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Isolation, identification, and antimicrobial activity of psychrophilic freshwater microalgae *Monoraphidium* sp. from Almaty region

Abstract. Microalgae is one of the potential biotechnologically valuable groups of photosynthetic microorganisms and represent the most promising resource for new products and applications. In this study, our efforts were focused on identification, isolation, and characterization of psychrophilic microalgae from the underinvestigated extreme environment. Three new isolates were isolated from the frozen Big Almaty Lake, but only for one of them the attempt to clean from bacteria and fungus was successful and it was obtained in algologically and bacteriologically pure form. The taxonomy and phylogeny of the green microalgae, was examined based on morphological (light and scanning electron microscopy) and sequence-based approach using universal molecular markers of ITS region and rbcL gene. Morphological and molecular characterization showed that this green microalgae is *Monoraphidium* sp. (strain ZBD-06). The antibacterial activity of the isolate was determined as well and 60% methanol extract of *Monoraphidium* sp. was used for antibacterial activity by disk diffusion assay and minimum inhibitory concentration using eleven different types of pathogenic bacteria including Gram positive (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228) and Gram negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Salmonella typhimurium* ATCC 14028, *Yersinia pseudotuberculosis* ATCC 911, and *Enterobacter cloacae* ATCC 13047). The zone of inhibition (10±2 mm in diameter with 10ul of extract and 20±2 mm in diameter with 30uL) was observed against *Klebsiella pneumoniae* ATCC 13883.

Key words: Almaty region, psychrophilic, microalgae, *Monoraphidium*, phylogenetic analysis, antimicrobial assay.

Introduction

Microalgae is one of the potential biotechnologically valuable groups of photosynthetic microorganisms and represent the most promising resource for new products and applications [1]. Recent decades have witnessed an increased appreciation of the role of microalgal diversity in ecosystem function and their significant value as source for important biological materials such as antibiotics, drugs, enzymes, herbicides, growth promoters and source of energy [2]. For transition toward a modern and sustainable bio-based economy, the researchers are harnessing the maximum potential of microalgae for providing solutions for addressing the diverse global challenges of the twenty-first century. Being instrumental for efficient nutrient recycling for modern agriculture and wastewater treatment systems [3], a viable alternative source of energy to replace the fossil fuels [4-5], valuable additive for food and animal feed products [6], serving as a raw material for amino acids, vitamins and productions of valuable bioactive compounds [7; 8], the unique attributes of microalgae has widened the scope of their utilization in nearly every research field.

Despite the widely acknowledged value of microalgae virtually in all fields of biotechnology, studies about their diversity, various ecological functions and properties from understudied environment are still scarce. Speculation that the number of still

undiscovered microalgal species exceeds such of known species has led researchers globally to search and collect new strains of microalgae, preserve, and explore their biotechnological potentials [9].

Owing to their extraordinary physiological, ecological, molecular and regulatory mechanisms, microalgae can be found in number of environments and are capable of surviving in extreme conditions [10]. Thus, the novel natural products with diverse biological activities, notably antibacterial, are expected to be obtained from microalgae, thriving in extreme conditions. It is important to understand the molecular and regulatory mechanism of such microalgae in order to use their biochemical, ecological, evolutionary and industrial potential. Therefore, over the past few decades, new strains of microalgae thriving in extreme conditions have been extensively studied through various culture-dependent and independent approaches in pursuit of novel biologically active molecules [11; 12].

The precise identification of microalgae is of great practical importance to unlock their possible biochemical potential and aid in understanding the basis of their biological activity. Studies of microalgae diversity have traditionally relied on morphological traits and various valuable and highly descriptive taxonomic identifiers, web-based guides, keys, manuals and manuscripts for microalgae [13-19]. Nevertheless, the misleading similarities of characteristic morphological features within microalgae do not enable a reliable identification of microalgae.

The recent advancement in molecular biology has revolutionized the taxonomic studies and offer a more consistently reliable and accurate method for identification. However, there is also a mounting evidence that sequence similarity-based approach alone is not superior to morphological taxonomy [20]. Therefore, reliable identification to species level can only be achieved using hybrid approaches, traditional taxonomic methods complemented with molecular diagnostic markers.

In the present study, investigations were focused on finding new strain of microalgae from extreme cold environment, able to demonstrate antimicrobial activity. Here we describe the discovery of a novel strain of *Monoraphidium* sp. The present study aims to report the morphological and molecular characterization of a psychrophilic green microalgae collected from glacial freshwater site, Big Almaty Lake in Almaty region of Kazakhstan, and examine the antibacterial activity of methanol extract of the isolate by performing disk diffusion and minimum inhibitory concentration (MIC) assays.

Materials and methods

Isolation and growth conditions. The microalgae was isolated from Big Almaty Lake by separating single microalgal cells from the mixed microbial suspension using capillary pipettes (repeated until single cell isolation) and cultivated in culture dishes containing BG-11 growth medium [21], with following composition (gL-^{1}): NaNO□, 1.5; K□HPO□, 0.04; MgSO□·7H$_2$O, 0.076; CaCl□·2H□O, 0.036; citric acid, 0.006; EDTA, 0.001; Na□CO□, 0.02; FeSO□, 0.006; trace elements solution: H□BO□, 2.86; MnCl□·4H□O 1.81; ZnSO□·7H□O, 0.222; CuSO$_4$·5H□O, 0.08; MoO□, 0.015; NaBO□.4H□O, 2.63; (NH□)□Mo□O□□·4H□O, 1; FeSO□.7H□O, 2.3; CaCl□·2H□O, 1.2; Co(NO□)□·2H□O, 0.02; EDTA, 10; and distilled water, 1.0 L.

