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Antibacterial activity of two soil cyanobacteria *Nostoc polludosum* and *Cylindrospermum licheniforme*

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Abstract

Antibacterial activity of two cyanobacterial strains i.e. *Nostoc polludosum* and *Cylindrospermum licheniforme* 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 were screened against two human pathogenic bacteria i.e. *S. aureus* and *E. coli*. Crude extracts of each strain showed the differential antibacterial response to test organisms. Crude extract only in Acetone of *Nostoc polludosum* showed antibacterial activity against *E. coli* and no antibacterial activity was noticed against *S. aureus*. Acetone extract of *Nostoc polludosum* showed maximum inhibition zone of 11.3±0.45 mm against *E. coli* which is near approximately twice the antibacterial activity due to the standard antibiotic. Crude extract, only in four organic solvents i.e. Ethanol, Methanol, Acetone & Petroleum ether of *Cylindrospermum licheniforme* showed antibacterial activity against *S. aureus* and *E. coli*, but in all extracts, the antibacterial activity was less than the standard antibiotic. Findings of experiment suggested that acetone extract of *Nostoc poludossum* was suitable for mining of antibacterial agent against *E. coli.*

Key words: Antibacterial activity, Cyanobacteria, *Nostoc polludosum*, *Cylindrospermum licheniforme*

Introduction

Cyanobacteria are well known, phototrophic prokaryote. They have the wide range of distribution including the extreme habitats of the world. Cyanobacterial pigments are now used as nutritional ingredients, natural dyes, cosmetics, pharmaceuticals and fluorescent markers in biomedical research (Venugopal et al., 2005). Algae are the rich source of antioxidants including ascorbic acid, reduced glutathione, phenols, and flavonoids (Wu et al., 2010). Cyanobacteria are the rich source of secondary metabolites and their potential in the pharmaceutical, immunomodulatory, bioregulatory and therapeutic use have been established (Nagle and Wedge, 2002; Volk and Furkert, 2006). Some screening results demonstrate cyanobacteria as the potential source of new antibiotic and pharmacologically active compounds (Browitzka, 1995; Fish and Codd, 1994; Jaiswal, 2011). Only a few no. of cyanobacteria isolated from Varanasi have been investigated for antibacterial properties. Hence, this experiment was designed to investigate antibacterial activity of two cyanobacteria *Nostoc polludosum* and *Cylindrospermum licheniforme* isolated from Varanasi, India.

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 were placed in sterile Petri dishes and moistened with sterilized BG-11 medium (Stanier, 1971) without nitrogen supplementation. The Petri dishes were 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 were used for isolation and purification of cyanobacterial strains (Stanier, 1971). Cyanobacterial strains were grown in BG-11 liquid medium without nitrogen supplementation 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

Identification of isolated cyanobacteria was done by morphological 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).

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 was subjected to extraction by mixing well in the organic solvent. 200 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. aureus* were used as 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 the equal distance in each Petri plates. Petri plates were incubated at 35^0 c for 24 hrs. Inhibition zone (Excluding the diameter of filter paper disk) produced around the disk was measured. The Same protocol was followed for standard antibiotic Ampicillin and concentration of standard antibiotic was 10 µg/ ml. Each experiment was performed in triplicate, in aseptic condition. The mean and standard error was calculated.

Results

Nostoc polludosum was isolated from agricultural fields of Varanasi, India. The cyanobacterial strain was filamentous, unbranched and heterocystous (Fig.– 1). Filaments were short and entangled to each other. Trichomes 3-3.5 µm broad, cells barrel shaped, 3.06-5.07 µm long, heterocyst spherical to subspherical, apical as well as intercalary, 4.6 - 5.4 µm broad, 4.6-8.4 µm long, spore oval, always formed away from heterocyst, 4-5.5 µm broad and 6-8 µm long (Fig. – 1) with the smooth wall. Morphological characters closely matched with *Nostoc polludosum* (Desikachary, 1959). Hence strain was identified as *Nostoc polludosum*.

