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EXPERIMENTAL ARTICLES

A Psychrotolerant *Caulobacter* sp. from Russian Polar Tundra Soil

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Abstract—train Z-0024, a psychrotolerant aerobic heterotrophic representative of the prosthecate bacteria of the genus *Caulobacter*, was isolated from a methanotrophic enrichment obtained from Russian polar tundra soil. The cells of the new isolate are vibrios (0.5–0.6 × 1.3–1.8 mm) with a polar stalk. The organism grows in a temperature range from 5 to 36°C, with an optimum at 20°C. The pH range for growth is from 4.5 to 7.0 with an optimum at pH 6.0. Strain Z-0024 utilizes a wide range of organic compounds: sugars, amino acids, volatile fatty acids, and primary alcohols. It tolerates a NaCl concentration in the medium of up to 15 g/l. The G+C content of DNA is 66.6 mol %. The 16S rRNA gene sequence analysis revealed that strain Z-0024 belongs to the cluster of Caulobacter species, showing a 98.8–99.2% sequence similarity to them. DNA–DNA hybridization revealed a low level of homology (24%) between strain Z-0024 and *C. vibrioides* ATCC 15252. The new isolate is described as *Caulobacter* sp. Z-0024.

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Key words: prosthecate bacteria, caulobacters, psychrotolerant bacteria, methanotrophic community.

The prosthecate bacteria of the genus *Caulobacter* are widespread in nature [1]. The sources of isolation of these bacteria are soil and fresh or marine water [2–6]. Caulobacters are typical representatives of oligotrophs and belong to the trophic group of dissipotrophs, occupying a specific position in the food chain [7]. Until recently, the taxonomy of caulobacters traditionally relied on morphological and physiological characteristics [8, 9]. Stahl et al. [10] were the first to sequence the 16S rRNA genes of several *Caulobacter* representatives. Their study revealed that caulobacters form two different lineages, one comprising freshwater and brackish-water forms, and the other comprising marine species. The development of modern molecular biology methods and the application of the polyphasic approach, which combines analysis of 16S rDNA sequences, analysis of lipids and immunological profiles, and characterization of NaCl tolerance, allowed the intrageneric taxonomy of *Caulobacter* and the position of this genus in the phylogenetic system of prokaryotes to be clarified [11]. In accordance with the results of that study, the species *C. henricii, C. fusiformis, C. vibrioides*, and *Mycoplana segnis* (*C. segnis* comb. nov.) form a paraphyletic group and belong to *Caulobacter* sensu stricto. *C. crescentus* is synonymous to *C. vibrioides.* Some of the freshwater species—*C. subvibrioides* subsp. *albus*, *C. henricii* subsp. *aurantiacus, C. bacteroides, C. intermedius, C. subvibrioides, C. variabilis*, and *Mycoplana bullata*—cluster with *Brevundimonas diminuta* and have been transferred to the genus *Brevundimonas* under the same species names. The marine species *C. maris* and *C. halobacteroides* differ from the other representatives of *Caulobacter* and members of *Brevundimonas* and were assigned to the new genus *Maricaulis* with the type species *Maricaulis maris.*

Representatives of the genus the *Caulobacter* are widespread geographically; in Russia, they occur in water reservoirs and soils from Crimea to the Polar zone [12]. The first tundra soil *Caulobacter* was isolated by B.V. Gromov from a culture of the alga *Chlorococcum* sp. [13] and described as a new strain of *C. vibrioides* on the basis of its morphological, physiological, and cultural features. Until recently, it was the only *Caulobacter* representative isolated from tundra soil.

The aim of the present work was to study the new psychrotolerant bacterium Caulobacter sp. Z-0024 isolated from the methanotrophic community of polar tundra soil of Russia.

MATERIALS AND METHODS

An enrichment culture of the methanotrophic bacterium *Methylobacter* sp., obtained from a sample of polar tundra soil taken in the surroundings of Vorkuta [14], was the source of strain Z-0024 isolation.

The isolation was carried out using agarized 2A ultrafreshwater medium, routinely employed for the cultivation of methanotrophic bacteria [14]. Peptone

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and yeast extract (0.01% each) were added to the medium as substrates. The incubation temperature was 5°C.

