INTRODUCTION

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INTRODUCTION

Cyanobacteria are one of the most primitive (Brock, 1973) and ubiquitous families of photosynthetic organisms. They are considered to be pioneers of early earth (Brock, 1973; Schopf, 1996). Cyanobacteria, strikingly similar to the present day counterparts were found fossilized in sedimentary rocks formed 3,500 million years ago (Bryant, 1994). They are members of the kingdom Prokaryote, division Gracilicutes (bacteria with gram negative cell wall), class Photobacteria, subclass Oxyphotobacteria and order Cyanobacteriales (Gibbons and Murray, 1978). The cyanobacteria are a remarkably widespread and successful group, colonizing fresh water, marine and terrestrial ecosystems, including extreme habitats such as Antarctic lakes, salt fields and hot springs (Fogg et al., 1973). Morphologically, physiologically and metabolically this group is one of the most diverse groups of prokaryotes (Codd, 1994). The rapid evolution of cyanobacteria in different water and land environments is related to their capacity for both aerobic and anaerobic photosynthesis. They have been known to occur in the sediments from the early Precambrian period as components of stromatolitic microbial mats (Schopf, 1983, 1992). This fact serves as a clue that these microorganisms might have played a major role in the evolution of an oxygenic environment (Fay, 1983). Their long evolutionary history is considered as a reason for the success of cyanobacteria in many habitats and wide ecological tolerance (Whitton and Potts, 2000).

Cyanobacteria are related to eubacteria in their prokaryotic cellular organization, possession of a gram negative cell wall made up of peptidoglycan and a gelatinous sheath liable to lysosome treatment, absence of sexual reproduction, tolerance to high temperature and susceptibility to antibiotics, high degree of adaptability and non-sexual genetic recombination.
They contain chlorophyll-a, carotene, xanthophylls, c-phycocyanin, allophycocyanin and c-phycoerythrin. The last two pigments can only be found in cyanobacteria (Benson, 1969). Their photosynthetic organ and mechanism of photosynthesis are similar to algae but, unlike eukaryotic microalgae, cyanobacteria do not possess membrane bound sub-cellular organelles like chloroplasts. The pigments are embodied in phycobilisomes, which are found in rows on the surface of the thylakoides (Douglas, 1994).

Cyanobacteria have several characteristics that allow them to outcompete more complex organisms. Due to minimal need for nutrients, cyanobacteria can inhabit a wide range of environments. Cyanobacteria are able to maintain photoautotrophic growth in relatively lower light intensities than their potential competitors (Reynolds and Walsby, 1975; Ganf et al., 1991). In addition, cyanobacteria have been shown to be both heterotrophic and photoheterotrophic and therefore able to survive in conditions of very low light intensity, such as at the bottom of euphotic zone of lakes and in lake sediments (Ganf and Oliver, 1982) and in caves (Prescott, 1968), or in places where there is no light at all. Some cyanobacteria not only survive high levels of visible light but also survive damage by near ultra violet light (UV), even being capable of utilizing these wave lengths for photosynthesis (Pearl et al., 1985; Tilzer, 1987). The amazing combination of properties found in algae and bacteria which these organisms exhibit, have been a source of fascination and attraction for many scientists.

A noteworthy feature of the cyanobacteria is the ability of some species to fix elementary nitrogen dissolved in water, and many species are capable of living in water with low levels of combined N₂. Using the enzyme nitrogenase, they convert N₂ directly into ammonium in aerobic conditions. Nitrogen fixing cyanobacteria are wide spread among filamentous, heterocyst forming genera such as Anabaena, Nostoc and Aphanizomenon (Ressom et
al., 1994; Adams, 2000). When cyanobacteria are present even in low numbers, their nitrogen-fixing capabilities may enhance the fertility of marine and freshwater environments; in fact, cyanobacteria are sometimes used to fertilize paddy (Singh, 1976; Bold and Wynne, 1985; Singh and Bisoyi, 1989). Cyanobacteria are most commonly found in neutral to alkaline surface waters (Moss, 1973) and are also able to utilize both free CO$_2$ and bicarbonate ions as a source of inorganic carbon for photosynthesis (Talling, 1976).

