Streptomyces thermospinisporus sp. nov., a moderately thermophilic carboxydotochrpic streptomycete isolated from soil

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A carboxydotochrpic actinomycete strain, AT10T (= DSM 41779T = KCTC 9909T), was the subject of a polyphasic study. The morphological and chemical properties of the strain were found to be consistent with its assignment to the genus Streptomyces. The organism formed a distinct phyletic line within the 16S rDNA Streptomyces tree, and DNA–DNA relatedness experiments further confirmed that it formed a distinct genomic species. The strain was also distinguished from related species using phenotypic properties. Strain AT10T, therefore, merits species status within the genus Streptomyces; the name Streptomyces thermospinisporus sp. nov. is proposed for this new taxon.

Keywords: Streptomyces thermospinisporus sp. nov., carboxydotochrpic streptomycete, 16S rDNA analysis, DNA–DNA relatedness

Carboxydotochrphy, the chemolithotrophic utilization of carbon monoxide or carbon dioxide, is not known as a common feature among actinobacteria (Bell et al., 1987; Gadkari et al., 1990; Meyer & Schlegel, 1983; Meyer et al., 1990). However, recent studies have indicated that large numbers of carboxydotochrpic actinomycetes are present in some soils, and that these organisms might comprise distantly related groups of moderately thermophilic actinomycetes (Falconer, 1988; O'Donnell et al., 1993). Initial chemotaxonomic and morphological studies on carboxydotochrpic actinomycetes suggested that they should be classified in the genus Streptomyces, although they were also shown to have distinct phenotypic properties (O'Donnell et al., 1993). To date, only an obligately carboxydotochrpic streptomycete, Streptomyces thermodiastaticus (Gadkari et al., 1990), and two facultatively carboxydotochrpic organisms, Streptomyces thermodiastaticus and Streptomyces thermodiastaticus (Kim et al., 1998), have been formally described. It is notable that the tools for carboxydotochrpic metabolism in S. thermodiastaticus are remarkably different from those of other carboxydotochrpic bacteria (Hugendieck & Meyer, 1992).

Most carboxydotochrpic actinomycetes fall into several phylogenetic groups within the 16S rDNA streptomycete clade, including one group that is close to Streptomyces thermodiastaticus, and another which shows a close affinity to Streptomyces thermodiastaticus. However, there are also strains that cannot be assigned to the genus Streptomyces on the basis of chemical properties (Falconer, 1988; O'Donnell et al., 1993); this implies that the true diversity of the carboxydotochrpic actinomycetes in natural habitats has still to be resolved.

Strain AT10T (= DSM 41779T = KCTC 9909T) is a facultatively carboxydotochrpic actinomycete that was isolated from garden soil (Falconer, 1988). The organism was examined for morphological and physiological properties, and its position in the 16S rDNA tree was determined.

Strain AT10T was maintained on modified Bennett’s agar plates (Jones, 1949) at 45 °C and as a suspension of spores and mycelial fragments in glycerol (20%, v/v) at −20 °C. The morphological, phenotypic and chemical properties of the organism were examined by following methods described in earlier studies (O’Donnell et al., 1993; Williams et al., 1983). The extraction of DNA and the PCR amplification and sequencing of the 16S rDNA were performed using procedures described previously (Kim et al., 1998). The resultant sequence was aligned with those of representative thermophilic streptomycetes and related strains (D.
The phylogenetic distances were calculated using several treeing algorithms, including the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) methods. Distances based on the Jukes–Cantor (1969) model were used for the least-squares and neighbour-joining algorithms. A bootstrap analysis (Felsenstein, 1985) was performed using 1000 resampled datasets based on the Jukes–Cantor distances and the neighbour-joining algorithm. *Nocardia asteroides* ATCC 19247T (nucleotide accession number Z3934) was used as the outgroup (Fig. 1). DNA–DNA relatedness values between strain AT10T and the type strains of *S. thermocarboxydovorans* and *S. thermodiastaticus* were carried out by the identification service at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), as described previously (Kim et al., 1999).

The strain forms a grey aerial spore mass, extensively branched aerial hyphae which carry long chains of spores, and substrate mycelia which lack distinctive pigments on both modified Bennett’s and inorganic salts-starch (ISP medium 4; Shirling & Gottlieb, 1966) agars. Soluble pigments are not produced. The spore chains are flexuous, and the spores have spiny ornamentation. The strain grows at moderately thermophilic temperatures, the optimal range being around 45 °C. It contains major amounts of LL-diaminopimelic acid, octahydrogenated menaquinones with nine isoprene units (MK-9[H₉]), and diphosphatidylglycerol and phosphatidylethanolamine (Falconer, 1988). The chemical and morphological properties of strain AT10T are clearly consistent with its assignment to the genus *Streptomyces* (Manfio et al., 1995; O’Donnell et al., 1993; Williams et al., 1989).

