

Dietzia papillomatosis sp. nov., a novel actinomycete isolated from the skin of an immunocompetent patient with confluent and reticulated papillomatosis

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An actinomycete isolated from an immunocompetent patient suffering from confluent and reticulated papillomatosis was characterized using a polyphasic taxonomic approach. The organism had chemotaxonomic and morphological properties that were consistent with its assignment to the genus *Dietzia* and it formed a distinct phyletic line within the *Dietzia* 16S rRNA gene tree. It shared a 16S rRNA gene sequence similarity of 98.3% with its nearest neighbour, the type strain of *Dietzia cinnamea*, and could be distinguished from the type strains of all *Dietzia* species using a combination of phenotypic properties. It is apparent from genotypic and phenotypic data that the organism represents a novel species in the genus *Dietzia*. The name proposed for this taxon is *Dietzia papillomatosis*; the type strain is N 1280^T (=DSM 44961^T=NCIMB 14145^T).

The monospecific genus *Dietzia* was proposed by Rainey *et al.* (1995) for actinomycetes previously classified as *Rhodococcus maris* Nesterenko *et al.* (1982). At the time of writing, the genus *Dietzia* consists of five species with validly published names: *Dietzia kunjomensis* (Mayilraj *et al.*, 2006); *Dietzia maris* (Rainey *et al.*, 1995), the type species; *Dietzia cinnamea* (Yassin *et al.*, 2006); *Dietzia natronolimnaea* (Duckworth *et al.*, 1998); and *Dietzia psychralcaliphila* (Yumoto *et al.*, 2002). The type strains of these species form a distinct 16S rRNA gene clade within the evolutionary radiation occupied by mycolic-acid-containing actinomycetes, that is, by organisms classified in the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997; Butler *et al.*, 2005; Soddell *et al.*, 2006; Yassin *et al.*, 2006).

D. maris strains have been isolated from the skin and intestinal tract of carp, from soil and from deep-sea sediments in the Pacific Ocean (Nesterenko *et al.*, 1982; Rainey *et al.*, 1995; Takami *et al.*, 1997; Colquhoun *et al.*, 1998), *D. cinnamea* was isolated from a perianal swab of a patient with a bone marrow transplant (Yassin *et al.*, 2006), *D. kunjomensis* from a cold desert soil (Mayilraj, *et al.*, 2006), *D. natronolimnaea* from a moderately saline and

alkaline East African soda lake (Duckworth *et al.*, 1998) and *D. psychralcaliphila* was isolated from a drain pool of a fish-egg-processing plant (Yumoto *et al.*, 2002). Species of the genus *Dietzia* have been reported as potential human pathogens in an immunocompetent patient (Pidoux *et al.*, 2001) and in immunocompromised patients (Berner-Melchior *et al.*, 1999; Yassin *et al.*, 2006).

The aim of the present investigation was to determine the taxonomic position of an actinomycete that had been isolated from the skin of an immunocompetent patient with confluent and reticulated papillomatosis and presumptively assigned to the genus *Dietzia* (Natarajan *et al.*, 2005). The isolate was the subject of a polyphasic taxonomic investigation which showed that it warrants recognition as a novel species of the genus *Dietzia*.

Strain N 1280^T was isolated from skin scrapings from a patient suffering from confluent and reticulated papillomatosis, as described by Natarajan *et al.* (2005). The organism was maintained on glucose-yeast extract agar (GYEA; Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20%, v/v) at –20 °C. Biomass required for chemotaxonomic and 16S rRNA gene sequence analyses was obtained by growing the novel strain in shake flasks of glucose-yeast extract (GYE) broth

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N 1280^T is AY643401.

for 5 days at 28 °C; cells were checked for purity and harvested by centrifugation. Cells for chemosystematic studies were washed twice in distilled water and freeze-dried; those for 16S rRNA gene sequencing were washed in NaCl/EDTA buffer (0.1 M EDTA, 0.1 M NaCl, pH 8.0) and stored at -20 °C until required.

The phylogenetic position of strain N 1280^T was determined by 16S rRNA gene sequence analysis. Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified products were carried out after Kim *et al.* (1998). The resultant 16S rRNA gene sequence (1437 nt) was aligned manually with corresponding sequences of representatives of the suborder *Corynebacterineae*, retrieved from the DDBJ/EMBL/GenBank databases, using the pairwise alignment option and 16S rRNA secondary structural information held in the program PHYDIT (available at <http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993); evolutionary distance matrices were prepared after Jukes & Cantor (1969). The topologies of the resultant unrooted trees were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using the CONSENSE and SEQBOOT options from the PHYLIP package.

