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ORIGINAL PAPER

Ferruginibacter profundus sp. nov., a novel member of the family *Chitinophagaceae*, isolated from freshwater sediment of a reservoir

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Abstract A Gram-negative, aerobic, non-motile, and rod-shaped bacterium, designated strain DS48-5-3^T, was isolated from a 48 m sediment sample taken from Daechung Reservoir, Republic of Korea. Comparative 16S rRNA gene sequence studies showed a clear affiliation of this strain to the *Bacteroidetes*, notably most closely related to *Ferruginibacter alkalilentus* HU1-GD23^T, *Ferruginibacter lapsinanis* HU1-HG42^T and *Ferruginibacter yonginensis* HME8442^T, showing 16S rRNA gene sequence similarities to the type strains of these species of 95.2–96.4 % similarity. The predominant ubiquinone was identified as MK-7. The major fatty acids were identified as iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and iso-$C_{15:1}$ G. The G+C content of the genomic DNA of strain DS48-5-3^T was determined to be 37.2 %. On the basis of polyphasic evidence, it is proposed that strain DS48-5-3^T should belong to a novel species, for which the name *Ferruginibacter profundus* sp. nov. (type strain DS48-5-3^T = KCTC 32478^T = JCM 19431^T), is proposed.

Keywords 16S rRNA gene · *Ferruginibacter profundus* · *Bacteroidetes* · Polyphasic taxonomy

Introduction

The genus *Ferruginibacter*, which was recently proposed by Lim et al. (2009), is affiliated to the family *Chitinophagaceae* of the phylum *Bacteroidetes*. And at the time of writing, the family *Chitinophagaceae* comprises 16 genera (http://www.bacterio.net/) and the genus *Ferruginibacter* comprises only three recognized species, *Ferruginibacter alkalilentus*, *Ferruginibacter lapsinanis* (Lim et al. 2009), and *Ferruginibacter yonginensis* (Lee et al. 2013), which were isolated from freshwater sediment and an artificial lake. The type species of this genus is *F. alkalilentus*. Here, we describe the characterization of a single strain we isolated, DS48-5-3^T, and propose that this strain represents the type strain of *Ferruginibacter profundus* sp. nov., based on a polyphasic approach, including the determination of its phenotypic and chemotaxonomic properties and a detailed phylogenetic investigation based on its 16S rRNA gene sequence.

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Materials and methods

Isolation, morphological and physiological characterization

Strain DS48-5-3^T was isolated from a sediment sample at a depth of 48 m collected using a KB core sampler (Wildco, USA) from the Daechung Reservoir in South Korea. 100 mg of the sediment sample was serially diluted in 0.85 % saline solution and then 100 µl aliquots of each serial dilution were spread on R2A agar (Difco) and incubated at 25 °C aerobically. A rust coloured colony, designated as DS48-5-3^T, was isolated after 6 days. The isolate was subcultivated on an R2A agar at 30 °C routinely and preserved in a glycerol solution (20 %, v/v) at −70 °C. For most experiments, all strains were cultivated on R2A agar (Difco) at 30 °C for 48 h. *F. alkalilentus* HU1-GD23^T, *F. lapsinanis* HU1-HG42^T and *F. yonginensis* HME8442^T were used as reference strains under the same conditions. The reference strains were received from the Korean Agricultural Culture Collection (KACC) and Korean Collection for Type Cultures (KCTC), respectively.

Cell morphology and motility were observed under a phase-contrast microscope (Nikon Optiphot, 1000× magnification) using cells grown on R2A agar for 2 days. Gram reaction was determined using a Gram stain kit (Becton–Dickinson) following the procedure of manufacturer. Oxidase and catalase activities were tested using 1 % tetramethyl-p-phenylenediamine (Tarrand and Groschel 1982) and 3 % H_2O_2, respectively. Growth at different temperatures (4, 8, 10, 15, 20, 30, 37, and 42 °C) and in different NaCl concentrations (1, 2, 3, 4, and 5 %) were investigated on R2A agar. Growth at different pH values (pH 5–10 at intervals of 1 pH unit) was determined on R2A broth using appropriate biological buffers: Na_2HPO_4/NaH_2PO_4 buffer and $Na_2CO_3/NaHCO_3$ buffer were used for pH values of 5–7 and pH values of 8–10, respectively (Bates and Bower 1956; Gomori 1955). Carbon source utilization tests, acid production tests and additional physiological tests were performed using API 20NE, API ID 32 GN, API 50CH, API ZYM galleries (bioMérieux), and GN2 MicroPlate (Biolog) according to the instructions of the manufacturer.

