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PARTIAL PURIFICATION OF BIOACTIVE COMPOUNDS FROM DIFFERENT CYANOBACTERIAL STRAINS AND ITS BIOLOGICAL POTENTIAL

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ABSTRACT

Cyanobacteria (blue-green algae) are rich sources of structurally novel and biologically active metabolites. The present study deals with the partial purification of bioactive compounds from the methanolic extract of seven cyanobacterial strains (*P.boryanum, Cylindrospermum, Nostoc muscorum, Phormidium, Anabaena variabilis, Oscillatoria* sp and *Chroococcus* sp.) by Thin Layer Chromatography (TLC). The active bands isolated from the chromatogram were tested for bioactivity against *Staphylococcus epidermidis*. The number of bands and its bioactivity varied with the type of cyanobacterial strains. Maximum number of bands for different class of compounds was obtained in case of *Chroococcus* (8 bands) and *N.muscorum* (7 bands). The tested bacterium responded differently to the active bands isolated. Band 2 from *N.muscorum* (NM-2) and *Chroococcus* (chro-2) showed maximum zone of inhibition

Keywords: antibacterial, antioxidant, bioactive compounds, cyanobacteria, TLC

INTRODUCTION

The present scenario of emergence of multiple drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial compounds with novel

mechanisms of action and diverse chemical structures for new and reemerging infectious diseases. Natural pharmaceutical compounds can be derived from Algae, Cyanobacteria, plants and other microbes. Most of these compounds are classified as secondary metabolites or secondary products and they originate from primary precursors. Secondary compounds are not believed to have any essential role in the basic life process of the organism. However, they are ecologically important in the reaction of the organism to the environment [1].

The rate of discovery from traditional microbial drug producers like actinomycetes, which are in the focus of pharmaceutical research for decades, is decreasing and it is the time to turn to cyanobacteria and exploit their potential. This is of paramount importance to fight increasingly resistant pathogens and newly emergent diseases [2]

Cyanobacteria are morphologically, physiologically, and metabolically very diverse group, which makes them as a promising group of organisms for research on drugs discovery. Cyanobacteria are considered to be a rich source of novel metabolites of a great importance from a biotechnological and industrial point of view. [3]. These organisms produce a range of compounds exhibiting antibacterial, antiviral, antituberculosis, antifungal, anti-inflammatory, antitumour and cytotoxic activities [4] [5] [6].

Marine cyanobacteria in particular have been considered a prominent source of structurally diverse and biologically active natural products [7]. Novel biochemically active natural products, with potential benefits against cancer, can be isolated from these organisms. Although several compounds were found to inhibit cell growth in a large variety of cancer cell line [8]. The production of bioactive compounds with commercial and medical applications has also increased interest in these organisms [9].

Due to the significant role of cyanobacteria, it was considered worthwhile to examine antimicrobial activities for possible biotechnological and pharmaceutical applications. There is a growing interest in natural additives as potential antimicrobials. In this study, TLC based separation of methanolic crude extract of *Anabaena doliolum, P.boryanum, Cylindrospermum, Nostoc muscorum* and *Phormidium* was performed and antibacterial activity of the active bands obtained after TLC was carried out. All the bands were bioassayed for antibacterial potential against *Staphylococcus epidermidis*

MATERIALS AND METHODS

Collection of cyanobacterial strains and growth conditions

Six axenic cyanobacterial strains (*Anabaena variabilis*, *Oscillatoria* sp., *Chroococcus* sp. *Nostoc* sp., *Plectonema boryanum* and *Scytonema* sp) were selected in the present study. *Anabaena variabilis* was obtained from Dr. Abhishek Chris, Department of Biological Sciences, Allahabad Agricultural Institute, Deemed University, Allahabad. Rest of the strains were isolated from rice paddy fields of different cities of UP. India. The strains were purified and maintained in the cyanobacterial culture room, Department of Biotechnology, Integral University, Lucknow. Strains were grown in BG-11 medium with or without extra supplementation of combined nitrogen depending upon the heterocystous and non heterocystous cyanobacteria used. Cyanobacteria were grown for twenty days in their respective growth media before experimental use and maintained in the culture room at 27 ± 2 ℃ under 75µmol $m^{-2}s^{-1}$ photon flux density (PFD) with a photoperiod of 14:10 h.

