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Research Article

ANTIBACTERIAL ACTIVITY OF TWO SOIL CYANOBACTERIA NOSTOC ELLIPSOSPORUM NDUPC002 AND NOSTOC SP. NDUPC003

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ABSTRACT

Antibacterial activity of two cyanobacterial strains i.e. *Nostoc ellipsosporum* NDUPC002 and *Nostoc* sp. NDUPC003 was studied. Both cyanobacterial strains were isolated from agricultural fields of Varanasi, India. Crude extracts of both strains in five solvents i.e. Ethanol, Petroleum ether, Acetone, Methanol, and Chloroform was screened against two human pathogenic bacteria i.e. *S. aureus* and *E. coli*. Crude extracts of each strain show differential antibacterial response to test organisms. Crude extract in organic solvents i.e. Ethanol, Petroleum ether, Methanol and Acetone of *Nostoc ellipsosporum* NDUPC002 showed antibacterial activity against *S. aureus* where as the extract in Petroleum ether and Methanol only showed antibacterial activity against *E. coli*. Ethanol extract of *Nostoc ellipsosporum* NDUPC002 showed the maximum antibacterial activity of 12.67±0.58 mm against *S. aureus*. Crude extract, only in three organic solvents i.e. Ethanol, Methanol & Acetone of *Nostoc* sp. NDUPC003 showed antibacterial activity against *S. aureus* and *E. coli* with maximum antibacterial activity (6.0±0.42 mm) in acetone extract against *E. coli*. Findings of the experiment suggest that ethanol extract of *Nostoc ellipsosporum* NDUPC002 can be used for mining of antibacterial agent against *S. aureus*.

INTRODUCTION

Cyanobacteria are an ancient group of morphologically diverse, gram negative photoautotrophic prokaryotes. They are cosmopolitan in distribution, including extreme habitats of the world. Some of the cyanobacteria are rich in essential nutrients like provitamins, minerals, proteins and polyunsaturated acids (Miranda *et al*., 1998). *Spirulina platensis* nutritionally rich cyanobacteria are harvested from centuries from Chad Lake in Africa and Texcoco Lake in Mexico for use as a source of food (Vonshak, 1997). Secondary metabolites are low molecular weight organic molecules that have diverse biological activities. They are not required for normal growth and development of organisms but facilitate the survival of the organism. Antibacterial, antifungal, anticancerous, immunosuppressants, herbicidal and cholesterol lowering properties of secondary metabolites are well established. Chemical nature of important secondary metabolites are polyketides, alkaloids, terpenoids, shikimate derived molecules and amino glycosides. Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial and antifungal (Krietlow *et al*., 1999; Mundt *et al*., 2001). Asthana *et al*. (2008) have isolated and characterized antibacterial entity from Antarctic cyanobacterium *Nostoc* CCC537. Culture crude extract in Chloroform 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* (Malathi *et al*., 2015). Reehana *et al*. (2012) studied the antibacterial properties of *Spirulina subssalsa* NTRI 02, *Oscillatoria pseudogeminata* NTRI 03 and *Phormidium corium* NTRI 04.

The emergence of antibiotic resistant bacteria is a serious problem to the whole world. Twenty-three thousand people of US died in 2013 due to infection of antibiotic resistance bacteria (CDC report 2013). Hence there is an urgent need for discovery of new antibacterial compounds. Cyanobacteria are the rich source of secondary metabolites and have great potential for drug discovery (Singh *et al*., 2005; Tan, 2007; Dixit and Suseela, 2013). The present study was framed to screen cyanobacteria isolated from Varanasi, India for its antibacterial properties.

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MATERIALS AND METHODS

Isolation, Purification, and cultivation of cyanobacteria

Soil samples were collected from agricultural fields of Varanasi and transported to Lab. Soil samples were powdered and utilized for enrichment studies in BG-11 medium without nitrogen supplementation. The soil samples placed in sterile Petri dishes and moist with sterilized BG-11 medium (Stanier, 1971) without nitrogen supplementation. The Petri dishes placed in culture room maintained at 28 ± 2^0 C and illuminated with fluorescent light of 12 Wm $^{-2}$. The Petri plates were regularly monitored for colonization and observed microscopically. Standard plating/ streaking techniques used for isolation and purification of cyanobacterial strains (Stanier, 1971). Cyanobacterial strains grown in BG-11 liquid medium without nitrogen supplementation (Stanier, 1971) 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 strain was viewed at 400x and 1000x using Olympus 21Xi microscope. The nature of filament, shape, and size of the vegetative cell, heterocysts, and Akinete was analyzed with the help of Magnus PRO Micromeasurement & Image analysis software and assigned to cyanobacterial species following taxonomic descriptions provided in the literature (Castenholz, 2001; Desikachary, 1959; Rippka *et al*., 1979). The identity of the isolates was further confirmed by sequencing of Partial 16S rRNA gene. The sequence of both strains was submitted to GenBank of NCBI with accession No.-JX912574.2 (*Nostoc ellipsosporum* NDUPC002) and JX966104.3 (*Nostoc* sp NDUPC003). The both strains were deposited at NAIMCC (NBAIM), Mau, India (Accession No. NAIMCC-C-000122 for *Nostoc ellipsosporum* NDUPC002 and NAIMCC-C-000123 for *Nostoc* sp NDUPC003).

