CYANOBACTERIA: A NOVEL SOURCE OF ANTIBIOTICS, NON-RIBOSOMAL POLYPEPTIDE SYNTHETASE (NRPS) AND POLYKETIDE SYNTHASE (PKS) – DRUG FOR THE EMERGING DISEASES.

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Abstract: One of the modern medical application is the ability to synthesize nonribosomally bioactive peptides and ketides from widely spread cyanobacteria. Which are cyclic or branched cyclic compounds containing non-proteinogenic amino acids, small heterocyclic rings and other usual modification in the peptide backbone. They are synthesized by multimodular enzymes from simple building blocks. The key principle is to study NRPS and PKS enzymes unveiled by Biochemical and Genetic Studies. This review focuses on how bioinformatics tools and software were used to predict the enzyme, substrate and structure of an individual compound.

Keywords: **NRPS, PKS, Cyanobacteria, Cyanotoxins, Secondary Metabolites.**

1. INTRODUCTION

Cyanobacteria are blue-green algae which are worldwide in distribution. Cyanobacterial secondary metabolites have attracted increasing scientific interest due to the bioactivity of many compounds in various test systems. Non-ribosomal Peptide Synthetase (NRPS) and genes are a very ancient part of the cyanobacterial genome and presumably have evolved by recombination and duplication events to reach the present structural diversity of cyanobacterial oligopeptides (Walker and Dohren 2006). These metabolites represent a widely distributed biomedically and biocontrol important class of natural products including antibiotics, siderophores, and anticancer as well as biopesticides that are considered as a novel source that can be used to defend ecological niche from competitors and to promote plant growth (Esmaeel *et al*., 2017).

Antibiotics: An antimicrobial agent purified from a microorganism that can kill or inhibit the growth of microorganisms, especially disease-causing microbes. **Siderophores:** a molecule which binds and transports iron in microorganisms. **Anticancer:** Otherwise called as antineoplastic, drugs are used to treat malignancies or cancerous growths. **Biopesticides:** The pesticides derived from natural materials such as animals, plants and bacteria are called biopesticides. From 1940s, Natural Products and their derivatives plays a major role in pharmaceutical industries, most of these are commercially available for frontline treatments in Human health, chiefly therapy for infectious disease, cancer, diabetes, etc., (Huang and Lin., 2017).

Toxin: A poisonous substance released by a plant or animal that are derived from microorganisms which are toxic or that acts as an antigen in a body. Polyketides (PKs) and non-ribosomal peptides (NRPs) are two major classes of secondary metabolites with diverse chemical structures and a valuable source of pharmaceutically important molecules (Khater *et al*., 2017). Cyanobacteria have the ability to produce a number of bioactive oligopeptides which are diverse in occurrence with exceptional structures. These peptides are synthesized by NRPS/PKS pathway like hepatotoxic microcystins / Trypsin-inhibitory cyanopeptolins (Ishida *et al.*, 2009).

In recent decades, negative human activity including Agricultural run-off, sewage, Industrial and Municipal wastes has led to increased eutrophication of water bodies and consequently to higher frequency and severity of harmful cyanobacterial blooms (Adamski *et al*., 2016).

Many bioactive secondary metabolites produced by cyanobacteria are highly toxic to other microorganisms, plants, animals and humans. George Francis in 1878 reported deaths of cattle and sheep after the consumption of water from Lake Alexandrina (South Australia) when it was covered by a toxic bloom of *Nodularia spumigena*. As a result, cyanobacterial toxins were classified as a health hazard to animals. The research on cyanotoxins focuses mainly on hepatotoxic microcystins and neurotoxic saxitoxins, while relatively little information relates to cytotoxic cylindrospermopsin (CYN). Cylindrospermopsin is produced by several freshwater species of cyanobacteria (*Anabaena bergii*, *A. lapponica*, *A. planctonica*, *Aphanizomenon flos-aquae*, *Aph. gracile*, *Aph. ovalisporum*, *Cylindrospermopsis raciborskii*, *Lyngbya wollei*, *Oscillatoria* sp., *Umezakia natans*, *Raphidiopsis curvata* and *R. mediterranea*)

occurring all over the world. Cylindrospermopsin is an alkaloid (415 Da) with a guanidine moiety built into a tricyclic system, so-called tricyclic guanidine combined with hydroxymethyluracil (Adamski *et al.*, 2016).

The information about microbial secondary metabolite biosynthesis are the blooming area in research. Gene clusters responsible for the biosynthesis of polyketides and non-ribosomal peptides, identified by the presence of polyketide synthases (PKS) or non-ribosomal peptide synthetases (NRPS) encoding genes, have received significant attention, resulting in the sequencing of hundreds of gene clusters. With the power, speed and low cost of next-generation sequencing methods, this number are expected to increase rapidly by at least an order of magnitude in the next few years (Conway and Boddy., 2013).

To take advantage of this wealth of data, it needs to be easily accessible and discoverable. Although the sequences themselves are available in National Center for Biotechnology Information (NCBI) databases they are frequently difficult to locate, partially because of the large amounts of information that these databases host. Standardized annotation for these biosynthetic gene clusters is not available.

With the rapid growth in bacterial genome sequencing, many new clusters are located within much larger genome sequence files and are occasionally unannotated, such as the antibiotic TA / Myxovirescin biosynthetic gene cluster in the *Myxococcus xanthus* genome. These problems are compounded by the fact that gene cluster discovery is being undertaken by researchers from diverse fields of expertise, including Chemistry, Biochemistry, Microbiology, Biotechnology and Drug discovery, all with differing standards for gene cluster annotation. Thus, it is no surprise that given these issues, it can be extremely challenging, time-consuming and often frustrating to find appropriate genes cluster in the NCBI database. Already available databases providing information about PKS/NRPS gene clusters from the sequence data this accelerate the research about the product from gene clusters (Conway and Boddy., 2013).

2.1 CYANOBACTERIA AND CYANOTOXINS

Cyanobacteria are prolific producers of notorious toxins called cyanotoxins (Calteau *et al*., 2014). Cyanotoxins are of different classification they are Hepatotoxins, Neurotoxins, Cytotoxins (Carmichael, 1989), Dermatotoxins, Irritant toxins (Rastogi *et al.*, 2015) and Endotoxins (Hudnell 2007). Harmful Algal Blooms (HABs) are a major problem in both Marine and Freshwater Environments. Increasing eutrophication of aquatic systems shifts in the equilibrium of the ecosystem. Water blooms are defined as Mass Occurrence of microalgae. Several bloom-forming algae are toxic, non-toxic algal blooms exert a negative influence on the environment. Decaying algal blooms may deplete the water of O_2, initiate the death of fish and animals and causing bad smells and low-quality water. In the marine environment, O_2 depletion will lead to producing extremely toxic sulfide. Cyanobacteria are a special group of bloom-forming microalgae. They are only oxygenic photoautotrophic prokaryotes. Three properties of Cyanobacteria to produce Algal bloom: (a) Buyont due to the possession of gas vesicles. (b) Capable of fixing N_2. (c) Low photosynthetic compensation points and a high affinity for light (stal *et al.*, 2003). A number of cyanobacterial based toxins had been identified and classified. They are as follows:

2.1.1 HEPATOTOXINS

Toxic chemical substances that are found naturally or in a laboratory environment that damages the liver. It also due to the side effect of medication or naturally as microcystins. Hepatotoxins are more commonly encountered by humans and other animals during cyanobacterial blooms. These are preferentially taken up by hepatocytes (functional cells of the liver), resulting in a range of ill effects. MCs and nodularins are both cyclic peptides and have similar toxicity mechanisms (Zurawell *et al*., 2005). They do not move across cell membranes, but can be actively transported by the bile acid transporter mechanism and hence preferentially attack hepatocytes (Gorham & Carmichael, 1988). i.e., Microcystins, Nodularins, cylindrospermopsin (Hudnell 2007).

