Neutrotolerant acidophilic *Streptomyces* species isolated from acidic soils in China: *Streptomyces guanduensis* sp. nov., *Streptomyces paucisporeus* sp. nov., *Streptomyces rubidus* sp. nov. and *Streptomyces yanglinensis* sp. nov.

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The taxonomic status of six neutrotolerant acidophilic streptomycetes isolated from acidic soils in Yunnan Province, China, was established using a polyphasic approach. The morphological and chemotaxonomic characteristics revealed that the isolates belong to the genus *Streptomyces*. Almost complete 16S rRNA gene sequences of the isolates were determined and aligned with available corresponding sequences of representatives of the family *Streptomycetaceae*; phylogenetic trees were inferred using four tree-making algorithms. The isolates formed a distinct, albeit heterogeneous, subclade in the *Streptomyces* 16S rRNA gene tree together with the type strain of *Streptomyces yeochonensis*, but were readily distinguishable from the latter using DNA–DNA hybridization and phenotypic data. It was evident from the genotypic and phenotypic data that the isolates belonged to four novel *Streptomyces* species, for which the following names are proposed: *Streptomyces guanduensis* sp. nov. (type strain 701^T=CGMCC 4.2022^T=JCM 13274^T), *Streptomyces paucisporeus* sp. nov. (type strain 1413^T=CGMCC 4.2025^T=JCM 13276^T), *Streptomyces rubidus* sp. nov. (type strain 13c15^T=CGMCC 4.2026^T=JCM 13277^T) and *Streptomyces yanglinensis* sp. nov. (type strain 1307^T=CGMCC 4.2023^T=JCM 13275^T); isolates 317 and 913 belong to this latter species.

Streptomycete systematics has been directed towards unravelling relationships between neutrophilic strains that, in culture, grow between pH 5.0 and 9.0 with an optimum around neutrality (Williams *et al.*, 1983; Manfio *et al.*, 1995; Liu *et al.*, 2005). In contrast, acidophilic actinomycetes, which share key chemotaxonomic and morphological properties with neutrophilic streptomycetes, have received little attention, though they are known to be common in terrestrial habitats with low pH values (Williams *et al.*, 1971; Khan & Williams, 1975; Goodfellow & Dawson, 1978; Seong *et al.*, 1993). It is of interest to clarify the taxonomy of these

organisms, not least because they are involved in the decomposition of fungal biomass in acid litters and soils (Williams & Robinson, 1981) and produce antifungal antibiotics (Williams & Khan, 1974) and acid-stable enzymes (Williams & Flowers, 1978; Williams & Robinson, 1981).

Acidophilic sporoactinomycetes can be assigned to two groups: neutrotolerant acidophilic strains grow from pH 4.5 to 7.5, with an optimum between pH 5.0 and 5.5; strictly acidophilic strains grow from pH 3.5 to 6.5, with an optimum around 4.5 (Williams *et al.*, 1971; Lonsdale, 1985). Members of the latter group have been assigned to the genus *Streptacidiphilus*, which currently contains four species (Kim *et al.*, 2003; Huang *et al.*, 2004). Neutrotolerant acidophilic actinomycetes form a taxonomically diverse group within the range of variation encompassed by the genus *Streptomyces* (Williams *et al.*, 1971; Lonsdale, 1985; Seong, 1992), but only one species has a validly published name, *Streptomyces yeochonensis* (Kim *et al.*, 2004). In the present study,

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 317, 701^T, 913, 1307^T, 13c15^T and 1413^T are AY882019, AY876942, AY882020, AY876940, AY876941 and AY876943, respectively.

six neutrotolerant acidophilic actinomycetes isolated from acidic soils were the subjects of a polyphasic study designed to clarify their taxonomic status. It was evident from the resultant data that the organisms represented four novel, but closely related, *Streptomyces* species.

