Massilia umbonata sp. nov., able to accumulate poly-β-hydroxybutyrate, isolated from a sewage sludge compost–soil microcosm

Marina Rodríguez-Díaz,[1,2]† Federico Cerrone,[3]† Mar Sánchez-Peinado,[3] Lucía SantaCruz-Calvo,[3] Clementina Pozo[1,3] and Jesús González López[1,3]

Correspondence
Clementina Pozo
clpozo@ugr.es

[1]Department of Microbiology, University of Granada, Granada, Spain

[2]Max-Planck-Institut für Marine Mikrobiologie, Celsiusstrasse 1, 28359 Bremen, Germany

[3]Water Research Institute, University of Granada, Granada, Spain

A bacterial strain, designated strain LP01^T, was isolated from a laboratory-scale microcosm packed with a mixture of soil and sewage sludge compost designed to study the evolution of microbial biodiversity over time. The bacterial strain was selected for its potential ability to store polyhydroxyalkanoates (PHAs) as intracellular granules. The cells were aerobic, Gram-stain-negative, non-endospore-forming motile rods. Phylogenetically, the strain was classified within the genus *Massilia*, as its 16S rRNA gene sequence had similarity of 99.2 % with respect to those of *Massilia albidiflava* DSM 17472^T and *M. lutea* DSM 17473^T. DNA–DNA hybridization showed low relatedness of strain LP01^T to the type strains of other, phylogenetically related species of the genus *Massilia*. It contained Q-8 as the predominant ubiquinone and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) as the major fatty acid(s). It was found to contain small amounts of the fatty acids $C_{18:0}$ and $C_{14:0}$ 2-OH, a feature that served to distinguish it from its closest phylogenetic relatives within the genus *Massilia*. The DNA G+C content was 66.0 mol%. Phylogenetic, phenotypic and chemotaxonomic data obtained in this study suggest that strain LP01^T represents a novel species of the genus *Massilia*, for which the name *Massilia umbonata* sp. nov. is proposed. The type strain is LP01^T (=CECT 7753^T=DSM 26121^T).

The genus *Massilia* was described by La Scola *et al.* (1998) on the basis of a single isolate from the blood of an immunocompromised patient (Gallego *et al.*, 2006). The genus seems to be ubiquitous, with species isolated from environmental samples of air, soil and water (Gallego *et al.*, 2006; Zhang *et al.*, 2006; Zul *et al.*, 2008; Weon *et al.*, 2008, 2009, 2010; Du *et al.*, 2012, Wang *et al.*, 2012) and also from human fluids (La Scola *et al.*, 1998; Lindquist *et al.*, 2003; Kämpfer *et al.*, 2012). Additional unidentified members of the genus *Massilia* have shown a capacity to degrade aromatic compounds (Bodour *et al.*, 2003; Khammar *et al.*, 2005), and other authors have illustrated the ability of non-pathogenic members of the genus to accumulate polyhydroxyalkanoates (PHAs) as intracellular granules (Cerrone *et al.*, 2011; Bassas-Galia *et al.*, 2012).

†These authors contributed equally to this work.

Abbreviations: PHA, polyhydroxyalkanoate; PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LP01^T is HM053474.

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

PHAs are potential alternatives to petroleum-based plastics, since chemically modified variants exhibit similar physical properties. Growing concern over environmental pollution has heightened interest in these biodegradable polymers over their chemically synthesized counterparts (Gumel *et al.*, 2012). PHAs are polyesters synthesized by micro-organisms under limiting nutrient conditions in the presence of an excess of carbon source, being accumulated as storage material inside microbial cells (Steinbüchel & Füchtenbusch, 1998; Reddy *et al.*, 2003). In fact, the diversity of monomeric components in accumulated PHA results in potentially diversified high-molecular-mass polymeric materials (Gumel *et al.*, 2012). Several studies have reported the synthesis of products of industrial interest, such as PHAs, by several non-pathogenic members of the genus *Massilia* using starch (*Massilia albidiflava*, *M. aurea*, *M. brevitalea*, *M. aerilata*, *M. lutea*, *M. dura* and *M. plicata*; Cerrone *et al.*, 2011) or glucose (*M. plicata*; Bassas-Galia *et al.*, 2012) as sole carbon sources.

In the present study, we describe the morphological, biochemical and phylogenetic characteristics of strain LP01^T, isolated from a mixture of soil and sewage sludge compost, which accumulates poly-β-hydroxybutyrate (PHB).

