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

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Six Gram-negative, non-gliding, rod-shaped bacterial strains isolated from freshwater sediment were subjected to polyphasic analyses to determine their taxonomic positions. Analysis of the 16S rRNA gene sequences of the six strains revealed that they represent two separate genomic species in a new lineage within the phylum *Bacteroidetes*, related to members of the family 'Chitinophagaceae', in which they were most closely related to members of the genus *Terrimonas* (93.4–90.5%). Two of the isolates, HU1-GD23^T and HU1-HG42^T, had a sequence similarity of 96.2%, with DNA G + C contents of 39.4 and 38.5 mol%, respectively. They possessed MK-7 as the predominant respiratory quinone and contained high amounts of iso-pentadecanoic acid and 3-hydroxy-iso-heptadecanoic acid in their cell envelopes, properties shared by members of the family 'Chitinophagaceae'. They were well differentiated from other members of the family 'Chitinophagaceae' by additional physiological and biochemical characteristics. In conclusion, strains HU1-GD23^T (=KCTC 22306^T=LMG 24312^T) and HU1-HG42^T (=KCTC 22305^T=LMG 24324^T) are considered to represent two novel species of a novel genus, *Ferruginibacter* gen. nov., for which the names *Ferruginibacter alkalilentus* sp. nov. and *Ferruginibacter lapsinanis* sp. nov., are proposed, respectively.

The phylum *Bacteroidetes* (Garrity & Holt, 2001) is phenotypically diverse and still undergoing considerable taxonomic revision. Several members formerly misclassified in the genera *Flexibacter* and *Cytophaga* have been transferred to the genus *Chitinophaga* (Kämpfer *et al.*, 2006), which originally belonged to the family *Crenotrichaceae* (Approved Lists 1980) that should not be used any more as its type genus, *Crenothrix*, turned out to belong to the phylum *Proteobacteria* (Stoecker *et al.*, 2006) [Rule 37a (1)]. A new family, 'Chitinophagaceae', was subsequently suggested to be created for the genus *Chitinophaga* on the basis of 16S rRNA gene phylogenetic analyses (Ludwig *et al.*, 2008), which also indicated that the genera *Terrimonas* (Xie & Yokota, 2006), *Niastella* (Weon *et al.*, 2006), *Niabella* (Kim *et al.*, 2007), *Flavisolibacter* (Yoon & Im, 2007) and others are phylogenetically classified in the same family. Sediment from a freshwater lake was investigated for bacterial culturability and diversity by Lim *et al.* (2008), and six novel isolates, HU1-GD23^T, HU1-GB11, HU1-ID41, HU1-IH3, HU1-

JE21 and HU1-HG42^T were found to be phylogenetically related to members of the family 'Chitinophagaceae'. On the basis of further taxonomic studies, we propose here that strain HU1-GD23^T along with four other strains and HU1-HG42^T represent two novel species of a novel genus, *Ferruginibacter* gen. nov. within the family 'Chitinophagaceae' of the phylum *Bacteroidetes*.

Sediment samples were collected from a littoral zone of Lake Hakha (36°17'N, 127°18'32''E), a typical freshwater lake in South Korea, and subjected to dilution-to-extinction cultivation (Button *et al.*, 1993) in 96-well plates, wherein cells were enriched on unsupplemented lake water media of low nutritional status for 3 months, followed by the detection of extinction wells, as described previously by Lim *et al.* (2008). Single colonies were isolated on 1:10-diluted R2A agar (Difco) plates to which bacterial suspension had been transferred from the wells containing pure cultures. The isolates were then routinely propagated aerobically on R2A agar at 30 °C for 5 days.

Colonies were directly applied to 16S rRNA gene PCR and amplified fragments were then sequenced using the primers 27F and 1100R (Lane, 1991), 341F and 534R (Muyzer *et al.*, 1993) and 907F (Lane *et al.*, 1985). Sequences were assembled with SEQMAN II 5.0 (DNASTAR) (Swindell &

The GenBank/EMBL/DDBJ accession numbers for the newly determined 16S rRNA gene sequences of strains HU1-GD23^T, HU1-IH3, HU1-HG42^T, HU1-JE21, HU1-ID41 and HU1-GB11 are FJ177530–177532 and FJ263932–263934, respectively.