2.1.2 NEUROTOXINS

They are the extensive class of exogenous chemicals or neurological insults that adverse the effect function in both developing a mature nervous tissue. They are poisonous/disruptive to nerve tissue that causes neurotoxicity (Rodgers *et al* 2017). The unique amino acid β-methylamino-L-alanine (BMAA) is previously known only to occur in cycads, nonflowering seed plants of ancient lineage. High doses of cycad neurotoxins to produce neurological disease to the people (Cox *et al*., 2003). i.e., Anatoxin – A, Anatoxin – A (S), Saxitoxin, β-methylamino-L-alanine (BMAA) (Hudnell 2007).

2.1.3 DERMATOTOXINS

A toxin produced by the microbes like cyanobacteria leads to damage Human or animal skin, mucous membrane leading to tissue necrosis. They can be natural chemicals, drugs or synthetic that is a harmful compound. They are also produced by certain spiders, jellyfish or bacteria. i.e., Lyngbyatoxin – A, Aplysiatoxin (Hudnell 2007).

2.1.4 CYTOTOXINS

The secondary metabolites produced by the cyanobacteria is harmful to the human or animal cell are cytotoxins. It is a quality of being toxic to cells. i.e., Cylindrospermopsin (Adamski *et al.*, 2016).

2.1.5 IRRITANT TOXINS (ENDOTOXINS)

A large number of a toxic substance that can cause pain in the urinary tract, abdominal cramps, digestive tract, vomiting and diarrhea. They are large molecules that are a combination of lipid – polysaccharide composed of O – antigen, the outer core and inner core joined by a covalent bond. The Gram-negative bacteria or cyanobacteria contain this endotoxin on its outer membrane. i.e., Lipopolysaccharides (Hudnell 2007).

3.1 ROLE OF EUTROPHICATION IN AQUATIC SYSTEMS AND TOXINS PRODUCTION

The term eutrophy is water containing optimum nutrients, enriched with organic and inorganic nutrients. The enriched nutrients particularly nitrogen and phosphorus, allow excessive growth of cyanobacteria and that condition is called Eutrophication. Cyanobacteria are the Earth's oldest oxygenic photoautotroph's alters the previous anoxic biosphere to evalution of higher plants and animals (Paerl and Otten, 2013).

The phosphorus concentration was found as a primary regulating factor for increased cyanobacterial growth and changes of genotypes, both of which were found to be closely related to the water temperature, signifying the role of eutrophication in the occurrence of toxic blooms (Rastogi *et al.*, 2015). Aquatic ecosystems throughout the world have been enriched with nutrients derived from urban/domestic, industrial and agricultural wastes as well as global climate change can play a major role in the global expansion of harmful algal blooms and toxin production, more frequently with toxic species, such as the *Microcystis aeruginosa* (Alvarez *et al.*, 2015).

The "harmful" aspect of these blooms, from an environmental context, begins with a loss of water clarity that suppresses aquatic macrophytes, and negatively affects invertebrate and fish habitats (Carmichael 2013). Dense cyanobacterial blooms can contain very high toxin concentrations, posing a major threat to birds, mammals and human health. Blooms may cause an increase in biological oxygen demand (BOD) and anoxia in the water bodies, and death of aquatic life (Van 2010).

Cyanotoxins are may cause serious, acute intoxication for Human affecting the nervous systems, dermal, digestive, endocrine, and hepatopancreatic. The liver toxins microcystins and cylindrospermospin, and the neurotoxins anatoxin-A and saxitoxins (Carmichael 2013). The general structure of microcystins is cyclo (D - Ala - X - D - MeAsp- Z- Adda – D – Glu - Mdha), (Azevedo *et al.*, 1994). Human health problem gastroenteritis by the toxin produced by *Microcystis* and *Anabaena* in drinking water from the reservoir (Codd *et al.*, 1999).

4.1 STRUCTURE

The structure of NRPS and PKS is often characterized from the particular cyanobacteria isolated from different environment like fresh and marine water and the major toxins produced by cyanobacteria have been elucidated. However, it is clear that cyanobacteria typically encode additional natural products (Calteau *et al*., 2014). The structure of NRPS and PKS determined by the module and the module is determined by the different arrangement of domains and secondary domains as shown in the figure: 1 (Schwarzer *et al*., 2003).

4.2 MECHANISM

The adenylation domain selects and activates the monomer transforming it into adenylate form. The thiolation / peptidyl carrier protein domain covalently binds the activated monomer to the synthetase. The condensation domain catalyzes the peptide bond formation between the residues linked to two adjacent modules. Finally, the thioester domain only present in the final module, release the peptide from the synthetase. The product can either be released as a linear compound or get transformed into a cycle peptide through an intramolecular reaction. The thioesterase domain can allow the enzyme to iterate the collinear biosynthesis many times especially in NRPS (Caboche *et al*., 2007).

The Secondary domains that allow residue modifications in many NRPSs i.e., Cyclization, Methylation Oxidation and epimerization domain leading to obtaining the D isomer of an amino acid can be encountered.. This mechanism can produce different variants of peptide that have the same structure but have different monomers at certain positions (Caboche *et al*., 2007).