In contrast to true algae, many species of planktonic cyanobacteria possess specialized intracellular gas vesicles. Stacks of minute (<300nm) proteinaceous hollow cylinders maintain a gas-filled space in the cell, which enable regulation of buoyancy of cells and colonies, and optimize their vertical position in the water bodies. This in turn enables them to find suitable niche for survival and growth. The buoyancy of some cyanobacteria is responsible for intensive formation of blooms at the surface of water. The process of nitrogen fixation and occurrence of gas vesicles are especially important for the success of noxious species of cyanobacteria. The slow growth rate of cyanobacteria in comparison to eukaryotic microalgae is compensated by a higher affinity for phosphorus and nitrogen, substantial phosphorus storage capacity, and low losses to grazing by zooplanktons as a result of the formation of large colonies (Reynolds, 1987).

The cyanobacteria also provide an extraordinarily wide ranging contribution to human affairs in every day life (Tiffany, 1958) and are of economic importance (Mann and Carr, 1992). Both the beneficial and detrimental features of the cyanobacteria are of considerable significance. They are important producers and their general nutritive value is high. They are a rich source of several phytopharmaceuticals (Schwartz et al., 1990). The nitrogen fixing species contribute globally to soil and water fertility (Rai,
However, cyanobacteria have the potential to produce mass population in natural and controlled water bodies. Such development leading to cyanobacterial blooms, scums and mats, is a common consequence of eutrophication. Abundant growth of cyanobacteria in water reservoirs creates severe practical problems for water supplies. Furthermore, cyanobacteria are well documented in being able to potentially synthesize a large number of low molecular weight compounds called cyanobacterial toxins or cyanotoxins.

Cyanobacterial blooms are usually observed during spring time or during late summer time. The following factors are responsible for the predominance of bloom forming cyanobacteria during the summer period: water temperature above 25°C, low light intensity in water, low N:P ratio and stability of the water column. A cyanobacterial bloom, occurring mainly under conditions of high water temperatures and reduced turbulence, show a buoyant migration to the water surface (Hutchinson, 1967; Reynolds and Walsby, 1975; Robarts and Zohary, 1987; Pearl, 1996). An explanation for cyanobacterial dominance may be their ability to control their buoyancy and to photosynthesize under low light conditions (Reynolds and Walsby, 1975). Many cyanobacteria are able to make controlled migrations to specific depths and thereby possess an advantage to obtain the otherwise unavailable nutrients retained there (Fogg, 1969; Reynolds and Walsby, 1975).

Most cyanobacterial blooms tend to occur in summer months, in temperate climates and it is reasonable to mention that elevated temperature would be a major factor in population growth and possible subsequent bloom formation (Tilman and Kiesling, 1984). Bloom forming cyanobacteria mainly belong to the genera Anabaena, Aphanizomenon, Anabaenopsis, Arthrospira, Cylindrospermopsis, Oscillatoria, Nodularia and Microcystis (Reynolds and Walsby 1975; Oliver and Ganf, 2000).
TOXIC CYANOBACTERIA

Cyanobacteria are the integral parts of many ecosystems and as such cause no problem. A small group of genera however, produce toxins which cause sporadic but repeated cases of animal poisoning (Schwimmer and Schwimmer, 1964, 1968; Carmichael, 1992 a, b). Because of wide spread eutrophication of lakes, ponds and some parts of oceans, cyanobacteria often form blooms, which lead to water hygienic problems (Henning and Kohl, 1981; Skulberg et al., 1984; Bell and Codd, 1994; Chorus and Bartram, 1999; Day et al., 2000). Several genera of cyanobacteria form toxic water blooms and different cyanobacterial toxins have been characterized (Carmichael, 1992c, 1994). Possibly the synthesis of highly active toxins is a defense option of cyanobacteria against attack by other organisms like bacteria, fungi, zooplankton and eukaryotic microalgae. Carmichael (1994) found that cyanobacterial toxins can be extremely harmful to zooplanktons that feed on cyanobacteria. They may be directly lethal or they may reduce the number of their offspring.

