Streptomyces gramineus sp. nov., an antibiotic-producing actinobacterium isolated from bamboo (*Sasa borealis*) rhizosphere soil

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Two actinobacterial strains, JR-43^T and JR-4, were isolated from bamboo (*Sasa borealis*) rhizosphere soil. The isolates produced grey aerial mycelium and a yellow soluble pigment on ISP 4. Microscopic observation revealed that strains JR-43^T and JR-4 produced *rectiflexibiles* spore chains with spiny surfaces. Both isolates had antibacterial activity against plant-pathogenic bacteria, such as *Xanthomonas campestris* LMG 568^T and *Xanthomonas axonopodis* pv. *vesicatoria* LMG 905. The isolates contained iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{16:0} as the major fatty acids and MK-9(H₈) and MK-9(H₈) as the major isoprenoid quinones. Phylogenetic analysis of the 16S rRNA gene sequences of strains JR-43^T and JR-4 showed that they grouped within *Streptomyces* cluster II and had highest sequence similarity to *Streptomyces seoulensis* NBRC 16668^T and *Streptomyces recifensis* NBRC 12813^T (both 98.2% 16S rRNA gene sequence similarity). DNA–DNA relatedness between strain JR-43^T and *S. seoulensis* NBRC 16668^T and *S. recifensis* NBRC 12813^T ranged from 31.42 to 42.92%. Based on DNA–DNA relatedness and morphological and phenotypic data, strains JR-43^T and JR-4 could be distinguished from the type strains of phylogenetically related species. They are therefore considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces gramineus* sp. nov. is proposed. The type strain is JR-43^T (=KACC 15079^T =NBRC 107863^T). Strain JR-43 (=KACC 15078 =NBRC 107864) is a reference strain.

The genus *Streptomyces* represents a group of micro-organisms that are widely distributed in nature. The genus was proposed by Waksman & Henrici (1943) for a group of aerobic, spore-forming actinobacteria, which are classified on the basis of morphological, chemotaxonomic and physiological characteristics and phylogenetic analysis. More than 500 *Streptomyces* species have been described, the largest number of any bacterial genus (Hain *et al.*, 1997; Euzéby, 2009). Streptomycetes are the most prolific producers of bioactive compounds such as antibiotics (Okami & Hotta, 1988; Berdy, 1995). Previously, we isolated 330 actinobacterial strains from bamboo forest soil and investigated their antibiotic activities against plant-pathogens such as the fungus *Botrytis cinerea* and the bacteria *Xanthomonas campestris*, *Xanthomonas axonopodis* pv. *vesicatoria* and *Bacillus cereus* (Lee & Whang, 2010). Strains JR-43^T and JR-4 were isolated

from a soil sample taken from Damyang, Jeonnam, South Korea, after 1 week of incubation at 28 °C on inorganic salts-starch agar [International *Streptomyces* Project (ISP) medium 4; Shirling & Gottlieb, 1966]. The isolates were stored at –86 °C in the presence of 20% (v/v) glycerol.

Physiological media were prepared according to the methods of the ISP (Shirling & Gottlieb, 1966). Czapek's agar was prepared according to Waksman (1961). Morphological and physiological characteristics were determined as recommended by Williams *et al.* (1989) and morphological observations of spores and mycelium were conducted using light microscopy and scanning electron microscopy. The strains were Gram-positive with the staining procedure of Gerhardt *et al.* (1994). Physiological tests were carried out at 28 °C unless otherwise indicated. D-Glucose, D-fructose, L-arabinose, inositol, D-mannitol, raffinose, L-rhamnose, sucrose and D-xylose were tested as sole carbon sources at concentrations of 1% (w/v) after filter sterilization. Growth on ISP 4 at 4, 10, 15, 20, 25, 30 and 37 °C, at pH 4–12 (at intervals of 1 pH unit) and with 0, 0.1, 0.5, 1, 2 and 3% (w/v) NaCl was examined in inorganic salts-starch broth after 21 days.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JR-43^T and JR-4 are HM748598 and HM748597, respectively.

