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Antibacterial and free radical scavenging potential of some cyanobacterial strains and their growth characteristics

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ABSTRACT

The present study deals with the growth characteristics of several cyanobacterial strains (Anabaena variabilis, Oscillatoria sp., Chroococcus sp. Nostoc sp., Plectonema boryanum and Scytonema sp.), evaluation of their antibacterial activity and free radical scavenging ability. The maximum specific growth rate was noticed in Nostoc sp. and Scytonema sp. followed by Plectonema boryanum, Chroococcus sp. and Oscillatoria sp. whereas, Anabaena variabilis exhibited minimum growth rate. Antibacterial activity of hexane and methanol extracts of cyanobacteria was assayed by disc diffusion method against Staphylococcus epidermidis bacterium. The tested bacterium responded differently to the types of extracts and cyanobacterial strains used. Hexane extracts exhibited more antibacterial potential as compared to methanol. Free radical scavenging activity by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) indicate the maximum antioxidant potential of P. boryanum followed by Scytonema sp. whereas, Nostoc sp. showed least activity. 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.

Key words: antibacterial, antioxidant, bioactive compounds, biomass, cyanobacteria, specific growth.

INTRODUCTION

With an increasing number of bacteria, fungi and viruses developing resistance to commercial antibiotic and its derivatives, the cyanobacteria hold great promise for novel medicines in

modern times as they are found to be rich source of structurally novel and biologically active metabolites [1, 2]. Secondary or primary metabolites produced by these micro-organisms may even prove to be potential bioactive compounds of interest in the pharmaceutical industry [3,4,5]. These include antibacterial compounds which in laboratory tests inhibited bacteria that are responsible for many deadly diseases in humans [6]. Apart from the threats imposed by the microorganisms, cancer and cardiovascular as chronic diseases are among the leading causes of death in the world, where oxidative stress induced by reactive oxygen species (ROS) is one of the foci related to these diseases. Oxidative stress is initiated by ROS, which are highly reactive oxidant molecules that seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation [7]. These free radicals are endogenously generated through regular metabolic activities, lifestyle, and diet. Hence, there is strong evidence that this damage may contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases [8].

The presence of certain substances acting as antioxidant or free radical scavenger may protect the body from the consequences of oxidative stress. Thus, antioxidants play an important role in the protection of cells against oxidative damage caused by ROS [9]. The occurrence of many compounds possessing antioxidant activity in biological systems in higher plants is well documented, while in microalgae little information is available [10].

Since very little attempt has been made to investigate the free radical scavenging and antibacterial potential of the extracts of cyanobacteria. An attempt has been made to screen the cyanobacterial extracts for their bioactive potential as they appear to be largely unexplored and represent a rich opportunity in the search of novel compound of pharmacological importance.

EXPERIMENTAL SECTION

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 °C under 75 μmol $m^{-2}s^{-1}$ photon flux density (PFD) with a photoperiod of 14:10 h.

Growth measurement

Chlorophyll *a* from each sample was extracted in 80% acetone and the content of the pigments was determined from absorbance at 663 nm using the method of Myers and Kratz [11]. Specific growth rate was calculated by using the equation $\mu = [\ln (B_f / B_i)] / 10$. Where B_i is the initial chlorophyll *a* and B_f is the chlorophyll *a* at the end of 10^{th} day of incubation. Productivity was

calculated from the equation P= (Xi -X0) / ti, where P = productivity (mg $L^{-1}day^{-1}$), X0 = initial biomass density (mg L^{-1}), Xi = biomass density at time i (mg L^{-1}) and ti =time interval (h) between X0 and Xi [10].

Extract preparation

Cyanobacterial strains grown for twenty days in the liquid medium were harvested and dried with the help of dryer at room temperature. The dried mass was extracted with hexane and methanol separately by using soxhlet extractor at 40 ^{O}C in order to extract non-polar and polar compounds [12]. The crude extracts were then filtered through Whatman No. 1 filter paper and extracts were subsequently dried under a fan at room temperature.

Test Bacteria

In the present study *Staphylococcus epidermidis* was used for testing antibacterial activity. These strains were 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.* [13]. The potency of each antibiotic was 10 µg per disc.

