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The *Streptomyces violaceusniger* clade: a home for streptomycetes with rugose ornamented spores

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Abstract The taxonomic status of 16 strains received as *Streptomyces hygroscopicus*, *Streptomyces melanosporofaciens*, *Streptomyces sparsogenes*, *Streptomyces sporoclivatus* and *Streptomyces violaceusniger* was evaluated in a polyphasic study. Eleven of the organisms formed a distinct clade in the *Streptomyces* 16S rRNA gene tree with the type strains of *Streptomyces asiaticus*, *Streptomyces cangkringensis*, *Streptomyces indonesiensis*, *Streptomyces javensis*, *Streptomyces malaysiensis*, *Streptomyces rhizosphaericus*, *Streptomyces yatensis* and *Streptomyces yogyakartensis*, the members of this group produced rugose ornamented spores in spiral spore chains. The eleven strains were assigned to three established and four novel species, namely *Streptomyces albiflaviniger* sp. nov., *Streptomyces demainii* sp. nov., *Streptomyces geldanamycininus* sp. nov., *Streptomyces griseiniger* sp. nov., and *Streptomyces hygroscopicus*, *Streptomyces melanosporofaciens* and *Streptomyces violaceusniger*. It is also proposed that *S. sporoclivatus* becomes a subjective synonym of *S. melanosporofaciens*. *S. sparsogenes* NRRL 2940^T, which produced ridged ornamented spores in spiral spore chains, formed a distinct phyletic line in the *Streptomyces* 16S rRNA gene tree and was readily distinguished from the other strains using a range of phenotypic properties. *S. violaceusniger* strains NRRL 8097, NRRL B-5799, NRRL 2834 and ISP 5182 fell outside the *S. violaceusniger* 16S rRNA gene clade and formed either smooth or ridged ornamented spores in either flexuous or spiral spore chains. These organisms were distinguished

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the tested strains are *S. albiflaviniger* NRRL B-1356^T (AJ391812), *S. auratus* NRRL 8097^T (AJ391816), *S. geldanamycininus* NRRL 3602^T (DQ334781), *S. griseiniger* NRRL B-1865^T (AJ391818), *S. hygroscopicus* NRRL 2387^T (AJ391820), NRRL 2339 (AJ391821) and NRRL B-1477 (AJ391819), *S. demainii* NRRL B-1478^T (DQ334782), *S. melanosporofaciens* NRRL B-12234^T (AJ391837), *S. phaeogriseichromatogenes* NRRL 2834^T (AJ391813), *S. phaeoluteichromatogenes* NRRL B-5799^T (AJ391814), *S. phaeoluteigriseus* ISP 5182^T (AJ391815), *S. sparsogenes* NRRL 2940^T (AJ391817), *S. sporoclivatus* NRRL B-24330^T (AJ 781369), *S. violaceusniger* ISP 5563^T (AJ 391823) and NRRL B-1476^T (AJ 391822).

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from one another and from their closest phylogenetic neighbors and were considered to merit species status as *Streptomyces auratus* sp. nov., *Streptomyces phaeoluteichromatogenes* sp. nov., *Streptomyces phaeogriseichromatogenes* sp. nov., and *Streptomyces phaeoluteigriseus* sp. nov., respectively.

Keywords Streptomycetes · Polyphasic taxonomy · Rugose-ornamented spores

Introduction

Taxonomic relationships within the genus *Streptomyces* have been clarified by the application of genotypic and phenotypic methods (Goodfellow et al. 1992; Manfio et al. 1995; Anderson and Wellington 2001). It is now apparent that many streptomycete type strains can be assigned to distinct multimembered species-groups, as exemplified by species classified in the *Streptomyces albidoflavus* (Lanoot et al. 2005), *Streptomyces griseus* (Liu et al. 2005), *Streptomyces violaceoruber* (Duangmal et al. 2005), *Streptomyces violaceusniger* (Sembiring et al. 2000) and *Streptomyces yeochonensis* (Xu et al. 2006) 16S rRNA gene clades. Polyphasic taxonomic studies based on representatives of validly described species show the genus to be overspeciated (Hatano et al. 2003; Lanoot et al. 2002, 2004; Liu et al. 2005) though complementary studies on unknown streptomycetes show that the taxon as a whole is underspeciated (Manfio et al. 2003; Xu et al. 2006).

Members of the *S. violaceusniger* 16S rRNA gene clade form a gray aerial spore mass and a grayish-yellow substrate mycelium on oatmeal agar, and produce aerial hyphae that differentiate into spiral chains of rugose ornamented spores (Sembiring et al. 2000; Ward and Goodfellow 2004). The clade currently contains 13 validly described species that include *S. hygroscopicus* (Jensen 1931) Labeda and Lyons 1991, *S. malaysiensis* Al-Tai et al. 1999, *S. melanosporofaciens* Arcamone et al. 1959, *S. sporoclivatus* Preobrazhenskaya et al. 1986, *S. violaceusniger* (Waksman and Curtis 1916) Labeda and Lyons 1991 and *S. yatensis* Saintpierre et al. 2003. The remaining species, *S. asiaticus*, *S. cangkringensis*, *S. indonesiensis*, *S. javensis*, *S. rhizosphaericus* and *S. yogyakartensis*, were proposed following a taxonomic study of *S. violaceusniger*-like strains isolated from the rhizosphere of *Paraserianthes falcataria* (Sembiring et al. 2000), a tropical leguminous tree used for improvement of poor soils and the supply of wood pulp for paper. Phenotypic tests are available to distinguish between species classified in the *S. violaceusniger* 16S rRNA gene clade (Saintpierre et al. 2003).

Putative members of the *S. violaceusniger* 16S rRNA gene clade are considered to be relatively easy to recognize on media designed to be selective for streptomycetes as they form colonies with a gray aerial spore mass that subsequently turns black and mucilaginous. However, in a pilot study strains previously identified as *S. hygroscopicus* and *S. violaceusniger* were shown to be misclassified as they fell outside the *S. violaceusniger* 16S rRNA gene clade (Ward and Goodfellow 2004), a finding in line with DNA:DNA relatedness (Labeda and Lyons 1991) and numerical taxonomic (Kämpfer et al. 1991) data which show that strains assigned to these species form a heterogeneous group. The organisms outside the *S. violaceusniger* 16S rRNA gene clade have been considered to merit species status as *S. auranticolor*, *S. phaeogriseichromogenes*, *S. phaeoluteichromogenes* and *S. phaeoluteigriseus* (Sembiring et al. 2000; Ward and Goodfellow 2004) though these names do not have any formal nomenclatural standing. This also applies to species assigned to the *S. violaceusniger* 16S rRNA gene clade and designated as *S. albiflaviniger*, *S. griseiniger* and *S. geldanamycinus* (Sembiring et al. 2000; Ward and Goodfellow 2004).

It is important to clarify the taxonomy of *S. hygroscopicus*, *S. violaceusniger* and related strains, not least because members of the *S. violaceusniger* 16S rRNA gene clade are a source of antibacterial and antifungal metabolites (DeBoer et al. 1970; Lam et al. 1990; Tripathi et al. 2004), biological control agents (Trejo-Estrada et al. 1998a, b; Chamberlain and Crawford 1999), enantioselective biocatalysts (Molinari et al. 2005) and immunosuppressants, including rapamycin (Vezina et al. 1975). Authenticated members of the *S. violaceusniger* 16S rRNA gene clade show the same pattern of

HPLC-detected secondary metabolites, namely eliaophylin, geldanamycin, nigericin and a polyene (Ward and Goodfellow 2004), a result consistent with those of earlier studies (Allen and Ritchie 1994; Fang et al. 2000).

The present study was designed to establish the taxonomic status and relationships of 16 strains assigned to species classified in the *S. violaceusniger* 16S rRNA gene clade and 5 strains received as *S. violaceusniger* that fall outside the clade (Sembiring et al. 2000; Ward and Goodfellow 2004). These organisms were the subject of a polyphasic study which confirmed that five of them merited species status outside the *S. violaceusniger* 16S rRNA gene clade, the remaining ones were assigned to three novel and three established species within the clade.

Materials and methods

Organisms and cultural conditions

Sixteen strains received as *S. hygroscopicus* NRRL B-1477, NRRL 2339 and NRRL 2387^T, *S. hygroscopicus* subsp. *geldanus* NRRL 3602^T, *S. melanosporofaciens* NRRL B-12234^T, *S. sparsogenes* NRRL 2940^T, *S. sporoclivatus* NRRL B-24330^T and *S. violaceusniger* ISP 5182, ISP 5563, NRRL B-1356, NRRL B-1476, NRRL B-1478^T, NRRL B-1865, NRRL B-5799, NRRL 2834 and NRRL 8097 were studied. The organisms were maintained on glucose-yeast extract-malt extract agar (DSMZ Catalogue 1998) at 4°C and as glycerol suspensions (20%, v/v) at –20°C. Biomass for the 16S rRNA gene sequencing analyses was prepared by growing the test organisms on a non-sporulating agar medium (Sanglier et al. 1992) for 14 days at 28°C.

Sequencing of 16S rRNA genes

Extraction of genomic DNA and PCR-amplification of 16S rRNA genes from the 16 strains, and from the type strain of *S. indonesiensis*, were carried out as described by Pitcher et al. (1989), using the modifications of Sembiring (2000). The amplified fragments were purified with Nucleospin Extraction kits (Biogen Ltd.) and sequenced directly using ABI PRISM^R BigDye^{TM} Terminator Cycle Sequencing kits (Applied Biosystems) and previously described oligonucleotide primers (Lane 1991; Chun and Goodfellow 1995). Sequencing gel electrophoresis was carried out and the nucleotide sequences automatically obtained by using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer. The 16S rRNA gene sequences were aligned manually with available streptomycete nucleotide sequences retrieved from the DDBJ/EMBL/GenBank databases, using the pairwise alignment option and 16S rRNA secondary structure information held in the PHYDIT program (available at http://plaza.snu.ac.kr/~jchun/phydit/).

Unrooted phylogenetic trees based on almost complete nucleotide sequences were inferred by using the least-squares (Fitch and Margoliash 1967), maximum-likelihood (Felsenstein 1981), maximum-parsimony (Fitch 1972) and neighbor-joining (Saitou and Nei 1987) tree-making algorithms from the PHYLIP software package (Felsenstein 1993). Evolutionary distance matrices were generated for the neighbor-joining and least-squares methods, as described by Jukes and Cantor (1969) and the resultant tree topologies evaluated in a bootstrap analysis (Felsenstein 1985) based on 1000 resamplings from the neighbor-joining dataset, using the SEQBOOT and CONSENSE programs from the PHYLIP package (Felsenstein 1993). The root position of the unrooted neighbor-joining tree was estimated using *Kitasatospora kifunensis* NBRC 15206^T (accession number AJ 852052) as the outgroup.