Strain LP01^T was isolated while studying the microbial biodiversity of soil samples amended with compost. The soil–compost mixture from which LP01^T was isolated had been incubated for 90 days at 5 °C using a laboratory-scale microcosm. The soil was a calcaric regosol taken from an olive grove near Guadalimar (Jaén, Spain). The sewage sludge compost was a co-compost obtained by the windrow method using trimming refuse from gardens and olive tree leaves (1 : 1). Composting lasted for a period of 8 months. Compost was kindly provided by Biomasa del Guadalquivir, SA (Granada, Spain), which is in charge of collecting, treating and recycling sewage sludge from the urban wastewater treatment plants of Granada and Motril (Granada, Spain).

The soil extract was prepared from the same soil used for the microcosm experiments by mixing it with distilled water (1 : 1, w/v) and subjecting the mixture to ultrasound in a sonicator (Selecta ultrasound) for 30 min. The supernatant was filtered through Whatman paper and then diluted 1 : 10 (v/v) with distilled water, and 0.01 % gellan gum (AppliedChem) was then added to obtain a semisolid soil extract. Tryptone soy gellan (TSG) plates were prepared with tryptone soy broth (TSB; Difco) (3 g l^{-1}) and solidified with gellan gum (10 g l^{-1}) and agar (Difco; 4 g l^{-1}).

Cultivation was accomplished by the method of dilution to extinction. Briefly, 5 g soil–compost mixture (about 10^9 c.f.u. g^{-1}) was diluted in serial volumes of soil extract until the theoretical number of bacteria reached 10^2 c.f.u. ml^{-1}. Five grams random microcosm sample was mixed in a blender bag containing 45 ml semisolid soil extract for 1 min (Masticator; IUL Instruments) and then subjected to ultrasound (Selecta ultrasound) for 10 s to detach bacteria from soil particles. The process was carried out twice. The final dilution medium included 25 µl Nile blue A solution [1 % (w/v) in distilled water; Sigma-Aldrich]. The dilutions were homogenized in a blender (Masticator; IUL Instruments) after each transfer. The final dilution was inoculated into a sterile microtitre plate, filling each well with a volume of 250 µl. The microtitre plate was sealed with a porous film (Breathe-Easy; Sigma-Aldrich) and incubated at 5 °C for 2 months. Fluorescence was measured just after inoculation of the microtitre plate and after 1 month of incubation using a fluorimeter (Fluostar Optima; BMG-Labtech) with excitation λ=620 nm and emission λ=660 nm; the gain was adjusted to 4095. Wells showing the maximum neat fluorescence were inoculated into Petri dishes containing 50 µl 1 % (w/v) Nile blue A and solid soil-extract medium. These plates were incubated under the same conditions as the microtitre plates and colonies that fluoresced under UV light were selected and isolated on TSG plates. Pure cultures were checked for potential PHA production under a phase-contrast microscope (BX40; Olympus). Strain LP01^T was selected for further characterization due to its remarkable large number of intracellular granules (Fig. S1a, available in IJSEM Online).

Confirmation of PHA production by strain LP01^T was achieved as described by Cerrone *et al.* (2011). For this, 250 ml Erlenmeyer flasks containing 50 ml ISP2 medium (Shirling & Gottlieb, 1966) amended with 4.0 g soluble starch l^{-1} instead of glucose were inoculated with strain LP01^T (10^6 c.f.u. ml^{-1}) previously grown in the same medium, and maintained under agitation (150 r.p.m.) and controlled temperature (30 °C) for 4 days. NMR was used to identify the biopolymers synthesized. Fig. S1(b) illustrates the amounts of biopolymer produced and the cell dry weight of strain LP01^T at each incubation time. NMR spectra of bioplastics obtained from strain LP01^T after 72 h of incubation and of pure PHB (Sigma-Aldrich) are shown in Fig. S1(c). Maximum production of PHA (22.6 %) was reached after 72 h of growth in flask trials and the NMR analysis confirmed that the bioplastics produced were homopolymers of PHB.

