Streptomyces baliensis sp. nov., isolated from Balinese soil

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The taxonomic positions of actinomycete strains ID03-0915^T and ID03-0825, isolated from soil on the Indonesian island of Bali, were examined using a polyphasic taxonomic approach. The morphological and chemotaxonomic characteristics of these organisms are typical of the genus *Streptomyces*. Phylogenetic analyses performed using almost-complete 16S rRNA gene sequences demonstrated that the strains were closely related to *Streptomyces glauciniger* and *Streptomyces lilacinus*. However, DNA–DNA hybridization and phenotypic characteristics revealed that the strains differed from known *Streptomyces* species. Therefore, we conclude that strains ID03-0915^T and ID03-0825 (=BTCC B-563) represent a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces baliensis* sp. nov. The type strain is strain ID03-0915^T (=BTCC B-608^T =NBRC 104276^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) and includes aerobic, spore-forming soil bacteria with high DNA G+C contents (69–78 mol%); rod-shaped spores originate from the substrate mycelium, LL-diaminopimelic acid (LL-A_2pm) is found in the cell wall and galactose and mannose are found in whole-cell hydrolysates. Although the genus *Streptomyces* contains more than 500 species (Hain *et al.*, 1997) with validly published names, the genus remains a target for screening for novel secondary metabolites.

In the course of an investigation of actinomycetes from Indonesia, strains ID03-0915^T and ID03-0825 were isolated from soil collected from the island of Bali. These isolates formed colonies typical of the genus *Streptomyces*. The aim of this study was to determine the taxonomic positions of strains ID03-0915^T and ID03-0825 by a polyphasic taxonomic approach that included phylogenetic analyses, chemotaxonomic characteristics, DNA–DNA hybridization and physiological properties.

The strains were isolated using the SDS/yeast extract method (Hayakawa & Nonomura, 1989) and humic acid/vitamin (HV) agar (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}). Morphological and chemotaxonomic studies were performed with the two isolates to confirm whether they exhibited properties similar to those of the genus *Streptomyces*. Morphology was observed under a light microscope and a scanning electron microscope (SEM model JSM-6060; JEOL) on yeast extract-starch (YS) medium or HV agar incubated for 14 days at 28 °C. Cultural and physiological characteristics were examined as described previously (Shirling & Gottlieb, 1966)

A_2pm isomers and whole-cell sugar patterns were analysed according to the procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1980). Fatty acid compositions were analysed by GC using the MIDI system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Isoprenoid quinones and polar lipids were examined as described previously (Schaal, 1985; Minnikin *et al.*, 1984; Tamura *et*

Abbreviation: A_2pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains ID03-0915^T (=NBRC 104276^T) and ID03-0825 (=BTCC B-563) are AB441718 and AB441719.

Detailed cultural properties of the novel strains are available as supplementary material with the online version of this paper.

al., 1994). Chromosomal DNA was extracted using the method described by Saito & Miura (1963) and the G+C content of the DNA was determined by HPLC as described by Tamura *et al.* (1994). DNA–DNA hybridization was performed fluorometrically in microdilution wells by using photobiotin as described by Ezaki *et al.* (1989).

The 16S rRNA gene was amplified by performing a PCR as described by Tamura & Hatano (2001). This was followed by direct sequencing using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) according to the manufacturer's protocol. The 16S rRNA gene sequences of the two strains were aligned with published sequences of species of the genus *Streptomyces* with validly published names available from EMBL/GenBank/DDBJ by using the CLUSTAL_X program (Thompson *et al.*, 1997). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms of the CLUSTAL_X 1.8 program (Thompson *et al.*, 1997) and MEGA version 3.1 (Kumar *et al.*, 2004). The topology of the constructed trees was evaluated by performing bootstrap analyses with 1000 replicates (Felsenstein, 1985).

DNA–DNA hybridization was performed between isolate ID03-0915^T and strains ID03-0825 and *Streptomyces glauciniger* NBRC 100913^T using the method described by Ezaki *et al.* (1989).

Isolates ID03-0915^T and ID03-0825 formed extensively developed, straight aerial hyphae that arose from the substrate mycelium and formed short or long spore chains (Table 1). In addition, the colonies exhibited well-developed, branched vegetative hyphae and white to light-bluish grey aerial mycelium. The substrate mycelium appeared yellow to yellowish brown when the isolates were grown on yeast extract/malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts/starch agar (ISP 4), glycerol asparagine agar (ISP 5) and tyrosine agar (ISP 7) (Supplementary Table S1, available in IJSEM Online). Furthermore, we found that melanin pigments were generated on ISP 7. Differential phenotypic characters with phylogenetically related *Streptomyces* species are shown in Table 1. On the basis of these phenotypic properties, the two isolates could be clearly distinguished from their closest phylogenetic neighbours.

Whole-cell hydrolysates of the test strains contained LL-A_2pm and lacked characteristic major sugars. The major menaquinones were MK-9(H_6), MK-9(H_8) and MK-9(H_4). The cellular fatty acid profile mainly comprised iso-$C_{16:0}$ (42–46.8 %) and iso-$C_{14:0}$ (13–14 %). The strains contained phosphatidylethanolamine and phosphatidylmethylethanolamine as the major polar lipids and phosphatidylglycerol as a minor polar lipid. The DNA G+C content of strain ID03-0915^T was 71.1 mol%. These morphological and chemotaxonomic features of the isolates were consistent with those of bacteria belonging to the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995).

