Filimonas lacunae gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from fresh water

Hatsumi Shiratori, Yudai Tagami, Tomoyo Morishita, Yuka Kamihara, Teruhiko Beppu and Kenji Ueda

Correspondence
Hatsumi Shiratori
siratori@brs.nihon-u.ac.jp

Life Science Research Center, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa 252-8510, Japan

A Gram-negative, strictly aerobic, motile, filamentous and viscous exopolymer-producing bacterium, designated strain YT21^T, was isolated from fresh water. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain YT21^T lies within a cluster containing the established genera *Segetibacter*, *Terrimonas*, *Niastella* and *Chitinophaga* in the phylum *Bacteroidetes*. However, the isolate represented a lineage distinct from these genera, with sequence similarities ranging from 88.9 to 91.8 %. The genomic G+C content was 45.2 mol%. The predominant isoprenoid quinone was MK-7. The major fatty acids were 15 : 0 iso, 17 : 0 iso 3-OH and 15 : 1 iso G. On the basis of morphological features and phenotypic and phylogenetic data, strain YT21^T represents a novel genus and species, for which the name *Filimonas lacunae* gen. nov., sp. nov. is proposed. The type strain of *Filimonas lacunae* is strain YT21^T (=NBRC 104114^T =DSM 21054^T).

Bacteria belonging to the phylum *Bacteroidetes* (Garrity & Holt, 2001) are widely distributed over a diverse range of ecological niches. They are routinely isolated from marine and fresh water, soil, plants and air (Bowman *et al.*, 1997; Buczolits *et al.*, 2002). This group of bacteria is characterized by the ability to degrade various biopolymers; hence, they are considered to be important members of the bacterial community involved in metabolic activities in various environments. Here, we carried out taxonomic characterization of a novel bacterial isolate (strain YT21^T), which belongs to the *Bacteroidetes*. We studied this strain because of its marked colony growth under a high-CO_2 atmosphere, since the taxonomic diversity of bacteria that require high levels of CO_2 for growth has not been fully studied (Ueda *et al.*, 2008). Based on the distinct physiological and phylogenetic properties, we propose a new bacterial genus, in which YT21^T represents a novel species.

Strain YT21^T was isolated from shallow fresh water in Fujisawa city (Kanagawa, Japan). The sample was diluted with saline solution (0.9 %, w/v) and spread on 1 : 100 modified nutrient agar no. 2 [containing (g l^{-1}): Bacto peptone, 0.1; meat extract, 0.1; NaCl, 0.05; agar, 20.0 (pH 7.0–7.2)] after serial dilution with saline solution. Chemicals were purchased from Kokusan unless indicated otherwise. Plates were incubated at 30 °C for 10 days under a 5 % CO_2 atmosphere using a CO_2 incubator (model 5400; Napco). Colonies were then replica-plated onto Luria–Bertani (LB) agar [containing (g l^{-1}): Bacto tryptone (Difco), 10.0; Bacto yeast extract (Difco), 5.0; NaCl, 5.0; agar, 15.0 (pH 7.6)] and checked for dependence on a high-CO_2 atmosphere. One of the isolates, strain YT21^T, grew effectively in the CO_2 incubator but not in a normal incubator. In order to characterize its physiological properties, strain YT21^T was grown on 1 : 2-diluted LB agar (pH 7.0) and R2A agar (pH 7.0; Difco). The culture was maintained as a glycerol suspension (20 %, w/v) or by using a Microbank (Pro-Lab Diagnostics) at −80 °C.

Cell morphology was observed under a Axioskop 2 optical microscope (Carl Zeiss Microimaging) and a JEM-1200EX electron microscope (JEOL), using cells that had been grown for 72 h at 30 °C. For transmission electron microscopy, cells were fixed with 2.0 % (v/v) glutaraldehyde and 2.0 % (v/v) osmium tetroxide. Samples were embedded in epoxy resin (Epon 812). Ultrathin sections were prepared using an LKB-8800 ultramicrotome. Specimens were stained with uranyl acetate solution (2.0 % uranyl acetate and lead citrate) and exposed to carbon vapour prior to observation. The Gram reaction was performed following the method described by Bartholomew & Mittwer (1952). Gliding motility was checked by observing the edges of colonies formed on LB and R2A agar. Oxidase was tested using oxidase reagent (bioMérieux) according to the instructions of the manufacturer. Catalase activity was determined by assessing bubble production in 3.0 % (w/v) H_2O_2. Carbon source

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YT21^T is AB362776.

