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EXPERIMENTAL ARTICLES

Magnetospirillum aberrantis sp. nov., a New Freshwater Bacterium with Magnetic Inclusions

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Abstract—A new strain of spirilla, SpK, was isolated from the bottom sediments of Ol'khovka River near Kislovodsk (Caucasus). The bacteria (0.4 ±1.5 μm) were motile, with polar flagella. They grew within the temperature range from 20 to 45°C, with the optimum at 31°C. The pH growth optimum was at 6.5–6.9. The main type of metabolism was respiratory, chemoorganotrophic. The organism was microaerophilic, with the growth optimum at 1–5% O_2 in the gas phase. Catalase activity was absent, while oxidase activity was detected. Good growth occurred in media with various organic acids, especially acetate and fumarate. Sugars and alcohols were not utilized. Importantly, the strain did not grow on casein hydrolysate and grew well on glycerol. The bacteria contained the RuBisCo cbbm gene (form II). Thiosulfate, ammonium, and ferrous iron were not used as electron donors for autotrophic growth. Unlike sulfate, thiosulfate, ferric iron, or perchlorate, nitrate could be used as an electron acceptor for photoheterotrophic growth. Strain SpK was characterized by the ability to form small, dense intracellular granules (30–40 nm) occurring in clusters or short chains. These inclusions were shown to have magnetic properties. Unlike magnetosomes, the granules did not form long chains. Invaginations of vesicular membranes similar to those found in the known magnetosome-forming microorganisms were observed. The DNA G + C content was 62.6 mol %. Ubiquinone Q_{10} was present. The main fatty acids were 18:1ω7 (58.19%), 16:0 (19.23%), 16:1ω7 (11.12%), and 18:0 (1.91%). Polyhydroxybutyrate and polyphosphates were the storage compounds. Analysis of the 16S rRNA gene sequence revealed that the strain belonged to the phylum *Alphaproteobacteria,* family *Rhodospirillaceae*, genus *Magnetospirillum*. Strain SpK formed an isolated cluster on the phylogenetic tree. The similarity between strain SpK and the known *Magnetospirillum* species was from 96.1 to 96.4%. Thus, the new microorganism was classified as a new species of the genus *Magnetospirillum, Magnetospirillum aberrantis* sp. nov.

Keywords: magnetotactic bacteria, taxonomy, 16S rRNA phylogeny, genus *Magnetospirillum, Magnetospirillum aberrantis* sp. nov., magnetosomes.

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Magnetotactic bacteria (MTB) possess a unique ability to form magnetic intracellular inclusions (magnetosomes). Due to the presence of magnetosomes, they are able to orient in the external magnetic field. Two types of MTB exist, those moving to the northern and to the southern geomagnetic pole or to the relevant poles of an external magnet. In microaerobic species, magnetosomes are usually comprised of the nanoparticles of magnetite Fe_3O_4, while in the strictly anaerobic ones they contain greigite Fe_3S_4. The crystals are single-domain structures 35 to 120 nm in size. The shape of these crystals may be rather diverse (hexagonal, rhombic, or toothlike) [1–3]. In the magnetosome, the crystals are surrounded by a CPM-like bilayer membrane.

Magnetotactic bacteria dwell mostly at the aerobic–anaerobic interface, especially at the boundary between the bottom sediments and near-bottom water. Both freshwater and marine forms are known among MTB, as well as halophiles and moderate thermophiles [2]. Most of the known species are microaerobes with respiratory metabolism. However, strictly anaerobic sulfate-reducing magnetotactic bacteria are also known [4]. Magnetotactic bacteria belong to various phylogenetic groups, mainly to the α-, β, γ-, and δ subgroups of the *Proteobacteria*, and to the phylum *Nitrospira* [5]. Magnetosomes were reported in archaea and in eukaryotic algae [6, 7]. Many magnetotactic bacteria were detected in the natural and laboratory ecosystems by cloning and subsequent analysis of the sequences of the 16S rRNA genes [8]. Few MTB species were obtained in pure cultures: magnetic cocci MC-1, magnetic vibrios MV-1, and three species of magnetic spirilla [3]. The genus *Magnetospirillum* includes three validly described species: *Magnetospirillum magneticum, M. magnetotacticum*, and *M. gryphiswaldense* [9]. The bacterial strain J10 phylogeneti-

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cally related to *M. gryphiswaldense* but exhibiting no magnetotactic properties is known [10]. The recently described perchlorate-reducing species *Magnetospirillum bellicus* also does not exhibit a magnetotactic reaction and does not possess the major genes responsible for magnetosome synthesis (*mam*I and *mam*L). No magnetosomes were revealed on the ultrathin sections of these microorganisms [11]. Many *Magnetospirillum* species have the gene encoding ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) form II (CbbM), one of the two key enzymes of the pentose phosphate cycle (Calvin cycle) for autotrophic CO_2 fixation. Autotrophic growth by oxidation of hydrogen, ferrous iron, or sulfide was shown for some magnetospirilla [10, 11]. The bacteria initially described as *Aquaspirillum polymorphum* are closely related to magnetospirilla [12]. Reclassification of this species within the genus *Magnetospirillum* was subsequently suggested [3]. Thus, the genus *Magnetospirillum* presently comprises both magnetotactic bacteria and those in which magnetosomes were not found.

In the present article, a new species of freshwater bacteria is characterized, *Magnetospirillum aberrantis* sp. nov., which forms irregular intracellular magnetite inclusions sharing some of the properties of magnetosomes.

