Streptomyces sedi sp. nov., isolated from surface-sterilized roots of Sedum sp.

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An endophytic actinomycete, strain YIM 65188T, was isolated from surface-sterilized tissue of Sedum sp. collected from Yunnan province, south-west China, and characterized by using a polyphasic approach. Analysis of the 16S rRNA gene sequence showed that strain YIM 65188T is a member of the genus Streptomyces and exhibited 97.5 and 96.3 % gene sequence similarities to Streptomyces specialis GW41-1564T and Streptomyces hainanensis YIM 47672T, respectively, whereas low sequence similarity values (<95.2 %) distinguished strain YIM 65188T from all other Streptomyces species with validly published names. Strain YIM 65188T had a unique menaquinone composition, with the predominant quinones being MK-11(H6) and MK-10(H6), with moderate amounts of MK-10(H8), MK-11(H8), MK-9(H8) and MK-10(H4). Similarly, the closest phylogenetic relative, S. specialis GW41-1564T, also had an unusual quinone composition, with the predominant menaquinones MK-10(H2) and MK-10(H4) and minor amounts of MK-9(H4) and MK-9(H6). The DNA–DNA hybridization value between strain YIM 65188T and S. specialis GW41-1564T was 42.7 %. On the basis of phenotypic and phylogenetic characteristics, strain YIM 65188T was identified as a novel species of the genus Streptomyces for which the name Streptomyces sedi sp. nov. is proposed, with YIM 65188T (=CCTCC AA 208020T =DSM 41942T) as the type strain.

The genus Streptomyces was proposed by Waksman & Henrici (1943) and currently encompasses more than 500 species with validly published names. It has been proposed that the genus is underspeciated (Sembiring et al., 2000; Kim & Goodfellow, 2002) and that the description of novel Streptomyces species needs to be based on a combination of genotypic and phenotypic data (Manfio et al., 1995, 2003; Atalan et al., 2000; Li et al., 2002).

Streptomycetes are still a rich source of useful compounds, notably antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents (Berdy, 2005). During a search for novel endophytic microbes, strain YIM 65188T was isolated from surface-sterilized plant tissues. The objective of the present study was to determine the taxonomic position of strain YIM 65188T.

Strain YIM 65188T was isolated from the surface-sterilized tissue of Sedum sp., which was collected from Yunnan province, south-west China. In the open, the plant was tagged and stored in a clean plastic bag until used (approx. 24 h). The plant sample was then washed thoroughly in running water to remove all soil. A sonication step, carried out at 160 W with an exposure time of 15 min, was employed to dislodge any soil and organic matter from the sample surface. The plant sample was then cut up with a sterilized knife. The tissue segments were surface-sterilized according to the method of Coombs & Franco (2003). The surface-sterilization process was verified by aseptic rolling of the surface-sterilized plant material on isolation medium and tryptic soy agar (Difco) and then incubating the plates at 28 °C for 7 days. The surface-sterilized tissues were then blended aseptically in a commercial blender and plated on sodium propionate agar (1:1: 1 g sodium propionate, 0.2 g L-asparagine, 0.9 g KH2PO4, 0.6 g K2HPO4, 0.1 g MgSO4.7H2O, 0.02 g CaCl2.2H2O, 18 g agar; pH 7.2) followed by incubation at 28 °C for up to 4 weeks. The purified strain was cultured routinely on YIM 38 medium [1:1: 4 g malt extract, 4 g yeast extract, 4 g glucose, vitamin mixture (0.5 mg each of thiamine–HCL, riboflavin, niacin, pyridoxine–HCL, inositol, calcium

Abbreviation: ISP, International Streptomyces Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 65188T is EU925562.
pantothenate and \( p \)-aminobenzoic acid, plus 0.25 mg biotin), 20 g agar; pH 7.2] at 28 °C and stored as a glycerol suspension (20%, v/v) at −70 °C.

Cultural characteristics were determined after 2 weeks incubation at 28 °C, according to the methods of the International Streptomyces Project (ISP; Shirling & Gottlieb, 1966). Czapek's agar and nutrient agar were prepared as described by Dong & Cai (2001). Colour determination was performed by using colour chips from the ISCC–NBS colour charts (standard samples, no. 2106; Kelly, 1964). After incubation on YIM 38 medium at 28 °C for 14 days, morphological properties were examined by using a light microscope (BH-2; Olympus) and a scanning electron microscope (Philips XL30; ESEM-TMP).

Carbon-source utilization was determined according to the methods of Shirling & Gottlieb (1966) and Locci (1989). Acid production from carbohydrates was assessed as described by Gordon et al. (1974). Growth at various temperatures, pH values and NaCl concentrations was examined according to Xu et al. (2005), using YIM 38 medium as the basal medium. Oxidase activity was determined from the oxidation of tetramethyl-\( \beta \)-phenyle-nediamine. Catalase activity was determined with 3% \( \text{H}_2\text{O}_2 \) according to standard methods. Other phenotypic characteristics were tested by using standard procedures (Goodfellow, 1971; Williams et al., 1983).