Chemotaxonomic characterization

Fatty acid methyl esters were saponified, methylated, extracted, and analysed (Sasser 1990) according to the protocol of the Sherlock Microbial Identification System (MIDI), using cells grown for 48 h on R2A agar at 30 °C. Standardization of the physiological age for the strains was done by the protocol given by MIDI (http://www.microbialid.com/PDF/TechNote_101.pdf). The extracts were analysed by GC (Hewlett Packard 6890) and identified by reference to the TSBA 6 database provided with the Sherlock software 6.1. Reference strains *F. alkalilentus* HU1-GD23^T, *F. lapsinanis* HU1-HG42^T, and *F. yonginensis* HME8442^T were analyzed following identical culture conditions. The menaquinones extracted from 100 mg of the freeze-dried cells with chloroform/methanol (2:1, v/v) and purified by using TLC on Kieselgel 60 F_{254} plates (20 × 20 cm, Merck) with petroleum ether/diethyl ether (9:1, v/v) as the solvent, and the extracts were purified and analysed by HPLC (Shimadzu) as described by Komagata and Suzuki (1987), using a YMC-Pack ODS-A column.

Molecular characterization

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of purified PCR products were performed according to Jin et al. (2013). The 16S rRNA gene sequences were aligned with published sequences retrieved from NCBI database (http://www.ncbi.nlm.nih.gov) using CLUSTAL X (Thompson et al. 1997) and edited using BIOEDIT (Hall 1999). The phylogenetic trees were reconstructed using the neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) algorithms in the MEGA 5 software (Tamura et al. 2011). The resultant tree topology was evaluated by the bootstrap analysis (Felsenstein 1985) based on 1,000 resampled data set.

DNA G+C content was analysed by HPLC after hydrolysis as described by Tamaoka and Komagata (1984) and non-methylated λ DNA (Sigma) was used as a standard.

Results and discussion

Strain DS48-5-3^T was observed to form visible colonies on R2A agar incubated at 30 °C within 48 h. Growth

was found to occur at temperatures ranging from 8 to 30 °C, but no growth was observed at 37 or at 4 °C. Growth was found to occur at pH 6–8, but no growth was observed at pH 5, 9 or 10. The colonies were observed to be rust coloured, convex and circular with entire edges. Cells were found to be Gram-negative, catalase- and oxidase-negative, non-motile and rod-shaped. The novel strain could be differentiated from *Ferruginibacter* species by the assimilation as carbon sources, the activity of enzymes and acid production. Strain DS48-5-3^T could be differentiated from *F. alkalilentus* HU1-GD23^T by not assimilating rhamnose and xylitol, presence of esterase (C4) and α-glucosidase, and absence of α-chymotrypsin, cystine arylamidase, β-glucosidase; from *F. lapsinanis* HU1-HG42^T by assimilating D-rhamnose and D-trehalose, presence of *N*-acetyl-β-glucosaminidase, esterase (C4), α-fucosidase, β-galactosidase, α-glucosidase, β-glucosidase, valine arylamidase, and not producing acids from glycogen, maltose, or starch; and from *F. yonginensis* HME8442^T by assimilating D-rhamnose and D-trehalose, presence of α-fucosidase, β-galactosidase, α-glucosidase, and absence of cystine arylamidase, β-glucosidase, and trypsin. Strain DS48-5-3^T is able to grow at 8 °C, unlike the other reference strains. Detailed physiological and biochemical characteristics are summarised in Tables 1, 2 and in the species description.

The almost-complete 16S rRNA gene sequence (EMBL accession number KF360048) of strain DS48-5-3^T was determined and compared with those of representative species within the genus *Ferruginibacter* and related genera. The 16S rRNA gene sequence similarity between strain DS48-5-3^T and its closest neighbours, *F. alkalilentus* HU1-GD23^T, *F. lapsinanis* HU1-HG42^T and *F. yonginensis* HME8442^T, were 95.2, 96.4 and 96.4 % respectively. Lower levels of sequence similarity were observed with the type strains of other neighbouring species in the family *Chitinophagaceae*. The 16S rRNA gene sequence clearly fell within the clade for the members of the genus *Ferruginibacter* (Fig. 1), also supported by the maximum likelihood and maximum parsimony trees (Fig S1).

