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TAXONOMIC DESCRIPTION

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Edaphobacter lichenicola sp. nov., a member of the family *Acidobacteriaceae* from lichen-dominated forested tundra

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Abstract

An isolate of aerobic, Gram-stain-negative, rod-shaped, non-motile and light-pink pigmented bacteria, designated SBC68^T, was obtained from slightly decomposed thalli of the lichen *Cladonia* sp. collected from the forested tundra of north-western Siberia. Cells of this isolate occurred singly, in pairs or in rosettes. These bacteria were acidophilic (optimum growth at pH 4.3–5.6) and mesophilic (optimum growth at 20–30 °C) but were also capable of growth at low temperatures, down to 7 °C. The preferred growth substrates were sugars, some organic acids and lichenan. The major fatty acids were iso-$C_{15:0}$, $C_{16:1}$ $\omega7c$, $C_{16:0}$, $C_{16:1}\omega7t$, and 13,16-dimethyl octacosanedioic acid. The only quinone was MK-8, and the G+C content of the DNA was 54.7 mol%. SBC68^T represented a member of the family *Acidobactericeae*; the closest taxonomically described relatives were *Edaphobacter dinghuensis* DHF9^T and *Granulicella aggregans* TPB6028^T (97.2 and 97.1 % 16S rRNA gene sequence similarity, respectively). In 16S rRNA gene-based trees, SBC68^T clustered together with species of the genus *Edaphobacter*. However, this isolate differed from all previously described species of the genus *Edaphobacter* with respect to the pink pigmentation, formation of cell rosettes and substrate utilization pattern. On the basis of these data, strain SBC68^T should be considered to represent a novel species of acidobacteria, for which the name *Edaphobacter lichenicola* sp. nov. is proposed. The type strain is SBC68^T (=DSM 104462^T=VKM B-3208^T).

INTRODUCTION

The genus *Edaphobacter* is part of the family *Acidobacteriaceae* of the phylum *Acidobacteria*. This genus accommodates mildly acidophilic, mesophilic, strictly aerobic chemoheterotrophs that multiply by binary fission. At present, it includes four species with validly published names, i.e. *Edaphobacter modestus*, *Edaphobacter aggregans* [1, 2], *Edaphobacter dinghuensis* [3] and *Edaphobacter acidisoli* [4]. These species are represented by Gram-negative, short, non-spore-forming, beige, ovoid rods that do not form a capsule and occur singly or in aggregates. Cell motility has been reported only for *E. modestus* [1]. The preferred growth substrates of these bacteria are sugars; hydrolytic capabilities are relatively weak. Species of the genus *Edaphobacter* have been isolated from alpine, temperate and subtropical acidic forest soils. In this study, we describe a novel member of this genus, which was obtained from lichen-dominated forested tundra.

Strain SBC68^T was isolated from slightly decomposed thalli of *Cladonia* sp. collected from the forested tundra (65° 30′ 59″ N, 72° 32′ 15″ E) in Nadym region, Yamalo–Nenets Autonomous Okrug, Russia. The collected thalli were ground in a mortar and the resulting material was suspended in 5 ml sterile water. This suspension was further spread-plated onto the medium solidified with 9 g phytagel (Sigma–Aldrich) and containing (per litre distilled water): 0.25 g xylan, 0.25 g starch, 0.05 g Bacto Tryptic Soy Broth without dextrose, 0.1 g NH_4NO_3, 0.04 g KH_2PO_4; pH was adjusted to 4.5 with 30 mg alginic acid l^{-1}. The plates were incubated at 20 °C for 4 weeks. Colonies that developed on these plates were screened using the *Acidobacteria*-specific PCR approach [5]. One particular type of colonies that were identified as belonging to members of the *Acidobacteria* was represented by small (1–3 mm in diameter after 4 weeks of incubation), circular, pinkish, translucent, mucous, convex colonies with entire edges and smooth surfaces. Cell material from these colonies was further re-streaked on the above described medium and the plates were incubated under the same conditions until the target bacterium, designated strain SBC68^T, was obtained as a pure culture.

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Keywords: *Acidobacteria*; *Acidobacteriaceae*; *Edaphobacter lichenicola* sp. nov.; *Cladonia* sp.; lichen thalli.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of *Edaphobacter lichenicola* SBC68^T is MG385062.

One supplementary table and one supplementary figure are available with the online version of this article.

