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Bioactive Compounds from Cyanobacteria and Microalgae: An Overview

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ABSTRACT Cyanobacteria (blue-green algae) are photosynthetic prokaryotes used as food by humans. They have also been recognized as an excellent source of vitamins and proteins and as such are found in health food stores throughout the world. They are also reported to be a source of fine chemicals, renewable fuel and bioactive compounds. This potential is being realized as data from research in the areas of the physiology and chemistry of these organisms are gathered and the knowledge of cyanobacterial genetics and genetic engineering increased. Their role as antiviral, anti-tumour, antibacterial, anti-HIV and a food additive have been well established. The production of cyanobacteria in artificial and natural environments has been fully exploited. In this review the use of cyanobacteria and microalgae, production processes and biosynthesis of pigments, colorants and certain bioactive compounds are discussed in detail. The genetic manipulation of cyanobacteria and microalgae to improve their quality are also described at length.

KEYWORDS cyanobacteria, microalgae, bioactive compounds, toxins, pigments, colorants.

I. INTRODUCTION

Cyanobacteria belong to the kingdom Monera and division Cyanophyta. They are among the most primitive forms of life on earth. Their cellular structure is simple prokaryote and performs photosynthesis, resembling plants but lack plant cell walls resembling primitive bacteria. These also resemble animals in having complex sugars like glycogen on their cell membrane. These include edible and toxic species. Edible blue green algae include *Nostoc, Spirulina* and *Aphanizomenon.* Cyanobacteria have the appeal of being a raw unprocessed food, rich in carotenoid, chlorophyll, phycocyanin, amino acid, minerals and many other bioactive components. The nutrient content depends on the location and environment in which the algae are grown. The environment includes altitude, temperature and sun exposure, which can greatly affect the lipid and pigment content in algae. Algae grown in canals and rivers differ from that from the sea. Prokaryotic photosynthetic microorganisms are rich in biologically active secondary metabolites. They are truly prokaryotic having no nuclear membranes, internal organelles and histone proteins associated with chromosomes. They are capable

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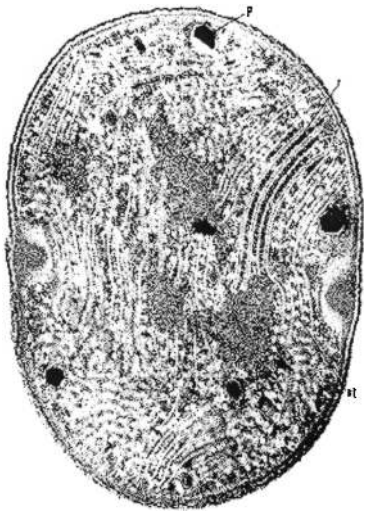

FIGURE 1 **Electron micrograph of cyanobacterium (Allen *et al.*, 1968).**

of using carbon dioxide as their sole carbon source employing the reductive pentose phosphate pathway or Calvin cycle (Stal and Moezelaar, 1997). They are larger than other bacteria and are mostly aquatic. As they are photosynthetic and aquatic, they are often called "blue-green algae." All cyanobacteria are unicellular (Figure 1), though many grow in colonies or filaments, often surrounded by a gelatinous or mucilaginous sheath, depending upon environmental conditions. Some of the filamentous colonies show ability to differentiate into three different cell types: vegetative cells, climate resistant spores and thick-walled heterocyst. In particular to divisions of microalgae, pyrrophyta (dinoflagellates) and cyanophyta are rich source of novel compounds and have been the subject of extensive investigation (Hayashi *et al.*, 1994). The cyanophyta division are widely classified and termed as cyanobacteria and not included in the category of algae because of their prokaryotic characteristics (Table 1).

1.1 Cyanobacteria as a Producer of Bioactive Compounds

Cyanobacteria have been identified as one of the most promising group of organisms from which novel

TABLE 1 **Principle groups of cyanobacteria.**

Classification of cyanobacteria			
Morphology	Reproduction	Order	Names (general)
Unicellular or Colonial	Binary fission = splitting in two	Chroococcales	*Gloeobacter* *Chroococcus* *Microcystis* *Synechocystis* *Merismopedia*
Unicellular or Colonial	Budding Multiple Fission = splitting in more than two parts	Chamaesiphonales	*Chamaesiphon* *Pleurocapsales* *Dermocarpa* *Xenococcus* *Pleurocapsa*
Filamentous	Trichome, which is a chain of cells	Nostocales	*Oscillatoria* *Microcoleus* *Lyngbya* *Phormidium* *Schizothrix* *Spirulina* *Plectonema*
Filamentous heterocystous	Trichome fragmentation = splitting of the chain of cells	Nostocaceae Rivulariaceae Scytonemataceae	*Anabaena* *Nostoc* *Cylindrospernum* *Calothrix* *Rivularia* *Scytonema*
Branched filamentous	Trichome fragmentation	Stigonematales	*Westiella* *Fisherella* *Stigonema* *Chlorogloeopsis*

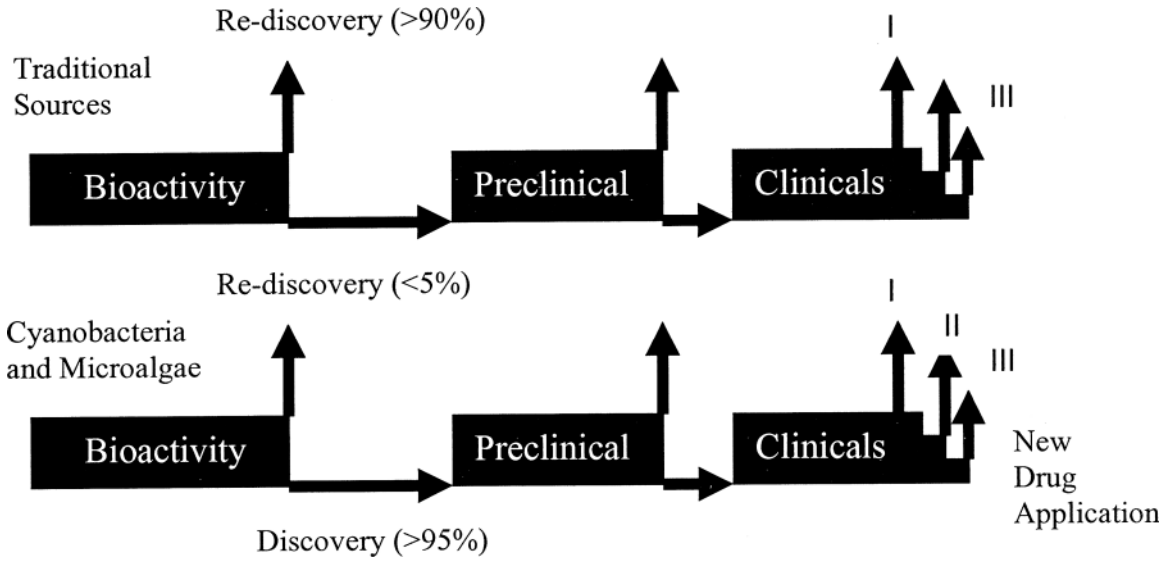

FIGURE 2 **Probability of new drug development rate (Olaizola, 2003).**

and biochemically active natural products are isolated. Cyanobacteria such as *Microcystis, Anabaena, Nostoc* and *Oscillatoria* produce a great variety of secondary metabolites. The only comparable group is *actinomycetes*, which has yielded a tremendous number of metabolites. The rate of discovery from traditional microbial drug producers like *actinomycetes* and *hyphomycetes*, which are in the focus of pharmaceutical research for decades, is decreasing and it is the time to turn to cyanobacteria and exploit their potential. This is of paramount importance to fight increasingly resistant pathogens and newly emergent diseases (Hayashi *et al.*, 1994). Because cyanobacteria are largely unexplored, they represent a rich opportunity for discovery; the expected rate of rediscovery is far lower than for other better-studied groups of organisms (Figure 2) (Olaizola, 2003). Cyanobacteria produce a wide variety of toxins and other bioactive compounds, which include 40% lipopeptides, 5.6% amino acids, 4.2% fatty acids, 4.2% macrolides and 9% amides (Figure 3). Cyanobacterial lipopeptides include different compounds like cytotoxic (41%), antitumor (13%), antiviral (4%), antibiotics (12%) and the remaining 18% activities include antimalarial, antimycotics, multi-drug resistance reversers, antifeedant, herbicides and immunosuppressive agents (Figure 4) (Burja *et al.*, 2001); besides the immune effect, blue green algae improves metabolism (Table 2). Blue green algae have a cholesterol-lowering effect in animals and humans. The level of the total cholesterol, LDL and VLDL cholesterol in rat serum was reduced when a high cholesterol diet was supplemented with blue green algae. It was found that adopohepatosis caused by a high cholesterol diet was cured by a diet supplemented with algae. This was due to the activity of lipoprotein lipase, an enzyme for metabolism of triglyceride rich lipoproteins (Iwata *et al.*, 1990). *Aphanizomenon flos-aquae* also shows hypocholesterolemic effect due to its chlorophyll content, which stimulates the liver function and decreases blood cholesterol level (Vlad *et al.*, 1995). *Aphanizomenon flos-aquae* inhibit the activity of a maltase and

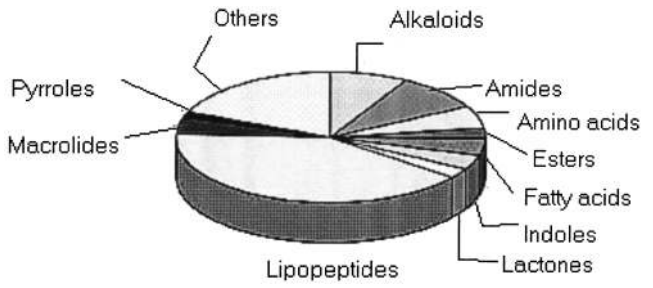

FIGURE 3 **Types of chemical compounds isolated from marine cyanobacteria (Burja *et al.*, 2001).**

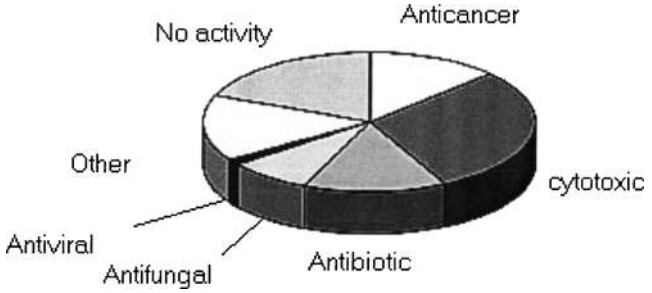

FIGURE 4 **Reported biological activities of marine cyanobacterial compounds (Burja *et al.*, 2001).**

