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Review

Photoprotective and biotechnological potentials of cyanobacterial sheath pigment, scytonemin

Shailendra P. Singh, Sunita Kumari, Rajesh P. Rastogi, Kanchan L. Singh, Richa and Rajeshwar P. Sinha*

Laboratory of Photobiology and Molecular Microbiology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005 India.

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Cyanobacteria are the main component of microbial populations fixing atmospheric nitrogen in aquatic as well as terrestrial ecosystems, especially in wetland rice-fields, where they significantly contribute to fertility as natural biofertilizers. Cyanobacteria require solar radiation as their primary source of energy to carry out both photosynthesis and nitrogen fixation. The stratospheric ozone depletion which has resulted in an increase in ultraviolet-B (UV-B; 280 - 315 nm) radiation on earth's surface has been reported to inhibit a number of photochemical and photobiological processes in cyanobacteria. However, certain cyanobacteria have evolved mechanisms such as synthesis of photoprotective compound scytonemin and their derivatives to counteract the damaging effects of UV-B. In addition this compound has anti-inflammatory and anti-proliferative potentials. This review deals with the role of scytonemin as photoprotective compound and its pharmacological as well as biotechnological potentials.

Key words: Cyanobacteria, biotechnology, ozone depletion, photoprotection, scytonemin, UV-B (280 - 315 nm) radiation.

INTRODUCTION

The continued depletion of stratospheric ozone layer due to anthropogenically released atmospheric pollutants such as chlorofluorocarbons (CFCs), chlorocarbons (CCs) and organo-bromides (OBS) has resulted in an increase in ultraviolet-B (UV-B; 280 - 315 nm) radiation reaching on to the earth's surface (Blumthaler and Ambach, 1990; Crutzen, 1992; Kerr and McElory, 1993; Lubin and Jensen, 1995; Kirchhoff, 1996). Ozone depletion has been reported in both Antarctic and Arctic regions, but it is most severe over the Antarctic, where ozone levels decline by more than 70% during late winter and early spring due to the well known phenomenon of polar vortex (Hoffman and Deshler, 1991; Smith et al.,

1992; von der Gathen et al., 1995). Recent studies suggested that for most of the world, the total column ozone loss has not been recovered (Weatherhead and Anderson, 2006).

Cyanobacteria are ubiquitous in distribution ranging from hot spring to the Antarctic and Arctic regions. The role of cyanobacteria in nitrogen fixation and thereby maintaining the fertility of rice paddy fields and other soils is well documented (Vaishampayan et al., 1992). They are also significant constituents of marine ecosystems and account for a high percentage of oceanic primary productivity. Absorption of solar energy to drive photosynthesis and nitrogen fixation exposes cyanobacteria to harmful ultraviolet radiation (UVR) in their natural habitats. Lethal doses of UVR reach deep into water column (Smith and Baker, 1979; Häder et al., 2007); down to a depth of 20 m in the clearest oceanic water and to a few centimeters in brown humic lakes and rivers (Kirk, 1994). The highly energetic UV-B radiation reaching the water column has both direct and indirect effects on cyanobacteria. The direct effect involves the denaturation of both DNA and RNA whereas its indirect effects include

*Corresponding author. E-mail: r.p.sinha@gmx.net. Tel: +91 542 2307147. Fax: + 91 542 2368174.

Abbreviations: CCs, Chlorocarbons; CFCs, chlorofluorocarbons; HPLC, high performance liquid chromatography; OBS, organobromides; PAR, photosynthetically active radiation; UVR, ultraviolet radiation.

production of reactive oxygen species (Karentz et al., 1991; Vincent and Roy, 1993; Bothwell et al., 1994; Vincent and Neale, 2000; Sinha and Häder, 2002; Häder and Sinha, 2005). In contrast, UV-A radiation which is not absorbed directly by the DNA, can still induce DNA damage either by producing a secondary photoreaction of existing DNA photoproducts or *via* indirect photosensitizing reactions (Hargreaves et al., 2007). In cyanobacteria, a number of physiological and biochemical processes such as survival, growth, pigmentation, photosynthetic oxygen production, motility, nitrogen metabolism, phycobiliprotein composition and $^{14}\text{CO}_2$ uptake have been reported to be affected by UVR (Sinha et al., 1995a, b; Sinha et al., 1996; Sinha et al., 1997; Sinha et al., 2005; Xue et al., 2005; Sinha and Häder, 2006; Häder et al., 2007).

