17 The Family *Dietziaceae*

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Abstract
Members of *Dietziaceae*, actinomycetes characterized by the presence of mycolic acids, especially high molecular weight 3-hydroxy fatty acids substituted in the two positions with a long alkyl branch, have previously been classified in the suborder *Corynebacterinae*. The suborder *Corynebacterinae* was originally placed in the order *Actinomycetales*. According to Bergey's road map of the Actinobacteria, the six families *Corynebacteriaeceae*, *Tsukumarellaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Segniliparaceae*, and *Dietziaceae* were removed from the order *Actinomycetales* and assigned to the order *Corynebacterales*. The family *Dietziaceae* comprises solely the genus *Dietzia* which consists of 13 species with validly published names. The morphology and physiology of *Dietzia* species is similar to that of *Rhodococcus equi*, which in the past often led to misidentification of *Dietzia* strains by traditional identification techniques. Nowadays molecular based methods like 16S rRNA gene sequencing can be used to discriminate *Dietzia* strains from the type strain of *Rhodococcus equi*. This is of ecological significance, as members of *Dietzia* have been isolated from diverse environments including clinical specimens, which led to conclusion that *Dietzia* species may act as an opportunistic pathogen.

Taxonomy: Historical and Current

Short Description of the Family and the Genus *Dietzia*

Dietziaceae Rainey, Ward-Rainey and Stackebrandt 1997, 486[VP], emend. Zhi, Li and Stackebrandt 2009, 595[VP]

N.L. fem. n. *Dietzia*, type genus of the family; -aceae, ending to denote a family; N.L. fem. pl. n. *Dietziaceae*, the *Dietzia* family.

The family *Dietziaceae* was proposed by Rainey et al. (1997) in the course of the hierarchical classification system of the *Actinobacteria*. The family description was mainly based upon the phylogenetic position and the presence of defined 16S rRNA gene sequence signature oligonucleotides. Rainey and colleagues defined the following 16S rRNA signatures for members of the genus *Dietzia* (Rainey et al. 1995), the only genus within the family: positions 70–98 (U-A), 293–304 (G-U), 307 (U), 418–425 (U-A), 508 (U), 614–626 (U-G), 631 (G), 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). In 2009, an emended description of the family was published by

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_188,

Zhi et al. (2009) by extending the signature oligonucleotides considering all species of the genus *Dietzia* for which the names were validly published at that time. The pattern was specified for the following positions: 241 : 285 (U–G), 250 (U), 316 : 337 (C–G), 418 : 425 (U–A), 599 : 639 (C–G), 662 : 743 (C–G), 987 : 1218 (A–U), 1000 : 1040 (A–U), 1059 : 1198 (U–A), and 1115 : 1185 (C–G).

Type genus: *Dietzia*, Rainey et al. 1995, 33^{VP,} emend. Kämpfer et al. 2010, 394^{VP}

Diet'zi.a. M.L. dim. ending –ia,; M.L. fem. n. *Dietzia*, in honor of Alma Dietz, an American microbiologist.

Type species: *Dietzia maris* (Nesterenko et al. 1982; Rainey et al. 1995).

The taxon proposed by Rainey et al. 1995 was originally described as "*Flavobacterium maris*" (Harrison 1929) and later assigned to the genus *Rhodococcus* as *Rhodococcus maris* (Nesterenko et al. 1982). The type strain of the species has probably been isolated from soil and others strains from intestinal tract of carp (*Cyprinus carpi*).

Members of the genus *Dietzia* are aerobic, non-acid fast, non-spore-forming Gram-positive bacteria. They are mostly characterized by cocci that germinate into short rods or rod-shaped cells which may produce V-shaped forms and exhibit snapping division. Strains are chemoorganotrophic. Circular, convex yellow to orange or reddish to pink colonies are formed on agar media. The diagnostic amino acid of the A1γ type peptidoglycan is meso-diaminopimelic acid. The major cell wall sugars are arabinose and galactose. Short chain mycolic acids are present and have 34–40 carbon atoms. The long-chain cellular fatty acids are predominantly straight-chain saturated and monounsaturated fatty acids. Tuberculostearic acid is present. Polar lipids of most species consist of diphosphatidylglycerol (DPG), and/or phosphatidylglycerol (PG). A few species have phosphatidylethanolamine (PE) available and others are characterized by the presence of phosphatidylinositol (PI) or phosphatidylinositol mannoside (PIM).

The predominant dehydrogenated menaquinone with eight isoprene units is MK-8(H_2). Minor amounts of MK-7(H_2) or MK-9(H_2) were also detected in some of the *Dietzia* species. Phylogenetically, the genus is placed into the family *Dietziaceae*. According to Bergey's road map of the Actinobacteria, the six families *Corynebacteriaeceae*, *Tsukamurellaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Segniliparaceae*, and *Dietziaceae* were transferred from the order *Actinomycetales* to the new order *Corynebacterales* (Ludwig et al. 2012). The order *Actinomycetales* is now restricted to members of the family *Actinomycetaceae*. The DNA G+C content of *Dietziaceae* strains varies between 64 and 73 mol%. They have been isolated from various environmental habitats as well as from clinical specimen.

Phylogenetic Structure and Molecular Analyses

At present thirteen species with validly published names are included in the genus *Dietzia*. Representatives of the genus share 16S rRNA gene sequence similarity values of 99.8–98.0 % as compared to the type species *Dietzia maris*, with the exception of the type strains of the species *D. timorensis* and *D. papillomatosis*, which are distantly related to *D. maris* only, showing similarity values of 96.89 % and 96.52 %, respectively. As shown in Fig. 17.1, the three species *D. maris*, *D. schimae*,

