Dietzia alimentaria sp. nov., isolated from a traditional Korean food

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An actinobacterial strain, designated 72^T, was isolated from a traditional salt-fermented seafood in Korea. Colonies were coral red and cells were Gram-reaction-positive, non-motile rods. Strain 72^T grew with 0–10 % (w/v) NaCl, at pH 7–10 and at 15–37 °C. Optimum growth conditions were 2 % NaCl, pH 7.0 and 30 °C. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain 72^T belonged to the genus *Dietzia*. The major cellular fatty acids (>5 %) were $C_{16:0}$, summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), 10-methyl $C_{18:0}$, $C_{17:0}$, $C_{19:0}$ and $C_{18:1}\omega9c$. 16S rRNA gene sequence analysis and DNA–DNA hybridization, coupled with physiological and biochemical tests, revealed genotypic and phenotypic differences between strain 72^T and other members of the genus *Dietzia*. Based on these data, strain 72^T represents a novel species, for which the name *Dietzia alimentaria* sp. nov. is proposed. The type strain is 72^T (=JCM 16360^T =KACC 21126^T).

The genus *Dietzia* was proposed by Rainey *et al.* (1995) to accommodate *Rhodococcus maris* (Nesterenko *et al.*, 1982) and includes eight species at the time of writing. Members of the genus have been isolated from a number of different environments, including skin, hospitals, plant tissue, soil and seawater (Duckworth *et al.*, 1998; Rainey *et al.*, 1995; Yumoto *et al.*, 2002). *Dietzia maris* strains have been isolated from soil, seawater and hospital environments (Colquhoun *et al.*, 1998; Nesterenko *et al.*, 1982; Rainey *et al.*, 1995; Takami *et al.*, 1997), *Dietzia natronolimnaea* from an alkaline East African soda lake (Duckworth *et al.*, 1998), *Dietzia psychralcaliphila* from a drain pool of a fish-egg-processing plant (Yumoto *et al.*, 2002), *Dietzia cinnamea* from a perianal swab of a patient after bone-marrow transplantation (Yassin *et al.*, 2006), *Dietzia kunjamensis* from a cold desert soil (Mayilraj *et al.*, 2006), *Dietzia papillomatosis* from the skin of an immunocompetent patient with confluent papillomatosis (Jones *et al.*, 2008) and *Dietzia schimae* and *Dietzia cercidiphylli* from surface-sterilized plant tissues (Li *et al.*, 2008).

Strain 72^T was isolated from a traditional salt-fermented seafood made by fermenting fresh clams mixed with rock salt for 2 weeks (Suh & Yoon, 1987). The purified strain was cultured on tryptic soy agar (TSA; BBL) and marine agar (MA; BBL) at 30 °C for 5 days. Cell morphology was inspected by light microscopy (ECLIPSE 50*i*; Nikon) and transmission electron microscopy (JEM-1010; JEOL). Growth at 4, 15, 25, 30, 37 and 45 °C, with 0–10 % NaCl (at intervals of 1 %) and 15 and 20 % NaCl and at pH 2.0–13.0 (at intervals of 1 pH unit) was tested in marine broth (BBL). The Gram reaction was performed using the non-staining method described by Buck (1982). Hydrolysis of starch, gelatin and Tweens 20, 40, 60 and 80 (1 % on MA) was performed as described by Smibert & Krieg (1994). H_2S production, citrate utilization, the Voges–Proskauer reaction and the methyl red test were performed according to described methods (Goodfellow, 1986). Motility was examined using the method of Tittsler & Sandholzer (1936) in semi-solid agar (motility test medium; BBL). Enzyme activity and utilization of sole carbon sources were determined using the API ZYM, API 20NE and API 50 CH kits (bioMérieux) according to the manufacturer's instructions. Cells of strain 72^T were Gram-reaction-positive, non-motile rods, 1–1.5 μm in diameter. They were positive for catalase, but negative for oxidase. The isolate was positive for hydrolysis of Tweens 20, 40, 60 and 80. Growth occurred at pH 7–10 (optimum pH 7.0), with 0–10 % NaCl (optimum 2 % NaCl) and at 15–37 °C (optimum 30 °C). The species description gives further phenotypic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 72^T is GQ368824.

Two supplementary figures are available with the online version of this paper.

details and Table 1 provides a phenotypic comparison of strain 72^T with members of the genus *Dietzia*.

