Dietzia schimae sp. nov. and *Dietzia cercidiphylli* sp. nov., from surface-sterilized plant tissues

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Two actinobacterial strains, YIM 65001^T and YIM 65002^T, were isolated from surface-sterilized plant tissues collected from Yunnan Province, south-west China, and their taxonomic positions were determined by using a polyphasic approach. The DNA G + C contents of strains YIM 65001^T and YIM 65002^T were 71.9 and 72.6 mol%, respectively. The two strains had chemotaxonomic markers that were consistent with their classification in the genus *Dietzia*. Phylogenetic analysis based on almost-complete 16S rRNA gene sequences indicated that strain YIM 65001^T was related most closely to *Dietzia maris* DSM 43672^T and that strain YIM 65002^T was related most closely to *Dietzia natronolimnaea* CBS 107.95^T. Levels of 16S rRNA gene sequence similarity between strains YIM 65001^T and YIM 65002^T and the type strains of other recognized members of the genus *Dietzia* were 95.8–99.8 %. DNA–DNA hybridization experiments confirmed the separate genomic status of strains YIM 65001^T and YIM 65002^T. Strains YIM 65001^T and YIM 65002^T showed significant phenotypic differences between each other and their closest recognized neighbours. On the basis of their phenotypic and phylogenetic distinctiveness, the two novel isolates were identified as representing two novel species of the genus *Dietzia*, for which the names *Dietzia schimae* sp. nov. (type strain YIM 65001^T=CCTCC AA 207015^T=DSM 45139^T) and *Dietzia cercidiphylli* sp. nov. (type strain YIM 65002^T=CCTCC AA 207016^T=DSM 45140^T) are proposed.

The genus *Dietzia* was first proposed by Rainey *et al.* (1995) to accommodate organisms previously classified as *Rhodococcus maris* (Nesterenko *et al.*, 1982). Since *Dietzia maris* was originally isolated from halibut by Harrison (1929), members of the genus have been isolated from various habitats such as marine, soil and hospital environments (Colquhoun *et al.*, 1998; Duckworth *et al.*, 1998; Mayilraj *et al.*, 2006; Yassin *et al.*, 2006; Jones *et al.*,

2008). During an investigation into the diversity of endophytic actinobacteria from pharmaceutically important plants, two novel strains, designated YIM 65001^T and YIM 65002^T, were isolated from surface-sterilized plant tissues. The aim of the present study was to determine the taxonomic status of these organisms.

Samples of *Schima* sp. and *Cercidiphyllum japonicum* were collected from Yunnan Province, south-west China, and were surface-sterilized according to the method of Coombs & Franco (2003). Strain YIM 65001^T was isolated from the surface-sterilized stem of *Schima* sp., and strain YIM 65002^T was isolated from the surface-sterilized root of *C. japonicum* by using tap water-yeast extract agar medium (Coombs & Franco, 2003). The purified strains were cultivated and maintained on trypticase soy agar medium (TSA; Difco). Cell morphology was observed by using light

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM 65001^T and YIM 65002^T are EU375845 and EU375846, respectively.

Two-dimensional thin-layer chromatograms of phospholipids of strains YIM 65001^T and YIM 65002^T and a table detailing the fatty acid profiles of strains YIM 65001^T and YIM 65002^T and of the type strains of closely related *Dietzia* species are available as supplementary material with the online version of this paper.

microscopy (BH2; Olympus) and electron microscopy (JEM-1010; JEOL). Colony colour was determined by comparison with colour chips from the ISCC-NBS colour chart standard samples (Kelly, 1964).

Phenotypic properties, and growth at various temperatures, pH values and NaCl concentrations were examined as described by Xu *et al.* (2005) by growing the strains on trypticase soy broth (TSB) as the basal medium. Oxidase activity was determined from the oxidation of tetramethyl-*p*-phenylenediamine (Kovacs, 1956). Catalase activity was determined by use of 3 % H₂O₂, and bubble production was identified as indicating a positive reaction. API 50 CH test kits (bioMérieux) were used to investigate several physiological and biochemical characteristics according to the manufacturer's instructions. Hydrolysis of starch, gelatin, and Tweens 20, 40 and 80 was determined as described by Smibert & Krieg (1994). Nitrate reduction was determined according to Lányi (1987). The following tests were performed as described by Goodfellow (1986): Voges–Proskauer, methyl red, and the production of H₂S and indole. Cell morphology and physiological and biochemical test results are given in detail in Table 1 and in the species descriptions below.