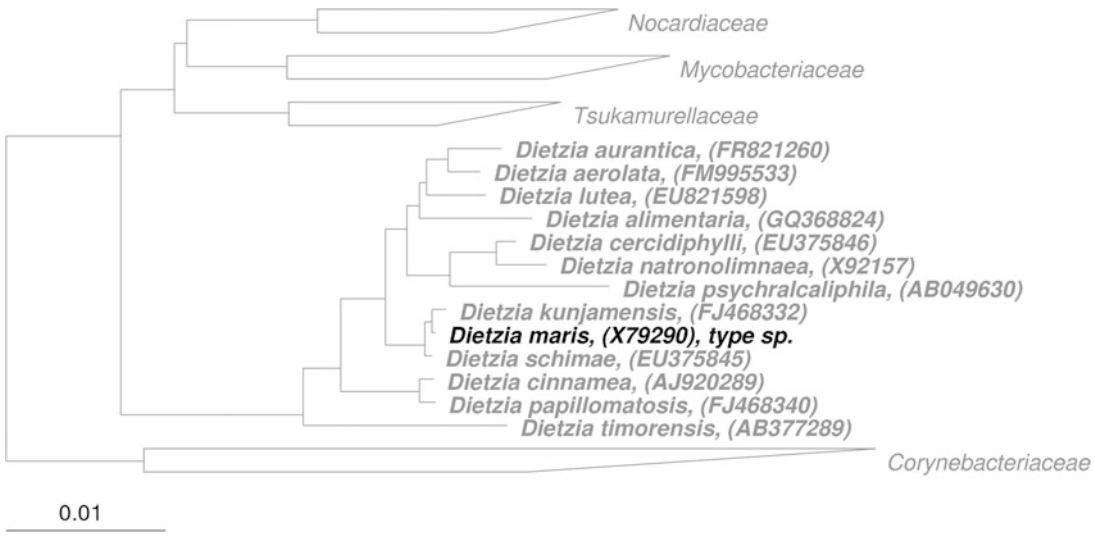

Fig. 17.1

Phylogenetic reconstruction of the family *Dietziaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. Scale bar indicates estimated sequence divergence

and *D. kunjamensis* are closely related. The 16S rRNA gene sequence of *D. schimae* differs only at three nucleotide positions as compared to that of *D. maris*. The sequence from the type strain of *D. kunjamensis* (AY972480) shows 14 additional nucleotides in the second part of the sequence and likely contains several sequencing errors. Analyses of two additional sequences, available at Genbank, confirmed the close relationship of *D. kunjamensis* to *D. maris* and *D. schimae*. However, *D. kunjamensis* could clearly be differentiated from *D. maris* and *D. schimae* by *gyr*B sequence analysis (Niwa et al. 2012). The *gyrB* protein sequence from *D. schimae* differs from *D. maris* at one position only, at which the amino acid alanine is replaced by valine. The genomic relatedness of *D. schimae* and the related phylogenetic neighbors *D. maris* and *D. kunjamensis* was determined by DNA:DNA reassociation studies (Li et al. 2008). DDH displayed low levels of DNA-DNA relatedness to both species (42.1 % and 44.0 %, respectively) and confirmed their separate species status. Further, a DNA-DNA relatedness value of 59.2 % between *D. kunjamensis* and *D. maris* was described by Mayilraj et al. (2006), as determined by the membrane filter method.

Figure 17.1 indicated also the close relationship of the two species *D. natronolimnaea* and *D. cercidiphylii*. Their 16S rRNA gene sequences are nearly identical (99.5 %), and the protein sequences of the DNA gyrase subunit B, as published by Niwa et al. 2012, also showed 100 % identity. However, DDH was determined according to a modified fluorometric micro-well method (He et al. 2005; Li et al. 2008) and led to detection of a low level relatedness (27.8 %) for *D. cercidiphylii* and *D. natronolimnaea*.

A third cluster is noticeable in *Fig. 17.1* which consists of *D. cinnamea* and *D. papillomatosis*. The 16S rRNA gene sequence of the type strain *D. papillomatosis* N1280 is accessible in Genbank as AY643401. The sequence shows several differences to *D. cinnamea*, especially behind the stretch >900 bp. The sequence of strain N1280 was reanalyzed in 2010 and submitted to Genbank again as FJ468340. This sequence showed significant differences to the original sequence (AY643401) and did not possess the various differences in the backmost part of the sequence and was found to be closely related to *D. cinnameae*. The close relationship was confirmed by *gyr* B sequence analysis (Niwa et al. 2012). The protein sequences of the DNA gyrase subunit B from both strains are nearly identical, but differ at one position, where isoleucine is replaced by valine. DDH studies have not been performed in order to confirm the separate species status of the type strains N1280 and IMMIB RIV-399.

Genome Analysis

Draft genome sequences from *Dietzia alimentaria* 72^{T} and *Dietzia cinnamea* strain P4 have been published in 2011 and 2012, respectively. *Dietzia alimentaria* strain 72 was originally derived from a traditional fermented Korean food called clam jeotgal. The genome of strain 72 has a G+C content of 67.34 % (Kim et al. 2011b). The genome sequence data are accessible via the SEED viewer (www.theseed.org, Overbeek et al. 2005). The 3,352,817 bp long genome includes 3,178 predicted protein-coding sequences, and 51 rRNA genes. The distribution of genes into subsystem categories shows that the highest numbers of genes are involved in carbohydrates (281), followed by genes coding for amino acid and derivatives (261), cofactors, vitamins, prosthetic groups, pigments (230) and fatty acids, lipids, isoprenoids (159). The whole-genome shotgun project has been deposited in GenBank under the accession number AGFF01000000.

The draft genome sequence of strain *Dietzia cinnamea* strain P4 has been deposited in GenBank under the accession number AEKG00000000. The 3,555,295 bp long genome contains 55 rRNA genes, including 50 tRNA genes. The G+C content is 70.96 %. In total, 3,593 genes were predicted, of which 3,538 were protein-coding genes: 62.82 % of the genes could be assigned to a putative function, and 72 % of these could be assigned to clusters of orthologous groups 2,587 protein coding genes with COGs are indicated in the Integrated Microbial Genomes platform (IMG, Markowitz et al. 2009). The highest number of genes is involved in amino acid transport and metabolism (220), followed by genes coding for lipid transport and metabolism (216), energy production and conversion (213), inorganic ion transport and metabolism (189). *Dietzia cinnamea* P4 derived from a study on hydrogen carbon degraders in tropical rainforest soil. A third draft genome sequence became available in 2013 (Diep et al. 2013). Strain *Dietzia* UCD-THP has originally been isolated from a residential toilet handle and shows the largest genome with 3,915,613 bp and a G+C content of 69.5 %. The whole-genome shotgun project has been deposited in GenBank under the accession number AOSR00000000: 3,614 protein-coding sequences and 50 non-coding RNAs were predicted within the RAST-Server-based annotation (Aziz et al. 2008).

Phenotypic Analysis

Phenotypic properties that distinguish *Dietzia* species from another are indicated in *Table 17.1*. Characteristics specific for the genus have been listed above.

