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Antifungal and antibacterial activity of some microalgae collected from lake "La Izvor" (Chisinau)

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Abstract

Antibacterial and antifungal activity of 8 isolated microalgae strains from Lake "La Izvor" (Chisinau) was studied. Ethanolic biomass extracts were tested against five strains of pathogenic bacteria and fungi. Thus, according to the results of the experiments, the isolated strains of microalgae are able to synthesize metabolites that have antifungal and antibacterial activity against the tested phytopathogens. It should be noted that the obvious antibacterial activity showed the strains of *Chlorella vulgaris*, *Oscillatoria planctonica* and *Spirulina major* with the largest areas of inhibition of the growth of pathogenic bacteria *Bacillus subtilis* B-117, *Xanthomonas campestris* 8003b and *Erwinia caratovora* 8982.
The cyanobacteria *Oscillatoria planctonica*, *Spirulina major* and the microalga *Chlorella vulgaris* had a significant inhibitory effect on the growth of the pathogenic fungi *Alternaria alternata, Aspergillus niger, Botrytis cinerea* and *Fusarium solani*.

Keywords: antibacterial and antifungal activity, microalgae, cyanobacteria, phytopathogens.

1. Introduction

Algae are a group of photosynthetic organisms that inhabit mostly aquatic habitats, including lakes, rivers, oceans, and even in wastewater. They can tolerate a wide range of temperatures, salinities and pH values; different light intensities; and can live alone or in symbiosis with other organisms (Muhammad Imran, 2018).
Actually, microalgae and cyanobacteria are attracting more and more attention from researchers due to their potential application in various fields of biotechnology. They are an ideal resource for biofuel production, especially biodiesel and biogas. Recently, there has been a growing interest in using algae along with bacteria, fungi, plants in removing, degrading or reducing organic pollutants from aquatic systems. These microalgae bioremediation capabilities are useful for environmental sustainability (Mathimani, 2019; Das, 2011).
These microorganisms are increasingly being studied due to their high content of bioactive substances (polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds and antioxidants) with various biological activities such as antibacterial, antifungal, antiviral,

anticancer, antimicrobial, immunostimulatory, etc. (Brennan, 2010; Jaki, B, 2000; Ramamurthy, V 2012).

Cyanobacteria are quite promising for their use in various fields, such as pharmaceuticals, animal husbandry, biotechnology, cosmetology, etc. Cyanobacteria extracts have fungicidal, bactericidal and bacteriostatic effects, so more and more research is linked to the use of these microorganisms as an alternative source of antibiotics which is a mixture of fatty acids that inhibits the growth of both gram-positive and gram-negative bacteria. (Hend., 2017). An example is eicosapentaenoic acid (EPA), hexadecatrienoic acid, and palmitoleic acid isolated from *Phaeodactylum tricornutum*, which have shown antimicrobial activity against a methicillin-resistant strain of *Staphylococcus aureus* (Benkendorff et al., 2005). Similarly, unsaturated fatty acids derived from *Scenedesmus intermedius*, *Chaetoceros muelleri*, *Haematococcus pluvialis*, *Chlorococcum sp*. and *Skeletonema kastum* have antimicrobial activity against a wide range of gram-positive and gram-negative bacteria. In addition, organic extracts of *Euglena viridis* and *S. kastum* showed inhibitory activity against *Pseudomonas sp*. and *Listeria monocytogenes* (Das, 2010; Terekhova, 2009).

Microalgae and cyanobacteria also have antiviral and antifungal activity against a wide range of microorganisms. Gasemi et al. reported that methanol and hexane extracts from *Chlamydomonas reinhardtii, Chlorella vulgaris, Scenedesmus obliquus* and *Oocystic sp*. prevent the growth of pathogenic fungi such as *Aspergillus niger, Candida kefyr* and *Aspergillus fumigatus* (Ghasemi, Y, 2007)

Other studies have shown that biologically active compounds in microalgae inhibit the replication of viruses. For example, sulfated polysaccharides isolated from *Navicula directa* and *Chlorella autotrophica* inhibit the replication of the enzyme hyaluronidase of VHSV, ASFV, HSV 1 and 2 and influenza A virus (Yi Zheng, 2001). Calcium Spirulan (Ca-SP) is a sulfated polysaccharide isolated from the cyanobacterium Spirulina platensis that possesses the property of inhibiting the replication of several enveloped viruses, including herpes simplex virus type 1, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV. The antiviral effect of Ca-SP is due to the chelation of the calcium ion with sulfated groups.(Hayashi, 1996).

Thus, the aim of the study is to determine the antimicrobial activity of some microalgae strains isolated from the lake "La Izvor" (Chisinau municipality).