Transmission electron microscopy (Libra 120 Plus; Zeiss) was used to observe intracellular PHA granules after 72 h of growth on ISP2 medium amended with 4 g soluble starch l^{-1} as well as the cellular morphology. The type of flagellation was examined by negative staining of cells from 25 ml bacterial suspension incubated on a grid support film for 5 min; after two washing steps with ultrapure water, the sample was contrasted with an aqueous solution of 2 % uranyl acetate (1 min) (Fig. S2a). Detection of intracellular PHA granules was achieved as follows: cells were pre-fixed with 2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M cacodylate at 4 °C (24 h), washed, post-fixed in 1 % OsO_4 in a dark room at 4 °C (1 h) and dehydrated in a graded ethanol series (50, 70, 90 and 100 %). The sample was embedded in Epon resin and polymerized. Ultrathin sections were stained with uranyl acetate (1 %) plus lead citrate (Reynolds, 1963) before being observed (Fig. S2b).

Extraction of genomic DNA for amplification of the 16S rRNA gene of strain LP01^T was performed as described by Martín-Platero *et al.* (2007). The almost full-length 16S rRNA gene was amplified using primers fD1 and rD1 (Weisburg *et al.*, 1991) purchased from Sigma-Genosys. PCRs were run using a GeneAmp PCR system 2400 (Perkin-Elmer) following a temperature profile described previously (Vinuesa *et al.*, 1998). The amplification product was purified by agarose gel electrophoresis and extracted with a QIAEX II agarose gel extraction kit (Qiagen). The nucleotide sequence of the purified band was determined by the dideoxy chain-termination method, using an ABI-PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) and an ABI373 automated sequencer (Applied Biosystems).

Related taxa were identified using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012) and 16S rRNA gene sequences and levels of 16S rRNA gene sequence similarity were obtained. The nearly complete sequence of the 16S rRNA gene of strain LP01^T (approx. 1500 bp) was aligned with sequences from strains of closely

related species of the genus *Massilia* and other genera of the family *Oxalobacteraceae* by using the ClustalX2 program (Larkin *et al.*, 2007). The MEGA 5.0 package (Tamura *et al.*, 2011) was used to reconstruct phylogenetic trees by applying the neighbour-joining (NJ; Saitou & Nei, 1987), maximum-likelihood (ML) and maximum-parsimony (MP) (Kolaczkowski & Thornton, 2004) methods. Bootstrap analyses were based on 1000 resamplings (Felsenstein, 1985). The substitution models used were Kimura's two-parameter model for NJ and the Tamura–Nei model for ML. Strain LP01^T exhibited 16S rRNA gene sequence similarities of over 97.0 % with various species of the family *Oxalobacteraceae* (species of the genus *Massilia*, *Pseudoduganella violaceinigra* and *Duganella radicis*). However, the closest phylogenetic relatives were *M. albidiflava* DSM 17472^T, *M. lutea* DSM 17473^T and *M. dura* DSM 17513^T (99.2–98 % 16S

