Streptomyces ferralitis sp. nov., a novel streptomycete isolated from a New-Caledonian ultramafic soil

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The taxonomic position of an actinomycete isolated from an ultramafic soil in New Caledonia was determined using a polyphasic approach. The isolate, which was designated SFOp68^T, was shown to have chemical and morphological properties typical of streptomycetes. An almost complete 16S rRNA gene sequence of the isolate was generated and compared with sequences of representative streptomycetes. The 16S rRNA data not only supported the classification of the strain in the genus *Streptomyces*, but also showed that it formed a distinct phyletic line that was most closely related to one composed of the type strain of *Streptomyces rimosus*. The two organisms can be readily separated using a diverse range of phenotypic properties. It is proposed that strain SFOp68^T (=DSM 41836^T=NCIMB 13954^T) be classified in the genus *Streptomyces* as *Streptomyces ferralitis* sp. nov.

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) and currently contains nearly 600 species with validly published names. The taxon contains aerobic, Gram-positive actinomycetes that form an extensively branched substrate mycelium, produce aerial hyphae that typically differentiate into chains of spores, 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.*, 1989; Manfio *et al.*, 1995). It is evident that the genus is under-speciated (Sembiring *et al.*, 2000; Kim & Goodfellow, 2002) and that the description of *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). Members of novel streptomycete species are in great demand as a source of novel commercially significant, bioactive compounds (Bérdy, 1995; Dieter *et al.*, 2003).

Actinomycetes dominate bacterial communities in New-Caledonian ultramafic soils (Amir & Pineau, 1998). These infertile soils, which account for up to a third of the landmass in the country (Jaffré, 1976), have a high level of metal toxicity (due to the presence of high concentrations of chromium, cobalt, iron and nickel) and provide habitats for diverse novel actinomycetes that produce bioactive compounds (Saintpierre, 2001; Saintpierre *et al.*, 2003; Saintpierre-Bonaccio *et al.*, 2004). In the course of a screening programme designed to isolate novel bioactive actinomycetes from ultramafic soils, an actinomycete, designated SFOp68^T, was isolated and provisionally assigned to the genus *Streptomyces*. The aim of the present study was to determine the taxonomic status of the strain using genotypic and phenotypic procedures. The resultant data indicate that the organism should be classified as a novel species of *Streptomyces*, for which the name *Streptomyces ferralitis* sp. nov. is proposed.

Strain SFOp68^T was isolated from a 10^{-1} suspension of a ferralitic, oxidic, ultramafic soil that had been heat-pretreated at 100 °C for 15 min and then used to inoculate a chitin/vitamin B agar plate (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (100 μg ml^{-1}) and polymyxin (25 μg ml^{-1}) and incubated at 30 °C for 2 weeks. The soil sample had been collected from the 'Ouenarou' region in the southern part of the main island of New Caledonia [see Institut National Geographique map no. 4835 (Yaté), série orange, 7549·5 × 681·5]. The isolate was tested for purity, maintained on modified Bennett's agar (MBA; Jones, 1949) and preserved as a mixture of hyphae and spores in 20 % (v/v) glycerol at −20 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Streptomyces ferralitis* strain SFOp68^T is AY262826.

The isolate was grown on MBA (Jones, 1949) and peptone/yeast extract/iron agar [ISP 6 (Difco); Shirling & Gottlieb, 1966] plates at 30 °C for 14 days. The colour of the aerial spore mass, the pigmentation of the substrate mycelium and the colour of any diffusible pigment on MBA were recorded, as well as the ability to form melanin pigments on ISP 6 agar. Spore-chain morphology and spore ornamentation were observed by using a Cambridge Stereoscan 240 scanning electron microscope to study a culture grown on oatmeal agar [ISP 3 (Difco); Shirling & Gottlieb, 1966] for 3 weeks at 30 °C by following the procedure described by O'Donnell *et al.* (1993).

Most of the phenotypic tests were carried out using the media and methods described by Williams *et al.* (1983). The ability of the test strain to grow in MBA supplemented with erythromycin (4 μg ml^{-1}), gentamicin sulphate (10 μg ml^{-1}), penicillin (25 μg ml^{-1}), rifampicin (6 μg ml^{-1}), streptomycin sulphate (5 μg ml^{-1}), tetracycline hydrochloride (30 μg ml^{-1}) and vancomycin hydrochloride (10 μg ml^{-1}) was examined following incubation of plates at 30 °C for 7 days. Similarly, the organism was examined for its ability to grow in MBA supplemented with crystal violet (0·0002 %, w/v), phenol (0·01 %, w/v), potassium tellurite (0·005 %, w/v) and sodium chloride (5 %, w/v). Lipase activity was detected using Sierra's medium (Sierra, 1957) supplemented with Tween 80 (1 %, v/v). Standard procedures were used to determine the isomeric form of LL-diaminopimelic acid and any major whole-organism sugars (Staneck & Roberts, 1974). A standard procedure was also used to detect the predominant isoprenoid quinone (Minnikin *et al.*, 1984). The antimicrobial activity of the test strain was determined against a range of bacteria and fungi, as described by Saintpierre *et al.* (2003).