The six strains were isolated on an acidified isolation medium (Huang *et al.*, 2004) supplemented with cycloheximide and nystatin (each at 50 µg ml⁻¹). The isolation plates were inoculated with soil suspensions prepared using a dispersion and differential centrifugation procedure (Wang *et al.*, 2003). The soil samples (pH 5.1–6.5) were collected from pine-forest soils in Yunnan Province, China; isolates 317 and 913 were from Diaocenshan Mountain, isolates 701^T and 1413^T were from Guandu and isolates 1307^T and 13c15^T were from Yanglin. The strains were maintained as suspensions of mycelial fragments and spores in glycerol (20%, v/v) at -20 °C and on either yeast extract/malt extract agar (ISP medium 2; Shirling & Gottlieb, 1966) or oatmeal agar (ISP medium 3; Shirling & Gottlieb, 1966) that had been adjusted to an initial pH of 5.5 using a citric acid (0.1 M)/Na₂HPO₄ (0.2 M) buffer. Biomass for chemotaxonomic and molecular systematic studies was grown in shake flasks of acidified ISP medium 2 broth at 28 °C for between 3 and 6 days and then harvested by centrifugation. Cells for chemotaxonomic studies were washed twice in distilled water and Tris-EDTA buffer (10 mM Tris, 1 mM EDTA; pH 8.0) and stored at -20 °C until required.

Extraction of chromosomal DNA, PCR amplification and sequencing of 16S rRNA genes from the test strains were carried out using established procedures (Kim *et al.*, 1998). The resultant sequence data were aligned with those of members of representative *Streptomyces* species by using the CLUSTAL X 1.8 program (Thompson *et al.*, 1997) and the alignments were checked manually. Phylogenetic trees were generated by using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP 3.5c package (Felsenstein, 1993) and the TREECON program, version 1.3b (Van de Peer & De Wachter, 1994). Phylogenetic distances were calculated as described by Kimura (1980). The topology of the phylogenetic trees was tested by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining data using the SEQBOOT and CONSENSE programs from the PHYLIP suite (Felsenstein, 1993).

Almost complete 16S rRNA gene sequences (1411–1447 nt) were obtained for the six isolates. Preliminary phylogenetic analysis, which included almost complete full-length sequences of type strains of the family *Streptomycetaceae*, showed that the isolates fell within the evolutionary radiation encompassed by the genus *Streptomyces* (data not shown). The organisms shared 16S rRNA gene sequence similarities of more than 96.5% with many *Streptomyces* species with validly published names, while the corresponding values for streptacidiphili ranged from 94.4 to 95.6%, results which indicate that the isolates are bona fide streptomycetes. In an

analysis restricted to *Streptomyces* strains, the isolates formed a distinct phyletic line in the *Streptomyces* tree together with the type strain of *Streptomyces yeochonensis*, an organism previously shown to form a distinct lineage in the *Streptomyces* phylogenetic tree (Kim *et al.*, 2004).

Chemotaxonomic and morphological studies were carried out on the six isolates to establish whether they had properties consistent with their assignment to the genus *Streptomyces*. The strains were grown on acidified oatmeal agar plates at 28 °C for 14 days and then examined by eye to determine aerial spore-mass colour, substrate mycelial pigmentation and the colours of any diffusible pigments. The micromorphology of the mycelium was observed by light microscopy using the coverslip technique of Kawato & Shinobu (1959). Spore-chain morphology and spore-surface ornamentation were established by examining gold-coated dehydrated specimens using a FEI QUANTA electron microscope. Standard procedures were used for the extraction and analysis of fatty acids (MIDI system; <http://www.midi-inc.com>), diagnostic isomers of diaminopimelic acids (Lechevalier & Lechevalier, 1980; Hasegawa *et al.*, 1983), isoprenoid quinones (Collins, 1985; Wu *et al.*, 1989), polar lipids (Minnikin *et al.*, 1984) and sugars (Lechevalier & Lechevalier, 1980). The G+C content of the genomic DNA was determined by using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* CGMCC 1.365 as the control.

The morphological and chemical features of the isolates were consistent with assignment to the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995). The organisms formed an extensively branched substrate mycelium and aerial hyphae that carried smooth-surfaced spores in flexuous spore chains. They contained major amounts of LL-diaminopimelic acid and minor proportions of the corresponding *meso*-component in whole-organism hydrolysates, hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H₆), MK-9(H₈)] as the predominant isoprenologues and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine and phosphatidylinositol mannosides as the major polar lipids (phospholipid type II *sensu* Lechevalier *et al.*, 1977); they lacked mycolic acids and characteristic sugars. The fatty acid profiles consisted mainly of saturated straight-chain and iso- and anteiso-branched-chain components (fatty acid type 2c *sensu* Kroppenstedt, 1985). The DNA G+C composition of the isolates was within the range 70–75 mol%.

