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Antibacterial Activity of Paddy Fields Cyanobacteria

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ABSTRACT

Antibacterial activity of some heterocystous cyanobacteria from paddy fields in Warangal district of Andhra Pradesh was studied. Soil samples were collected from various regions of paddy fields of Warangal and cyanobacteria were isolated. Supernatants and methanolic extracts from biomass of 42 strains of cyanobacteria were isolated and screened against four strains of bacteria. Methanolic extracts and culture supernatants of 6 strains of cyanobacteria exhibited significant antibacterial effect. According to these results, it is concluded that *Anabaena* and *Stegonema* species have more potential for producing antimicrobial substance than other strains.

INTRODUCTION

Blue-green algae constitute an important group of microorganisms capable of fixing atmospheric nitrogen. Some fossil forms have also been discovered which date back to Precambrian periods. Cyanobacteria, which constitute a versatile group of microorganisms, occur in diverse habitats ranging from alkaline hot springs to permanent snow fields in the poles. Certain cyanobacteria have drawn much attention as prospective and rich sources of biologically active constituents and have been identified as one of the most promising groups of organisms to be able to produce bioactive compounds (Fish & Codd 1994, Schlegel et al. 1999). Cyanobacteria are known to produce metobolites with diverse biological activity such as antibacterial, antifungal, antiviral, anticancer, antiplasmodial, algicide, antiplatelet aggregation and immuno-suppressive activities (Browitzka 1995, Jaki et al. 2000, Kajiyama et al. 1998, Patterson & Carmeli 1992, Patterson et al. 1994, Gerwick et al. 1994, Papendorf et al. 1998, Papke et al. 1997, Rho et al. 1996, Koehn et al. 1992, Ghasemi et al. 2003). Pandey & Pandey (2000) has studied antibacterial properties of cyanobacteria about effective and eco-friendly approach to control bacterial leaf spot disease of chilli. Recently, Sanaa (2007) have studied bioactive allelochemical compounds from *Oscillatoria* species (Egyptian isolates).

Screening of cyanobacteria for antibiotics and others pharmacologically active compounds has received ever-increasing interest as potential source for new drugs (Fish & Codd 1994, Ostensvik et al. 1998, Schlegel et al. 1999). Cyanobacteria from local habitats seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms (Mundt et al. 2001). Cyanobacteria of Andhra Pradesh not yet been studied for antimicrobial activity. In order to find the potential of cyanobacteria for production of antibacterial compounds in paddy fields of Warangal district of Andhra Pradesh, 42 strains of heterocystous cyanobacteria were isolated and their potency was studied.

MATERIALS AND METHODS

Paddy field soil samples were collected from Warangal district. Fogg's medium (Fogg 1949) with without nitrogen source was used for raising enrichment cultures. After colonization, cyanobacteria were transferred to the same medium. Uni-algal cultures were prepared using subculturing methods (Kaushik 1987). Each isolated cyanobacterium was cultured in a 250 mL flask containing 100 mL of Fogg medium without shaking for 28 days. The incubation temperature was 26 ± 2°C and illumination at 2-3 K Lux with continuous fluorescent light.

The cultures were harvested after 28 days by centrifugation at 5000 rpm for 15 minutes. The aqueous supernatant was collected and the algal pellets were extracted with 15 mL of methanol, with shaking for 20 minutes. The culture supernatants and solvent extracts were dried under reduced pressure at 40°C and were stored at -4°C for further studies.

The bacteria used as test organisms were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* sp. and *Escherichia coli.* Dried extracts and supernatants were dissolved in 4 mL of their extraction solvent, and antimicrobial activity was determined by the disc method. Filter paper discs (6mm) were saturated with 150 µL of the test solution, dried under laminar air flow and placed on the Muller Hinton agar plate for organisms, which have been inoculated with a lawn of the test microorganisms. These plates were incubated at 37°C for 24 hrs. Discs treated with 50mL methanol was used as negative control and gentamycin discs were used (10µg) as positive control. The extracts and supernatants containing antibacterial components produced distinct, clear circular zones of inhibition around the disc and the diameters of clear zones were determined and used as an identification of antibacterial activity. The following formula was used for comparison of the antimicrobial activity of the sample with that of the standard (antimicrobial index).

$$\text{Antimicrobial index} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of standard}} \times 100$$

Identification of cyanobacteria was done with the help of published literature (Desikachary 1959, Anand 1989, Santra 1993).

