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Research Article

ANTIMICROBIAL ACTIVITY OF SOME CYANOBACTERIA

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ABSTRACT

Fifteen cyanobacteria belonging to *Anabaena*, *Nostoc*, *Scytonema* and *Microcystis* species isolated from various aquatic and terrestrial habitats were screened for antimicrobial activity. A disc diffusion assay was used to detect the antimicrobial activity of crude hydrophilic and lipophilic extracts as well as culture supernatants of cyanobacteria on solid agar medium. Methanolic extracts of *Anabaena* BT2, *Nostoc* Brf02, *Nostoc* Brf04 and *Scytonema* br1 at a concentration of 500 µg/disc showed prominent zone of inhibition against two Gram-negative bacteria (*Pseudomonas* sb1 and sb3). In liquid cultures addition of 500 µg/ml crude extracts produced 72-78 % growth inhibition of test bacteria. Out of all the cyanobacteria tested only the methanolic extract of *Microcystis* and chloroform fraction of *Scytonema* br1 showed significant anticyanobacterial activity against *Anabaena* BT2 and *Nostoc* pbr01 and antialgal activity against a green alga *Bracteacoccus*. In liquid cultures a concentration of 300 µg/ml of these extracts produced 73-85 % growth inhibition of the test organisms. None of the culture filtrates showed any antimicrobial activity.

Keywords: Crude extract, Antibacterial and Antialgal activity, Cyanobacteria, Zone of inhibition

INTRODUCTION

Cyanobacteria are a group of prokaryotes that originated 3.5 billion years ago and are known to occur in diverse habitats such as rice paddy fields, polluted waters of lakes, ponds and water reservoirs, hypersaline lakes, tree barks, rocks, stones, deserts, thermal springs etc. In the process of adaptation to these versatile ecological niches, they have developed certain mechanisms to produce a wide variety of secondary metabolites. The secondary metabolites produced by cyanobacteria are of considerable significance because of their unique structural features and biological activities. Screening of cyanobacteria and algae for antibiotics and other pharmacologically active compounds has received considerable attention during the past few decades[1-6].

Extensive screening programs from cyanobacterial biomass or from the laboratory cultures have led to the discovery of novel compounds with antimicrobial, antineoplastic and cytotoxic activities[7-12]. Kreitlow et al[13] investigated the hydrophilic and lipophilic extracts of cyanobacterial strains from fresh and brackish water, and two waterblooms, from the Baltic Sea for their antibiotic activities. Ghasemi et al[14] have surveyed antibacterial activities of microalgae of Northern Iran and identified a novel antimicrobial substance named parsiguine[15]. Production of antimicrobial active substance under various growth conditions from the cyanobacterium *Nostoc muscorum* and purification of the active components and elucidation of its chemical structure was done by El-Sheekh and coworkers[16]. Other workers have also confirmed the antimicrobial and cytotoxic activity of cyanobacterial strains[4,17].

Present study was undertaken with a view to study the antimicrobial activity of cyanobacteria isolated from diverse habitats. Crude methanolic and chloroform fractions from sixteen cyanobacterial strains were tested for their antibacterial and antialgal activity both on solid agar medium and liquid cultures.

MATERIALS AND METHODS

Organisms and growth conditions

Cyanobacterial genera (Fig. 1) collected from diverse habitats (Table 1) were isolated and made unialgal employing standard microbiological techniques. *Anabaena, Nostoc and Scytonema* were routinely grown in nitrogen-free BG-11 medium[18] whereas for growing *Oscillatoria* nitrate containing Parker's medium was used. All algal cultures were grown in a culture room maintained at 25±2^0 C and illuminated with white cool fluorescent light at an intensity of 14.4 Wm^{-2} for a 14/10 h light/dark cycle. The purity of the algal isolates was routinely checked by streaking on nutrient broth medium and microscopic observations. Fresh bloom samples comprising *Microcystis aeruginosa* were collected from a local pond and used for the preparation of crude extract. Tentative identification of the algal strains was done using morphological characteristics according to Desikachary[19]. Antialgal activity was tested against two cyanobacterial species *Anabaena* BT2 and *Nostoc* Pbr01 and a unicellular green alga *Bracteacoccus* sp. (an isolate from pond water and grown in nitrate containing BG-11 medium).