TABLE 2 **Biological activities found in different orders of cyanobacteria.**

Order	Compounds	Activities
Chroococcales (11)	36	Enzyme inhibitor, cytotoxic, cell-differentiation, tumor promoter, endotoxic, hepatotoxic (6)
Pleurocapsales (1)	2	Antifungal, No activity (2)
Oscillatoroales (15)	197	Antialgal, anticancer, anti-HIV, antifeedant, antifungal, anti-inflammatory, antimicrobial, antimitotic, antiproliferative, antiviral, brine shrimp toxicity, cytotoxic, cytoskeleton disruption, herbicidal, hepatotoxin, icthyotoxic, immunosuppressive, molluscidal, neurotoxic, no activity, PBDu binding, tumor promoter, protein kinase activator, skin irritant, sunscreen pigment, toxin (26)
Nostocales (41)	126	Anticancer, antifungal, antimalarial, anti-HIV, cardioactive, hepatotoxic, antimicrobial, antimitotic, anti-inflammatory, antiviral, cytotoxic, enzyme inhibitor, toxin, neurotoxin, pigment, no activity (16)
Stigonematales (16)	16	Antifungal, antibiotic, anticancer, antimitotic, cytotoxic, herbicidal, no activity (7)

The three cyanophyta orders (*Chroococcales, Oscillatoriales* and *Nostocales*) produce the majority of toxic compounds (Burja *et al*., 2001).

sucrase in the digestive tract of rats (Kushak *et al*., 1999). Valencia *et al.* presented evidence that *Aphanizomenon flos-aquae* accelerate recovery from mild traumatic brain injury (Valencia and Walker, 1999). The wild cyanobacteria, *Lyngbya*, grow in abundance in the shallow water bloom. The alkaloids are unexceptional, peptides are unusual, either linear type (made of L- amino acid, like the immunosuppressive microcolin) (Koehn *et al*., 1992) or cyclic and formed by both L- and D-amino acid like the antimicrobial hormothamnin of *Harmathamnion enteromorphoides* from carribbeans (Gerwick *et al*., 1992). Cyclodepsipeptides of L-amino acid are represented by the strongly ichthiotoxic antillatoxin (Orjala *et al*., 1995). Curacin is an unusual acetogenin entailing a thiazoline ring. It shows strong toxicity towards L1210 leukaemic cell lines and inhibits tubulin polymerization by binding at colchicine site (Lai *et al*., 1996).

II. HIGH VALUE METABOLITES FROM CYANOBACTERIA

2.1 Cyanovirin-N

Cyanovirin-N (CV-N) is a unique, 101 amino acid long, 11 kDa protein. It was discovered as a constituent of a cultured cyanobacterium, *Nostoc ellipsosporum*, and both the sequence and the 3-D structure of CV-N are unprecedented. CV-N potently and irreversibly inactivates diverse primary strains of HIV-1, including M-tropic forms involved in sexual transmission of HIV. CV-N also blocks cell-to-cell transmission of HIV infection. CV-N is directly virucidal (Burja *et al*., 2001). It is largely a β sheet protein with internal two-fold pseudosymmetry. The two sequences repeat (residues 1–50 and 51–101) sharing of 32% sequence identity. The crystal structure of cyanovirin-N was solved and, surprisingly, revealed a domain swapped dimmer.

The two repeats do not form separate domains since the overall fold is dependent on numerous contacts between them (Figure 5). Rather, two symmetrically related domains are formed by strand exchange between the two repeats (Yang *et al*., 1999). CV-N is extremely resistant to physicochemical degradation and can withstand treatment with denaturants, detergents and organic solvents, multiple freeze-thaw cycles, and heat with no apparent loss of antiviral activity. During the first step of HIV infection, the viral surface envelope glycoprotein gp120 interacts with the CD4 receptor of the host cell, upon which gp120 undergoes a conformational change sufficient to accommodate a subsequent interaction between gp120 and a member of the α and β chemokine receptor families, now commonly referred to as co-receptors. CV-N interacts in an unusual manner with the viral envelope, apparently binding with extremely high affinity to poorly immunogenic epitopes on gp120, and inhibits fusion of virus with CD4 cell membrane. It has a potent activity against all

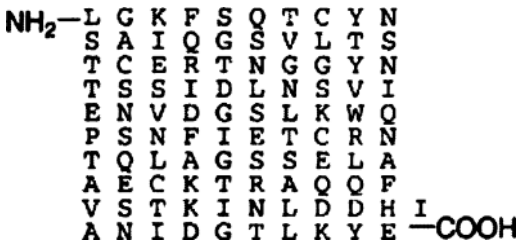

FIGURE 5 **Cyanovirin N amino acid sequence (Burja *et al*., 2001).**

immunodeficiency viruses (HIV-1, M- and T-tropic strains of HIV-1, HIV-2, SIV (simian) and FIV (feline) (Burja *et al.*, 2001). Recently, several patents have been filed to protect this new method of HIV prevention (Boyd, 2001, 2002, 2004). CV-N is under development as a topical (vaginal or rectal) microbicide to prevent sexual transmission of human immunodeficiency virus (HIV). CV-N is produced by recombinant *Escherichia coli* and purification resulted in monomeric protein (Colleluori, 2005). Vector containing pel-B signal peptide sequence is used for production of CV-N in high yield (Mori *et al.*, 1998). Production in yeast is also reported for the homologs of CV-N, which are active. The use of CV-N and its analogues could lead to an entirely new class of anti HIV drugs (Mori *et al.*, 2002).

2.2 Borophycin

Borophycin is a boron containing metabolite isolated from marine strains of cyanobacteria *Nostoc linckia* and *Nostoc spongiaeforme var. tenue* (Figure 6). It exhibits potent cytotoxicity against human epidermoid carcinoma and human colorectal adenocarcinoma cell lines and has been found to exhibit antimicrobial activity (Burja *et al.*, 2001).

2.3 Cryptophycin

Cryptophycin (Figure 7) first isolated from *Nostoc sp.* ATCC 53789 is a potent fungicide. It was also found to be very toxic and disregarded as natural product. It has also been isolated from *Nostoc sp.* GSV 224 and has exhibited potent cytotoxicity against human tumor cell lines. It shows good activity against a broad spectrum drug-sensitive and drug-resistant murine and human solid tumors (Burja *et al.*, 2001). Structure function study leads to cryptophycin, a semi-synthetic analogue with greater therapeutic efficiency and lower toxicity. Until now, none of the cryptophycin analogues have entered clinical trials. Cryptophycin-309, the glycinate of the chlorohydrin analog cryptophycin-296, emerged as superior over others. The mechanism of cytotoxicity of the cryptophycins is tubulin-interaction, with a disruption of tubulin-dynamics, resulting in apoptosis of tumor cells (Panda *et al.*, 1998).

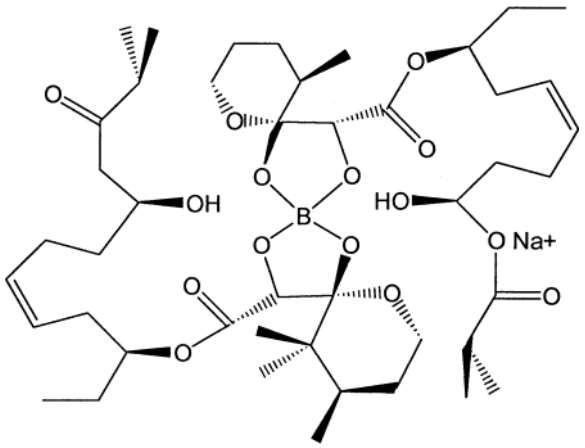

FIGURE 6 Chemical structure of Borophycin.

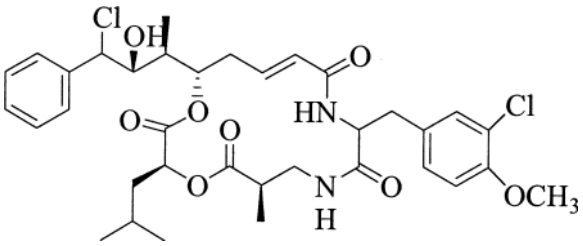

FIGURE 7 Chemical structure of Cryptophycin.

2.4 Lipopeptides

Approximately 68% of the natural products derived from cyanobacteria contain nitrogen. The natural products of many marine cyanobacteria contain an amino-acid derived fragment linked to fatty acid derived portion, forming compounds known as lipopeptides. Analysis of 424 marine cyanobacterial natural products shows that 40.2% are lipopeptides (cyclic or linear), 5.6% are of pure amino acid, 4.2% are fatty acids, 4.2% macrolides and 9.4% are amides. Lipopeptides are interesting and biochemically active, having cytotoxic, anticancer, antibiotic, enzyme inhibitor, antiviral and antifungal activities (Burja *et al.*, 2001). Hapalosin (Figure 8), a cyclic desipeptide isolated from the cyanobacteria, *Hapalosiphon welwitschii,* has a reversing activity against MDR (multi drug resistance) derived from P-glycoprotein (Kashihara *et al.*, 2000). Lipopeptides also

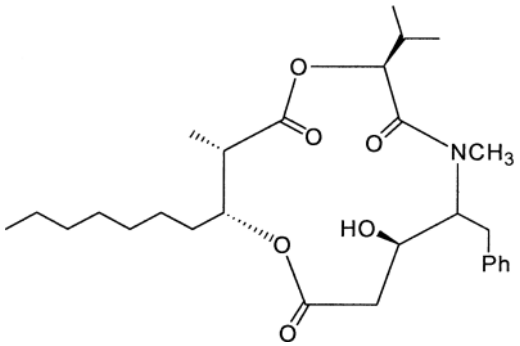

FIGURE 8 Chemical structure of Hapalosin.

have an affinity for liposomes and cell membranes and due to their low molecular weight they have an ability to pass through blood tissue and blood brain barrier leading to direct application as a drug delivery system (Burja *et al*., 2001).

2.5 Protease Inhibitors

Five classes of protease inhibitors have been reported from the toxic genera of cyanobacteria: they are micropeptins, aerugenosins, microginins, anabaenopeptins and microverdins. Serine protease inhibitors of micropeptin type are the most common inhibitors from cyanobacteria with more than fifty compounds. Some cyanopeptolins are specific inhibitors of serine proteases, including elastase, which is of critical importance in a number of diseases like lung emphysema, which is mediated by excessive action of elastase. Furthermore, it has been proposed that unphysiologically high levels of elastase activity are involved in myocardial damage and may cause a particular form of psoriasis. Cyanopeptolins are subjected to inhibition assays with commercial proteases, which are of medicinal relevance, like trypsin, thrombin, plasmin, papain and elastase (Grach-Pogrebinsky *et al*., 2003; Matern *et al*., 2001). Recently, Banyaside A and B was found to be the trypsin and thrombin inhibitor (Pluotno and Carmeli, 2005).

2.5.1 Scyptolin

These are cyclic desipeptides with elastase inhibiting activity, isolated from terrestrial cyanobacterium *Scytonema hofmanni pcc 7110*. These metabolites significantly inhibited porcine pancreatic elastase in invitro assays (Figure 9). *Scytonema julianum* has been reported as a potent inhibitor of platelet activating factor-induced platelet aggregation. Structural studies of this fraction indicated the existence of a phosphoglycoanalog of acyl-sphingosine. Two fractions identified as phosphoglycolipids include phosphoglyco-analog of acyl-acetylated sphingosine and the second one as a glyco-analog of phosphatidylglycerol (Antonopoulou *et al*., 2005). Natural elastase inhibitors might serve as valuable lead structures in pharmaceutical research dedicated to the development of more effective drugs (Matern *et al*., 2001). Three new protease inhibitors, such as planktopeptin BL1125, planktopeptin BL843 and planktopeptin BL1061, were isolated from *Planktothrix rubescens* (Figure 10). They are micropeptin type serine protease inhibitors. They were also found to be elastase and chymotrypsin inhibitors (Grach-Pogrebinsky *et al*., 2003).

