

Screening of terrestrial and freshwater halotolerant cyanobacteria for antifungal activities

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Abstract Cyanobacterial cultures tolerating 200 mmol l⁻¹ sodium chloride isolated from terrestrial and freshwater habitats of North Maharashtra region of India were evaluated for antifungal activity. Aqueous, methanol, *n*-propanol, and petroleum ether extracts of 40 cyanobacterial isolates belonging to nine genera were examined for inhibitory activity against five fungal pathogens. Eighteen isolates belonging to genus *Oscillatoria* dominated the population of halotolerant cyanobacterial cultures. Four antifungal bioassays viz. double layer agar method, disc diffusion assay, silica gel method, and minimum inhibitory concentration (MIC) were used to screen the cultures for antifungal activity. Among the solvents used, methanol extracts showed 34.9% inhibition followed by *n*-propanol, petroleum ether, and water exhibiting 30.2%, 18.6% and 16.2% inhibition, respectively. The double agar layer method was found to be a suitable method in preliminary screening for handling large number of cultures without extraction of compounds. However, in later screening experiments, silica gel method was seen to be advantageous over MIC and agar disc diffusion methods.

Keywords Antifungal activity · Halotolerant cyanobacteria · Screening · Solvent extraction

Introduction

Cyanobacteria belong to the prokaryotic photosynthetic group of organisms, with many species possessing nitrogen

fixation ability. Cyanobacteria growing under extreme environments offer the opportunity of growing and harvesting them in open outdoor ecosystems. Out of these, the halotolerant cyanobacteria being primary producers, proved to be useful for amelioration of salt affected soils (Kaushik 2007). Cyanobacteria have also been searched for other biotechnological potentials including pharmaceutically and agriculturally important bioactive compounds (Skulberg 2000; Soltani et al. 2005). In general, isolation of bioactive compounds from cyanobacteria is done with two objectives: (i) to discover new compounds and (ii) to understand the interactions of individual organisms within their natural communities (Schlegel et al. 1999).

Several species of cyanobacteria produce substances with antibiotic activity. They include *Tychonema bourrellyi*, *Aphanizomenon flos-aquae*, *Cylindrospermopsis* (Østensvik et al. 1998), *Anabaena*, *Calothrix*, *Leptolyngbya*, *Lyngbya*, *Microcystis*, *Nostoc*, *Phormidium*, *Pseudoanabaena*, *Synechococcus*, *Synechocystis*, *Tolypothrix* (Ördög et al. 2004). In spite of the studies carried out so far, many cyanobacterial compounds are still largely unexplored, thus giving a rich opportunity for discovery of new bioactive compounds. The expected rate of rediscovery is far lower than that for other better-studied group of organisms (Olaizola 2003). Cyanobacteria produce a wide variety of toxins and other bioactive compounds including 40% lipopeptides, 5.6% amino acids, 4.2% fatty acids, 4.2% macrolides, and 9% amides (Singh et al. 2005).

Numerous screening programs have revealed the potential of cyanobacteria in the production of novel antimicrobial compounds (Schlegel et al. 1999; Mian et al. 2003; Ghazala and Shameel 2005; Soltani et al. 2005). Antifungal effects from aqueous and organic solvent extracts of cyanobacteria have been reported in bioassays comprising selected fungal test organisms (Mian et al.

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2003; Ördög et al. 2004; Ghazala and Shameel 2005; Soltani et al. 2005). Numerous bioassays have been reported for screening of antimicrobial compounds, which include minimum inhibitory concentration (MIC) broth assay (Østensvik et al. 1998; Ördög et al. 2004), agar disc diffusion method (Soltani et al. 2005), agar well method (Ördög et al. 2004), double layer agar method (Schlegel et al. 1999), and silica gel plates (Mian et al. 2003; Quiroga et al. 2004; Rahmani et al. 2004).

The aim of the current venture was to investigate the antifungal activities of halotolerant cyanobacterial strains isolated from terrestrial and freshwater habitats of Maharashtra state, India. This paper reports the comparative evaluation of four methods of bioassay for visualizing antifungal activity.

