Dietzia cinnamea sp. nov., a novel species isolated from a perianal swab of a patient with a bone marrow transplant

A. F. Yassin,¹ H. Hupfer² and K. P. Schaal¹

Correspondence

A. F. Yassin

yassin@mibi03.meb.uni-bonn.de

¹Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, 53127 Bonn, Germany

²Kekulé-Institut für Organische Chemie und Biochemie der Universität Bonn, 53121 Bonn, Germany

The taxonomic status of a bacterium isolated from a perianal swab of a patient with a bone marrow transplant was characterized using a polyphasic taxonomic approach. Chemotaxonomic investigations revealed the presence of cell wall chemotype IV, short chain mycolic acids that co-migrated with those extracted from members of the genus *Dietzia*, and a dihydrogenated menaquinone with eight isoprene units as the predominant menaquinone. Generic assignment was confirmed by 16S rRNA gene sequencing. Comparative analysis of the 16S rRNA gene sequence showed that this isolate constitutes a distinct phyletic line within the genus *Dietzia*, displaying 97.5–98.7 % sequence similarity with *Dietzia* species with validly published names. The isolate could be distinguished from the type strain of *Dietzia maris* (1.6 % sequence divergence) and other species of the genus *Dietzia* by DNA–DNA hybridization, as well as by using a set of biochemical tests. Genotypic and phenotypic data show that the strain merits classification as a novel species of the genus *Dietzia* for which the name *Dietzia cinnamea* sp. nov. is proposed; the type strain is IMMIB RIV-399^T (= DSM 44904^T = CCUG 50875^T).

The genus *Dietzia* was first proposed by Rainey *et al.* (1995) to accommodate *Rhodococcus maris* (Nesterenko *et al.*, 1982). *Dietzia* strains are widely distributed in nature. *Dietzia maris* was originally isolated from halibut by Harrison (1929) and later from soil, skin and intestinal tracts of carp (Nesterenko *et al.*, 1982), deepest sea mud (Takami *et al.*, 1997) and sediments (Colquhoun *et al.*, 1998), and from a drain of a fish-product-processing plant (Yumoto *et al.*, 2002). *D. maris* has also been implicated in human diseases. Bemer-Melchior *et al.* (1999) were the first to report a case of bacteraemia due to *D. maris* infection associated with a catheter in an immunocompromised patient presenting with septic shock and pneumothorax. *D. maris* has also been isolated from a bone biopsy specimen from a hospitalized patient associated with a total hip prosthesis replacement (Pidoux *et al.*, 2001). In this paper, a bacterial strain, designated IMMIB RIV-399^T, isolated from a perianal swab of a human with a bone marrow transplant is described. Based on phylogenetic and phenotypic evidence, it is proposed that this strain should be classified as a representative of a novel species of the genus *Dietzia* for which the name *Dietzia cinnamea* sp. nov. is proposed.

Strains *D. maris* DSM 43672^T, *Dietzia natronolimnaea* DSM 44860^T and *Dietzia psychrhalcaliphila* DSM 44820^T were obtained from the DSMZ. All strains were cultured on Columbia agar supplemented with 5 % sheep blood agar and brain-heart infusion agar to determine their morphological characteristics. Pigment production was determined during growth at 37 °C for 7 days and observations were made at 24 h intervals. Air-dried smears at 24, 48 and 72 h intervals were Gram-stained to determine the Gram reaction and cell morphology. The Ziehl–Neelsen method as described by Kubica & Kent (1985) was used to determine acid-fastness. Growth temperature was determined by incubating cells at 27, 37 and 42 °C. The oxidase reaction was performed on filter paper moistened with a 1 % (w/v) aqueous solution of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine. Physiological properties of the strain were assessed using tests to determine hydrolysis of complex substrates as described previously (Gordon, 1966, 1967; Gordon & Mihm, 1957), as well as tests to determine carbon source utilization according to Yassin *et al.* (1995). The isomeric form of diamino-pimelic acid was determined by the methods of Becker *et al.* (1964) and whole-cell sugars were determined according to Lechevalier (1968). The acyl type of muramic acid was determined by the colorimetric method (Uchida & Aida, 1977). Lipids were extracted using acid methanolysis and mycolic acids were detected by TLC as described by

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB RIV-399^T is AJ920289.

