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Anoxynatronum buryatiense sp. nov., an anaerobic alkaliphilic bacterium from a low mineralization soda lake in Buryatia, Russia

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Abstract

An anaerobic alkaliphilic, proteolytic bacterium, strain Su22^T, was isolated from the bottom sediment of the alkaline low mineralization lake Sulphatnoe (Selenginsky district, Buryatia, Russia). A comparative analysis of the 16S rRNA gene sequence revealed that this bacterium was closely related to *Anoxynatronum sibiricum* Z-7981^T with a similarity of 98.1 %. Strain Su22^T differed from *A. sibiricum* Z-7981^T in its inability to use carbohydrates, peptone and amino acids as carbon sources. Strain Su22^T grew over a temperature range of 23–40 °C with an optimum at 30 °C and within the pH range 7.4–11.0 with an optimum at pH 9.6. Sodium cations stimulated the growth of the strain considerably with an optimal concentration at 0.76–1.09 M. The whole-cell fatty acid profile included $C_{17:1}\omega7c$, $C_{14:0}$ and $C_{16:0}$ ALDE. The G+C content was 46.1 mol%. Based on the DNA–DNA hybridization level (53.2 %) and phenotypical differences between strains Su22^T and Z-7981^T, the new isolate is thus considered to represent a novel species, for which the name *Anoxynatronum buryatiense* sp. nov. is proposed. The type strain is Su22^T (=VKM B-2510^T=CECT 8731^T).

Proteolytic anaerobic bacteria decompose biomass of primary producers and supply volatile fatty acids and ammonia to microbial community trophic chains. They are ubiquitous in harsh environments such as soda lakes. A number of different bacterial representatives, such as *Alkaliphilus* [1, 2], *Anaerovirgula* [3], *Anoxynatronum* [4], *Natranaerobius* [5], *Natronincola* [6, 7], *Spirochaeta* [8], *Anaerobranca* [9], *Tindallia* [10–12] and *Proteinivorax* [13], that are able to decompose proteins have been isolated from alkaline lakes of different locality. The majorities of these bacteria are alkaliphiles and belong to the order *Clostridiales*. Some of them, namely *Alkaliphilus peptidofermentans* and *Anoxynatronum sibiricum*, can grow on cyanobacterial biomass [2, 4]. Recently it has been conclusively demonstrated that the haloalkaliphilic bacterium *Proteinivorax tanatarense* in addition to cyanobacterial dead cells is able to use archaeal biomass as a carbon and energy source [14]. Photosynthesis and decomposition of the accumulated biomass of primary producers by alkaliphilic bacteria lead to the appearance of autochthonous organic matter and allow microbial communities of soda lakes to exist autonomously, carrying out the basic cycles of biogenic nutrients.

Strain Su22^T was a stable member of the sulfate-reducing enrichments from the bottom sediments of the alkaline low mineralization lake from which *Desulfonatronum lacustris* strain Su2 (=VKM B-2475^T) was isolated earlier [15]. Pure culture of Su22^T was obtained by serial dilutions using Hungate tubes with anaerobic medium AM1 containing (g l^{-1}): Na_2CO_3, 1.6; $NaHCO_3$, 0.6; KCl, 0.2; $MgCl_2\times6H_2O$, 0.1; NH_4Cl, 0.5; K_2HPO_4, 0.2; yeast extract, 2.0; $Na_2S\times9H_2O$, 0.25; trace element solution SL 10 (320 medium, DSMZ), 1.0 ml; vitamin solution (141 medium, DSMZ), 10.0 ml. Sterile sodium sulfide, carbonate and bicarbonate solutions were added to the medium before inoculations and pH was adjusted to 9.6. The culture purity was assessed by observing the uniform cell types under a phase-contrast microscope. The material for the examination were single colonies developed on the AM1 medium supplemented with Difco agar (15 g l^{-1}) under 100 % nitrogen atmosphere at 28–30 °C. Colonies appearing 2 weeks after plating were small, smooth, convex, glistening, non-pigmented and circular in shape with entire edges. *Anoxynatronum sibiricum* Z-7981^T=VKM B-2327^T [4] was used for comparative studies and grown in the AM1 medium amended with 5.0 g l^{-1} glucose at pH 9.0.

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Keywords: proteolytic bacteria; soda lake; alkaliphiles; *Anoxynatronum buryatiense*
Abbreviations: AHF, amorphous ferric hydroxide; ALDE, aldehyde; 10-OH, 10-hydroxy; OMe, methyl ester.
The GenBank accession number for the 16S rRNA gene sequence of strain Su22^T is EU315116.
Two supplementary tables and one supplementary figure are available with the online Supplementary Material.

