Dietzia timorensis sp. nov., isolated from soil

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An actinomycete strain, ID05-A0528^T, was isolated using the SDS-yeast extract pre-treatment method from soil under mahogany (*Swietenia mahogani*) trees in West Timor, Indonesia, and was examined by using a polyphasic taxonomic approach. Chemotaxonomic and phylogenetic characterizations demonstrated that the novel strain belongs to the genus *Dietzia*. 16S rRNA gene sequencing studies showed that the strain was related to *Dietzia cinnamea* (97.2 %). Results of phenotypic and phylogenetic analyses determined that strain ID05-A0528^T is different from the known species of the genus *Dietzia*. It is proposed that the isolate should be classified as a representative of a novel species of the genus *Dietzia*, with the name *Dietzia timorensis* sp. nov. The type strain is ID05-A0528^T (=BTCC B-560^T =NBRC 104184^T).

The genus *Dietzia* is a member of the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997) and encompasses eight species at the time of writing, including *Dietzia papillomatosis*, *Dietzia schimae* and *Dietzia cercidiphylli* (Jones *et al.*, 2008; Li *et al.*, 2008). Known species of the genus *Dietzia* were originally isolated from several sources, including clinical materials, such as an alkaline soda lake, a perianal swab, a drain pool of a fish-egg processing plant, soil, the skin of an immunocompetent patient, and plant tissue (Duckworth *et al.*, 1998; Yumoto *et al.*, 2002; Yassin *et al.*, 2006; Mayilraj *et al.*, 2006; Jones *et al.*, 2008; Li *et al.*, 2008). Some strains identified as representing species of the genus *Dietzia* show degradation of hydrocarbons, including *n*-alkanes (Rainey *et al.*, 1995; Chaillan *et al.*, 2004; Yumoto *et al.*, 2002). Additionally, Takeishi *et al.* (2006) reported xylanolytic strains of the genus *Dietzia* isolated from the hindgut and faeces of *Trypoxylus dichotomus* larvae. Hence, the discovery of additional species of this genus will help in understanding their ecological roles and provide bioresources for industrial applications, including bioremediation.

Strain ID05-A0528^T was isolated from a soil sample collected under mahogany trees in West Timor. The SDS-yeast extract pre-treatment method (Hayakawa & Nonomura, 1989) and humic acid-vitamin agar (Hayakawa & Nonomura, 1987) containing nalidixic acid (20 mg l^{-1}) were used in the isolation. The pre-treatment method was used to enhance the spore germination of actinomycetes and to decrease the number of non-filamentous bacteria on the isolation plates. The aim of the present study was to determine the taxonomic position of isolate ID05-A0528^T using a polyphasic approach.

The colonial properties of strain ID05-A0528^T were recorded from a modified Bennett's agar plate (Jones, 1949) that had been incubated for 14 days at 28 °C. Gram-staining was examined by using Hucker's method (Gerhardt, 1981). Motility was examined in hanging drops by light microscopy using culture grown on Bennett's agar plates. Morphology of the cells was observed using light microscopy. Tests for aesculin and arbutin hydrolysis (Williams *et al.*, 1983), nitrate reduction (Gordon & Mihm,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ID05-A0528^T is AB377289.

1962) and urea hydrolysis (Gordon *et al.*, 1974) were performed by using established procedures. Hydrolysis of complex substrates was examined by using well-established procedures (Gordon *et al.*, 1974; Isik *et al.*, 1999). The temperature for growth was determined at 5, 10, 15, 28, 37 and 45 °C on modified Bennett's agar plates (Jones, 1949). API 50 CH test kits (bioMérieux) were used to investigate several physiological and biochemical characteristics, according to the manufacturer's instructions. Diaminopimelic acid isomers and sugars in whole-cell hydrolysates were analysed based on the methods established by Hasegawa *et al.* (1983) and Schaal (1985), respectively. Standard procedures were also used for extraction and analysis of mycolic acids (Schaal, 1985), fatty acids (Tamura *et al.*, 1994), and isoprenoid quinones and polar lipids (Minnikin *et al.*, 1984), and compared with the appropriate controls. The acyl type of muramic acid was determined by the colorimetric method (Uchida & Aida, 1977). Chromosomal DNA from strain ID05-A0528^T was isolated and purified by the method of Saito & Miura (1963) with a minor modification (Hatano *et al.*, 2003). The G+C content (mol%) of DNA from the isolate was determined by HPLC, as described by Tamura *et al.* (1994).

