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Massilia tieshanensis sp. nov., isolated from mining soil

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A bacterial isolate, designated strain TS3^T, was isolated from soil collected from a metal mine in Tieshan District, Daye City, Hubei Province, in central China. Cells of this strain were Gram-negative, motile and rod-shaped. The strain had ubiquinone Q-8 as the predominant respiratory quinone, phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as the major polar lipids and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{16:0}$ and $C_{18:1}\omega7c$ as the major fatty acids. The G+C content was 65.9 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain TS3^T was most closely related to *Massilia niastensis* 5516S-1^T (98.5 %), *Massilia consociata* CCUG 58010^T (97.6 %), *Massilia aerilata* 5516S-11^T (97.4 %) and *Massilia varians* CCUG 35299^T (97.2 %). DNA–DNA hybridization revealed low relatedness between strain TS3^T and *M. niastensis* KACC 12599^T (36.5 %), *M. consociata* CCUG 58010^T (27.1 %), *M. aerilata* KACC 12505^T (22.7 %) and *M. varians* CCUG 35299^T (46.5 %). On the basis of phenotypic and phylogenetic characteristics, strain TS3^T belongs to the genus *Massilia*, but is clearly differentiated from other members of the genus. The strain represents a novel species, for which the name *Massilia tieshanensis* sp. nov. is proposed. The type strain is TS3^T (=CCTCC AB 2010202^T =KACC 14940^T).

The genus *Massilia* belongs to the family *Oxalobacteraceae*, class *Betaproteobacteria*, and was described by La Scola *et al.* (1998) on the basis of *Massilia timonae*, isolated from blood of an immunocompromised patient with cerebellar lesions. Subsequently, several strains belonging to *M. timonae* have been isolated from different patients (Sintchenko *et al.*, 2000; Lindquist *et al.*, 2003). At the time of writing, 18 species names have been validly published in the genus *Massilia*: *M. timonae* (type species), *M. dura*, *M. albidiflava*, *M. plicata*, *M. lutea*, *M. aurea*, *M. brevitalea*, *M. aerilata*, *M. niabensis*, *M. niastensis*, *M. jejuensis*, *M. consociata*, *M. oculi*, *M. alkalitolerans*, *M. varians*, *M. haematophila*, *M. suwonensis* and *M. flava*. Members of the genus have been isolated from various habitats such as air, soil, water and humans with different health problems (La Scola *et al.*, 1998; Xu *et al.*, 2005; Zhang *et al.*, 2006; Gallego *et al.*, 2006; Zul *et al.*, 2008; Weon *et al.*, 2008, 2009, 2010; Kämpfer *et al.*, 2008, 2011, 2012; Wang *et al.*, 2012). *M. alkalitolerans* (Xu *et al.*, 2005), *M. varians*, *M. haematophila* (Kämpfer *et al.*, 2008) and *M. suwonensis* (Weon *et al.*, 2010) were originally assigned to the genus *Naxibacter*. However, according to data reported by Kämpfer *et al.* (2008) and Weon *et al.* (2010), there are no genus-specific distinctions between *Massilia* and *Naxibacter*. Kämpfer *et al.* (2011) proposed to transfer all *Naxibacter* species to the genus *Massilia*. The typical characteristics of members of *Massilia* are aerobic metabolism, Gram-negative staining behaviour, motile, rod-shaped cells, summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{16:0}$ as major fatty acids and ubiquinone Q-8 as the predominant respiratory quinone, with relatively high DNA G+C contents (62–68.9 mol%). The major polar lipids have been determined for some species of the genus as phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol (Kämpfer *et al.*, 2011, 2012, 2008; Wang *et al.*, 2012; Weon *et al.*, 2010).

In a previous study, 45 arsenic-resistant bacteria were isolated from mining soil in Tieshan District (30° 12′ N 114° 53′ E), Daye City, Hubei Province, central China (Cai *et al.*, 2009). The arsenic concentration of the soil was 337.2 mg kg^{-1} (Cai *et al.*, 2009) and the pH was 7.4. Concentrations of arsenic and other heavy metals are extremely high in the water, soil and sediments of this area due to the smelting of gold, copper and iron for many years (Zhang *et al.*, 2005). By analysis of partial 16S rRNA gene sequences (about 1300 bp), these bacteria were preliminarily identified as members of the genera *Acinetobacter*, *Delftia*, *Arthrobacter*, *Microbacterium*, *Aeromonas*, *Acidovorax*, *Ensifer*, *Janibacter*, *Janthinobacterium*, *Klebsiella*, *Kocuria*, *Micrococcus*, *Shewanella* and *Thauera*

Abbreviation: MIC, minimium inhibitory concentration.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TS3^T is HM130516.

