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Reclassification of *Streptomyces hygroscopicus* strains as *Streptomyces aldersoniae* sp. nov., *Streptomyces angustmyceticus* sp. nov., comb. nov., *Streptomyces ascomycinicus* sp. nov., *Streptomyces decoyicus* sp. nov., comb. nov., *Streptomyces milbemycinicus* sp. nov. and *Streptomyces wellingtoniae* sp. nov.

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A polyphasic study was undertaken to determine the taxonomic status of six strains received as *Streptomyces hygroscopicus*. The strains had chemotaxonomic and morphological properties typical of members of the genus *Streptomyces* and formed distinct phyletic lines in the *Streptomyces* 16S rRNA gene tree. These strains were distinguished from one another and from phylogenetically close neighbours using a combination of phenotypic properties. The combined genotypic and phenotypic data showed that all six strains form distinct centres of taxonomic variation within the genus *Streptomyces*. The following novel species are proposed to accommodate the strains: *Streptomyces aldersoniae* sp. nov. (type strain DSM 41909^T =NRRL 18513^T), *Streptomyces angustmyceticus* sp. nov., comb. nov. (type strain DSM 41683^T=NRRL B-2347^T), *Streptomyces ascomycinicus* sp. nov. (type strain DSM 40822^T =NBRC 13981^T), *Streptomyces decoyicus* sp. nov., comb. nov. (type strain DSM 41427^T =NRRL 2666^T), *Streptomyces milbemycinicus* sp. nov. (type strain DSM 41911^T =NRRL 5739^T) and *Streptomyces wellingtoniae* sp. nov. (type strain DSM 40632^T =NRRL B-1503^T).

The taxonomy of the genus *Streptomyces* has been clarified and extended by the application of genotypic and phenotypic procedures to representatives of formally described and putatively novel species (Manfio *et al.*, 1995, 2003; Lanoot *et al.*, 2002, 2004). 16S rRNA gene sequence data show that the type strains of many *Streptomyces* species can be assigned to 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* (Goodfellow *et al.*, 2007) and *Streptomyces yeochonensis* (Xu *et al.*, 2006) clades. The *S. violaceusniger* clade encompasses strains that produce a grey aerial spore mass and a greyish-yellow substrate mycelium on oatmeal agar, form aerial hyphae that differentiate into spiral chains of rugose-ornamented spores (Sembiring *et al.*, 2000; Goodfellow *et al.*, 2007; Kumar & Goodfellow, 2008), show the same pattern of HPLC-detected metabolites, namely elaiophylin, geldanamycin, nigericin and an uncharacterized polyene (Ward & Goodfellow, 2004), and give a characteristic amplification product with taxon-specific primers (Kumar *et al.*, 2007).

The *S. violaceusniger* 16S rRNA gene clade currently contains 22 species with validly published names (Kumar & Goodfellow, 2008), delineated mainly by using a combination of DNA–DNA relatedness and standard phenotypic data (Sembiring *et al.*, 2000; Goodfellow *et al.*, 2007). The taxon includes two well-known species, *Streptomyces hygroscopicus* and *S. violaceusniger*, which have been considered to be particularly rich sources of novel antibiotics (Strohl, 2004). However, some strains labelled *S. hygroscopicus* have been misclassified, as they belong to novel species classified within and outside the *S. violaceusniger* clade (Goodfellow *et al.*, 2007; Kumar & Goodfellow, 2008). It is important to classify *Streptomyces* strains correctly, not least to determine the extent to which streptomycete taxonomy can be used as a surrogate for chemical diversity and hence influence bioprospecting strategies (Bull *et al.*, 2005).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the tested strains are EU170123 (*S. aldersoniae* NRRL 18513^T), EU170119 (*S. angustmyceticus* NRRL B-2347^T), EU170121 (*S. ascomycinicus* DSM 40822^T), EU170127 (*S. decoyicus* NRRL 2666^T), EU170126 (*S. milbemycinicus* NRRL 5739^T) and EU170124 (*S. wellingtoniae* DSM 40632^T).

