Heliimonas saccharivorans gen. nov., sp. nov., a member of the family *Chitinophagaceae* isolated from a mineral water aquifer, and emended description of *Filimonas lacunae*

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Two isolates, with optimum growth temperature and pH of about 30 °C and 6.0–7.0, were recovered from a borehole head of a mineral water aquifer in Portugal. The closest relatives based on 16S rRNA gene sequence analysis were species of genera of the family *Chitinophagaceae*. Strains L2-4^T and L2-109 formed translucent colonies and non-motile pleomorphic cells. Strains were strictly aerobic, and oxidase- and catalase-positive. The major fatty acids of strains L2-4^T and L2-109 were 17 : 0 iso 3-OH, 15 : 0 iso and 15 : 1 iso G. The major polar lipids were phosphatidylethanolamine, one unidentified phospholipid, four unidentified aminophospholipids, four unidentified aminolipids and three unidentified polar lipids. Menaquinone 7 was the only respiratory quinone. The G+C content of the DNA of strains L2-4^T and L2-109 was 42.0 and 41.4 mol%, respectively. Based on 16S rRNA gene sequence analysis, physiological and biochemical characteristics, strains L2-4^T (=CECT 8122^T=LMG 26919^T) and L2-109 (=CECT 8121=LMG 26920) are considered to represent a novel species of a new genus, for which the name *Heliimonas saccharivorans* gen. nov., sp. nov. is proposed. The type strain of *Heliimonas saccharivorans* is L2-4^T. Due to additional results obtained in this study an emended description of *Filimonas lacunae* is provided.

Two bacterial strains, designated L2-4^T and L2-109, were retrieved from the borehole head of a mineral water aquifer in Portugal (40° 23′ N 8° 23′ W). Phylogenetic analysis allocated strains L2-4^T and L2-109 to the family *Chitinophagaceae* within the phylum *Bacteroidetes*. The family *Chitinophagaceae* comprises, at the time of writing, 14 genera, namely *Chitinophaga* (Sangkhobol & Skerman, 1981), *Ferruginibacter* (Lim *et al.*, 2009), *Filimonas* (Shiratori *et al.*, 2009), *Flavihumibacter* (Zhang *et al.*, 2010b), *Flavisolibacter* (Yoon & Im, 2007), *Flavitalea* (Wang *et al.*, 2011), *Hydrotalea* (Kämpfer *et al.*, 2011), *Lacibacter* (Qu *et al.*, 2009), *Niabella* (Kim *et al.*, 2007), *Niastella* (Zhang *et al.*, 2010a), *Parasegetibacter* (Zhang *et al.*, 2009), *Sediminibacterium* (Qu & Yuan, 2008), *Segetibacter* (An *et al.*, 2007) and *Terrimonas* (Xie & Yokota, 2006), many of which comprise only one or two species. In this study we report the isolation and characterization of two strains representing a novel species of a new genus within the family *Chitinophagaceae*.

Abbreviation: RAPD, random amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Heliimonas saccharivorans* L2-4^T and L2-109 are JX458466 and JX475453, respectively.

Samples were obtained from the artesian aquifer through the sampling port at the head of a 150 m stainless steel borehole in central Portugal. The artesian water vented at a temperature of 28.0 °C with a pH of 5.9. The major cations (in mg l^{-1}) were: Na^+, 10.2; K^+, 1.7; Ca^{2+}, 0.9; and Mg^{2+}, 2.4. The major anions (in mg l^{-1}) were: Cl^-, 8.6; SO_4^{2-}, 2.0; HCO_3^-, 25.1; F^-, 0.1; NO_3^-, 1.4; and $H_2PO_4^-$, 0.2. The port was sterilized with the flame of a portable gas torch and the water was then turned on for 10 min before sampling. Water samples were maintained at 4 °C for about 6 h before filtration.

Aliquots of 500 ml were filtered through membrane filters (Gelman type GN-6); strains L2-4^T and L2-109 were isolated from the same sample of borehole head water. Strain L2-4^T was isolated from a filter (0.1 μm pore size) placed on the surface of solidified R2A agar (Difco) containing cycloheximide (10 mg l^{-1}) to inhibit the growth of fungi. Strain L2-109 was isolated from a filter (0.2 μm pore size) placed on the surface of one-tenth

strength R2A agar (Difco) containing cycloheximide (10 mg l^{-1}) and to which 15 g agar l^{-1} (Oxoid) was added. The plates were wrapped in plastic to prevent evaporation and incubated at 22 °C for up to 21 days. Cultures were purified by subculturing and the isolates stored at −70 °C in R2A broth with 15 % (w/v) glycerol.

Random amplified polymorphic DNA (RAPD) analysis was used as a primary method to group the isolates as described by Wiedmann-al-Ahmad *et al.* (1994) (data not shown). A total of 267 isolates were recovered from the borehole head. The isolates belonged primarily to the class *Alphaproteobacteria*, mainly from the orders *Sphingomonadales* and *Caulobacterales*. All isolates were grouped using RAPD analysis (data not shown). Strains L2-4^T and L2-109 had the same RAPD pattern and probably represent the same clone.

