

## NOTE

***Streptomyces avermitilis* sp. nov., nom. rev., a taxonomic home for the avermectin-producing streptomycetes**

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**The taxonomic status of '*Streptomyces avermitilis*' strain MA-4680 was established using a polyphasic approach. Strain MA-4680 formed a distinct phyletic line in the 16S rDNA streptomycete tree, and it was evident from the almost complete 16S rDNA sequence data that it was most closely related to *Streptomyces cinnabarinus*, *Streptomyces griseochromogenes*, *Streptomyces resistomycificus* and *Streptomyces viridochromogenes*. However, strain MA-4680 was readily distinguished from the type strains of these species by using a range of phenotypic properties, notably morphological and pigmentation features. The combined genotypic and phenotypic datasets indicate that the organism forms a recognizable centre of variation within the genus *Streptomyces*. It is proposed that '*Streptomyces avermitilis*' be formally recognized as a species of *Streptomyces*. The type strain is MA-4680<sup>T</sup> (ATCC 31267<sup>T</sup> = NCIMB 12804<sup>T</sup> = NRRL 8165<sup>T</sup>).**

**Keywords:** *Streptomyces avermitilis*, avermectins, 16S rDNA sequencing, polyphasic taxonomy

'*Streptomyces avermitilis*' was described by Burg *et al.* (1979) to accommodate a soil isolate which produced potent anthelmintic agents, the avermectins (Egerton *et al.*, 1979). The isolate, strain MA-4680, formed spiral chains of spherical to oval-shaped, smooth surfaced spores on aerial hyphae and showed cultural and physiological characteristics consistent with its assignment to the genus *Streptomyces*. However, '*S. avermitilis*' was not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), and still has no formal taxonomic status. It is now known that the genome of '*S. avermitilis*' strain ATCC 31267 consists of at least 8.7 Mbp, which forms a linear chromosome (Omura *et al.*, 2001). The detection of 25 kinds of secondary metabolite gene clusters in this organism provides fascinating insights into the intrinsic diversity of secondary-metabolite production in streptomycetes.

In the present study, '*S. avermitilis*' strain MA-4680 was examined for a range of genotypic and phenotypic properties and found to represent a distinct centre of taxonomic variation within the genus *Streptomyces*.

Strain MA-4680 (NCIMB 12804) was maintained on

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The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence of *Streptomyces avermitilis* strain MA-4680 is AF145223.

modified Bennett's agar (Jones, 1949) at 28 °C and as glycerol suspensions (20%, v/v) at –20 °C. Biomass for the chemical and molecular systematic studies was obtained by growing the strain in shake flasks of modified Bennett's broth at approximately 150 r.p.m. for 3–4 days at 28 °C, and harvesting by centrifugation. The biomass for the chemotaxonomic studies was washed in distilled water and freeze-dried, whereas that required for the molecular systematic investigation was washed in NaCl/EDTA buffer (0.1 M EDTA, pH 8.0, 0.1 M NaCl) and stored at –20 °C until needed.

The colonial and pigmentation properties of strain MA-4680 were examined on modified Bennett's and inorganic salts-starch agar (ISP medium 4; Shirling & Gottlieb, 1966) after 14 days at 28 °C. Biochemical, degradative and growth properties of the organism were acquired using the media and methods described by Williams *et al.* (1983). The isomeric form of diaminopimelic acid (A<sub>2</sub>pm) was determined by TLC of a whole-organism hydrolysate as described by Stanek & Roberts (1974). A standard procedure was also used to detect the predominant menaquinones (Minnikin *et al.*, 1984). 16S rDNA amplification and sequencing were carried out as described by S. B. Kim *et al.* (1998). The resultant sequence was aligned



**Table 1.** Phenotypic properties that differentiate strain MA-4680<sup>T</sup> from representatives of the most phylogenetically related species

Strains: 1, *S. avermitilis* MA-4680<sup>T</sup>; 2, *S. cinnabarinus* ISP 5467<sup>T</sup>; 3, *S. corchorusii* ISP 5340<sup>T</sup>; 4, *S. griseochromogenes* ISP 5499<sup>T</sup>; 5, *S. resistomycificus* ISP 5133<sup>T</sup>; 6, *S. viridochromogenes* ISP 5110<sup>T</sup>.

