Taxonomic study of a chromomycin-producing strain and reclassification of *Streptomyces cavourensis* subsp. *washingtonensis* as a later synonym of *Streptomyces griseus*

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A chromomycin-producing actinomycete, strain AP19, was isolated from a sample of faeces collected from Foping national nature reserve in China. Chemotaxonomic and morphological properties indicated that the novel isolate was a member of the genus *Streptomyces*. Phylogenetic analyses based on an almost complete 16S rRNA gene sequence of the strain and on the 120-nt nucleotide variable γ-region of this molecule revealed that it was closely related to *Streptomyces griseus* ISP 5236T and *Streptomyces cavourensis* subsp. *washingtonensis* ATCC 27732T. DNA-DNA relatedness values among these strains were above 70 %. *Streptomyces cavourensis* subsp. *washingtonensis* could be readily distinguished from *Streptomyces cavourensis* ATCC 14889T by differing BOX-PCR fingerprinting patterns, relatively low 16S rRNA gene sequence similarity and a low DNA-DNA relatedness value. It is proposed, therefore, that *Streptomyces cavourensis* subsp. *washingtonensis* is a later synonym of *Streptomyces griseus*.

Conventional classification methods for the identification of species within the genus *Streptomyces* have mainly relied on the morphological and phenotypic characteristics of the organisms. During the last decade, molecular biological methods such as 16S rRNA gene sequencing and BOX-PCR fingerprinting have had an increasing impact on streptomycete taxonomy (Kim & Goodfellow, 2002; Kim et al., 2004; Lanoot et al., 2004; Saintpierre et al., 2003; Williams et al., 1983). Molecular systematic data show that the genus is clearly overspeciated (Hatano et al., 2003; Lanoot et al., 2002, 2004; Saintpierre et al., 2003; Williams et al., 1983). As exemplified by the proposal that *Streptomyces lipmanii* LMG 20047T, *Streptomyces wilmorei* LMG 21046T, *Streptomyces griseus* subsp. *alpha* LMG 19953T and *Streptomyces griseus* subsp. *cretosus* LMG 19946T should be recognized as heterotypic synonyms of *Streptomyces microflavus* LMG 19527T (Lanoot et al., 2005).

The present study describes the taxonomic position of a chromomycin-producing strain, AP19, isolated from faeces. The sample of faeces was collected from Foping national nature reserve, Shanxi province, China. Phenotypic and phylogenetic data showed that the novel strain was closely related to *Streptomyces cavourensis* subsp. *washingtonensis* NRRL B-8030T and *Streptomyces griseus* ISP 5236T. In the phylogenetic tree based on 16S rRNA gene sequences, these strains formed a clade in the *Streptomyces* tree, indicating that they could belong to the same taxon.

Strain AP19 was isolated on Gause’s synthetic agar plates supplemented with 2 μg potassium dichromate. The plates were seeded with a faeces sample suspension and incubated at 28 °C for 14 days. The isolate was maintained on Gause’s synthetic slopes at 4 °C and as glycerol suspensions (20%, v/v) at −20 °C. All other strains used in this study, namely *Streptomyces alboviridis* NRRL B-3633T, *Streptomyces cavourensis* ATCC 14889T, *S. cavourensis* subsp. *washingtonensis* ATCC 27732T, *Streptomyces erumpens* NRRL B-3163T, *Streptomyces fulvoroceus* NRRL B-24329T, *Streptomyces griseus* AS 4.1419T, *S. griseus* ATCC 13273, *S. griseus* subsp. *solvificiens* NRRL B-1561T and *Streptomyces microflavus* AS 4.1428T, were grown on medium 65 agar (0.4 % w/v glucose, 0.4 % w/v yeast extract, 1 % w/v malt extract, 0.2 % w/v CaCO3; final pH 7.2) and incubated at 30 °C. For large-scale strain preparation, a 250 ml flask with 50 ml medium 65 broth (0.4 % w/v glucose, 0.4 % w/v yeast extract, 1 % w/v malt extract; final pH 7.2) was shaken for 36 h at 30 °C.