Micrographs showing cultural and morphological characteristics of strain YT21^T are available as supplementary material with the online version of this paper.

utilization and enzyme activities were tested by using API 20NE and API ZYM test strips (bioMérieux) and GN2 MicroPlates (Biolog). The API strips were incubated for 2–3 days at 30 °C. The API ZYM strips and Biolog GN2 MicroPlates were checked for results after 4 h or 72 h incubation at 30 °C, according to the manufacturer's instructions. To test biochemical characteristics, the methods of Tindall *et al.* (2007) were used for the following tests: hydrolysis of agar, casein, starch, DNA, chitin (1.0 %, w/v), tyrosine (0.5 %, w/v) and powdered cellulose (type D; Advantec) (1.0 %, w/v). The temperature range (5, 10, 20, 25, 28, 30, 35, 37 and 40 °C) and salinity tolerance (0, 0.5, 1, 2, 3, 5 and 7 % NaCl, w/v) for growth were determined after 3 days cultivation in R2A broth. The pH range was assessed at 1.0 pH unit intervals between pH 4.0–10.0 and at pH 7.5 by using R2A broth containing 25 mM citrate/phosphate buffer or 25 mM Tris/HCl. Growth on TSA agar (Difco) and MacConkey agar (Oxoid) was also studied at 30 °C. Growth under anaerobic conditions was checked using an Anoxomat Mark II system (Mart Microbiology). The test for flexirubin-like pigments was conducted by soaking with 20 % (w/v) KOH (Fautz & Reichenbach, 1980; Reichenbach *et al.*, 1980). Congo red adsorption was tested by using R2A-Congo red agar (25 mg Congo red l^{-1}).

Colonies of YT21^T were non-translucent, light yellow and flat, exhibiting irregular edges on agar plate of R2A and LB. Cells of YT21^T were filamentous (Fig. 1) and Gram-reaction negative. YT21^T grew strictly aerobically and exhibited a marked gliding motility (Supplementary Fig. S1, available in IJSEM Online). Motility was stimulated by the introduction of air containing 5 % CO_2 and high humidity. YT21^T grew on TSA agar but not on MacConkey agar. Although colonies were not coloured on R2A-Congo red agar, the isolate produced a viscous extracellular component that remained in the supernatant after centrifugation. Other physiological characteristics of YT21^T are summarized in the species description. A comparison of differential characteristics with related type strains is shown in Table 1.

Chemotaxonomic characterization of YT21^T was carried out using cells cultured on R2A agar for 72 h at 30 °C. The major respiratory quinone was determined to be MK-7, using the HPLC method as described by Nishijima *et al.* (1997). Fatty acid methyl esters of YT21^T were extracted and analysed according to the standard protocol of the Sherlock Microbial Identification System (MIDI version 5.0). As shown in Table 2, the major fatty acids of YT21^T were 15 : 0 iso, 17 : 0 iso 3-OH and 15 : 1 iso G, which accounted for 66.1 % of the total fatty acids. The fatty acid profile of YT21^T differed from those of the type strains of other recognized species belonging to phylogenetically related genera.

DNA was extracted by using a bacterial genomic DNA purification kit (Edge Biosystems). The DNA G+C content of YT21^T, determined by an HPLC method (Mesbah & Whitman, 1989), was 45.2 mol% (mean of three replicates). The 16S rRNA gene sequence was determined by PCR amplification with a bacterial domain-specific primer set, 27F/1492R (Wang *et al.*, 2007). PCR was performed on a T1 Thermocycler (Biometra) with Ex Taq polymerase (TaKaRa Bio). The PCR protocol consisted of an initial denaturation period of 4 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C and a final extension period of 3 min at 72 °C. PCR products were stored at 4 °C until further processing. The 16S rRNA gene fragment was sequenced directly by using the BigDye Terminator version 3.1 cycle sequencing kit on an ABI 3130 Genetic Analyzer (Applied Biosystems). The 16S rRNA gene sequence of YT21^T (consisting of 1493 bp) was compared with sequences from the GenBank/EMBL/DDBJ databases, using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Strain YT21^T showed highest similarities with *Niastella yeongjuensis* GR20-13^T (sequence identity 91.7 %) and *Chitinophaga sancti* IFO 15057^T (92.4 %). Alignment and phylogenetic analysis were performed using CLUSTAL W (Thompson *et al.*, 1994), SEAVIEW (Galtier *et al.*, 1996) and MEGA 3.1 (Kumar *et al.*, 2004). An evolutionary

