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Ecology and Biodiversity Analysis of Cyanobacteria

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Abstract

Cyanobacteria are a group of extraordinarily diverse Gram-negative prokaryotes that originated 3.5 billion years ago. Their diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colourless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkalophilic, planktonic to barophilic, freshwater to marine including hyper saline. They are found both free living and as endosymbionts. They are considered to be one of the potential organisms which can be useful to mankind in various ways. A number of important advances have occurred in cyanobacterial biotechnology in the recent years. World wide attention is drawn towards cyanobacteria for their possible use in food, feed, fuel, fertilizer, colourant, production of various secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals, pharmacological probes and the pollution abatement. Only a few cyanobacterial strains (including Spirulina) have been well characterized or exploited commercially. Basic research is needed to identify new cyanobacterial strains of high value products, strain improvement using molecular tools for rapid growth rate, ability to withstand varied environment conditions and the enhancement synthesis of high value products. This review is intended to focus on the biodiversity of cyanobacteria in various environments, recent application and the new development they are diversifying the directions or commercial exploitation.

Keywords: Cyanobacteria, environment, strains.

Introduction

Cyanobacteria are a photosynthetic prokaryotes and are grouped under the Gram-negative bacteria and their morphology varies from unicellular to multicellular. Some species have unique cells, called heterocysts that are capable of fixing atmospheric nitrogen. They are found in a wide variety of habitats, namely, fresh water, marine water, moist soil, saline and sodic soil as well as in thermophilic and psychrophilic conditions and in symbiotic associations. They are primarily photoautotrophic while some are capable of heterotrophic and mixotrophic growth. Cyanobacteria are a large and morphologically diverse group of phototrophic prokaryotes, which occur in almost every habitat on earth. This versatility may explain the remarkable lack of morphological change seen in 3.5-billion-year-old fossilized cyanobacteria and their modern day counterparts. Their long evolutionary history has been marked by key geochemical and biotic transition, including the creation of the development and proliferation of metabolically complex microbial and higher eukaryotic life forms. The cyanobacteria, which are capable of photosynthetic growth, have been used as a model organism for the study of oxygenic photosynthesis of higher plants because they have relatively simple genetic systems compared with higher

plants in which both the nuclear and chloroplast genomes are involved. The availability of genetic engineering technology based on the transformable characteristics also facilitated the wide use of these microorganisms, for elucidation of gene function. The sequence of the entire genome has so far been reported for Haemophilus influenzae (1.83 Mb) and Mycoplasma genitalium (0.58 Mb), but both are heterotrophic microorganism. Cyanobacterial biomass has been considered since long as an alternative source of protein that could supplement conventional food and feed production. There is historical evidence that blue-green algae were harvested, dried and eaten by the Aztecs of Tenochtitlan (Mexico City) at the time of the Spanish conquest (Farrar, 1966).

Taxonomy of cyanobacteria

The taxonomy system which developed during the 19th century was based almost entirely on morphology and International Code of Botanical Nomenclature take various papers published during the period 1886-1892 as the starting point for the valid publication of names of filamentous forms. Rather surprisingly, the starting point for the morphologically less complex forms is much earlier (Linnaeus, 1753), although there was of course little understanding of their diversity at that time. A number of "floras" summarizing the known species of cyanobacteria (the botanical name) in particular regions have been published during the 20th century. Several of these provide a lot of information about species occurring elsewhere in the world. In any case it is clear that many species have a very wide distribution. The best flora is that of Geitler (1932), which focused on Central Europe, but is still an essential source of information for those wanting to name the organisms in any part of the world. Desikachary's (1959) flora for India could have reached a very wide audience because it is written in English, but unfortunately has long been out of print.

Ecology of rice fields and soils

Light: Cyanobacteria growing on the soil surface often show dark colourations blue-black, brown, red brown or red. The darker colours usually result from the presence of brown sheaths surrounding the typical photosynthetic trichome and this colour is much more pronounced in populations in open than in shaded positions. The pigment responsible for the brown colouration, scytonemin, absorbs strongly in the near ultra-violet region of the spectrum and the evidence strongly suggests that scytonemin production is an adaptive strategy for photoprotection against short wavelength solar irradiation. The darker colour may also enhance warming of the substratum at times are ecologically important for some populations, as discussed by Belnap and Harper (1995). There are many reports of cyanobacteria occurring at depths some way below the surface in agricultural and other soils, but it is not clear to what extent natural populations are able to persist or even increase in the absence of light. Some strains can also photoheterotrophically (Khoja and Whitton 1971, Rippka et al., 1979), but Calothrix marchica was the only one of four Spanish ricefield soil isolates capable of doing so (Prosperi et al., 1992).