The G+C content of the genomic DNA was determined to be 37.2 mol %. The predominant respiratory quinone was identified as menaquinone MK-7. The predominant fatty acids were identified as iso-$C_{15:0}$ (33.3 %), iso-$C_{17:0}$ 3-OH (16.5 %) and iso-$C_{15:1}$ G (15.9 %). The major fatty acids in strain DS48-5-3^T were consistent with those of *Ferruginibacter* species, although there were differences in the proportions of

Table 1 Phenotypic and chemotaxonomic characteristics that distinguish strain DS48-5-3^T from other *Ferruginibacter* species

Characteristics	1	2	3	4
Urease	−	−	+	−
Gelatine hydrolysis	+	+	−	+
Carbon utilization				
Citrate	−	−	+	−
L-Glutamic acid	−	−	−	+
L-Proline	−	−	−	+
D-Raffinose	+	+	−	−
Rhamnose	−	+	−	−
L-Threonine	−	−	−	+
D-Trehalose	+	+	−	−
Xylitol	−	+	−	−
Enzyme activities				
N-Acetyl-β-glucosaminidase	+	+	−	+
α-Chymotrypsin	−	+	−	−
Cystine arylamidase	−	+	−	+
Esterase (C4)	+	−	−	+
α-Fucosidase	+	+	−	−
β-Galactosidase	+	+	−	−
α-Glucosidase	+	−	−	−
β-Glucosidase	−	+	−	+
Trypsin	−	−	−	+
Valine arylamidase	+	+	−	+
Acid production				
Glycogen	−	−	w	−
Maltose	−	−	+	−
Starch	−	−	+	−
DNA G+C content (mol%)	37.2	39.4[a]	38.5[a]	35.8[b]

Strains 1, DS48-5-3^T; 2, *F. alkalilentus* HU1-GD23^T; 3, *F. lapsinanis* HU1-HG42^T; 4, *F. yonginensis* HME8442^T. All data were from the present study unless indicated. All strains were positive for aesculin hydrolysis, acid phosphatase, alkaline phosphatase, leucine arylamidase, and naphthol-AS-BI-phosphohydrolase. All strains were negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esterase lipase (C8), α-galactosidase, β-glucuronidase, lipase (C14), α-mannosidase

w, weakly positive; +, positive; −, negative

[a] Data taken from Lim et al. (2009)

[b] Data taken from Lee et al. (2013)

certain fatty acids (Table 2). Therefore, when taken together, the chemotaxonomic data and results of the phylogenetic analysis support the affiliation of strain DS48-5-3^T to the genus *Ferruginibacter*.

On the basis of 16S rRNA gene dissimilarity to related taxa and the phylogenetically distinct position, together with differentiated phenotypic characteristics and genomic relatedness, strain DS48-5-3^T is considered to represent a novel species of the genus *Ferruginibacter*, for which the name *Ferruginibacter profundus* sp. nov., is proposed.

Description of *Ferruginibacter profundus* sp. nov.

Ferruginibacter profundus (L. masc. adj. *profundus* from the deep)

Cells are Gram-negative, non-motile and rod-shaped. Colonies are circular and convex with glossy surface and rust coloured when grown for 2 days at 30 °C on an R2A agar. Good growth is observed on R2A agar at 25–30 °C. Growth occurs at 8–30 °C, but not at 4 or 37 °C. Optimal pH for growth is 7.0; growth occurs at pH 6.0–8.0, but not at pH 5.0 or 9.0. No growth occurs in the presence of 1 % (w/v) NaCl. No growth on Luria–Bertani (LB) agar, MacConkey agar, nutrient agar (Difco) or tryptic soy agar (TSA). Oxidase and catalase tests are negative. Positive for aesculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for nitrate and nitrite reduction, indole production, glucose fermentation, arginine dihydrolase, and urease (as determined by API 20NE test strip). Positive for D-raffinose and D-trehalose, but negative for all other substrates with the API 20NE, API ID 32GN test strips and GN2 MicroPlate tests. Positive for the following enzyme activities (as determined by API ZYM test strip): *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, esterase (C4), α-fucosidase, α-glucosidase, β-galactosidase, leucine arylamidase, naphtol-AS-BI-phosphohydrolase, and valine arylamidase; but negative for the following enzyme activities: α-chymotrypsin, cystine arylamidase, esterase lipase (C8), α-galactosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, and trypsin. Acid is produced from aesculin to 5-ketogluconate (weakly), but not from *N*-acetylglucosamine, adonitol, amygdalin, DL-arabinose, DL-arabitol, arbutin, cellobiose, dulcitol, erythritol, fructose, D-fucose, L-fucose, galactose, gentiobiose, gluconate, glucose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, lactose, D-lyxose, maltose, mannitol, mannose, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xylose, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, starch sucrose, D-tagatose, trehalose, D-turanose, xylitol, D-xylose, or L-xylose. The predominant menaquinone is MK-7. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and iso-$C_{15:1}$ G. The DNA G+C content of the type strain is 37.2 mol % (determined by HPLC).

