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CYANOBACTERIAL SECONDARY METABOLITES

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ABSTRACT

Cyanobacteria inhabit a range of diverse and extreme habitats and have potential to produce an elaborate array of secondary metabolites with unusual structures and potent bioactivity. This review summarizes several classes of cyanobacterial secondary metabolites some of which pose a threat to human or animal health in aquatic ecosystems because of their acute toxicity. This class includes hepatotoxins (microcystins and nodularins), neurotoxins (saxitoxin and anatoxins) and irritant toxins (lipopolysachharides). Another important class includes the phytohormones (IAAs, cytokinin and gibberillin-like compounds) and iron-chelators (schizokinen, anachelin and synechobactins) which besides exerting profound effect on the productivity of the ecosystem have also potential for use as medicine for treatment of persistent metal toxicity. Several cyclic peptides and depsipeptides such as cyanopeptolins, micropeptin and oscillaeptin which have been categorized as protease inhibitors make cyanobacteria unattractive as a food source to grazers and help in their survival. UV-screening compounds such as scytonemin and mycosporin-like amino acids (MAAs) produced by cyanobacteria inhabiting habitats exposed to intense solar radiation may find use in development of artificial sunscreens.

KEYWORDS

Cyanobacteria, Secondary metabolites, Cyanotoxins, Microcystins, Protease inhibitors.

INTRODUCTION

Cyanobacteria are an ancient group of photosynthetic prokaryotic organisms and are thought to be the first organisms to carry out oxygenic photosynthesis. These organisms can inhabit a range of habitats including freshwater, marine and soil environments, as well as extreme habitats such as hot springwaters, and Arctic and Antarctic environments[1,2]. Under eutrophic conditions these organisms are able to form intense blooms. The bloom-forming process can be caused by increased levels of nutrients like P and N. Cyanobacteria have a number of special features, and besides their ability for dinitrogen fixation, many of them have long been recognized as producers of a wide array of secondary metabolites which allow the group to dominate under systems of high herbivory and extreme nutrient and light conditions[3,4] (Fig.1).

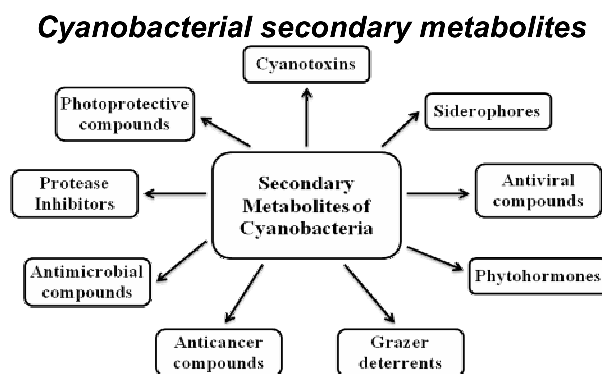

Figure 1
Secondary metabolites of cyanobacteria.

Cyanobacterial secondary metabolites represent a vast diversity of structures and have been isolated from a number of cyanobacterial genera from different geographical locations. During the last two decades, cyanobacterial secondary metabolites have attracted the attention of researchers mainly due to two reasons; (i) acute toxicity of toxins produced by several bloom-forming cyanobacteria in freshwater system and their harmful effect on animals and human health, and (ii) potential therapeutic use of several secondary metabolites[5-7]. The secondary metabolites from cyanobacteria include a range of compounds showing animal toxicity and antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, antiviral, antitumor and cytotoxic activities[3,8]. More than 800 secondary metabolic compounds belonging to several classes of substances including enzyme inhibitors, photosynthesis inhibitors, antimicrobial, antimitotic, immunosuppressures and antitumor depsipeptides have been isolated and classified and named on the basis of chemical structure, bioassay method and their toxicological targets (Fig. 2)[6,9,10]. Many of the oligopeptides produced by cyanobacteria are synthesized by large multifunctional enzyme complexes consisting of non-ribosomal peptide synthetases (NRPSs) and polyketide synthase (PKS) modules[11].

Classification of cyanotoxins

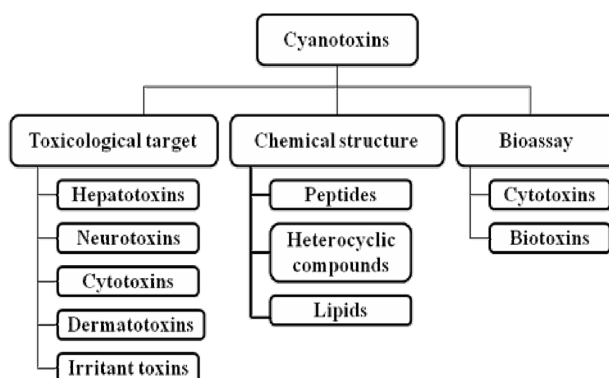

Figure 2
Classification of cyanotoxins on the basis of chemical structure, bioassay and toxicological target.

CYANOTOXINS

Several genera of cyanobacteria such as *Microcystis, Anabaena, Nostoc, Oscillatoria, Cylindrospermum, cylindrospermopsis, Aphanizomenon* and *Nodularia* are known to produce toxins. Based on their toxicological targets cyanobacterial toxins can be classified into five groups: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins[12] (Table 1). On the basis of the bioassay methods, the cyanotoxins are grouped into two categories; cytotoxins and biotoxins[13]. Another class of cyanobacterial poisons is mainly produced by marine cyanobacteria and cause inflammatory activity of skin due to contact and gastrointestinal inflammation upon the ingestion of contaminated water. Details of all the toxin groups are given below separately.

1. Hepatotoxins:

The most commonly encountered toxicosis by cyanobacteria includes hepatotoxicosis involving the hepatotoxins. These toxins mainly damage the hepatocytes of the liver thus owe their name[14]. Death occurs in few hours to few days after initial exposure due to intrahepatic haemorrhage and hypovolemic shock. These are transported preferentially into the hepatocyte and cause an increase in liver weight upto 100% in small animals[15,16]. Hepatotoxins constitute a family of cyclic peptide variants and are classified on the basis of number of amino acids.

Microcystin:

Microcystins (MCs) are produced by members of several cyanobacterial genera including *Microcystis, Anabaena, Planktothrix (Oscillatoria), Anabaenopsis, Nostoc* and *Hapalosiphon* and are most frequently associated with hepatotoxicity in mammals and other vertebrates[17].