Character	1	2	3	4	5	6
<b>Morphology and pigmentation</b>						
Aerial spore mass colour on oatmeal agar	Grey	Red	Light greyish yellowish brown	Grey	Grey	Green
Spore-chain arrangement	Spiral	Flexuous	Incomplete spirals/straight	Spiral	Spiral/looped	Spiral
Spore-surface ornamentation	Smooth	Smooth	Smooth	Spiny	Smooth	Spiny
Melanin pigments on peptone yeast extract iron agar	+	+	–	+	+	–
<b>Degradation of:</b>						
Adenine	+	+	+	+	+	–
Arbutin	–	+	–	–	–	+
Elastin	–	+	–	+	+	+
Starch	+	–	+	+	–	–
Xanthine	+	+	–	+	–	+
<b>Growth on sole carbon sources (1%, w/v):</b>						
Adonitol	–	+	–	+	+	–
L-Arabinose	+	–	+	–	+	+
Dextrin	+	+	–	–	–	–
Fructose	+	–	+	+	+	–
meso-Inositol	+	–	+	+	+	+
Melezitose	–	+	–	+	+	–
Sucrose	–	+	+	+	+	–
Xylose	+	–	–	–	+	+

The biochemical, carbon source and degradation test data on the marker strains are taken from Williams *et al.* (1983), and data on the morphological and pigmentation properties are from Shirling & Gottlieb (1968, 1969, 1972).

of *S. cinnabarinus*, *S. griseochromogenes*, *S. resistomycificus* and *S. viridochromogenes* by using a combination of phenotypic properties. The differential morphological and pigmentation features, which were expressed on oatmeal and peptone-yeast iron agars, are especially significant as it has been shown that these properties can be weighted for the delineation of members of phylogenetically related streptomycete species (Labeda & Lyons, 1991; Labeda *et al.*, 1997; S. B. Kim *et al.*, 1998; B. Kim *et al.*, 2000; Manfio *et al.*, 2001). It is evident from both the phylogenetic and phenotypic data that ‘*S. avermitilis*’ should be formally recognized as a species of the genus *Streptomyces*.

**Description of *Streptomyces avermitilis* sp. nov., nom. rev.**

*Streptomyces avermitilis* (a.ver.mi'ti.lis. N.L. adj. *avermitilis* avermectin producer).

The description is based on data taken from this and an earlier study (Burg *et al.*, 1979). Aerobic, Gram-

positive, mesophilic actinomycete which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long, compact spiral chains which become more open as the culture ages. The spore chains consist of 15 or more spherical to oval-shaped spores with smooth surfaces. Sporulation occurs on standard media such as egg albumin, glycerol-asparagine, inorganic salts-starch and oatmeal agars. A grey aerial spore mass is formed on oatmeal agar; the colony reverse is dark brown to tan. Melanin pigments are produced on peptone yeast extract iron agar and brown diffusible pigments on a range of standard media. The culture grows well at 28 and 37 °C, but does not grow at 50 °C. In addition to the properties shown in Table 1, it metabolizes casein but not tyrosine, liquefies gelatin, and uses glucose, maltose, mannose, rhamnose, but not cellulose, as sole carbon sources for energy and growth. Avermectins, a family of 16-membered antiparasitic macromolecules, are produced. Isolated from a soil sample collected at Kawana, Ito City, Shizuoka Prefecture, Japan. The type strain is *Streptomyces avermitilis* MA-4680<sup>T</sup> (ATCC 31267<sup>T</sup> = NCIMB 12804<sup>T</sup> = NRRL 8165<sup>T</sup>).

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