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EXPERIMENTAL ARTICLES

Pelosinus baikalensis sp. nov., an Iron-Reducing Bacterium Isolated from a Cold Freshwater Lake

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Abstract—An obligately anaerobic bacterium, strain Bkl1^T, was isolated from an enrichment culture of iron-reducing bacteria (IRB) obtained from a sample of the bottom sediments of the cold freshwater Lake Baikal (Russia). Cells of the strain were Gram-stain-negative, motile, spore-forming straight rods (0.6–0.7 × 2.0–7.0 μm) with a fermentative metabolism. Strain Bkl1^T grew in the temperature range from 7 to 38°C (optimum 20°C) and at pH 7.0–9.5 (optimum pH 7.6). The novel isolate was capable to reduce ferric citrate (FC), anthraquinone-2,6-disulfonate (AQDS) and Cr(VI) in the presence of lactate as carbon source. Ferrihydrite was not reduced in the absence of AQDS. Based on its 16S rRNA gene sequence, strain Bkl1^T was affiliated to the family *Sporomusaceae* and, more specifically, to the genus *Pelosinus*. The strain was most closely related to *Pelosinus fermentans* DSM 17108^T (99.2%) and *P. propionicus* DSM 13327^T (99.1%). Genome relatedness indexes revealed that the average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values between strain Bkl1^T and its closest phylogenomic relative (*P. fermentans* DSM 17108^T) were 93.3 and 54.2%, respectively. The G + C content of the genome of strain Bkl1^T was 39.1 mol % and its size was 5.32 Mb with 4939 protein-coding genes. The predominant fatty acids in cell walls were $C_{15:1}$, $C_{17:1}$, and $C_{16:1}$. Based on the phylogenetic analyses and phenotypic differences between the novel isolate and type strains of the genus *Pelosinus*, strain Bkl1^T (=VKM B-3511^T = JCM 39258^T) is proposed to represent a novel species *Pelosinus baikalensis* sp. nov.

Keywords: Lake Baikal, metal reduction, taxonomy, *Pelosinus* sp.

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Pelosinus spp. are fermentative firmicutes that were prominent members of microbial communities at contaminated subsurface sites in multiple locations (Bowen De León et al., 2015). *Pelosinus fermentans* strain R7^T DSM 17108^T was isolated as a representative iron-reducing bacterium from Russian kaolin clays as the new species for a new genus (Shelobolina et al., 2007). At present, there are three species of the genus *Pelosinus* with validly published names (https://lpsn.dsmz.de/genus/pelosinus). Representatives of all species of the genus are obligately anaerobic chemo-organotrophs and mesophiles, which do not produce acetate from $H_2 + CO_2$. They are capable of reducing Fe(III) and Cr(VI) in the presence of fermentable substrate (Boga et al., 2007; Moe et al., 2012). *Pelosinus* spp. dominated in electron acceptor-limited enrichments that used lactate as the main carbon source and electron donor and inocula from contaminated groundwater samples drawn from the U.S. Department of Energy's H-100 well in Hanford, WA (Mosher et al., 2012). Sixteen isolates obtained from the chemostat studies via fluorescence-activated cell sorting (FACS) were identified as being 99 to 100% identical to *P. fermentans* DSM 17108^T at the 16S rRNA gene level. At the time of publication, genomes of the five strains (*P. fermentans* JBW45, *P. fermentans* A11, *P. fermentans* B4, *Pelosinus* sp. strain HCF1, and *Pelosinus* sp. strain UFO1) were sequenced and assayed, in addition to the three type species of the genus *Pelosinus* that have been validly described. All strains reduced soluble Fe(III) with lactate as the electron donor. *P. fermentans* strain A11 was notable as being the only isolate able to reduce hexavalent uranium (Bowen De León et al., 2012; Ray et al., 2018).

In this paper, we describe a novel strain of *Pelosinus* sp. isolated from the bottom sediments of the Lake Baikal, Russia (N 55°30.94′, E 109°46.58′).

MATERIALS AND METHODS

Isolation and culture conditions. The samples of upper bottom sediments used in this work were collected during the 2009–2010 expeditions of the Limnological Institute, Siberian Branch, Russian Acad-

emy of Sciences (LIN SB RAS) on RV G.Yu. Vereshchagin using a Mir submersible (Zemskaya et al., 2012). The samples were collected at the areas of oil and gas release and were black oily sludges containing hydrocarbons from the depth of 409 m. Water temperature at the bottom was ~4°C. The average pH was 7.03.