The cellular fatty acid composition was determined as described by Sasser (1990), by using the Microbial Identification System (MIDI). For other chemotaxonomic analyses, freeze-dried cells were obtained from cultures grown in TSB for 5 days at 28 °C. The amino acids and sugars of whole-cell hydrolysates were determined by using TLC as described by Stanek & Roberts (1974). Phospholipids were extracted and identified according to standard procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Menaquinones were extracted (Collins *et al.*, 1977) and separated by HPLC (Tamaoka *et al.*, 1983). The presence of mycolic acids was demonstrated by using TLC (Minnikin *et al.*, 1980). The G + C content of the genomic DNA was determined by using the HPLC method according to Mesbah *et al.* (1989).

Strains YIM 65001^T and YIM 65002^T contained *meso*-diaminopimelic acid as the diamino acid. Whole-cell hydrolysates were rich in arabinose and galactose. For the two strains, the predominant menaquinone was MK-8(H₂) (100 % for both). Mycolic acids were present, and these co-migrated with those of *Dietzia kunjamensis* K30-10^T; it was thus inferred that the mycolic acids in strains YIM 65001^T and YIM 65002^T contained 33–40 carbon atoms (Mayilraj *et al.*, 2006). Polar lipid analysis showed that strains YIM 65001^T and YIM 65002^T contained diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. However, strain YIM 65002^T also contained phosphatidylinositol mannoside, which was absent from strain YIM 65001^T (see Supplementary Fig. S1 available in IJSEM Online). The DNA G + C contents of strains YIM 65001^T and YIM 65002^T were 71.9 and 72.6 mol%, respectively. These values are consistent with those of recognized members of the genus *Dietzia*, which range from 66.1 to 73.0 mol%

(Rainey *et al.*, 1995; Duckworth *et al.*, 1998; Yumoto *et al.*, 2002; Yassin *et al.*, 2006; Mayilraj *et al.*, 2006). The fatty acid profiles of the two novel strains are described in detail in Supplementary Table S1 (available in IJSEM Online) and in the species descriptions below.

Genomic DNA extraction, amplification and 16S rRNA gene sequencing were performed as described by Li *et al.* (2007). Phylogenetic analysis was performed by using MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances and *K*_{nuc} values were calculated and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987). The topology of the resultant tree was evaluated by bootstrap analysis of the neighbour-joining data based on 1000 resamplings (Felsenstein, 1985). The phylogenetic positions of strains YIM 65001^T and YIM 65002^T revealed that these two taxa were closely associated with the genus *Dietzia* but formed separate subclades with their most closely related neighbours (Fig. 1). A 16S rRNA gene sequence similarity value of 98.1 % was found between strains YIM 65001^T and YIM 65002^T. Strain YIM 65001^T showed 16S rRNA gene sequence similarities of 99.8, 99.0, 97.9, 97.8, 97.3 and 96.1 % to *D. maris* DSM 43672^T, *D. kunjamensis* K30-10^T, *Dietzia cinnamea* IMMIB RIV-399^T, *Dietzia natronolimnaea* CBS 107.95^T, *Dietzia psychrhalcaliphila* ILA-1^T and *Dietzia papillomatosis* N 1280^T, respectively. Strain YIM 65002^T displayed 16S rRNA gene sequence similarities of 99.5, 98.7, 98.2, 97.6, 97.3 and 95.8 % to *D. natronolimnaea* CBS 107.95^T, *D. psychrhalcaliphila* ILA-1^T, *D. maris* DSM 43672^T, *D. cinnamea* IMMIB RIV-399^T, *D. kunjamensis* K30-10^T and *D. papillomatosis* N 1280^T, respectively.

Genomic relatedness between strains YIM 65001^T and YIM 65002^T and their most closely related phylogenetic neighbours, i.e. the type strains of *D. maris*, *D. natronolimnaea*, *D. psychrhalcaliphila* and *D. kunjamensis*, was determined according to the DNA–DNA reassociation method described by He *et al.* (2005). Strain YIM 65001^T displayed low levels of DNA–DNA relatedness to YIM 65002^T (43.2 %), *D. maris* DSM 43672^T (42.1 %), *D. kunjamensis* K30-10^T (44.0 %), *D. natronolimnaea* CBS 107.95^T (53.3 %) and *D. psychrhalcaliphila* ILA-1^T (51.1 %). Strain YIM 65002^T displayed a moderate level of DNA–DNA relatedness to *D. kunjamensis* K30-10^T (59.6 %), and lower levels to *D. psychrhalcaliphila* ILA-1^T (42.7 %), *D. natronolimnaea* CBS 107.95^T (27.8 %) and *D. maris* DSM 43672^T (32.9 %). These data further supported the conclusions drawn by Duckworth *et al.* (1998) and Yassin *et al.* (2006) that representatives of *Dietzia* species with 16S rRNA gene sequence similarities of greater than 98 % share DNA–DNA relatedness values well below 70 %, the recommended threshold value for the delineation of genomic species (Wayne *et al.*, 1987). *D. cinnamea* IMMIB RIV-399^T is allocated to Risk Group 2 and restrictions are placed on its use and distribution. However, strains YIM 65001^T and YIM 65002^T could be distinguished easily from their closest relatives based on differences in several