The present study was designed to clarify further the taxonomic standing of strains classified as *S. hygroscopicus*. To this end, *S. hygroscopicus* NRRL 18513 (Yao & Hamill, 1993), *S. hygroscopicus* subsp. *angustmyceticus* NRRL B-2347^T (Yüntsen *et al.*, 1956), '*S. hygroscopicus* subsp. *aureolacrimosus*' NRRL 5739 (Haber *et al.*, 1991), *S. hygroscopicus* subsp. *decoyicus* NRRL 2666^T (Vavra *et al.*, 1959), *S. hygroscopicus* subsp. *glebosus* NRRL B-3248^T (Ohmori *et al.*, 1962), *S. hygroscopicus* subsp. *hygroscopicus* DSM 40632 (Tresner & Backus, 1956) and *S. hygroscopicus* subsp. *hygroscopicus* DSM 40822 (Hütter, 1967) were the subject of a polyphasic taxonomic study. The resultant data show that the eight strains form distinct centres of taxonomic variation in the genus *Streptomyces* that can be equated with species, albeit ones that fall outside the *S. violaceusniger* 16S rRNA gene clade.

The strains were maintained on oatmeal agar (DSMZ, 1998) slopes at 4 °C and as mycelial fragments and spores in glycerol (20 %, v/v) at −20 °C. Biomass for 16S rRNA gene sequencing was prepared by growing the organisms in shake flasks of tryptic soy broth at 160 r.p.m. for 7 days at 28 °C prior to harvesting by centrifugation.

Genomic DNA extraction, PCR amplification and sequencing of 16S rRNA genes from the strains was carried out with minor modifications (Kumar *et al.*, 2007) of the procedure described by Goodfellow *et al.* (2007). The resultant sequence data were aligned using the PHYDIT program (available at http://plaza.snu.ac.kr/~jchun/phydit/) and the alignments were checked manually. Using the sequence data, a BLAST search (Altschul *et al.*, 1997) of GenBank was performed to detect the closest relatives of each of the strains; the sequences included those of representatives of the *S. violaceusniger* clade. Phylogenetic trees were inferred using four tree-making algorithms (least-squares, maximum-likelihood, maximum-parsimony and neighbour-joining) drawn from the PHYLIP 3.5c suite of programs (Felsenstein, 1993). Evolutionary distance matrices were prepared for the least-squares and neighbour-joining methods, as described by Jukes & Cantor (1969). A bootstrap analysis was performed (Felsenstein, 1985) with 1000 resamplings of the neighbour-joining dataset by using the SEQBOOT and CONSENSE programs from the PHYLIP package. Almost-complete 16S rRNA gene sequences (1441–1450 nt) were obtained for the strains. It can be seen from Fig. 1 that none of the strains were closely related to members of the *S. violaceusniger* 16S rRNA clade, including the type strain of *S. hygroscopicus*, or with any of their closest phylogenetic neighbours.

The strains were examined for their ability to produce pigments on oatmeal and peptone-yeast extract-iron agars, and for spore-chain morphology and spore-surface ornamentation using previously described procedures (Shirling & Gottlieb, 1966; Sembiring *et al.*, 2000; Goodfellow *et al.*, 2007). They were also examined for their ability to grow on modified Bennett's, glucose-yeast extract-malt extract, glycerol-asparagine, inorganic salts-starch, tyrosine and yeast extract-malt-extract agars and for the presence of LL-diaminopimelic acid (LL-A_2pm) in whole-organism hydrolysates after Staneck & Roberts (1974) and probed using the *S. violaceusniger* clade-specific primers, as described by Kumar *et al.* (2007). The strains grew well on glucose-yeast extract-malt extract, glycerol-asparagine, tyrosine and yeast extract-malt extract agars, and produced whole-organism

