Streptomyces indicus sp. nov., an actinomycete isolated from deep-sea sediment

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The taxonomic position of an actinomycete isolated from deep-sea sediment from the Indian Ocean was determined by using a polyphasic approach. The presence of iso-C15:0, anteiso-C15:0, iso-C16:0, iso-C17:0 and anteiso-C17:0 as the major cellular fatty acids, LL-diaminopimelic acid as the characteristic diamino acid, and MK-9(H6, H8 and H4) as the major menaquinones supported the affiliation of strain IH32-1T to the genus Streptomyces. Comparison of 16S rRNA gene sequences showed that strain IH32-1T exhibited highest similarities to the type strains of Streptomyces globosus (97.6 %) and Streptomyces toxytricini (97.6 %). However, DNA–DNA relatedness values between strain IH32-1T and the type strains of S. globosus and S. toxytricini were determined as 55.2 ± 4.7 and 38.3 ± 2.5 %, respectively. Based on its chemotaxonomic, phenotypic and genotypic characteristics, strain IH32-1T is considered to represent a novel species in the genus Streptomyces, for which the name Streptomyces indicus sp. nov. is proposed. The type strain is IH32-1T (=DSM 42001T=CGMCC 4.5727T).

The genus Streptomyces (Waksman & Henrici, 1943) is a rich source of novel bioactive, commercially significant compounds (Watte et al., 2001). Apart from terrestrial habitats, large populations of actinomycetes have been shown to exist in the marine environment (Mincer et al., 2002; Jensen et al., 2005; Maldonado et al., 2005; Gontang et al., 2007). Even though the abundance and diversity of actinomycetes decrease with increasing depth (Stach et al., 2003), the genus Streptomyces is believed to be the dominant population in the sediment community.

The genus Streptomyces currently encompasses nearly 600 species with validly published names and it has been pointed out that the boundary for species delineation in this genus seems to be >97 % 16S rRNA gene sequence similarity (Kämpfer & Labeda, 2006). High 16S rRNA gene sequence similarities between members are a major problem when describing a novel species within the genus Streptomyces. Judging whether a strain represents a novel species and what its closest relatives are should be decided on the basis of individual sequence similarities, rather than the position of its sequence in a 16S rRNA gene phylogenetic tree (Kämpfer et al., 2008).

During a screen for novel bioactive metabolites from actinobacteria from deep-sea sediment, strain IH32-1T was isolated from a deep-sea (2434 m) sediment sample collected from the Indian Ocean (25.3206° S, 70.0402° E) using modified HV medium (humic acid 1.0 g, KCl 1.7 g, FeSO4, 7H2O 0.01 g, Na2HPO4 0.5 g, MgSO4, 7H2O 0.5 g, CaCO3 0.02 g, thiamine 0.5 mg, nicotinic acid 0.5 mg, pantothenic acid 0.5 mg, p-aminobenzoic acid 0.5 mg, riboflavin 0.5 mg, vitamin B6 0.5 mg, inositol 0.5 mg, biotin 0.25 mg, water 250 ml, seawater 750 ml, agar 18 g, pH 7.2) and incubated at 25 °C. Morphological observations of spores and mycelia on Gause’s synthetic agar (Atlas, 1993) at 28 °C for 12 days were made by light microscopy (Olympus microscope CX21) and scanning electron microscopy (Philips-Fei, model XL30 ESEM-TMP). Cultural characteristics were determined after 7–20 days incubation at 28 °C by methods used in the International Streptomyces...
Project (ISP) (Shirling & Gottlieb, 1966). Colours were assessed according to colour chips in the ISCC-NBS Color Charts Standard no. 2106 (Kelly, 1964). Biomass for chemical and molecular analyses was prepared by culturing in liquid ISP2 broth for 3–5 days at 28°C in a rotary shaker (180 r.p.m.) and then harvesting by centrifugation at 1000 g for 10 min.

The isolate grew well on ISP2 and ISP4 agar media, but moderately on other media. The colour of aerial mycelium was white, whilst the colour of substrate mycelia was medium-dependent (Supplementary Table S1, available in IJSEM Online). At maturity, the aerial mycelium developed well on Gause’s synthetic agar. The spore chains were long, straight to flexuous chains, but formed a compact coil at the end. The spores were elliptical and non-motile with smooth surfaces (Supplementary Fig. S1).

Amplification of the 16S rRNA gene sequence was performed as described by Cui et al. (2001). The almost-complete 16S rRNA gene sequence (1488 nt) of strain IH32-1T was obtained and submitted for BLAST analysis against the GenBank and EzTaxon databases (Altschul et al., 1997; Chun et al., 2007). The 16S rRNA gene sequences of representative related actinomycetes were aligned by using CLUSTAL_X (Thompson et al., 1997) and phylogenetic trees were constructed by the neighbour-joining (NJ) and maximum-parsimony (MP) methods using the MEGA 3.1 software (Kumar et al., 2004; Saitou & Nei, 1987). The topology of the NJ tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 replications. The highest 16S rRNA gene sequence similarities between the isolate and documented strains in the databases were 97.6 % (35 nucleotide differences out of 1462) to Streptomyces globosus LMG 19896T (=CGMCC 4.1969T) and 97.6 % (35 nucleotide differences out of 1459) to Streptomyces toxytricini NBRC 12823T (=CGMCC 4.1734T), whilst sequence similarities to all other strains were <97.5 %. The NJ and MP trees (Fig. 1) showed the close phylogenetic association of strain IH32-1T with certain other Streptomyces species and indicated a unique position in the 16S rRNA gene-based phylogenetic tree. Because of the low 16S rRNA gene sequence similarity of strain IH32-1T to other Streptomyces species, only S. globosus CGMCC 4.1969T and S. toxytricini CGMCC 4.1734T were included in a comparative study.