Extraction of genomic DNA and PCR amplification and sequencing of a 16S rRNA gene from strain SFOp68^T was achieved using previously described procedures (Kim *et al.*, 1999). The resultant rRNA gene sequence was aligned manually using the PHYDIT program (Chun, 1995) against corresponding sequences of members of the family *Streptomycetaceae* (Kim *et al.*, 2003) retrieved from the DDBJ, EMBL and GenBank databases. Unrooted phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated as described by Jukes & Cantor (1969). Tree topologies were evaluated by a bootstrap analysis of the neighbour-joining dataset, based on 1000 resamplings, using the SEQBOOT and CONSENSE programs from the PHYLIP package. A partial nucleotide sequence (120 nt) of the tested strain based on the variable γ-region was compared with corresponding nucleotide sequences of *Streptomyces* strains retrieved from GenBank. A phylogenetic tree based on these sequences was generated using the neighbour-joining algorithm.

The chemical and morphological properties of isolate SFOp68^T are consistent with its classification in the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995). The organism forms an extensively branched substrate mycelium, aerial hyphae that differentiate into chains of spores, contains LL-diaminopimelic acid in the wall peptidoglycan, lacks characteristic major sugars and has octahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue. The assignment of the strain to the genus *Streptomyces* is also supported by the results of the 16S rRNA gene sequence studies.

Comparison of the almost complete 16S rRNA nucleotide gene sequence of strain SFOp68^T (1490 nt) with corresponding streptomycete sequences clearly shows that the organism forms a distinct phyletic line in the *Streptomyces* 16S rRNA gene tree irrespective of the tree-making algorithm used (Fig. 1). The isolate was most closely related to the type strain of *Streptomyces rimosus*: the two strains shared a 16S rRNA gene sequence similarity of 97·9 %, a value which corresponds to 31 nt differences at 1434 sites. The organism also shared relatively high 16S rRNA gene sequence similarity values with the other organisms shown in Fig. 1, notably with the type strains of *Streptomyces violaceusniger* (97·8 %), *Streptomyces yogyakartensis* (97·7 %), *Streptomyces javensis* (97·5 %), *Streptomyces albofaciens* (97·4 %), *Streptomyces auranticolor* (97·4 %), *Streptomyces cangkringensis* (97·4 %), *Streptomyces griseiniger* (97·4 %), *Streptomyces hygroscopicus* (97·4 %) and *Streptomyces phaeoluteogriseus* (97·4 %). DNA–DNA relatedness studies were not carried out between strain SFOp68^T and any of these organisms, as representatives of other pairs of *Streptomyces* species with similarly low 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). The sharp separation of strain SFOp68^T from representatives of *Streptomyces* species with validly published names was underpinned by the results from the 120 nt sequence analysis (data not shown) as, once again, the organism was found to be most closely related to the type strain of *Streptomyces rimosus*.

Strain SFOp68^T forms smooth, ornamented spores in loops and spirals (Fig. 2) and does not produce melanin pigments on peptone/yeast extract/iron agar; these properties are also shown by *Streptomyces rimosus* ISP 5260^T (Shirling & Gottlieb, 1968). However, the two organisms have markedly different phenotypic profiles: only the *Streptomyces rimosus* strain degrades elastin, uses adonitol, L(+)-arabinose, D(+)-cellobiose, D(+)-lactose, D(+)-melibiose and sodium citrate as sole carbon sources and grows in the presence of 5 % (w/v) sodium chloride (Williams *et al.*, 1983). However, isolate SFOp68^T, unlike the *Streptomyces rimosus* strain, grows at 45 °C.

It is evident that isolate SFOp68^T can be distinguished from *Streptomyces* species with validly published names,