Determination of DNA: DNA relatedness

Total genomic DNA for estimation of DNA-DNA relatedness values between nine pairs of organisms known to share high 16S rRNA gene sequences and two pairs of control strains was isolated from strains grown in Tryptone Soy broth for 7 days at 28°C. Cells were resuspended in 500 µl of 0.5 × TE buffer (pH8), approximately 400 µg of sterile glass beads added, and the preparations ribolised for 20 s at speed setting 4.0; 2.5 µl of lysozyme (50 mg/ml) and 5 µl of proteinase K (20 mg/ml) were added prior to incubation at 37°C for 2 h. The resultant preparations were centri-

fuged, the supernatants separated and incubated at 75°C for 15 min then cooled to room temperature; 20 µl of RNAse (20 mg/ml) solution was added before incubation at room temperature for 2 min. DNA was precipitated from the samples by adding 200 µl of cold 99.5% ethanol and purified using a GenElute™ Bacterial Genomic DNA Extraction kit (Sigma-Aldrich, St. Louis, U.S.A); the resultant DNA samples were eluted with 200 µl of sterile distilled water (pH8). DNA-DNA relatedness experiments were carried out between the selected pairs of strains (Table 1) by measuring the divergence between the thermal denaturation midpoint of homoduplex DNA and heteroduplex DNA (ΔT_m), as described by Gonzalez and Saiz-Jimenez (2005).

Partial RNA polymerase β-subunit gene sequencing

Genomic DNA for sequencing of a ~300 base pair segment of the *rpo*B (RNA polymerase β subchain) gene was isolated from strains grown on yeast extract-malt extract agar plates (ISP medium 2; Shirling and Gottlieb 1966) using UltraClean™ Microbial DNA Isolation kits (MoBio Laboratories Inc., Solana Beach, CA). The partial *rpo*B genes were amplified by using a modification of the procedure described by Kim et al. (2004) with the omission of loading buffer from the reaction mixture and amplicon clean-up performed using UltraClean™ PCR kits (MoBio Laboratories Inc.). Sequencing followed the procedure of Kim et al. (2004), the resultant partial *rpo*B sequences were deposited in GenBank under accession numbers DQ241992 through to DQ242018. The sequences were aligned against published sequences for taxa classified in the family *Streptomycetaceae* within ARB (Ludwig et al. 2004) and a phylogenetic tree constructed using the neighbor-joining method (Saitou and Nei 1987); the stability of the resultant groupings were estimated by bootstrap analysis (Felsenstein 1989). A maximum-likelihood phylogenetic tree was prepared after Felsenstein (1989), as modified by Olsen et al. (1994).

Table 1 Relationships found between closely related members of the *S. violaceusniger* 16S rRNA clade based on ΔT_m (°C) values together with approximate corresponding figures expressed as percentage DNA: DNA relatedness value

Strains	ΔT_m (°C)	DNA:DNA relatedness (%)	Nucleotide base differences /total number of 16S rRNA nucleotides
S. asiaticus DSM 41761^T versus *S. cangkringensis* DSM 41769^T	5.6	62	5/1449
S. cangkringensis DSM 41769^T versus *S. rhizosphaericus* DSM 41670^T	6.3	58	6/1449
S. cangkringensis DSM 41769^T versus *S. violaceusniger* NRRL B-1865	6.1	59	6/1449
S. hygroscopicus subsp. *geldanus* NRRL 3602^T versus *S. sporoclivatus* NRRL B-24330^T	7.0	55	11/1460
S. hygroscopicus subsp. *geldanus* NRRL 3602^T versus *S. violaceusniger* NRRL B-1865	6.9	56	17/1447
S. indonesiensis DSM 41759^T versus *S. rhizosphaericus* DSM 41670^T	5.8	61	4/1449
S. indonesiensis DSM 41759^T versus *S. rhizosphaericus* DSM 41670^T	5.8	61	4/1449
S. indonesiensis DSM 41759^T versus *S. violaceusniger* NRRL B-1865	5.6	62	4/1449
S. melanosporofaciens NRRL B-12234^T versus *S. hygroscopicus* subsp. *geldanus* NRRL 3602^T	6.6	57	9/1446
S. melanosporofaciens NRRL B-12234^T versus *S. sporoclivatus* NRRL B-24330^T	2.0	90	3/1448
S. melanosporofaciens NRRL B-12234^T versus *S. violaceusniger* NRRL B-1865	6.6	57	18/1448
S. violaceusniger NRRL B-1865 versus *S. rhizosphaericus* DSM 41670^T	6.1	59	6/1449

Morphology and cultural characteristics

The strains were grown on oatmeal (ISP medium 3; Küster 1959) and peptone-yeast extract-iron agar plates (ISP medium 6; Shirling and Gottlieb 1966) at 28°C for 14 and 4 days, respectively. The oatmeal agar plates were examined by eye to determine aerial spore mass color, substrate mycelial pigmentation and the color of any diffusible pigments; colors were recorded using National Bureau of Standards (NBS) Color Name Charts (Kelly 1958; NBS 1964). The peptone-yeast extract-iron agar plates were examined to see whether the strains produced melanin pigments.

Spore-chain morphology was determined by light and scanning electron microscopy (SEM) of 14-day-old cultures grown at 28°C on inorganic salts/starch agar (ISP medium 4; Difco). Spore-chain morphology was observed using a Nikon Optiphot binocular light microscope fitted with long working distance objectives; spore-chains were assigned to the morphological categories proposed by Pridham et al. (1958). Spore-surface ornamentation was determined on SEM preparations using a Cambridge Stereoscan 240 instrument; ornaments were assigned to either the categories of Tresner et al. (1961) or to the rugose section of Dietz and Mathews (1971). Light and SEM preparations were also examined for the presence of sclerotia, fragmentation of the substrate mycelium and for the formation of spores on the substrate mycelium. Aerial spore mass color, substrate mycelial pigmentation and the production of diffusible pigments were recorded on several agar media (Tables 2 and 3) after incubation for 14 days at 28°C.

Numerical taxonomy

The 24 tested strains consisted of the 16 organisms and 8 type strains of *Streptomyces* species known to produce rugose spores. The organisms were examined for 142 unit characters using the media and methods described by Williams et al. (1983). In addition, all the strains assigned to the *S. violaceusniger* 16S rRNA gene clade were examined for 19 enzymes using API® ZYM kits (bioMérieux, Inc, Durham, USA), following the manufacturer's guidelines. Unit characters were scored either plus (1) or minus (0) and the resultant data examined using the NTSYSpc program (version 2.0; Numerical Taxonomy and Multivariate Analysis System: Rohlf 1998) and the simple matching coefficient (S_{SM}; Sokal and Michener 1958), which includes both positive and negative matches. Clustering was achieved using the unweighted pair group method with arithmetic averages algorithm (UPGMA; Sneath an Sokal 1973). The final data matrix contained differential information on 24 strains and 92 unit characters. A S_{SM}, UPGMA analysis was carried out on the 19 strains assigned to the *S. violaceusniger* clade using 56 differential unit characters and the results presented as a dendrogram (Fig. 3).

Results

16S rRNA gene sequences

Eleven out of the 16 strains were assigned to the *S. violaceusniger* 16S rRNA gene clade, including the type strain of *S. sporoclivatus* (Fig. 1). The taxonomic integrity of the clade is supported by results from all of the tree-making algorithms and by a bootstrap value of 88% in the neighbor-joining analysis. The members of the clade shared 16S rRNA gene similarities within the range 97.8–100%.

The almost complete 16S rRNA gene sequences obtained for *S. violaceusniger* strains NRRL B-1476 and ISP 5563 were identical. *Streptomyces violaceusniger* NRRL B-1356 shared a 16S rRNA gene similarity with each of these strains of 99.7%, a value that corresponds to 4 nucleotide (nt) differences at 1449 locations. Similarly, *S. hygroscopicus* NRRL 1477, NRRL 2339 and NRRL 2387^T shared 16S rRNA gene similarities within the range 99.8% and 99.9%, values equivalent to up to two nt differences at 1465 sites. *Streptomyces violaceusniger* NRRL B-1478^T shared 16S rRNA gene similarities with the *S. hygroscopicus* strains within the range 99.7% to 99.9%, values which corresponded to between 2 and 4 nt differences at 1449 locations.

Other organisms which shared high 16S rRNA gene similarities included the type strains

Table 2 Color grouping and morphological characteristics of the tested strains*

Strain code	Strain name	Oatmeal agar			Inorganic salts/starch agar	
		Aerial spore mass color	Reverse color	Soluble pigment color	Spore chain morphology	Spore surface ornamentation
Members of the Streptomyces violaceusniger clade						
NRRL 2387^T	*S. hygroscopicus*	Gray	Grayish-yellow	None	Spiral	Rugose
NRRL 2339	*S. hygroscopicus*	Gray	Grayish-yellow	None	Spiral	Rugose
NRRL B-1477	*S. hygroscopicus*	Gray	Orange	Orange	Spiral	Rugose
NRRL 3602^T	*S. hygroscopicus* subsp. *geldanus*	Gray	Grayish-yellow	None	Spiral	Rugose
NRRL B-12234^T	*S. melanosporofaciens*	Gray	Yellow	None	Spiral	Rugose
NRRL B-24330^T	*S. sporoclivatus*	Gray	Grayish yellow	None	Spiral	Rugose
NRRL B-1356	*S. violaceusniger*	White	Yellow	None	Spiral	Rugose
NRRL B-1476	*S. violaceusniger*	Gray	Grayish-yellow	None	Spiral	Rugose
NRRL B-1478^T	*S. violaceusniger*	Gray	Grayish-yellow	None	Spiral	Rugose
ISP 5563	*S. violaceusniger*	Gray	Grayish-yellow	None	Spiral	Rugose
NRRL B-1865	*S. violaceusniger*	Gray	Grayish-yellow	None	Spiral	Rugose
Strains outside of the Streptomyces violaceusniger clade						
NRRL 2940^T	*S. sparsogenes*	Gray	Grayish-yellow	None	Spiral	Ridged
ISP 5182	*S. violaceusniger*	Gray	Yellowish-brown	Yellow	Flexuous	Smooth
NRRL 2834	*S. violaceusniger*	White	Yellow	None	Flexuous	Smooth
NRRL B-5799	*S. violaceusniger*	Brown	Yellow	Yellow	Flexuous	Smooth
NRRL 8097	*S. violaceusniger*	Gray	Grayish-orange	Orange	Spiral	Smooth

