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Proposal of *Vibrionimonas magnilacihabitans* gen. nov., sp. nov., a Curved Gram Negative Bacterium Isolated From Lake Michigan Water

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Proposal of *Vibrionimonas magnilacihabitans* gen. nov., sp. nov., a Curved Gram-Stain-Negative Bacterium Isolated from Lake Water

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Microbial profiling of freshwater environments using 16S rRNA sequence-based methods has found that the microbial flora from this environment is primarily affiliated with the *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Verrucomicrobia*, Obsidian Pond-10 and Planctomycetes (Zwart et al., 2002). Typically, *Betaproteobacteria* are the most common, but *Actinobacteria* and *Bacteroidetes* can be nearly as common (Cottrell et al., 2005 ; Van der Gucht et al., 2005 ; Allgaier & Grossart, 2006 ; Newton et al., 2006 ; Schauer et al., 2005 ; Eiler & Bertilsson, 2004). Characterization of the microbial community in potable water by molecular-based sequencing methods found that potable water contained a bacterial community dominated by members of the *Alphaproteobacteria* and *Betaproteobacteria* (Santo Domingo et al., 2003 ; Williams et al., 2004).

Located in the upper Midwest of the USA is a group of five lakes that are collectively known as the Great Lakes. The Great Lakes contain approximately 22600 km 3 of fresh water or about 22% of the world's fresh water, making them the largest source of fresh water in

the world. The second largest lake, Lake Michigan, contains approximately 4900 km 3 of fresh water and is an important source of potable water as well as being used for recreation and commerce.

Very little is known about the microbial community associated with Lake Michigan water. To our knowledge there has only been one published study that has characterized this microbial flora. This study reported that *Betaproteobacteria* were predominant, followed by *Bacteroidetes* and *Alphaproteobacteria* (Mueller-Spitz et al., 2009). Characterization of the microbial community found in Lake Michigan potable water has, to our knowledge, not been reported, although Albert et al. (2010) did report the isolation of the novel *Alphaproteobacteria*, *Labrys wisconsinensis* from Lake Michigan potable water. Recently, Albert et al. (2013) reported the isolation of a novel *Bacteroidetes*, *Sphingobacterium psychroaquaticum* from Lake Michigan potable water.

During the taxonomic investigation of the bacterial population in Lake Michigan water collected from the Port Washington, Wisconsin community water system on August 11, 2008 and August 2, 2007, two strains were isolated and designated MU-2 T and MU-1, respectively. The strains were initially isolated by plating the water samples on plate count agar (PCA) (Difco) and incubating them at 32 °C for five days. These two strains were subjected to detailed characterization in order to identify their taxonomic positions.

The isolation of DNA and generation of PCR products for 16S rRNA analysis were performed using the following protocol. Sequencing of the 16S rRNA gene was accomplished by extracting the DNA from a pure culture using the PowerSoil DNA Isolation Sample kit (MoBio Laboratories). PCR amplification was performed using EconoTaq PLUS 2X Master Mix, which included the Taq polymerase (Lucigen). The bacterial primers that were utilized in this experiment were 27F (5'-AGAGTTTGATCATGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') as suggested by Lane (1991) . PCR was performed using the EuBact method. PCR cycling parameters were a hot start of 94 °C (2 min), denaturing temperature of 94 °C (30 s), annealing temperature of 55 °C (30 s) and elongation temperature of 72 °C (1 min) for 30 cycles before a final elongation of 72 °C for 4 min (Fode-Vaughan et al., 2001). The PCR products were sent to the