III. TOXINS FROM CYANOBACTERIA

3.1 Hepatotoxins

They are the most commonly encountered toxins involving cyanobacteria and include the cyclic peptides microcystin and nodularin. *Microcystis aeruginosa* and *Nodularia spumigena* synthesize toxins destructive to liver cells. These two species produce seven amino acid peptide microcystin and five amino acid peptide nodularin (Figures 11, 12), respectively (Burja *et al*., 2001). To date over 50 different variants of microcystins have been isolated from the species of *Anabaena*, *Hapalasiphon*, *Microcystis*, *Nostoc* and *Oscillatoria.*

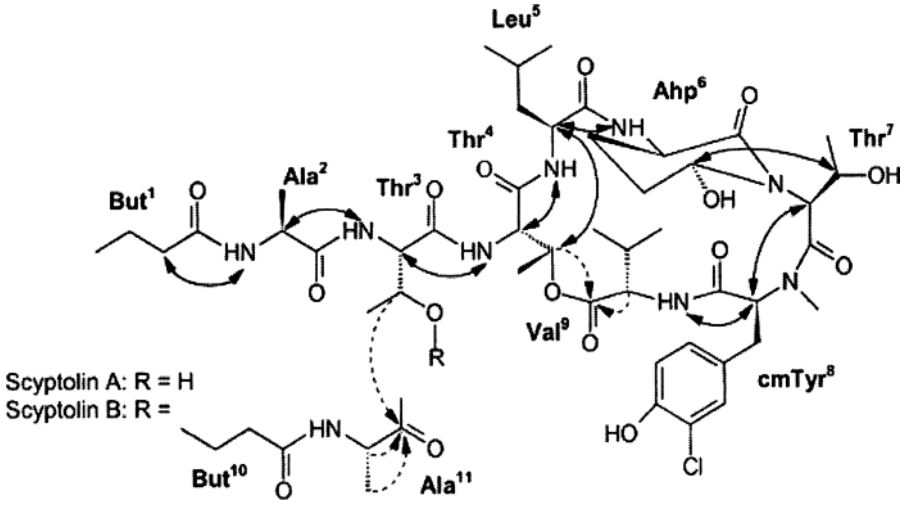

FIGURE 9 Chemical structure of Scyptolin.

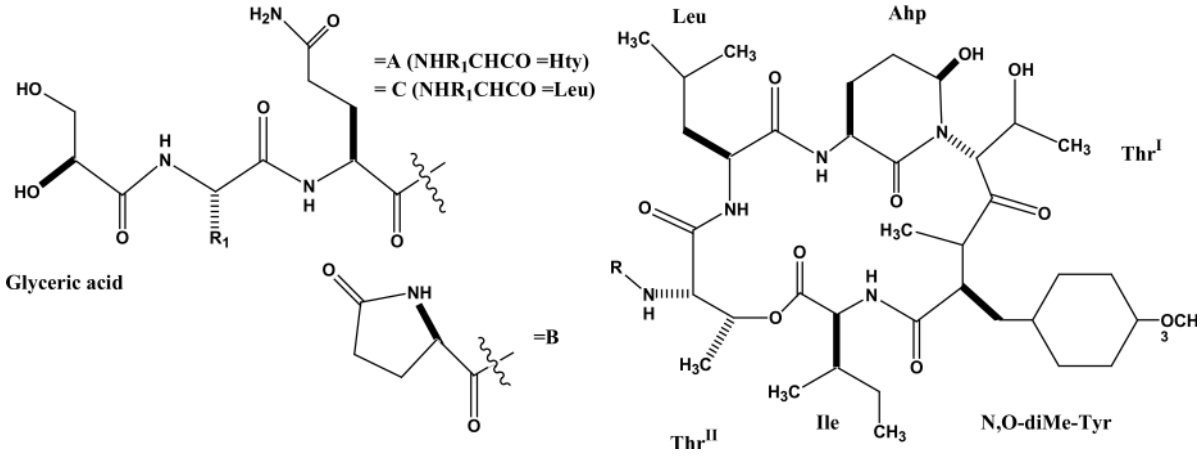

FIGURE 10 Chemical structure of Planktopeptin.

Microcystin-LR inhibits serine-threonine protein phosphatases 1 (PP1) and 2A (PP2A) with Ki values below 0.1 nM. Microcystin-LR may prove to be a useful probe for the study and identifying cellular processes, which are mediated by protein phosphatases due to their effect on cytoskeleton, they are now being used as tools to probe the working of this cellular scaffolding (Honkanen *et al.*, 1990). Microcystins are biosynthesized in the cyanobacterium *Microcystis aeruginosa* by a mixed gene cluster encoding non-ribosomal peptide synthetase and polyketide synthetase (Shimizu, 2003). One of the most toxic genera of cyanobacteria belonging to the order Oscillatoroales is *Lyngbya,* which are filamentous cyanobacteria abundant within tropical and subtropical waters. They are responsible for the synthesis of cytotoxic compounds such as antillatoxin, aplysiatoxin, debromoaplysiatoxin and lyngbyatoxin A, B and C.

The extraordinary chemical diversity seen in cyanobacteria *Lyngbya majuscula* is especially pronounced in the tropical marine species *Lyngbya majuscula.* Approximately 30% of all the natural products isolated from marine cyanobacteria have been isolated from this particular cyanobacterium. The list includes a wide variety of chemical structures including nitrogen containing compounds, polyketides, lipopeptides and many others. The latest addition to this list is a spectacular cyclic peptide, wewakazole. Its macrocyclic peptide ring is composed of six heterocycles, three oxazoles and three pyrrolidine rings (Shimizu, 2003). Biological activities of *Lyngbya majuscula* are very diverse and the compounds include potent protein kinase C activators and tumor promoters like lyngbyatoxins and aplysiatoxins (microlides). Curacin A is an unusual acetogenin entailing a thiazoline ring and is a good inhibitor of microtubulin assembly (Figure 13). Originally purified as a major lipid component of a strain of the cyanobacterium *Lyngbya majuscula* isolated in Curacao, curacin A is a potent inhibitor of cell growth and mitosis, binding rapidly and tightly at the colchicine site of tubulin. It shows strong cytotoxicity towards L1210 leukaemic cell lines and inhibits tubulin polymerization by binding to it (Burja *et al.*, 2002).

3.2 Kalkitoxin

This is a neurotoxin with five stereo centers (Figure 14). It blocks sodium channels preventing the nerves

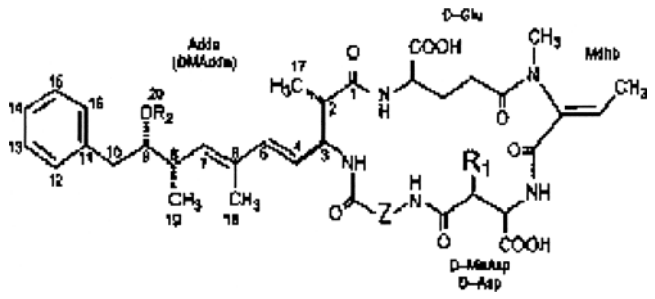

FIGURE 11 Chemical structure of Microcystin.

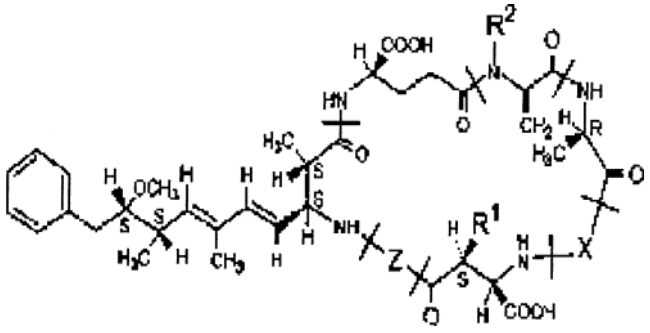

FIGURE 12 Chemical structure of Nodularin.

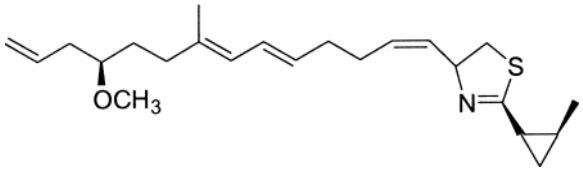

FIGURE 13 **Chemical structure of Curacin A.**

from firing off their electrical signals. Topiramate helps to suppress epileptic attacks largely by blocking sodium channels. Painkillers like lidocaine are sodium channel blockers. Kalkitoxin could treat these disorders including neurodegenerative diseases by selectively activating and blocking sodium channels, Kalkitoxin is a useful pharmaceutical compound and a valuable tool to understand the working of sodium channels and the effect of disease on them. (Wu *et al*., 2000).

3.3 Antillatoxin

This lipodesipeptide toxin is an extremely potent ichthyotoxin (Figure 15). Its activity is comparable to that of brevetoxin and involves the activation of voltage-gated sodium channels. However, the study indicates that the binding site for antillatoxin is different from the known sites for brevetoxins and other sodium channels activators such as batrachotoxin and α-scorpion toxin. Thus it should be possible to use antillatoxin as a new site-specific molecular probe for the sodium channel. It is intriguing that out of two potent ichthyotoxins from *L. majuscula* one is a sodium channel blocker and other an activator (Burja *et al*., 2002; Li *et al*., 2001).

There is a resemblance of cyanobacterial metabolites to those in *Streptomyces.* Metabolites isolated from *L. majuscula,* aplysiatoxin is basically the same as those of found in *Streptomyces*. Therefore, it would not be surprising that they are biosynthesized in the same manner as *Streptomyces* metabolites, whose biosynthetic genes have been extensively studied. Consistent with this, the non-ribosomal peptides found in cyanobacteria are synthesized in the same manner as those of *Streptomyces* and other eubacteria by non-ribosomal peptide synthetases (NRPSs) (Shimizu, 2003). Non-ribosomal peptide synthetases offer opportunity to design biocatalysts for producing novel products. The enzymes have a modular organization and synthesize the peptides on a protein template. NRPSs can synthesize polypeptides with fewer than about 50 amino acids, which can be assembled by peptide synthetases just as other compounds (fatty acids) are linked by other synthetases. A modular sequence structure of peptide synthetases has been shown to be responsible for the sequential and amino acid specific elongation of peptide chains. The order and the number of modules at the gene level determine the structure of the product. The specific combination of modules and various functional domains within the peptide synthetase determine the structure and hence activity of the peptide product. Thus exchange and rearrangement of peptide synthetase modules offer opportunity to design biocatalysts for producing novel products (Neilan *et al.,* 1999). By enzymatic biocatalysis techniques, multiplication of compound leads is possible by taking known compounds. By creating new compound leads (Olaizola, 2003), new compounds with desirable characteristics are produced with more potency and less toxicity (Figure 16). Polyketide synthetases are also involved in the biosynthesis of certain cyanobacterial compounds. Microcystins cyclic heptapeptides that contain unusual alkyl residue (characteristic feature of many cyanobacterial peptides) are biosynthesized in *Mycrocystis aeruginosa* by a mixed gene cluster encoding NRPS and polyketide synthetase (PKS). The *Lyngbya majuscula* lipopeptides are also assumed to be biosynthesised by similar NRPS/PKS clusters (Shimizu, 2003).