Materials and methods

Cultivation of halotolerant cyanobacteria

Halotolerant cyanobacterial cultures were isolated from diverse habitats such as saline soils (sandy loam and black cotton soils), fresh waters (water springs and stagnant water bodies), paddy fields, and an alkaline lake (Lonar lake) of North Maharashtra region of India. Cyanobacterial cultures were isolated by enriching water or soil samples in BG11/Gerloff medium (Kaushik 1987) amended with 200 mmol l⁻¹ sodium chloride. Axenic cultures of filamentous cyanobacteria were obtained by repeated liquid transfer of small amounts of material followed by antibiotic treatment (streptomycin, chloramphenicol, and penicillin (10 mg ml⁻¹) (Kaushik 1987). Unicellular cultures were purified by successive transfer from liquid to solid media. Isolates were grown axenically in 100 ml of BG11 or Gerloff's medium containing 200 mmol l⁻¹ sodium chloride at pH 7.8 and 24°C temperature in 500 ml Erlenmeyer flasks under continuous illumination at 2000 lux.

Preparation of culture extracts

Cyanobacterial biomass was harvested after 10 and 25 days of incubation for unicellular and filamentous cultures, respectively. Biomass was separated by centrifugation of 100 ml culture broth at 5000g for 15 min (Sorvall, USA, Model RC2). Water extracts were made by resuspending cyanobacterial biomass in distilled water (10 mg ml⁻¹) and ultrasonication at 20 KHz frequency (Sonics and Materials Inc., USA) for 2 min. The biomass was also extracted simultaneously with organic solvents viz. methanol, *n*-propanol, and petroleum ether. The cell mass was

separated by centrifugation at 5000g for 20 min and the extraction procedure was repeated thrice. The pooled supernatants were dried at 40°C under reduced pressure in a rotary evaporator (Büchi Rotavapor R.214, Switzerland). The dried extracts were resuspended in 3 ml of each solvent and preserved at 4°C till further use in antifungal assays.

Isolation of test cultures

Five phytopathogenic fungal cultures viz. *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum musae*, *Fusarium oxysporum*, and *Paecilomyces lilacinus* isolated from diseased plants and seeds were used in the study. Identification of these cultures was carried out from Agharkar Research Institute, Pune, India. Test cultures were grown on 50 ml Sabouraud's dextrose agar of composition (in g l⁻¹) peptone, 10, dextrose, 20, and agar, 15 at pH 5.6 for sporulation in 500 ml Erlenmeyer flasks. Spore suspension was prepared by pouring 5 ml sterile 0.01% triton X-100 solution, vortexing for 1 min and sieving through cheesecloth. Final inoculum density of 10⁶ spores ml⁻¹ was calibrated using a hemocytometer.

Screening of isolates for antifungal activity

Double layer agar method

Four to six cyanobacterial cultures were spot inoculated on Petri plates containing 15 ml growth medium (either BG11 or Gerloff's medium) solidified with 1.5% bacteriological agar and incubated for 3–5 days at 24 ± 3°C under illumination of light (2000 lux). The spot grown cultures were overlaid with a 10 ml of double strength Sabouraud's dextrose medium containing 1% agar and 0.1 ml spore suspension containing 10⁶ spores ml⁻¹ of fungal test cultures. The plates were incubated under dark for 3 days at 30°C. Antifungal activity was assessed by measuring the diameter of clearance zone around the cyanobacterial colony (Schlegel et al. 1999).

Disc diffusion assay

Whatman No.1 paper discs (6 mm) were saturated with 50 µl of cyanobacterial extracts, dried, and placed on Sabouraud's dextrose agar plates, prior inoculated with 0.1 ml fungal spore suspension (1 × 10⁶ spores ml⁻¹). Antifungal activity was assessed by measuring the diameter of growth inhibition zone after incubation at 30°C for 48 h.

Silica gel method

Antifungal activities of selected cyanobacterial extracts were checked using silica gel plate. Slurry of silica gel (S.D. Fine Chemicals, Mumbai, India) prepared in water was spread as a thin layer in the base of Petri dish (9" × 9"). The plates were activated at 90°C for 1 h. Cyanobacterial extracts were spot inoculated on the silica gel plate. Fungal spore suspensions (1×10^6 spores ml⁻¹) prepared in sterile Sabouraud's broth were spread over the silica gel using glass spreader and plates were incubated under moist conditions at 30°C for 3 days. Antifungal activity was measured as diameter of inhibition zones on the silica gel plate.