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Methanosarcina gilichinskyana sp. nov., a novel methanogenic archaeon isolated from Holocene permafrost, North East Russia

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Abstract:	A mesophilic, non-motile, non-spore-forming, irregular coccoid methanogen, designated JL01T, was isolated from Holocene permafrost of the Kolyma lowland in the Russian North East Arctic. The cells were 1.0-1.5 μm in diameter, occurred in small aggregates and stained Gram-positive. Strain JL01T was a strict anaerobe and grew on methanol, acetate and methylamines as energy and carbon sources. Optimum conditions for growth were 24-28 oC, pH 6.8-7.3 and 0.075-0.1 M NaCl. The DNA G+C content was 39.2 mol%. On the basis of 16S rRNA gene sequence comparisons with known methanogens, strain JL01T was affiliated with the genus Methanosarcina and was most closely related to Methanosarcina mazei S-6T (similarity 99.5%) and Methanosarcina soligelidi SMA-21T (similarity 99.4%). However, no significant DNA-DNA relatedness was observed between strain JL01T and the type strain of M. mazei. We propose that strain JL01T (=VKM B-2370T=JCM 31898T) represents a novel species, with the name Methanosarcina gilichinskyana sp. nov.

Methanosarcina gilichinskyana sp. nov., a novel methanogenic archaeon isolated from Holocene permafrost, North East Russia

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Abbreviations: ME, Minimum Evolution; NJ, Neighbor-Joining; TGGE, temperature gradient gel electrophoresis

Nucleotide sequence accession number:

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *mcr*A gene sequences of strain JL01^T are AF519802 and KY368727, respectively.

The effect of the antibiotics on strains JL01^T and *M. mazei* S-6^T growth was determined by transferring 5-ml aliquots of cultures to the fresh MSG medium containing one of the following antibiotics: (l^{-1}) chloramphenicol (10 mg), bacitracin (10 mg), polymyxin (10 mg), vancomycin (2000 mg), erythromycin (1000 mg), kanamycin (2000 mg) and penicillin G (2000 mg). Tests were performed in duplicate with a non-antibiotic control for 1 week at the optimum temperature. Strains JL01^T and *M. mazei* S-6^T were sensitive to chloramphenicol and polymyxin. The addition of bacitracin slowed the growth of strain JL01^T but did not affect *M. mazei* S-6^T growth. Both strains were resistant to penicillin, vancomycin, erythromycin and kanamycin.

The G+C content of DNA and DNA-DNA relatedness tests were performed spectrophotometrically as described by De Ley *et al*. [34], and modified by Huß *et al*. [35]. A model spectrophotometer Pye Unicam SP 1800 equipped with a thermoprogrammer and hermetically sealed thermocuvettes was used. The G+C contents of DNA in strains JL01^T and *M. mazei* S-6^T were 39.2 and 42.3 mol%, respectively (mean±SD of 4 determinations). *M. barkeri* strain MS^T VKM B-1635^T was used as control. The results of DNA-DNA hybridization (3 replications) indicated only 26.2±2.7% relatedness (mean±SD of 3 determinations) between strains JL01^T and *M. mazei* S-6^T.

Thus, strain JL01^T differed from its closest relatives in cell morphology (absence of individual coccus forms and positive Gram-staining) and in physiological properties (Table 1). Unlike *M. soligelidi* SMA-21^T and *M. mazei* S-6^T, the new strain did not use H_2 and CO_2 as energy and carbon sources. Despite of high 16S rRNA gene sequence similarity between strain JL01^T and *M. mazei* S-6^T, the DNA-DNA hybridization indicated only 26% genomic relatedness. According to the minimal standards of a new methanogenic taxa description [23], and based on phylogenetic and phenotypic differences, a novel species of the genus *Methanosarcina*, *Methanosarcina gilichinskyana* sp. nov. is proposed with type strain JL01^T.

Description of *Methanosarcina gilichinskyana* sp. nov.

Methanosarcina gilichinskyana (gi.li.chin.sky.a'na. N.L. fem. adj. gilichinskyana, named after David Gilichinsky, the pioneer of permafrost microbiology studies).

The cells are Gram-positive non-motile non-spore-forming irregular cocci. Occur in aggregates and resistant to lysis by 1% SDS in distilled water. Strict anaerobe. Methane is produced from methanol, acetate and methylamines. H_2/CO_2, formate, 2-propanol, 2-butanol, butyrate, ethanol or propionate are not catabolized. Penicillin, vancomycin, erythromycin and kanamycin did not affect the growth.

Growth occurs at 10–37 °C (optimum, 24–28 °C), at pH 5.5–9.0 (optimum, pH 6.8–7.3), and in the presence of NaCl concentrations from 0.01 to 0.2 M (optimum, 0.075–0.1 M). The

DNA G+C content of the type strain is 39.2 mol%.

