FEMS Microbiology Letters, 367, 2020, fnaa158

doi: 10.1093/femsle/fnaa158
Advance Access Publication Date: 5 October 2020
Research Letter

RESEARCH LETTER – Taxonomy & Systematics

'*Candidatus* Oscillochloris kuznetsovii' a novel mesophilic filamentous anoxygenic phototrophic *Chloroflexales* bacterium from Arctic coastal environments

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One sentence summary: Arctic coastal environments harbor the novel filamentous multicellular phototrophic Chloroflexi bacterium.

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Editor: Aharon Oren

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ABSTRACT

Chloroflexales bacteria are mostly known as filamentous anoxygenic phototrophs that thrive as members of the microbial communities of hot spring cyanobacterial mats. Recently, we described many new *Chloroflexales* species from non-thermal environments and showed that mesophilic *Chloroflexales* are more diverse than previously expected. Most of these species were isolated from aquatic environments of mid-latitudes. Here, we present the comprehensive characterization of a new filamentous multicellular anoxygenic phototrophic *Chloroflexales* bacterium from an Arctic coastal environment (Kandalaksha Gulf, the White Sea). Phylogenomic analysis and 16S rRNA phylogeny indicated that this bacterium belongs to the *Oscillochloridaceae* family as a new species. We propose that this species be named '*Candidatus* Oscillochloris kuznetsovii'. The genomes of this species possessed genes encoding sulfide:quinone reductase, the nitrogenase complex and the Calvin cycle, which indicate potential for photoautotrophic metabolism. We observed only mesophilic anaerobic anoxygenic phototrophic growth of this novel bacterium. Electron microphotography showed the presence of chlorosomes, polyhydroxyalkanoate-like granules and polyphosphate-like granules in the cells. High-performance liquid

Received: 30 June 2020; **Accepted:** 29 September 2020

chromatography also revealed the presence of bacteriochlorophylls *a*, *c* and *d* as well as carotenoids. In addition, we found that this bacterium is present in benthic microbial communities of various coastal environments of the Kandalaksha Gulf.

Keywords: anoxygenic phototrophic bacteria; *Chloroflexi*; *Chloroflexota*; *Chloroflexales*; *Oscillochloris*; Arctic; the White Sea; Kandalaksha Gulf

INTRODUCTION

The Arctic environment harbors unique microbial diversity, which has remained poorly explored due to the great scale of the region. However, many microbiologists have taken on the challenge of conducting research in high-latitude environments because the Polar regions have great significance for our biosphere (Stibal, Jacobsen and Häggblom 2018). The White Sea is an important northern region that has attracted the attention of microbiologists over the last decade. Recent studies of the Kandalaksha Gulf within the White Sea have revealed many meromictic lakes and partially isolated lagoons that enable diverse anoxygenic phototrophic bacteria, mainly green sulfur bacteria and purple bacteria, to thrive (Lunina *et al.* 2016; Savvichev *et al.* 2018; Zhiltsova *et al.* 2018; Grouzdev *et al.* 2018a, 2019b). However, knowledge of anoxygenic phototrophic *Chloroflexi* (*Chloroflexota*) in the ecosystems of this region has remained highly limited.

The majority of phototrophic *Chloroflexota* bacteria belong to the *Chloroflexales* order (Grouzdev *et al.* 2018b, Thiel 2018). These bacteria are filamentous multicellular anoxygenic phototrophic bacteria that thrive in extreme environments, such as hot springs (Hanada 2014; Imhoff 2017). The main body of knowledge regarding *Chloroflexales* is based on studies of thermophilic representatives. High-quality genomic sequences, in particular, are mainly only available for thermophilic *Chloroflexales*. This fact has seriously limited studies of such important topics as the evolution of photosynthesis (Ward *et al.* 2018). In order to overcome this limitation, we have made an effort to carry out the isolation, morphological characterization and genomic sequencing of newly discovered mesophilic *Chloroflexales* representatives (Gorlenko *et al.* 2014; Grouzdev et al. 2018b, 2019a; Gaisin *et al.* 2019a, b). Our approach was designed to characterize each *Chloroflexales* bacterium in the enrichment culture due to the difficulty involved in isolating the axenic culture. The sequencing of a metagenome of highly enriched cultures has allowed for the assembly of high-quality genomes of *Chloroflexales* bacteria as well as the assignment of a genome-based taxonomic name in the *Candidatus* category. As a result, we have characterized new bacteria such as '*Ca*. Chloroploca asiatica', '*Ca*. Viridilinea mediisalina', '*Ca*. Oscillochloris fontis' (Gorlenko *et al.* 2014; Grouzdev *et al.* 2018b; Gaisin et al. 2019a, b). These bacteria have been isolated from the aquatic environments of Siberia and the Chukotka Peninsula. In the present work, we applied the same approach for the study of a novel *Chloroflexales* bacterium from the partially isolated lagoons of the Kandalaksha Gulf. We described this novel bacterium '*Candidatus* Oscillochloris kuznetsovii' based on the genomic and phenotypic data presented herein. The present work comprises the first comprehensive characterization of a *Chloroflexales* strain from the Arctic region.