The tested strains and the type strain of *S. yeochonensis* formed several lineages in the *S. yeochonensis* 16S rRNA gene sequence subclade (Fig. 1). Isolates 317, 913 and 1307^T share sequence similarities within the range 99.7–100%, values that correspond to 2 and 0 nt differences at 1404 and 1411 locations, respectively. It is also interesting that this taxon was highlighted by all of the tree-making algorithms and was supported by a 100% bootstrap value in the neighbour-joining analysis. Strain 1413^T is loosely

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987), based on almost complete 16S rRNA gene sequences, showing the phylogenetic relationships between the isolates and related species of the genus *Streptomyces*. Asterisks indicate branches that were recovered using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. Numbers at nodes indicate levels of bootstrap support (%) based on 1000 resampled datasets; only values above 50 % are given. Bar, 0.01 substitutions per nucleotide position.

associated with this group, sharing its closest similarity with isolate 1307^T; the two strains shared a 16S rRNA gene sequence similarity of 98.6 %, a value equivalent to 20 nt differences at 1414 locations. Similarly, strain 13c15^T was loosely associated with the type strain of *S. yeochonensis* (Table 1). The final isolate, strain 701^T, was most closely related to isolate 13c15^T; the two organisms shared a sequence similarity of 97.8 %, a value that corresponds to 31 nt differences at 1396 sites.

The fluorescent microplate method of Ezaki *et al.* (1989) was used to determine DNA–DNA relatedness values between the following strains: isolates 701^T, 1307^T, 13c15^T, 1413^T and *S. yeochonensis* NRRL B-24245^T. Photobiotin-labelled DNA from each of the strains was individually hybridized with single-stranded unlabelled DNA samples of the remaining strains, non-covalently bound to microtitre wells. The hybridization experiments were carried out under stringent conditions in 50 % formamide at 50 °C, and the DNA

Table 1. 16S rRNA gene sequence similarities and DNA–DNA relatedness values between the species in the *S. yeochonensis* 16S rRNA gene sequence subclade

Values above the diagonal are DNA–DNA relatedness values (%), whereas values below the diagonal are 16S rRNA gene sequence similarities (%).

Strain	701 ^T	1307 ^T	13c15 ^T	1413 ^T	NRRL B-24245 ^T
701 ^T		23.1	21.4	14.9	18.7
1307 ^T	97.5		23.8	25.9	21.0
13c15 ^T	97.8	97.9		19.9	43.0
1413 ^T	97.1	98.6	97.9		35.0
<i>S. yeochonensis</i> NRRL B-24245 ^T	97.4	98.0	98.7	98.2	

hybridization rates were detected according to Christensen *et al.* (2000) by using a FLUOstar OPTIMA microplate reader (BMG LABTECH). Mean percentage DNA–DNA relatedness values were calculated from quadruplicate hybridization experiments and expressed as means of the corresponding reciprocal values. It was evident from the resultant data that all of the strains tested belong to different genomic species, as they shared DNA–DNA relatedness values between 14.9 and 43.0% (Table 1) – well below the 70% cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987). The same method was used to determine the corresponding relatedness values between isolates 317, 913 and 1307^T: they shared relatedness values of more than 89%.

S. yeochonensis NRRL B-24245^T and all of the isolates were examined for a range of phenotypic properties, using

established procedures (Williams *et al.*, 1983; Seong *et al.*, 1993), albeit with media adjusted to pH 5.5. The organisms were also examined for their ability to produce melanin pigments on acidified peptone/yeast extract/iron agar (ISP medium 6; Shirling & Gottlieb, 1966) and tyrosine agar (ISP medium 7; Shirling & Gottlieb, 1966) plates after 14 days at 28 °C. It is evident from Table 2 that the strains gave phenotypic profiles that matched the lineages detected in the *S. yeochonensis* 16S rRNA gene sequence subclade. In particular, it is apparent that isolates 317, 913 and 1307^T have identical phenotypic profiles. All of the isolates showed a greater capacity than the *S. yeochonensis* strain to utilize carbon sources for energy and growth.

It is evident from the genotypic and phenotypic data that the six neutrotolerant acidophilic isolates can be assigned to four novel species in the genus *Streptomyces*. Consequently,

Table 2. Phenotypic properties that separate organisms assigned to distinct lineages in the *S. yeochonensis* 16S rRNA gene sequence subclade

Strains: 1, isolate 701^T; 2, isolates 317, 913 and 1307^T; 3, isolate 13c15^T; 4, isolate 1413^T; 5, *S. yeochonensis* NRRL B-24245^T. +, Positive; +^W, weakly positive; –, negative.