2. Methodology

From the lake "La Izvor", were selected 8 microalgae strains for study and research. The cultures were isolated by inoculation on liquid and agarized mineral media. Hydroalcoholic extracts (60-70%) from microalgae biomass were used to determine the antimicrobial activity against phytopathogenic cultures of bacteria and fungi from The National Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology (CNMN) from Chisinau. Phytopathogenic bacteria *Bacillus subtilis* B-117, *Xantomonas campestris* 800 3b, *Corynebacterium miciganense* 13 a, *Agrobacterium tumefaciens* (*Rhizobium radiobacter*) 8628, *Erwinia caratovora* 8982 and strains of pathogenic fungi *Alternaria alternate, Botrytis cinerea*, *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus niger* were used as reference cultures. On the agar medium distributed in Petri dishes and inoculated with phytopathogenic culture, were formed wells where ethanolic extracts (60-70%) of the microalgae cultures and cyanobacteria studied were introduced. Petri plates were incubated for 48-72 hours at 30 ° C.

The diameter of the growth inhibition zone of the reference phytopathogenic strains was determined. (Gritskevich, 2017)

3. Results and discussion

3.1. Determination of antifungal activity of isolated microalgae strains from "La Izvor" lake

Data from the scientific literature confirm that most biologically active compounds contained in seaweed can be used as therapeutic agents. Cox and colleagues confirmed that phenolic compounds isolated from dried seaweed extracts are responsible for their antimicrobial properties. (Ertürk O., 2011;Cox, S, 2010) Currently, the extraction of new compounds from macroalgae and microalgae has confirmed their biocidal activity as antifungal agents. Antifungal compounds inhibit spore germination and suppress the early stages of mycelium growth (Sastry V., 1994; Yi Zheng, 2001). The antifungal activity of extracts from chlorophytes, diatoms and dinoflagellates is manifested by affecting the activity of fungal enzymes. Cordeiro et al. confirmed that chitinases and β-1,3-glucanase (which can affect pectinase function) are indeed recognized as natural antifungal proteins widely found in plants and seaweed. It has been reported in the literature that crude extracts of *P. gymnospora* and *C. frutic* have shown the same efficacy as the fungicide nystatin (Ertürk O., 2011; Cox, S., 2010)

Thus, a stage of the research was the study of the ability of strains isolated from microalgae and cyanobacteria to synthesize metabolites with antagonistic activity against phytopathogenic fungi.

Thus, as a result of our study, (Table 1) it was established that the 8 isolated microalgae strains showed antifungal activity against the phytopathogenic strain *Alternaria alternata*.

Table 1. Antifungal activity of microalgae isolated from the water of La Izvor lake

Microalgae sp	**Fungus culture test, diameter of the inhibition zone (mm)**				
	Aspergillus niger	*Alternaria alternate*	*Botrytis cinerea*	*Fusarium solani*	*Fusarium oxysporum*
Oscillatoria planctonica	21	20	22	20	0
Chlorella vulgaris	20	21	23	**24**	0
Nostoc verrucosum	0	22	0	21	0
O. brevis	**40**	20	0	0	0
O. acutissima	22	**24**	**24**	20	**30**
Spirulina major	0	**25**	22	21	0
Anabaena variabilis	0	20	21	22	0
Aphanizomenon flos aquae	0	22	0	0	0

An obvious inhibitory effect (diameter of the inhibition zone -24mm, respectively 25mm), was presented by *Oscillatoria acutissima* and *Spirulina major*. Extracts from the biomass of the cyanobacteria *O. planctonica*, *O. brevis* and *O. acutissima* and from the microalgae *Chlorella vulgaris*, showed a growth suppressing effect of *Aspergillus niger*, of which the cyanobacterium *O. brevis* a larger diameter of the inhibition zone (40 mm.). A significant inhibitory effect on the phytopathogenic crop *Botrytis cinerea* was observed at biomass

extracts of *Oscillatoria planktonica, O. acutissima*, (24 mm). *Chlorella vulgaris, Spirulina major* and *Anabaena variabilis* showed an inhibitory action on the growth of the pathogenic fungus *Fusarium solani*. On the *Fusarium solani* presented an inhibitory effect also extracts from *Oscillatoria planktonica, O. acutissima, Spirulina major*, *Anabaena variabilis* and *Nostoc verrucosum*. *Chlorella vulgaris* showed a more pronounced action (24 mm).
An inhibitory action on the growth of the fungus *Fusarium oxysporum* was also presented by the biomass extract of *Oscillatoria acutissima* (Ø inhibition zone of 30 mm).
The images below show the areas of inhibition of the growth of microalgae isolated from the lake "La izvor".

