**Lacisediminihabitans profunda** gen. nov., sp. nov., a member of the family *Microbacteriaceae* isolated from freshwater sediment

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**Abstract** A novel Gram-stain-positive bacterial strain, CHu50b-6-2$^T$, was isolated from a 67-cm-long sediment core collected from the Daechung Reservoir at a water depth of 17 m, Daejeon, Republic of Korea. The cells of strain CHu50b-6-2$^T$ were aerobic non-motile and formed yellow colonies on R2A agar. The phylogenetic analysis based on 16S rRNA gene sequencing indicated that the strain formed a separate lineage within the family *Microbacteriaceae*, exhibiting 98.0%, 97.7% and 97.6% 16S rRNA gene sequence similarities to *Glaciibacter tibetensis* KCTC 29148$^T$, *Frigoribacterium faeni* KACC 20509$^T$ and *Lysinibacter cavernae* DSM 27960$^T$, respectively. The phylogenetic trees revealed that strain CHu50b-6-2$^T$ did not show a clear affiliation to any genus within the family *Microbacteriaceae*. The chemotaxonomic results showed Blz type peptidoglan containing 2, 4-diaminobutyric acid (DAB) as the diagnostic diamino acid, MK-10 as the predominant respiratory menaquinone, diphosphatidylglycerol, phosphatidylglycerol, and an unidentified glycolipid as the major polar lipids, anteiso-C$\text{_{15:0}}$, iso-C$\text{_{16:0}}$, and anteiso-C$\text{_{17:0}}$ as the major fatty acids, and a DNA G + C content of 67.3 mol%. The combined genotypic and phenotypic data showed that strain CHu50b-6-2$^T$ could be distinguished from all genera within the family *Microbacteriaceae* and represents a novel genus, *Lacisediminihabitans* gen. nov., with the name *Lacisediminihabitans profunda* sp. nov., in the family *Microbacteriaceae*. The type
strain is CHu50b-6-2T (= KCTC 49081T = JCM 32673T).

Keywords Lacisediminihabitans · Lacisediminihabitans profunda · CHu50b-6-2 · Sediment

Introduction

Since Park et al. (1993) proposed the family Microbacteriaceae, 56 genera have been described validly in this family at the time of writing (http://www.bacterio.net/; Parte 2018). Members of the family Microbacteriaceae are widely distributed in nature including soil, freshwater, groundwater, cyanobacterial mats, the rhizosphere and phyllosphere of plants, air and ice samples, ponds in Antarctica, sludge, seawater, sediment, seaweed, and seafood (Dias and Bhat 1962; Männistö et al. 2000; Reddy et al. 2003; Lee 2007; Kim et al. 2008; Kim and Lee 2011; Shin et al. 2011; Jang et al. 2012; Park et al. 2012; Schumann et al. 2012; Jin et al. 2013; Lai et al. 2015). During an investigation on iron and sulfur oxidizing microbial diversity in the sediment of a eutrophic freshwater reservoir (Jin et al. 2017), a strain designated CHu50b-6-2T was isolated from the freshwater sediment of the Daechung Reservoir. Herein, we describe the phylogenetic, genetic, phenotypic and chemotaxonomic characteristics of this novel strain, which is proposed to represent a new genus within the family Microbacteriaceae by using a polyphasic approach.

Materials and methods

Isolation, morphological and physiological characterization

Strain CHu50b-6-2T was recovered from a 67-cm-long sediment core (36° 22′ 30″ N, 127° 33′ 58″ E) collected from the Daechung Reservoir at a water depth of 17 m in Daejeon, South Korea. 1 g sediment sample was applied to serial dilution method. A 100 µl sub-sample (10⁻⁶ or 10⁻⁷) of the suspended material was spread onto modified 1/10 R2A agar (L⁻¹: 0.05 g peptone, 0.05 g yeast extract, 0.05 g casamino acid, 0.05 g dextrose, 0.05 g soluble starch, 0.03 g K₂HPO₄, 0.005 g MgSO₄, 0.03 g sodium pyruvate, and 15 g agar) and incubated at room temperature (25 °C) for 4 weeks. One yellow colony, designated as CHu50b-6-2T, was isolated and subcultivated on R2A agar at 30 °C for further analysis. The colony characteristics were determined after growing for 5 days at 30 °C on R2A agar. Gram staining was performed using a Gram stain kit (Becton–Dickinson) and 3% KOH solution. The cell morphology and motility were examined under a phase-contrast microscope (Nikon Eclipse 80i microscope, 1000 × magnification) and a transmission electron microscope (CM20, Philips; Netherlands) after negative staining with 2% (w/v) uranyl acetate using cells grown for 48 h on R2A agar.

