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Antibacterial Activity of Cylindrospermum Sp. NDUPC005 and Cylindrospermum Sp. NDUPC006 Isolated from Varanasi, India

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ABSTRACT

Two cyanobacterial strains, i.e., Cylindrospermum sp. NDUPC005 and Cylindrospermum sp. NDUPC006 were isolated from soil collected from agricultural fields of Varanasi, India. Strains were identified by morphological and molecular methods. Antibacterial activity of crude extracts in five organic solvents, i.e., Acetone, ethanol, petroleum ether, chloroform, methanol of both strains against two human pathogenic bacteria, i.e., E. coli and S. aureus was studied. Differential variation in antibacterial response was noted against test organisms. Crude extract in all organic solvents of Cylindrospermum sp. NDUPC005 showed antibacterial activity against *S. aureus* whereas the extract only in methanol showed antibacterial activity against E. coli. Crude extract, in four organic solvents, i.e., Ethanol, methanol, petroleum ether, acetone of Cylindrospermum sp. NDUPC006 showed antibacterial activity against S. aureus whereas the extract in petroleum ether and acetone showed antibacterial activity against E. coli. Ethanol extract of Cylindrospermum sp. NDUPC006 showed the maximum antibacterial activity of 12.33±0.58 mm against S. aureus. Findings of the experiment suggest that ethanol extract of Cylindrospermum sp. NDUPC006 can be used for mining of antibacterial agent against *S. aureus*.

INTRODUCTION

Cyanobacteria are an ancient group of photosynthetic prokaryotes. The role of cyanobacteria in fertility of agriculture fields is well established. Cyanobacteria are the known source of secondary metabolites. Antimicrobial activities of cyanobacteria have been published in many research papers. Saiheb [1] have reported antibacterial activities of fifteen cyanobacteria from Libya. Thummajitsakul et al., [2] studied the antibacterial nature of *Phormidium* sp. and *Microcoleus* species. Thillairajasekar et al., [3] studied antibacterial activity of *Trichodesmium erythraeum*. Many researchers have isolated and characterized bioactive agents from cyanobacteria. Ghasemi et al., [4] have isolated and characterized Parsiguine, a novel antimicrobial substance from *Fischerella ambigua*. Heptadecane and tetradecane have been isolated from *Spirulina platensis* [5], Asthana et al., [6] have isolated and characterized antibacterial entity from Antarctic cyanobacterium *Nostoc* CCC537. Discovery of new antibacterial compounds is necessary to cope up the continuous increase of multi drug resistant bacteria. Cyanobacteria may prove the potential source for new antibacterial agents. *Cylindrospermum* sp. is very common soil cyanobacteria. Reports of antibacterial activity of these cyanobacteria are very less. Hence, this experiment was designed to study antibacterial activities of two *Cylindrospermum* species isolated from agricultural fields of Varanasi, India.

MATERIALS AND METHODS

Isolation, Purification, and cultivation of cyanobacteria

Soil samples were collected, powdered, placed in sterile Petri dishes and enriched withsterilized nitrogen free BG-11 medium [7]. The Petri dishes placed in culture room maintained at 28 ± 2^0 C and illuminated with fluorescent light of 12 Wm $^{-2}$. Cyanobacterial colonies developed in Petri plates were observed microscopically. Standard plating/ streaking techniques used for isolation and purification of cyanobacterial strains [7]. Cyanobacterial strains grown in BG-11 liquid medium without nitrogen supplementation [7] in a culture room maintained at a temperature of 28 ± 2^0 C and illuminated with fluorescent light of 12 Wm $^{-2}$.

Identification of cyanobacteria

Cyanobacteria were identified by morphological as well as molecular methods. The strains were viewed at 400x and 1000x using Olympus 21Xi microscope. The morphological characters, i.e., nature of filament, shape, and size of the vegetative cells, heterocysts, and Akinete was studied. Characters were analyzed with the help of Magnus PRO Micro-measurement & Image analysis software. Strains were assigned to cyanobacterial species following taxonomic descriptions provided in the literature [8-10]. The identity of the isolate *Cylindrospermum* sp. NDUPC005 was further confirmed by sequencing of Partial 16S rRNA gene. The sequence of strain was submitted to GenBank of NCBI with accession No.- KJ396069 (*Cylindrospermum* sp. NDUPC005).

Preparation of cyanobacterial extract

Biomass of 25 days old, cultures was used for the preparation of cyanobacterial extract. Biomass was harvested by centrifugation at 5000 rpm for 10 minutes, and dried in the hot-air oven at 60^0 C for 24 hrs. 250 mg biomass of each strain was mixed in 10 ml of solvents, i.e., Methanol, Ethanol, Petroleum ether, Acetone, chloroform and left overnight in freeze then centrifuged and filtered the extract. The filtrate of each strain was evaporated to dryness at 40^0C and again dissolved in 1 ml of respective solvents.