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University of Chicago Cancer Research Center for sequencing. DNA sequence data were analysed and assembled using the FinchTV (Geospira) and Vector NTI (Invitrogen) software and consensus sequences were assembled. Vector sequences were removed using a program designed internally for high-throughput analysis. The program uses the Basic Local Alignment Search Tool (blast) to match vector sequences in the UniVec Database (http://www.ncbi.nlm.nih.gov/VecScreen/UniVec.html) with the sample sequences in a manner identical to VecScreen (http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html). Those sequence sections matching the vector were removed from the sample sequences prior to sequencing to increase the accuracy and quality of the sequencing process (Altschul et al., 1997). A distance matrix based on the Kimura two-parameter algorithm was constructed using the Phylogeny Inference Package (phylip, version 3.68) dnadist program (Felsenstein, 2005). Neighbour-joining (Fig. 1), maximumparsimony (Fig. S1, available in IJSEM Online) and maximumlikelihood trees (Fig. S2) were reconstructed using phylip neighbour, dnapars and dnaml respectively. Consensus trees were generated with the phylip consensus program, using the extended majority rule. The resulting trees were visualized using FigTree, version 1.1.2 (Rambaut, 2008) and were found to be similar. Tree topology was evaluated by bootstrap analyses performed using the phylip seqboot program to generate 100 resamples.

The two strains MU-2 T and MU-1 shared 99.7% 16S rRNA gene sequence similarity. This implied relationship at the species level was confirmed by enterobacterial repetitive intergenic consensus (ERIC)-PCR-generated genomic fingerprinting carried out according to the method of Wieser & Busse (2000) . Both strains exhibited identical banding patterns indicating that they are representatives of a single species (Fig. 2). Since random amplified polymorphic DNA (RAPD)-PCR is considered to be more specific than ERIC-PCR, the relationship between the two strains was examined by genomic fingerprinting using four different RAPD-PCR primers. As shown in Fig. S3, fingerprints for each primer were identical for the two strains. These results are in agreement with ERIC fingerprinting and strongly indicate that the two strains are clonally related.

The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon–e server (http://eztaxon-e-ezbiocloud.net/; Kim et al., 2012). The 16S rRNA gene sequence of MU-2 T (FJ816610) exhibited highest similarities with *Sediminibacterium ginsengisoli* DCY13 T (94.4%), Sediminibacterium salmoneum NJ-44 T (93.6%), *Hydrotalea flava* CCUG 51397 T (93.1%), *Hydrotalea sandarakina* AF-51 T (91.8%), *Flavisolibacter gingsengiterrae* Gsoil 492 T (91.5%) and *Flavisolibacter ginsengisoli* Gsoil 643 T (91.5%). The detailed phylogenetic relationship between strain MU-2 T and related species is presented in Fig. 1 . Examination of the phylogenetic relationship (Figs 1 , S1 and S2) between strains MU-2 T and MU-1 and related strains indicates that strains MU-2 T and MU-1 form a distinct phylogenetic lineage within the family *Chitinophagaceae*.

Phenotypic characterization of the two strains was accomplished by examination of physiological, biochemical and chemotaxonomic traits. The physiological and biochemical characteristics reported in Table 1 were determined as follows. For strains MU-2 T and MU-1, all tests were performed at 25 °C, except for growth temperature range, using 48–72 h old cultures, grown on R2A (Difco). Incubation times and temperatures used for Sediminibacterium ginsengisoli KCTC 12833 T and Sediminibacterium salmoneum NBRC 103935 T were the same except that all tests were performed using cells grown on plate count agar (PCA) (Difco). Inoculum for tests utilizing cell suspensions was prepared in phosphate buffered water (Maturin & Peeler, 2001). Motility testing, catalase activity, oxidase testing, Gram staining, gelatin and casein hydrolysis and nitrate reduction testing were performed using standard procedures (Smibert & Krieg, 1994). Capsule staining was performed using the method of Murray et al. (1994). Temperature range for growth of strains MU-2 T, MU-1, Sediminibacterium salmoneum NBRC 103935 T and Sediminibacterium ginsengisoli KCTC 12833 T was determined by visual examination for growth on R2A plates. Prior to inoculation, the plates were incubated at the test temperature for 24 h. After inoculation, plates were incubated for up to ten days. Ability to utilize various carbohydrates as growth substrates was evaluated by using the method of Isalm et al. (2007), with modifications as described by Albert et al. (2010). Tubes containing 5 ml growth media were incubated with shaking aeration for up to 10 days. Growth was visually compared with that of controls,

which did not contain a carbohydrate source. Aerobic acid production was determined using the procedure described by Albert et al. (2013) with modifications. Phenol red (0.0025%) was used as the pH indicator and the growth medium was based upon R2A (Difco) with modifications, including not adding the soluble starch, sodium pyruvate and glucose.