In order to identify this isolate, the 16S rRNA gene sequence of SBC68^T was determined. PCR-mediated amplification of the 16S rRNA gene was performed using primers 9f and 1492r and reaction conditions described by Weisburg *et al.* [6]. Phylogenetic analysis was carried out using the ARB program package [7]. The trees were reconstructed using distance-based (neighbour-joining), maximum-likelihood (DNAml) and maximum-parsimony methods. The significance levels of interior branch points obtained in neighbour-joining analysis were determined by bootstrap analysis (1000 data re-samplings) using PHYLIP [8]. The comparative 16S rRNA gene sequence analysis revealed that SBC68^T represents a member of subdivision 1 of the *Acidobacteria* and is a member of the family *Acidobacteriaceae* (Fig. 1). Highest similarity (99 %) of 16S rRNA gene sequence from SBC68^T was observed with a number of environmental clone sequences that had been retrieved in cultivation-independent studies from a highland grassland soil under lichen and moss crusts (GenBank number JN023153, unpublished study), a boreal pine forest soil (FJ624919, FJ624920, unpublished study), and a woodland soil (HQ599055, [9]). The closest taxonomically described relatives of SBC68^T were *Edaphobacter dinghuensis* DHF9^T and *Granulicella aggregans* TPB6028^T (97.2 and 97.1 % 16S rRNA gene sequence similarity, respectively). In 16S rRNA gene-based trees, however, SBC68^T consistently clustered together with species of the genus *Edaphobacter* independently of the algorithm used for the tree reconstruction (Figs 1 and S1, available in the online version of this article) but branched separately from all taxonomically described representatives of this genus. Based on these results, the present study was initiated in order to characterize SBC68^T and to determine its taxonomic position.

Once selected for the taxonomic study, strain SBC68^T was maintained on medium MB containing (per litre distilled water): 0.5 g glucose, 0.1 g Bacto Casamino acids, 0.1 g yeast extract, 0.04 g $MgSO_4.7H_2O$, 0.02 g $CaCl_2.2H_2O$, 0.2 g KNO_3 and 9 g phytagel, pH 4.6–5.0 and was sub-cultured at 2–3 week intervals.

Morphological observations and cell-size measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). For preparation of ultrathin sections, cells of the exponentially growing culture were collected by centrifugation and pre-fixed with 1.5 % (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 6.5) for 1 h at 4 °C and then fixed with 1 % (w/v) OsO_4 in the same buffer for 4 h at 20 °C. After dehydration in an ethanol series, the samples were embedded into Epon 812 epoxy resin. Thin sections were cut on an LKB-4800 microtome, stained with 3 % (w/v) uranyl acetate in 70 % (v/v) ethanol, and then were stained with lead citrate [10] at 20 °C for 4–5 min. The specimen samples were examined with a JEM-1011 (JEOL) transmission electron microscope. SBC68^T was represented by non-spore-forming, non-motile, short rods that stained Gram-negative, reproduced by binary fission and occurred singly, in pairs or in rosettes (Fig. 2a). Copious production of extracellular polysaccharides, which is typical for all members of the genus *Granulicella* [11], was not observed. Formation of rosettes most commonly occurred in old

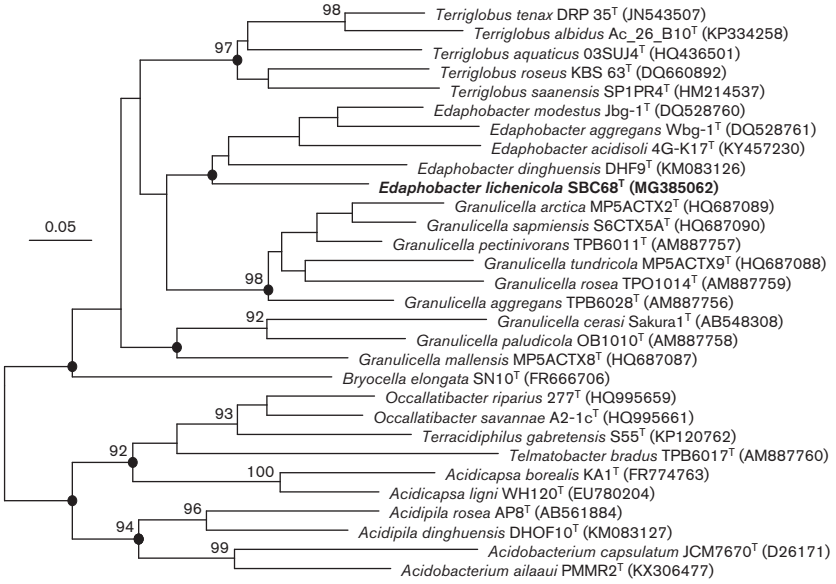

Fig. 1. Unrooted 16S rRNA gene-based neighbour-joining tree showing the phylogenetic relationship of SBC68^T to representative members of the family *Acidobacteriaceae*. The significance levels of interior branch points obtained in neighbour-joining analysis were determined by bootstrap analysis (1000 data re-samplings). Bootstrap values of >90 % are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.05 substitutions per nucleotide position.

