e-ISSN No.: 2582-4228

Journal of Indian Association for Environmental Management

Journal homepage: www.http://op.niscair.res.in/index/php/JIAEM/index

Overview of Toxic Cyanobacteria and Dinoflagellates and mcy Gene Clusters Responsible for Cyanotoxin Production

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Submitted: Dec. 08, 2021 Revised: April 21, 2021 Accepted: April 27, 2021

Abstract: Overview of toxic cyanobacteria is related to cyanotoxinin this research article. Cyanobacterial bloom occur in oligotrophic to mesotrophic conditions in waterbodies and related with environmental factors and nutrient concentrations. Analysis of microcystins, and health hazard due to cyanotoxin of *Microcystis aeurignosa* and *Anabaena* sp., *Planktothrix agardhii* were highlighted here. It is observed that cyanobacteria, dinoflagellate toxins are of hepatotoxic and neurotoxic nature and protect cells against planktivores.Toxin from *Aphanizomenonflos-aquae*, marinedinoflagellate *Gonyaulax* sp., *Alexandrium* sp., *Gymnodinium* sp., and *Pyrodinium* sp., (saxitoxins-STX) were causing paralytic shell fish poisoning (in human being).Microcystins oligopeptide structures have several congeners in cyanobacteria as biotoxin were observed inseveral species of cyanobacteria except cylindrospermopsin, (a guanidine alkaloid-cytotoxin). During recent years, molecular biologist's identified *mcyA* to *mcyE* gene clusters that synthesize microcystins in toxic cyanobacteria. Studies of degenerate primers directed to conserved functional motifs (exons –open reading frames)of *mcyABC* to *mcyDE* gene clusters. Mutational studies were conducted to locate microcystin synthetase coding gene sequences of in *mcyB* and *mcyD* genes. Presence *mcyE* gene sequences in multiple numbers toproportionate toxic factor of microcystins production in toxic*Microcystis* and *Anabaena* strains in Lakes were reported. Microcystins inhibits eukaryotic serine/threonine protein phosphatase 1 and 2A functions and result in hepatotxic tumor promoter. Earlier researchers have studied various aspects of toxic cyanobacteria i.e. genetic markersbased quantitative real time polymerase chain reaction (QRT-PCR), production of nonribosomally synthesis of cyclic heptapeptide of microcystins (ribosomes involve in translation process of protein synthesis-elsewhere) Picomolar concentration microcystin congeners can be detected using Mass M/Z (i.e. mass to charge ratio) in Matrix Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry analysis (MALDI-TOF-MS). *Microsystis* toxin can be destroyed in chlorine under pH dependent condition such that chlorine residual at least 0.5mg/l should be present after contact time of 30 minute.Ozone is also preferred disinfectant for water supplies where microcystin and anatoxin may affect water quality. Microcystins causes chronic toxicity. Symptoms of hepatotoxintoxicity are weakness, loss of appetite (anorexia), pallor of intestine mucous membranes, vomiting, cold extremities and diarrhoea. Anatoxinan irreversible inhibitor of cholinesteraseis also known for acute toxicity i.e. staggering, muscle fasciculation, gasping and convulsion.

Keywords: Cyanobacteria, Dinoflagellate, Microcystins, *mcyABC* and *mcyDE* gene clusters, QRT-PCR technique, MALDI-TOF-MS analysis

I. INTRODUCTION

Cyanobacteria cell characteristics is prokaryotic, photosynthetic pigment located in thylakoid adjacent to membrane and outer sheath, metachromatin granules (phosphate storage), binary fission mode of reproduction, akinetes, necretic cells (special cells that separates akinetes in filament) provide natural predominance to cyanobacteria over other eukaryotic planktonic algal groups in fresh water through-out the year.

The cyanobacterialbloom are consequently appeared after rainy season and species grow throughout the post monsoon and die off in summer. Where-ever cyanobacterial bloom

persists and the reason behind this is slow growth rate of cyanobacteria and high affinity to nitrogen and phosporus [1]. At elevated temperature cyanobacterial cell survival depends upon release of secondary metabolites to surrounding water. Increase of extracellular toxin was observed at ≤50µg/L chlorophyll concentration in cyanobacteria, in other terms, cyanobacteria survival under high photosynthesis and/orrelease of secondary metabolites thus prevent cell lysis in unfavorable growth conditions [2].

