Dietzia aurantiaca sp. nov., isolated from a human clinical specimen

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A Gram-positive, coccoid, non-endospore-forming actinobacterium (strain CCUG 35676^T) was isolated from cerebrospinal fluid from a 24-year-old woman in Gothenborg, Sweden. Based on pairwise 16S rRNA gene sequence similarity studies, strain CCUG 35676^T was shown to belong to the genus *Dietzia* and was most closely related to the type strains of *Dietzia aerolata* (99.3 %), *Dietzia lutea* (98.8 %), *Dietzia schimae* (98.5 %), *Dietzia maris* (98.5 %), *Dietzia alimentaria* (98.3 %) and *Dietzia cercidiphylli* (98.0 %). The major menaquinone was MK-8(H₂). Major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, an unidentified aminophospholipid (APL1), an unidentified phospholipid (PL1) and unidentified glycolipids (GL1 and GL3). Numerous other lipids were also detected. The fatty acid profile, comprising C_{16:0}, C_{17:0}, C_{18:1ω9c} and 10-methyl-C_{18:0} as major fatty acids, supported the affiliation of strain CCUG 35676^T to the genus *Dietzia*. On the basis of the results of physiological and biochemical tests and DNA–DNA hybridizations, a clear phenotypic and genotypic differentiation of strain CCUG 35676^T from the most closely related *Dietzia* species is possible. Strain CCUG 35676^T represents a novel species, for which the name *Dietzia aurantiaca* sp. nov. is proposed, with CCUG 35676^T (=JCM 17645^T) as the type strain.

At the time of writing, the genus *Dietzia* comprised 12 species with validly published names: *Dietzia maris* (Rainey *et al.*, 1995), the type species; *Dietzia natronolimnaea* (Duckworth *et al.*, 1998); *Dietzia psychrhaliphila* (Yumoto *et al.*, 2002); *Dietzia kunjamensis* (Mayilraj *et al.*, 2006); *Dietzia cinnamea* (Yassin *et al.*, 2006); *Dietzia schimae* and *Dietzia cercidiphylli* (Li *et al.*, 2008); *Dietzia papillomatosis* (Jones *et al.*, 2008); *Dietzia lutea* (Li *et al.*, 2009); *Dietzia timorensis* (Yamamura *et al.*, 2010); *Dietzia aerolata* (Kämpfer *et al.*, 2010); and *Dietzia alimentaria* (Kim *et al.*, 2011). Members of these species have been isolated from quite different sources, including various environmental and clinical materials (Duckworth *et al.*, 1998; Yumoto *et al.*, 2002; Yassin *et al.*, 2006; Mayilraj *et al.*, 2006; Kämpfer *et al.*, 2010).

An orange-pigmented strain, CCUG 35676^T, was isolated on blood agar (Oxoid) at 37 °C from cerebrospinal fluid from a 24-year-old woman in Gothenborg, Sweden. The strain showed a *Dietzia*-like colony morphology. CCUG

35676^T was further cultivated on tryptone soy agar (TSA) at 28 °C. Cellular morphological properties and Gram-reaction were observed by phase-contrast microscopy as described previously (Kämpfer & Kroppenstedt, 2004).

The 16S rRNA gene sequence of strain CCUG 35676^T (a continuous stretch of 1390 bp) was studied as described previously (Kämpfer & Kroppenstedt, 2004). Sequence alignment and analysis of the data were performed using the ARB software package (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURF 100 database (release August 2009; Pruesse *et al.*, 2007). Genetic distances based on pairwise comparisons were calculated with the ARB program and clustering was performed with the neighbour-joining method (Fig. 1) and with the maximum-likelihood method with fastDNAmI (Olsen *et al.*, 1994) without filters (Supplementary Fig. S1, available in IJSEM Online). In both trees, strain CCUG 35676^T clustered with *D. aerolata* Sjl4a^T.