The 16S rRNA gene from strain ID05-A0528^T was amplified by PCR following the procedures described by Tamura & Hatano (2001) and directly sequenced using an ABI Prism BigDye Terminator cycle sequencing kit (PE Applied Biosystems) and an automatic DNA sequencer (model 3130 Genetic Analyzer; PE Applied Biosystems). The 16S rRNA gene sequence obtained was aligned with reference sequences of members of the genus *Dietzia* available from EMBL/GenBank/DDBJ by using the CLUSTAL_X program (Thompson *et al.*, 1997). A phylogenetic tree was inferred using neighbour-joining tree algorithms (Saitou & Nei, 1987). Topography of the reconstructed tree was evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985).

The chemotaxonomic characteristics of isolate ID05-A0528^T were consistent with its assignment to the genus *Dietzia*. The whole-cell hydrolysate contained *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). The major menaquinone was MK-8 (H_2), in accordance with recognized species of the genus *Dietzia*. Strain ID05-A0528^T contained phosphatidylglycerol and trace amounts of phosphatidylinositol. Muramic acid residues of the peptidoglycan were *N*-acetylated. In addition, TLC revealed that the strain contained mycolic acids, which are equivalent in mobility (R_f=0.45) to those of *Dietzia natronolimnaea* NBRC105040^T; it was thus inferred that the mycolic acids in strain ID05-A0528^T contained 34–38 carbon atoms (Duckworth *et al.*, 1998). $C_{16:0}$ (48 %), $C_{18:1}\omega9c$ (39 %) and 10-methyl $C_{18:0}$ (8 %) were present as major cellular fatty acids. Colonies were moderately orange-yellow.

The almost complete 16S rRNA gene sequence (1455 nt) of isolate ID05-A0528^T was compared with sequences from recognized species of the genus *Dietzia*. Results of phylogenetic tree reconstruction based on the neighbour-joining method showed that the novel strain formed a monophyletic clade with known species of this genus (Fig. 1). 16S rRNA gene sequence similarities to known species of the genus *Dietzia* were 95.5–97.2 %. The closest phylogenetic relative was *Dietzia cinnamea*, sharing 97.2 % sequence similarity with strain ID05-A0528^T. DNA–DNA hybridization between strain ID05-A0528^T and the nearest phylogenetic neighbours was not attempted, since Stackebrandt & Ebers (2006) recommend a 16S rRNA gene sequence similarity threshold of 98.7–99 % as the point at which DNA–DNA reassociation experiments should be mandatory for testing the genomic uniqueness of a novel isolate(s). Physiological and biochemical characterization of strain ID05-A0528^T was also performed for comparison with recognized species of the genus *Dietzia* (Table 1).

On the basis of physiological and genotypic data, strain ID05-A0528^T represents a novel species within the genus *Dietzia*, for which the name *Dietzia timorensis* sp. nov. is proposed.

Description of *Dietzia timorensis* sp. nov.

Dietzia timorensis (ti.mo.ren′sis. N.L. fem. adj. *timorensis* pertaining to West Timor, Indonesia, from where the organism was first isolated).