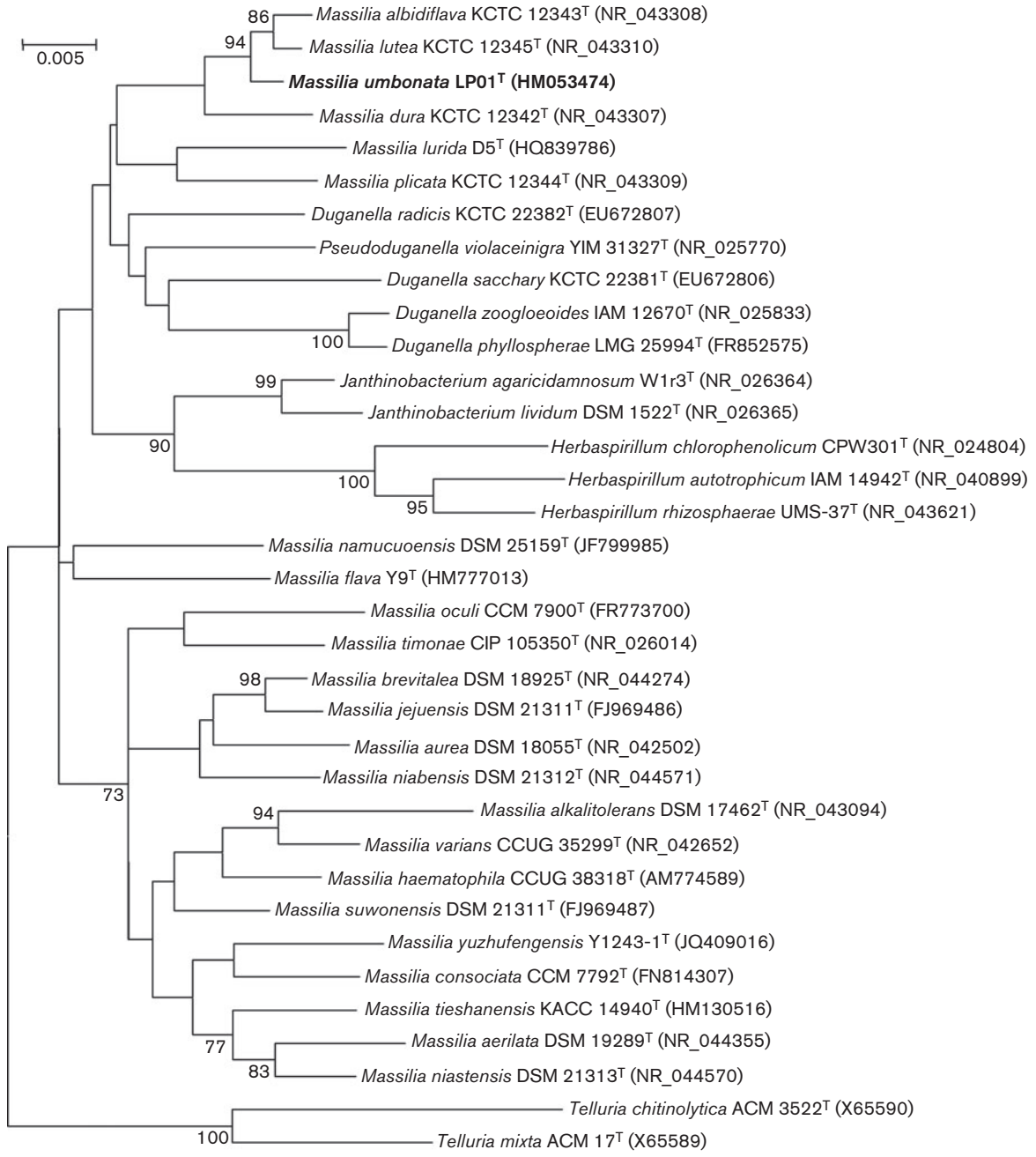

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence comparison of strain LP01^T and its closest relatives, obtained by the neighbour-joining algorithm. Numbers at nodes are bootstrap percentages based on 1000 resamplings. Bar, 0.005 changes per nucleotide position.

rRNA gene sequence similarity, with >98.7 % coverage). Clusters of LP01^T with these three strains were retrieved in the NJ phylogenetic tree (Fig. 1) and also the ML and MP phylogenetic trees (Figs S3 and S4).

DNA–DNA hybridizations, DNA G+C content and profiles of fatty acids, respiratory quinones and polar lipids were determined at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). Descriptions of the methods used for these techniques can be found in the section for identification of the DSMZ web page (http://www.dsmz.de/services/services-microorganisms/identification.html).

Mean values for DNA–DNA hybridization between strain LP01^T and *M. albidiflava* DSM 17472^T, *M. lutea* DSM 17473^T and *M. dura* DSM 17513^T were 33.3±6.0, 49.2±2.1 and 22.9±2.8 %, respectively. Strain LP01^T therefore does not belong to any of these species of the genus *Massilia*, according to the recommendation of a threshold value of 70 % DNA–DNA relatedness for the definition of bacterial species (Wayne *et al.*, 1987). The DNA G+C content of strain LP01^T was 66.0 mol%. The predominant respiratory quinone for strain LP01^T was Q-8 (97 %); Q-9 (1 %) and an unidentified minor compound were also detected. The major fatty acids were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{16:0}$. Moderate amounts of $C_{18:1}\omega7c$, $C_{10:0}$ 3-OH, $C_{12:0}$ and $C_{14:0}$ 2-OH were also found. In addition, small amounts of $C_{10:0}$, $C_{18:0}$, $C_{16:1}\omega5c$ and $C_{18:1}\omega9c$ were detected. Table 1 shows the fatty acid profiles of strain LP01^T and its closest relatives. The predominant polar lipids of strain LP01 were phosphatidylglycerol and phosphatidylethanolamine. Additionally, three unknown phospholipids were detected (Fig. S5).