MATERIALS AND METHODS

Culture isolation and maintenance

For the isolation of *Chloroflexales* bacteria, a small piece of microbial mat was gently homogenized and added to a semi-solid medium (0.5–0.7% agar) consisting of the following components (per liter of distilled water): KH_2PO_4 = 0.20 g, NH_4Cl = 0.20 g, $MgCl_26H_2O$ = 0.20 g, KCl = 0.30 g, NaCl = 10.0 g, $Na_2S_2O_3$ = 0.30 g, Na_2SO_4 = 0.30 g, $CaCl_22H_2O$ = 0.05 g, $NaHCO_3$ = 0.60 g, Na_2S9H_2O = 0.70 g, yeast extract = 0.05 g, sodium acetate = 0.10 g, trace element solution = 1 mL and Pfennig's vitamin solution = 1 mL (Pfennig and Lippert 1966). The tubes with medium were adjusted to a pH of 8.0–8.2 and incubated at 28°C in near-infrared light with a wavelength of 740 ± 10 nm (narrowband light-emitting diodes).

The enrichment of phototrophic *Chloroflexota* bacteria was accomplished using the solidified medium via cycles of isolation of a single colony and serial dilution followed by transfer to the fresh medium. Experiments for optimal NaCl and sulfide concentration were performed using the semi-solid medium (0.2–0.3% agar) in glass vials with rubber stoppers under full-range light (3800 lux). Growth of *Chloroflexales* bacteria under different culture conditions was determined by measuring the optical density of the cell suspension at 750 nm using a KFK-3-ZOMZ photometer (USSR).

Metagenomic sequencing, binning and phylogenetic analysis

Genomic DNA was isolated from the enrichment cultures as described previously (Grouzdev *et al.* 2018b). DNA libraries were constructed with the NEBNext DNA library prep reagent set for Illumina per the kit's protocol. DNA sequencing was accomplished using the Illumina HiSeq 1500 platform with single-end 220-bp reads. A total of 2854 502 reads were obtained and deposited in the NCBI SRA database under the accession number SRR11714372. 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 were assembled for all samples 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 v2.2.4 (Wu, Simmons and Singer 2016) and MyCC (Lin and Liao 2016). The three bin sets were supplied to DAS Tool v1.0 (Sieber *et al.* 2018) for consensus binning to obtain the final optimized bins. Genome bins were assessed for completeness and contamination using CheckM v1.0.12 (Parks *et al.* 2015). The genome of ZM16–3 was deposited in DDBJ/ENA/GenBank under the accession number RYFE00000000. Identification of protein-coding sequences and their annotations was performed using the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova *et al.* 2016).

Pairwise average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values were calculated using the ANI calculator (Varghese *et al.* 2015) and the genome-to-genome distance calculator v2.1 (Meier-Kolthoff *et al.* 2013), respectively, with BLAST+ for genome alignments (Camacho *et al.* 2009). Average amino acid identity (AAI) values were calculated using CompareM v0.0.23 (https://github.com/dparks1134/CompareM) with default blastp parameters (i.e. *e*-value ≤ 0.001, % identity ≥ 30% and alignment length ≥ 70%). The pairwise percentage of conserved proteins (POCP) was calculated using the runPOCP.sh

script (Pantiukh and Grouzdev 2017), which was based on a previously published approach (Qin et al. 2014).

Phylogenomic analysis of the *Chloroflexales* genomes was conducted using a concatenated alignment of 120 single-copy phylogenetic marker genes obtained using GTDB-Tk v0.3.3 (Chaumeil et al. 2019). Maximum-likelihood trees 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. 2018a). Maximum parsimony and neighbor-joining trees were reconstructed using MPBoot (Hoang et al. 2018b) and MEGA7 (Kumar, Stecher and Tamura 2016), respectively.