The extraction of genomic DNA for 16S rRNA gene sequence determination, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products were carried out as described previously (Rainey *et al.*, 1996). Pairwise similarities of the almost-complete sequence of strain L2-4^T were determined using the online EzTaxon-e database and analysis functions (Kim *et al.*, 2012). The phylogenetic dendrogram (Fig. 1) showing the relationships between strain L2-4^T and related taxa was reconstructed by the neighbour-joining method using the MEGA 4.0 software package (Tamura *et al.*, 2007).

Almost-complete 16S rRNA gene sequences 1456 and 1291 nt in length were determined for strains L2-4^T and L2-109, respectively. Pairwise 16S rRNA gene sequence similarity between strains L2-4^T and L2-109 was 100 %. Phylogenetic analysis showed that the new isolates clustered with the family *Chitinophagaceae* in the phylum *Bacteroidetes* (Fig. 1). 16S rRNA gene sequence comparisons showed that the sequence of strain L2-4^T shared pairwise similarities in the range 88.2–91.6 % with species of genera of the family *Chitinophagaceae*. These included species of the genera *Flavisolibacter* (91.5–91.6 %), *Terrimonas* (90.6–91.4 %), *Filimonas* (91.4 %) and *Niastella* (90.9–91.2 %). These similarity values along with the branching of strain L2-4^T in the phylogenetic tree clearly demonstrate that this strain does not belong to a previously described genus. From a phylogenetic standpoint, based on 16S rRNA gene sequence comparisons strain L2-4^T represents a novel species of a new genus within the family *Chitinophagaceae* and L2-109 is a clone of the same strain.

Hydrotalea flava CCUG 51397^T and *Filimonas lacunae* DSM 21054^T were used for comparative purposes. Unless stated otherwise, all biochemical and tolerance tests were performed as described previously (Albuquerque *et al.*, 2011) in R2A broth or on R2A agar plates incubated at 30 °C for up to 5 days. Cell morphology and motility were examined by phase-contrast microscopy. Gliding motility was examined as described by Bernardet *et al.* (2002). The growth temperature range of the strains was examined with 5 °C increments between 5 and 50 °C by measuring the turbidity (610 nm) of cultures incubated in 300 ml metal-capped Erlenmeyer flasks, containing 100 ml R2A broth.

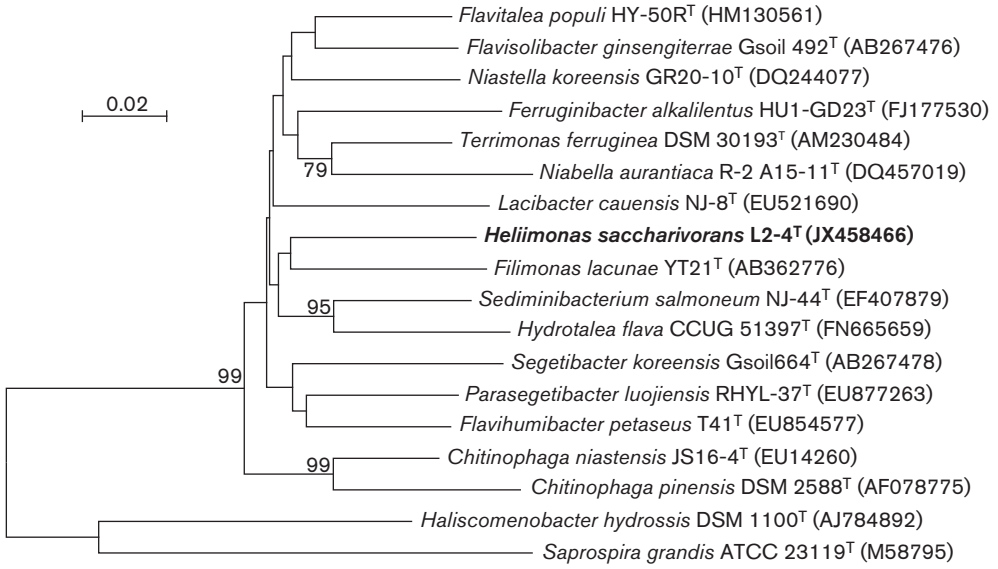

Fig. 1. Phylogenetic dendrogram based on 16S rRNA gene sequence comparisons. The phylogenetic relationships were reconstructed using the MEGA 4 software package and the neighbour-joining method (Tamura *et al.*, 2007). The main cluster supported by a bootstrap value of 99 % contains the type species of each of the genera of the family *Chitinophagaceae* and the novel strain L2-4^T. The two outgroup organisms are representatives of the family *Saprospiraceae*. Bar, 2 inferred nucleotide substitutions per 100 nt.

The pH range for growth was determined at 30 °C in the same medium using 50 mM MES, HEPES, TAPS and CAPSO over a pH range from 4.0 to 10.0 with intervals of 0.5 pH units. Growth in salt-containing medium was determined visually in R2A broth with additional NaCl ranging between 0.0 and 5.0 % (w/v) with intervals of 0.5 %. Anaerobic growth was assessed in the same medium, containing KNO_3 (1.0 g l^{-1}), incubated in anaerobic chambers (GENbox anaer; bioMérieux).