It is apparent from Fig. 1 that strain N 1280^T belongs to the *Dietzia* 16S rRNA gene clade, an association supported by the three tree-making algorithms and by a 100 % bootstrap value in the neighbour-joining analysis. The novel strain was most closely related to the type strain of *D. cinnamea*: the two organisms had a 16S rRNA gene sequence similarity of 98.3 %, a value that corresponds to 24

nucleotide differences at 1437 locations. Lower similarity values were recorded for the type strains of *D. kunjamenis* (95.6 %), *D. maris* (96.4 %), *D. natronolimnaea* (95.5 %) and *D. psychrocaliphila* (94.6 %). DNA–DNA relatedness studies were not carried out between these strains as the type strains of *D. kunjamenis* and *D. maris* share a high 16S rRNA gene sequence similarity value, but have a DNA–DNA relatedness value of only 59.2 % (Mayilraj *et al.*, 2006), a figure well below the 70 % guideline recommended for the delineation of bacterial species (Wayne *et al.*, 1987).

Strain N 1280^T was examined for key chemotaxonomic markers to establish if it had chemical properties that were characteristic of *Dietzia* strains. Standard procedures were used to determine the diagnostic isomers of diaminopimelic acid (A₂pm; Stanek & Roberts, 1974), fatty acids (Sutcliffe, 2000), isoprenoid quinones (Collins, 1994), muramic acid type (Uchida *et al.*, 1999), mycolic acids (Hamid *et al.*, 1993), polar lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Hasegawa *et al.*, 1983). The organism contained *meso*-A₂pm, arabinose and galactose in whole-organism hydrolysates (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), *N*-acetyl muramic acid, dihydrogenated menaquinones with eight isoprene units [MK-8(H₂)] as the predominant isoprenologue and a minor amount of MK-7(H₂), straight-chain saturated, unsaturated and tuberculostearic acids (fatty acid type 1b *sensu* Kroppenstedt, 1985) as major fatty acids, phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as major polar lipids, and mycolic acids that co-migrated with those from *D. maris* DSM 43672^T. This chemotaxonomic profile is consistent with classification of the novel strain in the genus *Dietzia* (Goodfellow & Maldonado, 2006).

The novel strain and *D. cinnamea* DSM 44904^T, *D. kunjamenis* DSM 44907^T, *D. maris* DSM 43672^T, *D.*

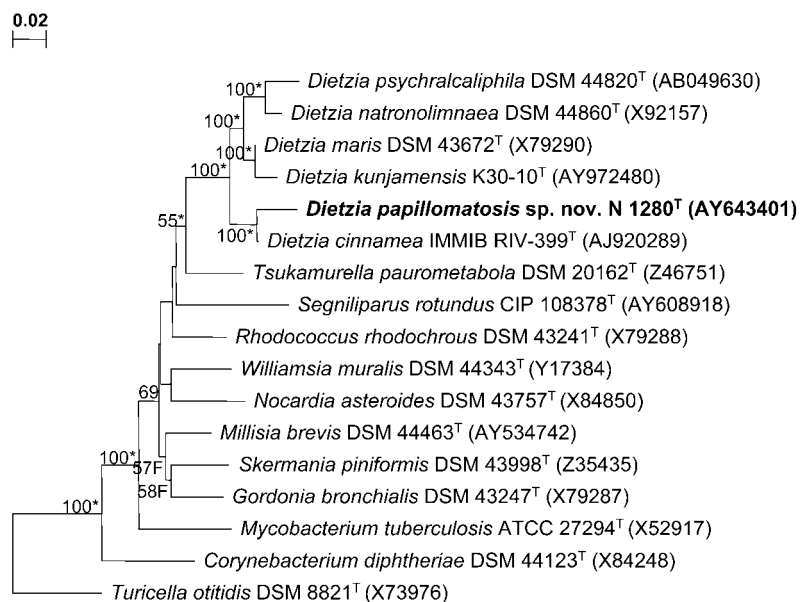


Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on a nearly complete 16S rRNA gene sequence of strain N 1280^T showing its position in the *Dietzia* clade. Asterisks indicate branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. F indicates branches that were also recovered using the least-squares method. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. Bar, 0.02 substitutions per nucleotide position.

