FEMS Microbiology Letters, 366, 2019, fnz097

https://doi.org/10.1093/femsle/fnz097
Advance Access Publication Date: 4 May 2019
Research Letter

RESEARCH LETTER – Taxonomy & Systematics

‘*Candidatus* Oscillochloris fontis’: a novel mesophilic phototrophic Chloroflexota bacterium belonging to the ubiquitous *Oscillochloris* genus

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One sentence summary: We present the results of a study on phylogenetic and functional diversity of Oscillochloris bacteria and description of ‘Candidatus Oscillochloris fontis’ bacterium from the Chukotka Peninsula.

Editor: Aharon Oren

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ABSTRACT

We present the results of a study of mesophilic anoxygenic phototrophic Chloroflexota bacteria from Mechigmen hot spring (the Chukotka Peninsula) and Siberia. According to 16S rRNA phylogenetic analysis, these bacteria belong to *Oscillochloris trichoides*. However, sequencing the draft genome of the bacterium from the Chukotka and analysis of the average nucleotide identity, as well as *in silico* DNA-DNA hybridization, reveal that this bacterium belongs to a novel species within the *Oscillochloris* genus. We, therefore, propose ‘*Candidatus* Oscillochloris fontis’ as a novel taxon to represent this mesophilic alkaliphilic anaerobic anoxygenic phototrophic bacterium. Spectrophotometry and high-performance liquid chromatography analysis show that the bacterium possesses bacteriochlorophylls c and a, as well as lycopene, β-carotene and γ-carotene. In addition, transmission electron microscopy shows the presence of chlorosomes, polyhydroxyalkanoate- and polyphosphate-like granules. The genome of ‘*Ca.* Oscillochloris fontis’ and the Siberian strains of *Oscillochloris* sp. possess the key genes for nitrogenase complex (*nif*H) and ribulose-1,5-bisphosphate carboxylase/oxygenase (*cbbL*), as previously described for *O. trichoides* DG-6. The results presented here, and previously published data, show that *Oscillochloris* bacteria from different aquatic environments have the potential for CO_2 and N_2 fixation. Additionally, we describe a new primer system for the detection of RuBisCo form I.

Received: 30 March 2019; **Accepted:** 2 May 2019

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Keywords: phototrophic bacteria; Chloroflexi; Oscillochloris; nitrogenase; RuBisCo; *cbbL*

INTRODUCTION

Oscillochloris trichoides is the best studied mesophilic member of *Chloroflexales* order, a group that includes the majority of the phototrophic representatives of Chloroflexi (Chloroflexota) phylum. The name *O. trichoides* was first assigned to a filamentous anoxygenic phototrophic chlorosome-containing bacterium that was isolated from a microbial mat on sludge in a freshwater lake (Gorlenko and Korotkov 1979). However, molecular identification of the bacterium was not accomplished at that time. Later, Keppen and colleagues isolated and described the neotype *O. trichoides* DG-6 from sediment in a Caucasian spring that contained sulfide (Keppen, Baulina and Kondratieva 1994). Genomic sequencing of *O. trichoides* DG-6 has revealed a genomic basis for the eco-physiological features of this bacterium (Kuznetsov *et al.* 2011). The most notable features being the autotrophic carbon fixation via the Calvin cycle (Ivanovsky *et al.* 1999) and an active nitrogenase complex (Keppen *et al.* 1989). These features have rendered *O. trichoides* DG-6 a unique research object among other *Chloroflexales*.

Despite the interesting eco-physiological potential of *O. trichoides* DG-6, insight into the adaptive evolution of this mesophilic *Chloroflexales* bacterium is limited by the low availability of interrelated genomic and phenotypic data about the mesophilic members of the order. Recently, the genomes of new mesophilic *Chloroflexales,* '*Candidatus* Viridilinea halotolerans', '*Ca.* Viridilinea mediisalina', and '*Ca.* Chloroploca asiatica' have become available (Gorlenko *et al.* 2014; Grouzdev *et al.* 2018, 2019; Gaisin *et al.* 2019). However, *O. trichoides* DG-6 remains the single member of the *Oscillochloris* genus which limits any comparative genomic analysis. In order to address this limitation, we completed the genomic sequencing of a novel *Oscillochloris* bacterium from a Mechigmen hot spring of the Chukotka Peninsula. In addition, we studied the phenotypic features of this bacterium. Because of the lack of axenic culture, we here describe this bacterium in the *Candidatus* category with the proposed name '*Ca.* Oscillochloris fontis' Chuk17. Furthermore, we also isolated and analyzed *Oscillochloris* strains from Alla, Umhey, and Kuchiger hot springs in the Barguzin Valley, Siberia, for better understanding of the phylogenetic and functional diversity of *Oscillochloris* bacteria.

