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Cyanobacteria Characteristics and Methods for Isolation and Accurate Identification of Cyanotoxins: A Review Article

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Abstract

Cyanobacteria are bacteria found in different ecosystems, such as lakes and rocks. These bacteria, capable of photosynthesis, are important sources of oxygen. However, some cyanobacterial strains can produce toxins, which are harmful to humans and animals. Therefore, collection of epidemiological and surveillance data on cyanobacterial toxins in the environment is vital to ensure a low risk of exposure to toxins in other organisms. For presentation of accurate data on environmental cyanobacterial toxins, it is essential to understand their characteristics, including taxonomy, toxin proteins, and genomic structures, and determine their environmental effects on bacterial populations and toxin production. Taxonomy, which is the scientific classification of organisms, is important in identifying species producing toxins. The structure of toxin proteins and their stability in the environment allow researchers to detect toxins with analytical methods and discuss their limitations. On the other hand, identifying toxins via molecular typing enables researchers to investigate toxic cyanobacteria by detecting toxin-encoding genes and toxin gene expression. Meanwhile, environmental factors, such as nutrient level, light intensity, and biotic factors, allow researchers to predict the suitable time and location for accurate sampling. In this review, these cyanobacterial features, which are important for accurate detection of cyanobacterial toxins, will be discussed.

Keywords: Cyanobacteria, Cyanotoxin, Hepatotoxin, Neurotoxin, Cytotoxin, Dermatotoxin, Cyanobacterial Taxonomy, Methods of Identification

1. Introduction

Cyanobacteria or "blue-green algae" are prokaryotes, which mainly receive their nutrients through photosynthetic processes. They are highly adaptable to the environment and can be found in soil, rocks, and most water bodies, ranging from hot springs to the cold water of Antarctic lakes and low-nutrient freshwater environments. As part of the aquatic environment ecology, cyanobacteria play an important role in the ecosystem maintenance. Photosynthesis of bacteria provides oxygen, while nitrogen-fixing cyanobacteria provide atmospheric nitrogen for other organisms.

Cyanobacteria are also important as potential sources of renewable energy and natural products. However, excessive growth of these bacteria forms visible cyanobacteria or cyanobacterial blooms in the water environment. The blooms can cause several problems, such as unpleasant odour and taste, and most importantly, toxin production (1). Cyanobacterial toxins are generally categorized into 4 major groups based on their toxicological effects: hepatotoxin (microcystin and nodularin), neurotoxin (anatoxin-a, anatoxin-a(s), and saxitoxin), cytotoxin (cylindrospermopsin), and dermatotoxin (aplysiatoxin and lyngbyatoxin A) (2).

Consumption or direct contact with cyanobacterial toxins can cause severe health problems. For instance, microcystin production led to the death of 60 dialysis patients in Brazil (3), hospitalisation of 148 children in Palm Island, Australia (due to cylindrospermopsin [CYN] toxicity) (4), and several cases of animal death. Some efforts have been made to analyse the health risks associated with cyanotoxin. In 1999, the world health organization (WHO) included cyanobacterial toxins as threatening compounds in drinking and recreational water guidelines (5).

Toxin detection in water bodies is important to prevent similar toxicities. The earliest record of toxic cyanobacteria was reported by Francis in Australia (6). Most primary studies in this area detected toxic cyanobacteria through toxicity analysis using mouse cell bioassays due to lack of

structural and molecular information on toxins. Only after several decades, toxin structures, genome codes, and biochemical pathways were identified, making the detection of toxic cyanobacteria faster and easier.

Toxic cyanobacteria have been detected in more than 65 countries worldwide. Despite various reports on cyanobacterial toxicity, studies on these toxins are still limited. Overall, 90% of research on these bacteria has been conducted in only 10 countries (2), and many countries have yet to include cyanobacterial toxins in their drinking and recreational water policies due to lack of monitoring. This in turn causes unknown health risks due to toxin exposure. Therefore, detection of toxins and toxin-encoding genes of cyanobacteria are crucial in every country to improve the epidemiological data and clarify the status of these toxins.

Use of detection methods is dependent on an understanding of bacterial morphology and characterisation of toxins and toxin gene clusters (7). As environment influences the dynamics of cyanobacterial population and accumulation (8), the sampling point and time for toxic cyanobacteria surveillance are important. Overall, without a proper understanding of the characteristics of cyanobacteria, limitations in the methods cannot be evaluated. Therefore, the results obtained in the experiments may not be reliable. This article aimed to review cyanobacterial features (Figure 1), which are important for accurate detection of cyanobacterial toxins.