Plasterer, 1997) and compared with all 16S rRNA gene sequences available in the GenBank database. Alignment of sequences was carried out with CLUSTAL_X (Thompson *et al.*, 1997). Gaps at the 5' and 3' ends and ambiguous bases were removed from the alignment using BIOEDIT (Hall, 1999). Phylogenetic trees based on comparison of 1283 bases were constructed by the neighbour-joining (Saitou & Nei, 1987) and the maximum-parsimony (Fitch, 1971) algorithms using MEGA version 3.1 (Kumar *et al.*, 2004). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983) and bootstrap values were based on 1000 replications (Felsenstein, 1985). Chromosomal DNA was extracted and purified using Qiagen Genomic-tip system 100/G for determination of G+C content, which was measured as described by Mesbah *et al.* (1989) using reverse-phase HPLC (Younglin).

Cell morphology and motility were examined by phase-contrast microscopy. Catalase and oxidase activities were determined by using standard methods (Cowan & Steel, 1965). Gram test was performed according to Buck (1982). Presence of flexirubin-like pigment was investigated by flooding culture plates with 20% (w/v) KOH (Fautz & Reichenbach, 1980). Substrate assimilation was tested on a defined liquid medium consisting of basal salts (I^{-1} : 1.8 g K_2HPO_4 , 1.08 g KH_2PO_4 , 0.5 g NaNO_3 , 0.5 g NH_4Cl , 0.1 g KCl , 0.1 g MgSO_4 , 0.05 g CaCl_2), vitamin mixture (Widdel & Bak, 1992), trace element solution SL-10 (Pedersen & Ekendahl, 1990) and selenite/tungstate solution (Tschech & Pfennig, 1984). The pH was adjusted to 6.8 and the medium was then supplemented with a single carbon source that had been filter-sterilized. Additional substrate-utilization and biochemical characters were determined using API 32GN, API 20NE, API 20E and API ZYM galleries, according to the manufacturer's protocol

(bioMérieux). Results of API tests except for API ZYM were read after 10 days incubation. DNase activity was detected on DNase test agar (Scharlau) by flooding culture plates with 1 M HCl. Hydrolysis of starch, chitin, aesculin and skim milk were examined according to Atlas (2004). Degradation of cellulose and xylan were also studied (Ten *et al.*, 2004). Anaerobic growth was tested on R2A medium containing 1 g thioglycolate l^{-1} in a serum bottle with the headspace substituted by nitrogen gas. Nitrate reduction was carried out both aerobically and anaerobically in a serum bottle supplemented with 10 mM KNO_3 as an electron acceptor, and was monitored by an ion chromatograph (Metrohm) equipped with a conductivity detector and anion exchange column. Isoprenoid quinones were determined as described by Komagata & Suzuki (1987) using Sep-Pak Vac silica cartridges (Waters). For quantitative analysis of cellular fatty acid compositions, cells were harvested after 3 days incubation at 28 °C on R2A, based on the culture conditions used for some members of the family 'Chitinophagaceae' that cannot grow on TSA (Weon *et al.*, 2006; Qu & Yuan, 2008). Cellular fatty acid methyl esters were prepared and identified following the protocol of the Sherlock Microbial Identification System (MIDI). All the phenotypic tests described above were performed more than twice with 5 days incubation, unless otherwise specified.

As shown in Fig. 1, the six new isolates were phylogenetically located within a cluster consisting of members of the genus *Terrimonas* and other recently validated genera in the family 'Chitinophagaceae'. This *Terrimonas* cluster has been displayed as a separate branch from the highly robust cluster comprising only members of the genus *Chitinophaga* (Kämpfer *et al.*, 2006; Kim *et al.*, 2007; Yoon & Im, 2007; Ludwig *et al.*, 2008). Comparison of 16S rRNA gene sequences showed that strains HU1-GD23^T and

Fig. 1. Neighbour-joining bootstrap consensus tree, showing relationships between the six new isolates and phylogenetic members of the family 'Chitinophagaceae'. Bootstrap values of 50% or more (using 1000 replications) are indicated at branch points. Filled circles represent that the corresponding nodes were also recovered in maximum-parsimony trees. Accession numbers are shown in parentheses. Bar, 0.02 substitutions per nucleotide position.

HU1-HG42^T had a sequence similarity of 96.2 %, forming a coherent cluster that shared considerably lower similarities with members of the genera *Terrimonas* (93.4–90.5 %), *Flavisolibacter* (92.1–91.6 %) and *Niastella* (91–90.6 %). Strain HU1-GD23^T and four more isolates that were tightly clustered together showed more than 99.6 % similarity to each other, indicating they belong to the same genomic species (Fig. 1). DNA G+C contents of strain HU1-GD23^T and HU1-HG42^T were 39.4 and 38.5 mol%, respectively, close to those of previously identified members of ‘*Chitinophagaceae*’ (40.4–49.8 mol%). Strain HU1-GD23^T and the other four strains showed a very narrow range of G+C content (39.4–39.5 mol%), supporting their phylogenetic affiliation in the same genomic species.