Enrichment and isolation were performed using anaerobic techniques (Hungate, 1969). The basal growth medium (MI) contains (g L^{-1}): $NaHCO_3$, 2.5; NaCl, 1.0; KH_2PO_4, 0.68; $MgCl_2 \cdot 6H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.1; NH_4Cl, 1.0; yeast extract (Difco), 0.2; trace element solution SL-10 (medium 320; DSMZ) 1.0 mL; vitamin solution (Wolin et al., 1963), 10.0 mL. To obtain IRB enrichments, the sediment (1 g) was added to 100 mL of the medium MI. Sodium lactate (20 mM) served as the carbon source and as the electron donor, while FC (30 mM) served as the electron acceptor. Medium preparation and cultivation were carried out under anoxic conditions with 100% N_2 in the gas phase; pH of the medium was 7.0–7.2. Incubation was carried out for 30 days in the dark at 15°C. Uninoculated mineral medium was used as the chemical control. Fe(III)-reducing enrichment culture was maintained by periodic transfers with lactate and FC in our laboratory for 2 years. After month of incubation in the enrichment culture was formed 4.0–5.0 mM of Fe(II). A pure culture, designated strain Bkl1^T, was obtained by the dilution method on anaerobic medium MI with lactate (20 mM). The purity of the strain was verified by microscopy. For comparative purposes, reference strain *P. fermentans* DSM 17108^T was obtained from the DSMZ, Brauschweig, Germany and grown in 311c medium (www.dsmz.de) with glucose as the carbon source.

Phylogenetic analysis and whole-genome sequencing analyses. For the 16S rRNA gene sequencing, DNA was prepared and purified as described by Marmur (1961). The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG) and 1492R (5'-TACGGYTACCTTGTTACGATT). The PCR product was purified using a Wizard PCR Preps DNA Purification System. The sequencing reactions were performed using a CEQ Dye Terminator Cycle Sequencing kit according to the protocols provided by the manufacturer and analyzed in a Beckman Coulter CEQ 2000 XL automatic DNA sequencer. The NCBI GenBank BLAST utility (Benson et al., 1998) was used to reveal the closest relatives of strain Bkl1^T. Phylogenetic trees were reconstructed using three different methods: the neighbor-joining, maximum likelihood, and minimum-evolution methods implemented in MEGA7 software (Kumar et al., 2016). The phylogenetic trees were evaluated by bootstrap analysis based on 1000 replications. The consensus tree was inferred by using the neighbor-joining method based on the general time-reversible model. Bootstrap values obtained for trees using the maximum-likelihood/minimum-evolution/neighbor-joining methods are shown at branch points. The 16S rRNA gene sequence of strain Bkl1^T was deposited in the GenBank under accession number MW805760.

To further clarify the phylogenetic assignment of strain Bkl1^T, its genome was sequenced. Genomic DNA preparation and sequencing were performed by the BioSpark Company (Troitsk, Russia). Genomic DNA was isolated with the FastDNA spin kit (MP Biomedicals, USA) by the column method with deposition on silica gel. The libraries were synthesized using KAPA HyperPlus kits (Kapa Biosystems, USA) in accordance with the manufacturer's recommendations. Sequencing was performed on the Illumina NovaSeq 6000 platform, and a paired-end library with a total of 6707822 reads and a read length of 101 bp was obtained. The quality of the reads was controlled with FastQC v. 0.11.5 (Andrews, 2010). The reads were edited and filtered using Trimmomatic v. 0.36 (Bolger et al., 2014) with adapter clipping (adapter library TruSeq3-PE-2). *De novo* genome assembly was performed using the metaSPAdes genome assembler (version 3.13.0) (Bankevich et al., 2012). Potential contamination was checked used CheckM v. 1.0.18 (Parks et al., 2015). CheckM demonstrated high completeness (100%) and low contamination (5.95%). The genomes of all other type strains within the genus *Pelosinus* were sequenced previously, and the data were available in the NCBI. For the overall genome relatedness index (OGRI), the dDDH values between strain Bkl1^T and the type strains of the genus *Pelosinus* were calculated using the Genome-to-Genome Distance Calculator available at http://ggdc.dsmz.de. The values of ANI were calculated for members of the genus *Pelosinus* using a web service JSpecies WS (Richter et al., 2015).

The phylogenetic tree on the basis of genome-wide core genes was reconstructed using program SpeciesTree v2.2.0 from the KBase suite (Arkin et al., 2018), built on the principle of combining several other algorithms: FastTree 2 (Price et al., 2010), GBLOCKS (Talavera and Castresana, 2007), and the KBase database. The app allows to construct a species tree using a set of 49 core, universal genes defined by COG (Clusters of Orthologous Groups) gene families. It combines the genomes provided by the user with a set of closely related genomes selected from the public KBase genomes import of RefSeq. Relatedness is determined by alignment similarity to a select subset of 49 COG domains. The curated alignments have been trimmed using GBLOCKS to remove poorly aligned sections of the MSA. The MSAs are then concatenated. A phylogenetic tree is reconstructed using, (version 2.1.10, a method to quickly estimate approximate maximum likelihood phylogeny) with the genome(s) provided by the user and the set of genomes identified as similar in the previous step. FastTree2 is used with the fastest setting.

