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Review Paper

Cyanobacteria and Its Application

NINGARAJ DALAWAI, K. M. HARINIKUMAR, K. N. KRUPA AND H. B. MANOJ KUMAR
Department of Plant Biotechnology, College of Agriculture, UAS, GKVK, Bengaluru-560065

ABSTRACT

Cyanobacteria are characterized by rapid photoautotrophic growth and high speed of biomass accumulation. Cyanobacteria are widely distributed Gram-negative bacteria with a long evolutionary history and the only prokaryotes that perform plant-like oxygenic photosynthesis. Cyanobacteria possess several advantages as host for biotechnological applications, The use of photosynthetic cyanobacteria to directly convert carbon dioxide to biofuels are now considered as important renewable energy alternatives for petroleum-based fuels, like biofuels, biogas, bioethanol or biodiesel. Equipped with the ability to degrade environmental pollutants and remove heavy metals, cyanobacteria are promising tools for bioremediation and waste water treatment. In field of agriculture, cyanobacteria is used as nitrogen fixing bio fertilizer, bioremediation CO_2 sequestration and biofuel production. In this review, the potential of cyanobacteria as sources of energy, bioactive compounds, high-value chemicals and tools for aquatic bioremediation and recent progress in applications are discussed.

ALGAE are the important food links in the aquatic ecosystems. Being autotrophic and members of the first tropical level, their major role in water is to capture solar energy to drive the ecosystem. The magnitude of primary production is the leading factor in deciding the tropic structure of water bodies. Several species of algae have been found in tropical condition such as those in India. It provides favourable environment for luxuriant growth of these organisms in natural ecosystem such as different water bodies.

Cyanobacteria are common in eutrophic nature. Being favoured by stable and nutrient enriched water, they may constitute an important part of phytoplanton communities. The Indian subcontinent studies are limited to phytoplankton of large rivers and streams even though their spatial and temporal variations have been studied (Nantiyal *et al*., 1997).

The names cyanobacteria and blue-green algae (Cyanophyceae) are valid and compatible systematic terms. This group of micro-organisms comprises unicellular to multicellular prokaryotes that possess chlorophyll *a* and perform oxygenic photosynthesis associated with photosystems I and II (Castenholz and Waterbury, 1989).

Human society has an insatiable appetite for fuels and today's supply of liquid fuels worldwide is almost completely dependent on petroleum. Bioenergy production has recently become a topic of intense interest due to increased concern regarding limited petroleum-based fuel supplies and the contribution of the use of these fuels to atmospheric CO_2 levels. Finding sufficient supplies of clean energy for the future is society's one of the most daunting challenges and is intimately linked with global stability, economic prosperity and quality of life. This leads to interesting questions and debate over the choice of new fuels, produced from new raw materials, to complement or replace present petroleum-based fuels (Posten and Schaub, 2009).

Taxonomy of Cyanobacteria

The cyanobacteria are amongst the most taxonomically challenging, yet species rich, lineages of microbes (Perkerson *et al.*, 2011). Originally classified based solely on morphology, wholesale revisions of the cyanobacteria were proposed by the International Code of Botanical Nomenclature, which relied on a series of papers published from 1886 to 1892 as a starting point for taxonomy.

In 1978, the Subcommittee on the Taxonomy of Phototrophic Bacteria of the International Committee on Systematic Bacteriology (ICSB) unanimously submitted to the ICSB a proposal according to which the nomenclature of cyanobacteria (blue-green algae) should be governed by the provisions of the International Code for Nomenclature of Bacteria (Bacteriological Code, ICNB) as of 1979. That date

was proposed as the deadline for the publication of a list of approved names for these prokaryotes (Stanier *et al*., 1978).

Two major attempts have been made to change the overall cyanobacterial classification system. One challenge in untangling the systematics of the cyanobacteria is the extensive phenotypic plasticity evidenced in some lineages. For eg., heterocystous species only produce heterocysts in nitrogen limited conditions, while, other morphological features may be altered in phosphorous limited conditions. Therefore, a cultured species may have a different phenotype from a field specimen, due to the environmental conditions in which it was cultured (Casamatta *et al.,* 2003). Hypothesizing that the vast biodiversity of the cyanobacteria was really only the result of a few taxa that exhibit a tremendous amount of phenotypic plasticity, Drouet and Daily (1956) used only a few simple morphological characteristics for taxonomy, resulting in the total number of proposed species of the day being reduced from over 2000 to only 62 and they proposed a greatly simplified taxonomic treatment of the group, largely based on an exhaustive comparison of published descriptions and herbarium specimens ; they recognized only six genera and 18 species.

Komarek and Anagnostidis (2005) proposed the other major revision to the classification system, suggesting many name changes, especially in the *Oscillatoriales*. They advocated a system of smaller, monophyletic genera identifiable using morphological, genetic or ecological apomorphies (Johansen & Casamatta, 2005). It is their revision which forms the basis of modern cyanobacterial systematics.

Komarek and Komarkova (2006) stated the application of modern ecological, ultra-structural and molecular methods, aided by the cultivation of numerous cyanobacterial morphotypes, has substantially changed our knowledge of these organisms. It has led to major advances in cyanobacterial taxonomy and criteria for their phylogenetic classification. Molecular data provide basic criteria for cyanobacterial taxonomy, however, a correct phylogenetic system can not be constructed without combining genetic data with knowledge from the previous 150 years research of cyanobacterial diversity. Thus, studies of morphological variation in nature and modern morphological, ultrastructural, ecophysiological and biochemical characters need to be combined in a polyphasic approach.