Three supplementary figures are available with the online version of this paper.

(Cai *et al.*, 2009). One strain, previously named *Janthinobacterium* sp. TS3, was chosen for study because of its undefined taxonomic position.

Strain identification was performed according to the recommendations for characterization of prokaryote strains of Tindall *et al.* (2010). All physiological and biochemical tests were performed at 28 °C unless otherwise mentioned. Cellular morphology was observed by light microscopy and transmission electron microscopy after 24 h of growth on R2A medium (Difco; in 1 l distilled water, 0.5 g yeast extract, 0.5 g proteose peptone, 0.5 g Casamino acids, 0.5 g glucose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g dipotassium phosphate, 0.05 g magnesium sulfate). For transmission electron microscopy, cells were negatively stained with 1 % (w/v) phosphotungstic acid. The motility of bacterial cells was observed on R2A swarming agar (0.3 %, w/v). Oxidase activity was measured using the API 20 NE test kit (bioMérieux) according to the manufacturer's instructions. Catalase activity was tested using young cells mixed with 3 % (v/v) H_2O_2 and monitoring for the production of oxygen bubbles (Gallego *et al.*, 2006). Gram-staining was performed as described by Gerhardt *et al.* (1994) combined with the KOH lysis method (Ryu, 1938). Methyl red and Voges–Proskauer tests and H_2S production were analysed as described by Smibert & Krieg (1994). Hydrolysis of casein, aesculin, gelatin, urea, starch, Tween 80, DNA, L-tyrosine, chitin from crab shells and CM-cellulose was examined as described by Cowan & Steel (1965). Enzyme activities, other biochemical characteristics and utilization of carbohydrates were determined using API ZYM, API 20 NE and API ID 32 GN kits, respectively, according to the manufacturer's instructions (bioMérieux). API ZYM test strips were read after 5 h of incubation at 37 °C. The API 20 NE and API ID 32 GN tests were examined after incubation at 28 °C for 48 h and 72 h, respectively.

Growth at 4, 20, 28, 32, 37 and 42 °C, pH 4–10 and 0, 1, 2, 3, 4 and 5 % (w/v) NaCl was assessed after 7 days of incubation in R2A broth (Difco). For pH tests, the broth was buffered with citrate/phosphate (pH 4.0–4.5), 10 mM MES (pH 5.0–6.0), 10 mM PIPES (pH 6.5–7.0) or 10 mM Tris/HCl (7.5–10) and adjusted with NaOH or HCl. Growth was monitored by measuring the OD_{600}. Anaerobic growth was examined by incubation in an anaerobic chamber (Mitsubishi Gas Chemical Co, Inc.) at 28 °C for 7 days on R2A agar. Antibiotic susceptibility tests were performed by spreading bacterial suspensions on culture plates and applying filter-paper discs containing different antibiotics according to the manufacturer's recommendation (Hangzhou Microbial Reagent Co., Ltd). Susceptibility was scored for inhibition zone diameters above 10 mm. All tests were repeated at least twice. The minimium inhibitory concentration (MIC), defined as the lowest metal(loid) concentration that completely inhibited growth, was determined for TS3^T as described by Lim & Cooksey (1993). Triplicate samples from single bacterial colonies were grown overnight at 28 °C with shaking at 160 r.p.m. Next, 2 % original culture was inoculated into 5 ml aliquots of R2A broth, each supplemented with serial concentrations of $NaAsO_2$, $CuSO_4 . 5H_2O$, $K_2(C_4H_2O_6Sb)_2 . 3H_2O$, $ZnSO_4 . 7H_2O$, $NiSO_4 . 6H_2O$ and $3CdSO_4 . 8H_2O$, to examine susceptibility to As^{3+}, Cu^{2+}, Sb^{3+}, Zn^{2+}, Ni^{2+} and Cd^{2+}, respectively. Growth was measured as OD_{600} after incubation at 28 °C with shaking at 160 r.p.m. for 7 days.

