Massilia aerilata sp. nov., isolated from an air sample

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A novel aerobic, Gram-negative, rod-shaped, motile bacterium, designated strain 5516S-11^T, was isolated from air samples collected in the Suwon region of the Republic of Korea. Analysis of the 16S rRNA gene sequence indicated that the organism belongs to the genus *Massilia*; the highest sequence similarity (97.2 %) was found with respect to *Massilia aurea* DSM 18055^T. Cells of strain 5516S-11^T contained ubiquinone Q-8 as the predominant isoprenoid quinone and possessed summed feature 3 ($C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH; 35.2 %), $C_{16:0}$ (30.6 %) and $C_{18:1}\omega7c$ (11.7 %) as the major fatty acids. DNA–DNA hybridization revealed 32 % relatedness between strain 5516S-11^T and *M. aurea* DSM 18055^T. The G+C content of the DNA of strain 5516S-11^T was 68.9 mol%. It is clear from the genotypic and phenotypic data presented that strain 5516S-11^T represents a novel species of the genus *Massilia*, for which the name *Massilia aerilata* sp. nov. is proposed. The type strain is 5516S-11^T (=KACC 12505^T =DSM 19289^T).

The genus *Massilia* was first proposed with the species *Massilia timonae* for a strain isolated from the blood of an immunocompromised patient with cerebellar lesions (La Scola *et al.*, 1998). Since then, five further species have been described: *Massilia dura*, *M. albidiflava*, *M. plicata*, *M. lutea* and *M. aurea* (Zhang *et al.*, 2006; Gallego *et al.*, 2006). The genus was characterized as comprising motile, rod-shaped bacteria containing $C_{16:0}$ and summed feature 3 as the major fatty acids and Q-8 as the predominant isoprenoid quinone and showing relatively high G+C contents (62–67 mol%).

In the course of a study on the bacterial populations in air samples, we isolated a yellow-coloured bacterial strain. Air samples were collected in the Suwon region by using an MAS-100 air sampler (single-stage multiple-hole impactor; Merck) that contained Petri dishes with R2A medium (BBL) supplemented with cycloheximide (Sigma) at 200 μg ml^{-1}. After sampling, plates were incubated at 28 °C for 5 days and strain 5516S-11^T was recovered.

Cell morphology and motility were observed with phase-contrast microscopy and transmission electron microscopy, using cells negatively stained with 0.5 % uranyl acetate after growth on R2A for 2 days. The methods of Smibert & Krieg (1994) were used to determine the following: Gram stain, catalase and oxidase activities and hydrolysis of CM-cellulose, casein, chitin from crab shells, DNA, hypoxanthine, pectin, starch, Tween 80, tyrosine and xanthine. The optimum pH for growth was examined in R2A broth adjusted to various pHs (pH 4–10, in increments of 1.0 pH unit). The tolerance of strain 5516S-11^T to various NaCl concentrations (0, 1, 2, 3 and 5 %, w/v) was tested in R2A broth. The optimum temperature for growth of the strain was examined at various temperatures (5–45 °C, in increments of 5 °C) on R2A agar. Growth under anaerobic conditions was determined after incubating the strain in a GasPak (BBL) jar at 30 °C for 15 days. Physiological and biochemical properties were investigated further using API ZYM, API 20NE and API ID 32GN kits (bioMérieux). Commercial tests were generally performed according to the manufacturer's instructions. The API ZYM test strip was read after 4 h incubation at 37 °C and the other API test strips were examined after 5 days at 28 °C. Phenotypic data for strain 5516S-11^T and recognized *Massilia* species are compared in Table 1.

Whole-cell fatty acids were analysed according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Isoprenoid quinones were extracted and analysed as described by Groth *et al.* (1996). The DNA G+C content was determined by means

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5516S-11^T is EF688526.

Table 1. Differential phenotypic characteristics of strain 5516S-11^T and type strains of *Massilia* species