Characteristic	1	2	3	4	5
Appearance on oatmeal agar					
Aerial hyphae	Sparse	Abundant	Abundant	Sparse	Abundant
Aerial spore-mass colour	White to grey	Grey	Mahogany	Grey	Grey
Melanin production on tyrosine agar	–	–	–	+	–
Degradation of (% w/v):					
Aesculin (0.1)	–	+	+	+	+
Casein (1.0)	–	–	–	+	+
Hypoxanthine (0.4)	–	+	–	–	–
L-Tyrosine (0.4)	–	–	–	–	–
Xylan (0.4)	–	+	+	–	–
Growth on sole carbon sources (at 1%, w/v)					
L-Arabinose	+	+	+	+ ^W	–
D-Fructose	+	+	+	–	–
Glycerol	–	+	+	–	–
D-Mannose	+	+	+ ^W	–	–
myo-Inositol	+ ^W	–	–	–	–
D-Raffinose	+	+	+	–	–
L-Rhamnose	+	+	–	+	–
L-Sorbose	+	+	+ ^W	+	–
D-Sucrose	+	+	+	–	–
D-Trehalose	+	+	–	+	–
D-Xylose	+	+	+	–	–
Growth on sole carbon sources (at 0.1%, w/v)					
L-Alanine	–	–	–	+ ^W	–
L-Arginine	–	–	+ ^W	+	–
L-Phenylalanine	+	–	–	+	–
Growth at:					
pH 4.5	+	+	+ ^W	+ ^W	+
pH 7.5	–	–	–	+ ^W	+

we propose the names *Streptomyces guanduensis* sp. nov. for isolate 701^T, *Streptomyces paucisporeus* sp. nov. for isolate 1413^T, *Streptomyces rubidus* sp. nov. for isolate 13c15^T and *Streptomyces yanglinensis* sp. nov. for isolates 317, 913 and 1307^T.

Description of *Streptomyces guanduensis* sp. nov.

Streptomyces guanduensis (gu.an.du.en'sis. N.L. masc. adj. *guanduensis* belonging to Guandu, the source of the soil from which the type strain was isolated).

Aerobic, Gram-positive, non-motile, neutrotolerant, acidophilic streptomycete that forms branched substrate and aerial hyphae. Smooth-surfaced spores are borne in flexuous spore chains. Deep-brown colonies that carry a white to grey aerial spore mass are formed on oatmeal agar, ISP medium 9 supplemented with glucose (1 %, w/v) and on yeast extract/malt extract agar. Diffusible pigments are not formed and melanin pigments are not produced on peptone/yeast extract/iron agar or tyrosine agar. Growth occurs at temperatures between 20 and 37 °C, but not at 15 °C, and at pH values from 4.5 to 7.0, but not at pH 3.5. Degrades Tween 80, but not adenine, guanine, starch or xanthine. D-Cellobiose, D-galactose, D-glucose, D-inulin, D-lactose, D-mannitol, D-salicin (each at 1 %, w/v), adipic acid and L-phenylalanine (each at 0.1 %, w/v) are used as sole carbon sources for energy and growth, but adonitol, D-sorbitol (each at 1 %, w/v), L-alanine, DL-aminobutyric acid, L-arginine, α -L-aspartic acid, L-cysteine, L-valine, sodium acetate, sodium citrate and sodium oxalate (each at 0.1 %, w/v) are not. Does not use L-alanine, L-arginine, α -L-aspartic acid, L-glutamic acid, L-isoleucine or L-phenylalanine (each at 0.1 %, w/v) as sole carbon and nitrogen sources. Does not grow in the presence of 5 % (w/v) NaCl. The organism is sensitive to filter-paper discs soaked in the following ($\mu\text{g ml}^{-1}$ unless indicated): acetylspiramycin (15), carbenicillin (10), cephalothin (30), ciprofloxacin (5), doxycycline hydrochloride (30), erythromycin (15), josamycin (15), kanamycin sulphate (30), minocycline hydrochloride (30), neomycin sulphate (30), ofloxacin (5), rifampicin (5), streptomycin sulphate (10), tetracycline hydrochloride (30) and tobramycin sulphate (10), but not to amoxicillin (10), ampicillin (10), azithromycin (30), aztreonam (30), penicillin G (10 IU ml^{-1}) or sulfamethoxazole (25). Additional properties are shown in Table 2. The DNA G+C content is 72.7 mol%. Chemotaxonomic properties are typical of members of the genus *Streptomyces*.