2. Cyanobacterial Taxonomy

Cyanobacteria phylum consists of greenish-blue bacteria due to its chlorophyll pigments and has different forms and structures. The bacteria are either unicellular or in a filamentous form, determined by the mode of reproduction. The unicellular forms are often seen as unicellular cocci, while the filamentous forms are filamentous or rod-shaped. Many filamentous cyanobacteria are capable of forming heterocysts or akinetes in a specific environment. Heterocysts are formed in the absence of nitrogen, especially in clean water environments, allowing cyanobacteria to fix nitrogen from the atmosphere and survive.

Certain nonheterocystous cyanobacterial species, such as *Filamentous trichodesmium*, *Lyngbya*, *Oscillatoria*, *unicellular Gloeothece*, and *Cyanothece* species, can fix nitrogen under aerobic conditions (9). Meanwhile, akinetes are resting-state cells, used as a survival strategy, similar to bacterial endospores. They are formed under harsh conditions, such as low temperature, drought, high salt level, and iron depletion (10). Both forms have thick cell walls, a trait to distinguish cyanobacterial species using microscopic evaluation. Besides the characteristics of heterocysts and akinetes, presence or absence of sheath, true or false branching, and cell size are also used for identification of cyanobacteria (11).

Previously, cyanobacterial taxonomy was mainly dependent on the described morphological characteristics. However, some morphological data are contrary to molecular results; in other words, cyanobacterial species have a similar morphology, but distinct 16S ribosomal RNA (rRNA) DNA sequences (11). In addition, relying on only morphological observations in cyanobacterial detection can lead to misidentification, as cyanobacteria are capable of changing their taxonomical characteristics. Researchers reported that about 50% of cyanobacterial cultures contradicted their taxonomical descriptions (12).

Therefore, considering the recent changes in taxonomy, a combination of molecular, biochemical, and ultrastructural patterns of thylakoids and ecology is required for cyanobacterial identification (11). The molecular analysis for cyanobacterial taxonomy uses 16S rRNA as a marker for identification and classification of cyanobacteria genera (or higher orders). Moreover, 16S rRNA can distinguish different habitats of cyanobacteria, which have similar morphological appearances (11). Through reevaluation of taxonomy, eight orders have been established: Gloeobacterales, Synechococcus, Spirulinas, Chroococcales, Pleurocapsales, Oscillatoriales, Chroococcidiopsis, and Nostocales (13).

3. Cyanobacterial Toxins

As previously stated, harmful cyanobacterial toxins or cyanotoxins are categorized into 4 major groups, based on their toxicological effects. For accurate cyanotoxin detection, it is essential to understand its structure and stability in the environment. Analytical or immunological detection methods, such as high-performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA), are used to detect toxins in the environment, based on their structure (14).

Meanwhile, direct detection of toxin-encoding genes via molecular typing includes conventional polymerase chain reaction (PCR) for identifying toxic cyanobacterial strains, reverse transcriptase PCR (RT-PCR) and DNA hybridization for detection of gene expression, and quantitative PCR (qPCR) for identification of the initial quantity of toxin genes. These molecular approaches require an understanding of cyanotoxin gene structure and regulation, which are distinctive for each toxin (15).

3.1. Hepatotoxins

Hepatotoxins, capable of destroying liver cells, include microcystin and nodularin. These toxins have particular

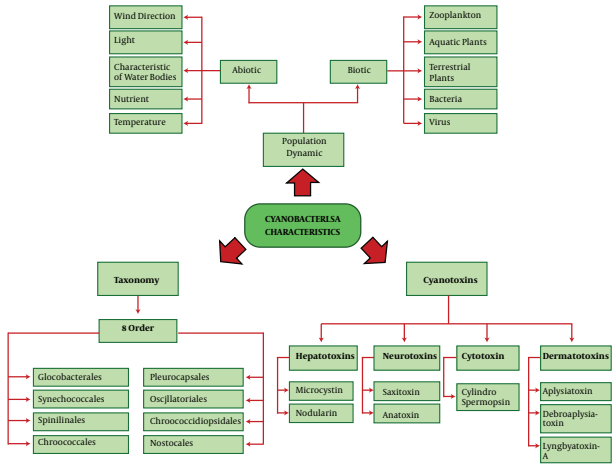

Figure 1. Summary of Cyanobacteria Characteristics for Accurate Identification and Epidemiological Study of Toxic Cyanobacteria

cyclic peptide structures, which facilitate the inhibition of eukaryote proteins, phosphatase 1 and 2A, involved in dephosphorylation of amino acid serine or threonine in liver cells. Inhibition of toxin change to proteins can lead to excessive phosphorylation of the filament structure (16) and cause cytoskeleton instability, which in turn results in cell death.