Fluorescence *in situ* hybridization, light and transmission electron microscopy

Fluorescence *in situ* hybridization (FISH) was accomplished using previously described methods (Pernthaler et al. 2001). The cells were fixed in 3% paraformaldehyde for 1.5 h. The probe ZM16–3 (5′-ac-gct-ccg-tac-cgc-cta-cgt-acg-3′) was designed using Decipher and included Cy3 dye (Wright et al. 2014). This probe was checked using probeCheck (Loy et al. 2008). A universal bacterial probe, EUB338 (5′-gct-gcc-tcc-cgt-agg-agt-3′) with FAM dye, was used as a positive control (Amann et al. 1990). The sample was imaged under a Nikon Eclipse Ti (Nikon, Japan) light microscope.

The cell morphology displayed in Fig. 1 was imaged using an Olympus BX41TF phase-contrast microscope (Olympus, Japan). Transmission electron microscopy was accomplished as described previously (Gaisin et al. 2019b) using the Libra-120 transmission electron microscope (Carl Zeiss, Germany).

Analysis of pigment composition

The absorption spectra of the membrane fractions of 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. HPLC of the pigment extracts was accomplished as described previously (Ashikhmin, Makhneva and Moskalenko 2014) using an Agilent Zorbax SB-C18 (4.6 × 250 mm) column (Agilent, Santa Clara, California, US). The device used for HPLC (Shimadzu, Japan) consisted of LC-10ADVP pump with FCV-10ALVP module, a detector with SPD-M20A diode matrix, and CTO-20AC thermostat. The column was balanced with the acetonitrile/water/ethyl acetate mixture (69.3%:7.7%:23%, v/v) as described previously (Gaisin et al. 2019b). The analysis was performed at 22°C, and the pigment concentrations were calculated with the LCsolution program (Shimadzu, Japan) as described previously (Gaisin et al. 2019b). The molar extinction coefficients for the corresponding pigments were as follows: 145/mM/cm, 75/mM/cm, 50/mM/cm, 74/mM/cm and 76/mM/cm for colored carotene, phytofluene, phytoene and bacteriochlorophylls *c*, *d* and *a*, respectively.

RESULTS AND DISCUSSION

Morphology and basic physiology

We isolated the novel mesophilic green filamentous multicellular *Chloroflexales* bacterium ZM16–3 from a partially isolated lagoon on the cape Zeleny Mys (66°31'46.2'N 33°05'38.4'E) in the Kandalaksha Gulf of the White Sea (Fig. 1A and 1B). The source of the isolation was a thin cyanobacterial mat on black, smelly (apparently sulfide-rich) sediment collected from the tidal zone of the lagoon in 2016 (Fig. 1C). The total mineralization of the interstitial fluid from the mat (15‰) was lower than the mineralization of the water in the lagoon (22–28‰). The interstitial fluid in the tidal zone was apparently diluted by fresh water from the wetland surrounding the lagoon.

The novel bacterium was isolated and characterized in the present work as a stable enrichment culture of *Chloroflexales* bacterium ZM16–3. The morphology of the novel bacterium was typical for representatives of the *Chloroflexales* order. *Chloroflexales* bacterium ZM16–3 formed green, spherical colonies in the deep parts of the tubes with the solidified medium. The cells formed long, unbranched filaments of variable length (Fig. 1D). The average cell length was 4 ± 1 μm (n = 25). The average cell diameter was 1 ± 0.1 μm (n = 35). Some cells with long intracellular inclusions were two times longer (9 ± 2 μm; n = 5) than the average cell length (Fig. 1E). Electron microphotographs of ultrathin cross-sections revealed that the cells contained chlorosomes, polyhydroxyalkanoate-like granules and polyphosphate-like granules (Fig. 1F and 1G). The polyhydroxyalkanoate-like granules occupied a significant volume of the cells on the electron microphotographs.

The growth of *Chloroflexales* bacterium ZM16–3 was observed only in anaerobic conditions under illumination. The best yield of the cells was observed at an NaCl concentration of 5 g/L (Figure S1A, Supporting Information). The bacterium was resistant to a high sulfide concentration, as growth was observed at Na_2S9H_2O concentration of 1 g/L (Figure S1B, Supporting Information). At present, we can conclude that *Chloroflexales* bacterium ZM16–3 is a sulfide-tolerant anaerobic anoxygenic phototrophic bacterium.