Water and desiccation: Air-dried terrestrial and soil cyanobacteria can survive prolonged dry periods, as has been shown in many studies. The most frequently mentioned genera are Anabaena, Aulosira, Cylindrospermum, Frischerella, Lyngbya, Nostoc, Plectonema and Stigonema. The ability can make it relatively easy to store field samples, enrichment cultures or even pure cultures in a dry state. Those genera which form akinetes (Anabaena) do so largely as a response to nutrient or other limitation rather than desiccation (Whitton 1987). Physiological features of desiccation-tolerant cyanobacteria have been reported in a number of studies. For instance, two drought resistant strains accumulated sugars to high concentrations when metric water stress was applied, whereas two strains not showing drought resistances did not do so (Hershkovitz et al., 1991). Although many soil and desert cyanobacteria are highly tolerant of desiccation, they require being rewetted thoroughly for full metabolic processes to resume.

Salinity: There are many accounts of saline (Singh 1961, Ali and Sandhu 1972) and semiarid (Smith *et al.*, 1990) soils containing a rich cyanobacterial

flora. Where the soil becomes thoroughly wetted intervals, crusts of photosynthetic at microorganisms frequently occur at or near the surface and cyanobacteria are usually important components of such crusts. Singh (1950) proposed that enhancement of cyanobacterial growths on saline (usar) soils in India could provide a means of improving soil quality and eventually reversing the trend to increased salinity. Microcoleus to be the most widespread appears cyanobacterium on saline surfaces throughout the world (Whitton 1990), where it can play an important part in stabilization of the underlying soil. Buttars et al., (1998) have developed a method for inoculating sterilized soil crusts, which involves the escape of Microcoleus from alginate beads and its subsequent growth and add to the more conventional methods suggested by Singh (1961).

pH: Cyanobacteria are infrequent below pH 6.0 in most temperate soils; they were, for instance, entirely absent below pH 5.4 in several samples from Ireland (Dooley and Houghton 1973). However, there are situations where they occur at substantially lower pH values. These include tropical soils (Moore 1963) and the edges of small pools. Values down to pH 4.2 or even slightly lower have been recorded in both cases. Nevertheless, the possible occurrence of cyanobacteria at highly acidic sites still requires through re-evaluation. Cyanobacteria may also influence the pH due to their metabolic activity. In addition to the marked changes that can take place in paddy fields and temporary pools overlying soil, they can also occur within surface crusts. Localized values of 10, 2-3 units above the soil pH have been found in desert crusts as a result of photosynthetic activity (Garcia-Pichel and Belnap 1996).

Biodiversity of freshwater cyanobacteria: Cyanobacteria, belonging to the order Chroococcales, and families Oscillatoriaceae and Nostocaceae occur ordinarily as planktonic forms. Several species grow in abundance and colour the entire body of water, forming the so-called waterblooms. Huber-Pestalozzi (cited by Desikachary) lists as many as 41 genera of 251 species of cyanobacteria that occur as freshwater planktons. *Microcystis* is one of the dominant organism that is associated with almost permanent blooms in tropical freshwaters that are exposed to constant sunshine, warmth and nutrients like phosphate, silicate, nitrates, CO_2 and lime. Formation of cyanobacterial blooms in freshwater bodies is essentially due to the buoyant nature of these organisms. Buoyancy is imparted by the gas vacuoles and the rate of surface accumulation of these organisms is dependent upon the number of gas vacuoles within their cells.