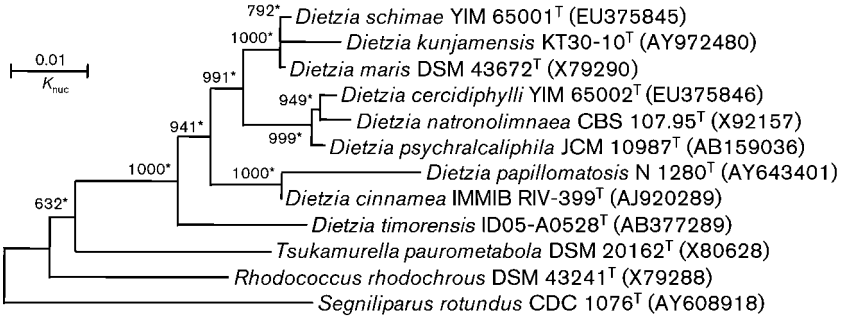

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of strain ID05-A0528^T to species of the genus *Dietzia*. The tree was reconstructed using the neighbour-joining method and K_{nuc} values (Saitou & Nei, 1987). Asterisks indicate branches of the tree that were also recovered using the minimum evolution and maximum-parsimony methods (Takahashi & Nei, 2000). Bar, 0.01 nucleotide substitution rate (K_{nuc}).

Table 1. Differential characteristics of strain ID05-A0528^T and type strains of species of the genus *Dietzia*

Strains: 1, ID05-A0528^T; 2, *Dietzia cinnamea* IMMIB RIV-399^T; 3, *D. maris* DSM 43672^T; 4, *D. schimae* YIM 65001^T; 5, *D. psychralcaliphila* ILA-1^T; 6, *D. cercidiphylli* YIM 65002^T; 7, *D. natronolimnaea* CBS 107.95^T; 8, *D. kunjamensis* KT30-10^T; 9, *D. papillomatosis* N 1280^T. +, Positive; −, negative. Data for reference strains from Li *et al.* (2008).

Characteristic	1	2	3	4	5	6	7	8	9
Urea hydrolysis	−	+	+	−	+	+	+	−	+
Nitrate reduction	−	+	+	+	−	−	−	+	+
Growth temperature range (°C)	10–37	22–45	10–45	10–45	10–37	10–37	10–37	10–37	10–37
Utilization as sole carbon source									
D-Adonitol	+	−	−	−	+	−	−	−	+
L-Arabinose	+	−	−	−	−	+	−	−	+
Cellobiose	+	−	−	+	+	−	+	+	+
L-Fucose	+	+	−	−	−	−	−	+	+
Inositol	+	−	−	−	+	−	−	−	+
Maltose	+	+	+	−	+	+	+	−	+
Raffinose	+	−	−	−	+	−	+	+	+
Salicin	+	+	−	−	−	−	−	−	+
Trehalose	+	−	−	−	+	−	+	+	+
D-Tagatose	+	+	−	−	−	+	−	−	+

Aerobic, Gram-positive, non-motile actinomycete that forms circular, convex, glistening, moderately orange-yellow colonies. Cells are rod- and coccoid-shaped. Diffusible pigments are not produced. Aesculin is hydrolysed. Nitrate is not reduced. Arbutin and urea are not hydrolysed. Does not degrade adenine, casein, elastin, hypoxanthine, testosterone, tyrosine, uric acid or xanthine. Grows at 10, 15, 28 and 37 °C, but does not grow at 5 or 45 °C. Growth occurs in the presence of 0–7 % NaCl (w/v). Utilizes aesculin, cellobiose, D-fructose, D-glucose, glycerol, lactose, D-mannose, sucrose, D-adonitol, amygdalin, L-arabinose, D-arabinose, L-arabitol, D-arabitol, arbutin, dulcitol, erythritol, L-fucose, D-fucose, D-galactose, gentiobiose, glycogen, inositol, inulin, D-lyxose, maltose, D-mannitol, melezitose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, *N*-acetylglucosamine, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, trehalose, turanose, xylitol, L-xylose and D-xylose. Contains *meso*-diaminopimelic acids, and arabinose and galactose are present in whole-cell hydrolysates. The acyl type of the glycan chain of the peptidoglycan is acetyl. The major fatty acids are $C_{16:0}$, $C_{18:1}\omega 9c$ and 10-methyl $C_{18:0}$. Mycolic acids are present. The polar lipid profile consists of phosphatidylglycerol and trace amounts of phosphatidylinositol. MK-8 (H_2) is the major menaquinone. The G+C content of DNA of the type strain is 65.5 mol%.

The type strain, ID05-A0528^T (=BTCC B-560^T =NBRC 104184^T), was isolated from a soil sample collected from under mahogany (*Swietenia mahogani*) trees on West Timor in Indonesia.

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