Strains: 1, 5516S-11^T; 2, *M. albidiflava* DSM 17472^T; 3, *M. aurea* DSM 18055^T; 4, *M. dura* DSM 17513^T; 5, *M. lutea* DSM 17473^T; 6, *M. plicata* DSM 17505^T; 7, *M. timonae* DSM 16850^T. Data were obtained in this study unless indicated. All strains are catalase-positive. In API 20NE test strips, all strains are positive for hydrolysis of aesculin and gelatin but negative for indole production, glucose fermentation and arginine dihydrolase. In API 20NE test strips, all strains assimilate D-mannose and maltose but not D-mannitol or capric acid. In API ZYM test strips, all strains are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase but negative for lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive; (+), weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7
Isolation source	Air	Soil	Water	Soil	Soil	Soil	Blood
Oxidase	+	+[a*]	(+)[b]	+[a]	+[a]	−[a]	+[c]
Starch hydrolysis	−	+[a]	+[b]	−[b]	+[b]	+[b]	+[c]
Nitrate reduction	+	+	−	−	−	−	−
Urease	+	−	−	−	−	−	−
β-Galactosidase	−	+	+	+	+	−	+
Assimilation of:							
D-Glucose	+	+	+	+	−	+	+
L-Arabinose	+	+	−	+	+	+	+
N-Acetylglucosamine	−	+	+	−	+	−	−
Potassium gluconate	−	+	−	+	+	+	+
Adipic acid	−	+	+	−	+	−	−
Malic acid	+	−	+	+	−	+	+
Trisodium citrate	−	−	+	−	−	−	+
Phenylacetic acid	+	−	−	−	−	−	+
Enzyme activities							
Cystine arylamidase	+	−	−	−	−	−	+
α-Galactosidase	−	+	−	+	−	+	−
β-Galactosidase	−	+	(+)	−	−	−	−
β-Glucosidase	−	+	−	−	−	−	(+)
DNA G+C content (mol%)	68.9	65.3[a]	66.0[b]	65.9[a]	63.3[a]	65.1[a]	62–67[c]

*Data from: *a*, Zhang *et al.* (2006); *b*, Gallego *et al.* (2006); *c*, Lindquist *et al.* (2003).

of HPLC analysis of deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco).

The major fatty acids of strain 5516S-11^T were summed feature 3 ($C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH; 35.2 %), $C_{16:0}$ (30.6 %) and $C_{18:1}\omega7c$ (11.7 %) (Table 2). The predominant isoprenoid quinone was Q-8. The DNA G+C content of strain 5516S-11^T was 68.9 mol%.

The 16S rRNA gene was amplified by using a PCR with primers fD1 and rP2 (Weisburg *et al.*, 1991) on colonies; the entire PCR fragment was directly sequenced (Hiraishi, 1992). The closest known relatives of the novel isolate were determined by performing GenBank/EMBL/DDBJ database searches. After multiple alignments of data by CLUSTAL W (Thompson *et al.*, 1994), the software package MEGA, version 3.1 (Kumar *et al.*, 2004), was used for all analyses. Phylogenetic dendrograms were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with bootstrap values based on 1000 replications. DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG High Prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

A phylogenetic analysis based on 16S rRNA gene sequences showed that strain 5516S-11^T falls within the genus *Massilia* (Fig. 1). Strain 5516S-11^T exhibited 16S rRNA gene sequence similarity values of 95.1–97.2 % with respect to the type strains of recognized *Massilia* species, the highest similarity value (97.2 %) being found with the sequence of *M. aurea* DSM 18055^T. Although the two recognized species of the genus *Telluria* also clustered with members of the genus *Massilia*, their phylogenetic position was unstable (low bootstrap values) (Fig. 1). Strain 5516S-11^T showed lower levels of sequence similarity (<95 %) with respect to the two *Telluria* species than with respect to *Massilia* species. The maximum-parsimony tree also supported the clustering of 5516S-11^T and *M. aurea* DSM 18055^T shown in the neighbour-joining tree. The value for DNA–DNA hybridization between strain 5516S-

Table 2. Fatty acid compositions of strain 5516S-11^T and type strains of *Massilia* species

Strains: 1, 5516S-11^T; 2, *M. albidiflava* DSM 17472^T; 3, *M. aurea* DSM 18055^T; 4, *M. dura* DSM 17513^T; 5, *M. lutea* DSM 17473^T; 6, *M. plicata* DSM 17505^T (data from Zhang *et al.*, 2006); 7, *M. timonae* DSM 16850^T. All data except those for *M. plicata* DSM 17505^T were obtained in this study. All strains except *M. albidiflava* DSM 17472^T were grown at 28 °C on R2A for 2 days; cells of *M. albidiflava* DSM 17472^T were harvested after growth at 28 °C on trypticase soy agar for 2 days. –, <1 % or not detected.

Fatty acid	1	2	3	4	5	6	7
$C_{10:0}$ 3-OH	4.7	7.0	6.0	5.5	5.7	10.1	4.6
$C_{12:0}$	3.4	5.3	4.4	3.9	4.0	7.1	3.3
$C_{12:0}$ 2-OH	–	–	–	–	–	1.6	2.2
$C_{14:0}$	1.9	2.6	2.0	1.1	1.2	–	–
$C_{14:0}$ 2-OH	2.5	2.4	2.5	2.6	2.9	6.1	–
$C_{16:0}$	30.6	23.4	27.2	27.5	26.6	25.1	30.5
$C_{17:0}$ cyclo	6.1	–	–	–	–	–	3.7
$C_{18:1}\omega7c$	11.7	7.4	7.92	7.03	7.8	11.7	7.87
Summed feature 3*	35.2	46.0	48.6	52.0	51.1	36.9	47.0

*Summed feature 3 included $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

11^T and *M. aurea* DSM 18055^T was 32 %. This level of relatedness was significantly lower than the threshold accepted as the phylogenetic definition of a species (Wayne *et al.*, 1987).