Sequence similarity calculations on the basis of a pairwise comparison indicated that the closest relatives of strain CCUG 35676^T were the type strains of *D. aerolata* (99.3 %), *D. lutea* (98.8 %), *D. schimae* (98.5 %), *D. maris* (98.5 %), *D. alimentaria* (98.3 %) and *D. cercidiphylli* (98.0 %).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCUG 35676^T is FR821260.

A supplementary figure is available with the online version of this paper.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from GenBank/EMBL (accession numbers are given in parentheses). The phylogenetic tree was constructed using the ARB software package (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURF 100 database (release August 2009; Pruesse *et al.*, 2007). Tree building was performed using the neighbour-joining method. Bootstraps were calculated with 1000 replications; only values >50% are shown. The sequence of *Tsukamurella paurometabola* ATCC 8368^T was used as an outgroup. Bar, 0.1 nt substitutions per nucleotide position.

Strains of other *Dietzia* species revealed 16S rRNA gene sequence similarities <98.0%. Similar values were obtained for strain CCUG 35676^T and the aforementioned species by using the pairwise similarity calculations based on the algorithm of the EzTaxon program (Chun *et al.*, 2007), with variation of 0.1% at the most. The type strain of *D. psychrocaliphila*, which showed only 97.0% sequence similarity to strain CCUG 35676^T on the basis of pairwise comparisons, showed 98.2% similarity with the algorithm of the EzTaxon program.

For quinone and polar lipid analyses, cells were grown in PYE medium (0.3% peptone from casein, 0.3% yeast extract, pH 7.2). Quinones and polar lipids were extracted and analysed as described previously (Tindall, 1990a, b; Altenburger *et al.*, 1996). Quinones were analysed by HPLC as described by Stolz *et al.* (2007). Total lipids were detected with molybdatophosphoric acid and further characterized by staining with ninhydrin (specific for free amino groups), molybdenum blue (specific for phosphate groups) and α -naphthol (specific for sugars). The quinone system of CCUG 35676^T was composed of menaquinones MK-8(H₂) (86%), MK-7(H₂) (12%) and MK-9(H₂) (2%). A quinone system with dihydrogenated menaquinone MK-8(H₂) as the predominant was given in the original genus description (Rainey *et al.*, 1995) and this trait has also been reported for other *Dietzia* species (Jones *et al.*, 2008; Kämpfer *et al.*, 2010; Yamamura *et al.*, 2010). Major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol,

an unidentified aminophospholipid (APL1), an unidentified phospholipid (PL1) and unidentified glycolipids (GL1 and GL3; the latter exhibits an orange colour indicating a pigment that contributes to pigmentation of strain CCUG 35676^T) (Fig. 2). Furthermore, moderate to minor amounts of phosphatidylinositol mannoside, a third glycolipid and 11 unidentified lipids that could not be stained with any of the specific spray reagents applied were detected. This polar lipid profile contained several components that were also reported to be present in the most related species, *D. aerolata* (Kämpfer *et al.*, 2010), e.g. phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, one phosphatidylinositol mannoside, glycolipid GL1 (corresponding to GL2 of *D. aerolata*) and lipid L2 (corresponding to L2 of *D. aerolata*). However, these two profiles were not identical. Both contained additional lipids that were not found in the profile of the other strain. These variations in the polar lipid profile indicate that strain CCUG 35676^T should not be assigned to the species *D. aerolata*.

Fatty acid extraction and analysis were done as described previously (Kämpfer & Kroppenstedt, 1996). MIDI software version 6.0 was used for peak identification. The fatty acid profile of strain CCUG 35676^T was very similar to the profiles already described for closely related *Dietzia* species, but some minor differences could be observed (Table 1). However, the typical major fatty acids, i.e. C_{16:0}, C_{17:0}, C_{18:1 ω 9c} and 10-methyl-C_{18:0}, could also be detected in strain CCUG 35676^T.