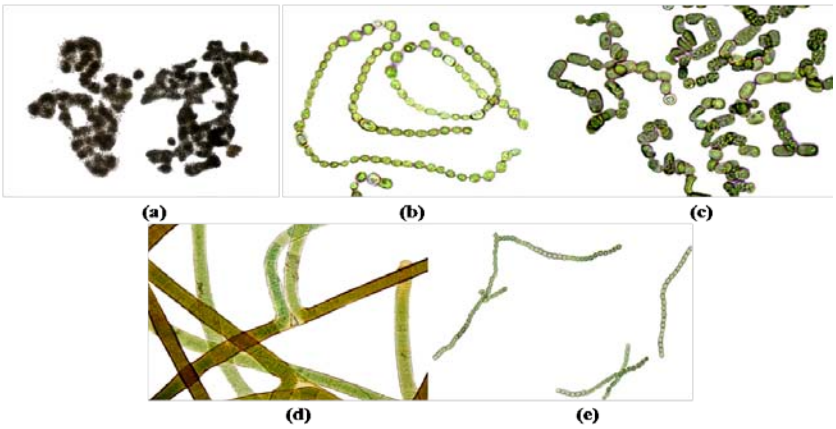

Fig. 1: Photomicrographs of cyanobacteria (a) *Microcystis* sp. (b) *Nostoc* Brf04 (c) *Nostoc* Brf02 (d) *Scytonema* Br1 and (e) *Anabaena* BT2.

Test of antibacterial activity was performed using pure cultures of two *Pseudomonas* strains (sb1 and sb3) and a *Bacillus* sp. isolated from local soil. These were regularly subcultured and maintained in nutrient broth medium in a bacteriological incubator at 37 ^{0}C.

Preparation of crude extracts

For the preparation of crude extracts 4-6 weeks old cyanobacterial cultures or fresh bloom samples were used. Cyanobacterial cells (5-10 g fresh weight) were harvested by centrifugation at 8000 rpm and were collected and weighed on pre-weighed dry filter paper whereas aqueous supernatant was collected, dried and stored at 4 ^{0}C. In general, extraction was carried out in 50 % methanol by sonicating cyanobacterial cells for 5 min at 8 output and 60 % duty cycle at 4 ^{0}C. Sonicated suspension was stirred for 12 h at room temperature. After centrifugation at 10,000 rpm for 5 min the resulting pellet was re-extracted 2-3 times. The supernatants were mixed and further partitioning with chloroform resulted in an aqueous methanol fraction and a chloroform fraction. The separated fractions were evaporated to dryness, weighed and stored at 4^{0}C till further use or dissolved in minimal volume of 1 % methanolic distilled water or chloroform and desired concentration of crude extract was prepared for further use. Likewise, culture supernatant were dried and dissolved in 1 % methanolic distilled water and used.

Table 1: Collection sites and source of cyanobacteria

Organism	Site of collection
Anabaena Brf01	Rice-field (Buxar)
Anabaena Brf02	Rice-field (Buxar)
Anabaena Brf03	Rice-field (Buxar)
Anabaena BT2	Rice-Field (BHU, Varanasi)
Nostoc Pbr01	Bare rocks (Pachmani)
Nostoc Brf02	Rice-field (Buxar)
Nostoc Pbr03	Bare rocks (Pachmani)
Nostoc Brf04	Rice-Field (Buxar)
Nostoc Pbr05	Bare rocks (Pachmani)
Nostoc Kpw06	Polluted water (Kanpur)
Nostoc Kpw07	Polluted water (Kanpur)
Nostoc Ktw08	Polluted water (Kanpur)
Nostoc brown	Rice-Field (BHU, Varanasi)
Oscillatoria sp.	Pond (Laatbhairav, Varanasi)
Microcystis aeruginosa	Pond (Laxmi Kund, Varanasi)
Scytonema Br1	Wall & Terrace (Konark temple, Puri)