Most important HABs were observed due to presence of cyanobacterial species *Anabaena*, *Microcystis* and *Aphanizomenon* in U.S. Fresh waters [3]. Baseline frequencies of human illnesses associated with Harmful algal blooms (HABs) including the shellfish poisoning, *Karenia* (neurotoxic shellfish poisoning, and aerosolized Florida red tide respiratory syndrome), *Dinophysis* and *Prorocentrum* (diarrhetic shellfish poisoning), *Gambierdiscus* sp. (Ciguatera fish poisoning) and brevetoxins associated with Florida red tides in marine waters.

Toxic algae surveillance and cyanobacteria in harmful algal bloom (HAB)

The reason is few epidemiologic studies related to climate variability and harmful algal bloom were designed to systematically assess these toxin effect [4]. Further research is need to be conducted on climate variability HABs, ocean acidification and human health [5]. It was obstructed by the Intergovernment panel on Climate Change (IPCC) projection of climate change impacts on HAB-related illnesses. Later on, the two working groups were formed on HAB-related disease surveillance, created as harmful bloom-related disease surveillance system (HABISS-HAB related illnesses) and the Centers for Disease Control and Prevention (CDC) in U.S. [5,6,7]. Saxitoxins is a toxin from *Aphanizomenon flos-aquae* and marine organism Dinoflagellate *Gonyaulax* sp., *Alexandrium* sp., *Gymnodinium* sp., and *Pyrodinium* sp., were causing paralytic shell fish poisoning (saxitoxin binds to voltage-gated sodium channels within cell membranes) (PSPs) exclusively in Australian waters; saxitoxins (STX) and its documented 58 analogues are environmental neurotoxic alkaloids responsible for the human illness, paralytic shellfish poisoning (PSP) [8,9]; Three theories have been postulated to explain the origin of saxitoxin in dinoflagellates [10]; STX evolved in dinoflagellates after a single horizontal gene transfer (HGT) event with cyanobacteria [11,12].

Toxins as class of secondary metabolites

Toxic *Microcystis* spp., *Planktothrix* sp. and *Anabaena* sp. belong to cyanobacteria are known to produce hepatotoxin - a cyclic heptapeptideof microcystins, [13,14,15]. Generally, intracellular cyanotoxins concentrations were affected by environmental factors such as light, temperature and nutrient concentrations [15].Carmichael [16] reviewed the cyanobacterial toxins and classified them as microbial secondary metabolites (biotoxins and cytotoxin) and biotoxins further divided in to hepatoxins and neurotoxins Cyanotoxin are produced by strains within the genera *Microcystis*, *Oscillatoria*, *Nodularia*, *Cylindrospermopsis*, *Anabaena* (hepatotoxin producing species-strain) than other strains of *Oscillatoria*, *Anabaena*, *Aphanizomenon*, and *trichodesmium* (neurotoxinsproducing strains) (Plate-A). No acute poisoning to human, such as occur in case paralytic shellfish poisoning.The invisible toxins in water furthermore notknown to concentrate in any other vectors i.e. shellfish in human food chain[16]. This lead to assumption water quality directly related to cyanobacterial bloom and eutrophication, where-ever large growths or blooms become more common in our freshwater supplies. In cyanobacteria, however neurotoxin was limited in distribution, especially in paddy fields, [16]. Toxin structures of cyanobacteria have classified (I) hepatotoxins as cyclic heptapeptide of *M. aeruginosa*-microcystins, *N. spumigena* -a pentapeptide of Nodularin, *C. raciborskii*- cylindrospermopsin, guanidine alkaloid linked to hydroxymethyl uracil, (II)Neurotoxins as *A. flos-aquae* - anatoxin-a and anatoxin a(s) and *A. flos-aquae*-saxitoxin,[17,18,19,20,21]. Internal changes from hepatotoxins resulted into intrahepatic haemorrhages and hypovolaemic shock. Hepatocyte necrosis with destruction of sinusoidal endothelium followed by intrahepatic haemorrhages is based on increase in liver weight as a fraction of body weight (up to 100% in small animals tested in the laboratory) as well as on hepatic haemoglobin and iron concentrations that account for blood loss sufficient to induced irreversible shock (tumor formation). In animals that live longer i.e. a few days, hepatic insufficiency may develop to a degree that becomes incompatible with life. Toxicity of microcystins is mediated through active transport of microcystins in to hepatocytes by bile acid anion transport system subsequently inhibition of eukaryotic serine/threonine protein phosphatases 1 and 2A in the liver cells. Protein phosphatases control cell structure and function [23]. Death occurs within a few hours to a few days after initial exposure and may be preceded by coma, muscle tremors and force expiration of air.Neurotoxin symptoms of toxicosis in field cases of wild and domastic animals include staggering, muscle fasciculation gasping, and convulsion opistothonos (birds). Anatoxin-a(s) is an irreversible inhibitor of cholinesterase and toxicity compared to arganophosphate (OP), diisopropil- flurophosphate (DFP) and showed that antx-a(s) is about 22 times more potent than DFP. Death is most probably due to respiratory arrest and occurs within minute or a few hours vary with doses in the species and prior food consumption. Later on cyanotoxins classified in to five modified categories (I) hepatotoxins (II) cytotoxins (III) neurotoxins (IV) dermatotoxins (V) Irritant toxins [22,23,24].

