Streptomyces specialis sp. nov.

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A Gram-positive, non-endospore-forming bacterium (GW41-1564^T) was isolated from soil. Comparison of 16S rRNA gene sequences showed that strain GW41-1564^T is a member of the genus *Streptomyces*, exhibiting highest similarities with *Streptomyces hainanensis* YIM 47672^T (97.8 %) and *Streptomyces cacaoi* subsp. *cacaoi* NBRC 12748^T (97.5 %). Strain GW41-1564^T could be distinguished from any other *Streptomyces* species with validly published names by sequence similarity values less than 97.5 %. Strain GW41-1564^T exhibited an unusual quinone system, with the predominant compounds MK-10(H₄) and MK-10(H₆) and smaller amounts of MK-9(H₄) and MK-9(H₆). The type strain of the most closely related species, *S. hainanensis* YIM 47672^T, also contained an unusual quinone system composed of MK-9(H₆) and MK-9(H₈) in addition to MK-9(H₄) and MK-10(H₀), whereas the type strain of the second most closely related species, *S. cacaoi* NBRC 12748^T, contained a quinone system, composed of MK-9(H₆) and MK-9(H₈), typical of *Streptomyces*. The polar lipid profile of GW41-1564^T consisted of the predominant compound diphosphatidylglycerol, moderate amounts of phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol and minor to trace amounts of two phosphatidylinositol mannosides and several unknown lipids, and the major fatty acids were iso-C_{16:0}, anteiso-C_{17:1}ω9c and anteiso-C_{17:0}. The results of physiological and biochemical tests allowed further phenotypic differentiation of strain GW41-1564^T from the related species *S. hainanensis*. Strain GW41-1564^T clearly merits species status, and we propose the name *Streptomyces specialis* sp. nov., with the type strain GW41-1564^T (=DSM 41924^T =CCM 7499^T).

The genus *Streptomyces*, proposed by Waksman & Henrici (1943), currently encompasses nearly 600 species with validly published names. *Streptomyces* species are aerobic, Gram-positive actinomycetes and most of them are able to form an extensively branched substrate mycelium and also to produce aerial hyphae that typically differentiate into chains of spores. *Streptomyces* species have LL-diaminopimelic acid but no characteristic sugars in the cell wall (wall chemotype 1 *sensu* Lechevalier & Lechevalier, 1970) and possess DNA rich in G + C (Williams *et al.*, 1983; Manfio *et al.*, 1995). Although some authors have found some evidence that the genus is underspeciated (Sembiring *et al.*, 2000; Kim & Goodfellow, 2002), the major problem in describing novel species is the high 16S rRNA gene sequence similarity. Kämpfer & Labeda (2006) pointed out that, although 16S rRNA gene sequence data should be

used as a framework for presentation of species and species groups, the boundary for species delineation in this genus seems to be higher than 97 %. As a consequence, 16S rRNA gene sequence data cannot serve as the sole basis for species delineation within the genus *Streptomyces*, and the problems of the insufficient resolution capacity of this molecule are evident. In particular, conclusions derived from 'simple' treeing methods (like the neighbour-joining method) should be regarded with caution, given that a tree is only a visual aid to place a novel species in its approximate neighbourhood. The judgement of what is a species and which are its closest relatives should be made on the basis of individual sequence similarities, rather than the position of a sequence in a tree.

In this regard, it was agreed earlier (Kämpfer & Labeda, 2003) that the proposal of novel species within the genus *Streptomyces* should only be accepted on the basis of very careful studies done with sufficient practice and considering all other species. However, this is almost impossible,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GW41-1564^T is AM934703.

given the large number of species. The description of *Streptomyces* species must be based on a combination of genotypic and phenotypic data (Manfio *et al.*, 1995, 2003; Atalan *et al.*, 2000; Li *et al.*, 2002) and, if sufficient evidence is provided that an unknown is clearly different in both genotypic and phenotypic features, novel species can be described.