Microscopy methods. The cell morphology was examined using phase-contrast microscopy (Olympus BX41) at ×1300 magnification and a JSM-6510LV scanning electron microscope (JEOL, Japan). For scanning electron microscopy analysis (SEM), samples were fixed in a solution of 1.5% (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) at 4°C for 1 h. The cells were washed three times in the same buffer and post-fixed in 1% OsO_4 in 0.05 M cacodylate buffer (pH 7.2) for 3 hours at 20°C. After dehydration in a series of ethanol solutions of increasing concentration (from 30 to 100% for 20 minutes at each stage), the cells were additionally soaked in *tert*-butanol (Sigma-Aldrich) in two shifts of 20 minutes at 26°C. Next, the samples were frozen in *tert*-butanol, and the freeze-drying procedure was carried out in a JFD-320 unit (JEOL, Japan) following the manufacturer's recommendations. Dried samples were sputtered with gold in a JFC 1100 spraying machine (JEOL, Japan). For ultrathin sectioning, cells were harvested by centrifugation and fixed in a solution of 1.5% (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) at 4°C for 1 hour. The cells were washed and then fixed as described for SEM. The preparation was dehydrated using a series of different ethanol concentrations and embedded in Epon 812 epoxy resin. The ultrathin sections were mounted on grids and post-stained in 3% (w/v) uranyl acetate in 70% (v/v) ethanol for 30 minutes, and afterward, they were additionally stained in lead citrate following Reynolds methodology (Reynolds, 1963).

Effects of pH, temperature, and NaCl. All experiments described in this article were performed under strictly anaerobic conditions. Kinetic parameters of growth were determined in medium MI with 20 mM lactate at different temperatures (0, 5, 7, 10, 20, 27, 32, 38, and 55°C), pH values (6.0, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0), and NaCl concentrations (0, 1.0, 5.0, 10, 20, 50, 100, and 150 g L^{-1}). To study the pH dependence, the following buffer solutions were used: pH 6.0 (5.0 mL 0.2 M CH_3COOH, 95.0 mL 0.2 M CH_3COONa); pH 7.0 and 7.5 (50 mL 0.1 M KH_2PO_4, 29.1 mL 0.1 M NaOH, water to 100 mL and 50 mL 0.1 M KH_2PO_4, 40.9 mL 0.1 M NaOH, water to 100 mL, respectively); pH 8.0 and 8.5 (7.5 g L^{-1} KCl, 6.2 g L^{-1} H_3BO_3, 3.9 mL 0.1 M NaOH and 7.5 g L^{-1} KCl, 6.2 g L^{-1} H_3BO_3, 10.1 mL 0.1 M NaOH, respectively); pH 9.0 (12.5 g L^{-1} $NaHCO_3$, 2.0 g L^{-1} Na_2CO_3); pH 9.5 (1.85 g L^{-1} $NaHCO_3$, 1.2 g L^{-1} Na_2CO_3); pH 10.0 (2.76 g L^{-1} $NaHCO_3$, 1.84 g L^{-1} Na_2CO_3). Sterile buffer solutions were added to the medium before inoculation. The dependence of growth on NaCl content was determined on the medium of the following composition (g L^{-1}): $KHCO_3$, 2.5; K_2HPO_4, 0.68; $MgSO_4 \cdot 7H_2O$, 0.2; $(NH_4)_2SO_4$, 1.0; yeast extract (Difco), 0.02; trace element solution SL-10 (medium 320; DSMZ), 1.0 mL; vitamin solution (Wolin et al., 1963), 10.0 mL. NaCl at concentrations from 0 to 150 g L^{-1} was added separately to each vial with the medium before inoculation. All the tests were done in triplicate and confirmed by growth with two subsequent transfers.

Electron donor and acceptor utilization studies. The ability to reduce thiosulfate (20 mM), sulfite (10 mM), sulfate (10 mM), elemental sulfur (2 g L^{-1}), nitrate (10 mM), AQDS (2.0 mM), Cr(VI) in the form of a dichromate (50 mM) and ferrihydrite (10 mM) was added as amorphous iron(III) oxide, prepared by titration of acidic $FeCl_3$ solution with 10% (w/v) NaOH to pH 7.0 (Lovley et al., 1993) was assessed in Hungate tubes containing anaerobic medium MI supplemented with 20 mM acetate as the electron donor and carbon source.