Cyanobacteria in freshwaters have been reported from sea level to high altitudes. They are abundant in temple tanks, ponds in the hill ranges and water lakes like Kodaikanal lake, Ooty lake and Yercaud lake situated at altitudes up to 2200 m. Huber-Pestalozzi (1938) has listed species of genera, Anabaena, Anabaenopsis, Aphanizomenon, Arthrospira, Coelospaerium, Gloeotrichia, Lyngbya, Microcystis, Nostoc, Nodularia, Oscillatoria, Spirulina and Gomontina to form water blooms. The water blooms are a nuisance for drinking water supplies since they choke flow in sand filters and are reported to cause mortality of fish and animals. The principal toxic species are Aphanizomenon flos-aquae, Microcystis **Microcystis** aeruginosa, flos-aquae, Gloeotrichia echinulata and several species of Anabaena. Cyanobacteria also occupy a variety of terrestrial environments. Soil is one of the most potential habitats for algal growth particularly in moist or waterlogged conditions. They play a significant role in maintaining soil fertility and in soil reclamation. Various workers have studied the Indian rice field for cyanobacteria during the past half century. Waterlogged rice field is an ideal habitat for these cyanobacteria which are capable of nitrogen fixation. These include species of Anabaena, Aulosira. Calothrix, Cylindrospermum, Gloeocapsa, Nostoc, *Rivularia, Scytonema* and *Tolypothrix*. N₂-fixing cyanobacteria have also been reported in sugarcane and maize field in India. Besides nitrogen fixation by heterocystous and few nonheterocystous forms, certain other cyanobacterial species such as Lyngbya, Microcoleus, Porphyrosiphon and Schizothrix often form crusts on soils. The crust formation interferes with

soil erosion, increases retention of rainwater and reduces the water loss by evaporation. Summarizing past studies of cyanobacterial distribution in rice fields of tropical and temperate countries, it can be concluded that cyanobacterial constitute about 15% of the total algal flora in the tropics and about 2% in the temperate climate.

Biodiversity and endemism: Biodiversity is the variety of life: the different plants, animals and micro-organisms, their genes and ecosystems of which they are a part. It is home to more than one million species of plants and animals, many of which are found nowhere else in the world. The biodiversity of polar cyanobacteria present a subject of considerable debate and uncertainty. In large part this reflects the inadequacy of current taxonomic criteria for these organisms. Broady (1996) pointed to the lack of consistent taxonomic criteria in analyses of the Antarctic microflora in general, but particularly with respect to the cyanobacteria. Few studies of Polar regions to date have used the criteria of Anagnostidis and Komarek (1988) that are based on morphological features and attributes of strains in culture. The slow rates of speciation by cyanobacteria in general in combination with the efficient dispersal abilities of this group of microorganism, and the relatively young age of ice-free environments in the Arctic and Antarctic, suggest that endemism is likely to be rare amongst polar cyanobacteria. Most of the forms identified to date appear to be cosmopolitan taxa. However, the morphological simplicity of cyanobacterial makes a high level of genetic variability. For example, Phormidium autumnale is a commonly uncounted species in both polar regions, and recent studies on lakes in the Bylot Island region of the Arctic (lat. 73 °N) demonstrated that several isolates which conformed to the morphological criteria for this taxon differed greatly in their pigment and growth characteristics.

Biodiversity of marine cyanobacteria: Of the estimated area of 150 million sq km of the earth, about 70.68% is occupied by oceans. However, of all the total photosynthetic productivity of 555.2 billion tons of dry weight/ year on earth, only 34.4% is contributed by the oceans. India has a

vast coastline of over 7500 km: in addition it has many lakes, ponds, puddles, brackish water areas and a tropical climate that results in abundance of natural populations of varied organisms. Cyanobacteria are widespread and abundant in most marine habitats. Their ability to grow in seawater is presumably related to a preference for alkaline conditions and an ability to tolerate high salt concentrations. The resistance, which many species show towards osmotic shock, extremes of temperature and reducing condition, suits their existence in the variety of intertidal habitats. Desikachary (1959) suggested that probably 20% of all known cyanobacteria occur in saline conditions and majority of them are truly marine. However, little work has been done to understand the cyanobacterial biodiversity of marine environments of India. There is several reports number of species from different part of the world including brines. Thajuddin and Subramanian (1992) have made a detailed survey of marine cyanobacterial biodiversity of a continuous stretch of over 2660 km off the coast line from Tirakol of Goa state, Tamil Nadu and from Cape Comorin to Bhimunipattanam of Andhra Pradesh encompassing the regions such as the Arabian Sea, Indian Ocean, Palk Bay, Strait, Gulf of Mannar and Bay of Bengal including Andaman and Nicobar and Lakshadweep Islands. This survey included coverage of not only the shore, deeper sea but also stagnant seawater ponds and puddles, backwater and saltpans.