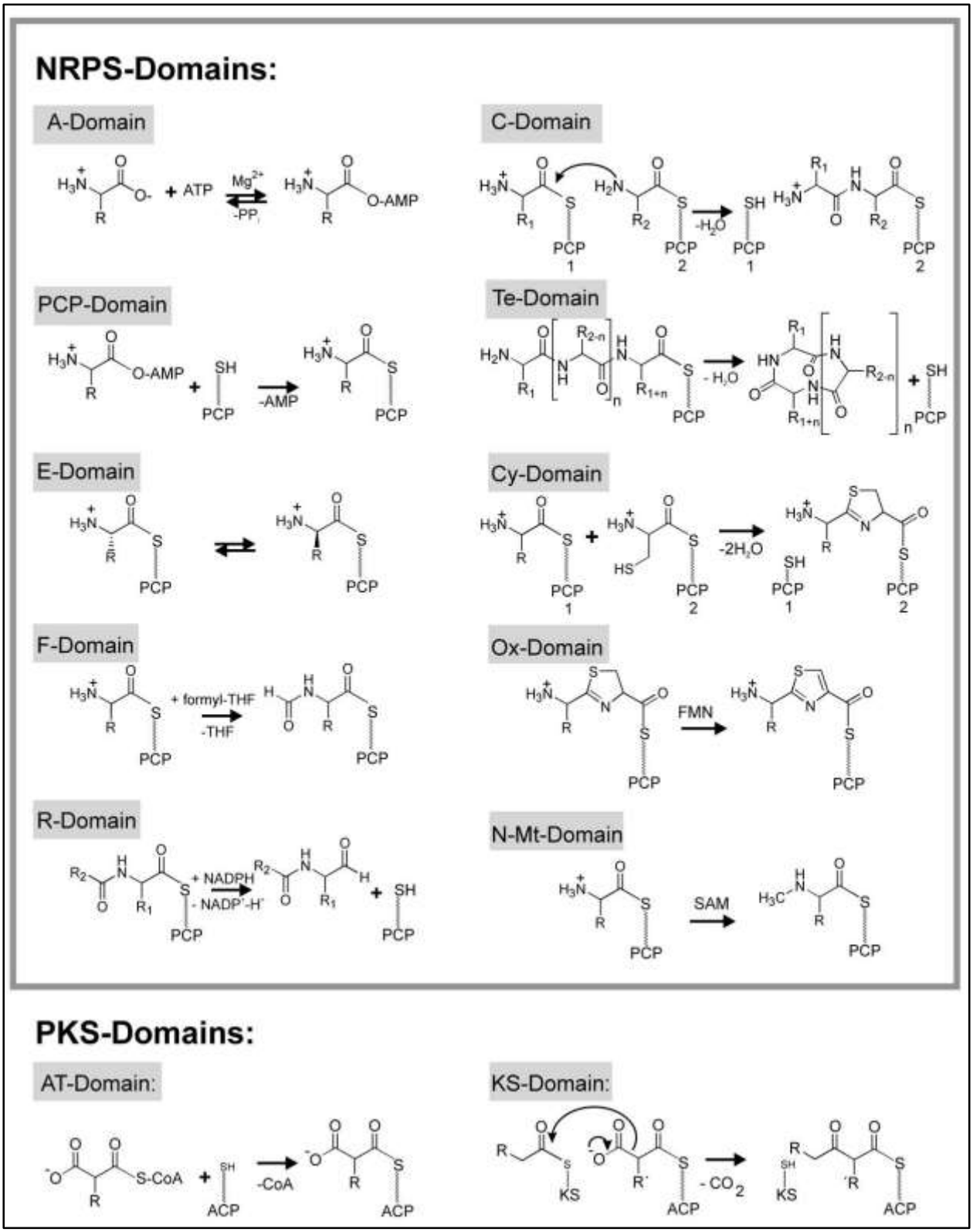

Figure 1: Structure of NRPS and PKS Domains and Reactions (Schwarzer *et al.*, 2003).

4.3 POLYPEPTIDE CHAIN LENGTH AND AMINO ACID SIDE CHAINS:

NRPS peptide chain length is minimally defined by the number of A domains present in the megasynthetase. As the terminal carboxylate remains uncoupled, for n A domains there should be n–1 condensation domains. Practically, an excess of C domains may

indicate other condensation reactions in addition to peptide homologations, including N-acylation, coupling to polyketide biosynthesis, or coupling to other (amino) acyl-S-T species. Initiation modules may be comprised of an A–T didomain, in the simplest case. If the first module is a tri-domain, C–A–T, this may indicate ligation of the first amino acid to the donor acyl-S–T chain. A thiolation domain initiating an ORF may suggest an initiating module that accepts an activated CoA ligand. If the T domain is prefaced by a CoA–ligase domain (AL), the likelihood is high that this module is initiating. Terminating modules are usually terminally punctuated by a Thioesterase (Te) domain, which releases the nascent peptide via macrocyclization or hydrolysis, or in some cases a Reduction (Re) domain, which reduces the terminal thioester to the aldehyde oxidation state (Bachmann and Ravel, 2009).

5 NONRIBOSOMAL PEPTIDE SYNTHETASE (NRPS) AND POLYKETIDE SYNTHASE (PKS)

The entire cyanobacterial lineage as 70% of the cyanobacterial genomes which contains the polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) pathways or hybrid. Cyanobacteria paves way about 5% of their genomes for these pathways, with an average of five NRPS / PKS clusters per genome. A large modular enzymes in which modules incorporate building blocks into the growing chain like on an assembly line. Interestingly, (Calteau *et al*., 2014).

These are the four (4) main domains catalyzes the specific activation groups: They are as follows:

1. Adenylation Domain,
2. Thiolation Domain,
3. Condensation Domain,
4. Thioester Domain.

5.1 ADENYLATION DOMAIN

Adenylation domains catalyze the specific activation of carboxyl groups of amino acids, imino acids or hydroxyl acids, as well as various carboxylic acids. They are the primary specification step for the amino acid sequence of the completed peptide. In a catalytic site the entry of specific amino acid is achieved. An analysis of the phenylalanine binding pocket of the activating domain of the first module of gramicidin S synthetase has led to an amino acid contact residue code permitting the prediction of substrates in NRPS adenylation domains. This specificity-conferring code has been confirmed in a variety of correlations of NRPS genes with known peptide product structures and may make it possible to predict unknown products. Although this non-ribosomal code is a good predictor of substrate selection, it is not the only mechanism of control. Thiolation, as well as condensation domains, are also involved in the specific form of a particular amino acid sequence.

About 200 adenylation domains have been identified in nucleotide sequences so far in cyanobacteria. They are generally integrated into NRPS systems or represent acyl-CoA synthetases. Upon alignment, 10 core motifs (A1–A10) can be easily identified in most cyanobacterial adenylation domains representing consensus sequences that can also be found in fungal systems (Welker and Dohren., 2006). ATP-PPi exchange assay is used to test the amino acid specific adenylation can be studied by hterologously expressed adenilation domains. i.e., Bar D incorporates L-Leucine but activates 3-chloroleucine and valine as well (Chang *et al*., 2002). The leucine-specific adenylation domain of McyB of *Microcystis aeruginosa* activates isoleucine and valine as well, but never observed in microcystins. Likewise, the first adenylation domain of Nos A activates Val, Ile and Leu when it is expressed in *Escherichia coli*, but Leu is not found in nostopeptolide (Welker and Dohren., 2006).

5.2 THIOLATION DOMAIN

The key role of these domains is in the transport of intermediates, which requires specific interaction with the activating adenylation domain and the corresponding condensation domains for aminoacyl and peptidyl elongation cycles. Intermediate modifications the transport also requires interactions with oxidation domains, epimerization domains, methyltransferase domains, reduction domains or thioesterase domains in terminating cyclization reactions. Acylation or aminoacylation of the ‘swinging arm’ cofactor 40-phosphopantetheine is considered the covalent transport principle in NRPSs and PKSs. These domains are generally identified as a signature sequence by the conserved 40-phosphopantetheine attachment site and the signature sequence is post-translationally modified by protein phosphopantetheinyl transferases (Welker and Dohren, 2006).

5.3 CONDENSATION DOMAIN

The condensation domain of about 450 amino acids has been functionally characterized in the gramicidin S/ tyrocidine synthetase systems. The current functional interpretation proposes, by analogy to the ribosomal system, that an aminoacyl (A-site) and a peptidyl site (P-site) receive the activated intermediates. The aminoacylated carrier proteins (thiolation domains) resemble charged tRNAs, and the condensing site, the peptidyl transferase region. As a prototype of a C-domain, the crystal structure of an isolated C-domain of the vibriobactin biosynthetic system, Vib H, has been determined. The Vib H-structure revealed a novel topology and is a monomer consisting of two subdomains. Alignments confirm the structure to be representative of the NRPS condensation domains, the related epimerization domains, and cyclocondensation domains. The downstream carrier, which transports the initiating acyl residue or the peptidyl intermediate, will bind to the C-terminal face of this domain with the pantetheinyl arm extending into the solvent channel. The upstream carrier with the acceptor compound, usually an aminoacyl residue generally binding in trans to the condensation domain, would approach from the opposing open end of the domain, and both pantetheinyl arms would extend into the solvent channel to facilitate peptide bond formation. A survey of about 160 cyanobacterial condensation domains reveals that their core sequences are

very similar to those derived from *Bacillus* domains (Von Dohren *et al* 1999). Upon alignment by CLUSTAL, domains group into functionally related types and not into subsections or genera. This has been observed before and correlates with a similar analysis of adenylation and thiolation domains. Obvious clusters are the related heterocyclization and epimerization domains and functionally related domains of systems producing homologous peptides (Welker and Dohren, 2006).

5.4 THIOESTER DOMAIN

The ending domain is a thioesterase (TE), which releases the product by catalyzing a lactonization. The TE domain is located in the termination module and catalyzes peptide release by either hydrolysis or macrocyclization (Strieker 2010).