MATERIALS AND METHODS

Isolation and maintenance of cultures

'*Ca.* Oscillochloris fontis' Chuk17 was isolated from a low temperature zone of a thermal mineral spring (24°C, pH 8.0, salinity 4g L^{-1}) in the Mechigmen group of springs located on the Chukotka Peninsula, Russia (65°48'23.9'N, 173°23'49.9'W). The enrichment culture was isolated from a brown-green microbial mat using a semi-solid medium (0.5–0.7% agar) consisting of the following components per liter: KH_2PO_4 = 0.20g, NH_4Cl = 0.20g, $MgCl_26H_2O$ = 0.20g, KCl = 0.30g, NaCl = 5.00g, $Na_2S_2O_3$ = 0.30g, Na_2SO_4 = 0.30, $CaCl_22H_2O$ = 0.05g, $NaHCO_3$ = 1.00g, Na_2S9H_2O = 0.50g, soytone = 0.05, yeast extract = 0.05g, sodium acetate = 0.10g, and 1 ml of Pfennig's trace-element solution (Pfennig and Lippert 1966) and each vitamin solution. The pH of the medium was adjusted to 8.0–8.5. Initially, tubes containing the medium were placed at 28°C in near-infrared light with a wavelength of 740 ± 10 nm from narrowband light-emitting diodes to eliminate both cyanobacteria and purple bacteria. In addition, Al-1, Um-3 and Ku-3 cultures of *Oscillochloris* sp. were isolated from Alla, Umhey and Kuchiger hot springs (Barguzin Valley, Siberia), respectively. These bacteria were isolated using a semi-solid medium (0.5%–0.7% agar) consisting of the following components per liter: KH_2PO_4 = 0.40g, NH_4Cl = 0.50g, $MgCl_26H_2O$ = 0.40g, KCl = 0.50g, NaCl = 0.50g, $Na_2S_2O_3$ = 0.50g, $CaCl_22H_2O$ = 0.30g, $NaHCO_3$ = 0.50g, Na_2S9H_2O = 1.00g, yeast extract = 0.10g, sodium acetate = 0.50g and 1 ml of Pfennig's trace-element solution and each vitamin solution. The each vitamin solutions consisting of the following components per 100 ml: Thiamin = 0.005g, calcium pantothenate = 0.005g in acidic solution (diluted HCl, pH 3); biotin = 0.002g, 4-aminobenzoic acid = 0.005g, nicotinic acid = 0.005g, pyridoxine = 0.010g in alkaline solution (0.1 N NaOH); folic acid = 0.002g, riboflavin = 0.005g, cobalamin = 0.500g in neutral solution (deionized water). The pH of the medium was adjusted to 8.0–8.5. When other phototrophic bacteria had been eliminated, each *Oscillochloris* culture was maintained at a temperature of 28°C under a full light range of 2000 lux.

We conducted experiments to determine the optimal temperature, NaCl concentration, and pH value for growing '*Ca.* Oscillochloris fontis' Chuk17. These experiments were performed using 0.2%–0.3% agar medium in glass vials with rubber stoppers under full-range light (3800 lux). The response of the bacteria to alterations in culture conditions was tracked by measuring the optical density of the cell suspension at 740 nm using a KFK-3-ZOMZ photometer (ZOMZ, USSR). The optimal temperature was determined using a gradient incubator at 21–51°C; optimal pH value was determined at 30°C; and optimal NaCl concentration was determined at 30°C and pH 9.0.

DNA extraction, metagenomic sequencing, binning and phylogenetic analysis

Total genomic DNA from the enrichment culture of '*Ca.* Oscillochloris fontis' Chuk17 was isolated according to previously described methods (Grouzdev *et al.* 2018) and was sequenced using the Illumina HiSeq 1500 platform with single-end 220-bp reads. Libraries were constructed with the NEBNext DNA library prep reagent set for Illumina, per the kit's protocol. A total of 2400,518 reads were obtained from Chuk17. Raw reads were quality checked with FastQC v0.11.7 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and low-quality reads were trimmed using Trimmomatic v0.36 (Bolger, Lohse and Usadel 2014). Trimmed reads for all samples were assembled using metaSPAdes v3.12.1 (Nurk *et al.* 2017) at the default settings. Metagenome binning was performed using three different binning algorithms: BusyBee Web (Laczny *et al.* 2017), MaxBin 2.0 version 2.2.4 (Wu, Simmons and Singer 2016) and MyCC (Lin and Liao 2016). The three bin sets were supplied to DAS Tool 1.0 (Sieber *et al.* 2018) for consensus binning to obtain the final optimized bins. Genome bins were assessed for completeness and contamination using CheckM 1.0.11 (Parks *et al.* 2015). The metagenomic bin of '*Ca.* Oscillochloris fontis' was uploaded to the RAST server for gene prediction and annotation (Aziz *et al.* 2008).

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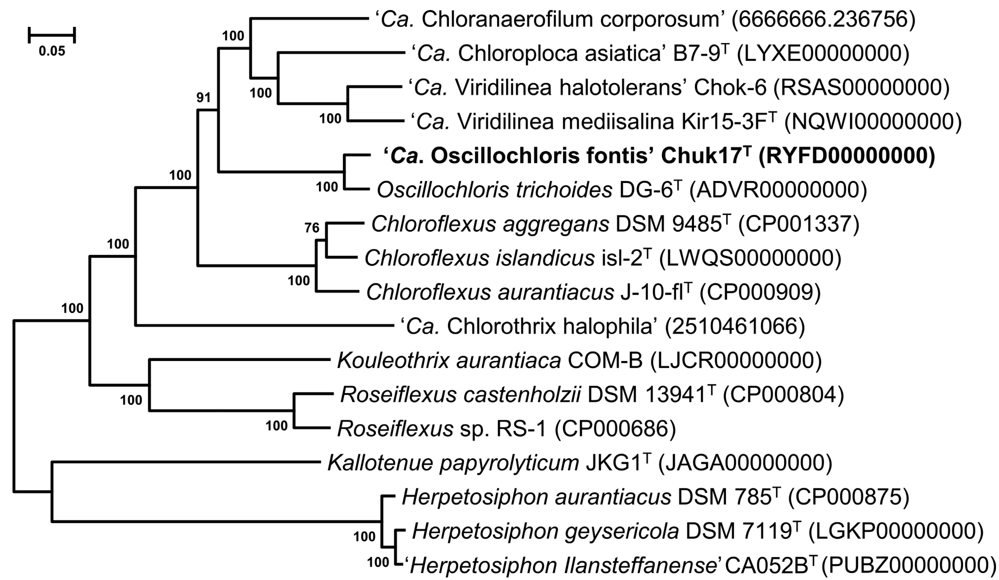

Figure 1. Maximum-likelihood phylogenetic tree inferred from a comparison of concatenated Chloroflexia core proteins showing the position of the Chuk17. Phylogenetic analysis was performed with an LG + F + I + G4 substitution model based on 37 016 amino acid positions'; the scale bar represents amino acid substitutions per site.