Two supplementary figures and two supplementary tables are available with the online version of this paper.

The 16S rRNA gene was amplified using the universal primers 27F [5'-AGAGTTTGATC(AC)TGGCTCAG-3'] and 1492R [5'-ACGG(CT)TACCTTGTACGACTT-3'] (Weisburg *et al.*, 1991) and nucleotide sequences were determined with an automated sequencer (model 377; Applied Biosystems). The 16S rRNA gene sequences of strains JR-43^T and JR-4 (1422 nt) were compared with corresponding sequences of representative reference strains of the genus *Streptomyces*. Identification of the closest phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic trees were generated using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms in the PHYLIP package (Felsenstein, 2005). Sequence distances were calculated using the F84 model (Felsenstein, 1984). A bootstrap analysis to evaluate the stability of the trees was performed using a consensus tree based on 1000 randomly generated trees. To determine genomic relatedness, DNA–DNA hybridization was performed using the modified method of Ezaki *et al.* (1989).

Cellular fatty acids of cells grown in tryptic soy broth (Difco) for 5 days at 28 °C were prepared, separated and identified according to the instructions for the Microbial Identification System (Microbial ID) using the Microbial Identification software package and the TSBA 40 database version 4.0 (Sasser, 1990). For chemotaxonomic analysis, freeze-dried cells were obtained from cultures grown in

inorganic salts-starch broth in a shaking incubator at 120 r.p.m. and 28 °C for 14 days. Menaquinones were extracted and purified according to Collins (1985) and analysed by HPLC. The isomer of diaminopimelic acid in the cell wall and the whole-cell sugars were analysed 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). Cell biomass for DNA extraction was obtained after growth in tryptic soy broth at 28 °C for 5 days. Chromosomal DNA was isolated by the method of Saito & Miura (1963) and the G+C content was determined by HPLC, as described by Mesbah *et al.* (1989).

Strains JR-43^T and JR-4 grew well on various media, such as 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. Grey aerial mycelium formed on ISP 2 and 4. Aerial mycelium did not form on ISP 6 or nutrient agar. Different diffusible pigments were produced on different media and melanin was produced on ISP 6 (Table S1, available in IJSEM Online). The isolates produced *rectiflexibiles* spore chains with spiny-surfaced spores (Fig. S1) and degraded cellulose, gelatin, starch and casein and utilized D-fructose, D-glucose, inositol, D-mannitol, raffinose, L-rhamnose, sucrose and D-xylose (Table 1). The predominant menaquinones were MK-9(H₈) and MK-9(H₆). The polar lipid pattern consisted of phosphatidylethanolamine, hydroxyphosphatidylethanolamine,

Table 1. Some characteristics that differentiate strains JR-43^T and JR-4 from closely related members of the genus *Streptomyces*

Strains: 1, *Streptomyces gramineus* sp. nov. JR-43^T; 2, *S. gramineus* sp. nov. JR-4; 3, *S. recifensis* NBRC 12813^T; 4, *S. seoulensis* NBRC 16668^T. Data were taken from this study. All strains have grey aerial mycelium on ISP 4 and *rectiflexibiles* spore chain morphology and are positive for degradation of cellulose and casein and utilization of D-glucose, D-mannitol, raffinose, sucrose and D-xylose. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4
Substrate mycelium colour (ISP 4)*	I	I	WH	I
Spore ornamentation†	SP	SP	SM	SM
Melanin production (ISP 6)	+	+	–	–
Soluble pigments	+	+	–	–
Degradation of:				
Gelatin	+	+	–	+
Starch	+	+	–	+
Maximum NaCl concentration for growth (% w/v)	3	3	1	2
Growth at 37 °C	+	+	–	+
pH range for growth	4–8	4–9	4–8	4–8
Utilization of:				
L-Arabinose	–	–	+	–
D-Fructose	+	w	+	+
Inositol	+	+	–	–
L-Rhamnose	+	+	–	+

*I, Ivory; WH, white.

