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Antiquaquibacter oligotrophicus gen. nov., sp. nov., a novel oligotrophic bacterium from groundwater

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Abstract

In this study, a Gram-stain-positive, non-motile, oxidase- and catalase-negative, rod-shaped, bacterial strain (SG_E_30_P1^T) that formed light yellow colonies was isolated from a groundwater sample of Sztaravoda spring, Hungary. Based on 16S rRNA phylogenetic and phylogenomic analyses, the strain was found to form a distinct linage within the family *Microbacteriaceae*. Its closest relatives in terms of near full-length 16S rRNA gene sequences are *Salinibacterium hongtaonis* MH299814 (97.72% sequence similarity) and *Leifsonia psychrotolerans* GQ406810 (97.57%). The novel strain grows optimally at 20–28°C, at neutral pH and in the presence of NaCl (1–2 w/v%). Strain SG_E_30_P1^T contains MK-7 and B-type peptidoglycan with diaminobutyrate as the diagnostic amino acid. The major cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{14:0}$, and the polar lipid profile is composed of diphosphatidylglycerol and phosphatidylglycerol, as well as an unidentified aminoglycolipid, aminophospholipid and some unidentified phospholipids. The assembled draft genome is a contig with a total length of 2897968 bp and a DNA G+C content of 65.5 mol%. Amino acid identity values with it closest relatives with sequenced genomes of <62.54 %, as well as other genome distance results, indicate that this bacterium represents a novel genus within the family *Microbacteriaceae*. We suggest that SG_E_30_P1^T (=DSM 111415^T=NCAIM B.02656^T) represents the type strain of a novel genus and species for which the name *Antiquaquibacter oligotrophicus* gen. nov., sp. nov. is proposed.

INTRODUCTION

The family *Microbacteriaceae*, belonging to the order *Microbacteriales*, class *Actinomycetes* within the phylum *Actinomycetota*, contains 69 genera at the time of writing and two *Candidatus* genera (https://lpsn.dsmz.de/family/microbacteriaceae). The members of this family are chemoorganotrophic, and their metabolism is primarily respiratory with oxygen as the terminal electron acceptor. They are aerobic, microaerophilic or facultative anaerobic [1]. The natural habitats of the members of this family are various terrestrial and aquatic environments. Some species are associated with plants [2], animals [3], algae [4] and fungi [5], as well as clinical specimens [1]. In this study, a novel genus of this family isolated from a groundwater sample in Hungary is described based on a polyphasic approach.

METHODS

Strain SG_E_30_P1^T was isolated from groundwater sampled 206 m above sea level at Sztaravoda (47.698221° N 19.047062° E), Hungary. The spring is located within the Dunazug mountains, where the water comes from a confined aquifer formed by agglomerates of andesite. The water sample was aseptically collected from the spring's outflow into a sterile, glass bottle according

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Keywords: *Antiquaquibacter oligotrophicus*; *Microbacteriaceae*; polyphasic description.

Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; GBDP, genome BLAST distance phylogeny.

The accession number for the 16S rRNA gene sequence of strain SG_E_30_P1^T is OQ835024. The whole genome shotgun project has been deposited at NCBI under the accession number CP085036.1.

Two supplementary figures and one supplementary table are available with the online version of this article.