The nearly complete 16S rDNA sequence (1522 nucleotides) obtained for strain AT10T was compared with corresponding streptomycete sequences; the strain was found to be most closely related to *S. thermocarboxydovorans* DSM 44296T, another carboxydrotrophic organism. These organisms shared a 16S rDNA similarity level of 99.2%, which corresponds to 11 nucleotide differences out of 1414 positions. The strain was closely related to *S. thermodiastaticus* DSM 40573T and *Streptomyces bluenis* ISP 5564T, sharing 16S rDNA similarity values of 98.5% (21 nucleotide differences) and 97.7% (32 nucleotide differences) with these organisms, respectively.

The close relationship between strain AT10T and the type strain of *S. thermocarboxydovorans* was supported by the results from all three treeing algorithms.

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**Fig. 1.** Neighbour-joining tree for strain AT10T and members of related streptomycete species based on nearly full 16S rDNA sequences (1414 positions). The phylogenetic distances were based on the Jukes & Cantor (1969) model. Asterisks indicate branches that were also recovered using the least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) algorithms. Numbers at the nodes indicate the levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets using Jukes–Cantor distances; only values over 70% are given. The scale bar corresponds to 0.01 substitutions per nucleotide position. ATCC, American Type Culture Collection, Manassas, VA, USA; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; ISP, International Streptomyces Project; NRRL, ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA.

Kim et al., 1996; S. B. Kim et al., 1998; B. Kim et al., 2000), and phylogenetic analyses were performed using PHYLIP package version 3.5c (Felsenstein, 1993). Three
Table 1. Phenotypic properties separating strain AT10<sup>T</sup> from related Streptomyces

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<td>Blue</td>
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Table 1. Phenotypic properties separating strain AT10<sup>T</sup> from related Streptomyces species

and by a bootstrap value of 88% based on the neighbour-joining method (Fig. 1). The strain was also found to share a DNA–DNA relatedness value of 72%, a value well below the cut-off level of 80% that has been proposed for the recognition of genomic species of Streptomyces (Labeda, 1993, 1998; Labeda & Lyons, 1992). The corresponding DNA–DNA relatedness level between strain AT10<sup>T</sup> and S. thermodiastaticus DSM 40573<sup>T</sup> was also 72%. Strain AT10<sup>T</sup> can also be distinguished from these and other closely related species by using a combination of phenotypic properties (Table 1). It is evident, therefore, that AT10<sup>T</sup> represents a new centre of taxonomic variation within the genus Streptomyces; the name Streptomyces thermospinisporus sp. nov. is proposed for this taxon.

Description of Streptomyces thermospinisporus sp. nov.

Streptomyces thermospinisporus (ther.mo.spi.ni.spo’rus. Gr. adj. thermos hot; L. adj. spinosus spiny; M.L. n. spora a spore; N.L. adj. thermospinusporus heat-loving, spiny spores).

The description is based on the data taken from this and earlier studies (Falconer, 1988; O’Donnell et al., 1993). Gram-positive, aerobic, moderately thermophilic, facultatively carboxydrotrophic actinomycete which forms extensively branched aerial and substrate hyphae. Aerial hyphae differentiate into flexuous chains of spores that show spiny ornamentation. The aerial spore mass is grey, but the substrate mycelium has no distinctive pigments. Soluble pigments are not produced, nor are melanin pigments formed on peptone/yeast extract/iron agar. The organism, which can use carbon monoxide and carbon dioxide as sole sources of carbon for energy and growth, contains major amounts of L-diaminopimelic acid, octahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue, and major amounts of diposphatidylglycerol and phosphatidyethanolamine. The molar G+C content of the genomic DNA is 73.6 mol%. Nitrate is reduced, and elastin, starch and L-tyrosine are degraded. Growth is inhibited in the presence of phenol (0–1%, w/v), phenyl ethanol (0–3%, v/v) and sodium azide (0–0.2%, w/v). meso-Inositol, mannitol, raffinose, sucrose and sodium pyruvate are used as sole carbon sources when the organism is grown heterotrophically. The type, and only, strain is AT10<sup>T</sup> (= DSM 41779<sup>T</sup> = KCTC 9909<sup>T</sup>), which was isolated from garden soil.

Acknowledgements

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References