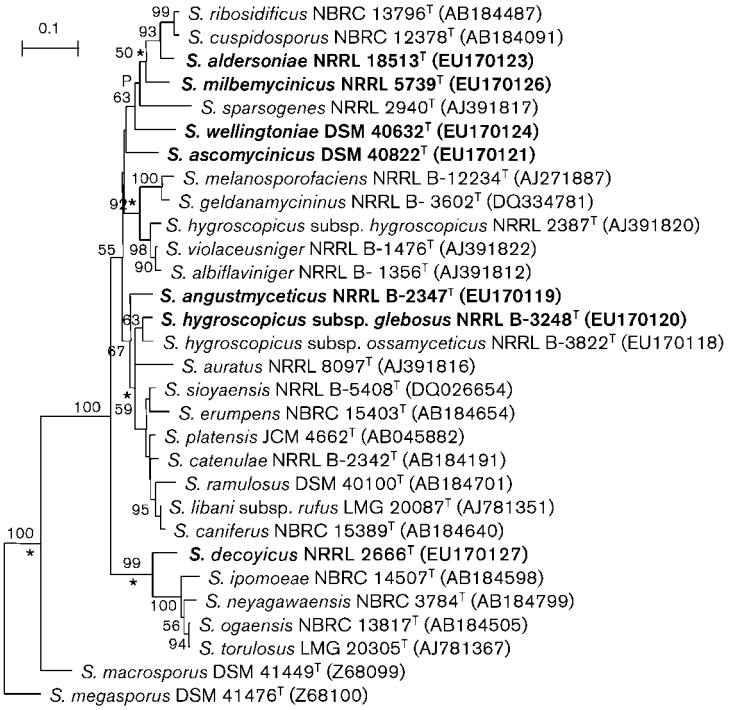

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) showing relationships between the tested strains and between them, representatives of the *Streptomyces violaceusniger* 16S rRNA gene clade and phylogenetically close *Streptomyces* strains based on almost-complete 16S rRNA gene sequences. Asterisks denote branches that were also recovered using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. P indicates branches that were recovered using the maximum-parsimony method. Bar, 0.1 substitutions per site. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets. The tested strains are given in bold. GenBank accession numbers are given in parentheses.

hydrolysates rich in LL-A_2pm. They did not form a grey aerial spore mass on oatmeal agar and produced neither spiral chains of rugose-ornamented spores nor the diagnostic PCR amplification product with the clade-specific primers. These results indicate that the organisms have properties consistent with their assignment to the genus *Streptomyces*, but not with their inclusion in the *S. violaceusniger* 16S rRNA gene clade (Manfio *et al.*, 1995; Kumar & Goodfellow, 2008).

The six organisms and the type strains of *Streptomyces cuspidosporus* and *Streptomyces torulosus* were examined for a range of phenotypic properties known to be of value in streptomycete systematics (Atalan *et al.*, 2000; Xu *et al.*, 2006; Goodfellow *et al.*, 2007). To this end, they were examined for phenotypic properties using media and methods described by Williams *et al.* (1983). The chemical and morphological properties of the organisms were consistent with their classification in the genus *Streptomyces*. All of the strains hydrolysed aesculin and arbutin and used L-arginine as a sole source of carbon and nitrogen, but not aminobutyric acid, DL-aspartic acid, DL-norleucine or L-norvaline. The assignment of the strains to the genus *Streptomyces* is in line with the 16S rRNA gene sequence data.

The strains formed well-delineated phyletic lines in the *Streptomyces* 16S rRNA gene tree (Fig. 1). Four of the strains, namely *S. hygroscopicus* NRRL 18513, '*S. hygroscopicus* subsp. *aureolacrimosus*' NRRL 5739 and *S. hygroscopicus* subsp. *hygroscopicus* strains DSM 40632 and DSM 40822, belong to or are most closely associated with members of the *S. cuspidosporus* clade, sharing their highest 16S rRNA gene similarities with the type strain of *S. cuspidosporus* (97.6–99.0 %, values which correspond to between 17 and 35 nt differences). The closest relationship found between the four tested strains was that between *S. hygroscopicus* NRRL 18513 and '*S. hygroscopicus* subsp. *aureolacrimosus*' NRRL 5739; these organisms shared a 16S rRNA gene sequence similarity of 98.6 %, a value that corresponds to 22 nt differences over 1444 locations. In contrast, the most distantly related organisms, *S. hygroscopicus* NRRL 18513 and *S. hygroscopicus* subsp. *hygroscopicus* DSM 40822, shared a 16S rRNA gene sequence similarity of 97.6 % (35 nt differences over 1449 locations). In addition, *S. hygroscopicus* NRRL 18513 was relatively closely related to the type strain of *Streptomyces ribosidificus*: these organisms shared a 16S rRNA gene sequence similarity of 99.0 %(14 nt differences over 1444 positions). DNA–DNA relatedness studies were not carried out between the four tested strains or between them and the type strains of *S. cuspidosporus* and *S. ribosidificus* as the respective 16S rRNA gene similarity values are well below the 99.4 % level at which *Streptomyces* strains have been shown to belong to different genomic species (Liu *et al.*, 2005; Xu *et al.*, 2006; Goodfellow *et al.*, 2007; Sun *et al.*, 2007).