Antibacterial assay

The antibacterial assay was performed by agar well diffusion method [14]. 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 in an. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.

Determination of free radical scavenging activity

The DPPH radical scavenging capacity of cyanobacterial strain was determined following the method of Williams *et al.*, [15] modified by Miliauskas *et al.*, [16]. 3 mL of freshly prepared DPPH• solution in methanol (6×10^{-5} M) was mixed with 100 μL of methanolic extracts of cyanobacteria. The samples were incubated for 20 min at 37 °C in a water bath, and then the decrease in absorbance at 515 nm was measured (*A*E). A blank sample containing 100 μL of methanol in the DPPH• solution was prepared, and its absorbance was measured (*A*B). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

% inhibition = [(*A*B-*A*E)/*A*B] × 100

where *A*B = absorbance of the blank sample, and *A*E = absorbance of the cyanobacterial extract.

Statistical analysis

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

RESULTS AND DISCUSSION

The results of the present study show growth characteristics of six cyanobacterial strains, their antibacterial and free radical scavenging potential. The strains were examined for their growth pattern in terms of chlorophyll a content (Fig. 1a, b, c, d and e). All the growth phases as well as specific growth rate and productivity showed variation among the strains.

Table 1. Specific growth rate and biomass production (dry mass) of six cyanobacterial strain

Microorganisms	Specific growth rate μ_{max} (day^{-1})	Productivity P_{288} (mg dry massL^{-1} day^{-1})
Anabena variabilis	0.08 ± 0.005	4.3 ± 0.03
Oscillatoria sp.	0.07 ± 0.003	3.8 ± 0.05
Chrococcus sp.	0.09 ± 0.003	6.2 ± 0.08
Nostoc sp.	0.15 ± 0.008	6.8 ± 0.06
Plectonema boryanum	0.13 ± 0.005	8.9 ± 0.08
Scytonema sp.	0.15 ± 0.008	5.2 ± 0.10

The data refer to mean value of three replicates ± SE.
μmax =maximum specific growth rate
P_{288}= productivity in terms of dry mass measured after 288h = 12 days.

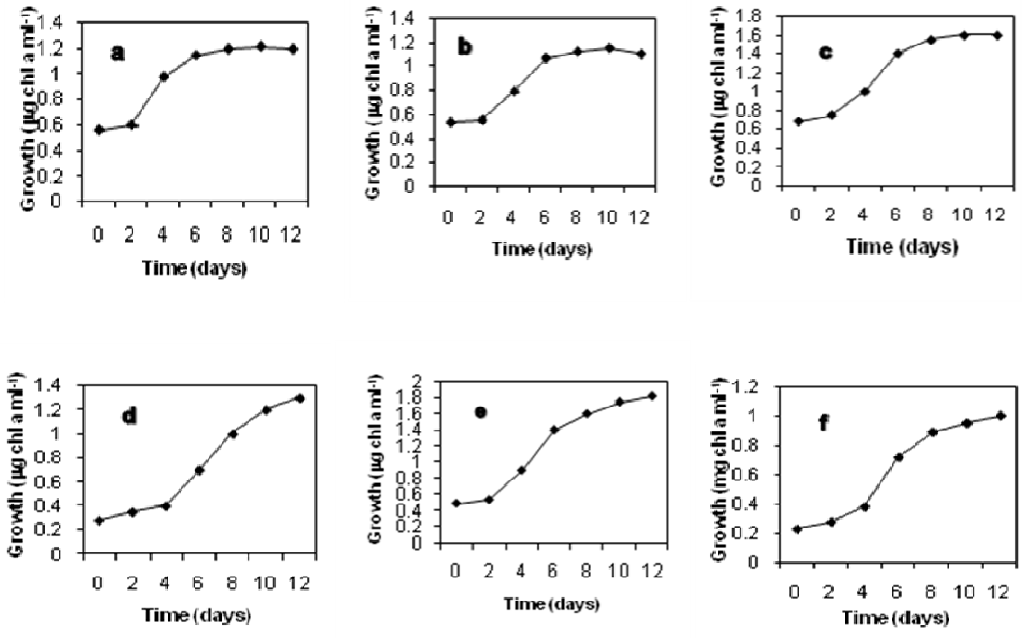

Fig. 1: Growth characteristic of *Anabaena variabilis* (a), *Oscillatoria* sp. (b), *Chroococcus* sp. (c) *Nostoc* sp. (d), *Plectonema boryanum* (e) and *Scytonema* sp (f).