Detection and partial purification of antibacterial entity

Extract preparation

For the detection and purification of antibacterial compounds dried cyanobacterial strains (1gm) was homogenized in 10 ml methanol (HPLC grade, Merck, India) and was extracted on a rotatory shaker in an Eherlyenmeyer flask at 40 rpm overnight [10]. The crude extracts were then filtered through Whatman No. 1 filter paper and concentrated in vacuum at 40 °C using a rotary evaporator. The concentrated extract was then dried aseptically with the help of drier. The methanol extract thus obtained was redissolved in 1ml methanol.

Partial purification of the antibacterial entity

For the TLC analysis, 25 µl of the extract was loaded on the TLC (Silica gel 60) plates (Merck, Germany). The solvent ratio used for the separation of the compounds was Chloroform: methanol (4:1, v:v). UV-transillumination of the plates at 365 nm revealed several bands. These bands were eluted separately with a minimum amount of methanol. All the bands were bioassayed for antibacterial potential against *Staphylococcus epidermidis* (NCIM 2493) (National Chemical Laboratory, Pune, India) by disc diffusion method on Mueller-Hinton (MH) agar [11].

Test Bacteria

In the present study *Staphylococcus epidermidis* was used for testing antibacterial activity. The strain was obtained from the

National Chemical Laboratory (NCL), Pune, India.

Antibiotic sensitivity testing

The antibiotics Erythromycin, Amoxicillin and Chloramphenicol were used to test the sensitivity of bacterial strain by the standard disc diffusion method of Baur *et al.* [11].The potency of each antibiotic was 10µg per disc.

Antibacterial assay

The antibacterial assay was performed by agar well diffusion method [11]. The 0.1 ml of diluted inoculum (10^5 CFU/ ml) of test bacteria was spread on Mueller-Hinton (MH) agar plates. Wells of 5 mm diameter were punched into the agar medium and poured with 25 µl of extract prepared in DMSO (10 mg ml-1). DMSO without extract was used as blank. After incubation for 24 h at 37 °C. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.

Statistical analysis

The Data were statistically analyzed by using spss version 10. The values are mean ± S.E. of three measurements.

RESULTS AND DISCUSSION

In the present study, the antibacterial activity of the different bands of the thin layer chromatogram of the methanolic extract of seven cyanobacterial strains (*P.boryanum, Cylindrospermum, Nostoc muscorum, Phormidium, Anabaena variabilis, Oscillatoria* sp and *Chroococcus* sp.) was tested against *Staphylococcus epidermidis.*

The TLC results of methanolic extract of *P. boryanum* indicate the presence of four bands under ultra violet light (365 nm) illumination (Figure 1). Out of these four bands (PB-1, PB-2, PB-3 and PB-4) only one band PB-3 with Rf value – 0.52 was found to be active against *S. epidermidis* with 10 mm zone of inhibition (Table I).

The TLC analysis revealed the presence of two bands in case of *cylindrospermum* (Cyli-1 and Cyli- 2) with Rf value- 0.08 and 0.58, respectively. One band Cyli-1 has shown 10 mm zone of inhibition against *S. epidermidis* (Table II).