During the characterization of organisms isolated from different soils, strain GW41-1564^T was recovered from 1 g of a soil sample on mannitol-rifampicin agar [containing (l⁻¹): mannitol, 10 g; yeast extract, 7 g; Casamino acids, 2 g; peptone (Bacto), 1 g; NaCl, 1 g; CaCO₃, 0.2 g; nystatin, 100 mg; rifampicin, 5 mg] for 6 weeks at 27 °C. The strain was maintained on DSMZ medium 65 (<http://www.dsmz.de/media/med065.htm>) at 25 °C and showed a pale-yellow-coloured substrate mycelium that fragmented easily into irregular rod-shaped cells. The isolate grew well on all tested ISP media (ISP1–5; Shirling & Gottlieb, 1966) after 2 weeks incubation at 28 °C. On all media, a pinkish-white aerial mycelium and a pale-yellow substrate mycelium were produced. The isolate produced a black pigment on all tested ISP media. Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 25 °C on DSMZ medium 65. The strain was Gram-positive with the staining procedure of Gerhardt *et al.* (1994). Spores were elliptical or short rods (Fig. 1).

The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Sequence analysis was performed using the ARB software package (Ludwig *et al.*, 2004) and also the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated (distance options according to the Kimura-2 model) and clustering with the

Fig. 1. Micrograph of strain GW41-1564^T showing curved chains of spores after 3 weeks growth at 30 °C on DSMZ medium 65. Spore length is approximately 1 µm.

neighbour-joining, maximum-parsimony and maximum-likelihood algorithms was performed by using bootstrap values based on 1000 replications. The almost-complete 16S rRNA gene sequences of all strains were compared by sequence similarity calculations after a neighbour-joining analysis (Saitou & Nei, 1987). The results of these calculations indicated that the closest relatives of strain GW41-1564^T were *Streptomyces hainanensis* YIM 47672^T (97.8%) and *Streptomyces cacaoi* subsp. *cacaoi* NBRC 12748^T (97.5%). All other *Streptomyces* species showed lower sequence similarity to strain GW41-1564^T (<97.5%). With all treeing methods, strain GW41-1564^T clustered clearly with *S. hainanensis* YIM 47672^T. *S. cacaoi* subsp. *cacaoi* and other related species like *Streptomyces aminophilus*, *S. violaceusniger*, *S. yogyakartensis* and *S. hygrosopicus* were found in adjacent clusters. A maximum-likelihood tree is shown in Fig. 2. *S. hainanensis* was described by Jiang *et al.* (2007); this species was also described as occupying a unique position in the 16S rRNA gene sequence-based tree. *S. cacaoi* subsp. *cacaoi* was shown to be an earlier heterotypic synonym of *S. aminophilus* (Lanoot *et al.*, 2002). Because of the low 16S rRNA gene sequence similarity of GW41-1564^T to other *Streptomyces* species, *S. cacaoi* subsp. *cacaoi* DSM 40057^T was included in a comparative study. However, DNA–DNA hybridization studies were not carried out between strain GW41-1564^T and these organisms, as representatives of other pairs of *Streptomyces* species with similarly low 16S rRNA gene sequence similarities (Sembiring *et al.*, 2000; Kim & Goodfellow, 2002; Manfio *et al.*, 2003) show relatedness values below the 80% cut-off point recommended for the recognition of genomic species of *Streptomyces* (Labeda & Lyons, 1992; Labeda, 1993, 1998).

For quinone and polar lipid analyses, cells were grown in liquid DSMZ medium 65 until the majority of the CaCO₃ was resolved. Menaquinones were extracted as described by Tindall (1990a) and analysed by HPLC according to Stolz *et al.* (2007) and extraction and analysis of polar lipids was performed as described by Tindall (1990b). Strain GW41-1564^T exhibited a quinone system with the predominant compounds MK-10(H₄), MK-10(H₆), MK-9(H₄), MK-9(H₆) and MK-10(H₈) in the ratio 58:16:15:4:3. Minor amounts (<2%) of MK-10, MK-10(H₂) and MK-11(H₆) were also detected. Analysis of an independently grown biomass of GW41-1564^T resulted in a similar quinone system, also consisting of the major compounds MK-10(H₄), MK-10(H₆), MK-9(H₄) and MK-9(H₆) in the ratio 61:24:8:6. The quinone system of *S. cacaoi* subsp. *cacaoi* DSM 40057^T consisted of MK-9(H₆) and MK-9(H₈) in the ratio 53:35 and minor amounts (<10%) of MK-8(H₆) and MK-9(H₂). *S. hainanensis* YIM 47672^T also contained an unusual quinone system, composed of MK-9(H₆) and MK-9(H₈) in addition to MK-9(H₄) and MK-10(H₆) (Jiang *et al.*, 2007), which is atypical in comparison with those reported for other *Streptomyces* species.

