Kitasatospora viridis sp. nov., a novel actinomycete from soil

Zhiheng Liu,¹ Carlos Rodríguez,² Liming Wang,¹ Quingfeng Cui,¹ Ying Huang,¹ Erika T. Quintana² and Michael Goodfellow²

Correspondence

Michael Goodfellow
m.goodfellow@ncl.ac.uk

¹State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

²School of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

The taxonomic position of a rhizosphere isolate, strain 52108a^T, was determined using a polyphasic approach. The strain was found to have chemical and morphological properties consistent with its assignment to the genus *Kitasatospora*. An almost complete 16S rRNA gene sequence determined for the strain was aligned with corresponding sequences of representatives of the genus *Kitasatospora* and related taxa using three tree-making algorithms. The organism formed a distinct phyletic line within the *Kitasatospora* clade and was most closely related to *Kitasatospora arboriphila* (98.9%), *Kitasatospora kifunensis* (99.0%), *Kitasatospora paracochleata* (98.4%) and *Kitasatospora terrestris* (98.2%), but was readily distinguished from representatives of these species using a combination of phenotypic properties. The combined genotypic and phenotypic data show that the strain should be classified in the genus *Kitasatospora* as a novel species. The name proposed is *Kitasatospora viridis* sp. nov., with the type strain 52108a^T (=AS 4.1878^T=DSM 44826^T).

The genus *Kitasatospora* (originally *Kitasatosporia*) was proposed by Ōmura *et al.* (1982); its members were transferred to the genus *Streptomyces* by Wellington *et al.* (1992), and the genus was re-established by Zhang *et al.* (1997). The taxon is now widely recognized and encompasses 18 species: *Kitasatospora arboriphila* Groth *et al.* 2004, *K. azatica* (Nakagaito *et al.* 1993a) Zhang *et al.* 1997, *K. cheerisanensis* Chung *et al.* 1999, *K. cineracea* Tajima *et al.* 2001, *K. cochleata* (Nakagaito *et al.* 1993a) Zhang *et al.* 1997, *K. cystarginea* Kusakabe and Isono 1992, *K. gansuensis* Groth *et al.* 2004, *K. griseola* Takahashi *et al.* 1985, *K. kifunensis* (Nakagaito *et al.* 1993b) Groth *et al.* 2003, *K. mediocidica* Labeda 1988, *K. niigatensis* Tajima *et al.* 2001, *K. nipponensis* Groth *et al.* 2004, *K. paracochleata* (Nakagaito *et al.* 1993a) Zhang *et al.* 1997, *K. paranensis* Groth *et al.* 2004, *K. phosalacinea* Takahashi *et al.* 1985, *K. putterlickiae* Groth *et al.* 2003, *K. setae* Ōmura *et al.* 1983 [species name corrected by Ōmura *et al.* (1985)], the type species, and *K. terrestris* Groth *et al.* 2004.

An actinomycete isolate, strain 52108a^T, was isolated from rhizosphere soil [pH 4.4–4.5, as determined using the method of Reed & Cummings (1945)] of wild tea plants (*Camellia oleifera*) growing on the campus of Jiangxi

Agricultural University, Jiangxi Province, China. Soil suspensions prepared using a dispersion and differential centrifugation procedure (Wang *et al.*, 2003) were plated onto an acidified selective isolation medium supplemented with actidione and nystatin (Huang *et al.*, 2004) and the preparations were incubated at 28 °C for 3 weeks. The organism was maintained on oatmeal agar (ISP medium 3; Küster, 1959) slants, adjusted to pH 5.5, at 4 °C and as suspensions of spores in glycerol (20%, v/v) at –20 °C.

Spore chain morphology was observed on acidified oatmeal agar following incubation for 2 weeks at 28 °C, using the coverslip technique of Kawato & Shinobu (1959); growth on the coverslip was fixed and examined following the methods of Zhou *et al.* (1998). Spore surface ornamentation was observed by examining gold-coated, dehydrated specimens using a Cambridge Stereoscan 240 scanning electron microscope following the procedure described by O'Donnell *et al.* (1993). Spore suspensions for biochemical, degradative, nutritional and physiological tests were prepared using the procedure described by Hopwood *et al.* (1985) and the tests were carried out using media and methods described by Williams *et al.* (1983). Strain 52108a^T and *K. paracochleata* strain DSM 41656^T were examined for their ability to grow on oatmeal agar adjusted to pH 3.5, 4.5, 5.5, 6.5, 7.0 and 8.0 using a citric acid/disodium hydrogen phosphate buffer system (McIlvaine, 1921).