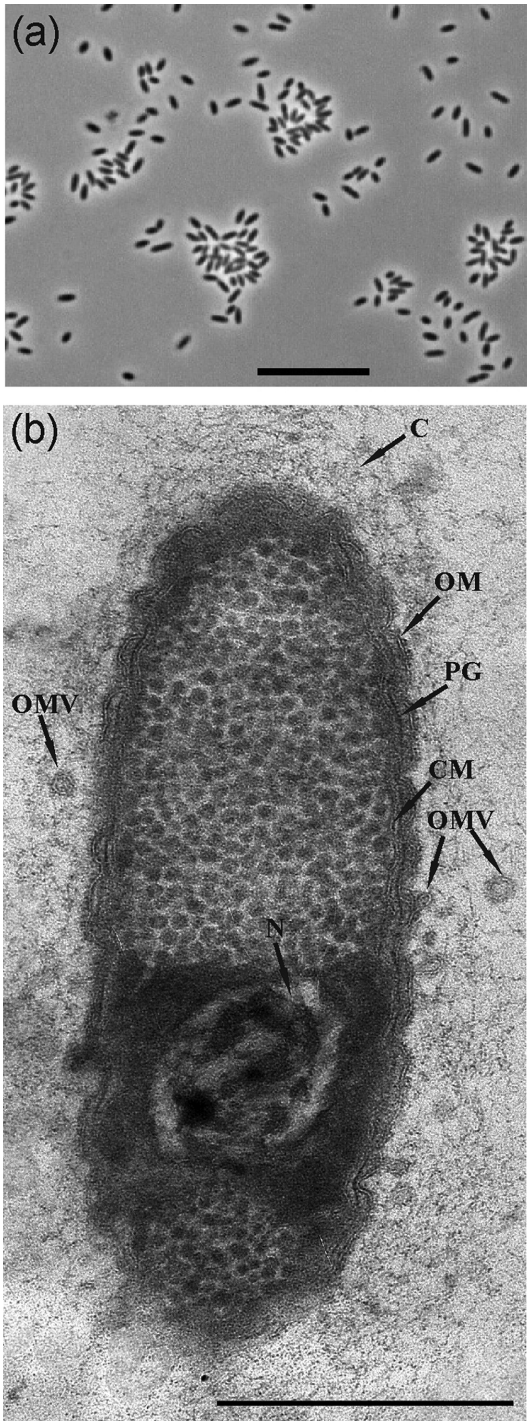

Fig. 2. (**a**) Phase-contrast image of cells of SBC68^T grown for 14 days on solid medium MB. (**b**) Electron micrograph of an ultrathin section of a cell. CM, cytoplasmic membrane; OM, outer membrane; PG, peptidoglycan layer; N, nucleoid; MV, membrane vesicles. Bars, 5 µm (a); 0.5 µm, (b).

(2–3 weeks) cultures. Cells were relatively small, 1.3–2.7 µm long and 0.4–0.7 µm wide. Electron microscopy revealed a cell-wall structure typical of Gram-negative bacteria. The cytoplasmic membrane, peptidoglycan layer and outer membrane were evident in ultrathin sections (Fig. 2b). We also revealed the presence of numerous outer-membrane vesicles, ranging in size from 50 to 100 nm (Fig. 2b).

Physiological tests were performed using batch cultures grown in liquid medium MB. Growth of SBC68^T was monitored by nephelometry at 410 nm using a 'Specol' spectrophotometer (Carl Zeiss) for 14 days under a variety of conditions, including temperatures of 2–37 °C, pH 3.0–7.9 and NaCl concentrations of 0–4.0 % (w/v). Incubations at various temperatures were made under static conditions in triplicate; OD_{410} was determined after 2 weeks of incubation. Variations in pH were achieved by using MES (pH 4.0–6.5) and MOPS (pH 6.5–7.9) buffer systems. The pH range of 3–4 was achieved by adjusting the medium pH with 0.1 M H_2SO_4. SBC68^T was capable of growth at pH values between 3.4 and 7.0 (with an optimum at pH 4.3–5.6) and at temperatures between 7 and 37 °C (with an optimum at 20–30 °C). The specific growth rate displayed by SBC68^T at 22 °C and pH 4.8 was about 0.04 h^{-1}; T_d=17.33 h. NaCl inhibited growth at concentrations above 1 % (w/v).

