Dietzia kunjamentsis sp. nov., isolated from the Indian Himalayas

S. Mayilraj,¹ K. Suresh,¹ R. M. Kroppenstedt² and H. S. Saini³

Correspondence

S. Mayilraj

mayil@imtech.res.in

¹Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Sector 39-A, Chandigarh, 160 036, India

²DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1B, 38124 Braunschweig, Germany

³Department of Microbiology, Guru Nanak Dev University (GNDU), Amritsar, 143 005, India

A coral-red-pigmented actinobacterium, strain K30-10^T, was isolated from a soil sample from a cold desert of the Indian Himalayas. Chemical and phenotypic properties of strain K30-10^T were consistent with its classification in the genus *Dietzia*. It showed 97.9% 16S rRNA gene sequence similarity to *Dietzia maris* MTCC 7011^T; similarities to the type strains of three other species of the genus, *Dietzia natronolimnaea*, *Dietzia psychrhalcaliphila* and *Dietzia cinnamea*, were 94.4–96.0%. The DNA–DNA relatedness between K30-10^T and the closely related strain *D. maris* MTCC 7011^T was 59.2%. The DNA G + C content of strain K30-10^T was 67.0 mol%. Based on physiological and biochemical tests and genotypic differences between strain K30-10^T and its closest phylogenetic relatives, it is proposed that this strain represents a novel species, *Dietzia kunjamentsis* sp. nov.; the type strain is K30-10^T (= MTCC 7007^T = DSM 44907^T = JCM 13325^T).

The genus *Dietzia* was proposed to accommodate organisms previously classified as *Rhodococcus maris* (Rainey *et al.*, 1995). Members of the genus *Dietzia* are distributed in a variety of habitats like marine, soil and hospital environments (Rainey *et al.*, 1995; Colquhoun *et al.*, 1998; Duckworth *et al.*, 1998; Yassin *et al.*, 2006). At the time of writing, the genus comprises four species, namely *Dietzia maris* (Rainey *et al.*, 1995), *Dietzia natronolimnaea* (Duckworth *et al.*, 1998), *Dietzia psychrhalcaliphila* (Yumoto *et al.*, 2002) and *Dietzia cinnamea* (Yassin *et al.*, 2006).

Strain K30-10^T was isolated from a soil sample collected from Kunjam Pass, a cold desert of the Indian Himalayas. After primary isolation on tryptic soya agar medium (TSA; HiMedia), the strain was maintained as glycerol stocks at –70 °C. The type strains *D. maris* MTCC 7011^T (= DSM 43672^T), *D. natronolimnaea* MTCC 7014^T (= DSM 44860^T), *D. psychrhalcaliphila* MTCC 7010^T (= DSM 44820^T) and *D. cinnamea* MTCC 7506^T (= DSM 44904^T) were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Morphological properties, motility, Gram staining, acid-fast staining and endospore staining were studied according to standard protocols (Murray *et al.*, 1994). Gram reaction was determined using the HiMedia Gram Staining kit according

to the manufacturer's instructions. Physiological tests like growth at different temperatures, pH and NaCl concentrations (under neutral pH) were examined by growing the strain on basal tryptic soy broth medium (TSB). Utilization of various substrates as sole carbon sources was tested using Biolog GP2 MicroPlates in accordance to the manufacturer's instructions, except that TSA was used instead of Biolog Universal Growth agar medium. The inoculated plates were incubated for 24 h and the results were read with a MicroPlate Reader using Microlog 4.2 software. Catalase and urea hydrolysis were determined as described by Cowan & Steel (1965). Hydrolysis of casein, gelatin, Tween 80 and starch, and the oxidase reaction (using *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride) were determined as described by Smibert & Krieg (1994). Nitrate reduction was determined as described by Lanyi (1987). Acid production from various sugars (at 0.5%) was tested on minimal medium (MgSO₄·7H₂O, 0.5 g; NaCl, 0.5 g; FeSO₄·7H₂O, 0.01 g; K₂HPO₄, 1.0 g; yeast extract, 0.2 g; distilled water, 1 l; 0.2% solution bromocresol blue used as indicator). Cell-wall sugars and diagnostic amino acids were determined as described by Stanek & Roberts (1974). Phospholipids and menaquinones were extracted and analysed as described by Minnikin *et al.* (1984). The presence of mycolic acids was demonstrated by TLC (Minnikin & Goodfellow, 1976) and the mycolic acid pattern was determined according to Klatte *et al.* (1994). The glycolic acid content of the bacterial cell wall was determined as described by Uchida & Aida (1984). For cellular fatty acid analysis, all strains were grown on TSA at 30 °C for

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain K30-10^T is AY972480.