Light intensity was observed as a possible stress inducing factor but not primary factor in release of cyanotoxin [25,26]. Light intensity of red and blue region of spectrum enhance the growing biomass of cyanobacteria. Cyanobacterial cells with photosynthetic active were released lower concentration of toxin between 3 and 19ºC as compared to reduced photosynthesis and release of higher concentration of toxin at temperature between 20 and 25ºC [27,28,29]. Temperature has described hot and toxic based on the generalized linear mixed

modeling in case Planktothrix agaradhii as 36% increase in microcystins release at temperature ≥20 ºC, [30]. This may be considered as the cyanobacterial bloom prediction and persistence. Inverse relationship exists between free microcystins concentration increase with decreasing biomass of P. agardhii [31,32].

Chronic and Acute Toxicity

These species of cyanobacteria form bloom or floting scums in surface waters. Environmental factors play important role in cyanobacteria persistance in ponds, lakes, and rivers.Microcystins are reported directly lethal to zooplankton, reduce the feeding activity, or influence the community structure of zooplankton, however dose dependent effect is highly variable and inconsistent between zooplankton species [33]. Daphnia has preferentially fed on green algae and the diatom *Melosira granulata* and seldom feeding on toxic cyanobacteria under depletion of edible food [34].

Cyanobacterial toxins was caused many animal deaths and had also been implicated in cases of human illness in U.S.A., Australia, China, Brazil, and Great Britain [35]. In this context, microsystins toxicity depends upon lipophilicities and polarities of reactive groups to organisms [36]. One cyanobacterial species strains may be toxic or nontoxic in same habitat e.g., toxic and nontoxic *Microcystis aeruginosa* genotype described based on molecular methods [37,38,39,40].

These toxins, were more frequently occurred in different strains of *Microcystis* sp.,*Oscillatoria* sp., *Planktothrix* sp., *Nostoc* sp., *Anabaena* sp., *Nodularia* sp. and *Umezakia* sp. One of the cytotoxic alkaloids from *Cylindrospermopsis raciborskii* (tropical species) inhibit protein synthesis although also cause liver and extensive kidney injury which occurs in rivers, lakes and water supply reservoirs. Cylindrospermopsin is a tricyclic guanidine linked to hydroxymethyl uracil with a molecular weight of 415 daltons and is stable to boiling temperature of water [41,42].