Anaerobic growth was determined by visually monitoring for growth in plate count broth (Difco). Prior to inoculation, screw capped tubes were filled with plate count broth and incubated under anaerobic conditions for 48 h. Anaerobic conditions were generated by using H 2 and CO 2 GasPaks (BBL) in anaerobe jars. Cell suspensions were used to inoculate the tubes containing the test medium. After inoculation the tubes were placed in the anaerobe jars and anaerobic conditions were regenerated using the H 2 and CO 2 GasPaks. Ten days later the tubes were removed from the anaerobic conditions and visually examined for growth. Turbidity was used to indicate growth. If turbidity was present tubes were streaked to PCA to determine the identity of the bacterium present.

Results for the physiological and biochemical characterization performed as part of this study are reported in Table 1 . Examination of the results for strains MU-2 Tand MU-1 shows that the results for the two strains are nearly identical, except for the ability to assimilate d-galactose. Strain MU-2 T readily assimilated d-galactose, while strain MU-1 was weakly positive for d-galactose assimilation. When compared with members of the genus Sediminibacterium both similarities and differences were observed. Differences included the production of a salmon pink pigment by Sediminibacterium salmoneum NBRC 103935 T and Sediminibacterium ginsengisoli KCTC 12833 T, while strains MU-2 T and MU-1 are colourless to light tan (Fig. S4). Strains MU-2 T and MU-1 were aerobically able to produce acid from dgalactose, d-rhamnose and d-xylose while both Sediminibacterium salmoneum NBRC 103935 T and Sediminibacterium ginsengisoli KCTC 12833 T did not. Sediminibacterium salmoneum NBRC 103935 T and Sediminibacterium ginsengisoli KCTC 12833 T were able to hydrolyse gelatin, albeit weakly in the case of the former, while both strains MU-2 T and MU-1 did not. When compared with previously published results, this study found primarily similar results for Sediminibacterium ginsengisoli KCTC 12833 T and for Sediminibacterium salmoneum

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NBRC 103935 T. Differences in growth temperature range, anaerobic growth, [NaCl] tolerance and carbohydrate assimilation were probably due to differences in methodology.

Analysis of mol% G+C was performed by DSMZ Identification Services. The G+C content was determined using DNA purified on hydroxyapatite according to the method of Cashion et al. (1977) from French-pressed cells grown in 1/10th strength BHI broth (Difco) for 72 h at 25 °C . The DNA was hydrolysed and processed according to the protocol of Mesbah et al. (1989) . The resulting deoxyribonucleosides were analysed by HPLC (Tamaoka & Komagata, 1984). Standards included phage lambda DNA and three DNAs from published genome sequences representing a G+C range of 43–72 mol%. G+C values were calculated using the ratio of deoxyguanosine and thymidine as described by Mesbah et al. (1989) . The mol% G+C content of strain MU-2 T was found to be 45.2, which is higher than the 40.2 reported for the nearest phylogenetic relative of MU-2 T, *Sediminibacterium ginsengisoli* KCTC 12833 T.

