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Antimicrobial activity of pesticide adapted cyanobacteria on fungal pathogens of rice

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ABSTRACT

Organic extracts (Chloroform, ethyl acetate and hexane) of three normal and pesticide adapted strains of blue green algae (BGA) were investigated for their antifungal activity against the fungal pathogens of rice. The pesticide adapted strains showed better antifungal activity when compared with the normal strains. The maximum zone of inhibition was 14mm, 21mm, and 22mm for Fusarium fujikori, Rhizoctonia oryzae and Curvularia oryzae. The percentage of activity increased when compared with the control strains were 12.3, 5.3 and 6.6percentage repectively.

Keywords: Rice, Fungal pathogens, adapted Strains, Blue green algae.

INTRODUCTION

Water which covers about 70% of earth's surface is home to thousands of living things a potential source for many bioactive compounds [1]. Concurrently the soil inhabitants produce useful bioactive natural products which include many important antibiotics and drugs [2]. Cyanobacteria are one such ancient inhabitant, blue green algae (BGA) a well known biofertilizer. Cyanobacteria are one of the largest and most important groups of algae on the earth [3]. In particular nitrogen fixing cyanobacteria are vital photosynthetic microorganisms that contribute to soil fertility by fixing atmospheric nitrogen they also maintain ecosystem stability [4]. The agronomic importance of free living cyanobacteria as biofertilizer is observed [5]. Some strains in the fields release small amounts of nitrogenous polypeptides during the active growth period. The dried cyanobacterial culture improved rice yields, increased soil organic content and increased nitrogen levels. After green revolution there is an extensive use of plant protecting chemicals used for better yields. These chemicals increased the yield but showed a long term

affect on the nitrogen fixing cyanobacteria. The fungicides like carbendazim, copper oxychloride, difenoconazole and other are extensively used for prevention of some fungal pathogens. So the present study deals with the antifungal activity of control strains of cyanobacteria and pesticide adapted strains.

MATERIALS AND METHODS

Isolation of BGA from soil

Collection and isolation of cyanobacteria from soil were made in accordance with Rippka (1988). Cyanobacteria were obtained from different selected paddy fields. The samples were collected at random from the top layer of each location. The BGA were maintained auxenic in BG 11 medium [6],[10]. The cultures received the light intensity of 14.4W/m^2 with light and dark regime of 16:8. Out of the isolates selected strains were used for the adaptation.

Adaptation of BGA to fungicide

Different methods of adaptation are followed for the test. Primarily all the isolated strains were grown in the modified BG 11 medium with known concentration of fungicide (0.5µg/ml-4.0µg/ml) and the cells which survived were taken on to an agar bilipid layer technique and the resistant strains are used for the study. Maximum yellowing and no observed effect concentration (NOEC) were used for the separation of adapted strains.

Test organisms

The rice fungal pathogen strains *Fusarium fujikori* (MTCC 4649), *Rhizoctonia oryzae* (MTCC 2162), *Curvularia oryzae* (MTCC 3726) were used in the study which were obtained from Microbial type culture collection, Institute of Microbial technology, Chandigarh.

Screening of BGA for antifungal activity

The pure cultures were screened for the antifungal activity by agar well diffusion method on potato dextrose agar (PDA). The dried algal masses of exponentially growing stage of isolates were extracted with aqueous, methanol chloroform and hexane (ratio 1:5 g.mL^{-1}) for 24 hrs. Three to five multiple extractions of the biomasses were done and the extracts were pooled and the volumes were maintained. The extracts were preserved at 4^0c and used for further antidermatophytic activity studies. 50 µ l of the extract was placed in the wells made on potato dextrose agar plates seeded with the test fungal cultures. The plates were incubated at 28^0c and observed for appearance of zones of inhibition after 72hrs [7-9, 11-12].

