were ribose and glucose as diagnostic sugars. No mycolic acids were detected. The polar lipid profile of strain CD-1T contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylethanolamine containing hydroxylated fatty acids, nihydrin-positive glycospholipid, an unknown phospholipid and glycolipids (Fig. S3). The major menaquinone of strain CD-1T was MK-7 (97.8 %). The fatty acid profile consisted of major amounts of iso-C15 : 0 (34.2 %), C16 : 0 (16.2 %), anteiso-C15 : 0 (12.6 %), iso-C17 : 0 (11.9 %) and iso-C16 : 0 (9.9 %) (Table S1). The DNA G+C content of strain CD-1T was 48.8 mol%.

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′-GAGTTTGATCCTGGCTCAG3′) and 1525r (5′-AGAGTTTGATCCTGGCTCAG3′) as described by Rainey et al. (1996). The PCR product was purified with a Gel extraction kit (BIOMEGA) and sequenced on an automatic DNA sequencer (model 3730xl; Applied Biosystems). An almost full-length 16S rRNA gene sequence
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). Multiple alignments with sequences from all recognized species in the family Thermoactinomycetaceae were carried out using CLUSTAL X (Thompson et al., 1997).

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.
model (Kimura, 1980). Bootstrap analysis was performed by using 1000 neighbour-joining datasets (Felsenstein, 1985). The 16S rRNA gene sequence (1426 bp) analysis of strain CD-1T indicated that it was affiliated to the family Thermoactinomycetaceae and showed highest similarity to Lihuaxuella thermophila YIM 77831T (95.69 %), Thermoactinomyces daqus H-18T (95.49 %), Laceyella putida KCTC 3666T (95.05 %) and Thermoactinomyces vulgaris KCTC 9076T (95.01 %). In the neighbour-joining tree (Fig. 1) based on the 16S rRNA gene sequences of all recognized species in the family Thermoactinomycetaceae showing the evolutionary divergence between strain CD-1T and the type strains of these species, strain CD-1T formed a cluster with the species of the genus Thermoactinomyces that was supported by all the treeing algorithms applied. The maximum-parsimony tree also showed a similar result (Fig. S4). The DNA–DNA relatedness value between CD-1T and its closest phylogenetic neighbours, Lihuaxuella thermophila JCM 18059T, Thermoactinomyces daqus DSM 45914T, Laceyella putida JCM 8091T, Thermoactinomyces vulgaris JCM 3162T and Thermoactinomyces intermedius JCM 3312T were 22.8 ± 3.6, 33.3 ± 1.8, 24.7 ± 2.9, 29.4 ± 1.2 and 30.0 ± 1.7 % (±, SD), respectively (Table S2), all well below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne et al., 1987), showing that CD-1T represents a different genomic species. The DNA–DNA hybridization values with type strains of the species of genus Thermoactinomyces that was supported by all the treeing algorithms applied. The maximum-parsimony tree also showed a similar result (Fig. S4).

Table 1. Differential characteristics of strain CD-1T and its closest phylogenetic neighbours

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<tr>
<td>DNA G+C content (mol%)</td>
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<td>49.1</td>
<td>48.0</td>
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<td>55.6</td>
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strains of species of the genera *Laceyella* and *Lihuaxuella* were lower than those observed with type strains of species of the genus *Thermoactinomyces*.

Strain CD-1T formed well-developed aerial mycelium, the G+C content of the genomic DNA was 48.8% and the absence of phosphatidylmethylthanolamine made it clearly distinct from *Lihuaxuella thermophila* YIM 77831T. The predominant menaquinone components of strain CD-1T differed from those of the genus *Laceyella*. Strain CD-1T formed long sporophores, but all of its closest phylogenetic neighbours formed short sporophores. The phylogenetic analysis, chemotaxonomic characteristics and morphological characteristics of strain CD-1T indicated that it belongs to the genus *Thermoactinomyces* and can be distinguished from its nearest phylogenetic neighbours (Table 1). 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 *Thermoactinomyces guangxiensis* sp. nov.

**Description of Thermoactinomyces guangxiensis sp. nov.**

*Thermoactinomyces guangxiensis* (guang.xi.en’sis. N.L. masc. adj. *guangxiensis* pertaining to Guangxi Zhuang Autonomous Region, south-western China, where the type strain was isolated).

Aerobic, thermophilic, Gram-positive, filamentous actinomycete. Grows well on HV medium, Sauton’s agar, Bennett’s agar, ISP3 and ISP4, exhibited moderate growth on Gause’s asparagine agar and ISP7, and poor growth on water agar and potato agar, but no growth on ISP2, ISP5, ISP6 or Czapek’s media. No diffusible pigment on water agar and potato agar, but no growth on ISP2, ISP3 and ISP4, exhibited moderate growth on Gause’s agar, ISP5, ISP6 or Czapek’s media. The whole-cell hydrolysates contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, and the whole-cell sugars were ribose and glucose as diagnostic sugars. No mycolic acids were detected. The polar lipid profile contained diophosphatidylglycerol, phosphatidylglycerol, phosphatidyethanolamine, phosphatidylethanolamine containing hydroxylated fatty acids, ninyhdrin-positive glycolipid, an unknown phospholipid and glycolipids. The predominant menaquinone was MK-7. The fatty acid profile consisted of major amounts of iso-C_{15:0} anteiso-C_{15:0} and iso-C_{16:0}.

The type strain, CD-1T (ATCC 2630^T=CGMCC 4.7156^T), was isolated from a mushroom compost in Nanning, Guangxi Zhuang Autonomous Region, south-western China. The DNA G+C content of the type strain is 48.8 mol%.

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**References**


