Streptomyces graminilatus sp. nov., isolated from bamboo litter

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A Gram-stain-positive, novel actinobacterium, designated strain JL-6T, was isolated from the litter of a bamboo (Sasa borealis) forest in Damyang, Korea. Strain JL-6T had white-grey, smooth, cylindrical spores that were borne in straight, long spore-chains. The novel strain grew aerobically at 15–28 °C (optimum, 28 °C), pH 4.0–8.0 (optimum, pH 5.5) and with 0–1.5 % (w/v) NaCl.

The cell-wall peptidoglycan contained LL-diaminopimelic acid, glutamic acid, alanine and glycine. The predominant menaquinones were MK-9(H₆) and MK-9(H₈). Whole-cell hydrolysates mainly contained glucose and ribose. Phosphatidylinositol and phosphatidylcholine were the diagnostic phospholipids. The G+C content of the genomic DNA was 72.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain JL-6T belonged to the genus Streptomyces with sequence similarities ranging from 97.3 % to 98.3 %. However, DNA–DNA hybridization between JL-6T and the closest related strain, Streptomyces turgidiscabies, ATCC 700248T and other closely related species in the genus Streptomyces showed <50 % relatedness. Based on these observations, strain JL-6T is proposed to represent a novel species of the genus Streptomyces, for which the name Streptomyces graminilatus sp. nov. is proposed. The type strain is JL-6T (=KACC 16470T=NBRC 108882T).

Members of the genus Streptomyces are ubiquitous microorganisms living mostly in the soil and environments such as seawater, sediments and plant surfaces. Members of the genus Streptomyces have a wide range of metabolic abilities and potential applications in the production of antibiotics, enzymes, enzyme inhibitors, vitamins and bioactive compounds, with importance in the food, agriculture and pharmaceutical industries (McCarthy & Williams 1992; Lazzarini et al. 2000; Bérdy, 2005). Previously, we investigated the diversity of actinobacteria from bamboo (Phyllostachys bambusoides, Phyllostachys nigro var. henonis, Phyllostachys nigra f. punctata and Sasa borealis) forest soils in Korea. Out of 330 isolates separated from litter, humus and rhizosphere soils of each bamboo forest (Lee & Whang, 2010), Streptomyces gramineus JR-43T was isolated from a bamboo (Sasa borealis) rhizosphere soil (Lee et al., 2012). For the actinobacterial distribution of isolates on the litter of Sasa borealis, Streptomyces was the major actinobacteria, showing >97 % distribution rate. One of these isolates, designated strain JL-6T, represented as a novel species of the genus Streptomyces and was subjected to a taxonomic investigation.

Strain JL-6T was isolated from litter taken from a bamboo forest in Damyang, Chonnam, South Korea. The litter was washed aseptically in sterilized water using a sonic oscillator (VCX750; SONIC Vibra-Cell) for 40 s at 30 W followed by using the dilution-plating method on starch-casein agar (Küster & Williams, 1964) at pH 5.5 and incubated at 28 °C for 7 days. The isolated strain was stored at −86 °C in the presence of 20 % (v/v) glycerol. Morphological, physiological and biochemical properties of strain JL-6T were investigated by following the standard protocol of the International Streptomyces Project (Shirling & Gottlieb, 1966, 1968a, b, 1969) and the methods of Williams et al. (1989). Morphological observations of spores and mycelia of strain JL-6T grown on ISP 3 at 28 °C for 10 days were made by scanning electron microscopy (Hitachi). Growth at various temperatures, pH and NaCl concentrations were examined according to Lee et al. (2012), using inorganic salts-starch agar.

Morphological observation of a 10 days culture of strain JL-6T grown on oatmeal agar (ISP 3 medium) revealed that strain JL-6T had the typical characteristics of the genus Streptomyces. Aerial mycelium and substrate mycelium were well-developed without fragmentation. Long chains...
of spores were straight, cylindrical, smooth-surfaced and non-motile (Fig. 1). The mycelium of strain JL-6T was well-developed on most media tested including yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7), nutrient agar and Czapek's agar. No diffusible pigment was detected in test media and melanin was not produced on peptone-yeast extract-iron agar (Table S1, available in IJSEM Online). Strain JL-6T was able to grow at between pH 4.0 and pH 8.0, with an optimum of pH 5.5. The growth temperature was 15–28°C with an optimum at 28°C. Strain JL-6T grew in presence of 0–1.5 % NaCl (w/v) and no growth was observed over 2.0 % NaCl. The strain degraded casein and cellulose but not starch or gelatin. As the sole carbon source, strain JL-6T utilized D-glucose, myo-inositol, D-mannitol, raffinose, L-rhamnose, sucrose, L-arabinose and D-xylose. Differentiating characteristics of strain JL-6T from type strains of closely related species in the genus *Streptomyces* are shown in Table 1.