The type (and only) strain, 701^T (= CGMCC 4.2022^T = JCM 13274^T), was isolated from Dashao pine-forest soil in Guandu, Yunnan Province, People's Republic of China.

Description of *Streptomyces paucisporeus* sp. nov.

Streptomyces paucisporeus (pau.ci.spo're.us. L. adj. *paucus* few; N.L. adj. *sporeus* spored; N.L. masc. adj. *paucisporeus* few spored, forming few spores).

Aerobic, Gram-positive, non-motile, neutrotolerant, acidophilic streptomycete that forms branched substrate and aerial hyphae. Khaki-coloured colonies that carry a white to grey aerial spore mass are formed on acidified oatmeal agar, yeast extract/starch agar and yeast extract/malt extract agar. A few spores with smooth surfaces are borne in short flexuous spore chains. Diffusible pigments are not formed, though melanin pigments are produced on tyrosine agar but not on peptone/yeast extract/iron agar. Growth occurs at temperatures between 20 and 37 °C, but not at 15 °C, and at pH values from 4.5 to 7.5, but not at pH 3.5. Degrades Tween 80, but not adenine, guanine, starch or xanthine. D-Cellobiose, D-galactose, D-glucose, D-mannitol, D-sorbitol (each at 1 %, w/v), L-alanine, L-arginine, α -L-aspartic acid, L-cysteine, L-glutamic acid, L-phenylalanine and L-valine (each at 0.1 %, w/v) are used as sole carbon sources for energy and growth, but adonitol, inulin, D-lactose (each at 1 %, w/v), adipic acid, DL-aminobutyric acid, sodium acetate, sodium citrate and sodium oxalate (each at 0.1 %, w/v) are not. Does not use L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-isoleucine or L-phenylalanine (each at 0.1 %, w/v) as sole carbon and nitrogen sources. Does not grow in the presence of 5 % (w/v) NaCl. The organism is sensitive to filter-paper discs soaked in the following ($\mu\text{g ml}^{-1}$ unless indicated): azithromycin (30), doxycycline hydrochloride (30), erythromycin (15), josamycin (15), kanamycin sulphate (30), minocycline hydrochloride (30), neomycin sulphate (30), streptomycin sulphate (10), tetracycline hydrochloride (30) and tobramycin sulphate (10), but not to filter-paper discs soaked in acetylspiramycin (15), amoxicillin (10), ampicillin (10), aztreonam (30), carbenicillin (10), cephalothin (30), ciprofloxacin (5), ofloxacin (5), penicillin G (10 IU ml^{-1}), rifampicin (5) or sulfamethoxazole (25). Additional properties are shown in Table 2. The DNA G+C content is 74.8 mol%. Chemotaxonomic properties are typical of the genus *Streptomyces*.

The type (and only) strain, 1413^T (= CGMCC 4.2025^T = JCM 13276^T), was isolated from Dashao pine-forest soil in Guandu, Yunnan Province, People's Republic of China.

Description of *Streptomyces rubidus* sp. nov.

Streptomyces rubidus (ru'bi.dus. L. masc. adj. *rubidus* dark red).

Aerobic, Gram-positive, non-motile, neutrotolerant, acidophilic streptomycete that forms branched substrate and aerial hyphae. Smooth-surfaced spores are borne in flexuous spore chains. Mahogany-coloured substrate mycelium, sparse aerial hyphae and diffusible pigments are formed on oatmeal agar and yeast extract/malt extract agar. Melanin pigments are not produced on tyrosine agar or peptone/yeast extract/iron agar. Growth occurs at temperatures between 20 and 37 °C, but not at 15 °C, and at pH values from 4.5 to 7.0, but not at pH 3.5. Starch and Tween 80 are degraded, but adenine, guanine and xanthine are not. D-Cellobiose, D-galactose, D-glucose, D-lactose, D-mannitol, D-salicin (each at 1 %, w/v), L-arginine and sodium citrate

