Screening for Antimicrobial Agent Production of Some Microalgae in Freshwater

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Abstract

In this study, 10 microalgae strains isolated from different freshwater reservoirs situated in various topographies in Turkey were tested in compliance with the agar-well diffusion method for their antimicrobial agent production on various organisms (Bacillus subtilis ATCC 6633, Bacillus thuringiensis RSKK 380, Bacillus cereus RSKK 863, Bacillus megaterium RSKK 578, Yersinia enterocolitica ATCC 1501, Escherichia coli ATCC 11230, Staphylococcus aureus ATCC 25923, Micrococcus luteus NRLL B-4375, Micrococcus flavus, Pseudomonas aeruginosa ATCC 29212, Saccharomyces cerevisiae ATCC, Candida albicans ve Candida tropicalis). The findings in this study reveal that activity is maintained against antimicrobial activity of acetone and ether extracts on Gram-negative bacteria; methanol extracts on Gram-positive bacteria; ethanol extracts on both Gram-positive and Gram-negative organisms. Chloroform extracts, on the other hand, were not found to reveal any antimicrobial activity. In addition, Oscillatoria sp. ve Chrococcus sp. were found to have antifungal activity on yeasts.

INTRODUCTION

One potential commercial application of microalgae derived compounds that has, as yet, received little attention is the area of pharmaceuticals, antibiotics and other biologically active compounds. Both cell extracts and extracts of the growth media of various unicellular algae (e.g. Chlorella vulgaris, Chlamydomonas pyrenoidosa) have been proved to have antibacterial activity in vitro against both Gram-positive and Gram-negative bacteria. It has also been reported that a wide range of in vitro active antifungal activities are obtained from extracts of green algae, diatoms and dinoflagellates. Microalgae, such as Ochromonas sp., Prymnesium parvum, and a number of blue-green algae produce toxins that may have potential pharmaceutical applications (Borowitzka and Borowitzka, 1992).

Biologically active substances were proved to be extracted by microalgae. Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity. Temperature of incubation, pH of the culture medium, incubation period, medium constituents and light intensity are the important factors influencing antimicrobial agent production (Noaman et al., 2004).

It is the main objective of this study to test 10 microalgae isolated from various freshwater reservoirs situated in Turkey for their antimicrobial activities on various microorganisms and optimize the conditions for the production of an antimicrobial agent by microalgae.

MATERIALS AND METHODS

CULTURING AND GROWTH CONDITIONS

Collection and isolation of microalgae were made in compliance with Rippka (1988). Micro algae were obtained from Mogan and Burdur Lakes, Kurtbo?azi Dame and from the waste waters of a sugar refinery.

Algae were made to increase in number by having been left at BG11 nutrition medium less than 3000 lux light intensity, for 16 h under illumination and 8 h under darkness. Algae were harvested approximately after a 15 day production period.

TEST ORGANISMS

The bacteria and yeast strains (Bacillus subtilis ATCC 6633, Bacillus thuringiensis RSKK 380, Bacillus cereus RSKK 863, Bacillus megaterium RSKK 578, Yersinia enterocolitica ATCC 1501, Escherichia coli ATCC 11230, Pseudomonas aeruginosa ATCC 29212, Micrococcus flavus, Micrococcus luteus NRLL B-4375, Staphylococcus aureus HU 25923, Candida albicans, Candida tropicalis, Saccharomyces cerevisiae and Saccharomyces cerevisiae

2SI TP(3-2)) used in this study were obtained from Culture Collections of the Biotechnology Laboratory at the Department of Biology in Gazi University.

While the bacteria strains were incubated into nutrient broth throughout 24 h, the yeast strains were incubated into YEPD throughout 48 h.

DRY WEIGHT DETERMINATION

The cells were separated from culture filtrate by centrifugation and then washed several times with distilled water. Biomass were transferred to a pre-weighed dry filter paper using a clean spatula then placed in an oven at 60 °C overnight to reach a fixed weight.

PREPARATION OF THE EXTRACTS

Algal mass from an axenic exponential culture of the microalgae strains growing in BG11 were separated from the culture medium by centrifugation and there pellets were dried at 60 °C for 24 h. According to the methods of Khan et al. (1988) and Vlachos et al. (1996) the methanol extract to be prepared, dry algal mass (ratio 1:15 g/ml) was extracted throughout 24 h. After the extraction phase being separated, this method was used for the chloroform, acetone, ethanol and ether respectively. All of the extracts were preserved at +4 °C.

INHIBITORY EFFECT BY THE AGAR-WELL DIFFUSION METHOD

Antibacterial and antifungal activities of microalgae were tested by agar-well diffusion method. Besides, the antimicrobial activity of microalgae was compared with antibiotics (erythromycin, vancomycin and tetracycline) and fungicide (flucanazole).

