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EXPERIMENTAL ARTICLES

Candidatus 'Chloroploca asiatica' gen. nov., sp. nov., a New Mesophilic Filamentous Anoxygenic Phototrophic Bacterium

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Abstract—Five phylogenetically similar monocultures of mesophilic filamentous anoxygenic phototrophic bacteria (FAPB) were isolated from microbial mats of low-mineral (5–28 g/L) alkaline lakes in Buryat Republic, Transbaikalia and Mongolia, as well as from biofilms of an alkaline sulfide spring (3 g/L) of the Umhei hydrothermal system (Buryat Republic). New isolates were characterized by short trichomes (15–30 μm long and ~1 μm in diameter), straight, curved, or wavy, surrounded by a thin iron-sorbing mucous sheath. Gliding motion of the trichomes was not observed. The trichomes formed bunches consisting of several filaments. Trichomes multiply by the separation of short fragments or single cells from the parental trichome. The cells in the filaments were elongated; they contained chlorosomes, gas vesicles, poly-β-hydroxybutyrate granules, and small polyphosphate inclusions. Bacteria contained bacteriochlorophylls *c* and a and γ-carotene. Absorption maxima of the pigments in the cells were observed at 462, (shoulder at 515), 742, 805, and 863 nm. The organisms were strict anaerobes capable of photoautotrophic growth with sulfide as an electron donor. Elemental sulfur emerged into the medium as a result of sulfide photooxidation. The organisms were tolerant to sulfide (up to 8 mM). Best growth occurred at pH 8.0, 3–15 g/L NaCl, and 1–5 g/L sodium bicarbonate. According to phylogenetic analysis, the 16S rRNA gene sequences of the FAPB isolates formed a separate cluster most closely related to the species cluster of the family *Oscillochloridaceae*, suborder *Chloroflexinae*, order *Chloroflexales*, class *Chloroflexi*. The differences with the closest 16S rRNA gene sequences of the known FAPB were 9–10%. The formal description of a new taxon, *Candidatus* 'Chloroploca asiatica' gen. nov., sp. nov., is provided.

Keywords: soda lakes, alkaliphilic phototrophic communities, mesophilic filamentous anoxygenic phototrophic bacteria, *Chloroflexi* phylogeny

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Filamentous anoxygenic phototrophic bacteria (FAPB) were initially found in microbial mats of thermal springs [1]. During the subsequent 40 years, two species of chlorosome-containing FAPB belonging to a new genus *Chloroflexus* were described (*Cfl. aurantiacus* and *Cfl. aggregans*), as well as two new FAPB genera with two species not containing chlorosomes, *Heliothrix oregonensis* and *Roseiflexus castenholzii* [2–4]. Thus, the species diversity of thermophilic FAPB is not high.

Recent molecular genetic studies revealed that members of the genera *Chloroflexus, Heliothrix*, and *Roseiflexus* formed deep phylogenetic branches at the family level in the *Chloroflexi* cluster, indicating early divergence within this ancient phylum [5–7].

Mesophilic *Chloroflexus*-like bacteria (CLB) morphologically and physiologically similar to thermophilic *Cfl. aurantiacus* were isolated from freshwater environments in 1975 [8, 9]. Two new genera with three species of freshwater FAPB containing gas vesicles were described at the same period: *Chloronema giganteum*, *Oscillochloris chrysea*, and *Osc. trichoides* [10–12]. Only *Osc. trichoides* strains were obtained as pure cultures. Research on their physiology and phylogeny resulted in the description of a new family, *Oscillochloridaceae* [5]. Investigation of a number of freshwater FAPB enrichment cultures isolated from Spanish meromictic lakes made it possible to modify the characterization of the family *Oscillochloridaceae*; according to the results of comparison of the 16S rRNA gene sequences, it also included *Chloronema giganteum* [13].

FAPB were revealed in algo–bacterial mats from marine and hypersaline environments, as well as in microbial mats from continental saline and soda lakes [14–17]. Filamentous anoxygenic bacteria formed close associations with marine mat-forming cyanobacteria *Microcoleus chthonoplastes*. High FAPB diversity was revealed in marine and hypersaline environments [15, 18]. At least two new CLB phenotypes

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Table 1. Characterization of the sources of isolation of *Candidatus* 'Chloroploca asiatica'

Isolate	Source name	pH	Mineralization, g/L	Temperature, °C	Region	Coordinates
Um-3	Umhei sulfide spring	9.2	3	18	Barguzin Valley, Buryat Republic	54°59.5′ N 111°07.5′ E
B11-1	Lake Orongoisoe	9.1	6	18.2	Ivolginsky region	51°31.57′ N 106°58.52′ E
A35-1	Sulfatnoe	9.3	5	16.1	Selenga region, Buryat Republic	51°32.5′ N 107°02.5′ E
B7-9^T	Lake Doroninskoe	9.8–10.2	23–28	11.5	Transbaikalia	51°25.5′ N 112°28.5′ E
M50-1	Chuchyn-Nur	9.3	5	20	Eastern Mongolia	49°31.66′ N 114°39.25′ E

were described in saline habitats, one of which, an inhabitant of hypersaline lagoons, was validated as a new taxon, *Candidatus* 'Chlorotrix halophile' [19]. Analysis of the 16S rRNA gene sequences demonstrated that *Candidatus* 'Chlorotrix halophile' formed a deep phylogenetic branch within the order *Chloroflexales*. The only isolate of *Candidatus* 'Chlorotrix halophile' is presently maintained as an enrichment culture.

Attempts at isolation of pure cultures of most mesophilic FAPB from freshwater and saline environments proved unsuccessful. Thus, only a few strains of freshwater *Osc. trichoides* are presently available as pure cultures. These organisms were shown to be capable of photoautotrophic growth using the Calvin cycle, unlike thermophilic *Cfl. aurantiacus*, which fix CO_2 via the hydroxypropionate cycle [20–22].