The incredible variety of niches inhabited by toxic cyanobacteria indicates a high degree of biological adaption which has enabled these organisms to thrive and compete effectively in nature. One reason toxic cyanobacteria have been so successful in wide spectrum of niches is their ability to produce a unique range of defensive secondary metabolites, the cyanobacterial toxins (cyanotoxins). Toxic cyanobacteria are well known for their ability to produce cyanotoxins, which have been responsible for numerous animal deaths (Schwimmer and Schwimmer, 1968; Carmichael et al., 1985; Beasley et al., 1989; Kuiper-Goodman et al., 1999). Of more than 50 genera of cyanobacteria, at least 19 of them, comprising 41 species, have been shown, to possess toxic properties (Scott, 1991). The best characterized genera are Microcystis, Anabaena, Aphanizomenon, Cylindrospermopsis,
Gloeotrichia, Lyngbya, Nodularia and Oscillatoria (Scott, 1991; Carmichael, 1992 a) as it is these genera which have been most frequently implicated in poisoning animals and causing illness in humans (Carmichael, 1992 a, b).

Of the cyanobacterial blooms tested to date, 50-75% have been toxic (Codd, 1995). In fact, toxicities of blooms of the same species can vary markedly both geographically and with time (Carmichael and Gorham, 1981). At least 46 species have been shown to cause toxic effects in vertebrates (Sivonen and Jones, 1999). In cyanobacterial blooms often only one species comes up to more than 95% of the population. Though this has been interpreted as a result of competition between species, the dominance of one species could be a hint for the formation of metabolites with cyanobacterial activity. The common toxic cyanobacteria in fresh water are Microcystis spp., Cylindrospermopsis raciborskii, Planktothrix (syn. Oscillatoria) rubescens, Synechococcus spp., Planktothrix (syn. Oscillatoria) agardhii, Gloeotrichia spp., Anabaena spp., Lyngbya spp., Aphanizomenon spp., Schizothrix spp. and Synechocystis spp. Among the toxic cyanobacteria, Microcystis is the most common genera producing microcystins, a group of toxins with strong hepatotoxicity (Carmichael, 1994). Acute hepatotoxic and chronic hepatocarcinogenic effects of microcystins have been studied intensively in mammals (Dawson, 1998; Dietrich and Hoeger, 2005) and human health risks resulting from the presence of microcystin in drinking and recreational water have been recognized (Codd et al., 2005). While initially toxicity appeared to be restricted to planktonic cyanobacteria, benthic forms which form mats in water bodies have also been shown to be toxic (Edwards et al., 1992; Carmichael et al., 1997; Metz et al., 1997).

Growth of cyanobacteria occurs all year round in some tropical lakes, however in temperate regions growth and blooms of cyanobacteria exhibit a characteristic seasonality (Reynolds and Walsby, 1975). Filamentous forms
such as *Aphanizomenon*, *Gloeotrichia* and *Nodularia* are first to appear, while colonial forms such as *Microcystis* generally appear later (Ganf and Oliver, 1982). All these genera overwinter in the sediment, either as akinetes or spores in the case of *Anabaena* and *Aphanizomenon* or as vegetative colonies in the case of *Microcystis* (Reynolds and Walsby, 1975). The ability to switch from photoautotrophy to heterotrophy, based on stored carbohydrate, combined with a reduction in respiration rate, is the likely reason that these cyanobacteria can survive in the bottom sediments for several months over winter (Reynolds and Walsby, 1975).

Contamination of water by toxic blooms of cyanobacteria has occurred widely in many regions of the world. Anthropogenic eutrophication can exacerbate the risks, allowing toxic cyanobacteria to grow unchecked and resulting in harmful algal blooms with potentially serious economic and health related impacts. The blooms are frequently found in bodies of warm, stagnant water such as drinking water reservoirs and ponds that serve as drinking holes for domestic or wild animals. Contact with or ingestion of water containing cyanobacterial cells or toxins can cause skin irritations, allergic responses, blistering of mucosa, hay fever symptoms, diarrhoea, acute gastroenteritis, and liver and kidney damage (Ressom et al., 1994; Falconer, 1994; Bell and Codd, 1996; Pilotto et al., 1997; Codd, 2000).