6 TOXINS AND CYANOBACTERIA

Marine cyanobacteria have yielded an amazing assortment of chemically diverse and biologically relevant natural products. Several promising clinical candidates coming from the marine environment can be traced to metabolic processes of cyanobacteria, including dolastatin and scytonemin. In this regard, the previous investigations of a Curacüao collection of *Lyngbya majuscula* resulted in a highly active antiproliferative and cytotoxic compound, curacin A (Chang *et al.,* 2004). Marine macro and microorganisms are an important source of biologically active secondary metabolites. An array of natural products with great potential for clinical application in a variety of disease indications have been isolated from marine organisms, such as the Bengamides, Milnamides, Hemiasterlins, Dolastatins, Bryostatins and Discodermolide (Chang *et al.,* 2002).

6.1 MICROCYSTIN

A new nitrogen-fixing cyanobacterium. **Chemical formula:** $C_{49}H_{74}N_{10}O_{12,}$ *Nostoc* sp. CENA88 has been found to produce the microcystin variant [Dha7] MCYST-YR. This variant was first identified in *Microcystis aeruginosa* strain CALU 972 isolated from a water bloom of lake Kroshnosero, Russia (Sivonen *et al* 1990; Sivonen et al., 1992b). The demethyl MCYST-YR variant has been found only in Microcystis strains, and then to our knowledge, this is the first report of its occurrence in another cyanobacterial genus. All the *Nostoc* microcystin-producing isolates described so far synthesize different microcystin variants (Bajpai *et al.,* 2009; Beattie *et al.,* 1998; Kaasalainen *et al.,* 2009; Mohamed *et al.,* 2006; Oksanen *et al.,* 2004; Sivonen *et al.,* 1990, 1992a). However, demethyl MCYSTs have already been documented in the genus *Nostoc*, with the Mdha replaced by dehydrobutyrine (Dhb) (Beattie *et al.,* 1998). The lack of N-methyl group in a microcystin has been reported as a consequence of the absence of the entire N-methyl transferase (NMT) domain in the mcyA gene in some strains of *Planktothrix* (Kurmayer *et al.,* 2005) and Anabaena (Fewer *et al.,* 2008). However, specific point mutations altering amino acid residues in the cofactor SAM-binding site of NMT domain of Microcystis strains also was reported as a possible cause of demethyl microcystin production (Tooming-Klunderud *et al.,* 2008). A NMT domain (422 aa) occurs only in the first adenylation domain of McyA, inserted between the core motifs A8 and A9 (Tillett *et al.,* 2000), and catalyses the transfer of the S-methyl group from S-adenosylmethionine to the a-amino group of the thioesterified amino acid (Sieber and Marahiel, 2005).

6.2 NODULARINS

Nodularins are mainly produced by *Nodularia spumigena*. **Chemical formula:** $C_{41}H_{60}N_8O_{10}$. Since first being described in 1988, approximately 10 variants have been discovered. Nodularin gene cluster is a representative example of the domain deletion mechanism. Nodularins and microcystins are produced by cyanobacteria and show several similarities regarding their chemical structure and biological activity (Rantala *et al*., 2004). Nodularin gene cluster has evolved from the microcystin gene cluster through deletion of two modules. Thus nodularin metabolites lack two amino acids in position 3 (Welker and Von Dohren 2006).

6.3 CYLINDROSPERMOPSIN

In 1992 it was shown that the hepatotoxin was an alkaloidal polyketide, cylindrospermopsin is most generally synthesized by *Cylindrospermopsis raciborskii*, having the molecular composition. **Chemical formula:** $C_{15}H_{21}N_5O_7S$. Detailed spectral analysis of 1 led to the identification of its gross structure and relative stereochemistry but its absolute configuration is still unknown (Burgoyne *et al*., 2000).

6.4 ANATOXIN – A AND ANATOXIN – A (S)

Chemical formula: Anatoxin – A - $C_{10}H_{15}NO$, Anatoxin – A (S) - $C_7H_{17}N_4O_4P$ cyanobacteria may produce neurotoxins that have been responsible for lethal poisonings of mammals and birds, including anatoxin - A (s). This toxin is a natural organophosphate which irreversibly inhibits acetylcholinesterase (AChE), similar to organophosphorous and carbamate insecticides and some chemical warfare agents. When acetylcholinesterase is inhibited, the neurotransmitter acetylcholine is no longer hydrolyzed in the synapse, the postsynaptic membrane cannot be repolarized, and nerve influx is blocked. Anatoxin - A (s) is highly toxic for mammals when the toxigenic cyanobacteria produce mass populations in drinking water (Devic *et al*., 2002).

6.5 SAXITOXIN

Saxitoxins are a class of chemically related neurotoxins, also known as paralytic shellfish toxins (PSTs) due to their association with seafood, predominantly filter-feeding shellfish. Paralytic shellfish toxins are the causative agents of paralytic shellfish poisoning (PSP), which is globally the most widespread alga-derived shellfish poisoning, causing detrimental public health effects and substantial annual damage to the fishing and aquaculture industries (Tebrineh *et al., 2010*). Saxitoxin or trialkyltetrahydropu rine (**Chemical formula:** $C_{10}H_{17}N_7O_7$) was first isolated from shellfish *Saxidomus giganteus* Desh and its name was borrowed for neurotoxin. SXT and its analogs enter the shellfish organism together with toxic algae and accumulate in the organs and tissues without harming the animals. Also, it is known that many aquatic inhabitants from zooplankton to fish and whales are PST sources, however, shellfish filtrat ing plankton and herbivorous crustacea are the major transvector for PST spreading (Moustafa *et al*., 2009).

6.6 β-METHYLAMINO-L-ALANINE (BMAA):

BMAA is concentrated in the developing reproductive tissues of the cycad *Cycas micronesica*, averaging 9 μg/g in the fleshy seed sarcotesta and a mean of 1,161 μg/g BMAA in the outermost seed layer. The unique amino acid β-methylamino-L-alanine (BMAA) is previously known only to occur in cycads, nonflowering seed plants of ancient lineage. Discovered in the genus Cycas by Vega and Bell, BMAA is a non-protein amino acid and structurally appears as a methylated alanine. The source of BMAA in cycads has been unknown. However, given its profound neurotoxic properties, BMAA may function in cycads as a chemical deterrent to herbivory. Because the indigenous Chamorro people consume tortillas made from cycad seed flour, Spencer and his coworkers suggested that BMAA might be a cause of amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS-PDC), a progressive neurological disease of the Chamorro with aspects of amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinsonism dementia. However, argued that massive amounts of flour would have to be consumed to generate a progressive neurological disease among the Chamorro people. (Cox *et al*., 2003).

6.7 Lyngbyatoxin – A:

Source of Lingbyatoxin – A from *Lyngbya majuscula* are potent skin irritants (Welker and Dohren 2006). Identified as aetiological agent of acute toxic dermatitis since 1950s (Testai et al 2016). Lyngbyatoxin A possible gastro intestinal inflammatory toxin (Carmichael 2013).

7 Medical Applications of Peptides:

Table 1: Bioactivity of Nonribosomal synthesized Compounds.