Strain YIM 65188\(^{T} \) grew well on nutrient agar, with moderate growth on yeast extract–malt extract agar (ISP 2), inorganic salts–starch agar (ISP 4) and Czapek's agar, and poor growth on oatmeal agar (ISP 3) and glycerol–asparagine agar (ISP 5). White aerial mycelia formed more slowly on ISP 2, ISP 4 and Czapek's agar than on nutrient agar. Aerial mycelia were not formed on ISP 3 or ISP 5 media. The vegetative mycelia were yellow–white in colour on ISP 3, ISP 4, ISP 5 and Czapek's agar, light grey–yellow on ISP 2 agar medium, and pale orange–yellow on nutrient agar. Soluble pigments were not produced on any of the media tested. Spore chains were of the Spirales type, spores were elliptical or short rods and the spore surface was smooth (Fig. 1). Detailed physiological results are given in Table 1 and in the species description.

For chemotaxonomic analysis, freeze-dried cells were obtained from cultures grown in YIM 38 broth for 1 week at 28 °C. The isomer of diaminopimelic acid and sugars of whole-cell hydrolysates were determined by using TLC as described by Staneck & Roberts (1974). Phospholipids were identified according to published procedures (Minnikin et al., 1979; Collins & Jones, 1980). Menaquinones were extracted (Collins et al., 1977) and separated by HPLC (Tamaoka et al., 1983). The G+C contents of the genomic DNAs were determined by the HPLC method according to Mesbah et al. (1989).

Strain YIM 65188\(^{T} \) contained \( LL \)-diaminopimelic acid as the diamino acid. Whole-cell hydrolysates contained glucose, galactose and madurose. Diphosphatidylglycerol was the major phospholipid, with moderate amounts of phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol and minor to trace amounts of two phosphatidylinositol mannosides and four unknown phospholipids. According to Kim et al. (2003), the predominant menaquinones of members of the genus *Streptomyces* are MK-9(30H) and MK-9(3-C). Strain YIM 65188\(^{T} \) has an unusual quinone composition, with the predominant menaquinones being MK-11(30H), MK-10(30H), MK-10(30H), MK-11(30H), MK-9(30H), MK-12(30H), MK-

**Table 1. Physiological characteristics that differentiate strain YIM 65188\(^{T} \) from its closest phylogenetic neighbours, *S. specialis* GW41-1564\(^{T} \) and *S. hainanensis* YIM 47672\(^{T} \)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<tr>
<td><strong>Starch hydrolysis</strong></td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td><strong>Utilization as sole carbon source:</strong></td>
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</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>Aesculin</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>D-Fructose</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Inositol</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Maltose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Trehalose</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td><strong>Utilization as sole nitrogen source:</strong></td>
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<tr>
<td>Adenine</td>
<td>−</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>L-Asparagine</td>
<td>−</td>
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<tr>
<td>L-Cysteine</td>
<td>+</td>
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<tr>
<td>L-Histidine</td>
<td>+</td>
<td>−</td>
<td>ND</td>
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<tr>
<td>Hypoxanthine</td>
<td>−</td>
<td>+</td>
<td>ND</td>
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<tr>
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<td>−</td>
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<tr>
<td>Xanthine</td>
<td>−</td>
<td>+</td>
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</table>

**Fig. 1.** Scanning electron micrograph of spore chains of strain YIM 65188\(^{T} \) after growth on YIM 38 medium at 28 °C for 14 days. Bar, 5 \( \mu \text{m} \).
11(H$_4$), MK-9(H$_6$) and MK-10(H$_4$) in the ratio 27:23:10:9:6:5:4:4. Minor amounts of MK-12(H$_6$) were detected. In order to verify the result, analysis of an independently grown biomass of YIM 65188$^T$ was carried out. A similar quinone profile was observed, which was composed of the compounds MK-11(H$_6$), MK-10(H$_6$), MK-10(H$_4$), MK-11(H$_4$), MK-11(H$_6$), MK-10(H$_6$), MK-9(H$_8$) and MK-9(H$_6$) in the ratio 27:24:11:9:8:7:7:7. The quinone system of *Streptomyces specialis* GW41-1564$^T$ was investigated simultaneously, and was found to consist of MK-10(H$_4$), MK-10(H$_6$), MK-9(H$_6$), MK-9(H$_4$) and MK-10(H$_2$) in the ratio 40:30:18:8:3. This result is consistent with the description by Kämpfer et al. (2008). *Streptomyces hainanensis* YIM 47672$^T$ has also been reported to contain an unusual quinone system, composed of MK-9(H$_2$), MK-10(H$_6$), MK-9(H$_6$) and MK-9(H$_8$) in the ratio 45:27:14:14 (Jiang et al., 2007). The surprising quinone system with predominant MK-10 and MK-11 derivatives in strain YIM 65188$^T$ is significantly different from those of *S. specialis* GW41-1564$^T$ and *S. hainanensis* YIM 47672$^T$, which are also unique among streptomycetes so far. The G+C content of the genomic DNA from strain YIM 65188$^T$ was 71.0 mol%.