Antimicrobial bioassay

Antibacterial activity of the crude extract on solid agar medium was tested by agar disc diffusion method using 5 mm diameter filter paper discs. Agar was inoculated with a standardized quantity of the suspension of the test organisms. Filter paper discs were loaded with known amount of extract to get a concentration of 100, 300, 500 and 700 µg/disc. Control discs received only the solvent. Discs were allowed to remain at room temperature until complete solvent evaporation and then placed on the seeded agar plates. The inhibition zone (from periphery of disc to periphery of zone) was measured after 24 h incubation at 37 ºC. Same procedure was followed for testing the antialgal activity and appearance of zone of inhibition if any was observed after two weeks of growth. For testing antialgal activity of the extracts in liquid medium exponentially growing cultures were inoculated into culture flasks containing 40 ml medium to give a whole cell optical density of 0.06-0.09 at 663 nm. Thereafter, known amount of the filter-sterilized extract was added to each flask. Control flasks received only the solvent. Growth was measured by extracting chlorophyll *a* at 3 d intervals lasting upto 15 days. Antibacterial activity in liquid medium was determined in the same way as for antialgal assay. Growth Inhibition of bacteria was determined by measuring O.D. of culture at 600 nm at 4 h intervals upto 24 h. All tests were performed under sterile conditions in triplicate and repeated three times.

RESULTS AND DISCUSSION

In the present investigation 16 cyanobacterial strains belonging to *Anabaena, Nostoc, Microcystis, Oscillatoria and Scytonema* have been screened for their antialgal and antibacterial activities (Table 2).

Table 2: Antimicrobial activity of crude extracts of cyanobacteria

Organism	Nature of the extract	Zone of inhibition (mm)[a]					
		Antibacterial activity[b] against			Antialgal activity[b] against		
		Pseudomonas sb 1	*Pseudomonas* sb3	*Bacillus*	*Anabaena* BT2	*Nostoc* Pbr01	*Bracteacoccus* sp.
Nostoc Brf02	Methanol	7.33±0.33	8.67±0.89	-	-	-	-
Nostoc Brf04	Methanol	6.67±0.88	-	4.0±0.57	-	-	-
Scytonema Br1	Methanol	5.0±0.58	-	-	-	-	-
Anabaena BT2	Methanol	9.67±0.57	7.63±0.33	-	-	-	-
Microcystis bloom	Methanol	-	-	-	6.0±0.58	7.0±0.57	5.67±0.33
Scytonema Br1	Chloroform Fraction	-	-	-	10.67±0.67	3.67±0.33	7.33±0.33

[a]Values are mean ± Standard Error.

[b]Concentration used for antibacterial and antialgal activity was 500 µg/disc and 300 µg/disc respectively.

In order to do this methanol and chloroform fractions of these cyanobacteria have been tested against three bacterial strains belonging to *Pseudomonas* (sb1, sb3) and *Bacillus* whereas for testing antialgal activity two cyanobacteria namely *Anabaena* BT2 and *Nostoc* sp. and a unicellular green alga *Bracteacoccus* sp. have been employed as test organisms. Among the cyanobacterial species tested antibacterial activity was found to be associated only with methanolic extract of four strains viz., *Nostoc* Brf02, *Nostoc* Brf04, *Scytonema* Br1 and *Anabaena* BT2 (Fig. 1, Table 2). Maximum size of inhibition zone (9.67 mm) was observed with 500 µg/disc concentration of methanol extract of *Anabaena* BT2 whereas smallest zone of inhibition (4.0 mm) occurred with *Nostoc* Brf04

extract against *Bacillus* sp. (Table 2). In all these cases maximum zone of inhibition was observed at a concentration of 500 µg/disc and no further increase in the size of the zone occurred upon further increasing the concentration upto 700 µg/disc. Antibacterial activity of these four species against *Pseudomonas* strains was also evident in liquid cultures at 300 µg/ml and 500 µg/ml concentrations (Fig. 2).