Carbohydrate utilization was carried out according to the method of Kämpfer et al. (1991) and the basal medium was supplemented with 0.02 % yeast extract. Gelatin liquefaction, starch hydrolysis, milk coagulation and peptonization, melanin pigment production, nitrate reduction and H2S production were tested using the methods of Goodfellow (1971) and Gordon et al. (1974). The temperature range for growth was determined on modified Bennett agar (Atlas, 1993) at 4, 10, 15, 20, 28, 37 and 45°C for 1–2 weeks.

Freeze-dried cells were hydrolysed with 6 M HCl (Lechevalier & Lechevalier, 1980) and 0.5 M HCl...
Table 1. Phenotypic properties that distinguish strain IH32-1T from related *Streptomyces* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Production of diffusible pigment on ISP2</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aerial mycelium colour on:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast extract agar (ISP2)</td>
<td>White</td>
<td>Moderate brown</td>
<td>Absent</td>
</tr>
<tr>
<td>Oatmeal agar (ISP3)</td>
<td>White</td>
<td>Light brown</td>
<td>Light reddish brown</td>
</tr>
<tr>
<td>Inorganic salts–starch agar (ISP4)</td>
<td>White</td>
<td>Dark yellowish pink</td>
<td>Greyish reddish orange</td>
</tr>
<tr>
<td>Czapek’s agar</td>
<td>White</td>
<td>Pale yellowish pink</td>
<td>Pale yellowish pink</td>
</tr>
<tr>
<td>H2S production</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>w</td>
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<tr>
<td>Gelatin liquefaction</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>D-Xylose</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>−/w</td>
<td>−</td>
</tr>
<tr>
<td>Inositol</td>
<td>−/w</td>
<td>+/w</td>
<td>+</td>
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<tr>
<td>Raffinose</td>
<td>+/w</td>
<td>+/w</td>
<td>−</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+/w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>−/w</td>
<td>+</td>
<td>−/w</td>
</tr>
<tr>
<td>Diagnostic sugars*</td>
<td>rib, glu, gal</td>
<td>rha, rib, glu, gal</td>
<td>rha, rib, glu, gal</td>
</tr>
</tbody>
</table>

*gal, Galactose; glu, glucose; rha, rhamnose; rib, ribose.
species and can be distinguished phenotypically from its closest relatives, *S. globosus* and *S. toxytricini* (Table 1). On medium ISP3, the aerial mycelium colour of strain IH32-1T is white, while the colour of the aerial mycelia of *S. globosus* and *S. toxytricini* is brown. The aerial mycelium and substrate mycelium colour are also different on ISP2, ISP4, potato agar, nutrient agar and Czapek's agar. Nitrate reduction and gelatin liquefaction by strain IH32-1T were positive, whereas the corresponding reactions for *S. globosus* and *S. toxytricini* were weak or negative. Based on the above differences and low DNA–DNA relatedness values, it can be concluded that strain IH32-1T represents a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces indicus* sp. nov.

**Description of Streptomyces indicus** sp. nov.

*Streptomyces indicus* (in’di. cus. L. masc. adj. indicus of India, Indian, referring to the Indian Ocean, from where the strain was isolated).

Aerobic, Gram-positive actinomycetes with branching aerial mycelium and substrate mycelium. Non-motile, ellipsoidal spores with smooth surfaces are borne in straight to flexuous chains (compact coil at the ends). On most test media, aerial mycelium colour is white and no diffusible pigments are produced. Grows well at 20, 28 and 37 °C, but does not grow at 42 or 10 °C. D-Galactose, glucose, L-arabinose, maltose, sucrose, trehalose, D-xylose and fructose can be utilized as sole carbon sources. Tests for gelatin liquefaction, nitrate reduction and milk coagulation and peptonization are positive and tests for H$_2$S production and melanin production are negative. Optimum NaCl concentration for growth is 3 % (w/v); optimum pH for growth is 7.2. Cell wall contains LL-DAP. Whole-cell hydrolysates contain ribose, galactose and glucose. Major menaquinones are MK-9(H$_4$), MK-9(H$_6$) and MK-9(H$_8$); minor amounts of MK-9(H$_2$) are also present. Predominant phospholipids are PE and DPG. Major fatty acid components are iso-C$_{15:0}$ anteiso-C$_{15:0}$ iso-C$_{16:0}$ iso-C$_{17:0}$ and anteiso-C$_{17:0}$. The G+C content is 71.3 ± 1.0 mol%.

The type strain, IH32-1T (=DSM 42001$^T$=CGMCC 4.5727$^T$), was isolated from deep-sea sediment from the Indian Ocean.

**Acknowledgements**

Jun Xu is indebted to the China Ocean Mineral Resources Research and Development Association fund (DYXM-115-02-2-B), a grant from the State Key Laboratory of Ocean Engineering (GKZD10045) and the National High Technology R&D Program of China (2007AA091904).

**References**