Toxin producing cyanobacteria flourish at higher pH has indicated the low (D_{ow}) value (Octanol-Water partition). Studies have shown that cyanotoxin consumed via gastro-intestinal route than those of large size of microcystin - LR as accumulated through passive diffusion across the membrane.At higher basic range pH 8-12 as compared at pH 1, thus have preference of entry via feeding and rare chance of accumulation of toxin in zooplankton. [43]. Oxidative stress can signal for greater release of cyanotoxin in active cells [44, 45]. Cyanobacterial bloom occur in oligotrophic to mesotrophic conditions in waterbodies. Model study carried out showing free microcystin concentration was influenced by intracellular phosphorus content of cyanobacterial biomass [32,46,47]. At high light levels of phosphorus, hepatotoxic strains and their toxin concentration did not decrease as did in case of the levels of anatoxin-a. Lack of phosphorus had no effect on anatoxin-a levels but affect microsystins, as result decrease amount of hepatotoxins produced [48]. In cyanobacteria species, production and release of microcystins were varied one strain to another strain and to the toxicity test organisms under the same laboratory conditions. Cultural and toxicity to dephnids studies were shown uptake of *Microcystis aeruginosa*, *Anabaena variabilis* and *Anabaena circinalis* via oral uptake caused acute to chronic toxicity to Daphnids [49].

Cyanotoxin Stuctures

Common variants of microcystin (MC-LR, MC-YR, MC-RR and MC-LA) were observed molecular weight vary about 1000 daltons, [16,36]. Variation in aminoacids in hepatotoxins forms 50-70 congeners structure of microcystins viz. Microsystin - LR, YR, RR, (structures of MCs; variations of A- Alanine, L-Lucine, R- Arginine, Y-Tyrosine in position 2 and 4) [16,50]. The hepatotoxin of *Microcystis* sp.,*Oscillatoria* sp., *Planktothrix* sp., *Nostoc* sp., *Anabaena* sp., cyanobacterial species contain microcystins and reactive aminoacids stucture of microcystin having unique hydrophobic aminoacid(3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) ADDA chain, D-Glu (D-Glutamate-iso),Mdha (Methylodehydroalanine), D-Ala (D-alannine), L-Leu (L-leucine), MeAsp (D-erythro-B-methyloaspartic acid, L-Arg (L-arginine) cyclic structure and variables position presenceoccur in *Microcystis* sp.for hepatotoxicity [50,51,52]. Another cyanobacteria *Nodularia* sp. is hepatotoxic and nodularin contains five amino-acids instead of seven five aminoacids in microcystin variables i.e. ADDA chain with D-glutamate (D-Glu), L-arginine (Arg) and D-B-methyl aspartic acid (MeAsp) and D-N-Methyl-dehydrobutyrin (Mdhb).

Cyanobacteria genera *Anabaena circinalis* and *Anabaena flos-aquae* strain NRC 525-17, also contained neurotoxin (anatoxin-a, homoanatoxin-a; anatoxin-a(s), secondary amine, 2-acetyl- 9-azabicyclo(4-2-1)non-2-ene (MW = 165), [53] anatoxin-a(S) is a unique phosphate ester of a cyclic N-hydroxyguanine (MW = 252) [54], Acute toxic and highly neurotoxins have indicated short biological half-lives and organism dies in few minutes to hours however are not widely spread as microcystins in water bodies.

Molecular biological aspects of toxin production

Studies on origin and evolution of group I introns preceding sequences in tRNA leu/UAA is proof of triplate origin and evolution of group is highlighted in early 1990. ManytRNA

introns prior placed to aminoacid codon suggesting that each family has its own evolutionary phylogenetic tree history. It hashowever, not confined to introns t RNA Leu/UAA universally present in this lineage and traced in four representative i.e. Plastids, *Nicotina tabacum*, *Marchantia polymorpha* (liverwort), and *Chlorella vulgaris* [55,56,57].The other initiation of mRNA recognizing t RNA^{fmet} intron (i.e. Present in number of filamentous and few unicellular cyanobacteria has sporadic distribution in cyanobacteria implying that lateral transmission of genes and many gains and losses were occurred durign cyanobacterial evolution),however t RNA fmet in cyanobacteria is shown common presence in bacterial progenitor and plastids [58]. Introns are known as very ancient bacterial origin and mobile genetic elements are observed to carryout horizontal tranfer elements[59,60]. Transposition of introns are frequent events and split genes arose by late insertion of introns into orignially uninterepted genes [61]. It has suggested in members of group I and II introns of t RNA lariat of cyanobacteria and were capable and shown autocatalytic activity in vitro [62,63,64]. Initial work on molecular biology is extensively used of 16S rRNA gene for cyanobactrial identification,[65,66,67].