Taxonomic concepts for generic and infra generic ranks are re-evaluated in light of combined phenotypic and molecular criteria. Despite their usefulness in experimental studies, the limitations of using strains from culture collections for systematic and nomenclatural purposes are highlighted. The need for a continual revision of strain identification and proper nomenclatural practice associated with either the bacteriological or botanical codes is emphasized. Recent advances in taxonomy are highlighted in the context of prospects for understanding cyanobacterial diversity from natural habitats and the evolutionary and adaptational processes that cyanobacteria undergo.

Abed *et al.* (2002) described a new genus of moderately halophilic, halotolerant and thermophilic cyanobacteria with very thin trichomes. The four strains included in the genus were isolated from benthic microbial mats in a man-made hypersaline pond. Trichomes were around 1μm thick, with small constrictions at the crosswalls and diffluent colourless sheaths. Thylakoids were parallel to the cell wall, but thylakoids and nucleoid were often ex-centrically arranged within the cytoplasm with respect to the main trichome axis.

Information on cyanobacterial taxa present in rice fields and other agro-ecosystems in Europe is still scarce and differs significantly from that obtained in Asiatic countries, especially concerning different climatic conditions and pesticide application methodologies (Ariosa *et al*., 2006; Zancan *et al*., 2006). On the other hand, the diversity, ecology and genetic heterogeneity among planktonic Anabaena like and Aphanizomenon-like morphospecies require a deeper characterization, since there is neither a clear phylogenetic nor a morphological separation (Hindak, 2000; Rajaniemi *et al*., 2005; Stuken *et al*., 2009).

Growth media and condition of Cyanobacteria

Most cyanobacteria grow well in the laboratory both in liquid culture and on agar-solidified media. Because some strains do not produce colonies from

single cells with high efficiency on agar-solidified media, various modifications of procedures for the preparation of solid media have been developed in different laboratories (Allen, 1968).

The microalgal growth in pure culture is mainly determined by biotic and abiotic factors. Abiotic factors are like light, temperature, the concentration of nutrients, oxygen and CO_2, pH and mineral composition of the medium *etc.* while biotic factors influences algal growth in culture media like viruses,fungi and bacteria (Mata *et al*., 2010).

Chen and Zhang (1997) mentioned that mixotrophic culture is a potential mode for mass production of cyanobacteria by using heterotrophic capability of them. This type of culture medium can achieve high cell densities and synthesize light-induced products such as photosynthetic pigments and was especially suitable for the production of high value bioactive compounds (Ducat *et al*., 2011).

Abreu *et al*. (2012) also observed higher specific growth rates, final biomass concentrations and productivities of lipids, starch and proteins by *Chlorella vulgaris* under mixotrophic conditions using cheese whey powder as a carbon source when compared with photoautotrophic conditions.

To develop an effective process, a significant step is to search, sort and identify hyper-lipid-producing strains of cyanobacteria. Successful isolation can lead to the selection of appropriate cyanobacterial strains with relevant properties for specific culture conditions and products. Culture condition optimization of the selected strains is of fundamental importance to the success of the process. Patel *et al*. (2014) obtained 130.76 per cent more lipid production compared with the control by *Synechocystis* sp. PCC 6803 using optimized culture conditions.

Isolation of cyanobacteria

The extension of the techniques of microbiology to the study of blue-green algae has been slow and relatively few representatives of the group have so far been obtained in pure culture (Koch, 1964). The isolation of these organisms from natural sources would be facilitated if cultures could be enriched in them by methods which effectively counter-selected eucaryotic algae. One such method was discovered by Beijerinck (1902), who showed that blue-green algae of the Nostoc type can be selected by using a mineral medium devoid of a combined-nitrogen source.

Subsequent studies (Allen, 1952; Fogg, 1956) suggest that the ability to fix nitrogen is largely restricted to blue-green algae belonging to the Nostocaceae and is by no means universal in the members of this class. Consequently, Beijerinck's enrichment method, although completely effective in eliminating competition from eukaryotic algae, yields an extremely restricted fraction of the total blue-green algal microflora. Apart from the nitrogen-fixers, the blue-green algae seem to have nutritional requirements very similar to those of other algae, so that the possibility of devising other enrichment methods based on the principle of nutritional selection does not appear to be promising.

Wilson *et al.*(1993) described five marine cyanophages propagated on *Synechococcus* sp. strain VWH7803 were isolated from three different oceanographic provinces during the months of August and September 1992. The five cyanophage isolates were found to belong to two families, Myoviridae and Styloviridae, on the basis of their morphology observed in the transmission electron microscope. DNA purified from each of the cyanophage isolates was restricted with a selection of restriction endonucleases and three distinguishably different patterns were observed. DNA isolated from the Myoviridae isolate from Woods Hole had a unique restriction pattern. Southern blotting analysis revealed that there was a limited degree of homology among all cyanophage DNAs probed, but clear differences were observed between cyanophage DNA from the Myoviridae and that from the Styloviridae isolates. Polypeptide analysis revealed a clear difference between Myoviridae and Styloviridae polypeptide profiles, although the major, presumably structural, protein in each case was 53 to 54 kDa.

Traditional isolation techniques include the use of a micropipette for isolation under a microscope or cell dilution followed by cultivation in liquid media or agar plates (Duong *et al*., 2012).

Cyanobacteria are a highly diverse group in relation to form, function and habitat (Neilan *et al.*, 1995). Current cyanobacterial systematics relies on the observation of morphological characters. They designed general primers to the phycocyanin operon (*cpc* gene) and developed a PCR which allows the amplification of a region of this gene, including a variable intergenic spacer sequence. Because of the specificity of this PCR for cyanobacterial isolates, the assay is appropriate for the rapid and reliable identification of strains in fresh water samples. Successive restriction endonuclease digestion of this amplification product, with a total of nine enzymes, yielded many identifying DNA profiles specific to the various taxonomic levels of cyanobacteria. The restriction enzyme profiles for *Msp*I, *Rsa*I, and *Taq*I were conserved for strains within each of the eight genera (40 strains) studied and clearly discriminated among these genera. Intrageneric delineation of strains was revealed by the enzymes *Alu*I, *Cfo*I, and *Hae*III for members of the genus *Microcystis*, while strains of genus *Anabaena* were differentiated by the digestion patterns provided by *Alu*I, *Cfo*I, and *Scr*FI. Phenetic and cladistic analyses of the data were used to infer the genetic relatedness and evolution of toxic and bloom-forming cyanobacteria.