The cell growth was checked on R2A agar, trypticase soy agar (TSA; Difco), Luria–Bertani (LB; Difco) medium, and nutrient agar. The growth temperature range was checked by incubating at 4, 8, 15, 20, 30, 37, and 45 °C on R2A agar. Salt tolerance was performed by adding different concentrations of NaCl to R2A agar. The pH growth was determined in R2A broth with a pH range of 5–11 at intervals of 1 pH unit. Different biological buffers were applied to adjust the pH values: Na₂HPO₄/NaH₂PO₄ buffer for pH 5–7 and Na₂CO₃/NaHCO₃ buffer for pH 8–11 (Bates and Bower 1956; Gomori 1955). The oxidase activity was checked using 1% tetramethyl-p-phenylenediamine (Tarrand and Groschel 1982), and the catalase activity was checked using 3% H₂O₂. We used the API 20NE, ID 32 DN, API ZYM kits (bioMérieux), and Biolog GN2 MicroPlate to determine carbon source utilization and to do enzyme activity assays as well as additional physiological tests following the manufacturer’s instructions. Duplicate antibiotic-susceptibility tests were conducted using filter-paper discs (6 mm) containing the following: amikacin (30 µg ml⁻¹), ampicillin/sulbactam (20 µg ml⁻¹, 1:1), chloramphenicol (30 µg ml⁻¹), erythromycin (30 µg ml⁻¹), gentamicin (30 µg ml⁻¹), kanamycin (30 µg ml⁻¹), lincomycin (15 µg ml⁻¹), nalidixic acid (30 µg ml⁻¹), rifampicin (30 µg ml⁻¹), spectinomycin (25 µg ml⁻¹), streptomycin (25 µg ml⁻¹), teicoplanin (30 µg ml⁻¹), tetracycline (30 µg ml⁻¹), and vancomycin (30 µg ml⁻¹). The discs were placed on R2A plates spread with a culture of strain CHu50b-6-2T and were then incu-
bated at 30 °C for 2 days. Susceptibility was recorded as positive at zones with diameters greater than 10 mm.

Chemotaxonomic characterisation

For fatty acid profiling, strain CHu50b-6-2T was cultured on R2A agar for 48 h to the late exponential phase. Harvesting of the cell mass was standardized in the instruction of MIDI (http://www.microbialid.com/PDF/TechNote_101.pdf). Separation and identification of the fatty acids were performed by GC (Hewlett Packard 6890), and the TSBA 6 database provided the Sherlock software 6.1. Extraction of isoprenoid quinone was carried out following the method described by Komagata and Suzuki (1987), and the analysis was done by HPLC (Shimadzu) with an YMC-Pack ODS-A column. Extraction and identification of polar lipids were done using two-dimensional TLC following the method described by Tindall (1990). The isomer of diaminopimelic acid (DAP) in the cell wall was analyzed using the method described by Hasegawa et al. (1983). The cell-wall peptidoglycan was extracted and identified using TLC after hydrolysis with 6 M HCl at 100 °C for 18 h (Komagata and Suzuki 1987). Genomic DNA was extracted using a commercial genomic DNA-extraction kit (FastDNA™ SPIN kit). The purity of the extracted DNA was then examined on a ND2000 spectrometer (Nanodrop Technologies, Inc.). DNA G + C contents (mol%) were analyzed by HPLC after hydrolysis as described by Tamaoka and Komagata (1984). Three reference strains were used: Glaciihabitans tibetensis KCTC 29148 T was obtained from the KCTC (Korean Collection for Type Cultures), Frigoribacterium faeni KACC 20509T from the KACC (Korean Agricultural Culture Collection), and Lysinibacter cavernae DSM 27960T from the DSMZ (German Collection of Microorganisms and Cell Cultures).

