Streptomyces polyantibioticus sp. nov., isolated from the banks of a river

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As part of an antibiotic-screening programme, an actinomycete, designated strain SPR^T, was isolated from soil collected from the banks of the Umgeni River, KwaZulu-Natal Province, South Africa. The isolate produced branching vegetative mycelia with sporangiophores bearing sporangia developing at a late stage of growth. The sporangia contained smooth, almond-shaped, non-motile spores. Strain SPR^T exhibited antibiosis against various Gram-positive and Gram-negative bacteria, including *Enterococcus faecium* VanA (a vancomycin-resistant strain), *Mycobacterium aurum* A+ and *Escherichia coli* ATCC 25922. The chemotaxonomic characteristics of the strain, with the exception of the phospholipid pattern, corresponded with those of the members of the family *Streptomycetaceae* Waksman and Henrici 1943. Furthermore, phylogenetic analysis based on 16S rRNA genes showed that the strain was closely related to members of the genus *Streptomyces*, which supports its classification in the family *Streptomycetaceae*. Thus strain SPR^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces polyantibioticus* sp. nov. is proposed. The type strain is SPR^T (=DSM 44925^T=NRRL B-24448^T).

Tuberculosis has become the leading cause of natural death in South Africa, killing 218 per 100 000 people in the population per annum, striking most victims in their most economically productive years. South Africa is one of the 22 most burdened countries in the world (listed fourth) for this disease, having an average incidence rate of 940 tuberculosis cases per 100 000 people in the population per annum (WHO, 2008). To compound the problem, it is estimated that 44% of all tuberculosis patients are also positive for the human immunodeficiency virus (50% in 2003; 61% in 2005; WHO, 2008).

The genus *Streptomyces* was first described by Waksman & Henrici (1943). After a period of classification and reclassification of genera such as *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Microellobosporia*, *Kitasatoa* and *Streptoverticillium* (Anderson & Wellington, 2001), the genus *Streptomyces* now consists of more than 540 species with validly published names (Euzéby, 2008). The systematics of the genus *Streptomyces* at species level, however, is still in a state of confusion and

the genus is believed to be overspeciated (having by far the highest number of species with validly published names) (Groth *et al.*, 1999).

As part of a screening programme for antitubercular antibiotic-producing actinomycetes, an actinomycete with unusual morphology was isolated from soil collected from the banks of the Umgeni River (KwaZulu-Natal Province, South Africa).

Strain SPR^T was isolated on Middlebrook 7H9 agar (Becton Dickinson; supplemented with 10 mM glucose; albumin-glucose-catalase supplement omitted) after incubation at 28 °C for 7 days. Following isolation, strain SPR^T was maintained on Middlebrook 7H9-glucose agar.

Antimicrobial activity was determined using the sloppy-agar overlay technique, whereby the isolate was stab-inoculated with sterile toothpicks into Middlebrook 7H9-glucose, Czapek solution (Atlas, 2004) and yeast extract-malt extract (ISP 2 medium; Shirling & Gottlieb, 1966) agar plates, in duplicate. Plates were incubated for 10 days at 30 °C (and the duplicate set at 37 °C) and overlaid with 6 ml Luria sloppy agar (Sambrook *et al.*, 1989) containing the test bacterium. Activity was tested against various Gram-positive and Gram-negative bacteria.

Strain SPR^T was cultured in 500 ml Hacène's medium (containing, l⁻¹ distilled water: 5.0 g glucose, 4.0 g yeast extract, 10.0 g malt extract and 1.0 g NaCl, pH 7.0; Hacène & Lefebvre, 1995) for 10 days at 30 °C on a rocking shaker.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SPR^T is DQ141528.

A Southern blot showing the rRNA operon copy number of strain SPR^T is available as a supplementary figure with the online version of this paper.

The culture was filtered through a coffee filter (1 × 4-sized filters; House of Coffees). The mycelial mass of the isolate was extracted with methanol and the antibacterial activity was determined by means of bioautography (Betina, 1973).

The morphological characteristics of strain SPR^T were determined using standard methods (Locci, 1989). The isolate was grown on Middlebrook 7H9-glucose agar for 19, 63 and 90 days at 30 °C and the morphology was observed under a light microscope and by cryo-scanning electron microscopy.

