Streptomyces sasae sp. nov., isolated from bamboo (Sasa borealis) rhizosphere soil

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A novel strain of Gram-staining-positive actinobacterium, designated strain JR-39T, was isolated from the rhizosphere soil of bamboo (Sasa borealis) sampled in Damyang, Korea, and its taxonomic position was investigated by a polyphasic approach. The isolate formed flexuous chains of spores that were cylindrical and smooth-surfaced. Strain JR-39T grew at 4–37 °C (optimum 28 °C). The pH range for growth was pH 5–10 (optimum pH 6–8) and the NaCl range for growth was 0–5 % (w/v) with optimum growth at 1 % NaCl. The cell-wall peptidoglycan contained LL-diaminopimelic acid, glutamic acid, alanine and glycine. Whole-cell hydrolysates mainly contained glucose, mannose, ribose and rhamnose. Predominant menaquinones were MK-9 (H6), MK-9 (H8) and MK-9 (H4). The major cellular fatty acids were anteiso-C15 : 0, iso-C16 : 0, iso-C15 : 0 and iso-C14 : 0. The G+C content of the DNA was 72.3 ± 0.3 mol%. Phylogenetic analyses based on 16S rRNA gene sequence analysis indicated that strain JR-39T belonged to the genus Streptomyces, showing the highest sequence similarity to Streptomyces panaciradicis 1MR-8T (99.4 %), Streptomyces capoamus JCM 4734T (98.8 %), Streptomyces galbus DSM 40089T (98.7 %), Streptomyces longwoodensis LMG 2009T (98.7 %), Streptomyces bungoensis NBRC 15711T (98.7 %) and Streptomyces rhizophilus JR-41T (98.7 %). However, DNA–DNA hybridization assays, as well as physiological and biochemical analyses, showed that strain JR-39T could be differentiated from its closest phylogenetic relatives. On the basis of the phenotypic and genotypic characteristics, strain JR-39T represents a novel species for which the name Streptomyces sasae sp. nov. is proposed. The type strain is JR-39T (=KACC 17182T=NBRC 109809T).

Bamboos are some of the fastest-growing plants in the world, due to a unique rhizome-dependent system (Ghavami, 1995). Bamboos (more than 70 genera and about 1000 species) occur naturally in tropical, subtropical and temperate regions of all the continents in the world, from sea level to 4000 m (Isagi et al., 1997, 2004; Kleinhenz et al., 2003). Among the countries of the Asia-Pacific region, China has the highest bamboo diversity (626 species), followed by India, Japan, Vietnam and South Korea (Bystrickova et al., 2003). The bamboo area in Damyang has 1797 ha of bamboo forest equal to 25.5 % of South Korea’s total bamboo habitat. In a previous study, we investigated phylogenetic characteristics of bacterial populations in bamboo (Phyllostachys bambusoides, Phyllostachys nigra var. henonis, Sasa borealis and Phyllostachys nigra f. punctata) forest soils in Damyang. We isolated a lot of actinobacteria strains from bamboo forest soil, and reported several novel species (Lee et al., 2012; Lee & Whang, 2014a, b, c). The present investigation was designed to establish the taxonomic position of strain representing the genus Streptomyces isolated from bamboo (Sasa borealis) rhizosphere soil. On the basis of data from a polyphasic study, isolate JR-39T represents a novel species of the genus Streptomyces, for which we propose the name Streptomyces sasae sp. nov.

Strain JR-39T was isolated from bamboo (Sasa borealis) rhizosphere soil in Damyang, Korea in 2007. Strain JR-39T grew well on starch casein agar (SCA; Küster & Williams, 1964) with the pH adjusted to 5.5 with HCl.

Abbreviation: ISP, International Streptomyces Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JR-39T is HQ267987.

Two supplementary tables and three supplementary figures are available with the online Supplementary Material.
which was incubated at 28 °C for 7 days. Bacterial culture of the isolated strain was stored at −86 °C in the presence of 20% (v/v) glycerol solution.