The polar lipid profile of strain GW41-1564^T consisted of the major compound diphosphatidylglycerol, moderate

Fig. 2. Phylogenetic analysis of selected *Streptomyces* species most closely related to strain GW41-1564^T based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses) constructed after multiple alignment of data by the ARB program and CLUSTAL_x (Thompson *et al.*, 1997). Clustering with the maximum-likelihood method was performed by using the ARB software package (Ludwig *et al.*, 2004).

amounts of phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol and minor to trace amounts of two phosphatidylinositol mannosides, of which one was visible only after α -naphthol detection and not after detection with molybdatophosphoric acid, one unknown phospholipid, one unknown aminolipid and three unknown polar lipids (Fig. 3). The profile corresponds to phospholipid type 2 (Lechevalier *et al.*, 1977; Minnikin *et al.*, 1977), and similar profiles have been reported for numerous *Streptomyces* species.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain GW41-1564^T was clearly different from those of *S. hainanensis* YIM 47672^T and *S. cacaoi* subsp. *cacaoi* DSM 40057^T (Table 1), and showed many fatty acids in smaller amounts that are not found in most other *Streptomyces* species (Kämpfer, 2006).

Results of the physiological characterization showed that strain GW41-1564^T is able to utilize few carbon sources. Detailed results of the physiological characterization (according to Kämpfer *et al.*, 1991) are given in the species description, and a summary of differentiating tests from most closely related species is shown in Table 2.

It is evident that strain GW41-1564^T is clearly different from all other *Streptomyces* species. It shares less than 97.8% 16S rRNA gene sequence similarity with other streptomycetes and it can be distinguished phenotypically/chemotaxonomically from its nearest relatives *S. hainanensis* and *S. cacaoi* based on striking differences in the fatty acid profile, regarding the absence/presence of iso-C_{14:0} and iso-C_{16:1} H and significant quantitative differences in the contents of iso-C_{15:0}, anteiso-C_{15:0} and

anteiso-C_{17:1} ω 9c, and the quinone system, with MK-10 derivatives predominating, which is unique to date among streptomycetes. For this reason, we describe a novel species, for which we propose the name *Streptomyces specialis* sp. nov.

Fig. 3. Polar lipid profile of GW41-1564^T after two-dimensional TLC and detection with molybdatophosphoric acid. PE, Phosphatidylethanolamine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL1, unknown phospholipid; AL1, unknown aminolipid; L1–3, unknown polar lipids.

Table 1. Major fatty acids (%) of type strains of species of the genus *Streptomyces* that grouped into the same cluster on the basis of 16S rRNA gene sequence similarity

Strains: 1, GW41-1564^T; 2, *S. hainanensis* YIM 47672^T; 3, *S. cacaoi* subsp. *cacaoi* DSM 40057^T. Strains GW41-1564^T and DSM 40057^T were grown on trypticase soy broth at 28 °C for 7 days prior to fatty acid analysis. Data for *S. hainanensis* YIM 47672^T were from Jiang *et al.* (2007); the strain was grown under identical conditions prior to fatty acid analysis (Kämpfer & Kroppenstedt, 1996). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; capital letters indicate unidentified double-bond positions and *cis* isomers are indicated by the suffix *c*. –, Not detected; NR, not reported.

Fatty acid	1	2	3
C _{15:0}	0.2	NR	3.6
C _{16:0}	3.5	13.7	4.1
C _{17:0}	0.2	5.6	1.0
C _{18:0}	0.3	1.2	–
iso-C _{14:0}	–	NR	6.5
iso-C _{15:0}	0.6	1.1	6.9
anteiso-C _{15:0}	1.5	NR	32.1
anteiso-C _{15:1} A	–	NR	1.3
iso-C _{15:1} G	–	NR	0.6
iso-C _{16:1} G	–	NR	1.6
iso-C _{16:1} H	8.0	NR	–
iso-C _{16:0}	33.1	30.9	24.3
iso-C _{17:0}	1.2	2.8	3.6
anteiso-C _{17:0}	26.4	10.8	12.5
iso-C _{18:1} H	0.4	NR	–
iso-C _{18:0}	0.4	NR	–
C _{17:0} cyclo	0.7	1.4	–
C _{17:1} ω 8 <i>c</i>	0.7	7.1	–
iso-C _{17:1} ω 9 <i>c</i>	1.5	1.4	–
anteiso-C _{17:1} ω 9 <i>c</i>	11.2	NR	1.1
C _{18:1} ω 9 <i>c</i>	1.3	4.9	–
Summed feature 3*	3.3	2.1	–
Summed feature 5*	4.5	9.7	0.5
C _{17:0} 10-methyl	1.2	NR	–

