Streptomyces fenghuangensis sp. nov., isolated from seawater

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An actinomycete, designated strain GIMN4.003T, was isolated from seawater collected in Sanya, China. It produced white aerial mycelium and yellow substrate mycelium on Gause’s synthetic agar medium no. 1. The substrate mycelium colour was not sensitive to pH. Scanning electron microscopy observations revealed that GIMN4.003T produced straight to flexuous spore chains of rough to warty spores. L-L-Diaminopimelic acid was present in the cell-wall hydrolysate. Based on chemotaxonomy and morphological features, strain GIMN4.003T was identified as a member of the genus Streptomyces. Melanin was not produced. No antimicrobial activity was detected against Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Penicillium citrinum or Candida albicans. Analysis of the 16S rRNA gene sequence revealed that the highest sequence similarity was to Streptomyces radiopugnans R97T (99.0 %). However, DNA relatedness between GIMN4.003T and S. radiopugnans DSM 41901T was low (41.24 ± 1.47 %). Furthermore, the morphological, physiological and biochemical characteristics of strain GIMN4.003T were different from those of S. radiopugnans DSM 41901T and the type strains of other closely related Streptomyces species. On the basis of its physiological and molecular properties, it is evident that strain GIMN4.003T (= CCTCCM 208215T = NRRL B-24801T) represents the type strain of a novel species within the genus Streptomyces, for which the name Streptomyces fenghuangensis sp. nov. is proposed.

The marine biosphere is one of the richest habitats of micro-organisms. Marine microbes, particularly Streptomyces species, are considered important for their secondary metabolites and enzymes with novel properties (Sabu, 2003). Marine streptomycetes are widely recognized as a potential source of new drug candidates (Jensen et al., 2005). Members of the genus Streptomyces are known to produce a wide variety of bioactive compounds, antibiotics and enzymes (Koshy et al., 1997; Balagurunathan, 2004). The isolation and screening of novel streptomycetes as sources of novel natural products, bioactive compounds and therapeutic enzymes are therefore essential. Strain GIMN4.003T was isolated from seawater collected in Sanya, China, in August 2007. Seawater samples were collected from five stations at Sanya and were stored in sterile 1 l bottles. The samples were stored under environmental conditions and processed within 24 h in a laboratory; water samples (1 ml) were inoculated onto Gause’s synthetic agar medium no. 1 (Atlas, 1993), prepared with artificial seawater, and incubated for 5–7 days at 28 °C.

International Streptomyces Project (ISP) media were prepared according to the methods of Shirling & Gottlieb (1966). Morphological and physiological characteristics were determined as recommended by Williams et al. (1989). Morphological observations of spores and mycelium were conducted by light microscopy (Leica DM RAR) and scanning electron microscopy (Hitachi S-3000N). Physiological tests were carried out at 28 °C (unless otherwise indicated). D-Glucose, sucrose, D-fructose, D-xylene, L-arabinose, inositol, mannitol, L-rhamnose and raffinose were tested as sole carbon sources at 0.1 % (w/v); all carbon sources for carbon-utilization tests were filter-sterilized. Colour determination was referenced against the Methuen Handbook of Colour (Kornerup & Wanscher, 1978).

†These authors contributed equally to this work.

Abbreviations: DAP, diaminopimelic acid; ISP, International Streptomyces Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GIMN4.003T is GU356598.
Analysis of the diaminopimelic acid (DAP) isomer and the whole-cell sugar composition followed the procedure described by Hasegawa et al. (1983) with the exception that dried cells were used instead of colonies from agar plates. Fully hydrolysed cell-wall material was prepared from approximately 10 mg (dry weight) cells by the hot trichloroacetic acid trypsin rapid-screening method of Schleifer & Kandler (1972). Fatty acid methyl esters were prepared by the trimethyl sulphonium hydroxide method (Butte, 1983) and analysed by GC (6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Menaquinones were extracted according to the method of Collins et al. (1977) and analysed by HPLC (Kroppenstedt, 1985). The genomic DNA base composition of strain GIMN4.003T was determined in 0.1 x SSC by the method of Mandel & Marmur (1968). Genomic DNA was extracted (Cui et al., 2001) and the 16S rRNA gene was amplified by PCR using universal bacterial 16S rRNA gene primers. The forward primer F27 (5’-AGAGTTTGATCCTGGCTCAG-3’) and reverse primer 1522R (5’-AAGGAGGATCCAGCCGCA-3’) were adapted from primers pA and pH of Edwards et al. (1989). The 16S rRNA gene was sequenced with an automated capillary DNA sequencing system (ABI 3730) and ABI Big Dye Terminator cycle sequencing kit. DNA relatedness studies were done by the method described by De Ley et al. (1970).