RESULTS

Identification of the potential antidermatophytic cyanobacteria:

The isolates which show potential antifungal activity were identified up to genus level using morphological characteristics as described in Desikachary (1959). These three potential isolates were identified as members of *Oscillatoriaceae* and *Scytonemataceae.* The microscopic images of three isolates are presented in Fig 1. The three isolates were designated as 1-A, 1-B and 1-C against for convenience during study.

Antifungal activity

Inhibitory effect by agar-well diffusion method:

The antifungal activity of cyanobacteria 1-A, 1-B and 1-C against test fungi were shown in Table-1. As the results reveal three adapted strain (A) isolates produced a significant inhibition zones and thus antifungal activity. In our study we observed that only chloroform extracts of the isolates exhibited potential antifungal activity (Fig-1). The maximum inhibition zone was observed with isolate 1-A on *Curvularia oryzae* (22mm), *Fusarium fujikori* (14mm) and with 1-C isolate on *Rhizoctonia oryzae* (16mm).

DISCUSSION

The predominant cyanobacteria isolated from rice fields soils in our present study were identified as member of Oscillatoriaceae. A large number of soil cyanobacterial isolates extracts and their extracellular products have been found to have antiactivity. In this study pH 7.6, temperature 35±2 C and 15days of incubation were chosen for the cyanobacteria as Noaman et al(2004) have reported the above conditions to be the best for growth and antimicrobial agent production. Antimicrobial activity of cyanobacteria could be explained by the presence of cyclic peptides, alkaloids and lipopolysaccharides. (Katircioglu H et al; 2006). There are number of reports on antibiotic and other pharmacological effects from cyanobacteria but, to the best of our knowledge no other reports or data are available on the antifungal activity of metabolites extracted from cyanobacteria. The present study is a primary screening report on the antifunfgal activity by the selected isolates

Fig 1: Microscopic images of three cyanobacterial isolates, 1-A, 1-B and 1-C. under (1000X). Antiactivity of the cyanobacterial isolates against 1-A, 1-B against *Fusarium fujikori* 2(a), *Rhizoctonia oryzae* 2(b) and against *Curvularia oryzae* by 2-C by agar well diffusion

