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, algicidal 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,
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, algicidal 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). Several 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 (Arãoz 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:

\[ C = 12 \times A_{440} \]

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:

\[ C = \frac{[A_{660} - 0.474(A_{420})]}{5.34} \]

where, \( C \) – concentration, \( A_{660} \) – absorbance at 660 nm, \( A_{420} \) – 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 10⁶ cfu ml⁻¹ (Duraipandian 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 10⁵ 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
determined at 620 nm using NB as control. The lowest concentration that inhibited visible growth of the tested organisms was recorded as the MIC (Table 1).

2.8. Agar well diffusion assay

The agar well diffusion method was used to test the antimicrobial effect of different extracts of *Synechococcus* spp. PCC7942 exposed to different durations to UV-B radiation (Okeke et al., 2001; Perez et al., 1990). All media plates (9 cm in diameter) were prepared with nutrient agar. After agar solidification, the well (7 mm in diameter) was cut from the agar to produce a total of four wells per agar plate. For the test, three doses of extract (25, 50, 100 µg/well) were prepared using 99.5% analytical Dimethyl Sulphoxide (DMSO) as an organic solvent. Streptomycin (30 µg), gentamycin (10 µg), doxycycline (30 µg), ampicillin (10 µg) and tetracycline (10 µg) were used as positive standard antibiotics. 100 µl (10^5 cfu) of each diluted microbial suspension was inoculated on nutrient agar plates using a sterile cotton swab. The inoculums were allowed to dry for 5 min. Then, 100 µl of each extract solution, blank (DMSO) and positive control was added separately to each well of the agar plate and allowed to diffuse at room temperature for 15–20 min. After incubation at 37°C for 24 h, all plates were examined for any zones of growth inhibition and the diameter of these zones was measured. The assay was repeated three times for each extract. The anti-microbial effect was recorded as the mean diameter of the resulting inhibition zones of growth in millimeters.

3. Results and discussion

The general trend of the inhibition by different extracts of *Synechococcus* spp. PCC7942 showed that the inhibition of bacterial growth by aqueous extract was least followed by isopropanol extract and methanol extract except in the case of *S. aureus* in which the inhibition was more by the isopropanol extract than the methanol extract. This was in accordance with the fact that most of the bioactive compounds showing antibacterial activity are soluble in methanol. The overall result also showed that the inhibition of bacterial growth was highest by the extracts of the cultures exposed to UV-B stress for a 24 h duration followed by the cultures exposed to UV-B stress for 12 h whereas it was least in the case of the control cultures. Thus, it is clear that UV-B stress of 24 h led to the production of the antibacterial phytochemicals in

| Pathogen                  | Minimum inhibitory conc. (mg ml⁻¹) | Duration of UV-B exposure |
|---------------------------|------------------------------------|---------------------------|
|                           | Cont.* | Aq.* | Isopropanol | Methanol | Aq.* | Isopropanol | Methanol | Aq.* | Isopropanol | Methanol |
|                           | 12 h    | 24 h    | 12 h    | 24 h    | 12 h    | 24 h    | 12 h    | 24 h    | 12 h    | 24 h    |
| *E. coli*                 | –       | 15.5 ± 0.57 | 10.5 ± 0.37 | –       | 12.0 ± 0.81 | 8.0 ± 0.57 | –       | 12.5 ± 0.35 | 6.5 ± 0.11 |
| *S. aureus*               | 16.0 ± 0.32 | 13.5 ± 0.22 | 11.0 ± 0.58 | 15.5 ± 0.33 | 11.5 ± 0.42 | 8.0 ± 0.75 | 16.5 ± 0.49 | 12.5 ± 0.57 | 6.0 ± 0.36 |
| *K. pneumoniae*           | 15.0 ± 0.32 | –       | 10.0 ± 0.22 | 14.5 ± 0.38 | 12.5 ± 0.82 | 8.0 ± 0.55 | 15.5 ± 0.99 | 12.5 ± 0.56 | 6.0 ± 0.48 |
| *P. aeruginosa*           | –       | 10.0 ± 0.45 | –       | 8.0 ± 0.67 | –       | –       | 8.0 ± 0.67 | –       | 6.0 ± 0.66 |
| *E. aerogenes*            | 15.0 ± 0.44 | 13.5 ± 0.25 | 10.0 ± 0.57 | 13.5 ± 0.58 | 12.5 ± 0.62 | 8.0 ± 0.89 | 15.5 ± 0.90 | 12.5 ± 0.34 | 6.0 ± 0.24 |

* conc. – concentration, cont. – control, Aq. – aqueous.