To enumerate the growth of microalgae after successful direct isolation procedure and observable growth, the microalgae culture was transferred to separate 250-mL flask containing 100 mL of liquid BG-11 medium. The enrichment culture was kept at 22°C ±2 for about 20 days under fluorescent light with a photoperiod of 12 h, with shaker speed 100±10 rpm.

Later the microalgae were plated onto BG-11 agar medium (supplemented with antibiotics), which was placed at 22°C ±2 until single colonies of same size and similar appearance emerged. The isolate was further purified by selecting a single colony and re-streaked, followed by microscopic examination, and individual pure colony was placed in autoclaved liquid medium to obtain axenic monoculture.

Identification of microalgal species. Light microscopic examination. The preliminary morphological identification was done under a light microscope, with a system of image capturing (MicroOptix OPTIX C600, Austria). Morphological identification was carried out based on some prominent morphological features for differentiation such as cell size (length to width ratio) and shape, solitary or colonial, presence or absence of mucilage etc., mentioned in the guidelines of description keys, taxonomic literature, manuals, manuscript and photo gallery available for microalgae species identification [15; 19; 21-24].

Sample preparation for scanning electron microscopy. After determination of the genera according to the microscopic features, the SEM technique was carried out at Electron Microscope Laboratory, Gazi University, Ankara, Turkey. The cells were washed with phosphate buffered saline (PBS) to remove culture medium and then they were collected by centrifugation at 1000xg for 5 min. After that, cells were fixed with 5% glutaraldehyde, dehydrat-

ed in ascending order of 20, 40, 60, 80 and 100% ethanol. After critical point drying with CO□ (Polaron CPD 7501), samples were coated with gold in a Polaron SC 502 sputer coater. The coated specimen was observed with JEOL JSM 6060 LV at accelerating voltage 10kV and SEM images were digitally produced.

Molecular identification of algal species. For molecular identification, the microalgal cell pellet (approximately 30mg) was obtained from culture medium by centrifugation at 6,000 rpm for 10 min and genomic DNA of strain was extracted using Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, D6005), according to the manufacturer instructions. The quality and quantity of DNA samples were measured with the Spectrophotometer Thermo Scientific™ NanoDrop 2000. DNA extracts were stored at -20 °C. The ribosomal ITS region and rbcL gene sequences were amplified using the universal primer pair, listed in Table 1, chosen from published studies with microalgae from class Chlorophyceae.

The 50 µL PCR reaction mixture was composed of 27.75 µL of dH_2O, 10 µL of GoTaq 5X PCR buffer, 6 µL $MgCl_2$ 25 mM, 1 µL dNTPs 10 mM, 0.25 µL of GoTaq DNA polymerase (5 U/µL) (Promega, USA), 1 µL of each primer (10 µM) and 3.0 µl of DNA template (30-50 ng/µL). The PCR amplification protocol used for both markers was: 95 °C for 2 min, 35 cycles of 95 °C for 50 sec, primer-annealing temperature 49 °C for 30 sec and 72°C for 1 min, with a final extension at 72 °C for 10 min.

Table 1 – List of Primer Sequences used for amplification of rbcL and ribosomal ITS region of Isolate

Primer	Sequence (5′-3′)	Type	Source
ITS1	TCCGTAGGTGAACCTGCGG	Forward	[25]
ITS4	TCCTCCGCTTATTGATATGC	Reverse	
ITS1	AGGAGAAGTCGTAACAAGGT	Forward	[25]
ITS4	TCCTCCGCTTATTGATATGC	Reverse	
ITS5	GGAAGTAAAAGTCGTAACAAG	Forward	[25]
ITS4	TCCTCCGCTTATTGATATGC	Reverse	
rbcL	GCTGGWGTAAAAGATTAYCG	Forward	[26]
	TCACGCCAACGCATRAASGG	Reverse	

Each PCR product of about 600 bp was cloned using a pUCm-T cloning vector (Bio Labs) and cDNA of strain was sequenced in both directions by Sanger sequencing (Macrogen Europe, Amsterdam, Netherland). The resulting ITS1/ITS4, ITS5/ITS4 and rbcL forward and reverse DNA sequences of ZBD-06 18S were aligned using sequence alignment editor Bioedit version 6.0 and search for similar sequences was carried out with the Basic Local Alignment Search Tool in the GenBank database of the National Center for Biotechnology Information (NCBI). Fifteen sequences having the highest similarity to each query sequence were obtained and used for phylogenetic analyses with *Isochrysis galbana* strain (JX393298) as an outgroup (accession numbers of these sequences are indicated before the name of each strain on Figure 4). Multiple sequence alignment was carried out using the MUSCLE program with default parameters integrated in Molecular Genetics Analysis (MEGA) 6.06. The Phylogenetic tree was built by the Neighbor-Joining method (Kimura-2-Parameter algorithm) using bootstrap value (n = 1000 replicates) to determine the statistical reliability of the obtained topologies.