**Streptomyces violaceusniger* ISP 5182 was the only strain to produce melanin pigments on peptone-yeast extract-iron agar

of *S. asiaticus* and *S. cangkringensis* (99.7% similarity; 5 nt differences), *S. cangkringensis* and *S. rhizosphaericus* (99.6% similarity; 6 nt differences) and *S. melanosporofaciens* and *S. sporoclivatus* (99.8% similarity, 3 nt differences). *Streptomyces hygroscopicus* subsp. *geldanus* NRRL 3602^T showed its closest 16S rRNA gene similarities with the type strains of *S. melanosporofaciens* (99.7%, 9 nt differences) and *S. sporoclivatus* (99.3%, 11 nt differences). High 16S rRNA gene similarities were also found between *S. violaceusniger* NRRL B-1865 and *S. cangkringensis* NRRL 41769^T (99.6%, 6nt differences), *S. indonesiensis* DSM 41759^T (99.7%, 4 nt differences) and *S. rhizosphaericus* DSM 41670^T (99.6%, 6 nt differences). The remaining members of the *S. violaceusniger* 16S rRNA gene clade had relatively low similarities both with one another and with the above mentioned strains. The strains which fell outside the *S. violaceusniger* 16S rRNA gene clade, namely, *S. sparsogenes* NRRL 2940^T and *S. violaceusniger* ISP 5182, NRRL 2834, NRRL B-5799 and NRRL 8097 shared relatively low 16S rRNA gene similarities with one another (range 95.7–98.3%) and with members of the *S. violaceusniger* clade (range 95.3–97.6%).

DNA:DNA relatedness studies

With a single exception the DNA:DNA relatedness studies carried out by thermal denaturation on phylogenetically close strains revealed differences in melting temperature above the cut-off point recommended for the delineation of genomic species ($\Delta T_m > 5.0°C$; Wayne et al. 1987), as shown in Table 1. It is evident from these results that the type strains of *S. melanosporofaciens* and *S. sporoclivatus* belong to a single genomic species (ΔT_m 2.0°C; corresponding to approximately 90% relatedness) through the remaining pairs are quite closely related with ΔT_m values within the range 5.6–7.0°C which corresponds to approximately 62–55% relatedness.

Partial RNA polymerase β-subunit gene sequences

The results based on the partial *rpo*B sequences are shown in Fig. 2. It is apparent that

Table 3 Growth and cultural characteristics of the tested strains

Strains	Growth and cultural characteristics	Modified Bennett's agar	Glucose-yeast extract-malt extract agar	Glycerol asparagine agar	Inorganic salts starch agar	Tyrosine agar	Yeast extract-malt extract agar
Members of the Streptomyces violaceusniger clade							
S. hygroscopicus NRRL 2387^T	Growth:	++	+++	+++	+++	+++	++
	Aerial mycelium:	White: Sparse	Gray: Abundant	White: Sparse	White-gray: Abundant	White: Abundant	White-gray: Moderate
	Reverse colour:	Colourless	Brown	Colourless	Grayish-yellow	Yellow	Brown
	Soluble pigment:	None	Brown	None	None	None	Brown
S. hygroscopicus NRRL 2339	Growth:	+	++	++	+++	++	++
	Aerial mycelium:	None	White-gray Sparse	None	White-gray: Moderate	White: Moderate	White: Sparse
	Reverse colour:	Colourless	Brown	Colourless	Yellow-orange-gray	Yellowish-white	Brown
	Soluble pigment:	None	Brown	None	None	None	Brown
S. hygroscopicus NRRL B-1477	Growth:	++	++	++	+++	++	++
	Aerial mycelium:	White: Sparse	White-gray: Moderate	None	White-gray-brown: Abundant	White: Moderate	White: Sparse
	Reverse colour:	Yellow	Orange-brown	Colourless	Orange-gray	Light brown	Brown
	Soluble pigment:	None	Brown	None	None	None	Brown
S. hygroscopicus subsp. *geldanus* NRRL 3602^T	Growth:	+++	+++	++	+++	+++	+++
	Aerial mycelium:	White: Sparse	Brownish-white: Abundant	Gray: Sparse	White-gray-brown: Abundant	White: Abundant	White: Abundant
	Reverse colour:	Yellow	Brown	Colourless	Orange-brown	Dark brown	Yellow
	Soluble pigment:	Yellow	Brown	None	None	Reddish-brown	Yellow
S. melanosporofaciens NRRL B-12234^T	Growth:	++	++	+	++	+++	++
	Aerial mycelium:	White: Sparse	Gray: Moderate	White: Sparse	White-gray: Moderate	White-gray: Abundant	Gray: Moderate
	Reverse colour:	Yellow	Light brown	Light brown	Yellow	Brown	Light brown
	Soluble pigment:	None	None	None	None	Brown	None
S. sporoclivatus NRRL B-24330^T	Growth:	++	++	++	++	++	+++
	Aerial mycelium:	None	White: Moderate	None	White: Abundant	White gray: Moderate	White: Moderate
	Reverse colour:	Light yellow-orange	Brown	Yellow	Orange brown	Light yellow-brown	Brownish orange
	Soluble pigment:	None	None	None	None	None	None
S. violaceusniger NRRL B-1356	Growth:	+++	+++	+	+	+++	+++
	Aerial mycelium:	White: Sparse	Gray white: Abundant	White: Sparse	White-gray: Sparse	White: Abundant	White-gray: Abundant
	Reverse colour:	Yellow	Brown	Colourless	Orange-gray	Orange	Brown
	Soluble pigment:	None	None	None	Orange	Yellow	Orange

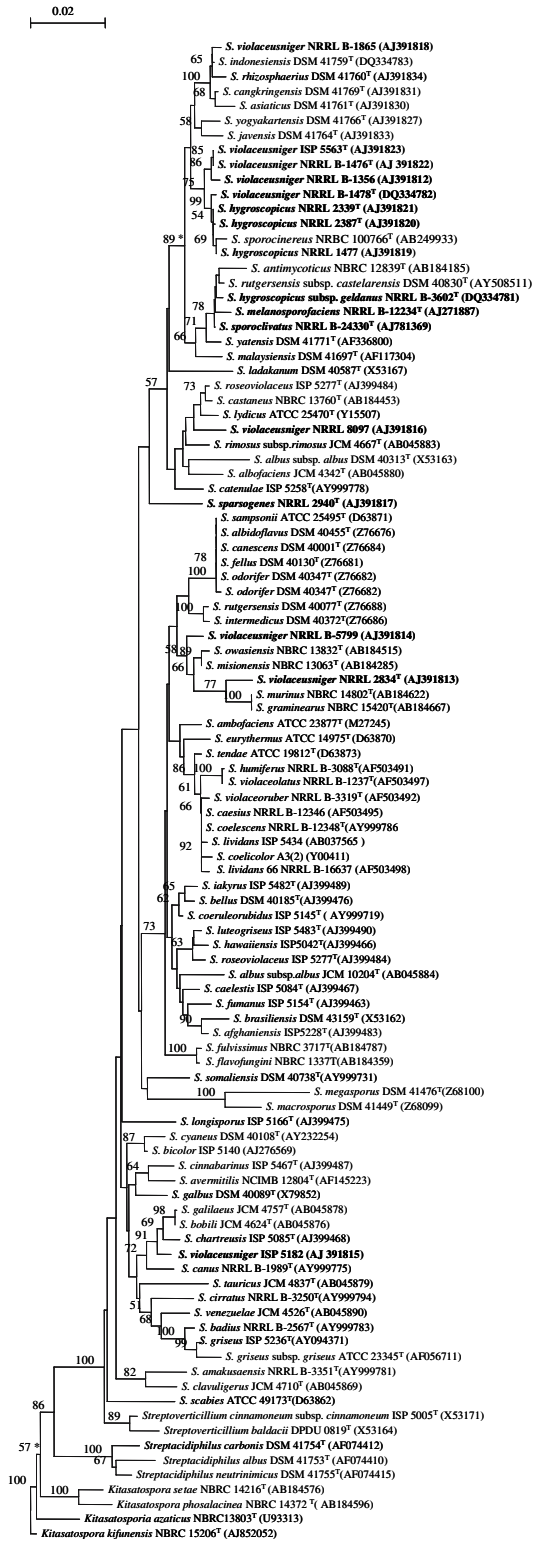
Table 3 continued

Strains	Growth and cultural characteristics	Modified Bennett's agar	Glucose-yeast extract-malt extract agar	Glycerol asparagine agar	Inorganic salts starch agar	Tyrosine agar	Yeast extract-malt extract agar
S. violaceusniger NRRL B-1476	Growth:	+	+++	++	+++	+++	+++
	Aerial mycelium:	White: Sparse	White-brown: Moderate	None	White-gray: Abundant	White-brown: Abundant	White: Moderate
	Reverse colour:	Colourless	Brown	Colourless	Yellowish-gray	Brown	Yellow
	Soluble pigment:	None	None	None	None	Reddish-brown	Yellow
S. violaceusniger NRRL B-1478^{T}	Growth:	+++	+++	++	+++	+++	+++
	Aerial mycelium:	White: Abundant	White-gray: Abundant	White: Moderate	White-gray: Abundant	White-gray: Abundant	White-gray: Abundant
	Reverse colour:	Brownish-black	Brownish-black	Colourless	Grayish-yellow	Light brown	Brown
	Soluble pigment:	Brown	Brown	None	Orange yellow	Grayish yellow	Yellow
S. violaceusniger ISP 5563	Growth:	+	+++	++	+++	+++	+++
	Aerial mycelium:	White: Sparse	White-brown: Abundant	None	White-gray: Abundant	White-brown: Abundant	White: Moderate
	Reverse colour:	Colourless	Brown	Colourless	Grayish-yellow	Brown	Yellow
	Soluble pigment:	None	None	None	None	Reddish-brown	Yellow
S. violaceusniger NRRL B-1865	Growth:	+	+++	++	+	++	+++
	Aerial mycelium:	White: Sparse	White-gray: Sparse	White: Sparse	Gray-white: Sparse	Whitish-gray: Moderate	White: Sparse
	Reverse colour:	Colourless	Yellow	Yellow	Yellow-orange	Brown	Yellow
	Soluble pigment:	None	None	None	Orange	None	Yellow
Strains outside of the Streptomyces violaceusniger clade							
S. violaceusniger ISP 5182	Growth:	++	+++	+++	++	++	+
	Aerial mycelium:	None	White: Sparse	None	Gray: Moderate	Gray: Moderate	White: Sparse
	Reverse colour:	Colourless					
	Soluble pigment:	None	Brown	Brown	Yellow-orange-gray	Dark brown	Brown
			None	None	Orange	Dark brown	Brown
S. violaceusniger NRRL 2834	Growth:	+++	++	++	+++	+++	+++
	Aerial mycelium:	None	Grayish-white: Sparse	White: Moderate	White: Abundant	Gray-brown: Abundant	White: Moderate
	Reverse colour:	Colourless	Yellow-brown	Yellow	Yellow	Whitish-brown	Yellow
	Soluble pigment:	None	None	None	None	Reddish-brown	Yellow