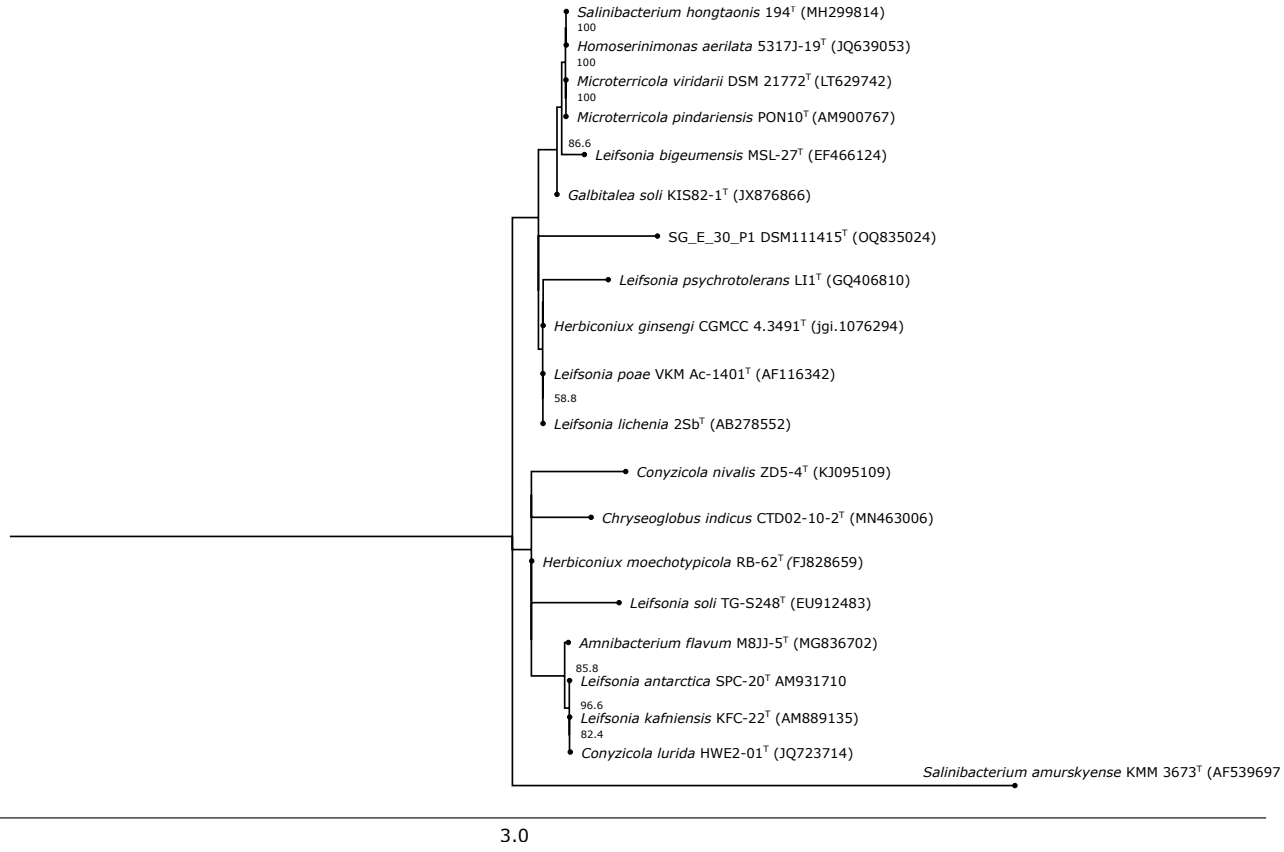

Fig. 1. Maximum-likelihood phylogenetic tree based on complete 16S rRNA gene sequences showing the phylogenetic position of strain SG_E_30_P1^T with closely related taxa. The outgroup was *Escherichia coli* (MG1655; not shown). Numbers at nodes indicate the percentage of 1000 bootstrap replicates.

to ISO 19458:2006 standards and transferred to 4°C on 6 September 2018. The temperature of the water was 8.6°C, its pH was 8.09, and the conductivity was 454 µS cm^{-1}.

To isolate the bacterial strain SG_E_30_P1^T, a new medium (named M5) was developed using 0.05 g l^{-1} yeast extract, 0.05 g l^{-1} proteose peptone, 0.05 g l^{-1} casamino acids, 0.05 g l^{-1} glucose, 0.05 g l^{-1} soluble starch, 0.03 g l^{-1} sodium pyruvate, 0.03 g l^{-1} K_2HPO_4 and 0.005 g l^{-1} $MgSO_4{\cdot}7H_2O$, adding 15 ml l^{-1} growth factor solution (composition, 0.5 g sodium acetate, 0.5 g sodium formiate, 0.5 g sodium succinate, 0.5 g L-D-glucosamine and 0.5 ml glycerin, dissolved in 100 ml distilled water) and 15 ml l^{-1} trace element solution (2 g $FeSO_4{\cdot}7H_2O$, 0.03 g H_3BO_3, 0.1 g $MnCl_2{\cdot}4H_2O$, 0.19 g $CoCl_2{\cdot}6H_2O$, 0.024 g $NiCl_2{\cdot}6H_2O$, 0.002 g $CaCl_2{\cdot}2H_2O$, 0.144 g $ZnSO_4{\cdot}7H_2O$, 0.036 g $Na_2MoO_4{\cdot}2H_2O$ and 5.2 g EDTA dissolved in 1 l distilled water. pH was adjusted to pH 7.0–7.2 and tap water was added to the medium until the final volume of 1 l was reached, and then finally autoclaved at 121°C for 20 min. The media were solidified with either agar or gellan gum, respectively [6]. Isolation happened randomly after enrichment of 50 ml water sample in 250 ml of M5 media for 2 weeks using polyurethane foam-based traps [7]. Plates were incubated (9 days at 25°C). Isolates from the different samples were purified and grouped based on their MALDI-TOF profile [8], and the group representatives and ungrouped bacterial strains were subjected to 16S rRNA gene sequencing. As the strain was found to grow on Reasoner's 2A (R2A) medium [9] as well, it was routinely maintained on this medium containing 1.5% agar (pH 7) at 23°C by subculturing every month to a freshly prepared medium.

In order to observe the colony morphology, incubation at 28°C for 3 days was performed on R2A agar medium [9]. Single cells were observed in native preparations and after Gram-staining using light microscopy [10] and by transmission electron microscopy (7100 Hitachi) [11].