3.1.1. Microcystin

Microcystin is the most prevalent and routinely monitored cyanotoxin, which has been intensively studied in comparison with other toxins. This toxin has been the most commonly found toxin in cyanobacterial blooms, and unlike other toxins, it has been associated with human fatality in Brazil (2). Researchers have also related the high incidence of liver cancer in China to the consumption of microcystin-contaminated water (17). In fact, the earliest cyanobacteria, detected as microcystin producers, were Microcystis species. Later, researchers identified Planktothrix, Nostoc, Anabaena, Nodularia, Phormidium, and Chroococcus species, capable of producing microcystins (18).

Microcystin is structurally the most variable cyanotoxin, consisting of about 90 different isoforms. Therefore, application of analytical methods, such as HPLC, can be especially difficult (19). Nevertheless, considering major similarities among microcystins, cyclic peptide structures, and presence of several conserved proteins (16), they can be used as immunological targets for detection.

In the environment, microcystin is stable during chemical hydrolysis and extremely high temperatures (> 300°C) (5); therefore, it may accumulate in water bodies from several days to years (20). However, microcystin is easily degraded through strong oxidation molecules, such as ozone (5), and break down by aquatic bacteria, such as Sphingomonas and Pseudomonas aeruginosa (Gagala, 2012 #83). Consequently, direct detection of this toxin in the environment may be less reliable.

Researchers have introduced molecular typing, considering its higher accuracy in the detection of toxic cyanobacteria. Tillett and colleagues (2000) were the first to characterise microcystin gene biosynthesis, using gene cloning and sequencing. The toxin was encoded in 55-kb microcystin synthetase (*mcyS*) gene cluster. In this regard, a previous study showed that Planktothrix species lost their toxicity when up to 90% of *mcyS* gene cluster was removed (21). However, loss of intergenic region in the gene cluster may have no effects on the expression of toxin proteins (22).

The gene cluster consists of 2 operons and is encoded by 10 genes. The *mcyD* gene encodes polyketide synthase (PKS), while *mcyE* and *mcyG* genes encode hybrid nonribosomal peptide synthetase (NRPS) and PKS. Moreover, *mcyJ*, *mcyF*, and *mcyI* genes transcribe proteins for tailoring, *mcyH* gene encodes proteins involved in transporting toxins, and *mcyA-C* gene encodes 3 NPRSs (18). For detection of toxin-producing genes, most researchers have targeted *mcyE* gene from the *mcyS* gene cluster (7), as its presence can immediately confirm toxic cyanobacteria in the environment (23).

Other researchers have also shown the effectiveness of other genes, such as *mcyC* and *mcyD*, in detection of hepatotoxic cyanobacteria (24). However, depending on only toxin-encoding gene amplification may lead to unreliable results regarding the presence of toxins, as the gene cluster may not be expressed by the cell. For instance, up to

21% of *P. rubescens* strains with *mcyS* genes tested negative for microcystin production (7), while some strains only had fragments of toxin gene cluster or mutations had occurred within these genes, which made them unable to express toxin genes (25). Therefore, in addition to identifying gene-encoding toxins, detection of gene expression and toxins is equally important for accurate surveillance of toxic cyanobacteria.

3.1.2. Nodularin

Nodularin is produced specifically by planktonic Nodularia species, such as *N. spumigena* (26), and has a similar structure to microcystin (16) due to its cyclic structure (7 different structures). Two structural isoforms comprise variations at 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (ADDA) residues, which directly affect the toxic level of toxins (26).

In 1997, Twist and Codd (27) observed that pure nodularin remained stable in sunlight and under dark conditions after 9 days. However, in the presence of Nodularia cells, 70% and 55% of toxins degraded in sunlight and darkness, respectively. This finding indicates that toxin-degrading compounds were released from the bacteria and that direct extracellular toxin detection in the environment or toxin purification should be performed prior to complete degradation of toxin. Consequently, nodularin gene cluster, *nda*, is characterised by sequencing toxic *N. spumigena* strains to detect potential toxic Nodularia species (26).