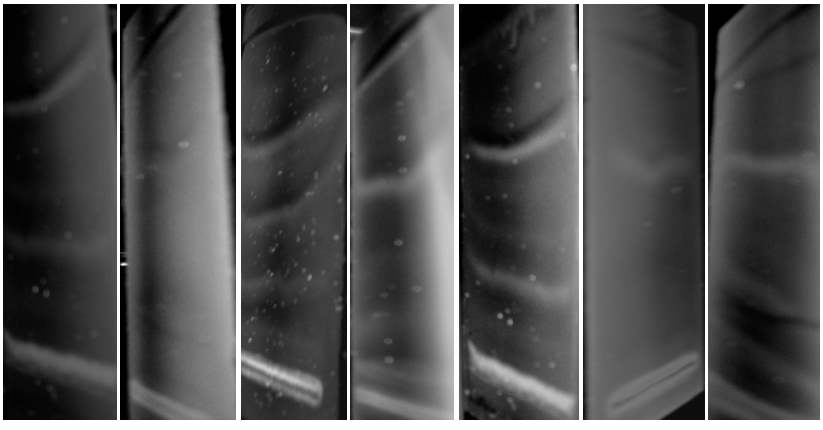
The antibacterial activity of bands (NM-1, NM-2, NM-3, NM-4, NM-5, NM-6 and NM-7) isolated from methanol extract of *Nostoc muscorum* is shown in Table III. From all the bands isolated only three bands (NM-2, NM-3 and NM-4) with Rf value 0.19, 0.32 and 0.43 respectively, were found to be active against *S. epidermidis*.

The results of antibacterial activity of six bands isolated from methanol extract of *Phormidium* are shown in Table IV. Out of six bands only one band Phor- 2 (Rf value-0.30) has shown antibacterial activity with 9mm inhibitory zone against *S. epidermidis.*

TLC analysis of methanol extract of *Anabaena virabilis* revealed the presence of eight bands when observed under UV illumination (Figure 2). The Table V result of antibacterial activity, showed the presence of two active bands (AV-2 and AV-3) which were found to be effective against *S. epidermidis* with inhibition zone of 9 and 8 mm, respectively. However, other bands (AV-1, AV-4, AV-5, AV-6, AV-7 and AV-8) have not shown any activity against *S. epidermidis*.

Methanol extract of *Oscillatoria sp.* when analysed by TLC showed the presence of three bands (OS-1, OS-2 and OS-3) with Rf value 0.17, 0.23 and 0.48, respectively (Figure 2). Out of these three bands, two bands (OS-1 and OS-2) were found to be effective against *S. epidermidis* with inhibitory zone of 6 and 9 mm, respectively (Table VI)

TLC analysis of *Chroococcus* methanolic extract showed the presence of eight bands under UV illumination (Figure 2). Antibacterial analysis of all these bands, revealed that only two bands Uni-1 and Uni-2 (Rf vaue- 0.10 and 0.02) were active against *S. epidermidis* with zone of inhibition 8 and 10 mm, repectively (Table VII).



(a) (b) (c) (d) (e) (f) (g)

Figure 1: TLC separation of methanolic crude extract of (a) *P.boryanum*, (b) *Cylindrospermum*, (c) *Nostoc muscorum* (d) *Phormidium* (e) *Anabaena variabilis* (f) *Oscillatoria* (g) *Chroococcus* using Chloroform: Methanol (4:1) as mobile phase, visualized under UV light 365 nm.

Table I: Antibacterial activity of bands isolated through TLC from *P.boryanum* against *S. epidermidis*

Strain	Zone of inhibition (mm) Bands	Rf value	PB* (100µg /disc)
P.boryanum	PB-1	0.166	ND
	PB-2	0.291	ND
	PB-3	0.525	10mm
	PB-4	0.75	ND

PB*- Bands eluted from TLC of methanol extract of *P.boryanum*

Table II: Antibacterial activity of bands isolated through TLC from *Cylindrospermum* against *S. epidermidis*

Strain	Zone of inhibition (mm) Bands	Rf value	Cyli* (100µg /disc)
Cylindrospermum	Cyli-1	0.08 cm	10 mm
	Cyli-2	0.584 cm	ND

Cylin*- Bands eluted from TLC of methanol extract of *Cylindrospermum*

Table III: Antibacterial activity of bands isolated through TLC from *Nostoc muscorum* against *S. epidermidis*