Genome, phylogeny and FISH

The total DNA of the enriched culture of *Chloroflexales* bacterium ZM16–3 was sequenced to assemble the genome of the target bacterium. Analysis of all the assembled scaffold from the entire dataset revealed that only one member of the *Chloroflexi* (*Chloroflexota*) phylum was present in the culture. A metagenome-assembled genome of the *Chloroflexota* bacterium was used for the following analysis. The final assembled 6269 253-bp-long genome comprised 127 scaffolds, with an N_{50} value of 134 699 bp and an average coverage of 35× . The genome sequence contained 5190 genes, of which 5068 were protein-coding sequences, 72 were pseudogenes and 50 coded RNAs. The genome of *Chloroflexales* bacterium ZM16–3 was 1.8–1.9 Mb larger and had a G + C composition more than 6% higher than the genomes of other *Oscillochloris* species (Table 1). The genome of this bacterium possessed genes of type I sulfide:quinone reductase, the nitrogenase complex (*nifHBDK*) and the Calvin cycle (form I RuBisCo), similar to the genome of *O. trichoides* DG-6. This allow suppose that *Chloroflexales* bacterium ZM16–3 able to photoautotrophic metabolism like *O. trichoides* DG-6.

The genome of *Chloroflexales* bacterium ZM16–3 also contained the complete 16S rRNA gene sequence. On the 16S rRNA gene phylogenetic tree (Fig. 2A), *Chloroflexales* bacterium ZM16-3 belonged to a clade of the *Oscillochloridaceae* family (Keppen et al. 2000). The 16S rRNA gene sequence of *Chloroflexales* bacterium ZM16–3 exhibited 94.3% and 93.5% similarity to the sequences of the most closely related species '*Ca*. Oscillochloris fontis' Chuk17 and *Oscillochloris trichoides* DG-6^T, respectively, and less than 91.8% similarity to the other species of *Chloroflexales*. The similarity levels were close to 94.5%, which is the border value for separation into different genera (Yarza et al. 2014); therefore,