Good growth was observed on yeast extract/malt extract agar (ISP 2), Gause’s synthetic agar medium no. 1, glucose-asparagine agar (Waksman, 1961) and Czapek’s solution agar (Waksman, 1961). Moderate growth was observed on oatmeal agar (ISP 3), glycerol-asparagine agar (ISP 5) and starch agar (Waksman, 1961). Poor growth was observed on inorganic salts/starch agar (ISP 4). Diffusible pigments were produced only on ISP 5 medium and Gause’s synthetic agar medium no. 1 (Table 1).

Morphological features were observed on Gause’s synthetic agar medium no. 1 (Xiao et al., 2009) and ISP 2. Cultures were incubated for 2 weeks at 28 °C. Strain GIMN4.003T had characteristics typical of the genus Streptomyces. Microscope studies revealed a straight to flexuous mycelium without verticils. The aerial mycelium produced straight to flexuous spore chains. Spores were rough to warty (Fig. 1). The colour of the spore mass was grey, and the strain produced white aerial mycelium and yellow to yellowish-white vegetative mycelium. Melanin was not produced on tyrosine agar (ISP 7).

Chemotaxonomic tests showed that the cell wall contained L-DAP, indicating that it was of cell-wall type I (Lechevalier & Lechevalier, 1970). Whole-cell hydrolysates contained glucose. The predominant menaquinones were MK-10(H2) (63.22 %), MK-9(H6) (33.23 %), MK-10(H4) (2.16 %) and MK-9(H6) (1.01 %). Fatty acid analysis showed that strain GIMN4.003T contained straight-chain and iso- and anteiso-branched fatty acids; the major cellular fatty acids (≥4 %) were iso-C16:0 (39.30 %), anteiso-C15:0 (24.20 %), iso-C14:0 (12.10 %), iso-C16:1 H (6.21 %), anteiso-C17:0 (5.43 %) and iso-C15:0 (4.63 %). The G+C content of the genomic DNA was 72.94 mol%.

The morphological and physiological characteristics of strain GIMN4.003T, for example its cell-wall type, whole-cell sugar pattern, fatty acid profile and menaquinones, were in line with its assignment to the genus Streptomyces (Williams et al., 1989; Manfio et al., 1995).

A partial 16S rRNA gene sequence (1427 nt) was determined for strain GIMN4.003T. Sequence comparison with representatives of the family Streptomycetaceae confirmed that strain GIMN4.003T is closely related to members of the genus Streptomyces (BLAST analysis; Altschul et al., 1997). It was 97.0–99.67 % similar to the 16S rRNA gene sequences of Streptomyces species. High 16S rRNA gene sequence similarity was found with Streptomyces radiopugnans R97T (GenBank accession no. DQ912930; 99.0 %). Streptomyces macrosporus DSM 41449T (AB184616; 98.1 % similarity over 1399 bases) and Streptomyces megarotans NBRC 14749T (AB184617; 97.0 % similarity over 1394 bases) showed lower similarity.

### Table 1. Cultural characteristics of strain GIMN4.003T on various media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth and sporulation</th>
<th>Colour of:</th>
<th>Production of diffusible pigments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aerial mycelium</td>
<td>Substrate mycelium</td>
</tr>
<tr>
<td>ISP 2</td>
<td>Good</td>
<td>White</td>
<td>Yellowish white</td>
</tr>
<tr>
<td>ISP 3</td>
<td>Moderate</td>
<td>White</td>
<td>Grey</td>
</tr>
<tr>
<td>ISP 4</td>
<td>Poor</td>
<td>None</td>
<td>Greyish white</td>
</tr>
<tr>
<td>ISP 5</td>
<td>Moderate</td>
<td>Greyish white</td>
<td>Yellowish white</td>
</tr>
<tr>
<td>Glucose-asparagine agar</td>
<td>Good</td>
<td>White</td>
<td>Yellow</td>
</tr>
<tr>
<td>Gause’s synthetic agar medium no. 1</td>
<td>Good</td>
<td>White</td>
<td>Yellow</td>
</tr>
<tr>
<td>Czapek’s agar</td>
<td>Good</td>
<td>Yellowish white</td>
<td>White</td>
</tr>
<tr>
<td>Starch agar</td>
<td>Moderate</td>
<td>Orange–yellow</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

ISP media (Shirling & Gottlieb, 1966) are described in the text. The colour of mycelium was compared according to Kornerup & Wanscher (1978). The strain gave the same result for growth and for sporulation on all tested media.
**Streptomyces fenghuangensis** sp. nov.