| Pathogen                  | Zone of inhibition (mm) | Duration of UV-B exposure |
|---------------------------|------------------------|---------------------------|
|                           | Cont.* | Aq.* | Isopropanol | Methanol | Aq.* | Isopropanol | Methanol | Aq.* | Isopropanol | Methanol |
|                           | 12 h    | 24 h    | 12 h    | 24 h    | 12 h    | 24 h    | 12 h    | 24 h    | 12 h    | 24 h    |
| *E. coli*                 | –       | 4       | 6       | 10      | 12      | –       | 6       | 8       | 10      | 14      | –       | 6       | 8       | 10      | 11      |
| *S. aureus*               | 4       | 6       | 6       | 10      | 4       | 10      | 6       | 6       | 8       | 10      | 6       | 10      | 8       | 8       | 10      | 12      |
| *K. pneumoniae*           | 4       | 6       | –       | 10      | 11      | 4       | 6       | 2       | 5       | 12      | 13      | 6       | 8       | 2       | 4       | 12      |
| *P. aeruginosa*           | –       | –       | 10      | 12      | –       | –       | 4       | 6       | 14      | 15      | –       | –       | 8       | 8       | 12      |
| *E. aerogenes*            | 2       | 4       | 2       | 4       | 2       | 4       | 4       | 6       | 6       | 7       | 8       | 10      | 4       | 8       | 8       | 10      |

* cont. – control, Aq. – aqueous.

Inhibitory effects of the cyanobacterial compounds with exposure to UV-B radiation
Synechococcus spp. PCC7942 and these compounds were best extracted in methanol followed by isopropanol and least extracted in the water. The overall result showed stress, solvent and dose-dependent antibacterial activity as 100 μl of the dose gave better inhibition as compared to 75 μl dose (Table 2).

The zones of inhibition of E. coli, K. pneumonia and P. aeruginosa and E. aerogenes were larger in methanolic extracts as compared to isopropanolic and aqueous extracts in the case of both the normal as well as test strain (Fig. 2). In E. coli maximum zone of inhibition was observed in the methanol extract of a culture exposed to 6 h of UV-B stress (14 mm). S. aureus showed maximum inhibition in the presence of isopropanol extract of cultures exposed to 24 h of radiation (12 mm). K. pneumonia showed maximum inhibition in the methanol extract of cultures exposed to a 24 h stress (14 mm). P. aeruginosa showed maximum inhibition in the methanol extract of cells exposed to 12 h of stress. In the case of E. aerogenes, maximum zone of inhibition was observed in the methanol extract exposed to 24 h of UV-B stress (12 mm) (Table 2). In earlier reports, antibacterial activity was found to be associated only with the methanolic extract of four strains of Nostoc, Scytomena and Anabaena (Yadav et al., 2012).

The methanolic extract of Anabaena variabilis and Anabaena fertilissima exhibited good antibacterial activity against S. aureus whereas the aqueous extract showed no activity (Bhattacharya et al., 2013).

These results are in accordance with the previously reported results by researchers regarding the antimicrobial potential of cyanobacteria. Cyanobacteria have shown activity toward Bacillus subtilis, Bacillus thuringiensis, Bacillus megaterium, E. coli, P. aeruginosa, Candida tropicalis and Saccharomyces cerevisiae (Volk and Furkert, 2006). It was reported that extracts of Oscillatoria, Phormidium and Lyngbya obtained by different solvents exhibited antimicrobial activity on both Gram positive and Gram negative organisms (Ozdemir et al., 2004; Sethubati and Prabu, 2010). The strains of Synechococcus elongatus, Synechocystis sp., Amphiprora paudosa, Chaetoceros muelleri, and Porphyridium cruentum can potentially be used to control Gram (+) and Gram(−) bacteria (Sanchez-Saavedra et al., 2010). Stress plays an important role in the production of bioactive metabolites from organisms. Antibacterial activity of twenty bacterial strains isolated from ten different stressed agro-ecological niches of eastern Uttar Pradesh region has been reported (Singh et al., 2009).

All the strains tested for antimicrobial activity were resistant to gentamycin, doxycycline and ampicillin and were sensitive toward streptomycin and tetracycline except S. aureus which showed sensitivity toward all the standard antibiotics. The zones of inhibition of tested strains in the presence of streptomycin were larger as compared to those shown by the extracts of Synechococcus spp. PCC7942. Streptomycin proved to be the best antibiotic against all the tested bacterial strains in the present study. The largest zone of inhibition was shown by E. aerogenes against streptomycin (27 mm) followed by E. coli (18 mm), S. aureus (15 mm), P. aeruginosa (13 mm) and K. pneumoniae (12 mm) respectively (Table 2). The zones of inhibition shown by tested bacterial strains were larger in the presence of cyanobacterial extracts as compared to tetracycline. E. coli showed a zone of inhibition of 14 mm in the methanol extracts of cultures exposed to a 12 h stress (tetracycline 10 mm). The methanol extract exposed to 24 h of UV-B showed 14 mm of inhibition zone in the case of K. pneumoniae (tetracycline 8 mm). P. aeruginosa showed an inhibition zone of 15 mm in the presence of methanol extract of cells exposed to 12 h of UV-B stress (tetracycline 10 mm) while the zone of inhibition in E. aerogenes was 12 mm in the case of methanol extract exposed to 24 h of UV-B stress (tetracycline 9 mm). S. aureus showed more inhibition in its growth by both the tested antibiotics as compared to cyanobacterial extracts.