The *nda* gene cluster is a 48-kb gene, consisting of 9 open reading frames (ORFs), *ndaA* to *ndaI*. The gene cluster contains 2 regulatory promoter regions, which transcribe genes in the opposite direction. By using a sequence relative to microcystin gene cluster, *mcy*, the *nda* gene cluster encodes putative functions: *ndaC* for PKS; *ndaE* for O-methyltransferase; *ndaF* for hybrid PKS/NPRS complex molecule; ndaI for putative ATP-binding cassette transporter; and *ndaA* and *ndaB* for putative NPRS molecule. The ndaCDEF encodes enzymes for ADDA molecule biosynthesis, while ndaFGHAB encodes enzymes important for peptide synthesis, cyclization, and transport.

Different target genes have been established for amplification and detection of nodularin-encoding genes, including PKS/NPRS hybrid molecule (28) and PKS site (29). Moreover, 16s rRNA of Nodularia species can distinguish nontoxic and toxic species (30). However, researchers found that nontoxic Nodularia species from Australia could encode both PKS and NPRS genes, which are vital to toxin synthesis (26), thus showing the lack of gene expression. This observation shows that evaluation of gene expression and translation of toxin proteins are important for accurate toxin detection.

3.2. Neurotoxins

Cyanotoxins, such as saxitoxin, anatoxin-a, and anatoxin-a(s), are neurotoxins, which mainly affect the human and animal nervous systems through different mechanisms.

3.2.1. Saxitoxin

Saxitoxin or paralytic shellfish toxin is a trialkyltetrahydropurine toxin, consisting of 30 different isoform structures. It affects the nervous system by blocking voltage-gated sodium channels of neuron cells. It also affects the heart cells by blocking calcium channels and lengthening the gating of potassium channels in the cells (16). The sxt gene cluster encodes proteins, which are important for biosynthesis of saxitoxin. Different from other cyanotoxins, saxitoxin intoxication occurs mainly through seafood consumption, such as seashells, as the toxin accumulates in the food chain (16). In the environment, saxitoxin is stable and able to accumulate in freshwater environments for 9 to 28 days, depending on its variant (31).

The *sxt* gene cluster encodes proteins important for biosynthesis of neurotoxic saxitoxin. This gene has distinctive genome sizes, depending on the cyanobacterial species. In *C. raciborskii*, *sxt* gene is 35-kb long, while *sxt* gene clusters in Anabaena and Aphanizomenon species are about 28-kb long (32), with most gene sets similar to Anabaena and Aphanizomenon species. The main genes commonly found in all *sxt* genes include *sxtA* gene encoding PKS, *sxtG* encoding transferase, and *sxtF* and *sxtM* encoding putative transporters. Moreover, *sxtY*, *sxtZ*, and sxtR genes, which are important for gene expression regulation, are unique to *C. raciborskii* (32).

Similar to other toxins, genes encoding PKS and PS proteins, which are important for toxin formation, are targeted for identification of saxitoxin-producing cyanobacteria (33). However, Ballot et al. (33) showed that nonsaxitoxin-producing cyanobacterial strains encode PKS protein in saxitoxin, indicating the loss of toxicity in cells, possibly due to the loss of all or part of *sxt* gene cluster.

3.2.2. Anatoxin

Another cyanobacterial neurotoxin, known as anatoxin-a, is a potent nicotinic acetylcholine receptor agonist. It is a receptor in nerve cells, involved in muscle contraction signals. Binding of anatoxin-a to the receptor leads to muscle fasciculation, gasping, seizure, and possibly death due to respiratory arrest in humans and animals. The anatoxin-a structure includes a secondary amine, encoded in the *ana* gene cluster (34). This toxin is produced by cyanobacteria, such as Aphanizomenon (35) and Oscillatoria species (36).

The *ana* gene cluster, responsible for the production of anatoxins, is highly similar among cyanobacterial species, whereas it shows different gene arrangements (34). Research by Mejean and Mann (36) showed that *ana* gene cluster, or *ks2*, in Oscillatoria species is a 29-kb DNA sequence. This gene consists of 4 or 5 operons, encoding 15 genes. The *anaB* to *anaG* genes form 1 cluster, while *anaA* and putative *cyclase* gene, *orf1*, are separated from the main cluster.