Bioactivity	Natural Product	Source	References
Anti-mitotic	Dolastatin	*Anabaena* sp.	(Yang *et al.*, 2017)
Anti-microbial	Ambiguine isonitriles	*Fischerella ambigua*	(Mo *et al.*, 2009)
Anti-obesity	Yoshinone A, B	*Leptolyngbya* sp.,	(Blunt *et al.*, 2016)
Inhibit HeLa cell line	Kurhamide	*Moorea bouillonii*	(Blunt *et al.*, 2016)
Neurotoxic	Antillatoxin	*Lyngbya majuscula*	(Rastogi *et al.*, 2015)
Antineoplastic Agent	Aplysiatoxin, Lynbyatoxin	*Lyngbya majuscula*	(Rastogi *et al.*, 2015)
Antiproliferative Agent	Curacin A	*Lyngbya majuscula*	(Rastogi *et al.*, 2015)
Neurotoxin	Kalkitoxin	*Lyngbya majuscula*	(Rastogi *et al.*, 2015)
Anticancer	Vinblastine, Vincristine	*Catharanthus roseus*	(Rastogi *et al.*, 2015)
Anticancer	Somocystinamide A, Tanikolide Malyngolide	*Lyngbya majuscula,* *Lyngbya majuscula,* *Lyngbya majuscula,*	(Nunnery *et al.*, 2010)

Anti-protozoal	Viridamide A, B Almiramide	*Oscillotoria nigro-viridis,*	(Nunnery *et al.,* 2010)
Anti-inflammatory	Bis-Bromoindoles	*Rivularia* sp.,	(Nunnery *et al.,* 2010)
Anti-malaria	Gallinamide A	*Symploa* sp.,	(Nunnery *et al.,* 2010)
Anti-leishmanial	Dragonamide E	*Lyngbya majuscula,*	(Nunnery *et al.,* 2010)
Anticancer	Microviridin, Dolastin	*Microcystis aeruginosa*	(Wase and Wright 2008)
Tumour Promotor	Microcystin	*Microcystis aeruginosa*	(Wase and Wright 2008)
Antifungal	Lyngbyabellin B	*Lyngbya majuscula*	(Wase and Wright 2008)
Antimicrobial	Microcolin A	*Lyngbya majuscula bouilloni*	(Wase and Wright 2008)
Antiviral, Anti-inflammatory	Laxaphycins A & B	*Lyngbya majuscula harvey*	(Wase and Wright 2008)
Neurotoxic skin irritant toxin	Homodolastatin 16, Curacin A	*Lyngbya majuscula,*	(Wase and Wright 2008)
Antigrazers, Alkaline Phosphatase	Lyngbyabellins A, B	*Lyngbya majuscula,*	(Wase and Wright 2008)
Antifeedant	Aurilides B & C	*Lyngbya majuscula,*	(Wase and Wright 2008)
Neurotoxin	Kalkitoxin	*Lyngbya majuscula,*	(Wase and Wright 2008)
Cytotoxic	Lyngbyatoxins B & C	*Lyngbya majuscula,*	(Wase and Wright 2008)
Antineoplastic	Acutiphycin, Didehydroacutiphycin	*Oscillatoria acutissima*	(Wase and Wright 2008)
Anticancer	Cryptophycin	*Nostac* sp.	(Wase and Wright 2008)
Cytotoxic	Nostophycin	*Nostac* sp.	(Wase and Wright 2008)
Antifungal	Nostocyclamide	*Nostac* sp.	(Wase and Wright 2008)
Antibiotic	Nostocyclin	*Nostac* sp.	(Wase and Wright 2008)
Antifungal	Nostodione	*Nostac commune*	(Wase and Wright 2008)
Antibiotic	Microsporine	*Nostac commune*	(Wase and Wright 2008)
Antimitotic cytotoxic	Diterpenoid	*Nostac commune*	(Wase and Wright 2008)
Anti-HIV / Antiviral	Cyanovirin	*Nostac ellipsosporium*	(Wase and Wright 2008)

Cytotoxic	Cylindrospermopsin	*Cylindrospermopsis raciborskii*	(Wase and Wright 2008)
Neurotoxin	Antillatoxin	*Lyngbya majuscula*	(Wase and Wright 2008)
Antimolluscicidal	Barbamide	*Lyngbya majuscula*	(Wase and Wright 2008)
Antitumour	Digalactosyl diacylglycerol	*Phormodium tenue*	(Wase and Wright 2008)

8 GENOME MINING SOFTWARE

8.1 GAPPED BLAST OR PSI-BLAST

The original BLAST program often finds several alignments involving a single database sequence which, when considered together, are statistically significant. The ability to generate gapped alignments has been added. Overlooking any one of these alignments can compromise the combined result. By introducing an algorithm for generating gapped alignments, it becomes necessary to find only one rather than all the ungapped alignments subsumed in a significant result. The new gapped alignment algorithm uses dynamic programming to extend a central pair of aligned residues in both directions. Gapped and PSI_BLAST find in **https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE=Proteins&PROGRAM=blastp&RUN_PSIBLAST=on** (Altschul *et al*., 1997).

8.2 2metDB

2metDB / SecmetDB is a standalone tool (web server locally installed on the user's machine) that offers the possibility to mine for PKS and NRPS biosynthetic gene clusters in whole genome protein fasta files. The Algorithms used are the same than at the PKS / NRPS web server / Predictive Blast Server. **http://secmetdb.sourceforge.net/** (Bechmann and Ravel, 2009).

8.3 antiSMASH

The antibiotics and Secondary Metabolites Analysis Shell (antiSMASH) is the bioinformatics tool we use to provide analysis on clusters. antiSMASH can scan a cluster's sequence and determine the most likely pathway type for that cluster (Conway and Boddy., 2013). The database is a fully automated pipeline to mine bacterial and fungal genome data for secondary metabolite biosynthetic gene clusters (BGCs). The small molecules encoded by these BGCs often have various bioactivities including antimicrobial, anti-cancer etc., Therefore they are lead compounds for many drugs like antibiotics.

An antiSMASH web is free of cost and does not require any login details or Registration. Users voluntarily provide an e-mail address for sending information and the Result link gets expired in 30 days. Upload microbial genome sequences (in FASTA format). The prediction of products by Biosynthetic pathways possible for NRPS/PKS gene clusters. Products in antismash are identified using Hidden Markow Models (HMMs) of protein motifs for key biosynthetic enzymes. Once the cluster detection is identified a secondary metabolite cluster of a certain type of specific analysis module is run. Lantipeptide- specific chemical core structure analysis to the existing set of NRPS/ PKS core prediction tools. Profile Hidden Markov Models (pHMMs) describe key biosynthetic enzymes of 24 secondary metabolites class detectable by antiSMASH (Blin *et al*., 2013). To generate a more detailed analysis of the pathway and the prediction of the product in given cluster is determined. The latest version of antiSMASH self-containing plugins that are loaded the users PYTHON-PATH at run-time (Blin *et al*., 2014).

The new medicine is developed by the great potential of microbial secondary metabolites. *In silico* characterization of each gene cluster is laborious process when compared to the laboratory research work. The manual annotation is very labor-intensive and time-consuming, leading incomplete annotations. An antiSMASH Fasta format Submission in **http://antismash.secondarymetabolites.org/** (Medema *et al*., 2011).

8.4 NaPDoS

Adenylation domain substrate specificity predictions for NRPS enzymes were made using NRPSpreditor2. Annotations were refined manually using CD-search, BLASTP and InterProScan to identify conserved domains (Calteau *et al.,* 2014). NaPDoS server can be found in **http://napdos.ucsd.edu/** (Bechmann and Ravel 2009).

8.5 HIERARCHICAL CLUSTERING

A Hierarchical Clustering analysis on the presence/absence pattern of all CFs found in the cyanobacterial genomes was done using the MeV software (v4.8: **http://mev-tm4.sourceforge.net**) and the following parameters: Pearson correlation, ordering optimization on the species, average linkage clustering (Calteau *et al.,* 2014).