Fig. 2. Polar lipid profile of CCUG 35676^T displayed after two-dimensional TLC and detection with molybdatophosphoric acid. Directions of the first and second dimensions are indicated in the bottom left-hand corner. PG, Phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; L1–L11, unknown polar lipids; GL1, 2, 3, unknown glycolipids; PL1, unidentified phospholipid; APL1, unidentified aminophospholipid.

Table 1. Fatty acid profiles of strain CCUG 35676^T and type strains of *Dietzia* species

Strains: 1, CCUG 35676^T; 2, *D. aerolata* Sj14a^T; 3, *D. schimae* YIM 65001^T; 4, *D. cercidiphylli* YIM 65002^T; 5, *D. psychrocaliphila* ILA-1^T; 6, *D. natronolimnaea* CBS 107.95^T; 7, *D. kunjamenis* K30-10^T; 8, *D. maris* DSM 43672^T; 9, *D. lutea* DSM 45074^T. Values are percentages of total fatty acids; all data were obtained with the MIDI system under the same conditions. Data for taxon 2 from Kämpfer *et al.*, (2010); data for taxa 3 to 8 from Li *et al.* (2008); data for taxon 9 from Li *et al.* (2009). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. —, Not detected.

Fatty acid	1	2	3	4	5	6	7	8	9
C _{10:0}	—	—	—	—	—	—	—	—	—
C _{12:0}	—	—	—	—	—	—	—	—	—
C _{13:0}	—	—	0.2	—	—	—	—	—	—
C _{13:0} 3-OH/iso-C _{15:1} H	—	—	0.5	—	—	—	—	—	—
C _{14:1} ω 5 <i>c</i>	—	—	0.2	—	—	—	—	—	—
C _{14:0}	0.9	0.5	2.9	1.2	0.8	1.0	0.5	0.8	1.0
C _{13:0} 2-OH	—	—	0.5	—	—	—	—	—	—
C _{15:0}	2.5	—	—	—	—	—	—	—	3.7
iso-C _{15:1}	—	—	—	0.4	—	—	—	—	—
iso-C _{15:1} G	—	—	0.9	—	0.1	—	—	—	—
anteiso-C _{15:1} A	—	—	0.4	—	—	—	—	—	—
C _{15:1} ω 8 <i>c</i>	—	—	0.8	—	0.2	—	—	0.2	—
C _{15:1} ω 5 <i>c</i>	—	—	0.4	—	—	—	—	0.4	—
iso-C _{16:0}	—	—	—	1.6	—	—	—	—	—
C _{16:1} ω 9 <i>c</i>	—	—	—	0.5	0.8	—	—	—	3.8
C _{16:1} ω 6 <i>c</i> /C _{16:1} ω 7 <i>c</i>	9.8	5.6	25.8	18.7	10.9	33.0	12.9	10.6	6.7
10-methyl-C _{16:0}	0.5	—	0.8	1.1	0.3	0.7	—	0.6	—
C _{16:0}	21.7	22.7	22.2	18.9	16.9	14.1	13.0	15.3	15.4
anteiso-C _{17:1} ω 9 <i>c</i>	—	—	—	0.9	—	—	—	—	—
C _{17:1} ω 8 <i>c</i>	—	5.0	—	3.7	7.5	—	8.7	17.2	12.2
C _{17:1} ω 7 <i>c</i>	0.4	—	19.2	2.2	—	—	7.6	—	—
C _{17:1} anteiso B/iso I	—	—	6.1	—	1.7	—	—	—	—
iso-C _{17:0}	—	—	—	0.2	—	—	—	—	—
anteiso-C _{17:0}	—	—	—	0.9	—	—	—	—	—
C _{17:0}	25.9	14.0	—	—	13.8	—	8.8	13.2	22.4
10-methyl-C _{17:0}	1.8	0.7	2.0	0.4	0.6	0.8	1.5	5.3	2.9
C _{18:1} ω 7 <i>c</i>	—	—	—	3.5	—	4.5	—	—	1.7
C _{18:1} ω 9 <i>c</i>	17.2	19.7	—	27.9	—	15.7	27.3	13.9	10.2
C _{18:3} ω 6 <i>c</i> (6,9,12)	—	—	—	1.4	—	—	—	—	—
C _{18:0}	7.9	6.9	—	—	13.9	—	—	12.3	8.0
10-methyl-C _{18:0}	18.8	13.2	13.4	17.9	9.7	30.2	19.8	10.3	7.8
iso-C _{19:0}	—	1.1	—	—	—	—	—	—	—
anteiso-C _{19:0}	—	1.7	—	—	—	—	—	—	—
C _{19:0}	4.4	8.4	—	—	9.6	—	—	—	2.6
C _{20:4} ω 6,9,12,15 <i>c</i>	1.9	1.4	—	—	11.7	—	—	—	—
C _{20:1} ω 9 <i>c</i>	3.9	—	—	—	—	—	—	—	—
iso-C _{20:0}	—	0.9	—	—	—	—	—	—	—
C _{20:0}	0.4	—	—	0.2	—	—	—	—	—