Average nucleotide identity (ANI) and *in silico* DNA-DNA hybridization (DDH) values were calculated using the ANI calculator (Rodriguez-R and Konstantinidis 2016) and GGDC genome-to-genome distance calculators (Meier-Kolthoff *et al.* 2013), respectively. Core genes from the complete genomes of '*Ca.* Oscillochloris fontis' Chuk17 and other strains of *Chloroflexia* were extracted using the USEARCH program (Edgar 2010) with a 50% sequence identity cut-off within BPGA (Chaudhari, Gupta and Dutta 2016), and their concatenated amino acid sequences were aligned using MAFFT (Katoh 2002). The core proteins were specified in Table S1 (Supporting Information). The aligned sequences were then cleaned with gBlocks (Castresana 2000). Maximum-likelihood trees of core proteins were inferred in IQ-Tree using the LG + F + I + G4 model recommended by ModelFinder (Kalyaanamoorthy *et al.* 2017) and branch supports were estimated using UFBoot2 (Hoang *et al.* 2018).

Identification of 16S rRNA, *nif*H and *cbb*L genes in the Siberian *Oscillochloris* sp.

Genomic DNA from the *Oscillochloris* sp. Al-1, Um-3 and Ku-3 cultures was isolated using previously described methods (Gaisin *et al.* 2015). The 16S rRNA gene sequences were amplified and sequenced using Cfl68f/Cfl1231r primers (Gaisin *et al.* 2015). The *nif*H (it encodes nitrogenase iron protein) and *cbb*L (it encodes ribulose bisphosphate carboxylase (RuBisCo) large chain) genes were identified using PCR and relevant sequencing. Specifically, *nif*H was identified using previously published primers and temperature-time PCR profiles (Marusina *et al.* 2001) while the primers and temperature-time profile for *cbb*L are here published for the first time.

We designed primers for detection of *cbb*L using the nested-PCR: first step (forward primer (297F) 5'—RTYGGNAAYGTNTTYGGNT-3' and reverse primer (1135R) 5'—TNCCNCCNCCRAACTG-3'), second step (forward primer (548F) 5'—GAYYTYRYBAARGAYGAYGA-3' and reverse primer (948R) 5'—TCNCCYTCNARYTTNCCNAC-3'). The temperature-time profile for the first step: 95°C–3 min; next 5 cycles 95°C–30 s, 58°C–40 s, 72°C–1 min; next 35 cycles 95°C–30 s, 52°C–40 s, 72°C–1 min.; final elongation–72°C–7 min. The temperature-time profile for the second step: 94°C–4 min; next 35 cycles 94°C–1 min, 56°C–1 min, 72°C–1 min; final elongation–72°C–7 min.

The PCR products were purified using agarose gel and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit in a 3730 DNA Analyzer (Applied Biosystems, USA). Nucleotide and amino acid sequences were aligned using the MAFFT (Katoh 2002), and the aligned sequences were cleaned with gBlocks (Castresana 2000). Maximum-likelihood trees of core proteins were inferred in IQ-Tree using the LG + F + I + G4 model recommended by ModelFinder (Kalyaanamoorthy *et al.* 2017) and branch supports were estimated using UFBoot2 (Hoang *et al.* 2018).

Data availability

The raw metagenomic sequences are available at the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under accession number SRR8543842. The draft genome sequence of '*Ca.* Oscillochloris fontis' Chuk17 is available at GenBank under accession number RYFD00000000. Sequences of 16S rRNA, *nif*H and *cbb*L from the Siberian strains are also available at GenBank under accession numbers MK607224–MK607226, MK616372–MK616374, and MK616369–MK616371, respectively.

Pigment composition analysis

The absorption spectra of the membrane fraction from the cultures in 10 mM Tris-HCl buffer (pH 8.0), acetone-methanol (7:2), and petroleum ether extracts were analyzed using a Cary 50 spectrophotometer (Varian, Australia) at a wavelength range of 360–960 nm. Pigments were analyzed with HPLC using an Agilent Zorbax SB-C18 (4.6 × 250 mm) column (Agilent, USA) as described elsewhere (Ashikhmin, Makhneva and Moskalenko 2014). The HPLC device (Shimadzu, Japan) consisted of an LC-10ADVP pump with an FCV-10ALVP module, a detector with an