Growth of strain SG_E_30_P1^T was assessed on R2A agar slants (pH 7) at different temperatures (4, 7, 20, 35, 40 and 45°C). Tolerance to NaCl and pH was assessed by using R2A broth (pH 7) at different concentrations (0, 1, 2, 3, 4 and 5% NaCl) and at different pH values (pH 3, 4, 5, 6, 7, 8, 9, 10 and 11), respectively. Catalase and oxidase activity, oxidative and fermentative degradation of glucose, indole production, casease, urease, gelatinase, DN-ase and phosphatase activities, hydrolysis of starch

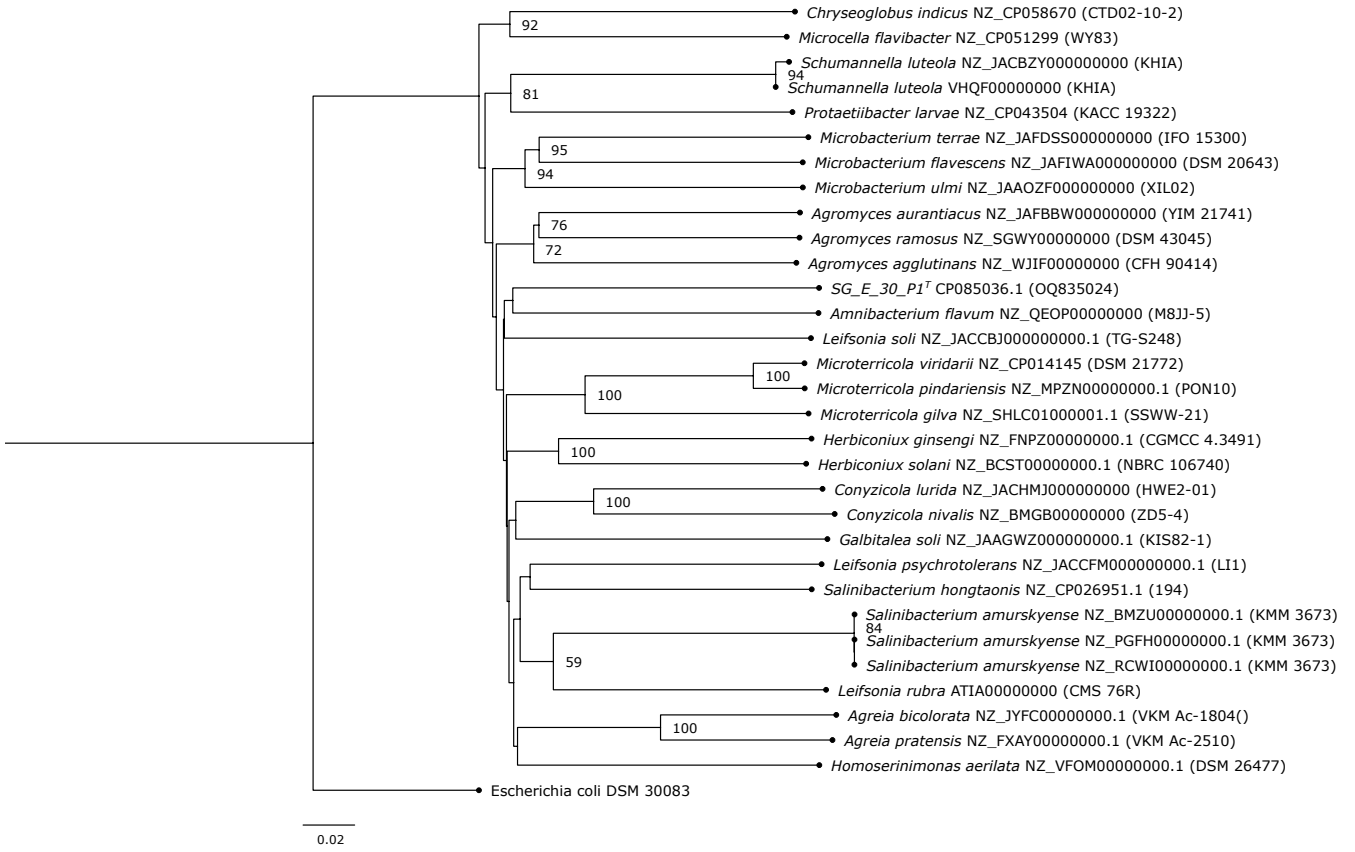

Fig. 2. Balanced minimum-evolution tree of SG_E_30_P^T and type strains of closely related taxa based on their genomic sequences. The tree was reconstructed based on data from the Type (Strain) Genome Server. The branch lengths are scaled in terms of GBDP distance formula d_5. The outgroup was *Escherichia coli* MG1655 (data not shown). The numbers above branches are GBDP pseudo-bootstrap values. Values under 50 are not given. Bar, 0.02 substitutions per site.

and Tween 80, production of H_2S from peptone, NO_3^- reduction to NO_2, N_2 and NH_3 were checked following the protocols of Tóth *et al.* [12]. Additional physiological and biochemical tests were performed using API ZYM and AP 50CH kits (bioMérieux) according to the manufacturer's instructions. Chemotaxonomic analyses for peptidoglycan, isoprenoid quinones, polar lipids and fatty acids were performed following the protocols of Tóth *et al.* [13].