Small DNA noncoding fragment i.e. not transcribe to functional amino acid adjusant to entron is called primer sequence is used for amplification of monocistronic gene (In PCR studies, primers are nothing but similar noncoding sequences of genes in DNA frame designed for molecular studies). Amplification of peptide synthetase genes have achieved by ligation of degenerate primers directed to conserved functional motifs of these peptide synthetase complexs. RPAs are observed to employ the level of mcyB and mcyD transcription under each condition. Both *mcyB* and *mcyD* transcript lavels are found to increase under highlight intensities and red light. Correlation between 16S rRNA and high light intensity have shown that *mcyB* and *mcyD* transcript expressions have occurred in early and mid growth phases not the late growth phase of *M. aeruginosa*.

This Microcystin gene clusters is operan *mcy* gene composed of nine to ten genes depending on taxa.In *Microcystis aeruginosa, mcyB* and *mcyD* genes are known to produce enzymes peptide synthetase and polyketide synthetase [68]. Mutations within *mcyB* and *mcyD* was resulted in loss of toxicity [69]. Relative position of primers was used to generate RNase protection assay (RPA probe) within *mcyB* (peptide synthatase and *mcyD* polyketide synthetase).

Diversity of microcystin-producing cyanobacteria *M. aeruginosa* and *P. agardhii* were studied using sequence analysis of *mcyA* gene fragments in the western basin of Lake Erie [70]. Instead of translation mechanism the study revealed that Microcystin is produced by microcystin synthetase enzyme of five gene structure although four genes responsible for production of microcystin variants, on *mcy* template via a thio-template mechanism. Nonribosomal peptide synthetase gene consists of modules that are built up of domains. Each module code contain adenylation, thiolation and condensation domainsfor synthesis of amino acids in order of gene sequences arranged in modules that incorporated in to growing peptide chain (in translation of gene transcript i.e. use of ribosome). Adenylation domain function is in recognition of the specific amino acid and after the amino acid is activated to its acyl adenylate, the amino acyl adenylate is transferred to 4-phosphopantetheine carrier within the tailoring enzyme of thiolation domain. Peptide bond formation between two activated amino acids is mediated in the condensation domain and sequence of several amino acids was formed. 10 conserved motif (i.e.10 amino acid residues lining the substrate binding pocket) within the adenylation domain designated A1 to A10 and were located within the region believed to be responsible for substrate specificity. Multiple copies ofmicrocystinsynthetase gene E (*mcyE*) directly related with concentrations of microcystins in *Microcystis* and *Anabaena* genera [71].

All known microcystin producing variants of D-glutamate and carboxyl group of the glutamate side chain have been differentiated as toxic strains. However, microcystin variants with an esterified carboxyl group did not cause toxic effect in mice. Therefore, the *mcyE*gene that encodes the glutamate activating adenylation domain can be used as a surrogate for microcystin-producing cyanobacteria [72,73].

Microcystinsynthetase gene clusters *mcyABC* and *mcyDE* code for different unit of general microcystin structure with different X - Z amino acid variants (-D-Ala-L-X-erythro-*β*-methyl-D-iso Asp-L-Y-ADDA-D-isoGlu-*N*-methyldehydro-Ala). Non ribosomal peptide synthesized using thiotemplate function of large peptide synthases and product defined as secondary metabolites.

The identification of toxic and nontoxic strains of *Microcystis* was studied [74], based on N-methyl transferase (NMT) domain of microcystin synthetase gene *mcy*A of *Microcystis* toxigenicity and phylogeny. A genetic probes developed and directed to the mcy B gene and to adenylation domains with microcynthetase gene cluster, 55kb of DNA encoding six (coding sequence) *mcy*A-E and G, with another four open reading frame mcy-f and H-J placed in the chromosome, using PCR. Insertion and inactivation of mcy B of *Microcystis aeruginosa* strain (pcc 7806) resulted in loss of microcystin synthesis [75,76]. The method was followed quantitative - real time PCR to amplify gene *mcy* A.

