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Research Article

ANTIMICROBIAL ACTIVITY OF CYANOBACTERIA ISOLATED FROM SHAHID RAJAEE HYDROTHERMAL FRESHWATER FISH POOL IN THE CITY OF SARI

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ABSTRACT
Cyanobacteria are rich source of primary and secondary metabolites which are important in biotechnology and pharmaceutical industries. The aim of the present study was to collect and identify 2 cyanobacteria (Lyngbya sp, Osilatoria sp) from Shahid Rajaee Hydrothermal freshwater fish pool in the city of Sari, Iran. Agar diffusion method was used to test antibacterial and antifungal activity on human pathogenic bacteria and fungi (Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Streptococcus mutants, Escherichia coli, Klebsiella pnuemoniae, Saccharomyces cerevisiae and Candida albicans). The antimicrobial activity of the named cyanobacteria was compared using different solvents (ethanol, acetone, diethyl ether and methanol). It was shown that ethanol and methanol extracts of Osillatoria have the highest antibacterial activity. Additionally, it was found that Oscillatoria sp and Lyngbya sp have antifungal activity against Candida albicans. Diethyl ether extracts of Oscillatorya sp and Lyngbya sp had the highest antifungal activity. Additionally, diethyl ether extract of the cyanobacterium Lyngbya sp had the highest antibacterial activity against E. Coli.

Keywords: *Antimicrobial Agents, Freshwater, Cyanobacteria, Lyngbya sp*

INTRODUCTION
Cyanobacteria are common inhabitants of soil, fresh and marine waters and they are excellent source of vitamins and proteins (Carmichael, 1981). Bacteria, fungi and viruses are ubiquitous and there is always a risk of resistance to antibiotics and derivatives forming. Cyanobacteria are a great choice for new drug development and they are a rich source of biologically active primary and secondary metabolites (Namikoshi and Rinehart, 1996). The metabolites of these microorganisms are important material with high biological activity used in pharmaceutical industries (Seleghim *et al.*, 2007). These metabolites have newly been studied. Recent research shows the presence of biologically active substances in water which have anti-cancer, anti-microbial, anti-inflammatory and other pharmacological activities (Seleghim *et al.*, 2007; Borowitzka, 1992). Cyanobacteria have gained great attention for use in the food and fuel industry and they also produce secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals, pharmacological probes which are associated with toxic, hormonal, anti-cancer and anti-microbial activities (Namikoshi and Rinehart, 1996). They are found everywhere around the world and are active in water and soil. They are known as blue-green algae (Elgorashi and van Staden, 2004). In the past few decades, several classes of aquatic cyanobacteria have been studied for their antibacterial and antifungal activity in the pharmaceutical industry (Takamatsu *et al.*, 2003). They have been proven useful for various applications, especially as new therapeutic agents for various diseases (Harada *et al.*, 2002).Secondary metabolites of cyanobactria have toxic properties and are anti-bacterial, anti-fungal, anti-yeast and anti-cancer (Carmichael, 1992). Biologically active substances are extractable from microalgae. Many species of cyanobacteria are known for producing anti-bacterial, anti-fungal, anti-viral and anti-cancer substances. Their survival is affected by many factors including incubation temperature, PH, kind of medium, incubation period, medium composition and light intensity (Noaman *et al.*, 2004). In the recent decades, cyanobacterial biotechnology has made great advances. The properties of their secondary metabolites are not fully clear in nature. The purpose of this study was to isolate cyanobacteria from water and test their antimicrobial activity.

MATERIALS AND METHODS

Sample Collection:

Cyanobacterial samples: samples of water were collected containing cyanobacteria from numerous sites of Shahid Rajaee Hydrothermal fish pool in the city of Sari, Iran, Jan 2012. Isolation and identification was performed using standard microbiological methods.

Isolation of Cyanobacteria:

Two genera of cyanobacteria (Oscillatoria sp and Lyngbya sp) were selected for this study. The two strains were isolated from Shahid Rajaee Hydrothermal freshwater fish pool in the city of Sari, Iran. After purification, the strains were stored in a room for cyanobacteria, Department of Microbiology, Islamic Azad University, northern Tehran branch, Tehran, Iran.