PKS gene in the *ana* gene cluster is amplified for identifying potential anatoxin-producing cyanobacteria. Although the *ana* gene cluster has a highly similar structural organization in different species, Ballot and Fastner (35) noted the unsuitability of primers in amplifying PKS protein genes from Ocsillatoria species for the detection of toxic Aphanizomenon species. Therefore, several primers should be selected for identification of gene-encoding anatoxins to prevent false negative results.

On the other hand, anatoxin-a(s) shows a similar toxicity mechanism to anatoxin-a. However, unlike anatoxin-a, the structure of anatoxin-a(s) consists of unique phosphate esters of cyclic N-hydroxy guanidine (37). Another neurotoxin, jamaicamide, produced by cyanobacteria, Lyngbya majuscule, is also found to have sodium channels blocking activity and fish toxicity. The jamaicamide structure consists of alkynyl bromide, vinyl chloride, a β-methoxy eneone system, and a pyrrolinone ring (38).

3.3. Cytotoxin

Cytotoxin has various effects on human and animal cells. This toxin potentially causes hepatotoxic and neurotoxic effects and even leads to tumour development. The main cytotoxin produced by cyanobacteria is CYN. This toxin is a polyketide-derived alkaloid, containing guanidine and sulfate groups (37). The toxicity of CYN depends on the inhibition of cytochrome P450, glutathione molecule, and protein synthesis (4).

Cytotoxin has been documented in all continents, and therefore, it is a threat to public health (4). *C. raciborskii* is the first cyanobacterium, identified as a CYN producer. Other cyanobacterial species, identified as CYN producers, include *Aphanizomenon ovalisporum*, *Anabaena bergii*, *Raphidiopsis curvata*, *Aphanizomenon flos-aquae*, *Anabaena lapponica*, *Lyngbya wollei*, and *Oscillatoria* sepcies (39).

The release of toxins into the extracellular environment occurs mainly during declining blooms. The extracellular toxin is extremely susceptible to heat and sunlight and can be degraded easily, with 90% of toxin broken down in 2 to 3 days when exposed to light (5). Therefore, detection of CYN toxin directly from the environment should be done immediately after toxin release or the results may not represent the risk of toxin to humans and animals.

Comparison between nontoxic and toxic *C. raciborskii* genome sequences shows several genome differences. The most important differences between toxic and nontoxic *C. raciborskii* genomes is the *cyr* gene cluster, which encodes important molecules in CYN production. The *cyr* gene cluster encodes 15 ORFs, which are as follows: *cyrA* or *aoaA* gene encoding amidinotransferase; *cyrB* gene encoding hybrid NRPS/PKS, and *cyrC*, *cyrE*, *cyrD*, and *cyrF* genes encoding PKS. Other genes include amidohydrolases (*cyrG* and *cyrH*), tailoring reaction (*cyrI*, *cyrJ*, and *cyrN*), putative transport (*cyrK*), regulation (*cyrO*), and 2 transposase (*cyrM* and *cyrL*) genes (21).

To detect potential CYN-producing *C. raciborskii*, researchers target different genes for molecular typing, such as PKS (40) and *cyrJ* genes (4). However, researchers have also found that CYN is regulated at the protein translation level due to the lack of correlation between gene transcript abundance and toxin concentration. Therefore, regulation of intracellular CYN level occurs at the protein level. However, factors affecting toxin biosynthesis require further research, as no standardized study has been conducted on environmental factors and toxin biosynthesis (37).

3.4. Dermatotoxins

Dermatotoxins, including aplysiatoxin, debromoaplysiatoxin, and lyngbyatoxin-A, are cyanotoxins mainly affecting the skin. Aplysiatoxin and debromoaplysiatoxin have phenolic bislactones, synthesized by Lyngbya majuscula. These toxins are strong skin irritants, causing skin rashes and blistering. While lyngbyatoxin-A is an indole alkaloid produced by marine benthic cyanobacteria (*L. majuscule* and freshwater *L. wollei*), it can cause dermatitis and inflammation of oral and gastrointestinal tissues (41). Among cyanotoxins, dermatotoxins are the least examined toxins, accounting for less than 2% of all studies on cyanotoxins in 2013 (2); therefore, information on these toxins is limited and further studies are required.

3.5. Identification of Cyanotoxins and Toxic Cyanobacterial Strains

For detection of toxins in the environment, their stability and degradation should be identified to allow researchers to determine the reliability of the results. Generally, toxins in the environment are readily degraded. Therefore, direct detection in the environment may show false negative results, as the toxin has been degraded. In addition, detection of toxins with HPLC is difficult, especially for toxins with different variants (19). Additionally, as cyanotoxins exist as intracellular toxins, analytical approaches are inapplicable, for they can only detect free toxins in the environment and fail to detect cell-bound toxins.

