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^T (ATCC 31267^T = NCIMB 12804^T = NRRL 8165^T).

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 (Ōmura *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

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 (A₂pm) 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

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

manually with corresponding almost-complete sequences of representative *Streptomyces* species retrieved from the DDBJ, EMBL and GenBank databases by using the AL16S program (Chun, 1995). Phylogenetic trees were inferred by using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated as described by Jukes & Cantor (1969). Tree topologies were evaluated by a bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset by using the SEQBOOT and CONSENSE programs from the PHYLIP package. The root position of the tree based on the neighbour-joining method was estimated by using four outgroup organisms, *Arthrobacter globiformis* (sequence accession no. M23411), *Bacillus subtilis* (K00637), *Escherichia coli* (J01695) and *Nocardia asteroides* (Z36934), as described by Swofford & Olsen (1990). A partial nucleotide sequence (120 nucleotides) of the 16S rDNA of strain MA-4680 based on the variable γ -region was compared with the corresponding nucleotide sequences of *Streptomyces* strains retrieved from GenBank. A phylogenetic tree based on these sequences was generated using the neighbour-joining algorithm.

Burg *et al.* (1979) considered that strain MA-4680 had morphological properties consistent with its classification in the genus *Streptomyces*. In particular, the organism formed spiral chains of smooth-surfaced, spherical to oval-shaped spores on aerial hyphae. The results of the present study underpin this earlier assignment as the test strain contained major amounts of LL-A₂pm in whole-organism hydrolysates and hexahydrogenated menaquinones with nine isoprene units (MK-9[H]₆) as the predominant isoprenoid quinone, properties typically shown by streptomycetes (Williams *et al.*, 1989; Manfio *et al.*, 1995).

Comparison of the almost-complete 16S rDNA sequence (*E. coli* positions 58–1481; Brosius *et al.*, 1978) of '*S. avermitilis*' MA-4680 with the corresponding sequences of representative strains of the genus *Streptomyces* showed that the organism formed a distinct phyletic line within a diffuse subclade circumscribed in the neighbour-joining analysis but not in the least-squares and maximum-likelihood runs (Fig. 1). The '*S. avermitilis*' strain was found to be most closely related to the type strains of *Streptomyces cinnabarinus* and *Streptomyces griseochromogenes*; it showed 16S rDNA similarities with these strains of 98.7 and 99.0%, respectively, values that corresponded to 19 and 16 nucleotide differences out of 1406 sites. 16S rDNA similarities and nucleotide differences within these ranges have been recorded for the type strains of several streptomycete species (Sembiring *et al.*, 2000; Saintpierre *et al.*, 2002), some of which are known to have low DNA–DNA relatedness values, i.e. values well below the 70% cutoff point recommended for the

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rDNA sequences showing the position of *S. avermitilis* in the streptomycete tree. Asterisks indicate branches that were also found using the least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1993) treeing algorithms. The numbers at the nodes indicate levels of bootstrap support based on 1000 resamplings of the neighbour-joining data; only values of 60% or above are given. The scale bar corresponds to 0.01 substitutions per nucleotide position.

delineation of genomic species (Wayne *et al.*, 1987). Strain MA-4680 was also shown to be quite closely related to the type strains of *Streptomyces resistomycificus* and *Streptomyces viridochromogenes*; the 16S rDNA similarities shared with these organisms were 98.4 and 98.3% respectively, values that were equated with 23 and 24 nucleotide differences. The sharp separation of strain MA-4680 from representatives of validly described species of *Streptomyces* was underpinned by the results from the 120 nucleotide sequence analysis (data not shown). The organism was most closely, albeit loosely, associated with the type strain of *Streptomyces corchorusii* but can readily be distinguished from the latter by using a combination of phenotypic properties (Table 1).

It is also evident from Table 1 that the '*S. avermitilis*' strain can be distinguished readily from the type strains

Table 1. Phenotypic properties that differentiate strain MA-4680^T from representatives of the most phylogenetically related species

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

Character	1	2	3	4	5	6
Morphology and pigmentation						
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	+	+	—	+	+	—
Degradation of:						
Adenine	+	+	+	+	+	—
Arbutin	—	+	—	—	—	+
Elastin	—	+	—	+	+	+
Starch	+	—	+	+	—	—
Xanthine	+	+	—	+	—	+
Growth on sole carbon sources (1 %, w/v):						
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^T (ATCC 31267^T = NCIMB 12804^T = NRRL 8165^T).

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