Dietzia aerolata sp. nov., isolated from the air of a duck barn, and emended description of the genus *Dietzia* Rainey *et al.* 1995

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A Gram-stain-positive, coccoid, non-endospore-forming actinobacterium (Sj14a^T) was isolated from the air of a duck barn on tryptone soy agar after filter sampling. On the basis of 16S rRNA gene sequence similarity studies, strain Sj14a^T was shown to belong to the genus *Dietzia* and was most closely related to *Dietzia schimae* (98.7%), *Dietzia cercidiphylli* (98.4%) and *Dietzia maris* (98.6%). The major menaquinone was MK-8 (H₂). The polar lipid profile included the major components diphosphatidylglycerol, phosphatidylglycerol, an unknown polar lipid and two unknown glycolipids. Phosphatidylinositol and two phosphatidylinositol mannosides, as well as several other unknown lipids, were also detected. The polyamine pattern contained the major compounds spermine and spermidine. The fatty acid profile, containing C_{16:0}, C_{17:0}, C_{18:1ω9c} and 10-methyl C_{18:0} as major fatty acids, supported the affiliation of strain Sj14a^T to the genus *Dietzia*. The results of physiological and biochemical tests and DNA–DNA hybridizations allowed a clear phenotypic differentiation of strain Sj14a^T from the most closely related species of the genus *Dietzia*. Strain Sj14a^T represents a novel species, for which the name *Dietzia aerolata* sp. nov. is proposed, with the type strain Sj14a^T (=DSM 45334^T =CCM 7659^T).

The genus *Dietzia* was proposed by Rainey *et al.* (1995) to accommodate actinomycetes previously classified as *Rhodococcus maris* (Nesterenko *et al.*, 1982). At the time of writing, the genus *Dietzia* consists of eight species with validly published names: *Dietzia maris* (Rainey *et al.*, 1995), the type species; *Dietzia natronolimnaea* (Duckworth *et al.*, 1998); *Dietzia psychrphiliphila* (Yumoto *et al.*, 2002); *Dietzia kunjamensis* (Mayilraj *et al.*, 2006); *Dietzia cinnamomea* (Yassin *et al.*, 2006); *Dietzia schimae* and *Dietzia cercidiphylli* (Li *et al.*, 2008); and *Dietzia papillomatosus* (Jones *et al.*, 2008).

An orange pigmented strain, Sj14a^T, was isolated on tryptone-soy agar (TSA) at 26 °C from the air collected in a duck barn.

The barn investigated contained 2500 ducks (each 2 months old). Bioaerosol samples were collected according to German standard procedures (VDI 4252-Blatt 2) on

gelatin filters by sampling 500 l air at a rate of 30 l min⁻¹. Subsequently, filters were dissolved and decimally diluted in 0.9% NaCl (up to 10⁻⁸). For cultivation, TSA plates were inoculated with 0.1 ml of each dilution in triplicate. Strain Sj14a^T was isolated from a plate inoculated from the first decimal dilution. The concentration of strain Sj14a^T, calculated from the numbers of colonies with an identical morphology on plates of the same dilution, was approximately 10³ c.f.u. per m³ air.

Morphological properties, Gram-staining and cell morphology were observed by phase-contrast microscopy as described previously (Kämpfer & Kroppenstedt, 2004).

The 16S rRNA gene was analysed as reported by Kämpfer *et al.* (2003). Multiple sequence alignment and analysis of the data were performed by the neighbour-joining (Fig. 1) and maximum-parsimony (data not shown) methods using the software package MEGA (molecular evolutionary genetics analysis) version 4 (Tamura *et al.* 2007). Genetic distances were calculated according to the Kimura-2 model. Bootstrap analyses were based on 1000 replications. The 16S rRNA gene sequence of strain Sj14a^T was a continuous stretch of 1388 bp. Sequence similarity calculations after a neighbour-joining analysis indicated that the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Sj14a^T is FM995533.

