

Cyanobacterial biodiversity and potential applications in biotechnology

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Cyanobacteria (also known as blue–green algae) 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 alkylphilic, planktonic to barophilic, freshwater to marine including hypersaline (salt pans). 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 mariculture, food, feed, fuel, fertilizer, colourant, production of various secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals, pharmacological probes and 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 environmental conditions and enhancement of synthesis of high value products. This review is intended to focus on the biodiversity of cyanobacteria in various environments, recent application and new developments that are diversifying the directions for commercial exploitation.

CYANOBACTERIA, also referred to as blue–green algae, are the pioneer oxygenic phototrophs on earth whose distribution around the world is surpassed only by bacteria¹. Fossil evidence points to their presence in geographically diverse regions during the Precambrian (2 to more than 3.5 billion years ago). They 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 (and presumably physiological) change seen in 3.5-billion-year-old fossilized cyanobacteria and their modern day counterparts¹. Their long evolutionary history has been marked by key geochemical and biotic transitions, including the creation of oxygenic photosynthesis², a prerequisite for the development and proliferation of metabolically complex microbial and higher eukaryotic life forms. Indeed, the plastids of higher

plants and other photosynthetic eukaryotes are thought to have possibly arisen from a single common ancestor, which is a result of an endosymbiosis between a phagotrophic host and a cyanobacterium³.

Cyanobacteria were originally considered as algae because of their microscopic morphology, pigmentation and oxygen-evolving photosynthesis in which photosystems, PS II and PS I are connected in series. The genome size of cyanobacteria, representative of all major taxonomic groups, lies in the range, 1.6×10^9 to 8.6×10^9 Da which is comparable to that of other bacteria (1.0 to 3.6×10^9 Da)⁴. Cyanobacteria are known to occur in oxic and anoxic environments. Several species can switch to the typical bacterial anoxygenic photosynthesis using sulphide as electron donor; other species assimilate sugars and organic compounds in presence of light (photoheterotrophy)⁵. In dark, cyanobacteria gain energy by respiring endogenous carbohydrates, which are accumulated in the light. However, under anoxic conditions some species maintain this requirement by fermentation whereas in a few cases chemo-organotrophy is found. Cyanobacteria can grow under very low water potential; such species can resist desiccation and grow in arid environments (deserts) and or can tolerate high salinity to grow in hypersaline ponds⁶.

In many environments, cyanobacteria are the primary producers at the base of the food web of the ecosystem, viz. marine waters (Figure 1 a–d); hypersaline (Figure 1 e); brackish waters (Figure 1 f); soda lakes, freshwater, paddy fields, soils (Figure 1 g, h); deserts, cave walls, hot springs, polar regions and other extreme environments. Cyanobacteria are symbionts of a variety of other organisms, viz. the marine diatom *Rhizosolenia*, leaves of *Azolla* and the roots of *Cycas*⁷.

Morphological diversity

Cyanobacteria are basically microscopic, although large colonies or mats are quite conspicuous^{8–10}. Coccoid species occur as single cells (Figure 2 a), colonies or masses of various shapes (Figure 2 b–g) wherein cells are arranged in rows resulting in a flat plate (Figure 2 c), or are arranged radially in spherical colonies (Figure 2 f). Cell numbers may range from few to many. Colonies may be loosely attached to the substrata without polarity (Figure 2 i) or remain firmly attached with a distinct base and apex (Figure 2 h, j). However, these are enclosed in a gelatinous sheath that varies in consistency and thickness.