The resulting sequences were deposited in the GenBank database for accession number. A living axenic culture of ZBD-06 was deposited in the Collection for Microalgae Cultures at the Biotechnology Lab, Al-Farabi Kazakh National University.

Antibacterial assay. Preparation of crude extract. The microalgae crude extract was obtained according to the method reported in previous study [27]. The cell density was counted with hemocytometer for quantifying the biomass and microalgal cells was harvested from 5 mL axenic monoculture by spun at 3500 rpm for 30 min. Subsequently, the pellet was washed with phosphate-buffered saline (PBS). In the next step, the solution was sonicated (twenty cycles of 30 s each) with a Soniprep Ultrasonicator (Sartorius Labsonic), and re-centrifuged at 3500 rpm for 30

min to remove cell debris. Afterwards, 60% methanol was added to the pellet in 1:5 w/w ratio of the initial biomass concentration. The resulting sample was re-centrifuged at 3500 rpm for 20 min. The obtained supernatant was utilized to assess the antimicrobial activity, and cell suspension density (number of algal cells in one mL) at the start of the extraction process.

Evaluation of antimicrobial activity. Antibacterial activity of microalgae crude extract against eleven bacterial strains, namely, Gram-positive (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228) and Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Salmonella typhimurium* ATCC 14028, *Yersinia pseudotuberculosis* ATCC 911, and *Enterobacter cloacae* ATCC 13047) were examined by disk diffusion assay. Pure colonies of test bacteria were obtained from the Microbiology Laboratory, Karadeniz Technical University. Turkey. The Mueller-Hinton agar (pH 7.2-7.4) plates were inoculated with broth cultures diluted to 0.5 turbidity (~1.5×10□ cells·mL^{-1}). Discs containing 10 µL of methanol extract of the isolate were placed on filter paper disc on the surface of an agar plate, pre-inoculated with bacterial suspension to be tested. Paper discs with only methanol and antibiotic (rifampicin) were used as negative controls. Finally, the plates were incubated at 37 °C for 16-24 h. The inhibition zones were measured in mm. All experiments were carried out three times with similar results.

Determination of minimum inhibitory concentration (MIC). The crude extract were then added in serial dilution to Mueller-Hinton Broth culture in test tubes and bacterial strains were inoculated at the same concentration used for disk diffusion method. The tubes were incubated at 37°C for 24-48 h and then examined for bacterial growth. The MIC values were obtained from the lowest concentration of the extract where the tubes remained clear, indicating that the bacterial growth was completely inhibited at this concentration.

Results and discussion

Isolation of new strain of microalgae. Microalgal strain ZBD-06 was isolated from water samples collected from Big Almaty Lake (43°0506′ N 76°9850′ E), a natural alpine reservoir, located in the Trans-Ili Alatau mountains, on south of Almaty, a city in the southeastern region of Kazakhstan. The glacial water reservoir lake, extended approximately 1.6 km in length and 1 km in width, is used for drinking water needs of the Almaty city.

Figure 1 – Map and location of sampling site of the isolate – Big Almaty Lake

Six algological samples were taken from this lake, the water temperature at the time of sampling was + 2 °C, pH 7.3. Water samples were taken from the coast of lake directly from the surface and from the water column (depth – 20 cm).

As a result, 4 accumulative cultures were obtained from the selected samples, of which 2 microalgae cultures were isolated by successive reseeding. Various concentrations of mixture antibiotics from wide-spectrum antibiotics against Gram-positive and Gram-negative bacteria and antifungal antibiotics was used to clean the isolated cultures from bacteria, because the concomitant microflora reacted differently to different antibiotics. Thus by treatment with a mixture of antibiotics, only 1 strain of microalgae was obtained in a bacteriologically pure form.

Morphological characterization. The light microscopy and scanning electron microscopy visualization of single cell of microalgal strain ZBD-06 revealed the cell size (Figure 2) varying from 4-6 μm in length and 0.78-1.23 μm in diameter. Solitary cells were predominantly observed having croissant shape to crescent shape with varying degree of curvature, rounded ends, occasionally appearing colonial in irregular arrangement. Solitary cells exhibited mucilage and wrinkled cell surface but no mucilaginous colonies were observed. Chloroplast occupied the majority of the cell volume. Such cell morphology shows that the ZBD-06 resembles the characterization of genus *Monoraphidium* and *Ankistrodesmus*.

However, the strain was preliminarily identified as member of the genus *Monoraphidium*, for demonstrating cells dimensions and shape variations similar to *Monoraphidium subclavatum* as described in previous studies [15; 21; 28]. Both genus, classified under the family Selenastraceae, are similar in morphology, however the *Ankistrodesmus* differs by having larger cell dimensions, more or less straight cells, joined by mucilage to form colonies of various shapes, while cells of *Monoraphidium* are distinguished from other genera in the family being strongly curved and lacking mucilage [23]. By assessment of all the morphological characteristic of the isolated microalgae, as illustrated in the microphotograph of the specimen under 40x magnifications (Figure 2) the suspected species was *Monoraphidium subclavatum*.