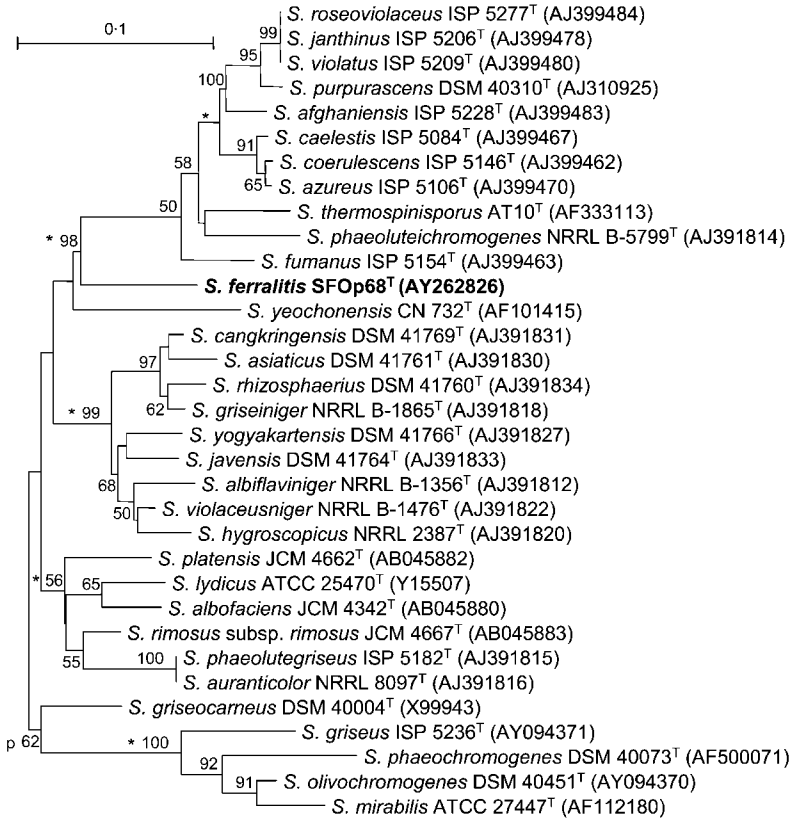

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987), based on nearly complete 16S rRNA gene sequences, showing the position of strain SFOp68^T in the streptomycete tree. Asterisks indicate branches that were also recovered using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms; p indicates a branch formed using the maximum-parsimony treeing method. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50 % are given. Bar, 0·1 nucleotide substitutions per nucleotide position.

notably *Streptomyces rimosus*, using a combination of genotypic and phenotypic properties. It is proposed, therefore, that this organism be given species status in the genus *Streptomyces* as *Streptomyces ferralitis* sp. nov.

Description of *Streptomyces ferralitis* sp. nov.

Streptomyces ferralitis (fer.ra′li.tis. N.L. gen. n. *ferralitis* of ferralite, denoting the type of soil from which the type strain was isolated).

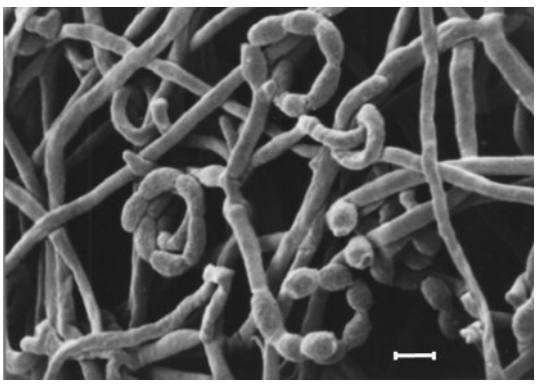

Fig. 2. Scanning electron micrograph of strain SFOp68^T showing looped to spiral chains of smooth-surfaced spores after 3 weeks growth at 30 °C on oatmeal agar. Bar, 1 μm.

Aerobic, Gram-positive actinomycete that forms an extensively branched substrate mycelium and aerial hyphae that differentiate into looped or spiral chains of spores. The spore chains consist of up to 15 barrel-shaped spores with smooth surfaces. A brown substrate mycelium and a white aerial spore mass are formed on MBA. Melanin pigments are not produced on peptone/yeast extract/iron agar. The culture grows well at 20 and 45 °C, but does not grow at 10 °C. Metabolizes casein, hypoxanthine, L-tyrosine, urea and xanthine, but not adenine, elastin, gelatin, guanine, starch, Tween 80 or xanthine. D(+)-Galactose, D(+)-glucose, D(+)-mannitol, D(+)-mannose and D(+)-trehalose are used as sole carbon sources for energy and growth, but adonitol, D-arabinose, D(+)-cellobiose, D(+)-melibiose and sodium citrate are not. The organism is resistant to penicillin (25 μg ml^{-1}), but does not grow in the presence of erythromycin (4 μg ml^{-1}), gentamicin sulphate (10 μg ml^{-1}), rifampicin (6 μg ml^{-1}), streptomycin sulphate (5 μg ml^{-1}), tetracycline hydrochloride (30 μg ml^{-1}), vancomycin hydrochloride (10 μg ml^{-1}), crystal violet (0·0002 %, w/v), phenol (0·01 %, w/v), potassium tellurite (0·005 %, w/v) or sodium chloride (5 %, w/v). It shows activity against clinical isolates of *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and a *Corynebacterium* strain, but not against *Fusarium oxysporum*, *Bacillus*, *Erwinia*, *Escherichia coli*, *Klebsiella pneumoniae* or *Pseudomonas aeruginosa* strains.

The type strain, SFOp68^T (=DSM 41836^T=NCIMB

13954^{T}), was isolated from a ferralitic, oxidic ultramafic soil collected at the southern end of the main island of New Caledonia. The species description is based upon a single strain and hence serves as the description of the type strain.

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