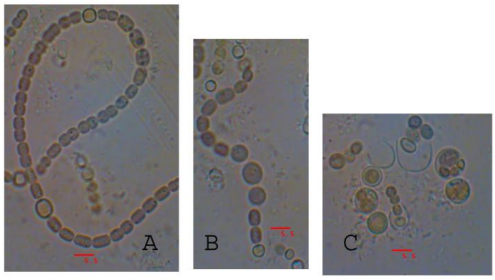

Fig. 1 Micrograph of *Nostoc polludosum* (scale bar 5.5µm).
(**A**- filament with intercalary heterocyst, **B**- filament with spores and **C-** Germination of spores)

Cylindrospermum licheniforme was isolated from agricultural fields of Varanasi, India. The cyanobacterial strain was filamentous, unbranched and heterocystous (Fig.–2). Heterocyst is always restricted to both apical ends of filaments (Fig. –2). Trichomes, 4-5 µm broad, more or less run parallel, pale blue green & constricted at cross wall, cells quadrate to cylindrical, 4-7 µm long, heterocyst oblong, 4-6 µm broad and 7-15 µm long, spores just after the heterocyst, ellipsoidal, oblong to ventricose elliptic, 09-14 µm broad, 20-30 µm long with reddish brown epispore.

Morphological characters closely matched with *Cylindrospermum licheniforme* (Desikachary, 1959). Hence strain was identified as *Cylindrospermum licheniforme*.

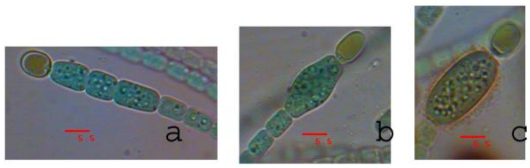

Fig. 2 Micrograph of *Cylindrospermum licheniforme* (scale bar 5.5µm). (**a**- filament with heterocyst, **b**- filament with young spore and **c-** Mature spore)

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 the standard 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 chloroform of *Nostoc polludosum* did not show antibacterial activity against *E. coli* (Table-1). Acetone extract of *Nostoc polludosum* only showed the maximum antibacterial activity of 11.3±0.45 mm (Table-1) against *E. coli*. Crude extract, only in four organic solvents i.e. Ethanol, Methanol, Ptroleum ether & Acetone of *Cylindrospermum licheniforme* showed antibacterial activity against *S. aureus* and *E. coli* (Table-1) with maximum antibacterial activity (5±0.85mm) in acetone extract against *E. coli* (Table-1).

Table: 1 Antibacterial activity of various extracts of *Nostoc polludosum* and *Cylindrospermum licheniforme* on *S. aureus* and *E. coli*

Cyanobacteria	Organic solvents & Antibiotic	Effective Zone of Inhibition (In mm)	
		S. aureus	***E. coli***
Nostoc polludosum	Ethanol	NZ	NZ
	Petroleum ether	NZ	NZ
	Acetone	NZ	11.3±0.45
	Methanol	NZ	NZ
	Chloroform	NZ	NZ
Cylindrospermum licheniforme	Ethanol	3.0	NZ
	Petroleum ether	2.0	NZ
	Acetone	5±0.85	NZ
	Methanol	NZ	5±0.65
	Chloroform	NZ	NZ
Control	Ampicillin	7.33±0.86	6.35±0.76

± Represent standard Error and NZ for no zone of inhibition

Discussion

Production of antibacterial, antifungal and antialgal agents by cyanobacteria are well established. Most of them are secondary metabolites and may be extracellular (Jaki et al., 1999, 2000a) or intercellular (Jaki et al., 2000b; Asthana et al., 2006). Helen Diana et al., (2014) studied antibacterial activity of ten strains of cyanobacteria against seven human pathogenic bacteria. Madhumathi et al. (2011) reported antibacterial and antifungal activity of *Oscillatoria latevirens, Phormidium corium, Lyngbya martensiana, Chrococcus minor,* and *Microcystis aeruginosa*. *Calothrix braunii* exhibited maximum antifungal activity 12.66 mm against *A. fumigatus* in the Hexane extract and antibacterial activity was found high 17.66 mm in Chloroform extract against *S. aureus* (Malathi et al., 2015). Reports of antibacterial nature of cyanobacteria are continuously increasing. Antibacterial nature of cyanobacteria was the strain and organic solvent dependent in all investigations. *Nostoc polludosum* and *Cylindrospermum licheniforme* were isolated from agricultural fields of Varanasi and screened with extract in five organic solvents i.e. Ethanol, Petroleum ether, Acetone, Methanol and Chloroform against two human pathogenic strains of bacteria. Bactericidal activity of *Cylindrospermum licheniforme* was less than the standard antibiotic. *Nostoc polludosum* showed bactericidal activity only in acetone extract against one of most common human pathogenic bacteria *E. coli*

and it was near about double to the standard antibiotic. Findings of experiment suggested that acetone extract of *Nostoc poludossum* was suitable for mining of potent antibacterial agent against *E. coli.*

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