Massilia oculi sp. nov., isolated from a human clinical specimen

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A Gram-negative, rod-shaped, non-spore-forming bacterium (strain CCUG 43427A^T) was isolated from a patient suffering from endophthalmitis and its taxonomic position was studied. 16S rRNA gene sequence analysis indicated that this strain was a member of the genus *Massilia*. Strain CCUG 43427A^T was most closely related to the type strains of *Massilia timonae* (97.4 % 16S rRNA gene sequence similarity) and *Massilia aurea* (97.2 %); levels of similarity to the type strains of all other recognized *Massilia* species were below 97.0 %. Chemotaxonomic data [Q-8 as major ubiquinone; phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as major polar lipids; and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0}, C_{18:1}ω7c, C_{12:0} and C_{10:0} 3-OH as major fatty acids] supported the affiliation of the isolate to the genus *Massilia*. Levels of DNA–DNA relatedness of strain CCUG 43427A^T with *M. timonae* CCUG 45783^T and *M. aurea* AP13^T were 60.6 % (reciprocal, 55.8 %) and 58.1 % (reciprocal, 34.0 %), respectively. Strain CCUG 43427A^T could be differentiated from its closest phylogenetic neighbours based on a range of phenotypic characteristics. Strain CCUG 43427A^T is therefore considered to represent a novel species of the genus *Massilia*, for which the name *Massilia oculi* sp. nov. is proposed. The type strain is CCUG 43427A^T (=CCM 7900^T).

The genus *Massilia* was proposed by La Scola *et al.* (1998) based on the description of a fastidious, slowly growing bacterium isolated from blood culture of a 25-year-old man with common variable immunodeficiency. The name was validly published in 2000 (La Scola *et al.*, 2000). At the time of writing, the genus comprised 11 recognized species (La Scola *et al.*, 1998; Gallego *et al.*, 2006; Zhang *et al.*, 2006; Zul *et al.*, 2008; Weon *et al.*, 2008, 2009, 2010) isolated from various sources. Members of the genus are characterized as aerobic, Gram-negative, motile, non-spore-forming rods with iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, C_{18:1}ω7c, C_{16:0} and C_{10:0} 3-OH as characteristic fatty acids. Species of the genus *Naxibacter* were transferred to the genus *Massilia* on the basis of both molecular and phenotypic data (Kämpfer *et al.*, 2011).

In 2000, strain CCUG 43427A^T was isolated in Linköping, Sweden, from the eye of a patient suffering from endophthalmitis. This strain was preliminarily identified as *Massilia*-like and colonies were beige in colour on blood agar (Oxoid) and nutrient agar (Oxoid) at 37 °C. Subcultivation for

further analyses was done on tryptone soy agar (TSA; Oxoid) at 28 °C for 48 h.

Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000 magnification, with cells grown for 3 days at 28 °C on TSA. The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Phylogenetic analysis was performed by using the ARB software package (version December 2007; Ludwig *et al.*, 2004) after multiple alignment of data with the ARB alignment tool and with the SILVA SSURF 100 database (release August 2009; Pruesse *et al.*, 2007). Distance (pairwise distances) calculations and tree construction with the neighbour-joining and maximum-likelihood methods with fastDNAm1 (Olsen *et al.*, 1994) were performed with the ARB software package. Clustering with the neighbour-joining method was performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain CCUG 43427A^T (1450 bp) was compared based on similarity calculations, which indicated that its closest relatives were the type strains of *Massilia timonae* (97.4 % similarity) and *Massilia aurea* (97.2 %). Levels of 16S rRNA gene sequence similarity to the type strains of all other recognized *Massilia* species were

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain CCUG 43427A^T is FR773700.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences showing the position of strain CCUG 43427A^T among sequences available from EMBL (accession numbers are given in parentheses). The phylogenetic tree was constructed by using the ARB software package (version December 2007; Ludwig *et al.*, 2004), after multiple alignment of the data with the ARB alignment tool and the SILVA SSURef 100 database (release August 2009; Pruesse *et al.*, 2007). Tree building was performed by using the maximum-likelihood method with fastDNAmI (Olsen *et al.*, 1994) without filters. Bar, 0.1 nt substitutions per nucleotide position.

below 97 %. A maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences is shown in Fig. 1.