Dietzia maris Rainey, Klatte, Kroppenstedt and Stackebrandt 1995, 33^{VP}; *Rhodococcus maris* Nesterenko, Nogina, Kasumova, Kvasnikov and Batrakov 1982, 11

mar'is. L. gen. n. *maris*, of the sea.

Gram-positive coccoid cells which germinate into short rods. Cells may exhibit snapping division and V-forms. Cells are 0.6–1.0 μm in diameter and 1.0–2.0 μm in length. Colonies grown on nutrient agar are raised, butyrous, glistening, and circular with an entire edge. Catalase activity is detectable.

Table 17.1

Phenotypic properties and other characteristics of the type strains of *Dietzia*

Characteristic	*Dietzia aerolata*	*Dietzia alimentaria*	*Dietzia aurantiaca*	*Dietzia cercidiphylii*	*Dietzia cinnamea*	*Dietzia kunjamensis*	*Dietzia lutea*	*Dietzia maris*	*Dietzia natronolimnaea*	*Dietzia papillomatosis*	*Dietzia psychralcaliphila*	*Dietzia schimae*	*Dietzia timorensis*
Colony color	Orange-yellow	Coral-red	Orange	Red-orange	Yellow-orange	Coral-red	Orange-yellow	Orange	Coral red	Orange	Coral red	Pink	Orange-red
Cell morphology	Coccoid	Rods	Coccoid	Rods, V-forms	Rods, V-forms	Coccoid, rods	Coccoid, rods, V-forms	Short rods, V-forms	Short rods, V-forms	Coccoid, rods, V-forms	Rods, snapping type division	Rods, V-forms	Coccoid, rods
Growth temperature (°C)	10–30	15–37	4–37	10–37	22–45	10–37	10–45	10–40	10–37	10–37	5–30	10–45	10–37
NaCl tolerance (%)	nd	0–10	nd	10	12	5	15	5–7	10	8	10	15	7
pH range	nd	7–10	5.5–12.5	6.0–9.0	nd	7–10	5.0–9.0	nd	6–10	nd	7–10	6–9	nd
Carbon source utilized:													
Adonitol	–	–	–	–	–	–	–	–	–	+	+	–	+
L-Arabinose	–	–	+	+	–	+	+	–	–	+	–	–	+
Cellobiose	–	–	+	–	–	+	–	–	+	+	+	+	+
D-Fructose	–	w	+	+	nd	–	+	+	–	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	nd	+	nd	+	–	+	–	–	+	–	–	+	+
Maltose	–	+	–	+	+	–	–	+	+	+	+	–	+
D-Mannose	+	+	–	+	nd	+	+	–	+	+	–	+	+
N-acetylglucosamine	–	+	+	–	nd	+	–	–	+	nd	–	–	+
Raffinose	–	–	nd	–	–	–	–	–	+	+	+	–	+
Sucrose	–	+	+	w	–	+	–	–	+	+	+	+	+
Trehalose	–	+	–	–	–	+	–	–	+	+	+	–	+
Predominant menaquinones	MK-8(H_2), MK-7(H_2)	MK-8(H_2)	MK-8(H_2), MK-7(H_2)	MK-8(H_2)	MK-8(H_2), MK-7(H_2)	MK-8(H_2)	MK-8(H_2)	MK-8(H_2)	MK-8(H_2)	MK-8(H_2), MK-7(H_2)	MK-8(H_2)	MK-8(H_2)	MK-8(H_2)
Major polar lipids	DPG, PG, PI, PIM	DPG, PG, PI, PIM	DPG, PG, PI	DPG, PG, PI, PIM	DPG, PG, PE	DPG, PG, PI	DPG, PG, PI, PIM, PE	DPG, PG, PE	DPG, PG, PE	DPG, PG, PE	nd	DPG, PG, PI	PG, PI (minor)
GC content (mol%)	nd	64.7	nd	72.6	72.3	67	70.5	73	66.1	nd	69.6	71.9	65.5

Strains: *D. aerolata* Sj14a^T (Data from Kämpfer et al. 2010); *D. alimentaria* 72^T (Kim et al. 2011); *D. aurantiaca* CCUG 35676^T (Kämpfer et al. 2012); *D. cercidiphylli* YIM 65002^T (Kim et al. 2011b); *D. cinnamea* IMMB RIV-399^T (Yassin et al. 2006); *D. kunjamensis* K30-10^T (Mayilraj et al. 2006); *D. lutea* YIM 80766^T (Li et al. 2009); *D. maris* IMV 195^T (Nesterenko et al. 1982; Rainey et al. 1995, Lie et al. 2008); *D. natronolimnaea* 15LN1^T (Duckworth et al. 1998; Kim et al. 2011b); *D. papillomatosis* N 1280^T (Jones et al. 2008); *D. psychralcaliphila* ILA-1^T (Yumoto et al. 2002; Koerner et al. 2009); *D. timorensis* ID05-A0528^T (Yamamura et al. 2010)

+, positive; –, negative; *nd* not determined. Some characteristics may differ from the original description of the strain, due to variation within the methods used for biochemical testing as published in other studies (see below)

Does not attack casein, cellulose, hypoxanthine, starch, tyrosine, and xanthine are not affected, but tween 80 is decomposed. Able to reduce nitrate. Acid is produced from glycerol, but not from galactose, inositol, mannitol, sorbitol, sorbose, and xylose. Additional properties are shown in Table 17.1. Utilizes butyrate, fumarate, and succinate in addition. Able to grow with C8 and C13 *n*-alkanes. Composition of whole cellular fatty acids is listed in Table 17.2. The type strain was originally deposited as strain IMV 195 = DSM 43672 = ATCC 35013.

Dietzia aerolata Kämpfer, Langer, Martin, Jäckel and Busse 2010, 395[VP]

ae.ro.la'ta. Gr. n. *aer* air, L. fem. part. adj. *lata* carried; N.L. fem. part adj. *aerolata*, airborne.

Gram-positive coccoid cells, 1.0–1.5 μm in diameter. Positive for catalase and oxidase activity. Phenotypic properties and other characteristics are summarized in Table 17.1. Menaquinone MK-9(H_2) is detectable in minor concentrations only (~2 %). Does not contain phosphatidylethanolamine within the polar lipid profile. The polyamine pattern consists of spermin and spermidine. Composition of whole cellular fatty acids is given in Table 17.2. The type is strain Sj14a = DSM 45334 = CCM 7659.