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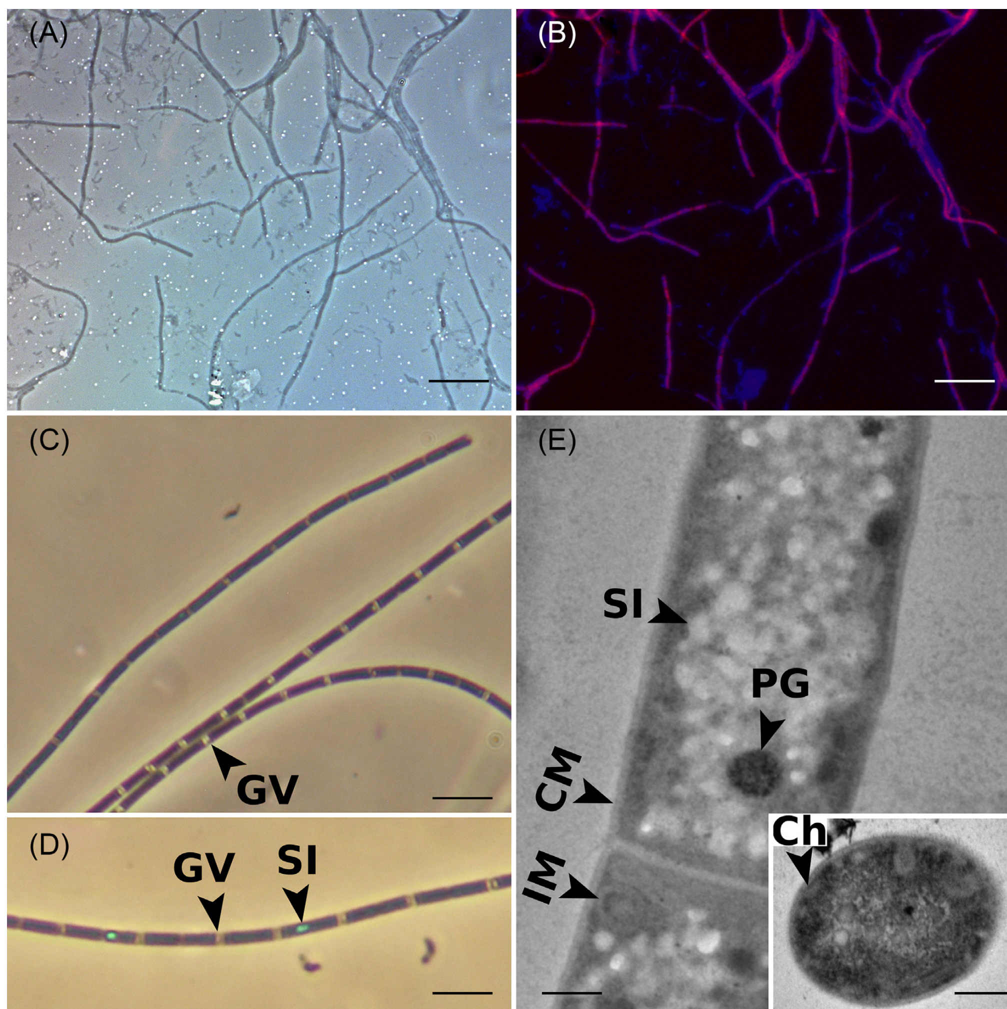

Figure 2. Fluorescence *in situ* hybridization, morphology, and ultrastructure of '*Ca.* Oscillochloris fontis' Chuk17. Phase-contrast image (**A**) and overlay of FISH probe (red) and DAPI (blue) images (**B**). Gas vesicles and optical light volume (see insertion) in phase-contrast image of cells (**C**, **D**). Transmission electron micrographs of cells in ultrathin cross-section (**E**). CM = cytoplasmic membrane; Ch = chlorosome; IM = intracellular membrane; SI = storage inclusion; GV = gas vesicle; and PG = polyphosphate-like granule. Bars = 10 μm (**A** and **B**); 5 μm (**C**); 2 μm (**D**); 100 nm (**E**) and 200 nm (**insertion**).

SPD-M20A diode matrix, and a CTO-20AC thermostat. The column was balanced with an acetonitrile/water/ethyl acetate mixture (69.3:7.7:23) which passed through the column in the first 3 min and was then linearly substituted with ethyl acetate over 37 min; subsequently, pure ethyl acetate was passed through the column for an additional 3 min. The feed rate of all solvents was 1.0 ml/min, and analysis was performed at a constant temperature of 22°C. The pigment concentration was calculated with the LC-solution program (Shimadzu, Japan). The initial peak area for each pigment on the HPLC chromatogram was divided by the molar extinction coefficient for the corresponding pigment: 145 $mM^{-1}cm^{-1}$, 74 $mM^{-1}cm^{-1}$ and 76 $mM^{-1}cm^{-1}$ for carotenes, bacteriochlorophylls *c* and *a*, respectively (Britton 1995; Olson *et al.* 2007). Then, using the obtained values, the molar ratio (mol%) of each pigment in their total mixture was calculated, as well as the molar ratios for each type of pigments (carotenoids or bacteriochlorophylls).

Fluorescence *in situ* hybridization

The correlation between phenotype and the genome of '*Ca.* Oscillochloris fontis' Chuk17 was assessed by fluorescence *in situ* hybridization (FISH) according to previously described methods (Pernthaler *et al.* 2001). The cells were fixed in 3% paraformaldehyde for 1.5 h, and the probe, Chuk17 (5′-GC-CTC-CAG-TCG-TCT-CGT-3′), was designed using Decipher and included Cy3 dye (Wright *et al.* 2014). The probe was then evaluated using probeCheck (Loy *et al.* 2008). A universal bacterial probe, EUB338 (5′-GCT-GCC-TCC-CGT-AGG-AGT-3′) with Cy3 dye, was used as a positive control (Amann *et al.* 1990). DAPI (4′,6-diamidino-2-phenylindole) was used for staining cellular DNA. The sample was analyzed using a Zeiss Axio Imager D1 (Carl Zeiss, Germany), an epi-fluorescence microscope.

Light and transmission electron microscopy

Cell morphology was observed using an Olympus BX41TF phase-contrast microscope (Olympus, Japan). Cells were collected by centrifugation for 3 min at 16 000 g and fixed using 2.5% glutaraldehyde in a PBS buffer (0.1 M sodium phosphate buffer containing 0.85% NaCl, pH 7.2) at 4°C. After rinsing three times in the PBS buffer, the sample was post-fixed in 1% OsO_4 for 1 h, then serially dehydrated with increasing ethanol concentrations and 100% acetone and embedded in Epon resin (Sigma, USA). Cell