Table 1
Cyanobacterial toxins, their source, chemical nature and effect

Toxin	Source	Chemical nature	Effect	LD_{50}
Hepatotoxins				
Microcystins	*Anabaena, Anabaenopsis, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Planktothrix,*	Cyclic heptapeptide	Inhibition of protein phosphatases (PP1 and PP2A)	50-160µg/kg
Nodularin	*Nodularia*	Cyclic pentapeptide	Inhibition of protein phosphatase (PP1 and PP2A)	50 µg/kg
Neurotoxins				
Anatoxin-a	*Anabaena, Aphanizomenon, Cylindrospermum, Oscillatoria, Phormidium, Rhaphidiopsis*	Alkaloid	Binds irreversibly to the nicotinic acetylcholine receptors	375 µg/kg
Anatoxin-a (s)	*Anabaena*	Guanidine methyl phosphate ester	Inhibits acetylcholinesterase	200-250 µg/kg
Saxitoxins	*Aphanizomenon, Anabaena, Cylindrospermopsis , Lyngbya,Planktothrix*	Carbamate alkaloid	Binds and blocks the sodium channels in neural cells	10 µg/kg
Cytotoxins				
Cylindrospermopsins	*Anabaena, Aphanizomenon Cylindrospermopsis, Raphidiopsis, Umezakia*	Guanidine alkaloid	Inhibitor of protein biosynthesis and genotoxic	2.1 mg/kg
Dermatotoxins				
Lyngbyatoxin-a	*Lyngbya, Oscillatoria, Schizothrix*	Alkaloid	Cause erythema (dermatitis), blisters, and necrosis in mammals; potent tumor promoters	—
Aplysiatoxins	*Lyngbya, Oscillatoria, Schizothrix*	Alkaloid	Inflammatory agents, protein kinase C activators	0.3 mg/kg
Endotoxins				
Lipopolysaccharide	All cyanobacteria	Lipopolysaccharide	Inflammatory agent, gastrointestinal irritants	45 - 190 mg/kg

These are cyclic heptapeptides (909-1067 Da) having the general structure cyclo (-D-Ala^{1}-X^{2}-D-Me Asp^{3}-Z^{4}-Adda^{5}-D-Glu^{6}-Mdha^{7}) in which X and Z are variable L-amino acids, D-Ala and D-Glu are alanine and glutamic acid (in the D configuration), respectively, D-MeAsp is D-erythro-β-methylaspartic acid, Mdha is N-methyldehydroalanine and Adda is a novel amino acid (2S, 3S, 8S, 9S)- 3-amino-9-methoxy-2, 6, 8-trimethyl-10 phenyldeca-4, 6-dienoic acid [18] (Fig. 3a). More than 70 structural variants differing mainly at the two L-amino acids at the 2 and 4 positions are known[19] and majority of them are potent inhibitors of protein phosphatases 1 and 2 A.. The most common and toxic variant microcystin-LR (MC-LR) contains amino acids leucine (L) and arginine(R) at positions 2 and 4 respectively. Microcystin is synthesized nonribosomally via a giant enzyme complex comprising peptide synthetases, polyketide synthases (PKSs), and additional modifying enzymes, which are encoded by a *mcy* gene cluster. Nonribosomal peptide synthetases (NRPSs) are involved in the synthesis of linear, cyclic, and branched-cyclic peptides.

Modular PKSs (PKS type I) are multifunctional megasynthases organized into repeated functional units. PKSs assemble acyl coenzyme A monomers by using the core domains ketosynthase, acyltransferase, and acyl carrier protein[20]. Biosynthesis and extracellular concentration of microcystin have been reported to vary under diverse conditions of light, temperature and phosphate which might have implication for the toxicity of cyanobacterial blooms in an aquatic ecosystem. Microcystins accumulate in vertebrate liver cells due to active transport by a highly expressed unspecific organic anion transporter (bile acid carrier transport system). The LD_{50} (i.p. mouse) for the most toxic microcystin (MC) has been reported to be 50-160 μg Kg^{-1} body weight. Death of vertebrate animals is mostly the consequence of severe liver damage which starts with cytoskeletal disorganization and can include cell blebbing, cellular disruption, lipid peroxidation, loss of membrane integrity, DNA damage, apoptosis, necrosis, intrahepatic bleeding, and eventually death by hemorrhagic shock[21,22]. In mammals, the toxicity of microcystin is mediated through its strong binding to key cellular enzymes protein phosphatases (PP1 and PP2A) and they are reported to be the prominent tumor promoter both *in vivo* and *in vitro*[23]. Protein kinases and protein phosphatases play a major part in regulating cell division. Inactivation of protein phosphatases by hepatotoxins disturb the normal balance, resulting in cell proliferation and cancer production. However, these toxins do not seem to initiate a cell's progression towards becoming cancerous, but, once another factor has triggered early changes, the hepatotoxins promote development of further carcinogenic

Chemical structures of microcystin-LR and nodularin

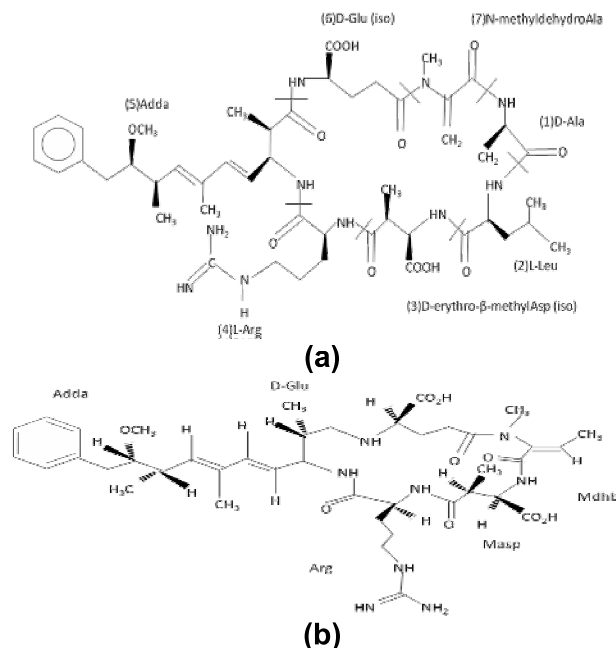

Figure 3

Chemical structures of hepatotoxins (a) microcystin-LR and (b) nodularin.

alterations. It has been suggested that the occurrence of extra-ordinary high rates of liver cancer in China may be correlated to the cyanobacterial toxins in drinking water. Yu et al. (1987)[24] have reported that in China, people who drank pond and ditch water had a higher risk of primary liver cancer than the people who drank well water. Carmichael et al. (1988)[25] have also reported that about 80 % of all ponds sampled in central China showed

dense cyanobacterial population, especially of *M. aeruginosa*. Besides showing animal toxicity microcystins are also known to cause various adverse effects on aquatic macrophytes such as inhibition of growth, photosynthesis and seed germination photosynthesis and induction of oxidative stress[26].

Nodularin:

Nodularin (NODLN) an another class of hepatotoxin, is a cyclic pentapeptide (824 Da) produced by species of *Nodularia* with a generalized structure cyclo (-D-MeAsp^1-L-Arg^2-Adda^3-D-Glu^4-Mdhb^5) where Mdhb is 2-(methylamino)-2-dehydrobutyric acid. Its structure is closely related to that of the microcystins having Mdhb$^{(5)}$ instead of Mdha$^{(7)}$, and lacking D-Ala$^{(1)}$and X$^{(2)}$[27] (Fig. 3b). About eight variants of nodularins are known. NODLN-Har, a structural variant of NODLN was isolated from *Nodularia* PCC7804, having a homoarginine in place of arginine[28] and two dihydronodularins have been chemically synthesized[29]. Nodularins are absorbed into the blood across the ileum, gill or lung membranes and preferentially accumulate in hepatocytes. NODLN is the inhibitor of the catalytic subunits of serine/threonine-specific protein phosphatases 1 (PP1) and 2A (PP2A) and its mode of action is very similar to that of the MCs[30], except that NODLN does not bind covalently to PP1 or PP2A.