The four strains assigned to the *S. cuspidosporus* clade can be distinguished from one another and from the type strain of *S. cuspidosporus*, their nearest phylogenetic neighbour, using a range of phenotypic properties (Table 1). The excellent congruence found between the 16S rRNA gene sequence and phenotypic data is consistent with the classification of all four strains within novel *Streptomyces* species. It is, therefore, proposed that *S. hygroscopicus* NRRL 18513, '*S. hygroscopicus* subsp. *aureolacrimosus*' NRRL 5739 and *S. hygroscopicus* subsp. *hygroscopicus* strains DSM 40632 and DSM 40822 be classified as the type strains of *Streptomyces aldersoniae* sp. nov., *Streptomyces milbemycinicus* sp. nov., *Streptomyces wellingtoniae* sp. nov. and *Streptomyces ascomycinicus* sp. nov., respectively.

S. hygroscopicus subsp. *decoyicus* NRRL 2666^T is loosely associated with the *Streptomyces ipomoeae* 16S rRNA gene clade (Fig. 1); it shows its highest 16S rRNA gene similarity to the type strains of *Streptomyces ogaensis* and *S. torulosus*. However, these three strains share relatively low 16S rRNA gene similarity of 98.4 %, a value which corresponds to 23 nt differences over 1443 positions. These strains can also be distinguished using a combination of phenotypic features (Table 1). The genotypic and phenotypic data show that strain NRRL 2666^T should be classified in the genus *Streptomyces* within a distinct species. The strain has to be named after the subspecific epithet in accordance with Rule 50a of the *International Code of Nomenclature of Bacteria* (Lapage *et al.*, 1992); although the epithet *decoyicus* is incorrectly formed and should be *decoyininicus*, such corrections are no longer permitted according to Rule 61 (De Vos & Trüper, 2000). Consequently, *Streptomyces hygroscopicus* subsp. *decoyicus* NRRL 2666^T should be designated the type strain of *Streptomyces decoyicus* (Vavra *et al.*, 1959) sp. nov., comb. nov.

As shown in Fig. 1, *S. hygroscopicus* subsp. *angustmyceticus* NRRL B-2347^T is closely related to *S. hygroscopicus* subsp. *glebosus* NRRL B-3248^T. These organisms share a 16S rRNA gene sequence similarity of 99.0 % (15 nt differences over 1447 locations) and can be separated readily using a range of phenotypic properties (Table 1). Consequently, it is proposed that *Streptomyces hygroscopicus* subsp. *angustmyceticus* NRRL B-2347^T be classified as the type strain of *Streptomyces angustmyceticus* (Yüntsen *et al.* 1956) comb. nov., sp. nov. by following Rule 50a of the *International Code of Nomenclature of Bacteria* (Lapage *et al.*, 1992). Correction of the epithet to *angustmycinicus* is not permitted under Rule 61 (De Vos & Trüper, 2000).

This study provides further evidence that the genus *Streptomyces* is underspeciated (Manfio *et al.*, 2003; Xu *et al.*, 2006; Goodfellow *et al.*, 2007), despite the large number of species it contains (http://www.bacterio.cict.fr/index.html).

Description of *Streptomyces aldersoniae* sp. nov.

Streptomyces aldersoniae (al.der.so′ni.ae. N.L. fem. gen. n. *aldersoniae* of Alderson, named in honour of Grace Alderson for her contributions to actinomycete systematics).

Table 1. Phenotypic properties that separate the tested *S. hygroscopicus* strains from one another and from phylogenetically close neighbours

Strains: 1, *S. hygroscopicus* subsp. *glebosus* NRRL B-3248^T; 2, *S. cuspidosporus* NBRC 12379^T; 3, *S. hygroscopicus* NRRL 18513; 4, '*S. hygroscopicus* subsp. *aureolacrimosus*' NRRL 5739; 5, *S. hygroscopicus* subsp. *hygroscopicus* DSM 40822; 6, *S. hygroscopicus* subsp. *hygroscopicus* DSM 40632; 7, *S. torulosus* LMG 20305^T; 8, *S. hygroscopicus* subsp. *decoyicus* NRRL 2666^T; 9, *S. hygroscopicus* subsp. *angustmyceticus* NRRL B-2347^T. Data were generated in this study. All the strains degraded adenine, grew on gelatin, used D-glucose, glycerol, maltose, D-mannose, sucrose and trehalose as sole carbon sources and were susceptible to ampicillin, cefoxitin, cephalosporin (all at 32 μg ml^{-1}) and fusidic acid (at 8 μg ml^{-1}). They were negative for allantoin hydrolysis, did not degrade cellulose, keratin, pectin, tributyrin, xanthine or xylan and did not use D-arabinose, amygdalin, arbutin, citric acid, dulcitol, inulin, pectin, propanol, pyruvic acid or L-sorbose as sole carbon sources.