Streptomyces fenghuangensis (feng.hu.ang.en'sis. N.L. masc. adj. *fenghuangensis* pertaining to Fenghuang, where the seawater was collected from which the type strain was isolated).

Aerobic, Gram-stain-positive, catalase-positive actinomycete that forms an extensively branched substrate mycelium which carries aerial hyphae that differentiate into straight to flexuous chains of spores with rough to warty surfaces. Milk coagulation, milk peptonization and starch hydrolysis are negative. Good growth on yeast extract/malt extract agar (ISP 2), and pale-yellow aerial spore mass on glycerol-asparagine agar (ISP 5). Substrate mycelium was grey to greyish-white on Gause's synthetic agar medium no. 1 and ISP 2, ISP 3, ISP 4, ISP 5 (Table 2).

The genotypic and phenotypic data presented clearly demonstrate that strain GIMN4.003T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces fenghuangensis* sp. nov. is proposed.

### Description of *Streptomyces fenghuangensis* sp. nov.

*Streptomyces fenghuangensis* sp. nov. is proposed.

It is evident from the phylogenetic analysis (Fig. 2) that strain GIMN4.003T forms a distinct phyletic line together with *S. radiopugnans* R97T, a relationship that is supported by both of the other tree-making algorithms employed and by a 91% bootstrap value in the neighbour-joining analysis. Despite the higher 16S rRNA gene sequence similarity between GIMN4.003T and *S. radiopugnans* R97T, DNA relatedness between strain GIMN4.003T and *S. radiopugnans* DSM 41901T was found to be 41.24 ± 1.47%, a value far below the 70% cut-off point recommended for the delineation of genomic species (Wayne et al., 1987). DNA–DNA hybridization studies therefore confirmed that strain GIMN4.003T represents a novel species within the genus *Streptomyces*. The result is in agreement with the results of the 16S rRNA gene sequence analyses.

Furthermore, *S. radiopugnans* DSM 41901T also differs from the new isolate in that it lacked characteristic sugars, and formed white to pale-grey aerial spore mass on Gause's synthetic agar medium no. 1 and yeast extract-malt extract (ISP 2) and pale-yellow aerial spore mass on glycerol-asparagine agar (ISP 5). Substrate mycelium was grey to greyish-white on Gause's synthetic agar medium no. 1 and ISP 2, ISP 3, ISP 4, ISP 5 (Table 2).

The genotypic and phenotypic data presented clearly demonstrate that strain GIMN4.003T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces fenghuangensis* sp. nov. is proposed.

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**Phenotypic properties of strain GIMN4.003T**

Phenotypic properties of strain GIMN4.003T were compared with those of its closest phylogenetic neighbours (Table 2) by using procedures described above. The results showed that GIMN4.003T has some characteristics that differed from those of its closest relatives; *S. radiopugnans* DSM 41901T, *S. macrosporus* NBRC 14748T and *S. megasporus* NBRC 14749T differed from strain GIMN4.003T in that they produced spiral spore chains and that diffusible pigments were not formed on any of the tested media (Table 2).

**Fig. 1.** Scanning electron micrographs of strain GIMN4.003T showing straight to flexuous (*rectiflexibles*) spore chains of rough to warty spores after growth on yeast extract/malt extract agar (ISP 2) for 14 days at 28 °C. Bars, 5 μm (a) and 2 μm (b).
Fig. 2. Unrooted neighbour-joining tree constructed from 16S rRNA gene sequences, showing phylogenetic relationships between strain GIMN4.003T and related *Streptomyces* species belonging to the major, minor and single-member clusters defined by Williams et al. (1983). *Actinomadura glomerata* IMSNU 22179T was used as the outgroup. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are given at nodes. The minimum evolution and maximum-parsimony methods were also used for tree reconstruction. Branches marked with an asterisk were conserved in all methods used. Bar, 0.01 % sequence divergence.