8.6 SBSPKSv2

SBSPKS chemical space. Traditional methods like microbial isolation and culturing combined with newer methods like genetic engineering and metagenomics have yielded >11000 PKs and NRPs also advances in sequencing technologies have exponentially increased the rate of discovery of new PKS and NRPS gene clusters. Of the 11000 PKs and NRPs discovered, a very small percentage

has its biosynthetic gene cluster known. Gene cluster discovery of these secondary metabolites can be facilitated by comparing them to characterized PKs, NRPs and their biosynthetic intermediates. Two essential requirements for such searches are a well-curated database containing characterized biosynthetic pathways of PKs and NRPs and suitable tool(s) to search and analyze the chemical structures of secondary metabolites and their biosynthetic intermediates. SBSPKS server found in **http://www.nii.ac.in/sbpks.html/** (Anand *et al*., 2010).

8.7 CLUSTSCAN PROFESSIONAL

ClustScan Professional is a commercial Java-based tool to identify PKS and NRPS gene clusters in genomic data. Clust Scan Database (CSDB) contains 170 clusters with highly annotated descriptions of Polypeptides, modules and domains from the DNA and Protein sequences are able to predict the product in a standard SMILE format (Diminic *et al*., 2013).

8.8 CLUSTERMINE360

The microbial PKS / NRPS database, ClusterMine360 (**http://www.clustermine360.ca/**), is organized around two key elements, the compound family and the gene cluster. A compound family is a grouping of compounds that have the same core structure. This term is used, as most gene clusters produce more than one compound, although they tend to be highly related. For example, the epothilone biosynthetic pathway produces four highly related polyketides, epothilones A–D, which differ by the presence or absence of a methyl group and an epoxide moiety.

The second major organization unit of the database is the gene cluster. Multiple clusters can be associated with a given compound family. For example, epothilone biosynthetic gene clusters have been sequenced from two strains of *Sorangium cellulosum* and erythromycin gene clusters have been sequenced from *Saccharopolyspora erythraea* and *Aeromicrobium erythreum*. Each cluster is associated with an NCBI nucleotide record. The NCBI record is used as the source for the lineage of the producing organism, including the phylum, genus and species. Links to primary literature references for the sequencing data are also retrieved from the NCBI record and displayed on the cluster's details page. Linked to each gene cluster is the annotation data for each gene in the cluster and each domain found in the PKS and NRPS encoding genes. These data are generated through antiSMASH analysis of each gene cluster. The domain sequences, extracted from the antiSMASH results, are also available from the gene cluster's details page (Conway and Boddy., 2013).

8.9 CLUSTERMINE360: A POWERFUL TOOL FOR PHYLOGENETIC ANALYSIS

To demonstrate the utility of the ClusterMine360 database, NRPS heterocyclization domains were selected and used for cluster analysis. Heterocyclization domains play a key role in NRPS biosynthesis, coupling acyl and peptidyl groups onto Cysteine, Serine and Threonine residues followed by cyclization of the associated side-chain to generate thiazol and oxazole rings. This occurs during the biosynthesis of non-ribosomal peptides, such as the antibiotic bacitracin, and mixed non-ribosomal peptide/ polyketides, such as the antimitotic agent epothilone and rhizoxin.

A FASTA file of 106 heterocyclization domains was downloaded and aligned using Multiple Sequence Comparison by Log-Expectation (MUSCLE). A phylogenetic tree was generated from the resulting alignment using the PhyML maximum likelihood method with the Whelan and Goldman (WAG) model of amino acid substitution and nearest neighbor interchange for the tree topology search. The tree shows that heterocyclization domains clustered by function, based on whether the domain used enzyme bound Cysteine, Serine or Threonine as its substrate. To evaluate which residues each heterocyclization domain used, the 'detail of cluster' function in the sequence repository was examined to identify the specificity of adenylation domain associated with the heterocyclization domain. Based on this analysis, the tree shows that Cysteine, Serine and Threonine specific heterocyclization domains all tree apart from each other (Conway and Boddy 2013).

9.1 AUTOMATION

Ensuring high data quality is time-consuming, and it makes database upkeep difficult. One of the most important requirements for the database was to integrate automation to make curation as easy as possible. As most of the data are populated automatically, external users are able to contribute without much risk to data quality. This semi-automatic curation also means that large amounts of data can be added to the database in a relatively short amount of time. The following steps occur once a cluster is added. First, the NCBI nucleotide database is queried to retrieve important information about the sequence, such as its description, the name and lineage of the organism it was isolated from and any sequencing references that are associated with the record. Once this information has been retrieved, the cluster is submitted to antiSMASH for analysis. The database automatically tracks the progress of the antiSMASH submission and proceeds to download the results when completed. The results are then parsed to retrieve information, such as the pathway types for that cluster, which is used to ensure that the pathway types of the linked compound family are correct.

Finally, if antiSMASH has identified any PKS/NRPS domains, the amino acid sequence of those domains will be stored in the database's sequence repository along with key information, such as domain substrate specificity, stereochemistry and activity of the domain, as applicable. In addition, when a compound family is added, it is searched against the PubChem database to retrieve Medical Subject Heading (MeSH) pharmacological identifiers that classify the compound's bioactivity. Simplified molecular-input line-entry system (SMILES) strings are also retrieved enabling users to search the database by substructure.

The typical time to complete these processes ranges from a few minutes to a few hours depending on server load. In addition to the automated processes above, we also incorporated some other features that make it particularly easy for users to add data. When a

compound family is added to the database, a wizard guides the user through the process of entering information on pathway types, synonyms and related families and helping the user in generating an image for the structure of the compound. To make it easy to associate an image, the ChemSpider database **(http://www.chemspider.com)** is queried to retrieve images that match the compound family name. Alternatively, an image can be generated from a user-supplied SMILES string. Similarly, when adding synonyms, potential synonyms are returned from ChemSpider and the user can easily select those that are applicable (Conway and Boddy., 2013).

9.2 SEQUENCE REPOSITORY

One of the unique aspects of this database is its sequence repository. The repository contains a large number of diverse PKS/NRPS domains extracted from the antiSMASH analysis of the clusters contained in the database. We have also included the ability to scan any NCBI nucleotide record and have the detected PKS/NRPS included in this repository. We believe that this repository will become an invaluable tool to those involved in identifying sequence homologies and bioprospecting. The sequences can be downloaded individually in FASTA format. Alternatively, all of the domains in a given cluster can be downloaded at once in a zip file. We have also included the ability to filter the domains based on a variety of criteria, following which they can be downloaded in a multi-sequence FASTA file. Importantly, the depth of information included in each sequence's header is exceptional. They are full of rich information, such as accession number, producing an organism, gene identifier, pathway type, domain type and any predicted properties of that domain. We have also included an option to output shortened headers for use with bioinformatics tools that have restrictions on the number of characters in the header (Conway and Boddy., 2013).

10 MEDICALLY RELEVANT NON-RIBOSOMAL PEPTIDES AND THE ASSOCIATED NRPSS

This is not meant to be a comprehensive review of all medically relevant NRPSs. Rather, NRPSs discussed below were chosen to highlight the basic enzymology of NRPSs and how subtle or extensive modifications to the basic C-A-T module repeat enable the formation of the enormous structural diversity seen in natural products using this type of enzymology. The order in which the natural products are discussed progress from the straightforward biosynthesis of the tripeptide backbone of β-lactam antibiotics to the more complicated enzymology involved in the biosynthesis of bleomycin, a molecule synthesized in large part by an NRPS that differs greatly from the classic C-A-T module architecture.