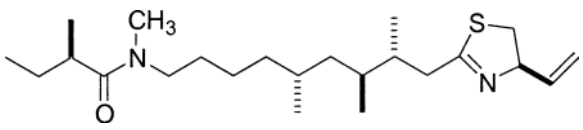

FIGURE 14 **Chemical structure of Kalkitoxin.**

3.4 Barbamide

Barbamide was isolated from a Curaçao strain of *L. majuscula* and is known to be molluscicidal. Although it is a small molecule, barbamide has complex structural and biosynthetic features, including a thiazole ring and a biosynthetically intriguing trichloromethyl group (Figure 17). The gene cluster of the *L. majuscula* producing barbamide has been reported in literature (Chang *et al*., 2002). The gene cluster (denoted as *bar*) contains 26 kb functional gene sequences, *bar* A–K. *Bar*A shows high homology to a peptidyl carrier protein of NPRS. *Bar*B1 and *bar*B2 (and possibly also *bar*C) are likely the candidates for the chlorination of a methyl group of leucine. *Bar*D activates trichloroleucine and L-valine in addition to L-leucine. The oxidative decarboxylation of trichloroleucine to trichlorovaleric acid may be carried out by *bar*J. This unusual truncation

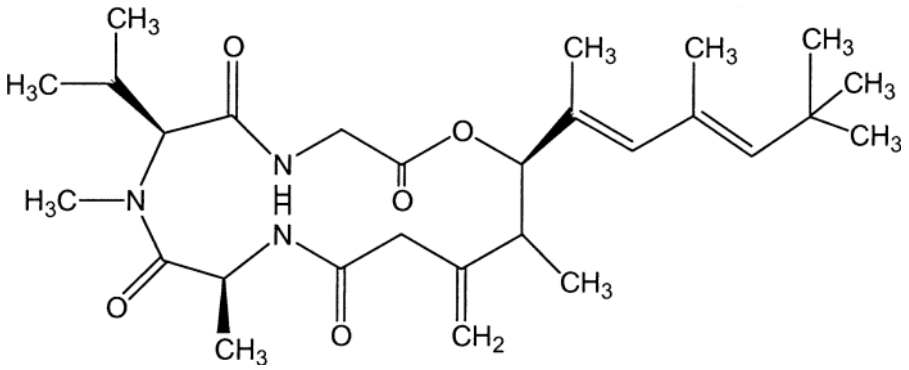

FIGURE 15 **Chemical structure of Antillatoxin.**

process is assumed to take place via the α-keto-acid intermediate. *Bar*E has features of both NPRS and PKS. Further stages in the biosynthesis include the condensation of the trichlorovaleriate moiety with a malonyl unit (*bar*F), O-methylation (*bar*F), peptide formation with phenylalanine and cysteine (*bar*G) and finally thiazolidine ring formation and oxidative decarboxylation (*bar*J, *bar*H and *bar*I) to complete the structure. Some of the gene functions and exact mechanism of biosynthesis are yet to be clarified (Shimizu, 2003).

3.5 Saxitoxin

Saxitoxins are neurotoxic alkaloids, which are known as paralytic shellfish poisons. The name saxitoxin was derived from the mollusk in which it was first identified. *Alexandrium catenella, A. minutum, A.ostenfeldi, A. tamarense, Gymnodinium catenatum* and *Pyrodinium bahamense* secrete saxitoxins. It is a polar compound and readily dissolves in water and lower alcohols but is insoluble in organic solvents (Figure 18). It is stable at neutral to acidic pH and at high temperature. This toxin blocks neuronal transmission by binding to the voltage gated Na^+ channels in nerve cells, thus causing a neurotoxic effect. Saxitoxin is highly toxic and kills a guinea pig at only 5 μg/kg when injected intramuscularly. The oral LD_{50} for human is 5.7 μg/kg. The human inhalation toxicity of aerosolized saxitoxin is estimated to be 5 mg/min.m^3 that can enter the body via open wounds. Saxitoxin is 1000 times more toxic than the potent nerve gas sarin. This neurotoxin specifically and selectively binds to the sodium channel in neural cells. Thus, it physically occludes the opening of Na^+ channels and prevents any sodium cation from going in or out of the cell. Since, neuronal transmittance of impulse and messages depends on depolarization of the cell, the action potential is stopped, impairing a variety of body functions, including breathing. The diaphragm may stop working and death may occur after cardiorespiratory failure.

Biosynthesis in freshwater cyanobacteria *Aphanizomenon flos-aquae* feeding experiments with $C^{13}$and ^{2}H-labelled precursor have shown that neosaxitoxin is biosynthesized from arginine and acetate and involves a claisen-type condensation between C_2 of arginine and C_1 of acetate (Shimizu *et al.*, 1984; Shimizu, 1986). The cyclic heptapeptide microcystin-LR is the major hepatatoxin associated with *Microcystis aeruginosa* (Carmichael *et al.*, 1988), which is a potent inhibitor of type 1 and type 2A protein phosphatases

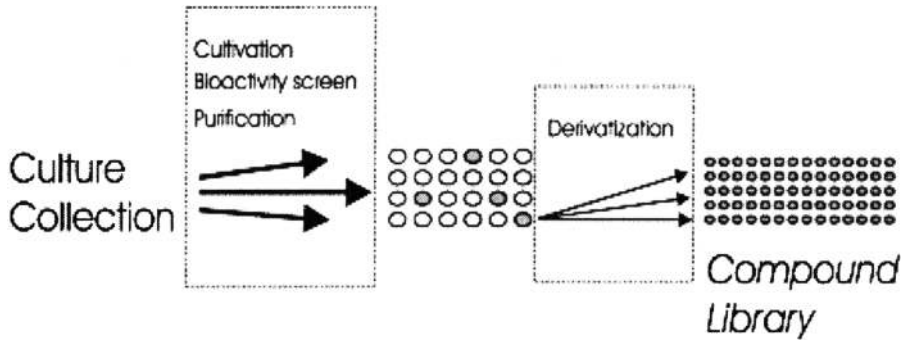

FIGURE 16 **Enzymatic biocatalysts techniques to multiply the number of bioactive leads in drug discovery (Olaizola, 2003).**

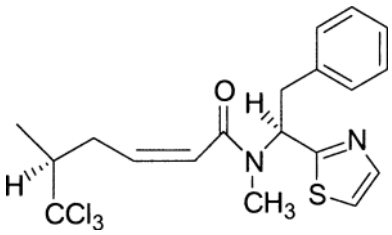

FIGURE 17 **Chemical structure of Barbamide.**

(Honkanen *et al.*, 1990) and has been implicated in net-pen liver disease, a common toxicopathic disease (Williams *et al.*, 1997). A stable isotope feeding experiment by the Moore group in Hawaii established the origin of the unusual (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl–4,6-decadienoic acid (Adda) and (2R,3S)-3-methyl asparatic acid (Masp) residue in Microcystin-LR (Moore *et al.*, 1999). The polyketide pathway involving a putative phenylacetyl-CoA starter unit and four Melonyl–CoA extensions, synthesizes the Adda unit. The Masp unit in microcystin-LR as well as cyclic pentapeptide nodularin were derived from acetate and pyruvate and probably involves the formation and rearrangement of citramalic acid; the synthesis of Masp is similar to biosynthesis of leucine and glutamic acid (Figure 19). The occurrence of many isoforms of the microcystin and the content of unusual and modified amino acids suggest that microcystin-LR is synthesised non-ribosomally by peptide syntheses (Marahiel *et al.*, 1997). In the presence of protein synthesis inhibitor chloroamphenicol, microcystin synthesis in the *M. aeruginosa* is not inhibited, thus supporting a non- ribosomal thio-template mechanism (Arment and Carmichael, 1996).

3.6 Anatoxins

Anatoxin-a and homoanatoxin-a (Figures 20, 21) are secondary amines and are postsynaptic depolarizing neuromuscular blocking agents (Carmichael *et al.*, 1977) that bind strongly to the nicotinic acetylcholine receptor (Spivak *et al.*, 1980). These compounds are potent neurotoxins, which cause rapid death due to respiratory arrest (the mouse LD_{50} is approximately 250 μg/kg) (Devlin *et al.*, 1977). Anatoxin-a produced by *Anabaene flos-aquae* (Gorham *et al.*, 1964) is a low molecular weight, water-soluble bicyclic compound and enters the body by inhalation, injection and exposure to high concentration through the skin. Natural anatoxin-a is a (+) stereoisomer and is more toxic. Homoanatoxin-a is structurally similar to anatoxin-a found in *Oscillatoria formosa* (Lilleheil *et al.*, 1997). Anatoxin-a (s) (Figure 22), a unique phosphate ester of a cyclic N-hydroxyguanidine moiety, is a potent neurotoxin (mouse LD_{50} is approximately 20–40 μg/kg) and is a cholinesterase inhibitor (Mahmood and Carmichael, 1986, 1987) and induces hypersalivation in mammals. Anatoxin is produced by fresh water cyanophyte *A. flos-aquae* (Matsunaga *et al.*, 1989). A feeding experiment with stable and radio labelled precursor established that the triaminopropane backbone and the guanidine unit in anatoxin are derived from L-arginine and the three methyl carbon arises from L-methionone or other donors to the tetrahydrofolate C_1 pool (Moore *et al.*, 1993) (Figure 23).

3.7 Brevitoxins

Brevitoxins are neurotoxins produced by *Ptychodiscus brevis*, from which the name is derived. These are lipophilic compounds with a molecular weight of approximately 900 Da (Baden, 1989). There are two classes of brevitoxins; the first contains eight 6-membered ring (type I brevitoxin) and the second class of brevitoxin has only 10 rings (type II brevitoxin) (Figure 24). Brevitoxin depolarizes the open voltage gated sodium (Na^+) ion channel in the cell wall, leading to the