Table 1. Phenotypic properties that differentiate strains ID03-915^T and ID03-0825 and their closest phylogenetic neighbours

Strains: 1, ID03-0915^T and ID03-0825; 2, *S. glauciniger* NBRC 100913^T; 3, *S. lilacinus* NBRC 12884^T; 4, S. *abikoensis* NBRC 13860^T. Data for *S. glauciniger* NBRC 100913^T were obtained in the present study; data for other reference strains were taken from Hatano *et al.* (2003). +, Positive; −, negative; NT, not tested; ND, no data. All strains utilize inositol.

Characteristic	1	2	3	4
Spore chain morphology*	RF	SP	VR	RF
Spore surface ornamentation	Warty	Smooth	Smooth	Smooth
Utilization of:				
Arabinose	+	+	−	−
Fructose	+	+	−	+/−
D-Mannitol	+	+	−	−
Raffinose	+	−	−	−
Rhamnose	+	+	−	−
Sucrose	+	+	−	−
D-Xylose	+	+	−	−
Decomposition of:				
Arbutin	+	NT	ND	ND
Urea	−	−	ND	ND
Tyrosine	+	−	ND	ND
Xanthine	−	−	ND	ND
Growth in 2 % NaCl	−	+	ND	ND
Growth at 37 °C	+/−	+	ND	ND

*RF, Rectiflexibiles; SP, spiral; VR, verticils.

The phylogenetic tree constructed using the neighbour-joining method demonstrated that the isolates formed a distinct phyletic line with reference strains of the genus *Streptomyces* (Fig. 1). This topology was supported by the results of phylogenetic analyses based on the maximum-likelihood and maximum-parsimony algorithms with a high bootstrap value. The two strains were most closely related to the type strains of *S. glauciniger* (98 %), *Streptomyces lilacinus* (97.6 %) and *Streptomyces abikoensis* (97.7 %).

Comparison of the phenotypic characteristics of strain ID03-0915^T and its close phylogenetic neighbours, *S. lilacinus* and *S. abikoensis*, revealed significant differences (Table 1). Based on the phylogenetic distance and phenotypic characteristics, *S. glaucinger* was selected for the DNA–DNA hybridization test. The reciprocal DNA–DNA relatedness between strains ID03-0915^T and ID03-0825 was 70 and 100 %, and the relatedness between ID03-0915^T and *S. glauciniger* NBRC 100913^T was 4–18 %. It has been recommended that strains exhibiting DNA–DNA relatedness values of less than 80 % with strains of known

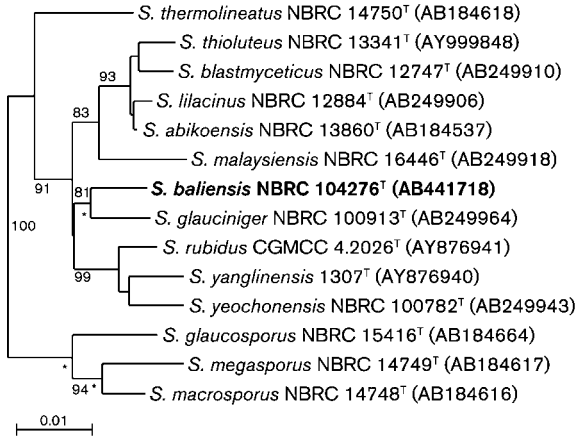

Fig. 1. Phylogenetic tree constructed by the neighbour-joining method (Saitou & Nei, 1987) using almost-complete 16S rRNA gene sequences, showing the relationships of strain NBRC 104276^T (=ID03-0915^T) with type strains of closely related *Streptomyces* species. Numbers on branches indicate percentage bootstrap values determined for 1000 replicates (only values above 60 % are indicated). Bar, 0.01 K_{nuc}. Asterisks indicate branches of the tree that were also recovered using the maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms.

Streptomyces species be recognized as belonging to distinct species of the genus (Labeda, 1993, 1996, 1998).

It is clear from the phenotypic and genotypic data that the two isolates should be classified within a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces baliensis* sp. nov.

Description of *Streptomyces baliensis* sp. nov.

Streptomyces baliensis (ba.li.en′sis. N.L. masc. adj. *baliensis* pertaining to the island of Bali, Indonesia, where the first strains were isolated).

Aerobic and strains Gram-positive. Forms well-branched vegetative hyphae and white to grey aerial mycelium. The substrate mycelium is pale yellow to yellow. The aerial mycelium is moderate white to grey on yeast extract/malt extract agar or oatmeal agar. Spore surface is smooth. Soluble pigments are not produced. Melanin pigments are produced on tyrosine agar. Glucose, galactose, mannose, melibiose and lactose are used as sole carbon sources. Grows at 10–30 °C with an optimum around at 28 °C; no growth at 37 °C. Optimum growth around pH 7.0. No growth in the presence of 2 % NaCl. Additional phenotypic properties are listed in Table 1. Type II phospholipids and menaquinones MK-9(H_6), MK-9(H_8) and MK-9(H_4) are detected. The major cellular fatty acids are iso-$C_{16:0}$ and iso-$C_{14:0}$. The G+C content of DNA of the type strain is 71.1 mol%.

Type strain is strain ID03-0915^T (=BTCC B-608^T =NBRC 104276^T), isolated from a soil sample collected in Eka karya Botanic Garden on Bali, Indonesia. Strain ID03-0825 (=BTCC B-563) is a second strain of the species.

Acknowledgements

This study was conducted under the Joint Research Project between Department of Biotechnology, National Institute of Technology and Evaluation, Japan, and the Indonesian Institute of Sciences (LIPI) representing Indonesian Government Research Institutes. The authors would like to thank Ms Yayoi Sakiyama for her assistance with the chemotaxonomic analyses, Mr Nobuyuki Goto for providing the 16S rRNA gene sequences and Dr Tomohiko Tamura for providing valuable suggestions.

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