FAPB are the most ancient phototrophic microorganisms known [6, 7, 22]. Discovery of mesophilic CLB makes it possible to suggest their involvement in formation of Precambrian stromatolites, which are known to have been formed at moderate temperatures in ancient shallow marine or lake water bodies [22]. Mesophilic CLB were found to play an important role in the functioning of benthic algo–bacterial communities in the present-day aquatic ecosystems which may be considered analogous to ancient water bodies [17]. For instance, the role of CLB in oxygen consumption by microbial mats and their active participation in the carbon and sulfur cycles in microcosms were established.

These findings indicate the importance of investigation of occurrence, diversity, and role of mesophilic CLB in natural environments.

The present work provides phenotypic and phylogenetic characterization of five mesophilic FAPB isolates obtained from microbial mat samples of four alkaline, low-mineral lakes in Buryat Republic, Transbaikalia, and Mongolia, as well as from one thermal alkaline spring of the Umhei hydrothermal system (Buryat Republic). Description of a new taxon *Candidatus* 'Chloroploca asiatica' gen. nov., sp. nov. is provided.

MATERIALS AND METHODS

Source of isolation. Four new FAPB monocultures were isolated from the samples of microbial mats from the coastal zone of soda lakes Doroninskoe (Transbaikalia, Russia), Sul'fatnoe and Orongoiskoe (Buryat Republic, Russia), and Chukhyn-Nyr (Mongolia) (Table 1). One more culture was isolated from the biofilms of a thermal spring in the Umhei hydrothermal system (Baikal rift zone, Barguzin valley, Buryat Republic). The samples were collected in September 2010–2012. Salinity and pH in the lakes and spring varied from 3 to 28 g/L and from 9.1 to 10.2, respectively. Trace amounts of sulfide were present in the water. Microbial mats were formed above sulfide-bearing sediments. Designations of the cultures, as well as the names, locations, and general characteristics of the sources of isolation are presented in Table 1.

Cultivation. The medium used contained the following (g/L): NH_4Cl, 0.5; KH_2PO_4, 0.5; $MgCl_2$, 0.2; NaCl, 5.0; KCl, 0.3; $NaHCO_3$, 2.0; yeast extract, 0.1; Na acetate, 0.5; $Na_2S \cdot 9H_2O$, 0.5; Pfennig trace elements solution, 1 mL/L; pH 8.0. Sterile solutions of $Na_2S \cdot 9H_2O$ (10%), $NaHCO_3$ (10%), yeast extract (5%), and sodium acetate (10%) were prepared separately and added to the medium prior to inoculation. Cultivation was carried out under anoxic conditions in 45-mL screw-capped vials. Isolation of pure cultures was carried out by terminal dilutions in agar medium (0.7 g/L) and subsequent transfer of the single colonies. The cultures were grown at 28°C and illuminated by incandescent bulbs (2000 lx).

Morphology and ultrastructure. Cell morphology was examined under an Olympus BX 41 phase contrast microscope. For electron microscopy, the material was fixed according to Kellenberger, dehydrated, and embedded in Epon. Ultrathin sections on formvar-coated copper grids were contrasted with the Reynolds reagent [9] and examined under a Jeol JEM–100C electron microscope (Jeol, Japan) at 80 kV.

Photosynthetic pigments. The pigment composition was determined from absorption spectra of cell suspensions in 50% glycerol within the 350–1100 nm

wavelength range (SF 56A, LOMO, Russia). Spectral characteristics of acetone–methanol (7 : 2) extracts of the cells were also examined.

Carotenoid pigments were analyzed by HPLC on an Agilent Zorbax SB-C18 column (5 μm × 4.6 mm × 250 mm (Agilent, United States) using the LC-solution software package (Shimadzu, Japan) [23]. Carotenoids were identified according to their retention times and spectral characteristics.

Physiological characteristics and growth conditions. Capacity for anaerobic growth under light and aerobic growth in the dark was determined for the FAPB isolates, as well as the NaCl concentrations, temperature, and pH optimal for their growth. Capacity for photoautotrophic and chemolithotrophic growth (in the dark) was assessed as growth on agar media without organic substrates and with sulfide (0.3 to 2.0 g/L) as the sole electron donor. Capacity for aerobic or microaerobic growth in the dark was determined by growth in the upper part of the agar stabs, where oxygen penetrated. In some experiments, organic substrates were added (0.5 g/L) as additional carbon sources, apart from CO_2.

Molecular genetic investigation. DNA was extracted from FAPB cultures using the CTAB method [24] with minor modifications.

Amplification of the 16S rRNA gene fragments was carried out using the primer system specifically developed for phototrophic *Chloroflexi*: ChiF (5'-TGGCTCAGGACGAACGCT-3') and ChiR (5'-AGTCGCGACCCCTGCCCT-3'). The reaction profile was as follows: first cycle, 9 min at 94°C, 1 min at 60°C, and 1 min at 72°C; subsequent 35 cycles: 1 min at 94°C, 1 min at 60°C, and 2 min at 72°C; and final elongation for 7 min at 72°CT. The application of this primer system made it possible to determine over 1300 nucleotides in the relevant genes of all four cultures.

The consensus sequence of the 16S rRNA gene (1400 bp) from the monoculture Um-3 was obtained by comparison of seven sequences of the inserts from the clonal library of the PCR fragments isolated from the total DNA. Amplification of the 16S rRNA gene fragment was carried out using the universal bacterial primers Univ27f and Univ1492r [25]. PCR products were purified by electrophoresis in 0.7% agarose gel and isolated using the Wizard SV Gel and PCR Clean-Up System kit (Promega, United States) according to the manufacturer's recommendations. The purified PCR products were cloned in a pGEM-T vector (pGEM-T Easy Vector System I, Promega, United States). The target insert was sequenced using the plasmid primers m13f and m13r and the universal bacterial primer Univ530f.