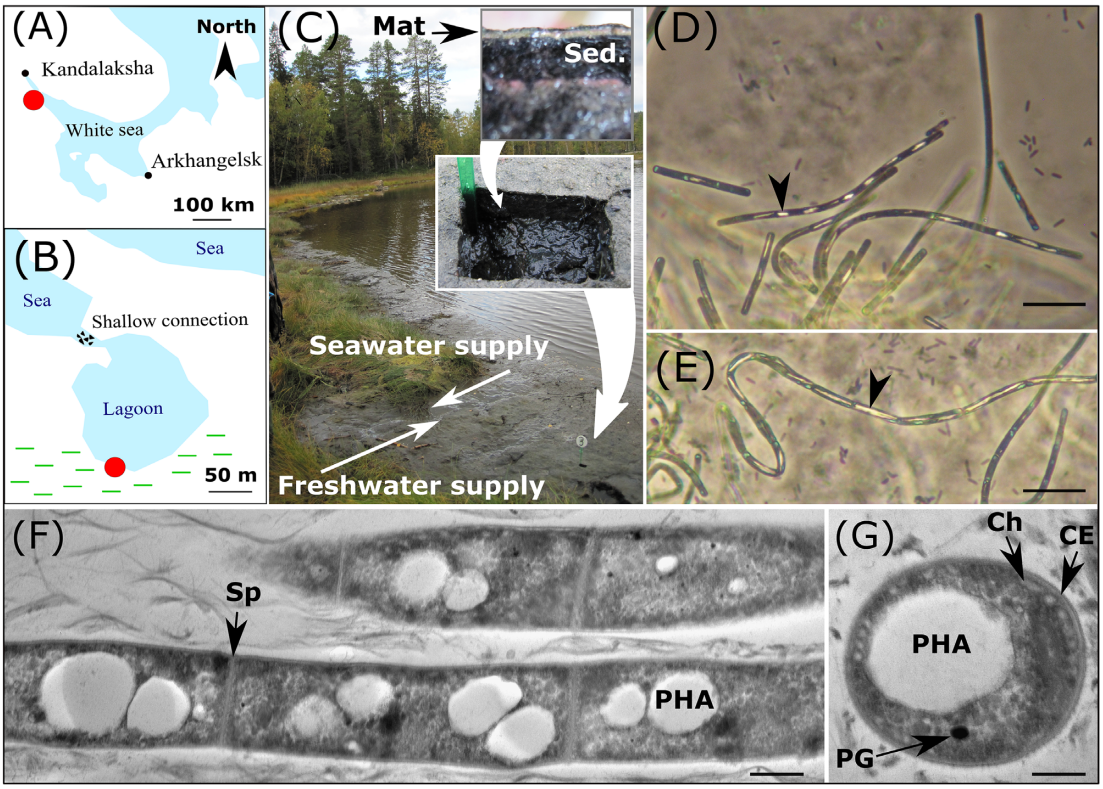

Figure 1. **(A)** Location of the sampling site on a large scale map and **(B)** on a small scale map of the lagoon. North direction is the same for both maps. The red dot points to the approximate location of the lake and sampling site. **(C)** Sampled mat and sampling site in a tidal zone of the lagoon. Sed. = the sediment. **(D)** Morphology of the green filamentous Chloroflexi bacterium ZM16–3 in transmission light. The arrow shows a bright intracellular inclusion. **(E)** Long, bright intracellular inclusions (arrow). (**F** and **G**) Transmission electron micrographs of cells in an ultrathin cross-section. CE = cell envelope; Ch = chlorosome; Sp = septum; PG = polyphosphate-like granule; PHA = polyhydroxyalkanoate granules. Bars = 10 μm (D and E); 400 (F) and 200 (G) nm.

Table 1. Genome statistics.

	ZM16–3 (GCA_004138175.2)		*Oscillochloris trichoides* DG-6 (GCF_000152145)		*Ca. Oscillochloris fontis* Chuk-17 (GCF_004138135)	
Attribute	Value	% of total	Value	% of total	Value	% of total
Genome size (bp)	6269 253	100.00	4373 075	100.00	4463 428	100.00
DNA coding (bp)	5463 563	87.15	3864 630	88.37	3920 492	87.84
DNA G + C (bp)	4088 254	65.21	2556 357	58.46	2614 697	58.58
DNA scaffolds	127	100.00	7	100.00	139	100.00
Total genes	5190	100.00	3549	100.00	3640	100.0
Protein coding genes	5068	97.65	3430	96.65	3515	96.57
RNA genes	50	0..96	54	1.52	60	1.65
Pseudo genes	72	1.39	65	1.83	65	1.79
Genes with function prediction	3834	73.87	2838	79.97	2884	79.23
Genes assigned to COGs	3596	69.29	2527	71.20	2664	73.19
Genes with Pfam domains	4094	78.88	2822	79.52	3005	82.55
Genes with signal peptides	468	9.02	272	7.66	290	7.97
Genes with transmembrane helices	1321	25.45	840	23.67	902	24.78

the results of sequences comparison did not allow us to unambiguously refer *Chloroflexales* bacterium ZM16–3 to the *Oscillochloris* genus.

Complete genomic sequence analysis was performed to confirm a new strain assignment at the genus and family level, as recommended by Chun and coauthors (Chun *et al.* 2018). The dDDH values of 20.1–20.2% were inferred from a comparison of *Chloroflexales* bacterium ZM16–3 genome against the reference genomes of *Oscillochloris* species. The ANI value of 75.3–75.5 and the aligned fraction (AF) of 0.53–0.54 were inferred from a comparison of the *Chloroflexales* bacterium ZM16-3 genome against the closely related *Ca. Oscillochloris fontis* Chuk17 and *Oscillochloris trichoides* DG-6^T. The ANI and dDDH values of the ZM16–3 genome indicated that this strain belongs to a new species.