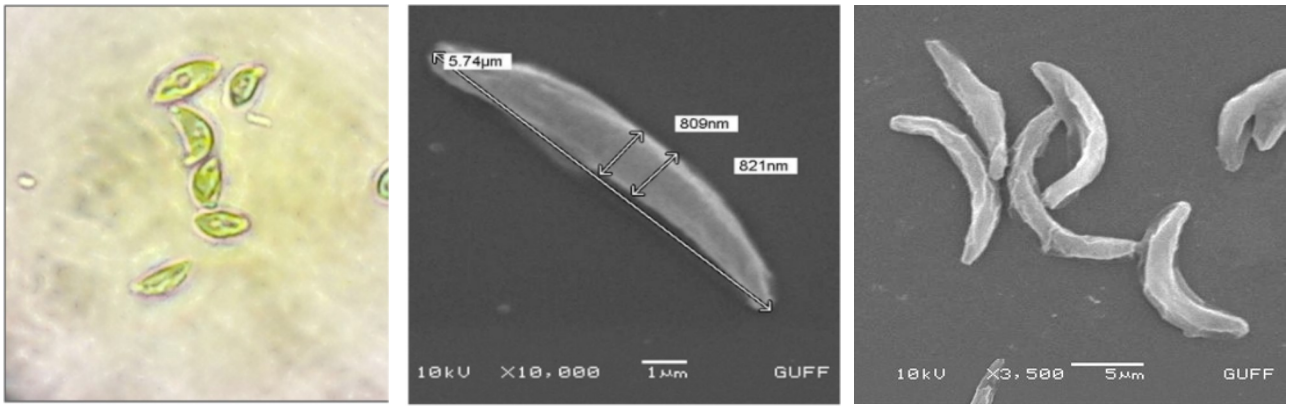


Figure 2 – The Light and Scanning Electron Microscope images of ZBD-06

Nevertheless, the morphological characteristics of the strain investigated in this study doesn't completely fit with the typical description of Monoraphidium at species level and, therefore, species-level identification could not be achieved based merely on morphological and ultrastructural characters. The identification results from the cell morphology were then verified by molecular identification through sequencing of the rbcL genes, ITS1-5.8S-ITS2 and 18S rDNA targeted region and alignment with sequences from the Genbank (NCBI).

Molecular identification and phylogenetic analysis. To perform the molecular identification of the isolate, the desired DNA fragments (~600 – bp) of isolate, amplified with universal primers of rbcL gene and ITS region were compared with the similar sequences in the database using BLAST online (http://www.NCBI.nim.nih.gov/). The similarity searches of the obtained amplicon sequences in the GenBank database were observed for the universal primer sets of ITS1/ITS4 and ITS5/ITS4, whereas the amplicon sequences generated from rbcL could not retrieved any blast match.

The blast result of only first set of primer ITS1/ITS4 was supportive and useful in species discrimination, while second set of primer ITS1/ITS4 and ITS5/

ITS4 resulted in database mismatches with macro algae, uncultured microorganism and microalgae completely conflicting with morphological description. As the results with the second set of primers were not satisfactory to infer generic identity, therefore, the phylogenetic analysis was performed only with the first set of ITS/ITS4 primer. This reduced success in retrieving accurate sequence matches can be attributed to the fact that the number of well-described microalgae species are still not significantly represented in Genbank. The limitations in molecular identification of some microalgae at the species-level implied that further work is required to establish efficient DNA barcode markers for revealing sufficient heterogeneity for species identification, maximizing the gene database, as well as employing combination of molecular and morphological methods for accurate identification of species with extreme morphological plasticity.

The fifteen sequence entries with highest similarity to the obtained sequence of isolate ITS region were observed and multiple alignment of the sequences were generated with the MUSCLE program. The phylogenetic tree was constructed with MEGA software version 6 [29] based on the evolutionary distances that were calculated by the Neighbor-Joining method [30] using Kimura-2-Parameter algorithm. Statistical evaluation of the tree topologies was performed by bootstrap analysis with 1000 re-samplings [31]. *Isochrysis galbana* strain (JX393298) was used as an outgroup. Figure 4 represents the Neighbour-Joining showing phylogenetic position of strain and related taxa based on ITS1-5.8S-ITS2 region sequence comparisons.

