Massilia niabensis sp. nov. and *Massilia niastensis* sp. nov., isolated from air samples

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Two bacterial isolates, designated strains 5420S-26^T and 5516S-1^T, were recovered from air samples collected in Suwon, Korea. Cells of both strains were aerobic, Gram-negative, motile rods. Phylogenetically, these strains were positioned within the radius of the genus *Massilia*. 16S rRNA gene sequence analysis showed that the strains shared 97.3 % sequence similarity and had sequence similarities of 94.9–98.1 % with respect to type strains of species belonging to the genus *Massilia*. In DNA–DNA hybridization tests, the two strains showed <39 % relatedness with respect to strains of closely related species of the genus *Massilia* and 27 % relatedness to each other. Both strains contained Q-8 as the predominant isoprenoid quinone and possessed summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) as the major fatty acid. Strain 5516S-1^T was found to contain the fatty acid $C_{20:0}$ (in small amounts), a feature that served to distinguish it from both 5420S-26^T and recognized members of the genus *Massilia*. The DNA G+C contents of 5420S-26^T and 5516S-1^T were 67.8 and 66.6 mol%, respectively. Phylogenetic, phenotypic and chemotaxonomic data accumulated in this study revealed that 5420S-26^T and 5516S-1^T represent novel species of the genus *Massilia*, for which the names *Massilia niabensis* sp. nov. (type strain 5420S-26^T =KACC 12632^T =DSM 21312^T) and *Massilia niastensis* sp. nov. (type strain 5516S-1^T =KACC 12599^T =DSM 21313^T) are proposed, respectively.

The genus *Massilia* was first proposed by La Scola *et al.* (1998) for an isolate from the blood of an immunocompromised patient with cerebellar lesions. Since then, other *Massilia* strains have been isolated from air, soil and water (Gallego *et al.*, 2006; Zhang *et al.*, 2006; Weon *et al.*, 2008; Zul *et al.*, 2008). The genus comprises aerobic, Gram-negative, motile, non-spore-forming rods or short rods. Summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{16:0}$ are the major fatty acids, Q-8 is the predominant isoprenoid quinone and the DNA G+C content is relatively high (63.3–68.9 mol%).

Air samples were collected in the Suwon region of Korea (37° 16′ 46″ N 126° 59′ 10″ E) by using an MAS-100 air sampler (single-stage multiple-hole impactor; Merck) that contained Petri dishes with R2A medium (Reasoner & Geldreich, 1985) supplemented with cycloheximide (Sigma) at 200 μg ml^{-1}. After incubation at 28 °C for 5 days, two bacterial strains, designated 5420S-26^T and 5516S-1, were isolated that were shown to be phylogenetically related to members of the genus *Massilia*.

After 24 h incubation at 28 °C, the cell morphologies of these novel strains were examined by means of light microscopy (Axio; Zeiss) and transmission electron microscopy (model 912AB; LEO). Phenotypic characteristics such as Gram staining, catalase activity, oxidase activity and hydrolysis of CM-cellulose, casein, chitin, DNA, hypoxanthine, pectin, tyrosine, Tween 80, starch and xanthine were performed using the methods of Smibert & Krieg (1994). The pH range for growth was determined in R2A broth adjusted to pH 4.0–10.0, in increments of 1.0 pH unit, with citrate/phosphate or Tris/HCl (Breznak & Costilow, 1994). Growth at 0, 1, 2, 3 and 5 % NaCl (w/v) was investigated in R2A broth. Growth at various

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 5516S-1^T and 5420S-26^T are EU808005 and EU808006.

temperatures (5–45 °C) was measured on R2A agar. Growth under anaerobic conditions was tested in GasPak (BBL) jars at 28 °C for 15 days. Physiological and biochemical properties were further determined with API 20NE, API ID 32GN and API ZYM strips (all from bioMérieux). Tests involving commercial systems 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.

The presence of isoprenoid quinones was investigated using HPLC, as described previously (Groth *et al.*, 1996). Fatty acid methyl esters were extracted and prepared using the standard protocol of the Microbial Identification System (MIDI; Microbial ID) with cells that had been grown on R2A for 48 h at 28 °C. DNA G+C contents were determined as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco).

Colonies were used for PCR amplification of the 16S rRNA genes, using primers fD1 and rP2 (Weisburg *et al.*, 1991); entire PCR fragments were sequenced directly (Hiraishi, 1992). The software package MEGA 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 using the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High Prime system (Roche) and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

Both strains were aerobic, mesophilic, catalase- and oxidase-positive bacteria comprising rod-shaped cells. They grew well on R2A and nutrient agar (Difco) but did not grow on trypticase soy agar (Difco) or MacConkey agar (Difco). Differential phenotypic characteristics of the isolates and recognized members of the genus *Massilia* are shown in Table 1.