The cellular fatty acid (CFA) profile was determined by growing strains MU-2 T, MU-1, Sediminibacterium salmoneum NBRC 103935 T and Sediminibacterium ginsengisoli KCTC 12833 T on R2A (Difco) at 25 °C for 48 and 72 h and then harvesting cells according to standard MIDI procedure (Sasser, 2001). After harvesting, bacteria were saponified, and the liberated fatty acids were methylated and analysed by capillary gas-liquid chromatography (model 6890, Hewlett Packard) by the Sherlock system (MIDI 4.5), according to the manufacturer's instructions. The CFA profiles of strain MU-2 T, MU-1, Sediminibacterium salmoneum NBRC 103935 T and Sediminibacterium ginsengisoli KCTC 12833 T are reported in Table 2 . For both strains MU-2 T and MU-1, there were four major cellular fatty acids, iso-C 15:1G, iso-C 15:0, iso-C 16:0 3-OH and iso-C 17:0 3-OH and moderate amounts of iso-C 16:0. For strain MU-2 T, after 48 and 72 h of growth these five fatty acids accounted for 73.6% and 72.3%, respectively.

When compared with the CFA profile for *Sediminibacterium ginsengisoli* KCTC 12833 T the CFA profile of strain MU-2 T revealed similarities in the primary fatty acids and differences in the secondary fatty acids. Both MU-2 T and *Sediminibacterium ginsengisoli* KCTC

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12833 T share three primary fatty acids, which are iso-C 15:1G, iso-C 15:0 and iso-C 17:0 3-OH. While iso-C 16:0 3-OH was a primary fatty acid in MU-2 T it was a secondary fatty acid in *Sediminibacterium ginsengisoli* KCTC 12833 T.

When compared with the CFA profile for *Sediminibacterium salmoneum* NBRC 103935 T the CFA profile of strain MU-2 T reveals both similarities and differences. Both strain MU-2 T and *Sediminibacterium salmoneum* NBRC 103935 T share three primary cellular fatty acids, iso-C 15:1G, iso-C 15:0 and C 16:0 3-OH. Although iso-C 17:0 3-OH, was a primary fatty acid in MU-2 T it was present at moderate amounts in *Sediminibacterium salmoneum* NBRC 103935 T. Results from this study for *Sediminibacterium ginsengisoli* KCTC 12833 T and *Sediminibacterium salmoneum* NBRC 103935 T are similar to those previously reported by Kim et al. (2013) and Qu & Yuan (2008), respectively.

Polar lipids, guinones and polyamines were extracted from cells grown on PYE medium (0.3% peptone from casein, 0.3% yeast extract, pH 7.2) at 28 °C. Polar lipids and guinones were extracted and analysed as described previously (Tindall, 1990a, b; Altenburger et al., 1996). Polyamines were extracted and analysed as described by Busse & Auling (1988). HPLC analyses were carried out using the equipment described by Stolz et al. (2007). The polyamine pattern of MU-2 T was composed of 81.5 μ mol g (dry weight) -1 symhomospermidine, 2.4 μ mol g (dry weight) -1 spermidine, 0.5 μ mol g (dry weight) -1 spermine and 0.1 µmol g (dry weight) -1 putrescine. The guinone system of MU-2 T consisted of menaguinone MK-7 (99%) and MK-6 (1%). The polar lipid profile (Fig. 3a) was composed of the major compound phosphatidylethanolamine (PE), moderate amounts of two unidentified glycolipids (GL1 and GL3), three polar lipids (L1, L3 and L4), one aminophospholipid (APL1), one aminolipid (AL3) and minor amounts of another glycolipid (GL2), an aminolipid (AL2), a phospholipid (PL2) and trace amount of three polar lipids (L5, L6 and L7) and an aminolipid (AL1). The presence of glycolipids is a distinguishing characteristic from the members of the genus Sediminibacterium .