Genomic DNA extraction was carried out as described previously (Cashion *et al.*, 1977). The nearly complete 16S rRNA gene sequence was amplified by PCR using primers Uni-27F and Uni-1492R (Wilson *et al.*, 1990). The purified PCR product was ligated into the pGEM-T Easy vector (Promega). DNA sequencing was performed by the Beijing Genomics Institute (Beijing, China). The 16S rRNA gene sequence was compared with available sequences in the NCBI GenBank database and EzTaxon server 2.1. Gene sequences were aligned with those of related species using CLUSTAL_X software (Thompson *et al.*, 1997). Phylogenetic analysis was performed using MEGA version 4.0 (Tamura *et al.*, 2007) and PhyML software (Guindon & Gascuel, 2003). Distance options according to the Kimura-2 model (Kimura, 1980) were determined and clustered was performed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms. The robustness of the tree topologies was evaluated by calculating bootstrap values using 1000 resamplings (Felsenstein, 1985).

For cellular fatty acid analysis, strain TS3^T and four type strains with high 16S rRNA gene sequence similarity (*M. niastensis* KACC 12599^T, *M. consociata* CCUG 58010^T, *M. aerilata* KACC 12505^T and *M. varians* CCUG 35299^T) were grown at 28 °C for 48 h on R2A agar until exponential phase (Weon *et al.*, 2009) and the fatty acids were analysed by GC (Hewlett Packard 6890) according to the instructions of the Sherlock Microbial Identification System [MIDI, Sherlock version 4.5 (0209B), TSBA40 4.10] (Sasser, 1990). Respiratory quinone analysis was performed by HPLC as described by Minnikin *et al.* (1984). Polar lipid analysis of strain TS3^T and type strains *M. niastensis* KACC 12599^T and *M. aerilata* KACC 12505^T was conducted under the same experimental conditions by two-dimensional TLC as described by Minnikin *et al.* (1984). The DNA G+C content was determined by HPLC according to the method of Mesbah *et al.* (1989). DNA–DNA hybridization analysis between strain TS3^T and each of the four type strains *M. niastensis* KACC 12599^T, *M. consociata* CCUG 58010^T, *M. aerilata* KACC 12505^T and *M. varians* CCUG 35299^T was performed by the thermal denaturation and renaturation method (Huß *et al.*, 1983).

The detailed results of morphological, physiological and biochemical characterization of strain TS3^T are given in the species description. Strain TS3^T shared some properties with the type species, *M. timonae*, and other *Massilia* species, but also showed some differences (Table 1). The

strain could be distinguished from related type strains in colony morphology, hydrolysis of casein, starch and L-tyrosine, reduction of nitrate and utilization of L-rhamnose, lactic acid, glycogen, 3-hydroxybenzoic acid, valeric acid, L-histidine, malic acid and potassium gluconate as carbon sources. In contrast to other *Massilia* species, colonies of strain TS3^T were rough on R2A agar, and they were difficult to scrape. The MICs of strain TS3^T for As^{3+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Sb^{3+} and Cd^{2+} were 4.0, 4.0, 0.8, 0.6, 0.4 and 0.2 mM, respectively. Among these metal(loid)s, the MIC for Cu^{2+} was much higher than those of four closely related type strains (4.0 mM, compared with 0.5 mM for *M. niastensis* KACC 12599^T, *M. aerilata* KACC 12505^T and *M. varians* CCUG 35299^T and 0.3 mM for *M. consociata* CCUG 58010^T).