However, cyanobacteria which are simultaneously exposed to visible and UV radiation have evolved certain mechanisms such as light dependent repair of UV-induced damage of DNA (Britt, 1995; Kim and Sancar, 1995; Pakker et al., 2000; Sinha et al., 2002; Häder and Sinha, 2005), accumulation of carotenoids and detoxifying enzymes or radical quenchers and antioxidants (Mittler and Tel-Or, 1991), and synthesis of photoprotective compounds such as mycosporine-like amino acids (MAAs) (Singh et al., 2008) and scytonemin (Karsten et al., 1998a, b; Sinha et al., 1998; Sinha et al., 1999; Richter et al., 2006; Sinha and Häder, 2008; Sinha et al., 2008; Rastogi and Sinha, 2009) to counteract the damaging effects of UVR. Cyanobacteria were present on the early earth when there was no oxygen in the atmosphere (Fischer et al., 2008) and thus the presence of UV-screening compounds such as MAAs and scytonemin might have played an important role in protecting these organisms from the lethal UVR (Cockell and Knowland, 1999). Thus, screening of UV-B and UV-A radiation is an important mitigation strategy in brightly lit habitats where organisms encounter intense solar radiation. Recently, scytonemin and its derivatives having absorption maxima in both UV-B and UV-A regions have received much attention for their putative role as UV-screening/absorbing compounds as well as their pharmacological potentials. In this review a brief account of the structure of scytonemin and its derivatives, their biosynthesis and photoprotection and pharmacological (biotechnological) potentials are presented.

STRUCTURE AND BIOSYNTHESIS OF SCYTONEMIN

This pigment was first reported by Nägeli (1849) in some terrestrial cyanobacteria and later termed scytonemin (Nägeli and Schwenderer, 1877). It is a yellow-brown lipid soluble pigment located in the extracellular polysaccharide sheath of some cyanobacteria (Geitler, 1932; Desikachary, 1959). It is a dimer composed of indolic and phenolic subunits having a molecular mass of 544 Da

(Figure 1A). The linkage between two subunits in scytonemin is an olefinic carbon atom that is unique among natural products. Hence, scytonemin possess a new ring system in nature for which Proteau et al. (1993) have proposed the trivial name 'the scytoneman skeleton'. Scytonemin exists in oxidized (green) and reduced (red) form (Garcia-Pichel and Castenholz, 1991) which was named as fuscochlorin and fusciorhodin, respectively, by Kyllim (1927, 1937). The existence of two forms of scytonemin depends upon the redox and acid-base conditions during the process of extraction. Recently, Bultel-Poncé et al. (2004) reported three new pigments such as dimethoxyscytonemin (Figure 1B), tetramethoxyscytonemin (Figure 1C) and scytonin (Figure 1D) from the organic extracts of *Scytonema* sp., which were derived from the scytoneman skeleton of the scytonemin, isolated from Mitaraka Inselberg, French Guyana. The structures of these new pigments were assigned mainly on the basis of ^1H and ^{13}C NMR and MS experiments.