Despite the existence of morphologically diverse cyanobacteria in a wide variety of terrestrial and aquatic habitats, work with these bacteria has been restricted to a relatively few representatives. Castenholz (1988) suggested that the techniques normally used to isolate cyanobacteria may severely limit the number of cyanobacterial species which can be readily cultured.

Agar, which is routinely used as a solidifying agent in bacteriological media, is known to contain impurities Krieg and Gerhardt (1981), and some of these are suspected to be responsible for the repeated observation that agar is inhibitory to the growth of some cyanobacteria (Allen and Gorham, 1981). Different approaches have been used in an attempt to lower or eliminate the growth-inhibitory effects of agar. These have included the use of low agar concentrations (Allen and Gorham, 1968 and Shirai *et al.*, 1989), agar-washing procedures (Allen *et al.* (1981), Krieg and Gerhardt (1981), the separate sterilization of agar and nutrient solutions (Allen, 1968) and the substitution of agarose or other alternative solidifying agents (Shirai *et al.*, 1989 and Thiel *et al.*, 1989).

Algae are photosynthetic thallophytesor lower plants which grow in water or on soil saturated with water. Blue green algae are distributed worldwide to enhance the fertility of many agricultural ecosystems. Blue green algae are a group of microalgae that can fix the atmospheric nitrogen. Isolation of these cyanobacteria from natural sources in pure form is essential step for their efficient use as biofertilizer (Mulani and Pawar, 2015). The isolation of pure cultures were done by selecting a single colony from mix cultures grown on selected media like BG-11, bold basal media, ASN III media as different BGA strains can grow on different media. The same media in solid form is used for further purification and sub-culturing

Identification of Cyanobacteria Species

Based on Morphology

Cyanobacteria exhibit the most diverse and complex morphology among all prokaryotic groups, perhaps indicating that they would have been the dominant life forms at some period of the early earth. External gross appearance under aquatic and moist conditions is often gelatinous, slimy and occasionally filamentous clusters, with colours ranging from dark green, blue-green, yellow, brown to black, and rarely red (Kulasooriya, 2011).

Cyanobacteria can be classified on the basis of morphology, cellular differentiation, Biochemical, Physiological and Genetic criteria. The taxonomy of cyanobacteria until now has been based mainly on their morphology and according to it they are classified into five orders: Chroococcales (I), Pleurocapsales (II), Oscillatoriales (III), Nostocales (IV) and Stigonematales (V). However, the morphology and other phenotypic characteristics of cyanobacteria can be dramatically influenced by environmental factors and stage of development (Suda *et al*., 2002 and Rajaniemi *et al*., 2005). Thus, classifications based on phenotypic characteristics do not represent natural grouping based on genetic data analysed.

According to Castenholz (1989) and Komarek and Anagnostidis (1989), the order Nostocales includes

filamentous cyanobacteria that are capable of cell differentiation in heterocysts, akinetes or reproductive trichomes (hormogonia). The genera *Anabaena, Aphanizomenon* and *Nostoc* belong to the family Nostocaceae by traditional classification (Komarek and Anagnostidis, 1989; Komarek, 2010) and subsection IV according to bacteriological classification (Rippka *et al*., 1979; Castenholz and Waterbury, 1989). This family is characterized morphologically by (i) isopolar filaments, (ii) absence of any branching (with the exception of certain anomalies), (iii) presence of heterocysts (with the exception of secondary derived genotypes) and (iv) facultative presence of typical paraheterocytic or apoheterocytic akinetes (Komarek, 2010).

Anabaena, an environmentally important cyanobacterium has been identified mainly on the basis of morphological characteristics, such as shape and size of trichomes, cell types, size and location of heterocysts and akinetes and plane(s) of division (Rippka *et al*., 1979).

According to the accepted morphological and ecological descriptions by Desikachary (1959), the genus *Anabaena* is identified based on the Presence of uniform trichomes, absence of sheath or presence of more or less diffluent sheath forming free or floccose or soft mucilaginous thallus. Heterocyst's, generally intercalary and presence of a single or series of spores near the heterocyst or between the heterocyst's.

Biodiversity

Cyanobacteria are genetically highly diverse, they occupy a broad range of habitats across all latitudes perhaps demonstrating the abilities of their pioneering ancestors as the earliest inhabitants of Earth. They are not only widespread in freshwater, marine and terrestrial ecosystems but also found in extreme habitats such as hot springs, hypersaline localities, freezing environments and arid deserts (Fogg *et al.*, 1973).

The majority of cyanobacteria are aerobic photoautotrophs. Their life processes require only water, carbon dioxide, inorganic substances and light. Photosynthesis is their principal mode of energy metabolism. In the natural environment, however, it is known that some species are able to survive long periods in complete darkness. Furthermore, certain cyanobacteria show a distinct ability for heterotrophic nutrition (Fay, 1965).

Cyanobacteria are often the first plants to colonise bare areas of rock and soil. Adaptations, such as ultraviolet absorbing sheath pigments, increase their fitness in the relatively exposed land environment. Many species are capable of living in the soil and other terrestrial habitats, where they are important in the functional processes of ecosystems and the cycling of nutrient elements (Whitton, 1992).