The range of potential growth substrates of SBC68^T was examined by replacing glucose in medium MB with the respective carbon sources at a concentration 0.05 % (w/v). Casamino acids were omitted from the medium and the concentration of yeast extract was reduced to 0.005 % (w/v). Cultivation was done in 30 ml vials containing 5 ml medium, which were incubated at 22 °C for 3 weeks on a shaker. All experiments were performed in triplicate. Enzymatic profiles, urease, β-galactosidase activity, indole production, abilities to hydrolyze gelatin and aesculin were examined with API ZYM and API 20NE kits (bioMérieux). A catalase test was carried out by a standard method [12]. Oxidase was tested using a REF 55 635 Oxidase Reagent (bioMérieux). The ability to grow under anaerobic conditions was tested in anaerobic jars by using AnaeroGen anaerobic system envelopes (Oxoid), which absorb atmospheric oxygen with the simultaneous generation of CO_2 (up to 9–13 %, v/v). SBC68^T was an obligately aerobic chemoheterotroph and did not grow under anoxic conditions. The preferred growth substrates were various sugars (see the species description), peptone, ethanol and several organic acids, such as glucuronate, succinate and oxalate. Good growth was also observed on lichenan and laminarin, while several other heteropolysaccharides, such as aesculin, locust bean gum and xylan, supported only a weak growth of these bacteria. Starch, pullulan, fucoidan, cellulose, pectin, chitosan, chitin and gelatin were not hydrolyzed. In general, SBC68^T displayed relatively low hydrolytic capabilities, which is one of the key features that differentiate members of the genus *Edaphobacter* from representatives of the genus *Granulicella* [11].

SBC68^T was catalase- and urease-negative and showed the following enzymatic activities (API ZYM test): alkaline and acid phosphatase, α-glucosidase, *N*-acetyl-β-glucosaminidase, esterase, leucine, valine and cystine arylamidase,

α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, esterase lipase, β-glucuronidase and β-glucosidase. The following enzymatic activities were not detected: lipase, trypsin, α-fucosidase and α-mannosidase. Indole production from L-tryptophan, fermentation of D-glucose and hydrolysis of L-arginine were also not detected.

For the analysis of total lipids (including fatty acids), cells of SBC68^T were grown in liquid medium MB and harvested during the late exponential growth phase. Lipids were analyzed after acid hydrolysis of the cell material following the procedure described by Kulichevskaya *et al.* [13]. The major fatty acids detected in SBC68^T were 13,16-dimethyl octacosanedioic acid (38.3 %), iso-$C_{15:0}$ (22.9 %), $C_{16:1}\omega7c$ (12.9 %), $C_{16:0}$ (10.9 %) and $C_{16:1}\omega7t$ (8.0 %) (Table 1). High amounts of iso-$C_{15:0}$ and $C_{16:0}$, as well as the membrane-spanning lipid 13,16-dimethyl octacosanedioic acid, are commonly found in members of the genera *Edaphobacter* and *Granulicella*, which are nearly indistinguishable on the basis of fatty acid composition [14] (Table S1). Yet, on the basis of the absence of many minor fatty acids, the fatty acid pattern in SBC68^T was closer to those in described species of the genus *Edaphobacter* (Table S1). The unsaturated fatty acid $C_{16:1}\omega7c$ has also been detected in all other described species of the genera *Edaphobacter* and *Granulicella* but SBC68^T contained relatively high amounts of $C_{16:1}\omega7t$ (Tables 1 and S1). The intact polar lipids (IPLs) in SBC68^T were analyzed as described by Moore *et al.* [15]. Unknown high-mass IPLs were the major constituents, while smaller amounts of phosphatidylethanolamine (PE), ornithine lipid (OL), phosphatidylglycerophosphate (PGP), phosphohexose (P-hex), and diphosphatidylglycerol were also detected (Table 2).

Isoprenoid quinones were extracted according to the methods of Collins [16] and analyzed using a tandem-type mass spectrometer LCQ ADVANTAGE MAX and a Finnigan Mat 8430 ionization mass spectrometer with atmospheric pressure chemical ionization (APCI). Similar to all currently described members of the family *Acidobacteriaceae*, SBC68^T contained menaquinone-8 (MK-8) as the predominant isoprenoid quinone.