Biomass for chemotaxonomic studies was grown in shake flasks of modified Bennett's broth (Jones, 1949), adjusted

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to pH 5.0, and incubated at 28 °C for 7 days. After centrifugation, the biomass was washed in distilled water and Tris/EDTA (0.03 M Tris/HCl, 0.1 M EDTA, pH 8.0) and stored at -20 °C until required. Standard chromatographic procedures were used to determine the diagnostic isomers of diaminopimelic acid (Staneck & Roberts, 1974), the acyl type of muramic acid (Uchida & Aida, 1977) and menaquinone (Collins, 1985; Wu *et al.*, 1989), polar lipid (Minnikin *et al.*, 1984) and whole-organism sugar patterns (Hasegawa *et al.*, 1983), using appropriate reference strains as controls. Non-hydroxylated fatty acids were extracted, purified, methylated, identified and quantified by GC using the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Extraction of genomic DNA, PCR amplification and direct sequencing of the 16S rRNA gene were carried out according to Kim *et al.* (1998), and the resultant almost complete sequence (1412 nt) was manually aligned with corresponding sequences of representatives of the genera *Kitasatospora*, *Streptacidiphilus* and *Streptomyces* using the pairwise alignment option and the 16S rRNA gene sequence secondary structural information from the PHYDIT program (Chun, 1995). Phylogenetic trees were inferred using the

least-squares, maximum-likelihood, maximum-parsimony and neighbour-joining tree-making algorithms from the PHYLIP 3.5c software package (Felsenstein, 1993) and the TREECON program (Van de Peer & De Wachter, 1994). The resultant unrooted tree topologies were evaluated by bootstrap analyses of the neighbour-joining method, based on 1000 resamplings, using programs from the PHYLIP package (Felsenstein, 1993). Genomic DNA was also used for PCR amplification of the nucleotide signature present in the 16S–23S rDNA region of members of the genus *Kitasatospora*, as described by Wang *et al.* (1996a, b).

Comparison of the almost complete 16S rRNA gene sequence of strain 52108a^T with the corresponding sequences of the marker strains showed that the isolate belongs to the *Kitasatospora* clade (Fig. 1). This assignment was confirmed by the positive detection of the diagnostic PCR product in the 16S–23S rRNA gene spacer region of strain 52108a^T. The organism shared closest 16S rRNA gene sequence similarity with the type strains of *K. kifunensis* (99.0%), *K. arboriphila* (98.9%), *K. paracochleata* (98.4%) and *K. terrestris* (98.2%). Lower 16S rRNA gene sequence similarities were found with the type strains of the remaining *Kitasatospora* species. DNA–DNA relatedness

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the position of strain 52108a^T in the *Kitasatospora* tree. Asterisks indicate branches that were also recovered using the least-squares, maximum-likelihood and maximum-parsimony tree-making algorithms; f, ml and p indicate branches that were formed using the least-squares, maximum-likelihood and maximum-parsimony algorithms, respectively. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50% are given. Bar, 0.02 nucleotide substitutions per nucleotide position. PT, Putative type strain (species name not yet validly published).

studies were not carried out between strain 52108a^T and its phylogenetically closest relatives as it has already been established that representatives of other *Kitasatospora* species with similar 16S rRNA gene sequence similarities, as exemplified by *K. paranensis* and *K. terrestris* (Groth *et al.*, 2004), share DNA–DNA relatedness values well below the 70 % cut-off point recommended for the delineation of bacterial species (Wayne *et al.*, 1987). The tested strain can be distinguished from its phylogenetically nearest relatives using a combination of phenotypic properties (Table 1). It is proposed that strain 52108a^T be assigned to the genus *Kitasatospora* as a novel species, with the name *Kitasatospora viridis* sp. nov.

Description of *Kitasatospora viridis* sp. nov.

Kitasatospora viridis (vi'ri.dis. L. fem. adj. *viridis* green, referring to the production of a green aerial spore mass).