Table 3 continued

Strains	Growth and cultural characteristics	Modified Bennett's agar	Glucose-yeast extract-malt extract agar	Glycerol asparagine agar	Inorganic salts starch agar	Tyrosine agar	Yeast extract-malt extract agar
S. sparsogenes NRRL 2940^T	Growth:	++	++	++	+	++	++
	Aerial mycelium:	None	Gray-yellow: Sparse	None	Grayish-yellow: Sparse	Grayish-yellow: Sparse	White: Sparse
	Reverse colour:	Colourless	Orange-brown	Colourless	Gray	Gray-black	Yellow-orange
	Soluble pigment:	None	None	None	None	Reddish-brown	Yellow
S. violaceusniger NRRL B-5799	Growth:	++	+++	++	++	+++	+++
	Aerial mycelium:	None	White-brown: Abundant	None	White-brown Moderate	Whitish-gray: Abundant	White-brown: Abundant
	Reverse colour:	Colourless	Yellow	Colourless	Gray-brown	Yellowish white-brown	Brown
	Soluble pigment:	None	None	None	None	None	None
S. violaceusniger NRRL 8097^T	Growth:	+++	+++	++	+++	+++	+++
	Aerial mycelium:	White: Sparse	Grayish-white: Abundant	White: Sparse	White-gray: Abundant	White-brown: Abundant	White Moderate
	Reverse colour:	Yellow	Brown	Colourless	Grayish-yellow	Brown	Brownish-orange
	Soluble pigment:	None	Yellow	None	None	Brownish-red	None

Key: +++: abundant growth; ++: moderate growth; +: poor growth; ±: sparse growth



◄**Fig. 1** Neighbor-joining tree showing relationships between the tested strains and between them and representatives of the genus *Streptomyces* based on almost complete 16S rRNA gene sequences. The asterisks denote branches that were recovered using the least- squares (Fitch and Margoliash 1967), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1972) tree-making algorithms. The numbers at the nodes indicate the levels of bootstrap support (%) based on a neighbor-joining analysis of 1,000 resampled datasets. The tested strains are given in bold. The arrow gives the estimated root position of the tree. The scale bar indicates 0.02 nucleotide substitutions per nucleotide position. GenBank accession numbers are given in parentheses

S. violaceusniger strains ISP 5182, NRRL B-5799 and NRRL B-8097 form single membered clusters. These organisms were found to be even more distant and separate from the other strains shown in Fig. 2 in the analysis of partial *rpo*B sequences available for the family *Streptomycetaceae* (data not shown). The remaining strains under study form two major clades, one of which encompassed *S. hygroscopicus* strains NRRL B-1477 and NRRL 2339 and *S. violaceusniger* strains NRRL B-1478 and NRRL 2834 and the other the rest of the tested strains, apart from those that formed single membered clusters. It is not clear why the partial *rpo*B sequence of *S. hygroscopicus* NRRL 2387^T is so different from those of *S. hygroscopicus* (formerly *S. endus*) NRRL 2339 and *S. hygroscopicus* NRRL B-1477.

It was determined experimentally that the third codon position of the *rpo*B sequences accounted for most of the variability and that the distance tree calculated without this position was very close to that determined from the amino acid translation of those sequences (data not shown). With the elimination of the third codon position, *S. violaceusniger* strains ISP 5182, NRRL 8097 and NRRL B-5799 are still recovered as single membered clusters, the remaining strains form clades corresponding to the results obtained for all of the codon positions.

Morphological and cultural characteristics

Most of the 16 tested strains grew well on a range of agar media, but only *S. violaceusniger* ISP 5182

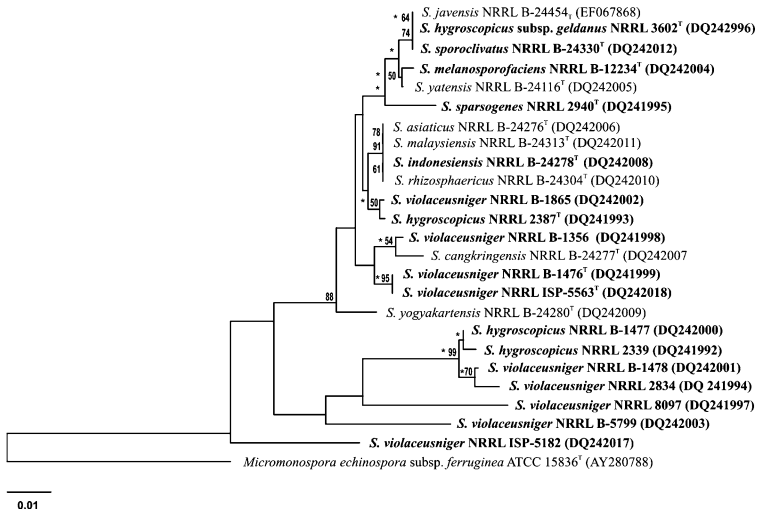


Fig. 2 Neighbor-joining tree showing relationships between the tested strains and representatives of the genus *Streptomyces* based on partial nucleotide sequences (301 bp) of the RNA polymerase β-subunit gene (*rpo*B). Percentages at the nodes represent levels of bootstrap support from 1,000 resampled datasets; values less than 50% are not shown. Asterisks indicate the corresponding branches that were also recovered in the maximum-likelihood tree. The tested strains are given in bold. The scale bar indicates 0.01 nucleotide substitutions per nucleotide position. GenBank accession numbers are given in parentheses

produced melanin pigments on peptone-yeast extract-iron agar (Tables 2, 3). Eleven of the strains formed rugose ornamented spores in spiral chains on inorganic salts/starch agar; three out of the nine *S. violaceusniger* strains produced flexuous spore chains composed of either smooth or ridged ornamented spores. S*treptomyces sparsogenes* NRRL 2940^{T} and *S. violaceusniger* NRRL 8097 produced ridged and smooth surfaced spores, respectively in spiral chains.

Numerical taxonomy

The 24 tested strains produced catalase; degraded DNA, elastin, gelatin, RNA, starch and tributyrin, but not keratin or pectin; grew on *meso*-erythritol, D-trehalose and D-xylose as sole sources of carbon, but not on aesculin, arbutin, D-arabinose, glucosamine, inulin, malonic acid, L-sorbose or pectin (all at 1%, w/v); grew in the presence of phenol (0.01%, w/v) and sodium chloride (4, 7 and 10%, w/v), but not at 4°C; and were resistant to ampicillin (32 and 64 μg ml^{-1}), cephalosporin (32 and 64 μg ml^{-1}), penicillin (10 and 20 μg ml^{-1}) and tetracycline hydrochloride (32 and 64 μg ml^{-1}), but sensitive to gentamicin sulphate, kanamycin sulphate, neomycin sulphate, streptomycin sulphate and tobramycin sulphate (all at 8 and 32 μg ml^{-1}).

It was clear from the initial numerical taxonomic analysis that the organisms fell into two aggregate groups, one encompassed all of the strains assigned to the *S. violaceusniger* 16S rRNA gene clade and the other organisms that fell outside the clade (data not shown). All of the strains assigned to the *S. violaceusniger* 16S rRNA gene clade hydrolyzed urea; produced hydrogen sulphide; degraded arbutin, but not chitin, xanthine or xylan; used D-mannitol and D-mannose as sole carbon sources (all at 1%, w/v), and L-valine (0.1%, w/v) as a sole carbon and nitrogen source. They also produced acid phosphatase, *N*-acetyl-β-glucosaminidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, but not cystine arylamidase, α-glucosidase, β-glucuronidase, α-fucosidase, lipase, α-mannosidase or valine arylamidase. The all positive and all negative properties were removed from the initial phenotypic database and the remaining 58 unit characters (Table 4) examined in a S_{SM}, UPGMA analysis.

Table 4. Phenotypic properties that separate members of the *Streptomyces violaceusniger* 16S rRNA gene clade

Phenotypic tests	*S. asiaticus* DSM 41761^T	*S. cangkringensis* NRRL 41769^T	*S. hygroscopicus* NRRL 23877^T	*S. hygroscopicus* NRRL 2339	*S. hygroscopicus* NRRL B-1477	*S. hygroscopicus* subsp. *geldanus* NRRL 3602^T	*S. indonesiensis* DSM 41759^T	*S. javensis* DSM 41764^T	*S. malaysiensis* DSM 41697^T	*S. melanosporofaciens* NRRL B-12234^T	*S. sporoclivatus* NRRL B-24330^T	*S. rhizosphaericus* DSM 41760^T	*S. violaceusniger* ISP 5563	*S. violaceusniger* NRRL B-1476	*S. violaceusniger* NRRL B-1356	*S. violaceusniger* NRRL B-1478^T	*S. violaceusniger* NRRL B-18653	*S. yatensis* DSM 41771^T	*S. yogyakartensis* DSM 41766^T
Enzyme activity:																			
Aesculin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
α-chymotrypsin	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
β-galactosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β-glucosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	+	+	+	-	+	+	+	-	-	-	+	-	-	+	+	-	+
Degradation tests(%, w/v):																			
Adenine (0.4)	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Casein (1)	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
Guanine (0.05)	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
Hypoxanthine (0.4)	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
Tween 20 (1%v/v)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Tween 40 (1%v/v)	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+
Tween 60 (1%v/v)	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
Tween 80 (1%v/v)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Uric acid (0.5%)	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+