Table 1. Phenotypic properties, mycolic acid chain length and G+C content of K30-10^T and type strains of *Dietzia* species

Strains: 1, strain K30-10^T; 2, *D. psychrhalcaliphila* MTCC 7010^T; 3, *D. maris* MTCC 7011^T; 4, *D. natronolimnaea* MTCC 7014^T; 5, *D. cinnamea* MTCC 7506^T. All data are from the present study. All were negative for: endospore production; H₂S production; indole; methyl red and Voges-Proskauer tests; hydrolysis of casein, DNA and starch; growth at pH 5; acid production from glucose, lactose, rhamnose and mannose; and utilization of α - and β -cyclodextrin, glycogen, inulin, mannan, *N*-acetyl-D-glucosamine, *N*-acetyl- β -D-mannosamine, amygdalin, D-arabitol, arbutin, D-cellobiose, D-galactose, D-galacturonic acid, D-gluconic acid, *myo*-inositol, α -D-lactose, lactulose, maltose, D-mannitol, D-melezitose, D-melibiose, methyl α -D-galactoside, methyl β -D-galactoside, methyl-D-glucose, methyl β -D-glucoside, methyl α -D-mannoside, D-raffinose, L-rhamnose, salicin, sedoheptulosan, D-sorbitol, stachyose, sucrose, xylitol, α -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α -ketoglutaric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D- and L-malic acid, succinamic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, D- and L-alanine, L-alanyl glycine, glycyl L-glutamic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, adenosine, 2'-deoxyadenosine, inosine, uridine, AMP, TMP, UMP, citrate and DL- α -glycerol phosphate. All were positive for: catalase; growth at pH 7–10; growth in 5% NaCl; and utilization of Tweens 40 and 80, D-fructose, α -D-glucose, D-mannose, palatinose, D-psicose, D-ribose, D-trehalose, acetic acid, β -hydroxybutyric acid, α -ketovaleric acid, propionic acid and pyruvic acid as carbon sources. +, Positive; –, negative; W, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5
Colony colour*	CR	CR	O	CR	O
Oxidase reaction	+	+	–	–	W
Nitrate reduction	+	–	+	–	+
Gelatin hydrolysis	–	W	W	–	+
Urea hydrolysis	–	–	–	–	+
Growth in NaCl at neutral pH:					
7 %	W	+	+	+	+
8 and 10 %	–	+	–	+	+
12 %	–	–	–	–	W
Growth at 5 °C	–	+	–	–	–
Growth at 42 °C	–	–	+	+	+
Acid production (aerobically):					
Arabinose	–	+	+	–	–
Cellobiose	–	+	–	–	+
Fructose	–	–	–	–	+
Galactose	–	–	–	–	+
Maltose	–	+	+	+	+
Mannitol	+	–	–	–	+
Raffinose	–	+	+	–	–
Salicin	–	–	–	–	+
Sorbitol	–	–	–	–	+
Sucrose	+	+	–	+	–
Xylose	–	+	–	–	+
Utilization as sole carbon source:					
Dextrin	+	+	–	+	–
L-Arabinose	+	+	+	–	+
L-Fucose	+	–	–	–	+
Gentiobiose	+	–	–	+	–
Maltotriose	+	–	–	–	+
Methyl α -D-glucoside	–	–	–	+	+
Sucrose	–	+	+	+	–
Succinic acid	–	–	–	–	+
D-Tagatose	–	–	–	+	+
Turanose	+	+	–	+	+
D-Trehalose	+	+	+	+	–
D-Xylose	+	–	+	–	+

Table 1. cont.

Characteristic	1	2	3	4	5
γ -Hydroxybutyric acid	+	—	+	—	—
Pyruvic acid methyl ester	—	+	—	—	—
Succinic acid monomethyl ester	+	—	+	+	+
L-Asparagine	+	—	—	—	+
L-Glutamic acid	+	—	—	—	+
L-Serine	—	—	+	—	—
Glycerol	+	—	+	—	—
Thymidine	+	—	—	—	—
D-Fructose 6-phosphate	+	—	+	+	+
α -D-Glucose 1-phosphate	—	—	—	—	+
D-Glucose 6-phosphate	—	—	—	—	+
Mycolic acid chain length	33–40	34–39	33–38	34–38	ND
DNA G + C content (mol%)	67.2	69.6	70.4	66.1	ND

*CR, Coral-red; O, orange.