Potentially fermentable substrates were examined in medium MI in the absence of an electron acceptor. The following substrates were tested: organic acids (20 mM), peptone (3 g L^{-1}), casamino acids (2 g L^{-1}), and alcohols (0.1%, v/v).

Growth was assessed by direct cell counting under a phase-contrast microscope. Bacterial biomass (measured as OD_{600} on the spectrophotometer ZEISS SPEKOL 221, Germany) was determined at the beginning and end of growth and all results were recorded after 5 days of incubation at 20°C. All the tests were in triplicate and confirmed by two transfers.

Analytical techniques. Sulfide was measured by the Pachmayr method (Cline, 1969). Nitrite was analysed according to Gries-Romijn van Eck (1966). Fe(III) reduction was determined colorimetrically by formation of a stable colored complex of Fe(II) with ferrozine (Viollier et al., 2000). Diphenylcarbazide reagent (DPC) was used to quantify Cr(VI) at 540 nm (Han et al., 2010). The AH_2DS reduced form was determined under anoxic conditions in 1-cm cuvettes purged with 100% N_2. AH_2DS concentration was determined on a Shimadzu spectrophotometer (Japan) by monitoring absorbance at 450 nm, using the extinction coefficient of 2.25 AU (Cervantes et al., 2000). Products of glucose fermentation in the culture medium were assayed with an HPLC system (Knauer, Germany). The analytical column was Inertsil ODS-3 (5 μm, 250 × 4.6 mm; Dr. Maisch GmbHs, Germany). Chromatography was carried out in 20 mM H_3PO_4 at 210 nm, at a temperature of 35°C and a pressure of 130 bar, resulting in an eluent flow rate of 1.0 mL per min. The products were identified using standard solutions of organic acids 1 g L^{-1} ("Sigma-Aldrich", USA) according to a retention time.

Chemotaxonomic analyses. For analysis of the cellular fatty acids, strain Bkl1^T and the related type strain *P. fermentans* DSM 17108^T were harvested at the late exponential growth phase after being cultivated for 5 days at 20°C in DSMZ 311c medium with glucose as the carbon source. Cellular fatty acids (CFA) profiles

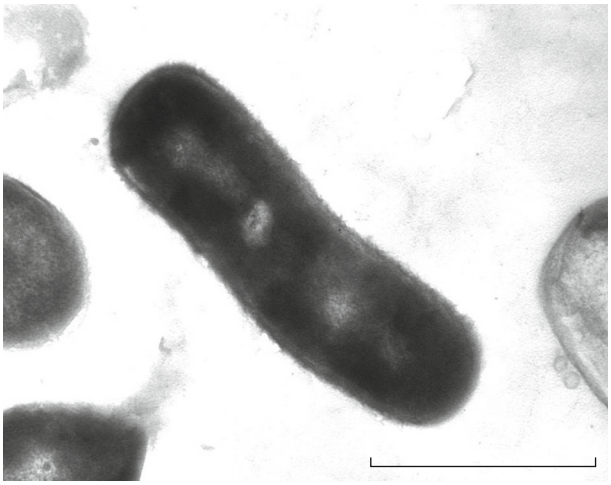

Fig. 1. Ultrathin section of a cell of strain Bkl1^T grown with lactate at 10°C showing cell walls and membranes. Bar, 1 μm.

were determined by GC-MS as described earlier by Slobodkina et al. (2020). Enzymatic activities of strain Bkl1^T and *P. fermentans* DSM 17108^T were determined using a set of enzymatic tests API ZYM (BioMerieux, France) according to the manufacturer's protocol.

RESULTS AND DISCUSSION

Isolation. The culture from the maximal dilution positive for growth (–5) was plated on the medium containing agar in the Petri dishes that were placed in the anaerobic jars (Oxoid). The colonies appearing on agar 5–7 days after the inoculation were circular, convex, cream coloured and 1–2 mm in diameter. Resulting from the serial transferred of the single colonies from the solid to liquid medium, a pure culture of anaerobic bacterium called strain Bkl1^T was isolated.

Phenotypic characterization. Cells of strain Bkl1^T were motile, straight to slightly curved rods (0.6–0.7 × 2.0–7.0 μm). In the late-exponential and stationary phases of growth, the rods formed terminal endospores. Gram staining was performed following a standard protocol (Smibert and Krieg 1994), and cells of the strain stained Gram-negative, which was confirmed by ultrathin sections (Fig. 1).