For quinone and polar lipid analyses, strain CCUG 43427A^T was grown for 48 h in shake flasks in nutrient broth at 180 r.p.m. at 28 °C. Respiratory quinones were extracted as

Fig. 2. Two-dimensional TLC of polar lipid extracts from strain CCUG 43427A^T stained with molybdotriphosphoric acid. AL, Unknown aminolipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipids.

described by Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1996). Polar lipids extracted according to the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC as described by Collins & Jones (1980). The polar lipid profile of strain CCUG 43427A^T contained phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol as major components (Fig. 2), which is similar to the profiles described for species of the genera *Naxibacter* and *Massilia* (Kämpfer *et al.*, 2008, 2011; Weon *et al.*, 2010). The detected unknown phospholipids PL1 and PL2 were also found in *Massilia consociata* (Kämpfer *et al.*, 2011), *Massilia haematophila* and *Massilia varians* (Kämpfer *et al.*, 2008), but the significant aminolipid AL1, found in *M. consociata*, *M. haematophila* and *M. varians*, could be detected only in minor traces. In contrast, the unknown aminophospholipid observed previously in some strains of the genus *Naxibacter*, now members of the genus *Massilia* (see Supplementary Fig. S1 in Kämpfer *et al.*, 2008), was not found in strain CCUG 43427A^T, but trace amounts of additional unknown phospholipids with low *R_f* values were detected.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). Fatty acids of cells of all strains under comparison were extracted after 48 h of growth on TSA. Given that colony development of the reference type strains was very similar, it can be assumed that they were in comparable growth phases.

Table 1. Cellular fatty acid compositions of strain CCUG 43427A^T and the type strains of recognized *Massilia* species

Strains: 1, CCUG 43427A^T; 2, *M. timonae* DSM 16850^T; 3, *M. aurea* DSM 18055^T; 4, *M. consociata* CCUG 58010^T; 5, *M. varians* KACC 13770^T; 6, *M. haematophila* KACC 13771^T; 7, *M. niastensis* KACC 12599^T (Weon *et al.*, 2009); 8, *M. aerilata* KACC 12505^T (Weon *et al.*, 2008); 9, *M. jejuensis* 5317J-18^T (Weon *et al.*, 2010); 10, *M. suwonensis* 5414S-25^T (Weon *et al.*, 2010); 11, *M. albidiflava* DSM 17472^T (Weon *et al.*, 2008); 12, *M. brevitalea* DSM 18925^T (Zul *et al.*, 2008; Weon *et al.*, 2009); 13, *M. dura* DSM 17513^T (Weon *et al.*, 2008); 14, *M. lutea* DSM 17473^T (Weon *et al.*, 2008); 15, *M. niabensis* KACC 12632^T (Weon *et al.*, 2009); 16, *M. plicata* DSM 17505^T (Zhang *et al.*, 2006); 17, *M. alkalitolerans* KACC 12188^T (Weon *et al.*, 2010). –, Present at <1% or not detected. Data for taxa 1–3 are from this study; data for taxa 4–6 are from Kämpfer *et al.* (2011).

Fatty acid	1	2*	3†	4	5‡	6‡	7	8	9	10	11	12	13	14	15	16	17
C _{10:0} 3-OH	9.6	4.6 (4.6)	5.9 (4.8)	4.3	3.3 (6.2)	3.0 (4.1)	5.5	4.7	6.1	5.4	7.0	4.9	5.5	5.7	6.6	10.1	3.2
C _{12:0}	7.7	4.4 (3.3)	9.6 (4.6)	3.4	3.7 (4.5)	3.3 (3.8)	4.7	3.4	5.9	4.4	5.3	5.0	3.9	4.0	8.9	7.1	3.3
C _{12:0} 2-OH	4.3	1.8 (2.2)	3.1 (1.9)	1.8	2.0 (3.1)	1.5 (2.1)	2.4	–	1.7	2.4	–	2.0	–	–	–	–	2.2
C _{14:0}	1.0	–	1.1 (0.6)	–	(0.7)	–	–	1.9	–	1.2	2.6	–	1.1	1.2	–	1.6	–
C _{14:0} 2-OH	–	–	–	–	–	–	–	2.5	–	–	2.4	–	2.6	2.9	–	6.1	–
anteiso-C _{15:0}	–	–	–	–	–	1.5	–	–	–	–	–	–	–	–	–	–	–
C _{16:0}	20.9	27.2 (30.5)	12.9 (36.8)	21.5	28.5 (23.6)	26.2 (25.2)	26.9	30.6	22.5	28.8	23.4	23.0	27.5	26.6	23.6	25.1	26.6
iso-C _{16:0}	–	–	–	2.2	– (1.0)	2.1	–	–	–	–	–	–	–	–	–	–	–
C _{17:0} cyclo	1.0	– (3.7)	–	–	– (11.4)	2.7 (9.6)	3.4	6.1	1.0	2.9	–	–	–	–	–	–	–
iso-C _{17:0}	–	–	–	–	–	1.6	–	–	–	–	–	–	–	–	–	–	–
iso-C _{17:1} ω ⁹ c	–	–	–	–	–	1.4	–	–	–	–	–	–	–	–	–	–	–
C _{18:1} ω ⁷ c	6.0	11.2 (7.9)	1.4 (2.5)	12.8	8.1 (8.0)	8.7 (10.4)	12.3	11.7	12.1	7.8	7.4	9.0	7.03	7.8	7.8	11.7	6.6
C _{20:0}	–	–	–	–	–	–	2.6	–	–	–	–	–	–	–	–	–	–
Summed feature 3§	48.2	49.2 (47.0)	58.6 (48.3)	54.0	51.7 (41.0)	44.7 (43.1)	38.1	35.2	49.0	45.8	46.0	54.2	52.0	51.1	46.0	36.9	55.2