†SM, Smooth; SP, spiny.

Fig. 1. Consensus phylogenetic tree based on 16S rRNA gene sequences showing the positions of strains JR-43^T and JR-4 among their phylogenetic neighbours. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. Bar, 0.005 substitutions per nucleotide position.

phosphatidylcholine, phosphatidylinositol and phosphatidylinositol mannoside (Fig. S2). The major cellular fatty acids were iso-C_{16:0}, iso-C_{14:0}, anteiso-C_{15:0} and iso-C_{15:0} (Table S2). The cell-wall peptidoglycan contained LL- and *meso*-diaminopimelic acid and glutamic acid, alanine and glycine. Whole-cell hydrolysates contained predominantly glucose. The DNA G + C content of strains JR-43^T and JR-4 was 70.5 and 69.2 mol%, respectively.

The 16S rRNA gene sequence analysis showed that strains JR-43^T and JR-4 were most closely related to *Streptomyces seoulensis* NBRC 16668^T and *Streptomyces recifensis* NBRC 12813^T (both 98.2 % 16S rRNA gene sequence similarity). In the phylogenetic tree (Fig. 1), the isolates formed a well-defined lineage in a cluster containing *S. seoulensis* NBRC 16668^T and *S. recifensis* NBRC 12813^T. The isolates exhibited high DNA–DNA relatedness with each other (87.5 ± 3.3 %) and low DNA–DNA relatedness with *S. recifensis* NBRC 12813^T ($31.42\text{--}37.87 \pm 0.7\text{--}2.2$ %) and *S. seoulensis* NBRC 16668^T ($32.34\text{--}42.92 \pm 1.5\text{--}2.9$ %).

It is concluded from the above results that strains JR-43^T and JR-4 should be recognized as members of a novel species of the genus *Streptomyces*.

Description of *Streptomyces gramineus* sp. nov.

Streptomyces gramineus (gra.mi.ne'us. L. masc. adj. *gramineus* of grass, referring to bamboo as the first isolation source).

Aerobic, Gram-stain-positive actinobacterium that forms extensively branched substrate mycelium and aerial hyphae that differentiate into *rectiflexibles* chains of spiny-surfaced spores. Growth occurs at 4–37 °C, at pH 4–8 and with 3 % (w/v) NaCl. Good growth occurs on all tested ISP media (2, 3, 4, 5, 6, 7), Czapek's agar and nutrient agar at 28 °C. Shows antibacterial activities against *Xanthomonas campestris* LMG

568^T and *Xanthomonas axonopodis* pv. *vesicatoria* LMG 905. The aerial mycelium is grey and the substrate mycelium is ivory on ISP 4. Melanin pigment is formed on peptone-yeast extract iron agar (ISP 6). Degrades casein, cellulose, gelatin and starch. Utilizes several compounds as sole carbon sources including D-fructose, D-glucose, inositol, D-mannitol, raffinose, L-rhamnose, sucrose and D-xylose. The cell-wall peptidoglycan contains LL- and *meso*-diaminopimelic acid and glutamic acid, alanine and glycine. Whole-cell hydrolysates mainly contain glucose. The predominant menaquinones are MK-9(H₈) and MK-9(H₈). The polar lipid pattern consists of phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and phosphatidylinositol mannoside. The fatty acids are iso-C_{16:0}, iso-C_{14:0}, anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{17:0}, C_{16:0}, anteiso-C_{17:0}, iso-C_{17:1}ω9c and iso-C_{16:1} H. The DNA G + C content is 69–71 mol%.

The type strain, JR-43^T (=KACC 15079^T =NBRC 107863^T), was isolated from bamboo (*Sasa borealis*) rhizosphere soil, collected from Damyang, Jeonnam, South Korea.

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