The fragments of the *puf*LM operon were amplified and sequenced using the previously developed and reported primers [26].

Amplification of the PFOR (pyruvate flavodoxin/ferredoxin oxidoreductase) gene fragments for detection of *Chloroflexus*-specific indel descriptors [27] was carried out using the specially designed primers PFOf (5'-GYKCDGAYGGYACBGTBGG-3') and PFOr (5'-GCRAAGAANSMSGTYTGCAT-3').

The composition of reaction mixtures for all amplification reactions was as follows: 1× Bio*Taq* DNA polymerase buffer (17 mM $(NH_4)_2SO_4$; 67 mM Tris-HCl, pH 8.8; 2 mM $MgCl_2$); 12.5 nmol of each dNTP, 50 ng template DNA; 5 pmol of each relevant primer; and 3 U Bio*Taq* DNA polymerase (Dialat, Russia).

Sequencing was carried out according to Sanger using the BigDye Terminator v. 3.1 Cycle Sequencing Kit on a DNA Analyzer 3730 automatic sequencer (Applied Biosystems, United States) according to the manufacturer's recommendations.

The sequences were edited using the BioEdit software package [28]. Comparison with the GenBank sequences was carried out using BLAST [http://www.ncbi.nlm.nih.gov/blast]. The presence of chimeric sequences was determined using the Pintail 1.0 software package [29]. Phylogenetic trees were constructed using the maximum likelihood algorithm implemented in the MEGA 5.1 software package [30].

The sequences were deposited to GenBank under accession nos: KJ605349–KJ605353 (16S pPHK), KJ944502–KJ944506 (*puf*LM), and KJ728535–KJ728538 (PFOR).

FISH analysis. For FISH confirmation of the phylotypes belonging to cultured filamentous phototrophic bacteria, Cy-3-labeled probe specific for the 16S rRNA gene sequences of the new FAPB was used. The probe sequence was as follows: 5'-ATGGTCGTATCGGCACGCCTCGCCAA-3'. Fixation and hybridization were carried out as described in [31].

RESULTS AND DISCUSSION

Cultural characteristics of the isolates. The FAPB formed rounded, uneven, olive-colored colonies 2–3 mm in diameter within agar media (0.4% agar). Stable growth occurred in agar medium with 0.2 to 2.5 g/L (0.5 to 8.0 mM) $Na_2S \cdot 9H_2O$. Growth in liquid media was possible in the presence of a solid phase, such as a plate of 2% agar at the bottom of the vial. In this case, loose biomass resembling a microbial mat was formed at the liquid–solid interface.

Morphology. Gram reaction of the cells was variable. The cells were elongated (0.5–0.7 × 1.0–3.0 μm), in short filaments (trichomes) 15–30 μm, covered with a thin sheath (Figs. 1d, 1f, 1g). Trichomes multiplied by the separation of short segments or single cells from the parental trichome. Under unfavorable conditions, the filaments were long due to incomplete separation of the short trichome fragments. Configuration of the trichomes (straight, wavy, or helical) was different in different isolates (Figs. 1a–1c). Short trichomes were morphologically similar to those of green sulfur bacteria *Chloroherpeton*. The trichomes