Biomass for the analysis of isoprenoid quinones and polar lipids was obtained by cultivation of SG_E_30_P1^T in R2A broth at 28°C for 2 days. Isoprenoid quinones were extracted following the protocol of Collins *et al.* [14], and further analyses were performed using HPLC (Shimadzu LC 20A) and electron-impact mass spectrometry (Singlequad 320) [15].

To perform the cellular fatty acids analyses, bacterial cells were cultivated for a total period of 24 h on R2A agar at 28°C. Fatty acid methyl esters were obtained following the protocol of Stead *et al.*[16], and samples were analysed using an Agilent 6850 chromatograph with the MIDI Microbial Identification System (library TSBA40, 4.10; Sherlock software package, version 6.1). Summed feature components were identified thereafter by GC/MS using a Singlequad 320 instrument (Varian).

Polar lipids were obtained following the protocol of Minnikin *et al.* [17] and separated by two-dimensional TLC to identify spots. Ninhydrin and Zinzadze reagents and molybdophosphoric acid were used following Rathsack *et al.* [18] (Fig. S2).

Genomic DNA was isolated following the protocol of Tóth *et al.* [13]. The 16S rRNA gene was amplified by PCR following the method of Tóth *et al.* [19]. Purification and Sanger sequencing of PCR products were performed by LGC Genomics (Berlin, Germany). The nearly full-length sequence of the 16S rRNA gene (1416 bp) was assembled using MEGA 10.0.5 software [20] and then aligned against the available sequences in the EzBioCloud database [21] using the SILVA project algorithm [22]. After the alignment was inspected, phylogenetic analysis was conducted using MEGA 10.0.5 software. Evolutionary distances were calculated using Kimura's two-parameter model [23]. Phylogenetic trees were reconstructed using the maximum-likelihood method [24].

Whole-genome sequencing of strain SG_E_30_P1^T was performed on a NovaSeq S4 platform (Illumina) using patterned flow cells and a reformulated NextSeq 2-colour SBS chemistry. The sequencing was performed at the US Department of Energy Joint

Table 1. Average amino acid identity (AAI), digital DNA–DNA hybridization (dDDH) and DNA G+C content values of the genome sequence of SG_E_30_P1^T and the reference genomes of its closest relatives

Strain	AAI (%)	dDDH (%)	DNA G+C content difference (mol%)	G+C content (mol%)
Amnibacterium flavum (MG836702)	60.2	20.2	3.09	68.6
Conyzicola nivalis (KJ095109)	64.6	19.3	2.62	68.2
Herbiconiux ginsengi (jgi.1076294)	60.6	20.1	2.88	68.4
Microterricola pindariensis (AM900767)	60.4	20.2	4.23	69.8
Salinibacterium hongtaonis (MH299814)	62.5	20.2	1.32	64.1
Leifsonia psychrotolerans (GQ406810)	59.2	19.9	1.31	64.2
Galbitalea soli (JX876866)	63.6	19	3.64	69.2

Genome Institute. Sequence read quality was checked by FastQC [25], and *de novo* assembly of raw reads was performed using the SPAdes 3.15.2 tool in careful mode [26]. The assembly and coverage depth qualities were evaluated by QUAST 5.1.0. Contigs with fewer than 500 nt were removed from the assembly. Analysis of the draft genome was done using RAST server version 2.0 [27].

A balanced minimum-evolution tree of SG_E_30_P^T and type strains of closely related taxa was reconstructed based on data from the Type (Strain) Genome Server (TYGS). The tree was created based on the intergenomic distances that were calculated from the genome BLAST distance phylogeny analysis (GBDP) with 100 pseudo bootstrap replicates using FastMe 2.0 with a BioNJ starting tree [28].

The 16S rRNA gene was obtained by PCR and Sanger sequencing. It was compared to the 16S rRNA gene from the genome sequence to ensure its authenticity and purity. In order to calculate the balanced minimum-evolution tree, closely related type strains were identified, and their whole genome sequences are obtained from TYGS [29].

RESULTS

The 16S rRNA gene sequence analysis of SG_E_30_P1^T indicated that the isolated bacterium was a member of the family *Microbacteriaceae*. The closest relative in terms of sequence similarity was *Salinibacterium hongtaonis* (accession no. MH299814; 97.77%), followed by *Leifsonia psychrotolerans* (accession no. GQ406810; 97.57%), *Herbiconiux ginsengi* (accession no. jgi.1076294; 97.48%), *Leifsonia bigeumensis* (accession no. EF466124; 97.48%) and *Leifsonia kafniensis* (accession no. AM889135; 97.48%).

Phylogenetic analysis of the 16S rRNA gene sequence of the novel strain grouped SG_E_30_P1^T together with the type species of two genera, *Galbitalea soli* (accession no. JX876866; 97.04% similarity) and *Amnibacterium flavum* (accession no. MG836702; (96.82% similarity) in a separate clade (Figs 1 and S1).

