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Streptomyces speibonae sp. nov., a novel streptomycete with blue substrate mycelium isolated from South African soil

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An actinomycete with blue substrate mycelium was isolated from a soil sample in Cape Town, South Africa, and designated strain PK-Blue^T. The colour of the substrate mycelium was not sensitive to changes in pH. The organism produced hairy spores in *Spirales*-type spore chains. Chemical taxonomy indicated that it belonged to the genus *Streptomyces*. Strain PK-Blue^T produced no diffusible pigments other than melanin, grew at 45 °C, did not degrade adenine and exhibited no antibacterial activity against *Enterococcus faecium*, *Escherichia coli* or *Pseudomonas aeruginosa*. Analysis of its 16S rRNA gene sequence and the results of physiological tests showed that strain PK-Blue^T (= DSM 41797^T = ATCC BAA-411^T) represents the type strain of a novel species of *Streptomyces*, for which the name *Streptomyces speibonae* sp. nov. is proposed.

Actinomycetes are widely distributed in terrestrial environments, from which they are easy to isolate. This fact, plus their broad metabolic capabilities and their ability to produce pigments and antibiotics, makes them fascinating and suitable subjects for undergraduate microbiology projects.

Streptomycetes may represent a considerable proportion of the actinomycete communities in soils (Elander, 1987). Despite the fact that streptomycetes have been studied extensively for many decades and many species have been described, from time to time, a novel streptomycete is isolated.

Strain PK-Blue^T was isolated by J. M. Pule and T. Kwetane on the Upper Campus of the University of Cape Town (South Africa) in August 2000, as part of their 3rd year BSc project on actinomycetes.

Morphological and physiological characteristics were determined as recommended by Williams *et al.* (1989), with the exception that antibiotic resistance was determined by incorporation of the antibiotics into Bennett's medium agar plates (Atlas, 1993) at the concentrations recommended instead of using antibiotic-impregnated filter discs. Non-standard antibiotics were tested at the following concentrations: 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}). Antimicrobial activity against *Enterococcus faecium*, *Escherichia coli* and *Pseudomonas aeruginosa* was determined using 5-day-old colonies of strain PK-Blue^T grown on nutrient agar (Williams *et al.*, 1989).

International *Streptomyces* Project (ISP) media were prepared according to the methods of Shirling & Gottlieb (1966). Tests for physiological characteristics were carried out at 28 °C (unless otherwise indicated) and results were read after the recommended incubation periods. All carbon sources for carbon-utilization tests were filter-sterilized. *meso*-Erythritol, glycerol, maltose, methyl α-D-glucoside, D(−)-ribose and L(−)-sorbose were tested as sole carbon sources at concentrations of 1 % (w/v). Sodium benzoate, sodium butyrate, sodium formate, sodium DL-malate, sodium maleate, sodium oxalate, sodium salicylate, sodium succinate and sodium L(+)-tartrate were tested as sole carbon sources at 0·1 % (w/v). DL-Citrulline, DL-ornithine and 4-amino-*n*-butyric acid were tested as sole nitrogen sources at 0·1 % (w/v).

Determination of the isomer of diaminopimelic acid (DAP) and the whole-cell sugar pattern were carried out as described by Hasegawa *et al.* (1983) with the exception that dried cells were used instead of colonies from agar plates. Fatty acid methyl esters were prepared by the trimethylsulphonium hydroxide method (Butte, 1983). The base composition of genomic DNA of strain PK-Blue^T was

Abbreviations: DAP, diaminopimelic acid; ISP, International *Streptomyces* Project.

The GenBank accession number for the 16S rRNA gene sequence of *Streptomyces speibonae* DSM 41797^T (= ATCC BAA-411^T) is AF452714.

determined in 0·1× SSC by the method of Mandel & Marmur (1968).

A 16S rRNA gene sequence of strain PK-Blue^T was amplified by PCR using universal bacterial 16S rDNA primers (forward primer adapted from primer fD1 of Weisburg *et al.*, 1991; reverse primer adapted from primer p1525r of Chun & Goodfellow, 1995). The 16S rDNA was sequenced using an ALFexpress DNA automated sequencer (Applied Biosystems) and an Amersham Pharmacia Biotech Cy5 Thermo Sequenase dye terminator kit.