DNA:DNA hybridization experiments against the type strains of the species *D. schimae* DSM 45139, *D. cercidiphylii* DSM 45140, and *D. maris* DSM 43672 resulted in a relatedness value of 28, 19, and 26 %, respectively.

Dietzia alimentaria Kim, Roh, Choi, Jung, Nam, Kim, Park, Shin and Bae 2011, 2255[VP]

a.li.men.ta'ri.a. L. fem. adj. *alimentaria*, pertaining to food.

Gram-positive, nonmotile rods, 1.0–1.5 μm in length. Catalase activity positive, but oxidase negative. No growth occurred at 45 °C. Hydrolysis of Tween 20, 40, 60, and 80 is positive, but casein and starch are not hydrolyzed. Other enzyme activities with positive reactions (Api ZYM) are esterase (C4), alkaline phosphatase, esterase lipase, and naphthol-AS-BI-phosphohydrolase. Additional characteristics are indicated in Table 17.1. Also able to utilize the following substrates as tested in the API 50CH kit: methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, arbutin, aesculin, salicin, inulin, starch and glycogen. Assimilates adipic acid, malic acid, and trisodium citrate (API 20NE). Composition of whole cellular fatty acids is listed in Table 17.2. The type strain is 72^T = JCM 1630 = KACC 21126.

DNA-DNA hybridization experiments (microarray technique) showed low level relatedness to the type strains of *D. maris* JCM 6166 (17.8 %), *D. schimae* DSM 45139 (18.5 %), *D. psychralcaliphila* DSM 44820 (21.3 %), *D. kunjamensis* JCM 13325 (17.0 %), *D. cercidiphylii* DSM 45140 (26.7 %), *D. natronolimnaeae* JCM 11417 (9.6 %), and *D. cinnamea* JCM 13663 (21.9 %).

Dietzia aurantiaca Kämpfer, Falsen, Frischmann and Busse 2012, 486[VP]

au.ran.ti.a'ca N.L. fem. adj. *aurantiaca*, orange-colored

Gram-positive coccid cells up to 1.5 μm in diameter. Oxidase and catalase activity is positive. Colonies grown on TSA are circular, convex, and pigmented. Phenotypic properties are shown in Table 17.1. Utilizes ribose, acetate, azelate, fumarate, glutarate, hydroxybutyrate, DL-lactate, malate, and pyruvate in addition. Composition of whole cellular fatty acids is listed in Table 17.2. The quinone system also consists of MK-9(H_2) ~ 2 %. The type strain is CCUG 3576 = JCM 17645.

DNA:DNA reassociation experiments with the following type strains were performed: *D. aerolata* Sj14a (15 %), *D. schimae* DSM 45139 (26 %), *D. cercidiphylii* DSM 45140 (34 %), *D. maris* DSM 43672 (28 %).

Dietzia cercidiphylli Li, Zhao, Zhang, Klenk, Pukall, Qin, Xu and Li 2008, 2552[VP]

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

Gram-positive short rods. Colonies on TSA are circular, smooth, opaque, and reddish orange. Cells may exhibit snapping division and V-forms. Positive for catalase activity, oxidase negative. Hydrolyses Tween 20, 40, 80 and urea, but not gelatin or starch. H_2S production and nitrate reduction negative. Biochemical characteristics and other properties are given in Table 17.1. Utilizes the following substrates in addition: arbutin, D-lyxose, D-mannose, potassium 5-ketogluconate, D-tagatose, and turanose. The whole cellular fatty acid profile is listed in Table 17.2. The type strain is YIM 65002 = CCTCC AA 207016 = DSM 45140.

DNA:DNA hybridization experiments displayed the following values for reassociation to. *D. kunjamensis* K30-10 (59.6 %), *D. psychralcaliphila* ILA-1(42.7 %), *D. natronolimnaea* CBS 107.95 (27.8 %), and *D. maris* DSM 43672 (32.9 %).

Dietzia cinnamea Yassin, Hupfer and Schaal 2006, 644[VP]

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

Gram-positive, rod-shaped cells with snapping division and V-forms. Colonies on BHI agar are smooth, and yellow pigmented. Catalase activity present, oxidase activity absent. Hydrolyzes testosterone and urea, but does not attack casein, gelatin, xanthine, hypoxanthine, or tyrosine. Comparative properties of the strain are indicated in Table 17.1. Assimilates acetate, 1, 2 propanediol as carbon source in addition. The fatty acid profile is shown in Table 17.2. Phosphatidylethanolamine is the diagnostic polar lipid. Type strain is strain IMMIB RIV-399 = DSM 44904 = CCUG 50875.

Table 17.2

Comparative analysis of whole cellular fatty acid compositions (%) for the various species within the genus *Dietzia*

Fatty acids (%)	*Dietzia aerolata*	*Dietzia alimentaria*	*Dietzia aurantiaca*	*Dietzia cercidiphylii*	*Dietzia cinnamea*	*Dietzia kunjamensis*	*Dietzia lutea*	*Dietzia maris*	*Dietzia natronolimnaea*	*Dietzia papillomatosis*	*Dietzia psychralcaliphila*	*Dietzia schimae*	*Dietzia timorensis*
C14:0	0.5	–	0.9	1.2	0.8	0.5	1.0	0.8	1.0	–	0.8	2.9	–
C15:0	–	–	2.5	–	8.3 (4.6)	–	3.7	– (6)	–	5.4	–	–	–
C16:0	22.7	15.5	21.7	18.9	28.9 (22.9)	13.0 (14.4)	15.4	15.3 (33)	14.1	21.1	16.9 (25)	22.2	48.0
C17:0	14.0	10.8	25.9	–	11.7 (4.0)	8.8 (12.9)	22.4	13.2 (6)	–	6.1	13.8	–	–
C18:0	6.9	–	7.9	–	2.3	–	8.0	12.3	–	–	13.9	–	–
C19:0	8.4	9.1	4.4	–	–	–	2.6	–	–	2.6	9.6	–	–
C16:1ω6c/ω7C	5.6	15.1	9.8	18.7	2.8	12.9 (2.5)	6.7	10.6 (13)	33.0	3	10.1 (18)	25.8	–
C17:1ω7c	–	4.9	2.2	2.2	5.3	7.6	–	–	–	–	–	19.2	–
C17:1ω8c	5.0	–	–	3.7	– (11.3)	8.7	12.2	17.2	–	2.7	7.5	–	–
C17:1 ai-B/i-I	–	–	–	–	–	–	–	–	–	–	1.7	6.1	–
C18:1ω7c	–	–	–	3.5	–	–	1.7	–	4.5	–	– (25)	–	–
C18:1ω9c	19.7	7.4	17.2	27.9	4.8 (13.5)	27.3	10.2	13.9 (18)	15.7	9.0	–	–	39
C20:1ω9c	–	4.5	3.9	–	–	–	–	–	–	–	–	–	–
C20:4ω6,9,12,15c	1.4	–	1.9	–	–	–	–	–	–	–	11.7	–	–
10-methyl C16:0	–	–	0.5	1.1	3.2	–	–	0.6	0.7	–	0.3	0.8	–
10-methyl C17:0	0.7	–	1.8	0.4	11.1	1.5	2.9	5.3	0.8	–	0.6	2	–
10-methyl C18:0	13.2	14.6	18.8	17.9	28.8 (20.7)	19.8 (10.5)	7.8	10.3	30.2	22.1	9.7 (22)	13.4	8