Phenotypic characteristics such as Gram staining, catalase and oxidase activities and hydrolysis of casein, Tween 80 and starch were assessed by using the methods of Smibert & Krieg (1994). API 20E, API 20NE, API 32E and API 50CH kits (bioMérieux) were used to determine the biochemical properties, carbohydrate utilization and enzyme activities of the novel strain. The kits were incubated for the times advised by the manufacturer at 30 °C. Colony morphology was determined after 3 days of growth on TSG at 25 °C. Growth at 4, 10, 28, 37, 40, 45 and 55 °C and at 0.1, 0.2, 0.3 and 0.4 % (w/v) NaCl was tested and pH tolerance range experiments were conducted in TSB medium (Difco) according to Breznak & Costilow (1994).

Strain LP01^T grew at 4–37 °C, with optimum growth at 28–30 °C, and it showed low tolerance to salinity (maximum growth at 0.3 % NaCl). It grew only under neutral/slightly alkaline conditions, optimal growth being observed at pH 7.5–8.0. Table 2 shows the phenotypic,

Table 1. Cellular fatty acid compositions of strain LP01^T and the type strains of closely related species

Strains: 1, LP01^T; 2, *M. albidiflava* DSM 17472^T; 3, *M. lutea* DSM 17473^T; 4, *M. dura* DSM 17513^T; 5, *M. plicata* KCTC 12344^T (data in columns 2–5 from Zhang *et al.*, 2006); 6, *M. lurida* KCTC 23880^T (Luo *et al.*, 2013); 7, *M. flava* KCTC 23585^T (Wang *et al.*, 2012); 8, *Pseudoduganella violaceinigra* DSM 15887^T (Kämpfer *et al.*, 2012); 9, *Duganella radicis* NCIMB 14476^T (Madhaiyan *et al.*, 2013). Values are percentages of total fatty acids; data in columns 1–7 were obtained from cells grown on TSB and data in column 9 were from cells grown on R2A agar (medium used to obtain the results shown in column 8 not known). –, Not present/not reported; TR, traces (<0.5%).

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated									
$C_{10:0}$	0.5	1.3	0.5	0.6	1.4	–	–	1.0	0.6
$C_{12:0}$	3.9	6.0	2.8	5.6	7.1	4.1	3.0	13.6	8.8
$C_{14:0}$	3.6	2.8	1.9	3.2	–	1.9	6.9	1.2	0.4
$C_{16:0}$	27.3	22.8	22.8	25.5	25.1	30.0	26.1	29.4	29.2
$C_{17:0}$ cyclo	–	–	–	–	–	2.4	–	–	11.9
$C_{18:0}$	0.7	–	–	–	–	–	3.2	–	0.2
Unsaturated									
$C_{16:1}\omega5c$	TR	–	–	–	–	–	–	–	–
$C_{18:1}\omega7c$	12.8	7.4	7.8	5.9	11.7	8.8	9.9	3.3	7.7
$C_{18:1}\omega9c$	TR	–	–	–	–	–	–	–	–
Hydroxy									
$C_{10:0}$ 3-OH	4.6	9.6	4.6	8.3	10.1	7.1	2.7	5.1	6.5
$C_{12:0}$ 2-OH	–	0.7	–	–	1.6	–	–	5.7	–
$C_{14:0}$ 2-OH	0.6	3.0	2.0	3.4	6.1	3.5	1.5	–	–
Summed features									
Summed feature 3*	45.5	46.4	54.7	46.7	36.9	38.3	35.8	32.6	3.8

*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.

Table 2. Differential characteristics among strain LP01^T and type strains of phylogenetically related species

Strains: 1, LP01^T; 2, *M. albidiflava* DSM 17472^T; 3, *M. lutea* DSM 17473^T; 4; *M. dura* DSM 17513^T; 5, *M. plicata* KCTC 12344^T (data in columns 2–5 from Zhang *et al.*, 2006); 6, *M. lurida* KCTC 23880^T (Luo *et al.*, 2013); 7, *M. flava* KCTC 23585^T (Wang *et al.*, 2012); 8, *Pseudoduganella violaceinigra* DSM 15887^T (Kämpfer *et al.*, 2012); 9, *Duganella radicis* NCIMB 14476^T (Madhaiyan *et al.*, 2013). +, Positive; −, negative; (+), weakly positive; ND, no data available. The data reported were obtained on the basis of the same or comparable conditions.