**Fig. 1.** Scanning electron micrographs of cells of strain JL-6T grown on oatmeal agar (ISP 3) for 10 days at 28°C. (a) Straight spore chains. (b) Spiny surfaces of spores. Bars, 5 μm (a) and 4 μm (b).

Cellular fatty acids were extracted from type strains of species of the genus *Streptomyces* grown in tryptic soy broth (Difco) for 5 days at 28°C according to the protocol of the Microbial Identification System (Microbial ID; MIDI) and identified by using the Microbial Identification software package (database TSBA 40, version 4.0; MIDI; Sasser, 1990). Peak identification (e.g. differentiating between C16:1ω9c and iso-C15:0 2-ОH in summed feature 3) was verified by GC-MS using an Agilent series 6890 GC system and 5973 mass-selective detector, equipped with a HP5 MS capillary column (30 m, 0.25 mm inner diameter, 0.25 mm film thickness) with helium as the carrier gas. For chemotaxonomic analysis, freeze-dried cells were obtained from cultures grown in starch-casein broth on a shaking incubator at 120 r.p.m. and 28°C for 14 days. The amino acids in the cell-wall peptidoglycan and analysis of the whole-cell sugars were performed as described by Lechevalier & Lechevalier (1970, 1980) and Stanek & Roberts (1974), respectively. Polar lipids were extracted and detected by the method of Minnikin *et al.* (1984). Menaquinones were extracted by the method of Collins (1985) and separated by HPLC. The G+C content of the genomic DNA of strain JL-6T was determined by the HPLC method according to Mesbah *et al.* (1989).

The major cellular fatty acids of strain JL-6T were iso-C15:0 (23.11%), iso-C16:0 (21.03%) and anteiso-C15:0 (10.82%). There were significant differences in the fatty acids profile of strain JL-6T compared to those of the reference strains, but like some of the reference strains, strain JL-6T did not contain hydroxyl fatty acids 3-ОH C17:0 and 3-ОH C18:0 (Table S2). The cell-wall peptidoglycan contained LL-diaminopimelic acid, glutamic acid, alanine and glycine. Whole-cell hydrolysates contained predominantly glucose and ribose. The polar lipid pattern consisted of phosphatidylcholine and phosphatidylinositol (Fig. S3). The predominant menaquinones were MK-9(H6) and MK-9(H8). The G+C content of the genomic DNA was 72.8 mol%.

Genomic DNA was extracted (Cui *et al.*, 2001) from the isolate and the 16S rRNA gene sequence was amplified as described by Lee *et al.* (2012). The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved by using the EzTaxon-e server (http://www.eztaxon-e.ezcloud.net/; Kim *et al.*, 2012). The 16S rRNA gene sequence of strain JL-6T was aligned with the published sequences of closely related bacteria with CLUSTAL W 2.1 software (Larkin *et al.*, 2007). Phylogenetic trees were reconstructed by using three different methods: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms within the MEGA5 software package (Tamura *et al.*, 2011). Evolutionary distance matrices for the neighbour-joining method were calculated using the algorithm of Kimura’s two-parameter model (Kimura, 1980). To evaluate the stability of the phylogenetic tree, a bootstrap analysis (1000 replications) was performed (Felsenstein, 1985).
Table 1. Some characteristics that differentiate strain JL-6<sup>T</sup> from the type strains of related species of the genus *Streptomyces*