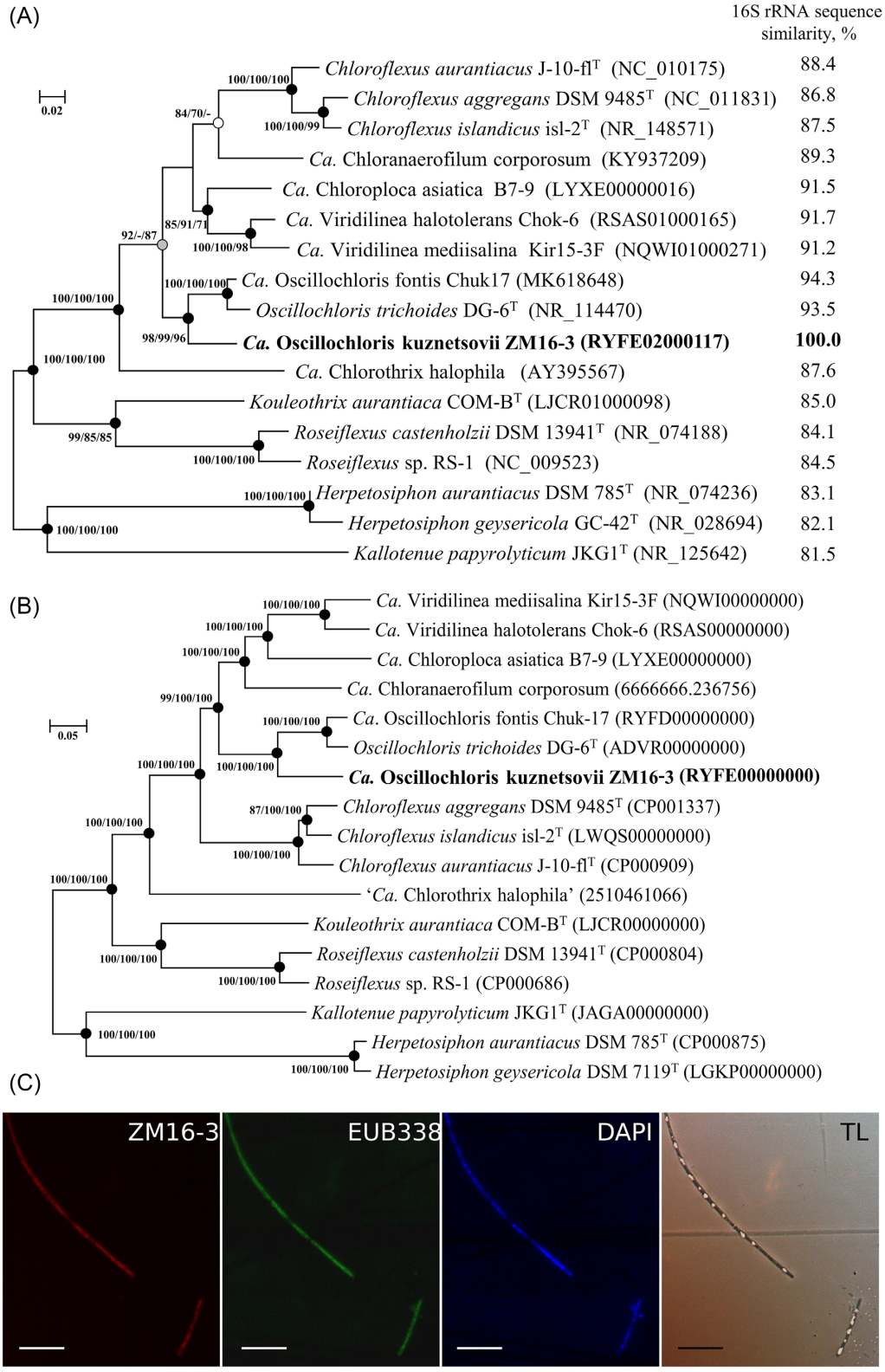

Figure 2. (A) Maximum-likelihood phylogenetic trees based on 16S gene sequences (1251 nucleotide sites) reconstructed with evolutionary model TN + F + I + G4 showing the position out 1395. The scale bar represents nucleotide substitutions per site. (B) Maximum-likelihood phylogenetic tree inferred from a comparison of concatenated *Chloroflexia* core proteins showing the position of '*Ca*. Oscillochloris kuznetsovii' ZM16–3. 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. (C) Fluorescence in situ hybridization using a Cy3-stained ZM16–3 probe against the 16S rRNA of '*Ca*. Oscillochloris kuznetsovii' ZM16–3 (red) and using a FAM-stained EUB338 probe against a wide range of bacteria (green). DNA was stained with DAPI (blue). TL: transmission light image. Bars = 10 μm.

The ANI and AF values of the genus demarcation boundaries were recently proposed as an ANI of ~74% and an AF of ~0.33 (Barco *et al.* 2020). Thus, according to its ANI and AF values, *Chloroflexales* bacterium ZM16–3 belongs to the *Oscillochloris* genus.

To determine the genome-based phylogenetic position of *Chloroflexales* bacterium ZM16–3, a phylogenomic tree was built based on 120 concatenated, conserved bacterial proteins (Fig. 2B). In contrast with the 16S rRNA gene tree, the phylogenomic tree had high bootstrap support for branches. On the phylogenomic tree, *Chloroflexales* bacterium ZM16–3 formed a separate lineage within the *Oscillochloris* genus. Although the ZM16–3 genome differed significantly from the genomes of other *Oscillochloris* species in terms of size and G + C composition, the AAI and POCP values of these species ranged from 74.6 to 74.8% and 65.4 to 65.8%, respectively (Tables S1 and S2, Supporting Information). According to the existing criteria, the AAI and POCP values of representatives of the same genus are 65–95% and higher than 50%, respectively (Qin *et al.* 2014; Konstantinidis, Rosselló-Móra and Amann 2017). However, this proposed threshold is not a strict requirement (Orata *et al.* 2018; Koziaeva *et al.* 2019). The use of lower boundaries of 65% for AAI and 50% for POCP would lead to the combination of all *Chloroflexinae* genomes into one family, but the use of values of 70% for AAI and 65% for POCP would support the separation of genera correlated with the branching of the phylogenomic tree. The obtained ANI, AAI and POCP values in combination with the lengths of the phylogenetic branches allowed us to propose *Chloroflexales* bacterium ZM16–3 as a new species of *Oscillochloris* in *Candidatus* status: '*Ca.* Oscillochloris kuznetsovii'.