Table 1. dDDH and ANI values (%) between strain Bkl1^T and closely related described strains the genus *Pelosinus*. The proposed and generally accepted species boundary for ANI and dDDH values are 95~96 and 70%, respectively (Chun et al., 2018)

Strain Bkl1^T in comparison with:	dDDH	ANI
P. fermentans DSM 17108^T	54.2	93.3
P. pronionicus TmPN$_3$^{T}	38.4	88.9
P. fermentans JBW45	76.9	97.1
P. fermentans A11	54.8	93.5
P. fermentans B4	54.4	93.3
Pelosinus sp. HCF1	54.3	93.3
Pelosinus sp. UF01	24.1	78.3

Phylogenetic and phylogenomic characterization. Phylogenetic analysis based on 16S rRNA gene sequences demonstrated that strain Bkl1^T was highly similar to the type strains of species of the genus *Pelosinus* (97.1–99.2%). The 16S rRNA gene sequence of strain Bkl1^T had the highest similarity (99.2%) with *P. fermentans* DSM 17108^T and *P. propionicus* DSM 13327^T (99.1%) and they were grouped in the phylogenetic tree (Fig. 2a). The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and RASTThe assembly yielded 165 contigs with a total size of 5 341 130 bp, N50 of 108 750 bp, and a sequencing depth of coverage of 123.6X. Finally, the genomic sequence of strain Bkl1^T was assembled in 159 scaffolds with a total size of 5.32 Mb and a G + C content of 39.1 mol %. The total number of genes was 5118, including 5037 coding sequences (4939 protein-coding sequences) and 98 pseudogenes. There were 81 RNA genes, including 13 rRNA genes (three 5S, three 16S, and seven 23S), 62 tRNA genes, and 6 noncoding RNA genes.

The dDDH and ANI between strain Bkl1^T and *P. fermentans* JBW45 were 76.9 and 97.1%, respectively (Table 1) and they were grouped in the phylogenetic tree basis of genome-wide core genes (Fig. 2b). However, strain JBW45 was not described as a type strain of a new speciesand at present, it is classified as *P. fermentans.* The small subunit (SSU) rRNA gene sequence of the isolate is 99% identical to the *P. fermentans* DSM 17108^T and *P. propionicus* DSM 13327^T gene sequences. Isolate JBW45 has an intermediate sequence near the 5' end of the SSU rRNA gene sequence, which is similar to those previously reported in *Pelosinus* species. The dDDH and ANI values between strain Bkl1^T and type strain *P. fermentans* DSM 17108^T were 54.2 and 93.3%, respectively (Table 1), indicating that strain Bk1^T belonged to the represent a novel species within *Pelosinus* genus (Chun et al., 2018).

Growth characteristics. Strain Bkl1^T could grow between 7 and 38°C, and the optimum growth was at 20°C. The optimum pH for growth was 7.0–7.5, with growth occurring between 6.0 and 8.0 The novel isolate grew at NaCl concentrations from 0 to 1.0% with an optimum at 0–0.5% (w/v). Na^+ and Cl^- ions were not obligatory components of the medium for the strain.

Strain Bkl1^T was capable of growth (at a concentration of 20 mM, unless stated otherwise) on lactate, pyruvate, glucose, fructose, sucrose, citrate, glycerol, peptone, Casamino acids and yeast extract. No growth