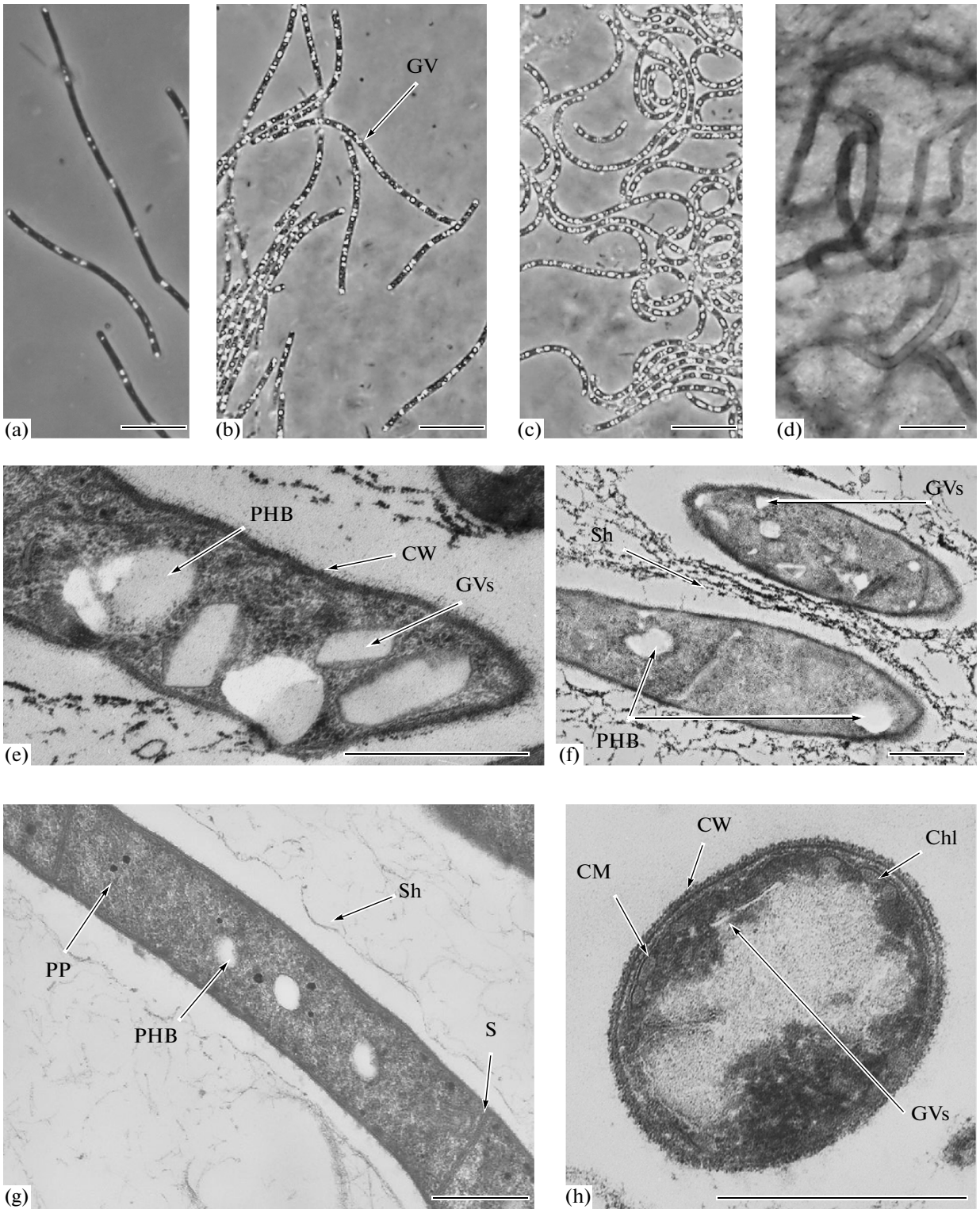

Fig. 1. Morphology (a–d) and ultrastructure (e–h) of the new filamentous bacterium *Candidatus* 'Chloroploca asiatica', isolates B7-9^T (a, e, f), M50-1 (c), and Um-3 (b, d, g, h). Scale bar, 5 μm (a–d) and 0.5 μm (e–h). Designations: GV, gas vacuoles, GVs, gas vesicles, PP, polyphosphates, PHB, poly-β-hydroxybutyrate, CW, cell wall, Chl, chlorosomes, CM, cytoplasmic membrane, S, septum, Sh, sheath.

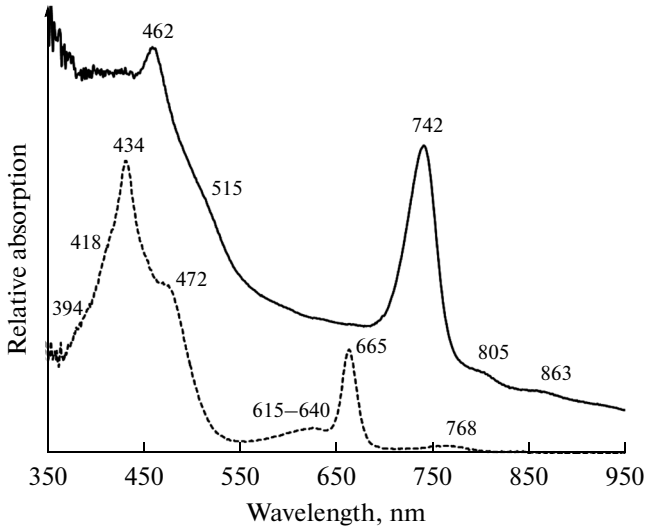

Fig. 2. Absorption spectra of whole cells (solid line) and acetone–methanol extract (broken line) of *Candidatus* 'Chloroploca asiatica' B7-9^T.

often formed bunches comprising several filaments. In the presence of excessive iron in the medium, finely dispersed iron sulfide was accumulated in the sheaths (Fig. 1d). In this case, the sheaths became thicker and wider and acquired dark gray coloration. The sheaths turned yellow after contact with air, probably due to chemical oxidation of iron sulfide. The sheaths seldom contained more than one trichome. The trichomes did not exhibit gliding motility. Trichome length and nonmotility differentiated the isolates from members of the genus *Oscillochloris*, which forms long straight filaments and is capable of relatively rapid gliding motility.

The cells of new isolates contained gas vacuoles located close to the septa. A similar type of gas vacuole localization has been described for *Oscillochloris* strains [12]. Under phase contrast gas vacuoles were very bright (Figs 1a–1c). Dim rounded inclusions (Figs. 1b, 1c) consisted of poly-β-hydroxybutyrate, a storage compound which was accumulated in high amounts in the cells grown in the media containing both acetate and bicarbonate.

Ultrastructure. On ultrathin sections, the cell wall structure was not typical of gram-negative bacteria (Figs. 1e–1h). No periplasmic space was observed between the cytoplasmic membrane and the cell wall (Fig. 1h). The outer membrane, characteristic of gram-negative bacteria, was not revealed. The cell envelope had several layers. FAPB isolated from saline environments (strain B7-9) had thicker cell walls (Fig. 1e) than the freshwater isolate Um-3 (Fig. 1h). The thin sheath had a loose fibrous structure and was located 0.1–0.2 μm or more from the cell wall (Figs. 1f, 1g).

The light-harvesting structures were chlorosomes typical for green sulfur bacteria and most FAPB (apart from the *Roseiflexus* and *Heliothrix* species) were located directly below the cytoplasmic membrane (Fig. 1h). The cytoplasm was granular, with large oval inclusions of low electron density typical of poly-β-hydroxybutyrate deposits (Figs. 1e–1g). Small dense intracellular granules were probably formed by polyphosphates (Fig. 1g). The cells in the trichomes were divided by diaphragmal ingrowth of the septa (Fig. 1g). Mesosome-like curled intracellular membrane structures were associated with the septa. Gas vesicles comprising the gas vacuoles were visualized as empty rhombic structures Figs. 1e, 1f). Some gas vesicles collapsed during the sample preparation and looked like slots (Fig. 1h).