natronolimnaea DSM 44806^T and *D. psychralcaliphila* DSM 44820^T were examined for a range of degradative properties using well-established procedures (Goodfellow, 1971; Isik *et al.*, 1999). Aesculin and arbutin hydrolysis were examined following Williams *et al.* (1983), allantoin hydrolysis was according to Gordon (1967), nitrate reduction was following Gordon & Mihm (1962) and urease production was as described by Rustigan & Stuart (1941). The oxidase reaction was performed on filter paper moistened with a 1% (w/v) aqueous solution of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine and catalase activity was demonstrated using 3% (v/v) hydrogen peroxide. Acid production from carbohydrates was carried out using media and methods described by Gordon *et al.* (1974) and utilization of sole carbon and sole carbon/nitrogen sources were determined after Stevenson (1967)

and Tsukamura (1966), respectively. Tolerance to pH, temperature and sodium chloride were established using GYEA plates that were incubated for up to 14 days. Resistance to lysozyme was determined after Gordon *et al.* (1974). It is evident from Table 1 that although the *Dietzia* strains have many properties in common, they can be distinguished from each other using a combination of phenotypic features.

The antibiotic sensitivity profile of strain N 1280^T was established by placing discs impregnated with antibiotics (Oxoid), six per plate, over GYEA plates and incubating them for 2 days at 30 °C prior to recording zones of inhibition around the discs. The colonial properties of the strain were examined on a modified Bennett's agar plate (Jones, 1949) after incubation for 5 days at 30 °C. Smears

Table 1. Phenotypic properties that distinguish strain N 1280^T and the type strains of *Dietzia* species

Strains: 1, N 1280^T; 2, *D. cinnamea* DSM 44904^T; 3, *D. kunjamensis* DSM 44907^T; 4, *D. maris* DSM 43672^T; 5, *D. natronolimnaea* DSM 44860^T; 6, *D. psychralcaliphila* DSM 44820^T. All data are from the present study. All strains hydrolysed allantoin, arbutin and urea, were catalase-positive, reduced nitrate, were resistant to lysozyme, degraded cellulose, starch, Tweens 40 and 60, and grew at 37 °C and pH 7 and 10. Adonitol, L-arabinose, arbutin, butane-1,3-diol, butane-1,4-diol, butane-1-ol, butane-2,3-diol, D-cellobiose, dextrin, ethanol, *meso*-erythritol, D-fructose, D- and L-fucose, D-galactose, D-gentiobiose, D-glucose, glycerol, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, D-melezitose, D-melibiose, propane-1,2-diol, propane-1,3-diol, D-raffinose, D-ribose, D-salicin, D-sorbitol, sucrose, D-tagatose, trehalose, turanose, D-xylose and D-xylitol were used as sole carbon sources by all strains [all at 1.0% (w/v) or 1.0% (v/v)]. Acetamide, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-leucine, L-isoleucine, DL-norleucine, L-norvaline, L-ornithine, DL-phenylalanine, L-proline, L-serine, L-thymidine, L-valine and urea were used as sole carbon and nitrogen sources. All strains were negative for aesculin hydrolysis, oxidase activity and degradation of casein, DNA, gelatin, hypoxanthine, pectin, RNA, xanthine and xylan. None of the strains utilized sodium adipate, sodium gluconate, sodium malonate, sodium oleate, sodium oxalate, sodium suberate or sodium succinate as sole carbon sources (all at 0.1%, w/v) or grew at pH 5.0. +, Positive; -, negative; w, weakly positive.

Characteristic	1	2	3	4	5	6
Colony colour	Orange	Orange	Coral red	Orange	Coral red	Coral red
Nitrite reduction	-	-	-	-	+	-
Degradation of:						
Chitin	+	-	+	+	+	+
Elastin	-	-	+	+	+	+
L-Tyrosine	+	-	-	+	-	+
Tributyrin	-	-	-	+	+	+
Uric acid	-	-	+	-	-	-
Acid production (aerobically) from:						
D-Fructose	+	+	-	+	+	+
D-Glucose	-	+	+	-	+	+
D-Mannose	-	+	-	-	+	-
D-Raffinose	-	-	-	w	w	w
Sucrose	-	+	+	+	+	+
Utilization as sole carbon and nitrogen sources:						
L-Arginine	-	+	+	-	-	+
L-Cysteine	+	+	+	+	+	-
Growth at:						
5 °C	-	-	-	-	-	+
10 °C	-	+	+	+	+	+
45 °C	-	-	+	+	+	+
Growth in the presence of:						
7% (w/v) NaCl	+	+	+	+	+	-
8% (w/v) NaCl	+	+	-	-	+	-

from this plate were prepared and Gram stained (Hucker's modification; Society for American Bacteriologists, 1957); they were also stained using a modification of the Ziehl-Neelson method (Gordon, 1967) to determine acid-alcohol-fastness. The isolate produced orange-pigmented colonies and was Gram-positive and non-acid-alcohol-fast.