Table 4 continued

Phenotypic tests	*S. asiaticus* DSM 41761^T	*S. cangkringensis* NRRL 41769^T	*S. hygroscopicus* NRRL 2387^T	*S. hygroscopicus* NRRL 2339	*S. hygroscopicus* NRRL B-1477	*S. hygroscopicus* subsp. *geldanus* NRRL 3602	*S. indonesiensis* DSM 41759^T	*S. javensis* DSM 41764^T	*S. malaysiensis* DSM 41697^T	*S. melanosporofaciens* NRRL B-12234^T	*S. sporoclivatus* NRRL B-24330^T	*S. rhizosphaericus* DSM 41760^T	*S. violaceusniger* ISP 5563^T	*S. violaceusniger* NRRL B-1476^T	*S. violaceusniger* NRRL B-1356	*S. violaceusniger* NRRL B-1478	*S. violaceusniger* NRRL B-1865	*S. yatensis* DSM 41771^T	*S. yogyakartensis* DSM 41766^T
Growth on sole carbon sources at 1% w/v																			
Adonitol	+	-	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	-	+
Amygdalin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
L(+)Arabinose	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+
D(+)Arabitol	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
D(+)Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+
Dextrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
D(-) Fructose	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+
L(-)Fucose	+	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+
α-Lactose	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+
D(+) Melezitose	-	-	-	-	-	+	-	+	-	+	+	-	-	-	+	-	-	+	+
Salicin	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+
D- sorbitol	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

Table 4 continued

Phenotypic tests	*S. asiaticus* DSM 41761^T	*S. cangkringensis* NRRL 41769^T	*S. hygroscopicus* NRRL 2387^T	*S. hygroscopicus* NRRL 2339	*S. hygroscopicus* NRRL B-1477	*S. hygroscopicus* subsp. *geldanus* NRRL 3602	*S. indonesiensis* DSM 41759^T	*S. javensis* DSM 41764^T	*S. malaysiensis* DSM 41697^T	*S. melanosporofaciens* NRRL B-12234^T	*S. sporoclivatus* NRRL B-24330^T	*S. rhizosphaericus* DSM 41760^T	*S. violaceusniger* I SP 5563^T	*S. violaceusniger* NRRL B-1476^T	*S. violaceusniger* NRRL B-1356	*S. violaceusniger* NRRL B-1478	*S. violaceusniger* NRRL B-1865	*S. yatensis* DSM 41771^T	*S. yogyakartensis* DSM 41766^T
Growth on sole nitrogen sources at 1% w/v																			
L(-) Alanine	+	-	-	+	-	-	-	+	+	-	-	-	+	+	-	+	+	+	+
α-Alanine	+	+	-	+	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+
L(-)Aminobutyric acid	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
L(-)Glycine	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
L(-)Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
L(-)Leucine	+	+	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-	+	+
DL(-)Methionine	-	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-
DL(-)Norleucine	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-
L(-)Phenylalanine	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	-	-	+
L(-)Serine	-	+	+	+	+	-	+	+	+	-	-	-	+	+	+	+	-	+	+
L(-) Tryptophan	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+

Table 4 continued

Phenotypic tests	*S. asiaticus* DSM 41761^T	*S. cangkringensis* NRRL 41769^T	*S. hygroscopicus* NRRL 2387^T	*S. hygroscopicus* NRRL 2339	*S. hygroscopicus* NRRL B-1477	*S. hygroscopicus* subsp. *geldanus* NRRL 3602	*S. indonesiensis* DSM 41759^T	*S. javensis* DSM 41764^T	*S. malaysiensis* DSM 41697^T	*S. melanosporofaciens* NRRL B-12234^T	*S. sporoclivatus* NRRL B-24330^T	*S. rhizosphaericus* DSM 41760^T	*S. violaceusniger* ISP 5563^T	*S. violaceusniger* NRRL B-1476^T	*S. violaceusniger* NRRL B-1356	*S. violaceusniger* NRRL B-1478	*S. violaceusniger* NRRL B-1865	*S. yatensis* DSM 41771^T	*S. yogyakartensis* DSM 41766^T
Growth at																			
pH 4.0	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-
pH5	-	+	+	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
pH 9	-	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	-
pH 10	+	+	-	-	-	-	+	-	+	-	-	+	+	-	-	+	-	+	-
10°C	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-
Antibiotics (μg/ml)																			
Cefoxitin (64)	-	+	+	-	+	+	-	+	-	+	-	+	+	+	-	-	-	-	+
Cephaloridine (32)	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+
Cephaloridine (64)	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+
Erythromycin (32)	+	+	-	-	-	-	+	+	+	-	+	+	+	-	-	-	+	+	+
Erythromycin (64)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Oleandomycin (32)	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Oleandomycin (64)	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	+	+
Chlortetracycline (4)	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	+	+	+
Chlortetracycline (8)	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	+	+	+
Doxycycline (4)	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
Rifampicin (32)	+	+	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
Rifampicin (64)	+	-	-	-	-	+	+	-	+	-	-	+	-	-	-	+	-	+	+
Lincomycin hydrochloride (32)	-	-	-	-	-	+	+	-	+	+	+	-	-	-	+	+	+	+	+
Lincomycin hydrochloride(64)	-	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-
Novobiocin (8)	+	+	-	-	+	+	+	+	-	+	-	+	+	+	-	-	+	+	+

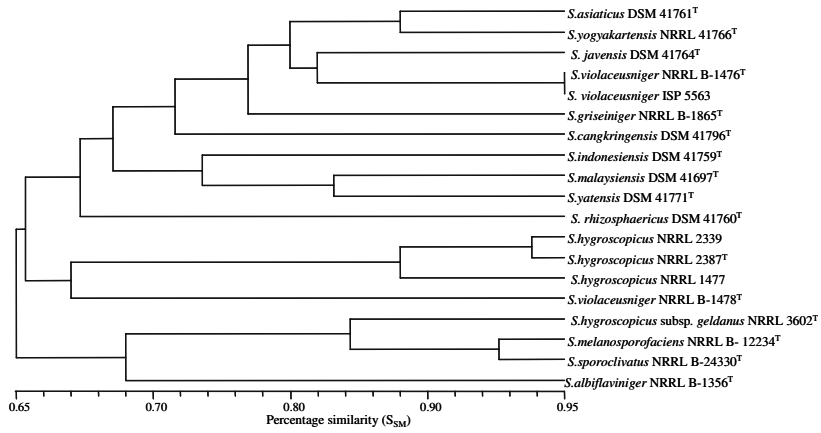


Fig. 3 Dendrogram showing relationships between members of the *Streptomyces violaceusniger* 16S rRNA gene clade based on an analysis of phenotypic properties using the S_{SM},UPGMA analysis

It is evident from Fig. 3 that the strains classified in the *S. violaceusniger* 16S rRNA gene clade can be assigned to 4 multimembered and 10 single-membered clusters defined at or above the 90% similarity (S-) level. Multimembered clusters were formed between the type strains of *S. asiaticus* and *S. yogyakartensis* at the 88% S- level, between *S. hygroscopicus* strains NRRL 1477, NRRL 2339 and NRRL 2387^T also at the 88% S- level, between *S. violaceusniger* strains NRRL B-1476 and ISP 5563 at the 100% S-level, and between *S. melanosporofaciens* NRRL B-12234^T and *S. sporoclivatus* NRRL B-24330^T just above the 95% S-level. The differential phenotypic properties of the strains assigned to the *S. violaceusniger* 16S rRNA gene clade are shown in Table 4; those marked with an asterisk distinguish between members of putatively novel and established species.

Discussion

The circumscription of *Streptomyces* species needs to be based on a judicious combination of genotypic and phenotypic data (Manfio et al. 1995, 2003; Atalan et al. 2000; Liu et al. 2005). It is, therefore, encouraging that good congruence was found between the phenetic and phylogenetic data as all of the organisms assigned to the *S. violaceusniger* 16S rRNA gene clade formed a distinct multimembered cluster based on the numerical taxonomic analysis of the differential phenotypic properties. In contrast, the five organisms found outside the *S. violaceusniger* 16S rRNA gene clade formed a heterogeneous group. These results provide further evidence that members of the *S. violaceusniger* 16S rRNA gene clade can be distinguished from all other streptomycetes using a range of phenotypic properties, notably by their ability to form spiral chains of rugose ornamented spores and distinctive pigments on oatmeal agar. The colonial properties are significant as streptomycete groups based on aerial spore mass color, colony reverse and diffusible pigment colors on oatmeal agar, and the ability to form melanin pigments on peptone-yeast extract-iron agar, are predictive as representatives of streptomycete color groups key out to either established or novel species based on chemotaxonomic and computer-assisted identification procedures (Goodfellow and Haynes 1984; Williams and Vickers 1988; Atalan et al. 2000; Manfio et al. 2003; Liu et al. 2005).

The taxonomic integrity of the *S. violaceusniger* 16S rRNA gene clade was supported by results from all of the tree-making algorithms and by a high bootstrap value in the neighbor-joining analysis, a result that confirms and extends those from previous studies (Sembiring et al. 2000; Saintpierre et al. 2003; Ward and Goodfellow 2004). The phylogeny of the strains under study based on partial *rpo*B sequences was not completely congruent with the tree computed from 16S rRNA gene sequences, a similar finding was reported by Rickert et al. (2007) in a molecular analysis of *Aureobacterium* and *Microbacterium* strains. It appears that partial *rpo*B sequences have value for distinguishing between members of

phylogenetically distant *Streptomyces*, but care must be taken in interpreting data for closely related streptomycetes.

It is evident from the 16S rRNA gene tree that some of the organisms have either identical or almost identical sequences; representatives of those with almost identical 16S rRNA gene sequences were examined in DNA:DNA relatedness studies using the thermal denaturation procedure of Gonzalez and Saiz-Jimenez (2005). The results obtained in comparisons between *S. violaceusniger* NRRL B-1865 and *S. hygroscopicus* subsp. *geldanus* NRRL 3602^T and *S. melanosporofaciens* NRRL B-12234^T were in good agreement with those reported by Labeda and Lyons (1991) who used a spectrophotometric procedure. Congruent results of a similar nature have been reported for *Phyllobacterium* strains (Jurado et al. 2005).