Minnikin *et al.* (1980); pyrolysis GC of mycolates was performed according to Yassin *et al.* (1993a). Non-hydroxylated fatty acids were purified, identified and quantified by GC as described by Yassin (1988). Phospholipids were extracted, purified and identified as described previously (Yassin *et al.*, 1993b). Menaquinones were extracted and purified according to Collins *et al.* (1977). Analyses of the menaquinones were recorded in positive ion mode on a Q-TOF 2 MS (Micromass) equipped with a nanospray source. Analytes were dissolved in acetonitrile and injected into the MS by glass capillaries (long type; Protona) using a capillary voltage of 950 V and a source block temperature of 80 °C. Instrument calibration was done with a mixture of sodium iodide and caesium iodide dissolved in 50 % aqueous 2-propanol. Collision energy was 35–45 eV at 0.7 bar. For the compounds under study, the major ions observed with electrospray were protonated pseudo-molecular ions, $[M+Na]^+$. The identity of menaquinones was verified by observing the diagnostic ion at m/z 187, which represents the 2-methylnaphthoquinone core.

DNA was isolated as described previously (Yassin *et al.*, 2000). The isolated DNA was then purified by chromatography on hydroxyapatite using the method of Cashion *et al.* (1977). G + C contents were determined by HPLC (Mesbah *et al.*, 1989) using 8 phage as reference. DNA–DNA hybridization was determined spectrophotometrically from renaturation rates (De Ley *et al.*, 1970; Huß *et al.*, 1983). Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and the purification of PCR products were carried out using procedures described previously (Rainey *et al.*, 1996). Purified PCR products were sequenced using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) according to the manufacturer. An Applied Biosystems 310 DNA Genetic Analyser was used for electrophoresis of the sequence reaction products. The 16S rRNA gene sequences of *D. maris* DSM 43672^T, *D. natronolimnaea* DSM 44860^T and *D. psychrhalcaliphila* DSM 44820^T determined in this study, as well as those of some representatives of the mycolic-acid-containing taxa retrieved from GenBank, were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the respective tools of the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony (Kluge & Farris, 1969), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981). An evolutionary distance matrix was calculated using the corrections of Jukes & Cantor (1969). Topologies of the resultant trees were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining database on 500 resamplings using the ARB package.

The almost complete 16S rRNA gene sequences of strain IMMIB RIV-399^T [1486 nt; 96.3 % of the *Escherichia coli* sequence (Brosius *et al.*, 1978)], *D. maris* DSM 43672^T (1483 nt), *D. natronolimnaea* DSM 44860^T (1484 nt) and *D. psychrhalcaliphila* DSM 44820^T (1485 nt) were determined in this study; the latter three were identical to sequences of

the same strains available from public databases under accession numbers X79270, X92157 and AB159036, respectively, and so the database sequences of these species were used in the comparative analyses. 16S rRNA gene sequence comparison showed clearly that isolate IMMIB RIV-399^T is a member of the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997) and it contained all signature nucleotides expected for this suborder. Furthermore, in our alignment, strain IMMIB RIV-399^T contained 11 of the 14 signature nucleotides defined for this family, whereas *D. natronolimnaea* DSM 44860^T and *D. psychrhalcaliphila* DSM 44820^T contained 12 of the 14 signature nucleotides defined for the family *Dietziaceae*. The isolation of a fourth *Dietzia* species allows signature nucleotides to be highlighted: the pattern of the 16S rRNA gene signatures for the genus *Dietzia* in the family *Dietziaceae* Rainey *et al.* 1997 now consists of 11 signature nucleotide rather than 14 signatures (Stackebrandt *et al.*, 1997). These are nucleotides at positions 70–98 (U–A), 293–304 (G–U), 307 (U), 418–425 (U–A), 661–744 (A–U), 771–808 (A–U), 824–876 (C–G), 825–875 (G–C), 843 (C), 1049–1198 (U–A) and 1122–1151 (A–U). Strain IMMIB RIV-399^T, *D. natronolimnaea* DSM 44860^T and *D. psychrhalcaliphila* DSM 44820^T share the same nucleotides at position 508 (C). *D. natronolimnaea* DSM 44860^T and *D. psychrhalcaliphila* DSM 44820^T have the same nucleotides at positions 614–626 (C–G). Strain IMMIB RIV-399^T differs from other *Dietzia* species in that it possesses U rather than G at position 631.