MATERIALS AND METHODS

Isolation and cultivation conditions. The samples were collected from the coastal bottom sediment of the Ol'khovka River in the city of Kislovodsk (N 43°56′07″, E 42°41′25″). Enrichment cultures were obtained by microaerobic incubation of the medium inoculated with the bottom sediments. The medium contained the following (g/l): KH_2PO_4, 0.4; NH_4Cl, 0.33; KCl, 0.33; $MgCl_2$, 0.33; Na_2SO_4, 0.25; $Na_2S_2O_3$, 0.25; $NaNO_3$, 0.33; $NaHCO_3$, 0.25, sodium acetate, 1.0; and yeast extract, 0.1, as well as Fe(III) citrate, 30 μM; resazurin, 0.5 mg/l; sodium thioglycollate, 50 mg/l; vitamin B_{12}, 15 μg/l; and trace elements, 1 ml/l [13]. The trace element solution was prepared with citric acid as the stabilizing agent. Iron was not included in the trace element mixture and was introduced into the medium as ferric or ferrous iron citrate, Mohr's salt, or iron quinate, depending on the goal of the experiments. The final iron concentration was from 10 to 50 μM. The optimal oxygen concentration was determined by cultivation in sealed Hungate tubes two-thirds filled with the nitrogen–oxygen mixture. The concentration of oxygen was from 1 to 20%. Magnetic separation was used to obtain pure bacterial cultures [14]. Pasteur pipettes filled with the medium and inoculated with the enrichment culture were placed behind a magnetic bar and incubated for 15–60 min at a room temperature. Every 15 min, the tip was cut off the pipette and its content was transferred into a 5-ml syringe with the medium. Bacteria were grown in the syringes under microaerobic conditions with a small air bubble at 30°C, pH 6.7. The same medium was used for the subsequent cultivation of the isolates. The pure culture was grown in 20- or 50-ml penicillin vials.

Physiological and biochemical characteristics. For determination of the spectrum of utilized organic substrates, acetate in the medium was replaced with 1 g/l of the relevant compound. The capacity for utilization of various nitrogen species was determined by supplementing the medium deprived of nitrogen compounds with ammonium compounds, sodium nitrate or nitrite, or amino acids. The optimal growth temperature was determined by cultivation at different temperatures (20, 30, 35, 40, and 45°C). The pH optimum for growth was determined by cultivation at different initial pH values (from pH 6 to pH 9), obtained by addition of 5% NaOH or 5% sulfuric acid. Capacity for growth was assessed after a week of incubation as OD_{650} determined on a KFK-3 photometer (Russia). Thiosulfate consumption was determined by iodometric titration according to the standard procedure [15].

The catalase and oxidase activities were determined by the standard techniques: addition of 3% H_2O_2 or 1% tetramethyl-N-phenylenediamine hydrochloride, respectively, to the concentrated cell suspension.

Morphology and cell structure. The cell morphology was investigated under an Olympus BX-41 light microscope and a JEM-100CXII transmission electron microscope (80 kV). The total preparations for electron microscopy were contrasted with 1% phosphotungstic acid. Ultrathin sections were prepared as described earlier [16]. For the isolation and purification of magnetic particles, ultrasound treatment was used (Virsonic 600, United States) with subsequent separation on magnetic columns (MACS Separation Columns, Germany). The images and diffraction pictures of the magnetosomes of strain SpK were obtained on a TECNAI G2 30 transmission electron microscope (300 kV). The elemental composition was determined by energy dispersion X-ray spectroscopy.

Fatty acids composition. The cellular fatty acids were analyzed by chromatography and mass spectrometry. Dry cell biomass (5 mg) was incubated for 3 h at 80°C in 0.4 ml of 1 N HCl in methanol (acid methanolysis). The formed fatty acid methyl ethers and other lipid components were extracted with hexane and analyzed on a Sherlock gas chromatograph (Microbial identification system, MIDI Inc., United States) [17].

Composition of isoprenoid quinones. Wet biomass was homogenized in a mortar with liquid nitrogen and extracted with cold acetone. Thin-layer chromatogra-

phy of the extract was carried out in the hexane–diethyl ether system (85 : 15). The UV adsorption bands with R_f 0.65–0.75 belonged to the menaquinones, while those with R_f 0.2–0.3 belonged to ubiquinones. The eluate was analyzed on an LCQ ADVANTAGE MAX tandem mass spectrometer with the source of chemical ionization at atmospheric pressure (APCI) [18].

DNA G + C content. DNA was isolated from the cultures according to Marmur [19]. The DNA G + C content was determined by thermal denaturation as described by Owen et al. [20].

Amplification and cloning of the gene fragments encoding 16S rRNA. DNA was isolated from bacterial biomass as described earlier [21].

The universal primer system [22] was used for amplification of the 16S rRNA gene fragments. The PCR products were isolated and purified in low-melt agarose using the Wizard PCR Preps reagent kit (Promega, United States) according to the manufacturer's recommendations. The purified PCR products were cloned using the pGEM-T Easy System reagent kit (Promega, United States) according to the manufacturer's recommendations.

Sequencing of the PCR products and cloned fragments was carried out on ABI PRISM 3100 and ABI 3730 automatic sequencers (Applied Biosystems, United States) using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, United States) according to the manufacturer's recommendations, using the universal primers.