The 1452 bp 16S rRNA gene sequence of strain TS3^T was analysed to determine its phylogenetic position. Strain TS3^T shared sequence similarities in the range 95.1–98.5 % with the type strains of the other 18 species of the genus *Massilia*. The most closely related type strains were *M. niastensis* 5516S-1^T (98.5 %), *M. consociata* CCUG 58010^T (97.6 %), *M. aerilata* 5516S-11^T (97.4 %) and *M. varians* CCUG 35299^T (97.2 %). A neighbour-joining tree based on 16S rRNA gene sequences revealed that strain TS3^T formed a small phyletic lineage within the genus *Massilia* containing *M. niastensis* 5516S-1^T, *M. aerilata* 5516S-11^T and *M. consociata* CCUG 58010^T (Fig. 1). Strain TS3^T showed 96.5 % 16S rRNA gene sequence identity to the type strain of the type species, *M. timonae*, and was grouped into a large cluster containing *M. timonae* and the other 17 *Massilia* species. The maximum-parsimony and maximum-likelihood trees (Fig. S1, available in IJSEM Online) supported the phylogeny obtained with the neighbour-joining analysis. In all three phylogenetic trees, the two *Telluria* species were grouped together with the *Massilia* species (Fig. 1); however, strain TS3^T shared lower 16S rRNA gene sequence similarities with the type strains of the two *Telluria* species (94 and 93 %, for *Telluria chitinolytica* and *T. mixta*, respectively). Kämpfer *et al.* (2011) suggested that the *Telluria* species may need to be carefully re-investigated. It is also interesting to note that *Duganella violaceinigra* YIM 31327^T was clustered with the *Massilia* species using the neighbour-joining and maximum-likelihood methods (Figs 1 and S1b), but it was grouped in another cluster containing *Duganella zoogloeoides* IAM 12670^T in the maximum-parsimony tree (Fig. S1a). However, *D. violaceinigra* YIM 31327^T showed only 95.9 % 16S rRNA gene sequence identity to strain TS3^T and had different major fatty acids ($C_{16:0}$ and $C_{12:0}$) compared with *Massilia* species.

Similar to the type species *M. timonae* and other members of the genus *Massilia*, strain TS3^T contained ubiquinone Q-8 (91.2 %) as the major respiratory quinone. The DNA G+C content of strain TS3^T was 65.9 mol%, which was within the G+C content range reported for *Massilia*. The major fatty acids were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 40.3 %), $C_{16:0}$ (21.1 %), $C_{18:1}\omega7c$ (15.2 %), $C_{12:0}$ (6.5 %), $C_{10:0}$ 3-OH (6.3 %) and $C_{12:0}$ 2-OH (5.3 %), in agreement with the characteristics of *Massilia* (Table 2). The major polar lipids of strain TS3^T were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol (Fig. S2), identical to the major polar lipids reported for *M. flava* Y9^T (Wang *et al.*, 2012), *M. consociata* CCUG 58010^T (Kämpfer *et al.*, 2011), *M. oculi* CCUG 43427A^T (Kämpfer *et al.*, 2012), *M. jejuensis* KACC 12634^T, *M. suwonensis* KACC 12635^T, *M. brevitalea* DSM 18925^T, *M. alkalitolerans* KACC 12188^T (Weon *et al.*, 2010), *M. varians* CCUG 35299^T and *M. haematophila* CCUG 38318^T (Kämpfer *et al.*, 2008). In addition, strain TS3^T also had a small amount of an unknown lipid (Fig. S2). *M. niastensis* KACC 12599^T and *M. aerilata* KACC 12505^T, closely related type strains for which no polar lipid data were available, were analysed in parallel in this study and showed the same major polar lipids as strain TS3^T (Fig. S2). In DNA–DNA hybridization tests, the relatedness between strain TS3^T and *M. niastensis* KACC 12599^T, *M. consociata* CCUG 58010^T, *M. aerilata* KACC 12505^T and *M. varians* CCUG 35299^T was 36.5 ± 0.6, 27.1 ± 2.6, 22.7 ± 1.3 and 46.5 ± 1.2 %, respectively. These DNA–DNA relatedness values were considerably less than 70 %, indicating that strain TS3^T represents a novel species distinct from the four tested reference strains (Wayne *et al.*, 1987).

In summary, the 16S rRNA gene sequence similarity, major fatty acids, the presence of Q-8 as the major respiratory quinone, the polar lipids and the DNA G+C content showed that strain TS3^T belonged to the genus *Massilia*. However, strain TS3^T showed clear differences in morphological, physiological/biochemical features and low DNA–DNA hybridization with the type strains of related species. On the basis of the phenotypic and phylogenetic characteristics, strain TS3^T should be classified within a novel species of the genus *Massilia*, for which the name *Massilia tieshanensis* sp. nov. is proposed.

Description of *Massilia tieshanensis* sp. nov.

Massilia tieshanensis (tie.sha.nen′sis. N.L. fem. adj. *tieshanensis* of or belonging to Tieshan, referring to the isolation of the type strain from mine soil in Tieshan, China).