Fig. 1 A

Fig. 1 B

Fig. 1 A Antagonistic activity of *Oscillatoria acutissima*, *Spirulina major* and *Anabaena variabilis* against *Botrytis cinerea*

Fig. 1 B Antagonistic activity of *Oscillatoria acutissima, Spirulina major* and *Anabaena variabilis* against *Botrytis cinerea*

3.2. Determination of antibacterial activity of isolated microalgae strains from "La Izvor" lake

Microalgae are a source of secondary metabolites with various important biological properties, including antibacterial activity. Secondary metabolites have an important role in the development of new pharmaceutical products, such as antibiotics, anti-inflammatory and anti-cancer drugs as well as against abiotic stress (Martínez-Ruiz et al 2022). Therefore, stress may play an important role in the synthesis of antibacterial substances in microalgae. For example, strains of *Dunaliella salina* collected from water contaminated with industrial and domestic waste produced more compounds with antibiotic activity than strains from areas with low pollution (Shannon M. 2021).
Microalgal compounds with antibacterial activity include fatty acids, glycolipids, phenols, terpenes, β-diketone and indole alkaloids However, most of the antibacterial activity is usually attributed to long-chain unsaturated fatty acids (Martínez-Francés et al 2018). Interest in microalgae as a source of antibiotics appears to have arisen when *Chlorella*, a genus of freshwater green microalgae capable of producing chlorine, which present an antibacterial compound capable of inhibiting the growth of both gram-positive and gram-negative bacteria. Since then, further studies have shown that some green microalgae produce substances that can kill or inhibit the growth of human pathogens (Alsenani F., 2021,). Antibacterial activity was observed in green microalgae from waters contaminated with high concentrations of metals. The results of this research indicated that extracts obtained from *Chlamydomonas sp*. were active against gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*. Studies have shown that *Dunaliella sp*, a green microalga that thrives in high salinity environments, increases its production of certain secondary metabolites such as β-carotene as

salinity increases by up to 14%, which may suggest an ability to produces metabolites with antibacterial activity. Regarding the antibacterial activity of unsaturated and saturated fatty acids from *Dunaliella primolecta* extracts, it was determined that γ-linolenic acid showed the highest activity against *S. aureus* (Shannon L. 2020, B. Digamber Rao 2015). Polyunsaturated aldehydes resulting from the degradation of free polyunsaturated fatty acids from *Coelastrum sp.*, *Scenedesmus quadricauda* and *Selenastrum sp*. have been shown to possess antibacterial activity, inhibiting the gram-negative bacterium *Serratia marcescens* which has been shown to cause a wide range of infectious diseases, including urinary, respiratory and biliary tract infections, peritonitis and pneumonia in hospital settings. (Shannon L. 2021).

Thus microalgae strains cultivated in different environments or in the presence of stressors will allow the discovery of new strains with the potential of antibacterial agents.

According to results of this study, it can be seen that all 8 strains of microalgae isolated from the lake "La Izvor" showed an antibacterial effect against 3 cultures of phytopathogenic bacteria: *Bacillus subtilis* B-117, *Xanthomonas campestris* 8003b and *Erwinia caratovora* 8982; but could not inhibit the growth of the phytopathogens *Corynebacterium miciganense* 13a and *Agrobacterium tumefaciens (Rhizobium radiobacter)* 8628.

As can be seen from the table 2, microalgae *Chlorella vulgaris* showed maximum antibacterial activity, showing the largest areas of growth inhibition at *Bacillus subtilis* B-117 (30 mm), *Xantromonas campestris* 8003b (26 mm) and *Erwinia caratovora* 8982 (25.5 mm) respectively.

Table 2 - Antibacterial activity of microalgae isolated from the "La Izvor" lake system, diameter (mm)

Microalgae sp.	**Bacterial culture tests, diameter of the inhibition zone, mm**				
	Bacillus subtilis B-117	*Xanthomonas campestris* 8003b	*Corynebacterium miciganense 13 a*	*Agrobacterium tumefaciens (Rhizobium radiobacter)* 8628	*Erwinia caratovora* 8982
Oscillatoria planctonica	27,5	26,5	0	0	21,5
Chlorella vulgaris	30	26,5	0	0	25,5
Nostoc verrucosum	12	13	0	0	11
Oscillatoria brevis	16	20	0	0	10,5
O.acutissima	19	12	0	0	16
Spirulina major	28	21	0	0	0
Anabaena variabilis	20	17,6	0	0	11
Aphanizomenon flos aquae	21	17	0	0	22

The cyanobacterium *O. planctonica* also showed significant activity in inhibiting the growth of cultures of *Bacillus subtilis* B-117 (27.5 mm) and *Xanthomonas campestris* 8003b (26.5 mm). *Spirulina major* showed an obvious antibacterial activity against the *Bacillus subtilis* B-117 strain (growth inhibition area - 28 mm).

4. Conclusions

The biomass extracts of some microalgae isolated from the lake "La izvor" possess antimicrobial activity on some phytopathogenic species of fungi and bacteria, among which it can be mentioned the extracts of *Chlorella vulgaris, O.acutissima, Spirulina major* that showed larger diameters of inhibition zones of the growth of pathogenic strains. So the microalgae studied proved to be potentially a new source of antimicrobial agents and the investigation continues in order to isolate new strains with such potential as well as to manipulate the cultivation conditions to allow an increased production of antibacterial compounds.

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