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

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An endophytic actinomycete, strain YIM 65188^T, 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 65188^T is a member of the genus *Streptomyces* and exhibited 97.5 and 96.3 % gene sequence similarities to *Streptomyces specialis* GW41-1564^T and *Streptomyces hainanensis* YIM 47672^T, respectively, whereas low sequence similarity values (<95.2 %) distinguished strain YIM 65188^T from all other *Streptomyces* species with validly published names. Strain YIM 65188^T 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), MK-11(H₄), MK-9(H₆) and MK-10(H₄). Similarly, the closest phylogenetic relative, *S. specialis* GW41-1564^T, also had an unusual quinone composition, with the predominant menaquinones MK-10(H₄) and MK-10(H₆) and minor amounts of MK-9(H₄) and MK-9(H₆). The DNA–DNA hybridization value between strain YIM 65188^T and *S. specialis* GW41-1564^T was 42.7 %. On the basis of phenotypic and phylogenetic characteristics, strain YIM 65188^T was identified as a novel species of the genus *Streptomyces* for which the name *Streptomyces sedi* sp. nov. is proposed, with YIM 65188^T (=CCTCC AA 208020^T =DSM 41942^T) 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 (Bérdy, 2005). During a search for novel endophytic microbes, strain YIM 65188^T was isolated from surface-sterilized plant tissues. The objective of the present study was to determine the taxonomic position of strain YIM 65188^T.

Strain YIM 65188^T 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 (TSA; 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 [l⁻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 [l⁻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 65188^T 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-*p*-phenylenediamine. Catalase activity was determined with 3 % H₂O₂ 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 Stanek & 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

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 µm.

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(H₆) and MK-9(H₈). Strain YIM 65188^T has an unusual quinone composition, with the predominant menaquinones being MK-11(H₆), MK-10(H₆), MK-10(H₈), MK-11(H₈), MK-9(H₈), MK-12(H₆), 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

Taxa: 1, strain YIM 65188^T; 2, *S. specialis* GW41-1564^T; 3, *S. hainanensis* YIM 47672^T (Jiang *et al.*, 2007). Characteristics were scored as follows: +, positive; –, negative; ND, undetermined.

Characteristic	1	2	3
Starch hydrolysis	–	–	+
Utilization as sole carbon source:			
Cellobiose	+	–	+
Aesculin	+	–	+
D-Fructose	+	–	–
Inositol	–	+	–
Maltose	+	–	–
D-Ribose	–	+	–
Trehalose	–	–	+
Utilization as sole nitrogen source:			
Adenine	–	+	ND
L-Asparagine	–	+	ND
L-Cysteine	+	–	ND
L-Histidine	+	–	ND
Hypoxanthine	–	+	ND
DL-Methionine	+	–	ND
L-Phenylalanine	–	+	ND
L-Threonine	–	+	ND
L-Valine	–	+	ND
Xanthine	–	+	ND

11(H₄), MK-9(H₆) and MK-10(H₄) in the ratio 27:23:10:10:9:6:5:4:4. Minor amounts of MK-12(H₄) 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(H₆), MK-10(H₆), MK-10(H₄), MK-11(H₄), MK-11(H₈), MK-10(H₈), MK-9(H₈) and MK-9(H₆) 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(H₄), MK-10(H₆), MK-9(H₆), MK-9(H₄) and MK-10(H₂) 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(H₄), MK-10(H₆), MK-9(H₆) and MK-9(H₈) 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 $\pm$ SD, 42.7 $\pm$ 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. Bar, one nucleotide substitution per 100 nt.

on ISP 2, ISP 3, ISP 4 and Czapek's agar (Jiang *et al.*, 2007) and YIM 65188^T does not produce soluble pigments on any of the media tested. Furthermore, as shown in Table 1, many differences between strain YIM 65188^T, *S. specialis* GW41-1564^T and *S. hainanensis* YIM 47672^T 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 65188^T 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-xylose are not utilized. L-Alanine, L-cysteine, L-cystine, L-histidine, DL-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, galactose and madurose are present in whole-cell hydrolysates (cell-wall type I). Phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol, with trace amounts of two phosphatidylinositol mannosides and four unknown phospholipids. The quinone system is composed of the major compounds MK-11(H₆) and MK-10(H₆) and moderate to minor amounts of MK-10(H₈), MK-11(H₈), MK-9(H₈), MK-11(H₄), MK-9(H₆) and MK-10(H₄). The G + C content of the genomic DNA of the type strain is 71.0 mol%.

The type strain, YIM 65188^T (=CCTCC AA 208020^T=DSM 41942^T), was isolated from a surface-sterilized sample of *Sedum* sp. collected in Yunnan province, south-west China.

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