Aeromicrobium flavum sp. nov., isolated from air

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A Gram-positive, non-motile, facultatively anaerobic actinobacterium, designated TYLN1^T, was isolated from an air sample from the campus of Wuhan University, China, and subjected to a polyphasic taxonomic study. Strain TYLN1^T grew optimally at pH 7.0 and 30 °C. Analysis of 16S rRNA gene sequence similarities revealed that the organism belongs to the genus *Aeromicrobium*. The G + C content of the strain was 73.3 mol%. The cell-wall diamino acid was LL-2,6-diaminopimelic acid, the predominant menaquinones were MK-9(H₄) and MK-8(H₄) and the predominant fatty acid was C_{18:1ω9c} (68.38 %). These chemotaxonomic data also support the affiliation of strain TYLN1^T to the genus *Aeromicrobium*. The values for DNA–DNA hybridization between strain TYLN1^T and the phylogenetically closest relatives *Aeromicrobium tamense* DSM 19087^T and *Aeromicrobium panaciterrae* DSM 17939^T were respectively 35 and 11.5 %. On the basis of the data from this study, the isolate represents a novel species, for which the name *Aeromicrobium flavum* sp. nov. is proposed. The type strain is TYLN1^T (=CCTCC AB 206046^T =DSM 19355^T).

The genus *Aeromicrobium* was proposed by Miller *et al.* (1991) and its description was later emended by Yoon *et al.* (2005). At the time of writing, the genus comprises seven species, *Aeromicrobium erythreum* (Miller *et al.*, 1991) (the type species), *A. alkaliterrae* (Yoon *et al.*, 2005), *A. fastidiosum* (Tamura & Yokota, 1994), *A. marinum* (Bruns *et al.*, 2003), *A. panaciterrae* (Cui *et al.*, 2007), *A. ponti* (Lee & Lee, 2008) and *A. tamense* (Lee & Kim, 2007). In our research into the isolation and identification of environmental microbes, we noticed that a strain named TYLN1^T formed a distinct branch within the *Aeromicrobium* clade, implying that it might represent a novel species. Polyphasic analysis of morphological, physiological, biochemical and chemotaxonomic properties was therefore undertaken to substantiate the result of the phylogenetic analysis.

An air sample was collected on the campus of Wuhan University using a Petri dish with TGY agar (a tryptone/glucose/yeast extract medium often used for growth of *Deinococcus* species; Brim *et al.*, 2003). After sampling, the plate was incubated at 30 °C for 1 week and strain TYLN1^T was recovered.

Chromosomal DNA preparation and 16S rRNA gene amplification were carried out according to the protocol of Niemann *et al.* (1997). Analysis of the 16S rRNA gene sequence was performed by using the software package MEGA version 3.1 (Kumar *et al.*, 2001) after multiple

alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). The model of Jukes & Cantor (1969) was used to compute evolutionary distances, based on which, a phylogenetic tree was built using the neighbour-joining method (Saitou & Nei, 1987) with bootstrap analysis of 1000 replications. Sequence-similarity calculations indicated that strain TYLN1^T showed the greatest degree of sequence similarity to the type strains of *A. tamense* (98.4 %), *A. panaciterrae* (97.1 %), *A. fastidiosum* (96.4 %), *A. alkaliterrae* (96.2 %), *A. marinum* (96.2 %) and *A. erythreum* (96.1 %). Lower sequence similarities (<95 %) were found with respect to members of all other genera shown in Fig. 1.

Based on 16S rRNA gene sequence similarity, DNA–DNA hybridization between strain TYLN1^T and the type strains of *A. tamense* and *A. panaciterrae* was assessed. DNA–DNA hybridization tests were carried out by Dr Cathrin Spröer (DSMZ, Braunschweig, Germany). DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). Strain TYLN1^T showed 35 % reassociation with *A. tamense* DSM 19087^T and 11.5 % with *A. panaciterrae* DSM 17939^T; these values are much lower than the recommended threshold of 70 % for

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain TYLN1^T is EF133690.

Fig. 1. Neighbour-joining tree, showing the phylogenetic positions of strain TYLN1^T and related taxa, based on 16S rRNA gene sequences. GenBank accession numbers are given. Bootstrap analysis was made with 1000 resamplings; percentages of support are shown at nodes. Bar, 1 % sequence divergence.

the delineation of genomic species (Wayne *et al.*, 1987). The results of the DNA–DNA hybridization experiments therefore indicate that strain TYLN1^T represents a novel species.