2. Neurotoxins:

On the basis of their structure, cyanobacterial secondary metabolites with neurotoxic activity can be grouped into two groups; (i) the alkaloids, including anatoxin-a and its analog homoanatoxin-a, anatoxin-a(s), and paralytic shellfish poisoning (PSP) toxins e.g., saxitoxin, gonyautoxin, and their derivatives, and (ii) the lipopeptides, including antillatoxins A and B, kalkitoxin, and the jamaicamides. Blooms of *Anabaena flos-aque*, which produce anatoxins, represent one of the most extreme cases of cyanobacterial poisoning. Neurotoxins mainly interfere with the normal functioning of the nervous system and cause very fast death within few minutes. They disrupt normal signaling between neurons and muscles in several ways, leading to death by causing paralysis of respiratory muscles followed by suffocation.

a. Alkaloid Neurotoxins:

So far three anatoxins (Figs. 4a,b,c) have been described namely, anatoxin-a (antx-a) (165 Da) and homoanatoxin-a(179 Da), these are alkaloids described as secondary amines whereas anatoxin-a(s) (252 Da) is a unique phosphate ester of a cyclic N-hydroxyguanidine structure[31]. Anatoxin-a, formerly known as the very fast death factor was the first toxin reported from a freshwater cyanobacterium to be chemically and functionally defined[31]. This toxin was first isolated from *Anabaena flos-aquae* but is also known to be produced by *Anabaena*, *Oscillatoria*, *Aphanizomenon*, *Cyclindrospermum* and in small amount even by *Microcystis* species[32]. It is a potent post-synaptic neuromuscular blocker with a LD_{50} (i.p.) value of 375 μg kg^{-1} body weight.

Antx-a mimics acetylcholine in structure and becomes bound to acetylcholine receptors on the muscle cell thereby triggering muscle contraction. However unlike acetylcholine it cannot be degraded by the enzyme acetylcholinesterase and this leads to exhaustion and overexcitation of muscle cells. It also causes muscle membrane depolarization and desensitization resulting in neuromuscular blockade. The novel, and the potential pharmacological applications of anatoxin-a have led numerous organic chemists to develop diverse strategies for total synthesis of anatoxin-a[33].

Chemical structures of neurotoxins

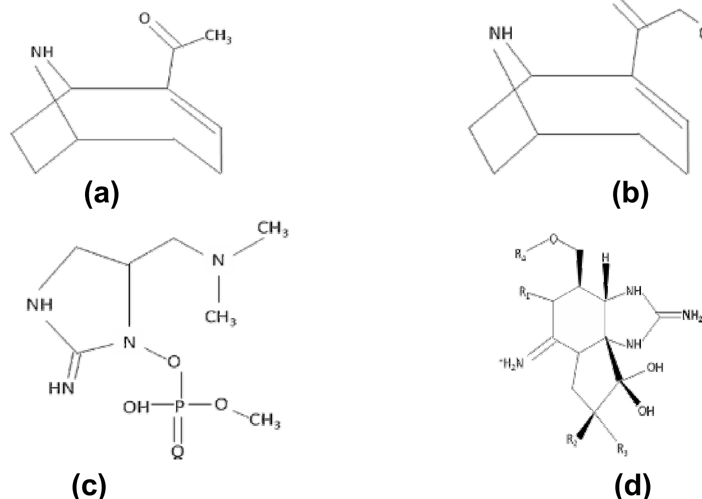

Figure 4

Chemical structures of neurotoxins (a) anatoxin-a, (b) homoanatoxin, (c) anatoxin-a(s) and (d) saxitoxin.

Anatoxin-a(s) is an unusual natural organophosphate, which irreversibly inhibits acetylcholinesterase and is reported to be 10 times more toxic to mice than anatoxin-a[34] with a LD_{50} (i.p.) value of 20 µg kg^{-1} body weight and a survival time of 10-30 min. Homoanatoxin a is a methyl homolog of antx-a with toxicity somewhat lesser than antx-a with a LD_{50} (i.p.) values of 200-250 µg kg^{-1} body weight.

Saxitoxins (299 Da) also known as paralytic shellfish poisons (PSPs) are produced by species of the genera *Aphanizomenon*, *Anabaena*, *Lyngbya*, and *Cylindrospermopsis*[35]. These are a group of alkaloid tricyclic compounds that are either non-sulphated (saxitoxins and neosaxitoxin), single sulphated (gonyautoxins), or doubly sulphated (C-toxins) and more than 20 structural analogues are known[36] (Fig 4d). Saxitoxin blocks neuronal transmission by binding to the voltage-gated Na^+ channels in nerve cells. These potent voltage-gated sodium channel antagonists can cause numbness, paralysis and death in mammals via respiratory arrest by blocking the channel opening, and stopping the inflow of sodium which leads to muscle paralysis and death by respiratory arrest[37,38]. The biotransformation of saxitoxin (STX) in shellfish is through epimerisation, decarbamoylation, and reductive elimination. Toxicity of saxitoxin has been investigated in mammals with mice LD_{50} i.p 10 µg kg^{-1} body weight. The STX is known to be accumulated in marine organisms, mostly in shellfish[39]. The transport of STX through the food chain and bioaccumulation of these toxins in zooplankton is an important mechanism for the availability of these toxins to higher trophic levels.The accumulation of STXs in fish from freshwater aquaculture was investigated and accumulated STXs have been monitored in liver and muscle samples of tilapia (*Oreochromis niloticus*) recently[40].

b. Lipopeptide neurotoxins:

The lipopeptides were first described as being acutely ichthyotoxic (antillatoxin A LC_{50}=0.1 µM, antillatoxin B LC_{50}=1 µM, kalkitoxin LC_{50}=0.7µM, jamaicamides A, B, and C LC_{100} ≥5 ppm) but later identified as neurotoxic[41-44]. Kalkitoxin and jamaicamide C are potent brine shrimp toxins with a LC_{50} of 0.17 µM and LC_{25}=10 ppm, respectively[42,44]. Kalkitoxin also inhibits cell division in sea urchin embryos (IC_{50} ≈0.025 µM). Unlike hepatotoxins, detailed studies on neurotoxins are lacking, there is need to make critical study on mode of action and molecular basis of sythesis of this important class of toxin.

3. Cytotoxins:

Cylindrospermopsin:

Cylindrospermopsin (CYN) is produced by several freshwater cyanobacteria, such as

Cylindrospermopsis raciborskii, Umezakia natans Aphanizomenon ovalisporum, Rhaphidiopsis curvata, and *Anabaena bergii*. CYN (415 Da) is a tricyclic guanidine derivative containing a hydroxymethyluracil group (Fig. 5)[45]. Only three structural variants of the cylindrospermopsin molecule have been described.

Cylindrospermopsin

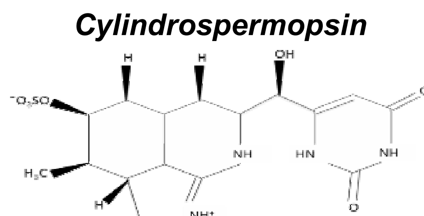

Figure 5
Chemical structure of cylindrospermopsin.

CYN and its analogs, deoxycylindrospermopsin[46] and 7-epi-cylindospermopsin[47], have been classified as hepatotoxins since exposure causes the greatest damage to the liver/hepatopancreas; however, effects are commonly observed in other organs as well, CYN and its analogs have also been generally classified as cytotoxins (i.e., substances toxic to cells). Other organs affected include kidneys, lungs, heart, stomach, and adrenal glands.CYN exposure results in the irreversible inhibition of protein synthesis, cellular necrosis, atrophy, DNA fragmentation, and tumor initiation or mutagenesis[12]. CYN is known to inhibit division of animal cells and the protein synthesis in eukaryotes, although the molecular mechanism is yet to be understood[48]. The alkaloid probably exerts its effect through DNA intercalation followed by strand cleavage. Recently histological and cytological alterations induced by CYN in roots of common reed (*Phragmites australis*) have been studied by Beyer et al (2009)[49]. Other studies demonstrated that the CYN induced growth inhibition of CHO-K1 cells is related to both microtubule and actin filament reorganization[50]. It has been revealed that CYN and deoxyCYN do not halt protein phosphatases but are a significant and permanent inhibitor of protein biosynthesis[51,52].