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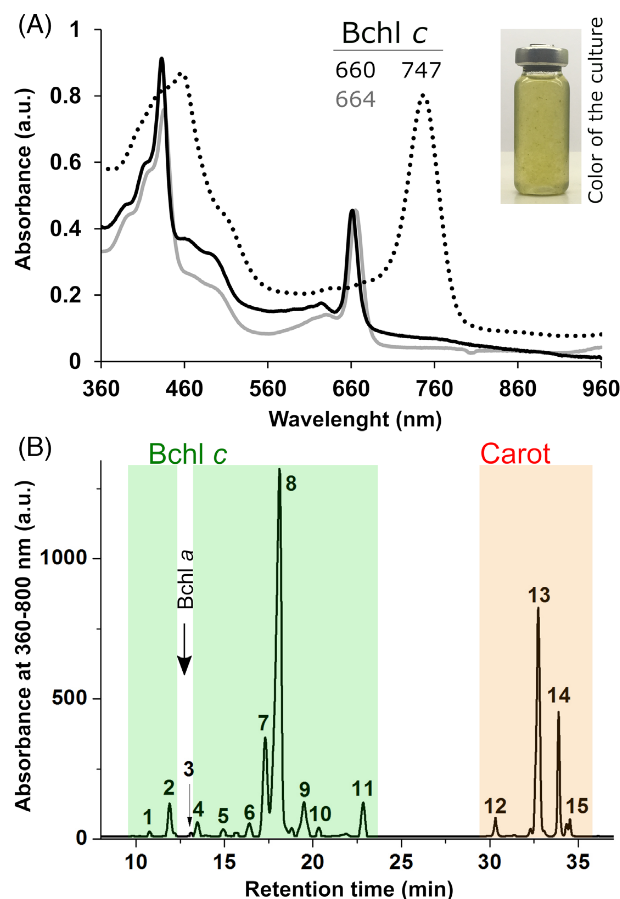

Figure 3. Cellular pigment composition of '*Ca.* Oscillochloris fontis' Chuk17. Absorption spectra for the membrane fraction in Tris-HCl buffer (dotted line), acetone-methanol (grey solid line) and petroleum ether extracts (black solid line) (**A**). HPLC chromatogram of the pigment extracted in acetone-methanol (7:2) detected at 360–800 nm (**B**). Identification of HPLC peaks: 1, 2, 4–11 = bacteriochlorophyll c; 3 = bacteriochlorophyll *a*; 12 = lycopene; 13 = γ-carotene; 14 = lycopene derivative and 15 = β-carotene. Bchl = bacteriochlorophyll; Carot = carotenes; and a.u. = absorbance units.

sections were contrasted by staining first with uranyl acetate and then with lead citrate according to Reynolds (1963) and analyzed with a Libra-120 transmission electron microscope (Carl Zeiss, Germany).

RESULTS AND DISCUSSION

Metagenome and phylogeny of '*Ca.* Oscillochloris fontis' Chuk17

Metagenomic sequence analysis of the highly enriched *Oscillochloris* culture from the Mechigmen hot spring showed that only one member of the Chloroflexota phylum was present. The high-quality metagenomic-assembled genome of '*Ca.* Oscillochloris fontis' Chuk17 is comprised of 139 scaffolds with an N50 value of 71 077 bp and an average coverage of 69 × ; the length and the G + C content of the genome are 4 463 428 bp and 58.6%, respectively. Completeness and contamination of the assembled genome were 99.1%, and 0.0%, respectively. The maximum-likelihood phylogenetic tree from the concatenated core proteins shows that '*Ca.* Oscillochloris fontis' Chuk17 belongs to a branch of *O. trichoides* DG-6 (Fig. 1). Further comparative genomic analysis revealed 85.5% ANI and 30.2% *in silico* DDH for '*Ca.* Oscillochloris fontis' Chuk17 versus *O. trichoides* DG-6. The value of these indexes fits the criteria of novel species (Goris *et al.* 2007) .

The minimum information about the genome sequence specification (Field *et al.* 2008) is summarized in Table S2 and Table S3 (Supporting Information). 4036 coding sequences, 3 rRNA and 48 tRNA genes were identified according to RAST annotation. Genomic statistics of '*Ca.* Oscillochloris fontis' in comparison with *O. trichoides* DG-6 show the close genomic content of both bacteria (Table S4; Supporting Information). The genome contains genes of the sulfide:quinone reductase, the RuBisCo form I, and the nitrogenase complex. Presence of genes of the sulfide:quinone reductase and the Calvin cycle indicate a potential of the bacterium for photoautotrophic metabolism.

Morphology and FISH

We designed FISH probes for using the 16S rRNA sequence from the '*Ca.* Oscillochloris fontis' Chuk17 genome. A strong signal was shown from the target probe which demonstrates a link between the analyzed genome and filamentous bacterium (Fig. 2A and B). Moreover, '*Ca.* Oscillochloris fontis' Chuk17 was observed to have a multicellular filamentous morphology (Fig. 2C and D), and the multicellular filaments are motile. Cells contain gas vesicles which appear as bright intracellular inclusions near cell-to-cell junctions (Fig. 2D). Cells without gas vesicles were also observed. Electron microphotographs of ultrathin cross-sections show the presence of chlorosomes, storage inclusions polyhydroxyalkanoate- and polyphosphate-like granules (Fig. 2E, Fig. S1; Supporting Information). These phenotypic features are typical for the *Oscillochloris* genus (Keppen, Baulina and Kondratieva 1994; Keppen *et al.* 2000).

Basic physiology

'*Ca.* Oscillochloris fontis' Chuk17 was grown in deep agar under the light. Growth in the dark under both anaerobic and aerobic conditions was not observed. The best yield of '*Ca.* Oscillochloris fontis' Chuk17 in the enrichment cultures was observed at 5–10 g L^{-1} NaCl, pH 8.5 and 28°C (Fig. S2; Supporting Information). The bacterium was tolerant of high sulfide concentrations with growth observed until a Na_2S9H_2O concentration of 1 g L^{-1} in the medium was reached. At present, we can, therefore, conclude that '*Ca.* Oscillochloris fontis' Chuk17 is a mesophilic alkaliphilic anaerobic anoxygenic phototrophic bacterium.