Streptomyces violaceusniger ISP 5563 and NRRL B-1476, its reported parent strain, gave identical 16S rRNA and partial *rpo*B gene sequences and an identical phenotypic profile. These results are in stark contrast to those reported by Labeda and Lyons (1991) who found that strains designated NRRL B-1476 and ISP 5563 shared a DNA: DNA relatedness value of only 46%. They also found that *S. violaceusniger* NRRL B-1476 and ISP 5563 shared DNA:DNA relatedness values of 39% and 97% with a strain designated *S. violaceusniger* NRRL B-1478 which they considered to be the type strain of the species. Labeda and Lyons (1991) attributed the apparently anomalous results between *S. violaceusniger* strains NRRL B-1476 and ISP 5563 to strains NRRL B-1476 and NRRL B-1478 having been switched when they were lyophilised on the same day in 1958. The ampoule submitted to E.B. Shirling, which was designated ISP 5563, was from this set of lyophilized cultures. However, this interpretation is not supported by the present data which show a commonality of properties between *S. violaceusniger* NRRL B-1476 and ISP 5563. These results are consistent with the original view that strain ISP 5563 is derived from strain NRRL B-1476. This means that the reference material sent directly from the International *Streptomyces* Project to the American Type Culture Collection, the Deutsche Sammlung von Mikroorganismen und Zellkulturen and the Japanese Collection of Microorganisms, and subsequently designated ATCC 27477, DSM 40563 and JCM 4092, respectively was derived from *S. violaceusniger* NRRL B-1476. It also means that *S. violaceusniger* NRRL B-1476 is the type strain of the species.

Streptomyces hygroscopicus strains NRRL B-1477, NRRL 2339 and NRRL 2387^T have almost identical 16S rRNA gene sequences and similar phenotypic profiles, results in good agreement with DNA:DNA relatedness data (Labeda and Lyons 1991). These workers found that *S. hygroscopicus* strains NRRL B-1477 and NRRL 2339 shared DNA relatedness values of 83% and 92%, respectively with DNA from *S. hygroscopicus* NRRL 2387^T. The minimum level of DNA relatedness between strains required to define a genomic species is recommended as 70% (Wayne et al. 1987) though extensive DNA:DNA studies of *Streptomyces* strains implied that genomic homologies greater than 80% may correspond to species level relatedness in this genus (Labeda 1993, 1998; Labeda and Lyons 1992). These data underpin the taxonomic integrity of *S. hygroscopicus* (Jensen 1931) Labeda and Lyons 1991 and provide further evidence that members of the *S. violaceusniger* clade can be assigned to species using a combination of 16S rRNA gene sequence and phenotypic data (Sembiring et al. 2000; Saintpierre et al. 2003).

The type strains of *S. melanosporofaciens* and *S. sporoclivatus* formed a distinct multimembered cluster in the numerical taxonomic study; *S. hygroscopicus* subsp. *geldanus* NRRL 3602^T was loosely associated with this taxon. These organisms formed a subclade in the 16S rRNA gene tree, and grouped together in the *rpo*B tree together with the type strains of *S. javensis* and *S. yatensis*. DNA:DNA relatedness studies are needed to resolve relationships between such phylogenetically close streptomycetes, as exemplified by studies on members of the *S. griseus* 16S rRNA gene clade (Liu et al. 2005). The type strains of *S. hygroscopicus* subsp. *geldanus* and *S. melanosporofaciens* belong to different genomic species though they share the relatively high DNA:DNA relatedness value of 64% (Labeda and Lyons 1991); these organisms can also be distinguished from one another and from the type

strain of *S. javensis* using a range of phenotypic characters (Table 4). In contrast, it is clear from the present study that *S. melanosporofaciens* NRRL 12234^T and *S. sporoclivatus* NRRL B-24330^T belong to the same genomic species; these organisms were grouped together using 16S-ITS RFLP fingerprint data (Lanoot et al. 2004). It is, therefore, proposed that *S. sporoclivatus* Preobrazhenskaya et al. 1986 should become a subjective synonym of *S. melanosporofaciens* Arcamone et al. 1957. It is evident from the genotypic and phenotypic data that *S. hygroscopicus* subsp. *geldanus* NRRL 3602^T merits recognition as a new species.

The type strains of *S. malaysiensis* and *S. yatensis* were loosely associated with members of the *S. melanosporofaciens* 16S rRNA gene subclade and with one another in the numerical phenetic study, but were sharply separated in the *rpo*B gene tree. *Streptomyces malaysiensis* NRRL B-24313^T had identical partial *rpo*B sequences with the type strains of *S. asiaticus*, *S. indonesiensis* and *S. rhizosphaericus* whereas *S. yatensis* NRRL B-24116^T was most closely related to the type strain of *S. melanosporofaciens*. The two strains can be separated from one another and from representatives of other species classified in the *S. violaceusniger* 16S rRNA gene clade using a range of phenotypic properties (Table 4). The type strains of *S. malaysiensis* and *S. melanosporofaciens* have been distinguished using 16S-ITS RFLP fingerprint data (Lanoot et al. 2004). The results of the present study underpin the taxonomic integrity of *S. malaysiensis* and *S. yatensis*.

Another group of closely related organisms include the type strains of *S. asiaticus*, *S. cangkringensis*, *S. indonesiensis* and *S. rhizosphaericus*. These organisms shared 16S rRNA gene similarities within the range of 99.2–99.7%, values that correspond to between 4 and 6 nt differences at 1449 locations. Apart from *S. cangkringensis* NRRL B-24177^T, these strains had identical partial *rpo*B sequences. However, DNA:DNA homology analyses performed by thermal denaturation revealed differences in melting temperature between representatives of these strains that were above the cut-off for the delineation of genomic species ($\Delta T_m > 5$°C; Wayne et al. 1987; Roselló-Mora and Amann 2001). The taxonomic integrity of these species is supported by the numerical taxonomic data as the type strains were recovered as distinct single membered clusters and can be separated using a combination of phenotypic properties.

The present genotypic and phenotypic data confirm and extend those of Labeda and Lyons (1991) in showing that strains identified as *S. violaceusniger* form a markedly heterogeneous group. Indeed, the recognition of *S. violaceusniger* NRRL B-1476^T as the type strain of the species necessitates the reclassification of strains received as *S. violaceusniger*. Strain NRRL B-1478 shares its highest 16S rRNA gene similarities with *S. hygroscopicus* NRRL 2339, NRRL 2387^T and NRRL B-1477, but has DNA:DNA similarities with the latter within the range 39–42% (Labeda and Lyons 1991). The sharp separation from the *S. hygroscopicus* strains is underpinned by the numerical taxonomic and the partial *rpo*B sequence data. It is evident, therefore, that *S. violaceusniger* NRRL B-1478 merits recognition as a new species within the *S. violaceusniger* 16S rRNA gene clade. The name proposed for this taxon is *Streptomyces demainii* sp. nov.

It is evident from 16S rRNA sequence data that *S. violaceusniger* NRRL B-1865 belongs to the *S. rhizosphaericus* subclade. It shares 16S rRNA gene similarities with the members of this taxon within the range 99.4–99.7, values that correspond to between 4 and 6 nt differences at 1449 locations. However, DNA:DNA homology analyses revealed ΔT_m differences (5.6–6.9°C; which corresponds to approximately 56–62% DNA:DNA relatedness) against representatives of the *S. rhizosphaericus* subclade well above the species level cut-off point ($\Delta T_m > 5$°C). The organism was most closely associated with the type strain of *S. hygroscopicus* in the partial *rpo*B analysis, but it is known that these strains belong to different genomic species as they share a DNA:DNA similarity of only 48% (Labeda and Lyons 1991). These workers found that the strain NRRL B-1865 shared its highest DNA: DNA homology value, 78%, with *S. violaceusniger* NRRL B-1356, albeit below the 80% cut-off point mentioned previously; these strains also have 16S rRNA gene sequences which differ by 20 nt. The combined genotypic and phenotypic

data are consistent with the classification of *S. violaceusniger* NRRL B-1865 as a separate species.

It is evident from the 16S rRNA gene sequence data that *S. violaceusniger* NRRL B-1356 is most closely related to *S. violaceusniger* NRRL B-1476^T. The two strains share a 16S rRNA gene similarity of 99.7%, but belong to different, albeit closely related, species on the basis of DNA:DNA pairing data (Labeda and Lyons 1991). In addition, *S. violaceusniger* NRRL B-1356 was sharply distinguished from all of the other members of the *S. violaceusniger* 16S rRNA gene clade in the numerical taxonomic and partial *rpo*B analyses. These results are consistent with *S. violaceusniger* NRRL B-1356 being described as a new species.

The present investigation provides further evidence that strains classified in the *S. violaceusniger* 16S rRNA gene clade have a combination of colonial and morphological properties that distinguish them from all other streptomycetes. Members of the clade share high 16S rRNA gene similarities but can be classified into species using a combination of genotypic and phenotypic data. The present data support the taxonomic status of all but one of the validly described species assigned to the clade, the exception, *S. sporoclivatus* Preobrazhenskaya et al. 1986 should be seen as a subjective synonym of *S. melanosporofaciens* Arcamone et al. 1959. It is also clear that *S. hygroscopicus* subsp. *geldanus* NRRL 3602 and *S. violaceusniger* NRRL B-1356 and NRRL B-1865 should be recognized as new species. It is proposed that these organisms be recognized as *S. geldanamycininus* sp. nov., *S. albiflaviniger* sp. nov., and *S. griseiniger* sp. nov., respectively, names used, or in case of the former epithet in line with that used, in previous studies (Sembiring et al. 2000; Ward and Goodfellow 2004). Members of these and the established species classified in the *S. violaceusniger* 16S rRNA gene clade can be distinguished from one another using a range of genotypic and phenotypic data. Descriptions of the new species assigned to the *S. violaceusniger* 16S rRNA gene clade are given below.

Description of *Streptomyces albiflaviniger* sp. nov.

Streptomyces albiflaviniger (al. bi. fla.vi. ni'ger. L. adj. *albus*, white; L. adj. *flavus*, yellow; L. adj. *niger*, black., N.L. adj. *albiflaviniger*, white, yellow and black colors).

The description is based on data taken from this and from a previous study (Labeda and Lyons 1991). Spore chains in *Spirales*; the spore surface is rugose. On oatmeal agar the aerial spore mass color is white becoming black and moist when mature; the reverse side of colonial growth is yellow. Brown, orange and yellow diffusible pigments are formed, but not melanin pigments. Additional morphological and pigmentation properties are shown in Table 3. The phenotypic properties of the organism are cited in the text and in Table 4. The G + C content of the DNA of the type strain is 70.5 mol% (as determined by the T_m method). The type strain is NRRL B-1356^T (=DSM 41598^T).

Description of *Streptomyces demainii* sp. nov.

Streptomyces demainii (de. mai. ni' i. N.L. gen. n. *demainii*, of Demain, named in honour of Arnold Demain, a celebrated actinomycete biologist).

