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Antibacterial activity of *Fischerella muscicola* NDUPC001

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Abstract: *Fischerella muscicola NDUPC001 was isolated from agricultural fields of Varanasi, India. The strain was characterized by morphological and molecular methods. Antibacterial activity of crude extracts in five organic solvents i.e., Acetone, ethanol, petroleum ether, Chloroform, methanol against two human pathogenic bacteria, i.e., E.coli and S. aureus was studied. Crude extracts showed the differential antibacterial response to test organisms. Crude extract in four organic solvents, i.e., Ethanol, Methanol, Petroleum ether, Acetone showed antibacterial activity against S. aureus where as the extract only in Petroleum ether showed antibacterial activity against E. coli. The methanol extract of Fischerella muscicola NDUPC001 showed the maximum antibacterial activity of 14.0±1.12 mm against S. aureus. Thin layer chromatography of crude extract in methanol indicated two types of compounds. Findings of the experiment suggest that Methanol extract of Fischerella muscicola NDUPC001 can be used for mining of antibacterial agent against S. aureus.*
Keywords: *Antibacterial activity, Cyanobacteria, Fischerella muscicola NDUPC001*

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I. Introduction

Cyanobacteria are a well-known source of biologically active metabolites. Antibacterial activities of cyanobacteria have been reported in crude extracts of various organic solvents. Bharat, N., et al., [1] reported antibacterial activities of Fresh water cyanobacteria *Lyngbya officinalis* NCCU-102, *Gleocapsa gelatinosa* NCCU-430, *Chroococcus* sp. NCCU-207, *Aulosira fertillisma* NCCU-444, *Anabaena ambigua* NCCU-160, *Hapalosiphon fontinalis* NCCU-339, *Anabaena* sp. NCCU-09, *Anabaena variabilis* NCCU-441, *Westiellopsis prolifica* NCCU-331, and *Scytonema* sp. NCCU-126. Thummajitsakul et al., [2] studied the antibacterial nature of *Phormidium* sp. and *Microcoleus* species. Thillairajasekar et al., [3] studied antibacterial activity of *Trichodesmium erythraeum*. Bioactive compounds isolated from cyanobacteria have shown antibacterial [4], antimalarial [5], antifungal, anticancer or cytotoxic [6] activities. Secondary metabolites are known for antimicrobial activities, and cyanobacteria are the rich source of Secondary metabolites. According to data of Marine Literature, [7] Three hundred twenty-six secondary metabolites from *Lyngbya* sp., Eighty-four from *Nostoc* sp., Eighty-two from *oscillatoria* sp. Thirty-nine from *Schizothrix* sp., Fifty from *Microcystis* sp. Thirty-five from *Synecococcus* sp. Twenty-eight from *Anabaena* sp. and only four from *Fischerella*. sp. have been isolated. Asthana et al., [8] have isolated and characterized antibacterial entity from Antarctic cyanobacterium *Nostoc* CCC537. Heptadecane and tetradecane have been isolated from *Spirulina platensis* [9], *Fischerella*. Sp is less explored for antibacterial properties. There are fewer reports of antibacterial activities of cyanobacteria isolated from Varanasi. Hence, *Fischerella muscicola* NDUPC001 isolated from agricultural fields of Varanasi, India was screened for its antibacterial properties.

II. Materials And Methods

2.1 Isolation, Purification, and cultivation of cyanobacteria

Cyanobacterium was isolated from soils of agricultural fields of Varanasi. Soil samples were powdered and placed in sterile Petri dishes. Petri dishes were moistened with the sterilized nitrogen free BG-11 medium [10] and placed in culture room maintained at 28 ± 2^0 C, illuminated with fluorescent light of 12 Wm $^{-2}$. The Petri plates were regularly monitored for colonization and observed microscopically. Cyanobacterial colonies developed on soil samples were isolated by repeated streaking on sterilized, nitrogen free BG-11 solid medium [10]. .Cyanobacterial strain grown in nitrogen free BG-11 liquid medium [10] in a culture room maintained at a temperature of 28 ± 2^0 C and illuminated with fluorescent light of 12 Wm $^{-2}$.

2.2 Identification of cyanobacteria

Cyanobacterium was identified by morphological as well as molecular methods. The strain was viewed at 400x and 1000x using Olympus 21Xi microscope. The morphological characters, i.e., nature of filament, shape, and size of the vegetative cell, heterocysts, and Akinete was noted. Size of cells was measured with the help of Magnus PRO Micromeasurement & Image analysis software. The strain was assigned to cyanobacterial species following taxonomic descriptions provided in the literature [11, 12 13]. The cyanobacterial isolate was further confirmed by sequencing of Partial 16S rRNA gene. The sequence of strain was submitted to GenBank of NCBI with accession No.-JX876898.2. The strain was deposited at NAIMCC (NBAIM), Mau, India (Accession No. NAIMCC-C-000121).