Catalase and oxidase activities and nitrate reduction were examined as described by Smibert & Krieg (1981). Additional characteristics were obtained using the API ZYM system (bioMérieux) and API 20 NE (bioMérieux) test strips incubated at 30 °C, following the manufacturer's instructions. Hydrolysis of casein, starch and aesculin was determined in R2A agar as described by Smibert & Krieg (1981). Chitin hydrolysis was tested using the method described by Skujins *et al.* (1965). The presence of flexirubin-type pigments was determined by flooding a small mass of bacterial cells with 20 % KOH according to Bernardet *et al.* (2002).

Single carbon source assimilation tests were performed in a medium composed of Degryse basal salts (Degryse *et al.*, 1978) to which 0.1 g yeast extract l^{-1} and 0.5 g ammonium chloride l^{-1} were added. Positive control cultures were grown in Degryse medium containing 2.5 g yeast extract l^{-1} and 2.5 g tryptone l^{-1} and negative controls were grown in minimal medium without a carbon source. The following filter-sterilized carbon and energy sources were tested: amino acid mixture as described by Hudson *et al.* (1989); Casamino acids (0.5 g l^{-1}); and single amino acids, carbohydrates, organic acids, polyols and *N*-acetyl-D-glucosamine, each at a final concentration of 2.0 g l^{-1}. Growth was also assayed in Degryse basal salts supplemented with the amino acid mixture (Hudson *et al.*, 1989) with and without 2.0 g fructose l^{-1}; Degryse basal salts supplemented with 0.5 g Casamino acids l^{-1} with and without 2.0 g fructose l^{-1}; Degryse basal salts supplemented with 1 g tryptone l^{-1}; Degryse basal salts supplemented with 1 g peptone l^{-1}; Degryse basal salts supplemented with 1 g yeast extract l^{-1}; Degryse basal salts supplemented with 1 g meat extract l^{-1}; and nutrient broth, plate count broth and trypticase soy broth (Difco). Growth of the strains was examined by measuring the turbidity of cultures incubated at 30 °C in 20 ml screw-capped tubes containing 10 ml medium for up to 5 days.

Strains L2-4^T and L2-109 formed irregular and translucent colonies on R2A medium, giving rise to Gram-stain-negative pleomorphic cells. Cells from a young culture had a rod-shaped morphology, while irregular and coccoid morphologies appeared with ageing. The optimum growth temperature was about 30 °C for both strains and the optimum pH was about 6.0–7.0. Nitrate and nitrite were not reduced. Anaerobic growth in the presence of nitrate was not observed. Other biochemical and physiological characteristics are listed in Table 1. During the assessment of carbon source assimilations, our results showed that strains L2-4^T and L2-109 assimilated only carbohydrates as single carbon source. Growth was not observed on single amino acids, the amino acid mixture of Hudson *et al.* (1989), Casamino acids, polyols or organic acids. Growth did not occur on basal salts medium supplemented with tryptone or peptone. Strains L2-4^T and L2-109 grew on basal salts medium supplemented with yeast extract or meat extract and in plate count broth, nutrient broth and trypticase soy broth. The organisms grew on basal medium supplemented with the amino acid mixture only when a sugar such as fructose was added to the medium, indicating that amino acids were used as nitrogen source but were not used as carbon source. The only explanation for this observation is that gluconeogenesis is not functional under the conditions examined, because organic acids in the presence of ammonium chloride and amino acids in the presence or absence of ammonium chloride do not support growth. On the other hand, the addition of a sugar and the amino acid did support growth of the organisms, which cannot otherwise synthesize sugars.

Cultures of strains L2-4^T, L2-109 and *Filimonas lacunae* YT21^T for polar lipids were grown in R2A medium at 30 °C for 2 days. Harvesting of the cultures, extraction of the lipids and two-dimensional TLC were performed as described previously (da Costa *et al.*, 2006). The reagents dodecamolybdophosphoric acid, molybdenum blue, ninhydrin and α-naphthol-sulphuric acid were used for testing the presence of total polar lipids, phospholipids, aminolipids and glycolipids, respectively (da Costa *et al.*, 2006).

Lipoquinones of strain L2-4^T were extracted from freeze-dried cells, purified by TLC and separated by HPLC (da Costa *et al.*, 2006; Tindall, 1989). The lipoquinones were separated by HPLC with a Gilson chromatograph by using a reversed-phase Hichrom 5 C18 column and methanol/heptane (10 : 2, v/v) as the mobile phase and were detected at 269 nm. Cultures of strains L2-4^T, L2-109, *Filimonas lacunae* YT21^T and *Hydrotalea flava* CCGU 51397^T for fatty acid analysis were grown on R2A plates, in sealed plastic bags submerged in a water bath at 30 °C for 48 h. Fatty acid methyl esters were obtained from fresh wet biomass and were separated, identified and quantified with the standard MIS Library Generation Software (Sherlock Microbial ID System, TSBA 6 database, version 6.0) as described previously (da Costa *et al.*, 2006).