4. Dermatoxins/ tumor promoters:

Lyngbya majuscula the marine cyanobacterium is the producer of lyngbyatoxins and aplysiatoxins which are inflammatory agents. Lyngbyatoxins A (LA) (Fig 6), B and C are indole alkaloids in nature while aplysiatoxins are phenolic bislactones consisting of aplysiatoxin (AT), debromoaplysiatoxin (DAT), and their less toxic anhydro variants[53]. Lyngbyatoxins and aplysiatoxins are regarded as dermatoxins[54], and tumor promoters[55].

Lyngbyatoxin A

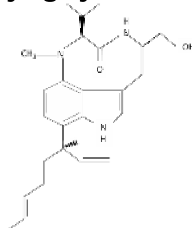

Figure 6
Chemical structure of lyngbyatoxin A.

Intraperitoneal injection of LA and AT to mice caused intestinal bleeding, stomach ulcers, and eventually death due to hemorrhagic shock[54]. Topical application of LA, AT and DAT resulted in dermatitis, blisters and necrosis in mammals. LA and DAT have the ability to accumulate in marine grazers (species of sea slugs)[56].

5. Irritant toxins:

Cyanobacterial lipopolysaccharides (LPS) are generally classified as endotoxins. Numerous genera of cyanobacteria including *Microcystis, Anabaena, Spirulina,* and *Oscillatoria* are known to produce toxic LPS. These are comprised of a carbohydrate polymer, a core oligosaccharide and an acylated glycolipid (lipid A). The latter segment is linked to toxicity in enteric, heterotrophic gram-negative bacteria, and found to be within or at the surface of the outer cell layer. Cyanobacterial LPS are known to be associated with numerous cases of human illness ranging from skin irritation to gastrointestinal tract and respiratory distress[57]. Co-administration of *Microcystis* LPS together with microcystin LR, decreased hepatotoxin potency[58]. Further research on health implications of cyanobacterial LPS in humans is required.

Genetic basis of toxin production in cyanobacteria:

Studies on the identification of biosynthetic genes responsible for the production of cyanotoxins were initiated with *M. aeruginosa*, a microcystin-LR producer. Microcystins are synthesized by the enzyme complex microcystin synthetase which includes nonribosomal peptide synthetase (NRPS), polyketide synthase (PKS) mixed NRPS/PKS and some additional tailoring enzymes. These are encoded by *mcy* gene cluster (55 kbp) containing 10 genes in *Microcystis* of which 6 large open reading frames (ORFs) (*mcy A-E* and *G*) are of a mixed NRPS/PKS nature together with four small ORFs (*mcy F* and *H-J*) with putative precursor and microcystin tailoring function. So far *mcy* cluster from *Microcystis*, *Plaktothrix and Anabaena*[59,60] and the closely related nodularin (*nda*) synthetase gene cluster from *Nodularia*[61] have been identified and sequenced and comparative analysis has shown significant differences with respect to number and arrangement and the localization and orientation of promoter region (Fig. 7).

Structural organization of the microcystin (mcy) gene clusters

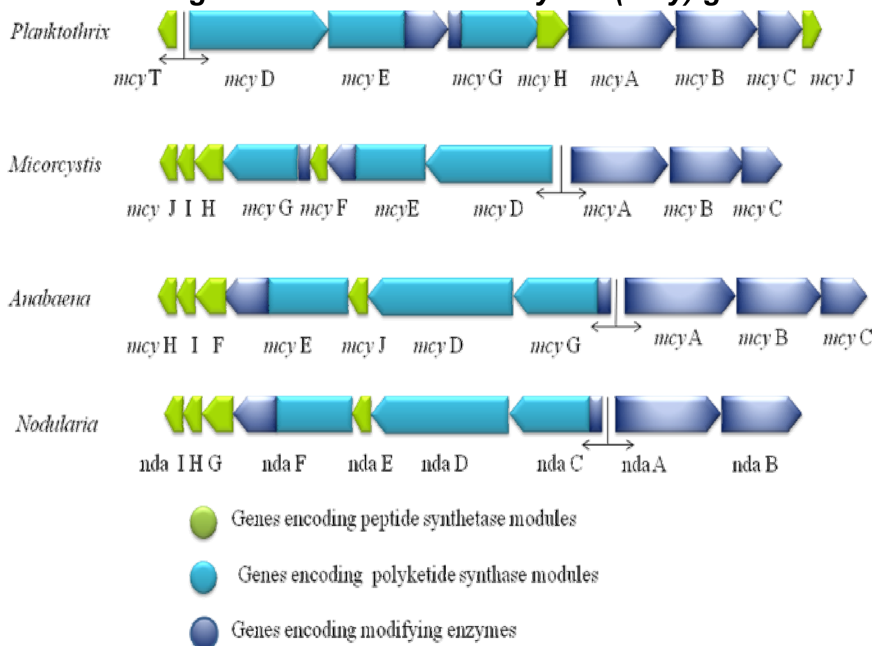

Figure 7

Structural organization of the microcystin (mcy) gene clusters from Microcystis, Planktothrix and Anabaena and of the nodularin (nda) synthetase gene cluster from Nodularia. Arrows indicate the direction of transcription, in which the (bi)-directional promoter sites have been indicated (scale not shown). (Modified from Kurmayer and Christiansen 2009[130]).

In *Microcystis* and *Anabaena* sp**.,** the genes are arranged into two divergently transcribed operons, however, the arrangement of genes within these operons differs between the two species. In *Planktothrix*, the *mcy* cluster also has a distinctive arrangement and lacks *mcyF* and *mcyI***.** Furthermore in *Planktothrix* the cluster contains an additional gene *mcy*T**,** upstream of the central promoter region.

The similarity of the *mcy* gene cluster between genera is remarkable. Except for the tailoring enzymes *mcyI* (a 2-hydroxy-acid hydrogenase)[62], *mcyF* (an aspartate racemase)[63], and *mcyT* (a type II thioesterase)[64], all other genes *mcy A B C D E G H J* are always present. Occurrence of *mcy* gene cluster in between genera can be explained on the basis of the horizontal transfer[61,65], vertical transfer[66,67]and gene loss hypothesis[68]. It has been suggested that the nodularin biosynthesis cluster evolved from the microcystin cluster by domain deletion.

Structure of cylindrospermopsin suggested a polyketide origin of the hepatotoxin and a screening of 13 *Cylindrospermopsis* strains revealed that the presence of PKS and NRPS genes could be correlated to cylindrospermopsin production[69]. Putative cylindrospermopsin biosynthesis gene cluster involving NRPS/PKS modules has been proposed but there is no biochemical proof for the role of this gene cluster in cylindrospermopsin biosynthesis. Similarly a saxitoxin (*sxt*) biosynthesis gene cluster consisting of 31 open reading frames has been described from *Cylindrospermopsis raciborskii*[70] but the conclusive proof for its involvement in saxitoxin biosynthesis is lacking.

DETECTION AND ANALYSIS OF CYANOTOXINS:

Several methods for screening, characterization and quantification of cyanobacterial toxins have been developed in recent years. Bioassay, chemical assay and immunoassay are main techniques of detection for the cyanotoxins.

