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Screening of Cyanobacteria from the soil of paddy field for biotechnological applications

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Cyanobacteria gained a lot of attention in recent years because of their potential applications in biotechnology. Cyanobacteria gained much attention as a rich source of bioactive compounds and considered as of the most promising groups of organisms. They produce metabolites with diverse biological activities such as antibacterial, antifungal, antiviral, anticancer, antiplasmodial, algicide and immunosuppressive are obtained. Soil cyanobacteria isolated from the rice paddy fields of different topographical locations across Manipur were evaluated by agar plate diffusion test for antifungal activity. Aqueous, petroleum and methanol extracts from cyanobacterial strains were examined for antifungal properties against three phytopathological fungi causing disease in rice. Of total cyanobacteria isolated, twenty cyanobacteria exhibited antifungal effects. The high inhibition of the pathogens was shown by methanol extracts.

Key words: Cyanobacteria, biofertilizer, rice fields, antifungal, rice diseases

INTRODUCTION

Increasing global population and a greater demand for food have led to more extensive farming practices resulting in increased disease (Lewis and Papavizas, 1991). Control of plant disease is a pressing issue for agriculture in the 21st century. Rapid and extensive use of agrochemicals to increase yield of rice and control plant diseases have disturbed the ecological balance which lead to contamination of groundwater, development of resistant races of pathogens and increased health hazards (Loper and Ischimura, 1991; Li *et al.*, 2007). Integrated nutrient management systems are needed to maintain agricultural productivity and protect the environment. Microbial inoculants are promising components of such management systems (Adesemoye and Kloepper, 2009). This has particular concern for countries like ours where agriculture is the main occupation. Among microorganisms, cyanobacteria (BGA) have a long history of usage in agriculture as biofertilizer (De, 1939; Watanabe *et al.*, 1951; Singh, 1961) and are known to enrich the nitrogen content of the rice fields (Patterson, 1996; Bergman *et al.*, 1997; Rai *et al.*, 2000; Whitton and Potts, 2000). Although the po-

tential of using cyanobacteria is well known, the attention has recently been focused on the biotechnological potentials of cyanobacteria for obtaining secondary metabolites (Carmichael, 1992). These cyanobacterial metabolites showed interesting and exciting biological activities which are important for the pharmaceutical industry and the agricultural sector (Bhadury and Wright, 2004; Dahms *et al.*, 2006). It is necessary to screen many cyanobacteria before suitable strain can be selected as biofertilizer and biocontrol agent (Kulik, 1995).

The aim of the current venture is to investigate the antifungal activities of cyanobacterial strains isolated from the local paddy fields of Manipur. These cyanobacteria could be use as biofertilizer and biocontrol inoculum for the improvement of rice plants.

MATERIALS AND METHODS

Isolation of Cyanobacteria

Soil samples were collected from different paddy-fields in different districts of Manipur. Soil samples in laboratory were cultured directly in N-free BG-11 media (Rippka *et al.,* 1979), after colonization, cyanobacteria were transferred to the same medium. Unialgal cultures were prepared using subculturing methods (Allen, 1968). Each isolated cyanobacterium was cultured in a 500 ml flask containing 150 ml of BG-11 medium without shaking for 30 days. The axenic cultures were grown in batches and maintained at 25°C ± 2 with a photonfluence rate of 50 $\mu mol.m^{-2}.s^{-1}$.

Test organisms

Standard strains of fungi (*Curvularia lunata*, *Fusarium moniliforme, Helminthosporium* sp.,) were used as test organisms. The fungal cultures were grown on potato dextrose agar (PDA) at pH 5.6 in 250 ml Erlenmeyer flasks at 25°C.

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 5000 rpm for 15 min. Water extracts were made by resuspending cyanobacterial biomass in distilled water (10 mg.ml-1) and treatment with CTAB (Cetyl trimethylammonium bromide) for 2 min. The biomass was also extracted simultaneously with organic solvents viz. methanol and petroleum. The cell mass was separated by centrifugation at 5000g for 20 min and extraction procedure was repeated thrice. The pooled supernatants were dried at 40°C under reduced pressure in a lyophilizer. The dried extracts were resuspended in 3 ml of each solvent and preserved at 4°C till further use in antifungal assays.

Antifungal bioassay

Dried extracts and supernatants were dissolved in 4 ml of their extraction solvent, and antifungal activity was determined by the disc method. Antifungal activity was evaluated by agar diffusion test (Lorain, 1996). Filter paper discs (6.4 mm) were saturated with 50 µl of the test solution, dried under laminar air flow and placed on the PDA plate which had been inoculated with a lawn of the test microorganisms. Plates were incubated at 25°C for a period of 24-48 hrs. for fungi. Discs treated with 50 µl methanol or petroleum extract were used as negative controls and fluconazole disc were used as positive controls. The extracts and supernatants containing antifungal components produced distinct, clear, circular zones of inhibition around the discs and the diameters of clear zones were determined and used as an antifungal activity.

RESULTS AND DISCUSSION

Numerous studies have shown that cyanobacteria produce substance with antifungal activities, thereby indicating a high potential of these primitive prokaryotes (Mian *et al.*, 2003). The screening of cyanobacteria for variety of antimicrobial activities have been reported by many researchers (Skulberg, 2000; Soltani *et al.*, 2005; Volk, 2005). A few studies have been done to screen cyanobacteria for production of antimicrobial substances from paddy fields.