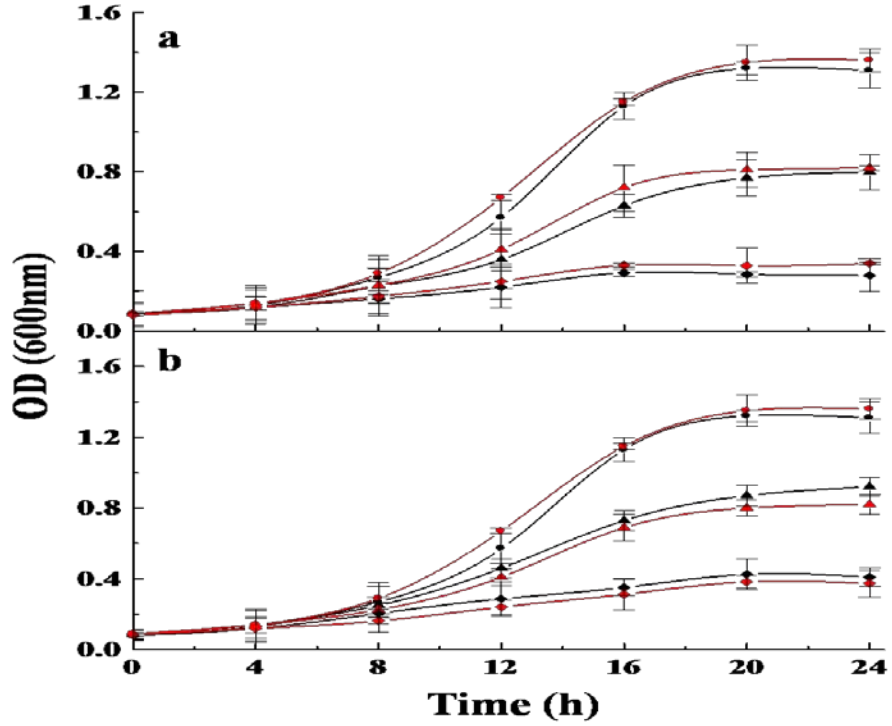

Fig. 2: Effects of varying concentrations of crude extract of *Anabaena* BT2 (a) and *Nostoc* Brf02 (b) on growth of *Pseudomonas* sb1 (–) and *Pseudomonas* sb3 (–)(●) control, (▲) 300 µg/ml and (◆) 500 µg/ml.