Preparation of Extracts

The two species of cyanobacteria used (Lyngbya and Oscilatoria) were collected from the Shahid Rajaee Hydrothermal freshwater fish pool in the city of Sari and grown in BG-11 medium (PH7). They were placed in vacuum and continuously lighted by 3500 LUX intensity. They were harvested in the stationary phase of growth (23 days), filtered and suspended in medium after collection of biomass. Biomass collection occurred in a hot air oven at 60°C for 1H. The algae were dried and placed in solvent mixture for 6hours and sonicated for 10 minutes and subsequently centrifuged at 4000 rpm for 5 minutes. The solution was centrifuged and supernatant dried. The mass was collected and re-weighed. Dry weight was used for antimicrobial extraction. One gram of each cyanobacterium was placed in 10 ml of acetone, ethanol, methanol and diethyl ether. The samples were mixed well and kept at room temperature for 60 minutes. The suspension was centrifuged at 3000 rpm for 5 minutes. The extracts were kept at +4°C. The dry mass was extracted with acetone, diethyl ether, methanol and ethanol separately using soxhlet extractor at 40 OC for extraction of non-polar and polar compounds (Elgorashi and van Staden, 2004) and resuspended in suitable solvent (methanol**,** acetone, diethyl ether, methanol and ethanol) for preparation of known concentration of solution for antimicrobial assaying. Extracts were kept at +4°C (Gonzalez *et al.,* 2001). The antimicrobial activity of cyanobacterial species such as Oscillatoria sp, Lyngbya sp was tested on microorganisms like Bacillus subtilis, Staphylococcus aureus, Streptococcus mutans, E. coli, Klebsiella pneumoniae, Saccharomyces cerevisiae and Candida albicans). These microorganisms were obtained from the Iranian Research Organization for Science and Technology. Bacterial strains were inoculated in nutrient broth and incubated at 37°C for 24 hrs. The yeast and fungal strains were also inoculated in glucose peptone broth and incubated at 30°C for 5 days.

Determination of the Inhibitory Effect of the Cyanobacteria Extracts:

Agar diffusion method was used for testing antibacterial and antifungal activity of the cyanobacteria extracts. 4 wells (6 mm) were constructed and filled with150ml of different solvents for obtaining extracts of cyanobacteria. Bacterial plates were incubated at 37°C for 24 hours and the fungi were incubated at 30°C for 3 days. Zone of growth inhibition was measured with calipers and test results were reported (Attaie, *et al.,* 1987). The antibacterial activity of the isolated cyanobacteria were studied and compared with standard antibiotics (erythromycin, tetracycline, and amoxicillin) and fungicides (itraconazole and polyoxylin).

RESULTS AND DISCUSSION

Samples of aquatic cyanobacteria were collected and identified based on the Bergey manual of determinative bacteriology. In total, 2 genera of cyanobacteria were isolated and identified (Table 1), Oscillatoria sp and Lyngbia sp. In the present study, two cyanobacteria were evaluated for antibacterial and antifungal activity using agar diffusion method (Table 1) and inhibition zones of cyanobacteria extracts obtained using four solvents were measured against the test organisms (Table 1). Both ethanol and methanol extracts of Oscillatoria sp showed an inhibitory effect on all bacteria tested. The maximum inhibition test of E. Coli was compared to other organisms. In general the maximum inhibition zone of 4