Characteristic	1	2	3	4	5	6	7	8	9
Cell width (μm)	0.6–0.8	1.8–2.0	1.8–2.0	0.6–0.8	0.6–0.7	0.6–0.9	0.6–0.8	2.0–3.0	0.32–0.39
Cell length (μm)	2.0–2.5	3.0–3.5	3.0–3.5	1.8–2.2	2.0–2.5	1.4–2.8	1.5–2.5	1.0–2.0	1.18–1.21
pH range	7.0–8.0	6.5–8.5	6.5–8.5	6.5–8.5	6.5–8.5	6.0–9.0	6.5–8.5	ND	4.0–10.0
Temperature range (°C)	4–37	10–45	10–45	10–45	10–45	10–37	10–45	28–30	15–37
Maximum NaCl tolerance (%)	0.3	1	1	1	1	0.5	1	ND	0.5
Flagellation	Polar	Lateral	Lateral	Lateral	Lateral	Lateral	ND	Polar	Polar
Oxidase	−	+	+	+	−	+	+	−	−
Nitrate reduction	−	+	(+)	+	(+)	−	+	−	+
Urease	−	−	−	+	−	−	−	−	−
Hydrolysis of:									
Aesculin	+	+	+	+	+	+	+	+	−
Gelatin	+	+	+	+	+	+	−	+	−
Starch	−	+	+	−	+	−	+	ND	−
Enzyme activities									
β-Glucosidase	+	+	+	+	+	−	−	+	−
β-Glucuronidase	+	+	+	+	−	−	−	+	−
N-Acetyl-β-glucosaminidase	−	+	+	−	−	−	−	+	−
α-Galactosidase	+	+	+	+	+	−	−	+	−
α-Glucosidase	+	−	+	+	+	−	+	+	−
β-Galactosidase	+	+	+	+	−	+	+	+	−
α-Maltosidase	+	+	−	−	−	ND	ND	+	−
Assimilation of:									
D-Glucose	(+)	+	−	+	(+)	+	+	+	+
L-Arabinose	+	+	−	+	+	+	+	−	+
D-Mannose	+	+	−	+	−	+	+	+	+
D-Mannitol	+	−	−	−	−	−	−	−	−
Maltose	+	+	−	+	−	+	+	+	+
N-Acetylglucosamine	(+)	+	−	−	−	+	−	−	+
Adipic acid	−	+	−	−	−	+	ND	−	ND
Malic acid	(+)	−	−	−	−	+	ND	+	ND
Trisodium citrate	−	−	−	(+)	−	+	ND	ND	ND
DNA G+C content (mol%)	66.0	65.3	63.3	65.9	65.1	65.9	68.7	62.8	54.9

biochemical and physiological characteristics that differentiate strain LP01^T from its closest relatives.

The molecular, chemotaxonomic, physiological and biochemical data obtained in this study indicate that strain LP01^T represents a novel species of the genus *Massilia* within the family *Oxalobacteraceae* of the order *Burkholderiales*, for which the name *Massilia umbonata* sp. nov. is proposed.

Description of *Massilia umbonata* sp. nov.

Massilia umbonata (um.bo.na′ta. N.L. fem. adj. *umbonata* umbonate from L. masc. n. *umbo -nis* a shield boss, referring to its colony morphology, resembling an embossed shield).

Cells are aerobic, Gram-stain-negative, non-endospore-forming rods (0.6–0.8 μm wide by 2.0–2.5 μm long), motile by a single polar flagellum and with intracytoplasmic granules when grown on ISP2 medium amended with soluble starch. Colonies grown on TSG for 3 days are yellowish and glossy, with an umbo surrounded by bosses, and round, entire edges that grow outwardly in decreasing waves. Grows at 4–37 °C with optimum growth at 28–30 °C and at pH 7–8 with optimum growth at pH 7.5–8.0. Grows at 0–0.3 % NaCl (optimal growth without NaCl). Positive for catalase and negative for oxidase. In the API 20E, API 20NE and API 32E kits, H_2S is not produced and nitrate is not reduced. Indole and acetoin are not produced. Aesculin and gelatin are hydrolysed, but not starch. Enzyme activities are positive for L-aspartate arylamidase, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase and α-maltosidase, but negative for arginine dihydrolase, lipase, lysine decarboxylase, *N*-acetyl-β-glucosaminidase, ornithine decarboxylase and