All the isolates were Gram-negative, strictly aerobic, non-sporulating and optimally grew at 30 °C and pH 7 in the absence of NaCl. None of the strains showed filamentous morphology, gliding motility, chitinase activity or a

flexirubin-positive reaction that have been shown to be differential characteristics for members of the genera *Chitinophaga*, *Flexibacter* and *Cytophaga* (Reichenbach, 1989a, b; Skerman, 1989). Lack of these traits is generally shared by non-*Chitinophaga* members of the family ‘*Chitinophagaceae*’ (Table 1). All the strains produced MK-7 as the major menaquinone. High levels of two iso-branched fatty acids (iso-C_{15:0} and iso-C_{17:0} 3-OH) were in good agreement with the cellular fatty acid profiles of previously described members of the family ‘*Chitinophagaceae*’. All the strains lacked *cis*-5-hexadecenoic acid (C_{16:1} ω5c) but possessed iso-pentadecenoic acid (iso-C_{15:1} G) abundantly, a character typically found in non-*Chitinophaga* species (Weon *et al.*, 2006, 2008; Xie & Yokota, 2006; An *et al.*, 2007; Kim *et al.*, 2007; Yoon & Im, 2007; Qu & Yuan, 2008). Strain HU1-GD23^T along with the four other isolates and HU1-HG42^T were distinguished from each other by quantitative and some minor qualitative differences found in the fatty acid composition; the difference in the amount of summed feature 3 (iso-

Table 1. Phenotypic characteristics of the *Ferruginibacter* gen. nov. and related genera phylogenetically belonging to the family ‘*Chitinophagaceae*’

Genera: 1, *Ferruginibacter* gen. nov.; 2, *Terrimonas* (data from Xie & Yokota, 2006); 3, *Flavisolibacter* (Yoon & Im, 2007); 4, *Niastella* (Weon *et al.*, 2006); 5, *Sediminibacterium* (Qu & Yuan, 2008); 6, *Niabella* (Kim *et al.*, 2007; Weon *et al.*, 2008); 7, *Segetibacter* (An *et al.*, 2007); 8, *Chitinophaga* (Sangkhobol & Skerman, 1981; Reichenbach, 1989a; Fujita *et al.*, 1996; Kämpfer *et al.*, 2006). All organisms are aerobic and Gram-negative. +, All species positive; –, all species negative; w, all species weakly positive; v, variable among species; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Filamentous cell shape	–	–	–	+	–	–	–	v
Cell length (μm)	1–5	1–3	0.6–6	10–50	1–1.2	0.8–1.5	1.6–2	0.6–80
Gliding motility	–	–	–	+	+	–	–	v
Flexirubin reaction	–	–	–	–	–	+	ND	+*
Growth at 37 °C	–	+	v	v	+	–	–	v
Growth on TSA	–	+	–	–	–	+*	–	+*
Acid produced from glucose	–	+	–	–	+	–	–	+*
Nitrate reduction	–	+	–	–	–	–	–	v
Catalase/oxidase activity	+ / +	w / +	v / v	– / v	+ / +	+ / v	+ / +	v / v
Hydrolysis of:								
L-Arginine	+	–	–	–	–	–	+	v
Chitin	–	–	–	+	–	–	–	v
Gelatin	+	+	+	+	ND	–	–	v
2-Naphthylcaprylate	–	+	+	–	ND	+	–	v
2-Naphthylphosphate (pH 5.4)	–	+	+	+	ND	+	+	+*
Starch	–	v	–	–	ND	–	–	v
Urea	+	–	–	–	–	–	+	v
Quinone system	MK-7	MK-7	MK-7	MK-7	ND	MK-7	MK-7	MK-7
DNA G+C content (mol%)	38.5–39.5	47.2–48.9	42.7–43	44.3–45.8	40.6	45	40.4	40.7–49.8
Major fatty acids†	iso-C _{15:0} , iso-C _{17:0} , 3-OH, iso-C _{15:1} G	iso-C _{15:0} , iso-C _{17:0} , 3-OH, iso-C _{15:1} G	iso-C _{15:0} , iso-C _{17:0} , 3-OH, (iso-C _{15:1} G)	iso-C _{15:0} , iso-C _{17:0} , 3-OH, iso-C _{15:1} G	iso-C _{15:0} , (iso-C _{17:0}), iso-C _{15:1} G	iso-C _{15:0} , iso-C _{17:0} , 3-OH, iso-C _{15:1} G	iso-C _{15:0} , iso-C _{17:0} , 3-OH, iso-C _{15:1} G, C _{16:1} ω5c	iso-C _{15:0} , (iso-C _{17:0}), 3-OH), C _{16:1} ω5c

*Data not available for some species.