Phylogenetic analysis. Primary analysis of the similarity between the nucleotide sequences of the 16S rRNA gene was carried out using the BLAST server (http://www.ncbi.nlm.nih.gov/blast). The sequences were aligned with the relevant sequences of related bacterial species using the CLUSTAL W software package [23]. The phylogenetic trees were constructed using the methods implied in the TREECON software package [24]. The sequences of the genes of the key Calvin cycle enzymes were determined based on the results of the annotation of the complete genome of the strain SpK (unpublished data).

RESULTS AND DISCUSSION

Phylogenetic analysis of the enrichment culture. A library of full-sized (Univ11F-Univ1492R, [22]) PCR fragments of 16S rRNA genes from the total DNA of the enrichment culture was created. The library contained 50 clones. Analysis of the clonal inserts revealed sequences closely related to *Azospira oryzae* (99%), *Dechlorosoma suillum* (99%), *Bacillus* sp. (99%), *Trichococcus pasteuri* (99%), *Trichococcus collinsi* (99%), *Nostocoida* type I sp. (99%), *Clostri-dium tunisiense* (99%), *Porphyromonadaceae* sp. (97%), *Azonexus* sp. (99%), and *Dechloromonas* sp. (99%). The sequence of one clone was related to the sequences of the known species of magnetotactic bacteria of the genus *Magnetospirillum* (*M. magneticum* MS-1, 96% and *M. gryphiswaldense*, 96%).

The goal of the subsequent work was isolation of a pure culture of the magnetospirillum from the studied enrichment.

Isolation of pure culture. The pure culture designated as strain SpK was obtained by several sequential stages of magnetic separation in the capillaries of the Pasteur pipettes adjacent to the north pole of a magnet. Comparative analysis of the 16S rRNA sequence of the strain SpK revealed its identity with the *Magnetospirillum*-like sequence identified in the enrichment.

Cultural properties. In Hungate tubes with semisolid medium (10 ml) and air (5 ml) in the gas phase, strain SpK grew 0.5 cm below the surface, an indication of their microaerobic nature. The bacteria were catalase-negative and oxidase-positive. On solid media (2% agar) in an anaerobic chamber (Oxoid, United States) with 95% N_2 and 5% O_2, round colonies 1–2 mm in diameter were formed. The colonies were white with a yellow tint. Bacteria of strain SpK grown on media with various iron sources and various levels of oxygen in the gas phase did not exhibit expressed magnetotaxis. During magnetic separation, the spirilla were probably concentrated in the sterile part of the capillary, beyond the cotton barrier, due to their high motility, independent on the magnetic field.

Morphology and ultrastructure of strain SpK. In pure culture, bacteria were spirilla-shaped (Figs. 1a, 1b). The cell diameter was 0.3–0.4 μm; the length varied from 1.5 to 5 μm. The cells were motile, with polar flagella (Fig. 1b). As a rule, a single flagellum was located at each end of the cell. Ultrathin sectioning revealed a multilayered cell wall typical of gram-negative bacteria (Fig. 2). The outer wavy cell-wall layer formed projections with a visible internal channel (Fig. 2a). Intracellular parietal membrane bubbles (~60 nm) formed by the CPM were sometimes observed (Figs 2b, 2c). On the total preparations and ultrathin sections, dense rounded granules (~100 nm), probably of polyphosphates, were observed, as well as unidentified medium-density inclusions of irregular hexagonal shape and almost electron-transparent inclusions (150 nm or more) similar to PBHB (poly-β-hydroxybutyrate) inclusions in morphology and density (Figs. 2c–2e).

Iron-containing intracellular inclusions. Strain SpK formed small dense granules, in clusters or in short chains (Figs 1c, 1f). Long chains characteristic of magnetosomes were not formed. These inclusions (30–40 nm) were of a rounded shape. Sometimes, the edges were visible, resembling the tetra- or hexahedral