Petri dishes with 10 ml of nutrient and Sabourd agar were prepared, previously inoculated with 0.1 ml of a 24 h broth culture of test bacteria and yeasts. Four wells (6 mm) were made and filled with 100 l extract. The inoculated plates were incubated for 24 h at 37 °C. After incubation, the diameter of the inhibition zone was measured with calipers (Attaie et al., 1987).

STATISTICAL ANALYSIS

Data were analyzed and treatments compared using the one-way ANOVA with 95% confidence limits ($p<0.05$).

RESULTS AND DISCUSSION

A large number of microalgal extracts and extracellular products have been found to have antibacterial activity. However, pH of the medium, incubation period and temperature of incubation were very important for the biosynthesis of antimicrobial agent products as secondary metabolites. Noaman et al., (2004) have reported that temperature 35 °C, pH 8 and 15 days of incubation were the best for growth and antimicrobial agent production. In this study, these were chosen as an optimum pH, incubation periods and temperature.

The present study is an endeavor towards antimicrobial agent production by some microalgae has screened for its biological activity against different species of bacteria and fungi and it was found that it has high biological activity against Bacillus subtilis ATCC 6633, Bacillus thuringiensis RSKK 380, Bacillus megaterium RSKK 578, Escherichia coli ATCC 11230, Pseudomonas aeruginosa ATCC 29212, Candida tropicalis and Saccharomyces cerevisiae.

Antibacterial and antifungal activity of microalgae (Chrococcus sp. Mogan, Chrococcus sp. Kurtbo?azi, Oscillatoria sp. ?.F., Oscillatoria sp. Burdur, Oscillatoria sp. Mogan, Oscillatoria limnosa 1966/1380, Anabaena sp. Burdur, Synechocystis aquatilis 1965/426, Chlorella vulgaris) against test microorganisms were shown in Table 1. The antimicrobial activity of test microorganisms against antibiotics and fungicides were shown in Table 2.

Figure 1

Table 1: Antimicrobial activity of extracts obtained from microalgae

Microalgae	Dry weight (g/l)	Test Microorganisms
Chrococcus sp. (Mogan	0.14±0.04	*B. subtilis* ATCC 6633
		P. aeroginosa ATCC 29212
Chrococcus sp.	0.19±0.05	*Candida tropicalis*
Oscillatoria sp. (Mogan	0.13±0.04	*P. aeruginosa* ATCC 29212
		Saccharomyces cerevisiae
		S. cerevisiae 2SI TP(3-2)
Synechocystis aquatilis	0.17±0.05	*B. subtilis* ATCC 6633
Oscillatoria sp. S.F.	0.15±0.04	*P. aeruginosa* ATCC 29212
		E. coli ATCC 11230
Anabaena sp. (Burdur	0.10±0.01	*B. megaterium* RSKK 578
		B. thuringiensis RSKK 380
Chlorella vulgaris	0.20±0.00	*B. megaterium* RSKK 578

Microalgae	Diameter of Inhibition Zone (mm)				
	CH-PE[a]	Ethanol	CH-	CH-	Acetone
Chrococcus sp. (Mogan	-	9.2±0.3	-	-	-
	10.2±0.2	-	-	-	13.0±0.0
Chrococcus sp.	-	10.4±0.3	-	-	-
Oscillatoria sp. (Mogan	9.8±0.0	11.2±0.0	-	-	9.2±0.4
	-	-	15.0±0.1	-	-
	-	-	8.8±0.0	-	-
Synechocystis aquatilis	-	8.8±0.1	-	-	-
Oscillatoria sp. S.F.	16.0±0.4	-	-	-	13.4±0.3
	-	7.2±0.4	-	-	-
Anabaena sp. (Burdur	-	21.2±0.2	-	-	-
	-	11.6±0.3	10.6±0.1	-	-
Chlorella vulgaris	-	8.6±0.0	10.4±0.2	-	-

[a]: petroleum ether extract [c]:Methanol extract [d]: Chloroform extract
-: No inhibition
Values are the means ± standard deviations of triplicate measurements.

Figure 2

Table 2: Diameters of inhibition zone (mm) exhibited against test microorganisms of standard antibiotics and fungicides.