Pigment composition

The '*Ca.* Oscillochloris fontis' Chuk17 culture is green in color. Spectral analysis of the membrane fraction re-suspended in Tris-HCl buffer resulted in an absorption spectrum with maxima at 457, and 747 nm, minor peaks at 637, 677 and 858 nm, as well as shoulders at 434 and 508 (Fig. 3A). In an acetone-methanol (7:2, v/v) mixture, the absorption spectrum of the pigment extract had peaks at 434, 629 and 664 nm and shoulders at 392, 419, 470 and 498 nm (Fig. 3A). The absorption spectrum of the pigment extract in petroleum ether extract had peaks at 432, 624 and 660 nm and shoulders at 392, 416, 466 and 496 nm (Fig. 3A). The HPLC elution profile of the pigment extract had 15 signals (Fig. 3B). Signal characteristics are presented in Table S5 (Supporting Information). We found ten forms

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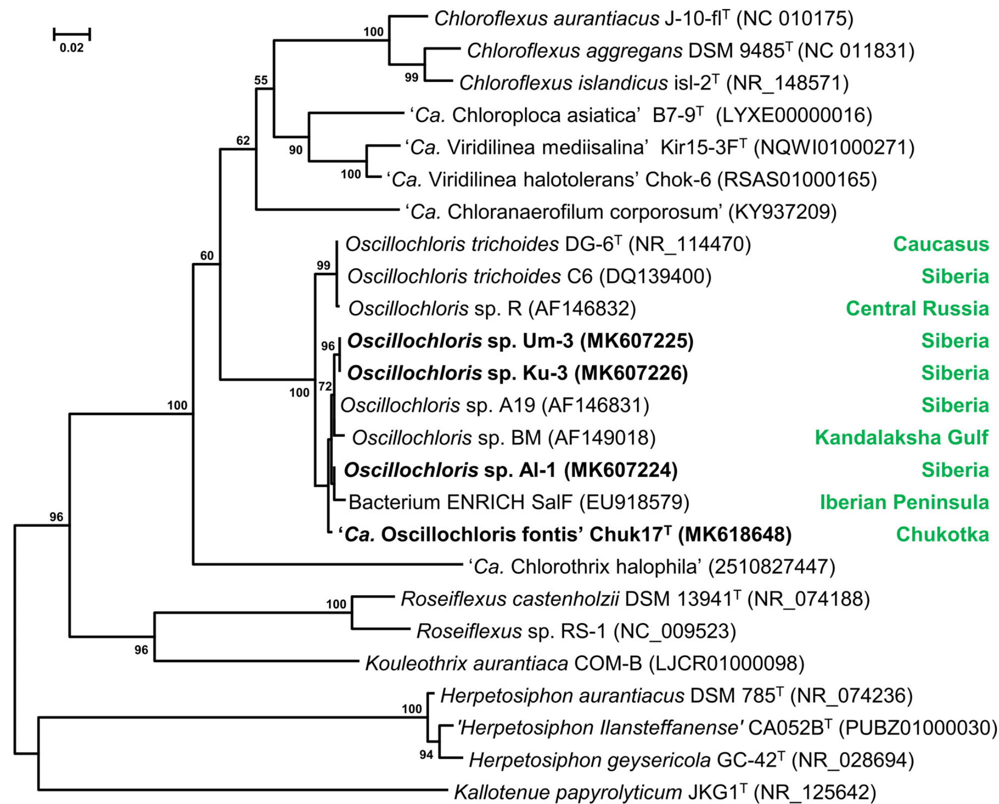

Figure 4. Maximum-likelihood phylogenetic trees based on 16S gene sequences (1251 nucleotide sites) reconstructed with evolutionary model TN + F + I + G4 showing the position of bacteria Chuk17, Al-1, Um-3, and Ku-3 in relation to taxonomically characterized members of the class Chloroflexia. The scale bar represents nucleotide substitutions per site.

Table 1. Eco-physiological traits of *Oscillochloris* bacteria from different source of isolation.

Organism	Geography	Source of isolation	Anaerobic	Phototrophic	*cbbL*	*nif*H	Source of information
Oscillochloris trichoides DG-6	Caucasus	Spring	+	+	+	+	This study
'Ca. Oscillochloris fontis' Chuk17	Chukotka	Spring	+	+	+	+	
Oscillochloris sp. Al-1	Siberia	Spring	+	+	+	+	
Oscillochloris sp. Um-3	Siberia	Spring	+	+	+	+	
Oscillochloris sp. Ku-3	Siberia	Spring	+	+	+	+	
Oscillochloris sp. R	Central Russia	Shallow river water	+	+	+	+	(Keppen *et al.* 2000; Turova *et al.* 2006)
Oscillochloris sp. C6	Siberia	Small body of water	+	+	+	+	

of bacteriochlorophyll *c*, as well as small amounts of bacteriochlorophyll *a*. Additionally, we found lycopene, its derivative, γ-carotene and β-carotene (4.3:23.7:69.0:3.0 mol%). The bacteriochlorophylls *c* and *a* accounted for 99.7% and 0.3 mol%, respectively. The bacteriochlorophylls and carotenoids accounted for 84.0% and 16.0 mol%, respectively. This data shows that '*Ca.* Oscillochloris fontis' Chuk17 cells, similarly to *O. trichoides* DG-6, contain only bacteriochlorophylls *c* and *a* as the main pigments of the photosystem.

Description of '*Ca.* Oscillochloris fontis'

'*Candidatus* Oscillochloris fontis' (fon′tis. L. masc. gen. n. *fontis* of a spring).