I. Classical method for screening of water bloom material and laboratory cultures or cell extracts for toxicity includes mouse bioassay. From the signs of poisoning it is possible to distinguish hepatotoxins from neurotoxins and even the different types of neurotoxins[13].
II. Biochemical method includes estimation of protein phosphatase and cholinesterase activity since activity of these enzymes is severely inhibited by microcystin and anatoxin-a[23, 71].
III. Immunological assay includes Enzyme linked immunosorbent assay (ELISA) and Radio immune assay (RIA), used frequently for the detection of cyanotoxins present even in a very minute quantity in the sample[72].
IV. NMR and mass spectroscopic methods such as FABMS/CID/ LC-MS are reported to be very useful for detection and determination of structure of cyanobacterial toxins because of their sensitivity and speed of analysis[73,74].
V. Recently, various molecular techniques mostly based on PCR are used for screening, identification and characterization of genes responsible for toxin production[75].
Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF) has been used for the detection of the low molecular mass anatoxin-a and homoanatoxin-a directly on cyanobacterial filaments of axenic strains of the genus *Oscillatoria*[76]. Ellman test is also frequently used for the detection of anatoxin-a(s)[77].

PHYTOHORMONES:

Another important feature of cyanobacteria in relation to secondary metabolite production is the ability to produce the phytohormones which was otherwise considered to be a sole trait of the plant kingdom. However recent studies show that cyanobacteria can also benefit plants by producing growth promoting regulators (PGRs)/hormones similar to IAA, cytokinin, gibberellin, ethylene, jasmonic acid or abscissic acids [78]. Glasshouse experiments conducted with a variety of vegetable crops together with cyanobacteria have clearly demonstrated the production of PGRs[79]. The ability of cyanobacteria to

produce the phytohormone indole-3-acetic acid (IAA) was demonstrated in free-living and symbiotically competent cyanobacteria that represent all morphotypes from the unicellular to the highly differentiated species. Production of IAA has been reported to be stimulated by exogenous addition of tryptophan in the medium[80]. Cyanobacterial genera such as *Anabaena*, *Anabaenopsis*, *Calothrix*, *Chlorogloeopsis*, *Cylindrospermum*, *Gloeothece*, *Nostoc*, *Plectonema*, *Haplosiphon* and *Synechocystis* have been reported to produce IAA[81]. The concentrated culture filtrates of three cyanobacterial strains *Calothrix ghosei*, *Hapalosiphon intricatus* and *Nostoc* sp. were able to enhance germination percentage, radicle and coleoptile length in studies with wheat seeds. TLC analyses of the filtrates revealed the presence of several amino acids, such as histidine and auxin like compounds. Gibberellin like substances has also been isolated from the cyanobacterium, *Phormidium foveolarum*[82], *Cylindrospermum* sp., *Anabaenopsis* sp.[83] and *Scytonema hofmanni*[84]. Recently screening and determination of cytokinins and auxin in five cyanobacterial strains namely *Anabaena* sp., *Oscillatoria* sp., *Phormidium* sp., *Chroococcidiopsis* sp. and *Synechosystis* sp. was made by Hussain and coworkers (2010)[85]. For screening, a rapid chromatographic method, ultra performance liquid chromatography and electrospray ionization-tandem mass spectrometry was developed for the simultaneous determination of cytokinins and indole-3-acetic acid (IAA). Unfortunately, sporadic reports are available on the occurrence of phytohormones in cyanobacteria and more attention is required to work in this fertile area of research.

PROTEASE INHIBITORS:

Equally important classes of cyanobacterial metabolites consist of protease inhibitors, which are known to interrupt the normal functioning of the digestive enzymes. Since their first description[86], numerous cyclic peptides and depsipeptides, which are efficient inhibitors of either tyrpsin or chymotrypsin, have been found in planktonic cyanobacteria. It has been hypothesized that ingested cyanobacterial filaments are partially digested in the gut of grazers, releasing the intracellular protease inhibitors and prevent the cleavage of protein, resulting in deficiency of essential or nonessential amino acid in diet of grazer[87]. Protease inhibitors can be produced by both toxic strains and non-toxic strains of *Microcystis, Anabaena, Planktothrix/Oscillatoria,* and *Nostoc*. It has been found that feeding inhibitory effect was independent of MCs production. Recently nostocarboline from *Nostoc* sp. has been identified as an inhibitor of acetylcholinesterase and trypsin[88-90]. Chemically protease inhibitors consist of depsipeptides and are characterized by an aminohydroxypiperidone (Ahp) unit, a N-methylated amino acid, a threonine, where the lactone ring is formed, and an attached peptidic side-chain, which is often terminated by either hydrophobic fatty acids or polar acids. Many of these depsipeptides have been isolated from cyanobacteria[3]. One of the first members described in the literature is the cyanopeptolins A-D[91] having basic amino acid (Arg in Cyanopeptolin A, Lys in Cyanopeptolin B, *N*-Me-Lys in Cyanopeptolin, *N,N*-Dime-Lys in Cyanopeptolin), Ahp, *N*-Me-Phe as well as a terminal hexanoic acid. Aminohydroxypiperodone is the key residue of protease inhibitors, and all active compounds have a 19 membered lactone ring. Namikoshi and Rinehart (1996)[5] classified serine protease inhibitors into four groups based on their structural characteristics. Cyclic depsipeptides with the unique amino acid 3-amino-6-hydroxy-2-piperidone (Ahp), which include variants of micropeptins, cyanopeptolins, oscillapeptins, nostopeptins, aeruginopeptins, and anabaenopeptilides are placed in group 1 (Fig. 8a).Tricyclic depsipeptides such as microviridins, belong to Group 2 (Figs. 8b,c,d). Group 3 (Fig. 8e) includes aeruginosins and microcins and are linear peptides with a unique amino acid unit 2-carboxy-6 hydroxyoctahydroindole (Choi). Anabaenopeptins and oscillamides belonging

to group 4 are cyclic peptides with an ureido linkage (Fig. 8f). It has been reported that the activity of a detoxification enzyme, soluble glutathione-S-transferase (sGST), is reduced on exposure to microcin SF608 (group 3) in *Daphnia magna*[92], where as microviridin J (group 2) caused lethal molting failure in *Daphnia pulicaria*[93]. Additionally, elastase, plasmin, tyrosinase, papain and thrombin which are serine or cysteine proteases are inhibited by secondary metabolites isolated from cyanobacteria. These protease inhibitors have numerous pharmaceutical and industrial (e.g., fungicide) applications[9].