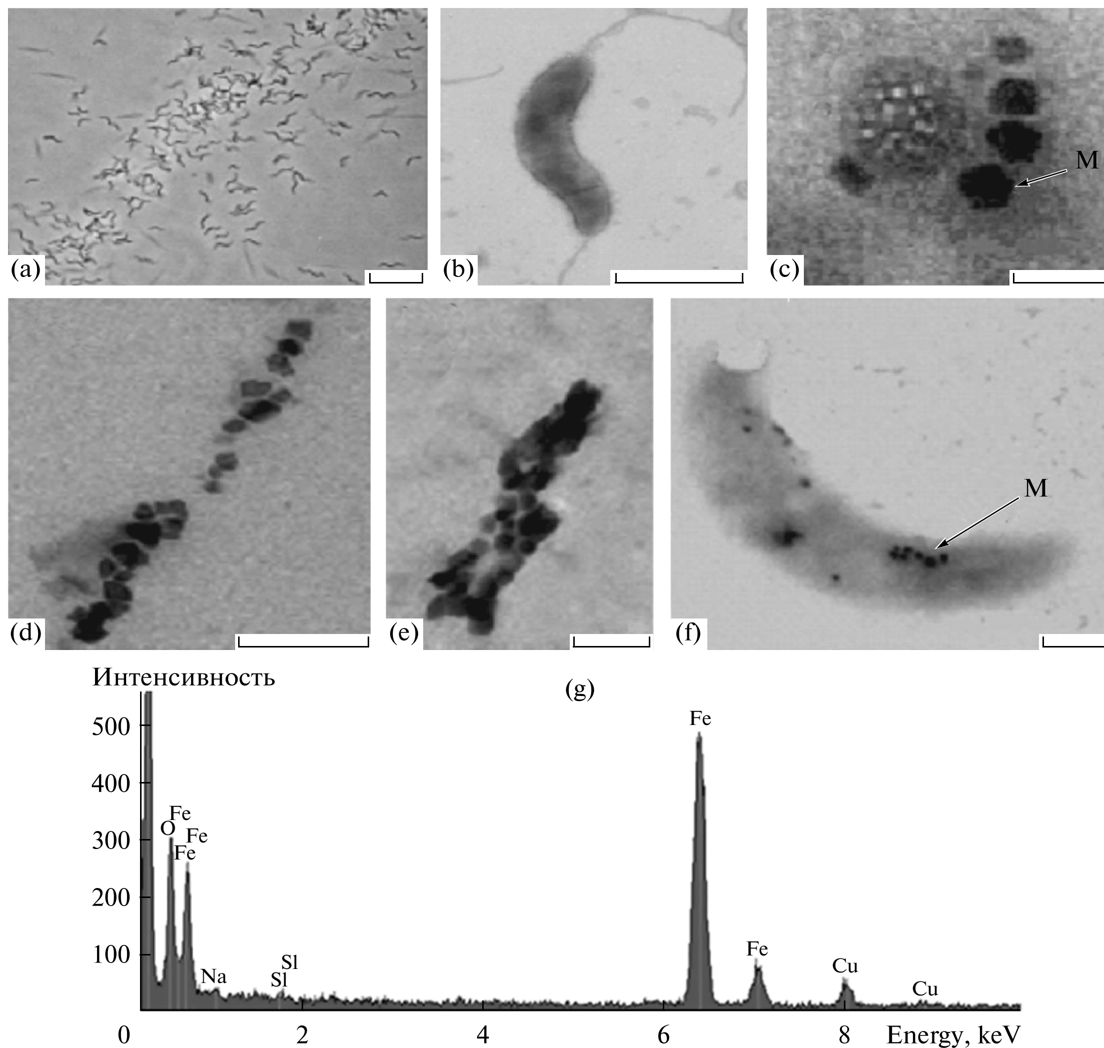

Fig. 1. Morphology of the cells and magnetic inclusions of *M. aberrantis.* Phase contrast (a). Electron microscopy, total samples: flagella at cell poles (b), intracellular magnetic inclusions (M) as clusters and short chains (c, f), magnetosome conglomerates of the type strain of *M. magnetotacticum* (d) and *M. aberrantis* (e). According to energy dispersion X-ray spectroscopy, the elemental composition of *M. aberrantis* mineral inclusions coincides with that of magnetite (g). Scale bar, 5 μm (a), 1 μm (b), 100 nm (e), 50 nm (c), and 200 nm (d), 0.3 μm (f).

crystals of immature magnetosomes [25]. The number of such inclusions per cell could be up to ten. No dense magnetic particles were found on ultrathin sections, probably due to their low abundance. However, the invaginations of vesicular membranes, similar to those reported for the magnetosome-forming microorganisms [26] were present. The parietal vesicles were ~60 nm in diameter (Figs 2b, 2c). In real magnetotactic bacteria, the chains of vesicles are gradually filled with magnetite, which forms regular crystals in the course of maturation. We found that the iron-containing inclusions of strain SpK possessed magnetic properties. The iron-containing particles isolated from the SpK cells on magnetic columns were morphologically similar to the magnetic particles of the type strain *M. magnetotacticum* (Figs 1e, 1f). The size of the individual particles varied from 30 to 40 nm.

Elemental composition of the inclusions. Energy dispersion X-ray spectroscopy revealed that the particles contained mostly iron and oxygen (Fig. 1g), and the diffraction picture correlated with the crystal lattice of magnetite. Thus, the isolated strain is able to synthesize nanometer-sized magnetic particles, which are not aligned in the fibrillar structures typical of the magnetosomes of true MTB.

Growth characteristics and physiological and biochemical properties of strain SpK. Aerobic heterotrophic metabolism is the main metabolic type of this microorganism. However, the operon (Mabe00289 and Mabe00290, our unpublished data) was found in the genomic DNA, which contained