Microcystins includes 16S rDNA amplification of peptide synthetase genes sequences includes use of forward primer (mcyE-F2) and genus-specific reverse primers for Microcystis (MicmcyE-R8) and Anabaena (AnamcyE-12R) were designed with mcy gene sequences of Anabaena sp. strain 90 [77]. Three standard Microcystis sp. strains GC260735, PCC7804 and PCC7941 and three Anabaena sp. strains 90, 315, and 202 A1 by using DNA amplification *mcy*E gene by standard primers (Sigma-Geno Sys Ltd.). The melting curve analysis of the *mcy*E QRT-PCR product (247bp) of the three *Microcystis* standard strains and three Anabaena standard strain were detected average melting temperature 81.5°C and 79.6°C was due to >40 nucleotide difference between *Microcystis* and *Anabaena* mcyE sequences of these modular enzyme complex. (QRT-PCR) technique. BLAST [78] and Bio Edit [79] following Quantitative Real Time Polymerase Chain Reaction using DNA binding SYBR green dye (Roche Diagnostics) produced highest mcyE gene copy *Microcystis* sp. strain PCC 7941 and *Anabaena* strain 202A1) and lowest mcy E gene copy with *Microcystis* sp. strain PCC7806 and *Anabaena* strain 315(Light cycler soft ware (version 3.5). Possible PCR-inhibitory contaminants, 16SrRNA gene primer was used to test DNAs which did not amplify products with mcyE primers in PCRs [66]. The presence or absence of the *mcy*E product was determined using 20 µl of amplification product and 1.5% agarose gel electrophoresis. The bands were stained with Ethidium bromide compared with known standard MW ledders and documented with Kodak CD 290 camera.

RNase protection assays (RPAs)

In this context further use of *rpo*CI (a discriminative marker, [80], in identification Toxic *Anabaena circinalis* was studied in Australian isolates. The nucleotide sequence analysis of (DNA-dependent RNA polymerase) *rpo*CI was characterized in *Anabaena circinalis* and gene sequences differentiated 24 *Anabaena* isolates, including 12strain of *A. circinalis* in environmental samples [81]. This observation was used in detecting *A. circinalis* dierctly from water sample without Laboratory culturing. The microcystin concentrations were correlated positively with the total of *mcyE* copy numbers in *Microcystis* and *Anabaena* from both Lake Tuusulanjarvi and Lake Hiidenvesi in Finland by quantitative real-time PCR [82].

Cyanotoxinswere confirmed targeting 16s rRNA and phycobiliprotein subunit (cpc), the genetic potential of *mcy*A and *mcy*E gene to produce microcystins in *Microcystis* sp. This molecular technique based on cylindrospermopsin synthatase gene *rpo*C1, Peptide synthase (PS) genes was also studied in *Cylindrospermopsis* sp. inwater reservoir at Anuradhapura, Srilanka [83]. A large multifunctional modular enzyme complex nonribosomal peptide synthetases (NRPS), Polyketides synthetases (PKS) and tailoring enzymes of thiotemplete activity is characteristics of cynotoxin producing cyanobacterial species *Anabaena*, *Microcystis* and *Planktothrix*. [84].

The above study revealed that sequence analysis of NMT domain has revealed two coherent groups based on the genomic region is immediately downstream of the *mcy* ABC cluster. In all 20-NMT positive strains contained an open reading frame of unknown function (*uma*-I) at conserved distance from *mcy* C. The PCR primers designed for N-methyl transferase (NMT) domain the microcystinsynthetase gene *mcy*A has probed in 35 *Microcystis* cultures. All nontoxic strains also contained *uma*-I, which is not co-transcribed with *mcyABC* as in toxic strains and thus planktonic cyanobacteria can be differentiated in to toxic and nontoxic strains within the genera in phylogenetic classification. In protein phosphatase (catalytic subunit of protein serine/threonine phosphatase-1) inhibition assay, 18 toxic strains have shown positive reaction to other17 nontoxic negative strains. Increased extracellular toxin is due to high light intensities and subsequent leakage of the peptides. However, the recent identification of a putative ABC transporter gene (*mcyH*) located upstream of *mcyE* may suggest the existence of a cytoplasmic or trans-membrane microcystin transport system [74]. Insertional mutagenesis in peptide synthetase gene resulted in loss of hepatotoxin production in Microcystis aeruginosa pcc 7806 [69].