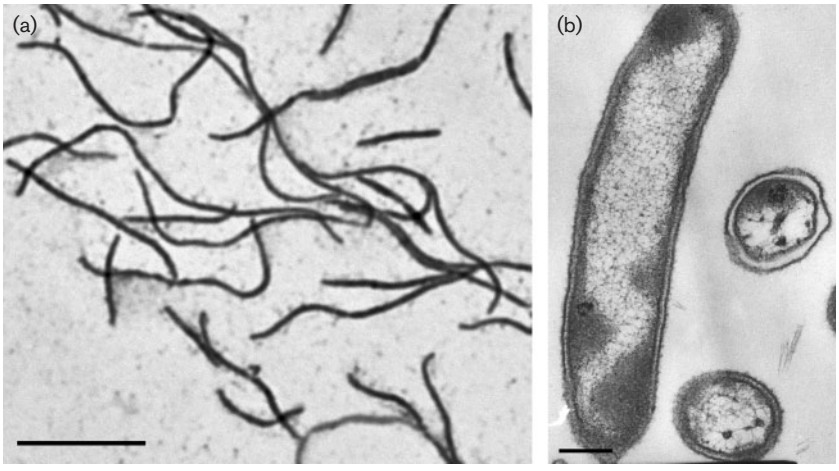

Fig. 1. Micrographs of strain YT21^T. (a) Optical micrograph showing filamentous cells grown in R2A broth. (b) Electron micrograph showing cross and longitudinal cell sections. Bars, 10 μm (a) and 0.2 μm (b).

Table 1. Phenotypic characteristics of strain YT21^T and type strains of related species

Strains: 1, YT21^T; 2, *Niastella koreensis* GR20-10^T; 3, *Niastella yeongjuensis* GR20-13^T; 4, *Terrimonas ferruginea* IAM 15098^T; 5, *Terrimonas lutea* IAM 15284^T; 6, *Chitinophaga pinensis* ACM 2034^T; 7, *Chitinophaga skermanii* CC-SG1B^T; 8, *Chitinophaga arvensicola* IAM 12650^T; 9, *Chitinophaga japonensis* NBRC 16041^T; 10, *Chitinophaga sancti* NBRC 15057^T; 11, *Chitinophaga filiformis* NBRC 15056^T; 12, *Chitinophaga terrae* KP01^T; 13, *Chitinophaga ginsengisegetis* DSM 18108^T; 14, *Chitinophaga ginsengisoli* DSM 18107^T; 15, *Segetibacter koreensis* DSM 18137^T. Data for reference strains were taken from Weon *et al.* (2006), Xie & Yokota (2006), Kämpfer *et al.* (2006), Kim & Jung (2007), Lee *et al.* (2007) and An *et al.* (2007). +, Positive; −, negative; W, weak; V, variable among studies; ND, no data available. All strains contain MK-7 as the major menaquinone.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Isolation source	Fresh water	Soil	Soil	Soil	Soil	Lake	Faeces*	Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil
Colony colour†	LY	LY	M	SR	Y	Y	Y	YO	YO	GY	GY	Y	Y	Y	Y
Cell length (μm)	5–30	10–50	10–40	1–3	1–4	<40	1–2	0.6–4.0	2–18	2–15	30–80	0.6–0.8	1.1–1.3	1.2–1.6	1.6–2.0
Gliding movement	+	+	+	−	−	+	−	V	+	+	+	−	−	−	−
Filamentous shape	+	+	+	−	−	+	−	−	+	+	+	−	−	−	−
Flexirubin reaction	−	−	−	−	ND	ND	+	+	+	+	+	ND	+	+	ND
Maximum NaCl concentration (%, w/v)	<1.0	ND	ND	1.0	1.0	ND	ND	2.0	2.0	1.0	0.3	ND	2.0	<1.0	3.0
Growth at 37 °C	−	+	−	+	+	+	+	−	+	−	+	+	+	+	−
Activity of:															
Catalase/oxidase	+/+	−/−	−/+	W/+	W/+	+/V	+/+	+/+	+/+	+/ND	V/+	+/+	−/−	+/+	+/+
Urease	−	−	−	−	−	+	+	−	ND	ND	ND	−	+	+	+
Chitinase	−	+	+	−	−	+	−	−	−	−	V	−	−	+	−
Cellulase	−	+	+	−	ND	−	ND	ND	−	+	ND	ND	ND	ND	ND
Hydrolysis of:															
Starch	−	−	−	+	−	−	−	V	−	+	−	ND	ND	ND	−
Casein	−	+	+	−	ND	+	−	ND	−	ND	−	ND	ND	ND	−
Gelatin	+	+	+	+	+	+	−	V	+	+	+	−	W	+	−
DNA	+	−	−	−	ND	−	+	+	+	ND	+	ND	−	−	−
Nitrate reduction	−	−	−	+	+	−	+	V	+	+	+	+	−	−	−
Major fatty acid	15:0 iso	17:0 iso 3-OH	15:0 iso	15:0 iso	15:0 iso	16:1ω5*c*	16:1ω5*c*	16:1ω5*c*	16:1ω5*c*	16:1ω5*c*	16:1ω5*c*	15:0 iso	15:0 iso	15:0 iso	15:0 iso
DNA G+C content (mol%)	45.2	45.8	44.3	48.9	47.2	45.2	40.7	46.0	49.8	43.3	45.0	46.3	47.1	48.4	40.4