†Components that exist in relatively small amounts (<10 %, >5 %) are indicated in parentheses.

C_{15:0} 2-OH and/or C_{16:1} ω7c) was particularly noticeable (Table 2). Differences in the physiological and biochemical characteristics listed in Table 3 also supported their affiliation to two separate species. Strain HU1-GD23^T and the other four isolates shared major phenotypic and biochemical traits with some variations as listed in Tables 2 and 3, and the species description, clearly demonstrating they belonged to a single species.

All of the above genotypic, phenotypic and chemotaxonomic data indicate a high degree of probability that strains HU1-GD23^T and HU1-HG42^T are members of a new lineage within the *Terrimonas* cluster (Fig. 1). Therefore, it is appropriate to classify strains HU1-GD23^T and HU1-HG42^T as separate species of a novel genus in the family 'Chitinophagaceae', for which the name *Ferruginibacter* gen. nov. is proposed, with the names *Ferruginibacter alkalilentus* sp. nov. and *Ferruginibacter lapsinensis* sp. nov. for strains HU1-GD23^T and HU1-HG42^T, respectively.

Description of *Ferruginibacter* gen. nov.

Ferruginibacter (Fer.ru.gi.ni.bac'ter. L. n. *ferrugo*, -inis colour of iron rust; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Ferruginibacter* rust-coloured rod)

Table 2. Cellular fatty acid composition (%) of *Ferruginibacter* species and *Terrimonas ferruginea*

Strains: 1, *Ferruginibacter alkalilentus* (5 strains included); 2, *Ferruginibacter lapsinensis* HU1-HG42^T; 3, *Terrimonas ferruginea* KCTC 2909^T. All the strains were subjected to the analysis after 3 days incubation at 28 °C on R2A. Values for *F. alkalilentus* are represented as mean values (±SD) for five strains with type-strain values indicated in parentheses. Summed feature 3=iso-C_{15:0} 2-OH and/or C_{16:1} ω7c. ECL, Unknown fatty acid of equivalent chain-length with indicated number of carbons.

Fatty acid	1	2	3
ECL 11.543	1.2 ± 0.4 (1.5)	—	—
iso-C _{13:0}	0.3 ± 0.4 (0.6)	3.2	0.9
ECL 13.565	1.6 ± 0.5 (2.1)	1.1	0.7
iso-C _{14:0}	0.2 ± 0.4	—	—
C _{14:0}	0.7 ± 0.5	—	0.8
iso-C _{15:1} G	21.7 ± 1.2 (22.3)	21.7	26.1
anteiso-C _{15:1} A	—	—	1.2
iso-C _{15:0}	30.2 ± 1.9 (30.4)	32.1	27.3
anteiso-C _{15:0}	1.6 ± 0.4 (2.0)	—	2.4
C _{15:0}	1.6 ± 0.5 (2.1)	—	4.1
iso-C _{16:0}	0.5 ± 0.6 (1.1)	—	—
anteiso-C _{16:0}	2.1 ± 0.1 (1.9)	1.5	1.2
Summed feature 3	4.4 ± 0.4 (4.0)	14.3	12.2
C _{16:0}	3.9 ± 0.9 (2.6)	3.3	3.2
iso-C _{15:0} 3-OH	9.1 ± 0.4 (8.8)	4.6	2.1
C _{15:0} 2-OH	—	—	1.0
iso-C _{16:0} 3-OH	2.6 ± 0.4 (3.2)	—	—
C _{16:0} 3-OH	4.5 ± 1.6 (2.1)	2.7	3.0
iso-C _{17:0} 3-OH	13.7 ± 1.8 (15.1)	15.6	14.0

Cells are Gram-negative, strictly aerobic, non-gliding, non-filamentous single rods. Colonies are rusty in colour. Flexirubin-like pigments are not found. Catalase and oxidase are positive. Nitrate is not reduced under either anaerobic or aerobic conditions. Predominant respiratory quinone is MK-7. Cellular fatty acids include large amounts of iso-C_{15:0} and iso-C_{15:1} G. The G+C content of chromosomal DNA is 38.5–39.5 mol%. Phylogenetically positioned within the family 'Chitinophagaceae', class 'Sphingobacteria', phylum Bacteroidetes. The type species is *Ferruginibacter alkalilentus*.