Chemical structure of protease inhibitors

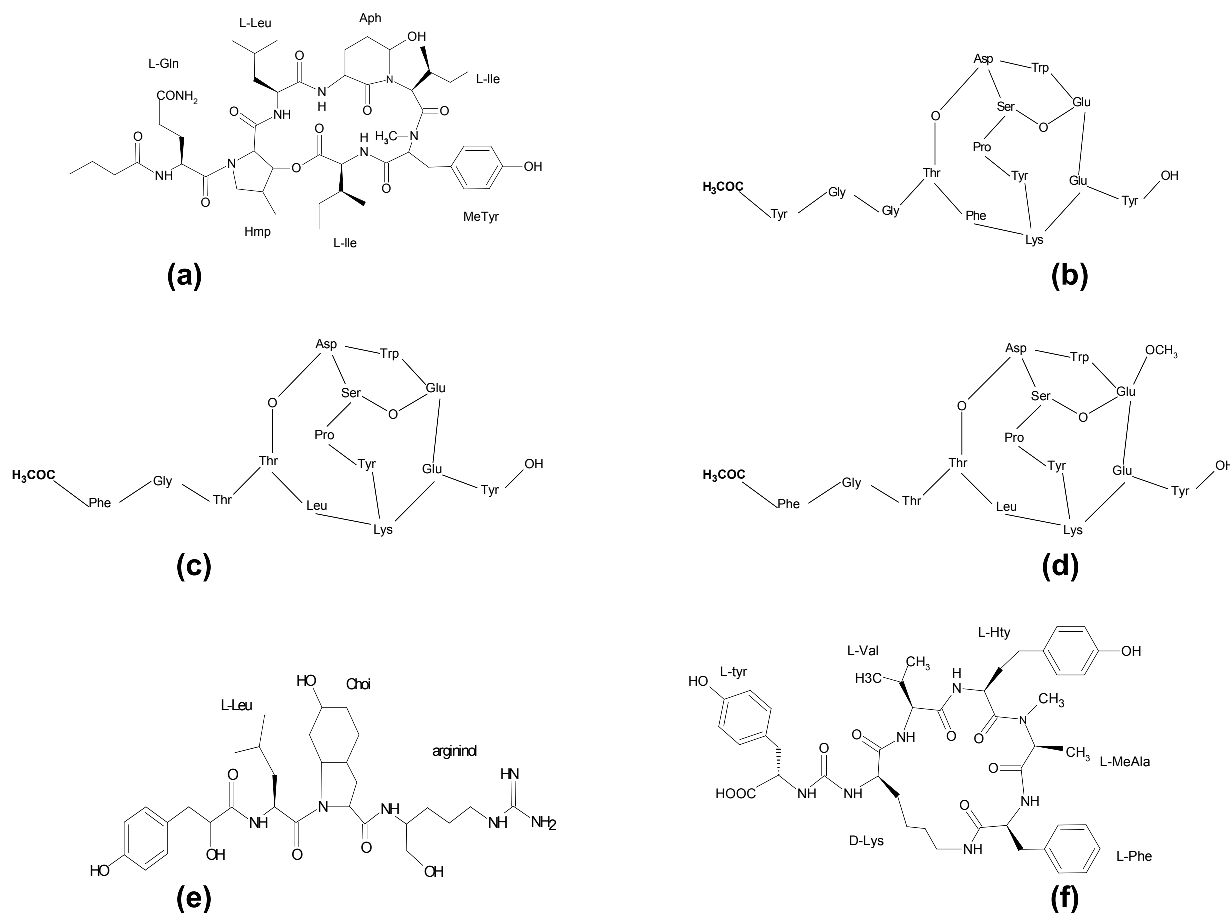

Figure 8

Chemical structure of various protease inhibitors isolated from cyanobacteria (a)Nostopeptin A (gp-1), (b) Microviridin A (gp-2), (c) Microviridin B (gp-2), (d)Microviridin C (gp-2), (e)Aeruginosin 298-A (gp-3), (f)Anabaenopeptin A (gp-4).

SIDEROPHORES:

Iron is the fourth most abundant element by weight in the Earth's crust, however its bioavailability is severely restricted due to the fact that in aqueous oxygen containing environments Fe^{2+} is quickly oxidized to Fe^{3+}. At physiological pH, Fe^{3+} forms insoluble or only poorly soluble hydroxides and hydroxy-aquo complexes[94], severely reducing the biological availability of iron. Iron serves as an essential component of heme and iron sulfur centers integrated into a variety of proteins that function in basic physiological processes such as photosynthesis, respiration, and nitrogen metabolism[95]. Several organisms have

developed mainly two sophisticated systems for iron acquisition. One involves utilization of iron chelating compounds including various siderophores and the other system involves reduction of ferric iron to ferrous iron by a plasma membrane redox system, followed by uptake using specific transporters[96]. Siderophores are low molecular weight, high-affinity Fe (III)-binding ligands secreted by microbes under conditions of iron stress to scavenge and transport iron. Siderophores bind to Fe^{3+} to form a ferrisiderophore complex which facilitates the transport of ferric ions into cells during periods of iron deficiency[97]. In cyanobacteria, siderophore-mediated iron uptake is a contributing factor in their ability to dominate eucaryotic algae. The first report on a cyanobacterial siderophore was schizokinen (Fig. 9a) isolated from the freshwater *Anabaena* PCC 7120[98]. Latter on, a complex siderophore, anachelin was isolated from an *Anabaena* species. Anachelin-1 and anachelin-2 (Figs. 9b,c) were isolated as the first genuine cyanobacterial siderophores from the freshwater cyanobacterium *Anabaena cylindrica*. Lately two complex siderophores, anachelin H containing a terminal salicylamide and anachelin 1, terminated by an oxazoline ring were isolated from biofilm-forming cyanobacterium, *Anabaena cylindrica* 1403-2a[99]. Three photoreactive and amphiphilic siderophores namely synechobactins A, B and C have been isolated from a marine *Synechococcus* sp. PCC 7002 by Ito and Butler (2005)[100].

Chemical structure of siderophores

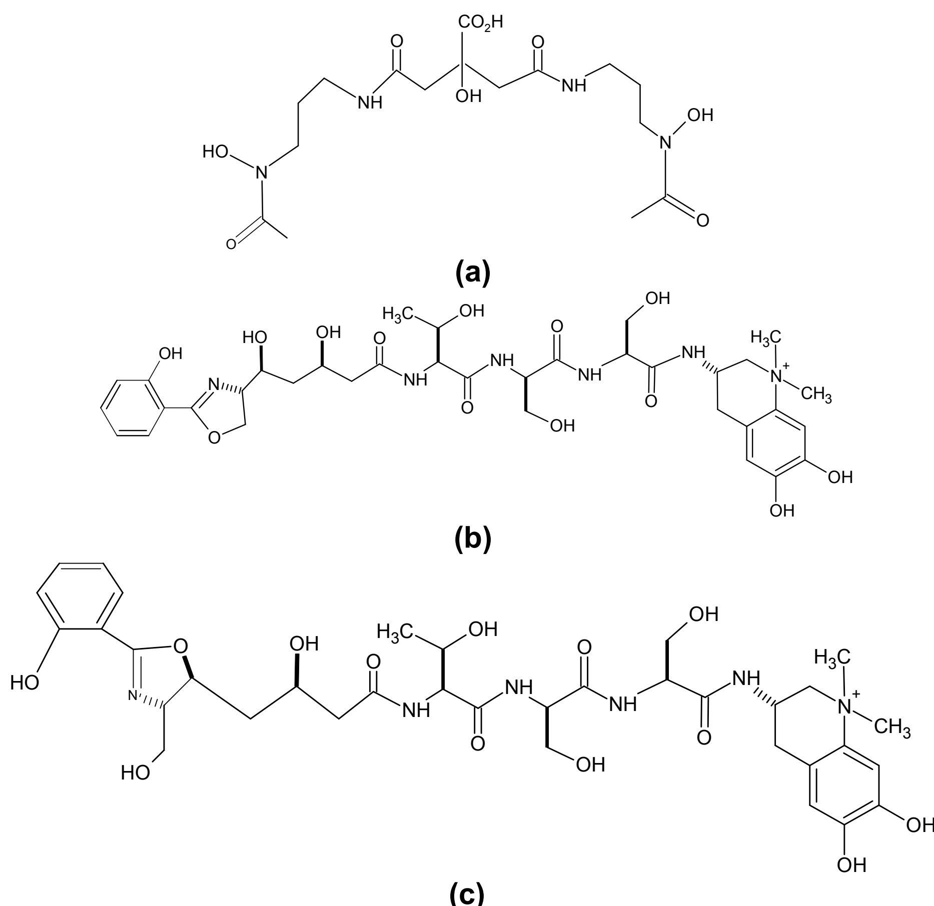

Figure 9

Chemical structure of siderophores (a) Schizokinen, (b) Anachelin 1 and (c) Anachelin 2.