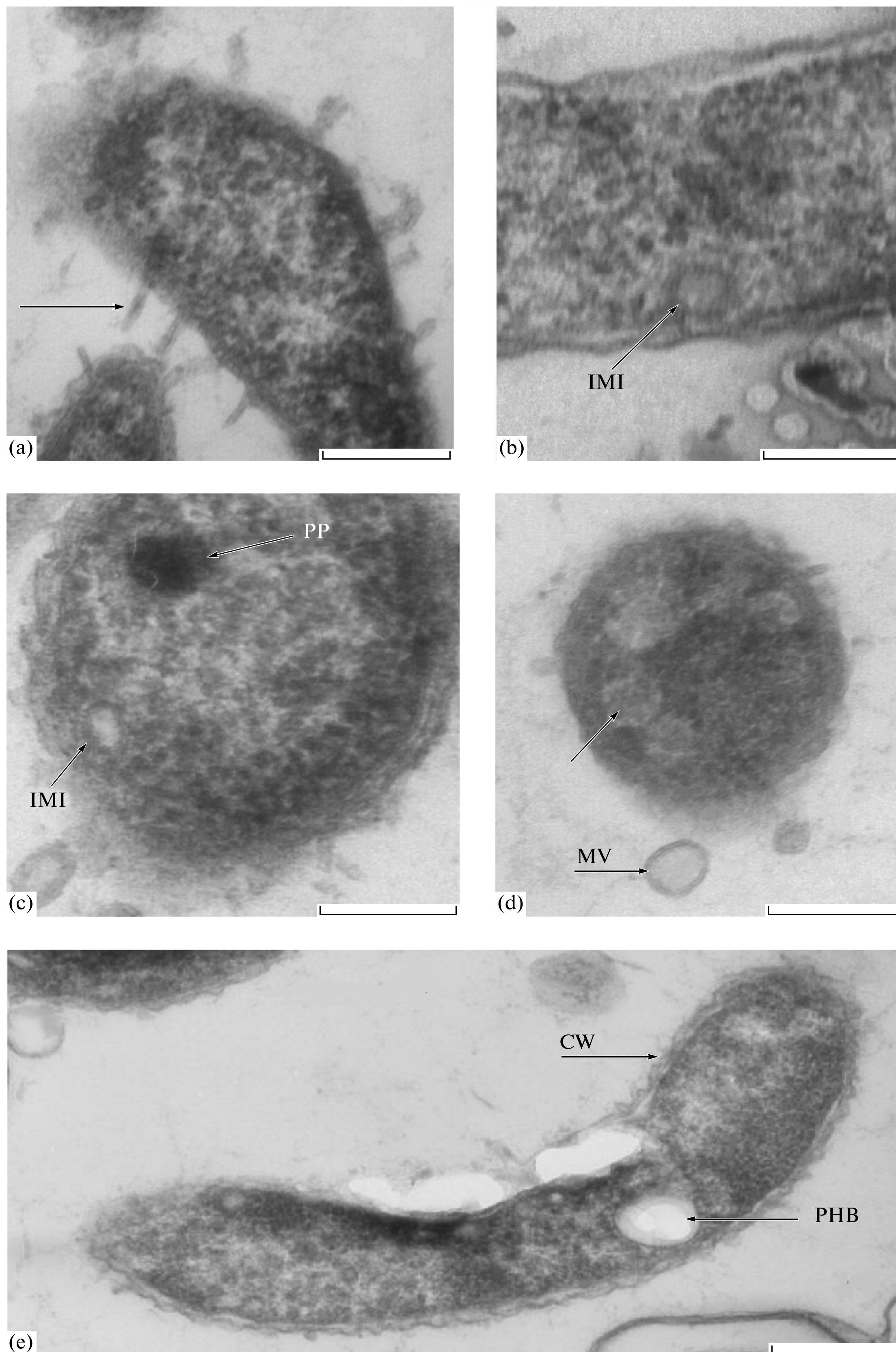

Fig. 2. Electron microscopy, ultrathin sections of *M. aberrantis*: three-layer cell wall, protrusions or pili (arrows) (a), intracellular CPM-associated membrane vesicles (MV) ~50–60 nm in size (b, c), intracellular membrane inclusions (IMI) (d), large polyphosphate (PP) granules (c), poly-β-hydroxybutyrate (PHB), and unknown large round inclusions (arrows) (e). Scale bar: 0.2 μm (a, e), 0.12 μm (b, c), and 0.15 μm (d).

both genes encoding the Calvin cycle key enzymes, rubulose-1,5-bisphosphate carboxylase/oxygenase and phosphoribulokinase. BLAST analysis revealed that these enzymes exhibited the highest similarity in the amino acid sequences to the analogous enzymes of *M. gryphiswaldense* MSR-1 (gi|144900524|emb, 73% and gi|144900525|emb, 87%). This finding indicated that strain SpK was probably capable of autotrophic growth. In the present work, this capacity was not investigated in detail. It was, however, determined that thiosulfate, ammonium, and ferrous iron could not be used as electron donors for autotrophic growth. The strain was found to be microaerobic, with the best growth occurring at 1–5% O_2. Catalase activity was absent, while oxidase activity was detected. When grown microaerobically with fumarate, bacteria reduced nitrate to nitrite. Although nitrite was further reduced, production of molecular nitrogen was not found. Sulfate, thiosulfate, ferric iron, and perchlorate could not be used as electron acceptors for photoheterotrophic growth of strain SpK. The bacteria grew within the temperature range from 20 to 45°C with optimal growth at 31°C. The best growth occurred at pH 6.5–6.9.

The content of magnetic particles in the cells did not depend on the iron source in the medium. Prolonged (six months) cultivation of strain SpK in the standard medium resulted in a significant decrease in their capacity for synthesis of magnetic particles. Partial or complete loss of capacity for magnetosome synthesis after prolonged cultivation has been reported for the typical MTB of the genus *Magnetospirillum* [27]. No dependence was revealed between formation of magnetic particles by strain SpK and the type of organic substrate.

Strain SpK grew well in the media with various organic acids, especially on acetate and fumarate. Sugars and alcohols were not utilized. Importantly, the strain did not grow on casein hydrolysate and grew well on glycerol (Table 1). Vitamins were not required for growth. Addition of yeast extract resulted in an insignificant increase of the biomass yield for acetate-grown cells.

Capacity for utilization of nitrogen compounds for biosynthesis was determined on the medium with fumarate. Ammonium salts and nitrates provided the best growth. Urea and most amino acids did not support growth.

Bacterial growth occurred in the media with sulfates as sulfur sources for the assimilation processes.

The capacity for oxidation of sulfide or hydrogen was not determined.

Composition of fatty acids and isoprenoid quinones. Generally, the fatty acid composition for strain SpK was similar to that of other *Magnetospirillum* species. Hexadecanoic and octadecenoic acids and derivatives thereof were predominant. Importantly, the strain contained over 50% of the octadecenoic acid (18: 1ω7) and did not contain 18:0 3OH (Table 2).