Diverse species having different ecological demands exhibit differential adaptations to the conditions at their source locality. The massive communities of *Prochlorococcus* in the oligotrophic deep oceans that live in close association with cyanophages enabling rapid lateral transfer of genomic material opens up new vistas on biological adaptation and evolution (Johnson *et al.,* 2006). The endolithic *Chroococcidiopsis* living as the only inhabitant in the extremely inhospitable habitat of the arid atacama desert provides a new tool for Astrobiology (Friedmann and Imre, 1982 and Wierzchos *et al*., 2006).

Microalgal biodiversity in of blue green algae or cyanobacteria in fresh and marine habitat has been investigated in India and abroad by many workers. The pioneer workers are Allen and Stanier (1967) who have cultured different aquatic blue green microalgae in laboratory.

Based on fossil records, Cyanobacteria have occurred as long ago as 2,600 to 3,500 million years (Myr), (Brocks *et al*., 1999). These earliest cyanobacteria are believed to have been played an important role in producing an oxygen-rich atmosphere on earth about 2,300 Myr ago (Blankenship, 1992). These organisms are identified according to their morphological characters such as morphology of vegetative cells, akinetes and heterocytes (Rajaniemi *et al*., 1992, Rippka *et al*., 1979).

Molecular characterization

In recent years, the use of molecular markers for identification of cyanobacteria in diverse niches has gained considerable significance. Molecular

datasets can effectively complement morphological characterization. Molecular markers such as restriction fragment length polymorphism (RFLP) and 16S rRNA, have been employed in phylogenetic analysis and characterization of cyanobacterial diversity (Giovannoni *et al*., 1990).

Due to existence of changeable morphology in these organisms, other techniques such as molecular techniques are used to improve cyanobacterial taxonomy. Several properties of the 16S rRNA gene, such as evolutionary properties and ubiquity have allowed it to become the most commonly used molecular marker to distinguish and establish relationships between cyanobacterial genera and species (Case *et al*., 2007).

The presence of highly conserved repetitive sequences in the genomes of microorganisms makes them highly useful for strain differentiation and diversity analysis. Repeat elements, such as short tandemly repeated repetitive (STRR), long tandemly repeated repetitive (LTRR) sequences (Mazel *et al*., 1990) or palindromic sequences such as highly iterated palindrome (Hip1) have been found in a number of cyanobacteria, especially heterocystous genera. PCR fingerprinting techniques based on such sequences have proved valuable in the identification and analysis of symbiotic cyanobacteria (Rasmussen and Svenning,1998). Molecular techniques have also been utilized to ascertain the genetic relationship of the genus Anabaena with genera such as Aphanizomenon and Nostoc (Gugger *et al*., 2002).

Sequencing of 16S rRNA while confirming the existence of several morphologically uniform and well-defined traditional genera has also placed similar morphotypes in distant positions in phylogenetic trees. Molecular data provide basic criteria for cyanobacterial taxonomy, but to construct a comprehensive phylogenetic system of cyanobacteria a combination with knowledge on their morphology, physiology and biochemistry is essential (Komarek and Komarkova, 2006).

The shared simple morphological characters as well as limitations of cultivation have made microscopic studies usefulness. One way to better characterize these morphologically similar species is to use molecular diversity information. It should also be noted that in studies where near-complete 16S rRNA gene sequences have been used, conflicts between morphological and molecular identification of some cyanobacterial sequences have been found (Hongmei *et al*., 2005).

Molecular techniques based on PCR amplification targeting conserved regions inside the 16S rRNA gene have allowed determination of phylogenetic affiliations among cyanobacteria and development of modern cyanobacterial taxonomy (Komarek and Komarkova, 2006).

Lachance (1981) investigated the genetic relatedness of 45 strains of heterocyst forming cyanobacteria assigned to eight genera by Rippka *et al.* (1979) and 19 undescribed strains of the same group by *in vitro* reassociation of radioiodinated deoxyribonucleic acids. The members of the genera *Nodularia, Cylindrospermum, Chlorogloeopsis* and *Fischerella* formed discrete clusters and showed intergeneric relatedness of less than 40 per cent, results consistent with the classification proposed by Rippka *et al.* (1979). The genus *Nostoc* was heterogeneous; four strains previously assigned to *Anabaena* appeared to belong to *Nostoc.* The genus *Calothrix* comprised four clusters with various degrees of internal homogeneity and two strains which showed low relatedness to any others. The general relatedness (*i.e*., relative binding) of heterocyst formers to various non-heterocystous cyanobacteria was on the order of 10 to 20 per cent.

The rapid and sensitive methods for the detection and genetic characterization of cyanobacteria have been developed based on DNA amplification techniques. Work describes the molecular methods that have been used to characterize cyanobacteria and their use as tools to identify toxin-producing strains. Different species and strains were compared using restriction fragment length polymorphism (RFLP) of amplified fragments of the phycocyanin gene and the 16S-23S rRNA internal transcribed spacer.

Kondo *et al.* (2002) stated that DNA base composition and DNA–DNA hybridization among the

cyanobacterial genus *Microcystis* were determined using nine axenic *Microcystis* strains, including the three 'morphological' species of *M. aeruginosa*, *M. viridis* and *M.wesenbergii*. These *Microcystis* spp. showed a similar DNA base composition (42<1–42<8 mol% GMC) and demonstrated more than 70 per cent DNA relatedness, confirming their synonym based on bacterial criteria.

Nubel *et al.* (1997) developed and tested a set of oligonucleotide primers for the specific amplification of 16S rRNA gene segments from cyanobacteria and plastids by PCR. PCR products were recovered from all cultures of cyanobacteria and diatoms that were checked, but, not from other bacteria and archaea. Gene segments selectively retrieved from cyanobacteria and diatoms in unialgal but nonaxenic cultures and from cyanobionts in lichens could be directly sequenced. In the context of growing sequence databases, this procedure allows rapid and phylogenetically meaningful identification without pure cultures or molecular cloning.