Pure culture of strain Z-0024 was cultivated in PYG medium (pH 6.7), routinely employed for the cultivation of caulobacters. This medium contains 0.05% peptone, 0.05% yeast extract, and 0.1% glucose [8].

The range of substrates utilized for growth was tested using a liquid medium similar to PYG, except that 0.1% of a tested compound was added instead of glucose. As possible substrates, we tested the carbohydrates arabinose, ribose, xylose, glucose, fructose, galactose, mannose, lactose, maltose, sucrose, and starch; the sugar alcohols dulcitol, sorbitol, and mannitol; the organic acid salts acetate, butyrate, propionate, pyruvate, fumarate, and succinate; the primary alcohols methanol and ethanol; and the amino acids DL-alanine, DL-glutamine, and DL-asparagine.

Bacterial growth in liquid medium was monitored turbidimetrically at a wavelength of 600 nm with Specol ZV (GDR) over 3–4 weeks of incubation at 20°C. The growth pH range was determined using media with the initial pH adjusted to a required value within the pH 4.0–7.8 range by the addition of 0.05 M solutions of Na_2HPO_4 or KH_2PO_4. The pH values were measured potentiometrically using an Expert 001 pH meter–ion meter (Russia). The effect of temperature on growth was determined in PYG medium within a temperature range of 5 to 42°C. The dependence of growth on the NaCl concentration was determined by evaluating growth at the optimal growth temperature (20°C) in PYG medium supplemented with NaCl at concentrations of 1 to 40 g/l.

The specific growth rates in the exponential phase of growth were calculated as $\mu = \Delta \ln x/\Delta t$, where x is the increase in optical density over the time interval Δt. The generation time was calculated as $t_d = \ln 2/\mu$.

Morphology of the isolate was studied using an Amplival phase-contrast microscope and a JEM-100C transmission electron microscope. Cells for morphological studies were grown in PYG medium. Whole-cell preparations for electron microscopy were negatively stained with a 1% uranyl acetate solution.

The methods of isolation and purification of DNA, estimation of its G+C content, and DNA–DNA hybridization were described earlier [15]. The 16S rDNA was amplified by PCR using universal bacterial primers [16], and the 16S rRNA gene sequencing was performed on an automatic 373A DNA Sequencer using an Applied Biosystems no. 402080 FS sequencing kit, according to the instructions of the manufacturer.

The 16S rRNA gene sequence was preliminary analyzed employing data and software from the Ribosomal Database Project (http://rdp.cme.msu.edu). Sequences were edited using the BioEdit software package (http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html). The16S rRNA gene sequence was aligned with the 16S rRNA gene sequences of the closest bacterial species using the CLUSTALW v 1.75 software package. Unrooted phylogenetic trees were constructed using the algorithms implemented in the TREECONW software package (http://bioc-www.uia.ac.be/u/yvdp/treeconw.html). Strain Z-0024 16S rRNA gene sequence was deposited in GenBank under the accession number DQ124680.

RESULTS AND DISCUSSION

The new representative of the prosthecate bacteria of the genus *Caulobacter*, strain Z-0024, was isolated from a psychrophilic methanotrophic enrichment obtained from Russian polar tundra soil at 5°C (Fig. 1a). The cells of strain Z-0024 had a morphology typical of caulobacters: they were rods with a complex life cycle that included motile and sessile stages. In the motile stage, the cells were rods with a single polar flagellum (Fig. 1b). In the sessile stage, cells were vibrios with a single polar stalk. During growth on PYG medium under optimal conditions, the cells measured $0.5–0.6 \times 1.3–1.8$ μm and had a short stalk with a diameter of 0.1–0.15 μm. At the distal end of the stalk, a holdfast occurred, which enabled cells to form a rosette structure (Fig. 1c). In 4 weeks of growth on PYG medium, strain Z-0024 produced salient, mucous, milk-white colonies measuring 2–3.5 mm in diameter and having even edges.