Sediminibacterium ginsengisoli KCTC 12833 T was characterized by a polyamine pattern consisting of 8.4 μ mol g (dry weight) -1

spermidine, 78.4 μ mol g (dry weight) -1 sym-homospermidine and 2.4 μ mol q (dry weight) -1 spermine. The guinone system was composed of menaquinone MK-7 (99%) and traces of MK-6 and MK-8 (each <1%). Both polyamine pattern and guinone system did not exhibit striking differences from the corresponding characteristics of strain MU-2 T. Kim et al. (2013) reported that the only respiratory quinone they identified in Sediminibacterium ginsengisoli KCTC 12833 T was MK-7, which is in accordance with our results. This study found that the polar lipid profile (Fig. 3b) of Sediminibacterium ginsengisoli KCTC 12833 T was composed of the major compound PE, moderate amounts of two unidentified aminolipids (AL2 and AL3), three unidentified polar lipids (L1, L3 and L4), an unidentified aminophospholipid (APL1) and one unidentified phospholipid (PL1), minor amounts of two polar lipids (L2 and L5), aminolipid (AL1) and an unknown phospholipid (PL2). These results are similar to those reported by Kim et al. (2013), but there were differences. Both studies reported PE as the major polar lipid and the presence of unknown polar lipids, with this study reporting five, while Kim et al. (2013) reported three. Both studies reported the presence of unidentified aminolipids (AL), although the number reported was different between the two studies, with the present study reporting three and Kim et al. (2013) reporting one. Additionally, Kim et al. (2013) reported two unknown aminophospholipids (APL) while the present study reported one and the present study found two unknown phospholipids (PL1 and PL2) which were not reported by Kim et al. (2013).

It is evident from the highly similar phenotype (biochemical, physiological traits and fatty acid profile) and identical genomic fingerprints that strains MU-2 T and MU-1 are representatives of the same species and are most probably clonally related. The presence of glycolipids differentiates strains MU-2 T and MU-1 from related genera (Table 1). On the basis of the phylogenetic, chemotaxonomic, physiological and biochemical evidence, it is concluded that strains MU-2 T and MU-1 represent a novel species in a new genus (Table 1) for which the name *Vibrionimonas magnilacihabitans* gen. nov., sp. nov. is proposed.

Description of Vibrionimonas gen. nov.

Vibrionimonas [Vi.bri.o.ni.mo'nas. N.L. n. vibrio -onis, a bacterial genus name of bacteria possessing a curved rod shape (Vibrio); L. fem. n. monas, a unit, monad; N.L. fem. n. *Vibrionimonas*, a vibrio(-like) monad, referring to curved rod-shaped bacterium].

Cells are curved rod-shaped, $3-4 \mu m$ in length and $1 \mu m$ wide and occur singly, in pairs or in short chains (Fig. S5). Gram-stainnegative. Aerobic, catalase- and oxidase-positive. The major cellular fatty acids are iso-C 15:1 G, iso-C 15:0, iso-C 16:0 3-OH and iso-C 17:0 3-OH with moderate amounts of iso-C 16:0. The polar lipid profile is composed of the major compound phosphatidylethanolamine (PE), moderate amounts of two unidentified glycolipids (GL1 and GL3), three polar lipids (L1, L3, L4), one aminophospholipid (APL1), one aminolipid (AL3) and minor amounts of another glycolipid (GL2), an aminolipid (AL2), a phospholipid (PL2) and trace amount of three polar lipids (L5, L6, L7) and an aminolipid (AL1) with the presence of glycolipids being characteristic of the genus. The primary respiratory quinone is menaquinone MK-7, while the major polyamine is *sym*homospermidine.

The type strain of the type species is *Vibrionimonas magnilacihabitans* MU-2 T.

Description of *Vibrionimonas magnilacihabitans* sp. nov.

Vibrionimonas magnilacihabitans (mag.ni.la.ci.ha.bitans. L. adj. magnus great; L. n. lacus, lake; L. masc. n. habitans, a dweller; N.L. masc. n. *magnilacihabitans*, great lake dweller, referring to the lake, Lake Michigan, one of the Great Lakes, from which the type species was first isolated).