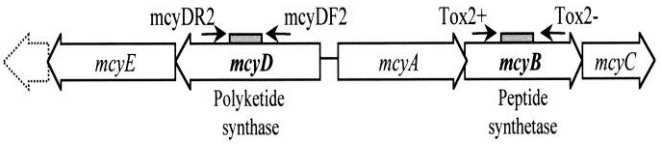

Figure-1: Microcystin synthetase gene clusters *mcyABC* and *mcyDE*. Studies revealed that mutations within *mcyABC* or *mcyDE* result in loss of toxicity. Relative positions of primers used to generate RPA probes within *mcyB* (peptide synthetase) and *mcyD* (polyketide synthase) are shown (25,76).

Limelight of Microcystin Detection, Assay nd Treatment

Detection of toxic *Microcystis aeruginosa* strains through molecular marker may be routinely used in monitoring application of aquatic ecosystems [85]. Planktonic cyanobacteria based on classification of prokaryotic linage of 16S rRNA gene of the evolutionary trees belong to one branch can contain both toxic and nontoxic strains within one cyanobacterial genus [74,86,87,88,89,90] and molecular studies were conducted based on isolation of toxic and nontoxic by Matrix Assisted Laser Desorption/Ionization-

Time of Flight Mass Spectrometry analysis (MALDI-TOF-MS) in the same mass occurrences [91,92].

The toxic and nontoxic strains The characterization methods of microcystins in toxic cyanobacteria areHigh Pressure Liquid Chromatography (HPLC), [92,93] and Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS). MALDI-TOF-MS extraction method of microcystin is not required microcystin standards and compared with earlier established mass values m/z (i.e. mass to charge ratio). The Cyanotoxin producing strains were detected positive ion MALDI-TOF mas spectra (*m/z* range, 600 to 1,100 Da) of three species *M. aeruginosa M. ichthyoblabe* and *Microcystis* sp. in Lake Wannsee [91]. However, microcystins and Nodularinwere directly detected using Solid Phase Extraction coupled on-line to Ultra-High Pressure Liquid Chromatography/Electrospray Tandem Mass Spectrometry (SPE – UHPLC – MS/MS) in water samples [94]. Different other linear and cyclic oligopeptides such as aeruginosins, anabaenopeptides, cyanopeptolins, anabaenopeptins and microginins are found within the genus *Microcystis*. HPLC method for determination of microcystins, which requires standards for comparison for the field strains of producing microcystins.

Intracellular microcystin content was measured using the colorimeteric PP2A inhibition assay. The assay was performed using a 5% inhibitory concentration of 6.72 µg/Liter for the microcystin LR standard using 0.5 µg of PP2A per ml and a limit of detection of 0.033 µg/Liter(33pM). Prior to the assay 1ml of cell suspension in water(supernatant removed) was freezed- thawed three times and diluted 1:100 to 1:8000. Sample were measured in duplicated in the same assay, and also in repeated assay. After adjusting each assay to percentages of protein phosphatase activities, the concentration of microcystin was calculated from as average standard curve of all assays. PP2A inhibitor (microcystin concentration was calculated for cells per milliliter of sample resulting in value reported a picomoles of microcystin per cell [95].

The findings summarized toxins are becoming increasingly recognized as human and animal health hazards. Symptoms of toxicity of hepatotoxin includes weakness, anorexia, and pallor of mucous membranes vomiting, cold extremities and diarrhea. Microcystin-LR induces oxidative DNA damage in HepG2 human cells at concentration 0.01µg/ml and become chronic exposure increases the risk of liver cancer, [96]. Microcystis aeruginosa extract in drinking water produce increased aberrant crypt foci in the colon, viz. preneoplastic colonic lesions in mice [97].