Minimum inhibitory concentration

The MIC was determined by inoculating 9 ml of Sabouraud's broth with 1 ml of various dilutions of cyanobacterial extracts and 0.05 ml of spore suspension (1×10^6 spores ml⁻¹) of fungal cultures. Set of tubes was incubated at 30°C for 3 days. Fungal growth in tubes was assessed visually. The MIC was determined as the lowest concentration of cyanobacterial extract resulting in complete inhibition of fungal growth in tubes after 3 days of incubation.

Filter sterilized fluconazole (Zuventus Healthcare Ltd, Mumbai, India), considered to be the standard antifungal antibiotic was used as the positive control at varying concentrations (50 to 500 µg ml⁻¹) in disc diffusion, silica gel, and MIC assays.

Results and discussion

Numerous studies have shown that cyanobacteria produce substances with antifungal activities, thereby indicating a high commercial potential of these primitive prokaryotes (Mian et al. 2003). Numerous studies report the screening of cyanobacteria for variety of antimicrobial activities (Skulberg 2000; Soltani et al. 2005; Volk 2005). The present study is an attempt to screen halotolerant cyanobacterial cultures isolated from terrestrial and freshwater habitats.

The cyanobacterial isolates were identified on the basis of morphological traits according to Desikachary (1959) and Castenholz and Waterbury (1989). Distribution of isolates in genera of cyanophyta is given in Table 1. Forty strains belonging to nine genera were isolated by culturing axenically either in BG.11 or Gerloff's medium containing 200 mmol l⁻¹ sodium chloride. Eighteen isolates belonging to genus *Oscillatoria* dominated the population of

Table 1 Halotolerant cyanobacterial isolates investigated in preliminary screening for antifungal activity

Genus	Number of isolates
<i>Aphanocapsa</i>	1
<i>Synechocystis</i>	2
<i>Synechococcus</i>	3
<i>Phormidium</i>	3
<i>Oscillatoria</i>	18
<i>Trichodesmium</i>	1
<i>Lyngbya</i>	5
<i>Nostoc</i>	4
<i>Anabaena</i>	3
Total	40

halotolerant cyanobacterial cultures. Genera *Aphanocapsa*, *Trichodesmium*, and *Synechocystis* were among the minority.

Screening by double layer agar method

Primary screening of isolates for antifungal activities was performed using double layer agar method (Fig. 1a). Table 2 shows the antifungal activities of cyanobacterial isolates against five fungal pathogens viz. *A. niger*, *A. flavus*, *C. musae*, *P. lilacinus*, and *F. oxysporum*. Among the cyanobacterial cultures tested, five isolates could inhibit growth of *A. flavus* while 12 isolates could inhibit growth of *C. musae*. Twenty cyanobacterial cultures appeared to possess inhibitory activity against *F. oxysporum*. From a set of 40 cultures tested, 20 isolates showed no activity against all of the fungal test cultures. Hyper-antifungal activity (>21 mm zone of diameter) was seen in case of three isolates. Based on the performance of cultures in agar double layer diffusion method, 10 cultures exhibiting zone of inhibition >15 mm were selected for further screening.

Screening by disc diffusion assay

Antifungal activities of selected ten cyanobacterial isolates extracted with methanol, *n*-propanol, and petroleum ether along with aqueous extracts were tested using disc diffusion method (Table 3). In all, 86 inhibitions were noted for 200 combinations of 10 cyanobacterial cultures, four extraction solvents, and five fungal test cultures. Among the solvents used, methanol extracts showed 34.9% inhibition followed by *n*-propanol, petroleum ether, and water extracts exhibiting 30.2, 18.6, and 16.2% inhibition, respectively. Out of five fungal test cultures, 12.8%