The description is the same as for the genus with the following additional characteristics. Capsules not produced. The [Nacl] growth range is 0-0.4%. The temperature range for growth is 15–40 °C. Cells tend to stick together and obtaining isolated colonies is difficult. Periodically, in heavy streak areas, zones of no growth can be

observed, although viable cells are present (Fig. S5). Grows well on PCA and R2A (Difco) but does not grow on TSA (Difco), TSBA (Difco), PIA (Difco) and MacConkey agar (Difco). Grows very slowly after several weeks of refrigerator storage. Viability during refrigerator storage is greater on R2A than on PCA. When grown on PCA colonies are colourless to light tan (Fig. S4). Growth is significantly enhanced by yeast extract, Casamino acids and phytopeptone. Acid is produced under aerobic conditions from cellobiose, d-galactose, d-glucose, lactose, maltose, d-mannose, raffinose, d-rhamnose, trehalose, sucrose and d-xylose but not from d-arabinose, fructose, d-mannitol, d-gluconate, d-glycerol, d-ribose and Tween 80. d-arabinose, cellobiose, d-galactose, d-glucose, lactose, d-mannitol and sucrose are assimilated while fructose and d-xylose are not. Casein gelation and starch are not hydrolysed.

The organism was isolated from a Lake Michigan water sample from south-eastern Wisconsin. Strain MU-2 T has been deposited with the DSMZ (Braunschweig, Germany) as DSM 22423 with the USDA Northern Regional Research Lab, Peoria, Illinois as NRRL B-59231. Strain MU-1 has been deposited with the DSMZ as DSM 21712 with USDA Northern Regional Research Lab as NRRL B-51334. The Gen Bank accession number for the 16S rRNA gene sequence of strain MU-2 T is FJ816610, while that for the corresponding sequence from strain MU-1 is EU559746. The DNA mol% G+C content of MU-2 T is 45.2.

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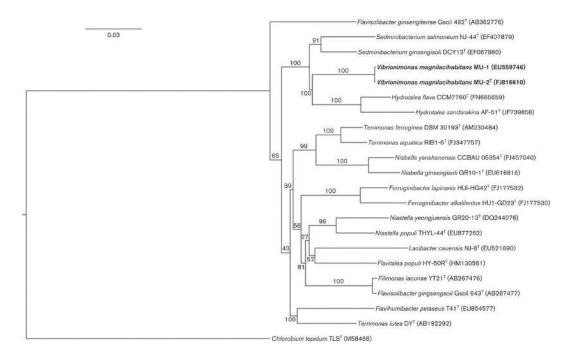


Fig. 1. Phylogenetic tree based on almost complete 16S rRNA gene sequences of the types strains of species related to MU-1 and MU-2T. The tree was reconstructed using the neighbour-joining tree reconstruction algorithm. Parameters used to construct the tree are discussed in the text.

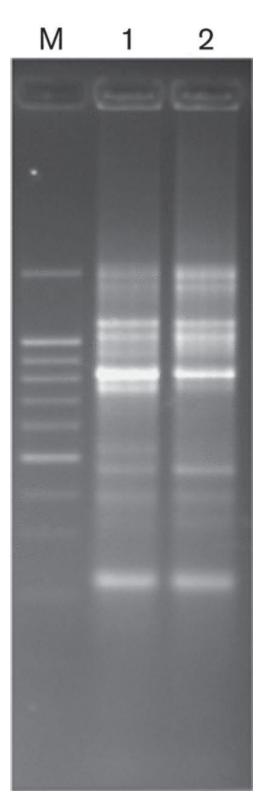


Fig. 2. ERIC-PCR generated genomic fingerprints of strains MU-1 (lane 1) and MU-2T (lane 2) and (M) 100 bp ladder.

