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 1280T (=DSM 44961T=NCIMB 14145T).

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 kunjamensis (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. kunjamensis 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 (Bemer-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 1280T 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.
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 1280T was deter-
determined by 16S rRNA gene sequence analysis. Isolation
of chromosomal DNA, PCR amplification and direct sequen-
cing 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 struc-
tural 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
(Fitch & Margoliash, 1967), neighbour-joining (Saitou &
Nei, 1987), maximum-likelihood (Felsenstein, 1981) and
organism sugars (Hasegawa et al., 1983). The organism
contained meso-A2pm, arabino and galactose in whole-
organism hydrolysates (wall chemotype IV sensu
Lechevalier & Lechevalier, 1970), N-acetyl muramic acid,
dihydrone- nated menaquinones with six isoprene units [MK-8(H2)]
as the predominant isoprenologue and a minor amount of
MK-7(H2), straight-chain saturated, unsaturated and tuber-
culosearic acids (fatty acid type 1b sensu
Kroppenstedt, 1985) as major fatty acids, phosphatidylethanolamine,
phosphatidylglycerol and diphosphatidylglycerol as major
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

It is apparent from Fig. 1 that strain N 1280T belongs to the
Dietzia 16S rRNA gene clade, an association supported by
the 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. cinnamnena: 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 strain of
D. kunjamensis
(95.6 %),
D. maris
(96.4 %),
D. natronolimnaea
(95.5 %) and
D. psychralcaliphila
(94.6 %). DNA–DNA relatedness
studies were not carried out between these strains as the
type strains of
D. kunjamensis
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 recom-
manded for the delineation of bacterial species (Wayne
et al., 1987).

Strain N 1280T 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; Staneck & 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

![Fig. 1.](image_url)

Fig. 1. Neighbour-joining tree (Saitou & Nei,
1987) based on a nearly complete 16S rRNA
gene sequence of strain N 1280T showing its
presence in the
Dietzia
clad. 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 nucleo-
tide position.
*natronolimnaea* DSM 44806<sup>T</sup> and *D. psychralcaliphila* DSM 44820<sup>T</sup> 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<sup>T</sup> 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>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
<th>Strain 5</th>
<th>Strain 6</th>
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</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Orange</td>
<td>Orange</td>
<td>Coral red</td>
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<td>Coral red</td>
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<tr>
<td>Nitrite reduction</td>
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<td>Degradation of:</td>
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<td>Chitin</td>
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<td>Elastin</td>
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<td>l-Tyrosine</td>
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<td>Tributyrin</td>
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<td>Uric acid</td>
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<td>D-Fructose</td>
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<td>D-Mannose</td>
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<td>D-Raffinose</td>
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<td>Sucrose</td>
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<td>l-Arginine</td>
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<td>Growth at:</td>
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<td>5 °C</td>
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<td>10 °C</td>
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<td>45 °C</td>
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<td>Growth in the presence of:</td>
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<td>7% (w/v) NaCl</td>
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<tr>
<td>8% (w/v) NaCl</td>
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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-Neelsen 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 1280T exhibited a rod–coccus life cycle: younger cultures exhibited snapping division and V-forms (1.0–0.2–0.4 μm in size).

It can be concluded from the genotypic and phenotypic data that strain N 1280T 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-sporo-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 isomyl 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 n-butyrate are used as sole carbon sources, but not 3,3-dimethylglutaric acid, sodium azelate, sodium citrate, sodium pinelate 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%), mono-unsaturated hexadecenoic acid (C16:1; 3.0%), septadecanoic acid (C17:0; 6.1%), mono-unsaturated septadecenoic acid (C17:1; 2.7%), mono-unsaturated 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 phosphatidyl-ethanolamine, phosphatidyglycerol and diphosphatidylglycerol. MK-8(H2) is the major menaquinone and MK-7(H2) is the minor one.

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

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