*Isolated from faeces of the millipede *Arthrosphaera magna*.

†GY, Golden yellow; LY, light yellow; M, milky; SR, salmon red; Y, yellow; YO, yellowish orange.

Table 2. Cellular fatty acid profiles of strain YT21^T and type strains of type species of related genera

Strains: 1, YT21^T; 2, *Segetibacter koreensis* DSM 18137^T; 3, *Niastella koreensis* GR20-10^T; 4, *Terrimonas ferruginea* IAM 15098^T; 5, *Chitinophaga pinensis* ACM 2034^T. Data for reference strains were taken from An *et al.* (2007), Xie & Yokota (2006), Weon *et al.* (2006) and Kämpfer *et al.* (2006). Major fatty acids are shown in bold. Unknown fatty acids are identified by their equivalent chain length.

Fatty acid	1	2	3	4	5
13:0 iso 3-OH	–	1.6	–	–	–
13:0 iso	0.1	–	–	0.5	–
13:1	–	–	–	0.2	–
14:0	0.2	1.0	–	0.5	0.7
14:0 2-OH	0.1	–	–	–	–
14:0 iso	–	–	–	1.1	–
15:0	–	1.1	–	2.7	0.4
15:0 2-OH	–	–	–	0.4	–
15:0 3-OH	–	–	–	0.3	–
15:0 iso	**25.2**	**23.4**	**26.8**	**28.4**	**30.4**
15:0 iso 3-OH	2.7	–	1.3	2.2	3.1
15:0 anteiso	0.2	3.5	4.9	0.6	–
15:1 iso G	**18.3**	–	**15.6**	–	–
15:1 iso	–	**14.4**	–	**26.2**	–
16:0	4.7	8.5	2.6	1.7	4.2
16:0 iso	0.3	–	1.0	1.4	–
16:0 iso 3-OH	1.0	–	–	0.7	0.4
16:0 2-OH	0.5	–	–	–	0.7
16:0 3-OH	3.9	–	1.3	2.5	1.2
16:1ω5*c*	3.0	12.5	–	–	**33.2**
16:1ω11*c*	–	–	–	–	1.9
17:0 2-OH	–	–	3.5	–	–
17:0 3-OH	–	–	1.2	–	–
17:0 iso	0.6	–	1.6	–	0.4
17:0 iso 3-OH	**22.6**	**14.5**	**29.4**	**15.3**	**11.5**
18:0	–	1.9	–	–	–
Summed feature 3*	13.6	6.6	4.3	11.2	7.7
Unknown 13.565	0.3	2.1	–	1.3	2.6
Unknown 14.959	–	–	–	–	0.3
Unknown 16.582	1.9	–	1.4	1.3	1.1

*Summed feature 3 contained 15:0 iso 2-OH and/or 16:1ω7*c*.

