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ORIGINAL ARTICLE

Alterations in the antibacterial potential of *Synechococcus* spp. PCC7942 under the influence of UV-B radiations on skin pathogens

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Abstract Marine organisms are seen as a source of novel drugs and the discovery of new pharmaceutical is increasingly in demand. Cyanobacteria are regarded as a potential target for this as antibacterial, antiviral, antifungal, algicide and cytotoxic activities have been reported in these organisms. They have been identified as a new and rich source of bioactive compounds belonging to diversified groups. Radiation in the UV-B range interferes with various metabolic reactions by generating free radicals and active oxygen species. These deleterious compounds are inactivated by antioxidants. Among them are the carotenoids and phycocyanin which protect against photodynamic action in different ways. Stress plays an important role in the production of bioactive metabolites from organisms. *Synechococcus* spp. PCC7942 was studied for antibacterial activity against various pathogenic bacteria resistant to a number of available antibiotics after being exposed to UV-B radiation. The antibacterial activity of *Synechococcus* spp. PCC7942 was studied on five potent skin pathogens. The highest antibacterial activity was seen the methanol extracts of 24 h UV-B exposed cultures of *Synechococcus* spp. PCC7942. It can be concluded that there was moderate antibacterial activity. Results showed stress, solvent and dose-dependent activity. This antibacterial activity might be due to the enhanced synthesis of carotenoids and phycocyanin under UV-B stress. The purpose of the present study was to relate the inhibitory effects of the cyanobacterial compounds specifically on skin pathogens with exposure to UV-B radiation as UV protecting compounds are already reported in these organisms.

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1. Introduction

Cyanobacteria have been identified as a new and rich source of bioactive compounds (Abarzua et al., 1999; Shimizu, 2003; Bhadury and Wright, 2004; Dahms et al., 2006). Isolated compounds belong to groups of polyketides, amides, alkaloids,

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fatty acids, indoles and lipopeptides (Burja et al., 2001). Secondary metabolites with antibacterial activity are widely produced by cyanobacteria (Dahms et al., 2006). These compounds are effective against Gram-positive and/or Gram-negative bacteria. The potential contribution of marine organisms to the discovery of new pharmaceuticals is increasingly challenging (Sponga, 1999; Skulberg, 2000). Antibacterial, antiviral, antifungal, algicide and cytotoxic activities have been reported (Rao, 1994; McDermott, 1998; Issa, 1999; Pushparaj, 1999; Schlegel, 1999; Schaeffer, 2000) in cyanobacteria.

Radiation in the UV-B range of approximately 300 nm interferes with various metabolic reactions, primarily by generating free radicals and active oxygen species (Foyer et al., 1994). These deleterious compounds are inactivated by antioxidants. Several natural products have the potential to exhibit antioxidative properties. Among them are the carotenoids, which protect against photodynamic action in different ways. Protection of photosynthetic reactions against UV-B damage was observed in Synechococcus PCC7942 and was dependent on carotenoid concentrations in the different transformants. The research report has suggested that carotenoids exert their protective function as antioxidants to inactivate UV-B-induced radicals in the photosynthetic membrane (Götz et al., 1999). UV radiations are shown to enhance the production of carotenoids whereas after an initial event characterized by phycobilisome degradation following UVR exposure to Nostoc cells, UV light induced the synthesis of new phycobiliproteins and the assembly of phycobilisomes (Aráoz and Häder, 1997).

An antimicrobial agent is produced by the cyanobacterium *Synechococcus leopoliensis* which was found to be active against the Gram-positive bacterium *Staphylococcus aureus* (Noaman et al., 2004). Marine Synechocystis and Synechococcus extracts induce apoptosis in eukaryotic cells and cause inhibition of Gram-positive bacteria (Martins et al., 2008).

2. Materials and methods

2.1. Maintenance and cultivation of cyanobacteria

The axenic culture of cyanobacterium *Synechococcus* spp. PCC7942 was obtained from the Centre for Biotechnology, University of Allahabad, Uttar Pradesh, India. Cultures of cyanobacteria were maintained in 250 ml flask with media at 2000–3000 lux light intensity, 25 ± 2 °C temperature and 14/10 h light and dark phases under aseptic conditions.

The test cultures were exposed to UV-B light (280–350 nm in range) for different durations by using UVB tube (Philip TEK 40 W; ACTINIC BL Reflector; 240 V (100–300 V) 50 Hz; made in Germany).

2.2. Estimation of carotenoids

For estimation of carotenoids 96% acetone was used as a solvent (Hellebust and Craigie, 1978). Absorbance of acetone extract was taken using 96% acetone as blank at 460 nm by UV Spectrophotometer. Carotenoid content was calculated using the equation:

$$C = 12 * A_{440}$$

where, C – concentration, A_{440} – absorbance of carotenoid at 440 nm.

2.3. Estimation of phycocyanin

Phycocyanin was estimated by the method of (Brody and Brody, 1961). Phycocyanin was extracted in 3 ml of 0.5 M, cold phosphate buffer at pH 7. The absorbance of the supernatant was recorded at 660 nm for phycocyanin and then at 620 nm in a spectrophotometer, phosphate buffer serving as blank. Phycocyanin content was calculated using the equation:

$$C = \frac{[A_{660} - 0.474(A_{620})]}{5.34}$$

where, C – concentration, A_{660} – absorbance at 660 nm, A_{620} – absorbance at 620 nm.

2.4. Extract preparation

Extractions were carried out successively with 1.5 ml of culture in isopropanol, methanol and water to extract compounds with increasing polarity. Solutions were sonicated with an ultra-sound probe (Vibra Cell 50 – Sonics & Materials Inc., Danbury, CT, USA) for 3 × 2 min on ice. The extract was concentrated in a rotavapour to a fine powder which was dissolved in DMSO. The sample was stored at −80 °C and was used in all experiments for determining the antimicrobial activity of *Synechococcus* spp. PCC7942.

2.5. Microbial strains used for the study

The extracts of *Synechococcus* spp. PCC7942 exposed to different durations of UV-B radiation were tested against five standard microorganisms which included Gram positive strain *S. aureus* (NCIM 2099) and Gram negative bacteria *Pseudomonas aeruginosa* (NCIM 5029), *Klebsiella pneumonia* (NCIM 2957), *Enterobacter aerogenes* (NCIM 5139) and *Escherichia coli* (NCIM 2065). These strains were obtained from NCIM, Pune.

2.6. Inoculum preparation

The test microorganisms were maintained at 4 °C on nutrient agar slants. Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth. The inoculated tubes were incubated without agitation for 24 h at 37 °C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 10^6 cfu ml^{-1} (Duraipandiyan et al., 2006).

2.7. Determination of in vitro antimicrobial effect broth dilution assay

The minimum inhibitory concentration (MIC) values were determined by using a modified macro-broth dilution technique (Ibrahim et al., 1997). Overnight culture of bacteria grown in nutrient both cultures were diluted 100-folds in nutrient broth (100 µl bacterial cultures in 10 ml of nutrient broth which contained 10^5 cfu of bacteria). Gradually increasing volumes of the extracts were added to test tubes containing the bacterial cultures to know the inhibitory concentration in a particular tube inhabiting the bacterial growth. The tubes were incubated at 37 °C for 18–24 h. The tubes were examined for visible turbidity and optical density of cultures was

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