Similarly to other magnetospirilla, only ubiquinone Q_{10} was found.

Taxonomic position. BLAST analysis of the 16S rRNA gene sequences (1452 bp) showed that the isolate belonged to the phylum *Alphaproteobacteria,* family *Rhodospirillaceae.* Together with the sequences of two uncultured magnetospirilla revealed in bacterial communities degrading chlorinated aromatic compounds, strain SpK formed an isolated cluster on the phylogenetic tree (Fig. 3). The similarity levels with the most closely related validly described species *M. bellicus* VDY EF405824, *M. gryphiswaldense*, and other magnetospirilla were 96.4, 95.4, and 94.9–96.1%, respectively (Table 3). Thus, the new isolate may be considered a member of the genus *Magnetospirillum.*

The chemical composition and other phenotypic characteristics of the new species fit the description of the genus *Magnetospirillum.* Like other magnetospirilla, the new microorganism is a microaerobe, contains ubiquinone Q_{10}, and has a similar fatty acid composition of the cell wall. The DNA G + C content is 62.6 mol %. However, strain SpK differs from the known *Magnetospirillum* species in some phenotypic properties. Unlike the true magnetotactic bacteria *M. magnetotacticum, M. magneticum*, and *M. gryphiswaldense*, they do not contain typical magnetosomes in chains, but synthesize irregularly located magnetic particles. Strain SpK may, therefore, be considered a transitional form between true MTB and bacteria capable of iron reduction and magnetite biomineralization, but not of magnetotaxis. The new organism differs also in the spectrum of utilized substrates, being unable to grow on casein hydrolysate and growing well on glycerol. Unlike the species *M. bellicus*, which also does not form magnetosomes, the new strain does not use perchlorates as electron acceptors. Strain SpK is capable of nitrate reduction with formation of nitrites. Nitrogen is not formed as the terminal product of nitrate reduction. Thus, the genetic phenotypic properties of strain SpK support its classification as a new species of the genus *Magnetospirillum.* We propose the species name *Magnetospirillum aberrantis* SpK sp. nov.

Description of the species *Magnetospirillum aberrantis* sp. nov. *abe'rrantis,* L. neut. adj. aberrant, different (from others).

The cells are spirilla, 0.3–0.4 μm in diameter and 1.5 μm or more long, motile, with polar flagella. The cells are gram-negative, with a wavy outer membrane forming pili and membrane vesicles. Under some conditions, sparse magnetic particles (30–40 nm), single

Table 1. Comparative physiological characterization of *M. aberrantis* SpK and the known *Magnetospirillum* species

Feature	Representatives						
	M. aberrantis	*M. bellicus* [11]	*M. magneto-tacticum* [9]	*M. gryph-iswaldense* [9]	*M. magneticum* [9]	J10 [10]	CC-26 [11]
Shape	Sp.[1]	Sp.	Sp.	Sp.	Sp.	Sp.	Sp.
Size, µm	0.3–0.4 × 1.5–5	0.5 × 3	0.4 × 4	0.7 × 4	0.5 × >3	0.5 × 4	0.5 × 5
Motility	+	+	+	+	+	+	+
Temperature range, °C	20–45	<10–42	15–37	ND[2]	ND	ND	ND
Temperature optimum, °C	31	42	30	28–34	28–34	ND	ND
pH range	ND	6.0–7.5	ND	ND	5.8–8.2	ND	ND
pH optimum	6.5–6.9	6.8	7.0–7.5	7.0–7.5	7.0–7.5	ND	ND
NaCl tolerance, %	ND	1.5	<1	ND	ND	ND	ND
Spore formation	–	–	ND	ND	ND	ND	ND
Catalase/oxidase activity	–/+	–	–	–	–	ND	ND
DNA G + C content, mol %	62.6	64.8	66.4	71	65.1	ND	ND
cld (chloride dismutase)	–	+	+	–	–	ND	ND
Magnetotaxis	–	–	+	+	+	–	–
Electron acceptors							
Oxygen	+	+	+	+	+	+	+
Perchlorate	-	+	ND	ND	ND	–	ND
Chlorate	ND	+	ND	ND	ND	–	ND
Nitrate	+	+	+	–	+	+	+
Nitrite	+	+	ND	ND	ND	ND	ND
N_2O	ND	+	ND	ND	ND	ND	ND
Sulfate	–	ND	ND	ND	ND	ND	ND
Thiosulfate	–	–	ND	ND	ND	ND	ND
Selenite	ND	–	ND	ND	ND	ND	ND
Arsenate	ND	–	ND	ND	ND	ND	ND
Fumarate	–	–	ND	ND	ND	–	ND
Malate	ND	–	ND	ND	ND	ND	ND
Fe(III) NTA[3]	–		ND	ND	ND	ND	ND
AQDS[4]	ND	–	ND	ND	ND	ND	ND
Electron donors							
Acetate	+	+	+	+	+	+	+
Propionate	+	+	–	ND	+	ND	+
Isobutyrate	ND	+	–	ND	ND	ND	+
Butyrate	+	+	–	ND	+	ND	+
Valerate	ND	+	ND	ND	ND	ND	–
Formate	ND	–	–	ND	ND	ND	–
Methanol	–	–	–	ND	ND	ND	–
Ethanol	–	+	–	ND	ND	ND	–
Catechol	ND	–	–	ND	ND	ND	–
Glycerol	+	–	–	ND	ND	ND	–

Table 1. (Contd.)