To cope with undetectable intracellular and unstable extracellular toxins, several companies, such as Abraxis, USA, have included a cyanobacterial cell lysis step in the cyanotoxin test kit for a higher accuracy of cytotoxin detection (42); therefore, detection of toxins bound in cyanobacterial cells is accommodated. However, these test kits are generally more expensive and less accessible, compared to molecular approaches.

Direct detection of toxin-encoding genes of cyanobacteria via PCR is generally easier and more cost-effective. Many target genes have been established for accurate detection. However, bacteria can regulate gene transcription and toxin production, based on their environment (see section 4) through several gene expression modifications. In addition, mutations may occur within these genes or fragments, or all toxin gene clusters may be lost; consequently, toxin gene clusters cannot express proteins (25). Confirmation of toxic cyanobacteria based on only amplification of toxin-encoding genes may lead to false positive results, as PCR does not confirm cyanotoxin gene expression.

Studies on gene expression should be conducted for further confirmation of toxic cyanobacteria in the environment. To investigate gene expression, researchers have developed methods, such as RT-PCR and DNA hybridization. These methods enable the detection of specific RNA molecules, which indicate gene expression.

4. Cyanobacteria Population Dynamics and Toxin Biosynthesis in the Environment

Even though cyanobacteria are highly adaptable to various environments, different ecologies may be the habitat for different species of cyanobacteria. Cyanobacteria are commonly found in freshwater environments, such as lakes, ponds, rivers, and reservoirs. The reservoirs comprise the main environmental concern, as humans are highly exposed through drinking water and recreational activities. Cyanobacteria from Oscillatoriales, Nostocales, and Chroococcales species are the main cyanobacteria found in freshwater environments. Meanwhile, *L. majuscule* is a potentially toxic marine cyanobacterium (41).

Cyanobacterial growth, species variations, and concentration in the environment are influenced by both abiotic and biotic environmental factors. Abiotic factors, such as wind and characteristics of water bodies (eg, depth, stream flow, and tides), affect cyanobacterial accumulation and concentration, while light intensity, nutrients, temperature, and biotic factors influence toxin biosynthesis and cyanobacterial populations, species, and strain variations.

4.1. Characteristics of Water Bodies and Wind Direction

Freshwater environments consist of 2 different habitats: benthic and planktonic habitats. The benthic habitat is the deepest region of freshwater environment, whereas the planktonic habitat is the upper region of the habitat. The benthic habitat is commonly inhabited by cyanobacteria lacking gas vacuoles, nontoxic Nodularia species (eg, *N. sphaerocarpa* and *N. harveyana*) (43), and a toxic benthic species, *Phormidium favosum* (44).

Meanwhile, the planktonic habitat is inhabited by cyanobacteria, consisting of gas vesicle organelles (enabling them to float). The planktonic cyanobacteria include Planktothrix (45), toxic Nodularia (43), Anabaena, Microcystis, Aphanizomenon, and Oscillatoria species (46). Therefore, samplings at different levels is important for accurate surveillance of toxic cyanobacteria.

In addition, for selecting the sampling point, it is important to take wind, water stream flow, and tides into consideration, as the concentrations of cyanobacteria change within hours, depending on these factors (8). The wide range of cyanobacterial concentrations in sampling may provide inaccurate data on the potential toxic hazards of cyanobacteria for occasional swimmers and the amount of toxins, potentially entering drinking water (5).

4.2. Light Intensity and Temperature

Many planktonic cyanobacteria regulate water buoyancy and position themselves for optimal light conditions by regulating the expression of gas vesicle genes (47). Alterations in buoyancy lead to cyanobacteria sinking during midday and floating at night (45). In addition to buoyancy regulation for optimal light conditions, specific cyanobacteria use phototaxis motility via gliding or twitching, as observed in Anabaena and Ocsillatoria species (gliding), as well as unicellular cyanobacteria, such as Synechocystis species (twitching) (48).

Light intensity is also involved in toxin release and bioproduction. By using PCR and RT-PCR, Gobler et al. (49) indicated that mcy gene cluster expression increases in Microcystis species during summer (abundant light intensity), but reduces during fall, similar to CYN release into the environment by *A. flos-aquae* (50). Light intensity also influences the amount of toxins released by benthic cyanobacteria, as shown in benthic Oscillatoria species (39).