The phylogenetic tree (Fig. 1) shows the position of strain IMMIB RIV-399^T within the radiation of representative phylogenetic groups of the suborder *Corynebacterineae*. The phylogenetic position of this organism, determined by the different tree algorithms (maximum-parsimony, neighbour-joining and maximum-likelihood), reveals that strain IMMIB RIV-399^T is closely associated with the genus *Dietzia* and represents a distinct subline within its members (Fig. 1). The determined sequence displays similarities of 97.5, 97.7 and 98.4 % to *D. natronolimnaea* DSM 44860^T, *D. psychrhalcaliphila* DSM 44820^T and *D. maris* DSM 43672^T, respectively. This result suggests that strain IMMIB RIV-399^T belongs to a genetically distinct *Dietzia* species that is closely related to *D. maris* (1.6 % sequence divergence). In view of the high levels of 16S rRNA gene sequence similarity between isolate IMMIB RIV-399^T and *D. natronolimnaea* DSM 44860^T, *D. psychrhalcaliphila* DSM 44820^T and *D. maris* DSM 43672^T, chromosomal DNA–DNA hybridization studies were performed to establish whether strain IMMIB RIV-399^T represents a distinct species. Strain IMMIB RIV-399^T displayed low levels of DNA–DNA reassociation with *D. natronolimnaea* DSM 44860^T (34.2 %), *D. psychrhalcaliphila* DSM 44820^T (35.7 %) and *D. maris* DSM 43672^T (40.3 %), results that are below the cut-off point recommended for the circumscription of bacterial genomic species by Wayne *et al.* (1987) and that confirm the separation of isolate IMMIB RIV-399^T from *D. natronolimnaea*, *D. psychrhalcaliphila* and *D. maris*.

Fig. 1. Neighbour-joining tree showing the position of strain IMMIB RIV-399^T (=DSM 44904^T=CCUG 50875^T) within the radiation of the mycolic acid-containing taxa. The tree was based on a comparison of sequences that were at least 90% complete (with regard to *E. coli* sequence). Bar, 10.0% sequence divergence.

Strain IMMIB RIV-399^T has morphological properties consistent with its assignment to the genus *Dietzia*. It is an aerobic organism that forms smooth, yellow-pigmented colonies on Columbia agar supplemented with 5% sheep blood. Cells are rod-shaped, exhibit snapping division, produce V-forms, stain Gram-positive and are not acid-fast. The physiological properties of strain IMMIB RIV-399^T are cited in the species description. Differences in some biochemical characteristics between strain IMMIB RIV-399^T and *D. maris* DSM 43672^T, *D. natronolimnaea* DSM 44860^T and *D. psychrhalcaliphila* DSM 44820^T, as determined in this study, are given in Table 1.

Chemotaxonomically, strain IMMIB RIV-399^T possesses chemical markers that support its assignment to the genus *Dietzia*. The cell wall contains *meso*-diaminopimelic acid as well as arabinose and galactose (i.e. wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). The muramic acid residues of the peptidoglycan are *N*-acetylated. One-dimensional TLC of whole-cell acid methanolysates of the organism revealed the presence of two lipid spots on the chromatogram. The lower one corresponded to mycolic acids, as identified by R_F value (0.48), and the higher spot corresponded to non-hydroxylated fatty acids. Pyrolysis GC of the purified mycolic acid methyl esters from strain IMMIB RIV-399^T released fatty acid methyl esters of C14:0, C15:0, C16:0, C17:0, C18:1 and C18:0 as pyrolysis cleavage products. GC analyses of the non-hydroxylated fatty acid methyl esters revealed the presence of tetradecanoate (0.8% total fatty acids), pentadecanoate (8.3%), *cis*-hexadecanoate (2.8%), hexadecanoate (28.9%), 10-methyl hexadecanoate (3.2%), heptadecanoate (5.3%), heptadecanoate (11.7%), 10-methyl heptadecanoate (11.1%), octadecanoate (4.8%), octadecanoate (2.3%) and tuberculostearic acid (10-methyl octadecanoate, 28.8%) as the major cellular fatty acid methyl esters. Polar lipid analysis showed that the organism contains phosphatidylethanolamine, phosphatidylglycerol

and diphosphatidylglycerol as the characteristic phospholipids (i.e. phospholipid type PII *sensu* Lechevalier *et al.*, 1977). MS analysis of the main component from strain IMMIB RIV-399^T shows a strong peak at m/z 741.55 attributable to $[M+Na]^+$ in the high mass region. This corresponds to a dihydrogenated menaquinone with eight isoprene units [MK-8(H₂)]. The second band shows a strong