To establish the phylogenetic position of strain Z-0024, we determined an almost complete 16S rRNA gene sequence, corresponding to *Escherichia coli* positions 38–1485. Comparison with 16S rRNA gene sequences available in GenBank revealed [16] that the new strain was a member of the cluster comprising strains of the species *C. vibrioides*, which has recently been extended to include strains of *"C. crescentus"* [11]. The level of similarity between strain Z-0024 and *C. vibrioides* strains was 98.8–99.2%. The level of similarity with *C. segnis* strains was also high (99.1–99.3%). The latter species belongs to the same cluster as *C. vibrioides*, although it still retains the status of a separate species. The level of 16S rRNA gene sequence similarity of strain Z-0024 with other species of the genus *Caulobacter* was significantly lower (96.8–98.5%) (Fig. 2).

As can be seen from Table 1, the new strain Z-0024 was similar to "*C. crescentus*" in its morphology and DNA G + C content. However, it was to a certain extent different from closely related species in its ecological and physiological features. Strain Z-0024 grew in a wide temperature range, from 5 to 36°C, with an optimum at 20°C, a value which is lower than the temperature optimal for the growth of *Caulobacter* species (Table 1). The generation time on PYG medium at optimal temperature was 1.2 days. At 30°C, the growth was significantly weaker, and at 36°C it was absent. Strain Z-0024 appeared to be psychrotolerant, exhibiting active growth at 5°C.

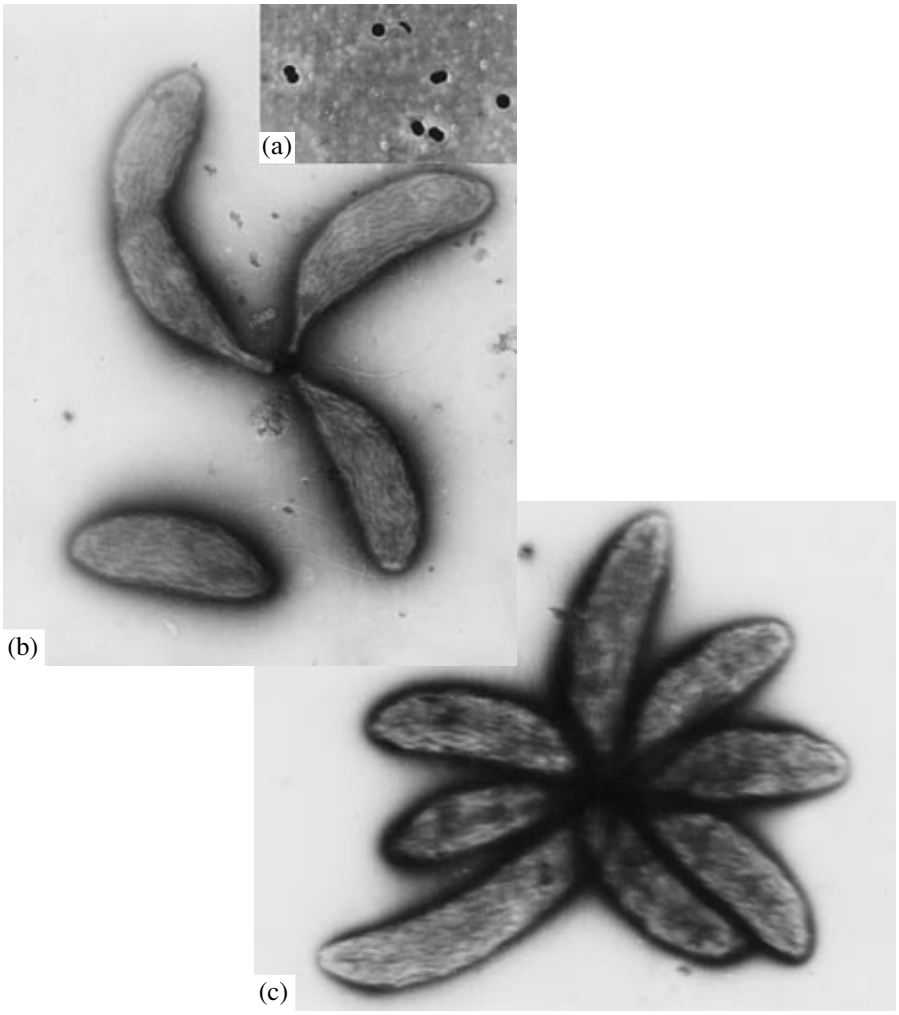

Fig. 1. Cells of Caulobacter sp. Z-0024 (a) in a methanotrophic enrichment grown at 5°C (phase-contrast, 1280×) and (b, c) after pure culture growth in PYG medium at optimal pH and temperature (transmission electron microscopy, 20000×).