In addition to light intensity, stress induced at specific temperatures also influences toxin release from the cells. For instance, Preußel and Wessel (50) indicated that a temperature of 25°C could significantly increase CYN production versus 20°C, which is in contrast to the reduced anatoxin-a level produced by Anabaena and Aph-

anizomenon species at high temperatures (37). Moreover, Conradie and Barnard (51) observed different frequencies of species in different seasons, where nontoxic Planktothrix species were dominant during autumn, while both nontoxic and toxic Microcystis species were prevalent during hot summer. Therefore, surveillance of cyanotoxins and toxic cyanobacteria should be conducted constantly when light intensity and temperature are favorable for cyanobacterial toxin production to maximize identification.

4.3. Nutrients

Cyanobacteria receive energy through photosynthetic processes, where essential nutrients, such as phosphorus, nitrogen, and iron, are required for cell growth (5). However, these nutrients may not influence bloom formation, as more cyanobacteria can utilise phosphate in phosphate-limited environments using alkaline phosphatise. In addition, many scientists have observed non-nitrogen-fixing cyanobacteria in nitrogen-limited and even nitrogen- and phosphorus-colimited fixation (52).

Cyanobacterial species and strain domination may be affected by different levels of nutrient use in different species. In this regard, Akcaalan et al. (53) observed *P. agardhii* domination in nutrient-rich water bodies, whereas *P. rubescens* was generally found in low-nutrient lakes. Meanwhile, growth of toxic Microcystis species showed a positive correlation with phosphorus concentration and a negative correlation with nitrate concentration in the environment (54). In addition, the nutrient level affected toxin gene expression, as shown in *mcy* gene expression, which improves as nitrogen and phosphorus levels increase (49).

In contrast, Gobler et al. (49) showed that phosphorus depletion could increase the concentration of anatoxin-a, and presence of nitrogen increased the level of toxins. The authors associated this finding to the high nitrogen level, leading to phosphorus depletion and increased toxin biosynthesis. However, the results contradicted previous research, which shows that nitrogen depletion increases the level of anatoxin-a biosynthesis in Anabaena and Aphanizomenon species (37). Therefore, transcriptional regulation of *anatoxin-a* gene clusters is yet to be investigated by researchers (37).

Meanwhile, previous studies have indicated that nitrate depletion increases saxitoxin production in the initial growth of heterocyst-forming *A. flos-aquae*. However, as the cells grow and are capable of fixing nitrogen from the environment, there are no significant differences in the production of saxitoxin between nitrate-depleted and nitrate-supplied media. Stucken and John (55) suggest that nitrogen does not directly affect toxin production in heterocyst-forming cyanobacteria, but instead growth of cyanobacteria is correlated with toxin biosynthesis.

On the other hand, Alexova and Fujii (56), using RT-PCR, reported the increased expression of toxin genes in *M. aeruginosa* in iron-depleted culture media. They also found that microcystin-producing cyanobacteria can uptake iron more than nontoxin encoding cyanobacteria. Subsequently, the importance of microcystin in iron metabolism is highlighted (56). However, Fujii and Rose (57) showed that differences in iron intake between nontoxic and toxic cyanobacteria were strain-specific, and not related to microcystin production, as shown in the analysis of iron intake in genetically modified nonmicrocystin-producing *M. aeruginosa* and microcystin-producing *M. aeruginosa* from the same strain.

4.4. Biotic Factors

Biotic factors also play an important role in cyanobacteria population and toxin production. Jang et al. (58) showed that increased number of zooplanktons, as the main predators of cyanobacteria, increased the production of microcystins. They also found that microcystin-producing cyanobacteria show better survival in combating zooplanktons. Research in this area has led to the theory that microcystin molecule expression is important to protect the cells against harsh conditions (58).

Meanwhile, many bacteria and viruses have shown anticyanobacterial characteristics and seem to influence the bloom dynamics of cyanobacteria. In addition, both aquatic and terrestrial plants are known to produce allelochemicals, as secondary metabolites, which either positively or negatively affect the surrounding organisms, such as microbes (59). Several studies have indicated that these chemicals may be natural inhibitors of cyanobacterial growth (60) (Table 1).

Subsequently, one single water body may exhibit different toxic or nontoxic strains of cyanobacteria every year (81), and different sampling points and depths may indicate different species and concentrations of cyanobacteria. In addition, cyanobacteria may exhibit distinct toxicities under different laboratory conditions (81), such as different culture media and light intensity.