| Characteristic | 1 | 2 | 3 | 4 | | |
|-----------------------------------|---|--|---|--|--|--|
| Cell shape | - Curved rod | Rod | Rod to curved | Rod | | |
| | | | rod | | | |
| Temperature range for growth | 15-40 °C | 10-40 °C | 10-35 °C | 20-37 °C | | |
| [NaCl] range (%) for growth | 0-0.4 | 0-1.1 | 0-0.5 | 0-4.0 | | |
| Pigmentation | None | Salmon pink | Salmon pink | Orange | | |
| Gelatin hydrolysis | _ | w+ | + | nd | | |
| Casein hydrolysis | _ | - | - | nd | | |
| Capsule formation | - | - | - | nd | | |
| Assimilation of carbon sources | | | | | | |
| d-arabinose | + | w+ | - | nd | | |
| Cellobiose | + | w+ | - | + | | |
| Fructose | - | w+ | - | w+ | | |
| d-galactose | + | + | + | w+ | | |
| d-glucose | + | + | + | - | | |
| d-mannitol | + | - | - | _ | | |
| Lactose | + | w+ | + | nd | | |
| Sucrose | + | + | + | - | | |
| Aerobic acid production | | | | | | |
| d-arabinose | - | - | - | nd | | |
| Cellobiose | + | - | + | - | | |
| Fructose | - | - | - | nd | | |
| d-galactose | + | - | - | nd | | |
| d-gluconate | - | - | - | nd | | |
| d-glucose | + | + | + | - | | |
| d-glycerol | - | - | - | nd | | |
| Lactose | + | - | + | _ | | |
| Maltose | + | - | + | - | | |
| d-mannose | + | + | + | - | | |
| Raffinose | + | - | + | - | | |
| d-rhamnose | + | - | - | nd | | |
| d-ribose | - | - | - | nd | | |
| Sucrose | + | - | + | - | | |
| Trehalose | + | + | + | - | | |
| Tween 80 | - | - | - | nd | | |
| d-xylose | + | - | - | - | | |
| Polar lipids | PE, GL1, GL3, L1, L3, L4, APL1, AL3 | PE, AL2, AL3, L1,L3, L4, APL1, PL1 | PE, AL, UL1, UL3, UL4, APL3 | PE, AL1, AL2, APL1, APL2, AL, L1, L2, L3, L4 | | |
| Major fatty acids | iso-C15:1G, iso- C15:0, iso-C16:0 3- OH, iso-C17:0 3-OH | iso-C15:1G, iso-C15:0, iso- C17:0 3-OH | iso-C15:1G, iso- C15:0, iso- C16:0 3-OH | iso-C15:1G, iso- C15:0, iso-C17:0 3-OH | | |

| Characteristic | 1 | 2 | 3 | 4 |
|---|-----------|----------------|--|--------------|
| Fatty acids present in moderate amounts | iso-C16:0 | iso-C16:0 3-OH | iso-C15:0 3-OH, iso-C17:0 3-OH iso-C14:0 | iso-C17:1ω9c |
| DNA G+C content (mol%) | 45.2 | 40.6 | nd | 42.0 |

Table 1. Morphological and phenotypic characteristics differentiating Vibrionimonas magnilacihabitans sp. nov. (MU-2T and MU-1) from *Sediminibacterium ginsengisoli* KCTC 12833T, *Sediminibacterium salmoneum* NBRC 103935T and *Hydrotalea flava* CCUG 51397T

1, Vibrionimonas magnilacihabitans MU-2T and MU-1; 2, *Sediminibacterium ginsengisoli* KCTC 12833T (data from this study) 3, *Sediminibacterium salmoneum* NBRC 103935T (data from this study). 4, *Hydrotalea flava* CCUG 51397 T (Kämpfer et al., 2011; Albuquerque et al., 2012). All species share the characteristics of being Gram-stain-negative, motile, catalase- and oxidase-positive, negative for capsule formation, anaerobic growth, nitrate reduction and assimilation of d-xylose. Strain MU-1 was weakly positive for the assimilation of d-galactose. All species did not produce aerobic acid from d-mannitol. +, Positive; -, negative; w+, weakly positive; nd, not determined. PE, phosphatidylethanolamine; AL, aminolipid; APL, aminophospholipid; GL, glycolipid; PL, phospholipid; L, other polar lipid; UL, unidentified lipid.