Molecular characterisation

The 16S rRNA gene was amplified by PCR as described previously (Ren et al. 2018) using the universal bacterial primer sets, 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′; Escherichia coli position 8–27) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′; E. coli position 1492–1510), were used (Lane 1991). The purified PCR products then were sequenced with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Whole genome was sequenced via the Illumina HiSeq platform. The genome was assembled by the CLC assembler (CLC-Assembly-Cell-5.1.1), and the gene annotation was performed by the PATRIC 3.5.36 (https://www.patricbrc.org). The average nucleotide identity (ANI) was calculated using OrthoANI tool in the EZBioCloud (Lee et al. 2016). To get the full 16S rRNA gene, the sequencing primers 27F, 785F (5′-GGA TTA GAT ACC CTG GTA-3′; E. coli position 8–27), 800R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′), and 1492R for the sequence analysis, were used (Lane 1991). The phylogenetic neighbors of strain CHu50b-6-2T were identified, and the pairwise similarities of the 16S rRNA gene sequences were calculated with EzBioCloud (Yoon et al. 2017). The retrieved 16S rRNA gene sequences were aligned using the CLUSTAL X program (Thompson et al. 1997). Evolutionary distances were calculated based on Kimura’s two-parameter model (Kimura 1980). Phylogenetic trees were reconstructed with MEGA version 7.0 (Kumar et al. 2016) applying the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum parsimony (Fitch 1971) algorithms. The bootstrap values were based on 1000 replicates (Felsenstein 1985). The housekeeping gene, recA gene encoding DNA recombinase A, was applied do delineate our strain more clearly from its close species. Housekeeping genes are useful for species identification as phylogenetic markers. The primer sets, recA-F (5′-GTT CTC YTT RCC CTG NCC-3′) and recA-R (5′-GAR TCS TCS GGW AAG ACB AC-3′), were used for amplifying and sequencing (Katayama et al. 2009). The PCR amplification conditions were as following: 95 °C for 5 min, 30 cycles of 95 °C for 1.5 min, 55 °C for 1 min and 72 °C for 1 min and final extension for 10 min at 72 °C. To determine genomic relatedness, DNA-DNA hybridisation experiment was carried out between strain CHu50b-6-2T and type strains of G. tibetensis, F. faeni and L. cavernae, which showed over 97% of 16S rRNA gene similarities to novel strain. The hybridisation test was carried out as described by Ezaki et al. (1989), and salmon sperm DNA (Sigma; D7656) was used as a control.
Results and discussion

Strain CHu50b-6-2T formed yellow colonies within 48 h on R2A agar at 30 °C. While cell growth occurred at temperatures ranging from 4 to 30 °C, no growth was observed at 37 °C. Growth was observed at pH 6 to 10, but no growth was observed at pH 5 or 11. The colonies were convex and circular with entire edges. The cells were found to be Gram-stain-positive, oxidase-negative, catalase-positive, non-motile, and rod shaped (Supplementary Fig. 1).

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Results and discussion

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The draft genome sequence of strain CHu50b-6-2T was deposited at DDBJ/EMBL/GenBank with the accession number PRJNA559971. The draft genome of strain CHu50b-6-2T was of 4,022,930 bp, containing 175 contigs, of which the largest was of 845,903 bp. The genome encoded 3975 genes, including 48 tRNAs and 7 rRNAs. The N50 value was 413,391 and the sequencing depth of coverage was 570X. The DNA G + C content calculated from the draft genome sequence was 67.3 mol% (Table S1). The ANI values of strain CHu50b-6-2T with G. tibetensis MP203T and F. faeni 801T were 73.1 and 73.4, respectively.