**Abbreviation:** DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AP19 is EF466100.

A figure showing the BOX-PCR patterns of strain AP19 and other closely related strains is available with the online version of this paper.
The morphological characteristics of strain AP19 grown on inorganic salt/starch agar at 28°C for 14 days were observed by light microscopy and scanning electron microscopy. The cultural, physiological and biochemical characteristics of the tested strains were determined as described by Williams et al. (1983). The diaminopimelic acid (DAP) isomer and whole-organism sugars pattern were analysed according to the method of Hasegawa et al. (1983). The G+C content of the DNA of the novel strain was determined using the thermal denaturation method (Marmur & Doty, 1962).

Genomic DNA was extracted and the 16S rRNA gene sequence was amplified as described by Chun & Goodfellow (1995). The PCR products were ligated into the PCR2.1-TOPO vector and transformed into Escherichia coli TOP 10. The 16S rRNA gene sequence of strain AP19, inserted into the plasmid vector, was sequenced on a model 3730 Applied Biosystems DNA sequencer.

The sequence was analysed along with the sequences of closely related reference organisms retrieved from the DDBJ/EMBL/GenBank databases. Sequence data were aligned with CLUSTAL_X software, version 1.8 (Thompson et al., 1997). Phylogenetic trees were constructed by using neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Edwards & Cavalli-Sforza, 1963) and maximum-parsimony (Kluge & Farris, 1969) algorithms with the MEGA 3 software package (Kumar et al., 2004). Evolutionary distance matrices were generated as described by Kimura (1980). Tree topologies were evaluated by bootstrap analyses based on 1000 resamplings (Fig. 1). The partial sequence covering the variable 3-region (120 nt, positions 158–277 according to the E. coli numbering system) of the 16S rRNA gene sequence of strain AP19 was also compared with the corresponding nucleotide sequences of type strains of the genus Streptomyces retrieved from GenBank. A phylogenetic tree based on these partial sequences was constructed using the neighbour-joining algorithm (Saitou & Nei, 1987) (Fig. 2).

BOX-PCR fingerprint patterns were analysed as described by Lanoot et al. (2004), apart from some modifications in the electrophoresis conditions. A 25 μl sample mixed with 5 μl loading dye (6 ×) was loaded on a 2.5% agarose gel (Biowest Agar, 10 × 10 cm). Every fourth well was loaded with 5 μl molecular ruler (200 bp). Electrophoresis was performed using 90 V, 60 mA for 210 min in 0.5 x TBE (0.54 % Tris, 0.27 % boric acid, 2 ml 0.5 M EDTA; pH 8.0).

The chemical and morphological properties of isolate AP19 were consistent with its assignment to the genus

![Phylogenetic tree based on 16S rRNA gene sequences from strain AP19 and other related organisms.](image-url)
**Streptomyces.** It formed extensive branching substrate mycelium and aerial hyphae with smooth-surfaced spores in rectiflexible spore chains. DAP analysis revealed the presence of LL-diaminopimelic acid in the cell-wall peptidoglycan. No characteristic sugar was detected in the whole-cell hydrolysates. The G+C content of the genomic DNA of strain AP19 was 71 mol%.

An almost complete 16S rRNA gene sequence (1478 nt) was determined for the novel strain. Primary sequence analysis with sequences of representatives of the family Streptomycetaceae confirmed that the novel isolate was closely related to species of the genus *Streptomyces*. In the phylogenetic tree based on almost complete 16S rRNA gene sequences (Fig. 1), sequence similarity values between strain AP19 and its nearest neighbours, namely *Streptomyces griseorubiginosus* NBRC 13047T, *S. alboviridis* NBRC 13013T, *S. globosus* NBRC 15874, *Streptomyces microflavus* NBRC 13062T and *Streptomyces fulvoruber* NBRC 15897T, were 99.93% (1 nt difference at 1476 sites), 99.93% (1 nt difference at 1475 sites), 99.93% (1 nt difference at 1473 sites), 99.93% (1 nt difference at 1478 sites) and 99.86% (2 nt differences at 1472 sites). Besides the strains mentioned above, two strains that shared many phenotypic characteristics with strain AP19, *S. cavoirensis* subsp. *washingtonensis* ATCC 27732T, and *S. cavorensis* subsp. *washingtonensis* ATCC 27732T, were separated from the nearest phylogenetic neighbours, *S. alboviridis* NRRL B-3633T, *S. microflavus* AS 4.1428T, *S. erumpens* NRRL B-3163T and *S. fulvoruber* NRRL B-24329T. Furthermore, distinct BOX-PCR fingerprinting patterns were found between *S. cavorensis* subsp. *washingtonensis* ATCC 27732T and *S. cavorensis* subsp. *washingtonensis* ATCC 27732T.

