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Antibacterial and toxicity evaluation of C-phycocyanin and cell extract of filamentous freshwater cyanobacterium – *Westiellopsis sps*

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Abstract. – In this study the culture filtrate and C-phycocyanin obtained from filamentous fresh water cyanobacterium *Westiellopsis* sps were tested for their antibacterial activity against three different bacterial cultures: *Bacillus subtilis*, *Pseudomonas sps* and *Xanthomonas sps*. The growth of all bacterial strains tested was inhibited by the culture filtrate and C-phycocyanin. The diameter of inhibition zones varied from 1.3 to 13.2 mm and from 2.2 to 13.1 mm for the culture filtrate and C-phycocyanin, respectively. It is therefore suggested that extracts from the *Westiellopsis sps* could be used traditionally in the treatment of bacterial infections. Bioassay studies of silkworm showed that there was no symptom of ill health after feeding the phycocyanin treated leaves and the body weight and silk gland weight of the silkworm increased with range of 65.0-102.6 mg and 209-240 mg respectively compared to the control.

Key Words:
Cyanobacteria, Phycocyanin, Antibacterial, Toxicity.

Introduction

Cyanobacteria (BGA) are prokaryotic photoautotrophs capable of carrying out photosynthesis and nitrogen fixation simultaneously. Although the potential of cyanobacteria as biofertilizer is well known, the attention has recently been focused on the biotechnological potentials of cyanobacteria for obtaining pharmacologically active secondary metabolites[1]. Recent investigation on biologically active secondary metabolites from cyanobacteria led to the identification wide range of compounds possessing antimicrobial, antiviral, antineoplastic and toxic properties[2-4]. The ability to produce antimicrobial substances may be noticed not only as a defensive instrument for the strains but also as a good source of new bioactive compounds from a pharmaceutical point-of-view[5]. The marine cyanobacterial group is well documented for their bioactive compounds possessing antimicrobial properties, while there are only few reports available with fresh water cyanobacteria. In this work, we report the antibacterial activity and the toxicity evaluation of the culture filtrate and C-phycocyanin of five different strains belongs to fresh water filamentous cyanobacterium group – *Westiellopsis sps*.

Materials and Methods

Production and Preparation of Material

The cyanobacterial cultures used in this study were obtained from Algal Biotechnology Laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, India. One ml of selected cyanobacterial cultures was well homogenized and transferred aseptically to 100 ml of sterilized nitrogen free BG-II medium[6]. Cultures were incubated in an illumination chamber at 28 ± 1°C with 3000 lux light intensity with periodical shaking. Cultures were grown until the late° exponential phase of growth (*i.e.* at 72 h). The purity of the cyanobacterial cultures was checked periodically by microscopic observation following[7] taxonomy guidelines.

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Preparation of Various Extracts of *Westiellopsis sps*

Cyanobacterial cell extracts were prepared following[8]. The phycobiliprotein C-phycocyanin was isolated following the method of Boussiba and Richmond[9] and estimated as described by Bennett and Bogorad[10]. In brief, Ten ml of cyanobacterial culture was homogenized and centrifuged at 5000 rpm for 5 min. The pellet was washed and suspended in 2.0 ml of 0.05 M phosphate buffer (pH 6.8). The aqueous phase containing cyanobacterial cells was subjected to freezing and thawing. Then the content was centrifuged at 5000 rpm for 5 min and then the supernatant was collected and stored in the refrigerator. Again the pellet was subjected to freezing and thawing till a colourless supernatant was obtained. The supernatant containing pigment was pooled and the final volume was recorded. The pigment absorption was measured at 615 and 652 nm in a Beckman DU-64 spectrophotometer against 0.05 M phosphate buffer as blank. The concentration of C-phycocyanin was calculated using the following formula. The phycobilin pigments C-phycocyanin content in the cyanobacterial cultures were expressed as µg per ml of the cyanobacterial cells.

$$\text{C-phycocyanin (PC)} = \frac{E_{615} - 0.474\,(E_{652})}{5.34}$$

(E_{615} and E_{652} are the absorbances at 615, 652 nm respectively)