urease. Carbohydrate assimilation is positive for L-arabinose, *o*-nitrophenyl *β*-D-galactopyranoside, D-mannitol, D-mannose and maltose, weakly positive for *N*-acetylglucosamine, D-glucose, gluconate and malic acid and negative for adipic acid, adonitol, L-arabinose, D- and L-arabitol, L-arginine, capric acid, cellobiose, galacturonate, 5-keto-D-gluconate, inositol, L-rhamnose, L-tryptophan, malonate, maltose, mannitol, palatinose, phenylacetic acid, sucrose, sorbitol, trehalose and trisodium citrate. In the API 50CH kit, acid production is positive from amygdalin, L-arabinose, cellobiose, D-fructose, inulin, maltose, D-mannose, melezitose, melibiose, mannitol, raffinose, rhamnose, sucrose, trehalose and D-xylose and weak from *N*-acetylglucosamine, D-galactose, gentiobiose, D-glucose, turanose, methylxyloside and starch. Acid production is negative from adonitol, D-arabinose, D- and L-arabitol, arbutin, dulcitol, erythritol, D- and L-fucose, gluconate, 2- and 5-keto-D-gluconate, methyl α-D-glucoside, glycerol, glycogen, *myo*-inositol, lactose, D-lyxose, methyl α-D-mannoside, ribose, salicin, sorbitol, L-sorbose, D-tagatose, L-xylose and xylitol. The predominant respiratory quinone is ubiquinone Q-8. The major fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{16:0}$; moderate amounts of $C_{18:1}\omega7c$, $C_{10:0}$ 3-OH, $C_{12:0}$ and $C_{14:0}$ are also found. In addition, small amounts (<1 %) of $C_{10:0}$, $C_{18:0}$, $C_{16:1}\omega5c$, $C_{18:1}\omega9c$ and $C_{14:0}$ 2-OH are detected. Phosphatidylglycerol and phosphatidylethanolamine are the two major membrane polar lipids. Two unknown phospholipids are also abundant.

The type strain is LP01^T (=CECT 7753^T=DSM 26121^T), isolated from a mixture of soil collected nearby Guadalimar (Jaén, Spain) and sewage sludge compost from Granada (Spain). The DNA G+C content of the type strain is 66.0 mol%.

Acknowledgements

We would like to acknowledge the technical work performed by Juan de Dios Bueno Pérez, David Porcel for the preparation of biological samples and the TEM service at the Centro de Instrumentación Científica (CIC, University of Granada, Spain). M. R.-D. thanks the Spanish Ministry for Science and Innovation for support through a Juan de la Cierva postdoctoral fellowship. This work was supported by the European Union project FP6-032967.

References

Bassas-Galia, M., Nogales, B., Arias, S., Rohde, M., Timmis, K. N. & Molinari, G. (2012). Plant original *Massilia* isolates producing polyhydroxybutyrate, including one exhibiting high yields from glycerol. *J Appl Microbiol* **112**, 443–454.

Bodour, A. A., Wang, J. M., Brusseau, M. L. & Maier, R. M. (2003). Temporal change in culturable phenanthrene degraders in response to long-term exposure to phenanthrene in a soil column system. *Environ Microbiol* **5**, 888–895.

Breznak, J. A. & Costilow, R. N. (1994). Physicochemical factors in growth. In *Methods for General and Molecular Bacteriology*, pp. 137–154. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Cerrone, F., Sánchez-Peinado, M., Rodríguez-Díaz, M., González-López, J. & Pozo, C. (2011). PHAs production by strains belonging to *Massilia* genus from starch. *Starch* **63**, 236–240.

Du, Y., Yu, X. & Wang, G. (2012). *Massilia tieshanensis* sp. nov., isolated from mining soil. *Int J Syst Evol Microbiol* **62**, 2356–2362.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Gallego, V., Sánchez-Porro, C., García, M. T. & Ventosa, A. (2006). *Massilia aurea* sp. nov., isolated from drinking water. *Int J Syst Evol Microbiol* **56**, 2449–2453.

Gumel, A. M., Annuar, M. S. M. & Heidelberg, T. (2012). Biosynthesis and characterization of polyhydroxyalkanoates copolymers produced by *Pseudomonas putida* Bet001 isolated from palm oil mill effluent. *PLoS ONE* **7**, e45214.

Kämpfer, P., Lodders, N., Martin, K. & Falsen, E. (2012). *Massilia oculi* sp. nov., isolated from a human clinical specimen. *Int J Syst Evol Microbiol* **62**, 364–369.