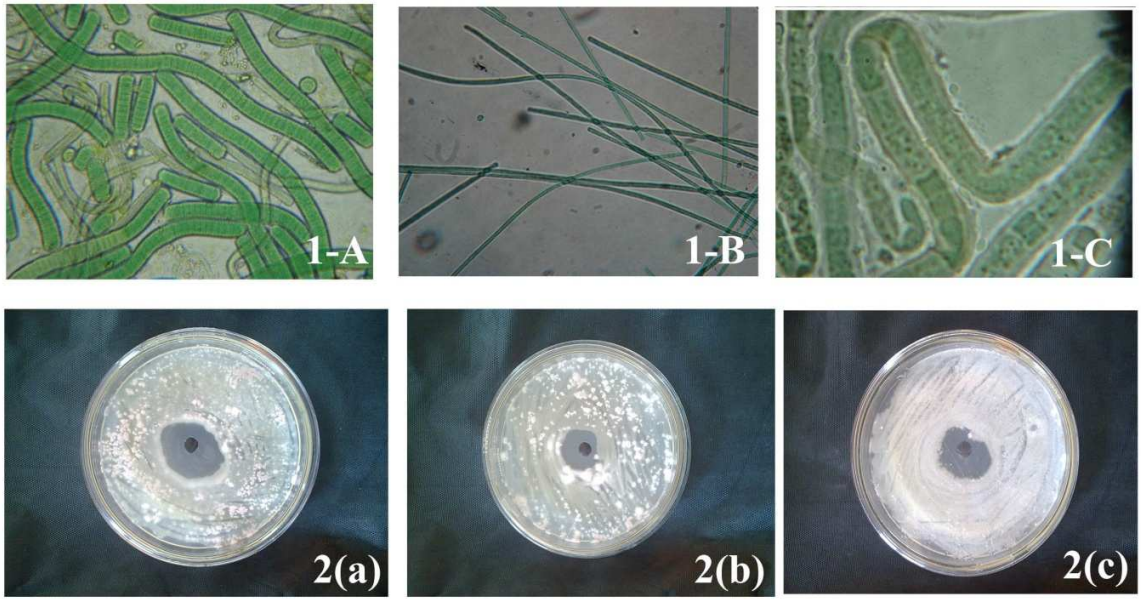
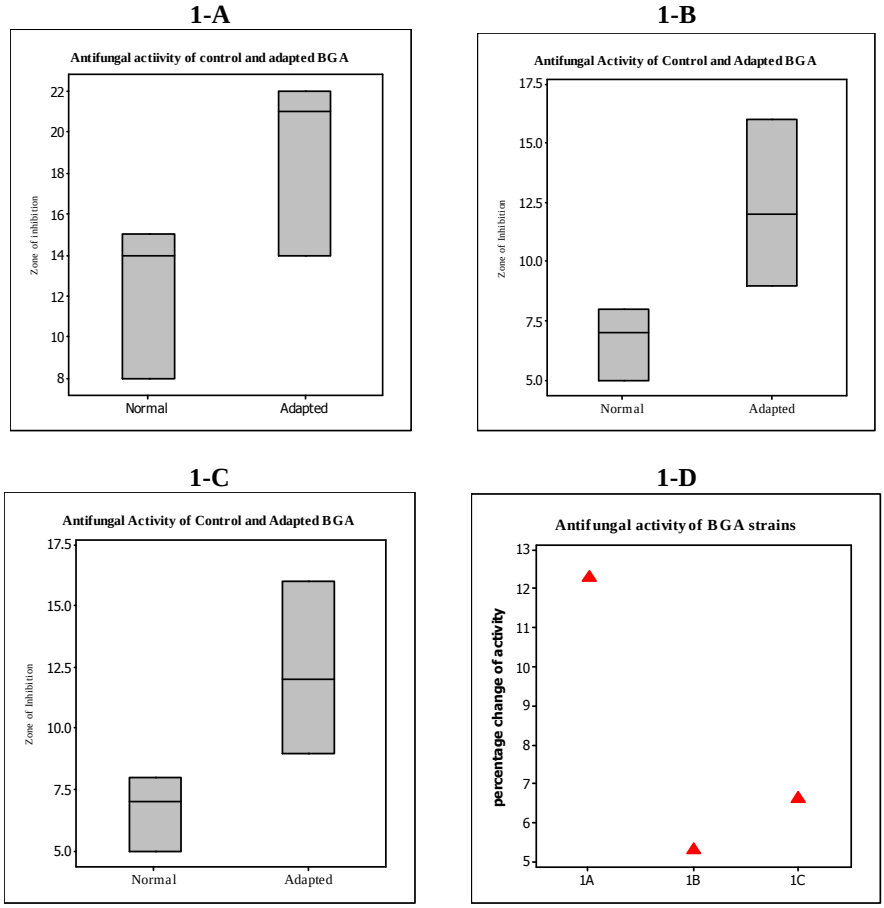


Table: Diameter of inhibition zone (mm) exhibited against test fungi by chloroform extracts of soil cyanobacterial isolates

Test fungi	Diameter of zone (mm)					
	1A		1B		1C	
	N	A	N	A	N	A
Fusarium fujikori	8±0.2	14±0.2	6±0.2	11±0.2	5±0.2	9±0.1
Rhizoctonia oryzae	14±0.3	21±0.3	4±0.1	7±0.1	8±0.1	16±0.2
Curvularia oryzae	15±0.1	22±0.1	6±0.3	14±0.3	7±0.3	12±0.1

** Values are the means ± standard deviations of triplicate measurements*

Fig 2: Box plot of antimicrobial activity of Adapted and control strains of Cyanobacterial isolates, 1-A, 1-B and 1-C and The percentage of change in antifungal activity of Normal and adapted strains 1-D



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