Pigment composition. All monocultures contained bacteriochlorophylls *c* and *a*, as well as carotenoid pigments. Absorption spectra of all the cultures were identical. Spectra of the live cells (Fig. 2) exhibited the following maxima: 462, (515—shoulder), 742, 805, and 863 nm. The presence of bacteriochlorophyll *c* was indicated by the peaks at 462 and 742 nm, while the peaks at 805 and 863 nm suggested the presence of bacteriochlorophyll *a*. The spectral characteristics of the pigments and the presence of the *puf*LM operon in all the cultures (see below) suggest type II reaction centers. Absorption maxima of the pigment–protein complexes (805 and 863 nm) were close to those of thermophilic *Chloroflexus* spp. The known *Oscillochloris trichoides* strains have a long-wavelength maximum at 854 nm.

The weakly pronounced peaks at 805 and 863 nm indicate low content of bacteriochlorophyll *a* in the cells of FAPB isolates. Low levels of bacteriochlorophyll *a* are characteristic of halophilic FAPB *Candidatus* 'Chlorothrix halophile' [32], while thermophilic *Chloroflexus* are relatively rich in bacteriochlorophyll *a*.

The spectra of acetone–methanol extracts (Fig. 2) confirmed the presence of bacteriochlorophylls *c* and *a* in the cells (maxima at 665 and 768 nm, respectively).

Carotenoid composition in the cells of new FAPB isolates was determined by HPLC (Fig. 3). The culture B7-9 from Lake Doroninskoe was found to contain mostly γ-carotene (99% of all carotenoids). It should be noted that γ-carotene is the predominant pigment in the cells of *Chloroflexus aggregans*, while *Chloroflexus aurantiacus*, *Oscillochloris trichoides*, and *Candidatus* 'Chlorothrix halophile' contain considerable amounts of both γ- and β-carotene [22, 31].

Physiological properties. Growth of the new FAPB isolates in the media used in the present work was slow, with the colonies forming after 7–14 days of incubation. Growth occurred only under illumination. Monocultures containing a single species of phototrophs were obtained by agar shake dilutions. Pure cultures were not obtained. Unicellular heterotrophic bacteria were constant satellites of FAPB. Growth of the monocultures in the medium with sulfide was better when both bicarbonate and acetate were present. While sequential transfers of the isolates in autotrophic media were possible for six months, the cell yield

decreased drastically afterwards. The temperature 25–32°C and pH 8.0 were the parameters optimal for growth. Although strain B7-9 was isolated from a saline soda lake (mineralization 28 g/L), all strains grew in the medium with 5 g/L NaCl and 2 g/L bicarbonate.

Molecular genetic properties. The oligonucleotide FISH probe specific for the 16S rRNA gene of the isolate Um-3 was constructed based on the results of comparison of the 16S rRNA gene sequences of all five cultures and of the known phototrophic members of the phylum *Chloroflexi*. Results of FISH analysis are presented on Fig. 4. Since the cells of *Osc. trichoides* DG-6 and *Cfl. aurantiacus* were not stained with the probe (data not shown), while the cells of the new FAPB exhibited reliable staining (Fig. 4c), all five isolates may be considered belonging to the same FAPB phylotype.

Comparison of the 16S rRNA gene sequences revealed that all five isolates formed a compact cluster among the phylotypes of the order *Chloroflexales* (Fig. 5). The sequences within this cluster differed by not more than 1%. The group of *Oscillochloris* spp. sequences was the closest relative, differing by 9–10%. The differences with members of the genera *Chloronema* and *Chloroflexus* were 12 and 11.2–11.8%, respectively. Thus, phylogenetic analysis of the 16S rRNA gene sequences suggested that the new isolates belonged to a new taxon of at least the genus level.

The sequences of the *puf*L gene encoding the reaction center protein PufL and the gene encoding pyruvate flavodoxin/ferredoxin oxidoreductase (PFOR) [7] were analyzed as additional differentiating characteristics. Comparison of the PufL amino acid sequences revealed that the new isolates formed a single phylotype remote from other phylotypes of the order *Chloroflexales* (Fig. 6). Analysis of the PFOR marker revealed differences between the new isolates and *Chloroflexus* species. Thus, the results presented on Fig. 7 show no insertion of four amino acid residues in PFOR positions 423–462 of the new isolates, which is characteristic of *Chloroflexaceae* members.

Thus, the studied new FAPB isolates exhibited certain phenotypic and phylogenetic differences from the known members of this group of phototrophic bacteria. According to the results of comprehensive phylogenetic analysis, all five monocultures belonged to the same new genus and species, for which the candidate status is proposed with the name *Candidatus* 'Chloroploca asiatica' gen. nov., sp. nov. The description is based on comparative investiga tion of five enrichment FAPB monocultures. The isolate B79 was studied in most detail.

Description of *Candidatus* 'Chloroploca asiatica' gen. nov., sp. nov.

Candidatus 'Chloroploca asiatica' (L. *candidatus*, candidate, designating the tentative taxonomic status. Chlo'ro.plo'ca Gr. adj. chloros green; Gr. fem. n. *ploke* a braid, a twist; M.L. fem *Chloroploca* green braid; asiatica, discovered in Asia. Chloroploca asiatica, green braid Asian).

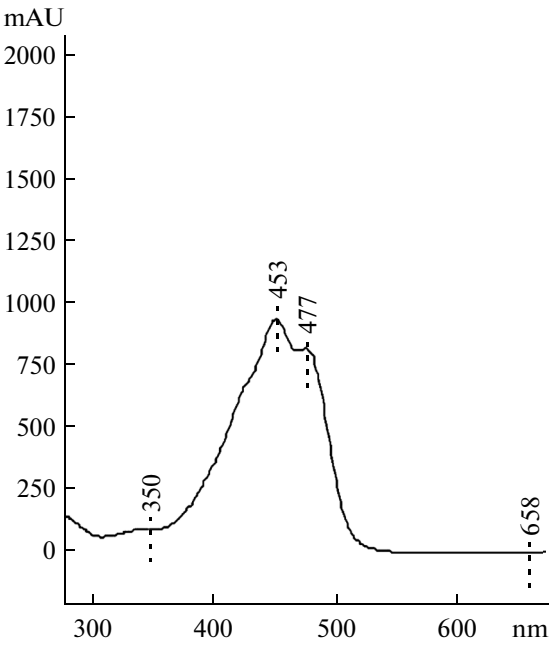

Fig. 3. Absorption spectrum of γ-carotene isolated from the cells of *Candidatus* 'Chloroploca asiatica' B7-9^T and identified by HPLC.