The variation in growth pattern could be explained on the basis of their morphological, nutritional or metabolic differences [8]. Similar to this Tiwari et al., [17] also observed the variation in the dry mass of some cyanobacterial strains isolated from different habitats of Uttar Pradesh under laboratory conditions. *Plectonema boryanum* exhibited greater productivity followed by *Nostoc* sp. whereas least productivity was observed in *Oscillatoria* sp. Greater productivity indicates the high cell density of the culture. The culture with high cell density have several advantages in industrial application as they reduce the expense of extraction and other downstream operations [18].

During the growth microorganisms synthesized metabolites of different nature that is accumulated in the cells or excreted into the medium for the support in growth and metabolic activities. The antimicrobials production ability may be significant not only as a defensive instruments for algae but also as a good source of novel bioactive compounds from a pharmaceutical point of view [19]. The antibacterial potential of the cyanobacterial strains was determined by disc diffusion method and results are presented in figure 2 and 3. Disc diffusion assay showed that *S. epidermidis* was sensitive to hexane extract of all the strains of cyanobacteria. The maximum sensitivity measured in terms of zone of inhibition was noticed against the hexane extract of *Scytonema* (19 mm) followed by *Plectonema boryanum* (17mm) and *Oscillatoria* (14 mm). However, *Nostoc* sp., *A. variabilis* and *Chrococcus* sp. showed inhibitory zone of 12, 12 and 11 mm against the test bacteria, respectively (Fig. 2). As compared to the hexane extract, methanol extract showed less antibacterial potential against the bacteria *S. epidermidis*. The zone diameter of 9, 9, 10, 10, 14 and 15 mm was recorded against *S. epidermidis* with the methanol extract of *Oscillatoria* sp., *Plectonema boryanum*, *A. variabilis*, *Chrococcus* sp. *Nostoc* sp. and *Scytonema* sp., respectively (Fig 3). The antibacterial activity of the extracts could be due to the presence of different chemical agents that may include flavonoids and triterpenoids and other compounds of phenolic nature or free hydroxyl group [18]. Zeeshan et al., [20] reported the presence of certain metabolites such as tannin, alkaloids, protein, and flavonoids in the extract of cyanobacteria. Singh and Chowdhary, [21] also identified antimicrobial agents in alga *Pithophora*.

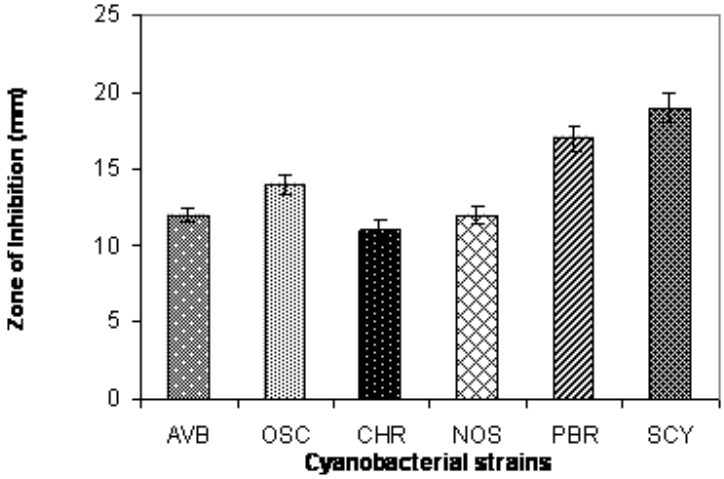

Fig. 2: Antibacterial activity of hexane extract of *Anabaena variabilis* (AVB), *Oscillatoria* sp. (OSC), *Chroococcus* sp. (CHR), *Nostoc* sp.(NOS), *Plectonema boryanum* (PBR) and *Scytonema* sp (SCY) against *S. epidermidis*. Values are means ± SE with *n* = 3