The type strain, DS48-5-3^T (=KCTC 32478^T =JCM 19431^T), was isolated from a sediment sample taken from Daechung Reservoir, South Korea. The EMBL

Table 2 Cellular fatty acid composition (%) of strain DS48-5-3^T and the type strains of related *Ferruginibacter* species

Fatty acids	1	2	3	4
$C_{13:0}$ iso	4.2	TR	TR	4.8
$C_{14:0}$ iso	TR	TR	–	1.2
$C_{14:0}$	TR	TR	1.2	TR
$C_{15:1}$ iso G	**15.9**	**15.4**	**11.0**	**19.8**
$C_{15:0}$ anteiso A	–	TR	–	1.2
$C_{15:0}$ iso	**33.3**	**38.5**	**47.5**	**20.0**
$C_{15:0}$ anteiso	1.2	2.5	1.0	3.5
$C_{16:1}$ iso G	TR	TR	–	1.3
$C_{16:0}$ iso	TR	TR	–	2.0
$C_{16:0}$	5.2	3.4	6.9	5.7
$C_{15:0}$ iso 3-OH	7.0	7.7	2.8	2.8
$C_{16:0}$ iso 3-OH	3.3	3.1	–	4.6
$C_{16:0}$ 3-OH	4.5	3.1	2.8	3.8
$C_{18:0}$	–	–	–	1.8
$C_{17:0}$ iso 3-OH	**16.5**	**17.6**	**16.0**	**9.0**
$C_{17:0}$ 2-OH	TR	1.0	–	TR
$C_{17:0}$ 3-OH	TR	–	–	4.6
Summed Feature 3[a]	4.1	2.8	7.8	7.7
Summed Feature 4[b]	–	–	–	1.3

For each strain the predominant fatty acid(s) (>10 %) are indicated in bold type

Strains 1, DS48-5-3^T; 2, *F. alkalilentus* HU1-GD23^T; 3, *F. lapsinanis* HU1-HG42^T; 4, *F. yonginensis* HME8442^T. All data were from the present study unless indicated. Cells of all strains were harvested after growth on R2A agar at 30 °C for 2 days. Fatty acids amounting to less than 1.0 % in all strains are not shown

TR, Trace (<1.0 %); –, not detected

[a] Summed feature 3 contains $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*

[b] Summed feature 4 $C_{17:1}$ iso I and/or $C_{17:1}$ anteiso B

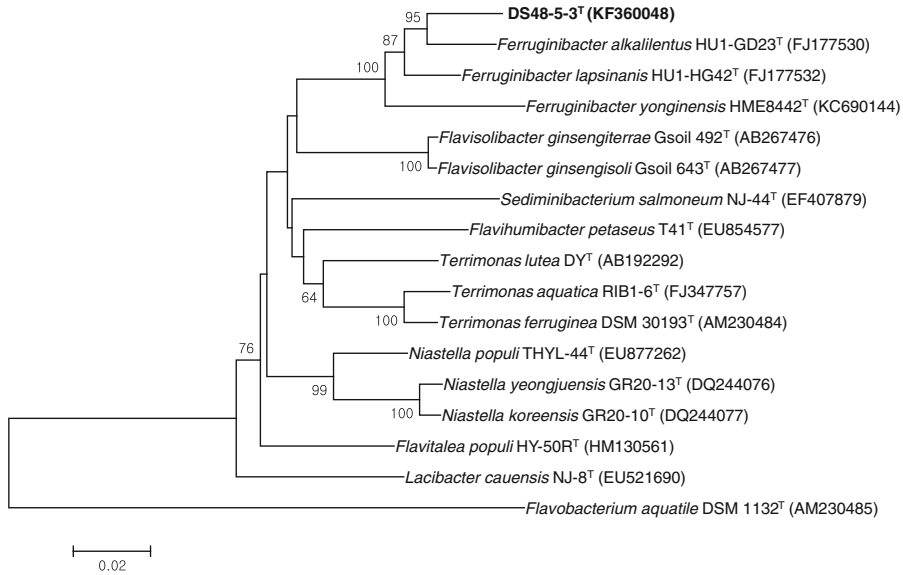

Fig. 1 A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the positions of strain DS48-5-3^T and related taxa. *Numbers* at branching points refer to *bootstrap values* (1,000 resamplings, only values above 50 % shown). *Bar* two substitutions per 100 nt positions

accession number for the 16S rRNA gene sequence of strain DS48-5-3^T is KF360048.

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