Reference strains *D. maris* JCM 6166^T, *D. kunjamensis* JCM 13325^T, *D. cinnamea* JCM 13663^T and *D. natronolimnaea* JCM 11417^T were obtained from the Japan Collection of Microorganisms and reference strains *D. schimae* DSM 45139^T, *D. cercidiphylli* DSM 45140^T and *D. psychralcaliphila* DSM 44820^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen. The 16S rRNA gene was amplified with PCR Pre-Mix (Solgent) using the colony PCR method (Baker *et al.*, 2003). The resulting PCR products were purified using a PCR purification kit (Qiagen) and sequenced using a BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) as described previously (Roh *et al.*, 2008). The almost-full-length 16S rRNA gene sequence was assembled using SeqMan software (DNASTAR). 16S rRNA gene sequences from strain 72^T and related taxa obtained from the NCBI database were aligned using CLUSTAL X version 1.8 (Thompson *et al.*, 1997), trimmed and converted to MEGA format for phylogenetic analysis. Phylogenetic consensus trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods. Phylogenetic relationships between the isolate and representative members of the genus *Dietzia* were determined using MEGA4 (Tamura *et al.*, 2007) and the topologies of the resultant trees were estimated using bootstrap analysis (Felsenstein, 1985) based on 1000 replicates. The phylogenetic relationships between strain 72^T and its relatives in the genus *Dietzia* are shown in Fig. 1. Strain 72^T exhibited 98.6, 98.5, 98.4, 98.1, 98.0, 97.9 and 97.5 % 16S rRNA gene sequence similarity with *D. maris* DSM 43672^T, *D. schimae* YIM 65001^T, *D. psychralcaliphila* ILA-1^T, *D. kunjamensis* K30-10^T, *D. cercidiphylli* YIM 65002^T, *D. natronolimnaea* CBS 107. 95^T and *D. cinnamea* IMMIB RIV-339^T, respectively.

DNA–DNA hybridization was performed in quintuplicate using microarrays according to Bae *et al.* (2005). Strain 72^T showed low levels of DNA–DNA relatedness with *D. maris* JCM 6166^T (17.8 ±2.9 %), *D. schimae* DSM 45139^T (18.5 ±1.1 %), *D. psychralcaliphila* DSM 44820^T (21.3 ±1.7 %), *D. kunjamensis* JCM 13325^T (17.0 ±2.6 %), *D. cercidiphylli* DSM 45140^T (26.7 ±8.3 %), *D. natronolimnaea* JCM 11417^T (9.6 ±2.7 %) and *D. cinnamea* JCM 13663^T (21.9 ±5.8 %).

Analysis of the cellular fatty acid composition of strain 72^T was performed according to the Sherlock Microbial Identification System (MIDI). Fatty acids were analysed by gas chromatography (Hewlett Packard 6890) with cells grown on TSA at 30 °C for 3 days. The fatty acids were $C_{16:0}$ (15.5 %), summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; 15.1 %), 10-methyl $C_{18:0}$ (14.6 %), $C_{17:0}$ (10.8 %), $C_{19:0}$ (9.1 %), $C_{18:1}\omega9c$ (7.4 %), $C_{17:1}\omega7c$ (4.9 %) and $C_{20:1}\omega9c$ (4.5 %); other fatty acids were present in small amounts (<4 %). The amino-acid composition of the cell-wall hydrolysate was determined using one-dimensional TLC on cellulose sheets (Bousfield *et al.*, 1985). The major polar lipids were extracted using the method of Xin *et al.* (2000), separated by two-dimensional TLC on a Merck silica gel 60 F_{254} glass-backed plate with chloroform/methanol/water (65 : 25 : 4, by vol.) in the first dimension and chloroform/acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.) in the second dimension and detected by spraying the plate with specific reagents, as described by Tindall (1990). The designations of all spots were according to Kämpfer *et al.* (2010). The polar lipids present were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, an unknown phospholipid, six unknown glycolipids, an unknown aminolipid and an unknown lipid (Supplementary Fig. S1, available in IJSEM Online). Mycolic acids were determined using one-dimensional TLC on cellulose sheets (Minnikin *et al.*, 1975; Supplementary Fig. S2). Cell-wall sugars were separated by one-dimensional TLC on a Merck silica gel 60 F_{254} glass-backed plate with n-butanol/water/pyridine/toluene (10 : 6 : 6 : 1, by vol.) using the method of Lechevalier (1968). The cell-wall sugars were arabinose and galactose. Quinone extraction and identification was performed according to the method of Komagata & Suzuki (1987); the major isoprenoid menaquinone of the isolate was MK-8(H_2).