Table 1. Differential characteristics between strains YIM 65001^T and YIM 65002^T and the type strains of recognized *Dietzia* species

Strains: 1, YIM 65001^T; 2, YIM 65002^T; 3, *D. psychralcaliphila* ILA-1^T; 4, *D. natronolimnaea* CBS 107.95^T; 5, *D. kunjamensis* K30-10^T; 6, *D. maris* DSM 43672^T; 7, *D. cinnamomea* IMMIB RIV-399^T; 8, *D. papillomatosis* N 1280^T. Data for all strains except *D. cinnamomea* IMMIB RIV-399^T and *D. papillomatosis* N 1280^T (Yassin *et al.*, 2006; Jones *et al.*, 2008) are from the present study. +, Positive; w, weakly positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Deep pink	Reddish orange	Soft red	Soft pink	Red	Deep orange	Yellow	Orange
Urea hydrolysis	–	+	+	+	–	+	+	+
Nitrate reduction	+	–	–	–	+	+	+	+
H ₂ S production	–	–	+	+	+	–	ND	ND
Tolerance to NaCl (%, w/v)	15	10	10	10	15	15	12	8
Growth temperature range (°C)	10–45	10–37	10–37	10–37	10–37	10–45	22–45	10–37
Utilization as sole carbon source								
D-Adonitol	–	–	+	–	–	–	–	+
Aesculin	+	–	–	–	–	–	–	ND
Amygdalin	–	–	+	+	–	+	ND	ND
D-Arabitol	–	–	+	–	–	–	ND	ND
L-Arabinose	–	+	–	–	–	–	–	+
Arbutin	–	+	–	–	–	–	ND	+
Cellobiose	+	–	+	+	+	–	–	+
L-Fucose	–	–	–	–	+	–	+	+
Gentiobiose	–	–	+	–	+	–	–	+
D-Glucose	+	+	–	+	+	+	+	+
Glycerol	+	–	w	+	–	–	–	+
Glycogen	–	–	–	–	+	–	ND	ND
Inositol	–	–	+	–	–	–	–	+
Inulin	–	–	–	–	+	–	ND	ND
D-Lactose (bovine origin)	+	+	+	+	+	–	–	+
D-Lyxose	–	+	–	–	+	–	–	ND
Maltose	–	+	+	+	–	+	+	+
D-Mannose	+	+	+	+	+	–	ND	+
Melezitose	–	–	+	+	w	+	–	+
Melibiose	–	–	+	+	+	–	ND	+
Methyl α-D-glucopyranoside	–	–	+	+	–	–	ND	ND
Methyl α-D-mannopyranoside	–	–	+	–	–	+	ND	ND
Methyl β-D-xylopyranoside	–	–	+	–	–	–	ND	ND
N-Acetylglucosamine	–	–	+	+	–	–	ND	ND
Potassium 5-ketogluconate	–	+	+	–	w	+	ND	ND
Raffinose	–	–	+	+	+	–	–	+
L-Rhamnose	–	–	–	–	+	–	–	ND
Salicin	–	–	–	–	–	–	+	+
Sucrose	+	w	+	+	+	–	–	+
D-Tagatose	–	+	–	–	–	–	+	+
Trehalose	–	–	+	+	+	–	–	+
Turanose	–	+	–	–	–	–	+	+
Xylitol	–	–	+	–	–	–	ND	+
D-Xylose	–	–	–	–	–	w	+	+
L-Xylose	–	–	–	–	–	–	+	ND

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships among strains YIM 65001^T and YIM 65002^T and related species. Bootstrap values (expressed as percentages of 1000 replications) of greater than 50 % are given at nodes. Bar, 1 substitution per 100 nt.

phenotypic characteristics (see Table 1). In view of these facts, we did not investigate the DNA–DNA relatedness of strains YIM 65001^T and YIM 65002^T with respect to *D. cinnamomea* IMMIB RIV-399^T. Given their genomic distinctiveness together with differences in some phenotypic properties, we suggest that strains YIM 65001^T and YIM 65002^T represent two novel species of the genus *Dietzia*, for which the names *Dietzia schimae* sp. nov. and *Dietzia cercidiphylli* sp. nov. are proposed, respectively.