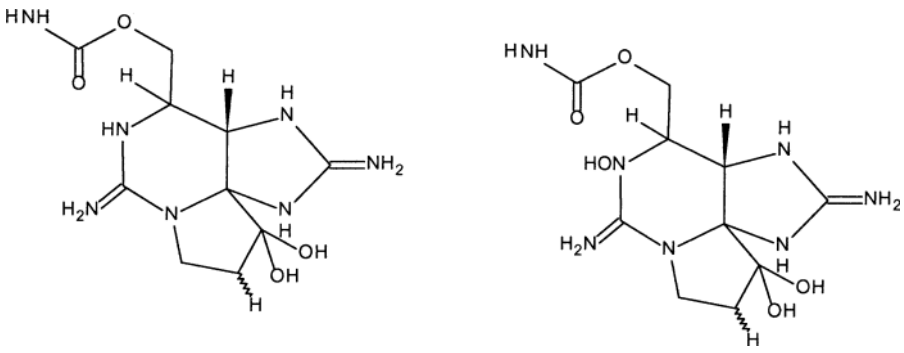

FIGURE 18 **Chemical structures of Saxitoxin and Neosaxitoxin.**

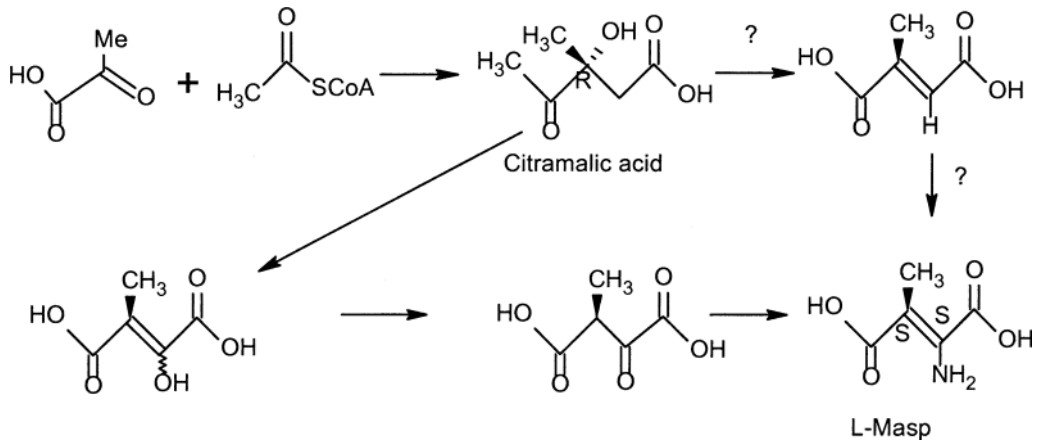

FIGURE 19 **Biosynthesis of Methyl aspartic acid unit (Moore, 1999).**

uncontrolled Na^+ influx into the cell (Baden, 1983). Brevitoxin binds to the ion channel of nerve and muscle tissue that selectively allows sodium to pass into the cell. The sodium channel opens during an action potential in response to the change in electrical potential across the cell membrane. Brevitoxin changes the threshold voltage at which opening occurs, thus making the sodium channel for uncontrolled influx, and consequently, the affected nervous and muscular cells are hyperexcited. Brevitoxins are usually stable in dry state and also in different solvents (acetone, acetonitrile, alcohol, ethyl acetate) including water where the half-life of the active material ranges from 4–6 months at pH 2-10 (Atchison *et al.*, 1986).

IV. BIOMODULATORY EFFECT

Many species of cyanobacteria have a biomodulatory effect. Oral doses of *Aphanizomenon flos-aquae* on healthy humans revealed a slight decrease in phagocytic activity of polymorph nucleated cells in in-vitro condition (Jensen *et al.*, 2000). This may indicate an anti-inflammatory rather than anti-phagocytic effect on human neutrophils. Mice fed on *spirulina* diet resulted in a slight increase in the phagocytic cells (Hayashi *et al.*, 1994). On feeding the blue-green algae, the spleenic leucocytes from chickens showed greater anti-tumor cell activity (Qureshi *et al.*, 1996). Human monocyte cell line THP-1 was used to study the mechanism of the immuno-stimulatory effect of brevitoxin, a novel polysaccharide, isolated from the crude extract of *Aphanizomenon flos-aquae* (Pugh *et al.*, 2001).

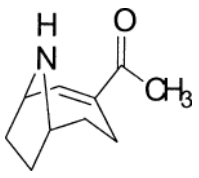

FIGURE 20 **Chemical structure of Anatoxin a.**

4.1 Effect on Specific Immunity

It is reported that immunized mice fed with an algae supplemented diet showed increased numbers of spleenic IgM antibody-producing cells. This finding is only true for primary immune response, as the IgG antibody production in the secondary immune response was hardly affected (Hayashi *et al.*, 1994). Blue-green algae do not seem to induce or enhance the food allergic IgE-dependent reaction. However, when ingested along with or before a potential antigen, they may enhance the IgA antibody level to protect against food allergies (Hayashi *et al.*, 1998). It was reported that by injecting the blue-green algae extract intraperitoneally one hour prior to allergic challenge, mortality induced by the anaphylactic compound decreased (Kim *et al.*, 1998; Yang *et al.*, 1997).

4.2 Effect on Leucocyte Trafficking

Studies by Jensen *et al.* (2000) showed that the blue-green alga *Aphanizomenon flos-aquae* triggers within two hours the migration of 40% natural killer cells. This effect is more pronounced in the long-term consumer.

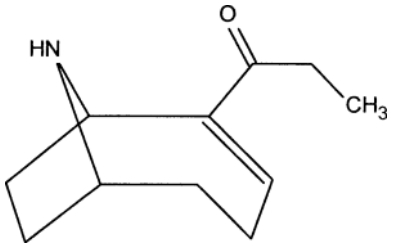

FIGURE 21 **Chemical structure of Homoanatoxin a.**

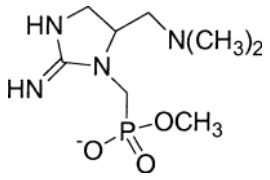

FIGURE 22 **Chemical structure of Anatoxin a(s).**

Aphanizomenon flos-aquae was shown to stimulate the mobilization of T and B cells.

4.3 Antiinflammatory Activity

Blue-green algae contain significant amounts of carotenoids (β-carotene, lycopene, lutein) having antioxidant properties. By the quenching action on the reactive oxygen species, these carotenoids also have anti-inflammatory activities. The anti-inflammatory activity of blue-green algae is also due to phycocyanin, a photo harvesting pigment. These proteins are 35 kDa water-soluble orange carotenoid proteins. Structural, biochemical, and genomic data on the orange carotenoid proteins and their paralogs help in revealing the functionality of these proteins in photoprotection (Kerfeld, 2004). C-phycocyanin is a free radical scavenger (Bhat and Madyastha, 2000) and has a significant hepatoprotective effect (Vadiraja *et al.*, 1998). The anti-inflammatory effect seemed to be a result of phycocyanin inhibiting the formation of leucotriene, an inflammatory metabolite of arachidonic acid (Romay *et al.*, 1999). *Aphanizomenon flos-aquae* decrease the level of arachidonic acid (Kushak *et al.*, 2000). *Aphanizomenon flos-aquae* contain significant amounts of omega-3–alpha linolenic acid which inhibit the formation of inflammatory postaglandins and arachidonate metabolite. *Spirulina* also contain significant amounts of omega-6-gamma linolenic acid.

4.4 Antiviral Effect

The protection of human lymphoblastoid T cells from the cytopathic effect of HIV infection with the