The cells are elongated (0.5–0.7 × 1.0–3.0 μm), forming short filaments (trichomes) 15–30 μm long, covered with a thin mucous sheath. The cells in the trichomes divide by diaphragmal ingrowth of the septa. In different isolates, the trichomes may be straight, wavy, or helical. Trichomes multiply by the separation of short segments or single cells from the parental trichome. The trichomes form bunches of several filaments. In the trichomes, cell length exceeds cell width three- to fivefold. The distance between the sheath and the cell wall is 0.1–0.2 μm or more. The sheath has a loose fibrous structure. Finely dispersed iron sulfide may accumulate in the sheaths. Two trichomes may occupy the same sheath in rare cases. No motility of the trichomes was detected. The cells contain gas vacuoles located close to the cell septa. Poly-β-hydroxybutyrate and small polyphosphate granules may be present as storage compounds. Gram staining is variable. The cell wall structure is not typical of gram-negative bacteria. The typical gram-negative outer membrane is not revealed. The cell envelope consists of several layers. Antennal photosynthetic structures (chlorosomes) are located below the cytoplasmic membrane. Bacteriochlorophyll *c* is the major pigment. Bacteriochlorophyll *a* is present in minor amounts. The main carotenoid is γ-carotene (at least 90%). Absorption maxima of the pigments in the cells are at 462, (515—shoulder), 742, 805, and 863 nm. The spectral characteristics of the pigments and the

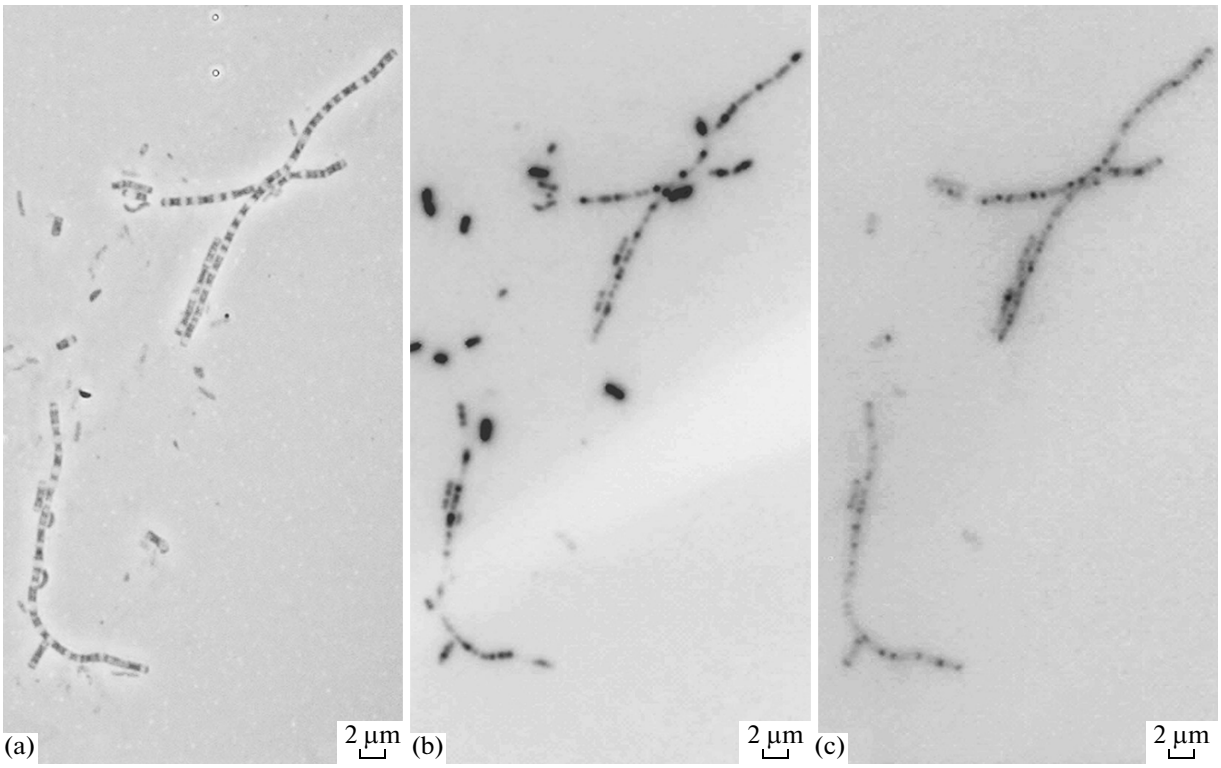

Fig. 4. Fluorescent in situ hybridization with the cells of isolate Um-3: visible light/phase contrast (a), nonspecific DNA staining (DAPI, UV excitation), negative image (b), and staining of the cells of the isolate Um-3 with CY-3-labeled oligonucleotide probe, negative image (c).