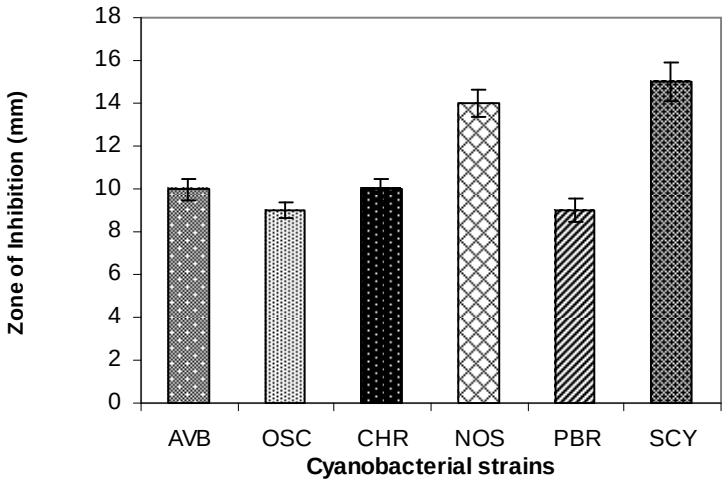

Fig. 3: Antibacterial activity of methanol extract of *Anabaena variabilis* (AVB), *Oscillatoria* sp. (OSC), *Chroococcus* sp. (CHR), *Nostoc* sp.(NOS), *Plectonema boryanum* (PBR) and *Scytonema* sp (SCY) against *S. epidermidis*. Values are means ± SE with *n* = 3

The antioxidant potential of the methanol extracts of different cyanobacteria was determined and the results are presented in figure 4. Among the extracts of different cyanobacteria, *Plectonema boryanum* and *Scytonema* sp. exhibited greater antioxidant activity as it was 30% and 27% inhibition of DPPH than the positive control (25 %) at 50 μg ml^{-1}. Other species which have shown potent radical scavenging activity include *Osillatoria sp., Chroococcus sp.,* followed by *Anabaena variabilis*, and *Nostoc* sp. (Fig.4).

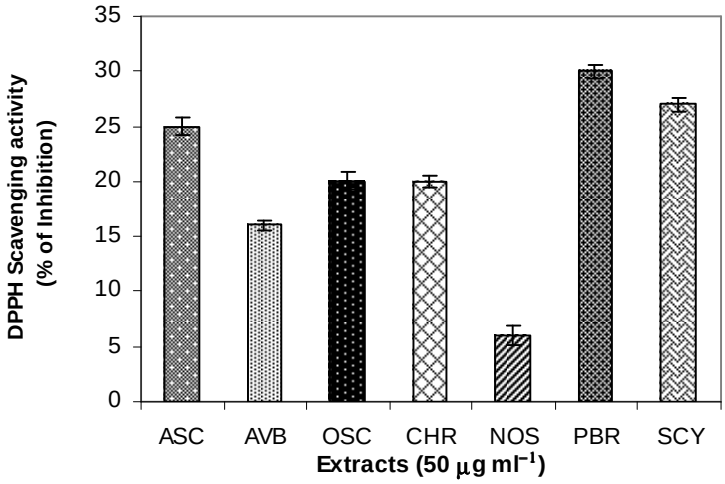

Fig. 4: Effects of cyanobacterial extracts and the ascorbic acid on the scavenging of DPPH. The data represent the percentage DPPH scavenging. All data are expressed as mean ± S.E. (n = 3). ASC = Ascorbic acid, AVB= *Anabaena variabilis*., OSC = *Oscillatoria* sp, CHR = *Chroococcus* sp., NOS= *Nostoc* sp., PBR= *Plectonema boryanum*., SCY= *Scytonema sp.*

Similar to this Abd El-Baky *et al.*, [22], observed pronounced anti-oxidant activity in a crude extracts of *Spirulina maxima*. *Spirulina* and its antioxidant activity are well documented by Abd El-Baky [23], Khan *et al.* [9] and Athukorela *et al.* [24]. The potent antioxidant activity of the

extract of different cyanobacteria might be due to the total phenolics, phycocyanin, triterpenoids present in the extracts [25].

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