An almost-complete 16S rRNA gene sequence of strain YIM 65188$^T$, comprising 1440 bp, was amplified by PCR and sequenced as described previously (Li et al., 2007). The resultant 16S rRNA gene sequence was aligned with corresponding sequences of representatives of the genus *Streptomyces* (retrieved from GenBank) by using CLUSTAL_X (Thompson et al., 1997). A phylogenetic tree was constructed by using the neighbour-joining tree-making algorithm (Saitou & Nei, 1987) from MEGA version 4.0 (Tamura et al., 2007). The PHYLIP version 3.6 software package was used to construct a maximum-likelihood tree (Felsenstein, 1981). The topologies of the resultant trees were evaluated by using bootstrap analysis (Felsenstein, 1985), based on 1000 resampled datasets.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 65188$^T$ belongs to the genus *Streptomyces* and, with both treeing methods, it formed a distinct clade with *S. specialis* GW41-1564$^T$ and *S. hainanensis* YIM 47672$^T$ (Fig. 2). Strain YIM 65188$^T$ showed 97.5 and 96.3% gene sequence similarities to *S. specialis* GW41-1564$^T$ and *S. hainanensis* YIM 47672$^T$, respectively. All other representatives of the genus *Streptomyces* exhibited lower sequence similarities to strain YIM 65188$^T$ (<95.2%). Genomic relatedness between strains YIM 65188$^T$ and *S. specialis* GW41-1564$^T$ was determined according to the DNA–DNA reassociation method described by He et al. (2005). The experiment was performed with three replicates. The low level of DNA–DNA relatedness (mean ± SD, 24.7 ± 1.9%) supported the hypothesis that these two strains belong to different genomic species.

Besides the genotypic evidence, strain YIM 65188$^T$ can also be distinguished from its closest relatives by additional phenotypic characteristics. *S. specialis* GW41-1564$^T$ produces a black soluble pigment on all media tested except for nutrient agar, whereas *S. hainanensis* YIM 47672$^T$ produces a light-brown to orange–yellow soluble pigment.

![Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain YIM 65188$^T$ and related and representative species of the genus *Streptomyces*. Bootstrap values (expressed as percentages of 1000 replications) >50% are given at nodes. Asterisks indicate clades that were conserved when the maximum-likelihood method was used to construct the phylogenetic tree.](image-url)
on ISP 2, ISP 3, ISP 4 and Czapek’s agar (Jiang et al., 2007) and YIM 65188T does not produce soluble pigments on any of the media tested. Furthermore, as shown in Table 1, many differences between strain YIM 65188T, S. specialis GW41-1564T and S. hainanensis YIM 47672T were detected during determination of carbon- and nitrogen-source utilization. On the basis of the data shown in the present study, we propose that strain YIM 65188T represents a novel species of the genus Streptomyces, for which the name Streptomyces sedi sp. nov. is proposed.

Description of Streptomyces sedi sp. nov.

Streptomyces sedi (se’di. N.L. gen. n. sedi of Sedum, the plant genus from which this species was isolated).

Forms extensively branched substrate mycelia; aerial mycelia differentiate into spiral spore chains. Spores are elliptical or short rods. Spore surface is smooth. Aerial mycelia are white; vegetative mycelia are yellow–white to light grey–yellow. No soluble pigment is produced. Growth occurs at 15–37 °C and pH 7.0–8.0. The NaCl tolerance range is up to 5 %. Catalase is produced. Negative for Voges–Proskauer and methyl red tests, oxidase reaction, production of H2S, nitrate reduction and milk coagulation and peptonization. Urea and Tweens 20, 40 and 80 are hydrolysed, but cellulose, gelatin and starch are not. Utilizes cellobiose, aesculin, D-fructose and maltose as sole carbon sources. Acid is produced from aesculin and maltose. D-Arabinose, dulcitol, erythritol, D-galactose, inositol, D-lactose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-ribose, sodium malate, D-sorbitol, L-sorbose, trehalose, xylitol and D-xyllose are not utilized. L-Alanine, L-cysteine, L-cystine, L-histidine, D,L-methionine, L-proline, L-serine and L-tyrosine can be used as sole nitrogen sources, but not adenine, L-arginine, L-asparagine, L-glutamic acid, glycine, hypoxanthine, L-lysine, L-phenylalanine, L-threonine, L-valine or xanthine. The diagnostic amino acid in the peptidoglycan is LL-diaminopimelic acid, and glucose,

Accomendations

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References