Description of *Dietzia schimae* sp. nov.

Dietzia schimae (schi'ma.e. N.L. gen. n. *schimae* of the plant genus *Schima*, isolated from a stem sample of *Schima* sp.).

Forms circular, smooth, opaque and deep pink colonies on TSA plates. Short, rod-shaped cells exhibit snapping division and produce V-forms. Aerobic, Gram-positive, non-motile, non-spore-forming and non-acid-fast. Catalase is produced. Negative for oxidase, Voges–Proskauer and methyl red tests, production of H₂S and indole, milk coagulation and peptonization, and hydrolysis of gelatin, urea and starch. Positive for nitrate reduction and hydrolysis of Tweens 20, 40 and 80. Tolerates up to 15 % NaCl and grows at temperatures between 10 and 45 °C, with an optimum growth temperature of 28 °C. Grows at pH 6.0–9.0 with optimum growth at pH 7.0. Utilizes aesculin, cellobiose, D-fructose, D-glucose, glycerol, D-lactose, D-mannose and sucrose, but not 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 or D-xylose. The diagnostic amino acid is meso-diaminopimelic acid, and arabinose and galactose are present in whole-cell hydrolysates. Phospholipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The predominant menaquinone is MK-8(H₂). Mycolic acids are present. Major fatty acids are C_{14:0}, C_{16:1ω6c}/C_{16:1ω7c}, C_{16:0}, C_{17:1ω7c}, C_{17:1} anteiso B/iso I, 10-methyl C_{17:0}, 10-methyl C_{18:0} (tuberculostearic acid), iso-C_{19:0} and anteiso-C_{19:0}. The DNA G + C content of the type strain is 71.9 mol%.

The type strain, YIM 65001^T (=CCTCC AA 207015^T = DSM 45139^T), was isolated from a surface-sterilized stem sample of *Schima* sp. collected from Yunnan Province, south-west China.

Description of *Dietzia cercidiphylli* sp. nov.

Dietzia cercidiphylli (cer.ci.di.phyl'li. N.L. gen. n. *cercidiphylli* of the plant genus *Cercidiphyllum*, isolated from root sample of *Cercidiphyllum japonicum*).

Forms circular, smooth, opaque and reddish orange colonies on TSA plates. Short, rod-shaped cells exhibit snapping division and produce V-forms. Aerobic, Gram-positive, non-motile, non-spore-forming and non-acid-fast. Catalase is produced. Negative for oxidase, Voges–Proskauer and methyl red tests, production of H₂S and indole, nitrate reduction, milk coagulation and peptonization, and hydrolysis of gelatin and starch. Positive for hydrolysis of urea, and Tweens 20, 40 and 80. Tolerates up to 10 % NaCl and grows at temperatures between 10 and 37 °C, with an optimum growth temperature of 28 °C. Grows at pH 6.0–9.0, with optimum growth at pH 7.0. Utilizes L-arabinose, arbutin, D-fructose, D-glucose, D-lactose, D-lyxose, maltose, D-mannose, potassium 5-ketogluconate, sucrose, D-tagatose and turanose, but not

D-adonitol, aesculin, amygdalin, D-arabinose, L-arabitol, D-arabitol, cellobiose, dulcitol, erythritol, L-fucose, D-fucose, D-galactose, gentiobiose, glycerol, glycogen, inositol, inulin, D-mannitol, melezitose, melibiose, methyl α -D-glucopyranoside, methyl α -D-mannopyranoside, methyl β -D-xylopyranoside, N-acetylglucosamine, potassium gluconate, potassium 2-ketogluconate, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, trehalose, xylitol, L-xylose or D-xylose. The diagnostic amino acid is meso-diaminopimelic acid, and arabinose and galactose are present in whole-cell hydrolysates. Phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannoside. The predominant menaquinone is MK-8(H₂). Mycolic acids are present. Major fatty acids are C_{14:0}, iso-C_{16:0}, C_{16:1} ω 6c/C_{16:1} ω 7c, 10-methyl C_{16:0}, C_{16:0}, C_{17:1} ω 8c, C_{17:1} ω 7c, C_{18:1} ω 7c, C_{18:1} ω 9c and 10-methyl C_{18:0} (tuberculostearic acid). The DNA G + C content of the type strain is 72.6 mol%.

The type strain, YIM 65002^T (=CCTCC AA 207016^T=DSM 45140^T), was isolated from a surface-sterilized root sample of *Cercidiphyllum japonicum* collected from Yunnan Province, south-west China.

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