*Data in parentheses from Weon *et al.* (2008).
†Data in parentheses from Gallego *et al.* (2006).
‡Data in parentheses from Weon *et al.* (2010).
§Summed feature 3 comprised C_{16:1}ω⁷c and/or iso-C_{15:0} 2-OH.

Table 2. Differential characteristics of strain CCUG 43427A^T and the type strains of recognized *Massilia* species

Strains: 1, CCUG 43427A^T; 2, *M. timonae* DSM 16850^T; 3, *M. aurea* DSM 18055^T; 4, *M. consociata* CCUG 58010^T; 5, *M. varians* KACC 13770^T (Weon *et al.*, 2010); 6, *M. haematophila* KACC 13771^T (Weon *et al.*, 2010); 7, *M. niastensis* KACC 12599^T (Weon *et al.*, 2009); 8, *M. aerilata* KACC 12505^T (Weon *et al.*, 2008); 9, *M. jejuensis* 5317J-18^T (Weon *et al.*, 2010); 10, *M. suwonensis* 5414S-25^T (Weon *et al.*, 2010); 11, *M. albidiflava* DSM 17472^T (Weon *et al.*, 2008); 12, *M. brevitalea* DSM 18925^T (Zul *et al.*, 2008; Weon *et al.*, 2009); 13, *M. dura* DSM 17513^T (Weon *et al.*, 2008); 14, *M. lutea* DSM 17473^T (Weon *et al.*, 2008); 15, *M. niabensis* KACC 12632^T (Weon *et al.*, 2009); 16, *M. plicata* DSM 17505^T (Weon *et al.*, 2008); 17, *M. alkalitolerans* KACC 12188^T (Weon *et al.*, 2010). Data for taxa 1–4 are from this study, except where indicated. (+), Weakly positive; ND, not determined.

Characteristic	1	2*	3*	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Oxidase	–	+ ^{a†}	(+) ^b	–	+ ^c	+ ^c	+	+	+	+	+ ^d	– ^e	+ ^d	+ ^d	+	– ^d	– ^f
Nitrate reduction	–	–	–	–	–	–	–	+	–	–	+	+ ^e	–	–	+	–	– ^f
Urease	–	–	–	–	–	–	–	+	–	–	–	– ^g	–	–	–	–	+ ^f
Hydrolysis of aesculin	+	+	+	+	+	–	+	+	+	–	+	+ ^g	+	+	–	+	+ ^f
Assimilation of:																	
D-Glucose	+	+	+	+	–	–	+	+	–	(+)	+	+ ^g	+	–	–	+	+
L-Arabinose	+	+	–	+	+	–	+	+	–	–	+	– ^g	+	+	–	+	+
Potassium gluconate	–	+	–	–	–	–	+	–	–	–	+	– ^g	+	+	–	+	–
Adipic acid	–	–	+	ND	–	+	–	–	–	+	+	+ ^g	–	+	–	–	–
Trisodium citrate	+	+	+	+	–	–	–	–	–	–	–	– ^g	–	–	–	–	+
L-Rhamnose	+	+	+	–	+	–	+	–	–	–	+	–	+	–	–	+	+
Suberic acid	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–
Lactic acid	–	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–
L-Alanine	+	+	+	–	+	–	+	+	–	–	+	–	+	–	–	+	+
Glycogen	ND	+	+	+	+	+	–	+	+	+	+	–	+	–	–	+	–
3-Hydroxybenzoic acid	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	+
L-Serine	+	+	+	–	+	–	–	+	–	+	+	–	–	–	–	+	+
Propionic acid	+	–	(+)	+	+	+	–	+	–	+	–	+	–	–	–	–	+
Valeric acid	ND	–	–	ND	–	+	–	–	–	+	–	+	–	–	–	–	–
L-Histidine	–	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–	+
3-Hydroxybutyric acid	+	+	+	+	+	+	+	+	–	+	+	+	+	–	–	+	+
4-Hydroxybenzoic acid	–	–	–	ND	–	+	+	–	–	–	+	–	+	–	–	–	–
L-Proline	+	+	+	+	+	–	+	+	–	+	+	+	+	+	–	+	+
DNA G+C content (mol%)	ND	62–67 ^a	66.0 ^b	ND	ND	ND	66.6	68.9	66.1	67.8	65.3 ^d	65.3	65.9 ^d	63.3 ^d	67.8	65.1 ^d	62.4±0.3 ^f

