Antibacterial activity of freshwater green microalgae from Almaty region

Huma Balouch [1*], *Zihni* Demirbag [2], *Mehwish* Durani [1], *Fariza* Sarsekeeva [3], *Aimeken* Nygymetova[3]

[1] Commission on Science and Technology for Sustainable Development in the South (COMSATS), Pakistan
[2] Department of Biology, Karadeniz Technical University, 61080, Trabzon, Turkey
[3] Department of Biotechnology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, 050040, Almaty, Kazakhstan

Abstract. The urgent search for new natural bioactive compounds has led to the investigation of freshwater microalgae in the Almaty region for their antibacterial properties. For this purpose, a study was carried out to investigate microalgae cultures from the division of *Clorophyta* for their potential to produce biologically active compounds (*Parachlorella kessleri, Monoraphidium griffithii, Ankistrodesmus falcatus, Nephrochlamys subsolitaria*), which also have the potential for wide use in other biotechnological applications. Methanol extracts of 4 strains of microalgae cultures against 11 strains of bacteria were used to evaluate the biological antibacterial activity. Significant antibacterial effects were observed, in particular the methanol extract of *P. kessleri* showed high activity against *B. subtilis, S. aureus* and *K. pneumoniae*; *N. subsolitaria* showed strong activity against *B. subtilis, P. aeruginosa* and *E. coli*. The results of GC/MS analysis confirmed the presence of important bioactive compounds (decamethylcyclopentasiloxane, octamethylcyclotetrasiloxane, hexamethylcyclotrisiloxane) in the microalgae extracts. The data obtained play an important role in the development of microalgae-based antibacterial drugs.

1 Introduction

Today, the problem of bacterial resistance to antibiotics is becoming increasingly serious, threatening the effectiveness of infection treatment and posing a global public health challenge. This rise in resistance highlights the need to find new sources of antimicrobial compounds. In the search for solutions, the study of natural objects and processes is attractive. In this context, microorganisms rich in active compounds, such as microalgae, have the potential to develop biotechnological products with antibacterial properties [1, 2].

These unicellular photosynthetic organisms play a vital role in maintaining the biosphere's ecological balance through the production of a wide array of metabolic outputs, such as enzymes, carbohydrates, fats and substances with antimicrobial properties [3, 4, 5].

Microalgae exhibit remarkable biodiversity and can adapt to various extreme environmental conditions. Under the influence of environmental stress, the internal mechanisms of microalgae change, with the possibility of synthesizing valuable metabolites with different characteristics [6, 7, 8]. In addition, microalgae show potential in combating resistant pathogens through the production of a variety of antimicrobial compounds. Studying the biologically active substances contained in new microalgae strains is one of the possible directions.

There is a lot of information in the literature about various microalgae and cyanobacteria exhibiting antibacterial activity. These are representatives of the genera *Coccomyxa, Chlorella, Dunaliella, Nannochloropsis, Cosmarium, Scenedesmus, Coelastrum, Selenastrum Spirulina, and Synechococcus* [9, 10, 11, 12]. Despite this, the production of antibacterial compounds based on microalgae has not yet been widely established in the world. One of the main obstacles to the production of antibacterial compounds is the insufficient productivity of secondary metabolites in microalgae strains, which is influenced by both genotype and environmental conditions. Research in this area is of practical interest and requires further fundamental discoveries and studies of new strains and their metabolites.

The aim of the study is to isolate strains of microalgae adapted to different extreme environmental conditions and to study their biological properties to determine their activity against bacteria. The scientific novelty lies in the identification of biologically active compounds in microalgae extracts that have potential for the development of new antimicrobial drugs.

Thus, this study contributes to the understanding of the potential of microalgae as a source of bioactive compounds and may contribute to the development of new strategies to combat infectious diseases.

2 Materials and Methods

From the microalgae collection of al-Farabi KazNU, which was obtained from freshwater bodies representing different environmental conditions (Almaty region, Kazakhstan) such as Lake Issyk, Lake Alakol with the highest salinity, Lake Balkhash and Big Almaty Lake with the lowest temperatures - as indicated in Table 1. Specific strains studied include *Monoraphidium griffithii* (ZBD-01), *Nephrochlamys subsolitaria* (ZBD-02), *Ankistrodesmus falcatus* (ZBD-03) and *Parachlorella kessleri* (ZBD-04) [13].

*Corresponding author: huma@comsats.org

Table 1 – Biological and Ecological Variables of Sampling Sites

Site code	*MAT (°C)*	*NaCl* gL^{-1}	*Saprobity index S*	*pH*	*Class of Salinity*
IL	4.1°C	2.34	1.25	7.97	IV
BAL	2°C	2.62	0.5	8.33	IV
AL	6.4°C	6.097	2.5	8.04	III

Microalgae are cultivated on a standard nutrient medium BG-11, at a temperature of 21-23°C, illumination of 100 µmol photons $m^{-2}s^{-1}$ and regular aeration until biomass of the required density is obtained. The resulting biomass was centrifuged (3000 g, 5 min) and gas chromatography/mass spectrometry (GC/MS) was performed.