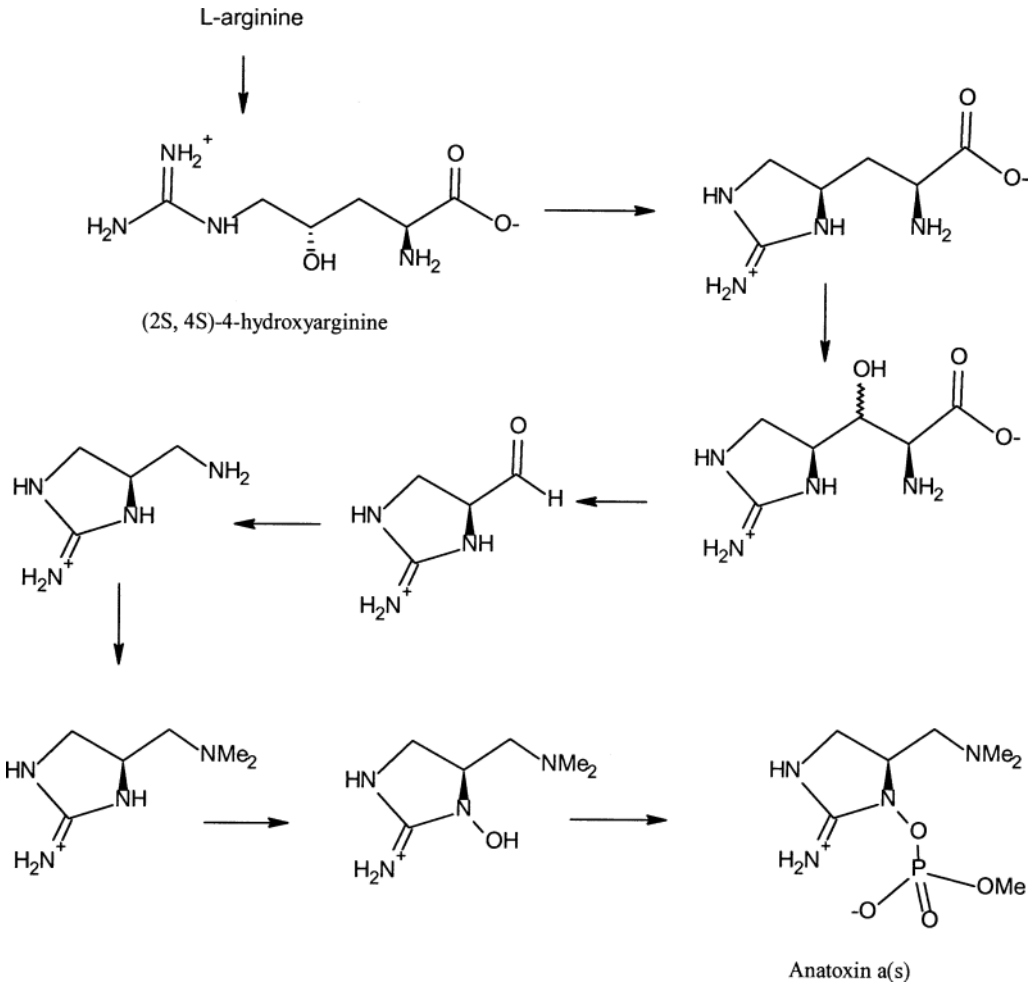

FIGURE 23 **Synthesis of anatoxin-a (s) from L-arginine.**

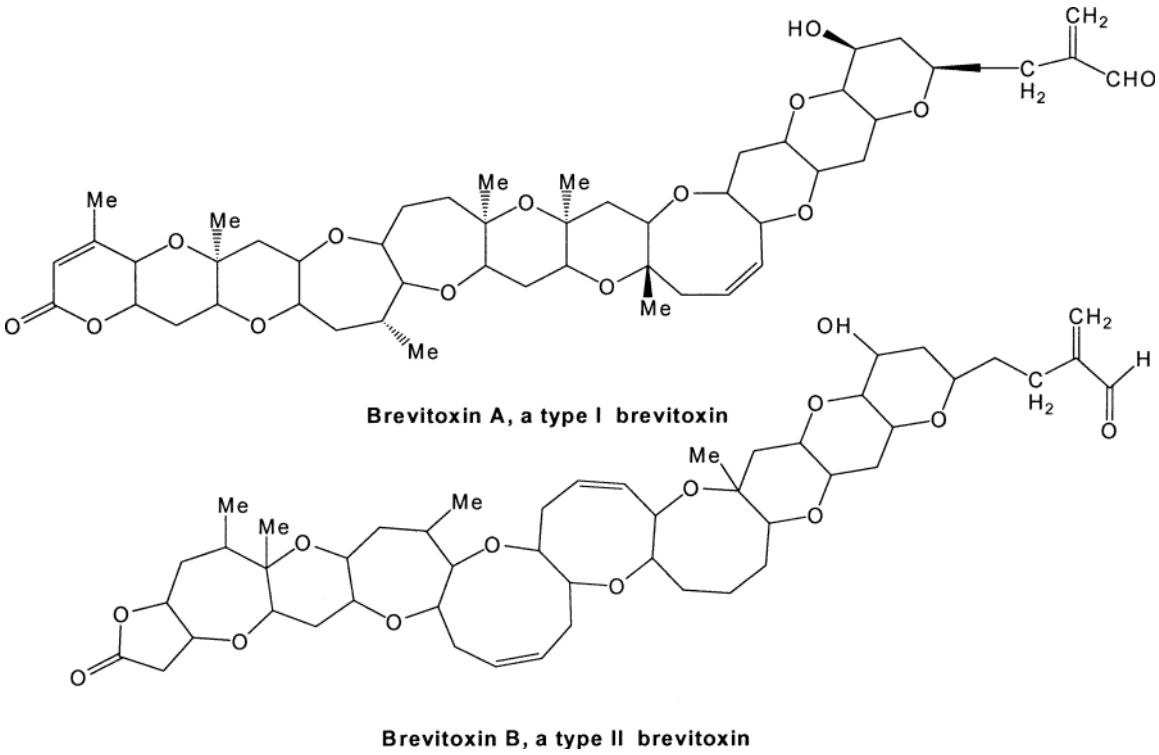

FIGURE 24 **Chemical structure of Brevitoxin.**

extract of blue-green algae (*Lyngbya lagerheimeii* and *Phormidium tenue* has been reported (Gustafson *et al.*, 1989). A new class of HIV inhibitors called sulfonic acid, containing glycolipid, were isolated from the extract of blue-green algae and the compounds were found to be active against the HIV virus. Cyanoviridin–N, isolated from blue-green algae, inactivates the strains of HIV virus and inhibits cell to cell and virus to cell fusion (Yang *et al.*, 1997). Calcium spirulan (Ca-SP), a novel sulphated polysaccharide, is an anti viral agent. This compound selectively inhibits the entry of enveloped virus (Herpes simplex, human cytomegalovirus, measles virus) into the cell (Hayashi *et al.*, 1996; Hayashi and Hayashi, 1996; Ayehunie *et al.*, 1998).

4.5 Antituberculosis Activity

The emergence of multidrug resistance strains of *Mycobacterium tuberculosis* has led to the discovery of new drugs from marine microorganisms. The alkaloid (+)-8-hydroxymanzamine A is characterized by a complex heterocyclic ring system attached to a β-carboline moiety. It was first isolated from a sponge *Puchypellium sp.* and then from the *Petrosiidae* genus. This alkaloid exhibits potent antituberculosis activity against *M. tuberculosis* H37Rv with minimum inhibitory dose of 0.91 μg/ml. Ircinol A is useful for in vivo assessment of *M. tuberculosis* as it shows less cytotoxicity and structural complexity as compared to a manzamine type alkaloid. Manzamine A inhibits *M. tuberculosis* at a concentration of 1.56 μg/ml. *Pseudomonas elisabethae* induces 97% growth inhibition for *M. tuberculosis* H37Rv at a concentration of 12.5 μg/ml. Litosterol is a C-19 hydroxysteroid isolated from *Litophyton viridis* and inhibits 90% growth of *M. tuberculosis* at a concentration of 3.13 μg/ml (Donia and Hamann, 2003) (Figure 25).

4.6 Anticancer Effect

The oral supplementation of *Spirulina fusiformis* resulted in regression of subjects with homogenous leukolakia (Mathew *et al.*, 1995). The extracts of *Spirulina* and *Dunaliella* inhibited the chemically induced carcinogenesis in model hamster buccal pouches (Schwartz and Shklar, 1987; Schwartz *et al.*, 1988). Studies have also showed that sulphated polysaccharide, calcium spirulans appears to inhibit tumor invasion and metastasis (Mishima *et al.*, 1998) of melanoma cells and inhibit the tumor invasion of basement membrane. *Aphanizomenon flos-aquae* extract containing a high concentration of phycocyanin inhibited the in vitro growth of one of four tumor cell lines tested, indicating the

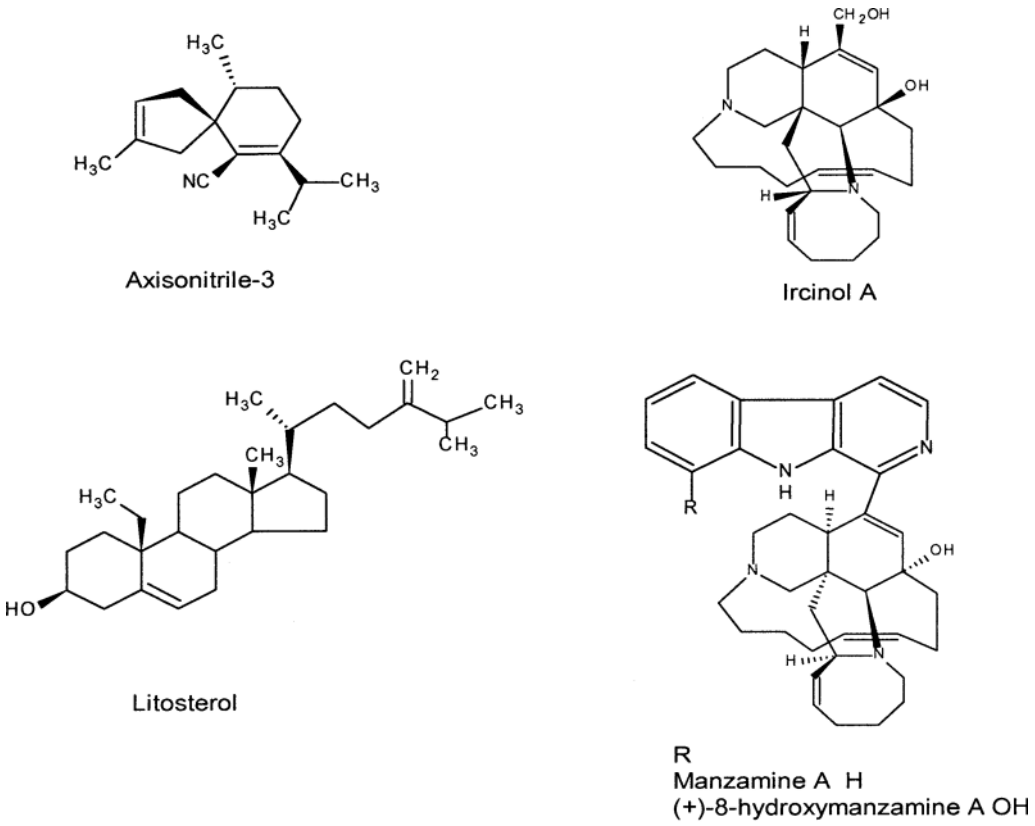

FIGURE 25 **Different antituberculosis drugs derived from cyanobacteria.**

sensitivity of cell lines to the phycocyanin. *Phormidium tenue* contain several diacylglycerols that inhibit chemically induced tumors on mice (Tokuda *et al.*, 1996). C-phycocyanin selectively inhibits COX-2, which is over expressed in breast cancer cells but has no effect on COX-1 (Reddy *et al.*, 2000).

4.7 Antihelmintic Activity

Dihydroxy tetrahydrofuran from the south Australian marine brown algae *Notheia anomala* exhibits selective nematodal activity against *Trichostorgyrus* with a lethal dose in 50% of LD_{50} of 9.9 μg/ml. This inhibits the development of eggs for the infective free-living stage. The sponge geodin A is macrocyclic polyketide lactam tetramic acid, showing an LD_{50} value of 1.0 μg/ml (Donia and Hamann, 2003). Jasplakinolide is a potent antiparasitic and antifungal agent and exhibits in-vitro 50% effective dose of less than 1 μg/ml against nematode *Nippostrongylus braziliensis.*

4.8 Antiprotozonal Activity

Pentavalent antimonials such as sodium stibogluconate and meglumine antimonite are used for the treatment of leishmaniasis. The most active cyclic peroxide (LD_{50} 0.29 μg/ml) causes the lysis of the cell membrane after 24 h at a concentration of 1 μg/ml and strikingly decreases mortality after 30 minutes (Berman, 1998).

4.9 Chemical Ecology of Cyanobacteria

There is a compilation of research regarding the chemistry and biochemistry of marine toxins and potential drug leads, but little experimental evidence exists to establish the full ecological significance of most of the cyanobacterial metabolites. Better understanding of ecological relationship and interactions that are present, in particular marine niches where the target microorganisms are found, allows mechanistic design of artificial media, which more closely resembles that micro niche and thus leads to a much greater percentage culturability. It is known that secondary metabolite production is sensitive to environmental factors and drug production sometimes disappears on repeated subculturing. Stable strains of drug producing cyanophytes are obtained using conventional techniques. Current research shows that cyanobacteria participate in symbiotic

relationships with several marine invertebrates and many are responsible for extremely active compounds previously attributed to marine invertebrates. Many marine natural products found in sponges and their predators closely resemble cyanobacterial metabolites. Recently, a cryptophycin type cytotoxin, arenastatin A was isolated from the sponge, *Dysidae arenaria.* Dolastatin 10, an anticancer drug which has been recommended for Phase-2 clinical trials, was initially isolated from the sea (*Dallabella auricularia*). Several dolastatin analogs like dolastatin 3, dolastatin 11, dolastatin 12, lyngbyastatin 1 and lyngbyastatin 2 have been isolated from *L. majuscula* and *Symploca hydnoides.* Cyanobacterial endosymbionts can now be genomically accessed as tunicates and sponges (source of anticancer drugs) are getting sequenced. It would be very interesting to determine whether symbiosis is the source of bioactive compounds, or individual members (Burja *et al.*, 2001). Recent estimates indicate that most of the newly approved drugs reported to date are of natural origin. In some cases demands are made by total synthesis of active metabolites, but in many cases this is not a viable option as synthesis may involve many steps, may be relatively expensive and produce low overall yield due to poor selectivity. The advantages of microorganisms are cultivability and a sustained supply of "targeted" metabolites is assured. Although there has been some interest in the exploitation of cultured cyanobacteria to develop pharmaceutical compounds, the study of these secondary metabolites and their controlled long-term production is still in its infancy. One of the major reasons why drug candidates do not make it to the world pharmaceutical market is the relatively small yield of the compounds available from natural stock, usually between 1 and 50 gm on a yearly basis. There is a need to develop a detailed procedure for the production of biochemically active, secondary metabolites from cyanobacteria. Bioprocess intensification strives to overcome this shortfall by developing detailed mechanistic growth kinetics for particular organisms and thereby design bioreactors based on an ecological approach. Bioprocess intensification involves optimizing fermentation yield via media composition and field strategies, dynamic control of physical conditions, induction genetics, immobilization, and bioreactor engineering. Since, cyanobacteria grow under photoautotrophic condition with carbon dioxide and light as a carbon and energy source respectively, it leaves relatively fewer, perhaps more critical, culture parameters to be manipulated for control of secondary metabolite production (Burja *et al.*, 2001, 2002). In bioprocess intensification, studies were carried out with *Lyngbya majuscula* for the production of lipopeptides and it was found that 1) growth conditions of *L. majuscula* had the greatest effect on secondary metabolite production; 2) growth rates for *L. majuscula* were increased when proper aeration system was maintained; 3) in contrast to many other classes of prokaryotes there is a direct correlation between growth and secondary metabolite production in cyanobacteria. Bioactive metabolites were produced either throughout the period of exponential phase in batch culture or synthesized during early and late exponential phase; 4) *L. majuscula* produced the greatest amount of wet material under larger surface area to volume ratio and inoculum to media ratio. The isolate thus being able to diffuse carbon dioxide through the medium faster (Burja *et al.*, 2002).