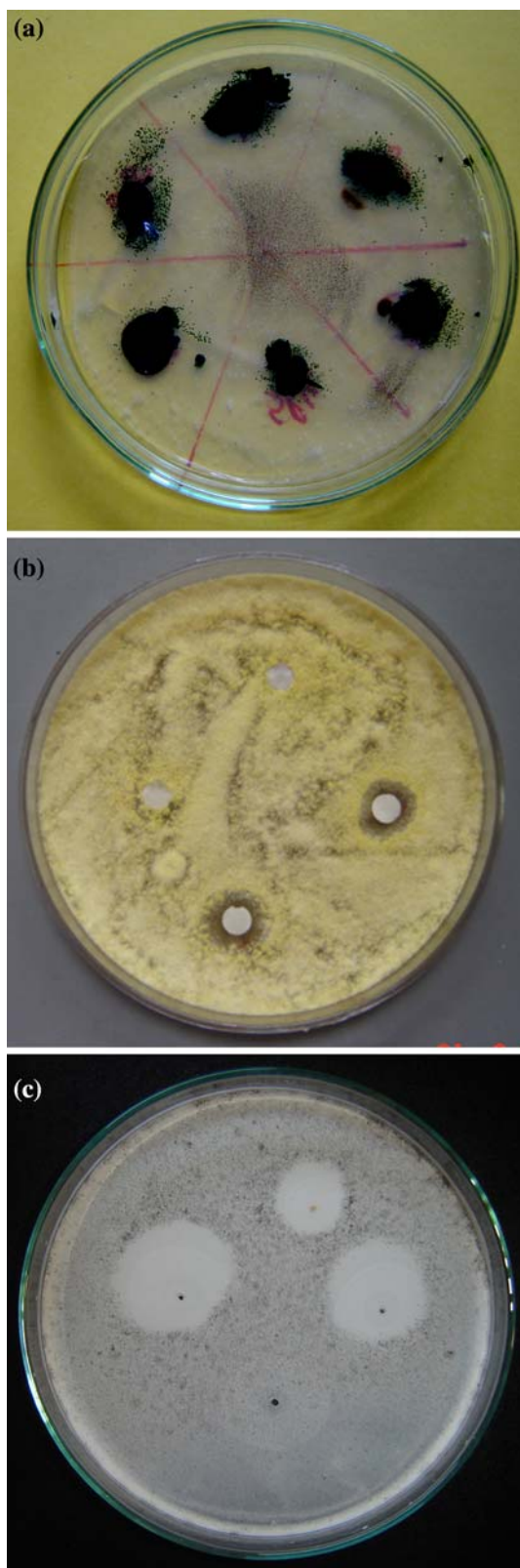


Fig. 1 Demonstration of the antifungal effect of *Oscillatoria limosa* NMU-31 against *Aspergillus flavus* in three different bioassay methods, (a) Double layer agar method; (b) Disc diffusion assay; and (c) Silica gel method

Table 2 Distribution of cyanobacterial cultures on the basis of antifungal activity against test organisms in double layer agar method

Test organism	Number of cyanobacterial isolates inhibiting growth of test organism					
	No zone	2–6 mm	7–10 mm	11–14 mm	15–20 mm	>21 mm
<i>Aspergillus niger</i>	27	1	4	3	3	2
<i>Aspergillus flavus</i>	35	2	0	0	2	1
<i>Colletotrichum musae</i>	30	5	5	5	2	0
<i>Paecilomyces lilacinus</i>	23	5	5	5	2	0
<i>Fusarium oxysporum</i>	20	3	8	9	0	0

inhibition was noted for *A. flavus* and a maximum of 24.4% inhibition was observed for *C. musae*. Highest number of inhibitions i.e. 14, was recorded for *Synechocystis* sp. NMU-17, whereas, lowest number of inhibitions i.e. 4 could be seen for *Nostoc commune* NMU-73 and *Anaebaena orientalis* NMU-79. A maximum zone of inhibition of 28 mm diameter was obtained for methanol extract of *Oscillatoria limosa* NMU-31 (Fig. 1b). Marginal antifungal activities (<10 mm diameter of inhibition zone) were obtained in case of 26 test combinations (Table 3).

Antifungal activities of cyanobacterial extracts were found to differ with the type of solvent used for extraction. Methanol, *n*-propanol, petroleum ether, ethanol, chloroform, and water were employed for the extraction antimicrobial compounds from cyanobacteria (Soltani et al. 2005). Maximum antifungal activities in case of methanol extraction as observed in the present study are in accordance with earlier reports (Østensvik et al. 1998; Soltani et al. 2005). Decreased activities obtained for aqueous and petroleum ether extracts may be due to the different polarities of antifungal substances.

