CHARACTERIZATION OF MICROCYSTIN FROM BLOOM FORMING FRESH WATER ALGAE

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Abstract : This study has been used to investigate the microcystin toxin production from the water samples contaminated with Cyanobacteria during the year 2019- 2020. The toxicity in the water samples are performed by classical method with some molecular characterization principles. This type of analysis is based on the functional group detection and their toxicity effect against bacteria. Algae play a vital role in the environment to produce oxygen and some point of overcrowding or blooming may produce toxin by the climatic conditions. These conditions are also very useful for the plant growth and the aquatic life survival. In this area, the algae flourish their growth along with toxins, which may cause the lethal effect in cattle's and also damage the neurons of the people who are consuming the toxin without filtration. The serious effects such as Alzheimer disease and Parkinson disease are caused by the cyanotoxin particularly microcystin.

IndexTerms – Toxin, microcystin, Toxicity, Neurogenic disorders.

I. INTRODUCTION

Cyanobacteria is referred as blue green algae which obtain energy from photosynthesis process [Sharma *et al*, 2011]. These are found in variety of environments ranging from fresh water to marine water. It has been adapted to survive in extreme environments also. Cyanobacteria are unicellular, colony forming, filamentous, sheet or even hollow spheres and have the potential to fix atmospheric nitrogen. Some filamentous species differentiated into vegetative, akinetes and heterocysts. Here heterocysts play an important role in nitrogen fixation by using nitrogenase enzyme. The Cyanobacteria were classified into five orders by morphological and it's various salient features. The first order Chroococcales is coccoid cells, reproduced by binary fission or budding. For e.g. *Aphanocapsa, Aphanotheca, Gloeocapsa, Merismopedia, Microcystis, Synechococcus, Synechocystis.* The second order Oscillatoriales is uniserrate, filamentous without heterocysts or akinetes. For e.g. *Lyngbya, Leptolyngbya, Microcoleus, Oscillatoria, Phormidium, Planktothrix*. Third order Nostacales are filamentous with heterocysts, divided by only one plane and false branching in genera such as *Scytonema, Anabaena, Aphanizomenon, Calothrix, Cylindrospermopsis, Nostoc, Scytonenma, Tolypothix*. Fourth order Stigonematales are divided by more than one plane, true branching and multiserrate forms heterocystsm e.g, *Stigonema*. [Archana, 2013].

Toxin producing Cyanobacterial harmful algal blooms (CHABs) has been increasing in worldwide and some species can produce neurotoxins, endotoxins and hepatotoxins [Hudnell, 2010]. These toxins are called as cyanotoxins and also have negative impacts on human beings and aquatic animals. These toxins are also classified into two broad categories: Neurotoxins (Anatoxin, Saxitoxin) and Hepatotoxins (Cylindrospermospin, Microcystins & Nodularin). The most widespread cyanobacterial toxins are microcystins and neurotoxins. Some species contain both neurotoxins and microcystin. These kinds of toxins cause liver infections (hepatotoxins), nerves problem (neurotoxins) and skin irritation (dermatotoxins). Bloom forming species are often found in most of the environments with favorable conditions such as warm, stable with high nutrient levels [Singh, *et al.*, 2011]. The most common toxic Cyanobacteria in fresh water are *Microcystis sp., Cylindrospermopsis racibroskii, Planktothrix (syn. Oscillatoria) rabescens, Lyngbya sp., Aphanizomenon sp., Nostoc sp., some Oscillatoria sp., and Schizothrix sp.*

Toxic Cyanobacteria are found in inlands and coastal environments [Mellisa *et al.*, 2013]. At least 46 species have shown their toxic effects in vertebrates. These are categorized as neurotoxins, endotoxins and hepatotoxins which are collectively known as cyanotoxins. These toxins often released by *Microcystis and Planktothrix* species can have negative impacts on human beings by causing liver infections (hepatotoxins), failure of the nervous system (neurotoxins) and skin irritation by dermatotoxins. Microcystins are found in most populations of *Microcystis sp.* these are almost toxic, but nontoxic strains also occur. Seventy structural analogues of microcystin have been identified and high microcystin content observed in planktothrix. Cyanobacteria are able to survive in extreme environments such as rocky shores, hot springs, droughts, nitrogen depleted and starvation places.