Strain PK-Blue^T was Gram-positive by Gram stain, did not grow under anaerobic conditions and gave a positive reaction in the catalase test. Light microscopy showed a branched mycelium without verticils. Scanning electron microscopy revealed *Spirales*-type spore chains with hairy spore sheaths (Fig. 1). Chemotaxonomic tests showed that the cell wall contained LL-DAP, indicating that it has cell-wall type I; no diagnostic sugars were detected in hydrolysates of whole cells. Fatty acid analysis showed that strain PK-Blue^T contained a high proportion of saturated straight-chain and iso- and anteiso-branched fatty acids: iso-15 : 0 (12·0 %), iso-16 : 0 (35·9 %), 16 : 0 (14·9 %) and anteiso-17 : 0 (9·4 %). The G+C content of the genomic DNA was 73·4 mol%.

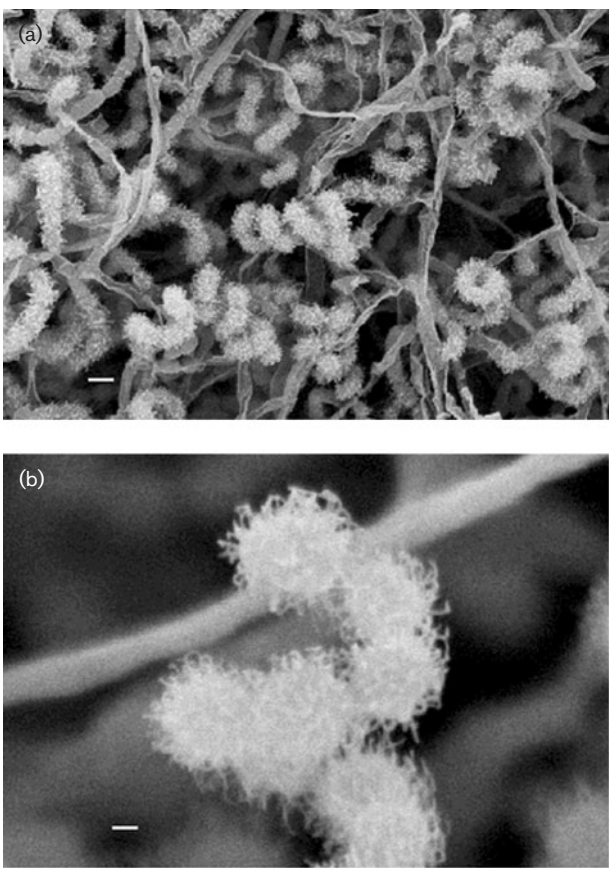

Fig. 1. Scanning electron micrographs of strain PK-Blue^T grown on inorganic salts-starch agar (ISP medium no. 4) at 28–30 °C for 14 days. *Spirales*-type spore chains with hairy spore sheaths are clearly evident. Bars, 1 μm (a) and 250 nm (b).

A 1490-bp 16S rRNA gene sequence was determined for strain PK-Blue^T. A standard nucleotide–nucleotide BLAST search (Altschul *et al.*, 1997) against the GenBank database using this sequence showed that it was most similar to the 16S rDNA sequences of '*Nocardioides thermolilacinus*' strains IFO 14336 and IFO 14335 (both 98 % similar over 1471 nt) and many species of *Streptomyces*. Although it was surprising that the highest similarity of the PK-Blue^T 16S rDNA was to '*N. thermolilacinus*' (strain PK-Blue^T does not have a *Nocardioides*-type life cycle), these organisms are considered to have been misidentified and are believed to be streptomycetes (Prauser, 1989).

A phylogenetic tree of *Streptomyces* 16S rDNA sequences was constructed by the neighbour-joining method of Saitou & Nei (1987) using CLUSTAL W (version 1.81) and MEGA (version 2.1; Kumar *et al.*, 2001) (Fig. 2).

The morphological and physiological characteristics of strain PK-Blue^T, as well as its cell-wall type, its whole-cell sugar pattern, its fatty acid profile and the sequence of its 16S rRNA gene, are consistent with the characteristics of members of the genus *Streptomyces*. Strain PK-Blue^T produced a blue substrate mycelium and a grey spore mass composed of hairy spores in *Spirales*-type spore chains. It produced melanin, but did not produce any other diffusible pigments.