The cyanobacterial isolates were identified on the basis of morphological traits according to Castenholz and Waterbury (1989). Distribution of isolates in genera of cyanobacteria is given in Table 1. Thirty strains were isolated by culturing axenically in BG 11 medium.

Antifungal activities of selected ten cyanobacterial isolates extracted with methanol, petroleum along

Table 1: Cyanobacterial isolates isolated during the research tenure

Isolate No.	Cyanobacterial species
1	*Anabaena spiroides* Klebahn
2	*A. oryzae* Fritsch
3	*A.variabilis* Kutz.
4	*A. torulosa (*Carm). Lagerh
5	*Aphanocapsa crassa* Ghose
6	*Calothrix scytonemicola* Tilden.
7	*Chroococcidiopsis* Geitler.
8	*Cyanotheca aeruginosa.* Nageli
9	*Dactylococcopies sp.* Hang.
10	*Dermocarpa olivacea* (Reinisch) Tilden
11	D. versicolor (Borzi) Geitler.
12	*Gloecocapsa compacta.* Kutz.
13	*G. punctata.* Nag.
14	*G. granosa (*Berk). Kutz.
15	*Gleotheca rupestris (*Lynb.) Bron.
16	*Microcystris flos-aquae* (Wilter) kirchmer.
17	*M. robusta* (Clark) Nygaard
18	*M. aeruginosa* Kutz.
19	*Microcoleus chthomoplastis* Thuret.
20	*Nostoc punctiforme (*Kutz.) Hairet.
21	*N. linkia* (Roth).
22	*N. moscorum* Ag.
23	*Oscillatoria* sp. Vacher
24	*Phormidium* sp. Kutz.
25	*Plectonema* sp. Thurnet.
26	*Spirulina* sp. Turpin en. Gardner.
27	*Synechococcus aeruginosa* Nag.
28	*Synechocystis elongatus*
29	*Scytonema* sp. Ag.
30	*Westiellopsis prolifica* janet.

with aqueous extracts were tested using disc diffusion method (Table 2). The antifungal activities of cyanobacterial extracts were found to vary with the type of solvents used for extraction. Out of 90 combinations of 10 cyanobacterial cultures with three extraction solvents, 60 inhibition were noted for the three fungal test cultures. The variation in antifungal activities could be due to different permeabilities of bioactive substances into the test organisms. Among the solvents used, methanol extracts showed 30 % followed by petroleum and aqueous extracts exhibiting 20 and 10 % inhibition, respectively. A maximum zone of inhibition of 28mm diameter was observed by methanol extract of *Nostoc moscorum* against the fungal pathogen *Curvularia lunata*. Maximum zone of inhibition (26

Table 2: Activity of cyanobacterial extracts against fungal test cultures by disc diffusion method

Isolate	Extract	Diameter of inhibition (mm)		
		A	B	C
Anabaena oryzae Fritsch	Aqueous	-	-	-
	Methanol	12	16	14
	Petroleum	10	8	12
Microcystris flos -aquae (Wilter) kirchmer	Aqueous	8	-	-
	Methanol	14	18	28
	Petroleum	10	10	?
M. robusta Clark	Aqueous	10	-	-
	Methanol	16	14	12
	Petroleum	8	9	10
M. aeruginosa Kutz.	Aqueous	10	-	-
	Methanol	20	18	18
	Petroleum	9	8	8
Nostoc punctiforme Kutz.	Aqueous	12	-	-
	Methanol	16	18	14
	Petroleum	?	12	10
N. linkia Roth.	Aqueous	8	-	-
	Methanol	16	18	18
	Petroleum	9	10	10
N. moscorum Ag.	Aqueous	12	-	-
	Methanol	28	14	16
	Petroleum	-	12	-
Spirulina sp.	Aqueous	10	-	-
	Methanol	16	18	16
	Petroleum	-	8	-
Synechococcus aeruginos	Aqueous	8	-	-
	Methanol	18	14	15
	Petroleum	10	-	-
Synechocystis elongatus	Aqueous	8	-	10
	Methanol	14	26	15
	Petroleum		-	12
Control, fluconazole (50 µg disc^{-1})	Methanol	20	22	20

A, *Curvularia lunata*; B, *Fusarium moniliforme*; C, *Helminthosporium oryzae*

and 28 mm) in case of *Fusarium moniliforme and Helminthosporium oryzae,* was exhibited by the methanol extract of *Synechocystis elongates* and *Microcystis flos-aquae,* respectively. Marginal antifungal activities (<12mm diameter of inhibition zone) were obtained in case of 23 test combinations (Table 2). Maximum antifungal activity by the methanol extracts as observed in the present study was in accordance with earlier reports (Ostensvik *et al.*, 1998; Soltani *et al.*, 2005). Decrease activities obtained for aqueous and petroleum extracts

may be due to the different polarities of antifungal substances. Fungal cultures used in present study are the phytopathogens causing major damage to agricultural crops in Manipur.

The results presented in this paper show that cyanobacteria possess promising antifungal activity. The cyanobacteria *Microcystis flos-aquae, Nostoc moscorum* and *Synechocystis elongates* with efficient antifungal activity may serve as promising biocontrol agents in the present day agricultural practices.

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