Scytonemin is thought to be synthesized from metabolites of aromatic amino acid biosynthesis and can be induced under high photon fluence rate (Garcia-Pichel and Castenholz, 1991). The effect of bright light in promoting the production of scytonemin could be prevented by treating the cells with formaldehyde, chloramphenicol and keeping the cultures at 4°C during the exposure to high light. The inhibition of scytonemin synthesis in the presence of chloramphenicol indicates that protein synthesis pathway is involved in its production. The effect of light quality have also been examined (Garcia-Pichel and Castenholz, 1991) and it has been found that UV-A treatment is very efficient in inducing the synthesis of scytonemin, whereas blue, green or red light at the same fluence rates do not cause any significant increase in scytonemin. Dillon et al. (2002) have investigated the effect of other correlated stress factors including heat, osmotic and oxidative stress on the synthesis of scytonemin in a cyanobacterium *Chroococcidiopsis* sp. The experiments were performed both in the presence and absence of UV-A irradiation. These experiments showed that both increase in temperature and oxidative stress in combination with UV-A, have a synergistic effect on high production of scytonemin. However, the osmotic stress causes a decrease in scytonemin synthesis both in the presence and absence of scytonemin-inducing irradiance. Thus, scytonemin induction may be regulated as a part of a complex stress response pathway in which multiple environmental signal affects its synthesis.

The particular region in the genome of *N. punctiforme* associated with scytonemin biosynthesis has recently been explored by Soule et al. (2007). The genomic region flanking the mutation revealed an 18-gene clusters (NpR 1276 to NpR1259) (Figure 2A) among which the gene clusters NpR1274-NpR1271 was recognized for their significance in scytonemin biosynthesis but NpR1273 was shown to be directly involved in scytonemin biosynthesis in *N. punctiforme*. All 18 genes were induced