Strains: *D. aerolata* Sj14a^T (Data from Kämpfer et al. 2010); *D. alimentaria* 72^T (Kim et al. 2011); *D. aurantiaca* CCUG 35676^T (Kämpfer et al. 2012); *D. cercidiphylli* YIM 65002^T (Li et al. 2008); *D. cinnamea* IMMB RIV-399^T (Yassin et al. 2006; Li et al. 2009); *D. kunjamensis* K30-10^T (Mayilraj et al. 2006; Li et al. 2009); *D. lutea* YIM 80766^T (Li et al. 2009); *D. maris* IMV 195^T (Rainey et al. 1995; Lie et al. 2009); *D. natronolimnaea* 15LN1^T (Li et al. 2009); *D. papillomatosis* N 1280^T (Jones et al. 2008); *D. psychralcaliphila* ILA-1^T (Yumoto et al. 2002; Li et al. 2009); *D. timorensis* ID05-A0528^T (Yamamura et al. 2010)

For some species, the values differ within the publications cited; so, differences are indicated in brackets

DNA:DNA hybridization studies revealed low levels of DNA relatedness to *D. natronolimnaea* DSM 44860 (34.2 %), *D. psychralcaliphila* DSM 44820 (35.7 %), and *D. maris* DSM 43672 (40.3 %).

Dietzia kunjamensis Mayilraj, Suresh, Kroppenstedt and Saini 2006, 1670[VP]

kun.ja.men'sis. N.L. fem. adj. *kunjamensis* pertaining to Kunjam Pass of the cold dessert of the Indian Himalayas.

Gram-positive, aerobic, nonmotile cells, which are coccoid or rod like. Cells are 1.0–1.2 in diameter and 1.1–2.0 μm in length. Colonies on TSA are small, smooth, glistening, and convex. Optimal growth temperature is 25 °C. Oxidase activity present, positive for nitrate reduction. Does not hydrolyze gelatin or urea. Acid is produced from mannitol. Phenotypic characteristics and other properties are given in Table 17.1. The following substrates can be utilized as carbon source as tested with the Biolog GP2 microplate system in addition: dextrin L-fucose, gentobiose, maltotriose, turanose, D-xylose, hydroxybutyric acid, L-asparagine, L-glutamic acid, glycerol, thymidine, and D-fructose-6-phosphate. The type strain is strain K30-10 = MTCC 7007 = DSM 44907.

DNA:DNA hybridization (membrane filter method) was performed against *D. maris* MTCC 7011, and the DNA relatedness was determined as 59.2 %.

Dietzia lutea Li, Chen, Zhao, Klenk, Pukall, Zhang, Tang, and Li 2009, 122[VP]

lu.te'a. L. fem. adj. *lutea*, orange-yellow colored.

Gram-positive, aerobic nonmotile cells which are coccoid or short rods (1.0–1.2 by 1.1–2.4 μm). Cells exhibit snapping division and V-forms. Colonies on TSA are circular, smooth, opaque, and convex. Catalase activity is positive, but negative for oxidase. Hydrolysis of Tween 20, 40, 80 is positive. API ZYM testing showed positive enzyme reactions for alkaline phosphatase, α-galactosidase, β-glucuronidase, and α-glucosidase. Utilizes the following substrates from the API 50CH kit: D-arabinose, aesculin, galactose, glycerol, inositol, D-lyxose, D-mannitol, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, L-rhamnose, D-ribose, L-sorbose, D- and L-xylose. Additional characteristics are shown in Table 17.1. The major fatty acids are given in Table 17.2. The type strain is strain YIM 80766 = KCTC 19232 = DSM 45074 = CCTCC AA 207008.

DNA:DNA hybridization experiments (fluorometric micro-well method) showed low level DNA relatedness to *D. maris* DSM 43672 (49.4 %), *D. kunjamensis* K30-10 (44.8 %), *D. natronolimnaea* CBS 107.95 (21.1 %), *D. psychralcaliphila* ILA-1 (30.2 %), *D. schimae* YIM 65001 (57.6 %), and *D. cercidiphylii* YIM 65002 (39.6 %).

Dietzia natronolimnaea Duckworth, Grant, Grant, Jones and Meijer 1998, 365[VP]; corrig. Duckworth et al. 1999

na.tro.no.lim.na'e.a. N.L. n. *natron* (arbitrarily derived from the Arabic n. natrun or natron) soda, sodium carbonate; N.L. fem. adj. *natronolimnaea*, of or from a soda lake (marsh).

Gram-positive, nonmotile rods (0.9–1.1 μm by 1.2–2.3 μm). Catalase activity is detectable, but oxidase activity not. Rod-shaped cells may exhibit snapping division and V-forms. Growth occurs in BHI medium and also on alkaline agar media. Colonies are circular, convex, glistening with entire margin. Optimal pH for growth is pH 9.0. Under neutral conditions, the following substrates are utilized within the API ATB 32N panel: acetate, fumarate, glutamate, succinate, mannitol, propionate, suberate, valerate, hydroxybutyrate, citrate, glycogen, L-serine, L-proline, L-asparagine, L-arginine, methionine, phenylalanine, L-glycine, and L-valine. Under alkaline conditions, fumarate, D-fructose, D-lactose, and D-xylose are not utilized. The following enzyme activities are detectable with the Api Zym kit: alkaline phosphatase, esterase (C4), esterase/lipase (C8), leucine arylamidase, cysteine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase. Additional characteristics are given in Table 17.1 including data from Li et al. 2009, which derived from API 50CH testing. Positive reactions also occurred for amygdalin, glycerol, D-melezitose, D-melibiose, and methyl α-D-glucopyranoside. Whole cellular fatty acid profile is shown in Table 17.2. The type strain is strain 15LN1 = CBS 107.95.