Equally interesting is the finding that many pathogenic microorganisms use NRPS enzymology to assemble small molecules that enable them to survive in the host environment and cause disease. For example, many pathogenic microorganisms access iron from the host environment by biosynthesizing, secreting, and reabsorbing iron-chelating molecules called siderophores. The production and reacquisition of these metabolites can be essential for an organism to cause disease. Due to this, there is intense interest in developing small molecule inhibitors of these NRPSs to provide a new target for drug development. Thus, NRPS enzymology has been harnessed by us to treat an infection but has also been harnessed by microorganisms to enable an infection. Due to space limitations, we will focus our attention on NRPSs that produce medically relevant drugs (Felnagle *et al.,* 2011).

11 CONCLUSION

This review is based on Secondary metabolites from cyanobacteria by NRPS and PKS Pathway. Research work in our Laboratory deals with cyanobacteria in association with cycads (Root nodules), Lichens, Bryophytes, *Gunnera Manicata* (Leaf nodules), etc., Samples from different location were collected and isolated then, it was cultured in the laboratory condition and biomass is obtained from this the compound characterized by using LC-MS, MALDI-TOF, FTIR, etc.,

References:

1. Adamski M, Zmudzki P, Chrapusta E, Bober B, Kaminski A, Zabaglo K, Latkowska E and Bialczyk J. 2016. Effect of pH and temperature on the stability of cylindrospermopsin. Characterization of decomposition products. Algal Research 15: 129–134.

2. Altschul S F, Madden T L, Schaffer A A, Zhang J, Zhang Z, Miller W and Lipman D J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research.25 (17): 3389–3402.

3. Anand S, Prasad MVR, Yadav G, Kumar N Shehara, J, Ansari M Z and Mohanty D. 2010. SBSPKS: structure based sequence analysis of polyketide synthases. Nucleic Acids Research. 38(2): W487-W496.

4. Alvarez X, Valero E and Picos J. 2015. Eutrophication, water quality and nutrient loading: blooms of green algae. Environmental Chemistry and Toxicology. 10th Iberian and 7th Iberoamerican Congress on Environmental Contamination and Toxicology: 181, 182.

5. Azevedo S M F O, Evans W R, Caimichael W W and Namikoshi M. 1994. First report of microcystins from a Brazilian isolate of the cyanobacterium Microcystis aeruginosa. Journal of Applied Phycology 6: 261-265.

6. Bachmann B O and Ravel J. 2009. Methods for In Silico Prediction of Microbial Polyketide and Nonribosomal Peptide Biosynthetic Pathways from DNA Sequence Data. Methods in Enzymology 458: 181 – 217.

7. Bajpai R, Sharma N K, Lawton L A, Edwards C and Rai A K. 2009. Microcystin producing cyanobacterium Nostoc sp. BHU001 from a pond in India. Toxicon 53: 587–590.

8. Beattie K A, Kaya K, Sano T and Codd G A. 1998. Three dehydrobutyrine containing microcystins from Nostoc. Phytochemistry 47: 1289–1292.

9. Blin K, Medema M H, Kazempour D, Fishbach M A, Breitling R, Takano E and Weber T. 2013. antiSMASH 2.0 – a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Research 41: W204-W212.

10. Blin K, Kazempour D, Wohlleben W and Weber T. 2014. Improved Lanthipeptide Detection and Prediction for antiSMASH. PLOS ONE 9 (2): 1-7.

11. Blunt J W, Copp B R, Keyzers R A, Munro M H G and Prinsep M R. 2016. Marine natural products. The Royal Society of Chemistry 33: 382–431.

12. Burgoyne D L, Hemscheidt T K, Moore R E, and Runnegar M T C. 2000. Biosynthesis of Cylindrospermopsin. Journal of Organic Chemistry 65: 152-156.

13. Caboche S, Pupin M, Leclere V, Fontaine A, Jacques P and Kucherov G. 2007. NORINE: a database of non-ribosomal peptides. Nucleic Acids Research 36: D326 – D331.

14. Calteau A, Fewer D P, Latifi A, Coursin T, Laurent T, Jokela J, Kerfeld C A, Sivonen K, Piel J and Gugger M. 2014. Phylum-wide comparative genomics unravel the diversity of secondary metabolism in Cyanobacteria. BMC Genomics 15(977): 1-14.

15. Carmichael W W. 1989. Freshwater Cyanobacteria (blue-green algae) Toxins. Natural Toxins characterization, Pharmacology and Therapeutics. Pergamon press: 3-16.

16. Carmichael W W. 2013. Human Health Effects from Harmful Algal Blooms: a Synthesis. International Joint Commission: 1 – 55.

17. Carmichael W W. 1989. Freshwater Cyanobacteria (blue-green algae) Toxins. Natural Toxins characterization, Pharmacology and Therapeutics. Pergamon press: 3-16.

18. Carmichael W W. 2013. Human Health Effects from Harmful Algal Blooms: a Synthesis. International Joint Commission: 1 – 55.

19. Chang Z, Sitachitta N, Rossi J V, Roberts M A, Flatt P M, Jia J, Sherman D H and Gerwick W H. 2004. Biosynthetic Pathway and Gene Cluster Analysis of Curacin A, an Antitubulin Natural Product from the Tropical Marine Cyanobacterium *Lyngbya majuscula*. Journal of Natural Products 67: 1356-1367.

20. Chang Z, Flatt P, Gerwick W H, Nguyen V A, Willis C L and Sherman D H. 2002. The barbamide biosynthetic gene cluster: a novel marine cyanobacterial system of mixed polyketide synthase (PKS) - nonribosomal peptide synthetase (NRPS) origin involving an unusual trichloroleucyl starter unit. Gene 296: 235–247.

21. Codd G, Bell S, Kaya K, Ward C, Beattie K and Metcalf J. 1999. Cyanobacterial toxins, exposure routes and human health. European Journal of Phycology 34(4): 405-415.

22. Conway K R and Boddy C N. 2013. ClusterMine360: a database of microbial PKS/NRPS Biosynthesis. Nucleic Acids Research. 41. Pp: D402-D407.

23. Cox P A, Banack S A, and Murch S J. 2003. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. PNAS 100(23): 13380-13383.

24. Devic E, Li D, Dauta A, Henriksen P, Codd G A, Marty J L, and Fournier D. 2002. Detection of Anatoxin-a(s) in Environmental Samples of Cyanobacteria by Using a Biosensor with Engineered Acetylcholinesterases. Applied and Environmental Microbiology 68(8): 4102–4106.

25. Dohren H V, Dieckmann R and Vrancic M P. 1999. The nonribosomal code. Chemistry & Biology, Elsevier Science Ltd. 6: R273–R279

26. Diminic J, Zucko J, Ruzic I T, Gacesa R, Hranueli D, Long P F, Cullum J and Starcevic A. 2013. Databases of the thiotemplate modular systems (CSDB) and their in silico recombinants (r-CSDB). Journal of Industrial Microbiology and Biotechnology 40: 653–659.

27. Esmaeel Q, Pupin M, Jacques P and Leclere V. 2017. Nonribosomal peptides and polyketides of *Burkholderia*: new compounds potentially implicated in biocontrol and pharmaceuticals. Environmental Science and Pollution Research: 1-14.

28. Felnagle E A, Jackson E E, Chan Y A, Podevels A M, Berti A D, McMahon M D, and Thomas M G. 2011. Nonribosomal Peptide Synthetases Involved in the Production of Medically Relevant Natural Products. NIH-PA. Molecular Pharmaceutics 5(2): 191–211.

29. Fewer D P, Klunderud A T, Jokela J, Wahlsten M, Rouhiainen L, Kirstensen T, Rohrlack T, Jakobsen K S, Sivonen K. 2008. Natural occurrence of microcystin synthetase deletion mutants capable of producing microcystins in strains of the genus Anabaena (Cyanobacteria). Microbiology 154: 1007–1014.

