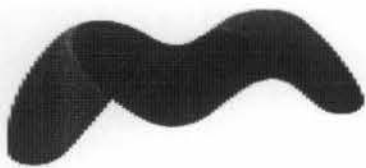
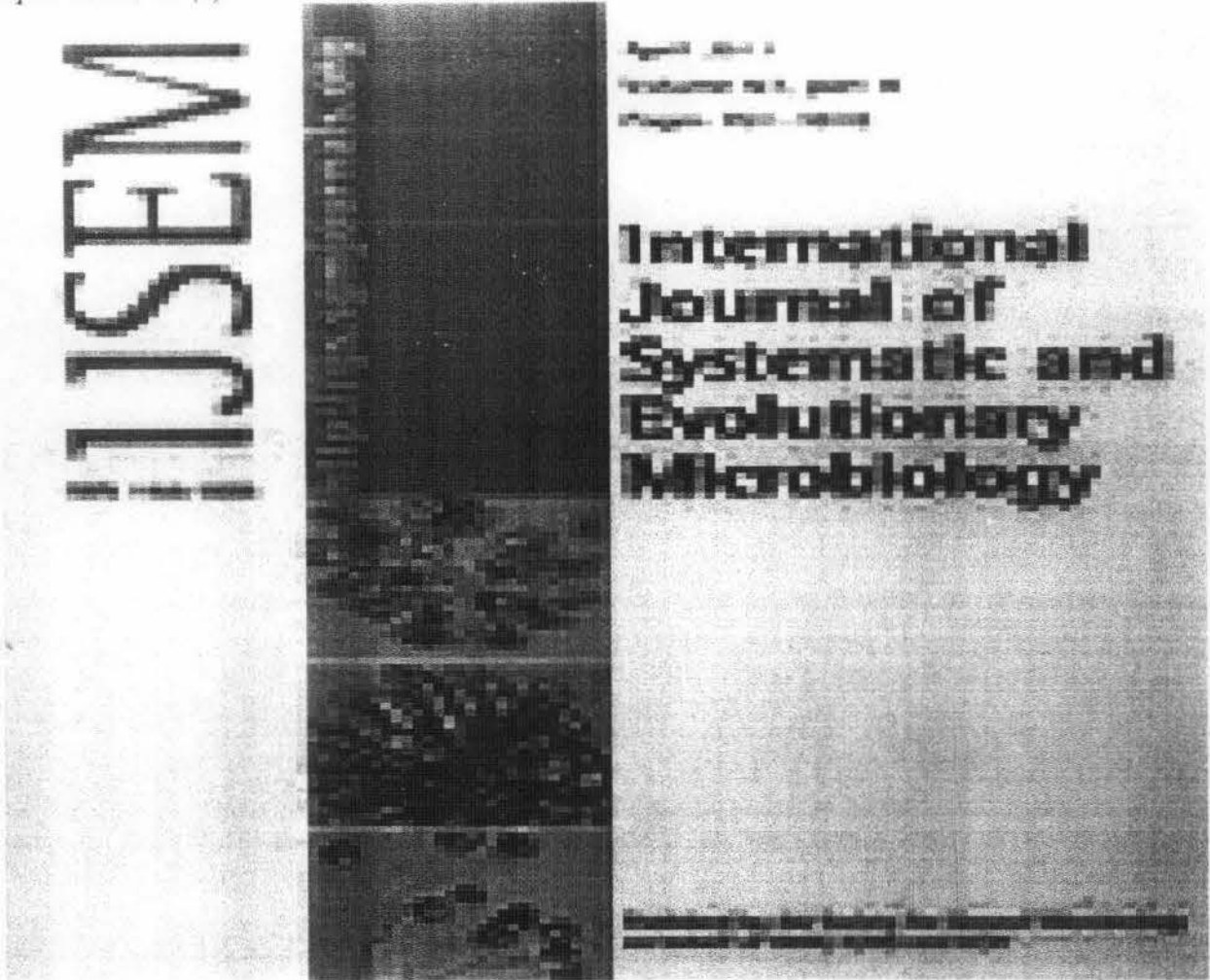


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Actinophytocola timorensis sp. nov. and *Actinophytocola corallina* sp. nov., isolated from soil

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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(H₄) 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.