The results of a comparative physiological/biochemical characterization using identical test conditions are given in Table 2 and the species description, on the basis of methods reported previously (Kämpfer *et al.*, 1991). DNA–DNA reassociation experiments were performed with CCUG 35676^T and *D. aerolata* Sj14a^T, *D. schimae* DSM 45139^T, *D. cercidiphylli* DSM 45140^T and *D. maris* DSM 43672^T, which resulted in values of 15, 26, 34 and 28 %, respectively, on the basis of the method of Ziemke *et al.* (1998).

The observed phenotypic differences and the genotypic differences in 16S rRNA gene sequences and DNA–DNA

relatedness values justify the proposal of a novel species to accommodate the isolate.

Description of *Dietzia aurantiaca* sp. nov.

Dietzia aurantiaca (au.ran.ti.a'ca. N.L. fem. adj. *aurantiaca* orange-coloured).

Coccoid cells, about 1.0–1.5 μ m in diameter. Gram-positive, oxidase-positive and catalase-positive showing an oxidative metabolism. Good growth occurs after 3 days of incubation on R2A agar, nutrient agar and TSA at 10–37 °C. Colonies

Table 2. Differential characteristics of strain CCUG 35676^T and type strains of *Dietzia* species

Strains: 1, CCUG 35676^T; 2, *D. aerolata* Sjl4a^T; 3, *D. schimae* YIM 65001^T; 4, *D. cercidiphylli* YIM 65002^T; 5, *D. psychrhalcaliphila* ILA-1^T; 6, *D. natronolimnaea* CBS 107.95^T; 7, *D. kunjamensis* K30-10^T; 8, *D. maris* DSM 43672^T; 9, *D. cinnamea* IMMIB RIV-399^T; 10, *D. lutea* DSM 45074^T; 11, *D. papillomatosis* N 1280^T. All data for taxa 1 to 3 are from this study; data for *D. cinnamea* IMMIB RIV-399^T are from Yassin *et al.* (2006); data for *D. papillomatosis* N 1280^T are from Jones *et al.* (2008); data for *D. lutea* are from Li *et al.* (2009); other data are from Li *et al.* (2008). +, Positive; w, weakly positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Colony colour	Deep orange	Deep orange	Deep pink	Reddish orange	Soft red	Soft pink	Red	Deep orange	Yellow	Orange yellow	Orange
Urea hydrolysis	ND	–	–	+	+	+	–	+	+	–	+
Nitrate reduction	ND	ND	+	–	–	–	+	+	+	–	+
H ₂ S production	ND	ND	–	–	+	+	+	–	ND	–	ND
Maximum NaCl tolerance (% w/v)	12	ND	15	10	10	10	15	15	12	15	8
Growth temperature range (°C)	4–37	10–30	10–45	10–37	10–37	10–37	10–37	10–45	22–45	10–45	10–37
Utilization as sole carbon source:											
D-Adonitol	–	–	–*	–*	+	–	–	–	–	–	+
D-Arabitol	ND	–	–*	–*	+	–	–	–	ND	–	ND
L-Arabinose	+	–	–*	+	–	–	–	–	–	+	+
Arbutin	–	–	–*	+	–	–	–	–	ND	–	+