Both strains contained ubiquinone Q-8 as the predominant isoprenoid quinone. In terms of fatty acid composition, strain 5420S-26^T contained summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 46.0 %), $C_{16:0}$ (23.6 %), $C_{12:0}$ (8.9 %), $C_{18:1}\omega7c$ (7.8 %) and $C_{10:0}$ 3-OH (6.6 %), while strain 5516S-1^T contained summed feature 3 (38.1 %), $C_{16:0}$ (26.9 %), $C_{18:1}\omega7c$ (12.3 %), $C_{10:0}$ 3-OH (5.5 %), $C_{12:0}$ (4.7 %), $C_{20:0}$ (2.6 %) and $C_{12:0}$ 2-OH (2.4 %). Comparisons of the fatty acid compositions of the novel isolates and recognized members of the genus *Massilia* are shown in Table 2.

For the phylogenetic analysis, 1398 and 1394 bp stretches, respectively, of the 16S rRNA gene sequences for strains 5420S-26^T and 5516S-1^T were used. The sequence similarity between 5420S-26^T and 5516S-1^T was 97.3 %. Strain 5420S-26^T showed 95.3–98.1 % sequence similarity with respect to the type strains of the genus *Massilia*, having the highest level of similarity with the sequence of *Massilia brevitalea* byr23-80^T (98.1 %). Strain 5516S-1^T shared sequence similarities in the range 94.9–98.1 % with respect to the type strains of the other species of the genus *Massilia*, the highest level of similarity being found with the sequence of *Massilia aerilata* KACC 12505^T (98.1 %). Both strains showed relatively low sequence similarities (<96 %) with respect to members of other genera. The neighbour-joining phylogenetic tree (Fig. 1) clearly showed both strains to be members of the genus *Massilia*, revealing that strains 5420S-26^T and 5516S-1 clustered with *Massilia aurea* AP13^T, *M. brevitalea* byr23-80^T and *M. aerilata* KACC 12505^T; the maximum-parsimony tree supported the phylogeny obtained with neighbour joining. In DNA–DNA hybridization tests, the relatedness between 5420S-26^T and *M. brevitalea* DSM 18925^T, *M. aurea* DSM 18055^T and strain 5516S-1^T was 39, 30 and 26 %, respectively. For 5516S-1^T, the values obtained with respect to *M. aerilata* KACC 12505^T and strain 5420S-26^T were 37 and 28 %, respectively.

The phylogenetic and DNA–DNA hybridization data showed that the novel isolates were members of the genus *Massilia*, but that they represented two genomic species separable from recognized *Massilia* species. Phenotypically, strain 5420S-26^T can be differentiated from 5516S-1^T on the basis of the former's ability to reduce nitrate, its inability to hydrolyse aesculin or gelatin and its inability to assimilate various substrates. In addition, strain 5420S-26^T differs from *M. brevitalea* DSM 18925^T in having oxidase, β-galactosidase and β-glucuronidase activities, lacking the ability to hydrolyse aesculin and casein and being unable to assimilate various substrates. Strain 5516S-1^T showed β-galactosidase activity, weak hydrolysis of starch, assimilation of *N*-acetylglucosamine and maltose and the absence of nitrate reduction, urease activity, casein hydrolysis, esterase lipase (C8) activity and α-glucosidase, unlike its closest relative, *M. aerilata* KACC 12505^T.

Therefore, on the basis of the phenotypic and genotypic data presented, strains 5420S-26^T and 5516S-1^T represent two novel species of the genus *Massilia*, for which the names *Massilia niabensis* sp. nov. and *Massilia niastensis* sp. nov., respectively, are proposed.

Description of *Massilia niabensis* sp. nov.

Massilia niabensis (ni.a.ben′sis. N.L. fem. adj. *niabensis* pertaining to NIAB, the National Institute of Agricultural Biotechnology, where taxonomic studies of the type strain were conducted).