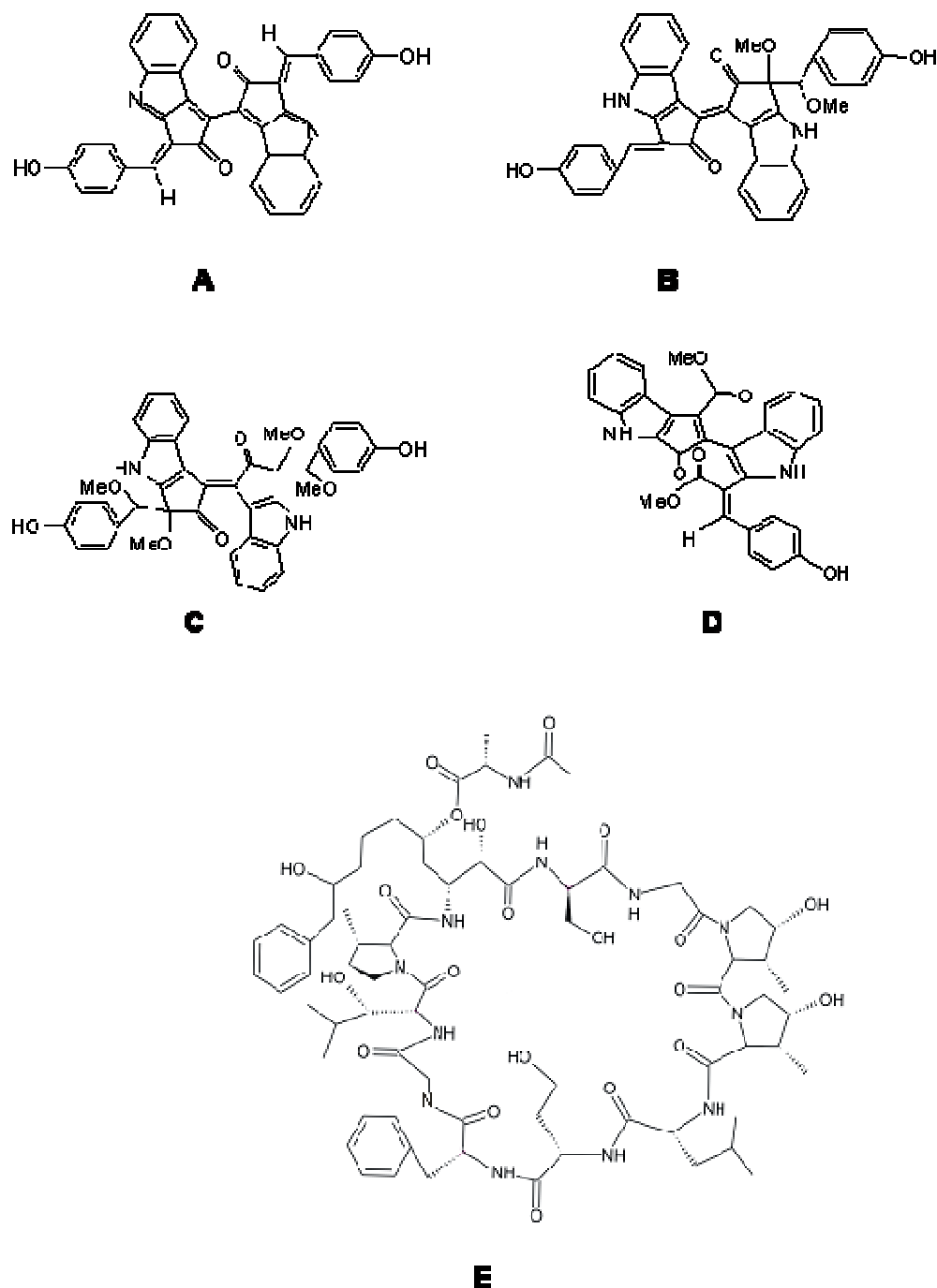


Figure 1. Chemical structures of (A) Scytonemin (B) Dimethoxyscytonemin (C), Tetramethoxyscytonemin (D) Scytonin and (E) Scytonemin A.

by UV-A irradiation, with relative transcription levels that generally peak after 48 h of continuous UV-A exposure. A five-gene cluster implicated in the process of scytonemin biosynthesis solely on the basis of comparative genomics was also upregulated. All of the genes in the 18-gene region were co-transcribed as part of a single transcriptional unit (Soule et al., 2009a). A comparison of these

clusters revealed that two major cluster architectures exist which appeared to have evolved through rearrangements of large sections, such as those genes responsible for aromatic amino acid biosynthesis and through the insertion of genes that potentially confer additional biosynthetic capabilities. Differential transcriptional expression analysis demonstrated that the entire gene

cluster is transcribed in higher abundance after exposure to UV radiation. The findings from an evolutionary phylogenetic analysis combined with the fact that the scytonemin gene cluster is distributed across several cyanobacterial lineages led to a proposal that the distribution of this gene cluster is best explained through an ancient evolutionary origin (Sorrels et al., 2009). Balskus and Walsh (2008) have presented the possible biosynthetic route for the scytonemin biosynthesis and identified the acyloin reaction as a key step in constructing the carbon framework of this ecologically and evolutionary important pigment (Figure 2B). They have also functionally characterized two enzymes encoded by ORF NpR1275 and NpR1276 from the gene cluster identified by Soule et al. (2007) which are involved in the initial step of scytonemin biosynthesis (Singh et al., 2009) (Figure 2B). However, the products of NpR1263 and NpR1269 ORFs are still to be functionally characterized. Soule et al. (2009b) suggested that two additional conserved clusters, NpF5232 to NpF5236 and a putative two-component regulatory system (NpF1277 and NpF1278), are likely involved in scytonemin biosynthesis and regulation, respectively, on the basis of conservation and location.

ROLE IN PHOTOPROTECTION

Cyanobacteria are the first photosynthetic oxygen evolving organisms that are thought to appear in Precambrian era. The presence of a UV-absorbing compound like scytonemin most probably helped them to survive from lethal effects of UV radiation when there was no stratospheric ozone layer. This assumption is supported by the fact that scytonemin has an *in vivo* absorption maximum at 370 nm whereas purified scytonemin shows maximum absorption at 386 nm, but it also absorb significantly at 252, 278 and 300 nm (Figure 3A).