The polar lipid pattern on TLC of strains L2-4^T and L2-109 consisted of phosphatidylethanolamine, one unidentified phospholipid, four unidentified aminophospholipids, four unidentified aminolipids and three unidentified polar lipids (Fig. 2). The only respiratory lipoquinone was menaquinone 7. The fatty acid composition of strains L2-4^T and L2-109 was dominated by 17 : 0 iso 3-OH, 15 : 0 iso and 15 : 1 iso G (Table 2). Other members of the family *Chitinophagaceae* have the same major fatty acids but in

Table 1. Differential characteristics between strains L2-4^T and L2-109 and the type strains of related species

Strains: 1, L2-4^T and L2-109; 2, *Filimonas lacunae* YT21^T (data from Shiratori *et al.*, 2009); 3, *Hydrotalea flava* CCUG 51397^T (Kämpfer *et al.*, 2011; Albuquerque *et al.*, 2012); 4, *Flavisolibacter ginsengiterrae* Gsoil 492^T (Yoon & Im, 2007); 5, *Lacibacter cauensis* NJ-8^T (Qu *et al.*, 2009); 6, *Terrimonas ferruginea* IAM 15098^T (Xie & Yokota, 2006); 7, *Niastella koreensis* GR20-10^T (Zhang *et al.*, 2010a). +, Positive result; −, negative result; W, weakly positive; ND, not determined. All single carbon source assimilations of strains L2-4^T and L2-109 as well as those of *Filimonas lacunae* YT21^T and *Hydrotalea flava* CCUG 51397^T were performed by the authors. Those that were identical to the corresponding characteristics in the original description were left as + or −; characteristics examined in this work alone are given in parentheses; characteristics that were different from those of the original description are given in parentheses after the results from the original publication. All of the organisms examined in this study assimilate D-fructose, D-galactose, D-glucose, cellobiose, D-lactose, maltose, D-mannose, melibiose, raffinose, D-rhamnose, sucrose, trehalose and D-xylose; none of the organisms assimilates D-ribose, D-sorbose, acetate, benzoate, citrate, formate, fumarate, gluconate, lactate, malate, pyruvate, succinate, L-alanine, L-arginine, L-cysteine, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, ethanol, L-arabitol, mannitol, methanol, *myo*-inositol or xylitol.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Pleomorphic	Filamentous	Rods	Rods	Rods	Rods	Filamentous
Gliding motility	−	+	−	−	+	−	+
Pigmentation	Translucent	Light yellow	Orange–yellow	Yellow	Orange	Salmon red	Light yellow
Catalase	+	+	+	−	+	W	−
Oxidase	+	+	+	+	+	+	−
NaCl tolerance (%)	⩽0.5	⩽1.0	⩽4.0	⩽3.0	⩽1.0	⩽1.0	ND
Growth at 37 °C	+	−	+	−	+	+	+
Hydrolysis of:							
Chitin	−	ND	ND	−	ND	−	+
Casein	+	−	ND	−	+	ND	+
Starch	+	−	ND	−	+	ND	−
Gelatin	−	+	ND	+	W	+	+
Aesculin	+	+	ND	+	ND	ND	+
Assimilation of:							
Glycogen	+	−	(+)	+	ND	+	−
L-Arabinose	+	+	−	+	−	−	−
L-Fucose	−	− (+)	(−)	+	ND	ND	−
N-Acetyl-D-glucosamine	+	− (+)	− (+)	+	ND	ND	−
L-Asparagine	−	− (+)	(+)	ND	−	ND	ND
L-Aspartate	−	− (+)	+	ND	ND	ND	ND
L-Glutamate	−	− (+)	(+)	ND	ND	ND	ND
L-Glutamine	−	(+)	(+)	ND	ND	ND	ND
L-Proline	−	− (+)	−	+	ND	ND	−
Glycerol	−	+	−	ND	−	−	ND
DNA G+C content (mol%)	42.0; 41.4	45.2	42.0	43.0	46.6	48.9	45.8

distinct relative proportions. The organism represented by strains L2-4^T and L2-109 has the highest levels of 17 : 0 iso 3-OH; 15 : 0 iso is the major fatty acid in all other type species of this family (Kämpfer *et al.*, 2011; Wang *et al.*, 2011).

DNA for the determination of the genomic G+C content was isolated as described by Nielsen *et al.* (1995). The G+C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989). The G+C content of the DNA of strains L2-4^T and L2-109 was 42.0 and 41.4 mol%, respectively.