Screening by silica gel method

Performance of disc diffusion method and silica gel method were compared using varying concentrations of methanol extracts of *O. limosa* NMU-31 and *Phormidium tenue* NMU-55 against test cultures, *A. niger* (Fig. 2) and *F. oxysporum* (Fig. 3). In both these methods, the diameter of inhibition zones was found to be increased with increasing concentrations of cell extracts. The regression coefficient of >0.89 were observed for both disc diffusion and silica gel methods. The slopes of regression lines were 0.24 and 0.34 for disc diffusion method for extracts of

Table 3 Activity of cyanobacterial extracts against fungal test cultures by disc diffusion method

Isolate	Extract	Diameter of inhibition zone (mm)				
		A	B	C	D	E
NMU-31, <i>Oscillatoria limosa</i>	Aqueous	8	– ^a	9	–	–
	Methanol	12	8	13	14	28
	<i>n</i> -propanol	14	–	10	16	12
	Petroleum ether	–	–	–	–	–
NMU-29, <i>Oscillatoria amphibia</i>	Aqueous	–	12	18	–	–
	Methanol	–	–	–	–	12
	<i>n</i> -propanol	10	9	14	–	13
NMU-64, <i>Oscillatoria ornata</i>	Aqueous	–	–	–	–	–
	Methanol	14	12	–	18	14
	<i>n</i> -propanol	–	–	12	–	12
NMU-17, <i>Synechocystis</i> sp.	Aqueous	10	8	12	14	–
	Methanol	16	15	13	26	12
	<i>n</i> -propanol	13	14	8	20	17
NMU-55, <i>Phormidium tenue</i>	Aqueous	14	–	16	–	8
	Methanol	18	–	–	11	14
	<i>n</i> -propanol	–	–	9	–	8
NMU-18, <i>Synechococcus elongates</i>	Aqueous	–	–	12	–	–
	Methanol	14	–	18	–	12
	<i>n</i> -propanol	12	11	–	15	–
NMU-70, <i>Lyngbya lutea</i>	Aqueous	8	–	–	–	–
	Methanol	14	–	12	–	10
	<i>n</i> -propanol	–	8	–	–	12
NMU-62, <i>Trichodesmium hildebrandtii</i>	Aqueous	–	–	8	–	–
	Methanol	8	–	14	–	10
	<i>n</i> -propanol	9	8	10	8	–
NMU-73, <i>Nostoc commune</i>	Aqueous	–	–	–	–	–
	Methanol	12	–	–	15	–
	<i>n</i> -propanol	–	–	–	–	–
NMU-79, <i>Anabaena orientalis</i>	Aqueous	–	–	–	–	–
	Methanol	–	–	–	8	7
	<i>n</i> -propanol	–	–	–	–	–
Control, fluconazole (50 µg disc ⁻¹)	Methanol	22	16	20	26	35

A, *Aspergillus niger*; B, *Aspergillus flavus*; C, *Colletotrichum musae*; D, *Paecilomyces lilacinus*; E, *Fusarium oxysporum*

^a No inhibition

O. limosa NMU-31 and *P. tenue* NMU-55, respectively, while with silica gel method they were 0.56 and 0.89. Increase in inhibition zone was more proportionate with concentration of cell extract in silica gel method in comparison with disc diffusion method (Fig. 1b and c).

The comparative assessment of disc diffusion and silica gel method of antifungal assays showed that silica gel method was a more appropriate method of the two. Elimination of solvent effect in bioassays was the main advantage with silica gel method. The method demands less quantity of bioactive compounds and results in proportionate increase in zone of inhibition. Sharp and clear

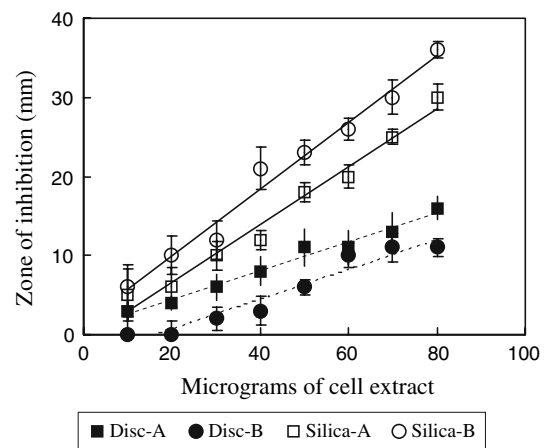


Fig. 2 Dose–response relationship of methanol extract (A), *Oscillatoria limosa* NMU-31 (■,□) and (B), *Phormidium tenue* NMU-55 (●,○) against test culture, *Aspergillus niger* in the antifungal bioassay performed in disc diffusion method (■,●) and silica gel method (□,○)