| Strains: | 1, JL-6<sup>T</sup>; 2, *Streptomyces turgidiscabies* ATCC 700248<sup>T</sup>; 3, *Streptomyces cacaoi* subsp. *asoensis* NRRL B-16592<sup>T</sup>; 4, *Streptomyces torulosus* LMG 20305<sup>T</sup>; 5, *Streptomyces scabiei* ATCC 49173<sup>T</sup>; 6, *Streptomyces tauricus* JCM 4837<sup>T</sup>. All data are from this study. All strains are positive for degradation of cellulose. +, Positive; −, negative; w, weakly positive.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Aerial spore mass colour on ISP 2</td>
<td>White–grey</td>
<td>Ivory</td>
<td>Ivory</td>
<td>Ivory</td>
<td>White</td>
<td>Red</td>
</tr>
<tr>
<td>Colour of reverse side of colony</td>
<td>Ivory</td>
<td>Ivory</td>
<td>Ivory</td>
<td>Ivory</td>
<td>Ivory</td>
<td>Yellowish-pink</td>
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<tr>
<td>Spore chain</td>
<td>Straight</td>
<td>Flexuous</td>
<td>Spirals</td>
<td>Spirals</td>
<td>Rectiflexibles</td>
<td>Spirals</td>
</tr>
<tr>
<td>Spore surface</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Knobby</td>
<td>Smooth</td>
<td>Smooth</td>
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<tr>
<td>Soluble pigments</td>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>Greenish-yellow</td>
<td>Red</td>
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<tr>
<td>NaCl for growth (%, w/v)</td>
<td>&lt;1.5</td>
<td>&lt;4</td>
<td>&lt;3</td>
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<td>pH range for growth</td>
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<td>+</td>
<td>−</td>
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<td>+</td>
<td>−</td>
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<tr>
<td>l-Arabinose</td>
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<td>w/−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
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<td>+</td>
<td>w/−</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>l-Rhamnose</td>
<td>+</td>
<td>w/−</td>
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<td>+</td>
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</tr>
<tr>
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<td>w/−</td>
<td>+</td>
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<td>d-Xylose</td>
<td>+</td>
<td>w/−</td>
<td>+</td>
<td>+</td>
<td>w/−</td>
<td>−</td>
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</tbody>
</table>

The almost-complete 16S rRNA gene sequence of strain JL-6<sup>T</sup> (1462 nt) was compared with those of members of the genus *Streptomyces* and related taxa. Strain JL-6<sup>T</sup> shared highest 16S rRNA gene sequence similarity with *Streptomyces turgidiscabies* SY9113<sup>T</sup> (98.4 %), *Streptomyces cacaoi* subsp. *asoensis* AS 4.1602<sup>T</sup> (98.1 %), *Streptomyces tauricus* ATCC 29340<sup>T</sup> (98.8 %), *Streptomyces torulosus* ATCC 27470<sup>T</sup> (98 %), *Streptomyces scabiei* RL-34<sup>T</sup> (97.6 %) and *Streptomyces cacaoi* NRRL B-16592<sup>T</sup> (97.2 %) and was clearly discriminated from the type strains of other species of the genus *Streptomyces* based on the levels of DNA–DNA relatedness, i.e. below the 80 % cut-off point recommended for the recognition of genomic species of *Streptomyces* (Labeleda & Lyons, 1992; Labeda, 1993, 1998).

The characteristics that differentiate strain JL-6<sup>T</sup> from the other species of the genus *Streptomyces* are summarized in Table 1. Strain JL-6<sup>T</sup> shared similar chemotaxonomic characteristics with members of the genus *Streptomyces* in terms of major fatty acids. However, strain JL-6<sup>T</sup> could be clearly discriminated from the type strains of other species of the genus *Streptomyces* based on the levels of DNA–DNA relatedness, i.e. below the 80 % cut-off point recommended for the recognition of genomic species of *Streptomyces* (Labeleda & Lyons, 1992; Labeda, 1993, 1998).

Therefore, on the basis of these polyphasic taxonomic evidences, we propose that strain JL-6<sup>T</sup> represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces graminilatus* sp. nov. is proposed.

### Description of *Streptomyces graminilatus* sp. nov.

*Streptomyces graminilatus* [g. ra.mi.ni.la’tus. L. n. *gramen*-inis grass (incl. bamboo); L. part. perf. *latus* carried (from elsewhere, meaning litter); *graminalitus* from the litter layer of bamboo, from where the type strain was isolated].

Aerobic, Gram-stain-positive, non-motile actinobacteria that forms extensively branched substrate mycelium and aerial hyphae. Produces long, straight chains of cylindrical...
Acknowledgements

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References


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