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete that forms an extensively branched, light-yellow substrate mycelium and a greenish aerial spore mass on acidified oatmeal agar. Aerial hyphae differentiate into

long, spiral chains of smooth-surfaced, cylindrical spores (1.0–1.2 × 0.7–0.8 µm). Starch is degraded, but not adenine, guanine, hypoxanthine, xanthine or xylan. Adonitol, (+)-D-cellobiose, dextran, (+)-D-galactose, (–)-D-gluconic acid, (+)-D-glucose, inulin, (+)-D-lactose, (+)-D-maltose, (+)-D-mannose, (+)-D-melezitose, (+)-D-melibiose, (–)-D-salicin, (–)-D-sorbitol, (+)-D-trehalose and xylitol are used as sole carbon sources for energy and growth, but not glycerol, *meso*-inositol or xylan (all at 1 %, w/v). Similarly, 2-aminoethanol, α-DL-aminobutyric acid, L-alanine, L-arginine, L-cysteine, L-glutamic acid, L-histidine, L-isoleucine, L-phenylalanine, L-threonine, L-valine, sodium oxalate and sodium pyruvate are used as sole carbon sources, but not adipic acid or L-aspartic acid (all at 0.1 %, w/v). 2-Aminoethanol, L-alanine, L-arginine, L-isoleucine and L-phenylalanine are used as sole sources of carbon and nitrogen for energy and growth. Growth occurs at 10–37 °C, but not at 4 or 45 °C. The pH range for growth is pH 4–7.0. Growth occurs in the presence (µg ml^{–1}) of amikacin (32), amoxicillin (32), ampicillin (32), cefalexin (32), cephaloridine (64), clindamycin (8), doxycycline hydrochloride (32), fusidic acid (16), gentamicin sulphate (16), kanamycin sulphate

Table 1. Phenotypic properties that separate strain 52108a^T from representatives of closely related *Kitasatospora* species

Taxa: 1, strain 52108a^T; 2, *K. arboriphila* HKI 0189^T (data from Groth *et al.*, 2004); 3, *K. kifunensis* DSM 41654^T (Groth *et al.*, 2004); 4, *K. paracochleata* DSM 41656^T (Nakagaito *et al.*, 1992); 5, *K. terrestris* HKI 0186^T (Groth *et al.*, 2004). All are positive for (+)-D-xylose. +, Positive/present; –, negative/absent; (+), weakly positive; NA, not analysed.

Characteristic	1	2	3	4	5
Spore chain morphology*	S	RA, RF, S	RF, S	S	RA, RF, S
Melanoid pigments	–	+	+	+	+
Growth at:					
10 °C	–	–	(+)	–	–
37 °C	+	+	–	–	+
Growth in the presence of NaCl (% w/v):					
2.5	+	–	+	+	+
3.0	+	–	–	–	(+)
3.5	+	–	–	–	–
pH for growth:					
4.0	+	–	–	+†	–
8.0	–	+	+	NA	+
Growth in the presence of sole carbon sources:					
(+)-D-Fructose	+	+	–	+	+
(–)-D-Mannitol	+	–	+	+	–
(+)-D-Raffinose	+	+	–	+	+
(+)-L-Rhamnose	+	–	–	+	(+)
(–)-D-Sucrose	+	–	+	+	(+)
Resistance to antibiotics (µg ml ^{–1}):					
Ampicillin (10)	+	–	(+)	NA	–
Lincomycin hydrochloride (2)	+	–	–	+	–
Novobiocin (5)	–	+	+	+	+
Penicillin G (10 IU)	+	–	–	+	–

*RA, *Retinaculiaperti*; RF, *rectiflexibiles*; S, *spirales*.

†Data from this study.

(16), lincomycin hydrochloride (16), midcamycin (4), neomycin sulphate (32), penicillin G (16 IU), streptomycin sulphate (16), tetracycline hydrochloride (32) and tobramycin sulphate (16), but is inhibited by erythromycin (8) and novobiocin (8). Sodium chloride is tolerated up to a concentration of 10% (w/v). Additional phenotypic properties are listed in Table 1. Cell wall contains both *meso*- and *LL*-diaminopimelic acid and *N*-acetylated muramic acid, and whole-organism hydrolysates are rich in galactose and glucose. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. The predominant isoprenologues are hexa- (76%) and octa- (17%) hydrogenated menaquinones with nine isoprene units. The major fatty acids are iso-C_{15:0} (19%), anteiso-C_{15:0} (18%), iso-C_{16:0} (18%), C_{16:0} (22%) and anteiso-C_{17:0} (8.0%).

The type strain, 52108a^T (=AS 4.1878^T=DSM 44826^T), was isolated from a soil sample taken from the roots of *Camellia oleifera* in Jiangxi Province, China.

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