DNA:DNA reassociation experiment against *D. maris* was performed with the membrane filter method using [35S] dCTP labeled DNA and revealed a low DNA relatedness of 8 %.

Dietzia papillomatosis Jones, Koerner, Natarajan, Perry and Goodfellow 2008, 71[VP]

pa.pil.lo.ma.to'sis. N.L. gen. n. *papillomatosis*, of papillamatosis.

Gram-positive, aerobic nonmotile rods or coccoid cells that show snapping division and V-forms. Colonies on modified Bennett's agar are convex, shiny, and pigmented. Tween 20, 40, and 80 are attacked, but not adenine. Degrades chitin and L-tyrosine. Utilizes isoamylalcohol as sole carbon source. Biochemical and other characteristics are listed in Table 17.1. Fumaric acid, hydroxyl benzoic acid, β-hydroxybutyric acid, sodium acetate, sodium benzoate, sodium *n*-butyrate, sodium propionate, sodium pyruvate, and sodium DL-malate are also used as carbon sources. Whole cellular fatty acid composition is given in Table 17.2. Growth is inhibited in the presence of bacitracin (10U), ciprofloxacin (5 μg/mL), cotrimoxazole (25 μg/mL), fusidic acid (10 μg/mL), and penicillin (1 μg/mL).

The type strain is strain N 1280 = DSM 44961 = NCIMB 14145.

Dietzia psychralcaliphila Yumoto, Nakamura, Iwata, Kojima, Kusumoto, Nodasaka and Matsuyama 2002, 89[VP]

psy.chral.ca.li.phil'a. Gr. adj. *psychros* cold; N.L. *alcali* alkali, from Arabic *alqali* potash soil; Gr. adj. *philos* friendly to; N.L. fem. adj. *psychralcaliphila* loving cold, alkaline environments.

Gram-positive, nonmotile rods. Cells are 0.8–1.0 μm in diameter and 1.0–2.2 μm in length. Cells may exhibit snapping division. Colonies are circular, glistening, and convex. Catalase and oxidase activity is present. Negative for indole or H_2S production, and hydrolysis of urea. Tween 20, 40, 60, and 80 can be hydrolyzed, but not casein, gelatin, or starch. Utilizes propionate, valerate, hydroxybutyric acid, pyruvate, acetate, *n*-butyrate, isobutyrate, ethanol, *n*-tridecane, *n*-pentadecane, *n*-hexadecane, *n*-eicosane, *n*-tetracosane, and pristine in addition to the substrates listed in ❯ *Table 17.1*. Composition of cellular fatty acid is given in ❯ *Table 17.2*. The type strain is strain ILA-1 = JCM 10987 = IAM 14896 = NCIMB 13777.

The level of DNA-DNA relatedness to *D. maris* and *D. natronolimnaea* was determined as 38.4 % and 49.7 %, respectively.

Dietzia schimae Li, Zhao, Zhang, Klenk, Pukall, Qin, Xu and Li 2008, 2552[VP]

schi'ma.e. N.L. gen. N. *schimae* of the plant genus *Schima*, isolated from stem of *Schima* sp.

Gram-positive, aerobic, and nonmotile rod-shaped cells which exhibit snapping division and V-forms. Catalase activity present, but negative for oxidase. Negative for hydrolysis of gelatin, urea, and starch, but able to hydrolyze Tween 20, 40, and 80. Positive for nitrate reduction. Utilizes aesculin, glycerol, in addition to the substrates listed in ❯ *Table 17.1*. The fatty acid composition of the type strain is shown in ❯ *Table 17.2*. The type strain is strain YIM 65001 = CCTCC AA 207015 = DSM 45139.

The type strain displayed low levels of DNA-DNA relatedness to *D. maris* DSM 43672 (42.1 %), *D. cercidiphylii* YIM 65002 (43.2 %), *D. kunjamensis* K30-10 (44 %), *D. natronolimnaea* CBS 107.95 (53.3 %), *D. psychralcaliphila* ILA-1 (51.1 %).

Dietzia timorensis Yamamura, Lisdiyanti, Ridwan, Ratnakomala, Sarawati, Lestari, Triana, Kartina, Widyastuti and Ando 2010, 452[VP]

ti.mo.ren'sis. N.L. fem. adj. *timorensis* pertaining to West Timor, Indonesia, from where the organism was first isolated.

Gram-positive, aerobic, nonmotile coccoid to rod-shaped cells. Colonies are circular, convex, and glistening. Aesculin is hydrolyzed; arbutin and urea are not hydrolyzed. Adenine, casein, elastin, hypoxanthine, testosterone, tyrosine, uric acid, and xanthine are not attacked. Utilizes the following substrates within the Api 50 CH-Kit: aesculin, glycerol, amygdalin, L-arabitol, D-arabitol, arbutin, dulcitol, erythrol, L-fucose, D-fucose, D-galactose, gentobiose, glycogen, inositol, inulin, D-lyxose, D-mannitol, melezitose, melibiose, methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, xylitol, L-xylose, and X-xylose. Additional characteristics are indicated in ❯ *Table 17.1*. Whole cellular fatty acid profile is summarized in ❯ *Table 17.2*.

Isolation, Enrichment, and Maintenance Procedures

Isolation and Enrichment

Dietzia maris IMV 195^T (Rainey et al. 1995), classified by Nesterenko et al. 1982 as *Rhodococcus maris*, and originally known as *Flavobacterium maris*, was isolated from soil. Additional strains have also been isolated from skin and intestinal tracts of carp (*Cyprinus carpio*). Growth occurs on nutrient agar, trypticase soy broth agar, or ISP2 medium (Shirling and Gottlieb 1966) incubated at 28 °C.