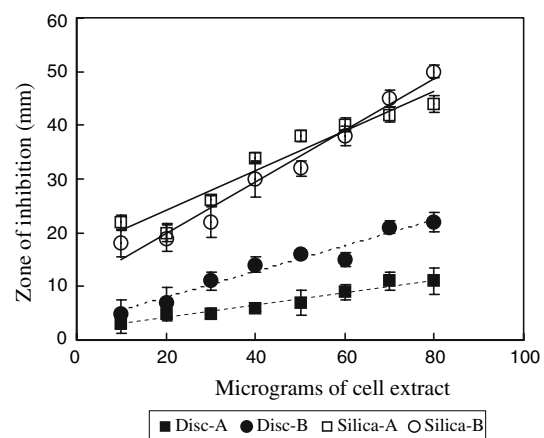


Fig. 3 Dose–response relationship of methanol extract (A), *Oscillatoria limosa* NMU-31 (■,□) and (B), *Phormidium tenue* NMU-55 (●,○) against test culture, *Fusarium oxysporum* in the antifungal bioassay performed in disc diffusion method (■,●) and silica gel method (□,○)

Table 4 Minimum inhibitory concentrations of the cyanobacterial extracts prepared in methanol

Isolate	Minimum inhibitory concentration (MIC) ($\mu\text{g ml}^{-1}$)				
	A	B	C	D	E
NMU-31, <i>Oscillatoria limosa</i>	259.20	324.00	194.40	194.40	64.80
NMU-64, <i>Oscillatoria ornata</i>	147.84	184.80	258.72	73.92	147.84
NMU-55, <i>Phormidium tenue</i>	58.88	353.28	412.16	176.64	117.76
NMU-18, <i>Synechococcus elongatus</i>	189.96	379.92	126.64	379.92	253.28
NMU-62, <i>Trichodesmium hildebrandtii</i>	225.00	315.00	90.00	270.00	180.00
Control, fluconazole	80.00	80.00	120.00	140.00	120.00

A, *Aspergillus niger*; B, *Aspergillus flavus*; C, *Colletotrichum musae*; D, *Paecilomyces lilacinus*; E, *Fusarium oxysporum*

zone of inhibitions could not be seen with agar diffusion methods due to increased diffusion of bioactive compounds with incubation time, whereas, in silica gel method, the extent of diffusion and dilution could be reduced and sharper zones were obtained.

Minimum inhibitory concentration

On the basis of antifungal activities obtained in disc diffusion assay, five cyanobacterial cultures were selected for determination of the MICs. Methanol extracts, recording maximum inhibitions (34%) in disc diffusion assay were used in further bioassays. Antifungal activity higher than that obtained for antibiotic fluconazole was observed in case of *P. tenue* NMU-55 against *A. niger*, *Trichodesmium hildebrandtii* NMU-62 against *C. musae*, *Oscillatoria ornata* NMU-64 against *P. lilacinus* and *O. limosa* NMU-31 and *P. tenue* NMU-55 against *F. oxysporum* (Table 4).

Skulberg (2000) has emphasized on exercising imagination and creativity in investigating organisms from untried biotopes and develop new methodologies to exploit them. Different methods reported so far for the screening of microbial cell extracts, cell free broth and plant extracts for antifungal activity mostly rely on disc diffusion, agar well diffusion and broth assays (Østensvik et al. 1998; Mian et al. 2003; Ördög et al. 2004). The double agar layer method reported here has more advantages in screening cyanobacterial cultures. In the present study 10 out of 40 cultures tested could be selected with a positive result. The success of the method lies in the fact that preliminary screening could test a large number of cultures in one step without extraction. Schlegel et al. (1999) reported screening of cyanobacterial isolates for antialgal-cyanobacterial activity using double layer agar technique. In the present study, the proportion of isolates with antifungal activity was between 12–50%. None of the cyanobacterial isolates exhibited inhibition of all test fungal cultures. The variation in antifungal activities could be due to different permeabilities of bioactive substances into the test

organisms. The production of bioactive compounds and expression of antimicrobial activity depends on physiological factors such as stage of growth and culture conditions (Schlegel et al. 1999). Two fold increase in antibiotic production under optimum light conditions have been reported by Chetsumon et al. (1994). Different environmental conditions may result in expression of entirely different suites of biological activities (Robles Centeno and Ballantine 1999). Fungal cultures used in present study are among the phytopathogens causing major damage to agricultural products in North Maharashtra region of India.