Rocky shores diversity: The type of substrata in the intertidal area influenced greatly the availability of benthic cyanobacterial biodiversity, Thajuddin and Subramanian (1992) observed that the shore in the Bay of Bengal was essentially sandy and therefore there were only 11 species of cyanobacteria whereas in the Gulf of Mannar region at several places the shores were rocky (sand stones) or coral reefs were present, as many as 145 species were observed which included both benthic and planktonic forms. Little and Renaunt *et al.*, (1973 & 1975) reported that the cyanobacterial community is most abundant on soft, porous rocks such as sand stones. A hard substratum is not essential for growth of

cyanobacterial mats. Cyanobacteria are apparently always present as epiliths, chasmolith and endoliths including sometimes as discrete well development cryptoendolith layer such as that described by Moul (1975), with *Oscillatoria nigroviridis*, *Lyngbya confervoides* and *Phormidium valderianum* at a depth of 6 mm inside the rock.

Sandy shores diversity: On sandy shores, the cyanobacterial population was very poor due to rough tides, absence of substratum, and poor nutrient content of water. In some areas, the stagnated sea water ponds and puddles showed rich populations of cyanobacteria in the form of thick mats, because these habitats remained undisturbed for relatively long periods. Lyngbya confervoides, L. martesiana, Microcoleus chthonoplastes, M. acustissimus, Oscillatoria salina, O. tenuis, Spirulina subsala sp. Labynithiformis, Pseudanabaena schemidleii were predominant in these mats.

Brackish water and estuarine diversity: Brackish water and mangroves forests are common along many shores in the tropics and subtropics, particularly where they are protected from severe wave action and major rivers enter the sea. Benthic cyanobacteria are abundant in mangrove environments. This is on account of rich organic muddy substratum, relatively stagnant shallow water conditions (15-30 ppt.). Thajuddin and Subramanian (1992) reported as many as 58 species of cyanobacteria belonging to 22 genera in brackish waters and mangrove habitats of the southern east coast of India.

Salt pans diversity: Thajuddin and Subramanian (1992) reported 50 species of 19 genera in salt pans with salinity of over 50 ppt. Nonheterocystous forms in general and the species belonging to the family of Oscillatoriaceae in particular were dominant; and some could grow at even 340 ppt salinity. However, this contention was subsequently disputed and the prevailing anaerobic conditions in the dark in these environments were believed to exclude the heterocystous forms. Hof and Fremy (1933) who studied the flora of salt waters divided algae into physiological groups, halotolerant and halophilic.

Feldmann (1951) divided cyanobacteria into euryhaline and stenohaline forms- those in brackish water as hyposaline; *Microcoleus chthonoplastes* is a euryhaline representative.

Cyanobacteria and extreme environments: Cyanobacteria have been reported to grow in brines, where they form thick mats at the bottom. Microcoleus chthonoplasts and Oscillatoria species together with other cyanobacterial components as minor partners, such as Aphanocapsa marina, Lyngbya aestuarii and Spirulina subsala are found in these mats. Thajuddin et al., (2002) have reported as many as 89 species from the east coast and 69 species from the west coast, of which 56 species were common in both the habitats. Of a total of 36 species in 16 genera recovered from salt pans of Pudakkottai District, Tamil Nadu, 18 species exhibited varying degree of salinity tolerance (45-90 ppt). Cyanobacteria have been reported from thermal waters all over the world. Mastigocoleus laminosus, Phomidium tenue and Synechococcus elongates var. amphigranulatus are the more common species in hot springs. Cyanobacteria can also tolerate low temperatures; *Phormidium* sp. has been reported from extensive ice layers in the Antarctic lakes. Taylor (1954) reported Calothrix and Rivularia as common cyanobacteria inhabiting marine Arctic area while Gloeocapsa and Nostoc were abundant in freshwaters. Endolithic cyanobacterial communities are able to trap and retain water on rock subsurface microenvironments. A recent account estimated that 700 taxa of non marine algae are present in Antarctica. The flora is dominated by species of Anabaena, Aphanocapsa, Calothrix, Chroococcidiopsis, Gloeocapsa, Lyngbya, Mastigocladus, Microchaete, Microcoleus, Oscillatoria, Phormidium, Plectonema, Pseudanabaena, Nodularia, Nostoc, Schizothrix, Scytonema, Stigonema, Synechococcus and Tolypothrix.