2.3 Preparation of cyanobacterial extract

Cyanobacterial biomass of stationary phase of growth (25 days old culture), was harvested by centrifugation and dried in the hot-air oven at 60^0 c for 24 hrs. The biomass extracted by mixing well in the organic solvent. 200 mg pellet of the 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 the strain was evaporated to dryness at 40^0c and again dissolved in 1 ml of respective solvents.

2.4 Antibacterial test of cyanobacterial extracts

The antibacterial activities of cyanobacterial extracts were determined by agar disk diffusion assay [14]. Two bacterial strains, i.e., *E.coli* and *S. aureus* were test organisms, and 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. Lawn of test pathogen was prepared by spreading 100 µl of the bacterial suspension with L- shaped spreader. Filter paper disks (6 mm), i.e., three loaded with 25 µl of extract and one filter paper disk loaded 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 37^0 c for 24 hrs. Inhibition zones were produced around the disks and measured excluding the diameter of filter paper disk. The antibacterial activity of standard antibiotic Ampicillin was also tested with the same protocol, and concentration of standard antibiotic was 10 µg/ ml. The mean and standard error was calculated.

2.5 Thin layer chromatography of bioactive compound

Thin layer chromatography was performed on a stripe of silica gel plate (Silica gel-60, Merck). 50µl of the sample (1mg/ml) was spotted manually on silica gel and run in mobile phase, hexane: ethyl acetate (8.5:1.5). Number of spots produced were counted.

III. Results

Fischerella muscicola NDUPC001 (Fig. 1) was isolated from agricultural fields of Varanasi, India and characterized by morphological as well as molecular methods (16S rDNA). The crude extracts of the strain in all the five organic solvents were used for screening antibacterial activity. *S. aureus* and *E. coli* were test organisms, and ampicillin was a standard antibiotic. Crude extracts showed the differential antibacterial response to test organisms (Table- 1). Crude extract in organic solvents, i.e., Acetone, Petroleum ether, Methanol and Ethanol of *Fischerella muscicola* NDUPC001 showed antibacterial activity against *S. aureus* where as the extract in Petroleum ether only showed antibacterial activity against *E. coli* (Table- 1). The methanol extract of *Fischerella muscicola* NDUPC001 showed the maximum antibacterial activity of 14.0±1.12 mm (Table-1) against *S. aureus*. Thin layer chromatography of crude extract in methanol was performed, and two separate spots were produced (Fig. 2) indicating two types of compounds in the extract.

IV. Discussion

Antibacterial activities of cyanobacteria are well established. Scores of researcher have reported antibacterial nature of cyanobacteria and the isolated variety of bioactive compounds. *Lyngbya* species have been searched extensively for secondary metabolites with the isolation of three hundred twenty-six secondary metabolites [7]. Research on antibacterial activities of *Fischerella* species is very less. Ghasemi et al., [15] have isolated and characterized Parsiguine, a novel antimicrobial substance from *Fischerella ambigua*. There are no reports of antibacterial activities of *Fischerella muscicola* . We studied antibacterial activities of *Fischerella muscicola* NDUPC001, isolated from agricultural fields of Varanasi. Significant antibacterial activity of14.0±1.12 mm (Table-1) against *S. aureus* was found. Thin layer chromatography analysis showed two types of compounds in methanol extract. Various studies have demonstrated that cyanobacteria produce the variety of antimicrobial compounds and some strains had more than one antimicrobial compounds. In our finding, the effective antibacterial agent against *S. aureus* is being produced by *Fischerella muscicola* NDUPC001 and Methanol is the best solvent for its extraction.

Table :1 Antibacterial activity of various extracts of *Fischerella muscicola* NDUPC001 on *S. aureus* and *E. coli* ±Represent standard Error and NZ for no zone of inhibition

S.N.	Organic solvents & Antibiotic	Effective Zone of Inhibition (In mm)	
		S. aureus	*E. coli*
1	Ethanol	5.0±0.86	NZ
2	Petroleum ether	3.33±0.54	10±0.58
3	Acetone	8.0±0.55	NZ
4	Methanol	14.0±1.12	NZ
5	Chloroform	NZ	NZ
6	Ampicillin	7.33±0.86	6.35±0.76

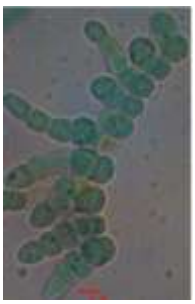

Fig.1 Micrograph of *Fischerella muscicola* NDUPC001 (scale bar 5µm).

Fig. 2 Thin layer chromatogram showing spots produced by cyanobacterial extract.

V. Conclusion

Some secondary metabolites having diverse antibacterial activity have been isolated and characterized from cyanobacteria. Crude extract in methanol of *Fischerella muscicola* NDUPC001 showed approximately double the antibacterial activity than the standard antibiotic against *S. aureus*. Two type compounds are present in the methanolic crude extract of *Fischerella muscicola* NDUPC001. Methanol was the best solvent for extraction of potent active compounds. Plan of this research work will be isolation and characterization of the active compounds.

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