Conclusions

The results presented in this paper show that halotolerant cyanobacterial cultures possess promising antifungal activities. The success of screening program was also found to be dependent on appropriate selection of bioassay method. The double agar layer method was a suitable method of preliminary screening for handling large number of cultures without extraction of compounds. However, further screening experiments proved that silica gel method was advantageous over MIC and agar disc diffusion methods.

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References

- Castenholz RW, Waterbury JB (1989) Oxygenic photosynthetic bacteria, group I. Cyanobacteria. In: Staley JT et al (eds) Bergey's manual of systematic bacteriology. Williams and Wilkins Co., Baltimore, pp 1710–1720
- Chetsumon A, Maeda I, Umeda F, Yagi K, Miura Y, Mizoguchi T (1994) Antibiotic production by the immobilized cyanobacterium, *Scytonema* sp. TISTR 8208, in a seaweed-type photoreactor. J Appl Phycol 6:539–543

- Desikachary TV (1959) Cyanophyta. Indian Council of Agricultural Research, New Delhi
- Ghazala B, Shameel M (2005) Phytochemistry and bioactivity of some freshwater green algae from Pakistan. *Pharm Biol* 43:358–369
- Kaushik BD (1987) Laboratory methods for blue-green algae. New Delhi Associated Publishing Company, New Delhi
- Kaushik BD (2007) Cyanobacteria for amelioration of salt affected soils. In: Somani LL, Bhandari SC (eds) Organic recycling and bioinoculants for sustainable crop production. Agrotech Pub Academy, Udaipur, pp 308–323. ISBN 818321066X
- Mian P, Heilmann J, Bürgi H-R, Sticher O (2003) Biological screening of terrestrial and freshwater cyanobacteria for antimicrobial activity, brine shrimp lethality, and cytotoxicity. *Pharm Biol* 41:243–247
- Olaizola M (2003) Commercial development of microalgal biotechnology: from the test tube to the marketplace. *Biomol Eng* 20:459–466
- Ördög V, Stirk WA, Lenobel R, Bancířová M, Strnad M, van Staden J, Szigeti J, Németh L (2004) Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *J Appl Phycol* 16:309–314
- Østensvik Ø, Skulberg OM, Underdal B, Hormazabal V (1998) Antibacterial properties of extracts from selected planktonic freshwater cyanobacteria—a comparative study of bacterial bioassays. *J Appl Microbiol* 84:1117–1124
- Quiroga EN, Sampietro AR, Vattuone MA (2004) In vitro fungitoxic activity of *Larrea divaricata* cav. Extracts. *Lett Appl Microbiol* 39:7–12
- Rahmani M, Ling CY, Meon S, Ismail HBM, Sukari MA (2004) The antifungal activity of *Glycosmis calcicola* and *G. rupestris* extracts. *Pharm Biol* 42:430–433
- Robles Centeno PO, Ballantine DL (1999) Effects of culture conditions on production of antibiotically active metabolites by the marine alga *Spyridia filamentosa* (Ceramiaceae, Rhodophyta). I. Light. *J Appl Phycol* 11:217–224
- Schlegel I, Doan NT, de Chazal N, Smith GD (1999) Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *J Appl Phycol* 10: 471–479
- Singh S, Kate BN, Benerjee UC (2005) Bioactive compounds from cyanobacteria and microalgae: an overview. *Crit Rev Biotechnol* 25:73–95
- Skulberg OM (2000) Microalgae as a source of bioactive molecules—experience from cyanophyte research. *J Appl Phycol* 12:341–348
- Soltani N, Khavari-Nejad RA, Yazdi MT, Shokravi S, Fernández-Valiente E (2005) Screening of soil cyanobacteria for antifungal and antibacterial activity. *Pharm Biol* 43:455–459
- Volk R-B (2005) Screening of microalgal culture media for the presence of algicidal compounds and isolation of two bioactive metabolites, excreted by the cyanobacteria *Nostoc insulare* and *Nodularia harveyana*. *J Appl Phycol* 17:339–347