Antibacterial test of cyanobacterial extracts

Agar disk diffusion assay [11] was used to determine the antibacterial activities of cyanobacterial extracts. The Bacterial strains of *E.coli* and *S. aureus* were test organisms. Both bacterial strains were obtained from Dept. of Endocrinology and Metabolism, IMS, BHU, Varanasi, India. The sterilized MHA medium was poured into Petri plates, allowed to cool and solidify. 100µl of bacterial suspension was poured in each Petri plates and spread with L- shaped spreader. Three filter paper disks (6 mm), saturated with 25µl of extract and one filter paper disk saturated with 25 µl of respective solvents, well dried in laminar flow, were placed at an equal distance in each Petri plate. Petri plates were incubated at 35ºC for 24hrs. Inhibition zone (Excluding the diameter of filter paper disk) produced around the disks were measured. The antibacterial activity of standard antibiotic Ampicillin was measured by following same protocol and concentration of standard antibiotic was 10µg/ ml. The mean and standard error was calculated.

RESULTS

Cylindrospermum sp. NDUPC005 and *Cylindrospermum* sp. NDUPC006 (Fig. 1) were isolated from agricultural fields of Varanasi, India and characterized by morphological as well as molecular methods (16S rDNA). The crude extract of each strain in five organic solvents, i.e., Ethanol, Petroleum ether, Acetone, Methanol, and Chloroform were used for screening antibacterial activity. *S. aureus* and *E. coli* were test organisms, and ampicillin was a control antibiotic. Differential variation in antibacterial response was noted against test organisms (Table- 1). Crude extract in all organic solvents of *Cylindrospermum* sp. NDUPC005 showed antibacterial activity against *S. aureus* whereas the extract only in methanol showed antibacterial activity against *E. coli* (Table- 1). Crude extract, in four organic solvents, i.e., Ethanol, Methanol, Acetone. Petroleum ether of *Cylindrospermum* sp. NDUPC006 showed antibacterial activity against *S. aureus*.

Table-1 Antibacterial activity of various extracts of *Cylindrospermum* Sp NDUPC005 and *Cylindrospermum* Sp NDUPC006 on *S. aureus* and *E. coli*

Cyanobacteria	Organic solvents & Antibiotic	Effective zone of Inhibition (In mm)	
		S. aureus	*E. coli*
Cylindrospermum Sp. NDUPC005	Ethanol	3.33±0.15	NZ
	Petroleum ether	4.33±0.58	NZ
	Acetone	8±0.65	NZ
	Methanol	10±0.55	7.33±0.51
	Chloroform	1±0.73	NZ
Cylindrospermum Sp. NDUPC006	Ethanol	12.33±0.58	NZ
	Petroleum ether	2.33±0.54	9±0.15
	Acetone	6.33±0.54	4±1
	Methanol	1.67±0.58	NZ
	Chloroform	NZ	NZ
Control	Ampicillin	7.33±0.86	6.35±0.76

± Represent standard Error and NZ for no zone of inhibition

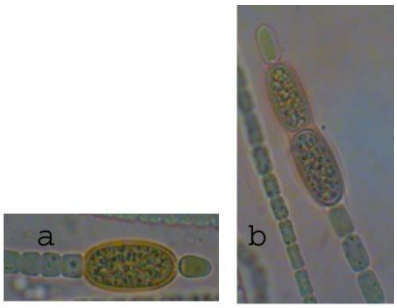

Fig. 1: Micrograph of (a) *Cylindrospermum* Sp. NDUPC005

(b) *Cylindrospermum* Sp. NDUPC006

Crude extract only in Petroleum ether and acetone showed antibacterial activity against *E. coli* (Table- 1). Ethanol extract of *Cylindrospermum* sp. NDUPC006 showed the maximum antibacterial activity of 12.67±0.58 mm (Table-1) against *S. aureus*.

DISCUSSION

Antibacterial activities of cyanobacteria are well established. A number of researchers have isolated and characterized the antibacterial agents from cyanobacteria. Asadi et al., [12] reported widespread spectrum antibacterial activity of *Microchaete tenera, Anabaena sp. Bory ISC55, Phormidium sp. and Chroococcus pallidus*. Chloroform extract of *Cylindrospermum majus* have shown significant antibacterial activity (17.33 mm inhibition zone) against *K. pneumoniae*, and meaningful antifungal activity in the aqueous extract against *A. fumigates* [13]. Reehana et al. [14] studied the antibacterial properties of *Spirulina subsalsa* NTRI 02, *Oscillatoria pseudogeminata* NTRI 03 and *Phormidium corium* NTRI 04. Extracts in five organic solvents, i.e., Ethanol, Petroleum ether, Acetone, Methanol and Chloroform of two cyanobacterial strains, i.e., *Cylindrospermum* sp. NDUPC005 and *Cylindrospermum* sp. NDUPC006 were screened against two human pathogenic strains of bacteria. The bactericidal activity was more against *S. aureus* in comparison to *E. coli* (Table-1). Bacteriocidal activity varies according to cyanobacterial strain and solvent used for extract (Table- 1). Ethanol extract of *Cylindrospermum* sp. NDUPC006 showed the maximum antibacterial activity of 12.33±0.58 mm (Table-1) against *S. aureus* which is approximately double the antibacterial activity due to the standard antibiotic. Antibacterial agents have been isolated and characterized in various cyanobacterial strains. In our findings, the effective antibacterial agent against *S. aureus* is being produced by *Cylindrospermum* sp. NDUPC006, ethanol is the best solvent for its extraction.

CONCLUSION

Crude extract in ethanol of *Cylindrospermum* sp. NDUPC006 showed approximately double the antibacterial activity than the standard antibiotic against *S. aureus*. Ethanol was the best solvent for extraction potent active compound. Plan of this research work will be isolation and characterization of the active compound.

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