The pH growth limits for strain Z-0024 were pH 4.5 and 7.0; the optimal pH was 6.0. The lower pH limit and the optimal pH were more acidic than values characteristic of earlier described *Caulobacter* representatives (Table 1). Thus, the temperature and pH characteristics of strain Z-0024 growth give evidence of the adaptation of the new *Caulobacter* representative to its natural habitat—swampy tundra soils with acidic pH values and low temperatures [18].

Strain Z-0024 utilized quite a wide substrate range, which included sugars, volatile fatty acids, amino acids, and primary alcohols (Table 2). Like *C. vibrio-*

Table 1. Characteristics of *Caulobacter* species [8, 17]

Species	Cell morphology	Colony color	DNA G+C content, mol %	pH optimum (range)	Temperature optimum, °C
C. vibrioides	Thin vibrios or egg-shaped cells (1.4–2.2 × 0.6–0.8 μm)	Colorless or yellowish	64–65	6.5 (6–9)	30
C. henricii (ATCC 15253^T)	Small vibrios (cells of some strains are smaller than 0.5 × 1 μm)	Yellow or orange	62–65	6.5 (6.1–7.8)	30
"*C. crescentus*" (CB2^T)	Thin vibrios (1.2–1.8 × 0.5–0.6 μm)	Colorless; the center becomes dark pink	62–67	6.5 (6.1–7.8)	30
Caulobacter sp. Z-0024	Vibrios (1.3–1.8 × 0.5–0.6 μm)	Milk-white	66.6	6.0 (4.5–7.0)	20

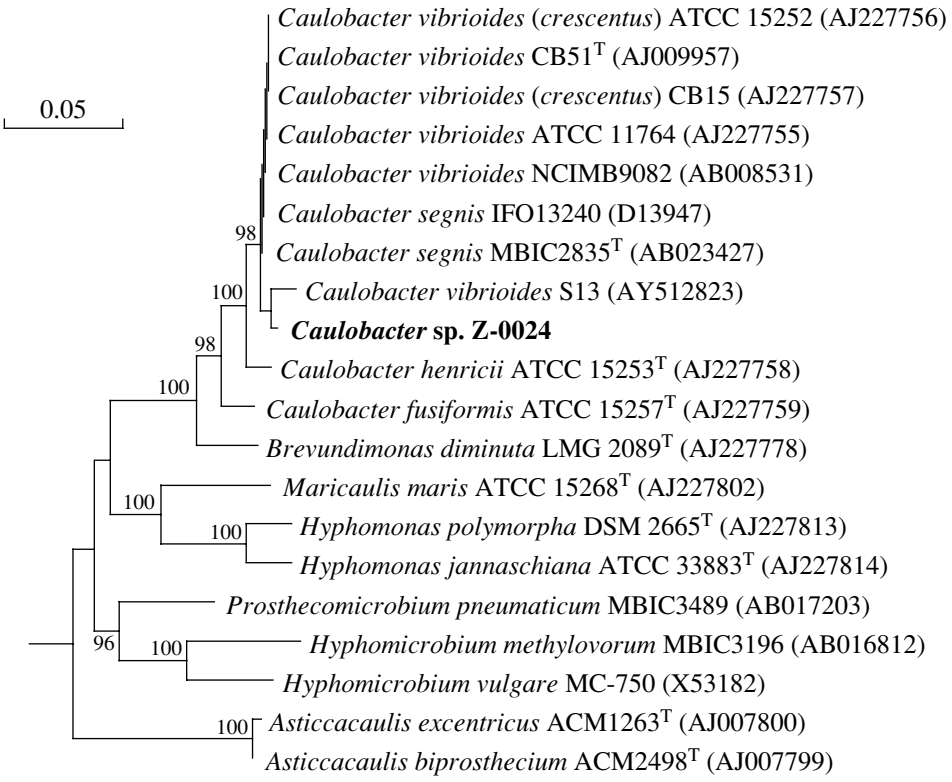

Fig. 2. Phylogenetic position of Caulobacter sp. Z-0024 as determined by comparative analysis of the 16S rRNA gene sequence. The scale bar represents the evolutionary distance corresponding to 5 substitutions per 100 nucleotides. Numerals show the statistic significance of the branching order as determined by bootstrap analysis (bootstrap values higher than 95 were considered significant).