BOX-PCR fingerprinting has been reported as a powerful tool to distinguish finer taxonomic relationships in the genus *Streptomyces* (Lanoot et al., 2004). The BOX-PCR fingerprinting patterns of strain AP19 and the closely related strain, *S. cavoirensis* subsp. *washingtonensis* ATCC 27732T were very similar (see Supplementary Fig. S1 in IJSEM Online). These two strains formed a phylogenetic cluster, joining the phylogenetic clade comprising *S. griseus* AS 4.1419T, *S. griseus* ATCC 13273 and *S. griseus* subsp. *solvificiens* NRRL B-1561T. It was also evident that strain AP19 and *S. cavoirensis* subsp. *washingtonensis* ATCC 27732T were separated from the nearest phylogenetic neighbours, *S. alboviridis* NRRL B-3633T, *S. microflavus* AS 4.1428T, *S. erumpens* NRRL B-3163T and *S. fulvoruber* NRRL B-24329T. Furthermore, distinct BOX-PCR fingerprinting patterns were found between *S. cavorensis* ATCC 14889T and *S. cavorensis* subsp. *washingtonensis* ATCC 27732T. This was consistent with the relatively low levels of 16S rRNA gene sequence similarity (98.9%) between these strains and the fact that they belonged to different clusters in the phylogenetic tree based on 16S rRNA gene sequences. These data indicate that strains AP19 and *S. cavorensis* subsp. *washingtonensis* ATCC 27732T can be classified as *S. griseus*.

A DNA–DNA hybridization study provided further evidence for the taxonomic classification of strain AP19. High DNA–DNA relatedness values, in the range from 75.2 to 94.2%, were found between strain AP19, *S. cavorensis* subsp. *washingtonensis* ATCC 27732T.
subsp. washingtonensis ATCC 27732T and S. griseus ATCC 13273 (Table 1). The DNA–DNA relatedness value between S. cavourensis subsp. washingtonensis ATCC 27732T and S. cavourensis ATCC 14889T was 51.3 %, which is below the 70 % cut-off point for recognition of genomic species (Wayne et al., 1987). Furthermore, S. cavourensis subsp. washingtonensis ATCC 27732T could be distinguished from S. cavourensis ATCC 14889T by distinct BOX-PCR fingerprinting patterns, different cultural characteristics on standard media and a set of physiological features (Table 2). Phylogenetic trees based on 16S rRNA gene sequences and the 120 nt c-region of the 16S rRNA gene in which S. cavourensis subsp. washingtonensis ATCC 27732T and S. griseus ISP 5236T formed a clade, distinct from S. cavourensis ATCC 14889T, also supported this assignment (Figs 1, 2).

Based on the genotypic and phenotypic evidence, Streptomyces cavourensis subsp. washingtonensis ATCC 27732T should be considered as a later heterotypic synonym of Streptomyces griseus ISP 5236T and strain AP19 should also be assigned to this taxon.

Acknowledgements

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References


### Table 1. DNA–DNA reassociation values between strain AP19 and some related species of the genus Streptomyces

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hybridization (%) with labelled DNA from:</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1. AP19</td>
<td>100</td>
</tr>
<tr>
<td>2. S. griseus ATCC 13273</td>
<td>94.2</td>
</tr>
<tr>
<td>3. S. cavourensis subsp. washingtonensis ATCC 27732T</td>
<td>75.2</td>
</tr>
<tr>
<td>4. S. cavourensis ATCC 14889T</td>
<td>54.8</td>
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### Table 2. Phenotypic characteristics of strain AP19 and some related strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanin production</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Production of diffusible pigments</td>
<td>Greenish yellow</td>
<td>Greenish yellow</td>
<td>Yellow</td>
<td>Pale orange–yellow</td>
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<tr>
<td>Degradation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cellulose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Guanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Xanthine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth on sole carbon source (1.0 %, w/v):</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adonitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>Production of antibiotic</td>
<td>Chromomycin</td>
<td>Chromomycin</td>
<td>Chromomycin</td>
<td>Flavensomycin</td>
</tr>
</tbody>
</table>
Synonymy of Streptomyces griseus