Many extremophiles have evolved to grow best at extremes of pH. Cyanobacteria are indeed present in acid lakes (pH 4.1-5.0) and have even found to dominate at low pH. This was confirmed by a recent study which demonstrated the

existence of filamentous cyanobacteria, *Chroococcus turgidus*, *Limnothrix* sp., *Mastigocladus* sp., *Oscillatoria* sp., *Spirulina* sp. (pH 2.9) and *Synechococcus* sp., (pH 4.0) in acid lake of Germany. Extreme alkaliphilic live in soils laden with soda (natron) or in soda lakes where the pH can rise to 12, but such organisms grow poorly at neutral pH. As many as 13 cyanobacteria alkaliphilic were reported to grow under alkaline conditions.

Molecular ecology of cyanobacteria: A review by Castenholz (1992) and the sister volume Bryant (1994) to the present one make clear how important is an understanding of cyanobacterial molecular biology not just as an aid to taxonomy, but for interpreting ecological phenomena in general. The widespread repetition in cyanobacteria of short sequences of DNA and its possible evolutionary significance are assessed. Suboptimal light and nutrient conditions result in a number of responses that strongly influences the physiology of the cell. The responses can be striking or subtle and subsequent changes take place rapidly or very slowly. The key role of light means that the signal transduction pathways that permit an appropriate adaptive response to be set in trait differ somewhat from most other prokaryotes. Cyanobacteria tend to show resistance to multiple environmental stresses, and it is probable that the response pathways to the different stimuli overlap. Caution is therefore needed in assuming that, because a particular stimulus brings about a characteristic morphogenetic response in the laboratory, it is the same stimulus which does so in nature. The ability of some cyanobacteria to withstand extremes of UV radiation and desiccation is aided by their capacity for efficient DNA repair. It will be of interest to understand if, and how, such repair plays a role in other responses to environmental stimuli such as nutrient limitation and cyanophage infection.

Cyanobacterial phylogeny and evolution: Cyanobacteria are a morphologically diverse group of photoautotrophic bacteria whose classification, in accordance with the Botanical (Anagnostidis and Komarek 1985) and Bacterial (Rippka *et al.*, 1979) codes, is almost entirely based on phenotypic traits. The current phylogenetic tree of cyanobacteria demonstrates that cyanobacteria lacking conspicuous morpho structural details such as those currently classified as Synechococcus, Synechocystis or Leptolyngbya are polyphyletic. Also, recent molecular data, mainly based on comparative analysis of 16S rRNA sequence show that this has resulted in an artificial classification, not reflective of the true relationships among the cyanobacteria. Analysis based upon a wide array of gene sequences have shown clearly that cyanobacteria constitute one of the 11 major eubacterial clades (phyla) and their similarities with other bacteria is clearly illustrated. Most systematics has also been utilized for understanding of phylogenetic divergence within cyanobacteria. They exhibit an array of thallus types that is unparallel among the prokaryotes and which approximates the morphological variation occurring in eukaryotic algal groups. It is no easier to delimit cyanobacterial species than it is to define most algal species. The biological species concept is not applicable to cyanobacteria (as is utilized for other algae) because true sexual reproduction is completely absent, although bacterial DNA exchange mechanisms such as transformation, conjugation are operative. Historically, therefore, morphological features have been used to define cyanobacterial taxa, including variations in cyanobacterial thallus structure. Most of these studies have shown that (Giovannoni et al., 1988; Wilmotte, 1994) that unicellular / colonial and unbranched filamentous forms are polyphyletic and it has been suggested that molecular changes required to maintain the integrity of filaments, as opposed to allowing separation of daughter cells after division, may be relatively slight. Hence, additional molecular studies are required to explain the occurrence of lineages containing both unicellular and filamentous taxa.

Major trends in cyanobacterial diversity assessment: Historically, considered to be algae, botanists and phycologists placed them into cyanophyceae or blue-green algae (Castenholz and Waterbury 1989), based on the International Code of Botanical Nomenclature. The taxonomy of cyanobacteria was therefore determined by morphological characters such as presence of sheath around individuals or colonies, pigmentation, trichome width, cell division planes, cell shape, cell dimensions or cell numbers in a colony besides the basic thallus structure i.e. unicells, colonies, psuedofilaments, filaments-unbranched / false branched or true branched with/ without heterocysts. The preliminary taxonomical treaties (Geitler 1932) therefore consisted of species differing even in a single character resulting in

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about a total of 1500 species in about 150 genera. However, some of these characters are variable with changing environment conditions. This provided the impetus for a drastic reduction, followed by consolidation of cyanobacterial species, based on ecophysiological criteria into only nine genera. Despite its simplicity, it received extensive criticism from classical taxonomists (Desikachary 1959; Anagnostides and Komarek 1985).

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