Strain PK-Blue^T differs from other streptomycetes that produce a blue substrate mycelium in several respects. Some strains of *Streptomyces griseoflavus* produce a blue substrate mycelium (Williams *et al.*, 1989), but this species differs from PK-Blue^T in producing *Retinaculiaperti* spore chains and a green or yellow spore mass and by its ability to hydrolyse pectin and degrade adenine. Most strains of *S. griseoflavus* do not produce melanin. *S. griseoflavus* does not degrade xanthine, is sensitive to vancomycin and does not grow at 45 °C or in the presence of 10 % NaCl. PK-Blue^T grows on L-valine as a sole nitrogen source and on sodium malonate as a sole carbon source. *S. griseoflavus* does not use either of these.

There are no full-length or almost-complete 16S rDNA sequences for *S. griseoflavus* (or any of its subjective synonyms: *Streptomyces cyanoalbus*, *Streptomyces hirsutus*, *Streptomyces pilosus*, *Streptomyces prasinopilosus* and *Streptomyces prasinus*) in the GenBank database. However, 119–121 bp sequences, from the variable α region of the 16S rRNA molecule, have been determined for each of these species (Kataoka *et al.*, 1997). These single sequences correspond to nucleotide positions 158–277 of the *Streptomyces ambofaciens* 16S rRNA sequence of the *rrnD* rRNA gene cluster (Pernodet *et al.*, 1989). The similarity of these sequences to the PK-Blue^T 16S rDNA sequence was

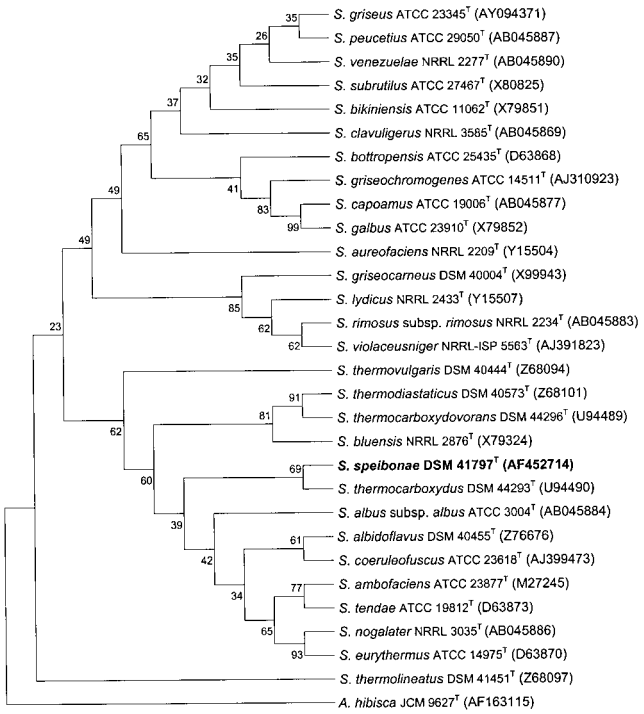

Fig. 2. Unrooted phylogenetic tree constructed from almost-complete *Streptomyces* 16S rRNA gene sequences, showing the relationship between *Streptomyces speibonae* sp. nov. and streptomycetes belonging to the major, minor and single-member clusters defined by Williams *et al.* (1983). The sequence of the 16S rRNA gene of *Actinomadura hibisca* JCM 9627^T was used as an outgroup. All sequences were edited to produce the longest sequence region common to all sequences (1440 bp). GenBank sequence accession numbers are given in parentheses. The tree was generated using the neighbour-joining method (CLUSTAL W version 1.81 and MEGA version 2.1) and includes bootstrap percentages based on an analysis of 1000 resampled datasets.

determined by pairwise alignment using the DNAMAN software (Lynnon BioSoft; version 4.13). The range of similarity is 83·2 % (*S. prasinopilosus* JCM 4404^T; accession no. D44119) to 96·7 % (*S. griseoflavus* JCM 4479^T; D44174). These differences eliminate the possibility that PK-Blue^T is a member of the *S. griseoflavus* species group.