Cell morphology was examined by phase-contrast and transmission electron microscopy. For transmission electron microscopy, cells were negatively stained with 1 % (w/v) phosphotungstic acid after air drying. Cells were irregular rods, 0.2–0.4 µm wide and 0.3–1.2 µm long (Fig. 2). No flagella were observed.

Growth at various NaCl concentrations (0.1–2 %, w/v) was investigated using LB broth. The pH range for growth was determined using LB broth that was adjusted to pH 5–10 at intervals of 1 pH unit. Growth at different temperatures (4, 11, 25, 28, 30, 37, 42 and 50 °C) was tested on LB agar. Gram staining was performed as described by Gerhardt *et al.* (1994). Antimicrobial susceptibility testing was performed by the agar-diffusion method using antibiotic-impregnated discs as described by Buczolits *et al.* (2002). Oxidation of *N,N,N,N*-tetramethyl *p*-phenylenediamine was used to determine oxidase activity. Catalase activity was determined using 3 % (v/v) H₂O₂ solution. Hydrolysis

of casein was tested as described by MacFaddin (1980). The ability to use different carbon sources was analysed with the Biolog GP2 Microplate system according to the instructions of the manufacturer. Other physiological and biochemical properties were tested using the API ZYM, API 20E and API 20NE systems (bioMérieux), according to the instructions of the manufacturer.

Strain TYLN1^T exhibited nitrate reduction activity, which has not been reported previously for species of the genus *Aeromicrobium* (Miller *et al.*, 1991; Tamura & Yokota, 1994; Bruns *et al.*, 2003; Yoon *et al.*, 2005; Cui *et al.*, 2007; Lee & Kim, 2007). The main physiological and biochemical properties are given in Table 1 and the species description. Features that differentiate strain TYLN1^T from *A. tamlense* are shown in Table 2.

The isomer type of the diamino acid in the cell-wall peptidoglycan was analysed using TLC according to the method described by Komagata & Suzuki (1987). Extraction of menaquinones and determination of the menaquinone composition were carried out as described by Collins (1985). The thermal denaturation method (Mandel & Marmur, 1968) was used to determine the G + C content of the genomic DNA spectrophotometrically (DU800 spectrophotometer; Beckman Coulter). Strain TYLN1^T contained LL-diaminopimelic acid as the diagnostic diamino acid in the cell wall. The major isoprenoid quinones were MK-9(H₄) (74.6 %) and MK-8(H₄) (25.4 %). The DNA G + C content was 73.3 mol%.

Preparation and analysis of cellular fatty acid methyl esters were performed using the Sherlock Microbial Identification System (version 6.0; MIDI), according to the instructions of the manufacturer. Strain TYLN1^T was grown for 24 h at 30 °C on trypticase soy broth (Difco) supplemented with Bacto agar (Difco). The cellular fatty acid profile of strain TYLN1^T was characterized by the presence (>1 %) of C_{18:1ω9c} (68.38 %), C_{18:0} (11.69 %), C_{16:0} (8.66 %), C_{16:0} 2-OH (5.26 %), 10-methyl C_{18:0} (2.28 %) and C_{16:1ω6c} (1.03 %) acids. The presence of C_{18:1ω9c} as the predominant component of the fatty acid composition is similar to *A. tamlense* but unlike other *Aeromicrobium* species (Miller *et al.*, 1991; Tamura & Yokota, 1994; Bruns *et al.*, 2003; Yoon *et al.*, 2005; Cui *et al.*, 2007; Lee & Kim, 2007).

Fig. 2. Transmission electron micrographs of cells of strain TYLN1^T. Cells were grown on LB agar plates at 30 °C for 16 h (a) and 48 h (b). Bars, 1 µm.

Table 1. Phenotypic characteristics of strain TYLN1^T and species of the genus *Aeromicrobium*

Taxa: 1, strain TYLN1^T (*A. flavum* sp. nov.); 2, *A. tamlense* (data from Lee & Kim, 2007); 3, *A. erythreum* (Miller *et al.*, 1991; Tamura & Yokota, 1994); 4, *A. fastidiosum* (Collins & Stackebrandt, 1989; Tamura & Yokota, 1994); 5, *A. panaciterrae* (Cui *et al.*, 2007); 6, *A. marinum* (Bruns *et al.*, 2003); 7, *A. alkaliterrae* (Yoon *et al.*, 2005). +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Irregular rods	Irregular rods	Irregular rods, cocci	Rods, cocci	Rods	Rods	Rods, cocci
Motility	–	–	–	+	–	–	+
Oxidase	+	–	+	+	–	+	–
Urease	w	–	–	–	–	ND	–
Nitrate reduction	+	–	–	ND	–	–	–
Growth at:							
10 °C	–	+	–	+	–	+	+
42 °C	–	+	–	–	–	–	–
Hydrolysis of:							
Casein	–	–	+	+	–	–	+
Aesculin	+	+	+	ND	ND	ND	–
DNA G + C content (mol%)	73.3	72.7	71–73	71–72	65.5	70.6	71.5