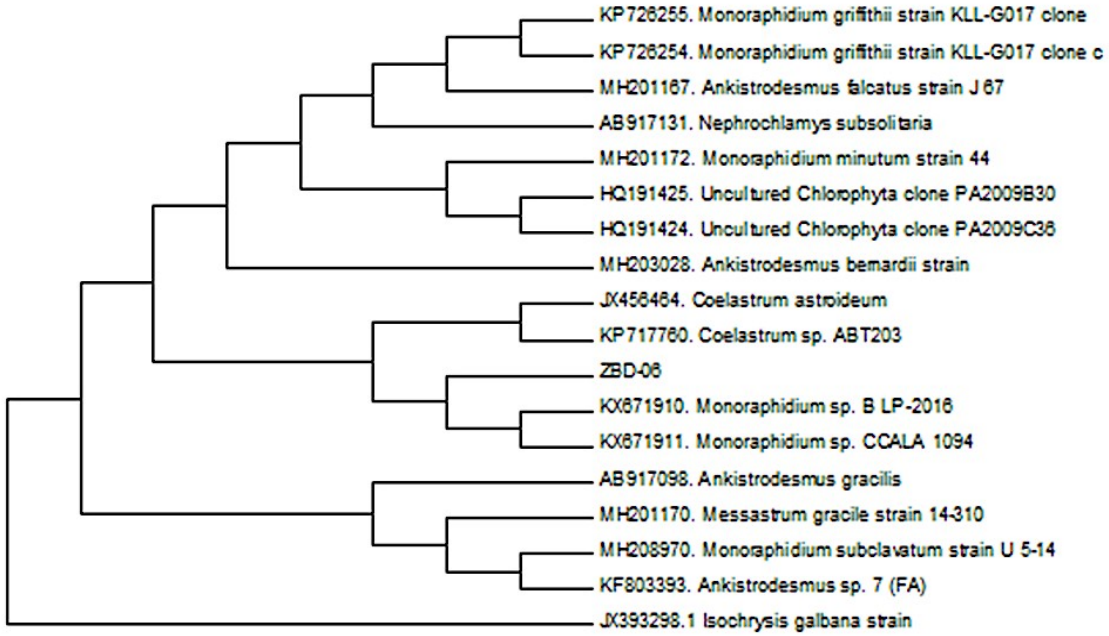


Figure 3 – Phylogenetic tree of the isolated microalgae and the closely related strains based on ITS1-5.8S-ITS2 region sequence comparisons

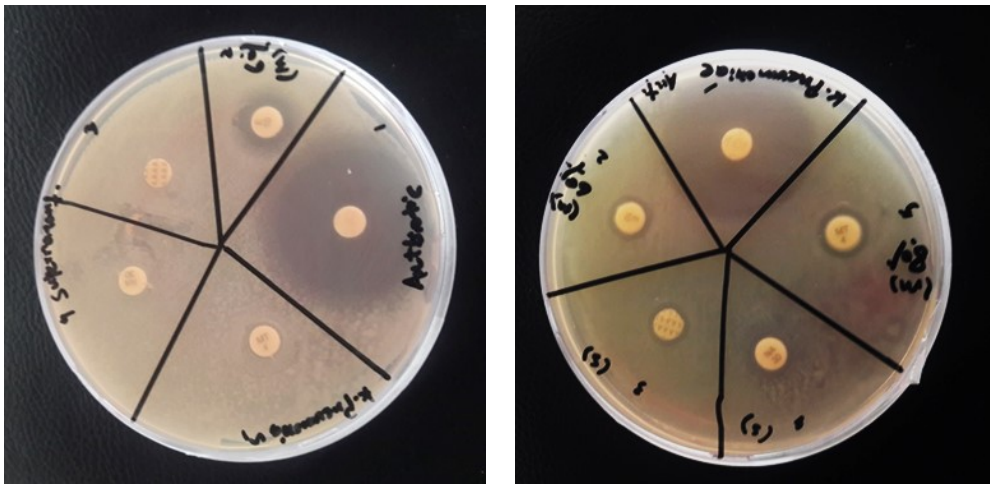
Phylogenetic tree consisted of basically three major clusters and one outgroup, each of which mostly contained different species of genus *Monoraphidium* and *Ankistrodesmus*. The phylogram shows that the ZBD-06 strain is closely related to *Monoraphidium sp*. B LP-2016 (score = 911; ident. = 93.33%), *Monoraphidium sp.* CCALA 1094 (score = 900, ident. = 93.28%) isolated from ice-covered lakes on James Ross Island (northeastern Antarctic Peninsula) [32].

The clustering of *Monoraohidium* sp. from Almaty region with *Monoaphidium* sp. from Antarctic region determines the presence of closely related extermophilic species of microalgae in geographically far distinct location (continental level). The phylogram also depicts the relatedness of the newly isolated native strain to *Coelastrum astroideum* (JX456464), however, both strains are morphologically different [33]. Other closely related species were from the genus of *Ankistrodesmus* and *Monoraphidium*

representing a polyphyletic group [34]. The overall morphological features and sequence-based phylogenetic analysis of ZBD-06 with one of the primers of ITS1 region presented a good correlation and served as important molecular target for identification and taxonomical position of the isolate to great extent based on the availability of sequence database. The obtained molecular result suggests the consideration of ZBD-06 strain as *Monoraphidium* sp. that was also based on light and SEM microscopic observation and the genus belongs to the family *Selenastracaceae* (*Cholorophyceae*). The ITS sequence of the isolate was submitted with accession number of MT178772 in NCBI database, respectively. The relative phylogenetic position based on the ITS1/ITS4 primer sequence might not determine species-level resolution for this species, but when matched with the results of morphological studies, it provided more reliable identification of the isolate.

Antibacterial assay. In this study, the 60% methanol extract of ZBD-06 was tested for antimicrobial activity against eleven bacterial strains, including Gram-positive (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228) and Gram-negative (*Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Salmonella typhimurium* ATCC 14028, and *Yersinia pseudotuberculosis* ATCC 911). The bacterial cultures in petri plates were incubated along with filter paper discs containing 10 and 30 µL of methanol extract of specimen (initial cell number 5.0 × 10□ cells/mL before extraction), for 16-24 h to determine the growth inhibitory effect in the form of inhibition zone.