Antimicrobial Testing

Antimicrobial activities of the C-phycocyanin and culture filtrate from *Westiellopsis sps* were measured by the paper disk diffusion method[11]. Briefly, sterile, 5 mm diameter of Whatman No. 42 filter paper discs (6 mm diameter) were impregnated with 40–50 µL of culture filtrate, C-phycocyanin at the concentration of 1µg/disc and air dried. The agar plates inoculated with the test organisms were incubated for 1 h before placing the extract impregnated paper discs on the plates. Following this, the sterile discs impregnated with the different extracts were placed on the agar plates. The bacterial plates were incubated at 30° ± 0.1°C for 48 h. After incubation, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters. All tests were performed in triplicate under sterile conditions.

Silkworm Toxicity Assay

The present study also included observations of the phycocyanin toxicity. Fifty number of second instar larvae of silkworm (*Bombyx mori*) were taken in a clean plastic tray (25 cm diameter and 8 cm height plastic trays). A quantity of 50 g of clean tender and chopped leaves of mulberry was taken and one g of the C-phycocyanin was dissolved in minimum quantity of water and thoroughly mixed. The leaves were allowed to air dry. After starvation for 5 hrs , the larvae were allowed to feed the treated leaves. Untreated leaves were also fed to another set of larvae which served as a control. Observations were made after 24 hrs for the worm movement, death rate, feeding rate, symptoms of ill health .The larval body weight of the silk worm was observed when the larvae passed on to the third instar from second instar.

Results

Cyanobacteria are recognized as a rich but not yet extensively studied source of pharmacological as well as structurally interesting secondary metabolites[12]. During the past few years the attention has been directed towards the searching of bioactive compounds from cyanobacteria.

Antibacterial Activity of the Culture Filtrate and C-phycocyanin of Filamentous Fresh Water Cyanobacteria *Westiellopsis sps*

In the present study phycocyanin pigment and culture filtrate of cyanobacterial cultures *viz.*, *Westiellopsis*-4A$_2$, *Westiellopsis*-ARM 48, *Westiellopsis*-HT-SGK-1, *Westiellopsis*-ST and *Westiellopsis*-PSG was tested against the pathogenic bacteria *viz.*, *Bacillus subtilis* and *Pseudomonas sps and Xanthomonas sp* and the results are shown in Table I. In general the inhibition of culture filtrate was more followed by the phycocyanin pigment. Among the five different *Westiellopsis* strains, culture filtrate from *Westiellopsis*- HT-SGK-1 (13.2 mm) and C-phycocyanin from *Westiellopsis*-PSG (13.1 mm) showed higher inhibition of *Bacillus subtilis*. The culture filtrate from *Westiellopsis*-PSG (9.6 mm) and C-phycocyanin from *Westiellopsis*-ARM 48 (9.5 mm) showed higher inhibition of *Xanthomonas*

Table I. Antibacterial activity of culture filtrate and C-phycocyanin from filamentous fresh water cyanobacteria *Westiellopsis sps*.

Cyanobacterial culture	Treatments	Inhibition annules (mm ± SEM)*		
		Bacillus subtilis	*Pseudomonas sps*	*Xanthomonas sps*
Westiellopsis-4A2	Culture filtrate	10.9 ± 1.3	2.6 ± 0.3	8.9 ± 0.2
	C-Phycocyanin	11.3 ± 0.0	2.2 ± 0.1	8.1 ± 1.4
Westiellopsis-ARM 48	Culture filtrate	12.7 ± 0.2	3.1 ± 0.0	9.5 ± 0.6
	C-Phycocyanin	11.3 ± 2.1	2.5 ± 0.1	8.6 ± 0.3
Westiellopsis-HT-SGK-1	Culture filtrate	13.2 ± 0.6	2.9 ± 0.2	8.3 ± 0.2
	C-Phycocyanin	11.6 ± 0.1	2.2 ± 0.1	7.8 ± 0.0
Westiellopsis-ST	Culture filtrate	11.6 ± 0.0	1.3 ± 0.3	5.9 ± 0.1
	C-Phycocyanin	10.2 ± 0.2	3.8 ± 0.1	8.1 ± 0.2
Westiellopsis-PSG	Culture filtrate	4.6 ± 0.1	4.3 ± 0.2	9.6 ± 0.3
	C-Phycocyanin	13.1 ± 1.2	2.3 ± 0.1	7.5 ± 0.1
Streptomycin		22 ± 0.0	15 ± 0.0	20 ± 0.0

*Data are presented as mean of three readings ± SEM.

sp among the different treatments. The *Pseudomonas sp* was more resistant showing the minimum inhibition annule (1.3-4.3 mm).

