Actinophytocola timorensis sp. nov. and *Actinophytocola corallina* sp. nov., isolated from soil

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Two actinomycete strains, ID05-A0653^T and ID06-A0464^T, were isolated from soils of West Timor and Lombok island, respectively, in Indonesia. 16S rRNA gene sequence analysis clearly demonstrated that the isolates belonged to the family *Pseudonocardiaceae* and were closely related to the genus *Actinophytocola*. Strains ID05-A0653^T and ID06-A0464^T exhibited 98.1 and 98.2 % 16S rRNA gene sequence similarity, respectively, with *Actinophytocola oryzae* GMKU 367^T. The isolates grew well on ISP media and produced white aerial mycelium. Short spore chains were formed directly on the substrate mycelium. The isolates contained *meso*-diaminopimelic acid, arabinose and galactose as cell-wall components, MK-9(H_4) as the sole isoprenoid quinone, iso-$C_{16:0}$ as the major cellular fatty acid and phosphatidylethanolamine as the diagnostic polar lipid. The DNA G+C contents of strains ID05-A0653^T and ID06-A0464^T were 69.7 and 71.2 mol%, respectively. On the basis of phenotypic characteristics, DNA–DNA relatedness and 16S rRNA gene sequence comparisons, strains ID05-A0653^T and ID06-A0464^T each represent a novel species of the genus *Actinophytocola,* for which the names *Actinophytocola timorensis* sp. nov. (type strain ID05-A0653^T =BTCC B-673^T =NBRC 105524^T) and *Actinophytocola corallina* sp. nov. (type strain ID06-A0464^T =BTCC B-674^T =NBRC 105525^T) are proposed.

The family *Pseudonocardiaceae* was originally proposed by Embley *et al.* (1988) for mycolateless cell-wall chemotype IV actinomycetes. The description of the family was later emended by Zhi *et al.* (2009) on the basis of 16S rRNA gene sequence analysis. The family currently includes the genera *Actinoalloteichus* (Tamura *et al.*, 2000), *Actinomycetospora* (Jiang *et al.*, 2008), *Allokutzneria* (Labeda & Kroppenstedt, 2008), *Amycolatopsis* (Lechevalier *et al.*, 1986), *Crossiella* (Labeda, 2001), *Goodfellowiella* (Labeda & Kroppenstedt, 2006; Labeda *et al.*, 2008), *Kibdelosporangium* (Shearer *et al.*, 1986), *Kutzneria* (Stackebrandt *et al.*, 1994), *Prauserella* (Kim & Goodfellow, 1999), *Pseudonocardia* (Henssen, 1957), *Saccharomonospora* (Nonomura & Ohara, 1971), *Saccharopolyspora* (Lacey & Goodfellow, 1975), *Sciscionella* (Tian *et al.*, 2009), *Streptoalloteichus* (Tomita *et al.*, 1987), *Thermobispora* (Wang *et al.*, 1996) and *Thermocrispum* (Korn-Wendisch *et al.*, 1995). Recently, the genus *Actinophytocola* (Indananda *et al.*, 2010) has been added to the

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains ID05-A0653^T and ID06-A0464^T are AB511315 and AB511316, respectively.

A supplementary figure and a supplementary table are available with the online version of this paper.

family; its first species was isolated from the roots of Thai glutinous rice plants. Genera of the family *Pseudonocardiaceae* are distinguishable from each other on the basis of morphological, chemotaxonomic and a few physiological characteristics and they form a distinct group on the basis of 16S rRNA gene sequence analysis. During an investigation of actinomycetes recovered from Indonesia, strains ID05-A0653^T and ID06-A0464^T were isolated from soil samples collected at West Timor and Lombok island, respectively. These two strains showed chemotaxonomic characteristics of the family *Pseudonocardiaceae* and morphological and chemotaxonomic characteristics of the genus *Actinophytocola* but were genotypically and phenotypically distinguishable from members of recognized *Actinophytocola* species.