Sivonen *et al.* (1992) described that Hepatotoxins (microcystins) from seven freshwater *Anabaena* strains originating from three different Finnish lakes and one lake in Norway were isolated by high-performance liquid chromatography and characterized by amino acid analysis and fast atom bombardment mass spectrometry. All strains produced three to seven different microcystins. A total of 17 different compounds were isolated, of which 8 were known microcystins.

Barker *et al.* (1999) explained the filamentous diazotrophic cyanobacterium *Nodularia* forms water blooms each year in the Baltic Sea. Filaments isolated from such water blooms vary in their trichome width, degree of coiling, and properties of their gas vesicles. To test the validity of such a phenotypic classification, they determined the nucleotide sequences for a region of the phycocyanin locus that includes a noncoding intergenic spacer (PC-IGS), the IGS between two adjacent copies of the *gvpA* gene (which encodes the main structural gas vesicle protein) and the rDNA internal transcribed spacer (rDNA-ITS), for 13 clonal *Nodularia* isolates. The complete 16S-rDNA sequence was determined for three isolates and was found to be identical in each of them.

Suda *et al.* (2002) explained a polyphasic approach to clarify the taxonomy of the water bloomforming oscillatorioid cyanobacteria. Seventy-five strains of oscillatorioid cyanobacteria were characterized by 16S rDNA sequence analysis, DNA base composition, DNA–DNA hybridization, fatty acid composition, phycobilin pigment composition, complementary chromatic adaptation, morphological characters, growth temperature and salinity tolerance.

The recent study by Sanchez *et al.* (2005) shows that the cyanobacterium *Microcoleus chthonoplastes* forms a consortium with heterotrophic bacteria present within the cyanobacterial sheath. They showed that this consortium was able to grow in the presence of crude oil, degrading aliphatic heterocyclic organo-sulfur compounds as well as alkylated monocyclic and polycyclic aromatic hydrocarbons. They characterized the oil-degrading consortium through the analysis of the 16S rRNA gene sequences. They also performed the study in cultures of *Microcoleus* grown in mineral medium and in cultures of the cyanobacterium grown in mineral medium supplemented with crude oil. The results indicate that most of the clones found in the polluted culture correspond to well-known oil degrading and nitrogen-fixing microorganisms, and belong to different phylogenetic groups.

Ouellette *et al.* (2006) studied on algae blooms, which include the toxic cyanobacterium *Microcystis*, have reoccurred in the Laurentian Great Lakes, most commonly in the western basin of Lake Erie. Whereas, the western basin is the most impacted by toxic *Microcystis* in Lake Erie, there has historically been little effort focused on identifying the spatial distribution of *Microcystis* throughout this lake. Therefore, to address this lack of knowledge, they have employed a polymerase-chain-reaction-based detection of genes required for synthesis of the toxin microcystin (mcyD and mcyB), as well as 16S rDNA fragments specific to either all *Microcystis* or all cyanobacteria.

Cultivation of cyanobacteria

Extensive studies have been carried out for the cultivation of different cyanobacteria and microalgae using a variety of cultivation systems ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks.

The most commonly used cultivation systems include shallow big ponds, tanks, circular ponds and raceway ponds (Oron *et al*., 1979). One of the major advantages of open ponds is that they are easier to construct and operate than most closed systems (Borowitzka, 1999). However, major limitations in open ponds include poor light utilization by the cells, evaporative losses, diffusion of CO_2 to the atmosphere and requirement of large amounts of water and land and low biomass productivity (Posten and Schaub, 2009).

Santillan (1982) identified an alternative to open ponds are closed ponds where the control over the environment is much better than that for the open ponds. Closed pond systems are more cost intensive than the open ponds, and considerably less than photobioreactors for similar areas of operation. It allows more species to be grown, it allows the species that are being grown to stay dominant, and it extends the growing season. Usually closed ponds are used in *Spirulina* cultivation

Closed bioreactors have some specific advantages (Pulz, 2001; Posten and Schaub, 2009). Firstly, they can distribute the sun light over a larger surface area, which can be up to 10 times higher than the footprint area of the reactor. Secondly, evaporation can be avoided. The only water loss is due to the water content in the wet cyanobacteria product. This allows for the cultivation of cyanobacteria also in arid areas, where classical terrestrial agriculture is not possible. Limiting factors are the high reactor costs and the need for auxiliary energy requirements. However, ongoing research in the reactor field is promising and will lead to cheaper and more energy-effective designs (Posten and Schaub, 2009).

The cultivation of cyanobacteria / microalgae in sewage and wastewater treatment plant is expected to bring double benefit to the environment since that they can be used to extract nutrients from waste water, and convert it to fats for biodiesel production and reduces pollution from the atmosphere. Unlike other algal-biofuel technologies this approach relies on 'wild algae' – *i.e.* algae that naturally colonize sewage ponds already (Metcalf and Eddy, 1980).

Matsunaga *et al*. (2005) stated that another economical way of cultivating cyanobacteria/ microalgae is sea water (salt water). The main nutrients needed for their growth is already present in seawater. Seawater is a solution of salts of nearly constant composition, dissolved in variable amounts of water. There are over 70 elements dissolved in seawater with six of them make up >99 per cent of all the dissolved salts; all occur as ions-electrically charged atoms or groups of atoms (Sodium, Chlorine, Magnesium, Potassium, Sulfate and Calcium).

Cyanobacteria are extensively cultivated for the production of biofertilizer, microbial protein, plant growth- promoting substances and rare chemicals such as carotein and enzymes. A chemostat constructed by Thomas (1973) was found suitable for the growth of filamentous photosynthetic microorganisms (e.g. *Anabaena).*

Large quantities of cyanobacterial biomass are needed to prepare biofertilizer for application to rice fields. Use of conventional fermenter vessels would result in cost escalation, whereas, open-air cultivation leads to the growth of unwanted microbes. To grow photoautotrophs one has to either use glass culture vessels with outside fights or use metal culture vessels provided with inside submersible lamps. Large volume glass vessels are very expensive.