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1} ω 7*c* and/or iso-C_{15:0} 2-OH. Summed feature 5 contained C_{18:2} ω 6,9*c* and/or anteiso-C_{18:0}.

Description of *Streptomyces specialis* sp. nov.

Streptomyces specialis (spe.ci.a'lis. L. masc. adj. *specialis* particular, special, because of the unusual quinone type and the separate phylogenetic position).

Aerobic, Gram-positive actinomycete that forms an extensively branched substrate mycelium and aerial hyphae that differentiate into spiral chains of spores. Spore chains consist of up to 15 spores. Spores are elliptical or short rods (Fig. 1). Grows well on all tested ISP media (ISP1–5; Shirling & Gottlieb, 1966) after 2 weeks incubation at 28 °C. On all media, a pinkish white aerial mycelium and a

Table 2. Physiological characteristics of strain GW41-1564^T and the type strains of *Streptomyces* species showing 16S rRNA gene sequence similarity >96.5 %

Strains: 1, GW41-1564^T; 2, *S. cacaoi* subsp. *cacaoi* NBRC 12748^T; 3, *S. yogyakartensis* NBRC 100778^T; 4, *S. hygroscopicus* NRRL 2387^T; 5, *S. violaceusniger* NRRL B-1478^T; 6, *S. hainanensis* YIM 47672^T. +, Positive; –, negative; (+) weakly positive; ND, no data available. Test results for *S. yogyakartensis* NBRC 100778^T, *S. hygroscopicus* NRRL 2387^T and *S. violaceusniger* NRRL B-1478^T were taken from Goodfellow *et al.* (2007) and results for *S. hainanensis* YIM 47672^T were taken from Jiang *et al.* (2007).

Test	1	2	3	4	5	6
Production of:						
β-D-Galactosidase	–	ND	+	–	+	ND
β-D-Glucosidase	(+)	+	–	–	+	ND
Aesculin hydrolysis	–	+	+	+	+	+
Growth on sole carbon sources						
Adonitol	+	–	+	–	+	–
L-Arabinose	–	–	+	–	+	–
Cellobiose	–	+	+	+	+	+
Dulcitol	–	ND	–	–	–	ND
D-Fructose	+	–	+	+	–	–
Melezitose	–	–	+	–	–	–
Salicin	+	ND	+	–	+	–
D-Sorbitol	(+)	–	–	–	–	–

pale yellow substrate mycelium are produced. Produces a black pigment on all tested ISP media. Good growth occurs on nutrient agar and DSMZ medium 65 at 25–30 °C. Major fatty acids are iso-C_{16:0}, C_{16:0}, anteiso-C_{17:1} ω 9*c*, anteiso-C_{17:0} and iso-C_{16:1} H. The quinone system is composed of the major compound MK-10(H₄) and moderate to minor amounts of MK-10(H₆), MK-9(H₄) and MK-9(H₆). In the polar lipid profile, diphosphatidylglycerol is predominant. Phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol are present in moderate amounts and phosphatidylinositol mannosides, an unknown aminolipid, an unknown phospholipid and three unknown polar lipids are present in minor to trace amounts. The following carbon sources are utilized: *N*-acetyl-D-glucosamine, D-glucose, D-gluconate, D-ribose, sucrose, adonitol, D-arabitol, D-sorbitol, *myo*-inositol, propionate, glutarate, butyrate, isobutyrate, isovaleric acid, L-arginine and L-asparagine. The following carbon sources are not utilized: L-arabinose, cellobiose, D-fructose, D-galactose, maltose, D-mannose, melibiose, L-rhamnose, salicin and D-xylose.

The type strain, GW41-1564^T (=DSM 41924^T =CCM 7499^T), was isolated from soil.

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