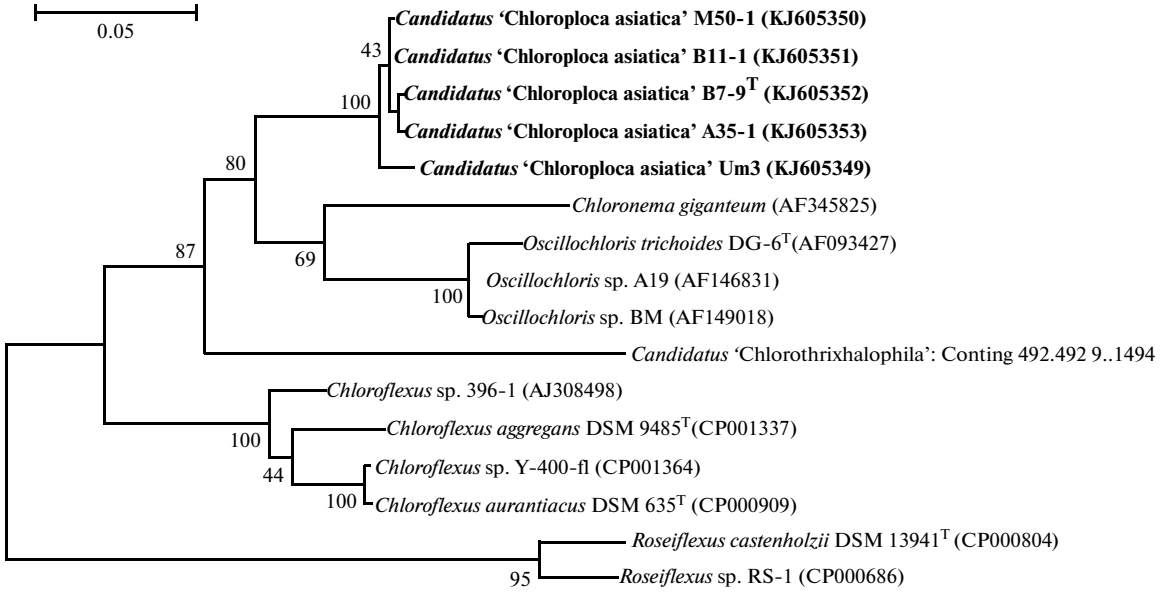

Fig. 5. Phylogenetic tree of the 16S rRNA gene sequences from the new isolates and the FAPB of the order *Chloroflexales*. The tree was constructed using the maximum likelihood algorithm with the application of the Tamura–Nei model. The branching order was determined by analyses of 500 alternative trees. Scale bar corresponds to 5 replacements per 100 nucleotides. Compared sequences were 1286 nucleotides long. The sequences determined in the present work are marked by bold underlined script.

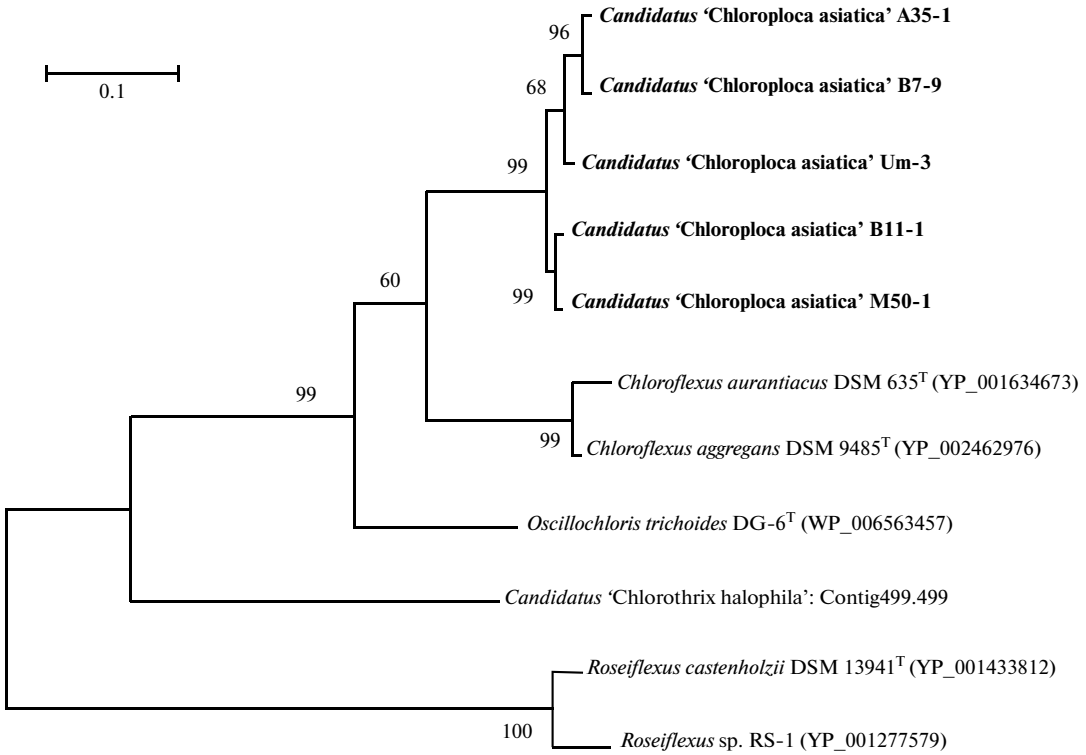

Fig. 6. Phylogenetic tree of PufL amino acid sequences from the new isolates and the FAPB of the order *Chloroflexales*. The tree was constructed using the maximum likelihood algorithm with the application of the Tamura–Nei model. The branching order was determined by analyses of 1000 alternative trees. Scale bar corresponds to 10 replacements per 100 amino acid residues. Compared sequences were 244 amino acids long. The sequences determined in the present work are marked by bold underlined script.