Whole-genome sequencing of strain SG_E_30_P1^T resulted in 433× genome coverage. The final genome assembly was 2895655 nt in length with one contig. The G+C content was 65.55 mol%. The N50 value was 2895552. There were 45 tRNA and three rRNA genes. The total CDS value was 2820. The sequence of the PCR product amplified from the 16S rRNA gene was identical to that obtained from the genome assembly, which verified the purity and identity of the final genome sequence. Raw sequence reads are deposited in the NCBI Sequence Read Archive and can be accessed through the BioProject ID PRJNA762240. The whole-genome sequence data was deposited into the GenBank database and is accessible under accession number CP085036.1. In order to calculate the balanced minimum-evolution tree, closely related type strains were identified, and their whole genome sequences are obtained from TYGS [29]. The phylogenomic tree analyses placed SG_E_30_P1^T within the family *Microbacteriaceae* (Fig. 2), where the novel genus was grouped with *Amnibacterium flavum* MG836702 and separated from all the other relatives.

The average average amino acid identity (AAI) and digital DNA–DNA hybridization (dDDH) values were determined between the genome sequences of SG_E_30_P1^T and those of the close relatives: *Leifsonia psychrotolerans* (GQ406810), *Galbitalea soli* (JX876866), *Amnibacterium flavum* (MG836702), *Conyzicola nivalis* (KJ095109), *Herbiconiux ginsengi* (jgi.1076294), *Microterricola pindariensis* (AM900767) and *Salinibacterium hongtaonis* (MH299814) [21, 30, 31]. The results are shown in Table 1. AAI and dDDH values [32] between strain SG_E_30_P1^T and its relatives were below the genus delineation thresholds. Accordingly, we propose that SG_E_30_P1^T represents a member of a new genus within the family *Microbacteriaceae*.

Analysis of the SG_E_30_P1^T genome through the RAST server 2.0 showed the presence of genes relating to resistance to different antibiotics and toxic compounds (copper homeostasis, cobalt-zinc-cadmium resistance, fluoroquinolone resistance and mercury reductase). Genes encoding for toxin-antitoxin systems were also found. The genome also contained ammonia, sulphur and phosphorus metabolism/assimilation genes. Moreover, genes involved in vitamin biosynthesis were present (thiamine, menaquinone,

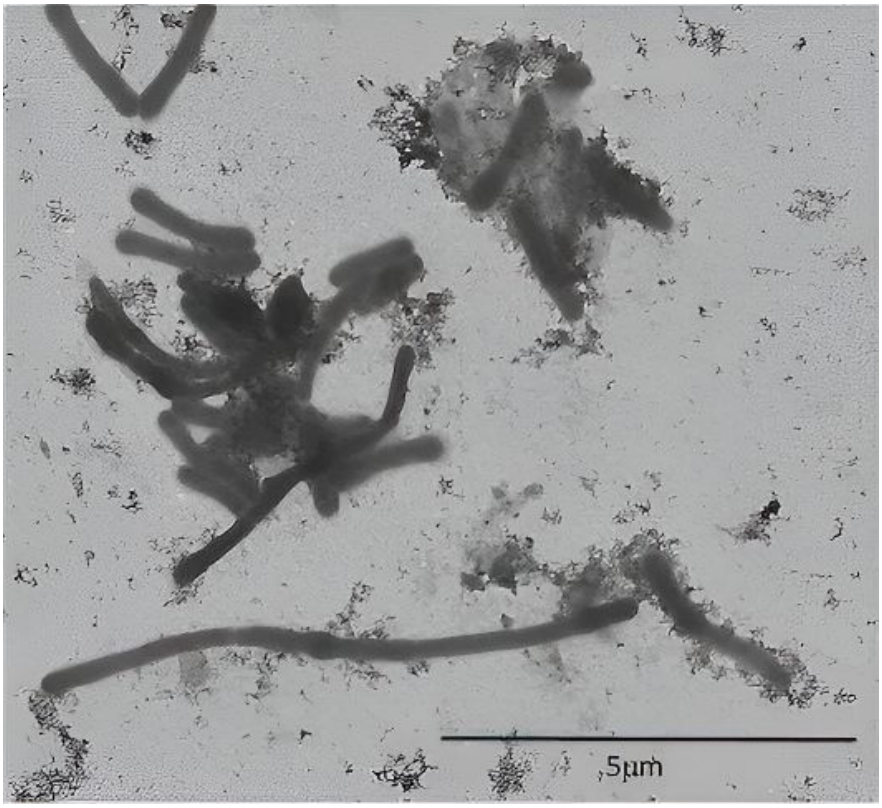

Fig. 3. Transmission electron microscope image of *Antiquaquibacter oligotrophicus* SG_E_30_P1^T

riboflavin and pyridoxin). Genes involved in the metabolism of aromatic and central aromatic intermediates were also present, among them those for salicylate and gentisate catabolism.

Based on the 16S rRNA gene sequence similarities observed in the phylogenetic tree, *Galbitalea soli* (JX876866) and *Leifsonia psychrotolerans* (GQ406810) were chosen as controls for the direct comparison of phenotypes. The cells of the novel bacterium were non-motile and stained Gram-positive. Flagellation and endospores were not observed. The cell shape varied from single rods to elongated forms (2–7µm) (Fig. 3). On R2A agar medium light-yellow pigment production was observed.