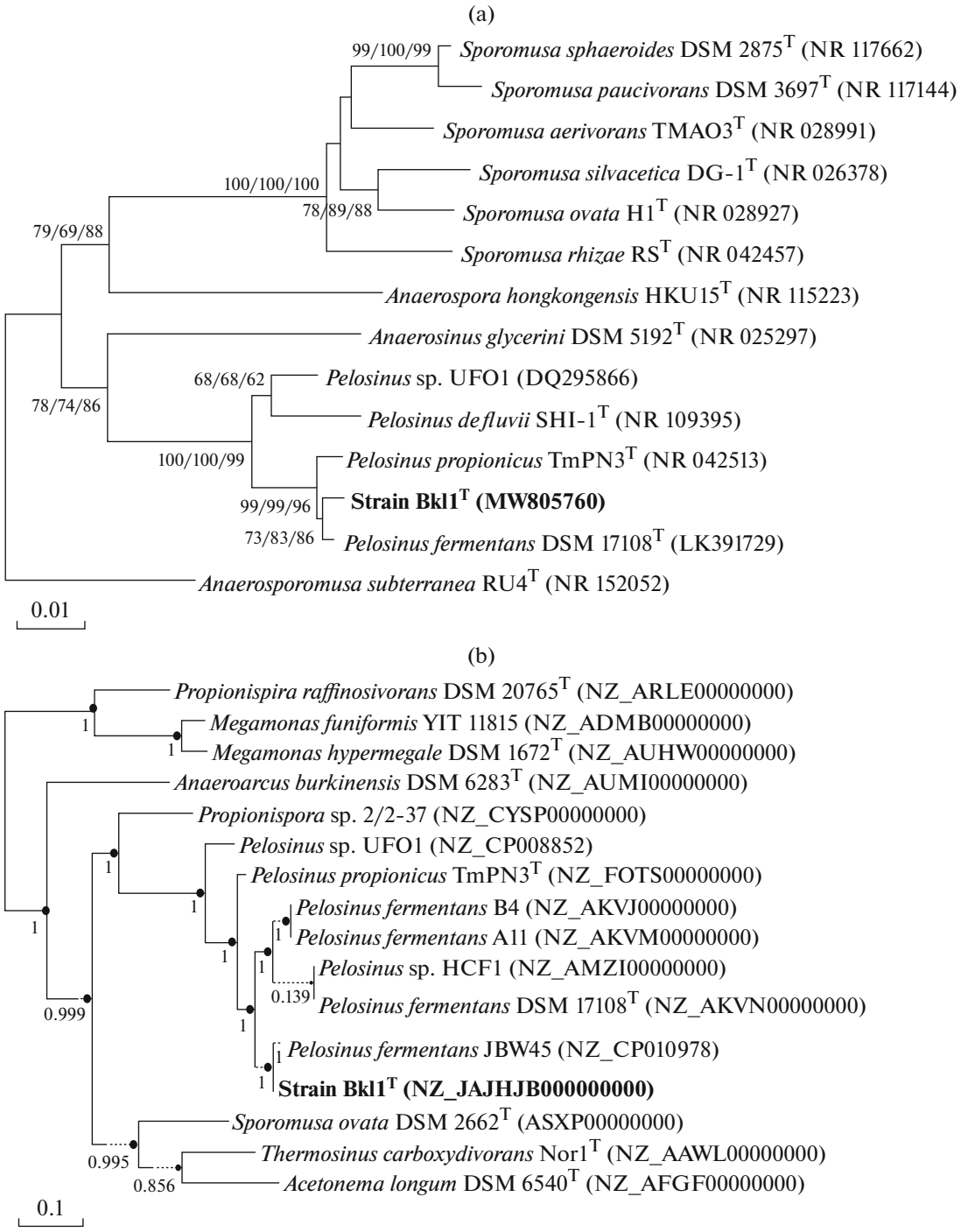

Fig. 2. Phylogenetic trees showing the position of strain Bkl1^T and the strains of related species of the family *Sporomusaceae* on the basis of 16S rRNA gene sequences (a), and (b) phylogenetic tree constructed from 49 orthologue groups (COG) from representative genomes and genome of strain Bkl1^T (SpeciesTree v.2.2.0). The tree on the basis of 16S rRNA gene sequences was reconstructed by using the neighbor-joining method. The bootstrap values (only values ≥50% shown) were obtained with the maximum-likelihood/minimum-evolution/neighbor-joining methods based on 1000 replicates. (a) Bar, 0.01 substitutions per nucleotide sequence position; (b) bar, 0.10 substitutions per nucleotide sequence position. WGS of the strains in NCBI are shown in parentheses.

was observed with ramnose, trehalose, ethanol and methanol. The strain did not grow autotrophically on H_2/CO_2 (80/20 (v/v), 1.5 atm). In comparison with *P. fermentans* DSM 17108^T, strain Bkl1^T use maltose for growth (Table 2). Propionate and acetate were the major products of glucose fermentation.

Table 2. Distinctive characteristics between strain Bkl1^T and type species of the genus *Pelosinus*

Characteristic	1	2	3	4
Cell shape	Straight rods, singly or in pairs	Slightly curved rods	Long rods cells, singly or in pairs	Straight or curved rods
Cell size, μm	0.6–0.7 × 2–7	0.6 × 2–6	0.5–0.7 × 2.2–12	0.6–1 × 2–7
Temperature range, °C	7–38	4–36	15–37	10–42
(optimum)	(20)	(22–30)	(30–37)	(30–37)
pH range	6.0–8.0	5.5–8.0	5.5–8.5	5.5–8.5
(optimum)	(7.0–7.5)	(7.0)	(6.5–7.0)	(7.0–7.5)
Growth on:				
Lactate	+	+	+	–
Maltose	+	–	ND	–
Rhamnose	–	–	+	–
Sucrose	+	ND	ND	–
AQDS + Lactate	+	+	ND	ND
Ferrihydrite (in the presence AQDS) + Lactate	+	–	ND	ND
Alkaline phosphatase	+	+*	ND	ND
Acid phosphatase	+	+*	ND	ND
Naphthol-AS-BI-phosphohydrolase	+	+*	ND	ND
Esterase (C4)	–	+*	ND	ND
Esterase lipase (C8)	–	+*	ND	ND
DNA G + C content, mol %	39.1	39.7	38.8	39.2
Source	Bottom sediments of Lake Baikal (Russia)	Subsurface primary kaolin deposits (Russia)	Intestinal tract of the termite *T. macrothorax*	Chlorinates solvent–contaminated groundwater (USA)