Characteristic	1	2	3	4	5	6	7	8	9
Morphology and pigmentation on oatmeal agar									
Aerial spore mass colour	Yellowish grey	Grey	Whitish grey, becoming black and moist	Grey	Dark grey	Reddish grey	Grey	Grey, becoming black and moist	Greyish white
Substrate mycelium colour	Yellowish brown	Greyish yellow	Orange	Olive grey	Yellowish brown	Reddish purple	Yellowish brown	Deep yellow	Deep yellow
Diffusible pigment	Orange–yellow	None	None	Pale olive	Orange	Reddish purple	None	None	None
Spore chains	Spirals	Loops or spirals	Loops	Loops or spirals	Loops or spirals	Loops or spirals	Spirals	Spirals	Spirals
Spore ornamentation	Smooth	Spiny	Smooth	Warty	Spiny	Smooth	Knobbly	Smooth	Smooth
Production of melanin pigments	−	−	−	−	−	−	+	−	−
Degradation tests (%, w/v)									
Casein (1 %)	+	+	+	−	+	+	+	+	−
Hypoxanthine (0.4 %)	+	−	−	−	−	+	−	+	+
Starch (1 %)	+	+	+	+	+	−	+	−	−
Tyrosine (0.5 %)	+	−	−	+	+	+	+	+	+
Uric acid (0.5 %)	+	−	−	−	−	+	−	+	+
Growth on sole carbon sources									
At 1 % (w/v)									
Adonitol	−	−	+	−	−	−	+	−	−
L-Arabinose	−	+	+	+	+	+	+	−	−
D-Arabitol	+	+	−	−	+	+	+	+	+
Cellobiose	+	+	+	−	+	+	−	+	+
Dextrin	+	−	+	−	+	+	−	+	+
meso-Erythritol	−	−	+	−	+	+	−	−	−
D-Fructose	+	+	+	−	+	+	+	+	+
L-Fucose	−	−	−	−	+	−	−	+	−
D-Galactose	+	+	+	−	+	+	+	+	+
Glycogen	+	+	+	−	+	+	−	+	+
myo-Inositol	+	+	+	+	+	+	+	+	−
α-Lactose	+	+	−	−	+	+	−	−	−
Melezitose	−	+	+	−	−	−	−	+	+
L-Rhamnose	−	+	−	+	+	+	+	−	−
D-Ribose	+	+	−	−	+	+	−	+	−
Raffinose	+	+	+	−	+	+	+	−	+
Salicin	+	+	+	−	+	−	−	−	−
D-Sorbitol	+	−	−	−	−	−	+	−	+
D-Xylose	+	+	+	+	+	+	+	+	−
At 1 % (v/v)									
Butane-1,4-diol	+	−	−	−	+	+	−	+	+
Growth on sole carbon and nitrogen sources at 1 % (w/v)									
L-Glutamic acid	+	+	+	−	+	+	+	+	+
L-Leucine	+	+	+	−	−	+	+	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9
L-Proline	+	+	+	−	+	+	+	+	+
Growth at/with:									
pH 4.0	−	−	−	+	+	−	−	−	−
pH 5.0	−	+	−	−	+	+	+	−	−
pH 9.0	+	+	−	−	+	+	+	+	+
pH 10.0	+	+	+	−	+	+	+	+	+
10 °C	−	+	+	−	+	−	+	−	+
37 °C	+	−	+	+	+	−	+	+	+
Phenol (0.01 % v/v)	+	+	−	+	+	+	+	+	−
NaCl (10 % w/v)	+	+	+	+	+	+	+	−	+
NaCl (13 % w/v)	+	−	−	−	−	+	−	+	+

The description is based upon data taken from this and from a previous study (Yao & Hamill, 1993). Spores (0.8×1.6 μm) are borne in loops with two to three turns. Spore surface smooth. Tween 60 is degraded. Does not use methanol or propanol as a sole carbon source (at 1 %, v/v). Sensitive to chlortetracycline hydrochloride (64 μg ml^{-1}). Other phenotypic properties are mentioned in the text or in Table 1. The G+C content of the DNA of the type strain is 72.0 mol%. Produces the polyether antibiotic A80789, which shows antimicrobial activity against Gram-positive and anaerobic bacteria.

