Streptomyces baliensis sp. nov., isolated from Balinese soil

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The taxonomic positions of actinomycete strains ID03-0915T 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 ID03-0915T 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 ID03-0915T (=BTCC B-608T =NBRC 104276T).

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, L,L-diaminopimelic acid (L,L-A2pm) 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 ID03-0915T 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 ID03-0915T 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).

A2pm 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: A2pm, diaminopimelic acid.

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequences of strains ID03-0915T (=NBRC 104276T) 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.
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 isolates 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 method (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 ID03-0915T and strains ID03-0825 and Streptomyces glauciniger NBRC 100913T using the method described by Ezaki et al. (1989).

Isolates ID03-0915T 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-A2pm and lacked characteristic major sugars. The major menaquinones were MK-9(H6), MK-9(H8) and MK-9(H4). The cellular fatty acid profile mainly comprised iso-C16:0 (42–46.8 %) and iso-C14:0 (13–14 %). The strains contained phosphatidylethanolamine and phosphatidylmethyl ethanolamine as the major polar lipids and phosphatidylglycerol as a minor polar lipid. The DNA G+C content of strain ID03-0915T 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).

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

### Table 1. Phenotypic properties that differentiate strains ID03-0915T and ID03-0825 and their closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tbody>
<tr>
<td>Spore chain morphology*</td>
<td>RF</td>
<td>SP</td>
<td>VR</td>
<td>RF</td>
</tr>
<tr>
<td>Spore surface ornamentation</td>
<td>Warty</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
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<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+/–</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Rhamnose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Decomposition of:</td>
<td></td>
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<td></td>
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<tr>
<td>Arbutin</td>
<td>+</td>
<td>NT</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Urea</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Tyrosine</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Xanthine</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Growth in 2 % NaCl</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Growth at 37 °C</td>
<td>+/-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*RF, Rectiflexibles; SP, spiral; VR, verticils.
**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* (bali.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 in extract agar or oatmeal agar. Spore surface is smooth. Soluble substrate mycelium is pale yellow to yellow. The aerial vegetative hyphae and white to grey aerial mycelium. The Aerobic and strains Gram-positive. Forms well-branched strains were isolated.

Streptomyces *baliensis* sp. nov. (Trad, E. 1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol 39, 224–229.