The cultivation of algae involves optimization of various factors like pH, alkalinity, salinity, aeration, temperature, light etc. The pH of the culture medium is one of the important factors in algal cultivation. It determines the solubility of carbon dioxide and minerals in the medium and directly and indirectly influences the metabolism of algae (Becker, 1994). Algae exhibit a clear dependency on the pH of the growth medium and different species vary greatly in their response to the pH. Among soil properties pH is a very important factor in growth, establishment and diversity of Cyanobacteria, which can generally be reported to prefer neutral to slightly alkaline pH for optimum growth.

Ciferri and Tiboni (1985) mentioned that *Spirulina* sp. could thrive pH range 7 to 11.3. Numerous reports of cyanobacteria in fresh water and

soil indicated that their diversity and abundance were greatest at higher pH (Kanniayan and Kumar, 2004).

Cyanobacteria prefer an optimum temperature between 30 and 35°C. Ray and Bagchi (2001) optimized the culture conditions for growth of *Oscillatoria laetevirens*, at a temperature of 28 ± 2°C and illumination by cool white fluorescent lamps of intensity 25 Wm^{-2}.

Biochemical Composition of Cyanobacteria

Cyanobacteria are impressive ecosystem engineers with an evolutionary history stretching back at least 2.15 billion years (Hayes *et al*., 2007). These oxygenic photoautotrophic prokaryotes are widely distributed in natural environments and constitute a major component of microbial populations in terrestrial and aquatic habitats worldwide. They are often referred to as 'miniature factories' of the biological world and represent an alternative source of a variety of bioactive compounds, lipids/fatty acids, proteins, enzymes, pigments and compounds of pharmaceutical and nutraceutical.

Microalgae represent a valuable source of a wide spectrum of lipids with different potential applications. Especially interesting are the polyunsaturated fatty acids, including the essential fatty acids linoleic acid, a-linolenic acid (ALA), g-linolenic acid (GLA), arachidonic acid (AA), and eicosapentaenoic acid (EPA). Essential fatty acids are precursors of prostaglandins and, as such, are becoming increasingly important in the pharmaceutical industry (Borowitzka, 1988 and Becker, 1994).

Cyanobacteria or blue-green algae are photosynthetic microorganisms that can be used to produce high-value compounds (Vincent, 2009). These include high protein content; capacity to synthesize all amino acids (and provide the essential ones to humans and animals); presence of carbohydrates composed of starch, glucose, sugars and non-digestible polysaccharides (agar, carrageenan and alginate); lipids in the form of glycerol and fatty acids of the ù-3 and ù-6 families; and a valuable content of many essentials vitamins, minerals and antioxidant substances. With this biochemical composition is not a surprise that this microorganism can be used as a food source for animal and humans (Gantar and Svircev, 2008).

Gantar and Svircev (2008) reported that dried microalgal biomasses typically contain 46–63 per cent protein, 8–17 per cent carbohydrates, and 4–22 per cent lipids, as well as a wide range of vitamins and other biologically active substances such as bioactive peptides and pigments.

Nostoc, an edible blue-green alga, is a cyanobacterium that has been grown and cultivated for medicinal uses for centuries (Gantar and Svircev, 2008). Recent studies have indicated that *Nostoc* contains cryptophycin, a compound that inhibits cancer cell growth, as well as anti-viral compounds (Cunningham and Joshi, 2010).

Filamentous cyanobacteria *Nostoc*, *Spirulina*, *Arthrospira*, *Anabaena*, *Aphanizomenon*, *Rivularia,* and many others are particularly attractive for the production of high quality biomass, because they represent a source of protein and a variety of chemicals and pharmaceuticals (Gantar and Svircev, 2008).

Cyanobacteria are commercially exploited in several countries for their valuable constituents particularly for proteins, pigments and polyunsaturated fatty acids. *Spirulina* is known for its high protein content and quality, and is considered to be a rich source of single cell protein (Reed *et al*., 1985).

Most reports on fatty acids in cyanobacteria indicate an acyl chain length of 18 or fewer carbon atoms, with 16:0, 16:1, 18:2, and ALA present as the major fatty acids. Based on their fatty acid composition, cyanobacteria have been classified into four groups. Strains in the first group contain only saturated fatty acids (SAFAs) and monounsaturated fatty acids (MUFAs), whereas strains in the other groups contain polyunsaturated fatty acids (PUFAs) in addition to SAFAs and MUFAs. The second group is characterized by the presence of ALA, the third group by GLA, and the fourth group by 18:4. Therefore, cyanobacteria are quite variable in their fatty acid composition, with marked differences occurring even within the same genus (Kenyon, 1972, Murata *et al*., 1992).

Biochemical studies in cyanobacteria (Holton *et al*., 1968) demonstrated a significant correlation between the morphological complexity of species and their fatty acid composition.

Algae accumulate a variety of carbohydrates as reserve or storage materials; these are often found in large amounts in the cell: the carbohydrates are polyglucans (starch), ß-1,3glucans, fructosans, inulin, sucrose, and poly hydroxy alcohols (Craigie, 1974).

Cyanobacteria contain significant quantities of lipids and some of them are also rich in essential fatty acids such as linoleic and gamma linolenic acids. Besides nutritional value, the fatty acids of cyanobacteria are generally used to clarify taxonomical problems (Li and Liu, 2001).

Fatty acids, in general are of commercial value and many are pharmaceutical agents. The cyanobacterium *Spirulina* has commercially exploited in several countries. It has been used as food for many centuries in Central America. For the past two decades, *Spirulina platensis* has been a focus of interesting among researchers in various fields because of its commercial importance as a source of proteins, vitamins, essential amino acids and fatty acids and more recently, for its potential in therapeutic effects (Belay *et al*., 1993).