Streptomyces anthocyanicus, *Streptomyces caeruleus*, *Streptomyces tricolor* and *Streptomyces violaceoruber* produce blue substrate mycelia (Williams *et al.*, 1989), but differ from PK-Blue^T in producing smooth spores and in the production of diffusible pigments. Strain PK-Blue^T produces *Spirales*-type spore chains, whereas *S. anthocyanicus* has *Retinaculiaperti*-type spore chains and *S. caeruleus* has *Rectiflexibiles*-type spore chains. *S. anthocyanicus*, *S. caeruleus* and *S. violaceoruber* do not produce melanin. Strain PK-Blue^T uses, as sole carbon sources, L(+)-arabinose, L(+)-rhamnose, D(+)-xylose (*S. caeruleus* does not use any of these) and D-mannitol (not used by *S. anthocyanicus*). Strain PK-Blue^T does not use salicin (*S. violaceoruber* is able to use this glucoside as a sole carbon source).

The single available 121 bp sequences for *S. anthocyanicus* JCM 5058^T, *S. tricolor* JCM 5065^T and *S. violaceoruber* JCM 4423^T, from the variable α region (Kataoka *et al.*, 1997), all show 89·3 % similarity to the PK-Blue^T 16S rDNA sequence by pairwise alignment (accession nos D44427, D44434 and D44135, respectively). A 451 bp 16S rDNA sequence of *S. violaceoruber* (AF434717) shows only 35·8 % similarity to the PK-Blue^T 16S rDNA sequence by pairwise alignment. These differences support the other evidence that strain PK-Blue^T is not a strain of *S. anthocyanicus*, *S. caeruleus*, *S. tricolor* or *S. violaceoruber*.

Strain PK-Blue^T also differs from other streptomycetes that produce hairy spores. *Streptomyces acrimycini* produces a green spore mass and no melanin (Williams *et al.*, 1989). *Streptomyces bambergiensis* has *Retinaculiaperti*-type spore chains and a green spore mass. It produces a pH-sensitive red–orange substrate mycelium and pH-sensitive red–orange diffusible pigments, but no melanin. This species is also sensitive to lincomycin (100 μg ml^{-1}) (Williams *et al.*, 1989).

Streptomyces capillispiralis differs from strain PK-Blue^T in producing a light brownish-grey spore mass and yellow–brown to brownish-black substrate mycelium. A brown diffusible pigment may be produced, but melanin is not produced. This species produces urease, but does not degrade starch or reduce nitrate (Williams *et al.*, 1989).

Streptomyces chromofuscus produces a yellow–brown substrate mycelium and is sensitive to oleandomycin (100 μg ml^{-1}). Most strains produce smooth spores. *Streptomyces flaveolus* and *Streptomyces pactum* produce yellow–brown substrate mycelia and yellow–brown diffusible pigments, but no melanin. *S. pactum* is also unable to use D(+)-xylose as a sole carbon source (Williams *et al.*, 1989).

Streptomyces finlayi differs from PK-Blue^T in producing *Rectiflexibiles*-type spore chains, a green substrate mycelium and no melanin. This species is sensitive to lincomycin (100 μg ml^{-1}) (Williams *et al.*, 1989).

Streptomyces geysiriensis differs from PK-Blue^T in not producing melanin. *Streptomyces glaucescens* produces a blue spore mass, a red–orange substrate mycelium and red–orange diffusible pigments. This species also lacks proteolytic activity (Williams *et al.*, 1989).

Streptomyces griseostramineus produces a green spore mass and greyish-yellow or greyish-yellow–green substrate mycelium. *Streptomyces prasinosporus* produces a green spore mass, yellow–brown substrate mycelium and no melanin on tyrosine agar. It is also unable to degrade tyrosine or to use L-arginine as a sole nitrogen source (Williams *et al.*, 1989).

Streptomyces viridoviolaceus differs from strain PK-Blue^T in producing a light brownish-grey to greyish-yellow–brown spore mass, reddish- or yellowish-brown substrate mycelium and orange or red, pH-sensitive diffusible pigments. Melanin pigment is not produced (Williams *et al.*, 1989).