Cells are approximately 1 μm in diameter and 2–4 μm long. Cells form unbranched multicellular filaments of variable lengths; the filaments are motile. Gas vesicles are present near cell-to-cell junctions. Storage inclusions, polyhydroxyalkanoate- and polyphosphate-like granules are present, as are chlorosomes. Cells form green colonies in agar medium and green biofilms in liquid. The absorption spectrum of the cellular membrane suspension exhibits maxima at 457, and 747 nm, minor peaks at 637, 677 and 858 nm, as well as shoulders at 434 and 508 nm. The photosynthetic pigments are bacteriochlorophylls *c* and *a*, as well as carotenoid: lycopene, a derivative of lycopene, β-carotene and γ-carotenes. In the enrichment culture, the best yield was observed at 5–10 g L^{-1} NaCl, pH 8.5 and a temperature of 28°C. There is anaerobic and phototrophic growth. The genome contains genes of the sulfide:quinone reductase, the ribulose-1,5-bisphosphate carboxylase/oxygenase, and the nitrogenase complex. The DNA G + C content is 58.62%. In comparison to *O. trichoides*

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DG-6, the ANI and DDH of this bacterium are 85.5% and 30.2%, respectively. The genome sequence is available in GenBank under the accession number RYFD00000000.

Functional and phylogenetic diversity of *Oscillochloris*

In addition to '*Ca*. Oscillochloris fontis' Chuk17, we isolated three enrichment cultures of *Oscillochloris* bacteria from Siberian sulfide-containing freshwater hot springs: *Oscillochloris* sp. Al-1, *Oscillochloris* sp. Um-3, and *Oscillochloris* sp. Ku-3 from the low-temperature zones of Alla, Umhey, and Kuchiger hot springs, respectively. Phylogenetic analyses of 16S rRNA gene sequences showed that these bacteria also belong to a cluster of *O. trichoides* (Fig. 4). These results, as well as previously published data, show that *Oscillochloris* bacteria can be found in disparate freshwater springs that contain sulfides in Spain, the Caucasus, Siberia, and the Chukotka Peninsula (Keppen, Baulina and Kondratieva 1994; Bañeras *et al*. 2009). Additionally, some *Oscillochloris* bacteria have been isolated from different sulfide-containing aquatic environments such as shallow rivers, the White Sea estuary, and a freshwater alkaline soda lake (Keppen *et al*. 2000). However, the ecological role of *Oscillochloris* bacteria in these habitats remains enigmatic.

Similar to *O. trichoides* DG-6, the genome of '*Ca*. Oscillochloris fontis' possesses genes for CO_2 and N_2 fixation, that is those for the Calvin cycle and nitrogenase complex (Keppen *et al*. 2000; Turova *et al*. 2006). We also detected key genes for both processes in the Siberian bacteria using *nif*H and *cbbL* primers and PCR with subsequent Sanger sequencing. Phylogenetic analysis showed that these Siberian bacteria, as with '*Ca*. Oscillochloris fontis' possess a type of NifH and CbbL proteins specific for *O. trichoides* DG-6 (Fig. S3A and S3B; Supporting Information). These, and previously published, data show that *Oscillochloris* bacteria from different sources possess the same traits including CO_2 and N_2 fixation (Table 1).

CONCLUSIONS

Genes for CO_2 and N_2 fixation are conservative genomic traits of bacteria belonging to the *Oscillochloris* genus. The evolution and adaptive significance of these traits in phototrophic Chloroflexota are still not clear. In light of this thesis, our new genomic data, which are enhanced by phenotypical and some physiological detail, will enable more robust comparative analysis in future research.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

ACKNOWLEDGMENTS

The authors are grateful to colleagues of the Laboratory of Microbiology at the Institute of General and Experimental Biology, Siberian Branch, RAS, Ulan-Ude for organizing the expedition to the Siberian hot spring. The DNA sequencing was partially performed using the equipment of the Collective Use Center 'Bioengineering' of the Federal Research Center of Biotechnology.

FUNDING

This study was supported by the Russian Foundation for Basic Research (research projects № 16–34–60071, 19–04-00423 and 17–04-01578) and Ministry of Science and Higher Education of the Russian Federation.

Conflicts of interest. None declared

REFERENCES

Amann RI, Binder BJ, Olson RJ *et al*. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl Environ Microbiol* 1990;**56**:1919–25.

Ashikhmin A, Makhneva Z, Moskalenko A. The LH2 complexes are assembled in the cells of purple sulfur bacterium *Ectothiorhodospira haloalkaliphila* with inhibition of carotenoid biosynthesis. *Photosynth Res* 2014;**119**:291–303.

Aziz RK, Bartels D, Best AA *et al*. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008;**9**:75.

Bañeras L, Gich F, Martinez-Medina M *et al*. New phylotypes of mesophilic filamentous anoxygenic phototrophic bacteria enriched from sulfide-containing environments. *Environ Microbiol Rep* 2009;**1**:86–93.

Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;**30**:2114–20.

Britton G. *Carotenoids*. Britton G, Liaaen-Jensen S, Pfander H (eds). Basel: Birkhäuser Basel, 1995.

Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000;**17**:540–52.

Chaudhari NM, Gupta VK, Dutta C. BPGA- an ultra-fast pan-genome analysis pipeline. *Sci Rep* 2016;**6**:24373.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460–1.

Field D, Garrity G, Gray T *et al*. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* 2008;**26**:541–7.

Gaisin VA, Burganskaya EI, Grouzdev DS *et al*. '*Candidatus* Viridilinea mediisalina', a novel phototrophic Chloroflexi bacterium from a Siberian soda lake. *FEMS Microbiol Lett* 2019, doi:10.1093/femsle/fnz043.

Gaisin VA, Kalashnikov AM, Sukhacheva M V *et al*. Filamentous anoxygenic phototrophic bacteria from cyanobacterial mats of Alla hot springs (Barguzin Valley, Russia). *Extremophiles* 2015;**19**:1067–76.