30. Genuario D B, Silva-Stenico M E, Welker M, Beraldo-Moraes L A, Fiore M F. 2010. Characterization of a microcystin and detection of microcystin synthetase genes from a Brazilian isolate of Nostoc. Toxicon 55: 846–854.

31. Hudnell K H. 2007. Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms. Advances in Experimental Medicine and Biology: 17-35.

32. Huang T and Lin S. 2017. Microbial Natural Products: A Promising Source for Drug Discovery. Journal of Applied Microbiology and Biochemistry 2 (1): 1-3.

33. Ishida K, Christiansen G, Yoshida W Y, Kurmayer R, Nativitat-Valls M W, Bonjoch J, Hertweck C, Borner T, Hemscheidt T and Dittmann E. 2007. Biosynthesis and Structure of Aeruginoside 126Aand 126B, Cyanobacterial Peptide Glycosides Bearing a 2-Carboxy-6-Hydroxyoctahydroindole Moiety. Chemistry and Biology 14: 565–576.

34. Kaasalainen U, Jokela J, Fewer D P, Sivonen K and Rikkinen J. 2009. Microcystin production in the tripartite cyanolichen Peltigera leucophlebia. Molecular Plant-Microbe Interactions Journal 22: 695–702.

35. Khater S, Gupta M, Agrawal P, Sain N, Prava J, Gupta P, Grover M, Kumar N and Mohanty D. 2017. SBSPKSv2: structure-based sequence analysis of polyketide synthases and non-ribosomal peptide synthetases. Nucleic Acids Research 45: W72–W79.

36. Klunderud A T, Mikalsen B, Kristensen T and Jakobsen K S. 2008. The mosaic structure of the mcyABC operon in Microcystis. Microbiology 154: 1886–1899.

37. Kurmayer R, Christiansen G, Gumpenberger M, Fastner J. 2005. Genetic identification of microcystin ecotypes in toxic cyanobacteria of the genus Planktothrix. Microbiology 151: 1525–1533.

38. Medema M H, Blin K, Cimermancic P, Jager V, Zakrzewski P, Fischbach M A, Weber T, Takano E and Breitling R. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Research 39: W339–W346.

39. Mo S, Krunic A, Chlipala G and Orjala J. 2009. Antimicrobial ambiguine isonitriles from the cyanobacterium *Fischerella ambigua*. Journal of Natural Products 72(5): 894–899.

40. Mohamed Z A, Sharouny H M and Ali W S. 2006. Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigation canals. Egypt Toxicon 47: 584–590.

41. Moustafa A, Loram E J, Hackett D J, Anderson M D, Plumley F G, Bhattacharya D. 2009. Origin of Saxitoxin Biosynthetic Genes in Cyanobacteria. PLoS ONE. 4(6): 1-10.

42. Nunnery J K, Mevers E and Gerwick W H (2010). Biologically active secondary metabolites from marine Cyanobacteria. Current Opinion in Biotechnology 21(6): 787–793.

43. Oksanen I, Jokela J, Fewer D P, Wahlsten M, Rikkinen J, and Sivonen K. 2004. Discovery of rare and highly toxic microcystins from lichenassociated cyanobacterium Nostoc sp. strain IO-102-I. Applied and Environmental Microbiology 10: 5756–5763.

44. Rantala A, Fewer D P, Hisbergues M, Rouhiainen L, Vaitomaa J, Borner T and Sivonen K. 2004. Phylogenetic evidence for the early evolution of microcystin synthesis. Proceedings of the National Academy of Sciences of the United States of America, 101: 568–573.

45. Rastogi R P, Madamwar D and Incharoensakdi A. 2015. Bloom Dynamics of Cyanobacteria and Their Toxins: Environmental Health Impacts and Mitigation Strategies. Frontiers in Microbiology 6(1254): 1-22.

46. Rodgers K J, Main B J and Samardzic K. 2017. Cyanobacterial Neurotoxins: Their Occurrence and Mechanisms of Toxicity. Neurotoxicity Research: 1-10.

47. Schwarzer D, Finking R and Marahiel M A. 2003. Nonribosomal peptides: from genes to products. Natural Product Reports 20: 275–287.

48. Sieber S A and Marahiel M A. 2005. Molecular mechanisms underlying nonribosomal peptide synthesis: approaches to new antibiotics. Chemical Reviews 105: 715–738.

49. Sivonen K, Carmichael W W, Namikoshi M, Rinehart K L, Dahlem A M and Niemela S I. 1990. Isolation and characterization of hepatotoxic microcystin homologs from filamentous freshwater cyanobacterium Nostoc sp. strain 152. Applied and Environmental Microbiology 56: 2650–2657.

50. Sivonen K, Namikoshi M, Evans W R, CarmichaelW W, Sun F, Rouhiainen L, Luukkainen R and Rinehart K L. 1992. Isolation and Characterization of a Variety of Microcystins from Seven Strains of the Cyanobacterial Genus Anabaena. Applied and Environmental Microbiology 58(8): 2495-2500.

51. Sivonen K, Namikoshi M, Evans W R, Fardig M, Carmichael W W and Rinehart K L. 1992a. Three new microcystins, cyclic heptapeptide hepatotoxins, form Nostoc sp. strain 152. Chemical Research in Toxicology 4: 464–469.

52. Sivonen K, Namikoshi M, Evans W R, Gromov B V, Carmichael W W, Rinehart K L. 1992b. Isolation and structures of five microcystins from a Russian Microcystis aeruginosa strainCALU972. Toxicon 30: 1481–1485.

53. Stal L J, Albertano P, Bregman B, Brockel K V, Gallon J R, Hayes P K, Sivonen K and Walsby A E. 2003. BASIC: Baltic Sea Cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea-responses to a changing environment. Continental Shelf Research 23: 1695 – 1714.

54. Strieker M, Tanovic A and Marahiel M A. 2010. Nonribosomal peptide synthetases: structures and dynamics. Structural Biology 20: 234–240.

55. Tebrineh J A, Mihali T K, Pomati F, and Neilan B A. 2010. Detection of Saxitoxin-Producing Cyanobacteria and Anabaena circinalis in Environmental Water Blooms by Quantitative PCR. Applied and Environmental Microbiology. 76(23): 7836–7842.

56. Testai E, Buratti F M, Funari E, Manganelli M, Nathalie-Arnich S V, Bire R, Fessard V, Sialehaamoa A. 2016. Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. European Food Safety Authority Supporting publication.

57. Tillett D, Dittmann E, Erhard M, Dohren H V, Borner T, Neilan B A. 2000. Structural organization of microcystin biosynthesis in Microcystis aeruginosa PCC7806: an integrated peptide-polyketide synthetase system. Chemical biology 7: 753–764.

58. Van D E. 2010. Out of balance – Implications of climate change for the ecological stoichiometry of harmful cyanobacteria. Gildeprint Drukkerijen. ISBN 978-94-61080-45-5: 1 – 144.

59. Yang G, Zhang Y, Lee N K, Cozad M A, Kearney S E, Luesch H and Ding Y. 2017. Cyanobacterial Sfp-type phosphopantetheinyl transferases functionalize carrier proteins of diverse biosynthetic pathways. Scientific Reports. 7(11888): 1-9.

60. Wase N V and Wright P C. 2008. Systems biology of cyanobacterial secondary metabolite production and its role in drug discovery. Expert Opinion on Drug Discovery 3(8): 903-929.

61. Welker M and Dohren H. 2006. Cyanobacterial peptides - Nature's own combinatorial biosynthesis FEMS Microbiology Reviews 30: 530–563.

62. Zurawell R W, Chen H, Burke J M, Prepas E E. 2005. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. Journal of Toxicology and Environmental Health 8: 1–37.