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–

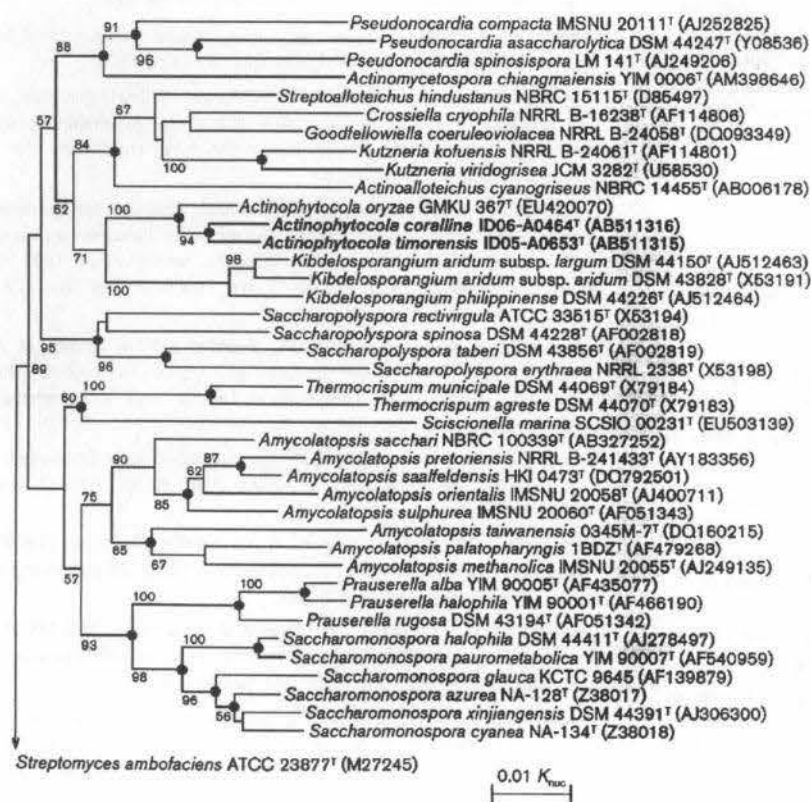
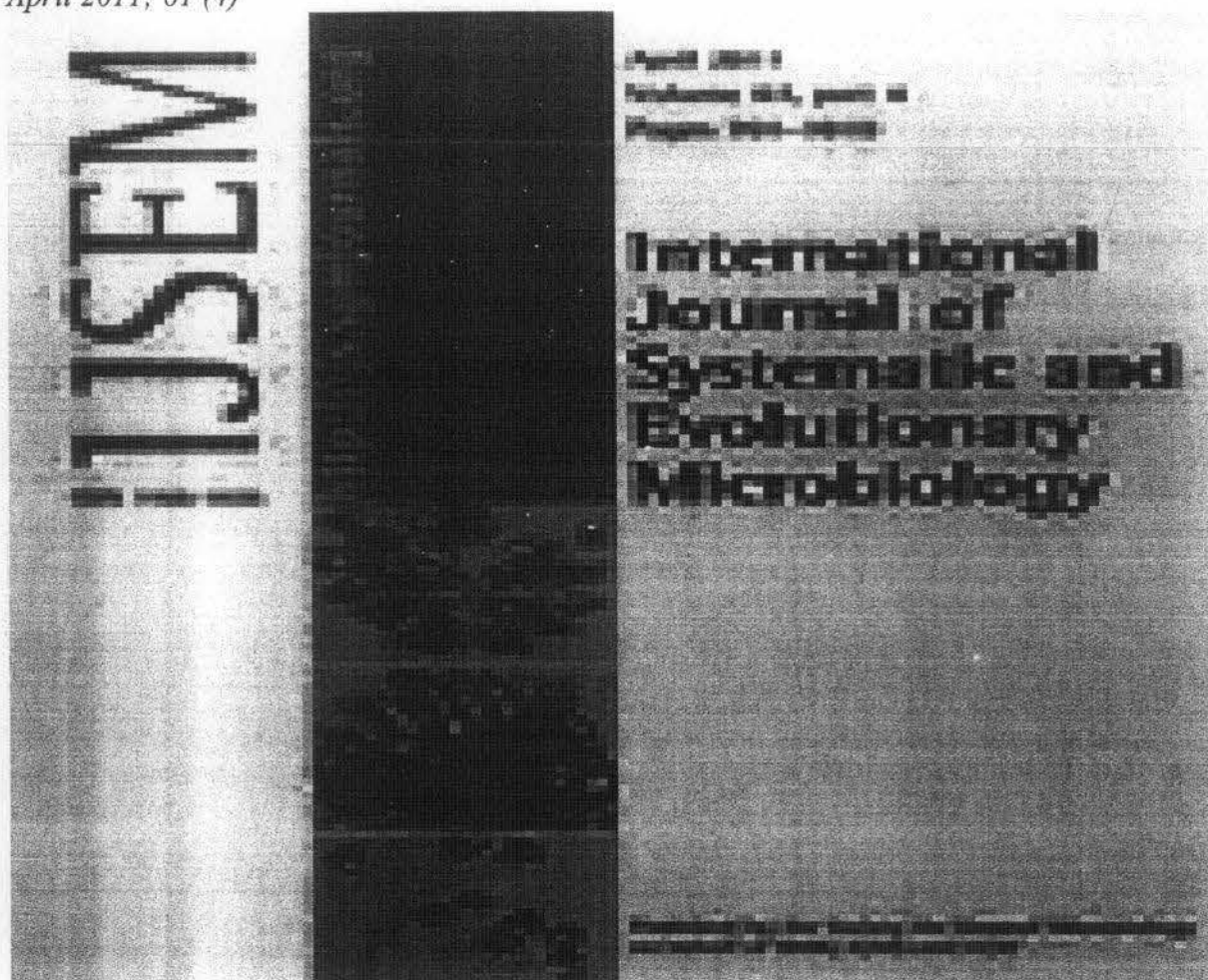


Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strains ID05-A0653^T and ID06-A0464^T within the family *Pseudonocardiaceae*. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. Filled circles indicate that the corresponding 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} .

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