The most susceptible microbes to specimen methanol extract were Gram-positive bacteria than Gram-negative bacteria. However, surprisingly the methanol extracts of *Monoraphidium* sp. ZBD-06 exhibited the notable antibacterial activity against *Klebsiella pneumoniae* ATCC 13883. The strain showed a positive response in an inhibition zone assay (10±2 mm with 10µl and 20±2 mm in diameter with 30µL) against Gram-negative bacteria *Klebsiella pneumoniae* ATCC 13883 (Figure 4, a and b). In order to confirm the antimicrobial action of strain, the Minimum Inhibitory Concentration (MIC) of the methanol extract was performed against the same bacterial isolate. The MIC value of the extract of ZBD-06 was observed at the first well (2-1).



(10 µL) (30 µL)

Figure 4 – The disk diffusion assay indicating antibacterial activity of methanol extract of *Monoraphidium* sp. ZBD-06

The results showed the potential presence of certain metabolites like phenols, flavonoids, terpenes or carbohydrates, which are responsible for antibacterial activity of the extract against Gram-negative bacteria. Earlier, the presence of phenols, tannins, and anthraquinones was determined in the methanol crude extract of *Monoraphidium contortum* [35].One previous study on assessment of antimicrobial potential of marine green microalgae *Desmococcus* (*D.*) *olivaceus* and *Chlorella* (*C.*) *vulgaris*, revealed that

the solvent choice influence the physical and chemical properties of the extract, such as high phenol content was only found in methanol extract, and amount of falvonoids, terpenes, carbohydrates and alkaloids differed when extracted with different solvents such as ethanol, methanol and chloroform and diethyl ether extract [36].

The multilayered and complex cell envelope of the Gram-negative bacteria is one of the major obstacles, which restrict the entrance of antibacterial agents [37]. However, certain biomolecules can damage the bacterial membrane component, such as terpenoid and phenolic compounds [38]. Antibacterial action of *Monoraphidium* sp. ZBD-06 against *Klebsiella pneumoniae* is possibly related with presence of high content of key molecule, which was specific in action for growth inhibition of the bacterium. Taking into account the isolation of the strain from the freezing lake and continued growth at 24 °C retaining optimal functionality of their macromolecules suggests that adaptation to extreme environment (low temperature) in this strain confers a particular ability to produce unique antifreeze proteins, intracellular molecule or membrane structure to protect the cell from freezing damage, and bioactive compounds of potential antibacterial activity [39; 40]. Another possible explanation of the antimicrobial activity of the studied microalgal extract can be attributed to its potential contents of fatty acids, as the psychrophilic microalgae maintain their membrane fluidity at low temperature by incorporating a higher level of polyunsaturated fatty acids (PUFAs) in membrane lipids [41], thereby making them a potential source of polyunsaturated fatty acids for antibacterial product.

The obtained result invokes the necessity of further exploring this great potential and specific action of the *Monoraphidium* sp. strain ZBD-06 by screening biotechnologically appealing bioactive molecules, investigating its source in the cell (chloroplast, cytoplasm, mitochondria, etc.) and deciphering the main biosynthetic pathways of the bioactive molecules. Because antimicrobial activity against *Klebsiella pneumoniae* have, to our knowledge, not previously been reported for Monoraphidium genus, the identification of novel bioactive metabolites may be possible in this strain, which probably either independently or synergistically act together against the bacterium.

Conclusion

A new strain of green microalgae was isolated from the Big Almaty Lake. This strain was identified using scanning electron microscopy and molecular identification as *Monoraphidium* sp. ZBD-06. According to morphology, these are single cells having the shape of a croissant or the shape of a crescent with varying degrees of curvature, rounded ends. Cells of algae with mucus and a wrinkled cell surface, with chloroplast, which occupies most of the cell volume.

The molecular analysis of this study highlighted the on-going challenge in identification of microalgae strains due to lack of availability of taxonomically curated DNA databases. For improving success in accurate specific or infra-specific identification of microalgae strain, more systematic studies using combined approach of morpho-taxonomy and metabarcoding using different molecular markers and deposition of more taxonomically accurate reference barcodes in curated databases are needed.

A study of the antibacterial activity of this microalgae culture showed that the extract of a new strain of psychrophilic microalgae *Monoraphidium* sp. ZBD-06 can yield pharmaceutically promising bioactive substances provided with antibacterial activity useful against Gram-negative bacteria *Klebsiella pneumonia*. However, further studies are needed to better evaluate the presence of antibacterial compounds in the isolated microalgae, and potential effectiveness of the crude extract as the antibacterial agent. It is also expected that additional screening of this under-explored strain may reveal a novel antimicrobial substance that have not yet described and identify yet more useful characteristics for biotechnology.

Acknowledgements

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References

1 Raja R., Hemaiswarya S., Kumar N.A., Sridhar S., Rengasamy R. (2008) A perspective on the biotechnological potential of microalgae. Crit Rev Microbiol., vol. 34, no. 3, pp. 77-88. https://doi.org/10.1080/10408410802086783.

2 Liu J., Sun Z., Gerken H. (2014) Recent advances in microalgal biotechnology. Potential applications of microalgae in wastewater treatments.

Published by OMICS Group eBooks, USA, ISBN: 978-1-63278-066-9.