Comparative 16S rRNA gene sequence analysis showed that strain TYLN1^T is most closely related to the genus *Aeromicrobium* of the family *Nocardiodaceae* (Fig. 1). Chemotaxonomic properties support the monothetic phylogenetic classification of strain TYLN1^T as a member of the genus *Aeromicrobium*. However, the isolate exhibited phenotypic, phylogenetic and genetic distinctiveness.

Taken together, the evidence provided by 16S rRNA gene sequencing, DNA–DNA hybridization, phenotypic characterizations and chemotaxonomic data indicates that

strain TYLN1^T represents a novel species, for which the name *Aeromicrobium flavum* sp. nov. is proposed.

Description of *Aeromicrobium flavum* sp. nov.

Aeromicrobium flavum (fla'vum. L. neut. adj. *flavum* yellow, referring to the colony colour).

Cells are Gram-positive, non-motile and facultatively anaerobic. Colonies are circular, smooth, entire and yellow pigmented. Growth occurs at 25–37 °C and at pH 5–10. Optimal growth at 30 °C and pH 7. Tolerates 4 % NaCl in the medium; the optimum NaCl concentration for growth is 1–2 %. Catalase- and oxidase-positive. Aesculin is hydrolysed, but not casein. In API 20E and API 20NE tests, urease is weakly positive. Gelatin liquefaction is observed. H₂S and indole are not produced. Nitrate is reduced to nitrite. Voges–Proskauer reaction is weakly positive. The following enzyme activities are not detected: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase. The fatty acid profile comprises C_{18:1}ω9c (68.38 %), C_{18:0} (11.69 %), C_{16:0} (8.66 %), C_{16:0} 2-OH (5.26 %), 10-methyl C_{18:0} (2.28 %) and C_{16:1}ω6c (1.03 %). Positive responses in the Biolog GP2 plate for maltose, D-psicose, D-ribose, sucrose, trehalose, turanose, D-fructose, α-D-glucose, acetic acid, α-ketovaleric acid, γ-hydroxybutyric acid, pyruvic acid, D-fructose 6-phosphate and glycerol; negative for α-cyclodextrin, dextrin, glycogen, inulin, mannan, Tween 80, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, amygdalin, D-xylose, L-arabinose, D-arabitol, cellobiose, L-fucose, L-rhamnose, D-mannose, L-alanyl glycine, arbutin, L-asparagine, 2'-deoxyadenosine, salicin, L-lactic acid, methyl α-D-mannoside, putrescine, sedoheptulosan, xylitol, glycyl L-glutamic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, thymidine and uridine. In API ZYM tests, esterase (C4), esterase lipase (C8), leucine arylamidase,

Table 2. Physiological characteristics that differentiate strain TYLN1^T from *A. tamlense* NRRL B-24466^T

Data were obtained in this study. +, Positive; w, weakly positive; –, negative.

Characteristic	Strain TYLN1 ^T	<i>A. tamlense</i> NRRL B-24466 ^T
Biolog GP2 plate response for:		
Tween 80	–	+
Uridine	–	+
Thymidine	–	+
L-Rhamnose	–	+
Dextrin	–	+
Pyruvic acid	+	–
γ-Hydroxybutyric acid	+	w
API ZYM test results		
Alkaline phosphatase	–	+
Acid phosphatase	–	+
Trypsin	–	w
Naphthol-AS-BI-phosphohydrolase	–	w
Esterase (C4)	+	–
β-Glucosidase	+	–

α -glucosidase and β -glucosidase are detected. Naphthol-AS-BI-phosphohydrolase, alkaline and acid phosphatase, valine arylamidase, cystine arylamidase, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase and trypsin tests are negative. Lipase (C14) is weakly positive. Resistant to penicillin and nalidixic acid and susceptible to tetracycline, chloramphenicol, kanamycin, vancomycin, erythromycin, rifampicin and tobramycin. The major menaquinones are MK-9(H₄) and MK-8(H₄). Cell-wall peptidoglycan is based on LL-2,6-diaminopimelic acid. The DNA G+C content of the type strain is 73.3 mol%.

The type strain, TYLN1^T (=CCTCC AB 206046^T =DSM 19355^T), was isolated from air.

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