Strains: 1, Strain Bkl1^T (data from this study); 2, *P. fermentans* DSM 17108^T (Shelobolina et al., 2007); 3, *P. propionicus* TmPN3^T (Boga et al., 2007; Moe et al., 2012); 4, *P. defluvii* SHI-1^T (Moe et al., 2012). +, positive; –, negative; ND, not determined.
* Obtained in our study.

With acetate as the electron donor and as the carbon source, strain Bkl1^T was not capable of utilizing sulfate, sulfite, thiosulfate, elemental sulfur, nitrate, AQDS, Cr(VI) and ferrihydrite as the electron acceptor.

Although strain Bkl1^T was isolated from an Fe(III)-reducing enrichment by cultivation with lactate and FC, this strain was not capable of respiratory growth on FC when 20 mM acetate was provided as the electron donor. Strain Bkl1^T was capable of reducing AQDS to AH_2DS only the presence of a fermentable substrate. Quinone reduction by the new strain was accompanied by changes in the color of the medium from yellow to bright orange, which did not occur in the abiogenic control. In the present of 20 mM lactate, nearly 4 mM Fe(II) and 0.8 mM AH_2DS were produced after 1 week of incubation at 20°C. Ferrihydrite was reduced only in the presence of AQDS and lactate (data not shown).

Strain Bkl1^T also reduced Cr(VI) in the presence of fermentable substrate. Figure 3 shows the nearly complete removal of 50 mM Cr(VI) by strain Bkl1^T within twenty days when lactate (20 mM) was provided as an electron donor. During the same time, the cell-free medium reduced about 60% of Cr(VI) due to the chemical oxidation of lactate. Biotic reduction of Cr(VI) in the absence of lactate did not occur.

Biochemical characteristics. Strain Bkl1^T and *P. fermentans* DSM 17108^T were characterized by high activity of alkaline and acid phosphatase, and naphthol-AS-BI-phosphohydrolase. In contrast to strain Bkl1^T, *P. fermentans* DSM 17108^T showed high activities of esterase (C4) and esterase lipase (C8) (Table 2).

The majority of fatty acids identified in strain Bkl1^T were straight saturated chains. The predominant fatty acids were $C_{15:1}$ (43.47%) and $C_{16:1}$ (10.36%). We detected saturated $C_{17:1}$ (20.71%) which is not characteristic for the species of *Pelosinus* genus (Table 3). However, $C_{15:0}$ found in the species of the genus

(*P. fermentans* DSM 17108^T—13.82%; *P. propionicus* DSM 13327^T—8.3%) was just 6.9% in the novel strain.

Potential ecophysiological role of strain Bkl1^T and implication for metal transformation. Microbiological and phylogenetic studies have shown that the microbial community an enrichment culture of IRB of the bottom sediments of the cold freshwater Lake Baikal includes fermentative bacteria that are involved in the oxidation of organic compounds. Kappler et al. (2004) demonstrated that fermenting bacteria represented the largest bacterial population in freshwater lake sediments and had an important role in humic acid reduction. The results suggested that humic acid-mediated reduction of poorly soluble Fe(III) oxides is an important reductive pathway in anoxic natural environments. Strain Bkl1^T was capable of reducing the humic acid analog, AQDS, in the presence fermentable substrate; in addition, AQDS mediated the reduction of the insoluble Fe(III)-oxide, ferrihydrite. These findings suggest that fermentative bacteria such as strain Bkl1^T could play an important role in multiple biogeochemical cycles in aquifer environments.

Genome annotation of strain Bkl1^T supported its abilities to metal transformation. The genome of strain Bkl1^T contained the genes encoding putative selenate reductase subunit *YgfK*, which would selenate reduction to selenite. Selenate reductase is part of an electron transport chain that generates an electrochemical gradient across the cytoplasmic membrane. We found genes encoding respiratory nitrate reductase subunit gamma *NarI*, two subunits nitrate reductase (beta, alpha) and genes encoding *NapC/NirT* family cytochrome *c*. Genes that encode chromate transport protein *chrA* (resistance to chromium compounds) were also found in the genome of the strain Bkl1^T, in contrast to genome *P. fermentans* DSM 17108^T.