Strains ID05-A0653^T and ID06-A0464^T were isolated on humic acid-vitamin (HV) agar (Hayakawa & Nonomura, 1987), using the SDS/yeast extract method (Hayakawa & Nonomura, 1989) and the rehydration and centrifugation method (Hayakawa *et al.*, 2000), respectively. The isolates were maintained on yeast extract-malt extract agar (ISP 2) and stored as glycerol suspensions (20 %, v/v) at –80 °C. Cell morphology of cultures grown on HV agar or NBRC medium 266 (yeast extract-starch agar) at 28 °C for 14–21 days was observed using light microscopy and scanning electron microscopy (model JSM-6060; JEOL). Cultural and physiological properties were characterized using several well-established procedures (Gordon *et al.*, 1974; Shirling & Gottlieb, 1966) and API tests (bioMérieux). For cultural characterization, the isolates were grown at 28 °C for 21 days on a series of ISP media, NBRC medium 266 and NBRC medium 231 (maltose-Bennett's agar). The colour of colonies was determined according to Rayner (1970). Growth with 0–6 % NaCl and the temperature range for growth were investigated using NBRC medium 266 for 21 days. Growth at pH 3.0–9.0 was determined using yeast extract-glucose broth.

Strains ID05-A0653^T and ID06-A0464^T formed a branched substrate mycelium, which fragmented into rod-shaped elements (0.5–0.8 × 0.8–1.2 μm), and relatively short aerial hyphae with chains of spores (Supplementary Fig. S1, available in IJSEM Online). Short spore chains were formed directly on the substrate mycelium (Supplementary Fig. S1). The isolates grew well on ISP 2, 3, 4, 5 and 7 and NBRC medium 266, and a white to yellowish-white aerial mycelium was observed on ISP 5 and 7 and NBRC medium 266. Strain ID05-A0653^T produced moderate orange–yellow soluble pigment and ID06-A0464^T produced coral soluble pigment on ISP 7. The isolates utilized glucose, D-fructose, lactose, maltose, D-mannitol, raffinose, rhamnose and trehalose as sole carbon sources. Additionally, strain ID05-A0653^T used adonitol, sucrose and xylose and strain ID06-A0464^T used D-sorbitol. Strain ID05-A0653^T grew at 15–37 °C and strain ID06-A0464^T grew at 15–28 °C (Table 1). Other phenotypic characteristics are given in Table 1 and the species descriptions.

Table 1. Phenotypic properties that distinguish strains ID05-A0653^T and ID06-A0464^T from their closest phylogenetic neighbour

Strains: 1, *A. timorensis* sp. nov. ID05-A0653^T; 2, *A. corallina* sp. nov. ID06-A0464^T; 3, *A. oryzae* NBRC 105245^T. Data were taken from this study and Indananda *et al.* (2010). None of the strains grow with 6 % NaCl. +, Positive; −, negative; ND, no data available.

Characteristic	1	2	3
Nitrate reduction	+	−	−
Use of sole carbon sources			
Adonitol	+	−	ND
D-Sorbitol	−	+	−
Sucrose	+	−	−
D-Xylose	+	−	−
Growth with/at:			
4 % NaCl	−	+	−
5 % NaCl	−	+	−
37 °C	+	−	−
pH 5	+	−	+
Soluble pigment on ISP 7*	Orange–yellow (44)	Coral (38)	None

*Values in parentheses correspond to colour codes from Rayner (1970).

Diaminopimelic acid isomers and sugars of whole-cell hydrolysates were analysed using procedures described by Hasegawa *et al.* (1983) and Schaal (1985), respectively. Polar lipids were extracted, examined by two-dimensional TLC and identified using methods described by Minnikin *et al.* (1984). Standard procedures were used for the extraction and analysis of mycolic acids (Schaal, 1985) and isoprenoid quinones (Minnikin *et al.*, 1984). Cellular fatty acid composition was determined using the Microbial Identification system (MIDI). Chromosomal DNA from strains was isolated and purified by the method of Saito & Miura (1963) with a minor modification (Hatano *et al.*, 2003). DNA G+C content was determined by HPLC, as described by Tamura *et al.* (1994). DNA–DNA hybridization was carried out as described by Kusunoki *et al.* (1991) using biotinylated DNA.