(0.1 %, w/v) are used as sole carbon sources for energy and growth, but adonitol, inulin, D-sorbitol (each at 1 %, w/v), adipic acid, L-alanine, DL-aminobutyric acid, α -L-aspartic acid, L-cysteine, L-phenylalanine, sodium acetate, sodium oxalate and L-valine (each at 0.1 %, w/v) are not. L-Alanine is used as a sole carbon and nitrogen source, but L-arginine, α -L-aspartic acid, L-glutamic acid, L-isoleucine and L-phenylalanine (each at 0.1 %, w/v) are not. Does not grow in the presence of 5 % (w/v) NaCl. Sensitive to filter-paper discs soaked in the following ($\mu\text{g ml}^{-1}$ unless indicated): azithromycin (30), aztreonam (30), carbenicillin (10), cephalothin (30), ciprofloxacin (5), doxycycline hydrochloride (30), erythromycin (15), josamycin (15), kanamycin sulphate (30), minocycline hydrochloride (30), neomycin sulphate (30), streptomycin sulphate (10), tetracycline hydrochloride (30) and tobramycin sulphate (10), but resistant to filter-paper discs soaked in acetylspiramycin (15), amoxicillin (10), ampicillin (10), ofloxacin (5), penicillin G (10 IU ml^{-1}), rifampicin (5) and sulfamethoxazole (25). Additional phenotypic properties are shown in Table 2. The DNA G+C content is 70.6 mol%. Chemotaxonomic properties are typical of members of the genus *Streptomyces*.

The type (and only) strain, 13c15^T (CGMCC 4.2026^T = JCM 13277^T), was isolated from a pine-forest soil in Yanglin, Yunnan Province, People's Republic of China.

Description of *Streptomyces yanglinensis* sp. nov.

Streptomyces yanglinensis (yang.lin.en'sis. N.L. masc. adj. *yanglinensis* belonging to Yanglin, the source of the soil from which the type strain was isolated).

Aerobic, Gram-positive, non-motile, neutrotolerant, acidophilic streptomycete that forms branched substrate and aerial hyphae. Grey-coloured colonies that carry an abundant white to grey aerial spore mass are formed on acidified oatmeal agar; blackish colonies bearing an abundant white to grey aerial spore mass are formed on ISP 9 agar supplemented with fructose, mannose or sucrose (each at 1 %, w/v). Smooth-surfaced spores are borne on flexuous spore chains. Diffusible pigments are not produced, and melanin pigments are not formed on peptone/yeast extract/iron agar or tyrosine agar. Growth occurs at temperatures between 20 and 37 °C, but not at 15 °C, and at pH values between 4.5 and 7.0, but not at pH 3.5. Degrades starch and Tween 80, but not adenine, guanine or xanthine. Adonitol, D-cellobiose, D-galactose, D-glucose, D-inulin, D-lactose, D-mannitol, D-salicin and D-sorbitol (each at 1 %, w/v) are used as sole carbon sources for energy and growth, but D-melezitose (at 1 %, w/v), adipic acid, L-alanine, DL-aminobutyric acid, L-arginine, α -L-aspartic acid, L-cysteine, L-phenylalanine, L-valine, sodium acetate, sodium citrate and sodium oxalate (each at 0.1 %, w/v) are not. L-Glutamic acid is used as a sole carbon and nitrogen source, but L-alanine, L-arginine, L-aspartic acid, L-isoleucine and L-phenylalanine (each at 0.1 %, w/v) are not. Does not

grow in the presence of 5 % (w/v) NaCl. Sensitive to filter-paper discs soaked in the following ($\mu\text{g ml}^{-1}$ unless indicated): cephalothin (30), doxycycline hydrochloride (30), erythromycin (15), josamycin (15), kanamycin sulphate (30), minocycline hydrochloride (30), neomycin sulphate (30), sulfamethoxazole (25) and tobramycin sulphate (10), but not to filter-paper discs soaked in acetylspiramycin (15), amoxicillin (10), ampicillin (10), azithromycin (30), aztreonam (30), carbenicillin (10), ciprofloxacin (5), ofloxacin (5), penicillin G (10 IU ml^{-1}), rifampicin (5), streptomycin sulphate (10) or tetracycline hydrochloride (30). Additional phenotypic properties are shown in Table 2. The DNA G+C content is 74.8 mol%. Chemotaxonomic properties are typical of the genus *Streptomyces*.

The type strain, 1307^T (= CGMCC 4.2023^T = JCM 13275^T), was isolated from a pine-forest soil in Yanglin, Yunnan Province, People's Republic of China.

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