| | | 1 | | 2 | 3 | | 4 | |
|-------------------------|------|------|------|------|------|------|------|------|
| | 48 h | 72 h |
| iso-C13:0 | 2.9 | 2.9 | 2.9 | 2.9 | 1.4 | 1.7 | 1.0 | 1.1 |
| iso-C14:0 | 1.6 | 1.8 | 1.7 | 1.9 | 0.8 | 0.9 | 6.2 | 6.2 |
| iso-C15:1G | 15.2 | 14.8 | 15.6 | 14.8 | 22.0 | 23.2 | 24.7 | 23.2 |
| anteiso-C15:1A | 1.0 | 1.0 | 1.0 | 0.9 | 0.4 | 0.5 | 6.7 | 6.7 |
| iso-C15:0 | 31.1 | 29.2 | 29.8 | 27.7 | 30.8 | 24.0 | | |
| anteiso-C15:0 | 4.4 | 4.4 | 4.1 | 4.2 | 2.2 | 2.1 | 9.6 | 9.8 |
| iso-C16:0 | 6.1 | 6.0 | 5.7 | 5.5 | 1.0 | 0.7 | 1.5 | 1.4 |
| iso-C15:0 2-OH/C16:0ω7c | 2.1 | 2.4 | 2.2 | 2.6 | 3.2 | 4.0 | nd | nd |
| iso-C15:0 3-OH | 2.9 | 3.1 | 3.1 | 3.1 | 4.0 | 5.3 | 6.9 | 7.0 |
| C15:0 3-OH | 1.2 | 1.6 | 1.4 | 2.0 | 1.0 | 1.5 | 0.7 | 0.7 |
| iso-C16:0 3-OH | 8.5 | 10.2 | 9.1 | 10.3 | 4.8 | 6.1 | 11.3 | 11.9 |
| C16:0 3-OH | 1.6 | 1.7 | 1.7 | 1.8 | 2.2 | 2.6 | 1.7 | 1.8 |
| iso-C17:0 3-OH | 12.7 | 12.1 | 13.2 | 12.3 | 20.2 | 20.4 | 5.7 | 5.7 |
| C17:0 2-OH | 1.2 | 1.2 | 1.1 | 1.2 | 0.8 | 0.9 | 1.4 | 1.5 |
| | | | | | | | | |

Table 2. Cellular fatty acid profile of strains MU-2T, MU-1, Sediminibacterium

 ginsengisoli KCTC 12833T and Sediminibacterium salmoneum NBRC 103935T

Strains: 1, MU-2T; 2, MU-1; 3, *Sediminibacterium ginsengisoli* KCTC 12833T;4, *Sediminibacterium salmoneum* NBRC 103935T. All data from this study. Strains were grown on R2A (Difco) at 25 °C for 48 and 72 h. nd, Not detected.

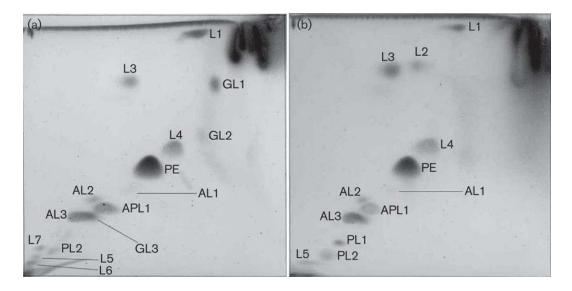


Fig. 3. Polar lipid profile of (a) MU-2T and (b) *Sediminibacterium ginsengisoli* KCTC 12833T after two-dimensional TLC and detection with molybdatophosphoric acid. PE, Phosphatidylethanolamine; PL1–2, unidentified phospholipids; AL1–3, unidentified aminolipids; APL1, unidentified aminophospholipid; GL1–3, unidentified glycolipids; L1–7, polar lipids not stainable with reagents that detect a phosphate group, an amino group or a sugar moiety.