Cells are aerobic, Gram-negative rods (0.6–0.9 × 1.4–4.0 μm) that are motile by means of single polar flagella. Colonies are yellowish white, round and convex with clear margins. Grows well on R2A and nutrient agar, but does not grow on trypticase soy agar or MacConkey agar. Catalase- and oxidase-positive. Grows at 5–35 °C (optim-

Table 1. Differential properties of strains 5420S-26^T and 5516S-1^T and type strains of recognized members of the genus *Massilia*

Strains: 1, 5420S-26^T; 2, 5516S-1^T; 3, *M. aerilata* KACC 12505^T; 4, *M. albidiflava* DSM 17472^T; 5, *M. aurea* DSM 18055^T; 6, *M. brevitalea* DSM 18925^T; 7, *M. dura* DSM 17513^T; 8, *M. lutea* DSM 17473^T; 9, *M. plicata* DSM 17505^T; 10, *M. timonae* DSM 16850^T. Data for reference strains were taken from Zul *et al.* (2008) (*M. brevitalea* DSM 18925^T) and Weon *et al.* (2008) (remaining strains) unless otherwise indicated. In API 20NE test strips, all of the strains are negative for indole production, glucose fermentation and arginine dihydrolase. In API ZYM test strips, all of the strains are positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), trypsin, α-chymotrypsin, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive; (+), weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10
Catalase/oxidase	+/+	+/+	+/+	+/+[a*]	+/(+)[b]	(+)/−	+/+[a]	+/+[a]	+/−[a]	+/+[c]
Nitrate reduction	+	−	+	+	−	+	−	−	−	−
Urease	−	−	+	−	−	−[d]	−	−	−	−
β-Galactosidase	+	+	−	+	+	−[d]	+	+	−	+
Hydrolysis of:										
Aesculin	−	+	+	+	+	+[d]	+	+	+	+
Casein	−	−	+	+[e]	+[e]	+	+[e]	+[e]	+[e]	ND
Gelatin	−	+	+	+	+	−	+	+	+	+
Starch	+	(+)	−	+[e]	+[e]	+	−[e]	+[e]	+[e]	+[e]
Assimilation of:										
D-Glucose	−	+	+	+	+	+[d]	+	−	+	+
L-Arabinose	−	+	+	+	−	−[d]	+	+	+	+
D-Mannose	−	+	+	+	+	−[d]	+	+	+	+
D-Mannitol	−	−	−	−	−	−[d]	−	−	−	−
N-Acetylglucosamine	−	+	−	+	+	−[d]	−	+	−	−
Maltose	−	+	+	+	+	+[d]	+	+	+	+
Potassium gluconate	−	+	−	+	−	−[d]	+	+	+	+
Capric acid	−	−	−	−	−	−[d]	−	−	−	−
Adipic acid	−	−	−	+	+	+[d]	−	+	−	−
Malic acid	−	+	+	−	+	+[d]	+	−	+	+
Trisodium citrate	−	−	−	−	+	−[d]	−	−	−	+
Phenylacetic acid	−	−	+	−	−	−[d]	−	−	−	+
Enzyme activities										
Esterase lipase (C8)	+	−	+	+	+	+[d]	+	+	+	+
Cystine arylamidase	−	+	+	−	−	−[d]	−	−	−	+
α-Galactosidase	−	−	−	+	−	−[d]	+	−	+	−
β-Glucuronidase	+	−	−	−	−	−[d]	−	−	−	−
α-Glucosidase	+	−	+	−	(+)	+[d]	−	−	−	−
β-Glucosidase	−	−	−	+	−	−[d]	−	−	−	(+)
DNA G+C content (mol%)	67.8	66.6	68.9	65.3[a]	66.0[b]	65.3	65.9[a]	63.3[a]	65.1[a]	64.6[f]

*Data taken from: *a*, Zhang *et al.* (2006); *b*, Gallego *et al.* (2006); *c*, Lindquist *et al.* (2003); *d*, this study; *e*, Zul *et al.* (2008); *f*, La Scola *et al.* (1998).

ally at 28 °C) on R2A, at pH 7–9 (optimally at pH 7) and 0–1 % NaCl. Positive for nitrate reduction and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis and gelatin hydrolysis (API 20NE test strips). Degrades hypoxanthine, starch, tyrosine and Tween 80, but does not degrade casein, chitin, CM-cellulose, DNA, pectin or xanthine. Does not assimilate any of the substrates embedded in the API 20NE and API ID 32GN test strips. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase and α-glucosidase, but negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM test strips). The major fatty acids (>10 %) are summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{16:0}$. The predominant ubiquinone is Q-8. The DNA G+C content of the type strain is 67.8 mol%.

The type strain, 5420S-26^T (=KACC 12632^T =DSM 21312^T), was isolated from an air sample collected from Suwon, Republic of Korea.

Description of *Massilia niastensis* sp. nov.