Cells are Gram-negative, non-spore-forming, rod-shaped (0.3–0.6 × 1.2–2.0 μm) and motile with flagella (Fig. S3). Colonies are circular, convex, viscous, translucent, straw-yellow, 1.0–2.0 mm in diameter with entire edges on nutrient agar plates after 36 h of incubation. Some differences are observed using R2A medium, on which colonies are opaque, yellowish and rough. Grows on nutrient agar, R2A and trypticase soy agar, but does not grow on MacConkey agar. Cannot grow under anaerobic conditions. Aerobic growth can be observed at 10–40 °C, with optimum growth at 28 °C. The pH range for growth is 5.0–9.0, with optimum growth at pH 7.0. Growth does not require NaCl, but the type strain can tolerate 1 % (w/v) NaCl. Oxidase- and catalase-positive. H_2S is not produced.

Table 1. Differential characteristics of strain TS3^T and type strains of other species of the genus *Massilia*

Data in columns 1–6 are from this study unless indicated and were obtained after growth under the same conditions; data in parentheses were taken from Weon *et al.* (2009). Strains: 1, TS3^T; 2, *M. niastensis* KACC 12599^T; 3, *M. consociata* CCUG 58010^T; 4, *M. aerilata* KACC 12505^T; 5, *M. varians* CCUG 35299^T; 6, *M. haematophila* CCUG 38318^T; 7, *M. suwoensis* 5414S-25^T (unless indicated, data from Weon *et al.*, 2010); 8, *M. albidiflava* DSM 17472^T; 9, *M. timonae* DSM 16850^T; 10, *M. lutea* DSM 17473^T (unless indicated, data in columns 8–10 from Weon *et al.*, 2008); 11, *M. niabensis* KACC 12632^T (Weon *et al.*, 2009); 12, *M. aurea* DSM 18055^T (Weon *et al.*, 2008); 13, *M. jejuensis* 5317J-18^T (Weon *et al.*, 2010); 14, *M. brevitalea* DSM 18925^T (Zul *et al.*, 2008; Weon *et al.*, 2009); 15, *M. dura* DSM 17513^T (Weon *et al.*, 2008); 16, *N. alkalitolerans* KACC 12188^T (Weon *et al.*, 2010); 17, *M. plicata* DSM 17505^T (Weon *et al.*, 2008); 18, *M. oculi* CCUG 43427A^T (Kämpfer *et al.*, 2012). *M. niastensis* KACC 12599^T and *M. aerilata* KACC 12505^T are the deposited strains in KACC corresponding to *M. niastensis* 5516S-1^T and *M. aerilata* 5516S-11^T, respectively. +, Positive; W, weakly positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Nitrate reduction	+	−	−	+	−	−	−	+	−	−	+	−	−	+	−	−[a]*	−	−
Urease	−	−	−	+	−	−	−	−	−	−	−	−	−	−[b]	−	+[a]	−	−
Hydrolysis of:																		
Aesculin	+	+	− (+)	+	W	−	−	+	+	+	−	+	+	+[b]	+	+[a]	+	+
Starch	+	W	+	−	+	+	−	+[c]	+	+[c]	+	+[c]	W	+	−[c]	−[a]	+[c]	ND
Casein	+	−	+	+	+	+	+	+[c]	ND	+[c]	−	+[c]	−	+	+[c]	ND	+[c]	ND
Gelatin	+	+	+	+	W	W	+	+	+	+	−	+	−	−	+	−[a]	+	ND
Assimilation of:																		
D-Glucose	+	+	+	+	+ (−)	−	W	+	+	−	−	+	−	+[b]	+	+	+	+
L-Rhamnose	−	+	−	−	+	−	−	+	+	−	−	+	−	−	+	+	+	+
Lactic acid	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−
Glycogen	+	−	+	+	+	+	+	+	+	−	−	+	+	−	+	−	+	ND
3-Hydroxybenzoic acid	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−
Valeric acid	+	−	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	ND
L-Histidine	+	−	−	+	+ (−)	−	+	−	+	−	−	−	−	−	−	+	−	−
Potassium gluconate	+	+	−	−	−	−	−	+	+	+	−	−	−	−[b]	+	−	+	−
Malic acid	−	− (+)	+	+	+	+	+	−	+	−	−	+	−	+[b]	+	ND	+	ND
N-Acetylglucosamine	−	− (+)	−	−	−	−	−	+	−	+	−	+	−	−[b]	−	ND	−	ND
Phenylacetic acid	−	−	−	+	−	+	+	−	+	−	−	−	−	−[b]	−	ND	−	ND
Enzyme activities																		
β-Galactosidase	−	+	−	−	+	−	−	+	+	+	+	+	−	−	+	+	−	ND
α-Galactosidase	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	+	ND
α-Glucosidase	+	−	−	+	+	+	+	−	−	−	+	W	+	+	−	+	−	ND
β-Glucosidase	+	+ (−)	−	−	+	−	−	+	W	−	−	−	−	−	−	+	−	ND
β-Glucuronidase	−	−	W	−	−	−[d]	−	−	−	−	+	−	−	−	−	ND	−	ND
Cystine arylamidase	−	+	−	+	+	−[d]	W	−	+	−	−	−	−	−[b]	−	ND	−	ND
DNA G+C content (mol%)	65.9	66.6	ND	68.9	ND	ND	67.8	65.3[e]	62–67[f]	63.3[e]	67.8	66.0[g]	66.1	65.3	65.9[e]	62.4±0.3[a]	65.1[e]	ND