Methanol extracts of microalgae strains were prepared according to established protocols [14, 15]. The protocol consisted of several steps, starting with the dissolution of the centrifuged sediment in phosphate-buffered saline (PBS), followed by the destruction of biomass cells using ultrasound for 20 cycles (1 cycle - 30 seconds) and the addition of 60% methanol solution (5:1).

The disc diffusion method was used to evaluate the antimicrobial activity of the obtained microalgae extracts against the following bacteria *B. subtilis, E. faecalis, S. aureus, S. epidermidis, E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, S. typhimurium, Y. pseudotuberculosis* and *E. cloacae*, which were classified into gram-positive (Gram+) and gram-negative (Gram-) bacteria (the strains were provided by Karadeniz Technical University, Turkey, Microbiology Laboratory). First, agar dishes containing Mueller-Hinton medium with optimal neutral pH were prepared and inoculated with bacterial cultures reaching a certain density of about 1.5×10^8 cells-ml^{-1}. Filter paper discs soaked in 10 µl of methanol extract were then placed on the agar surface. As a control, discs soaked in methanol and the bactericidal antibiotic rifampicin alone were used. After incubation (37°C, 1 day), the bacteria were examined for zones of inhibition and measured. The whole process was repeated 3 times for the 4 microalgae strains tested to minimize the influence of random factors.

The minimum inhibitory concentration (MIC) of microalgae extracts (diluted tenfold in Mueller-Hinton broth) was determined. After dilution, bacterial cultures were added to the test tubes and incubated (1-2 days, 37°C) while monitoring changes in bacterial growth. The MIC was defined as the lowest concentration of extract at which no bacterial growth was observed. The initial concentration of microalgae cells used to determine the MIC was expressed per milliliter, based on the use of 6 g raw weight of isolate for the extraction process.

3 Results and discussion

Investigation of the antibacterial properties of green microalgae involved subjecting methanol-based extracts from different microalgae isolates to a disc diffusion assay targeting eleven different bacterial strains. The results detailed in Table 2 and illustrated in Figure 1 show that the four microalgae strains showed different antibacterial activities against *B. subtilis, S. aureus* (Gram+) and *E. coli, K. pneumonia* and *P. Aeruginosa* (Gram-). Strong antibacterial activity against *B. subtilis* was observed in microalgae *N. subsolitaria* and *P. kessleri*, manifested as zones of inhibition with a diameter of 16 mm. In addition, a significant antibacterial effect against *S. aureus* and *K. pneumonia* (Gram-) was demonstrated by *P. kessleri*. Other microalgae strains had weaker antibacterial effect against *E. coli* and *K. pneumonia*. *N. subsolitaria* had a pronounced inhibitory effect on *E. coli* with a slightly reduced effect on *P. aeruginosa*.

Table 2 – Evaluation of Antibacterial Effects of Microalgal Isolates on Specific Bacterial Pathogens Using the Disk Diffusion Approach

Isolates	Species	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumonia*	MIC values (microalgal cells/mL)
ZBD-04	*Parachorella kessleri*	+++	++	–	–	+	1.2×10^8
ZBD-01	*Monoraphidium griffithi*	–	–	+	–	+	0.90×10^8
ZBD-02	*Nephrochlamys subsolitaria*	+++	–	++	+	–	0.70×10^8
ZBD-03	*Ankistrodesmus falcatus*	–	–	+	–	+	0.45×10^8

Note: no antibacterial activity (-), zone of inhibition less than 2 mm (+), zone of inhibition greater than 4 mm (++), zone of inhibition greater than 6 mm (+++).

Based on the results of MIC analysis, methanol extracts from a quartet of strains ZBD-01, ZBD-02, ZBD-03 and ZBD-04 demonstrated the ability to inhibit the proliferation of previously tested bacterial strains. The extracts at concentrations of 10-15 mg/ml showed bacterial inhibition (7-18 mm) with minimal biomass utilisation. These results are notably akin to those derived from other microalgae species [16, 17, 18] and are in the vicinity of outcomes for methanol extracts from more substantial macroalgae biomass [19, 20]. *P. kessleri* exhibited the most significant antibacterial effect against *B. subtilis* and *S. aureus*, with a lesser effect noted against *K. pneumoniae*. The MIC for *N. subsolitaria*, indicating its effectiveness against *B. subtilis, E. coli,* and

P. aeruginosa, was found in the second level of dilution. Conversely, *A. falcatus* displayed minimal antibacterial action against *E. coli* and *K. pneumoniae*, with its MIC detected in the initial dilution stage. Similarly, the initial dilution tube revealed the MIC for *M. griffithi* against *E. coli* and *K. pneumoniae.* Figure 1 details the MIC values for the methanol extracts of these four microalgae strains as determined in the dilution tests.