Standard physiological tests were performed as described by Locci (1989). ISP media were prepared as described by Shirling & Gottlieb (1966). Antibiotic resistance was determined by incorporating antibiotics into Bennett's medium agar plates (Atlas, 2004) at the recommended concentrations (Locci, 1989). The concentrations of the non-standard test antibiotics were as follows: capreomycin (20 µg ml⁻¹), cefotaxime (100 µg ml⁻¹), D-cycloserine (50 µg ml⁻¹), kanamycin (10 µg ml⁻¹) and viomycin (8 µg ml⁻¹). Physiological characteristics were determined after growth at 30 °C (unless otherwise stated) for the recommended incubation periods. All carbon sources used for carbon-utilization tests were filter-sterilized and tested at the concentrations recommended by Locci (1989) and Shirling & Gottlieb (1966). Utilization of organic acids and resistance to lysozyme were determined as described by Gordon *et al.* (1974).

Standard techniques were used for the determination of catalase and oxidase activities. Staining was performed using the standard Gram-staining technique. Acid-fast staining (with 1 % sulphuric acid in the decolorization step) and acid-alcohol-fast staining (Ziehl-Neelsen) were performed using standard methods.

The freeze-dried cells used in chemotaxonomic tests were obtained from a 500 ml ISP 2 culture of strain SPR^T, which was cultivated on a rocking shaker at 30 °C for 5 days. The diaminopimelic acid isomer and the whole-cell sugar pattern were determined using the method of Hasegawa *et al.* (1983), with the exception that freeze-dried cells were used instead of colonies from agar plates.

The fatty acid methyl esters were prepared according to Chou *et al.* (1998) and were analysed by GC on a Zebron GC column (ZB-1; 0.25 mm by 30 m). The temperature of the column was fixed at 210 °C and the temperature of the injector was programmed to increase from 150 to 220 °C at a rate of 4 °C min⁻¹. The peaks were identified by means of comparison with fatty acid methyl ester standards (MIX GLC-80; Supelco). Mycolic acids were isolated and analysed as described by Minnikin *et al.* (1975). Phospholipids were isolated as described by Minnikin *et al.* (1984) and analysed by two-dimensional TLC on silica gel 60 F₂₅₄ (Merck) plates, as described by Komagata & Suzuki (1987). Menaquinones were analysed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig,

Germany), using an HPLC separation method. The peptidoglycan type was determined as described by Komagata & Suzuki (1987); TLC was used for the analysis. The G+C content of the DNA was determined in 1.0 × SSC by using the thermal denaturation method described by Mandel & Marmur (1968).

The 16S rRNA gene was amplified using PCR with the bacterial 16S rRNA gene primers F1 (Cook & Meyers, 2003) and R6 5'-AAGGAGGTGTTCAICC-3' [modified from primer p1525r of Chun & Goodfellow (1995); I=inosine]. PCR conditions were as described by Cook & Meyers (2003). The amplified DNA was purified for sequencing using a QIAquick PCR purification kit (Qiagen). For the phylogenetic analysis, reference strains were chosen from the BLAST results (Altschul *et al.*, 1997) and the top hits from the identity analysis of the EzTaxon server (Chun *et al.*, 2007). For the construction of phylogenetic trees, the software package MEGA (version 3.1; Kumar *et al.*, 2004; <http://www.megasoftware.net/>) was used. Unrooted phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution and maximum-parsimony methods (Takahashi & Nei, 2000) and then evaluated by means of bootstrap resampling (based on 1000 replications).