Test Bacteria	Erythromycin	Vancomycin	Tetracycline	Flucanazole
Bacillus cereus RSKK 863	23	12	17	ND
Bacillus megaterium RSKK 578	18	12	19	ND
Bacillus thuringiensis RSKK 380	R	R	R	ND
Bacillus subtilis ATCC 6633	29	19	19	ND
Pseudomonas aeruginosa ATCC 29212	R	R	4	ND
Yersinia enterocolitica ATCC 1501	R	R	16	ND
Escherichia coli ATCC 11230	R	R	R	ND
Micrococcus flavus	31	20	31	ND
Micrococcus luteus NRLL-B, 4375	31	16	23	ND
Staphylococcus aureus ATCC 25923	17	8	15	ND
Saccharomyces cerevisiae	ND	ND	ND	2
Saccharomyces cerevisiae 2SI TP(3-2)	ND	ND	ND	3
Candida albicans	ND	ND	ND	15
Candida tropicalis	ND	ND	ND	12

ND: Not detected, R: Resistant

As the results reveal, while acetone and ether extracts exhibit antimicrobial activity on gram negative bacteria; methanol extracts exhibit that activity on Gram-positive bacteria. On the other hand, ethanol extracts exhibited antimicrobial activity on both Gram-positive and Gram-negative organisms. Yet, the chloroform extracts did not exhibit antimicrobial activity. Oscillatoria sp. ve Chrococcus sp. exhibited antifungal activity on yeasts. The methanol extract, obtained from Oscillatoria sp. Mogan, showed antifungal activity on S. cerevisae and S. cerevisae 2SI TP (3-2).

In general, activities both against Gram-negative and Gram-positive bacteria in ethanol extracts were observed. The only activity against C. tropicalis was found also in ethanol extracts obtained from Chrococcus sp. Kurtbo?azi. An activity was determined in acetone and ether extract only against P. aeruginosa ATCC 29212. No activity was determined in chloroform extract against test bacteria. However, the microalgae differ significantly in their activity against test microorganisms ($F=1.123$; df:10, $p<0.05$).

When the effects of extracts obtained from microalgae were compared with the antibiotics used in the study, ether and acetone extracts obtained from Chrococcus sp. Mogan, ether and acetone extracts obtained from Oscillatoria sp. (?.F.) and ethanol extract obtained from Oscillatoria sp. Mogan (Table 1) exhibited antibacterial activity on Pseudomonas aeroginosa ATCC 29212. Ethanol extract obtained from Anabaena sp. (Burdur) exhibited antibacterial activity on B. megaterium RSKK 578 and B. thuringiensis RSKK 380. In this study, the antimicrobial activity of microalgae could be explained by the presence of cyclic peptides, alkaloids and lipopolysaccharides.

Both cell extracts and extracts of the growth media of various unicellular algae have been proved to have antibacterial activity in vitro against both Gram-positive and Gram-negative bacteria. Microalgae such as Ochromonas sp., Prymnesium parvum, and a number of blue green algae produce toxins that may have potential pharmaceutical applications (Borowitzka, 1995).

There are a number of reports by many authors on antibiotic and other pharmacological effects from cyanobacteria. The genius from cyanobacteria, generally studied on an antimicrobial activity are Nostoc sp.(Knübel et al., 1990; Bloor and Englan, 1991; de Mule et al., 1991), Scytonema sp.(Chetsumon et al., 1993; 1994; 1995; Stewart et al., 1988; Ishida et al., 1997), Microcystis sp.(Carmichael et al., 1988), Oscillatoria sp. (Bagchi et al., 1990; Barchi et al., 1984) and Phormidium sp. (Fish and Codd, 1994).

The extracts of 12 cyanobacterial strains were investigated for their antibiotic activities against 7 microorganisms by Kreitlow et al. (1999). All cyanobacterial samples, extracts from 7 species were concluded to inhibit the growth of at least one of the Gram-positive bacteria Micrococcus flavus, Staphylococcus aureus and Bacillus subtilis. Finally, the hexane and dichloromethane extracts were shown to exhibit antimicrobial effects.

Bagchi et al. (1990) originally proposed that natural algaecides could effectively be applied in control of toxic algal blooms.

de Mule et al. (1991) determined that the methanol extracts of Nostoc muscorum revealed antibacterial activity on Sclerotinia sclerotiorum. Ishida et al. (1997) observed that kawaguchipeptin B, isolated from Microcystis aeruginosa and inhibited the growth of Staphylococcus aureus. It was observed that thermo tolerant cyanobacterium Phormidium sp. inhibited the growth of Staphylococcus aureus and Candida albicans (Fish and Codd, 1994). Barchi et al. (1984) found out that liphophilic extracts of Oscillatoria accutissima were anti-neoplastic and toxic activities. Özdemir et al. (2001) synthesized found that the Spirulina extracts, obtained from various solvents exhibited antimicrobial activity on both Gram-positive and Gram-negative organisms.

As is in the studies reported, it was observed that the extracts obtained from various solvents used in this study had antibacterial and antifungal activities, and that these extracts could be much more effective when compared with

contemporary antibiotics and fungicides.

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