PCR-based assay demarcated that 10cells/ml indicative of toxic bloom well before cell density reaches to 2000/ml i.e. recommended by Australian drinking water standard, [98]. Chorination is effective means of desturction of microcystins. Chlorinating agents' calcium and sodium hypochlorite reported not effective and required at large dose rates, and higher pH [99]. Peptide hepatotoxins extreme rapid destroyed by ozone. And in combination with peroxide destruction is even faster, [100]. Elevated doses of ozone are required to destroy microcystins within the intact cyanobacterial cells.

He, Xue xiang et al., (2017) were observed varied differenceson ELISA cross reactivity of microcystin ADDA and protein variables using ELISA kitand distraction congeners by chlorinated mixtures[101]. Comparison of microcystin detection of two analytical methods ELISA and LC-MS/MS with or without chlorination and finding reveals in chlorinated water sample(n-21) microcystins different extents of degradation of microcystins (MC-RR>MC-LR>MC-LA>MC-LF) weredetected but found no MC-YR and MC-LY residues.Free chlorine reactivity with the protein variables with increasing pH (pH6>pH8>pH10) was highly dependent on speciation of the oxidant rather than the amino-acids side chain, (single letter code-A-Alanine, F-Phenylalanine, L-Leucine, R-Arginine,Y-Tyrosine).Temperature, oxidation dose and the background matrix, though not as significant also contributed to the removal of microcystins except tyrosine congener were not dependent on pH and speciation of the oxidant.

II. CONCLUSION

US EPA has recommended ELISA for water treatment utilities as a primary analytical tool for the quantification of total microcystins (MCs) in raw and treated water. With the standards of microcystins/Nodularin-ADDA, ELISA test kit is an indirect competitive ELISA kit and the detection mechanism is primarily through the microcystin ADDA functional groups in toxin and the immobilized microcystins-aminoaacids analogue for the binding sites of the polyclonal sheep antibodies in solution.

Toxic cyanobacteria cause illnesses ranging from acute pneumonia and gastroenteritis. Cyanobacterial toxins acts as defence or deterrent agents against planktivores [102]. High alert level of 20,000cells/ml abloom contain sufficient toxin to be human health hezard, [103]. Toxicity of hepatotoxin should be treated with cyclosporine-A, rifampin and silymarine and neurotoxin is curable by Atropine [104]. Water safety guideline for drinking water USEPA, drinking water which containing less then 1 µg of microcystins has approved drinking water purpose.

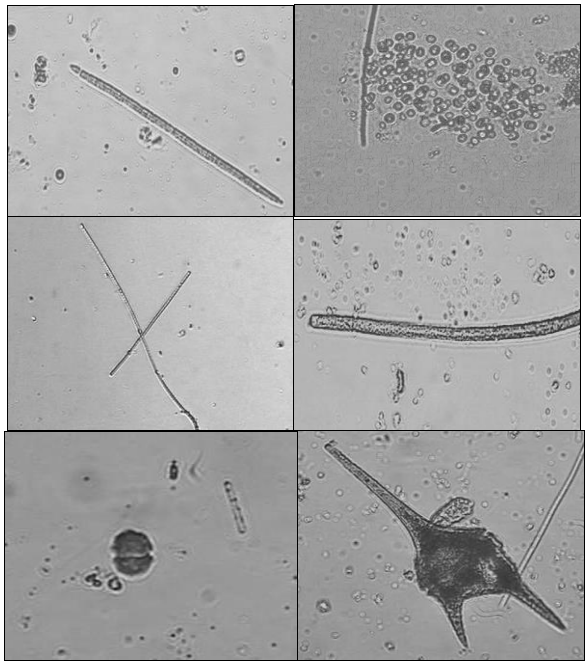

Plate-A: (1) *Microcystis aerginosa* (2) *Cylindrospermopsis ruciborskii* (3) *Planktothrix agardhii* (4) *Oscillatoria* sp. (5) *Peridinium* sp. (6) *Ceratium* sp.

III. ACKNOWLEDGEMENT

Author thanks to Director, NEERI, Nagpur for giving permission to publish this review paper. Toxic cyanobacteria recent research on cyanobacterial molecular studies in early 90's and health risks studies carried out by various authors in different parts of the world is gratefully acknowledged.

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