mm was observed in the extract of diethyl ether of Lyngbya against the E.Coli and the minimum inhibition zone of 0.4 mm was observed by the acetone extract of Lynbya sp against S. cerevisae. Zone of growth inhibition depends on the genus of the cyanobacteria, the type of solvent used and tested against the test microbes. Regarding antibacterial effects, the results clearly showed that the methanol extract of Oscillatoria sp had the highest antimicrobial activity against E.coli. It was shown that the acetone extract of Oscillatoria sp had no activity against Klebsiella pneumonia, B. subtilis and E. coli. Cyanobacteria Lyngbya extracts from various solvents had positive effect on Bacillus subtilits and S. cerevisaea and a negative effect on Streptococcus mutans and K. pneumonia, S. aureus, and C. albicans. These results are in harmony with those obtained by another study (Volk and Furkert, 2006) (Table 1). The results further showed that ethanol and methanol are the best solvent for extraction of antibacterial and antifungal activities of Oscillatoria sp and acetone and methanol are the best solvent for extraction of antibacterial and antifungal agents from Lyngbya sp (Table 1).Our results agreed with studies by other researchers (Moreau *et al.*, 1988). In many studies, researchers have shown that acetone extracted cyanobacteria have antimicrobial activity against E. coli and Bacillus subtilis (Mule *et al.*, 1991). In this study, it was found that diethyl ether extract of Oscillatorya sp and Lyngbya sp showed the largest inhibition zone on the test fungi. Antimicrobial activity of the microorganisms tested against standard antibiotics and fungicides are reported in Table 2. In this study, it was shown that standard antibiotics were more effective than Cyanobacteria extracts on Bacillus subtilis, E. coli and Klebsiella pneumonia. Yet, the cyanobacteria extract bactericidal effects against S. aureus was higher than standard antibiotics. The antifungal effect of cyanobacteria extracts was more positive than the standard antifungals against Sacchromyces cerevisiae. While itraconazole and polynoxylin, did not show any effect against the tested fungi. In this report, it was observed that the extracts obtained from various solvents with anti-bacterial and anti-fungal activity are more effective than standard antibiotics and fungicides. Other researchers have examined the antimicrobial properties of cyanobacteria. Indifferent studies, the antibacterial and antifungal effects of cyanobacteria on pathogenic microorganisms have been examined. For example Oscillatoria anguistisima antimicrobial effect was reported in (Issa, 1999). Anabaena, Oscillatoria antimicrobial effect was reported in 1999 by (Kreitlow *et al.*, 1999) and Spirulina platensis antimicrobial effect was reported in (Kaushik and Goyal, 2008) and Chauhan. The authors also reported that extracts of cyanobacteria from various genera in different solvents had different positive effects against both gram-positive and gram-negative organisms. This is in agreement with the results we obtained.

Table 1: Antibacterial and antifungal activities of different cyanobacteria extracts Diameter of inhibition zone (cm)

Algal species	Organic solvent	B. subtilis	S. aureus	S. mutans	E. coli	K. pneumonia	S. cerevisae	C. albicans
Oscillatoriasp	Ethanol	2	2.6	2.8	1.8	1.8	1.6	1
	Acetone	R	2.5	2. 7	R	1	1.5	R
	Diethyl ether	1.8	R	1.3	1	R	1.2	1.7
	Methanol	0.8	1.1	2.5	3.7	1.6	1.1	0.5
Lyngbyasp	Ethanol	2.1	1.4	R	1.2	R	1	0.7
	Acetone	1.1	1.6	1.8	1.2	1.8	0.4	r
	Diethyl ether	1.5	r	1.2	.4	R	1.2	1
	Methanol	1.8	2.0	1.	r	1	1.4	0.8

R – Resistant; ND – Not Detected

Table 2: Diameters of inhibition zone (cm) exhibited by test microorganisms against standard antibiotics and fungicides Diameter of inhibition zone (cm)

Standard antibiotics Standard fungicides

Test organism	*Erythromycin*	*Tetracycline*	*Amoxicillin*	*Itraconazole*	*Polynaxylin*
Bacterial species					
Bacillus subtilis	*3.1*	*3.2*	*5.0*	*ND*	*ND*
S. aureus	*r*	*r*	*r*	*ND*	*ND*
S. mutans	*3.1*	*2.4*	*2.8*	*ND*	*ND*
E. coli	*4.7*	*4.3*	*3.6*	*ND*	*ND*
Klebsiella pneumoniae	*3.2*	*R*	*2.2*	*ND*	*ND*
Fungal species					
Saccharomyces cerevisiae	ND	ND	ND	R	R
Candida albicans	ND	ND	ND	2.1	1.6

R – Resistant; ND – Not Detected

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