The evidence for the photoprotective role of scytonemin has been shown in a number of cyanobacteria from various harsh habitats (Sinha et al., 1999; Garcia-Pichel and Castenholz, 1991; Hunsucker et al., 2001; Garcia-Pichel et al., 1992; Gröniger et al., 2000). The relevance of UV sunscreens such as scytonemin for the protection have also been reported from cyanobacterial lichens such as *Collema*, *Gonohymenia* and *Petulla*, growing in high light intensity habitats and it is shown that scytonemin is located extracellularly in the sheath of the outer thallus part (Büdel et al., 1997). Solar radiation is not always required for the production of scytonemin because this pigment has been reported to be synthesized in cyanobacterium *Calothrix* deficient in Fe or Mg and grown under low illumination (Sinclair and Whitton, 1977). The UV-sunscreen role of scytonemin has been demonstrated in terrestrial cyanobacterium *Chlorogloeopsis* sp. by Garcia-Pichel et al. (1992). In cyanobacterial cultures, 5% of the cellular dry weight is contributed by the scytonemin while it may be higher in naturally occurring

cyanobacteria (Castenholz, 1997). Fleming and Castenholz (2007) have shown that when the cells were hydrated for two days, in between desiccation periods had high scytonemin synthesis compared to the cells which were hydrated for one day in *Nostoc punctiforme*, but in *Chroococcidiopsis* periodic desiccation inhibits scytonemin synthesis. Fleming and Castenholz (2008) suggested that the greater the restriction in nitrogen accessibility, the greater the production of scytonemin.

The HPLC analysis of dried, yellow-brown, leathery mats of cyanobacterium, *Lyngbya* sp. (Figure 3B) harboring the bark of a number of mango trees (Figure 3C) have shown the presence of UV-absorbing compound scytonemin (Sinha et al., 1999). Pentecost (1993) has examined a correlation between UV flux and scytonemin content in population of *Scytonema* and *Rivularia* sp. He found a variable and even negative correlation between UV and scytonemin in *Scytonema*, but a positive correlation in *Rivularia*. The high amount of scytonemin is required for uninhibited photosynthesis under high UV flux in a monospecific population of *Calothrix* sp. (Brenowitz and Castenholz, 1997). The absorption spectra of methanolic extracts of the terrestrial cyanobacterium, *Tolypothrix byssoidea* showed a prominent absorption at 260 and 384 nm, corresponding to the presence of scytonemin (Adhikary and Sahu, 1998). The presence of scytonemin in cyanobacterial sheath has been reported to reduce the entry of UV-A radiation in the cell by 90% (Garcia-Pichel et al., 1992; Proteau et al., 1993). The scytonemin is highly stable and perform its screening activity without any further metabolic investment even under prolonged physiological inactivity (e.g. desiccation) when other ultraviolet protective mechanisms such as active repair of biosynthesis damaged cellular component would be ineffective (Sinha et al., 1999; Brenowitz and Castenholz, 1997; Ehling-Schulz et al., 1997).

PHARMACOLOGICAL (BIOTECHNOLOGICAL) POTENTIALS OF SCYTONEMIN

A number of cyanobacteria produce the pigment scytonemin in their sheath (Figure 3C). This pigment has been found to act as ultraviolet sunscreen, with the greatest absorption in the spectral range of UV-A and therefore may have application in sunscreens (Proteau et al., 1993; Rastogi and Sinha, 2009). Production of this pigment in certain cyanobacteria is believed to be the earliest developed mechanism of ultraviolet protection, more ancient than the flavonoids or melanins (Garcia-Pichel, 1998). Its ring structure, the "scytoneman skeleton", is unique among natural products and is thought to stem from the condensation of tryptophan- and phenylpropanoid-derived subunits and also closely related to nostodione A (Proteau et al., 1993). Other attractive structural features include its lack of chirality, multiple