The type strain, DSM 41909^T (=NRRL 18513^T), was isolated from soil collected in Manioka, New Guinea.

Description of *Streptomyces angustmyceticus* (Yüntsen *et al.* 1956) sp. nov., comb. nov.

Streptomyces angustmyceticus [an.gust.my.ce′ti.cus. N.L. n. *angustmycinum* angustmycin, an antibiotic; L. masc. suff. *-icus* adjectival suffix used with various meanings; N.L. masc. adj. *angustmyceticus* (sic) related to angustmycin, referring to the ability of the organism to produce angustmycin].

Basonym: *Streptomyces hygroscopicus* subsp. *angustmyceticus* Yüntsen *et al.* 1956.

The description is based upon data taken from this and from previous studies (Yüntsen *et al.*, 1956; Dietz & Mathews, 1962). Smooth, ornamented spores (0.8×1.6 μm) are borne in spirals with two to three turns. Degrades Tween 40 and uric acid. Does not use methanol or propanol as a sole carbon source (at 1 %, v/v). α-Alanine, L-alanine, L-arginine, L-glycine, L-histidine, L-isoleucine, L-ornithine, L-serine, L-threonine and L-valine are used as sole nitrogen sources for energy and growth (at 1 %, w/v). Sensitive to doxycycline hydrochloride, gentamicin sulphate, kanamycin sulphate, neomycin sulphate, streptomycin sulphate and tobramycin sulphate (at 8 μg ml^{-1}) and erythromycin, lincomycin hydrochloride, oleandomycin phosphate and rifampicin (at 32 μg ml^{-1}). Other phenotypic properties are mentioned in the text or in Table 1. The G+C content of the DNA of the type strain is 70.2 mol%. Produces angustmycin A, B and C; the latter shows antitubercular activity.

The type strain is DSM 41683^T (=NRRL B-2347^T).

Description of *Streptomyces ascomycinicus* sp. nov.

Streptomyces ascomycinicus (as.co.my.ci′ni.cus. N.L. n. *ascomycinum* ascomycin, an antibiotic; L. masc. suffix *-icus* adjectival suffix used with various meanings; N.L. masc. adj. *ascomycinicus* related to ascomycin, referring to the ability of the organism to produce ascomycin).

The description is based upon data taken from this and from a previous study (Hütter, 1967). Rough spores with spiny surfaces are borne in loops ($0.5–0.7 \times 0.5–1.2$ μm) with two to three turns. Grows well on modified Bennett's agar. Tweens 40, 60 and 80 are degraded. Does not use methanol or propanol as a sole carbon source (at 1 %, v/v). α-Alanine and D-glycine are used as sole nitrogen sources for energy and growth (at 1 %, w/v). Sensitive to cephaloridine hydrochloride (32 μg ml^{-1}), chlortetracycline hydrochloride (64 μg ml^{-1}), erythromycin, gentamicin sulphate, kanamycin sulphate, lincomycin hydrochloride, neomycin sulphate and novobiocin (4 μg ml^{-1}) and to doxycycline hydrochloride, oleandomycin phosphate, rifampicin, streptomycin sulphate, tetracycline hydrochloride and tobramycin sulphate (8 μg ml^{-1}). Other phenotypic properties are mentioned in the text or in Table 1. The G+C content of the DNA of the type strain is 70.2 mol%. Produces ascomycin, which has an immunosuppressant action.

The type strain, DSM 40822^T (=NBRC 13981^T), was isolated from soil collected in Sannomiya, Kobe City, Japan.

Description of *Streptomyces decoyicus* (Vavra *et al.* 1959) sp. nov., comb. nov.

Streptomyces decoyicus [de.co′yi.cus. N.L. n. *decoyininum* decoyinine, an antibiotic; L. masc. suff. *-icus* adjectival

suffix used with various meanings; N.L. masc. adj. *decoyicus* (sic) related to decoyinine, referring to the ability of the organism to produce decoyinine].

Basonym: *Streptomyces hygroscopicus* subsp. *decoyicus* Vavra *et al.* 1959.