Physiological media were prepared according to the methods approved by the International Streptomyces Project (ISP; Shirling & Gottlieb, 1966). Nitrate reduction was determined for cultures grown in nitrate broth (Conn & Breed, 1919). Physiological characteristics were determined as recommended by Williams et al. (1989); and morphological observations of spores and mycelia were conducted by scanning electron microscopy (Hitachi High-Technologies). Gram staining was performed by the Hucker method (Gerhardt et al., 1994). Growth at various temperatures, pH and NaCl concentrations was examined according to the methods of Lee et al. (2012) by using SCA. Strain JR-39T formed straight to flexuous (Rectiflexibles) spore chains with cylindrical, smooth-surfaced spores (Fig. 1). Strain JR-39T grew well on yeast extract–malt extract agar (ISP 2), oatmeal agar (ISP 3), ISP 4, glycerol–asparagine agar (ISP 5), peptone–yeast extract–iron agar (ISP 6) and tyrosine agar (ISP 7). Strain JR-39T formed grey and white aerial mycelium on ISP 2, 3, 4, 5 and ISP 7 and no aerial mycelium was observed on ISP 6. Melanin was observed on ISP 6 and ISP 7. The cultural characteristics of the isolates are shown in Table S1 (available in the online Supplementary Material). The temperature range for growth on SCA at pH 7.0 was 4–37 °C and no growth was observed at 40 °C. Strain JR-39T grew well with up to 3.0% (w/v) NaCl and poorly with 5.0% NaCl. The pH range for growth was from pH 5 to 10 (optimal range was pH 6–8). Strain JR-39T degraded casein, cellulose, gelatin and starch but not aesculin or urea. Strain JR-39T was able to utilize d-fructose, d-glucose, d-mannitol, raffinose, l-rhamnose, sucrose, l-arabinose and d-xylene as sole carbon sources. The physiological characteristics of strain JR-39T are listed in the species description and in Table 1.

Genomic DNA was extracted (Cui et al., 2001) from the isolate and the 16S rRNA gene sequencing of strain JR-39T was performed as described previously (Lee et al., 2012). The phylogenetic neighbours were identified and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon-e server (Kim et al., 2012). The 16S rRNA gene sequence 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 the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms within the MEGA5 program (Tamura et al., 2011). Evolutionary distance matrices for the neighbour-joining method were calculated using the algorithm of the Kimura two-parameter model (Kimura, 1980). To evaluate the stability of the phylogenetic tree, a bootstrap analysis (1000 replications) was performed (Felsenstein, 1985).

The nearly complete 16S rRNA gene sequence (1444 nt) of strain JR-39T was aligned with those of closely related species of the genus Streptomyces. The 16S rRNA gene sequence similarities between strain JR-39T and members of the genus Streptomyces ranged from 98.5 to 99.3%. The isolate was most closely related to Streptomyces panaciradica 1MR-8T (99.4%), Streptomyces capoanus JCM 4734T (98.8%), Streptomyces galbus DSM 40089T (98.7%), Streptomyces longwoodensis LMG 20096T (98.7%), Streptomyces bungoensis NBRC 1571T (98.7%), Streptomyces rhizophilus JR-41T (98.7%), Streptomyces corchorusii NBRC 13032T (98.6%), A. Streptomyces olivaceoviridis NBRC 13066T (98.6%) and Streptomyces canarius NBRC 13431T (98.5%), respectively. Phylogenetic analysis revealed that strain JR-39T belonged in the genus Streptomyces and formed a separate lineage with Streptomyces panaciradica 1MR-8T in the cluster containing Streptomyces rhizophilus JR-41T (Figs. 2, S1 and S2). Neighbour-joining (Fig. 2) and maximum-parsimony methods (Fig. S2) also resulted in highly similar tree topologies, with strain JR-39T forming a distinct phyletic line with the most closely related type strain, Streptomyces panaciradica 1MR-8T.

Cellular fatty acids of strain JR-39T grown in trypticase soy broth (Difco) for 5 days at 28 °C were prepared and separated according to the instructions for the Microbial Identification System (MIDI) and were identified by using the Microbial Identification software package (MIDI, database TSBA 40, version 4.5; Sasser, 1990). For chemotaxonomic analysis, freeze-dried cells were obtained from a culture grown in starch casein broth (SCB) on a shaking incubator at 120 r.p.m. and 28 °C for 14 days. Identification of the isomer of dianaminopimelic acid in the cell wall and analysis of whole-cell sugars were performed as described by Lechevalier & Lechevalier (1970, 1980) and Staneck & Roberts.
Polar lipids were extracted and detected according to the method of Minnikin et al. (1984). Menaquinones were extracted as described by Collins (1985) and were separated by HPLC. The G+C content of the genomic DNA was determined by the HPLC method according to Mesbah et al. (1989). The major cellular fatty acids were anteiso-C₁₅ : 0, iso-C₁₆ : 0, iso-C₁₅ : 0 and iso-C₁₄ : 0 (Table S2). The cell-wall peptidoglycan contained LL-diaminopimelic acid, glutamic acid, alanine and glycine. Whole-cell hydrolysates contained predominantly glucose, mannose, ribose and rhamnose. The polar lipid pattern consisted of phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and an unknown aminophospholipid. (Fig. S3). The predominant menaquinones were MK-9 (H₆), MK-9 (H₈) and MK-9 (H₄). The G+C content of the genomic DNA of strain JR-39T was 72.3 mol%.