Massilia niastensis (ni.as.ten′sis. N.L. fem. adj. *niastensis* pertaining to NIAST, the National Institute of Agricultural

Table 2. Cellular fatty acid compositions (%) of strains 5420S-26^T and 5516S-1^T and type strains of recognized members of the genus *Massilia*

Strains: 1, 5420S-26^T; 2, 5516S-1^T; 3, *M. aerilata* KACC 12505^T; 4, *M. albidiflava* DSM 17472^T; 5, *M. aurea* DSM 18055^T; 6, *M. brevitalea* DSM 18925^T; 7, *M. dura* DSM 17513^T; 8, *M. lutea* DSM 17473^T; 9, *M. plicata* DSM 17505^T; 10, *M. timonae* DSM 16850^T. All data except those for *M. plicata* DSM 17505^T (data from Zhang *et al.*, 2006) were obtained in this study. All strains except *M. albidiflava* DSM 17472^T and *M. brevitalea* DSM 18925^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, while cells of *M. brevitalea* DSM 18925^T were harvested after growth at 15 °C for 2 days on R2A. –, Not detected or <1 %.

Fatty acid	1	2	3	4	5	6	7	8	9	10
$C_{10:0}$ 3-OH	6.6	5.5	4.7	7.0	6.0	4.9	5.5	5.7	10.1	4.6
$C_{12:0}$	8.9	4.7	3.4	5.3	4.4	5.0	3.9	4.0	7.1	3.3
$C_{12:0}$ 2-OH	–	2.4	–	–	–	2.0	–	–	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	–
$C_{16:0}$	23.6	26.9	30.6	23.4	27.2	23.0	27.5	26.6	25.1	30.5
$C_{17:0}$ cyclo	–	3.4	6.1	–	–	–	–	–	–	3.7
$C_{18:1}\omega7c$	7.8	12.3	11.7	7.4	7.92	9.0	7.03	7.8	11.7	7.9
$C_{20:0}$	–	2.6	–	–	–	–	–	–	–	–
Summed feature 3*	46.0	38.1	35.2	46.0	48.6	54.2	52.0	51.1	36.9	47.0

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprised $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

Science and Technology, where taxonomic studies of the type strain were conducted).

Cells are aerobic, Gram-negative rods (0.6–0.8 × 1.5–5.0 μm) that are motile by means of single polar flagella. Colonies are ivory-coloured, round and convex with clear margins. Grows well on R2A and nutrient agar, but does not grow on trypticase soy agar or MacConkey agar. Catalase- and oxidase-positive. Grows at 5–40 °C (optimally at 28 °C) on R2A, at pH 6–8 (optimally at pH 7) and 0–1 % NaCl. Positive for aesculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease (API 20NE test strips). Degrades hypoxanthine, starch and tyrosine, but does not degrade casein, chitin, CM-cellulose, DNA, pectin, Tween 80 or xanthine. Assimilates D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine, maltose, potassium gluconate, malic acid, L-rhamnose, itaconic acid, sodium acetate, L-alanine, 3-hydroxybenzoic acid, salicin, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline, but not D-mannitol, capric acid, adipic acid, trisodium citrate, phenylacetic acid, D-ribose, inositol, sucrose, suberic acid, sodium malonate, lactic acid, potassium 5-ketogluconate, glycogen, L-serine, melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine or potassium 2-ketogluconate (API 20NE and API ID 32GN test strips). Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for esterase lipase (C8), lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosi-

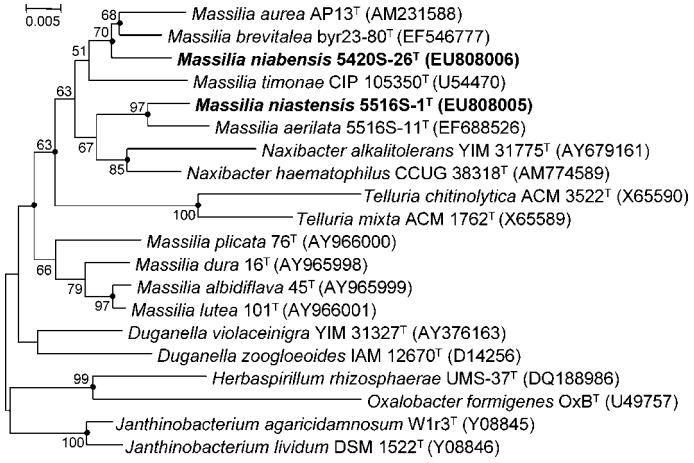

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strains 5420S-26^T and 5516S-1^T with respect to closely related species. Numbers at nodes indicate bootstrap percentages (based on 1000 replications); only values >50 % are shown. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.005 accumulated changes per nucleotide.

dase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM test strips). The major fatty acids (>10 %) are summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{16:0}$ and $C_{18:1}\omega7c$. The predominant ubiquinone is Q-8. The DNA G+C content of the type strain is 66.6 mol%.

The type strain, 5516S-1^T (=KACC 12599^T =DSM 21313^T), was isolated from an air sample collected from Suwon, Republic of Korea.

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