Description of *Pelosinus baikaliensis* sp. nov. *Pelosinus baikalensis* (bai.ka'l.en'sis N.L. neut. adj. baikalensis pertaining to the Lake Baikal, referring to the site where the type strain was isolated).

Cells are Gram-stain-negative, motile, spore-forming straight rods (0.6–0.7 × 2.0–7.0 μm), obligately anaerobic. Growth occurs between 7 and 38°C (optimum 20°C). The ranges pH for growth are from 6.0 to 8.0 and the optimum growth is achieved at pH 7.0–7.5. Range of NaCl concentration for growth is 0 to 1.0% (optimum 0–0.5% (w/v)). Utilizes a broad range of carbon sources for growth, such as glucose, fructose, maltose, sucrose, pyruvate, lactate, citrate, peptone, Casamino acids, yeast extract, and glycerol. Does not utilize rhamnose, trehalose, ethanol, methanol. Propionate and acetate were the major products of glucose fermentation. Capable of using Fe(III), AQDS, and Cr(VI) as an electron sink in the presence of lactate. Does not respire anaerobically with sulfate, sulfite, thiosulfate, elemental sulfur, nitrate, AQDS, Cr(VI) and ferrihydrite. Able to reduce ferrihydrite only in the presence of AQDS. Does not grow autotrophically. Strain Bkl1^T is characterized by high activity of alkaline and acid phosphatase, and naphthol-AS-BI-phosphohydrolase. The G + C content of the genome of strain Bkl1^T is 39.1 mol % and its size is

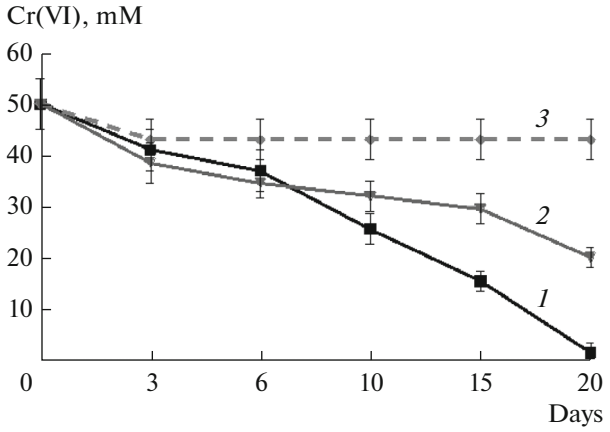

Fig. 3. Reduction of Cr(VI) (50 mM) by strain Bkl1^T in the presence/absences, 20 mM lactate after 20 days of incubation at 20°C. (*1*) Cr(VI) + lactate + cells; (*2*) Cr(VI) + lactat – cells; (*3*) Cr(VI) – lactate + cells. Symbols are means of triplicate cultures, and error bars indicate ± standard deviation.

Table 3. Cellular fatty acid composition of strain Bkl1^T in comparison with *P. fermentans* DSM 17108^T

Fatty acids, %	1	2
$C_{10:0}$	1.13	2.49
iso-$C_{11:0}$	ND	2.08
$C_{11:0}$	4.16	3.46
$C_{13:0}$	0.61	1.02
$C_{13:1}$	1.18	ND
$C_{14:0}$	1.82	4.26
iso-$C_{15:0}$	ND	1.27
$C_{15:0}$	6.9	13.82
$C_{15:1}$	43.47	34.28
$C_{16:0}$	3.35	6.3
$C_{16:1}$	10.36	15.37
$C_{17:0}$	1.02	1.58
$C_{16:0}$ 3-OH	1.29	1.1
$C_{17:1}$	20.71	6.55
$C_{18:0}$	0.79	1.03
$C_{18:1}$	2.32	5.39
$C_{17:0}$ 3-OH	0.89	ND

Strains: 1, Strain Bkl1^T; 2, *P. fermentans* DSM 17108^T (data from this study). ND, not detected.

5.32 Mb with 4939 protein-coding genes. The predominant fatty acids in cell walls are $C_{15:1}$, $C_{17:1}$, and $C_{16:1}$.

The type strain is Bkl1^T (=VKM B-3511^T = JCM 39258^T), isolated from bottom sediments of the cold freshwater Lake Baikal (Russia). The accession number of the genome sequence is NZ_JAJHJB000000000 and MW805760 of the 16S rRNA sequence.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

DATA AVAILABILITY

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Bkl1^T is MW805760. The whole genome sequence of strain Bkl1^T is available at the NCBI (www.ncbi.nlm.nih.gov.) under accession number NZ_JAJHJB000000000.

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