The G+C content of strain 72^T and the reference strains *D. psychralcaliphila* ILA-1^T and *D. kunjamensis* K30-10^T was determined using SYBR Green I and real-time PCR (Gonzalez & Saiz-Jimenez, 2002). Genomic DNA from *Bacteroides finegoldii* DSM 17565^T and *Escherichia coli* K-12 (obtained from KCTC) was used for calibration. The DNA G+C content of the isolate was 64.7 mol%. The lowest genomic DNA G+C content observed thus far for the genus *Dietzia* is 66 mol% (Duckworth *et al.*, 1998) and the highest is 73 mol% (Rainey *et al.*, 1995). The DNA G+C content of strain 72^T is slightly lower than those reported for members of the genus *Dietzia* with validly published names.

On the basis of genotypic, chemotaxonomic and phenotypic data, strain 72^T represents a novel species of the genus *Dietzia*, for which the name *Dietzia alimentaria* sp. nov. is proposed.

Description of *Dietzia alimentaria* sp. nov.

Dietzia alimentaria (a.li.men.ta′ri.a. L. fem. adj. *alimentaria* pertaining to food).

Colonies are opaque, smooth, circular, convex, entire and shiny in appearance, with a coral-red pigment and approximately 1–2 mm in diameter after 5 days on marine agar at 30 °C. Cells are rods (1–1.5 μm wide), Gram-reaction-positive, non-motile, catalase-positive and oxidase-negative. Growth occurs at pH 7–10 (optimum pH 7.0), with 0–10 % NaCl (optimum 2 % NaCl) and at 15–37 °C (optimum 30 °C). No growth at 45 °C. Positive for hydrolysis of Tweens 20, 40, 60 and 80 but negative for hydrolysis of casein and starch. With API ZYM, positive for esterase (C4), alkaline phosphatase, esterase lipase and naphthol-AS-BI-phosphohydrolase and negative for lipase

Table 1. Differential characteristics of strain 72^T and type strains of species of the genus *Dietzia*

Strains: 1, *Dietzia alimentaria* sp. nov. 72^T; 2, *D. maris* DSM 43672^T; 3, *D. schimae* YIM 65001^T; 4, *D. psychralcaliphila* ILA-1^T; 5, *D. kunjamensis* K30-10^T; 6, *D. cercidiphylli* YIM 65002^T; 7, *D. natronolimnaea* CBS 107.95^T; 8, *D. cinnamea* IMMIB RIV-399^T. Data were obtained in this study. +, Positive; w, weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7	8
Colony colour*	CR	DO	DP	CR	R	RO	SP	Y
Growth temperature (°C)	15–37	10–45	10–45	10–37	10–37	10–37	10–37	22–45
Utilization of (API 50CH):								
Glycerol	−	w	−	+	−	−	+	−
Erythritol	−	−	−	−	−	−	+	w
D-Arabinose	−	+	−	+	−	+	+	−
L-Arabinose	−	w	−	+	−	−	−	−
D-Ribose	−	+	w	−	w	−	+	w
D-Xylose	−	+	−	−	+	w	−	+
L-Xylose	−	+	−	−	−	−	−	−
D-Adonitol	−	+	−	−	−	+	w	−
Methyl β-D-xylopyranoside	+	+	−	−	−	+	w	−
D-Galactose	−	+	−	−	w	−	+	−
D-Glucose	+	+	−	+	w	−	−	+
D-Fructose	w	+	+	+	+	+	−	+
D-Mannose	+	+	+	w	w	−	+	−
L-Sorbose	+	+	w	+	−	−	+	−
L-Rhamnose	+	+	+	+	+	−	+	−
Dulcitol	+	+	−	+	−	w	+	−
Inositol	w	+	+	+	w	−	−	−
D-Mannitol	w	+	+	+	−	−	−	−
D-Sorbitol	w	+	−	+	−	−	−	−
Methyl α-D-mannopyranoside	−	+	−	+	−	−	+	−
Methyl α-D-glucopyranoside	−	+	+	−	−	−	+	+
N-Acetylglucosamine	+	+	w	w	−	+	+	w
Amygdalin	−	w	w	+	−	−	w	−
Arbutin	+	+	−	w	+	−	−	−
Aesculin	+	+	+	+	+	+	−	−
Salicin	+	w	w	−	−	−	−	−
Cellobiose	−	+	+	w	w	+	−	−
Maltose	+	w	+	+	w	−	−	−
Lactose	+	w	+	−	+	−	−	−
Melibiose	−	+	−	−	+	+	−	+
Sucrose	+	+	+	+	+	+	−	+
Trehalose	+	+	+	−	−	+	−	−
Inulin	+	+	+	+	+	+	−	+
Melezitose	−	+	+	+	+	+	−	+
Raffinose	−	+	−	−	w	+	−	−
Starch	+	+	+	+	+	+	−	−
Glycogen	+	+	+	+	+	+	−	+
Xylitol	−	w	+	−	+	+	−	−
Gentiobiose	−	w	+	−	+	+	−	−
Turanose	−	+	−	+	+	−	−	−
D-Lyxose	−	−	−	−	+	−	+	−
D-Tagatose	−	−	−	+	+	−	−	−
D-Fucose	−	−	−	+	w	−	−	−
L-Fucose	−	−	−	+	+	−	−	−
D-Arabitol	w	−	−	w	+	−	−	−
L-Arabitol	−	−	−	+	+	−	−	−
Potassium gluconate	−	−	−	−	+	−	−	−
Potassium 2-ketogluconate	−	−	−	−	+	−	−	−
Potassium 5-ketogluconate	w	w	−	+	+	−	−	−