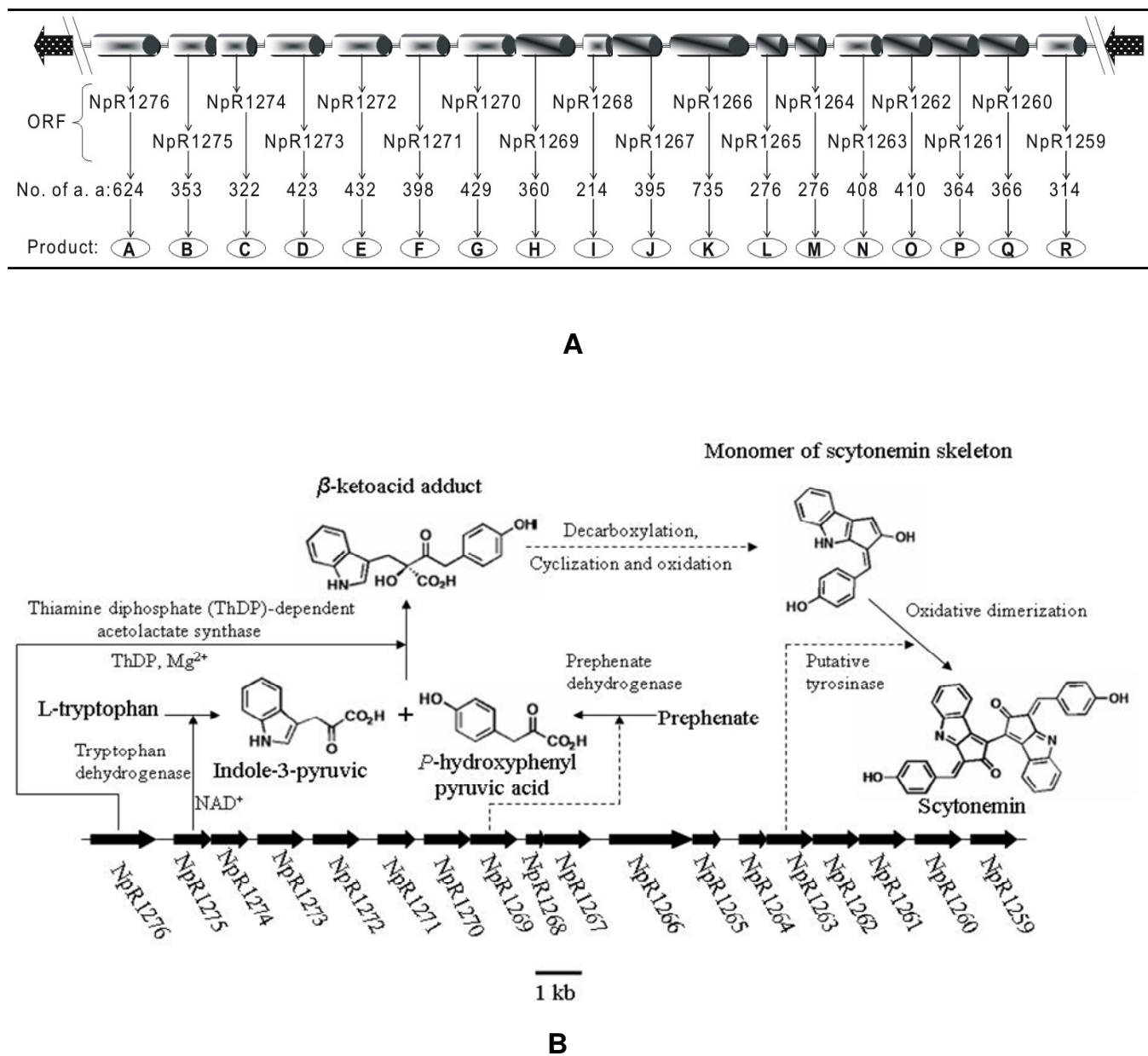
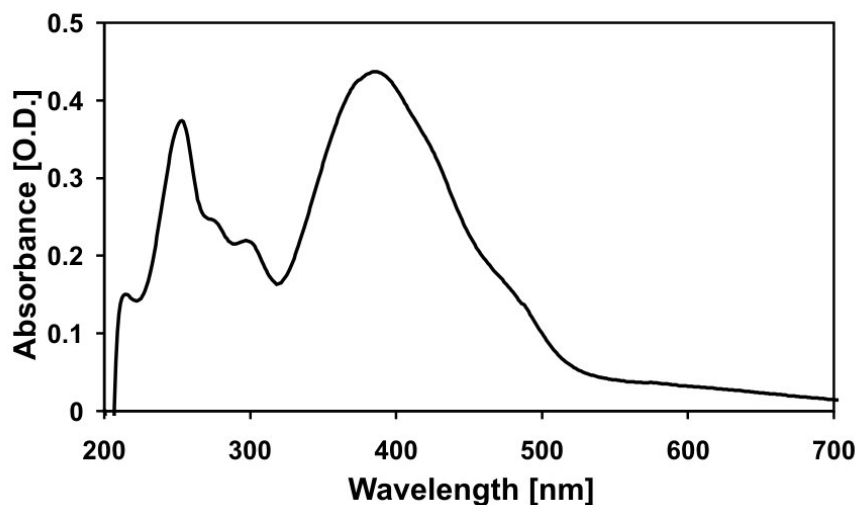