V. MICROALGAE

Microalgae belong to the subgroup of algae and are photosynthetic in nature and comprise several thousand species. They are classified as 1) cyanobacteria (BGA), 2) rhodophytes, (rhodophytes), 3) chlorophytes (chlorophytes), and 4) chromophytes (all others). The details of some of the bioactive compounds produced by microalgae are given below.

5.1 Omega 3-Polyunsaturated Fatty Acid

Omega 3-fatty acids like eicosapentanoic acid (EPA) and docosahexaenoic acid from microalgae have therapeutic importance. EPA is used in the treatment of heart and inflammatory disease. Omega 3-polyunsaturated fatty acids are also effective against rheumatoid arthritis and immunodeficiency disease. The annual worldwide demand of EPA is 300 tons. This is found in fish oil and microalgae. In microalgae it is found in the classes of *Bacillariophceae (diatoms) Chlorophyceae, Chrysophyseae, Cryptophyceae, Eustigamatophyceae* and *Prasinophyceae.* This product from algae is superior over fish oil in not having off flavors, is more pure, has a low cholesterol content and is inexpensive (Belarbi *et al.*, 2000).

5.1.1 Structure and Biosynthesis of EPA

The eicosanoids resemble with prostaglandins, thromboxane and leukotrienes. Arachidonic acid and

FIGURE 26 Chemical structure of EPA.

EPA are precursors of eicosanoid compounds (Figure 26). EPA has been reported to be a potential anticachexia and anti-inflammatory agent. EPA exhibits therapeutic activity against cardiovascular disease. EPA prevents atherosclerosis by decreasing the level of low-density lipoproteins (LDL). The biosynthesis occurs in two steps. In the first step, the de novo synthesis of oleic acid from acetate takes place followed by conversion of oleic acid to linoleic acid and α-linolenic acid and after a number of subsequent steps of desaturation and elongation, it forms PUFA including EPA (Figure 27). Biosynthesis starts with the carboxylation of acetyl Co-A to form acetate or pyruvate by the action of glycolytic enzyme and then the acetyl Co-A is converted to malonyl Co-A, which is used to derive a condensation reaction to extend the acetyl group to stearic acid and desaturate to oleic acid. *Aphanizomenon flos-aquae* contain omega 3-fatty acids, which inhibit the formation of inflammatory prostagladins and arachidonate metabolite. Spirulina also contain omega 3-linolenic acid. EPA performs many vital functions in the biological membrane and serves as a precursor of a variety of lipid regulators in cellular metabolism (Wen and Chen, 2003).

5.2 Cultivation of Microalgae for EPA Production

Microalgae are obligate photoautotrophs that require light for growth. A number of microalgae are also capable of heterotrophic growth having organic substrate as single carbon source. Different ways of cultivation of microalgae are as follows.

5.2.1 Photoautotrophic Cultivation System

There are three main ways to cultivate microalgae: a) open pond system; b) closed photobioreactor with natural sunlight; and c) closed photobioreactor with artificial illumination. Mass cultivation of microalgae takes place in an open pond where conditions are identical to the natural environment. Commercial scale-up is difficult in open ponds due to the contamination problem and the recovery is expensive. To overcome these problems, closed algal photobioreactors have been used, which are made of transparent material placed outdoors for illumination by natural light. Vessels have a high surface to volume ratio. It reduces the contamination, but the growth is suboptimal due to variation in temperature and light. Enclosed photobioreactors are similar to conventional photobioreactors, except they require light and carbon dioxide. Some have oxygen removal devices to reduce the toxic effect of high oxygen concentrations on algal growth. The disadvantage of these bioreactors is that they are difficult to scale-up and capital cost is very high (Pulz, 2001).

5.2.2 Heterotrophic Cultivation System

This can be a cost-effective alternative to photoautotrophic cultivation as sugar and organic acids are used as the sole carbon source for the cultivation. This mode eliminates the requirement of light, thus increasing the cell density and productivity. This mode can further be modified to fed-batch, chemostat culture, which further reduces the cost of EPA recovery. Heterotrophic production requires an organism that can divide in the dark and can grow on inexpensive and sterilizable media, can adapt quickly to new environments and can withstand hydrodynamic stresses in the fermenter and peripheral environments. Other factors influencing the production of EPA are culture age, carbon source, nitrogen source, C/N ratio, C/P ratio and environmental factors like temperature (low temperature favors high PUFA content), pH (a pH range of 6.0–8.8 with optimum of 7.6) and salinity.

5.2.3 Photobioreactor

Production of microalgal biomass is also possible in photobioreactors. Open-culture systems are almost always located outdoors and rely on natural light for illumination. Closed photobioreactors may be located indoors or outdoors, but the outdoor location is more common because it can make use of free sunlight (Grima *et al.*, 2003).

5.2.3.1 Open Pond System

The most common technical designs for open pond systems are cultivations driven by paddle wheels and usually operating at water depths 15–20 cm. At this water depth, biomass concentration up to 1000 mg L^{-1} and productivities up to 60–100 mg L^{-1} d^{-1} (10–25 g m^{-2} d^{-1}) are possible. They have the disadvantage of significant evaporative loss, diffusion of CO_2 into the atmosphere and permanent threats of contamination and pollution; therefore, maintenance of the desired algal population is difficult, productivity is light limited

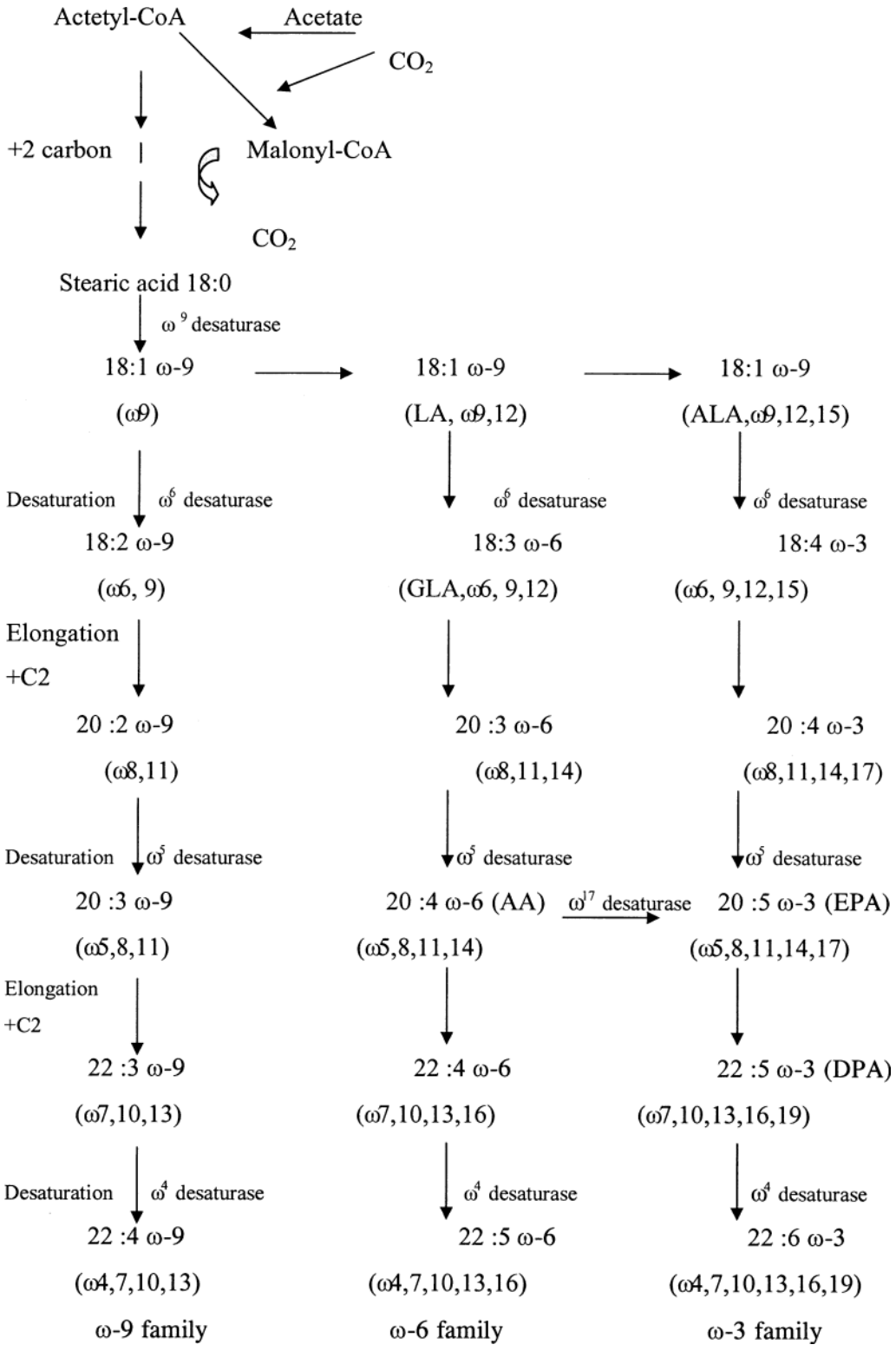

FIGURE 27 **Biosynthesis of three families of polyunsaturated fatty acids by microalgae.**

in higher thickness and a large surface area is required. Until recently, open ponds were the most important design principles for microalgal cultivation; however, the preparation of high value products from microalgae for application in pharmacy and cosmetics appears to be feasible only if closed photobioreactors with the ability to reproduce production conditions are used (Pulz, 2001).

5.2.3.2 Closed Photobioreactor

These are characterized by regulation and control of nearly all the biotechnologically important parameters as well as benefits: reduced contamination risk, no CO_2 losses, reproducibility of cultivation conditions, controllable hydrodynamics and temperature, and flexible technical design. Closed photobioreactors are of the following types: tubular system (glass, plastic, bags), flattened plate system, ultra-thin immobilized configurations. Vertical arrangement of horizontally running tubes and plates seems to be preferred for the reason of light distribution and appropriate flow. Important parameters for cyanobacterial cultivation are: species efficient light incidence into photobioreactor lumen, light path, layer thickness, O_2 release from the total system volume, CO_2/O_2 balance, salinity, nutrients, pH, temperature and turbulence (Pulz, 2001) (Table 3).

5.3 Green Microalgae

5.3.1 β-Carotene

Interest in green microalgae is currently focused on the *Dunaliella* complex, which provide the basics for the industrial production by culturing in the open air for the *trans*/*cis*-β-carotene. This is more liposoluble and has better quality as a free radical scavenger (Ben-Amotz and Avron, 1990) and a potential food additive for enhancing the color of the flesh of fish and egg yolk. It is used to improve health and fertility of the grain-fed cattle (Borowitzka and Borowitzka, 1987). Until 1980 the production of β-carotene was synthetic. During the 1970s researchers found that under nutrient stress, high salt and high light condition, the microalgae, *Dunaliella salina*, will accumulate up to the 14% of its dry weight as β-carotene. Now, β-carotene from *Dunaliella salina* is a substantially growing industry. Commercial utilization of microalgae (including cyanobacteria) is economically viable and there is a worldwide market for its derivatives (Olaizola, 2003).