Fluorescence *in situ* hybridization with a probe against the 16S rRNA gene from the assembled genome proved that the '*Ca.* Oscillochloris kuznetsovii' genome corresponds to the analyzed green filamentous multicellular *Chloroflexales* bacterium ZM16–3 bacterium in the culture (Fig. 2C).

Pigment composition

A spectral analysis of the membrane fraction resuspended in the Tris-HCl buffer resulted in an absorption spectrum with peaks at 416 and 755 nm, minor peaks at 670 nm and shoulders at 458 and 521 nm (Fig. 3A). In an acetone-methanol (7:2, v/v) mixture, the absorption spectrum of the pigment extract had peaks at 391, 418, 435, 615 and 665 nm and shoulders at 460 nm (Fig. 3A). The HPLC elution profile of the pigment extract had 21 signals (Fig. 3B). We found small amounts of bacteriochlorophyll *d* and *a* (0.1 and 0.3 mol%, respectively) in the pigment extract. However, the majority of the pigments were composed of bacteriochlorophyll *c* and γ-carotene (86.5 and 7.4 mol%, respectively). We also detected the presence of β-carotene, ζ-carotene, phytoene, phytofluene, lycopene and its derivatives. The molar ratios of all the pigments are presented in Table S3 (Supporting Information).

Distribution of '*Ca.* Oscillochloris kuznetsovii' ZM16-3 in the Kandalaksha Gulf

We found that '*Ca.* Oscillochloris kuznetsovii' ZM16–3 was detected previously as OTU1395 in environmental samples from the Kandalaksha Gulf (Burganskaya *et al.* 2019). We analyzed the 16S rRNA amplicon data published in the aforementioned work in order to understand the distribution of '*Ca.* Oscillochloris kuznetsovii' ZM16–3 in habitats of the Kandalaksha Gulf. Our analysis revealed that '*Ca.* Oscillochloris kuznetsovii' ZM16–3 was present in trace amounts (<0.5% of the total

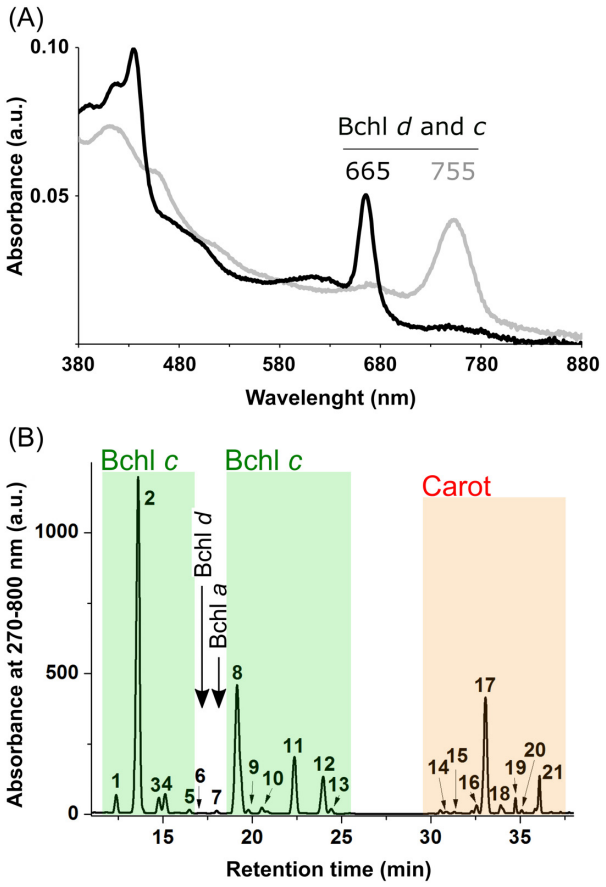

Figure 3. Cellular pigment composition of '*Ca.* Oscillochloris kuznetsovii' ZM16–3. (A) Absorption spectra for the membrane fraction of the Tris-HCl buffer (grey line) and acetone-methanol (black line). (B) HPLC chromatogram of the pigment extracted in acetone-methanol (7:2), detected at 270–800 nm. Identification of HPLC peaks: 1–5, 8–13 = bacteriochlorophyll *c*; 6 = bacteriochlorophyll *d*; 7 = bacteriochlorophyll *a*; 14 = lycopene; 15, 16 = lycopene derivatives; 17 = γ-carotene; 18 = ζ-carotene; 19 = β-carotene; 20 = phytofluene; 21 = phytoene. Bchl = bacteriochlorophyll; Carot = carotenes; a.u. = absorbance units.