Archana, *et al.,* (2013) suggested that cyanobacterial extracts of *Anabena variabilis* and *Synechococcus* elongates have shown significant antibacterial proportion towards *E-coli, Enterococcus, Klebsiella*. Singh, [2011] noted that the cyanobacteria from coastal region of oceanic water used for the production of antibacterial substances against bacteria using solvent extracts of Diethyl ether, Ethyl acetate and Ethanol. Microcystins are most frequently occurring and widespread cyanotoxin. They are cyclic heptapeptides containing a specific amino acid (ADDA) side chain which has been found only in microcystin. Different structural analogues of microcystin have been identified. The seven amino acids that are involved in the structure of a microcystin include a unique β-amino acid (ADDA). The LR form of microcystin has leucine and arginine, RR form has arginine and arginine, YR form

has tyrosine in R1 position and arginine in R2 position. For vertebrates, a lethal dose of microcystin (1µg<) causes death by liver necrosis within few hours to one or two days. An acute dose response of microcystin may damage liver in several ways.

II. MATERIALS AND METHODS

Isolation of Microcystin producing algae

The water samples were collected from nearby villages and different localities in and around Virudhunagar. By using light microscope, the morphology of the sample particularly microcystin producing algae were identified and also immediately inoculated in BG11 broth. These samples were kept in an algal incubator or wooden rack with the proper light source for 15-20 days. After incubation, the samples were allowed to dry and make a fine powder for future purpose.

1(a)

1(b)

Figure 1(a), showed a containers with water samples from various places (b) fresh media of BG11 with contaminated water samples

Extraction of Microcystin by Methanol

5 to 10 ml of water samples were added to the respectively labelled tubes with sterilized BG11 broth and kept in an incubator for 5 – 10 days. 200ml of both broth and water containing culture was filtrated via 0.45um nylon membrane filter or by what man No.1 filter paper in a vaccum condition. The algal blooms were deposited on the filter paper and the remaining liquids collected in a conical flask. The algal blooms or the filter discs were carefully collected and placed in a conical flask containing 10ml of methanol, 50 ml of distilled water with 0.1% of tween solution. These flasks were incubated in the absence of light source for 30 minutes at room temperature for 30 minutes and centrifuged at 4000rpm for 5 minutes. Finally, the supernatant was diluted with distilled water, at this point, the sample is ready to continue the assay studies [Metcalf *et al*., 2020].

Characterization of Microcystin by FTIR

Fourier Transform InfraRed Spectroscopy is used to elucidate the compositions of the derived samples. The components in the final products were analyzed by FTIR (BRUKER - ALPHA ECO- ATR, GERMANY). Here, there methanol was used as a solvent for predicting the purity of microcystin. This work aimed to study the toxic profile of commercial as well as drinking water supply. Therefore, this technique should be applied as the reference method for identifying the toxicity level in water samples.

Antimicrobial Activity for microcystin

The antimicrobial activity was analyzed by Mueller Hinton Agar plates for the extracted algal samples. The agar plates were spreaded with 0.1ml of methanol containing microcystin and kept in an incubator for 37^0C for overnight. This toxin effect is not yet published in papers. Some researchers did and published based on the basic level of work not in concentration dependent. Like that, we focused the concentration according to the disease causing ability and the death causing factors of fish [Funari, 2008] and [Mugilan *et al*., 2016].

III. RESULTS AND DISCUSSION

Morphology identification and cultivation

The algal sample was analyzed and showed a lengthy rod like structure with or without surrounding sheath by light microscopes. Ingredients like Ferric ammonium citrate, Disodium magnesium EDTA, Boric acid, Copper sulphate, Dipotassium hydrogen phosphate, Calcium chloride and Citric acid are used in BG11 broth which induces only the growth of fresh water algae. After 15 days, the algal sample was harvested and dried by dry heat method at 40 -50^0C. From the result, inferred that that the samples from Servaikaran patti, Ampasamuthram pond water, Ayyampalayam lake water samples showed the features like elongated tube without thick sheath surrounds the structure, which confirmed the algae of *Oscillatoria sp*. because of the climatic condition favors the species as well as *Microcystis* in some places. We have been identifying species of Oscillatoriales on the basis of colour, sheath, cell measurement, constriction, granulation and end cells. V.V.V.College hostel water, Virudhunagar kovil pond water, lake water from Aruppukottai, Veerapandi eri sample Showed the structure related to *Microcystis sp*. Here, We Knew that the climatic condition of Madurai and Virudhunagar area induce the accumulation of nutrient and contamination from various sources.