Dietzia natronolimnaea 15LN1^T (Duckworth et al. 1998) was isolated from littoral sediment of the East African soda lake (Lake Oloidien; little lake Naivasha), which is a moderately saline and alkaline soda lake with a pH of. 8.5. Strain 15LN1 was enriched in alkaline broth which contained the following compounds (g per liter): glucose 10.0, peptone 5.0, yeast extract 5.0, KH_2PO4 1.0, $MgSO_4 \times 7\ H_2O$ 0.2, NaCl 40.0, and Na_2CO_3 10.0. The strain grows also well on brain-heart-infusion agar (pH 9.0) incubated at 30 °C.

Dietzia psychralcaliphila ILA-1^T (Yumoto et al. 2002) was isolated from a drain of a fish egg processing plant. The strain represents a cold-adapted alkaline bacterium that utilizes petroleum hydrocarbons and was isolated on AT-medium that consisted of (g/L): KNO_3 5.0, KH_2PO_4 0.5, $MgSO_4 \times 7H_20$ 0.5, $FeSO_4 \times 7H_20$ 0.01, $CaCl_2 \times 2H_2O$ 0.02, $MnSO_4 \times n\ H_2O$ 0.001, $ZnSO_4 \times 7H_2O$ 0.0005, and agar 15.0 in 1 L 100 nM $NaHCO_3/Na_2CO_3$ buffer, supplemented with vaporized *n* -tetradecane as sole carbon source. Strain ILA-1 was isolated from AT medium after 1 month of aerobic incubation at 4 °C. The strain is also able to grow on R agar (pH 9.0) at 27 °C.

Dietzia cinnamea IMMB RIV-399^T (Yassin et al. 2006) was isolated from a perianal swab of a patient with a bone marrow transplant. Strain IMMB RIV-399 is able to grow on Columbia blood agar (5 % sheep blood), GPHF medium or brain-heart-infusion agar, incubated at 37 °C under aerobic conditions. GPHF medium (g/L): glucose 10.0, peptone from Casein 5.0, yeast extract (Oxoid) 5.0, beef extract (Oxoid) 5.0, $CaCl_2 \times 2H_2O$ 0.74, agar (Oxoid) 15.0; distilled water 1,000 mL, pH 7.2.

Dietzia kunjamensis K30-10^T (Mayilraj et al. 2006) was isolated from cold dessert soil, 45 cm below an ice glacier at 4,200 m at Kunjam Pass, Himachal Pradesh, India. Strain K30-10 was enriched on tryptic soy agar medium (TSA) incubated at 25 °C.

Dietzia papillomatosis N 1280^T (Jones et al. 2008) was isolated on glucose-yeast extract agar from skin scrapings of a patient suffering from confluent and reticulated papillomatosis. The strain is also able to grow on ISP2 agar or modified Bennett's agar after 5 days of incubation at 30 °C.

Dietzia cercidiphylli YIM 65002^T and *Dietzia schimae* YIM 65001^T (Li et al. 2008) were isolated from surface sterilized roots of *C. japanicum* and surface sterilized stem of *Schima* sp., respectively. Both strains were maintained on trypticase soy agar medium (TSA) at 28 °C.

Dietzia lutea YIM 80766^T (Li et al. 2009) was isolated from a soil sample collected from the Eastern dessert of Egypt. The soil sample was diluted in sterile water and after vigorous shaking for 30 min, an aliquot of the sample was spread-plated onto Horikoshi agar. Plates were incubated at 28 °C for 2 weeks. Strain YIM 80766 is also able to grow on tryptic soy agar medium (TSA).

Dietzia aerolata Sj14a^T (Kämpfer et al. 2010) was isolated on tryptone soy agar (TSA) at 26 °C from the air collected in a duck barn. Good growth occurs also on R2A agar and nutrient agar.

Dietzia timorensis ID05-A0528^T (Yamamura et al. 2010) was isolated from a soil sample collected under mahogany trees (*Swietenia mahogany*) in West Timor, Indonesia. After pretreatment with SDS yeast extract, the strain was enriched on Humic acid vitamin agar, but the strain is also able to grow on modified Bennett's agar incubated at 28 °C for 14 days or on trypticase soy broth agar.

Dietzia alimentaria 72^T (Kim et al. 2011a) was isolated on marine agar from a salt-fermented seafood sample, which was made by fermented clams mixed with rock salt. Growth occurs also on tryptic soy agar, incubated up to 5 days at 30 °C.

Dietzia aurantiaca CCUG 35676^T (Kämpfer et al. 2012) was isolated on blood agar from a cerebrospinal fluid sample from a 24-year-old woman in Gothenborg, Sweden. The strain grows also on tryptone soy agar, nutrient agar, or R2A agar.

Maintenance

Standard procedures can be applied for members of the genus *Dietzia*. Serial transfer of subcultures grown on appropriate media (every 6–8 weeks) is possible. Strains can also be achieved in glycerol stocks (50 % (v/v) stored at −20 °C or for better survivability at −80 °C). For long-term storage, freeze drying or storage in liquid nitrogen should be used. Detailed protocols are given in the Cabri guidelines, accessible at www.cabri.org.

Ecology and Pathogenicity

Strains from the genus *Dietzia* have been isolated from various environmental habitats around the world, but increasing numbers of isolates were also obtained from clinical specimen. *Dietzia maris* strains or its DNA were often detectable in soil and sediment, including petroleum- or oil-contaminated habitats (sequence accession numbers KC189154, KC514120, JF727664, EF619406; Al-Awadhi et al. 2012), were found in seawater, associated with red algae, the dinoflagellate *Pyrodinium bahamense*, soft corals or fishes, in spring water or activated sludge (HQ425656, EF469496, JF792051, Azanza et al. 2006, Ruckmani and Chakrabarti 2011, Sun et al. 2012). A few strains have been isolated from clean room environments in Brazil (FJ876398), from Phoenix associated spacecraft surfaces (USA) and also from the assembly building in Kourou (Ghosh et al. 2010; Moissl-Eichinger et al. 2012). One strain was isolated from snow and floor debris of internal surfaces from the Moon-1 Rover (JX571065). Further strains have been isolated from clinical material like blood (DQ386308, DQ286854), throat, or thoracic fluid (Niwa et al. 2012), bone biopsy (Pidoux et al. 2001) as well as from a patient with bacteremia (Dinakaran et al. 2012).