36 h and fatty acid methyl ester analysis was performed with the Sherlock Microbial Identification System (MIDI) as described previously (Pandey *et al.*, 2002).

Genomic DNA extraction, amplification and 16S rRNA gene sequencing were performed as described previously (Mayilraj *et al.*, 2005). DNA–DNA hybridization was performed each time with freshly isolated genomic DNA and was repeated three times by the membrane filter method (Tourova & Antonov, 1987). The genomic DNA G + C content was determined spectrophotometrically (Lambda

35; Perkin Elmer) using the thermal denaturation method (Mandel & Marmur, 1968). Phylogenetic analysis was carried out as described previously (Mayilraj *et al.*, 2005).

Chemical and phenotypic properties of strain K30-10^T were consistent with its classification in the genus *Dietzia*. Detailed characteristics are given in Table 1 and the species description. The fatty acid compositions of strain K30-10^T and the type strains of the four species of the genus *Dietzia* are shown in Table 2. The fatty acid methyl ester profile matches qualitatively with that of other species of the genus *Dietzia*. However, strain K30-10^T contained a significant amount of 17:1 *cis*-10 (*cis*-10-heptadecenoic acid, 7.41%), which was absent from the other species. The 16S rRNA gene sequence of strain K30-10^T generated in this work (1455 bases) was aligned with sequences of other species of the genus *Dietzia* retrieved from GenBank. Phylogenetic

Table 2. Fatty acid compositions of strain K30-10^T and type strains of *Dietzia* species

Strains: 1, strain K30-10^T; 2, *D. psychrocaliphila* MTCC 7010^T; 3, *D. maris* MTCC 7011^T; 4, *D. natronolimnaea* MTCC 7014^T; 5, *D. cinnamea* MTCC 7506^T. Values are percentages of total fatty acids; all data are from the present study.

Fatty acid	1	2	3	4	5
14:0	0.36	0.44	0.81	0.66	0.65
15:1 <i>cis</i> -10	—	—	0.24	—	0.46
15:0	2.88	1.63	14.53	0.79	4.66
16:1 <i>cis</i> -9	1.50	5.38	1.92	5.37	2.35
16:1 <i>cis</i> -10	6.00	3.78	5.53	8.29	12.17
16:0	14.43	25.65	17.04	28.50	22.97
10-Methyl 16:0	—	—	—	—	0.56
17:1 <i>cis</i> -9	8.85	9.26	25.46	3.49	11.43
17:1 <i>cis</i> -10	7.41	—	—	—	—
17:0	12.95	4.78	8.09	2.10	3.96
10-Methyl 17:0	0.99	—	4.23	—	5.23
18:1 <i>cis</i> -9	30.39	35.55	15.95	31.06	13.49
18:0	0.84	—	0.52	0.75	0.32
10-Methyl 18:0	10.51	13.52	4.63	18.26	20.65
19:1 <i>cis</i> -9	2.59	—	0.87	0.73	1.10

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences (1455 bases) showing the phylogenetic relationship between strain *Dietzia kunjamensis* sp. nov. K30-10^T and other species of the genus *Dietzia*. *Arthrobacter globiformis* DSM 20124^T was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are given at the nodes. Bar, 1% sequence variation.

analysis based on 16S rRNA gene sequences revealed that strain K30-10^T formed a separate branch within the lineage including *D. maris* (Fig. 1). Highest sequence similarity (97.9%) was found with *D. maris* DSM 43672^T, whereas sequences from other species of the genus *Dietzia* were 94.4–96.0% similar. DNA–DNA hybridization was performed in triplicate under optimal conditions to determine the genetic relatedness between K30-10^T and *D. maris* MTCC 7011^T. The DNA–DNA relatedness value between these two strains was 59.2% (mean of 62.4, 56.7 and 58.6%), which is well below the 70% cut-off value recommended for the delineation of bacterial species (Wayne *et al.*, 1987). The DNA–DNA relatedness between strain K30-10^T and strains of other *Dietzia* species was not determined, since it has been shown that organisms with more than 3% 16S rRNA gene sequence dissimilarity belong to different genomic species (Stackebrandt & Goebel, 1994). On the basis of genotypic and phenotypic data, strain K30-10^T is very clearly distinguishable from species most closely related to it. Therefore, strain K30-10^T represents a novel species of the genus *Dietzia*, for which the name *Dietzia kunjamenensis* sp. nov. is proposed.