UV-PROTECTIVE COMPOUNDS:

UV radiation (UVR) is an important component of solar radiation having many deleterious effects on biological system including cyanobacteria. In order to counteract the injurious effect of solar ultraviolet radiation cyanobacteria synthesize UV-protecting/screening compounds such as mycosporine-like amino acids (MAAs) and scytonemin[101]. Brief details of UV-protecting/screening compounds so far reported from cyanobacteria are presented below.

MAAs:

These molecules strongly absorb UV radiation, with the maximum absorbance between 310 and 360 nm and found in many cyanobacteria MAAs have been reported from cyanobacterial species growing in diverse habitats exposed to irradiance of solar radiation[102]. Their presence has also been reported from strains growing in Antarctica as well as from a community of halophilic species[103].

Chemical structure of MAAs

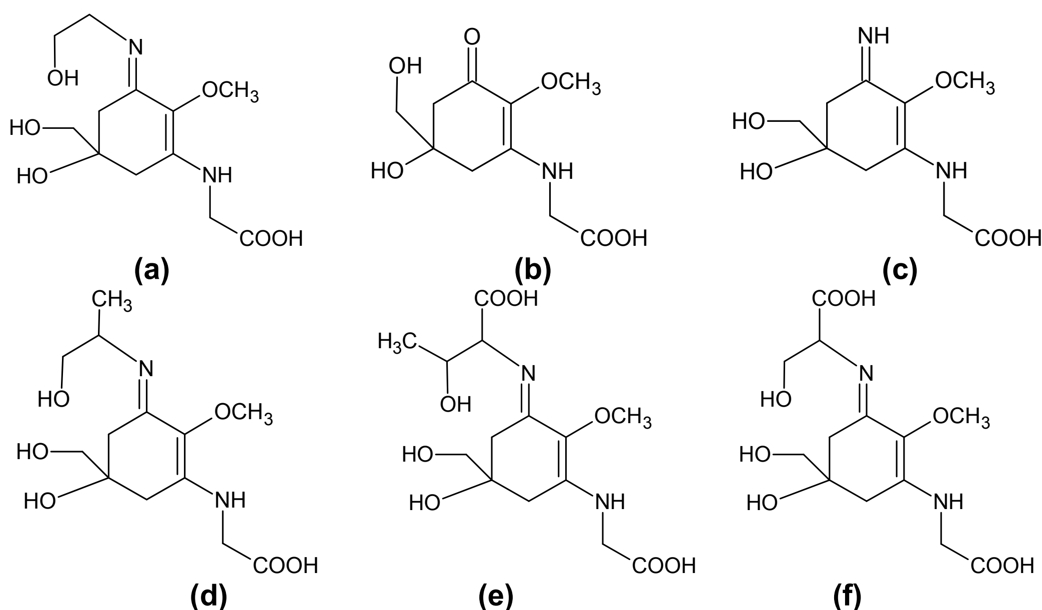

Figure 10
Chemical structure of some MAAs (a) Asterina-330 (330nm) (b) Mycosporine-glycine (310nm) (c) palythine (320nm) (d) Palythinol (332nm) (e) Porphyra-334 (334nm) (f) Shinorine (334nm). (Modified from Sinha and Häder 2008[101]).

MAAs are water-soluble, low molecular-weight (generally <400) colorless compounds, composed of either a cyclohexenone or a cyclohexenimine chromophores conjugated with the nitrogen substituent of an amino acids or its imino alcohol (Fig. 10a-f).

Most effective inducing wavelengths for MAA synthesis lies between 280 and 320 nm. In the rice-field cyanobacteria *Anabaena* sp., *Nostoc commune* and *Scytonema* sp. the production of shinorine, a bisubstituted MAA, was found to be increasing under natural solar radiation concentrations during the light periods whereas the constant values were found during dark periods[104]. *Microcystis aeruginosa* was found to produce the MAAs Porphyra-334 and shinorine[105]. A mixture of MAAs (i.e., P-334 + shinorine) extracted from the red alga *Porphyra umbilicalis* has been reported to suppress UV-induced aging in human skin[106]. Misonou et al. (2003)[107] have reported that MAAs can block the production of both 6-4 photoproduct and cyclobutane pyrimidine dimer formation. The biosynthesis of MAA in cyanobacteria is dependent on photosynthesis for the carbon source, as an

externally added carbon source was found to overcome the negative effect of DCMU on MAA biosynthesis. DCMU an inhibitor of the photosynthetic electron transport blocked the synthesis of MAAs in the dinoflagellate *Alexandrium excavatum*. These findings clearly suggest that MAA synthesis is closely linked to photosynthesis[108]. Growth media with a inorganic combined nitrogen source also enhanced MAA synthesis in comparison to medium without a nitrogen source. There is a general consensus that MAAs are synthesized from the early steps of the shikimate pathway. Favre-Bonvin *et al.* (1987)[109] suggested that the shikimate pathway intermediate, 3-dehydroquinate (DHQ), is the precursor for the six-membered carbon ring common to fungal mycosporines. MAAs are treated as true 'multipurpose' secondary metabolites, having many additional functions in the cell beyond their well-known UV screening role. They may serve as antioxidant molecules scavenging toxic oxygen radicals and can be accumulated as compatible solutes following salt stress, their formation is induced by desiccation or by thermal stress in certain organisms. They have also been suggested to function as an accessory light-harvesting compound in photosynthesis [110,111].

Scytonemin:

Scytonemin is a yellow-brown lipid-soluble pigment which has been reported from the extracellular polysaccharide sheaths of about 300 cyanobacteria. It has a molecular mass of 544 Da (Fig 11a) and is a dimeric pigment made up of indole and phenolic substances with an *in-vivo* absorption maximum at 370 nm. However purified scytonemin shows maximum absorbance at 386 nm.