Feature	Representatives						
	M. aberrantis	*M. bellicus* [11]	*M. magnetotacticum* [9]	*M. gryphiswaldense* [9]	*M. magneticum* [9]	J10 [10]	CC-26 [11]
Benzoate	ND	–	–	ND	ND	ND	+
Pyruvate	+	+	+	+	+	ND	+
Citrate	–	–	–	ND	ND	ND	-
Succinate	+	+	+	+	+	ND	+
Fumarate	+	+	+	ND	+	ND	+
Tartrate	+	ND	ND	ND	ND	ND	ND
Malate	ND	-	ND	ND	ND	ND	ND
Lactate	+	+	+	+	+	ND	+
Glucose	–	–	–	ND	ND	ND	–
Sucrose	–	–	ND	–	ND	ND	–
Fructose	–	–	–	ND	ND	ND	–
Arabinose	–	ND	ND	ND	ND	ND	ND
Maltose	ND	+	–	–	ND	ND	–
Soytone	–	ND	ND	ND	ND	ND	ND
Yeast extract	+	ND	ND	ND	ND	ND	ND
Casein hydrolysate	–	+	ND	ND	ND	ND	ND
Hydrogen	ND	+	ND	ND	ND	ND	ND
$FeCl_2$	ND	+	ND	ND	ND	–	ND
AHDS[5]	ND	+	ND	ND	ND	ND	ND
H_2S	ND	ND	ND	+	ND	+	ND
Thiosulfate	–	ND	ND	+	ND	+	ND
Methane	ND	–	ND	ND	ND	ND	ND
Urea	–	–	ND	ND	ND	ND	ND

Notes: [1]Sp., spirilla; [2]ND, not determined; [3]NTA, nitrilotriacetic acid; [4]AQDS, anthraquinone-2,6-disulfonate; [5]AHDS, 2,6-anthrahydroquinone disulfonate.

or in small clusters, are synthesized. CPM invaginations (not numerous) in the form of 60-nm bubbles are visible on ultrathin sections; their interior is of low electron density. The organism is microaerobic, with best growth occurring at 1–5% O_2 in the gas phase. Yellowish colonies 1–2 mm in diameter are formed on agar media. Growth in semisolid agar is in the form of a ring 3–5 mm below the surface. The organism is catalase-negative and oxidase-positive. The basic type of metabolism is chemoorganotrophy. The genes of RuBisCo form II and phosphoribulokinase, the key enzymes of the Calvin cycle, are present in the genome. The cells grow within the temperature range from 20 to 45°C with the optimum at 31°C. The optimal pH for growth is 6.5–6.9.

Various organic acids (malate, fumarate, butyrate, acetate, succinate, lactate, pyruvate, and propionate) and glycerol are utilized. Protein hydrolysates are not utilized. Vitamins are not required. Addition of yeast extract results in an insignificant increase in the biomass yield of acetate-grown cultures. During microaerobic growth, nitrate is reduced to nitrite. Nitrite is sub-

Table 2. Comparison of the fatty acid composition of *M. aberrantis* and the known *Magnetospirillum* species

Fatty acid	*M. aberratis*	*Aquaspirillum polymorphum* (strain 13961) [12]	*M. gryphiswaldense* [9]	*M. magnetotacticum* [9]	*M.bellicus* (VDY) [11]
12 : 0	0.44	ND	ND	2	3.25
12 : 1	ND	2	ND	ND	ND
14 : 1	ND		2	ND	ND
14 : 0 3OH	ND	66	66	58	
14 : 1ω5	0.13	ND	ND	ND	ND
14 : 0	0.62	4	3	1	0.37
16 : 1ω9	0.33	ND	ND	ND	ND
16 : 1ω7	11.12	ND	ND	ND	ND
16 : 1ω5	0.12	ND	ND	ND	ND
16 : 1		13	25	30	
16 : 0 3OH		6	18	39	0.75
16 : 0	19.23	20	7	25	12.01
3h14	1.74	ND	ND	ND	ND
18 : 1ω9	0.55	ND	ND	ND	ND
18 : 1ω7	58.19	ND	ND	ND	ND
18 : 1ω7c	ND				64.64
18 : 1	ND	58	56	41	
18 : 1 2OH	ND	ND	ND	ND	1.23
11 methyl 18 : 1ω7c	ND	ND	ND	ND	0.83
18 : 0 OH	ND	28	16	3	0.33
18 : 0	1.91	1	5	1	1.89
2h18 : 1	5.36	ND	ND	ND	ND
3h18 : 0	0.28	ND	ND	ND	ND

Table 3. Similarity levels between 16S rRNA sequences of different magnetospirilla species

	Magnetospirillum aberrantis SpK	*Magnetospirillum magneticum* AMB-1 AP007255	*Magnetospirillum bellicus* VDY EF405824	*Aquaspirillum polymorphum* DSM 9160 FJ562215	*Phaeospirillum molischianum* ATCC 14031 M59067	*Magnetospirillum magnetotacticum* DSM 3856 NR_026381	*Magnetospirillum gryphiswaldense* MSR-1 NR_027605	*Magnetospirillum magneticum* MGT-1 D17515
Strain SpK	ID*	0.950	0.964	0.961	0.911	0.949	0.954	0.947
Magnetospirillum magneticum AMB-1 AP007255 [9]	0.950	ID	0.952	0.946	0.936	0.998	0.954	0.996
Magnetospirillum bellicus VDY EF405824 [11]	0.964	0.952	ID	0.952	0.924	0.950	0.957	0.949
Aquaspirillum polymorphum DSM 9160 FJ562215 [12]	0.961	0.946	0.952	ID	0.923	0.944	0.972	0.943
Phaeospirillum molischianum ATCC 14031 M59067 [26]	0.911	0.936	0.924	0.923	ID	0.938	0.926	0.933
Magnetospirillum magnetotacticum DSM 3856 NR_026381 [9]	0.949	0.998	0.950	0.944	0.938	ID	0.952	0.994
Magnetospirillum gryphiswaldense MSR-1 NR_027605 [9]	0.954	0.954	0.957	0.972	0.926	0.952	ID	0.950

* ID – identical.