Venkataraman and Mahadevaswamy (1992) pointed out that good culture management with suitable strain is one of the basic needs to get promising yields with quality material on commercial scale. Mass cultivation of cyanobacteria is essentially a complex process involving a large number of variables for successful growth of essential requirements of the organism as possible. The limitations imposed in the cultivation process be due to physical factors like light, nutrients, temperature, pH and physiological (organism-environmental-interrelationship) and economic constraints.

Beneficial effects of Cyanobacteria

Cyanobacteria also known as blue-green algae, exhibit diversity in metabolism and structure also along with morphology and habitat. Moreover, cyanobacteria and microalgae have simple growth requirements and use light, carbon dioxide and other inorganic nutrients efficiently. These organisms also capable of both oxygenic photosynthesis and hydrogen production. Photo-biological production of H_2 by microorganisms is of great public interest because it promises a renewable energy carrier from nature's most plentiful resources: solar energy and water. It has been investigated to produce different feed stocks for energy generation like hydrogen (by direct synthesis in cyanobacteria), lipids for biodiesel and jet fuel production, hydrocarbons and isoprenoids for gasoline production and carbohydrates for ethanol production (Ueda *et al.,* 1996).

Studies by Singh *et al*. (2005) have indicated that BGA have antiviral, antitumor, antioxidant, anti-inflammatory, antiallergic, antidiabetic, and antibacterial properties as well as lipid lowering effects. In particular, inhibitory effects of BGA on hyperlipidemia, inflammation, and oxidative stress can contribute to the prevention of the development of CVD and non alcoholic fatty liver disease (NAFLD).

Cyanobacteria for food and nutritional value

Cyanobacteria are one of the beneficial organisms widely used in food industries and in few biotechnological applications. They store reserve food materials which can be used as the source of pigments, lipids, vitamins, proteins and certain secondary metabolites (Tan, 2007; Cardozo *et al*., 2007). In addition, cyanobacteria also have the capacity of fixing atmospheric nitrogen. These qualities make cyanobacteria the most successful and widespread group among the prokaryotes, occupying a wide range of terrestrial and aquatic environments.

Cyanobacterial protein has received worldwide attention for either as food supplement or as an alternative source of food. Some species of *Anabaena, Nostoc* and *Spirulina* are consumed as food due to their high protein and fibre content (Anupama, 2000).

The consumption of cyanobacterial and microalgal biomass as a human health food supplement is currently restricted to only a few species, e.g., *Spirulina* (Arthospira), *Chlorella*, *Dunalliella* and to a lesser extent, *Nostoc* and *Aphanizomenon* (Spolaore *et al*., 2006).

Alga has gained a particular interest as a bio-resource that can be used in food as well as energy production. Due to consisting of 60–70 per cent (wet basis) proteins, 12 amino acids, vitamins (A, B1, B2,

B6, B12, E, K) and minerals (iron, calcium, potassium, phosphorus, manganese, copper, zinc, magnesium), the microalgae is assumed to be useful for its nutritional qualities and curing properties (Dissa *et al*., 2010).

Cyanobacteria as fertilizers

Cyanobacterial and microalgal biomass are used as a plant fertilizer and to improve the water-binding capacity and mineral composition of depleted soils (Metting *et al*., 1990). Moreover the effluent generated during anaerobic digestion for biomethane production can also be used as a fertilizer.

Nitrogen (N) is generally the most difficult nutrient to manage for organic crop production, and N uptake is dependent on the amount of plant-available N supplied by the soil. In organic systems, the most important limiting factor appears to be N availability since soil N commonly exists in organic forms that are not available to plants. In managing plant nutrients over continuing crop cycles, farmers face the challenge of estimating the amount of the soil organic N resource that is made available for plant uptake overtime (Deenik, 2006).

Cyanobacteria as biofuel

Human society has an insatiable appetite for fuels and today's supply of liquid fuels world-wide is almost completely dependent on petroleum. Bioenergy production has recently become a topic of intense interest due to increased concern regarding limited petroleum-based fuel supplies and the contribution of the use of these fuels to atmospheric CO_2 levels. Finding sufficient supplies of clean energy for the future is society's one of the most daunting challenges and is intimately linked with global stability, economic prosperity and quality of life. This leads to interesting questions and debate over the choice of new fuels, produced from new raw materials, to complement or replace present petroleum-based fuels (Posten and Schaub, 2009).

Cyanobacteria can be developed as an excellent microbial cell factory that can harvest solar energy and convert atmospheric CO_2 to useful products. Fossil traces of cyanobacteria are claimed to have been found from around 3.5 billion years ago, and most probably played a key role in the formation of atmospheric oxygen, and are thought to have evolved into present-day chloroplasts of algae and green plants (Tamagnini *et al*., 2007).

Singh and Gu (2010) in their review article have compared the biodiesel yields from microalgae with other best oilseed crops. Biodiesel yield is 58,700 l/ha from microalgae containing only 30 per cent oil (w/w), compared to 1190 l/ha for rapeseed and canola. 1892 l/ha for jatropha, 2590 l/ha for karanj (*Pongamia pinnata*) 172 l/ha for corn; 446 l/ha for soybean; 1059 l/ha for peanut; 2689 l/ha for coconut; 5950 l/ha for oil palm.

Chisti (2007) discussed the economics and quality constraints of biodiesel from microalgae in his review paper. He pointed out that the cost of growing microalgae for biofuel production must be drastically reduced to compete directly with traditional energy sources. It is essential to consider the other roles cyanobacterial cultures can play concurrently with biofuel production and the long term benefits this entails.