Description of *Dietzia kunjamenensis* sp. nov.

Dietzia kunjamenensis (kun.ja.men'sis. N.L. fem. adj. *kunjamensis* pertaining to Kunjam Pass of the cold desert of the Indian Himalayas).

Cells are aerobic, Gram-positive, acid-fast, non-motile and non-endospore-forming. They are rod- and coccoid-like (1.0–1.2 × 1.1–2.0 µm) and occur in groups. Colonies on TSA are coral-red-pigmented, small, smooth, glistening and convex. Catalase is produced. Negative for H₂S production, indole, methyl red and Voges–Proskauer tests and hydrolysis of casein, DNA and starch. Tolerates up to 5% NaCl and grows at temperatures between 20 and 37 °C, with an optimum growth temperature of 25 °C. Grows at pH 7–10; optimum growth at pH 8.0. Other phenotypic characteristics and utilization of compounds as sole carbon sources are given in Table 1. The cell-wall amino acid is meso-diaminopimelic acid and the major cell-wall sugars are arabinose and galactose. The acyl type of the glycan chain of peptidoglycan is acetyl. Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated fatty acids, C_{16:0} (14.43%), C_{16:1} 10c (6.00%), C_{17:0} (12.95%), C_{17:1} 10c (7.41%), C_{18:1} 9c (30.39%) and 10-methyl C_{18:0} (tuberculostearic acid, 10.51%). Short-chain mycolic acids are present (33–40 carbon atoms). The polar lipids present are phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol. Major menaquinone is MK-8(H₂). The DNA G+C content of the type strain is 67.0 mol%.

The type strain is K30-10^T (=MTCC 7007^T=DSM 44907^T=JCM 13325^T), isolated from soil, 45 cm below an ice glacier, at 4200 m above sea level, Kunjam Pass, Himachal Pradesh, India.

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References

- Colquhoun, J. A., Heald, S. C., Li, L., Tamaoka, J., Kato, C., Horikoshi, K. & Bull, A. T. (1998). Taxonomy and biotransformation activities of some deep-sea actinomycetes. *Extremophiles* **2**, 269–277.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Duckworth, A. W., Grant, S., Grant, W. D., Jones, B. E. & Meijer, D. (1998). *Dietzia natronolimnaios* sp. nov., a new member of the genus *Dietzia* isolated from an East African soda lake. *Extremophiles* **2**, 359–366.
- Klatte, S., Rainey, F. A. & Kroppenstedt, R. M. (1994). Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordona* as *Gordona aichiensis* comb. nov. and *Gordona amarae* comb. nov. *Int J Syst Bacteriol* **44**, 769–773.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance–temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.
- Mayilraj, S., Prasad, G. S., Suresh, K., Saini, H. S., Shivaji, S. & Chakrabarti, T. (2005). *Planococcus stackebrandtii* sp. nov., isolated from a cold desert of the Himalayas, India. *Int J Syst Evol Microbiol* **55**, 91–94.
- Minnikin, D. E. & Goodfellow, M. (1976). Lipid composition in the classification and identification of *Nocardia* and related taxa. In *The Biology of the Nocardaceae*, pp. 160–219. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Murray, R. G. E., Raymond, N., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Pandey, K. K., Mayilraj, S. & Chakrabarti, T. (2002). *Pseudomonas indica* sp. nov., a novel butane-utilizing species. *Int J Syst Evol Microbiol* **52**, 1559–1567.
- Rainey, F. A., Klatte, S., Kroppenstedt, R. M. & Stackebrandt, E. (1995). *Dietzia*, a new genus including *Dietzia maris* comb. nov., formerly *Rhodococcus maris*. *Int J Syst Bacteriol* **45**, 32–36.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the

present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Tourova, T. P. & Antonov, A. S. (1987). Identification of microorganisms by rapid DNA-DNA hybridization. *Methods Microbiol* **19**, 333–355.

Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Yassin, A. F., Hupfer, H. & Schaal, K. P. (2006). *Dietzia cinnamea* sp. nov., a novel species isolated from a perianal swab of a patient with a bone marrow transplant. *Int J Syst Evol Microbiol* **56**, 641–645.

Yumoto, I., Nakamura, A., Iwata, H., Kojima, K., Kusumoto, K., Nodasaka, Y. & Matsuyama, H. (2002). *Dietzia psychrocaliphila* sp. nov., a novel, facultatively psychrophilic alkaliphile that grows on hydrocarbons. *Int J Syst Evol Microbiol* **52**, 85–90.