

Antimicrobial Activity of Cyanobacteria Isolated from Marine Water Resource

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The present study was aimed to collect and identify the cyanobacteria from kovalam beach, Tamilnadu, India. Totally three species of marine cyanobacteria were isolated and cultured in BG11 medium. According to their growth characteristics the three species were confirmed with the comparison of the control obtained from NFMC, Tiruchirapalli. They were *Oscillatoria salina*, *Oscillatoria boryana*, *Oscillatoria laetrivirans*. These cyanobacteria were tested for the production of antimicrobial agents against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, *E.coli* and *Klebsiella pneumoniae*. The antimicrobial activity of the extract were based on the gram character of the cells i.e., antimicrobial activity of ethanol and acetone on the gram negative bacteria and methanol extracts on gram positive bacteria were done.

Key words: Marine cyanobacteria, antimicrobial activity.

Cyanobacteria, the archaic photosynthetic microorganisms which have flourished on earth more than three billion years with some genera showing only minor morphological changes since that time.¹ This blue green algae belonging to the family cyanophyceae is so named because of its resemblance with the bacterial characteristics. Previously they were known as myxophyceae. Apart from the main feature that the cells lack true nuclei they show other features which, with the bacteria distinguish them from the eukaryota.² The bacteria and the cyanophyceae lack mitochondria, true vacuoles and endoplasmic reticula. Sterols are not detected in bacteria and the absence of sterols in cyanobacteria was an accepted fact. In some forms of cyanobacteria especially in *oscillatoria spp*

pseudovacuoles may be formed, these contribute towards their buoyancy by virtue of the gas that they contain. Cyanobacteria exhibit three morphological forms i.e. coccoid, unbranched filamentous and branched filamentous; and two functional group, i.e. heterocyst bearing, nitrogen fixing, filamentous forms and non heterocystous, non-nitrogen fixing filaments.³ The reproduction is mainly by fragmentation. They are photoautotrophic. The presence of antagonistic compound make them antibiotic. Although they are truly prokaryotic, cyanobacteria have an elaborate and highly organized system of internal membranes which function in photosynthesis.⁴ Marine microorganisms are rich source of structurally novel and biologically active metabolites⁵. Secondary and primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry.⁶

Oscillatoria is a genus of filamentous cyanobacterium which is named after its oscillation movement. They are already a subject of research

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into the natural production of butylated hydroxytoluene (BHT), an antioxidant, food additive and industrial chemical. *Oscillatoria spp.* also have known to produce vitamins, minerals, viridamines and anti protozoal activity.⁷ The aim of the present work was to study the antibacterial activity of cell extract of *Oscillatoria salina*, *Oscillatoria boryana* and *Oscillatoria laetrivirans* both gram positive and gram negative bacterial pathogens.

MATERIALS AND METHODS

Isolation of cyanobacteria

Oscillatoria spp., a marine cyanobacteria, autotrophic filamentous photosynthetic organism was chosen for this study. It was collected from the kovalam beach from three different places. The obtained culture was grown in the cyanobacterial medium. The growth characteristics were studied and the biochemical nature were compared with the cultures obtained from National facility of marine cyanobacteria (NFMC) tiruchirapally.

Culture media

The *oscillatoria spp.* were cultured in BG11 broth and enhanced with a nitrogen source like urea. This was provided with a light source and incubated for $25 \pm 2^\circ\text{C}$ in 1,500 lux with 12 hrs day/night cycle and was allowed to grow for 15-20 days.

Harvest

After the duration of 15-20 days, the mass was harvested using a sieve and was washed many times with tap water followed by distilled water to remove salts. The fresh weight of the mass was obtained using an electronic balance (Precisa 125A, Switzerland). This wet mass was used for the preparation of extract.

Extraction

The weighed wet mass was grounded in a pestle and mortar with 100% alcohol (distilled). The ground material was centrifuged at 10,000 rpm for 10 minutes at 4°C (Remi cooling centrifuge C24) and the supernatant was separated and collected. This process was repeated till the pellet turned grey or the supernatant turned colorless. The supernatant was pooled and filtered through crude filter paper, followed by Whatmann No.1 filter paper and then it was concentrated using a speed vacuum concentrator. Extractions were carried out

successively with ethanol, acetone, diethyl ether and methanol and all the three samples were refluxed until saturation (24h), and the respective extract were dried in oven at 50°C ^{8,9}. Subsequently, the residual extracts were suspended in the respective solvents to final concentration of 1 mg/20 μl .