The polar lipid profile of strain Sj14a^T and the fatty acid profiles of strain Sj14a^T and the type strains of species of the genus *Dietzia* are available as supplementary material with the online version of this paper.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses). Multiple alignment, distance calculations (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were performed by using the software package MEGA version 4 (Tamura *et al.* 2007). Bootstrap values based on 1000 replications are given as percentages at the branching points. All nodes in the neighbour-joining tree were also found in the maximum-parsimony tree. Bar, 0.005 substitutions per nucleotide position.

closest relatives of strain *Sj14a^T* were *Dietzia schimae* DSM 45139^T (98.7 %), *Dietzia cercidiphylli* DSM 45140^T (98.4 %) and *Dietzia maris* DSM 43672^T (98.6 %). 16S rRNA gene sequence similarity of strain *Sj14a^T* to the remaining species of the genus *Dietzia* ranged from 97.5 % to 98.2 %.

For polyamine, quinone and polar lipid analyses, cells were grown in PYE medium (Busse *et al.*, 2005). Polyamines were extracted and analysed by HPLC as described by Busse & Auling (1988) and Altenburger *et al.* (1997). Quinone and polar lipid extraction and analyses were carried out as described by Tindall (1990a,b) and Altenburger *et al.* (1996). HPLC equipment was as described by Stolz *et al.* (2007). The polyamine pattern of strain *Sj14a^T* consisted of the predominant amines spermine [0.33 µmol (g dry weight)⁻¹] and spermidine [0.14 µmol (g dry weight)⁻¹] and trace amounts of cadaverine and putrescine [<0.02 µmol (g dry weight)⁻¹]. To our knowledge, this is the first representative of the genus *Dietzia* that has been analysed for polyamines and hence the results cannot be compared with other representatives of the genus. The quinone system with the predominant compound menaquinone MK-8 [H₂] (83 %) is in agreement with the genus description (Rainey *et al.*, 1995); minor amounts of MK-7 [H₂] (14 %), MK-9 [H₂] (2 %), MK-7 (<1 %) and MK-8 (<1 %) were detected in addition. The polar lipid profile contained the major compounds diphosphatidylglycerol, phosphatidylglycerol, an unknown polar lipid and two unknown glycolipids. Phosphatidylinositol, a phosphatidylinositol mannoside, as well as an unknown aminolipid, were detected in moderate amounts. Additionally, minor amounts of a second phosphatidylinositol mannoside and other unknown lipids were detected (Supplementary Fig. S1, available in IJSEM Online). This polar lipid profile is not in accordance with the description of the genus *Dietzia* (Rainey *et al.*, 1995), which lists the lipids phosphatidylinositol and phosphatidylinositol mannosides as being absent and phosphatidylethanolamine as being present. However, the presence of

phosphatidylinositol or phosphatidylinositol and phosphatidylinositol mannoside and the absence of phosphatidylethanolamine has been reported for *D. schimae*, *D. cercidiphylli* (Li *et al.*, 2008) and *D. kunjamensis* (Mayilraj *et al.*, 2006). Hence, an emended description of the genus *Dietzia* is required, which is provided below.

Fatty acid analysis was performed as described by Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain *Sj14a^T* was similar to those of the other closely related species of the genus *Dietzia*, but some minor differences were observed (Supplementary Table S1). The typical major fatty acids C_{16:0}, C_{17:0}, C_{18:1ω9c} and 10-methyl C_{18:0} could be detected in strain *Sj14a^T*.

The results of the comparative physiological characterization using identical test conditions are given in Table 1 and the species description, with methods as described previously (Kämpfer *et al.*, 1991). DNA–DNA hybridization experiments were performed with strain *Sj14a^T* and the type strains *D. schimae* DSM 45139^T, *D. cercidiphylli* DSM 45140^T and *D. maris* DSM 43672^T, which resulted in relatedness values of 28, 19 and 26 %, respectively.

The phenotypic differences observed in fatty acid profiles and physiological tests, the presence of two phosphatidylinositol mannosides in the polar lipid profile, and the genotypic differences in 16S rRNA gene sequences and DNA–DNA pairing between strain *Sj14a^T* and the type strains of closely related species of the genus *Dietzia* clearly warrant the creation of a separate species.

Emended description of the genus *Dietzia* Rainey *et al.* 1995

In addition to the properties given in the genus description (Rainey *et al.*, 1995), members of the genus may contain the polar lipids phosphatidylinositol and phosphatidylinositol mannosides. Phosphatidylethanolamine may be absent.