The cells required for determination of cellular morphology of the organism were grown in shake flasks of GYE broth for 24, 48 and 72 h at 28 °C; cells were checked for purity at each of these times and harvested by centrifugation. The resultant preparations were fixed in 2% glutaraldehyde in Sorenson's phosphate buffer for 4 h proceeded by washing three times with 1 × phosphate buffer solution. Cell suspensions were inoculated on to separate coverslips coated with 0.025% poly-L-lysine, dehydrated in a graduated ethanol series (25–100%, v/v), critical-point-dried in CO₂, fixed on a specimen mount with Acheson Silver DAG, gold coated and examined using a Cambridge Stereoscan S40 scanning electron microscope. Strain N 1280^T exhibited a rod-coccus life cycle: younger cultures exhibited snapping division and V-forms (1.0–1.4 × 0.2–0.4 μm in size).

It can be concluded from the genotypic and phenotypic data that strain N 1280^T can be readily distinguished from the recognized *Dietzia* species and hence should be classified as a representative of a novel species in the genus *Dietzia*. The name proposed for this taxon is *Dietzia papillomatosis* sp. nov.

Description of *Dietzia papillomatosis* sp. nov.

Dietzia papillomatosis (pa.pil.lo.ma.to'sis. N.L. gen. n. *papillomatosis* of *papillomatosis*).

Aerobic, Gram-positive, non-motile, non-spore-forming, non-acid-alcohol-fast actinomycete that shows snapping division and V-forms and a rod-coccus life cycle. Circular, convex, shiny, orange-pigmented colonies are formed on modified Bennett's agar after growth for 5 days at 30 °C. Neither aerial hyphae nor diffusible pigments are formed. Degrades Tweens 20 and 80, but not adenine. Utilizes isoamyl alcohol as a sole carbon source for energy and growth (at 1%, v/v). Similarly, fumaric acid, *m*-hydroxybenzoic acid, DL-β-hydroxybutyric acid, sodium acetate, sodium benzoate, sodium *n*-butyrate, sodium propionate, sodium pyruvate and sodium DL-malate are used as sole carbon sources, but not 3,3-dimethylglutaric acid, sodium azelate, sodium citrate, sodium pimelate or sodium sebacate (all at 0.1%, w/v). Growth occurs in the presence of filter paper discs soaked in cephalixin (30 μg ml⁻¹), clindamycin hydrochloride (2 μg ml⁻¹), colistin (25 μg ml⁻¹), erythromycin (5 μg ml⁻¹), nalidixic acid (30 μg ml⁻¹), novobiocin (5 μg ml⁻¹) and tetracycline hydrochloride (10 μg ml⁻¹), but not in the presence of bacitracin (10 U), ciprofloxacin (5 μg ml⁻¹), cotrimoxazole (25 μg ml⁻¹), fusidic acid (10 μg ml⁻¹) or penicillin (1 μg ml⁻¹). Additional phenotypic properties are shown in Table 1. The cell-wall amino acid is *meso*-diaminopimelic acid and

the major cell-wall sugars are arabinose and galactose. The glycan moiety of the cell wall contains *N*-acetyl residues (*N*-acetylmuramic acid). Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely pentadecanoic acid (C15:0; 5.4%), hexadecanoic acid (C16:0; 21.1%), monounsaturated hexadecenoic acid (C16:1; 3.0%), septadecanoic acid (C17:0; 6.1%), monounsaturated septadecenoic acid (C17:1; 2.7%), monounsaturated octadecenoic acid (C18:1; 9.0%), tuberculostearic acid (22.1%), nonadecanoic acid (C19:0; 2.6%) and unidentified peaks with the retention times of 19.99 (10.6%), 21.61 (5.6%) and 21.88 (5.7%). The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. MK-8(H₂) is the major menaquinone and MK-7(H₂) is the minor one.

The type strain, N 1280^T (=DSM 44961^T=NCIMB 14145^T), was isolated from the skin of an immunocompetent patient with confluent and reticulated papillomatosis.

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