Genomic DNA was digested singly with restriction endonucleases: *SalI* and *ScaI* for the determination of 16S rRNA operon multiplicity, and *SalI*, *EcoRV*, *SspBI* and *SnaBI* for the determination of 23S rRNA operon multiplicity. The digested genomic DNA was electrophoresed on a 0.7 % agarose gel for 24 h. Copy numbers for both the 16S rRNA gene and the 23S rRNA gene were determined by means of Southern hybridization (Sambrook *et al.*, 1989). DNA probes labelled with digoxigenin-dUTP (Roche) were used. The probe for the determination of the 16S rRNA copy number was amplified from strain SPR^T by using the universal bacterial primers F1 and R5 (Cook & Meyers, 2003). The probe for the determination of the 23S rRNA copy number was amplified using the primers described by Wang & Zhang (2000): forward primer 5'-CCGATGAAGGACGTGGGA-3' (positions 46–63; numbering according to the *Streptomyces ambofaciens* ATCC 23877^T 23S rRNA gene) and reverse primer 5'-ACCAGTGAGCTATTACGC-3' (positions 1212–1195; numbering according to the *S. ambofaciens* ATCC 23877^T 23S rRNA gene; the last three nucleotides of the published primer were modified from GCG to CGC). Amplified, unlabelled 16S rDNA and 23S rDNA were used as positive controls. Hybridization and visualization were performed as recommended in the Roche DIG manual (<http://www.roche.com>).

From cryo-scanning electron microscopy (Fig. 1), it was clear that the strain goes through various stages of morphological development. After 19 days growth, *Rectiflexibiles*-type spore-chain morphology with smooth spores was clearly visible (Fig. 1a). After 63 days growth, the first signs of sporangium formation became apparent

Fig. 1. Cryo-scanning electron micrographs of strain SPR^T grown on Middlebrook 7H9-glucose agar at 30 °C for 19 days (a), 63 days (b) and 90 days (c). Clustered sporangiophores bearing mature sporangia are clearly visible in (c); smooth, almond-shaped spores are contained within the sporangia. Bars, 1 µm.

(Fig. 1b). After 90 days growth, sporangia borne on clustered sporangiophores were clearly visible (Fig. 1c). Straight-to-flexuous chains of spores were still visible at 90 days (this is not clearly shown in Fig. 1c). The spore sacs produced are clearly different from the pseudosporangia (which lack a surrounding membrane) observed in the two members of the now-defunct genus *Actinosporangium*, namely *Actinosporangium violaceum* and *Actinosporangium vitaminophilum* (now named *Streptomyces paradoxus* and *Streptomyces vitaminophilus*, respectively). Smooth, almond-shaped spores were visible within the sporangia. The sporangiophores were typically 11–18 µm long, the sporangia were 6–10 µm in diameter and the spores were approximately 2 µm long. The sporangiophores and sporangia had a ‘lollipop’ appearance and clustered in groups of four to six (Fig. 1c). When plates containing mature sporangia were flooded with water, no spore motility was observed under a light microscope.

A comparison of the chemotaxonomic characteristics of strain SPR^T with those of members of the sporangiate genera and the phylogenetically related non-sporangiate genera *Streptomyces* and *Kitasatospora* is shown in Table 1. LL-Diaminopimelic acid and glycine were detected in an amino acid analysis of strain SPR^T and the whole-cell sugar hydrolysate yielded galactose and glucose, with traces of ribose and mannose. Mycolic acids were not detected and the peptidoglycan was of the A3γ type [LL-diaminopimelic acid and glycine, as determined by TLC; Schleifer &

Kandler (1972)]. The predominant fatty acids were iso- and anteiso-branched (28 % iso-C_{16:0}; 18 % anteiso-C_{15:0}; 15 % C_{16:0}; 14 % anteiso-C_{17:0}; 10 % iso-C_{15:0}; 8 % iso-C_{17:0}), the profile being similar to those observed for *Streptomyces* strains. Phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside and two unknown phospholipids were detected by means of two-dimensional TLC analysis. The predominant menaquinones were MK-9(H₄) (60 %) and MK-9(H₆) (40 %); traces of MK-9(H₂) (<3 %) were also detected. The DNA G+C content was 74.4 ± 0.2 mol% when determined (in duplicate) in 1.0 × SSC.