Phylogenetic analysis based on 16S rRNA gene sequences showed that strains L2-4^T and L2-109 formed a distinct lineage sharing pairwise similarities in the range 88.2–91.6 % with species of genera of the family *Chitinophagaceae*. Moreover, the inability of these organisms to assimilate any amino acids, amino acid mixtures or proteins clearly distinguish strains L2-4^T and L2-109 from closely related species, such as the type strains of *Hydrotalea flava*, *Hydrotalea sandarakina* and *Filimonas lacunae*, which assimilate several amino acids (Albuquerque *et al.*, 2012; Kämpfer *et al.*, 2011; Shiratori *et al.*, 2009). Unfortunately, the last named species was described as being unable to assimilate amino acids using the Biolog GN2 microplate system, which does not assess assimilation, and the API 20NE system. In other closely related organisms such as *Flavisolibacter ginsengiterrae* Gsoil 492^T, *Terrimonas ferruginea* IAM 15098^T and *Lacibacter cauensis* NJ-8^T, for which the assimilation of only a few amino acids was examined, the results nevertheless indicate that these organisms assimilate some amino acids in the absence of sugars. We showed that *Filimonas lacunae* YT21^T could, in

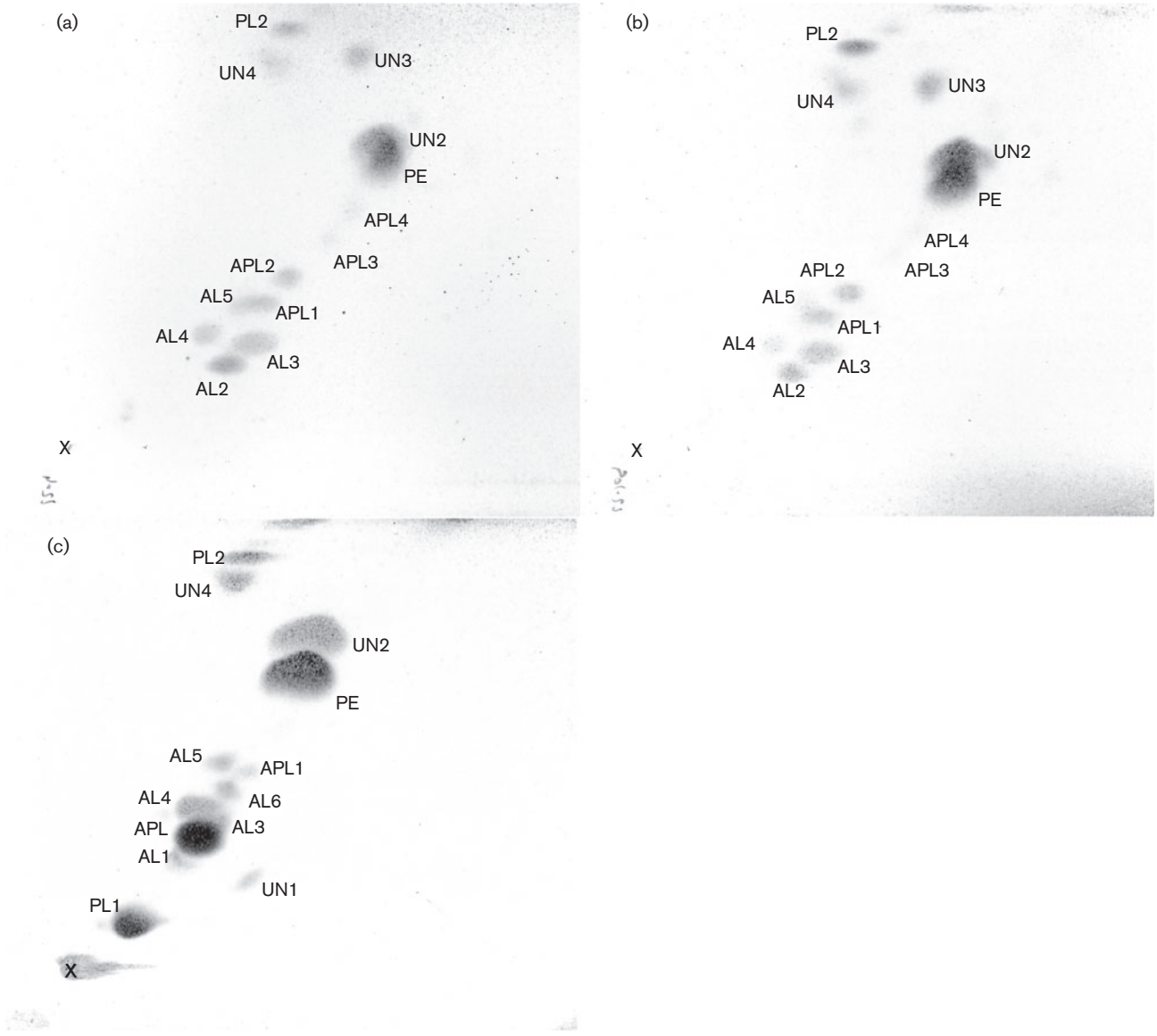

Fig. 2. Two-dimensional TLC of the total polar lipids of strains L2-4^T (a), L2-109 (b) and *Filimonas lacunae* YT21^T (c). AL1–5, unidentified aminolipids; APL1–5, unidentified aminophospholipids; PE, phosphatidylethanolamine; PL1 and 2, unidentified phospholipids; UN1–4, unidentified polar lipids.

fact, assimilate several amino acids using a traditional methodology where a single carbon source is added to a basal medium along with negative and positive controls. Due to the different single carbon source assimilation results and the absence of a polar lipid profile in the description of *Filimonas lacunae* YT21^T (Shiratori *et al.*, 2009), we emend the description of this organism. On the basis of chemotaxonomic and phenotypic characteristics, especially the absence of amino acid utilization, and the low 16S rRNA gene sequence similarity, as compared with other closely related organisms, we suggest that strains L2-4^T and L2-109 represent a novel species of a new genus for which the name *Heliimonas saccharivorans* gen. nov., sp. nov. is proposed.