Preparation of cyanobacterial extract

After the incubation of 25 days, cultures were harvested by centrifugation at 5000 rpm for 10 minutes, and biomass was dried in the hot-air oven at 60^0 c for 24 hrs. The biomass extracted by mixing well in the organic solvent. 250 mg pellet 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

The antibacterial activities of cyanobacterial extracts were determined by agar disk diffusion assay (Bauer *et al*., 1966). The Bacterial strains of *E.coli* and *S. aurious* were test organisms. Both bacterial strains were obtained from Dept. of Medicine, IMS, BHU, Varanasi, India. The sterilized MHA medium was poured into Petri plates, allowed to cool and solidify. 100 µl of bacterial suspension was poured in each Petri plates and spread with L- shaped spreader. Three filter paper disks (6 mm), saturated with 25 µl 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 35^0 c for 24 hrs. Inhibition zone (Excluding the diameter of filter paper disk) produced around the disks were measured. The Same protocol was followed for standard antibiotic Ampicillin and concentration of standard antibiotic was 10 µg/ ml. The mean and standard error was calculated.

RESULTS AND DISCUSSION

Nostoc ellipsosporum NDUPC002 and *Nostoc* sp NDUPC003 (Fig. 1) were isolated from agricultural fields of Varanasi, India and characterized by morphological as well as molecular methods (16S rDNA).

Figure 1 Micrograph of (A) *Nostoc ellipsosporum* NDUPC002 (B) *Nostoc* sp. NDUPC003

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 conventional antibiotic. Crude extracts of each strain show differential antibacterial response to test organisms (Table- 1). Crude extract in organic solvents i.e. Ethanol, Petroleum ether, Methanol and Acetone of *Nostoc ellipsosporum* NDUPC002 showed antibacterial activity against *S. aureus* where as the extract in Petroleum ether and Methanol only showed antibacterial activity against *E. coli* (Table- 1). Ethanol extract of *Nostoc ellipsosporum* NDUPC002 showed the maximum antibacterial activity of 12.67±0.58 mm (Table-1) against *S. aureus*. Crude extract, only in three organic solvents i.e. Ethanol, Methanol & Acetone of *Nostoc* sp NDUPC003 showed antibacterial activity against *S. aureus* and *E. coli* (Table- 1) with maximum antibacterial activity (6.0±0.42 mm) in acetone extract against *E. coli* (Table- 1).

Table 1 Antibacterial activity of various extracts of *Nostoc ellipsosporum* NDUPC002 and *Nostoc* sp. NDUPC003 on *S. aureus* and *E. coli*

Cyanobacteria	Organic solvents & Antibiotic	Effective Zone of Inhibition (In mm)	
		S. aureus	*E. coli*
Nostoc ellipsosporum NDUPC002	Ethanol	12.67±0.58	NZ
	Petroleum ether	3.0±0.46	7.0±0.65
	Acetone	10±0.82	NZ
	Methanol	2.33±0.73	6.0±0.86
	Chloroform	NZ	NZ
Nostoc sp NDUPC003	Ethanol	2.33±0.25	4.67±0.66
	Petroleum ether	NZ	NZ
	Acetone	NZ	6.0±0.42
	Methanol	3.0±0.73	NZ
	Chloroform	NZ	NZ
Control	Ampicillin	7.33±0.86	6.35±0.76

± Represent standard Error and NZ for no zone of inhibition

Cyanobacteria are the rich source of secondary metabolite with diverse antimicrobial activities. A large number of cyanobacteria from different habitats have shown antibacterial activity against wide range of human pathogenic bacteria. Ghasemi *et al*. (2003) have reported antibacterial activity of supernatant and methanolic extract of different strains of *Fischerella* sp., *Stigonema* sp., *Hapalosiphon* sp and *Nostoc* sp. against six human pathogenic bacteria. Yadav *et al*. (2012) studied the antibacterial activity of methanol and chloroform extract of different strains of *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp., *Microcystis aeruginosa* and *Scytonema* Br. 1 against six strains of human pathogenic bacteria. Madhumathi *et al*. (2011) reported antibacterial activity in Ethanol, Acetone, Diethyl ether and Methanol extract of *Oscillatoria latevirens*, *Phormidium corium*, *Lyngbya martensiana*, *Chrococcus minor* and *Microcystis aeruginosa* against eight strains of human pathogenic bacteria. Similarly, scores of researchers have reported antibacterial nature of cyanobacteria. In all findings, antibacterial activity was based on cyanobacterial strain and organic solvent used for extract. Two cyanobacterial strains i.e. *Nostoc ellipsosporum* NDUPC002 and *Nostoc* sp NDUPC003 were screened with extract in five organic solvents i.e. Ethanol, Petroleum ether, Acetone, Methanol and Chloroform against two human pathogenic strains of bacteria. The magnitude of bacteriocidal 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 *Nostoc ellipsosporum* NDUPC002 showed the maximum antibacterial activity of 12.67±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. Most of them are polyketides, alkaloids, terpenoids, shikimate derived molecules and amino glycosides. In our finding, the effective antibacterial agent against *S. aureus* is being produced by *Nostoc ellipsosporum* NDUPC002 and ethanol is the best solvent for its extraction.

CONCLUSION

Cyanobacteria are the rich source of secondary metabolites having diverse antibacterial activity. Some of them have been isolated and characterized. Crude extract in ethanol of *Nostoc ellipsosporum* NDUPC002 showed approximately double the antibacterial activity than the standard antibiotic against *S. aureus*. Ethanol was the best solvent for extraction potent active compound. Future plan of this research work will be isolation and characterization of the active compound.

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