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

Zhiheng Liu, Carlos Rodríguez, Liming Wang, Quingfeng Cui, Ying Huang, Erika T. Quintana and Michael Goodfellow

The taxonomic position of a rhizosphere isolate, strain 52108aT, 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 paracocleata (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 52108aT (= AS 4.1878T = DSM 44826T).


An actinomycete isolate, strain 52108aT, 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 52108aT and K. paracocleata strain DSM 41656T 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
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/Cl, 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 52108aT 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 52108aT. 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
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. terrestis* (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 x 0·7–0·8 \(\mu\)m). Starch is degraded, but not adonitol, guanine, hypoxanthine, xanthine or xylan. Adonitol, (+)-D-cellulobiose, 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, \(-\text{DL-}

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore chain morphology*</td>
<td>S</td>
<td>RA, RF, S</td>
<td>RF, S</td>
<td>S</td>
<td>RA, RF, S</td>
</tr>
<tr>
<td>Melanoid pigments</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 °C</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>37 °C</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth in the presence of NaCl (%; w/v):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2·5</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3·0</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td>3·5</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH for growth:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4·0</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+†</td>
<td>–</td>
</tr>
<tr>
<td>8·0</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Growth in the presence of sole carbon sources:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+)-D-Fructose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(+)-D-Mannitol</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>(+)-D-Raffinose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(+)-L-Rhamnose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>(+)-D-Sucrose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Resistance to antibiotics (µg ml(^{-1})):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin (10)</td>
<td>+</td>
<td>–</td>
<td>(+)</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Lincomycin hydrochloride (2)</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Novobiocin (5)</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Penicillin G (10 IU)</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

*RA, Retinaculapiertii; RF, rectiflexibles; S, spirales.
†Data from this study.
(16), lincomycin hydrochloride (16), midecamycin (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 L-Diaminopimelic acid and N-acetylated muramic acid, and whole-organism hydrolysates are rich in galactose and glucose. The major polar lipids are diphostathidylglycerol, phosphatidylethanolamine, phosphatidylglynositol and phosphatidylglynositol mannosides. The predominant isoprenologues are hexa- (76 %) and octa- (17 %) hydrogenated menaquiones 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.

**Acknowledgements**

This work was supported through the Royal Society–Chinese Academy of Sciences Exchange Scheme (grant no. Q 814). C.R. gratefully acknowledges receipt of a studentship from the Ecuadorian FUNDACYT (Foundation for Science and Technology) and an Overseas Research Studentship Award (UK). E.T.Q. gratefully acknowledges the financial support provided by the Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico City, Mexico) and an Overseas Research Studentship Award (UK). We are also indebted to Professor Gao Yongsheng for providing the soil samples.

**References**


Nakagaito, Y., Shimazu, A., Yokota, A. & Hasegawa, T. (1993a). *Streptomyces cochleatus* sp. nov., *Streptomyces paracochleatus* sp. nov. and *Streptomyces azaticus* sp. nov. In Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB, List no. 44. *Int J Syst Bacteriol* 43, 188–189.