The description is based on data taken from this and from a previous study (Labeda and Lyons 1991). Spore chains are *Spirales*: the spore surface is rugose. On oatmeal agar the aerial spore mass color is gray, becoming black and moist when mature; the reverse side of colony growth is grayish-yellow. Melanin pigments are not formed. Additional morphological and pigmentation properties are shown in Table 3. The phenotypic properties of the organism are cited in the text and in Table 4. The G + C content of the DNA is 71.2 mol%, as determined by the T_m method. The type strain is NRRL B-1478^T (= DSM 41600^T).

Description of *Streptomyces geldanamycininus* sp. nov.

Streptomyces geldanamycininus (gel. da. na. my. ci. ni'nus N.L. neut.n. *geldanamycinum*, geldanamycin; L. suff. *-inus*, adjectival suffix used with the sense of belonging to or related to: N.L. masc. adj. *geldanamycininus*, related to geldanomycin, producing the antibiotic geldanamycin).

The description is based on data taken from this and from a previous study (Labeda and Lyons 1991). Spore chains are *Spirales*; the spore surface is rugose. On oatmeal agar the aerial spore mass color is grayish-brown and the substrate mycelium grayish-yellow. Melanin pigments are not produced. Additional morphological and pigmentation properties are shown in Table 3. The phenotypic properties of the organism are cited in the text and in Table 4. The G + C content of the DNA is 70.2 mol %, as determined by the T_m method. The type strain is NRRL 3602^T (=DSM 41894^T).

Description of *Streptomyces griseiniger* sp. nov.

Streptomyces griseiniger (gri'se.i.ni'ger. N.L. adj. *griseus*, gray; L. adj. *niger*, black; N.L. adj. *griseiniger*, gray-black).

The description is based on data taken from this and from a previous study (Labeda and Lyons 1991). Spore chains are *Spirales*; the spore surface is rugose. On oatmeal agar the aerial spore mass color is gray, becoming black and moist when mature; the reverse side of colonial growth is grayish-yellow. Melanin pigments are not formed. Additional morphological and pigmentation properties are shown in Table 3. The phenotypic properties of the organism are cited in the text and in Table 4. The G + C content of the DNA of the type strain is 70.2 mol%, as determined by the T_m method. The type strain is NRRL B-1865^T (=DSM 41895^T).

The five strains that fell outside the *S. violaceusniger* 16S rRNA gene clade formed distinct phyletic lines within the evolutionary radiation occupied by the genus *Streptomyces*. These organisms were also outside the zone of evolutionary radiation occupied by members of *S. violaceusniger* 16S rRNA tree in the *rpo*B tree, apart from the *S. sparsogenes* strain which was loosely associated with the *S. melanosporofaciens rpo*B subclade. The type strain of *S. sparsogenes* was assigned to the *S. violaceusniger* cluster by Williams et al. (1983), who recorded that it produced rugose ornamented spores. In contrast, Kämpfer et al. (1991) found that the organism was physiologically different from authentic *S. hygroscopicus* and *S. violaceusniger* strains. In the present study, *S. sparsogenes* NRRL 2940^T was shown to produce spiral chains of ridged ornamented spores and formed a single membered cluster outside the *S. violaceusniger* aggregate cluster that was circumscribed in the numerical taxonomic study. These results are in good agreement with those of Labeda and Lyons (1991) who found low levels of DNA:DNA relatedness between *S. sparsogenes* NRRL 2940^T and strains now known to belong to the *S. violaceusniger* 16S rRNA gene clade. The *S. sparsogenes* strain is most closely, albeit quite distantly, related to *S. fulvissimus* NBRC 3717^T; these organisms share a 16S rRNA gene similarity of 96.5%, a value that corresponds to 35 nt differences at 1443 locations. They can also be distinguished using morphological and pigmentation properties as the *S. fulvissimus* strain forms straight to flexuous chains of smooth surfaced spores and melanin pigments on peptone-yeast extract-iron agar (Shirling and Gottlieb 1969). It is timely to emend the description of *S. sparsogenes* in light of these results.

Emended description of *Streptomyces sparsogenes*

Streptomyces sparsogenes Owen et al. 1963, 772^{AL} (spar. so. gen'nes. L. part. adj. *sparsus*, scattered; Gr. v. *genmao*, to produce; N.L. part. adj. *sparsogenes* (sic), scattered producing, probably referring to the sparse formation of aerial mycelium).

The description is based on data taken from this and from previous studies (Shirling and Gottlieb 1969; Labeda and Lyons 1991). Spore chains are *Spirales* and spore surfaces ridged. Aerial hyphae when present may emerge from coremia-like structures. On oatmeal agar the aerial spore mass color is gray and the reverse

side of colonial growth grayish-yellow. Melanin pigments are not formed. Additional morphological and pigmentational properties are shown in Table 3. Aesculin is hydrolyzed, but not urea. Nitrate is reduced, but hydrogen sulphide is not produced. Adenine, arbutin, chitin, Tween 80 and xylan are degraded, but not guanine, hypoxanthine, tyrosine, uric acid or xanthine. Grows at pH 6.0 and pH 8.0 and at 25°C and 40°C, but not at 10°C. L-arabinose, D- and L-arabitol, D-maltose, D-mannose, D-raffinose and D-sucrose are used as sole carbon sources, but not butane- 1, 4 diol, D-cellobiose, citric acid, dextrin, D-fructose, L-fucose, D-galactose, D-glucose, *meso*-inositol, α-lactose, D-mannose, D-melezitose, methanol, propanol, pyruvic acid, L-rhamnose, D-ribose or salicin. α-Alanine, L-histidine, L-proline, L-serine and L-threonine are used as sole carbon and nitrogen sources, but not L-alanine, L-aminobutyric acid, L-arginine, L-glutamic acid, L-glycine, L-leucine, DL-methionine, DL-*nor*leucine, L-phenylalanine, L-tryptophane or L-valine. Grows in the presence of 13%, w/v NaCl. Resistant (μg ml^{-1}) to carbenicillin (32, 64), cephalosporin (32), cefoxitin (32, 64), cephaloridine (32, 64), chlortetracycline hydrochloride (4, 8), doxycycline hydrochloride (4, 8), tetracycline hydrochloride (64), rifampicin (32, 64), fusidic acid (8, 16), lincomycin hydrochloride (32, 64) and novobiocin (4), but is sensitive to erythromycin (32, 64), oleandomycin phosphate (32, 64) and novobiocin (8). Additional phenotypic properties are cited in the text. The G + C content of the DNA is 71.9 mol%, as determined by the T_m method. The type strain is NRRL 2940^T (=DSM 40356^T).

The four remaining strains received as *S. violaceusniger* are clearly misclassified as they do not form rugose ornamented spores, and fell outside the *S. violaceusniger* aggregate group defined in the initial numerical taxonomic study. These findings are in excellent agreement with DNA:DNA relatedness data which show that these organisms bear little relationship either to one another or to streptomycetes which form rugose spores (Labeda and Lyons 1991); these results are underpinned by the 16S rRNA gene sequence data and in the main by those from the partial *rpo*B studies.

Streptomyces violaceusniger ISP 5182 is most closely related to *S. bobili* JCM 4624^T and *S. galilaeus* JCM 4757^T; it shares 16S rRNA gene similarities of 99.42% and 99.45% with the latter, values which correspond to 9 nt differences at 1445 and 1446 locations, respectively. The *S. violaceusniger* and *S. bobili* strains can be can be readily distinguished as the former produces a gray aerial spore mass and a yellow-brown reverse colonial color on oatmeal agar, flexuous spore chains, but does not form melanin pigments whereas the latter may or may not form a sparse white aerial mycelium, but it does produce a grayish-yellow reverse color and melanin pigments (Williams et al. 1989). The *S. galilaeus* strain forms a brownish yellow to brownish red reverse color on oatmeal agar, but unlike the *S. violaceusniger* strain strain, it bears spores in spiral chains (Gauze et al. 1983).

Streptomyces violaceusniger NRRL 2834 is most closely related to *S. murinus* NBRC 14802^T and *S. graminearus* NBRC 15420^T; it shares 16S rRNA gene similarities of 98.7%and 98.8% with these organisms, values which correspond to to 18 nt differences at 1487 and 1444 locations, respectively. The *S. violaceusniger* and *S. murinus* strains can be distinguished readily as the former produces a white aerial mycelium and a yellow-reverse colonial color on oatmeal agar and flexuous spore chains whereas the latter is characterized by a gray or red aerial spore mass, grayish-yellow substrate mycelial pigments and spiral spore chains (Shirling and Gottlieb 1968). It can also be distinguished readily from the *S. graminearus* strain as the latter produces a gray aerial spore mass and a colorless to light lemon-yellow undersurface on oatmeal agar and spiral spore chains (Gauze et al. 1983).

Streptomyces violaceusniger NRRL B-5799 is most closely related to *S. misionensis* NBRC 13063^T; these organisms share a 16S rRNA gene similarity of 99.1%, which corresponds to 13 nt differences at 1443 locations. The two strains have many physiological properties in common, but were assigned to different subclusters within the *S. albidoflavus*/*S. griseus*/*S. antibioticus* cluster in the numerical taxonomic study of Kämpfer et al. (1991). They can be distinguished using colonial and morphological properties as the

S. violaceusniger strain forms a brown aerial spore mass, a yellow reverse colonial color and a yellow diffusible pigment on oatmeal agar and flexuous chains of smooth ornamented spores whereas the *S. misionensis* strain produces a gray-brown or light grayish red aerial spore mass and olive-brown or yellow-brown reverse colors and spiral chains of smooth or slightly warty spores (Shirling and Gottlieb 1969).

The remaining strain, *S. violaceusniger* NRRL 8097, is most closely related to the type strain of *S. roseoviolaceus*. These organisms share a 16S rRNA gene similarity of 98.8%, which is equivalent to 18nt differences at 1448 positions. They can also be distinguished by their colonial and morphological properties as the *S. violaceusniger* strain produces a gray aerial spore mass, grayish-orange reverse colonial pigments and an orange diffusible pigment on oatmeal agar, forms spiral chains of smooth ornamented spores, but does not produce melanin pigments. In contrast, *S. roseoviolaceus* ISP 5277^{T} forms a red aerial spore mass, a red to blue substrate mycelium and a violet–blue diffusible pigment, melanin pigments and spiral chains of spiny spores (Shirling and Gottlieb 1969).