Silkworm Toxicity Assay

Various members of cyanobacteria are very toxic to human beings and having poisonous effects towards animal[16]. The present study also included observations of the toxicity cyanobacterial pigment C-phycocyanin obtained from *Westiellopsis sps*. Toxicity of phycocyanin pigments was tested in a sensitive insect silkworm and the results were given in Table II. Phycocyanin pigment from the five cyanobacterial cultures *viz.*, *Westiellopsis*-ARM48, *Westiellopsis*-HT-SGK-1, *Westiellopsis*-4 A_2, *Westiellopsis*-PSG and *Westiellopsis*-ST mixed with the mulberry leaves fed to the silkworm larvae once in a day caused no toxicity. The movement of the worms was normal. There was no symptom of ill health and abnormal secretion. Hundred per cent survival was recorded in the worms in all treatments. The results also showed the body weight and silk gland weight of the silkworm increased with range of 65.0-102.6 mg and 209-240 mg respectively compare to the control.

Discussion

Antimicrobial activity of fresh water cyanobacteria – *Westiellopsis sps* not yet been studied and this screening program is among the first studies done for assessment of it's antibacterial activity. The recent investigations with cyanobacteria have demonstrated the antimicrobial effects of *Nostoc*[13], *Anabaena*, *Oscillatoria*, *Synechocystis*[14], *Oscillatoria angustissima* and *Calothrix parietina*[15] extracts against both Gram-positive and Gram-negative organisms. These reports are in agreement with our present study,

Table II. Effect of phycocyanin pigments on the bodyweight of silkworm.

Cyanobacterial culture	Body weight of silkworm (mg)	Percentage increase	Silk gland wt (mg)	Percentage increase
Westiellopsis-ARM 48	1436 ± 11.2*	98.8	750 ± 5.6*	240
Westiellopsis-HT-SGK-1	1324 ± 13.6*	83.3	680 ± 7.8*	209
Westiellopsis-4	1192 ± 9.3*	65.0	700 ± 11.3*	218
Westiellopsis-PSG	1463 ± 12.4*	102.6	720 ± 6.4*	227
Westiellopsis-ST	1296 ± 14.2*	79.50	690 ± 8.4*	213
Control	722 ± 12.3	–	220 ± 9.2	–

Values are mean ± SEM for fifty larva; *Statistically significant difference in comparison with the control group with $P < 0.001$.

since the extracts from cyanobacteria *Westiellopsis sps* had similar effects on the pathogenic microorganisms used in this study. The high proportion of antimicrobial producing strains may be associated with an ecological role, playing a defensive action to maintain their niche, or enabling the invasion of a strain into an established microbial community.

The toxicity tests are important for the algal products because, various members of cyanobacteria produce toxins like neurotoxins, hepatoxins and cytotoxins. In the present study phycocyanin pigment from all the five cyanobacterial cultures *viz., Westiellopsis*-ARM48, *Westiellopsis*-HT-SGK-1, *Westiellopsis*-4A$_2$, *Westiellopsis*-PSG and *Westiellopsis*-ST showed no toxicity in the silkworm toxicity assay. The results revealed that the phycocyanin pigments are safe.

Conclusion

Cyanobacteria are a promising but still unexplored natural resource possessing many bioactive compounds useful for the pharmaceutical, food and cosmetic industry. Of the new drugs approved between 1983 and 1994, up to 80% of antibacterial and anticancer drugs were derived from natural products[17]. The present study aims the preliminary investigation of antimicrobial and toxicity evaluation of *Westiellopsis sps*. This merits further and more detailed investigations.

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