In vitro antibacterial activity of laboratory grown culture of *Spirulina platensis*

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Received: 11 July 2007 / Accepted: 26 December 2007

Abstract The hexane, ethyl acetate, dichloromethane, methanol extracts and spent media (extracellular substances) were tested *in vitro* for their antibacterial activity for which one Gram-positive bacterium (*Staphylococcus aureus*) and four Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumoniae*) were used as test organisms. The methanol extract showed more potent activity than other organic extracts, spent medium of the culture exhibited little activity against *E. coli* only. No inhibitory effect was found against *Klebsiella pneumoniae*. The broth microdilution assay gave minimum inhibitory concentrations (MIC) values ranging from 1 to 512 µg/ml. The MIC of methanol extract against *S. aureus* and *E. coli* were 128 µg/ml and 256 µg/ml, respectively.

Keywords Antibacterial activity · *Spirulina platensis* · Minimum inhibitory concentration

Introduction

Pharmaceutical drug discoveries have for most of the past 40 years depended heavily on the process of empirically screening of large number of pure compounds to provide new leads. Historically, actinomycetes have been the most prolific producers of new bioactive metabolites. The cyanobacteria (blue-green algae) are among the oldest phototrophic organisms. Their cultivation without organic substrate can be an economical advantage over the microorganisms. An optimized production of relevant compounds under controlled culture condition is conceivable [1]. The search for cyanobacteria with antimicrobial activity has gained importance in recent years due to growing world wide concern about alarming increase in the rate of infection by antibiotic-resistant microorganisms. Biologically active substances were proved to be extracted by Cyanobacteria. Various strains of Cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial [2, 3], antifungal [4], cytotoxic [5], algacide [6], immunosuppressive [7] and antiviral activities [8]. The aim of study reported here was to investigate the antibacterial activity of different extracts of laboratory grown culture of *Spirulina platensis*.

Material and methods

Preparation of extracts

Spirulina platensis used in the study was collected from National Center for Conservation and Utilization of Blue-green Algae, Indian Agricultural Research Institute, New Delhi

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and maintained in BG-11 growth medium (pH 7) under rotatory conditions and illuminated continuously at a light intensity of 3500 LUX. At the stationary phase of growth (23 days) the culture was harvested, spent medium was collected after filtering the biomass. The collected biomass was dried in a hot air oven at 60°C for 1 h and then placed in solvent leave the mixture for 5 hrs at room temperature and sonicated for 10 min and then centrifuged at 4000 rpm for 10 min. After centrifugation supernatant was collected in a preweighed test tube then concentrated under nitrogen until it was completely dry, again weighed and then resuspended in the appropriate solvent to make the solution of known concentration for the antibacterial assay.

Test microorganisms

The microorganisms used in antibacterial assays were supplied by Institute of Microbial Technology (IMTECH). The species employed include pathogenic Gram-positive bacteria (*Staphylococcus aureus* MTCC-740) and Gram-negative bacteria (*Escherichia coli* MTCC-739, *Pseudomonas aeruginosa* MTCC-741, *Salmonella typhi*, MTCC-733 and *Klebsiella pneumoniae* MTCC-139). The bacterial strains were inoculated on Tryptone Soya Agar (TSA) and incubated for 24 h at 30°C then suspended in saline solution 0.85% NaCl and adjusted to yield approximately 1.0×10^8 – 1.0×10^9 cfu/ml by using spectrophotometer (25% transmittance at 530 nm). Media component were purchased from Hi Media, Mumbai, India. All the chemicals used were of analytical grade.

Antibacterial testing

Agar well Diffusion Assay: *In vitro* antibacterial activity of different crude extracts of *Spirulina platensis* was

evaluated using the agar well diffusion assay [9]. 100 µl of adjusted culture was mixed with 100 ml of Muller Hinton Agar (MHA) mixed well and poured 25 ml each into sterile petridishes (90 mm) this was allowed to solidify and then individual plates were marked for the organisms inoculated. After solidification plates were punched to make the well of 6 mm diameter with the help of sterile borer. 100 µl of respective cyanobacterial extracts were pipetted into the well in assay plates. Plates were incubated overnight at 37°C and all the plates were observed for the zone of inhibition, diameter of these zones were measured in millimeters. All the tests were performed under sterile conditions and repeated three times. The solvent control revealed no activity.

Broth micro dilution assay

Minimum inhibitory concentration of active crude extracts were determined by broth microdilution method as recommended by National Committee for Clinical Laboratories Standards [10]. The tests were performed in 96 wells microtiter plates, twofold dilutions of all extracts and standard antibiotic were made in Muller-Hinton Broth (MHB) ranging from 1 to 512 µg/ml. Each inoculum was prepared in the same medium at a density adjusted to a 25% transmittance turbidity standard (10^8 cfu/ml) and diluted to 1:100 for the assay. After inoculation, each well contained approximately 5×10^5 cfu/ml. The final volume in the wells was 200 µl. After 24 hrs of incubation at 37°C, the calculated amount of nitrogen dried cyanobacterial material present in the most diluted extract that inhibit a visible growth was defined as MIC.

Statistical analysis

The data of all the parameters were statistically analyzed and expresses as mean±Standard deviation.