distance matrix was generated according to Kimura's two-parameter model (Kimura, 1983) and phylogenetic trees were constructed with the neighbour-joining method (Saitou & Nei, 1987). To evaluate the topology of the resultant tree, bootstrap analysis (Felsenstein, 1985) was performed with 1000 resamplings. In the inferred phylogenetic tree (Fig. 2), YT21^T fell into the large cluster containing the genera *Chitinophaga*, *Niastella*, *Terrimonas* and *Segetibacter*, but it formed a distinct lineage of descent within this cluster. The 16S rRNA gene sequence identity between YT21^T and the type strains of related species (*Chitinophaga pinensis*, *Niastella koreensis*, *Terrimonas ferruginea* and *Segetibacter koreensis*) ranged from 88.9 to 91.8 %. These results suggest that strain YT21^T represents a novel genus within the *Bacteroidetes*.

Based on the polyphasic evidence described above, we conclude that YT21^T represents a novel genus and species of the phylum *Bacteroidetes*, for which the name *Filimonas lacunae* gen. nov., sp. nov. is proposed.

Description of *Filimonas* gen. nov.

Filimonas (Fi.li.mo′nas. L. n. *filum* a thread; Gr. fem. n. *monas* a unit, a monad; N.L. fem. n. *Filimonas* a thread-like monad).

Cells are Gram-negative, strictly aerobic, non-spore-forming, non-flagellated, gliding and filamentous. Oxidase- and catalase-positive. Flexirubin-type pigments are not formed and nitrate is not reduced. Negative for hydrolysis of chitin and cellulose. The major fatty acids are 15:0 iso, 17:0 iso 3-OH and 15:1 iso G. The major respiratory quinone is MK-7. Positioned phylogenetically in the phylum *Bacteroidetes*. The type species is *Filimonas lacunae*.

Description of *Filimonas lacunae* sp. nov.

Filimonas lacunae (la.cu′nae. L. gen. n. *lacunae* of a pool, referring to the isolation of the type strain from shallow fresh water).

Cells are 0.3–0.5 × 5–30 μm in size. Colonies are irregular, non-translucent and light yellow. Growth and motility on agar plate are stimulated by 5 % CO_2 and higher humidity. Viscous exopolymer is produced. Growth temperature range is 10–35 °C; optimum growth at 28–30 °C. The pH range for growth is pH 5.0–9.0; optimum growth at pH 6.0. Growth occurs at NaCl concentrations of up to 1.0 % (w/v). The detailed fatty acid profile is given in Table 2. Positive for hydrolysis of aesculin, gelatin, DNA and tyrosine, but not for hydrolysis of starch, casein or agar. Negative for nitrate reduction, indole production and glucose fermentation. Positive for arginine dihydrolase, urease, alkaline phosphatase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase. Weakly positive for esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, α-chymotrypsin and α-galactosidase. Negative for lipase (C14), trypsin, β-glucuronidase, α-glucosidase, α-mannosidase and α-fucosidase activities (API ZYM). The following compounds are utilized as carbon and energy sources (tested with API 20NE and Biolog GN2 MicroPlates): D-glucose, L-arabinose, D-mannose, maltose, D-galactose, dextrin and glycerol. The following compounds are not utilized: D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, α-cyclodextrin, glycogen, Tweens 40 and 80, *N*-acetyl-D-galactosamine, adonitol, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, gentiobiose, *myo*-inositol, α-lactose, lactulose, D-mannitol, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol,

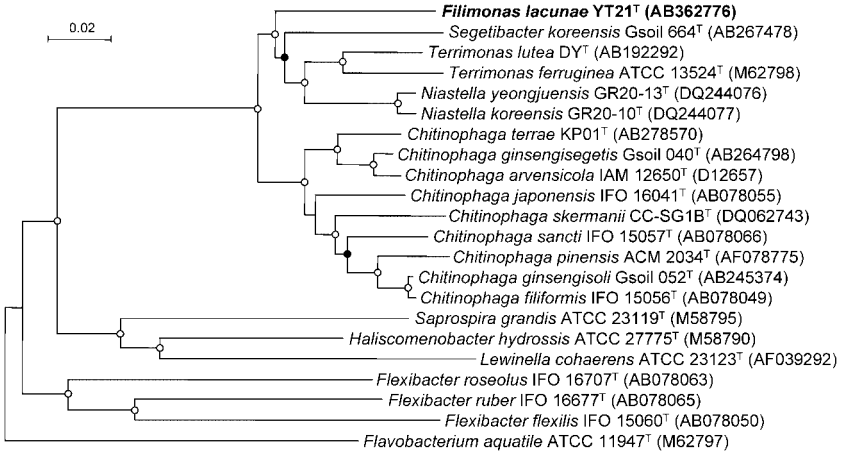

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain YT21^T with the representatives of the phylum *Bacteroidetes*. Bootstrap values (from 1000 resampled datasets) are indicated by open (>80 %) and closed (>60 %) circles at branching points. Bar, 0.02 substitutions per nucleotide position.