Table 2. Phenotypic properties of strain GIMN4.003T and its phylogenetic neighbours

Data were obtained in this study under the same conditions. +, Positive; w, weakly positive; −, negative; ai, anteiso-branched; i, iso-branched.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain GIMN4.003T</th>
<th><em>S. radiopugnans</em> DSM 41901T</th>
<th><em>S. macrosorbus</em> NBRC 141748T</th>
<th><em>S. megasporus</em> NBRC 141749T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of spore mass/substrate mycelium on:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISP 2</td>
<td>Grey/yellowish white</td>
<td>White/greyish white</td>
<td>Light grey/greyish grey</td>
<td>None/yellowish grey</td>
</tr>
<tr>
<td>ISP 3</td>
<td>Pinkish white/grey</td>
<td>None/greyish white</td>
<td>Olive/white</td>
<td>None/greyish white</td>
</tr>
<tr>
<td>ISP 4</td>
<td>Pale yellow/greyish white</td>
<td>None/grey</td>
<td>None/greyish white</td>
<td>None/white</td>
</tr>
<tr>
<td>ISP 5</td>
<td>Greyish white/yellowish white</td>
<td>Pale yellow/greyish white</td>
<td>White/white</td>
<td>Pale white/pale yellow</td>
</tr>
<tr>
<td>Spore-surface ornamentation</td>
<td>Rough to warty</td>
<td>Rough to warty</td>
<td>Warty</td>
<td>Warty</td>
</tr>
<tr>
<td>Spore-chain morphology</td>
<td>Straight to flexuous</td>
<td>Spiral</td>
<td>Spiral</td>
<td>Spiral</td>
</tr>
<tr>
<td>Colour of diffusible pigment on ISP 5</td>
<td>Blue</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Growth on sole carbon sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Raffinose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Inositol</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Major cellular fatty acids (&gt;4 %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i-C16:0</td>
<td>(39.3 %), ai-C15:0</td>
<td>i-C16:0 (35.0 %), ai-C15:0</td>
<td>i-C16:0 (38.8 %), ai-C15:0</td>
<td>i-C16:0 (42.5 %), ai-C15:0</td>
</tr>
<tr>
<td>i-C15:0</td>
<td>(24.2 %), i-C14:0</td>
<td>(15.6 %), ai-C15:0</td>
<td>(16.0 %), i-C16:1 H</td>
<td>(12.5 %), i-C16:1 H</td>
</tr>
<tr>
<td>i-C16:1 H</td>
<td>(6.2 %), ai-C17:0</td>
<td>(13.5 %), i-C15:0</td>
<td>(11.6 %), i-c14:0</td>
<td>(10.0 %), i-C15:0</td>
</tr>
<tr>
<td>i-C15:0</td>
<td>(5.4 %), i-C15:0</td>
<td>(9.9 %), i-C15:0</td>
<td>(8.9 %), ai-C17:0</td>
<td>(10.0 %), i-C14:0</td>
</tr>
<tr>
<td>Major cellular fatty acids (&gt;4 %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i-C16:0</td>
<td>(38.8 %), ai-C15:0</td>
<td>i-C16:0 (38.8 %), ai-C15:0</td>
<td>i-C16:0 (42.5 %), ai-C15:0</td>
<td>i-C16:0 (42.5 %), ai-C15:0</td>
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</tr>
<tr>
<td>i-C16:1 H</td>
<td>(6.2 %), ai-C17:0</td>
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</tr>
<tr>
<td>i-C15:0</td>
<td>(5.4 %), i-C15:0</td>
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<td>(8.9 %), ai-C17:0</td>
<td>(10.0 %), i-C14:0</td>
</tr>
</tbody>
</table>
hydrolysate. The predominant menaquinones are MK-10(H2), MK-9(H4), MK-10(H4) and MK-9(H6). The type strain contains straight-chain and iso- and anteiso-branched fatty acids and a proportion of unsaturated fatty acids. The major cellular fatty acids (>/4 %) are iso-C16:0, anteiso-C15:0, iso-C14:0, iso-C16:1, anteiso-C17:0 and iso-C15:0. No antimicrobial activity is detected against Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 6538, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231 or Penicillium citrinum AS 3.2788. The DNA G+C content of the type strain is 72.94 mol%.

The type strain, GIMN4.003^T (=CCTCCM 208215^T =NRRL B-24801^T), was isolated from seawater.

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References