Goris J, Konstantinidis KT, Klappenbach JA *et al*. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;**57**:81–91.

Gorlenko VM, Bryantseva IA, Kalashnikov AM *et al*. *Candidatus* "Chloroploca asiatica" gen. nov., sp. nov., a new mesophilic filamentous anoxygenic phototrophic bacterium. *Microbiology* 2014;**83**:838–48.

Gorlenko VM, Korotkov SA. Morphological and physiological features of the new filamentous gliding green bacteria *Oscillochloris trichoides* nov. comb. *Izv Akad Nauk SSSR Biol* 1979:848–57.

Grouzdev DS, Burganskaya EI, Krutkina MS *et al*. Genome Sequence of "*Candidatus* Viridilinea halotolerans" Chok-6, isolated from a saline sulfide-rich spring. *Microbiol Resour Announc* 2019;**8**:1–2.

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Grouzdev DS, Rysina MS, Bryantseva IA *et al*. Draft genome sequences of '*Candidatus* Chloroploca asiatica' and '*Candidatus* Viridilinea mediisalina', candidate representatives of the *Chloroflexales* order: phylogenetic and taxonomic implications. *Stand Genomic Sci* 2018;**13**:24.

Hoang DT, Chernomor O, von Haeseler A *et al*. UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol* 2018;**35**:518–22.

Ivanovsky RN, Fal YI, Berg IA. *et al*. Evidence for the presence of the reductive pentose phosphate cycle in a filamentous anoxygenic photosynthetic bacterium, *Oscillochloris trichoides* strain DG-6. *Microbiology* 1999;**145**:1743–8.

Kalyaanamoorthy S, Minh BQ, Wong TKF *et al*. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 2017;**14**:587–9.

Katoh K. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 2002;**30**:3059–66.

Keppen OI, Baulina OI, Kondratieva EN. *Oscillochloris trichoides* neotype strain DG-6. *Photosynth Res* 1994;**41**:29–33.

Keppen OI, Lebedeva N V., Troshina Y O *et al*. The nitrogenase activity of a filamentous phototrophic green bacterium. *Mikrobiologiya* 1989;**58**:520–1.

Keppen OI, Tourova TP, Kuznetsov BB *et al*. Proposal of *Oscillochloridaceae* fam. nov. on the basis of a phylogenetic analysis of the filamentous anoxygenic phototrophic bacteria, and emended description of *Oscillochloris* and *Oscillochloris trichoides* in comparison with further new isolates. *Int J Syst Evol Microbiol* 2000;**50** Pt 4:1529–37.

Kuznetsov BB, Ivanovsky RN, Keppen OI *et al*. Draft genome sequence of the anoxygenic filamentous phototrophic bacterium *Oscillochloris trichoides* subsp. DG-6. *J Bacteriol* 2011;**193**:321–2.

Laczny CC, Kiefer C, Galata V *et al*. BusyBee Web: metagenomic data analysis by bootstrapped supervised binning and annotation. *Nucleic Acids Res*. 2017; **45 (** W1**)**:W171–9.

Lin HH, Liao YC. Accurate binning of metagenomic contigs via automated clustering sequences using information of genomic signatures and marker genes. *Sci Rep* 2016;**6**:24175.

Loy A, Arnold R, Tischler P *et al*. probeCheck - a central resource for evaluating oligonucleotide probe coverage and specificity. *Environ Microbiol* 2008;**10**:2894–8.

Marusina AI, Boulygina ES, Kuznetsov BB *et al*. A system of oligonucleotide primers for the amplification of *nif*H genes of different taxonomic groups of prokaryotes. *Microbiology* 2001;**70**:73–8.

Meier-Kolthoff JP, Auch AF, Klenk H-P *et al*. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;**14**:60.

Nurk S, Meleshko D, Korobeynikov A *et al*. MetaSPAdes: a new versatile metagenomic assembler. *Genome Res* 2017;**27**:824–34.

Olson T Le, van de Meene AML, Francis JN *et al*. Pigment analysis of "*Candidatus* Chlorothrix halophila" a green filamentous anoxygenic phototrophic bacterium. *J Bacteriol* 2007;**189**:4187–95.

Parks DH, Imelfort M, Skennerton CT *et al*. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;**25**: 1043–55.

Pernthaler J, Glöckner FO, Schönhuber W *et al*. Fluorescence *in situ* hybridization (FISH) with rRNA-targeted oligonucleotide probes. *Methods Microbiol* 2001;**30**:207–26.

Pfennig N, Lippert K. Über das Vitamin B12-Bedürfnis phototropher Schwefelbakterien. *Arch Mikrobiol* 1966;**55**:245–56.

Reynolds ES. The use of lead citrate stain at high pH in electron microscopy. *J Cell Biol* 1963;**17**:208.

Rodriguez-R LM, Konstantinidis KT. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *Peer J Prepr* 2016;**4**:e1900v1.

Sieber CMK, Probst AJ, Sharrar A. *et al*. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. *Nat Microbiol* 2018;**7**:836–43.

Turova TP, Spiridonova EM, Slobodova NV *et al*. Phylogeny of anoxygenic filamentous phototrophic bacteria of the family *Oscillochloridaceae* as inferred from comparative analyses of the *rrs*, *cbb*L, and *nif*H genes. *Mikrobiologiia* 2006;**75**:235–44.

Wright ES, Yilmaz LS, Corcoran AM *et al*. Automated design of probes for rRNA-targeted fluorescence *in situ* hybridization reveals the advantages of using dual probes for accurate identification. *Appl Environ Microbiol* 2014;**80**:5124–33.

Wu YW, Simmons BA, Singer SW. MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics* 2016;**32** :605–7.

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