The type strain of *D. cinnamea* was isolated from a perianal swab sample of a patient with bone marrow transplant (Yassin et al. 2006), Together with *D. maris* and *D. papillomatosis*, the species *D. cinnamea* is in individual cases identified as or suspected to be an opportunistic pathogen. *D. cinnamea* was also isolated from a dog bite wound in an adult patient (Hirvonen et al. 2012). Additional strains of the species are described as hydrogen carbon degrader (von der Weid et al. 2007) or found to be associated with *Phaseolus vulgaris* (sequence accession no. HM355703) the common bean, with biofouling material (JF514328) or on fresco surface (KC429622). The type strain of *D. papillomatosis* was originally isolated from skin of an immunocompetent patient with papillomatosis (Jones et al. 2008). *D. papillomatosis* was for the first time detected in a case of infection in a 2-year-old boy with known syringomyelia (Rammer et al. 2013). Another strain affiliated to *D. papillomatosis* was originally isolated from oat bran and found to be able to produce folate (Herranen et al. 2010).

Pilares et al. 2010 have reanalyzed a set of clinical strains, which were originally identified as *Rhodococcus equi* or *Rhodococcus* like by traditional techniques using the API Coryne identification kit. Reanalysis was done by 16S rRNA sequence analysis. The study revealed that seven of the strains could be assigned to the genus *Dietzia*. Four strains were identified as *D. maris*, two strains as *D. natronolimnaea*, and one strain as *D. timorensis*. A similar study was published by Niwa et al. in 2012, reanalyzing 16 strains previously identified as *Rhodococcus equi*. Also this study showed that the strains have previously been misidentified by biochemical testing and could be assigned to the genus *Dietzia*, based on 16S rRNA – and gyrB gene sequence analysis. Most of the strains sequenced were members of the *D. cercidiphylii*/*D. natronolimnaea* cluster and five isolates were found to be related to the *D. maris*/*D. schimae* cluster. Additional reports showing that *D. cercidiphylii* strains have been isolated from humans are not available at present. The type strain of *D. natronolimnaea* has been isolated from an East African Soda Lake (Naivasha) (Duckworth et al. 1998). Additional strains have been isolated from alkaline groundwater (Tiago et al. 2004), sediment of Lonar Lake in India (Joshi et al. 2008), from waste water of a chemical plant in China (Jin et al. 2012), from soil in Japan (Ueda et al. 2001), and from reed periphyton (Rusznyák et al. 2008).

Table 17.3

16S rRNA gene sequences available in Genbank, which can be assigned to the genus *Dietzia*

Strain/DNA isolated from	Accession number	Reference
Oral cavity	GU430732	Dewhirst et al. 2010
Air sample, China	GU933571	Unpublished
Altitude wetland, Argentina	AM882683	Unpublished
Fresh water, South Korea	JQ687118	Unpublished
Arsenic ground water sediment	JX961606	Unpublished
Deep Sea sediment, China	HM222663	Unpublished
Sub-seafloor sediment	AB094465	Inagaki et al. 2003
Marine sediment	DQ344847	Biddle et al. 2005
Mangrove sediment, Thailand	AB818673	Unpublished
Permafrost ice	AB272789	Katayama et al. 2007
Sponge, South East India	DQ001306	Anand et al. 2006
Red algae	EU278344	Unpublished
Oil-pollution	DQ521380	Unpublished
Oil production, water	KC209818	Unpublished
Petroleum-contaminated soil	HM449701	Unpublished
Diesel fuel in saline environments	AY918101	Kleinsteuber et al. 2006
Solid waste from oil-shale industry	EF540468	Unpublished
Ciliate Collinia, endoparasite	EU090135	Unpublished
Swine effluent impacted environment	DQ337506	Unpublished
Bovine dung	GQ246709	Unpublished
Limestone quarries, India	FJ911544	Unpublished
Peritoneal fluid, clinical	FJ468338	Niwa et al. 2012
Blood, clinical	FJ468337	Niwa et al. 2012
Endodontic infection	AF481211	Munson et al. 2002
Horned beetle, larvae	AB266603	Takeishi et al. 2006
Plant root	JN120941	Kim et al. 2012
Smear ripened cheese	AJ969176	Unpublished
Uncultured clones:		
Waste water	AY438789	McGarvey et al. 2004
Waste, steel plant	EU151500	Freitas et al. 2008
Showerhead, swab sample	EU631293	Feazel et al. 2009
Soil	JF411345	Unpublished
Soil, phenol degrader	JN039338	Unpublished
Soil, petroleum contaminated	JN038211	Unpublished
Sediment	JQ178130	Rotaru et al. 2012
Volcanic deposits, Japan	AB366289	Lu et al. 2008
Crude oil	JN882176	Gong et al. 2012
Gypsum-treated oil sands Tailing Pond	HQ035378	Ramos-Padrón et al. 2011
Stink bug midgut	JQ927512	Zucchi et al. 2012
Rat gastrointestinal microbiota	DQ856787	Dalby et al. 2006
Vaginal microbiota	JF480085	Unpublished
Raw cow milk	EU029309	Raats et al. 2011
Cow teat, skin	JN834337	Verdier-Metz et al. 2012
Bioaerosol, hog lagoon	JQ478541	Unpublished

For *D. schimae* and *D. timorensis*, DNA sequences were only detectable from environmental samples (JQ282810, JQ409503, JX429817, JX429818 and HE578791). The same is true for *D. psychralcaliphia* strains, which have been isolated from deep sea sediment (Chen and Shao 2009), petroleum-contaminated soil (Mathe et al. 2012), or from canine dental plaque (Elliott et al. 2006).

Several sequences are presently available in GenBank which have not been assigned to one of the *Dietzia* species or which derived from not yet cultivable strains; these are summarized in *Table 17.3*. Many of these sequences are indicated as unpublished, but may be the data set entry was not updated from the authors.

Application

As shown above, strains of the genus *Dietzia* have mainly been isolated from various environmental habitats. They are common in soil and marine sediment and some of the strains are able to degrade hydrocarbons, which may play an important role for the bioremediation of hydrocarbon-contaminated environmental sites. *Dietzia cinnamea* P4 was isolated from tropical rainforest soil and is able to degrade hydrocarbons. In 2012, its whole genome sequence was published by Procópio et al. (2012) which offers the possibility to study the genetic background for degradation of *n*-alkanes in more detail.

As indicated in *Table 17.1*, all type strains of the genus *Dietzia* are able to produce pigments. The carotenoid canthaxanthin is responsible for the reddish color of the *Dietzia natronolimnaeae* colonies. Strain *D. natronolimnaea* HS-1 has extensively been used for optimization of canthaxanthin production in the past (Khodaiyan et al. 2007).

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