A 1459 bp 16S rRNA gene sequence was obtained for strain SPR^T. A BLAST search revealed 98 % sequence similarity with respect to *Streptomyces cavourensis* subsp. *cavourensis* NBRC 13026^T (97.88 % by pairwise local alignment in DNAMAN, version 4.13; Lynnon BioSoft), *Streptomyces caviscabies* ATCC 51928^T (97.03 % in DNAMAN) and *Streptomyces setonii* ATCC 25497^T (97.03 % in DNAMAN) and various other members of the genus *Streptomyces*. A search of the EzTaxon server revealed 99.5 % pairwise sequence similarity with respect to *S. cavourensis* subsp. *cavourensis* NBRC 13026^T (97.88 % in DNAMAN), *Streptomyces albolongus* NBRC 13465^T (97.81 % in DNAMAN), *Streptomyces celluloflavus* NBRC 13780^T (97.81 % in DNAMAN) and 99.4 % pairwise sequence similarity with respect to *Streptomyces griseobrunneus* NBRC 12775^T (97.81 % in DNAMAN). A phylogenetic tree

Table 1. Comparison of chemotaxonomic characteristics of strain SPR^T, sporangiate genera and the genus *Streptomyces*

Taxa: 1, *Actinomadura*; 2, *Actinoplanes*; 3, *Dactylosporangium*; 4, *Planomonospora*; 5, *Planobispora*; 6, *Spirillospora*; 7, *Streptosporangium*; 8, *Streptomyces*; 9, *Kitasatospora*; 10, strain SPR^T (*Streptomyces polyantibioticus* sp. nov.). Data are from Goodfellow (1989) and Locci (1989); data for the genus *Kitasatospora* are from Chung *et al.* (1999) and Groth *et al.* (2003). None of the genera possesses mycolic acids.

Characteristic	1	2	3	4	5	6	7	8	9	10
Diagnostic amino acid*	meso-DAP	meso-DAP + glycine	meso-DAP + glycine	meso-DAP	meso-DAP	meso-DAP	meso-DAP	LL-DAP + glycine	LL-DAP and meso-DAP	LL-DAP + glycine
Diagnostic sugars	Madurose	Xylose + arabinose	Xylose + arabinose	Madurose	Madurose	Madurose	Madurose	No diagnostic sugars	Galactose	No diagnostic sugars
Phospholipid pattern†	PI	PII	PII	PIV	PIV	PI, PII	PIV	PII	PII	PG, PE, PI, PIM and DPG§
Menaquinone pattern	MK-9(H ₄ ,H ₆ ,H ₈)	MK-9(H ₄), MK-10(H ₄)	MK-9(H ₄ ,H ₆ ,H ₈)	MK-9(H ₀ ,H ₂ ,H ₄)	MK-9(H ₂ ,H ₄)	MK-9(H ₄ ,H ₆)	MK-9(H ₂)	MK-9(H ₆ or H ₈)	MK-9(H ₂ ,H ₄ ,H ₆)	MK-9(H ₄ ,H ₆)
Fatty acid pattern‡	3a	2c	2c	3c	3c	3a	3c	2c	2c	2c
DNA G + C content (mol%)	65–77	72–73	71–73	72	70–71	71–73	69–71	69–78	69–76	74

*DAP, 2,6-diaminopimelic acid.

†Phospholipid pattern: PI, phosphatidylglycerol; PII, phosphatidylethanolamine; PIII, phosphatidylcholine (with variable proportions of phosphatidylethanolamine, phosphatidylmethylethanolamine and phosphatidylglycerol); PIV, phospholipids containing glucosamine (with variable proportions of phosphatidylethanolamine and phosphatidylmethylethanolamine).

‡Fatty acid pattern: 2c, iso- and anteiso-branched and saturated fatty acids; 3a, saturated, unsaturated, iso- (variable) and methyl-branched fatty acids; 3c, saturated, unsaturated, iso-, anteiso- (variable) and methyl-branched fatty acids.

§DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside.

of strain SPR^T, streptomycete strains and the type species of the genera *Kitasatospora* and *Streptacidiphilus* (Fig. 2) showed that the strain clustered with the type strain of *Streptomyces mauvecolor* and other members of the genus *Streptomyces*. The 16S rRNA gene sequence similarity

between strain SPR^T and *S. mauvecolor* was 98.80 % (by pairwise local alignment in DNAMAN). Phylogenetically, therefore, strain SPR^T clearly belongs to the genus *Streptomyces*. Table 2 lists phenotypic differences between strain SPR^T and its closest phylogenetic relatives.