To determine genomic relatedness, DNA–DNA hybridization was performed using the method of Ezaki et al. (1989). After the highest and lowest values for each sample were excluded, the mean was reported as the DNA–DNA relatedness value. Strain JR-39T exhibited low DNA–DNA relatedness (mean ± SD of triplicate determinations) with the type strains of Streptomyces panaciradicis 1MR-8T (52.7 ± 2.4 %), Streptomyces capoanus JCM 4734T (49.7 ± 3.2 %), Streptomyces bungoensis NBRC 15711T (47.4 ± 1.3 %), Streptomyces galbus DSM 40089T (48.2 ± 1.7 %) and Streptomyces longwoodensis LMG 20096T (47.8 ± 2.0 %). These values are below the 70 % cut-off point recommended for the assignment of organisms to the same species (Wayne et al., 1987).

On the basis of phylogenetic analyses and the morphological, physiological and chemotaxonomic data, as well as DNA–DNA hybridization, we propose that strain JR-39T represents a novel species of the genus Streptomyces, for which the name Streptomyces sasae sp. nov. is proposed.

**Description of Streptomyces sasae sp. nov.**

Streptomyces sasae (sa’sae. N.L. fem. gen. n. sasae of the bamboo Sasa borealis).
Table 1. Some characteristics that differentiate strain JR-39T from the type strains of related species of the genus *Streptomyces*

Strains: 1, JR-39T; 2, *Streptomyces panaciradicis* 1MR-8T; 3, *Streptomyces capoanus* ICM 4734T; 4, *Streptomyces galbus* DSM 40089T; 5, *Streptomyces longwoodesis* NBRC 14251T; 6, *Streptomyces bungoensis* NBRC 15711T; 7, *Streptomyces rhizophilus* JR-41T. All data were obtained in this study. All strains are positive for degradation of cellulose, negative for degradation of urea, and positive for utilization of D-glucose and D-mannitol. +, Positive activity and/or growth occurs; –, no activity and/or no growth; w/–, variable.

<table>
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<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Aerial spore mass colour on ISP 2</td>
<td>Grey</td>
<td>Grey</td>
<td>Red</td>
<td>Light ivory</td>
<td>Grey</td>
<td>None</td>
<td>Grey</td>
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<tr>
<td>Reverse side of colony on ISP 2</td>
<td>Brown</td>
<td>Light yellow</td>
<td>Ivory</td>
<td>Yellow</td>
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<tr>
<td>Diffusible pigment</td>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Spore chain</td>
<td>Flexuous</td>
<td>Straight or flexuous</td>
<td>Flexuous</td>
<td>Spirales</td>
<td>Spirales</td>
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<td>Spore surface</td>
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<td>Nitrate reduction</td>
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<td>Degradation of:</td>
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<td>Ascesulin</td>
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<td>Casein</td>
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<td>Starch</td>
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<td>+</td>
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<td>Growth at/with:</td>
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<td>Carbon utilization</td>
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<td>D-Arabinose</td>
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<tr>
<td>D-Fructose</td>
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<td>Raffinose</td>
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<td>+</td>
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<td>L-Rhamnose</td>
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<td>w/–</td>
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<td>w/–</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>w/–</td>
<td>–</td>
<td>–</td>
<td>w/–</td>
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Aerobic, Gram-stain-positive, non-motile actinobacterium that forms extensively branched substrate mycelium and aerial hyphae that differentiate into flexuous (Rectiflexibles) spore chains with cylindrical, smooth-surfaced spores. Growth occurs at 4–37 °C (optimum, 28 °C). The pH range for growth is pH 5.0–10.0 (optimum, pH 6.0–8.0). Growth occurs with 0–5 % (w/v) NaCl, but not with 7 % NaCl. Good growth occurs on all ISP media tested (ISP 2–7) at 28 °C. Melanin pigments are formed on peptone–yeast extract–iron agar (ISP 6) and tyrosine agar (ISP 7). Negative for nitrate reduction. Casein, cellulose, gelatin and starch are hydrolysed but aesculin and urea are not hydrolysed. Utilizes several compounds as sole carbon sources, including D-glucose, D-fructose, D-mannitol, raffinose, L-rhamnose, sucrose, L-arabinose and D-xylene. The cell-wall peptidoglycan contains LL-diaminopimelic acid, glutamic acid, alanine and glycine. Whole-cell hydrolysates mainly contain glucose, mannose, ribose and rhamnose. Predominant menaquinones are MK-9 (H8), MK-9 (H4) and MK-9 (H4). The polar lipid pattern consists of isoprenoid quinone analysis in bacterial classification and identification. In Chemical Methods in Bacterial Systematics, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

**Acknowledgements**

This work was supported by a grant from the Regional SubGenBank Support Program of Rural Development Administration, Republic of Korea.

**References**


hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.


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