Description of *Ferruginibacter alkalilentus* sp. nov.

Ferruginibacter alkalilentus (al.ka.li.len'tus. N.L. n. *alkali* from the Arabic word *al-qaliy*, the ashes of saltwort; L. adj. *lentus*, -a, -um tough, resistant; N.L. masc. adj. *alkalilentus* resistant to alkaline solution, referring to negative reaction for the flexirubin-KOH test)

Rod-like cells occur as single units, 0.3–0.5 µm in diameter, but vary in length and shape depending on age.

Table 3. Differential characteristics between *Ferruginibacter* species and *Terrimonas ferruginea*

Strains: 1, *Ferruginibacter alkalilentus* (5 strains included); 2, *Ferruginibacter lapsinensis* HU1-HG42^T; 3, *Terrimonas ferruginea* IAM 15098^T (Xie & Yokota, 2006). All strains are positive for hydrolysis of 2-naphthylphosphate (pH 8.5) and negative for production of H₂S and indole, hydrolysis of DNA, 6-bromo-2-naphthyl-α-D-galactopyranoside, 2-naphthyl-β-D-galactopyranoside, 6-bromo-2-naphthyl-β-D-glucopyranoside, naphthol-AS-BI-β-D-glucuronide, 6-bromo-2-naphthyl-α-D-mannopyranoside and 2-naphthylmyristate, acid production from inositol, mannitol and sucrose, and assimilation of dulcitol, fructose, galactose and glycerol. +, All strains positive; —, all strains negative; w, all strains weakly positive; v(+), strain-dependent and positive for type strain; v(w), strain-dependent and weakly positive for type strain; ND, no data available.

Characteristic	1	2	3
Growth on nutrient agar (Difco)	—	—	+
Highest NaCl concentration (% w/v) tolerated	<0.5	<0.5	1
Hydrolysis of:			
L-Leucyl-2-naphthylamide	—	+	+
p-Nitrophenyl-β-D-galactopyranoside	w	—	ND
Assimilation of:			
L-Arabinose	v(w)	—	—
Citrate	v(w)	+	—
Glucose	v(w)	—	+
Melibiose	v(+)	—	—
Raffinose	+	—	—
Rhamnose	—	—	+
Trehalose	+	—	—
D-Xylose	+	—	+

Cells of young cultures (2 days old) are straight, slender, 3–5 µm long, with slightly tapered ends; cells of 5-day-old cultures are curved or sometimes semicircle-shaped (strain-dependent), 1–3 µm long with rounded or tapered (strain-dependent) ends. Colonies grown on R2A at 30 °C for 5 days are circular and convex with an entire margin, 0.7–1.2 mm in diameter, translucent, glossy and rusty in colour, and tend to be slimy. Temperature range for growth is 18–30 °C; growth rate is strain-dependent. Cells grow within the pH range 6–8 in the presence of less than 0.5 % (w/v) NaCl. No growth on either MacConkey agar or nutrient agar (Difco). H₂S production, indole production, nitrite reduction and acetoin production from pyruvate are not observed. Aesculin, gelatin, skim milk and urea are hydrolysed, but hydroxyethylcellulose, DNA, starch and xylan are not. No acid is produced from amygdalin, L-arabinose, D-mannitol, D-melibiose, L-rhamnose or D-sucrose. Activity for arginine dihydrolase is present, but activities for lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are not. 2-Naphthylphosphate (pH 8.5), naphthol-AS-BI-phosphate (weakly) and *p*-nitrophenyl-β-D-galactopyranoside (weakly) are hydrolysed, but *N*-benzoyl-DL-arginine 2-naphthylamide, 2-naphthylbutyrate, L-cystyl-2-naphthylamide, *N*-glutarylphenylalanine 2-naphthylamide, 2-naphthyl-α-L-fucopyranoside, *o*-nitrophenyl-β-D-galactopyranoside, 2-naphthyl-α-D-glucopyranoside and L-valyl-2-naphthylamide are not. Hydrolysis of L-naphthyl-*N*-acetyl-β-D-glucosaminide is strain-dependent (negative for type strain). All strains can utilize D-raffinose, D-trehalose, xylitol (weakly) and D-xylose as sole carbon sources, but the following compounds are not assimilated: adipate, adonitol, L-alanine, amygdalin, benzoate, caprate, D-cellobiose, dextran, formate, D-fructose, L-fucose, fumarate, D-galactose, glutarate, glycogen, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, inulin, itaconate, 2-ketogluconate, 5-ketogluconate, DL-lactate, D-lactose, D-lyxose, malate, maleate, malonate, D-maltose, oxalate, phenylacetate, L-proline, propionate, pyruvate, D-ribose, L-rhamnose, salicin, L-serine, L-sorbose, suberate, succinate, tartrate, tyrosine, valerate and L-xylose. Utilization of *N*-acetyl-D-glucosamine, D-melibiose and D-sucrose (positive for type strain), acetate, L-arabinose, citrate, gluconate, D-glucose, inositol, D-mannitol, D-mannose and D-sorbitol (weakly positive for type strain) and D-arabinose (negative for type strain) are strain-dependent. Additional characteristics are listed in Tables 1–3 and the genus description, or described elsewhere in the text.