Fig. 2. Unrooted, neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain SPR^T among its phylogenetic neighbours. The 16S rRNA gene sequence of *Actinomadura madurae* NBRC 14623^T was used as an outgroup. All sequences were edited to the longest common region (1409 bp). GenBank sequence accession numbers are given in parentheses. Bootstrap percentages (based on 1000 replications) are shown at the nodes. Asterisks indicate clades that were conserved in neighbour-joining, minimum-evolution and maximum-parsimony trees. Bar, 1 nucleotide substitution per 100 nt.

Table 2. Comparison of phenotypic characteristics of strain SPR^T with those of *Streptomyces* species

Strains: 1, strain SPR^T; 2, *S. mauvecolor* NBRC 13854^T (data from Locci, 1989); 3, *S. albolongus* NBRC 23465^T (Shirling & Gottlieb, 1972); 4, *S. cavourensis* subsp. *cavourensis* NBRC 13026^T (Skarbak & Brady, 1978). w+, Weakly positive; ND, not determined.

Phenotypic characteristic	1	2	3	4
Spore-chain morphology	<i>Rectiflexibiles</i> ; sporangia	<i>Spirales</i>	<i>Rectiflexibiles</i>	<i>Rectiflexibiles</i>
Spore-surface ornamentation	Smooth	Spiny	Smooth	Smooth
Colour of aerial mycelium	White	Violet	White	White
Colour of substrate mycelium	Dark brown	ND	Yellow-brown	Yellow-brown
Utilization as sole carbon source				
L-Arabinose	—	+	+	w+ to +
D-Fructose	+	—	—	+
myo-Inositol	+	—	—	—
Raffinose	+	+	—	—
Salicin	+	+	ND	ND
D-Xylose	+	—	+	+

Strain SPR^T contains seven copies of the 16S and 23S rRNA genes (see Supplementary Fig. S1, available in IJSEM Online). Copy numbers of four or more have been detected in soil actinomycetes; this is not surprising, as Klappenbach *et al.* (2000) showed that a high rRNA gene copy number confers an advantage in fluctuating environments (such as soil), where it ensures an ability to utilize newly introduced resources quickly.

The chemical and phylogenetic characteristics of strain SPR^T were similar to those of members of the genus *Streptomyces*. The predominant menaquinones observed for strain SPR^T were MK-9(H₄,H₆); this profile differs from that of most streptomycetes [which contain MK-9(H₆,H₈)], but matches that of members of the genus *Actinomadura*. The tetrahydrogenated MK-9 menaquinone has been detected in some streptomycetes, including *Streptomyces hebeiensis* DSM 41837^T (Xu *et al.*, 2004), *Streptomyces scabrisporus* NRRL B-24202^T (Ping *et al.*, 2004), *Streptomyces scopiformis* A25^T (Li *et al.*, 2002), *Streptomyces sodiiphilus* CIP 107975^T (Li *et al.*, 2005), *Streptomyces thermocoprophilus* B19^T (Kim *et al.*, 2000) and *Streptomyces yeochonensis* NRRL B-24245^T (Kim *et al.*, 2004). With the exception of *S. scabrisporus* NRRL B-24202^T, none of these streptomycetes has MK-9(H₄) as the predominant menaquinone.

On the basis of the results of the polyphasic taxonomic analysis described here, strain SPR^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces polyantibioticus* sp. nov. is proposed.

Description of *Streptomyces polyantibioticus* sp. nov.

Streptomyces polyantibioticus (po.ly.an'ti.bi.o'ti.cus. Gr. adj. *poly* many; Gr. prep. *anti* against, in opposition to; Gr. n. *bios* life; L. suff. *-icus -a -um* suffix used in adjectives with the sense of belonging to, related to; N.L. masc. adj. *polyantibioticus* related to many antibiotics, referring to the ability to produce numerous antibiotics).

Produces brown substrate mycelium and fluffy, white aerial mycelium on inorganic salts-starch agar (ISP 4 medium). Leathery, dark-brown colonies are formed on ISP 2 medium, but no sporulation occurs. Brown substrate mycelium with white aerial mycelium is formed on oatmeal agar (ISP 3 medium). The colour of the substrate mycelium is not pH sensitive. Good growth is observed on Middlebrook 7H9-glucose agar with the formation of sporangia after prolonged incubation (90 days). A dark-brown, diffusible pigment is produced on glycerol-asparagine agar (ISP 5 medium) and melanin is produced on peptone-yeast extract-iron agar (ISP 6 medium) and tyrosine agar (ISP 7 medium).