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

Methyl red and Voges–Proskauer tests are negative. Hydrolyses casein and starch, but not L-tyrosine, CM-cellulose, chitin, DNA or Tween 80. Positive for nitrate reduction and hydrolysis of aesculin and gelatin, but negative for β-galactosidase, indole production, glucose fermentation, urease and arginine dihydrolase (API 20 NE test strip). Assimilates D-glucose, L-arabinose, D-mannose, potassium gluconate, lactic acid, sodium acetate, glycogen, valeric acid, sucrose, maltose, L-histidine, 3-hydroxybutyric acid and L-proline, but not L-rhamnose, D-ribose, L-fucose, D-mannitol, inositol, salicin, melibiose, D-sorbitol, L-alanine, L-serine, *N*-acetylglucosamine, adipic acid, malic

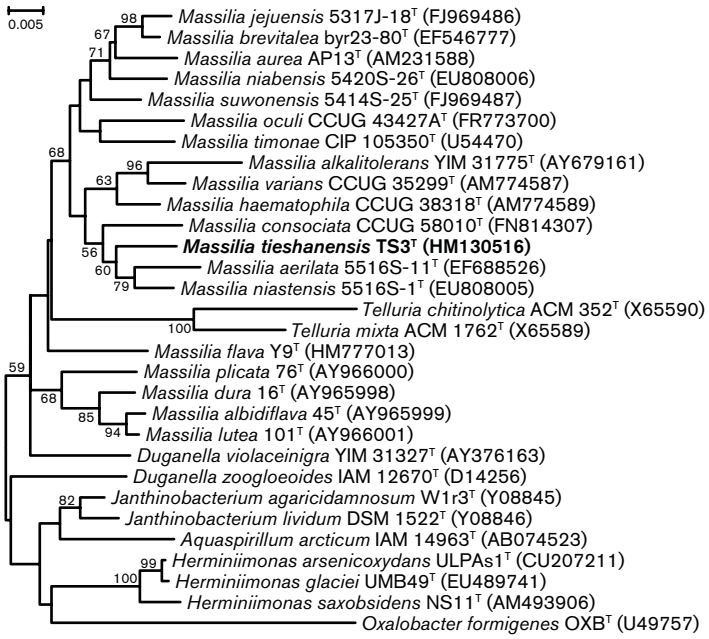

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences. The position of strain TS3^T is shown with respect to other closely related species. Numbers at nodes indicate bootstrap values, expressed as percentages of 1000 replications; values >50 % are shown. Bar, 0.005 accumulated changes per nucleotide.

acid, capric acid, trisodium citrate, propionic acid, itaconic acid, phenylacetic acid, sodium malonate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, potassium 2-ketogluconate or 4-hydroxybenzoic acid (API 20 NE and API ID 32 GN test strips). Also assimilates D-fructose, trehalose, lactose, D-galactose, cellobiose, raffinose, tartrate, pyruvate, β-hydroxybutyrate, L-arginine, creatine and L-threonine, but not succinate, glycerol, malate, L-cysteine, L-leucine or L-tryptophan. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine

Table 2. Cellular fatty acid compositions of strain TS3^T and the type strains of *Massilia* species