The type strain JL01^T (=VKM B-2370^T= JCM 31898^T) was isolated from Holocene permafrost of the Kolyma lowland, northeastern Russia. The GenBank accession numbers for the 16S rRNA and *mcr*A gene sequences of strain JL01^T are AF519802 and KY368727, respectively.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Rathayibacter oskolensis sp. nov., a novel actinobacterium from Androsace koso-poljanskii Ovcz. (Primulaceae) endemic to Central Russian Upland

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Abstract:	A rod-shaped, non-endospore-forming and non-motile bacterium, strain DL-329T, was isolated from the above-ground part a plant, Androsace koso-poljanskii Ovcz. (Primulaceae), the State Natural Reserve "Belogorie", Russia. On the basis of 16S rRNA gene sequence comparisons, the strain clustered with members of the genus Rathayibacter, showing the highest sequence similarity to R. tritici (98.89%), R. rathayi (98.82%), and R. festucae (98.82%). The DNA hybridization experiments demonstrated that strain DL-329T represents a separate genomic species. The results of comparative studies of physiological and chemotaxonomic characteristics, including cell-wall sugar patterns, polar lipid profiles, and the MALDI-TOF mass spectra of bacterial cells, allowed clear differentiation of VKM Ac-2121T from the recognized Rathayibacter species at the phenotypic level. Based on the data obtained, a new species, Rathayibacter oskolensis sp. nov., is proposed, with DL-329T (= VKM Ac-2121T = LMG 22542T) as the type strain.

Rathayibacter oskolensis sp. nov., a novel actinobacterium from *Androsace koso-poljanskii* Ovcz. (Primulaceae) endemic to Central Russian Upland

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Abbreviations:

ANI, average nucleotide identity; CB, corynebacterium medium; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; G, glycolipids; L, lipids; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; DDBJ, DNA Data Bank of Japan; ENA, European Nucleotide Archive; IMG/M, Joint Genome Institute Integrated Microbial Genomes & Microbiome Samples system; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; LMG, Laboratorium voor Microbiologie Universiteit Gent; VKM, All-Russian Collection of Microorganisms.

Footnotes:

The DDBJ/ENA/GenBank accession number for the 16S rRNA gene sequence of strain VKM Ac-2121^T is KX758084, and the genome sequence is available at DDBJ/EMBL/GenBank under the accession number FXBM00000000.

Three supplementary figures are available with the online version of this paper.

not observed. In older (5–7-day) cultures, coccoid and coccobacillar forms (0.6–0.8 μm in diameter) predominant; occur singly or in pairs, short chains or clumps. A marked life cycle could be observed on CB agar and on brain heart infusion agar (Conda, Spain). Colonies grown on R2A and CB agar are yellow-pigmented, circular, slightly convex, entire, opaque and butyrous.

Aerobic. Catalase-positive. Oxidase test reaction with tetramethyl-*p*-phenylenediamine is negative or weakly positive, depending on growth condition. Mesophilic; optimum growth temperature is 24–28ºC, no growth at 7 or 37ºC. L-arabinose, cellobiose, dulcitol, D-fructose, D-galactose, D-glucose, *meso*-inositol, inuline, lactose, lyxose, maltose, mannitol, mannose, melibiose, L-rhamnose, raffinose, salicin, sorbitol, sucrose, trehalose, turanose, D-xylose are used as carbon sources for growth in a mineral salt medium supplemented with 0.1% (w/v) of yeast extract. Adonitol, dextran, *meso*-erytritol, ribose and sorbose are not used as C-source in the same medium. Tween 40, Tween 60, Tween 80, starch, hypoxanthine, and xanthine are not hydrolyzed. H_2S is not produced from peptone. Susceptible to 2% (w/v) of NaCl. Growth occurs in the presence (10 μg ml^{-1}) of ampicillin, gentamicin, rubomicin, and streptomycin; growth is inhibited by levomycetin, metaciclin, and rifampicin in the same concentration.

The peptidoglycan is of the B group based on 2,4-diaminobutyric acid. The cell-wall sugar pattern includes glucose, mannose, rhamnose, galactose, and xylose. The major menaquinone is MK-10, with minor amount of MK-9. The predominant cellular fatty acids are represented by anteiso-15:0, anteiso-17:0 and iso-16:0. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and unidentified glycolipid, along with moderate or minor amounts of other unidentified components (two phospholipids, a few glycolipids and a lipid detectable only after staining for total lipids). The MALDI mass-spectrum of the type strain includes the following unique peaks (m/z): 2659, 3941, 4083, 4373, 4647, 4687, 4824, 6046, 6108, 6169, 7458. The genome size for the type strain is 3.95 Mbp. G+C content of DNA is 71.6 mol%.

The type strain is DL-329^T (= VKM Ac-2121^T = LMG 22542^T) was isolated from the above-ground part of a plant, *Androsace koso-poljanskii* Ovcz., growing on chalky soils, the State Natural Reserve “Belogorie”, Russia.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.