Khammar, N., Malhautier, L., Degrange, V., Lensi, R., Godon, J. J. & Fanlo, J. L. (2005). Link between spatial structure of microbial communities and degradation of a complex mixture of volatile organic compounds in peat biofilters. *J Appl Microbiol* **98**, 476–490.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kolaczkowski, B. & Thornton, J. W. (2004). Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. *Nature* **431**, 980–984.

La Scola, B., Birtles, R. J., Mallet, M. N. & Raoult, D. (1998). *Massilia timonae* gen. nov., sp. nov., isolated from blood of an immunocompromised patient with cerebellar lesions. *J Clin Microbiol* **36**, 2847–2852.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. & other authors (2007). CLUSTAL W and CLUSTAL_X version 2.0. *Bioinformatics* **23**, 2947–2948.

Lindquist, D., Murrill, D., Burran, W. P., Winans, G., Janda, J. M. & Probert, W. (2003). Characteristics of *Massilia timonae* and *Massilia timonae*-like isolates from human patients, with an emended description of the species. *J Clin Microbiol* **41**, 192–196.

Luo, X., Xie, Q., Wang, J., Pang, H., Fan, J. & Zhang, J. (2013). *Massilia lurida* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **63**, 2118–2123.

Madhaiyan, M., Poonguzhali, S., Saravanan, V. S., Hari, K., Lee, K. C. & Lee, J. S. (2013). *Duganella sacchari* sp. nov. and *Duganella radicis* sp. nov., two novel species isolated from rhizosphere of field-grown sugar cane. *Int J Syst Evol Microbiol* **63**, 1126–1131.

Martín-Platero, A. M., Valdivia, E., Maqueda, M. & Martínez-Bueno, M. (2007). Fast, convenient, and economical method for isolating genomic DNA from lactic acid bacteria using a modification of the protein "salting-out" procedure. *Anal Biochem* **366**, 102–104.

Reddy, C. S., Ghai, R., Rashmi & Kalia, V. C. (2003). Polyhydroxyalkanoates: an overview. *Bioresour Technol* **87**, 137–146.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* **17**, 208–212.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Steinbüchel, A. & Füchtenbusch, B. (1998). Bacterial and other biological systems for polyester production. *Trends Biotechnol* **16**, 419–427.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Vinuesa, P., Rademaker, J. L. W., de Bruijn, F. J. & Werner, D. (1998). Genotypic characterization of *Bradyrhizobium* strains nodulating endemic woody legumes of the Canary Islands by PCR-restriction fragment length polymorphism analysis of genes encoding 16S rRNA (16S rDNA) and 16S-23S rDNA intergenic spacers, repetitive extragenic palindromic PCR genomic fingerprinting, and partial 16S rDNA sequencing. *Appl Environ Microbiol* **64**, 2096–2104.

Wang, J., Zhang, J., Pang, H., Zhang, Y., Li, Y. & Fan, J. (2012). *Massilia flava* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **62**, 580–585.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.

Weon, H. Y., Kim, B. Y., Son, J. A., Jang, H. B., Hong, S. K., Go, S. J. & Kwon, S. W. (2008). *Massilia aerilata* sp. nov., isolated from an air sample. *Int J Syst Evol Microbiol* **58**, 1422–1425.

Weon, H. Y., Kim, B. Y., Hong, S. B., Jeon, Y. A., Koo, B. S., Kwon, S. W. & Stackebrandt, E. (2009). *Massilia niabensis* sp. nov. and *Massilia niastensis* sp. nov., isolated from air samples. *Int J Syst Evol Microbiol* **59**, 1656–1660.

Weon, H. Y., Yoo, S. H., Kim, S. J., Kim, Y. S., Anandham, R. & Kwon, S. W. (2010). *Massilia jejuensis* sp. nov. and *Naxibacter suwonensis* sp. nov., isolated from air samples. *Int J Syst Evol Microbiol* **60**, 1938–1943.

Zhang, Y. Q., Li, W. J., Zhang, K. Y., Tian, X. P., Jiang, Y., Xu, L. H., Jiang, C. L. & Lai, R. (2006). *Massilia dura* sp. nov., *Massilia albidiflava* sp. nov., *Massilia plicata* sp. nov. and *Massilia lutea* sp. nov., isolated from soils in China. *Int J Syst Evol Microbiol* **56**, 459–463.

Zul, D., Wanner, G. & Overmann, J. (2008). *Massilia brevitalea* sp. nov., a novel betaproteobacterium isolated from lysimeter soil. *Int J Syst Evol Microbiol* **58**, 1245–1251.