As shown in Tables 1 and 2, strain 5516S-11^T can be differentiated from other *Massilia* species on the basis of basic biochemical properties, assimilation profiles and the presence of a moderate amount (6.1 %) of $C_{17:0}$ cyclo. In particular, strain 5516S-11^T could be distinguished from its closest phylogenetic relative, *M. aurea* DSM 18055^T, by their very different assimilation patterns and enzyme activities; the former showed a negative reaction for starch hydrolysis and β-galactosidase and demonstrated nitrate reduction and urease activity. Therefore, on the basis of the phenotypic, phylogenetic and genetic data, strain 5516S-11^T should be classified within the genus *Massilia* as a member of a novel species, for which the name *Massilia aerilata* sp. nov. is proposed.

Description of *Massilia aerilata* sp. nov.

Massilia aerilata (ae.ri.la′ta. L. n. *aer* air; L. part. adj. *latus* -*a* -*um* carried; N.L. fem part. adj. *aerilata* airborne).

Cells are aerobic, Gram-negative, rod-shaped bacteria, 0.7–10 μm wide and 1.5–3.0 μm long, and are motile by means of single polar flagella. Colonies are light yellow, round and convex with clear margins. Grows well on R2A, nutrient agar (Difco) and trypticase soy agar (Difco) but does not grow on MacConkey agar (Difco). Catalase- and oxidase-positive. Grows at 5–35 °C (optimum, 28 °C) on R2A. The pH range for growth is 5–9 (optimum, pH 6–8). Grows with 0–1 % NaCl (w/v). Degrades casein, hypoxanthine, tyrosine and Tween 80, but does not degrade chitin, DNA, pectin, starch or xanthine. Assimilates L-alanine, L-arabinose, D-glucose, glycogen, L-histidine, 3-hydroxybutyric acid, malic acid, maltose, D-mannose, phenylacetic acid, L-proline and L-serine. Weakly assimilates propionic acid and sodium acetate. Does not assimilate *N*-acetylglucosamine, adipic acid, capric acid, L-fucose, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, inositol, itaconic acid, lactic acid, D-mannitol, melibiose, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, L-rhamnose, D-ribose, sucrose, sodium malonate, suberic acid, salicin, D-sorbitol, trisodium citrate or valeric acid (API 20NE and API ID 32GN test strips). The major fatty acids are summed feature 3 ($C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH; 35.2 %), $C_{16:0}$ (30.6 %) and $C_{18:1}\omega7c$ (11.7 %). The predominant ubiquinone is Q-8. The DNA G+C content of the type strain is 68.9 mol%. The species is most closely related phylogenetically to *M. aurea*.

The type strain, 5516S-11^T (=KACC 12505^T =DSM 19289^T), was isolated from an air sample collected from Suwon, Republic of Korea.

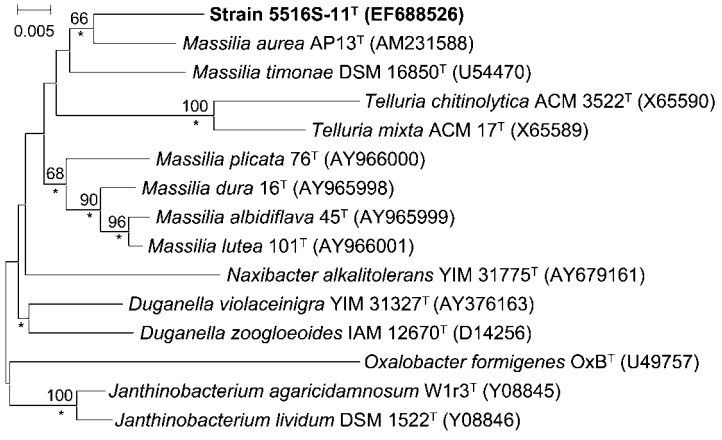

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain 5516S-11^T and closely related species. Asterisks indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bootstrap percentages are shown at branch points; percentages below 50 % are not indicated. Bar, 0.005 changes per nucleotide position.

Acknowledgements

This work was supported by a grant (no. 06-4-11-19-1) from the National Institute of Agricultural Biotechnology, Rural Development Administration, Republic of Korea.

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