Even though many researchers have suggested that environmental factors lead to variations in cyanobacterial genotype domination and diversity, further investigation is required to determine how these factors affect the dynamics of cyanobacteria. Through these external factors, researchers can predict possible species in an environment and identify the time of cyanobacterial blooms. Such predictions are crucial to accurate environmental sampling and assessment of possible limitations in the sampling methods.

Table 1. Summary of Organisms Controlling Cyanobacteria Population and Species Variations

Biotic Factors		Potential Active Compounds/Allelopathy Substances/Mechanisms	Tested Cyanobacteria Species	References
Predators	Zooplankton	Grazing	*Microcystis aeruginosa*	(58)
Viruses	Cyanophage	Infection	*Microcystis aeruginosa*	(61)
Bacteria	*Streptomyces neyagawaensis*	Unknown/antialga	*Microcystis aeruginosa*, *Anabaena cylindrica*, *Anabaena Xos-aquae*, and *Oscillatoria sancta*	(62)
	Bacillus cereus	Unknown/allelopathy	*Microcystis* sp.	(63)
		Cell-to-cell contact/lysis	*Aphanizomenon flos-aquae*	(64)
	Pseudomonas putida	Unknown/antishock	*Microcystis aeruginosa*	(65)
	Stenotrophomonas F6	Hydroquinone, cyclo-Gly-Pro/allelopathy	*Microcystis aeruginosa* and *Synechococcus* sp.	(66)
Aquatic	*Pistia stratiotes*	Unknown/oxidative damage	*Microcystis aeruginosa*	(59)
	Vallisneria spiralis	2-Ethyl-3-methylmaleimide, ionone/allelopathy	*Microcystis aeruginosa*	(67)
	Phragmites communis	Ethyl 2-methyl acetoacetate/ oxidation	*Microcystis aeruginosa*	(68)
	Cyperus alternifolius	Phenolic/ allelopathy	*Microcystis aeruginosa*	(69)
	Myriophyllum verticillatum	Unknown/ allelopathy	*Anabaena variabilis*	(70)
	Macrophyte species (n, 8)	Unknown	*Microcystis aeruginosa*	(71)
	Lindernia rotundifolia, *Hygrophila stricta*, and *Cryptocoryne crispatula*	Removal of nitrogen and phosphorus	Cyanobacteria	(72)
Terrestrial	*Artemisia annua*, *Conyza Canadensis*, and *Erigeron annuus*	Fatty acids and terpenoids (isoprenoids)/ allelopathy	*Microcystis aeruginosa*	(60)
	Leaf litter	Polyphenols/oxidative damage	*Microcystis aeruginosa*	(73)
	Oak trees	Tannins/allelopathy	*Microcystis aeruginosa* and *Anabaena* sp.	(74)
	Lantana camara	lantadene A and lantadene B (triterpenoids)/ allelopathy	*Microcystis aeruginosa*	(75)
	Chelidonium majus	Alkaloids/unknown	*Microcystis aeruginosa* and *Synechococcus* sp.	(76)
	Moringa oleifera	Photosynthesis inhibition	*Microcystis aeruginosa*	(77)
	Swinglea glutinosa	Citbrasine (alkaloid)/allelopathy	*Oscillatoria perornata*	(78)
	Ginkgo biloba	ginkgolic acids/allelopathy	*Microcystis aeruginosa*	(79)
	Solidago canadensis	Antioxidant molecules/oxidative damage	*Microcystis aeruginosa*	(80)

5. Conclusions

Although cyanobacteria are widely examined as future renewable resources of energy and natural products, certain cyanobacterial strains can synthesize toxins, which are potentially fatal to humans and animals. Therefore, identification of toxic cyanobacteria from the environment is important. Precise detection of toxic cyanobacteria requires an understanding of bacteria characteristics, including taxonomy and structure of toxins and toxin-encoding genes, as well as environmental regulations. The available information on these characteristics allows researchers to predict suitable periods for sampling, detect toxins with analytical methods, and identify genes encoding toxins via molecular techniques. This information also enables researchers to discuss limitations in the identifying process. However, some characteristics of cyanobacteria, such as dynamics of cyanobacterial growth, and environmental effects on cyanobacterial toxin production and expression are yet to be fully understood for accurate results on cyanotoxin detection.

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Footnote

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