sucrose, trehalose, turanose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, formic acid, D-galacturonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-, β- and γ-hydroxybutyric acids, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. The genomic G+C content of the type strain is 45.2 mol%.

The type strain, YT21^T (=NBRC 104114^T =DSM 21054^T), was isolated from shallow fresh water in Japan.

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References

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.

Bartholomew, J. W. & Mittwer, T. (1952). The Gram stain. *Bacteriol Rev* **16**, 1–29.

Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. (1997). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.

Buczolits, S., Denner, E. B. M., Vybiral, D., Wieser, M., Kämpfer, P. & Busse, H.-J. (2002). Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp nov. *Int J Syst Evol Microbiol* **52**, 445–456.

Fautz, E. & Reichenbach, H. (1980). A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.

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

Galtier, N., Gouy, M. & Gautier, C. (1996). SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput Appl Biosci* **12**, 543–548.

Garrity, G. M. & Holt, J. G. (2001). The road map to the *Manual*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 119–166. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

Kämpfer, P., Young, C. C., Sridhar, K. R., Arun, A. B., Lai, W. A., Shen, F. T. & Rekha, P. D. (2006). Transfer of [*Flexibacter*] *sancti*, [*Flexibacter*] *filiformis*, [*Flexibacter*] *japonensis* and [*Cytophaga*] *arvensicola* to the genus *Chitinophaga* and description of *Chitinophaga skermanii* sp. nov. *Int J Syst Evol Microbiol* **56**, 2223–2228.

Kim, M. K. & Jung, H. Y. (2007). *Chitinophaga terrae* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **57**, 1721–1724.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Lee, H. G., An, D. S., Im, W. T., Liu, Q. M., Na, J. R., Cho, D. H., Jin, C. W., Lee, S. T. & Yang, D. C. (2007). *Chitinophaga ginsengisegetis* sp. nov. and *Chitinophaga ginsengisoli* sp. nov., isolated from soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **57**, 1396–1401.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine+cytosine of DNA. *J Chromatogr* **479**, 297–306.

Nishijima, M., Araki-Sakai, M. & Sano, H. (1997). Identification of isoprenoid quinones by frit-FAB liquid chromatography–mass spectrometry for the chemotaxonomy of microorganisms. *J Microbiol Methods* **28**, 113–122.

Reichenbach, H., Kohl, W., Böttger-Vetter, A. & Achenbach, H. (1980). Flexirubin-type pigments in *Flavobacterium Arch Microbiol* **126**, 291–293.

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

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Tindall, J. B., Sikorski, J., Simbert, A. R. & Krieg, R. N. (2007). Phenotypic characterization and the principles of comparative systematics. In *Methods for General and Molecular Microbiology*, 3rd edn, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. A. Marzluf, T. M. Schmidt & L. R. Snyder. Washington, DC: American Society for Microbiology.

Ueda, K., Tagami, Y., Kamihara, Y., Shiratori, H., Takano, H. & Beppu, T. (2008). Isolation of bacteria whose growth is dependent on high levels of CO_2 and implications of their potential diversity. *Appl Environ Microbiol* **74**, 4535–4538.

Wang, X., Hoefel, D., Saint, C. P., Monis, P. T. & Jin, B. (2007). The isolation and microbial community analysis of hydrogen producing bacteria from activated sludge. *J Appl Microbiol* **103**, 1415–1423.

Weon, H. Y., Kim, B. Y., Yoo, S. H., Lee, S. Y., Kwon, S. W., Go, S. J. & Stackebrandt, E. (2006). *Niastella koreensis* gen. nov., sp. nov. and *Niastella yeongjuensis* sp. nov., novel members of the phylum *Bacteroidetes*, isolated from soil cultivated with Korean ginseng. *Int J Syst Evol Microbiol* **56**, 1777–1782.

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.