Extraction of Microcystin

After 10 -15 days the algal samples were filtered by Filter paper or a membrane disc. Here, the filter paper is our preferable material for the purification or separation of intracellular or extracellular microcystin. The materials like filter paper or disc were taken in a test tube or centrifuge tube and added with 10 ml of diluted methanol containing Tween. The mixture was kept in a room temperature without disturbance for 30minutes to excretion of microcystin from algal cell. The toxins were extracted twice from the algae.

Characterization of toxins by FTIR

There are yet no official methods or regulatory limits for the cyanotoxin groups. From figure 2, we inferred that the secondary amine with N-H stretching appears at 3300 – 3400 cm. Next peak appears at 2800 – 3000 cm which indicates the amine group. The stretching of O=C=O appears at 2300 – 2350 cm and the C=C appears at 1648 – 1658 cm which denotes the medium alkine group. Finally, the C-H stretching appears at 1450 – 1465 cm for alkane and the bond range of 1085 – 1150 cm predict the C-O stretch of an aliphatic group. This FTIR graph showed various peaks with different retention time corresponding to the group attached and adsorption maxima with standard value.

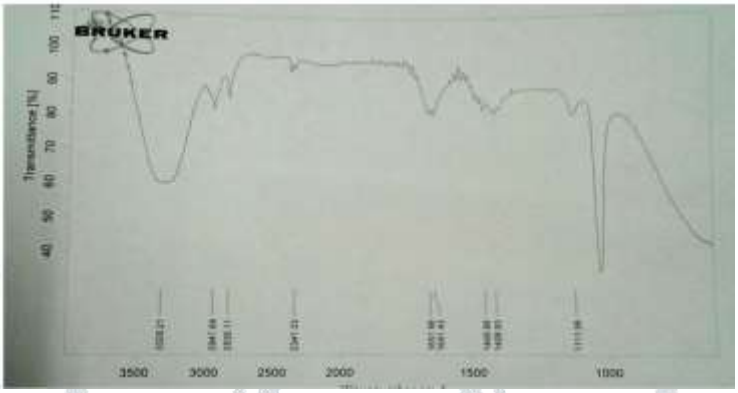

Figure 2 : FTIR analysis for the functional group detection

Antimicrobial Activity

The methanolic extracts of microcystin were produced clear zone formation against *E.coli*, *Pseudomonas* sp. and *Staphylococcus sp.*. This characteristic features are very important for determining the quality of water and also the efficacy of microcystin in water bodies. The types of microcystin and its structural forms in water bodies induce the tumor effects in liver. Disease causing ability in pond water, eri, lake and stagnant area are easily polluted by soil, water from industry, domestic water, waste refineries from beverage industry and sewage contamination etc., Here, the pathogenic as well as water borne organisms showed large zone formation towards the concentration of algal samples.

Table 1 Antimicrobial activity for microcystin

S.No.	Organism Name	Zone Diameter (cm or mm)	Resistance/ Sensitivity
1.	*Pseudomonas sp.*	3.5 or 35	Resistance
2.	*Vibrio sp.*	3 or 30	Resistance
3.	*Staphylococcus sp.*	2.5 or 25	Resistance
4.	*Escherichia coli*	3 or 30	Resistance
5.	*Proteus sp.*	2.8 or 28	Resistance

IV. CONCLUSION

The consumption of food, water and air for purpose of survival is increased in every year. Pond water systems were dominated by greenish and bluish mats of cyanobacteria and algae. Cyanobacteria cause health problems to humans and also to the environment after exposing longer period of solar radiation. These mats were capable of producing photoprotective compounds. This is due to consumption of drinking water and failure to control the algal mass in the water bodies. Studies on the mechanism of cell toxicity showed that microcystin interferes with cell structure and also in mitosis cell division. This may help in expanding the tumor promoting activity. These samples were rich in cyanobacteria. Eutrophication but also other environmental factors enhance bloom formation like temperature, pH. Under these circumstances, cyanotoxins can reach high concentrations in waters and might represent health and ecological risks [Codd *et al*., 2005]. These algae are not only causing health issue problem, but also breaking the food chain of water bodies. In China, liver tumors in humans may be associated with the presence of cyanotoxins in drinking water [Audrey et al., 2017]. Our recent research of microcystin, causes harmful effects to humans and environment, which is responsible for many issues regards health problems and disorders of protein modification in cattle's. In this situation, we have to rectify the problem by creating awareness and remove the blooms from the water bodies.

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