Cyanobacteria have already been engineered to produce a number of different biofuel related compounds (Machado and Atsumi, 2012). In one of the first examples of biofuel production in cyanobacteria, *Synechococcus elongatus* sp. strain PCC 7942 (*S. elongatus*) was successfully engineered to produce ethanol through the addition of a pyruvate decarboxylase and an alcohol dehydrogenase, redirecting carbon from pyruvate (Deng and Coleman, 1999). Cyanobacterial production of ethanol has since been significantly improved (Dexter and Fu, 2009; Gao *et al*., 2012).

There are several aspects of cyanobacterial biofuel production that have combined to capture the interest of researchers and entrepreneurs around the world. These include: (1) They are able to perform oxygenic photosynthesis using water as an electron donor, (2) They grow to high densities and have high per-acre productivity compared to typical terrestrial oil-seed crops. Consequently, mass cultivation for commercial production of cyanobacteria can be performed efficiently, (3) They are non-food based feedstock resources, (4) They use otherwise non-

productive, non-arable land, (5) They utilize wide variety of water sources (fresh, brackish, seawater and wastewater) (Tamagnini *et al*., 2007), and (6) They produce both biofuels and valuable coproducts.

Cyanobacterial biomass

Photosynthetic biomass is a promising resource for the generation of biofuels and other valuable bioproducts. However, rapid biomass production and high-yield conversion processes are essential for successful applications. Plant-derived lignocellulosic biomass is abundant but, due to the recalcitrant nature of this material, significant challenges have to be solved if this biomass is to be used for the microbial production of biofuels and bioproducts (Mamo *et al.*, 2013).

Whole-cell material from starch-enriched green microalgae and glycogen enriched cyanobacteria (Aikawa *et al*., 2013) has recently been used as feedstock for bioethanol production by yeast fermentation. These studies employed various enzymatic, chemical, and physical treatments (including drying, heating, acid and base-treatment) to liberate monomeric hexoses from the biomass.

Beyond that, the complete algal biomass can also be processed for syngas production followed by a fischer tropsch process, hydrothermal gasification for hydrogen or methane production, methane production by anaerobic digestion, and co-combustion for electricity production. Hence, cyanobacterial system could contribute to a sustainable bioenergy production. However, different biotechnical, environmental and economic challenges have to be overcome before energy products from this system can enter the market.

Lipid content of cyanobacteria

Oleaginous microorganisms (microalgae, fungi, yeast, and bacteria), with their ability to produce significant amounts of lipids, are an attractive source of oil suitable for biodiesel production (Bellou *et al*., 2014), especially since their fatty acid composition is often similar to that of common plants currently used as feedstock in biodiesel manufacturing (Vicente *et al*., 2009).

Cyanobacterial lipid could be one of the promising feedstocks for biodiesel production (Yang *et al*., 2011). As common photosynthetic prokaryotic organisms, cyanobacteria can be technologically developed as an excellent microbial cell factory that can harvest solar energy and transform atmospheric carbon dioxide to useful organic compounds (Parmar *et al*., 2011).

Cyanobacteria have basic growth requirements, and use carbon dioxide, light and other inorganic nutrients effectively (Parmar *et al*., 2011). However, some cyanobacteria and microalgae could use both organic carbon sources and carbon dioxide under mixotrophic conditions, and exhibit higher biomass and lipid yields.

The primary components of cyanobacterial biomass are proteins, carbohydrates, and lipids. Some species, such as *Spirulina* sp. and *Planktothrix rubescens*, are suitable for protein-rich biomass production, since their protein content is very high (Demirbas, 2011). Furthermore, some cyanobacteria-for example, *Nostoc muscorum*, are able to accumulate starch in high concentrations, and thus can be used as feedstock for hydrogen production. While the accumulation of lipids in cyanobacterial biomass is fairly low, their fatty acid composition is suitable for biodiesel production (Demirbas and Demirbas, 2011).

Fatty acids are used in membranes formation maintaining cell viability. Knowing the mechanisms of fatty acids synthesis provides a major economic effect on the achievement of the biotechnological processes in order to obtain the desired products. The importance as biotechnological objects and their use in biodiesel production was determined by the cyanobacteria specific fatty acids and their ability to modify lipid profile depending on the cultivation conditions. The biodiesel properties depends on the chemical structure of fatty acid methyl esters. Saturated fatty acids gives a more stable biodiesel than unsaturated fatty acids. Changing the cultivation conditions leads to fatty acids biosynthesis (Ludmila, 2006).

Cyanobacteria in agriculture

Cyanobacteria are important microorganisms in agricultural soils and especially in rice fields, where a large variety of them occur (Whitton, 2000). Cyanobacteria are oxygenic photoautotrophs and are known to produce a wide variety of secondary metabolites that may be important for a proper

functioning of agricultural soils. In particular, filamentous cyanobacteria play multiple roles in the enhancement of soil fertility and thus in the improvement of crop plants.

In the rhizosphere, cyanobacteria directly interact with plants through a variety of secondary metabolites. Phytohormones such as auxins are commonly used by rhizospheric microorganisms in order to interact with. In response, the plants secrete a chemically rich mixture of organic and inorganic compounds in the form of root exudates that mainly serve to attract and assist the growth of microorganisms in rhizosphere (Lambers *et al*., 2009).

Phototrophic, N-fixing cyanobacteria have been used as a biofertilizer in rice paddies for centuries and more recently, as a soil inoculum in dryland crops (Maqubela *et al*., 2010). Each season, paddy inoculation can add 30 kg of fixed N per hectare in flooded rice fields and increase grain yield by 10 to 20 per cent. In addition to supplying N, cyanobacteria can secrete plant growth hormones and build soil organic matter. The purpose of using cyanobacteria as an inoculum is to establish populations of cyanobacteria to increase biological N-fixation within the soil.

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