Strains: 1, TS3^T; 2, *M. niastensis* KACC 12599^T; 3, *M. consociata* CCUG 58010^T; 4, *M. aerilata* KACC 12505^T; 5, *M. varians* CCUG 35299^T; 6, *M. haematophila* CCUG 38318^T (data from Weon *et al.*, 2010); 7, *M. suwoensis* 5414S-25^T (Weon *et al.*, 2010); 8, *M. albidiflava* DSM 17472^T; 9, *M. timonae* DSM 16850^T; 10, *M. lutea* DSM 17473^T (data in columns 8–10 from Weon *et al.*, 2008); 11, *M. niabensis* KACC 12632^T (Weon *et al.*, 2009); 12, *M. aurea* DSM 18055^T (Gallego *et al.*, 2006); 13, *M. jejuensis* 5317J-18^T (Weon *et al.*, 2010); 14, *M. brevitalea* DSM 18925^T (Weon *et al.*, 2009); 15, *M. dura* DSM 17513^T (Weon *et al.*, 2008); 16, *M. alkalitolerans* KACC 12188^T (Weon *et al.*, 2010); 17, *M. plicata* DSM 17505^T (Zhang *et al.*, 2006); 18, *M. oculi* CCUG 43427A^T (Kämpfer *et al.*, 2012). Data in columns 1–5 are from this study. Values are percentages of total fatty acids; –, <1 % or not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
$C_{10:0}$ 3-OH	6.3	5.4	6.4	4.8	6.0	3.0	5.4	7.0	4.6	5.7	6.6	6.0	6.1	4.9	5.5	3.2	10.1	9.6
$C_{12:0}$	6.5	7.8	6.3	5.4	5.2	3.3	4.4	5.3	3.3	4.0	8.9	4.4	5.9	5.0	3.9	3.3	7.1	7.7
$C_{12:0}$ 2-OH	5.3	3.5	2.1	–	4.0	1.5	2.4	–	2.2	–	–	–	1.7	2.0	–	2.2	1.6	4.3
$C_{14:0}$	–	1.0	–	3.4	–	–	1.2	2.6	–	1.2	–	2.0	–	–	1.1	–	–	1.0
$C_{14:0}$ 2-OH	–	–	–	2.6	–	–	–	2.4	–	2.9	–	2.5	–	–	2.6	–	6.1	–
$C_{16:0}$	21.1	22.9	14.2	26.1	21.7	26.2	28.8	23.4	30.5	26.6	23.6	27.2	22.5	23.0	27.5	26.6	25.1	20.9
iso-$C_{16:0}$	–	–	–	–	–	2.1	–	–	–	–	–	–	–	–	–	–	–	–
$C_{17:0}$ cyclo	1.8	3.3	–	9.5	6.3	2.7	2.9	–	3.7	–	–	–	1.0	–	–	–	–	1.0
iso-$C_{17:0}$	–	–	–	–	–	1.6	–	–	–	–	–	–	–	–	–	–	–	–
iso-$C_{17:1}\omega 9c$	–	–	–	–	–	1.4	–	–	–	–	–	–	–	–	–	–	–	–
$C_{18:1}\omega 7c$	15.2	7.0	11.2	5.4	6.5	8.7	7.8	7.4	7.9	7.8	7.8	7.9	12.1	9.0	7.0	6.6	11.7	6.0
$C_{18:0}$	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
$C_{20:0}$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Summed feature 3*	40.3	46.9	57.8	40.1	48.5	44.7	45.8	46.0	47.0	51.1	46.0	48.6	49.0	54.2	52.0	55.2	36.9	48.2

*Summed feature 3 included $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH.

arylamidase, α-glucosidase, β-glucosidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM test strip). Sensitive to amoxicillin (20 μg), ampicillin (10 μg), cefotaxime (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), nalidixic acid (30 μg), neomycin (30 μg), nitrofurantoin (30 μg), novobiocin (30 μg), polymyxin B (25 μg), streptomycin (10 μg), penicillin (10 μg), tobramycin (10 μg) and trimethoprim (5 μg). 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 respiratory quinone is ubiquinone Q-8. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The DNA G+C content of the type strain is 65.9 mol%.

The type strain, TS3^T (=CCTCC AB 2010202^T =KACC 14940^T), was isolated from subsurface soil of a metal mine at Tieshan, Hubei Province, central China.

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