RESULTS AND DISCUSSION

The observations of culture supernatants and methanolic extracts of the isolated cyanobacteria that demonstrated antibacterial activity are shown in Tables 1 and 2. Supernatant and methanolic extract of 6 strains from 42 cyanobacterial strains showed significant antibacterial activity against the bacteria under the investigation, two of them were identified as *Anabaena* sps., two *Stegonema* sps., one *Scytonema* sp. and one *Hapalosiphon* sp. showing antibacterial activity. All the bacteria showed maximum inhibition for methanolic extract and supernatants of six strains of cyanobacteria.

The cyanobacteria such *Fischerella ambiqua* (Falch et al. 1995), *Fischerella musciola* (Hagmann et al. 1996), *Nostoc commune* (Jaki et al. 2000), *Scytonema hofmanni* (Pignatello et al. 1983), *Hapalosiphon fontinalis* (Moore et al. 1987), *Anabaena* sp. (Frankmolle et al. 1992), *Nostoc spongiaeforme* (Hirata et al. *1996*), *Microcystis aeruginosa* (Ishida et al. 1997) and *Phormidium* sp. (Fish & Codd 1994) have been reported as the main cyanobacteria to produce antimicrobial substances. Screening efforts aimed to identify antimicrobial agents in cyanobacteria have revealed several promising lead compounds. Some of these substances identified include Nostocyclyne A, Nostofungicidine, Kawaguchipeptin B, Nostocin A, Ambigol A and B, Hapalindoles and Scytophycins. A few studies have been done to screen cyanobacteria for production of antimicrobial substances from paddy fields. Probably the synthesis of highly active toxin is a defence option of cyanobacteria in these environments against other organisms like bacteria, fungi, viruses and other microorganisms. In one study, it was shown that cyanobacteria from paddy fields of northern Thai-

Table 1: Antibacterial activity of cyanobacteria methanolic extracts against bacteria as presented by inhibition zone diameter (in mm) and antimicrobial index (in parentheses).

Sample	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella* sp.	*Escherichia coli*
Control (Gentamycin)	16 (100)	15 (100)	20 (100)	20 (100)
A. variabilis Kutz	16 (100)	12 (85)	12 (80)	16 (80)
A. oryzae Fritsch	14 (87)	10 (71)	13 (65)	13 (65)
Stegonema ocellatum Born.et. Flah	16 (112)	12 (85)	14 (70)	12 (60)
Stegonema sp.	9 (56)	14 (100)	12 (60)	14 (70)
Scytonema simplex Bharadwaja	8 (50)	10 (71)	12 (60)	11(55)
Hapalosiphon luteolus West W. and G.S.	7 (43)	8 (57)	7(35)	8 (40)

Table 2: Antibacterial activity of cyanobacteria supernatants against bacteria as presented by inhibition zone diameter (in mm) and antimicrobial index (in parentheses).

Sample	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella* sp.	*Escherichia coli*
Control (Gentamycin)	16 (100)	15 (100)	20 (100)	20 (100)
A. variabilis Kutz	14 (87)	16 (106)	14 (70)	11 (55)
A. oryzae Fritsch	16 (100)	13 (86)	12 (60)	14 (70)
Stegonema ocellatum Born.et.Flah	14 (87)	18 (120)	13 (65)	12 (60)
Stegonema sp.	16 (100)	14 (93)	14 (70)	13 (65)
Scytonema simplex Bharadwaja	12 (75)	12 (80)	10 (50)	12 (60)
Hapalosiphon luteolus West W. and G.S.	12 (75)	12 (80)	10 (50)	8 (40)

land produce bioactive substances with antibiotic activity against *Bacillus substilis* (Chetsumon 1993). In the present investigation, out of 42 strains of cyanobacterial isolates, 6 strains showed significant *in vitro* antimicrobial activity. The proportion of the isolates with antibacterial activity was 15% respectively, which is comparable with those published earlier in other screening programs, 14% (Ghasemi et al. 2003), 11% (Flores & Wolk 1986) and 10% (Schlegel et al. 1999). *Anabaena*, *Stegonema*, *Scytonema* and *Hapalosiphon* species produce bioactive substances which may have potential for antibacterial activity. The results indicated that all the supernatants and methanolic extracts of *Anabaena* and *Stigonema* species have high activity against bacteria. Among the isolated cyanobacteria, *Hapalosiphon* species have the minimum activity against the test organisms. Among all the species studied in the investigation for antimicrobial activity, it seems *Stigonema* strains are being reported for the first time as producer of antimicrobial substances from India. The observations in the investigation indicate that the cyanobacteria display a potential application that leads to further work.

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