Phenotypic differences between SG_E_30_P1^T and its closest relatives are shown in Table 2.

SG_E_30_P1^T contained MK-7 as its sole respiratory quinone. Peptidoglycan structural analysis revealed it to be B type with a peptide sidechain of DAB:D-/L-Ala:L-DAB:D-Glu at a ratio of 1.6 Ala:1.0 Gly:1.0 Glu:0.6 DAB. The phospholipid profile of SG_E_30_P1^T differed from those of its closest relatives due to the presence of aminoglycolipid (AGL), aminophospholipid (APL) and phospholipid (PL). Diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) were detected in SG_E_30_P1^T and its closest relatives. The fatty acid profile showed the common presence of anteiso-$C_{15:0}$ (Table 2 and Fig. S1) in SG_E_30_P1^T and *Galbitalea soli* JX876866; however, some differences in the amounts of the components were present.

Based on the phenotypic data presented in Table 2, SG_E_30_P1^T is characterized by its inability to tolerate temperatures higher than 28°C. Features that differentiate this strain from its closest relatives include its inability to utilize valine arylamidase, β-galactosidase and α-glucosidase. The short to elongated rod shape of strain SG_E_30_P1^T further distinguishes it from its closest relatives.

Based on the phenotypic, chemotaxonomic, phylogenetic and phylogenomic data presented here, strain SG_E_30_P1^T represents a novel genus and species within the family *Microbacteriaceae*, for which the name *Antiquaquibacter oligotrophicus* gen. nov. is proposed.

DESCRIPTION OF *ANTIQUAQUIBACTER* GEN. NOV.

Antiquaquibacter (An.tiqu.a.qui.bac'ter. L. masc. adj. *antiquus*, ancient; L. fem. n. *aqua*, water; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Antiquaquibacter*, a rod from ancient water).

Table 2. Differential characteristics of strain SG_E_30_P1^T and its most closely related taxa

Strains: 1, SG_E_30_P1^T; 2, *Leifsonia psychrotolerans* GQ406810; 3, *Galbitalea soli* JX876866; 4, *Amnibacterium flavum* MG836702; 5, *Conyzicola nivalis* KJ095109; 6, *Salinibacterium hongtaonis* MH299814. Data presented here are based on analyses performed during this study, except of where indicated. +, Positive; –, negative; w+, weak positive reaction; ND, no data.

Characteristic	1	2	3	4‡	5§	6#
Isolation source	Groundwater	Soil*	Soil†	Surface sterilized bark of *Neriumindicum* Mill	Snow of the Zadang Glacier	Faeces of Tibetan antelopes
Colony pigmentation	Light yellow	Yellow	Light yellow	Vivid greenish yellow	Translucent yellow	Pale yellow
Shape	Short to elongated rod	Regular rod- or filament-shaped	Rod	Circular	Rod	Circular
Motility	Non-motile	Motile	Motile	ND	Non-motile	Non-motile
Temperature range for growth (°C)	4–28	4–35	4–45	4–37	0–40	4–30
Optimum temperature for growth (°C)	20–28	20–28	7–28	28	14	28
pH range for growth	6–8	5–7	6–10	6–9	6–10	4–10
Optimum pH for growth	7	6–7	8	7–8	6–8	7
NaCl tolerance (w/v%)						
NaCl tolerance range for growth (%, w/v)	0–2	0–1	0–2	0–4	0–6	0–10
Optimum NaCl growth (%, w/v)	1–2	1	0–1	0	0–2	0–1
Utilization as sole carbon source:						
L-Arabinose	–	+	–	–	+	+
Maltose		–	–	–	+	+
D-Ribose	–	+	–	w+	–	–
D-Xylose	–	+	–	w+	+	+
L-Rhamnose	–	+	–	–	+	–
Aesculin	–	–	+	+	+	+
Salicin	–	–	+	–	ND	–
Potassium 5-ketogluconate	+	–	+	–	ND	+
D-Fructose	–	–	–	–	+	+
D-Glucose	–	–	–	–	+	–
Mannose	–	–	–	–	+	–
Mannitol	–	–	–	–	+	+
Galactose	–	–	–	–	+	–

Continued

Table 2. Continued

Characteristic	1	2	3	4‡	5§	6#
Enzyme activities:						
Esterase (C4)	−	+	−	+	+	−
Valine arylamidase	−	+	+	+	+	+
Cystine arylamidase	−	+	−	+	+	+
Acid phosphatase	−	+	−	+	ND	−
β-Galactosidase	−	+	+	+	ND	+
α-Glucosidase	−	+	+	+	ND	+
β-Glucosidase	−	+	−	+	ND	+
Lipase	−	−	−	+	+	+
Naphthol-AS-BI-phosphohydrolase	−	−	−	+	−	−
Leucine	−	−	−	+	+	+
Arylamidase	−	−	−	+	+	+
Hydrolysis enzymes:						
Catalase	−	+	−	+	+	+
Casease	−	+	+	−	ND	ND
Phosphatase	−	−	+	+	+	−
Gelatinase	−	+	+	−	−	ND
Urease	−	−	−	−	−	ND
Nitrate reduction:						
NO_2	+	+	−	−	ND	−
Major fatty acids	anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$	iso-$C_{16:0}$, anteiso-$C_{15:0}$, anteiso -$C_{17:0}$	anteiso-$C_{15:0}$, iso-$C_{16:0}$	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$
Major polar lipids**	DPG, AGL, APL, PG, PL	PG, DPG*	DPG, PG, GL†	DPG, PG	DPG, PG	DPG, PG
DNA G+C content (mol%)	65.55	64.50*	69.19†	68.60	67.20	64.10