Emended description of *Filimonas lacunae* Shiratori *et al.* 2009

Characteristics are as given for the description of the species by Shiratori *et al.* (2009) plus the results obtained in this study. The following carbon sources are assimilated using Degryse basal salts basal medium: D-fructose, D-galactose, D-glucose, cellobiose, D-lactose, maltose, D-mannose, melibiose, raffinose, D-rhamnose, sucrose, trehalose, D-xylose, L-arabinose, L-fucose, *N*-acetyl-D-glucosamine, L-asparagine, L-aspartate, L-glutamate, L-glutamine, L-proline and glycerol. The following carbon sources are not assimilated: D-ribose, D-sorbose, glycogen, acetate, benzoate, citrate, formate, fumarate, gluconate, lactate,

Table 2. Mean fatty acid composition (%) of strains L2-4^T and L2-109 and the type strains of related species

Strains: 1, L2-4^T/L2-109; 2, *Filimonas lacunae* YT21^T; 3, *Hydrotalea flava* CCUG 51397^T. All were grown at 30 °C. All data from this study. Values shown are the mean ± SD of two to four analyses; TR, trace (<0.5 %); −, not detected. Values for fatty acids present at levels of less than 0.5 % are not shown.

Fatty acid	1	2	3
11 : 0 iso	0.5 ± 0.1	−	−
UN 11.980*	1.2 ± 0.2	TR	TR
UN 12.560†	−	−	1.3 ± 0.2
13 : 0 iso	0.6 ± 0.1	−	4.7 ± 0.2
UN 13.565‡	1.3 ± 0.9	2.2 ± 0.1	3.5 ± 0.4
14 : 0 iso	−	−	0.8 ± 0.1
14 : 0	TR	1.5 ± 0.1	TR
15 : 1 iso G	12.3 ± 0.3	19.9 ± 0.3	3.0 ± 0.1
15 : 0 iso	23.0 ± 0.9	20.3 ± 0.3	24.0 ± 0.4
15 : 0 anteiso	−	TR	2.8 ± 0.1
15 : 1ω6*c*	−	−	0.7 ± 0.1
15 : 0	0.5 ± 0.1	0.5 ± 0.1	2.2 ± 0.1
16 : 1 isoH	−	−	1.6 ± 0.1
16 : 0 iso	−	1.9 ± 0.2	3.7 ± 0.1
Summed feature 3§	3.0 ± 0.2	14.3 ± 0.4	7.9 ± 0.2
16 : 1ω5*c*	2.1 ± 0.1	3.3 ± 0.1	−
UN 15.939‖	−	−	0.5 ± 0.1
16 : 0	9.6 ± 0.3	5.6 ± 0.2	1.2 ± 0.1
15 : 0 iso 3-OH	1.3 ± 0.1	3.1 ± 0.1	3.5 ± 0.1
17 : 0 iso	2.3 ± 0.1	−	−
15 : 0 2-OH	−	TR	0.7 ± 0.1
Summed feature 9¶	−	−	6.0 ± 0.2
15 : 0 3-OH	−	−	1.5 ± 0.1
UN 16.582#	0.7 ± 0.1	1.5 ± 0.2	1.3 ± 0.1
17 : 0 iso	−	0.5 ± 0.1	0.8 ± 0.1
17 : 1ω8*c*	−	−	0.6 ± 0.1
17 : 1ω6*c*	−	TR	1.2 ± 0.1
16 : 0 iso 3-OH	−	1.2 ± 0.1	5.9 ± 0.2
16 : 0 3-OH	2.8 ± 0.1	4.7 ± 0.2	2.1 ± 0.1
17 : 0 iso 3-OH	37.7 ± 1.1	15.7 ± 0.6	14.4 ± 0.5
17 : 0 2-OH	TR	TR	1.4 ± 0.1

*Unknown fatty acid with an equivalent chain-length of 11.980.
†Unknown fatty acid with an equivalent chain-length of 12.560.
‡Unknown fatty acid with an equivalent chain-length of 13.565.
§Group of fatty acids (16 : 1ω7*c*/16 : 1ω6*c*/15 : 0 iso 2-OH) that could not be separated by this method.
‖Unknown fatty acid with an equivalent chain-length of 15.939.
¶Group of fatty acids (17 : 1 iso ω9*c*/16 : 0 10-methyl) that could not be separated by this method.
#Unknown fatty acid with an equivalent chain-length of 16.582.

malate, pyruvate, succinate, L-alanine, L-arginine, L-cysteine, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, ethanol, L-arabitol, mannitol, methanol, *myo*-inositol and xylitol. The polar lipid profile is composed of phosphatidylethanolamine, two unidentified phospholipids, two unidentified aminophospholipids, five unidentified aminolipids and three unidentified polar lipids.