Grows in the presence of 0.1% (but not 0.3%) 2-phenylethanol, 0.0001% crystal violet, 7% (but not 10%) NaCl and 0.1% phenol, but not in the presence of sodium azide (0.01%). Growth is also observed in the presence of lysozyme, capreomycin (20 µg ml⁻¹), cefotaxime (100 µg ml⁻¹), cephaloridine (100 µg ml⁻¹), D-cycloserine (50 µg ml⁻¹), penicillin G (10 IU ml⁻¹) and viomycin (8 µg ml⁻¹), but not in the presence of gentamicin (100 µg ml⁻¹), kanamycin (10 µg ml⁻¹), lincomycin (100 µg ml⁻¹), neomycin (50 µg ml⁻¹), oleandomycin (100 µg ml⁻¹), rifampicin (50 µg ml⁻¹), streptomycin (100 µg ml⁻¹), tobramycin (50 µg ml⁻¹) or vancomycin (50 µg ml⁻¹). Growth occurs at 4 and 30 °C and at pH 4.3, but not at 37 °C. Uses DL-α-amino-*n*-butyric acid, L-arginine, L-cysteine, L-histidine, L-hydroxyproline, L-phenylalanine, potassium nitrate, L-serine, L-threonine and L-valine as sole nitrogen sources; L-methionine is weakly utilized. Uses (+)-D-cellobiose, (–)-D-fructose, (+)-D-galactose, (+)-D-glucose, myo-inositol, (–)-D-lactose, (+)-D-mannose, (+)-D-melibiose, raffinose, salicin, sodium acetate (0.1%), sodium citrate (0.1%), trehalose and (+)-D-xylose as sole carbon sources [(–)-D-ribose is utilized weakly], but does not utilize adonitol, (+)-L-arabinose, inulin, (–)-D-mannitol, (+)-D-melezitose, (+)-L-rhamnose, sucrose or xylitol.

H₂S is produced and nitrate is reduced (weakly). Lecithinase and lipase activity are observed on egg-yolk agar, but protease activity is not observed. Pectin is hydrolysed, but hippurate is not. Degrades adenine, aesculin, arbutin, casein, DNA, hypoxanthine, L-tyrosine, Tween 80 and xanthine, but not allantoin, cellulose, gelatin, guanine, starch, urea or xylan. Able to utilize the organic acids, sodium acetate, sodium citrate, sodium formate, sodium gluconate, sodium DL-lactate, sodium DL-malate, sodium succinate and sodium (+)-L-tartrate. Sodium butyrate is utilized weakly and sodium benzoate, sodium maleate, sodium mucate, sodium oxalate, sodium salicylate and sodium sorbate are not utilized.

The predominant menaquinones are MK-9(H₄) and MK-9(H₆) and the phospholipids phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidyl-inositol mannosides and diphosphatidylglycerol are detected. Very strong antibiosis is exhibited (in agar overlays) against *Bacillus coagulans* ATCC 7050^T, *Enterococcus faecium* VanA (a vancomycin-resistant clinical isolate), *Enterococcus phoeniculicola* JLB-1^T, a *Micrococcus* species (clinical isolate), *Mycobacterium aurum* A+ and a *Streptococcus* species (clinical isolate); weak activity is exhibited against *Citrobacter braaki* strain 90 (clinical isolate), *Enterobacter cloacae* strain 67 (clinical isolate), *Escherichia coli* ATCC 25922 and *Klebsiella oxytoca* strain K52 [clinical isolate; resistant to Augmentin and cefuroxime]. Five anti-*M. aurum* A+ activity spots can be detected by bioautography of a methanol extract of the mycelial mass of a culture grown for 10 days with shaking at 30 °C in Hacène's medium [TLC, using ethyl acetate/methanol (100:15, v/v) as the mobile phase].

The type strain, SPR^T (=DSM 44925^T=NRRL B-24448^T), was isolated from the banks of the Umgeni River in KwaZulu-Natal Province, South Africa. The DNA G+C content of the type strain is 74.4 ± 0.2 mol% (1.0 × SSC).

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