The almost-complete 16S rRNA gene sequence (approximately 1461 nt) of strain CHu50b-6-2T was compared with those of representative species within the family Microbacteriaceae. Strain CHu50b-6-2T showed over 97% 16S rRNA gene sequence similarities with G. tibetensis, F. faeni, L. cavernae, F. endophyticum, Parafugoribacterium mesophilum, Cryobacterium arcticum, F. salinisoli, and Homoerminonas aerilata, and less than 97% with the remaining members of the family Microbacteriaceae. According to maximum-likelihood, neighbour-joining, and maximum-parsimony tree analysis (Fig. 1 & Supplementary Fig. S2), the isolate was phylogenetically distinct from closely related members of the family Microbacteriaceae, especially with G. tibetensis, F. faeni, L. cavernae with which it showed 98.0%, 97.7%, and 97.6% 16S rRNA gene sequence similarities, respectively. And the genomic delineation between strain CHu50b-6-2T and the type strains of G. tibetensis, F. faeni, and L. cavernae was supported by the DNA-DNA relatedness (the mean of triplicate experiments) data, for which our novel isolate showed DNA-DNA relatedness values of 31.2% (reciprocal 33.1%), and 22.5% (reciprocal 24.9%) with G. tibetensis KCTC 29148T, F. faeni KACC 20509T and L. cavernae DSM 27960T, respectively. For clearer delineation, the housekeeping gene, recA gene was applied as phylogenetic marker. The recA gene sequence of CHu50b-6-2T had 88.8%, 88.2%, 86.2%, 85.9%, and 85.4% similarities with Clavibacter michiganensis VKM Ac-1403T, Subtercola boreus DSM 13056T, Rathayibacter...
rathayi DSM 7485T, F. faeni KACC 20509T, and G. tibetensis KCTC 29148T respectively. Overall, phylogenetic analyses based on 16S rRNA, recA genes and UPGMA dendrogram based on the ANI values of genomic sequences revealed groups that are in good agreement with the currently recognized genera
The lower DNA-DNA hybridization values together with phylogenetic analysis revealed that strain CHu50b-6-2T could not be clearly assigned to any species of the genus *Glaciibacter, Frigoribacterium*, and *Lysinibacter*. The G+C content of the genomic DNA was 67.3 mol%, and the major fatty acids were anteiso-C_{15:0} (46.6%), iso-C_{16:0} (29.7%), and anteiso-C_{17:0} (14.5%) (Table 2). The major predominant respiratory menaquinone was MK-10. The polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), an unidentified glycolipid (GL), and three unidentified lipids (L1, L2, and L3) (Supplementary Fig. 3). It is noteworthy that strain CHu50b-6-2T had a (1000 resamplings, only values above 70% shown). *Beutenbergia cavernae* HKI 0122T (Y18378) was used as an outgroup. Bar, 1 substitutions per 100 nt
large amount of anteiso-C 17:0 (14.5%) compared to the closest members G. tibetensis KCTC 29148 T (3.6%), F. faeni KACC 20509 T (5.1%) and L. cavernae DSM 27960 T (1.8%), and a smaller amount of iso-C 14:0 (1.6%) compared to L. cavernae DSM 27960 T (22.2%). Although the overall polar lipid patterns were very similar, there were some differences in the unidentified phospholipids and the unidentified lipids between CHu50b-6-2 T and the species G. tibetensis, F. faeni and L. cavernae. Strain CHu50b-6-2 T had B1γ type peptidoglycan structure, which differed from phylogenetically related genera Glaciibacterius, Frigoribacterium, and Lysinibacter. Together with some other physiological results, it could be concluded that the strain CHu50b-6-2 T differs from the close species G. tibetensis, F. faeni and L. cavernae (Tables 1, 2). And also, strain CHu50b-6-2 T can be differentiated from closely related genera within the family Microbacteriaceae on the basis of its chemotaxonomic characteristics such as fatty acids, polar lipids, menaquinones, and G + C content (Table 3). Therefore, it should be considered that the strain is not accommodated in any of known genera within the family Microbacteriaceae.

On the basis of the phylogenetic position and genotypic, chemotaxonomic, and physiological differences, we propose that strain CHu50b-6-2 T should be assigned as a novel species within a new genus, Lacisediminihabitans gen. nov., with the name Lacisediminihabitans profunda sp. nov. within the family Microbacteriaceae.

The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumber for type strain CHu50b-6-2 T is GA00113.
Description of *Lacisediminihabitans profunda* gen. nov.

*Lacisediminihabitans* (L.a.ci.se.di.mi.ni.ha.bi’tans. L. n. *lacus* lake; L. n. *sedimeninis* sediment; L. masc. n. *habitans* an inhabitant; N.L. fem. n. *Lacisediminihabitans* an inhabitant of lake sediment).