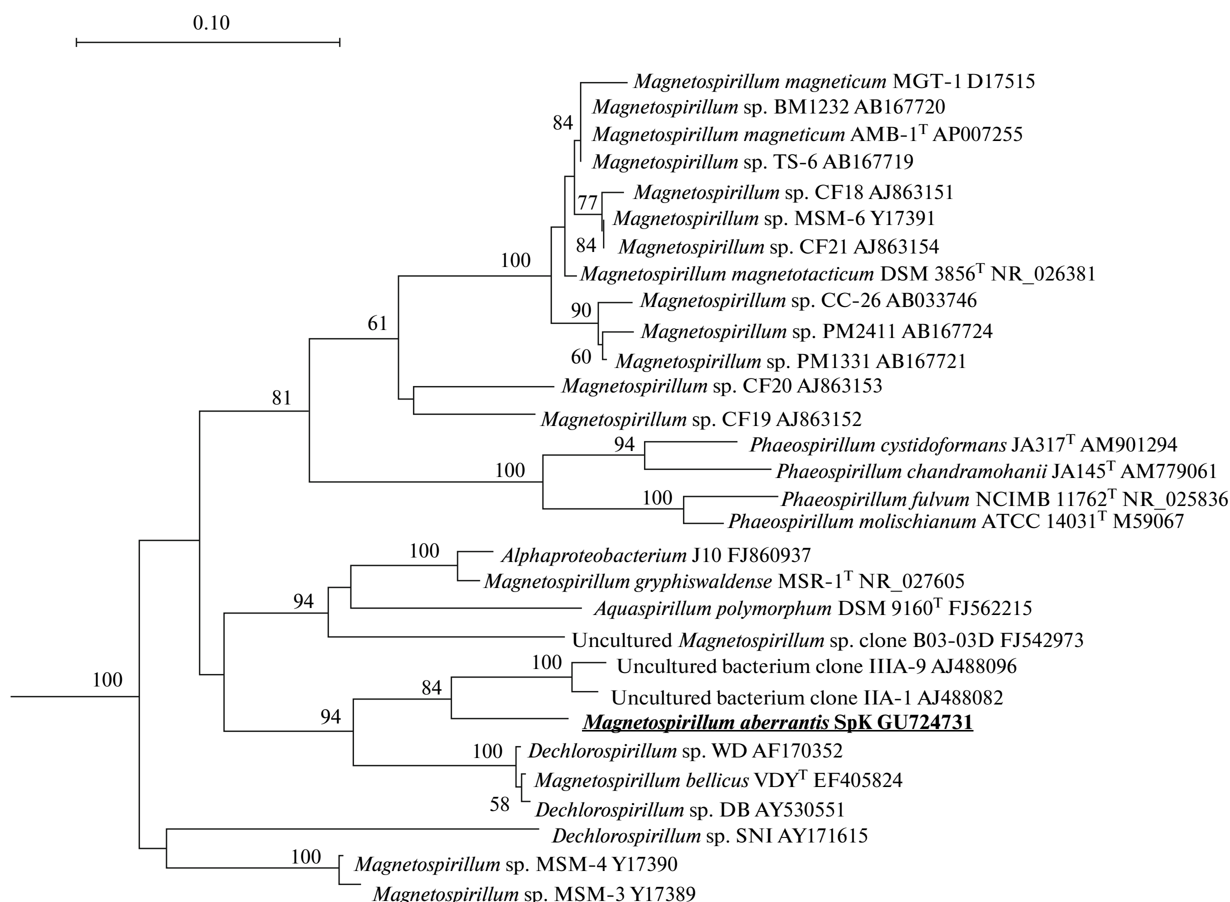

Fig. 3. Phylogenetic position of strain SpK. The numerals indicate the bootstrap values. The strain under study is in boldface. The evolutionary distance scale is given above. Neighbor-joining algorithm. The type strains are marked with *T*. The 16S rRNA gene sequence from the magnetite-containing coccus (GenBank no. L06456) was taken as an outgroup.

sequently reduced, although molecular nitrogen is not formed. Sulfate, thiosulfate, ferric iron, and perchlorate are not used as electron acceptors for photoheterotrophic growth.

The cells contain ubiquinone Q_{10}. The major fatty acids are 18:1ω7 (58.19%), 16:0 (19.23%), 16:1ω7 (11.12%), and 18:0 (1.91%).

The DNA G + C content is 62.6 mol %.

Poly-β-hydroxybutyrate and polyphosphates are the storage compounds.

The organism was isolated from the coastal bottom sediments of the Ol'khovka River near Kislovodsk (Russian Federation).

Type strain SpK is deposited in the VKPM B-11049.

The 16S rRNA gene sequence is deposited in GenBank; accession no. GU724731.

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