number of reads) in samples of microbial mats collected from the surfaces of sulfide-rich sediments from different ecotopes of the Kandalaksha Gulf, beside the lagoon of the cape Zeleny Mys. OTU1395 was detected in microbial mats derived from salt marshes located close to the capes Ermolinskaya Guba and Nilma Guba as well as a mat derived from the tidal zone of the meromictic lake Kislo-Sladkoe (Table S4, Supporting Information). Representative photographs of the sampling sites are shown in Figure S2 (Supporting Information). Surprisingly, OTU1395 was absent in reads corresponding to the site from which *Ca.* Oscillochloris kuznetsovii' ZM16–3 was isolated. Low number of reads were present in two other sites from the lagoon on Zeleny Mys. This finding could be explained by a combination of two factors: the low number of cells in the samples and bias of the DNA extraction procedure. Ultimately, we can conclude that *Ca.* Oscillochloris kuznetsovii' ZM16–3 is present in different mats of sulfide-rich sediments in the coastal environment of the Kandalaksha Gulf.

Description of *Ca.* Oscillochloris kuznetsovii' ZM16-3

'*Ca.* Oscillochloris kuznetsovii' ZM16–3 (kuz.net.so'vi.i. N.L. gen. n. kuznetsovii, of Kuznetsov, named in honor of Boris B.

Kuznetsov, who has significantly contributed to the study of mesophilic phototrophic Chloroflexi).

'*Ca*. Oscillochloris kuznetsovii' ZM16–3 cells are approximately 1.0 μm in diameter and 4.0 μm long. These cells form unbranched, multicellular filaments of variable lengths. Bright long intracellular inclusions, polyhydroxyalkanoate-like granules, polyphosphate-like granules and chlorosomes are present in the cells. Cells form green colonies in agar medium. The absorption spectrum of the cellular membrane suspension exhibits maxima at 416, 755 and 670 nm with shoulders at 458 and 521 nm. The photosynthetic pigments of these cells are bacteriochlorophylls *c*, *d* and *a*; γ-carotene; β-carotene; ζ-carotene; phytoene; phytofluene; lycopene; and its derivatives. Growth of this species occurs in the presence of light under anaerobic conditions supplemented with Na-sulfide, Na-bicarbonate and Na-acetate. This species does not exhibit growth in the absence of light, whether in aerobic or anaerobic conditions. The genome of this species possessed genes of type I sulfide:quinone reductase, the nitrogenase complex (*nifHBDK*) and form I RuBisCo. The DNA G + C content is 65.2. The 16S rRNA gene sequence of '*Ca*. Oscillochloris kuznetsovii' ZM16–3 exhibited 94.3% and 93.5% similarity to the sequences of the most closely related species '*Ca*. Oscillochloris fontis' Chuk17 and *O. trichoides* DG-6^T, respectively. In comparison of '*Ca*. Oscillochloris kuznetsovii' ZM16–3 versus *O. trichoides* DG-6^T the ANI and DDH value of 75.5% and 20.2%, respectively. Both genome sequence and 16S rRNA gene sequence of '*Ca*. Oscillochloris kuznetsovii' ZM16–3 are available in GenBank under the accession number RYFE00000000 and RYFE02000117, respectively.

DATA AVAILABILITY

The raw metagenomic sequences are available at the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under accession number SRR11714372. The draft genome sequence of *Ca*. Oscillochloris kuznetsovii' ZM16–3 is available at GenBank under accession number RYFE00000000.

ACKNOWLEDGMENTS

The authors are grateful to all the staff of the White Sea Biological Station of Moscow State University for providing an excellent research facility on the Kandalaksha Gulf. We are also particularly grateful to our colleagues Elena Krasnova and Dmitry Voronov from Moscow State University for their invaluable help in organizing our sampling at Zeleny Mys. VG is grateful to Dr. Michal Koblížek for his support during preparation of the final version of the manuscript.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

FUNDING

This study was supported by the Russian Foundation for Basic Research (research projects number 19–04-00423) and Russian Ministry of Science and Higher Education.

Conflicts of interests. None declared.

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