Table 1. Differential characteristics of IMMIB RIV-399^T, *D. maris* DSM 43672^T, *D. natronolimnaea* DSM 44860^T and *D. psychrhalcaliphila* DSM 44820^T

Strains: 1, IMMIB RIV-399^T; 2, *D. maris* DSM 43672^T; 3, *D. natronolimnaea* DSM 44860^T; 4, *D. psychrhalcaliphila* DSM 44820^T. All strains utilized acetate, D-glucose, testosterone and urea. None of the strains utilized acetamide, adenine, adipic acid, adonitol, casein, cellobiose, iso-amylalcohol, L-alanine, L-arabinose, 2,3-butanediol, elastin, aesculin, D-galactose, gelatin, guanine, *m*-hydroxybenzoate, *p*-hydroxybenzoate, hypoxanthine, *myo*-inositol, lactate, lactose, melezitose, ornithine, proline, serine, D-sorbitol, raffinose, rhamnose, tyrosine, xanthine or D-xylose. w, Weakly utilized after 3 weeks incubation. The utilization of various substrates as sole sources of carbon and energy is shown.

Substrate	1	2	3	4
2,3-Butanediol	—	—	+	—
Citrate	—	+	+	w
<i>meso</i> -Erythritol	—	+	—	—
Gluconate	—	+	—	—
Maltose	+	—	—	—
Mannitol	—	+	—	—
Paraffin	—	+	+	+
1,2-Propanediol	+	—	w	w
Sucrose	—	—	+	—
Trehalose	—	—	+	—

peak at m/z 673·50, attributable to $[M+Na]^+$ in the high mass region. This corresponds to a dihydrogenated menaquinone with seven isoprene units [MK-7(H_2)]. The results of triplicate determinations gave a DNA G+C content for strain IMMIB RIV-399^T of 72.3 ± 0.4 mol%.

It is apparent from the genotypic and phenotypic data that strain IMMIB RIV-399^T represents a novel species of the genus *Dietzia*, for which the name *Dietzia cinnamea* is proposed.

Description of *Dietzia cinnamea* sp. nov.

Dietzia cinnamea (cin.na.me'a. L. fem. adj. *cinnamea* of/ from cinnamon referring to the colour of the cellular biomass).

Forms smooth, yellow-pigmented colonies on agar media. Cells are rod-shaped, exhibit snapping division and produce V-forms. Gram-positive and not acid-fast. Aerobic, catalase-positive, oxidase-negative. Grows at temperatures of 22–45 °C. Has the salient chemotaxonomic characteristics of the genus *Dietzia*. The glycan moiety of the cell walls contains *N*-acetyl residues (*N*-acetylmuramic acid). Cleavage of mycolic acids by pyrolysis releases fatty acids of C14:0, C15:0, C16:0, C17:0, C18:1 and C18:0 as the major products. The fatty acid profile mainly consists of straight-chain saturated, unsaturated and 10-methyl branched fatty acids. MK-8(H_2) is the major menaquinone component and MK-7(H_2) is the minor one. The phospholipid type is PII, with phosphatidylethanolamine as diagnostic phospholipid. Hydrolyses testosterone and urea, but not adenine, casein, elastin, aesculin, gelatin, guanine, hypoxanthine, tyrosine or xanthine. Assimilates acetate, D-glucose, maltose and 1,2-propanediol as carbon sources, but not adonitol, adipate, iso-amylalcohol, L-arabinose, 2,3-butanediol, cellobiose, citrate, *meso*-erythritol, D-galactose, gluconate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, *myo*-inositol, lactate, lactose, mannitol, melezitose, raffinose, rhamnose, D-sorbitol, sucrose, trehalose or D-xylose. Unable to utilize acetamide, L-alanine, arginine, gelatin, ornithine, proline or serine as simultaneous carbon and nitrogen sources.

The type strain of *Dietzia cinnamea*, IMMIB RIV-399^T (=DSM 44904^T=CCUG 50875^T), was isolated from a perianal swab of a patient with a bone marrow transplant.

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