ides, strain Z-0024 utilized arabinose, xylose, ribose, glucose, fructose, lactose, and sucrose, but not maltose; it hydrolyzed starch and grew on the amino acids DL-alanine, DL-glutamine, and DL-asparagine. Like *"C. crescentus,"* strain Z-0024 grew on methanol and ethanol. Like *"C. crescentus"* and *"C. henricii"*, strain Z-0024 utilized butyrate, acetate, and fumarate. However, by contrast with closely related species, it did not utilize pyruvate.

Strain Z-0024 grew within a NaCl concentration range of 1 to 15 g/l; 20 g/l of NaCl completely inhibited growth. Earlier, it was reported that the *Caulobacter* species *"C. crescentus," C. vibrioides*, and *C. fusiformis* can tolerate only 10 g/l NaCl [11].

The DNA G+C content of *Caulobacter* strain Z-0024 was 66.6 mol %.

DNA–DNA hybridization of strain Z-0024 with the type strain of the type species, *Caulobacter vibrioides* ATCC 15252, revealed only 24% homology. This hybridization level confirmed that strain Z-0024 belonged to the genus *Caulobacter*, representing another species. The isolation of a single psychrotolerant strain did not allow us to describe a new species of the genus *Caulobacter.* Among the strains that we found in the methanotrophic communities from the tundra soils of Vorkuta, the Chukchi Peninsula, and the Yugorskii Peninsula, strain Z-0024 is the first *Caulobacter* representative that possesses a set of features enabling it to adapt to cold habitats—the acidic swampy soils of tundra.

Characteristics of *Caulobacter* sp. Z-0024

Morphologically, the cells are rods which have a dimorphic life cycle. In the motile stage, they have a single polar flagellum. In the sessile stage, cells are vibrios 0.5–0.6 μm in width and 1.3–1.8 μm in length, possessing a polar stalk with a diameter of 0.1–0.15 μm. Multiplication occurs by cell division. Colonies are milk-white, salient, and mucous, with a smooth surface and even edges, of viscous consistency.

No water-soluble pigment is produced. The temperature range for growth is 5–36°C, with the optimum at 20°C. The growth at 5°C is quite good. The pH range for growth is 4.5–7.0, with the optimum at pH 6.0. The metabolism is aerobic and heterotrophic. A wide range of organic compounds, including sugars, volatile fatty acids, and alcohols, are utilized. The DNA G+C content is 66.6 mol % (Tm). Up to 15 g/l of NaCl can be toler-

Table 2. Substrate utilization by *Caulobacter* species

Substrates	*C. vibrioides*	"*C. crescentus*"	*C. henricii*	*Caulobacter* sp. Z-0024
Carbohydrates:				
arabinose	+	±	±	+
ribose	+	–	±	+
xylose	+	+	+	+
glucose	+	+	+	+
galactose	+	+	+	ND
mannose	+	+	±	ND
fructose	+	–	±	+
lactose	+	+	±	+
maltose	+	+	+	–
sucrose	+	+	+	+
starch	+	±	+	+
Amino acids:				
DL-alanine	+	+	+	+
DL-asparagine	+	+	+	+
DL-glutamine	+	+	+	+
Organic acid salts:				
acetate	–	+	+	+
butyrate	±	+	+	+
pyruvate	±	±	±	–
succinate	+	–	+	+
fumarate	+	±	+	+
propionate	ND	ND	ND	+
Primary alcohols:				
methanol	–	+	–	+
ethanol	–	+	±	+

Note: "+" means that a substrate is utilized; "±" means that a substrate is utilized by some strains; "–" denotes lack of substrate utilization; "ND" stands for "not determined".

ated. The isolation source is soil of the southern tundra zone in European Russia.

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