Antibacterial assay

Antibacterial activities of the cyanobacterial extracts were tested by agar well diffusion method. Nutrient agar plates were inoculated with 100 μl of a 24h broth culture of the test bacteria. Two wells of (6mm) were made and filled with 100 μl extract. The inoculated plates were incubated for 24h at 37°C .¹⁰ The diameter for zone of inhibition was measured with calipers and the results were recorded. The antimicrobial activity of cyanobacteria with standard antibiotics were compared (erythromycin, tetracycline and amoxicillin).

RESULTS AND DISCUSSION

Three species of Cyanobacteria of *Oscillatoria spp.* were isolated namely, *Oscillatoria salina*, *Oscillatoria boryana* and *Oscillatoria laetrivirans*. The results clearly indicated that the inhibition factor mainly depended on three main things i.e, type of the cyanobacterial species, type of the solvent and the tested bacterial species. The results showed that the acetone extract of *Oscillatoria laetrivirans* and the *Oscillatoria boryana* showed highest activity in *E.coli* and *Bacillus subtilis*¹¹. The ethanol extract of *Oscillatoria laetrivirans* also showed a rise in antibiotic activity against *Streptococcus mutans*¹². These solvents showed moderate activity with *Klebsiella Pneumoniae*. Methanolic extract of *Oscillatoria laetrivirans* also showed sufficient activity against *Streptococcus mutans*¹³. *Oscillatoria salina* showed considerable antibacterial activity and its diethyl ether extract showed antibacterial activity against *E.coli*. The cyanobacteria *Oscillatoria spp.* are a group of organisms which plays a major role in photo production of bio fuels, ammonia, vitamins, toxins, therapeutic substances, aqua or animal feed¹⁴. Cyanobacteria are used as good bio fertilizers also. They have been flourished today and begun to reap the benefits of molecular biology to enhance their performance.¹⁵

Table 1. Antibacterial activities of cyanobacterial extracts

Cyanobacteria		Diameter of Inhibition Zone				
		Bacteria				
		<i>B.subtilis</i>	<i>E.coli</i>	<i>S.aureus</i>	<i>S.mutans</i>	<i>K.pneumoniae</i>
<i>Oscillatoria Laetrivirans</i>	Ethanol	2.2	2.0	2.4	3.2	2.6
	Acetone	3.0	3.8	R	1.2	1.0
	Diethyl Ether	2.2	R	2.0	2.5	2.3
	Methanol	1.0	1.5	2.0	3.3	1.8
<i>Oscillatoria Salina</i>	Ethanol	2.5	1.6	1.8	2.2	2.2
	Acetone	1.5	2.0	2.2	1.6	2.2
	Diethyl Ether	2.2	2.6	R	1.8	1.8
	Methanol	1.8	2.0	1.2	R	1.8
<i>Oscillatoria Boryana</i>	Etahnol	2.0	1.2	1.0	1.8	2.5
	Acetone	1.8	3.4	1.0	2.2	1.0
	Diethylether	1.0	2.0	2.0	R	2.0
	Methanol	R	1.8	1.2	1.8	1.5

Table 2. Diameter of zone of inhibition(cm)of the test organism against standard antibiotics

Bacterial Spp.	Test organisms	Diameter of Inhibition zone(cm)		
		Standard Antibiotics		
		Amoxicillin	Tetracycline	Erythromycin
	<i>Bacillus subtilis</i>	4.2	2.6	3.3
	<i>Streptococcus mutans</i>	3.0	3.0	2.8
	<i>Escherichia coli</i>	3.5	4.4	4.6
	<i>Klebsiella pneumoniae</i>	2.6	R	3.0
	<i>Staphylococcus aureus</i>	R	R	R

R-Resistant

Table 1 clearly indicates that the antibacterial activities of all the extracts using various solvents showed optimistic results. Acetone extract of *Oscillatoria boryana* on *E.coli* and the diethyl ether extract of *O.laetrivirans* on *B.subtilis* showed higher zone of inhibition, where as *K.pneumoniae* and *S.mutans* showed considerable antibacterial activity. The antimicrobial action of the cyanobacterial extract against the standard are shown in Table 2. It was inferred from both the tables that the cyanobacterial extract were comparatively effective.

CONCLUSION

The antibacterial activity with cyanobacteria using various solvents were done. The cyanobacterial extracts show considerable antibacterial effect on the organisms and they are

effective than the present contemporary antibiotics.

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