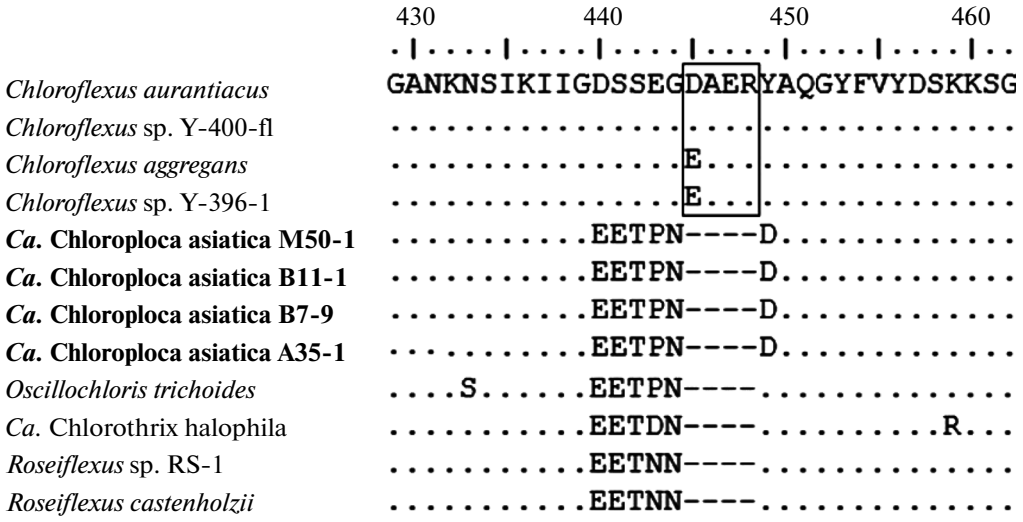

Fig. 7. Specific insertion in the sequence of the PFOR phylogenetic marker.

presence of the *puf*LM operon in all the cultures (see below) are indicative of the presence of type II reaction centers. The organisms are anaerobes, obligate phototrophs capable of photoautotrophic growth coupled to sulfide oxidation. The isolates are tolerant to sulfide (grow well with 8 mM sulfide). The organisms

Table 2. Comparative characterization of *Candidatus* 'Chloroploca asiatica' and other cultured FAPB

Features	*Candidatus* 'Chloroploca asiatica'	*Osc. trichoides* [5, 12]	MCLO [15]	*Candidatus* 'Clilorothrix halophila' [19, 31]	*Cfl. aurantiacus* [1]	*Cfl. aggregans* [3]
Cell diameter, μm	0.7–1.0	1–1.5	1.0–3.0	2.0–2.5	0.5–1.0	1.0–1.5
Trichome length, μm	15–30	Indefinite length	Indefinite length	Indefinite length	Indefinite length	Indefinite length
Sheath	+	–/+	–/+	–/+	–	–
Gas vacuoles	+	+	–	–	–	–
Chlorosomes	+	+	+	+	+	+
Color of cell suspension	Yellow-green	Green	Yellow-green	Yellow-green	Orange-green	Green, Orange-green
Bacteriochlorophyll	*c*, *a*	*c*, *a*	*c*, *a*	*c*,(*d*?), *a*	*c*, *a*	*c*, *a*
Absorption maxima of bacteriochlorophylls, nm	742, 805, 863	748, 852	753, 815, 850, 894	759, 850	750, 805, 860	740, 803, 868
Major carotenoids	γ-Carotene	β and γ-carotene	ND	γ-Carotene	β and γ-carotene	γ-Carotene and its derivatives
pH optimum	8.0	7.7–8.0	7.5–8.0	7.5	8.0–8.5	7.0–9.0
Temperature optimum, °C	25–32	28–30	28–30	35–38	50–60	50–60
Salinity, growth range, %	0.3–1.5	Freshwater	3–5	5–12	Freshwater	Freshwater
Photoautotrophic growth	+	+	n.d.	+	+	–
Photoorganoheterotrophic growth	–	–	+	n.d.	+	+
Growth in the dark	–	–	n.d.	–	+	+
Aerobic growth O_2	–	–	n.d.	–	+	+
N_2 fixation	n.d.	+	n.d.	n.d.	n.d.	–
Storage compounds	PHB, PP	PHB, PP	n.d.	PHB	PP	PHB, PP
Major quinones	n.d.	MK-10	n.d.	n.d.	MK-10, MK-4	MK-10
DNA G+C ratio, mol %	n.d.	59.2	n.d.	n.d.	53.1–54.9	56.7–57.0

Designations: "+" and "–" stand for the presence or absence of a feature, respectively; ND, not determined; n.d., no data; PHB, poly-β-hydroxybutyrate; PP, polyphosphates.

are mesophilic (20–32°C), halotolerant (grow at 3 to 15 g/L NaCl), and alkali tolerant (pH optimum 8.0).

Habitats include microbial mats of soda lakes and epibioses in thermal sulfide springs (mineralization 3–28 g/L).

Sources of isolation of the monocultures are the following: coastal microbial mat of Doroninskoe soda lake, mineralization 28 g/L, Zabaykalsky krai, Russia (B7-9); microbial mat of the Oronujiskoe soda lake, mineralization 6 g/L, Buryat Republic, Russia (B11-1); microbial mat of the Sul'fatnoe soda lake, mineralization 5 g/L, Buryat Republic, Russia (A35-1); microbial mat of the Chukhyn-Nur soda lake, mineralization 5 g/L, Eastern Mongolis (M50-1); and microbial biofilm in the Umhei thermal sulfide spring, mineralization 3 g/L, Buryat Republic, Russia (Um-3).

All five cultures are very close in their 16S rRNA gene sequences, differing by not more than 2%, and form a separate branch on the phylogenetic tree of the suborder *Chloroflexinae*. The new phylotypes differ from the closest relatives by 9–10%, which agrees with the genus level of the new taxon.

The sequences were deposited to GenBank under accession nos: KJ605349–KJ605353 (16S rRNA), KJ944502–KJ944506 (*puf*LM), and KJ728535–KJ728538 (PFOR).

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