*Data from Ganzert *et al.* [33].
†Data from Kim *et al.* [34].
‡Data from Tuo *et al.* [35].
§Data from Gu *et al.* [36].
#Data from Li *et al.* [37].
**AGL, aminoglycolipid; APL, aminophospholipid; DPG, diphosphatidylglycerol; GL, glycolipid; PG, phosphatidylglycerol; PL, phospholipid.

Cells are Gram-stain-positive, elongated, non-motile, oxidase-negative and catalase-negative. The cells contain MK-7. The predominant fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{14:0}$. The major polar lipids are DPG, AGL, APL, PG and PL.

Based on of the results of phylogenetic analysis the genus is part of the family *Microbacteriaceae* within the phylum *Actinomycetota*. The type species is *Antiquaquibacter oligotrophicus*.

DESCRIPTION OF *ANTIQUAQUIBACTER OLIGOTROPHICUS* SP. NOV.

Antiquaquibacter oligotrophicus (o.li.go.tro'phi.cus. Gr. masc. adj. *oligos*, few; Gr. masc. adj. *trophikos*, nursing, ending or feeding; N.L. masc. adj. *oligotrophicus*, oligotrophic, referring to the low nutrient content of the isolation site).

Possesses the following properties in addition to those given for the genus: grows well on R2A agar and M5 media [6]. Colonies on R2A agar are small, circular and light yellow in colour. Cells are non-motile and elongated. Growth occurs at 4–28°C (optimum, 20–28°C), pH 6–8 (optimum, pH 7) and with NaCl concentrations of 0–2%. Positive for NO_3^- reduction and negative for casease, phosphatase, gelatinase and urease, as well as starch, indole and H_2S production. Negative for acid production from any carbon sources in the API 50CH system except potassium 5-ketogluconate. In the API ZYM kit, positive results for esterase lipase (C 8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase.

The type strain, SG_E_30_P1^T (=DSM 111415^T=NCAIM B.02656^T), was isolated from a water sample collected from Sztaravoda groundwater in Hungary. The DNA G+C content of the type strain is 65.5mol% (calculated from the genome sequence). The GenBank accession number for the 16S rRNA gene sequence is OQ835024. The whole genome shotgun project has been deposited in the NCBI database under the accession number SAMN21381085 and under Bioproject number PRJNA762240.

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Author contributions
M.T.: writing the manuscript, sampling, cultivation and data analysis. W.B.W., N.C.K. and T.W.: whole genome sequencing. J.W. and M.N.-S.: chemotaxonomic analysis. G.A.: bioinformatics for genome sequencing. B.K.: electron microscopic investigations. E.T.: conception, phenotypic analysis and taxonomic supervision.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Evtushenko LI. Microbacteriaceae. In: *Bergey's Manual of Systematic of Archaea and Bacteria*. 2015. pp. 1–14.
2. Behrendt U, Ulrich A, Schumann P, Naumann D, Suzuki KI. Diversity of grass-associated *Microbacteriaceae* isolated from the phyllosphere and litter layer after mulching the sward; polyphasic characterization of *Subtercola pratensis* sp. nov., *Curtobacterium herbarum* sp. nov. and *Plantibacter flavus* gen. nov., sp. nov. *Int J Syst Evol Microbiol* 2002;52:1441–1454.
3. Kämpfer P, Rainey FA, Andersson MA, Nurmiaho Lassila EL, Ulrych U, *et al*. *Frigoribacterium faeni* gen. nov., sp. nov., a novel psychrophilic genus of the family *Microbacteriaceae*. *Int J Syst Evol Microbiol* 2000;50 Pt 1:355–363.
4. Alvarado P, Huang Y, Wang J, Garrido I, Leiva S. Phylogeny and bioactivity of epiphytic Gram-positive bacteria isolated from three co-occurring antarctic macroalgae. *Antonie van Leeuwenhoek* 2018;111:1543–1555.
5. Cardinale M, Grube M, Berg G. *Frondihabitans cladoniiphilus* sp. nov., an actinobacterium of the family Microbacteriaceae isolated from lichen, and emended description of the genus *Frondihabitans*. *Int J Syst Evol Microbiol* 2011;61:3033–3038.
6. Toumi M, Abbaszade G, Sbaoui Y, Farkas R, Ács É, *et al*. Cultivation and molecular studies to reveal the microbial communities of groundwaters discharge located in Hungary. *Water* 2021;13:1533.
7. Kalwasińska A, Felfoldi T, Walczak M, Kosobucki P. Physiology and molecular phylogeny of bacteria isolated from alkaline distillery lime. *Pol J Microbiol* 2015;64:369–377.
8. Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, *et al*. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin Biochem* 2011;44:104–109.
9. Reasoner DJ, Geldreich EE. A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* 1985;49:1–7.
10. Thorn K. A quick guide to light microscopy in cell biology. *Mol Biol Cell* 2016;27:219–222.
11. Golding CG, Lamboo LL, Beniac DR, Booth TF. The scanning electron microscope in microbiology and diagnosis of infectious disease. *Sci Rep* 2016;6:26516.
12. Tóth EM, Schumann P, Borsodi AK, Kéki Z, Kovács AL, *et al*. *Wohlfahrtiimonas chitiniclastica* gen. nov., sp. nov., a new gammaproteobacterium isolated from *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). *Int J Syst Evol Microbiol* 2008;58:976–981.
13. Tóth EM, Vengring A, Homonnay ZG, Kéki Z, Spröer C, *et al*. *Phreatobacter oligotrophus* gen. nov., sp. nov., an alphaproteobacterium isolated from ultrapure water of the water purification system of a power plant. *Int J Syst Evol Microbiol* 2014;64:839–845.

14. Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
15. Groth I, Schumann P, Rainey FA, Martin K, Schuetze B, *et al*. *Demetria terragena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* 1997;47:1129–1133.
16. Stead DE, Sellwood JE, Wilson J, Viney I. Evaluation of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. *J Appl Bacteriol* 1992;72:315–321.
17. Minnikin DE, Collins MD, Goodfellow M. Fatty acid and polar lipid composition in the classification of *Cellulomonas, Oerskovia* and related taxa. *J Appl Bacteriol* 1979;47:87–95.
18. Rathsack K. Reclassification of *Aurantimonas altamirensis* (Jurado et al. 2006), *Aurantimonas ureilytica* (Weon et al. 2007) and *Aurantimonas frigidaquae* (Kim et al. 2008) as members of a new genus, *Aureimonas* gen. nov., as *Aureimonas altamirensis* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2006;61:2722–2728.
19. Tóth E, Szuróczki S, Kéki Z, Kosztik J, Makk J, *et al*. *Brevundimonas balnearis* sp. nov., isolated from the well water of a thermal bath. *Int J Syst Evol Microbiol* 2017;67:1033–1038.
20. Stecher G, Tamura K, Kumar S. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Mol Biol Evol* 2020;37:1237–1239.
21. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
22. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, *et al*. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;41:D590–6.
23. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
24. Gascuel O, Steel M. Neighbor-joining revealed. *Mol Biol Evol* 2006;23:1997–2000.
25. Leggett RM, Ramirez-Gonzalez RH, Clavijo BJ, Waite D, Davey RP. Sequencing quality assessment tools to enable data-driven informatics for high throughput genomics. *Front Genet* 2013;4:288.
26. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
27. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, *et al*. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:1–15.
28. Lefort V, Desper R, Gascuel O. FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. *Mol Biol Evol* 2015;32:2798–2800.
29. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182.
30. Rodriguez-R LM, Konstantinidis KT. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ Preprints* 2016. DOI: 10.7287/peerj.preprints.1900v1.
31. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
32. Luo C, Rodriguez-R LM, Konstantinidis KT. MyTaxa: an advanced taxonomic classifier for genomic and metagenomic sequences. *Nucleic Acids Res* 2014;42:1–12.
33. Ganzert L, Bajerski F, Mangelsdorf K, Lipski A, Wagner D. *Leifsonia psychrotolerans* sp. nov., a psychrotolerant species of the family Microbacteriaceae from Livingston Island, Antarctica. *Int J Syst Evol Microbiol* 2011;61:1938–1943.
34. Kim S-J, Lim J-M, Ahn J-H, Weon H-Y, Hamada M, *et al*. Description of *Galbitalea soli* gen. nov., sp. nov., and *Frondihabitans sucicola* sp. nov. *Int J Syst Evol Microbiol* 2014;64:572–578.
35. Tuo L, Yan XR, Li FN, Yang C, An MB, *et al*. *Amnibacterium flavum* sp. nov., a novel endophytic *actinobacterium* isolated from bark of *Nerium indicum* Mill. *Int J Syst Evol Microbiol* 2019;69:285–290.
36. Gu Z, Liu Y, Xu B, Wang N, Shen L, *et al*. Description of *Conyzicola nivalis* sp. nov., isolated from glacial snow, and emended description of the genus *Conyzicola* and *Conyzicola lurida*. *Int J Syst Evol Microbiol* 2017;67:2818–2822.
37. Li J, Lu S, Jin D, Yang J, Lai X-H, *et al*. *Salinibacterium hongtaonis* sp. nov., isolated from faeces of Tibetan antelope (*Pantholops hodgsonii*) on the Qinghai-Tibet Plateau. *Int J Syst Evol Microbiol* 2019;69:1093–1098.