The description is based upon data taken from this and from previous studies (Vavra *et al.*, 1959; Dietz & Mathews, 1969). Spores (0.5–0.7 × 0.5–1.2 μm) are borne in spiral chains with two to three turns. Spore surface smooth. Grows well on modified Bennett's agar. Tweens 40 and 60 are degraded. Does not use methanol or propanol as a sole carbon source (at 1 %, v/v). α-Alanine, L-glycine, L-histidine, L-isoleucine, DL-methionine, L-ornithine, L-phenylalanine, L-threonine and L-valine are used as sole nitrogen sources (at 1 %, w/v). Sensitive to doxycycline hydrochloride, erythromycin, gentamicin sulphate, kanamycin sulphate, neomycin sulphate, oleandomycin phosphate, rifampicin, streptomycin sulphate, tetracycline hydrochloride and tobramycin sulphate (8 μg ml^{-1}), cephaloridine hydrochloride (32 μg ml^{-1}), chlortetracycline hydrochloride (64 μg ml^{-1}), fusidic acid (16 μg ml^{-1}), lincomycin hydrochloride and novobiocin (4 μg ml^{-1}), and penicillin G (20 μg ml^{-1}). Other phenotypic properties are mentioned in the text or in Table 1. The G+C content of the DNA of the type strain is 71.2 mol%. Produces decoyinin and psicofuranine; the latter shows antitumour activity.

The type strain, DSM 41427^T (=NRRL 2666^T), was isolated from garden soil.

Description of *Streptomyces milbemycinicus* sp. nov.

Streptomyces milbemycinicus (mil.be.my.ci′ni.cus. N.L. n. *milbemycinum* milbemycin, an antibiotic; L. masc. suff. *-icus* adjectival suffix used with various meanings; N.L. masc. adj. *milbemycinicus* related to milbemycin, referring to the ability of the organism to produce milbemycin).

The description is based upon data taken from this and from previous studies (Aoki *et al.*, 1976; Haber *et al.*, 1991). Aerial hyphae develop from a finely branched substrate mycelium to form whorls with spirals or loops. Chains of 10–50 warty spores (0.6–0.9 × 1.1–1.5 μm) are formed. Grows well on modified Bennett's agar, Czapek-sucrose, inorganic salts-starch and maltose-tryptose agars, and from 18 to 55 °C. Does not use methanol or propanol as a sole carbon source (at 1 %, v/v) or grow in the presence of 0.1 % (v/v) phenol. Sensitive to gentamicin sulphate, kanamycin sulphate, neomycin sulphate, streptomycin sulphate and tobramycin sulphate (8 μg ml^{-1}), erythromycin, lincomycin hydrochloride, rifampicin and oleandomycin phosphate (32 μg ml^{-1}) and doxycycline hydrochloride (4 μg ml^{-1}). Other phenotypic properties are mentioned either in the text or in Table 1. The G+C content of the DNA of the type strain is 71 mol%. Produces milbemycin.

The type strain is DSM 41911^T (=NRRL 5739^T).

Streptomyces wellingtoniae sp. nov.

Streptomyces wellingtoniae (wel.ling.to′ni.ae. N.L. fem. gen. n. *wellingtoniae* of Wellington, named in honour of Elizabeth Wellington for her contributions to streptomycete systematics).

The description is based upon data taken from this and a previous study (Tresner & Backus, 1956). Smooth spores (0.5–0.7 × 0.5–1.2 μm) borne in loops and spirals with one or two turns. Grows well on modified Bennett's agar. Degrades casein, hypoxanthine and Tweens 40 and 60, but not starch. Does not use methanol or propanol as a sole carbon source (at 1 %, v/v). α-Alanine, L-alanine, L-asparagine, L-isoleucine, L-histidine, DL-methionine, L-ornithine, L-phenylalanine, L-serine, L-threonine and L-valine are used as sole nitrogen sources for energy and growth (all at 1 %, w/v). Sensitive to doxycycline hydrochloride, gentamicin sulphate, kanamycin sulphate, neomycin sulphate, streptomycin sulphate and tobramycin sulphate (8 μg ml^{-1}) and erythromycin, lincomycin hydrochloride, rifampicin and oleandomycin phosphate (32 μg ml^{-1}). Other phenotypic properties are mentioned either in the text or in Table 1. The G+C content of the DNA of the type strain is 72 mol%.

The type strain is DSM 40632^T (=NRRL B-1503^T).

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