Table 1 Antibacterial activity of different extracts of *Spirulina platensis*

Microorganisms	Diameter of effective zone of inhibition (mm)					Standard
	Organic extracts				Extracellular Substances	
	Hexane	Ethyl acetate	Dichloromethane	Methanol	Spent medium Ciprofloxacin	
<i>S.aureus</i>	8.29±.2	4.45±1.3	11.38±.18	15.21±.1	–	25.33±.15
<i>E.coli</i>	–	–	–	12.42±.47	7.26 ±.1	23.46±.4
<i>P.aeruginosa</i>	–	–	–	11.55±.38	–	21.35±.22
<i>K.pneumoniae</i>	–	–	–	–	–	17.35±.22
<i>S.typhi</i>	–	–	–	11.52±.18	–	28.44±.28

Results are the means of diameter values±standard deviation.–, No activity, ciprofloxacin (5 µg/ml) Effective zone of inhibition (total zone of inhibition-diameter of well)

Fig. 1 Zone of inhibition exhibited by different extracts of *Spirulina platensis*: 1, *S. aureus*; 2, *E. coli*; 3, *P. aeruginosa*; 4, *K. Pneumoniae*; 5, *S. typhi*; H, Hexane extract; E, ethyl acetate extract; D, dichloro-methane extract; M, methanolic extract; S, spent medium

Table 2 Minimum inhibitory concentration of active crude extracts of *Spirulina platensis*

Microorganisms	Minimum inhibitory concentration (µg/ml)				Standard
	Organic Extracts				
	Hexane	Ehtyle acetate	Dichloromethane	Methanol	
<i>S.aureus</i>	>512	>512	512±0	128±0	<1
<i>E.coli</i>	ND	ND	ND	256±0	<1
<i>P.aeruginosa</i>	ND	ND	ND	>512	<1
<i>S.typhi</i>	ND	ND	ND	>512	<1

Results are the means of values $\pm$ standard deviation, ND-not detected

Fig. 2 MIC analysis for *Spirulina platensis* extracts: A ninety six well microtiter plate showing the inhibition of growth

Rows: A-Hexane extracts against *S. aureus*.
 B-Ethyl acetate extract against *S. aureus*.
 C-Di chloro methane extract against *S. aureus*.
 D-Methanolic extract against *S. aureus*.
 E-Methanolic extract against *E. coli*.
 F-Methanolic extract against *P. aeruginosa*.
 G-Methanolic extract against *S. typhi*.
 H-Ciprofloxacin extract against *S. aureus*.
 Column: 1-Negative control (MHB)
 2-11 - Dilution range 512 $\mu\text{g/ml}$ to 1 $\mu\text{g/ml}$
 2-512 $\mu\text{g/ml}$, 3-256 $\mu\text{g/ml}$, 4-218 $\mu\text{g/ml}$
 5-64 $\mu\text{g/ml}$, 6-32 $\mu\text{g/ml}$, 7-16 $\mu\text{g/ml}$, 8-8 $\mu\text{g/ml}$
 9-4 $\mu\text{g/ml}$, 10-2 $\mu\text{g/ml}$, 11-1 $\mu\text{g/ml}$.
 12-Positive control (MHB+Culture)

Results and discussion

The antibacterial activity of different extracts of *Spirulina platensis* are presented in Table 1. All the extracts tested

exhibited different degree of antibacterial activity against tested microorganisms.

There is a growing interest in isolating antibacterial substances from cyanobacteria. In different studies, the an-

timicrobial effect of *Fischerella sp* [11] *Oscillatoria angustissima* and *Calothrix parietina* [12], *Anabaena*, *Oscillatoria*, *Pseudoanabaena*, *Synechocystis* [1], *Nostoc* [13], *Phormidium* [14] and *Fischerella ambigua* [15] extracts on some pathogenic microorganisms have been reported. They have also reported that the extracts extracted in different solvents were effective against both Gram-positive and Gram-negative organisms. This is in agreement with our findings, since the *Spirulina platensis* extracted in different solvents had similar effects on both types of organisms used in this study.

The results of antibacterial activity of *Spirulina platensis* are summarized in Table 1. In this case, all the extracts exhibited activity against *S. aureus*. The methanolic extract showed broad spectrum activity as highest effective zone of inhibition was recorded against *S. aureus* followed by *E. coli*, *P. aeruginosa* and *S. typhi*. However, extracts prepared in hexane, ethyl acetate and dichloromethane were found active only against *S. aureus*. In addition, no inhibitory effect was observed against *K. pneumoniae*. Interestingly, spent medium of this alga was found active against *E. coli* (Fig. 1).

The results of minimum inhibitory concentrations of *Spirulina platensis* extracts are shown in Table 2. The results showed that the MICs of methanolic extract were 128 µg/ml and 256 µg/ml against *S. aureus* and *E. coli*, respectively. However, the MIC of this extract was recorded >512 µg/ml against *P. aeruginosa* and *S. typhi*. The MIC of dichloromethane extract was found 512 µg/ml against *S. aureus*. The MIC of both the hexane and ethyl acetate extracts were also recorded more than >512 µg/ml (Fig. 2).

We have examined only crude extracts. Thus, the results do not indicate and defined antibacterial substance but the results obtained indicate a spectrum of antibacterial activity that provide support to some of the traditional uses of *Spirulina* and show that cyanobacteria bear potential use in pharmaceutical drug discovery.

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