Blooms of blue-green algae are potential hazard to human beings due to the fact that extensive growth of cyanobacteria in water supply reservoirs lasts for several months per year (Carmichael and Falconer, 1993). It is also important to note that mass occurrences of toxic cyanobacteria are not always associated with human activities causing pollution or "cultural eutrophication".
IMPORTANCE OF PRESENT STUDY

The toxic blooms of cyanobacteria develop and flourish worldwide, both in freshwater and in marine environments (Carmichael and Falconer, 1993). There is evidence that these toxic organisms are on the increase perhaps as a result of global pollution. The frequency, intensity and geographic distribution of toxic species in aquatic environment seem to be increasing in recent decades due to the proliferation of harmful cyanobacteria (Gago-Martinez et al., 2003).

One of the major problems arising from harmful cyanobacterial blooms is the production of cyanotoxins. The hepatotoxic microcystin are amongst the most frequently reported cyanotoxins (Sivonen and Jones, 1999), as they are not only associated with Microcystis blooms, but also with blooms of Anabaena, Nostoc and Oscillatoria (Bartram et al., 1999). These compounds represent, by different exposure routes, a significant health hazard to humans and livestock (Kuiper-Goodman et al., 1999), being involved in several intoxication episodes throughout the world, and even in death of humans exposed through haemodialysis (Jochimsen et al., 1998). The observed hepatotoxicity of microcystins is leading to acute liver failure via disruption of hepatocyte cytoskeletal components (Fastner et al., 1999). Furthermore, microcystins have been shown to act as tumor promoters (Nishiwaki-Matsushima et al., 1992) and have been considered as a major risk factor contributing to the high rate of hepatocellular carcinoma in south-east China (Ueno et al., 1996).

Health hazards of cyanotoxins have alerted water authorities and even the public, the need for detailed research on potentially harmful cyanobacteria. Indeed, an estimated 50% of cyanobacterial blooms contain toxins, and some of these toxins are not destroyed by the chlorine treatment or filtration methods often used by water treatment plants and can therefore be a
threat to humans (Dale and Yentsch, 1978). The ability of cyanobacterial population to produce potent toxins and examples of associated human and animal health problems have raised the position of cyanobacteria in the priorities for the management and production of water quality. Consequently the early detection in waters used for drinking is highly desirable so that measures to minimize or prevent exposures can be implemented. Reports of toxicity and associated health risks of cyanobacteria in waters used for domestic supply, agriculture and recreation have resulted in increased level of awareness and monitoring to detect potentially toxic species. In addition, blue green algae are used in many parts of the world as fertilizers, and several types are even used as side dishes (http) and as a dietary supplement in countries like the U.S.A. Furthermore, blue green algae are used to treat children with Attention Deficit Disorder (http) and also used for pets (http). About a million people in the U.S.A. and Canada consume dietary supplements containing blue-green algae (http).

Although in recent years, toxic cyanobacterial blooms have been reported with an increasing frequency in different countries worldwide, often associated with the production of microcystins, such toxic occurrences are poorly documented in India. Most work has been done in the field of water bloom ecology and taxonomy. Few studies have addressed on the cytotoxic effects of cyanobacteria on plant growth.

OBJECTIVES OF THE STUDY

The present study is aimed to assess the cytotoxicity of some selected cyanobacterial strains of algal blooms of water samples collected from a water reservoir of Calicut city, ‘Mananchira pond’, water bodies and garden pond of the Calicut University campus, rice fields and adjoining areas of Calicut and Malappuram Districts in a random way. The study also includes:
1. Characterization of potentially toxic cyanobacteria at species level based on field collection.

2. Isolation and identification of selected cyanobacterial strains in unicycleanobacterial cultures under laboratory conditions.

3. Cytotoxic activities of different dosages of crude extracts of toxic cyanobacteria are evaluated at different time intervals by using *Allium cepa* assay.

4. Cytotoxic potentiality of all the isolated toxic cyanobacteria will be analysed based on the clastogenic and non-clastogenic aberrations induced by them on *Allium cepa* root tip meristem.

5. Detection of the probable causes for all the clastogenic and non-clastogenic abnormalities.

6. Detection of the level of the toxicity of the studied samples of Cyanobacteria based on the results of the cytotoxic assays.