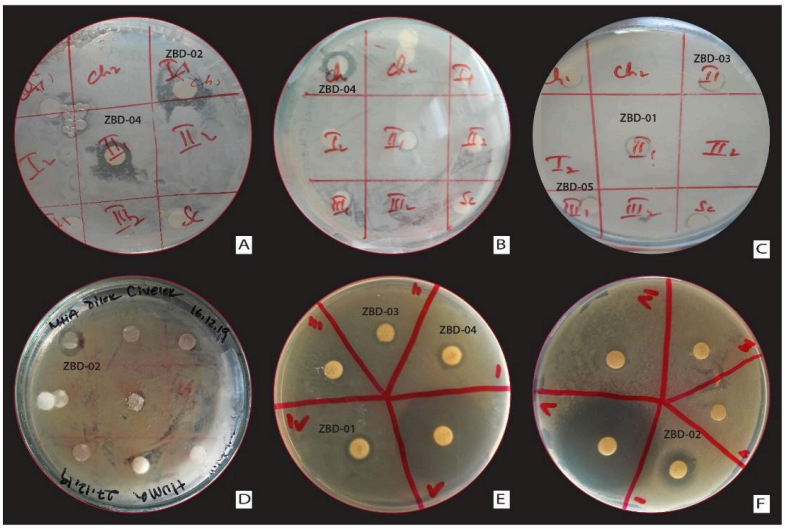

Fig.1. Effect of methanol extracts of microalgae on bacteria (A-*B. subtilis,* B-*S. aureaus*, C&D-*E. coli*, E-*K. pneumonia*, F-*P. aeruginosa).*

The GC/MS analysis findings of the studied strains unveiled the presence of significant constituents such as phenols, alkaloids, terpenes, and esters. This analysis of the methanol extract revealed the predominance of cyclotrisiloxane hexamethyl and cyclotrisiloxane octamethyl as the main compounds in all the strains studied, particularly in the microalgae *P. kessleri*.

Many species of microalgae are known for their ability to inhibit bacterial growth by producing a variety of compounds. These include basic substances such as proteins, carbohydrates and lipids, which are important for pathogen control. In addition, microalgae can synthesise secondary metabolites such as vitamins, pigments, polysaccharides, antibiotics, antioxidants and toxins [21, 22, 23]. Due to the presence of terpenes, tannins, saponins and carotenoids, microalgae have unique antibacterial properties, making them a valuable source of biologically active compounds. Over the past thirty years, a significant body of research has been devoted to exploring the antimicrobial properties of bioactive molecules derived from both freshwater and marine microalgae [24, 25]. However, detailed information regarding the precise antibiotic effects of these compounds and the mechanisms behind their antibacterial actions remains limited.

The result of the GC/MS study shown in Table 3 revealed the presence of several important compounds, highlighting the detection of specific phenols, alkaloids, terpenes and esters with antibacterial properties [26]. In particular, cyclotrisiloxane-hexamethyl and cyclotetrasiloxane-octamethyl were identified as the predominant compounds in the strains studied, with a particular content in *P. kessleri*. These results highlight the potential of these microalgae to produce bioactive molecules with antibacterial properties, underlining their importance in the search for natural antibacterial agents.

Table 3 – GC/MS Screening of secondary metabolites in microalgae strains

Strain	**Phenolic**	**Terpenes**	**Tannins**	**Flavonoids**	**Alkaloid**
Parachlorella kessleri	++	+	-	+	+++
Nephrochlamys subsolitaria	++	-	+	+	+++
Ankistrodesmus falcatus	+	-	-	-	++
Monoraphidium griffithi	+	+	-	-	++

The results of the antibacterial and MIC tests in this study highlight the significant antibacterial potential of the microalgae isolates studied, especially against *E. coli, P. aeruginosa* and *K. pneumoniae* (Gram-). This

differential effect of microalgae on Gram- and Gram+ bacteria probably depends on different cell wall structures [27].

4 Conclusion

The study of the antibacterial potential of microalgae represents an important area of research into the globally growing problem of antibiotic resistance in bacteria. The present study demonstrated promising antibacterial potential of four strains of microalgae, highlighting the importance of comprehending the composition of bioactive compounds within microalgae species. These data open up prospects for the development of effective antibacterial drugs based on microalgae, which is important for combating infectious diseases and overcoming the problem of antibiotic resistance. Further research into the mechanism of action of biologically active compounds in microalgae may provide the basis for the development of new ways of treating and preventing infections. Such research not only increases our knowledge of microalgae as a valuable resource, but also highlights the importance of biotechnology research in creating innovative solutions for modern medicine and industry.

Data Accessibility Statement:

The supporting data for the conclusions of this research are included within this document. Any additional data can be obtained from the lead author upon a justified request.

Conflict of Interest Statement:

The authors affirm that there are no conflicts of interest associated with this publication.

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