Strain	Zone of inhibition (mm) Bands	Rf value	NM* (100µg /disc)
Nostoc muscorum	NM-1	0.041 cm	ND
	NM-2	0.191 cm	10 mm
	NM-3	0.325 cm	6 mm
	NM-4	0.433 cm	5 mm
	NM-5	0.642 cm	ND
	NM-6	0.716 cm	ND
	NM-7	0.875 cm	ND

NM*- Bands eluted from TLC of methanol extract of *Nostoc muscorum*

Table IV: Antibacterial activity of bands isolated through TLC from *Phormidium* against *S. epidermidis*

Strain	Zone of inhibition (mm) Bands	Rf value	Phor* (100µg /disc)
Phormidium	Phor-1	0.114 cm	ND
	Phor-2	0.307 cm	9 mm
	Phor-3	0.189 cm	ND
	Phor-4	0.625 cm	ND
	Phor-5	0.772 cm	ND
	Phor-6	0.840 cm	ND

Phor*- Bands eluted from TLC of methanol extract of *Phormidium*

Table V: Antibacterial activity of bands isolated through TLC from *Anabaena virabilis* against *S. epidermidis*

Strain	Bands	Rf value	Zone of inhibition(mm) AV* (100µg /disc)
Anabaena virabilis	AV-1	0.083 cm	ND
	AV-2	0.125 cm	9 mm
	AV-3	0.416 cm	8 mm
	AV-4	0.7 cm	ND
	AV-5	0.75 cm	ND
	AV-6	0.891 cm	ND
	AV-7	0.961 cm	ND
	AV-8	0.933 cm	ND

AV*- Bands eluted from TLC of methanol extract of *Anabaena virabilis*

Table VI: Antibacterial activity of bands isolated through TLC from *Oscillatoria sp* against *S. epidermidis*

Strain	Bands	Rf value	Zone of inhibition(mm) OS* (100µg /disc)
Oscillatoria sp.	OS-1	0.173 cm	6 mm
	OS-2	0.234 cm	9 mm
	OS-3	0.486 cm	ND

OS*- Bands eluted from TLC of methanol extract of *Oscillatoria sp*.

Table VII: Antibacterial activity of bands isolated through TLC from *Chroococcus sp*. against *S. epidermidis*

Strain	Bands	Rf value	Zone of inhibition(mm) Chro* (100µg /disc)
Chroococcus sp.	Chro-1	0.104 cm	8 mm
	Chro-2	0.02 cm	10 mm
	Chro-3	0.28 cm	ND
	Chro-4	0.4 cm	ND
	Chro-5	0.48 cm	ND
	Chro-6	0.528 cm	ND
	Chro-7	0.584 cm	ND
	Chro-8	0.8 cm	ND

***Chro*- Bands eluted from TLC of methanol extract of *Oscillatoria sp*.**

Therefore, on the basis of above results the cyanobacterium *Anabaena doliolum* and *Tolypothrix* sp. were selected for biomass study under various carbon and nitrogen sources and light intensities so that the production of bioactive metabolites could be enhanced.

Cyanobacteria are photosynthetic prokaryots and produce a high variety of secondary metabolites that often have potent biological activities [12]. The aim of this study was to exploit the presence of a wide array of bioactive compounds in the crude methanolic extracts of seven cyanobacterial strains against gram positive *S.epidermidis* and to assess the biological potential of compounds partially purified by TLC. Maximum number of bands was observed in case of

Chroococcus (8) and *Anabaena variabilis* (8) followed by *N. muscorum* (7). [13] also reported the antimicrobial activity of isolated Beta glucan from *Chroococus turgidus* against three fungal and three bacterial pathogens.

The findings of [14] indicated that the freshwater cyanobacteria *Anabaenopsis* sp. extracts are promising sources of new bioactive and antioxidative natural products.

CONCLUSION

Though this kind of investigation creates quite a general view of cyanobacterial possibility to produce biologically active compounds still it points out the necessity of exploring cyanobacterial strains as potentially excellent sources of these substances and reveals the most prospective strains for further investigations.

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