It is evident that the four misclassified *S. violaceusniger* strains can be distinguished from one another using a combination of genotypic and phenotypic data thereby underpinning the DNA:DNA relatedness data of Labeda and Lyons (1991). In addition, they can be distinguished from their closest phylogenetic neighbors using predictive colonial and morphological characteristics. It is evident that these organisms should be recognized as novel *Streptomyces* species. It is proposed that *S. violaceusniger* NRRL 8097, NRRL 2834, NRRL B-5799 and ISP 5182 be classified as *S. auratus* sp. nov., *S. phaeogriseichromatogenes* sp. nov., *S. phaeoluteichromatogenes* sp. nov. and *S. phaeoluteigriseus* sp. nov., the latter three names were used information in earlier studies (Sembiring et al. 2000; Ward and Goodfellow 2004).

Description of *Streptomyces auratus* sp. nov.

Streptomyces auratus (au.ra' tus. L. masc. adj. *auratus*, gold colored).

The description is based on data taken from this and from an earlier study (Labeda and Lyons 1991). Spore chains are *Spirales* and the spore surface is smooth. On oatmeal agar the aerial spore mass color is gray and the substrate mycelium grayish-yellow; an orange diffusible pigment is produced, but not melanin pigments. Additional cultural and pigmentation properties are given in Table 3. The organism produces hydrogen sulphide, degrades adenine, arbutin, chitin, hypoxanthine, Tweens 40, 60 and 80, uric acid and xanthine, but not casein, guanine, tyrosine or xylan. It does not reduce nitrate or hydrolyze aesculin or urea. It grows from pH 5 to pH 10, at 25°C and 37°C, but not at 10°C or 40°C. Butan-1, 4 diol, D-cellobiose, citric acid, dextrin, D-fructose, L-fucose, D-galactose, D-glucose, *meso*-inositol, D-maltose, D-mannitol, D-mannose, D-melezitose, methanol, propanol, pyruvic acid, D-raffinose, L-rhamnose, D-ribose, L-salicin and D-sucrose are used as sole carbon sources, but not α-lactose. α- and L-alanine, L-aminobutyric acid, L-glutamic acid, L-glycine, L-histidine, L-proline, L-serine and L-threonine are used as sole carbon and nitrogen sources, but not aspartic acid, L-leucine, DL-methionine, DL-*nor*leucine, L-phenylalanine, L-tryptophane or L-valine. Growth occurs in the presence of 13%, w/v NaCl. Resistant (μg ml^{-1}) to carbenicillin (32, 64), cefoxitin (32), cephaloridine (32), chlortetracycline hydrochloride (4, 8), doxycycline hydrochloride (4), rifampicin (32, 64) and novobiocin (4, 8), but is sensitive to cefoxitin (64), cephaloridine (64), doxycycline hydrochloride (8), fusidic acid (8, 16), lincomycin hydrochloride (32, 64) and oleandomycin phosphate (32, 64). Additional phenotypic properties are cited in the text. The G + C content of the DNA is 65.6 mol %, as determined by the T_m method. The type strain is NRRL 8097^{T} (=DSM 41897^{T}).

Description of *Streptomyces phaeogriseichromatogenes* sp. nov.

Streptomyces phaeogriseichromatogenes (phae.o.-gri.se'i.chro.ma'to.ge.nes. Gr.adj. *phaeus*, brown;

N.L. adj. *griseus*, gray; Gr. n. *chroma-atos*, color; Gr. v. *genmao*, to produce; N.L. part. adj. *phaeogriseichromatogenes*, producing brown and gray colors).

The description is based on data taken from this and from an earlier study (Labeda and Lyons 1991). Spore chains are *Rectiflexibiles*; the spore surface is ridged. Aerial spore mass colors range from grayish-white to grayish-brown and the substrate mycelium is yellow to yellowish-brown, a yellow diffusible pigment is formed on yeast extract-malt extract agar, but melanin pigments are not produced. Additional cultural and pigmentation properties are shown in Table 3. Hydrogen sulphide is produced, aesculin is hydrolyzed, but not urea. Nitrate is not reduced. Casein, chitin, hypoxanthine, tyrosine, Tween 60, uric acid and xanthine are degraded, but not adenine, arbutin, guanine or xylan. Grows from pH 5 to pH 10, at 25 and 37°C, but not at 10°C or 40°C. L-arabinose, D-arabitol, butan-1, 4 diol, D-cellobiose, citric acid, dextrin, D-fructose, D-galactose, D-glucose, α-lactose, D-maltose, D-mannitol, D-mannose, D-ribose and D-sucrose are used as sole carbon sources, but not L-arabitol, L-fucose, *meso*-inositol, D-melezitose, methanol, propanol, pyruvic acid, D-raffinose, L-rhamnose or salicin. L-alanine, L-arginine, L-glutamic acid, L-histidine, L-leucine, DL-methionine, DL-*nor*leucine, L-proline and L-valine are used as sole carbon and nitrogen sources, but not α-alanine, L-aminobutyric acid, L-glycine, L-phenylalanine, L-threonine or L-tryptophane. Does not grow in the presence of 13%, w/v NaCl. Resistant (μg ml^{-1}) to cephalosporin (32), cefoxitin (32), cephaloridine (33, 64), doxycycline hydrochloride (4) and rifampicin (64), but not to cefoxitin (64), carbenicillin (32, 64), erythromycin (32, 64), oleandomycin phosphate (32, 64), chlortetracycline hydrochloride (4, 8), doxycycline hydrochloride (8), rifampicin (32, 64), fusidic acid (8, 16), lincomycin hydrochloride (32, 64) or novobiocin (4, 8). Additional phenotypic properties are cited in the text. The G + C content of the DNA is 71.2 mol %, as determined by the T_m method. The type strain is NRRL 2834^T (=DSM 40710^T).

Description of *Streptomyces phaeoluteichromatogenes* sp. nov.

Streptomyces phaeoluteichromatogenes (phae.o.-lu.te.i.chro.ma.to.ge.nes. Gr. adj. *phaeos*, dark, brown; L. adj. *luteus*, yellow; Gr. n. *chroma-atos*, color; Gr. v. *genmao*, to produce, N.L. part. adj. *phaeoluteichromatogenes*, producing brown and yellow colors).

The description is based on data taken from this and from an earlier study (Labeda and Lyons 1991). Spore chains are *Rectiflexibiles*; the spore surface is smooth. On oatmeal agar the aerial spore mass color is brown and the substrate mycelium yellow; a yellow diffusible pigment is produced. Melanin pigments are not formed. Additional cultural and pigmentation properties are shown in Table 3. Aesculin and urea are hydrolyzed. Does not produce hydrogen sulphide or reduce nitrate. Adenine, casein, chitin, hypoxanthine, tyrosine and Tween 80 are degraded, but not casein, guanine, uric acid, xanthine or xylan. Grows from pH 4 to pH 9 and at 25°C and 40°C, but not at 10°C. Uses L-arabinose, D- and L-arabitol, butan-1, 4 diol, D-cellobiose, citric acid, D-fructose, D-galactose, D-glucose, *meso*-inositol, D-mannitol, D-mannose, D-melezitose, methanol, propanol and pyruvic acid as sole carbon sources, but not dextrin, L-fucose, α-lactose, D-maltose, D-raffinose, L-rhamnose, D-ribose, D-salicin or D-sucrose. α-Alanine, L-glutamic acid, L-glycine, L-leucine, L-proline, L-serine and L-threonine are used as sole carbon and nitrogen sources, but not L-alanine, L-aminobutyric acid, L-histidine, DL-methionine, DL-*nor*leucine, L-phenylalanine, L-tryptophane or L-valine. Growth occurs in the presence of 13%, w/v NaCl. Resistant (μg ml^{-1}) to carbenicillin (32, 64), cephalosporin (32), cefoxitin (32, 64), cephaloridine (32, 64), chlortetracycline hydrochloride (4,8), doxycycline hydrochloride (4, 8), tetracycline hydrochloride (64), rifampicin (32, 64) and novobiocin (4), but is sensitive to erythromycin (32, 64), oleandomycin phosphate (32, 64), fusidic acid (8, 16) and lincomycin hydrochloride (32, 64). Additional phenotypic properties are cited in the text. The G + C content of the DNA is 69.8 mol %, as determined by the T_m method. The type strain is NRRL B-5799^T (=DSM 41898^T).

Description of *Streptomyces phaeoluteigriseus* sp. nov.

Streptomyces phaeoluteigriseus (phae.o.lu.te.i.gri. se' us. Gr. adj. *phaeos*, dark, brown; L. adj. *luteus*, yellow; N.L. adj. *griseus*, gray. N.L. adj. *phaeoluteigriseus*, brown, gray and yellow-colored).

The description is based on data taken from this and from an earlier study (Labeda and Lyons 1991). Spore chains are *Rectiflexibiles*; the spore surface is smooth. On oatmeal agar the aerial spore mass color is gray and the substrate mycelium yellowish-brown; a yellow diffusible pigment is produced, as are melanin pigments. Additional cultural and morphological properties are given in Table 3. Aesculin is hydrolyzed but not urea. Hydrogen sulphide is produced, but nitrate is not reduced. Adenine, arbutin, chitin, hypoxanthine, Tween 80 and xanthine are degraded, but not arbutin, guanine, tyrosine, uric acid or xylan. Grows from pH 4 to pH 10, and at 25°C and 37°C, but not at 10°C or 40°C. Adonitol, L-arabitol, citric acid and dextrin are used as sole carbon sources, but not L-arabitol, butan-1, 4 diol, D-cellobiose, D-fructose, L-fucose, D-galactose, D-glucose, *meso*-inositol, α-lactose, D-mannitol, D-mannose,D-melezitose, methanol, propanol, pyruvic acid, D-raffinose, L-rhamnose, D-ribose, salicin or D-sucrose. α-Alanine, L-histidine and L-threonine are used as sole carbon and nitrogen sources, but not L-alanine, L-aminobutyric acid, L-arginine, L-glutamic acid, L-leucine, DL-methionine, DL-*nor*leucine, L-phenylalanine, L-serine, L-threonine, L-tryptophane or L-valine. Growth occurs in the presence of 13%, w/v NaCl. Resistant (μg ml^{-1}) to carbenicillin (32, 64), cefoxitin (32, 64), cephaloridine (32), oleandomycin phosphate (32), chlortetracycline hydrochloride (4), tetracycline hydrochloride (64), fusidic acid (8, 16) and novobiocin (4), but is sensitive to cephalosporin (32), cephaloridine (64), erythromycin (32, 64), oleandomycin phosphate (64), chlortetracycline hydrochloride (8), doxycycline hydrochloride (4, 8), rifampicin (32, 64), lincomycin hydrochloride (32, 64) and novobiocin (8). Additional phenotypic properties are cited in the text. The G + C content of the DNA is 72.2 mol%, as described by the T_m method. The type strain is ISP 5182^T (=DSM 41896^T).

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