Cells are observed to be Gram-stain-positive, non-spore-forming, non-motile and rod-shaped. The predominant respiratory menaquinone is MK-10. The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and an unidentified glycolipid (GL). The major fatty acids are anteiso-C15:0, iso-C16:0, and anteiso-C17:0. The cell-wall peptidoglycan is B1z contains 2, 4-diaminobutyric acid as the diagnostic diamino acid. Phylogenetically, the genus belongs to the family *Microbacteriaceae* in the class *Actinobacteria*, being closely related to the genera *Clavibacter michiganensis* ATCC 33113T (AM849034), *Rathayibacter rathayi* DSM 7485T (VIVJ01000000), *CHu50b-6-2T* (VRMG000000000), *Glaciilhabitans arcticus* KACC 21151T (NZ_SISG000000000), *Glaciilhabitans tibetensis* CGMCC 1.12484T (PVTI01000000), *Diaminobutyricimonas aerilata* DSM 27393T (PGFF010000001), *Compostimonas suwonensis* DSM 25625T (PGFB000000000), *Cryobacterium psychrotolerans* CGMCC 1.5382T (FNU010000000), *Labeledella gwajienesis* KSW2-17T (RZGY010000000), *Frigoribacterium faeni* NBRC 103086T (BJUV000000000), *Frondihabitans australicus* DSM 17894T (RBKS000000000), *Ammibacterium kyonggiense* DSM 24782T (SOAM010000000), *Klugiella xanthotipulae* DSM 18031T (VFNP0100000), *Mycetocella saprophilus* NRRL B-24119T (JOEC000000000), *Aurantimicrobium minutum* KNC1T (AP017457), *Rhodoluna lacicola* MWHT-Ta8T (CP007490), *Pontimonas salivibrio* CL-TW6T (CP026923), *Beutenbergia cavernae* DSM 12333T (NC 012669).

**Figure 3** UPGMA dendrogram based on ANI values of genomic sequences showing the positions of strain CHu50b-6-2T among the type species within the family *Microbacteriaeae*.

Table 2 Cellular fatty acid compositions (%) of strain CHu50b-6-2T and related type strains

| Fatty acids | 1   | 2   | 3   | 4   |
|-------------|-----|-----|-----|-----|
| Iso-C14:0   | 1.6 | 5.9 | 3.4 | 22.2 |
| Iso-C13:0 3 OH | tr | tr | 2.6 | tr |
| Anteiso-C15:1 A | 2.7 | 1.5 | tr | tr |
| Iso-C15:0    | 3.1 | 1.7 | 3.8 | 1.0 |
| Anteiso-C15:0 | 46.6 | 57.9 | 38.0 | 27.2 |
| Iso-C14:0 3 OH | tr | tr | 1.1 | tr |
| C14:0 2 OH   | tr | 4.2 | 17.0 | tr |
| Iso-C16:0    | 29.7 | 21.5 | 23.9 | 45.6 |
| C16:0        | 1.3 | 2.7 | 3.1 | 1.6 |
| Anteiso-C17:0 | 14.5 | 3.6 | 5.1 | 1.8 |

Strains: 1, CHu50b-6-2T; 2, *G. tibetensis* KCTC 29148T; 3, *F. faeni* KACC 20509T; 4, *L. cavernae* DSM 27960T. All data were from present study. Cells of all strains were harvested after growth on R2A agar at 30°C for 48 h. tr, not detected or less than 1%
Glaciibacter, Frigoribacterium, and Lysinibacter. The type species is *Lacisediminihabitans profunda*.

Description of *Lacisediminihabitans profunda* sp. nov.

*Lacisediminihabitans profunda* (pro.fun’d.a. L. fem. adj. *profunda* from the deep).

In addition to the characteristics described above, the novel species has the following properties. Colonies on R2A are convex, circular with entire edges and yellow color. The cells are observed to be oxidase-negative but catalase-positive. Growth occurs on R2A at temperatures from 4 to 30 °C (optimum temperature 25–30 °C), but not at 37 °C. The pH range for growth is from pH 6–10 (optimum pH 7); however, there is no growth at pH 5 and 11. No growth was observed on TSA, LB, and NA media. The cells are positive for nitrate reduction and β-galactosidase but negative for aesculin hydrolysis, indole production, glucose fermentation, urease, arginine dihydrolase or gelatin hydrolysis (API 20NE test strip). The G + C content of the genomic DNA is 67.3 mol%.

The type strain is CHu50b-6-2 T (= KCTC 49081 T = JCM 32673 T) isolated from a 67-cm-long sediment core taken from the Daechung Reservoir, Republic of South Korea.

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Author contributions L.J. and H.G.L. designed the experiments; Y.Z. and C.Z.J. carried out the experiments; T.L., C.S.L., F.-J.J. and H.M.O. analyzed the results (phylogenetic and chemotaxonomic data); D.H.K. contributed to extract and analyze peptidoglycan and description and
discussion of characteristics of the peptidoglycan; Y.Z. and L.J. wrote the manuscript.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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