Many strains of cyanobacteria are filamentous and diazotropic, i.e., they can use atmospheric nitrogen as the sole nitrogen source (including *Anabaena*). Besides lowering the cost of the culture medium, this ability restricts the problem of contamination by other microorganisms. Moreover, the filamentous nature of these microorganisms facilitates the harvesting of biomass. Open cultures are performed in 1 m^2 pond of 30 cm maximal depth (Moreno *et al.*, 2003). Turbulence was provided by a rotating paddle wheel made up of three 28×32 cm paddles, operating at a rotating speed of 18 rpm. Pure CO_2 (at the flow rate required to keep pH at 8.5–9.0) was supplied, from sunrise to sunset, through a PVC porous tube placed on the bottom of the container. Cells were grown under semi-continuous regime;

TABLE 3 **Advantages and disadvantages of open and closed algal cultivation plants (Pulz, 2001).**

Parameter	Open pond	Closed system
Contamination risk	Extremely High	Low
Space required	High	Low
Water losses	Extremely High	Almost none
CO_2–losses	High	Almost none
Biomass quality	Not susceptible	Susceptible
Variability as to cultivatable species	Cultivation possibilities are restricted to a few algal varieties	High, nearly all microalgal varieties may be cultivated
Flexibility of production	Change of production between the possible varieties nearly impossible	Change of production without any problem
Reproducibility of production parameters	Dependant on exterior conditions	Possible within certain problems
Standardization	Not possible	Possible
Weather dependence	Absolute, production impossible during rain	Insignificant, because closed configurations allow production also during bad weather
Period until net production is reached after start or interruptions	Long, approximately 6–8 weeks	Relatively short, approximately 2–4 weeks
Biomass concentration during production	Low, approximately 0.1–0.2 g L^{-1}	High, approximately 2–8 g L^{-1}
Efficiency of treatment processes	Low, time consuming, large volume flows due to low concentrations	High, short time, relatively small volume flows

the cultures were diluted with fresh medium early in the morning to establish cell density value. The lower temperature limit of the culture was fixed at 30°C. The main factors determining the growth rate were pH, nutrient supply, temperature and the availability of light to the cells. Light availability to the cells depends on impinging irradiance, turbulence, culture depth, and cell density. The biomass productivity values achieved ranged from 9 g (dw) m^{-2} d^{-1} in winter to over 20 g (dw) m^{-2} d^{-1} in summer, indicating that they are strongly affected by temperature. The optimal value for cell density (which critically affects availability of light to the cell) was 0.1 g (dry biomass) L^{-1} for which biomass productivity was maximal. Maximal phycobiliprotein production, about 3 g m^{-2} d^{-1}, was recorded in the summer. The specific phycobiliprotein productivity values achieved in open cultures of *Anabaena sp.* ATCC 33047 were very high (mean annual values over 1 g m^{-2} d^{-1} for each allophycocyanin and phycocyanin), underlining the potential for commercial production of these pigments (Moreno *et al.*, 2003) (Table 4).

5.4 Red Microalgae

5.4.1 Phycobiliprotein

The red microalgae are characterized by their chlorophyll, a number of carotenoids as well as the phycocyanin and phycoerythrin (photosynthetic accessory pigment, collectively known as phycobiliproteins, which are red and blue). These algal pigments have the potential as natural colorants for use in food, cosmetics, and pharmaceuticals, particularly as substitutes of synthetic dyes. Phycobiliproteins are built up of bilins, which are open chain tetrapyrroles, covalently linked via one or two thioester to the cysteine residue in the apoprotein. Phycobiliproteins are water-soluble, absorb light in the visible region of 450–650 nm and are used in the food and cosmetic industry. Solutions of phycobilins were found to be stable at pH range 5–9. At lower pH value phycobilins precipitate and are sensitive to high temperature (Roman *et al.*, 2002). *Porphyridium cruentum* has also become of industrial interest for the production of eicosapentaenoic acid and other polysaturated fatty acids (Serval *et al.*, 1994). This organism also produces substantial amounts of toxic trichloroethylene and perchloroethylene (Abrahamsson *et al.*, 1995).

TABLE 4 **Relationship of dilution rate with different parameters.**

	Dilution rate	
Parameter	0.18 d^{-1}	0.26 d^{-1}
Standing cell density	1.9 g (DW) L^{-1}	1.3 g (DW) L^{-1}
Biomass productivity	9–10 g (DW) m^{-2} d^{-1}	9–10 g (DW) m^{-2} d^{-1}
Polysaccharide released	2.9 g L^{-1}	5.1 g L^{-1}

5.4.2 Phycocyanin

Spirulina plantensis is a blue-green microalgae that produces phycocyanin. This organism has been found to consume organic carbon substrate for heterotrophic and mixotrophic growth. Heterotrophic production is not suitable for the synthesis of phycocyanin.

Production in Fed Batch Culture

Production of algal cells is reported in fed batch mode (Chen and Zhang, 1997). They mentioned that algal cells can be grown in a 3.7 liter fermenter containing 2 g l^{-1} glucose until late exponential phase where the pH was adjusted between 9.5–10.5. Temperature was maintained at 30°C at 300 rpm and a flow rate of 100 l h^{-1}. Higher light intensities were found to be inhibitory and low light intensities reduced the cell mass production. In mixotrophic cultures, phycocyanin production increased constantly up to 107 mg g^{-1} dry cell in 250 h and in photoautotrophic cultures the content of phycocyanin was constant (135 mg g^{-1}). Recently, purification of C-phycocyanin has been reported from Cyanobacterial sp. (Patel *et al.*, 2005).

5.5 Polysaccharides

Microbial polysaccharides are attracting increasing interest for their potential applications in the food, cosmetic and pharmaceutical industries, competing with other natural polysaccharides obtained from plants and macroalgae. Among these, cyanobacterial extracellular polymeric substances (EPSs) (polysaccharidic in nature) present unique biochemical properties that make them interesting from the biotechnological point of view. Cyanobacteria produce complex exopolysaccharides composed of at least 10 different monosaccharides and are characterized by the presence of pentoses, which are usually absent in other polysaccharides of prokaryotic origin, and by their anionic nature, which is due to the presence of acidic sugars (glucuronic and/or galacturonic acids) and other anionic organic (acetyl, pyruvil) and inorganic (phosphate and sulphate) substituents. Besides the standard applications of microbial

EPSs as food coating, emulsifying and gelling agents, flocculants and hydrating agents, the anionic nature of cyanobacterial polysaccharides makes them interesting for biomedical applications. In the field of bioremediation, EPSs are used to remove toxic metals from polluted waters (Otero and Vincenzini, 2003).

VI. NEW AREAS OF RESEARCH FOR CYANOBACTERIAL METABOLITES

Several secondary metabolites, isolated from *L. majuscula* and other cyanobacterial species, possess synergistic activity, i.e. the activity of the combined extract is greater than its individual activity. Laxaphycin A and B, for example, first isolated from the terrestrial cyanobacteria *Anabaena laxa*, and also found in *L. majuscula*, exert a synergistic effect against *Candida albicans* and inhibit lymphoblastic cell lines. The natural occurrence of these lipopeptide associations in the same organism suggests that the complex might be involved in the cell growth regulation of the producer microorganism, or in the cell growth inhibition of competitor microorganisms. This synergistic effect can be a potentially novel area of research. Only a small fraction of all microbes present within the marine environment are cultivable via conventional methods and new techniques must be developed to produce natural products in unnatural ways. Combinatorial genetic engineering may be the prospective remedy to this problem (Burja *et al.*, 2001).

VII. OTHER APPLICATIONS OF CYANOBACTERIA

The microalgae industry has developed to its current status by providing a safe and nutritious product for the human supplement market as well as the animal and aquaculture feed market. The majority of this microalgal biomass is produced from *Spirulina plantesis*, *Chlorella* and *Aphanizimenon flos-aqua*. *Spirulina*, a vegetable based nutrient rich dilatory supplement, is a highly absorbable source of natural β-carotene, mixed carotenoids, phytonutrients, B vitamins, γ-linoleinic acid, proteins and essential amino acids. It has a positive effect on immune system and detoxification and has anti-inflammatory, antiviral and anticancer action. It is cultivated in an open pond system, where it can be grown free from contaminant algae as it is cultivated and thrives in very alkaline conditions, where competitor algae and other contaminants cannot grow and also pond ecology is balanced to support the growth of *Spirulina*.

Cyanobacteria are autotrophic prokaryotes, which carry out oxygenic photosynthesis and accumulate glycogen as a major form of stored carbon. A new gene was introduced into a cyanobacterium in order to create a novel pathway for fixed carbon utilization, which results in synthesis of ethanol (Deng and Coleman, 2003). As cyanobacteria have simple growth requirements, grow to high densities, use light, carbon dioxide and inorganic elements efficiently, production of ethanol by cyanobacteria is a potential system for bioconversion of solar energy and carbon dioxide into a valuable resource. Cyanobacteria can also be used as a carbon sequestration agent, where it can combine cyanobacteria from fossil fuel combustion systems and nutrients to give compounds with high commercial value (Olaizola, 2003).

VIII. CONCLUSION

Cyanobacteria are a promising but still unexplored natural resource offering a wealth of chemicals for lead compounds discovery and new drugs. Of the new drugs approved between 1983 and 1994, up to 80% of antibacterial and anticancer drugs were derived from natural products. Traditional microbial drug producers like *Actinomycetes* and *Hyphomycetes* have been in the focus of pharmaceutical research for decades. Now that the rate of discovery of interesting compounds in these classical source organisms is decreasing, it is time to turn to cyanobacteria and exploit their potential. Despite the complexity of the algal genome, the mechanism behind their enormous chemical diversity is being slowly unfolded. The biosynthetic information on the chemical structures unique to these organisms will be very valuable for gene manipulation aimed at creating new therapeutic agents, and in the near future they will achieve the same position as *streptomyces* and other actinomycetes we have today. Cyanobacteria produce a wide variety of toxins and other biomedically interesting bioactive compounds. They produce cyclic heptapeptide hepatotoxins, microcystins and pentapeptide nodularins and three types of neurotoxins: (homo) anatoxin-a, anatoxin-a(s) and saxitoxins. Cyanobacterial neurotoxins block neurotransmission and hepatotoxins are inhibitors of serine/threonine specific protein phosphatases (PP1 and PP2A). Several new cyclic or linear peptides and desipeptides are protease inhibitors.

Cyanobacteria are also known to produce antitumor, antiviral and antifungal compounds. Many of the pharmaceutically interesting compounds in cyanobacteria are peptides, including cyanobacterial toxins and important candidates for anti-cancer drugs. Peptide synthetases are common in cyanobacteria and responsible for the production of cyanobacterial hepatotoxins and other peptides. Polyketide synthetases are also involved in the biosynthesis of certain cyanobacterial bioactive compounds (e.g. microcystins). Photobioreactor technology has advanced to the point where it is relatively easy to scale up cultures to produce enough material for research purposes. Cyanobacteria also have good potential as a food. Dried *Anthrospira* (*Spirulina*) is sold in the market as a health food with annual sales estimated at 40 million US $. Cyanobacteria can also have application as fuel producers (ethanol and H_2). Many desirable chemicals are the products of secondary metabolism triggered under conditions not conducive to fast growth. For those chemicals to be produced by microalgae, one needs to develop new strains (faster growth, higher substrate tolerance, etc.) by classical selection or genetic manipulation so microalgal biomass can be produced consistently.

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