Description of *Heliimonas* gen. nov.

Heliimonas [Hel.i.i.mo′nas. N.L. n. *Helia* Hel (the goddess of Hell in Nordic mythology); L. fem. n. *monas* a unit, monad; N.L. fem. n. *Heliimonas* a unit (bacterium) isolated near Hell (i.e. isolated from a 150 m borehole)].

Forms pleomorphic cells that stain Gram-negative. Strictly aerobic, and catalase- and oxidase-positive. Major fatty acids are 17 : 0 iso 3-OH, 15 : 0 iso and 15 : 1 iso G; menaquinone 7 is the only respiratory quinone. The major polar lipids are phosphatidylethanolamine, one unidentified phospholipid and one unknown lipid; other amino lipids, aminophospholipids and unknown lipids are minor components. The type species is *Heliimonas saccharivorans*.

Description of *Heliimonas saccharivorans* sp. nov.

Heliimonas saccharivorans (sac.cha.ri.vo′rans. L. neut. n. *saccharum* sugar; L. part. adj. *vorans* devouring; N.L. masc. adj. *saccharivorans* sugar-devouring).

In addition of the characteristics reported for the genus, cells are pleomorphic without gliding motility. Colonies on R2A medium are faintly cream-coloured, translucent and irregular. Growth occurs between 10 and 45 °C; the optimum growth temperature is about 30 °C. The optimum pH for growth is between 6.0 and 7.0; growth does not occur at pH 4.0 or 9.5. Growth occurs only in medium with less than 0.5 % NaCl. In addition to the major polar lipids, four unidentified aminophospholipids, four unidentified aminolipids and three unidentified polar lipids are present. Negative for reduction of nitrate to nitrite, glucose fermentation and indole production. Flexirubin-type pigments are not detected. Positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase; weakly positive for esterase (C4) and esterase lipase (C8); and negative for lipase (C14), α-chymotrypsin, β-glucuronidase, arginine dihydrolase and urease. Casein, aesculin and starch are degraded; chitin and gelatin are not degraded. Assimilates L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycogen, D-lactose, maltose, D-mannose, melibiose, *N*-acetyl-D-glucosamine, raffinose, D-rhamnose, sucrose, trehalose and D-xylose but not L-fucose, D-ribose, D-sorbose, fumarate, gluconate, acetate, succinate, pyruvate, malate, benzoate, citrate, formate, lactate, L-valine, L-phenylalanine, L-serine, L-leucine, L-alanine, L-aspartate, L-asparagine, L-histidine, L-glutamine, L-isoleucine, L-lysine, L-glutamate, L-proline, L-threonine, L-methionine, L-arginine, L-glycine, L-tryptophan, L-cysteine, L-tyrosine, Hudson's amino acid mixture, glycerol, mannitol, L-arabitol, *myo*-inositol, ethanol, methanol or xylitol.

The type strain, L2-4^T (=CECT 8122^T=LMG 26919^T), and strain L2-109 (=CECT 8121=LMG 26920) were isolated from a deep mineral water aquifer in Portugal. The G+C content of the DNA of the type strain is 42.0 mol%.

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References

Albuquerque, L., Rainey, F. A., Nobre, M. F. & da Costa, M. S. (2011). *Schleiferia thermophila* gen. nov., sp. nov., a slightly thermophilic bacterium of the phylum '*Bacteroidetes*' and the proposal of *Schleiferiaceae* fam. nov. *Int J Syst Evol Microbiol* **61**, 2450–2455.

Albuquerque, L., Rainey, F. A., Nobre, M. F. & da Costa, M. S. (2012). *Hydrotalea sandarakina* sp. nov., isolated from a hot spring runoff, and emended descriptions of the genus *Hydrotalea* and the species *Hydrotalea flava*. *Int J Syst Evol Microbiol* **62**, 1603–1608.

An, D.-S., Lee, H.-G., Im, W.-T., Liu, Q.-M. & Lee, S.-T. (2007). *Segetibacter koreensis* gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes*, isolated from the soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **57**, 1828–1833.

Bernardet, J.-F., Nakagawa, Y., Holmes, B. & Subcommittee on the taxonomy of Flavobacterium and Cytophaga-like bacteria of the International Committee on Systematics of Prokaryotes (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.

da Costa, M. S., Nobre, M. F. & Wait, R. (2006). Analysis of lipids from extremophilic bacteria. In *Methods in Microbiology*, vol. 35, pp. 127–159. Edited by F. A. Rainey & A. Oren. Amsterdam: Elsevier.

Degryse, E., Glansdorff, N. & Piérard, A. (1978). A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. *Arch Microbiol* **117**, 189–196.