Table 1. Differential characteristics of strain Sj14a^T and the type strains of species of the genus *Dietzia*

Strains: 1, Sj14a^T, 2, *Dietzia schimae* YIM 65001^T; 3, *D. cercidiphylli* YIM 65002^T; 4, *D. psychrhalcaliphila* ILA-1^T; 5, *D. natronolimnaea* CBS 107.95^T; 6, *D. kunjamensis* K30-10^T; 7, *D. maris* DSM 43672^T; 8, *D. cinnamea* IMMIB RIV-399^T; 9, *D. papillomatosis* N 1280^T. All carbon source utilization data for strains 1–3 from this study; data for *D. cinnamea* IMMIB RIV-399^T from Yassin *et al.* (2006); data for *D. papillomatosis* N 1280^T from Jones *et al.* (2008); other data are from Li *et al.* (2008). +, Positive; w, weakly positive; –, negative; ND, not determined. DO, Deep orange; DP, deep pink; RO, reddish orange; SR, soft red; SP, soft pink; R, red; Y, yellow; O, orange.

Characteristic	1	2	3	4	5	6	7	8	9
Colony colour	DO	DP	RO	SR	SP	R	DO	Y	O
Urea hydrolysis	–	–	+	+	+	–	+	+	+
Nitrate reduction	ND	+	–	–	–	+	+	+	+
H ₂ S production	ND	–	–	+	+	+	–	ND	ND
Tolerance to NaCl (% w/v)	ND	15	10	10	10	15	15	12	8
Temperature range (°C)	10–30	10–45	10–37	10–37	10–37	10–37	10–45	22–45	10–37
Utilization as sole carbon source									
D-Adonitol	–	–*	–*	+	–	–	–	–	+
D-Arabitol	–	–*	–*	+	–	–	–	ND	ND
L-Arabinose	–	–*	+	–	–	–	–	–	+
Arbutin	–	–*	+	–	–	–	–	ND	+
Cellobiose	–	+	–*	+	+	+	–	–	+
Gentiobiose	ND	–	–	+	–	+	–	–	+
D-Glucose	+	+	+	–	+	+	+	+	+
Inositol	–	–*	–*	+	–	–	–	–	+
Inulin	–	–*	–	–	–	+	–	ND	ND
Lactose	ND	+	+	+	+	+	–	–	+
D-Lyxose	ND	–	+	–	–	+	–	–	ND
Maltose	–	–*	+	+	+	–	+	+	+
D-Mannose	+	+	+	+	+	+	–	ND	+
Melezitose	ND	–	–	+	+	W	+	–	+
Melibiose	–	–*	–*	+	+	+	–	ND	+
N-Acetylglucosamine	–	–*	–*	+	+	–	–	ND	ND
Raffinose	–	–*	–*	+	+	+	–	–	+
L-Rhamnose	–	–*	–*	–	–	+	–	–	ND
Salicin	–	–*	–*	–	–	–	–	+	+
Sucrose	–	+	+	+	+	+	–	–	+
D-Tagatose	ND	–	+	–	–	–	–	+	+
Trehalose	–	–*	–*	+	+	+	–	–	+
D-Xylose	–	–*	–*	–	–	–	W	+	+

*Results congruent with the results of Li *et al.* (2008) and Kämpfer *et al.* (1991).

Description of *Dietzia aerolata* sp. nov.

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

Coccoid cells, approximately 1–1.5 µm in diameter. Gram-stain-positive, oxidase-positive, catalase-positive showing an oxidative metabolism. Good growth occurs after 3 days of incubation on R2A agar and nutrient agar at 25–30 °C. The quinone system contains MK-8 [H₂] predominantly. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids and three unknown polar lipids. Phosphatidylinositol and two phosphatidylinositol mannosides as well as two unknown aminolipids and several unknown polar lipids are detectable. The polyamine pattern contains the major com-

pounds spermine and spermidine. Major fatty acids are C_{16:0}, C_{17:0}, C_{18:1}ω9c and 10-methyl C_{18:0}. Carbon source utilizations (including differentiating characteristics under identical conditions) are indicated in Table 1. D-Glucose and D-mannose are utilized, but many other sugar compounds are not utilized.

The type strain is Sj14a^T (=DSM 45334^T =CCM 7659^T), which was isolated from the air of a duck barn.

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