These results support the classification of strain PK-Blue^T as a novel species of *Streptomyces*, for which we propose the name *Streptomyces speibonae* sp. nov. Additional data from the phenotypic characterization of the strain are presented below

Description of *Streptomyces speibonae* sp. nov.

Streptomyces speibonae (spei.bo′nae. L. n. *spes -ei* hope; L. adj. *bonus* good; N.L. masc. adj. *speibonae* of good hope, to indicate Cape Town, the Cape of Good Hope, South Africa, the geographical location from which the type strain was isolated).

Aerobic, Gram-positive, catalase-positive actinomycete that forms a grey aerial mycelium and a blue substrate mycelium. The colour of the substrate mycelium is not pH sensitive. Verticils are not present. The mycelium does not fragment. *Spirales*-type spore chains with hairy spore sheaths are produced. No diffusible pigments are produced on glycerol-asparagine agar (ISP medium no. 5) or on any other medium. Melanin pigment is produced on both peptone-yeast extract-iron agar (ISP medium no. 6) and tyrosine agar (ISP medium no. 7). Although growth on inorganic salts-starch agar (ISP medium no. 4) is initially slow, very good growth with profuse sporulation is observed on this medium after 14 days. Very good growth occurs on yeast extract-malt extract agar (ISP medium no. 2). Good growth is observed on oatmeal agar (ISP medium no. 3) and moderate growth on Czapek solution agar (Atlas, 1993). Growth on glycerol-asparagine agar is poor. The substrate mycelium is blue on yeast extract-malt extract agar and oatmeal agar, but light grey on the Czapek and glycerol-asparagine media. The cell wall contains LL-DAP (cell wall type I). The whole-cell sugar pattern contains no diagnostic sugars. No antibiosis is exhibited against *Enterococcus faecium* (clinical isolate), *Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853. Grows in the presence of (μg ml^{-1} unless stated otherwise): cefotaxime (100), cephaloridine (100), D-cycloserine (50), lincomycin (100), oleandomycin (100), penicillin G (10 IU ml^{-1}), phenol (0·1 %), 2-phenylethanol (0·1 %), sodium chloride (10 %) and vancomycin (50) and at 45 °C but not at 4 °C, pH 4·3 or in the presence of sodium azide (0·01 %), capreomycin (20), gentamicin (100), kanamycin (10), neomycin (50), rifampicin (50), streptomycin (100), tobramycin (50) or viomycin (8). Uses DL-α-amino-*n*-butyric acid, 4-amino-*n*-butyric acid, L-arginine, DL-citrulline, L-cysteine, L-histidine, L-methionine, DL-ornithine, potassium nitrate, L-serine, L-threonine and L-valine as sole nitrogen sources, but not L-hydroxyproline or L-phenylalanine. Uses L(+)-arabinose, D(+)-cellobiose, D(−)-fructose, D(+)-galactose, glycerol, *meso*-inositol, lactose, maltose, D-mannitol, D(+)-mannose, L(+)-rhamnose, D(−)-ribose, sodium acetate, sodium butyrate, sodium DL-malate, sodium malonate, sodium propionate, sodium pyruvate, sodium succinate, sucrose (weak growth), trehalose and D(+)-xylose as sole carbon sources but not adonitol, *meso*-erythritol, inulin, D(+)-melezitose, D(+)-melibiose, methyl α-D-glucoside, raffinose, salicin, sodium benzoate, sodium citrate, sodium formate, sodium maleate, sodium oxalate, sodium salicylate, sodium L(+)-tartrate, L(−)-sorbose or xylitol. Tests for nitrate reductase and the production of H_2S are positive, but pectin is not hydrolysed. Lipase and lecithinase are produced on egg-yolk agar, but protease activity is not seen on this medium after the recommended 2 days of incubation (there is weak activity after 6 days). Degrades casein, DNA, gelatin, guanine, hypoxanthine, starch (weakly), Tween 80, L-tyrosine, xanthine and xylan but not adenine, allantoin or urea.

The DNA G+C content of the type strain, strain PK-Blue^T (=DSM 41797^T=ATCC BAA-411^T), is 73·4 mol%.

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