3 Brennan L., Owende P. (2010) Biofuels from microalgae–a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev., vol. 14, no. 2, pp. 557–577. doi:10.1016/j.rser.2009.10.009.

4 Markou G., Nerantzis E. (2013) Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. Biotechnol. Adv., vol. 31, no. 8, pp. 1532–1542. https://doi.org/10.1016/j.biotechadv.2013.07.011.

5 Priyadarshani I., Rath B. (2012) Commercial and industrial applications of micro algae–A review. Journal of Algal Biomass Utilization, vol. 3, no. 4, pp. 89-100.

6 Larkum A.W., Ross I.L., Kruse O., Hankamer B. (2012) Selection, breeding and engineering of microalgae for bioenergy and biofuel production. Trends in biotechnology, vol. 30, no. 4, pp. 198-205. https://doi.org/10.1016/j.tibtech.2011.11.003.

7 Muñoz-Ochoa M., Murillo-Álvarez J.I., Zermeño-Cervantes L. A., Martínez-Díaz S., Rodríguez-Riosmena R. (2010) Screening of extracts of algae from Baja California Sur, Mexico as reversers of the antibiotic resistance of some pathogenic bacteria. Eur. Rev. Med. Pharmacol. Sci, vol. 14, no. 9, pp. 739-747.

8 Pane G., Cacciola G., Giacco E., Mariottini G. L., Coppo E. (2015) Assessment of the antimicrobial activity of algae extracts on bacteria responsible of external otitis. Marine drugs, vol. 13, no. 10, pp. 6440-6452. https://doi.org/10.3390/md13106440.

9 Guiry, M.D. (2012) How many species of algae are there?. Journal of phycology, vol. 48, no. 5, pp. 1057-1063. https://doi.org/10.1111/j.1529-8817.2012.01222.

10 Pradhan J., Das S., Das B. K. (2014) Antibacterial activity of freshwater microalgae: A review. African Journal of Pharmacy and. Pharmacology, vol. 8, no. 32, pp. 809-818. https://doi.org/10.5897/AJPP2013.0002.

11 Sydney E.B., Schafranski K., Barreti B.R.V., Sydney A.C.N., Zimmerman J.F.D.A., Cerri M.L., Demiate I.M. (2019) Biomolecules from extremophile microalgae: from genetics to bioprocessing of a new candidate for large-scale production. Process Biochemistry, vol. 87, pp. 37–44. https://doi.org/10.1016/j.procbio.2019.09.012

12 Heidari F., Shariatmadari Z., Riahi H., (2019) Screening of Extremophile Microalgae Isolated from High Background Radiation Areas as Source of Bioactive Materials, Current Bioactive Compounds, vol. 15, pp. 1-8. https://doi.org/10.2174/1573407215666181219104518.

13 Canter-Lund H., Lund J.W.G. (1995) Freshwater algae: Their microscopic world explored. Biopress Ltd., Bristol. 360 p. ISBN 0-948737-25-5.

14 Dillard, G.E. 1999) Common Freshwater Algae of the United States. An Illustrated Key to the Genera (Excluding the Diatoms). J. Cramer in der Gebr. Bomtraeger Verlagsbuchhandlung, Berlin-Stuttgart., pp.173, 298 Figs. DM 58. ISBN 3-443-50026-9.

15 Edward G.B., David C.S. (2010) Freshwater algae identification and use as bioindicators. A John Wiley & Sons, Ltd, 101. ISBN-13: 978-0470058145.

16 Huynh, M., Serediak, N. (2006) Algae identification field guide agriculture and agric food canada. Agri-Environment Services Branch, Majesty the Queen in Right of Canada, pp. 40. ISBN 9781100183077-1100183078

17 John D.M., Whitton B.A., Brook A.J. (2002) Freshwater algal flora of the British Isles: An identification guide to freshwater and terrestrial algae. Cambridge University Press. 702 p. ISBN 0□521□77051□3.

18 Shayler H.A. and Siver P.A. (2006) Key to Freshwater Algae: A web-based tool to enhance understanding of microscopic biodiversity. Journal of Science Education and Technology vol. 15, no. 3, pp. 298-303. https://doi.org/10.1007/s10956-006-9016-4.

19 Whitford L.A., Schumacher G.J. (1973) A manual of freshwater algae (p. 324). Raleigh: Sparks.

20 Stoykova P., Stoyneva-Gärtner M., Uzunov B., Gärtner G., Atanassov I., Draganova P., Borisova, C. (2019) Morphological characterization and phylogenetic analysis of aeroterrestrial Vischeria/Eustigmatos strains with industrial potential. Biotechnology & Biotechnological Equipment, vol. 33, no. 1, pp. 231-242. https://doi.org/10.1080/13102818.2018.1561212.

21 Hindák, F. (1970) A contribution to the systematics of the family Ankistrodesmaceae (Chlorophyceae). Archiv für Hydrobiologie Supplement – Algological Studies 1, pp. 7-32.

22 Komárková – Legnerová J., (1969) The systematics and ontogenesis of the genera Ankistrodesmus Corda and Monoraphidium gen. nov. In: Studies in Phycology (Fott B, ed). E Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller) Stuttgart. pp. 75 – 144.