Hudson, J. A., Schofield, K. M., Morgan, H. W. & Daniel, R. M. (1989). *Thermonema lapsum* gen. nov., sp. nov., a thermophilic gliding bacterium. *Int J Syst Bacteriol* **39**, 485–487.

Kämpfer, P., Lodders, N. & Falsen, E. (2011). *Hydrotalea flava* gen. nov., sp. nov., a new member of the phylum *Bacteroidetes* and allocation of the genera *Chitinophaga*, *Sediminibacterium*, *Lacibacter*, *Flavihumibacter*, *Flavisolibacter*, *Niabella*, *Niastella*, *Segetibacter*, *Parasegetibacter*, *Terrimonas*, *Ferruginibacter*, *Filimonas* and *Hydrotalea* to the family *Chitinophagaceae* fam. nov. *Int J Syst Evol Microbiol* **61**, 518–523.

Kim, B.-Y., Weon, H.-Y., Yoo, S.-H., Hong, S.-B., Kwon, S.-W., Stackebrandt, E. & Go, S.-J. (2007). *Niabella aurantiaca* gen. nov., sp. nov., isolated from a greenhouse soil in Korea. *Int J Syst Evol Microbiol* **57**, 538–541.

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.

Lim, J. H., Baek, S.-H. & Lee, S.-T. (2009). *Ferruginibacter alkalilentus* gen. nov., sp. nov. and *Ferruginibacter lapsinanis* sp. nov., novel members of the family '*Chitinophagaceae*' in the phylum *Bacteroidetes*, isolated from freshwater sediment. *Int J Syst Evol Microbiol* **59**, 2394–2399.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Nielsen, P., Fritze, D. & Priest, F. G. (1995). Phenetic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. *Microbiology* **141**, 1745–1761.

Qu, J.-H. & Yuan, H.-L. (2008). *Sediminibacterium salmoneum* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic reservoir. *Int J Syst Evol Microbiol* **58**, 2191–2194.

Qu, J.-H., Yuan, H.-L., Yang, J.-S., Li, H.-F. & Chen, N. (2009). *Lacibacter cauensis* gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic lake. *Int J Syst Evol Microbiol* **59**, 1153–1157.

Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.

Sangkhobol, V. & Skerman, V. B. D. (1981). *Chitinophaga*, a new genus of chitinolytic *Myxobacteria*. *Int J Syst Bacteriol* **31**, 285–293.

Shiratori, H., Tagami, Y., Morishita, T., Kamihara, Y., Beppu, T. & Ueda, K. (2009). *Filimonas lacunae* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from fresh water. *Int J Syst Evol Microbiol* **59**, 1137–1142.

Skujins, J. J., Potgieter, H. J. & Alexander, M. (1965). Dissolution of fungal cell walls by a streptomycete chitinase and β-(1-3) glucanase. *Arch Biochem Biophys* **111**, 358–364.

Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, pp. 409–443. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Tindall, B. J. (1989). Fully saturated menaquinones in the archaebacterium *Pyrobaculum islandicum*. *FEMS Microbiol Lett* **60**, 251–253.

Wang, Y., Cai, F., Tang, Y., Dai, J., Qi, H., Rahman, E., Peng, F. & Fang, C. (2011). *Flavitalea populi* gen. nov., sp. nov., isolated from soil of a Euphrates poplar (*Populus euphratica*) forest. *Int J Syst Evol Microbiol* **61**, 1554–1560.

Wiedmann-al-Ahmad, M., Tichy, H. V. & Schon, G. (1994). Characterization of *Acinetobacter* type strains and isolates obtained from wastewater treatment plants by PCR fingerprinting. *Appl Environ Microbiol* **60**, 4066–4071.

Xie, C.-H. & Yokota, A. (2006). Reclassification of [*Flavobacterium*] *ferrugineum* as *Terrimonas ferruginea* gen. nov., comb. nov., and description of *Terrimonas lutea* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 1117–1121.

Yoon, M.-H. & Im, W.-T. (2007). *Flavisolibacter ginsengiterrae* gen. nov., sp. nov. and *Flavisolibacter ginsengisoli* sp. nov., isolated from ginseng cultivating soil. *Int J Syst Evol Microbiol* **57**, 1834–1839.

Zhang, K., Tang, Y., Zhang, L., Dai, J., Wang, Y., Luo, X., Liu, M., Luo, G. & Fang, C. (2009). *Parasegetibacter luojiensis* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from a forest soil. *Int J Syst Evol Microbiol* **59**, 3058–3062.

Zhang, K., Wang, Y., Tang, Y., Dai, J., Zhang, L., An, H., Luo, G., Rahman, E. & Fang, C. (2010a). *Niastella populi* sp. nov., isolated from soil of Euphrates poplar (*Populus euphratica*) forest, and emended description of the genus *Niastella*. *Int J Syst Evol Microbiol* **60**, 542–545.

Zhang, N. N., Qu, J. H., Yuan, H. L., Sun, Y. M. & Yang, J. S. (2010b). *Flavihumibacter petaseus* gen. nov., sp. nov., isolated from soil of a subtropical rainforest. *Int J Syst Evol Microbiol* **60**, 1609–1612.