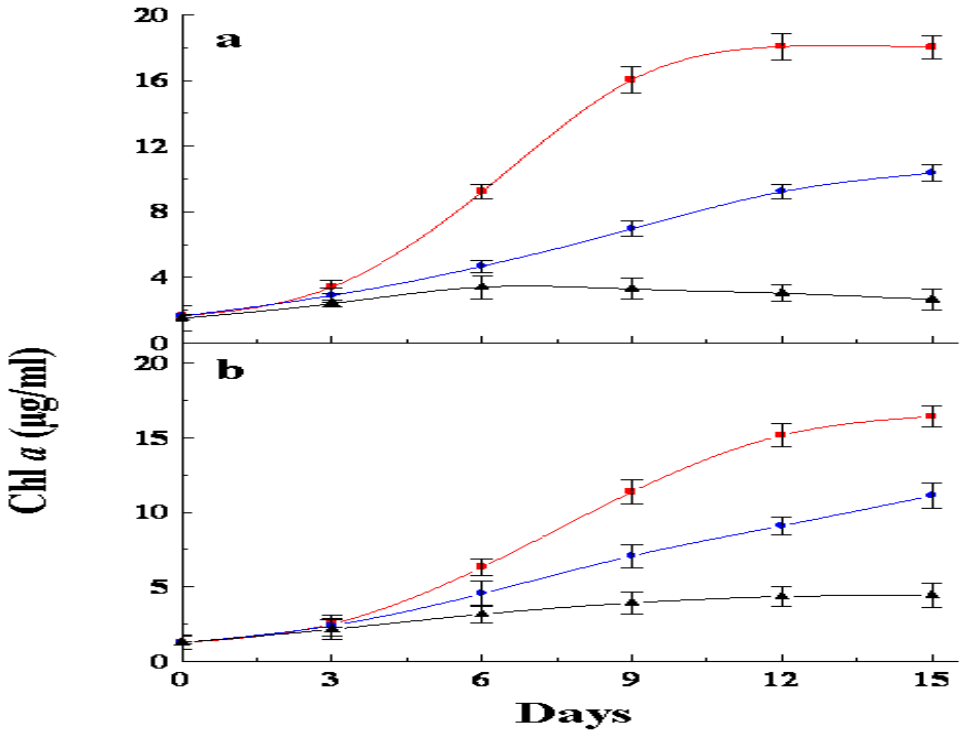

Fig. 3: Effect of varying concentrations of crude extract of *Microcystis* sp. on growth of *Bracteacoccus* (a) and *Anabaena* BT2 (b) in liquid culture. ■—■control; ●—●100 µg/ml; ▲—▲ 300 µg/ml.

A 30-40 % and 72-78 % growth inhibition was observed at 300 µg/ml and 500 µg/ml concentrations respectively (Figs. 2a and 2b). Our results of antibacterial activity of cyanobacterial extract are similar to those of Østensvik et al[20], Ghasemi et al[14] and Ghosh et al[21]. Soltani et al[22] used solvents with different polarities for the extraction of bioactive compounds from soil cyanobacteria isolated from the paddy fields of Iran. They also evaluated the antimicrobial activity of these extracts and found maximum inhibition with methanol extract. Kaushik and coworkers[23] also found highest effective zone of inhibition by methanol extract of *Anabaena variabilis* against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Other investigators have also showed the production of antimicrobial substances by cyanobacteria[2,3,17].

Out of all the strains tested, antialgal activity was found to be associated only with the methanol extract of *Microcystis* bloom and chloroform fraction of the *Scytonema* Br1. Paper discs loaded with 100, 300 and 500 µg crude extracts/disc produced the zone of inhibition against the cyanobacterial test organisms *Anabaena* BT2, *Nostoc* Pbr01 and the green alga *Bracteacoccus* (Table 2).

Maximum size of zone of inhibition (10.67 mm) was observed by chloroform fraction of *Scytonema* Br1 against *Anabaena* BT2 whereas methanolic extract of *Microcystis* also inhibited the growth of test organisms. In liquid cultures methanol extract of *Microcystis* at a concentration of 300 µg/ml produced 85 % and 73 % growth inhibition of *Bracteacoccus* and *Anabaena* BT2 respectively in compare to control after 15 days (Fig. 3).

Similarly chloroform fraction of *Scytonema* Br1 was found to exhibit 82 % and 77 % growth inhibition of *Bracteacoccus* and *Anabaena* BT2 respectively at 300 µg/ml concentration (Fig. 4). We could not observe any antibacterial or antialgal activity from the culture filtrates of any of the cyanobacteria tested. Several workers have demonstrated the growth inhibitory effect of whole cells or crude extracts of cyanobacteria on photosynthetic microorganisms[24-26]. A chlorine-containing allelochemical named cyanobacterin, and a closely related chemical hydroxycyanobacterin isolated from *Scytonema hofmanni* inhibited the growth of eukaryotic algae and other cyanobacteria[25]. The ability to produce compounds with antimicrobial activity probably helps the organism to compete with other organisms for survival.