23 Ramos G.J.P., Bicudo C.E.D.M., Góes-Neto A. Moura C.W.D.N. (2012) Monoraphidium and Ankistrodesmus (Chlorophyceae, Chlorophyta) from

Pantanal dos Marimbus, Chapada Diamantina, Bahia State, Brazil. Hoehnea, vol. 39, no. 3, pp, 421-434. https://doi.org/10.1590/S2236-89062012000300006.

24 Ramos G.J.P., Bicudo C.E.D.M., Góes-Neto A. Moura C.W.D.N. (2014) New additions of coccoid green algae to the phycoflora of Brazil and the Neotropics. Acta Botanica Brasilica, vol. 28, no. 1, pp. 8-16. https://doi.org/10.1590/S0102-33062014000100002.

25 White T. J., Bruns T., Lee S.J.W.T., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications, vol. 18, no. 1, pp. 315-322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1.

26 Saunders G.W., Kucera H. (2010) An evaluation of rbcL, tufA, UPA, LSU and ITS as DNA barcode markers for the marine macroalgae. Cryptogam, Algol, vol. 31, no. 4, pp. 487–528.

27 Pane G., Cacciola G., Giacco E., Mariottini G.L., Coppo E. (2015) Assessment of the antimicrobial activity of algae extracts on bacteria responsible of external otitis. Marine drugs, vol. 13, no. 10, pp.6440-6452. https://doi.org/10.3390/md13106440.

28 Nygaard G. (1977) New or interesting plankton algae with a contribution on their ecology. Det Kongelige Danske Videnskabernes Selskab, Biologiske Skrifter, vol. 21, no. 1, pp. 1-107.

29 Tamura K., Stecher G., Peterson D., Filipski A., & Kumar S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution, vol. 30, no. 12, pp. 2725-2729. https://doi.org/10.1093/molbev/mst197.

30 Saitou N, Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol, vol. 4, no. 4, pp. 406–25. https://doi.org/10.1093/oxfordjournals.molbev.a040454.

31 Felsenstein J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution, vol. 39, no. 4, pp. 783–91. https://doi.org/10.2307/2408678.

32 Nedbalová L., Mihál M., Kvíderová J., Procházková L., Řezanka T., & Elster J. (2017) Identity, ecology and ecophysiology of planktic green algae dominating in ice-covered lakes on James Ross Island (northeastern Antarctic Peninsula). Extremophiles, vol. 21, no. 1, pp. 187-200. https://doi.org/10.1007/s00792-016-0894-y.

33 Hentschke G.S., Prado J.F. (2012) Chlorococcales sl (Chlorophyceae) e Zygnematales (Zygnematophyceae) em um açude do Balneário do Lérmen, Rio Grande do Sul, Brasil. Iheringia. Série Botânica., vol. 67, no. 1, pp. 59-74.

34 Krienitz L., Bock C., Dadheech P. K., Pro¨schold T. (2011) Taxonomic reassessment of the genus Mychonastes (Chlorophy-ceae, Chlorophyta) including the description of eight newspecies. Phycologia, vol. 50, no. 1, pp. 89–106. https://doi.org/10.2216/10-15.1.

35 Ferreira C.R.S., Gama F.S., Faustino S.M.M., Almeida S.S.M.S. (2017) Study of the Secondary Metabolites, Cytotoxic and Antioxidant Activity of the Methanolic Monoraphidium contortum Crude Extract. Journal of Chemical and Pharmaceutical Research, vol. 9, no. 6, pp. 327-331.

36 Ramar D., Rajendran N., Pichai S. (2016) Phytochemical and antimicrobial activity of green microalgae from Vellar Estuary, southeast coast of India. Journal of Coastal Life Medicine, vol. 4, pp. 374-376. https://doi.org/10.12980/jclm.4.2016J5-167.

37 Bergsson G., Arnfinnsson J., Steingrímsson Ó., & Thormar H. (2001) In vitro killing of Candida albicans by fatty acids and monoglycerides. Antimicrobial agents and chemotherapy, vol. 45, no. 11, pp. 3209-3212. https://doi.org/10.1128/AAC.45.11.3209–3212.2001.

38 Helander I.M., Alakomi H.L., Latva-Kala K., Mattila-Sandholm T., et al. (1998) Characterization of the action of selected essential oil components on Gram-negative bacteria. Journal of agricultural and food chemistry, vol. 46, no. 9, pp. 3590-3595. https://doi.org/10.1021/jf980154.

39 Jung W., Gwak Y., Davies P.L., Kim H.J., Jin E. (2014) Isolation and characterization of antifreeze proteins from the antarctic marine microalga Pyramimonas gelidicola. Mar Biotechnol, vol. 16, no. 5, pp. 502–12. https://doi.org/10.1007/s10126-014-9567-y.

40 Varshney P., Mikulic P., Vonshak A., Beardall, J., Wangikar P.P. (2015) Extremophilic micro-algae and their potential contribution in biotechnology. Bioresource technology, vol. 184, pp. 363-372. https://doi.org/10.1016/j.biortech.2014.11.040.

41 Spijkerman E., and Wacker A. (2011) Interactions between P-limitation and different C conditions on the fatty acid composition of an extremophile microalga. Extremophiles, vol. 15, no. 5, pp. 597–609. https://doi.org/ 10.1007/s00792-011-0390-3.