The DNA base composition of the novel isolate was determined by thermal denaturation using a Cary-100 UV-VIS spectrophotometer (Varian) at a heating rate of 0.5 °C min^{-1}. The mol% G+C value was calculated according to the methods of Owen *et al.* [17]. DNA of *Escherichia coli* K-12 (G+C value 51.7 mol%) was used as the standard. The DNA G+C content of SBC68^T was 54.7 mol%.

In summary, the results of 16S rRNA gene sequence analysis indicated affiliation of SBC68^T with the members of the genus *Edaphobacter*. Several phenotypic characteristics, such as small cell size, absence of copious exopolysaccharide production, and relatively low hydrolytic capabilities, also differentiated this isolate from described species of the genus *Granulicella* [11] and supported its placement in the genus *Edaphobacter*. SBC68^T possessed a number of characteristics that clearly distinguished it from other described members of the genus *Edaphobacter* (Table 3). The cells of this acidobacterium formed rosettes and colonies were light-pink, which is not typical for other members of this genus. The low G+C content of genomic DNA and differences in the substrate utilization pattern also distinguished strain SBC68^T from other species of the genus *Edaphobacter*. Given these differences, we propose to classify the novel isolate as representing a novel species of genus *Edaphobacter*, for which the name *Edaphobacter lichenicola* sp. nov. is proposed.

Isolation from slightly decomposed thalli of *Cladonia* sp. and the capability of growth on lichenan, the characteristic

Table 1. Fatty acid composition (percentages; normalized on their sum) released after acid hydrolysis of cell material of SBC68^T in comparison with *E. aggregans* Wbg-1^T and *E. modestus* Jbg-1^T (data from [14]

The procedure used to analyze the lipids of *E. dinghuensis* DHF9^T and *E. acidisoli* 4GK17^T does not allow detection of iso-diabolic acid and the respective data, therefore, are not included in this comparison but their other common fatty acids are also iso-$C_{15:0}$, $C_{16:1}\omega7c$, and $C_{16:0}$. Strains: 1, SBC68^T; 2, *E. aggregans* Wbg-1^T; 3, *E. modestus* Jbg-1^T. Major components (>5 %) are indicated by bold type. –, Not detected.

Fatty acids	1	2	3
iso-$C_{13:0}$	2.2	–	–
$C_{14:1}\omega5c$	–	0.6	0.5
$C_{14:0}$	0.5	1.0	1.1
iso-$C_{15:0}$	**22.9**	**26.0**	**22.4**
$C_{16:1}\omega7c$	**12.9**	**23.7**	**21.5**
$C_{16:1}\omega7t$	**8.0**	–	2.1
$C_{16:0}$	**10.9**	**5.6**	**7.6**
iso-$C_{17:1}\omega7c$	0.4	1.1	0.3
iso-$C_{17:0}$	2.8	2.0	0.7
$C_{18:1}\omega7c$	–	–	0.2
$C_{18:0}$	0.5	–	0.8
13,16-dimethyl octacosanedioic (iso-diabolic acid)	**38.3**	**36.1**	**42.8**
iso-diabolic acid monoglycerol ether	0.6	3.5	–
iso-C_{15} monoglycerol ether	–	0.5	–

Table 2. Relative abundances of intact polar lipids of SBC68^T

The abundance is relative to major peak in the LC–MS base peak chromatogram: +++, base peak; ++, 50–100 % of base peak; +, 10–50 % of base peak. Note that the mass spectral response factors for different IPL groups can be quite different.

IPL	SBC68
Phosphatidylethanolamine	+
Ornithine lipid	+
Diphosphatidylglycerol (cardiolipin)	+
Unknown IPL (m/z 890, 412)	+
Phosphohexose	+
Unknown high masses	+++

Table 3. Major characteristics that distinguish SBC68^T from other described species of the genus *Edaphobacter*

Species: 1, strain SBC68^T (data from this study); 2, *E. dinghuensis* (data from [3]); 3, *E. modestus* (data from [1]); 4, *E. aggregans* (data from [1]); 5, *E. acidisoli* (data from [4]). +, Positive; w, weakly positive; −, negative; ND, not determined.