Whole-cell hydrolysates of the isolates contained *meso*-diaminopimelic acid, galactose, arabinose and small amounts of rhamnose. The phospholipid profile corresponded to type II and contained phosphatidylethanolamine, diphosphatidylglycerol and ninhydrin-positive phosphoglycolipids (Lechevalier *et al.*, 1977). The predominant menaquinone was MK-9(H_4) (100 %). The major cellular fatty acid was iso-$C_{16:0}$ (Supplementary Table S1). The DNA G+C contents of strains ID05-A0653^T and ID06-A0464^T were 69.7 and 71.2 mol%, respectively.

The 16S rRNA genes of the isolates were amplified by PCR as described by Tamura & Hatano (2001) and directly sequenced using an ABI Prism BigDye Terminator cycle

sequencing kit (Applied Biosystems) and an automatic DNA sequencer (3130 Genetic Analyzer; Applied Biosystems). The almost-complete 16S rRNA gene sequences of strains ID05-A0653^T and ID06-A0464^T (1476 nt) were aligned with reference sequences of the family *Pseudonocardiaceae* available from EMBL/GenBank/DDBJ using CLUSTAL X (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. Tree topologies were evaluated using the bootstrap resampling method with 1000 replicates (Felsenstein, 1981).

The neighbour-joining and maximum-likelihood phylogenetic trees based on 16S rRNA gene sequences showed that strains ID05-A0653^T and ID06-A0464^T formed a monophyletic cluster with *Actinophytocola oryzae* GMKU 367^T, which was supported by a bootstrap value of 94 % in the neighbour-joining tree (Fig. 1). 16S rRNA gene sequence similarity between the isolates was 98.5 %. 16S rRNA gene sequence similarity between strain ID05-A0653^T and *A. oryzae* GMKU 367^T was 98.1 % and between strain ID06-A0464^T and *A. oryzae* GMKU 367^T was 98.2 %. The isolates showed <95.3 % 16S rRNA gene sequence similarity to other members of the family *Pseudonocardiaceae*.

DNA–DNA relatedness between the isolates was 15–50 %. DNA–DNA relatedness between strain ID05-A0653^T and *A. oryzae* NBRC 105245^T was 5–7 % and between strain ID06-A0464^T and *A. oryzae* NBRC 105245^T was 6–7 %. These results were well below the 70 % cut-off value recommended for the assignment of bacterial strains to the same genomic species (Wayne *et al.*, 1987). The results of the 16S rRNA gene sequence analysis were supported by the DNA–DNA relatedness results.

Thus, on the basis of phylogenetic position, chemotaxonomic data and morphological features, we propose that strains ID05-A0653^T and ID06-A0464^T be classified in two novel species in the genus *Actinophytocola*, for which we propose the names *Actinophytocola timorensis* sp. nov. and *Actinophytocola corallina* sp. nov., respectively.

Description of *Actinophytocola timorensis* sp. nov.

Actinophytocola timorensis [ti.mo.ren′sis. N.L. fem. adj. *timorensis* pertaining to (West) Timor, Indonesia, from where the type strain was isolated].