The type strain, HU1-GD23^T (=KCTC 22306^T=LMG 24312^T), and four other strains, HU1-GB11 (=KCTC 22307=LMG 24310), HU1-ID41 (=KCTC 22308=LMG 24325), HU1-IH3 (=KCTC 22304=LMG 24326) and HU1-JE21 (=KCTC 22303=LMG 24327), were isolated from freshwater sediment.

Description of *Ferruginibacter lapsinensis* sp. nov.

Ferruginibacter lapsinensis (lap.si'na.nis. L. n. *lapsus* -us gliding; L. adj. *inanis* void; N.L. masc. adj. void of gliding motility)

Cells grown on R2A at 30 °C for 5 days are single, short rods, 1–2 µm long and 0.3–0.5 µm wide. Colonies are circular and convex with an entire margin, slimy, translucent, glossy, rusty in colour, and reach 1–2.2 mm in diameter after 5 days incubation. Growth occurs at 18–30 °C and at pH 6–8 with less than 0.5 % (w/v) NaCl. No growth on either MacConkey agar or nutrient agar (Difco). H₂S production, indole production, nitrite reduction and acetoin production from pyruvate are not observed. Aesculin, gelatin, skim milk and urea are hydrolysed, but hydroxyethylcellulose, DNA, starch and xylan are not. Acid is not produced from amygdalin, L-arabinose, D-mannitol, D-melibiose, L-rhamnose or D-sucrose. Activity for arginine dihydrolase is present, but activities for lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are not. L-Leucyl-2-naphthylamide, 2-naphthylphosphate (pH 8.5) and naphthol-AS-BI-phosphate (weakly) are hydrolysed, but *N*-benzoyl-DL-arginine 2-naphthylamide, 2-naphthylbutyrate, L-cystyl-2-naphthylamide, *N*-glutarylphenylalanine 2-naphthylamide, 2-naphthyl-α-L-fucopyranoside, 2-naphthyl-α-D-glucopyranoside, *o*-nitrophenyl-β-D-galactopyranoside, L-naphthyl-*N*-acetyl-β-D-glucosaminide and L-valyl-2-naphthylamide are not. Only citrate among the substrates tested is utilized as sole carbon source and the following compounds are not: acetate, adipate, adonitol, L-alanine, amygdalin, D-arabinose, benzoate, caprate, D-cellobiose, dextran, formate, D-fructose, L-fucose, fumarate, D-galactose, gluconate, *N*-acetyl-D-glucosamine, D-glucose, glutarate, glycogen, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, inositol, inulin, itaconate, 2-ketogluconate, 5-ketogluconate, DL-lactate, D-lactose, D-lyxose, malate, maleate, malonate, D-maltose, D-mannitol, D-mannose, D-melibiose, oxalate, phenylacetate, L-proline, propionate, pyruvate, D-raffinose, D-ribose, L-rhamnose, salicin, L-serine, D-sorbitol, L-sorbose, suberate, succinate, D-sucrose, tartrate, D-trehalose, tyrosine, valerate, xylitol and L-xylose. Additional characteristics are listed in Tables 1–3 and the genus description, or described elsewhere in the text.

The type strain, HU1-HG42^T (=KCTC 22305^T=LMG 24324^T), was isolated from freshwater sediment.

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