*Results of physiological/biochemical tests for the type strains of *M. timonae* and *M. aurea* were identical to those reported by Weon *et al.* (2008) for *M. timonae* and those reported by Gallego *et al.* (2006) for *M. aurea*.

†Data from: a, Lindquist *et al.* (2003); b, Gallego *et al.* (2006); c, Kämpfer *et al.* (2008); d, Zhang *et al.* (2006); e, Zul *et al.* (2008); f, Xu *et al.* (2005); g, Weon *et al.* (2009).

The fatty acid profiles of strain CCUG 43427A^T and the type strains of recognized *Massilia* species are shown in Table 1 (identified with Sherlock version 2.11, TSBA40 Rev. 4.1 database). No pronounced differences in the fatty acid profiles were found, although there were minor differences in the proportions of some components. All had summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and C_{16:0} as major components, as well as C_{18:1}ω7c, C_{10:0} 3-OH and C_{12:0} in moderate amounts. Additionally, C_{12:0} 2-OH, C_{14:0}, C_{14:0} 2-OH, anteiso-C_{15:0}, iso-C_{16:0}, C_{17:0} cyclo, iso-C_{17:0}, C_{17:1}ω9c and C_{20:0} were all found in some of the strains.

Results of the physiological characterization, determined as described previously (Kämpfer *et al.*, 1991), are given in the species description below and differential characteristics between strain CCUG 43427A^T and the type strains of recognized *Massilia* species are given in Table 2. At the species level, strain CCUG 43427A^T shared highest 16S rRNA gene sequence similarity (>97.0%) with the type strains of *M. timonae* and *M. aurea*, but shared <97% similarity with the type strains of all other recognized *Massilia* species. Levels of DNA–DNA relatedness [determined according to Ziemke *et al.* (1998)] between strain CCUG 43427A^T and *M. timonae* CCUG 45783^T and *M. aurea* AP13^T were 60.6% (reciprocal, 55.8%) and 58.1% (reciprocal, 34.0%), respectively.

On the basis of these results and phenotypic traits, we consider that strain CCUG 43427A^T represents a novel species of the genus *Massilia*, for which the name *Massilia oculi* sp. nov. is proposed.

Description of *Massilia oculi* sp. nov.

Massilia oculi (oc'ul.i. L. n. *oculus* -i an eye; L. gen. n. *oculi* of the eye, the source of the type strain).

Cells are non-spore-forming rods (approx. 2 µm long and 1 µm wide). Gram-negative, oxidase-positive, showing oxidative metabolism. Good growth occurs on R2A agar, TSA, peptone-yeast extract agar, nutrient agar and MacConkey agar at 25–30 °C. Growth on TSA occurs at 15–37 °C, but not at 10 or 45 °C. Growth in TSA broth occurs at pH 5.5–10.5. Colonies are beige, translucent and shiny with entire edges within 24 h of incubation, with a diameter of approximately 2 mm. The quinone system consists of ubiquinone Q-8. The major compounds in the polar lipid profile are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Moderate amounts of three unknown phospholipids and one unknown aminolipid are present. The major fatty acids are summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and C_{16:0}; moderate amounts of C_{18:1}ω7c, C_{10:0} 3-OH and C_{12:0} are also found. Test results for carbon source utilization and hydrolysis of chromogenic substrates are indicated in Table 2. Assimilates D-glucose, L-arabinose, trisodium citrate, propionic acid, 3-hydroxybutyric acid, L-alanine, L-proline and L-serine, but not potassium gluconate, L-rhamnose, suberic acid, lactic acid, 3-hydroxybenzoic acid

or L-histidine. Positive for aesculin hydrolysis, but negative for nitrate reduction, urease and oxidase.

The type strain is CCUG 43427A^T (=CCM 7900^T), isolated in Linköping, Sweden, from the eye of a patient suffering from endophthalmitis.

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