All the bacterial strains tested are potent skin pathogens and may cause serious infections. S. aureus produces a number of cellular and extracellular products, including exotoxins and coagulase that contribute to the pathogenicity of impetigo, especially when coupled with preexisting tissue injury. Skin and soft tissue infections (SSTIs) are common, and complicated SSTIs (cSSTIs) are the more extreme end of this clinical manifestation. It includes a deep-seated infection, a requirement for surgical intervention, the presence of systemic signs of sepsis, the presence of complicating co-morbidities, accompanying neutropenia, accompanying ischemia, tissue necrosis, burns and bites. S. aureus is the commonest cause of SSTI across all continents (Dryden, 2010). There are reports of fatal skin or soft tissue infections caused by E. coli that occurred in the postoperative course of liver transplantation (Janny et al., 2013). K. pneumoniae is one of the etiological organisms of erysipelas, and has also been found to cause more serious skin infections. K. pneumoniae was found to cause complicated skin (and soft tissue) infection of the extremities in adults sixteen years of age or older (Chang et al., 2008). Enterobacter is well adapted to cause nosocomial infections, as it is ubiquitous in the environment and can survive on skin and dry surfaces as well as replicate in contaminated fluids. In most cases, Enterobacter skin and soft-tissue infections are hospital-acquired and include cellulitis, fasciitis, myositis, abscesses, and wound infections. Enterobacter species can infect surgical wounds at any body site, and these infections are clinically indistinguishable from infections caused by other bacteria (Peleg and Hooper, 2010). Four cases of P. aeruginosa infections of intact skin have been reported. These cases illustrate the clinical spectrum of these cutaneous infections: localized, mild epidermal infections (the green nail syndrome and webbed space infections), moderately serious infections (cutaneous folliculitis and otitis externa), and, in immunocompromised patients, extremely serious infections including malignant otitis externa, perirectal infection, and ecthyma gangrenosum (Agger and Mardan, 1995).

Cyanobacteria proved to be an excellent source of natural metabolites, possessing antibacterial, antifungal, and cytotoxic–cytostatic activity. Cyanobacteria have a high growth rate and survive under extreme conditions in competitive natural environment due to their ability to synthesize antibiotic compounds (Patil et al., 2009). The chemical structures of these cyanobacterial metabolites have been determined, including polyketides, amides, alkaloids, lactones, peptides, and lipopeptides (Singh et al., 2005). An interesting consequence is that these are potent bioactive compounds and could be used for therapeutic purposes or as precursors for the synthesis of useful drugs. Cyanobacteria are regarded as good candidates for drug discovery, with applications in pharmaceuticals (Mundt et al., 2001). Marine cyanobacteria are known for the ability to produce cytotoxic compounds (Mayer, 2003; Shimizu, 2003).

The enhancement in the antibacterial potential of Synechococcus spp. PCC7942 under the influence of UV-B radiations may be attributed to the enhanced synthesis of
carotenoids and phycocyanin (Fig. 1). These pigments play an important role to combat the deleterious effects of UVR. These pigments have shown potent antibacterial effects on various bacterial pathogens. Carotenoids were reported to have antibacterial activity (Mahanom et al., 1990). The protective effect of β-carotene from green algae, Chlorococcum humicola has also been reported (Bhagavathy and Sumathi, 2010). The main natural resources of phycobiliproteins are the cyanobacterium Spirulina for phycocyanin. In Spirulina, phycocyanin is a phycobiliproteins; it is used against many bacterial infections and has anti-inflammatory, antioxidant and antiviral properties. It is effectively active against human pathogens such as Streptococcus sp., Staphylococcus sp., E. coli, Bacillus spp., and Pseudomonas spp. (Muthulakshmi et al., 2012; Murugan, 2012). Large quantities phycocyanin were isolated and partially purified from Anabaena cylindrica and filamentous fresh water cyanobacterium Westiellopsis spp., which was tested against Gram positive and Gram negative bacteria (Sabarinathan and Ganesan, 2008).

Figure 1  Synthesis of carotenoids and phycocyanin in Synechococcus spp. PCC7942 in UV-B stress.

Figure 2  Petriplates showing zones of inhibition of bacterial strains by the extracts of UV-B exposed cultures Synechococcus spp. PCC7942.

4. Conclusion

In the light of the important findings, we conclude that the present report is focused to study the antibacterial efficacy of UV-B treated cyanobacteria mainly pathogens causing skin diseases. Cyanobacteria produce an array of bioactive compounds which are well known UV protectants and the application of these compounds to produce new cosmetic ingredients is the need of the hour and much progress has already been made in this direction. They have shown antioxidant, anticancer and anti-skin aging effects. The intention behind the present study was to relate the inhibitory effects of the cyanobacterial compounds specifically on skin pathogens with the exposure to UV-B radiation. This study is one of its kind and to the best of our knowledge there is no previous finding in this area. This study can further pave the way for the scientists to deduce the mechanism behind this effect.
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