Figure 2. (A) Structure of genome associated with biosynthesis of scytonemin in *N. punctiforme*. The ORFs from NpR1276 to NpR1259 are labeled with particular proteins (A→TPP-requiring enzyme, B→Leucine dehydrogenase, C to F→Hypothetical proteins, G→Putative glycosyltransferase, H→Prephenate dehydrogenase, I→Dithiol-disulfide isomerase, J→3-Dehydroquinase synthase, K→Anthranilate synthase, L→Indole-3-glycerol phosphate synthase, M→Tryptophan synthase [α -subunit], N→Putative tyrosinase, O→Tryptophan synthase [β -subunit], P→Anthranilate phosphoribosyltransferase, Q→DAHP synthase, R→Hypothetical protein) of different amino acids. The ORFs in the downstream region of these clusters (NpR1269 to NpR1260) are predicted to encode a set of enzymes involved in the shikimic acid and aromatic amino acid biosynthesis pathways. Most of the upstream ORFs within these clusters (NpR1276 to NpR1270) encode products annotated as hypothetical proteins. Black dotted arrows show the nearest ORFs outside the genome cluster, and the hatch marks indicate a break in the distance scale. TPP: Thymine pyrophosphate; DAHP: 3-Deoxy-D-arabino-heptulosonate-7-phosphate (modified from Soule et al., 2007). (B) Biosynthetic route for the scytonemin and corresponding gene products involved in each step. Continuous arrow represents functionally characterized gene product while gene product indicated by broken arrow are still to be functionally characterized for their involvement in corresponding step (Adapted from Singh et al 2009).

dissection points and phenolic groups that could be easily modified. These attributes and its relation to other anti-proliferative agents make scytonemin a prime candidate

for investigating its potential utility as a pharmacophore with which new therapies targeting hyperproliferative disorders can be developed. The cyclic peptide, scytonemin



A

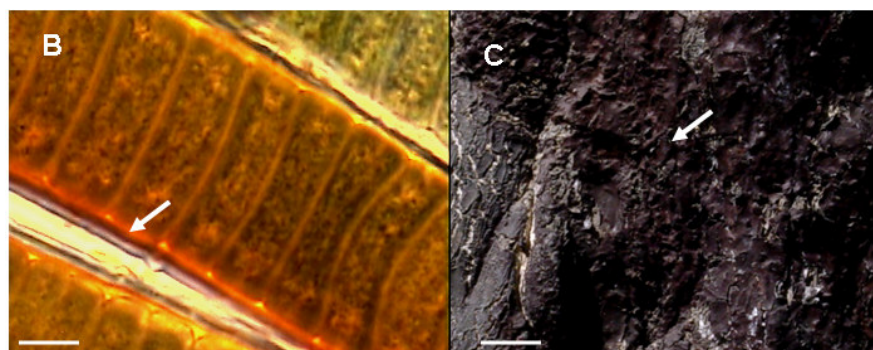


Figure 3. Absorption spectrum of scytonemin having peaks at 252, 278, 300 and 386 nm (A). Photographs showing the filaments of *Lyngbya* sp. (Bar = 10 μ m) with sheaths typically colored by the presence of yellow-brown UV-protective pigment, scytonemin (B). *Lyngbya* sp. harboring the bark of mango (*Mangifera indica*) trees (Bar = 5 mm) as a brown leathery mats (C).