*CR, Coral red; DO, deep orange; DP, deep pink; R, red; RO, reddish orange; SP, soft pink; Y, yellow.

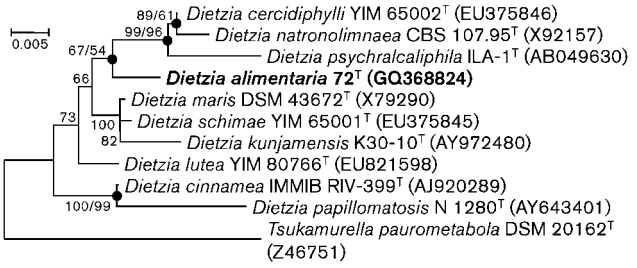

Fig. 1. 16S rRNA gene sequence-based phylogenetic consensus tree reconstructed using the neighbour-joining algorithm, showing the position of strain 72^T in the genus *Dietzia*. Filled circles indicate nodes that were also recovered using the maximum-parsimony algorithm. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes (neighbour joining/maximum parsimony). Bar, 0.005 accumulated changes per nucleotide.

(C14), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β- and α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase; acid phosphatase reaction is weak. With API 20NE, positive for reduction of nitrates to nitrogen, fermentation of D-glucose and assimilation of adipic acid, malic acid and trisodium citrate and weakly positive for assimilation of D-mannose; negative for production of indole, assimilation of potassium gluconate, urease and β-galactosidase activity (4-nitrophenyl β-D-galactopyranoside hydrolysis) and assimilation of D-glucose, L-arabinose, D-mannitol, *N*-acetylglucosamine, maltose, capric acid and phenylacetic acid. With API 50 CH, assimilates methyl β-D-xylopyranoside, D-glucose, D-mannose, L-sorbose, L-rhamnose, dulcitol, *N*-acetylglucosamine, arbutin, aesculin, salicin, maltose, lactose, sucrose, trehalose, inulin, starch and glycogen, assimilates D-fructose, inositol, D-mannitol, D-sorbitol, D-arabitol and potassium 5-ketogluconate weakly, but does not assimilate glycerol, erythritol, D- or L-arabinose, D-ribose, D- or L-xylose, D-adonitol, D-galactose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, cellobiose, melibiose, melezitose, raffinose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- or L-fucose, L-arabitol, potassium gluconate or potassium 2-ketogluconate. Whole-cell hydrolysates show the main cell-wall components to be *meso*-diaminopimelic acid, arabinose and galactose. The major fatty acids (>5 %) are $C_{16:0}$, summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), 10-methyl $C_{18:0}$, $C_{17:0}$, $C_{19:0}$ and $C_{18:1}\omega9c$. Mycolic acids are present. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, an unknown phospholipid, six unknown glycolipids, an unknown aminolipid and an unknown lipid. The major isoprenoid menaquinone is MK-8(H_2). The DNA G+C content of the type strain is 64.7 mol%.

The type strain, 72^T (=JCM 16360^T =KACC 21126^T), was isolated from a traditional salt-fermented seafood made of clam jeotgal from Sokcho, Korea.

Acknowledgements

We thank Dr J. P. Euzéby (Ecole Nationale Vétérinaire, France) for etymological advice. This work was supported by TDPAF (Technology Development Program for Agriculture and Forestry).

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