Good growth on several ISP media and produces orange–yellow pigments on ISP 7. Vegetative mycelium is orange–yellow to yellowish white in colour. Nitrate reduction is positive. Leucine aminopeptidase, acid phosphatase, β-galactosidase, α-glucosidase and *N*-acetyl-β-glucosaminidase are produced. Adonitol, arabinose, cellobiose, fructose, glucose, lactose, maltose, mannitol, raffinose, rhamnose, salicin, sucrose, trehalose and xylose are used as sole carbon sources, but sorbitol is not. Growth occurs at 15–

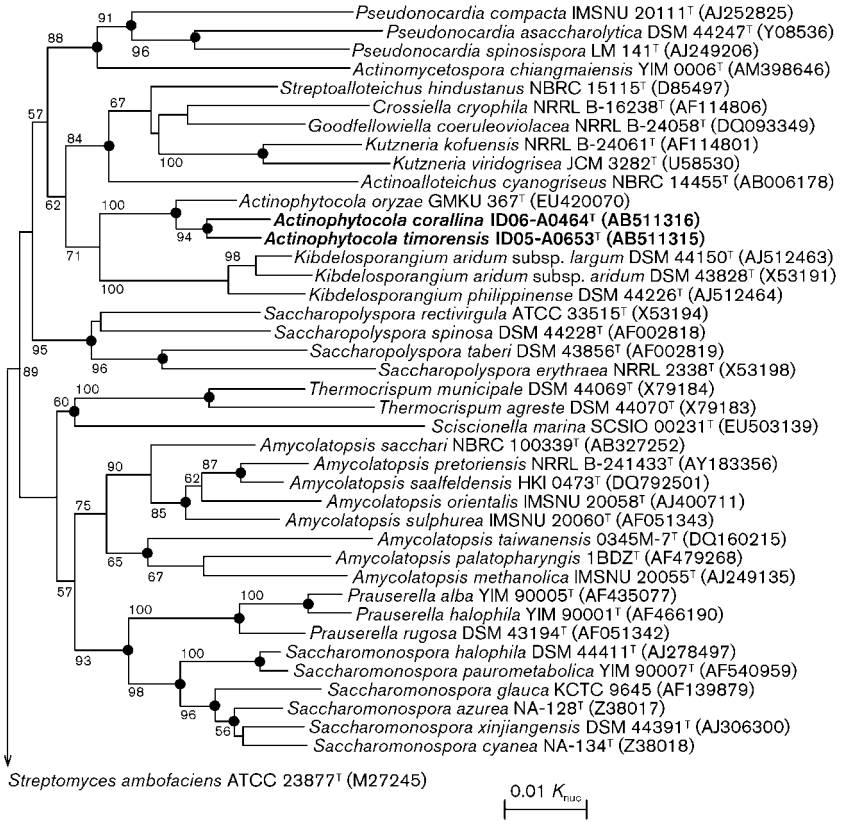

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strains ID05-A0653^T and ID06-A0464^T within the family *Pseudonocardiaceae*. Bootstrap values (>50 %) based on 1000 replicates are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. *Streptomyces ambofaciens* ATCC 23877^T was used as an outgroup. Bar, 0.01 K_{nuc}.

37 °C (optimum 25–28 °C); no growth occurs at 45 °C. No growth with 4 % NaCl. The DNA G+C content of the type strain is 69.7 mol%.

The type strain, ID05-A0653^T (=BTCC B-673^T =NBRC 105524^T), was isolated from soil of West Timor, Indonesia.

Description of *Actinophytocola corallina* sp. nov.

Actinophytocola corallina (co.ral.li′na. L. fem. adj. *corallina* coral red, because the organism produces coral-coloured soluble pigment).

Good growth on several ISP media and produces coral-coloured pigments on ISP 7. Vegetative mycelium is salmon-pink to yellowish-white in colour. Nitrate reduction is negative. Leucine aminopeptidase, acid phosphatase, α-glucosidase and *N*-acetyl-β-glucosaminidase are produced. Arabinose, cellobiose, fructose, glucose, lactose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol and trehalose are used as sole carbon sources, but sucrose and xylose are not. Growth occurs at 15–28 °C (optimum 25–28 °C); no growth occurs at 37 °C. No growth with 6 % NaCl. The DNA G+C content of the type strain is 71.2 mol%.

The type strain, ID06-A0464^T (=BTCC B-674^T =NBRC 105525^T), was isolated from soil of Lombok island, Indonesia.

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