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

Seung Bum Kim† and Michael Goodfellow

Department of Agricultural and Environmental Science, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

Author for correspondence: Seung Bum Kim. Tel: +82 42 860 4619. Fax: +82 42 860 4677. e-mail: sbk01@mail.kribb.re.kr

A carboxydrotrophic actinomycete strain, AT10^T (= DSM 41779^T = KCTC 9909^T), 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 AT10^T, 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., carboxydrotrophic streptomycete, 16S rDNA analysis, DNA–DNA relatedness

Carboxydrotrophy, 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 carboxydrotrophic actinomycetes are present in some soils, and that these organisms might comprise distantly related groups of moderately thermophilic actinomycetes (Falconer, 1988; Kim *et al.*, 1998). Initial chemotaxonomic and morphological studies on carboxydrotrophic 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 carboxydrotrophic streptomycete, *Streptomyces thermoautotrophicus* (Gadkari *et al.*, 1990), and two facultatively carboxydrotrophic organisms, *Streptomyces thermocarboxydovorans* and *Streptomyces thermocarboxyidus* (Kim *et al.*, 1998), have been formally described. It is notable that the tools for carboxydrotrophic metabolism in *S. thermoautotrophicus* are remarkably different from those of other carboxydrotrophic bacteria (Hugendieck & Meyer, 1992).

Most carboxydrotrophic 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 thermovulgaris*. 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 carboxydrotrophic actinomycetes in natural habitats has still to be resolved.

Strain AT10^T (= DSM 41779^T = KCTC 9909^T) is a facultatively carboxydrotrophic 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 AT10^T 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.

† Present address: Korean Collection for Type Cultures, Korea Research Institute for Bioscience and Biotechnology, 52 Oun Dong, Yusong, Daejeon 305-333, Republic of Korea.

The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence of *Streptomyces thermospinisporus* DSM 41779^T (= KCTC 9909^T) is AF333113.

Table 1. Phenotypic properties separating strain AT10^T from related *Streptomyces* species

1, Strain AT10^T; 2, *S. bluenensis*; 3, *S. thermocarboxydovorans*; 4, *S. thermodiastaticus*; 5, *Streptomyces thermoviolaceus* subsp. *apingens*; 6, *S. thermoviolaceus* subsp. *thermoviolaceus*; 7, *S. thermovulgaris*. Data for all strains apart from AT10^T were taken from previous studies (Goodfellow *et al.*, 1987; Kim *et al.*, 1998; Shirling & Gottlieb, 1969, 1972; Williams *et al.*, 1983).

Characteristic	1	2	3	4	5	6	7
Colour of aerial spore mass							
Spore chains	Grey <i>Rectiflexibiles</i> Spiny	Blue <i>Spirales</i> Rugose	Grey <i>Rectiflexibiles</i> Smooth	Brownish-grey <i>Spirales</i> Warty or spiny	Violet-grey <i>Retinaculiaperti</i> Warty	Grey-yellowish pink <i>Spirales</i> Warty	Greyish-brown <i>Spirales</i> Smooth
Spore surface	+	+	+	-	+	-	+
Nitrate reduction	+	+	+	+	+	+	+
Degradation of elastin	-	-	-	+	+	+	+
Growth in the presence of:							
Phenol (0.1%, w/v)	-	-	-	+	+	+	+
Phenyl ethanol (0.3%, v/v)	-	-	-	+	+	+	+
Sodium azide (0.02%, w/v)	-	-	-	+	+	+	+
Utilization of sole carbon sources							
<i>meso</i> -Inositol	+	+	-	+	+	-	+
Mannitol	+	+	-	+	+	+	+
Raffinose	+	+	-	+	-	-	+
Sucrose	+	+	-	+	-	-	+
Sodium pyruvate	+	-	+	+	+	+	+

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^T and *S. thermodiastaticus* DSM 40573^T was also 72%. Strain AT10 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^T 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. *thermospinisporus* 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 carboxydophilic 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 LL-diaminopimelic acid, octahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue, and major amounts of diphosphatidylglycerol and phosphatidylethanolamine. 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 (0.02%, 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^T (= DSM 41779^T = KCTC 9909^T), which was isolated from garden soil.

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