Cellobiose	+	–	+	–*	+	+	+	–	–	–	+
Gentiobiose	ND	ND	–	–	+	–	+	–	–	–	+
D-Glucose	+	+	+	+	–	+	+	+	+	+	+
Inositol	–	–	–*	–*	+	–	–	–	–	+	+
Inulin	–	–	–*	–	–	–	+	–	ND	–	ND
Lactose	ND	ND	+	+	+	+	+	–	–	–	+
D-Lyxose	ND	ND	–	+	–	–	+	–	–	+	ND
Maltose	–	–	–*	+	+	+	–	+	+	–	+
D-Mannose	–	+	+	+	+	+	+	–	ND	+	+
Melezitose	ND	ND	–	–	+	+	W	+	–	–	+
Melibiose	–	–	–*	–*	+	+	+	–	ND	–	+
N-Acetylglucosamine	+	–	–*	–*	+	+	–	–	ND	–	ND
Raffinose	ND	–	–*	–*	+	+	+	–	–	–	+
L-Rhamnose	–	–	–*	–*	–	–	+	–	–	+	ND
Salicin	–	–	–*	–*	–	–	–	–	+	ND	+
Sucrose	+	–	+	+	+	+	+	–	–	–	+
D-Tagatose	ND	ND	–	+	–	–	–	–	+	–	+
Trehalose	–	–	–*	–*	+	+	+	–	–	–	+
D-Xylose	–	–	–*	–*	–	–	–	W	+	+	+

*Results are congruent with those of Li *et al.* (2008) obtained with the method of Kämpfer *et al.* (1991).

grown on TSA are circular, convex and orange pigmented. Optimal temperature for growth is 30 °C; growth occurs at 4–37 °C but not at 4 or 50 °C. Optimal pH for growth is 7.0–8.0 and growth occurs at pH 5.5–12.5; however, it must be mentioned that growth at pH values >10 must be interpreted with care, because, even if the medium pH is adjusted to 12.5, CO₂ production will cause the pH to drop fairly quickly. The quinone system contains predominantly MK-8(H₂). Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, an unidentified aminophospholipid (APL1), an unidentified phospholipid (PL1) and unidentified glycolipids (GL1 and GL3). Moderate to minor amounts of phosphatidylinositol

mannoside, a third glycolipid and 11 unidentified lipids are also detected. Predominant fatty acids are C_{16:0}, C_{17:0}, C_{18:1 ω 9c} and 10-methyl-C_{18:0}. Also produces C_{15:0} and C_{20:1} in low amounts. Utilizes L-arabinose, cellobiose, D-glucose, fructose, N-acetyl-D-glucosamine, ribose, acetate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate and pyruvate, but not D-galactose, D-gluconate, L-rhamnose, trehalose, D-xylose, D-adonitol, *i*-inositol, maltitol, D-mannitol, sorbitol, caprate, 4-amino-butyrate, L-alanine, asparagine, citrate, L-leucine or proline. Further carbon source utilizations (including differentiating characters determined under identical test conditions) are indicated in Table 2.

The type strain is CCUG 35676^T (=JCM 17645^T), isolated from human cerebrospinal fluid.

Acknowledgements

We are grateful to Gundula Will and the CCUG staff for excellent technical assistance and the Blood Department of the PHLS for submitting the isolate to the CCUG.

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