Characteristic	1	2	3	4	5
Cell size (μm)	1.3–2.7×0.4–0.7	0.8–1.4×0.5–0.7	1.0–1.8×0.5–0.7	1.5–2.1×0.7–0.9	0.8–1.2×0.5–0.8
Cell rosettes	+	−	−	−	−
Motility	−	−	+	−	−
Colony colour	Pinkish	Beige	Beige	Beige	Beige
pH growth range	3.4–7.0	3.5–5.5	4.5–7.0	4.0–7.0	3.0–7.0
pH optimum	4.3–5.6	4.0	5.5	5.5	4.0–5.5
Temperature growth range (°C)	7–37	10–33	15–30	15–37	10–42
Optimum (°C)	20–30	28–33	30	30	28
NaCl tolerance range(%)	0–1.0	0–2.0	ND	ND	0–2.5
Utilisation of:					
Arabinose	−	+	+	–	+
Mannose	+	+	−	–	−
Raffinose	+	+	−	–	−
Aesculin	+	+	−	–	+
Mannitol	w	−	+	−	−
Glucuronate	+	−	−	+	ND
Malate	w	+	−	−	−
Trehalose	+	+	+	−	−
Xylose	+	+	+	−	−
Enzymic activities:					
Cystine arylamidase	+	−	−	−	+
N-acetyl-β-glucosaminidase	+	+	−	+	+
β-glucosidase	w	+	+	+	+
Esterase lipase	w	+	+	+	+
β-glucuronidase	w	+	−	−	+
Oxidase	+	−	+	−	+
Catalase	−	−	+	+	+
DNA G+C content (mol%)	54.7	57.7	55.8	56.9	57.2–57.6

component of lichen-derived phytomass [18, 19], indicate the involvement of SBC68^T-like acidobacteria in degradation of lichen-derived debris in tundra ecosystems. This is also supported by previous published reports of a high abundance of *Acidobacteria* in decaying lichen thalli [20] and peat sampled from lichen-dominated tundra wetlands [21].

DESCRIPTION OF *EDAPHOBACTER LICHENICOLA* SP. NOV.

Edaphobacter lichenicola (li.che.ni'co.la. L. masc. n. *lichen* lichen; L. masc. suff. *-cola* (from L. n. *incola*), an inhabitant; N.L. masc. n. *lichenicola* inhabitant of lichens).

Colonies are light pink. Cells are Gram-stain-negative, non-motile rods, 1.3–2.7 μm long and 0.4–0.7 μm wide, occur singly, in pairs or in rosettes. Carbon sources include glucose, lactose, lactulose, maltose, melibiose, cellobiose, galactose, fructose, leucrose, mannose, rhamnose, raffinose, ribose, sucrose, trehalose, xylose, salicin, glucuronate, succinate, oxalate and ethanol. Weak growth is observed on *N*-acetylglucosamine, melezitose, fucose, lactate, malate, pyruvate, gluconate, fumarate, mannitol and arbutin. Capable of hydrolyzing lichenan, laminarin, aesculin, locust bean gum, arabinogalactan, dextrin, peptone and xylan. Cannot utilize sorbose, methanol, acetate, butyrate, propionate, valerate, citrate, formate, galacturonate, dulcitol and sorbitol. Cannot hydrolyze starch, pullulan, fucoidan, cellulose, pectin, chitosan, chitin and gelatin. Shows the following enzyme activities: alkaline and acid phosphatase, α-glucosidase, *N*-acetyl-β-glucosaminidase, naphtol-AS-BI-phosphohydrolase, esterase, leucine, valine and cystine arylamidases, α-chymotrypsin, α- and β-galactosidase, esterase lipase, β-glucuronidase, β-glucosidase and cytochrome oxidase. (API ZYM test). The following enzymatic activities are not present: catalase, urease, lipase, trypsin, α-fucosidase and α-mannosidase. Negative for dissimilatory nitrate reduction, indole production, fermentation of D-glucose and hydrolysis of L-arginine and gelatin. Growth occurs at pH 3.4–7.0 (optimum at pH 4.3–5.6), and at temperatures between 7 and 37 °C (optimum at 20–30 °C). NaCl inhibits growth at concentrations above 1 % (w/v). The major cellular fatty acids are 13,16-dimethyl octacosanedioic acid, iso-$C_{15:0}$, $C_{16:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega7t$.

The type strain is SBC68^T (=DSM 104462^T=VKM B-3208^T), which was isolated from lichen thalli collected from the lichen-dominated forested tundra in Nadym region, Yamalo-Nenets Autonomous Okrug, Russia. The G+C content of the DNA of the type strain is 54.7 mol%.

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

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