A (Figure 1E), from a *Scytonema* sp. has also been shown to be a strong calcium agonist (Helms et al., 1988). In a screening effort designed to look for inhibitors of a cell cycle kinase human *polo*-like kinase, a serine/threonine kinase that plays an integral role in regulating the G2/M transition in the cell cycle, scytonemin was shown to be active, with an IC_{50} of 2 μ M (Stevenson et al., 2002a). Scytonemin also inhibits other cell cycle kinases with similar potency. In human T-cell leukemia Jurkat cells, scytonemin inhibited cell proliferation (IC_{50} = 7.8 μ M) and induced apoptosis in 24% of the cells (at 3 μ M). Scytonemin inhibited *polo*-like kinase 1 activity in a concentration-dependent manner with an IC_{50} of 2 μ M against the recombinant enzyme. Biochemical analysis showed that scytonemin reduced GST-*polo*-like kinase 1 activity in a time-independent fashion, suggesting reversibility and with a mixed-competition mechanism with

respect to ATP. Although scytonemin was less potent against protein kinase A and Tie2, a tyrosine kinase, it did inhibit other cell cycle-regulatory kinases like Myt1, checkpoint kinase 1, cyclin-dependent kinase 1/cyclin B and protein kinase C β 2 with IC_{50} values similar to that seen for *polo*-like kinase 1. Consistent with these effects, scytonemin effectively attenuated, without chemical toxicity, the growth factor- or mitogen-induced proliferation of three cell types commonly implicated in inflammatory hyperproliferation. Similarly, scytonemin (up to 10 μ M) was not cytotoxic to nonproliferating endotoxin-stimulated human monocytes. In addition, Jurkat T cells treated with scytonemin were induced to undergo apoptosis in a non-cell cycle-dependent manner consistent with its activities on multiple kinases. Scytonemin possesses both anti-inflammatory and anti-proliferative properties. The dual kinase inhibitory activity may be of

value therapeutically in acute and possibly chronic disorders where both inflammation and proliferation are prevalent. Limiting both neovascularization and the presence of inflammatory mediators at the affected site offers a broader spectrum of activity, which could hypothetically be more efficacious in the management of complex inflammatory disorders. Whether or not the ability of scytonemin to inhibit both inflammation and proliferation is due solely to its effects on these two kinases, scytonemin offers a novel pharmacophore, which may serve as a template for synthesizing more potent and selective inhibitors (Stevenson et al., 2002b).

CONCLUSION

In addition to the important role of scytonemin in cyanobacterial adaptation, this pigment certainly play a vital role in microbial communities exposed to high solar radiation (Proteau et al., 1993). The high concentration of scytonemin in many cyanobacterial sheaths might be providing significant protection to other microorganisms living within and beneath the upper layer of sheathed cyanobacteria. Scytonemin has also been identified and characterized as an antiproliferative pharmacophore that inhibits cell cycle kinases (Stevenson et al., 2002a). Because of the long term stability (Garcia-Pichel et al., 1992) of scytonemin, this compound can be used for understanding the evolutionary history of life in palaeobotanical studies. Attempts are being made to reconstruct the historical ozone and UV-B amount for periods prior to the modern instrumental records and prior to human impacts by analyzing the UV-absorbing pigments in herbarium specimens over a period of upto ~100 years (Huttunen et al., 2005). This attempt is still in a developmental stage, but show promise for the future. Extensive work is still required to explore the ecological, industrial and pharmaceutical importance of scytonemin that shows potent anti-inflammatory and anti-proliferative properties, which, combined with UV protection properties, is a promising combination for potential use as sunscreens.

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