Chemical structure of photoscreening compounds

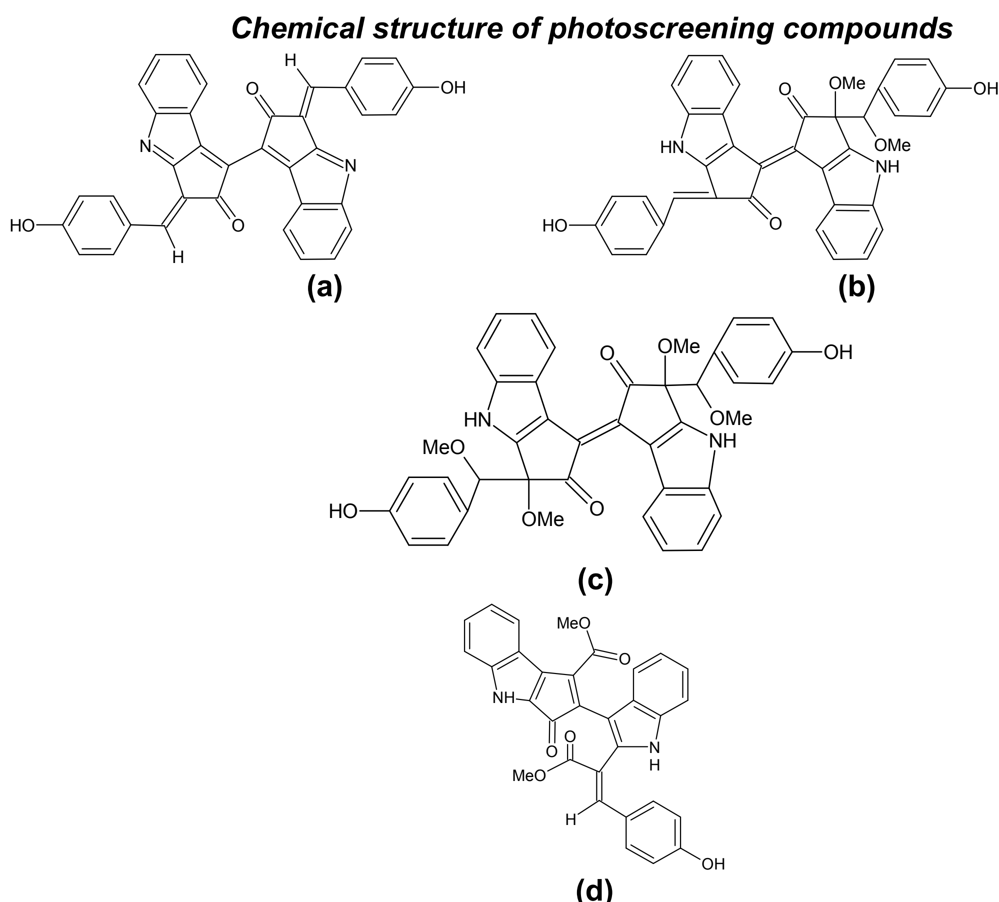

Figure 11

Chemical structure of photoscreening compounds (a) Scytonemin (b) dimethoxyscytonemin (c) tetramethoxyscytonemin, (d) scytonin. (Adapted from Sinha and Häder 2008[101]).

Three new pigments dimethoxyscytonemin, tetramethoxyscytonemin and scytonin (Figs. 11b,c,d) have also been isolated from the organic extracts of *Scytonema* sp.[112].

Strong evidence for the role of scytonemin as ultraviolet shielding compound has been presented in several cyanobacterial isolates and collected materials from diverse harsh habitats, mostly exposed to high irradiances[113]. Increase in temperature and oxidative stress in combination with UV-A, have a synergistic effect on high production of scytonemin[114]. Additionally, Fleming and Castenholz (2008)[115] have demonstrated the importance of nitrogen in the synthesis of scytonemin in the cyanobacterium *Nostoc punctiforme* PCC 73102. There is evidence which suggests that certain open reading frames (ORFs), NpR1274 to NpR1271 had a significant influence on scytonemin biosynthesis. NpR1273 is thought to be directly involved in scytonemin biosynthesis in *N. punctiforme*[116]. Scytonemin is a non-toxic, antiproliferative agent, and its unique chemical structure puts forward a potential scaffold for additional chemical modification that could be used to build up a new class of therapeutically valuable drugs in treating hyperproliferative disorders[117].

BIOTECHNOLOGICAL POTENTIAL OF CYANOBACTERIAL SECONDARY METABOLITES:

Role of cyanobacterial secondary metabolites in toxicity, as iron chelator, protease inhibitor, growth inhibitory as well as growth promoting properties have been well documented [4,6,12, 81]. Some metabolites such as microcystins, saxitoxins or anatoxins, are of global significance because of their toxicity while other display significant pharmaceutical potential[118]. These metabolites may be used for the development and application as algaecides, fungicides, herbicides and insecticides[119]. Biocidal activity against several phytopathogenic fungi has been observed from the *Anabaena* isolates wherein the hydrolytic enzymes produced by these genera play an important role as biocontrol agents[120]. It has been found that the cyanotoxins, anatoxin-a, microcystins and cylindrospermopsin obtained from *Anabaena*, *Microcystis* and *Cylindrospermopsis* respectively show larvicidal activity with >50 % mortality[119]. Cyanobacteria also contribute in production and accumulation of plant growth promoting substances. Their advantages over expensive synthetic phytohormones include broader spectrum of activity and optimum levels of biologically active molecules, which are needed for normal plant development *in-vivo* or *in-vitro*. A number of studies have demonstrated the growth promotion activity of cyanobacterial extracts on plant regeneration and plantlet formation[80,81]. Pharmaceutical and industrial applications of protease inhibitors have also been reported[9]. Recently, a compound nostocarboline, an acetyl- and butyrylcholinesterase, and trypsin inhibitor[88] from *Nostoc* 78-12A has been isolated which shows strong algicidal activity against both eukaryotic and prokaryotic phototrophs[121]. Different variants of aeruginosin have been isolated from the planktonic cyanobacterial genera such as *Microcystis*, *Nodularia* and *Planktothrix*[122,123]. The aeruginosins particularly inhibit serine proteases (trypsin, chymotrypsin, thrombin or elastase) and has been regarded as a promising drug candidate [123].

The iron acquisition by siderophores is supposed to be of great importance in molecular understanding of iron uptake by marine and freshwater organisms as well as surface modification in materials science[3]. Siderophores are of particular interest as possible drugs for use both in the treatment of iron overload and for conditions of both acute and chronic metal toxicity[124] as well as in the areas ranging from biomaterials to biosensors[125].

MAAs are true multipurpose secondary metabolites considered as natural photoprotectants that may be biotechnologically exploited in various ways[126]. Recently, protective effects of MAAs (such as shinorine, porphyra-334 and

mycosporine–glycine) on human fibroblast cells from UV irradiation and protection of the cells from UV-induced cell death has been investigated by Oyamada and coworkers (2008)[127]. These compounds are capable of effectively dissipating absorbed radiation as heat without producing reactive oxygen species (ROS)[128]. These compounds may be of great value in the development of artificial sunscreens and a source for future biotechnological research. Scytonemin was first characterized as small molecule inhibitor of polo-like kinase 1(PLK1), a serine/threonine kinase that plays an integral role in regulating the G2/M transition in the cell cycle[117]. Pharmacological potential of scytonemin as interesting antiinflammatory and antiproliferative activities and its unique chemical structure puts forward a potential scaffold for additional chemical modification that could be used to build up a new class of therapeutically valuable drugs in treating hyperproliferative disorders[117,129,130].

CONCLUSION

Cyanobacteria are natural inhabitants of fresh, brackish and marine waters, producing a diverse range of secondary metabolites[131,132]. All these metabolites make this prokaryotic group successful for the survival in vastly different habitats thereby interacting and dominating over microbes to mammals. Some metabolites such as cyanotoxins are reported widely and are of general concern due to human and animal health hazard. Protease inhibitors are compounds produced for deterrence purposes, unattractive food source for grazer. A number of metabolites from cyanobacteria are considerable for their growth promoting activity. Iron chelating compounds (siderophores) and compounds with UV screening properties in cyanobacteria population facilitate them for inhabiting in extreme conditions such as bare rocks or the open ocean. The sources and properties of these compounds have been highlighted in this review. Several cyanobacterial secondary metabolites such as cyanotoxins have significant pharmaceutical potentials comprising a rich source of natural cytotoxic compounds. Production of antimicrobial, anticancer, antiviral and enzyme inhibiting compounds may be utilized for the benefit of mankind that is conspicuous aims of biomedical research.

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