The results presented in this paper are only based on crude extracts and did not specify any defined antibacterial or antialgal substance. Nevertheless our results prove that terrestrial and freshwater cyanobacteria are a promising source of new bioactive natural products and there is a need to carry out extensive studies to identify the promising strains. A large scale production to obtain sufficient cyanobacterial biomass is the precondition for the isolation and characterization of the biologically active compounds. Further studies on fractionation, separation and preliminary characterization of active compound are in progress.

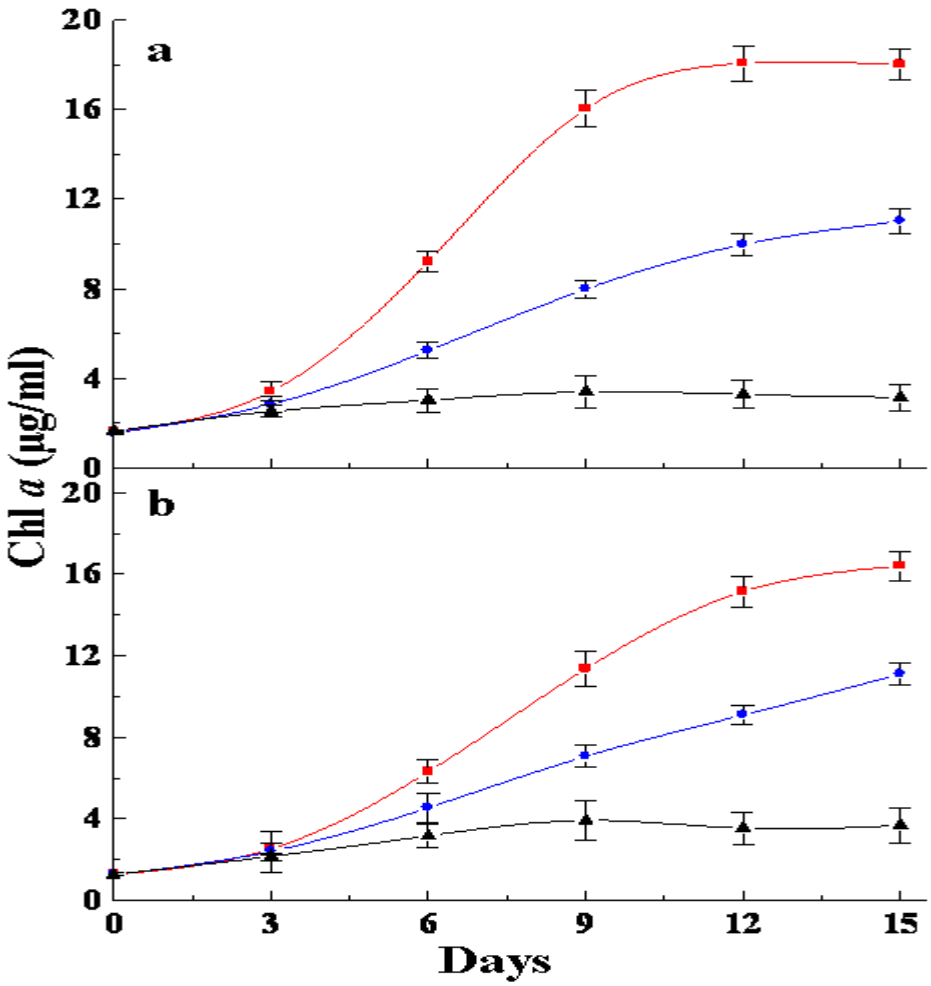

Fig. 4: Effect of varying concentrations of crude extract of *Scytonema* Br1 on growth of *Bracteacoccus* (a) and *Anabaena* BT2 (b) in liquid culture. control; 100µg/ml; 300 µg/ml.

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