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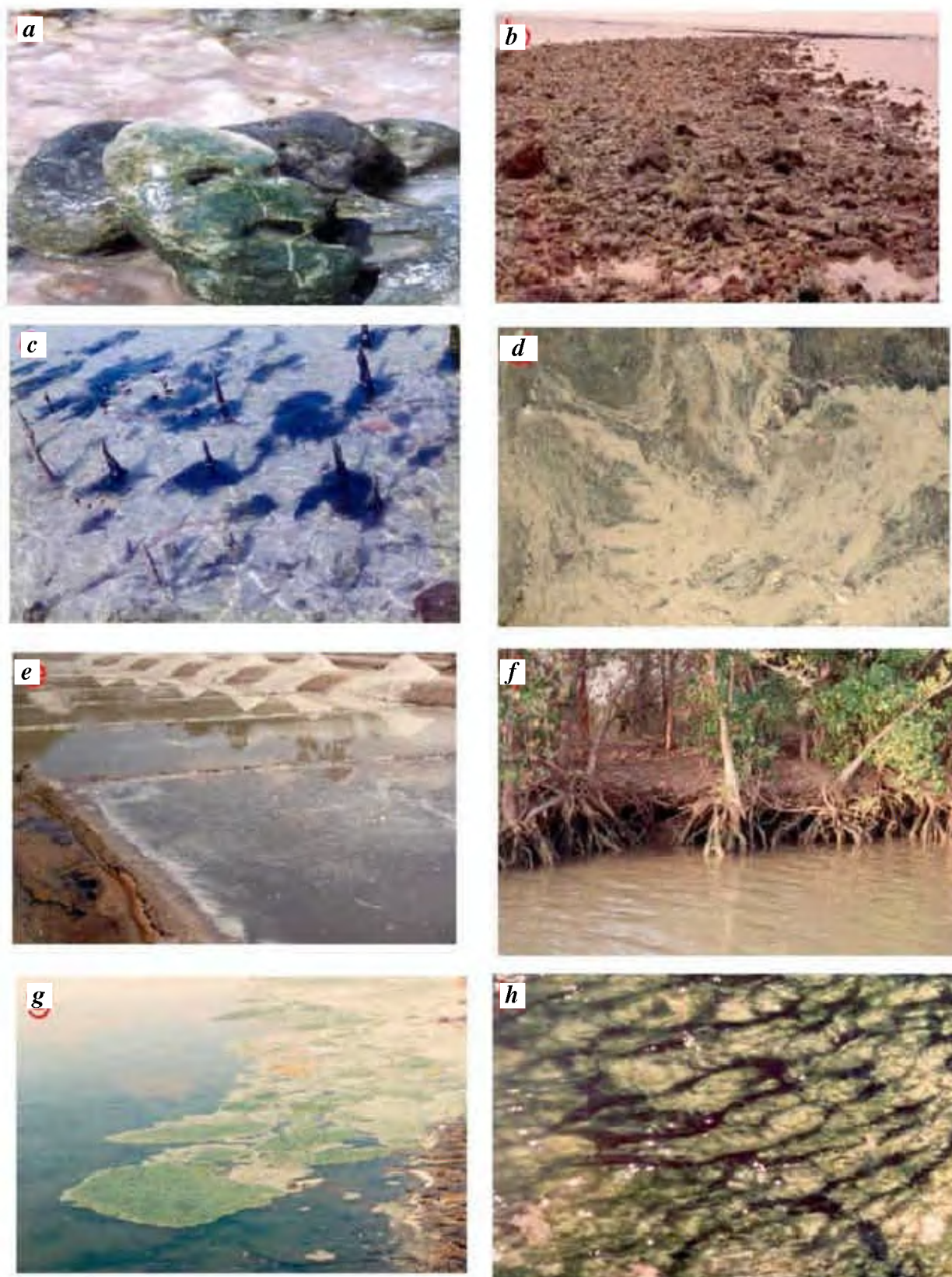


Figure 1 a–h. *a*, Epilithic cyanobacteria on stones of intertidal regions (Bay of Bengal); *b*, Coral reef of Kurusadai Island (Gulf of Mannar); *c*, *Lyngbya majuscula* on breathing roots of mangroves (South Andaman); *d*, A portion of the bloom of *Trichodesmium erythraeum* (Gulf of Mannar); *e*, A salt pan with thick cyanobacterial growth (Thondi, Palk Bay); *f*, Lagoon showing epiphytic cyanobacteria on the roots of mangroves in Muthupet (Palk Strait); *g*, Stagnant sea water puddle with cyanobacterial bloom in Point Calimere (Palk Strait); *h*, Cyanobacterial mat in the stagnant sea water in Nagappattinam (Bay of Bengal).

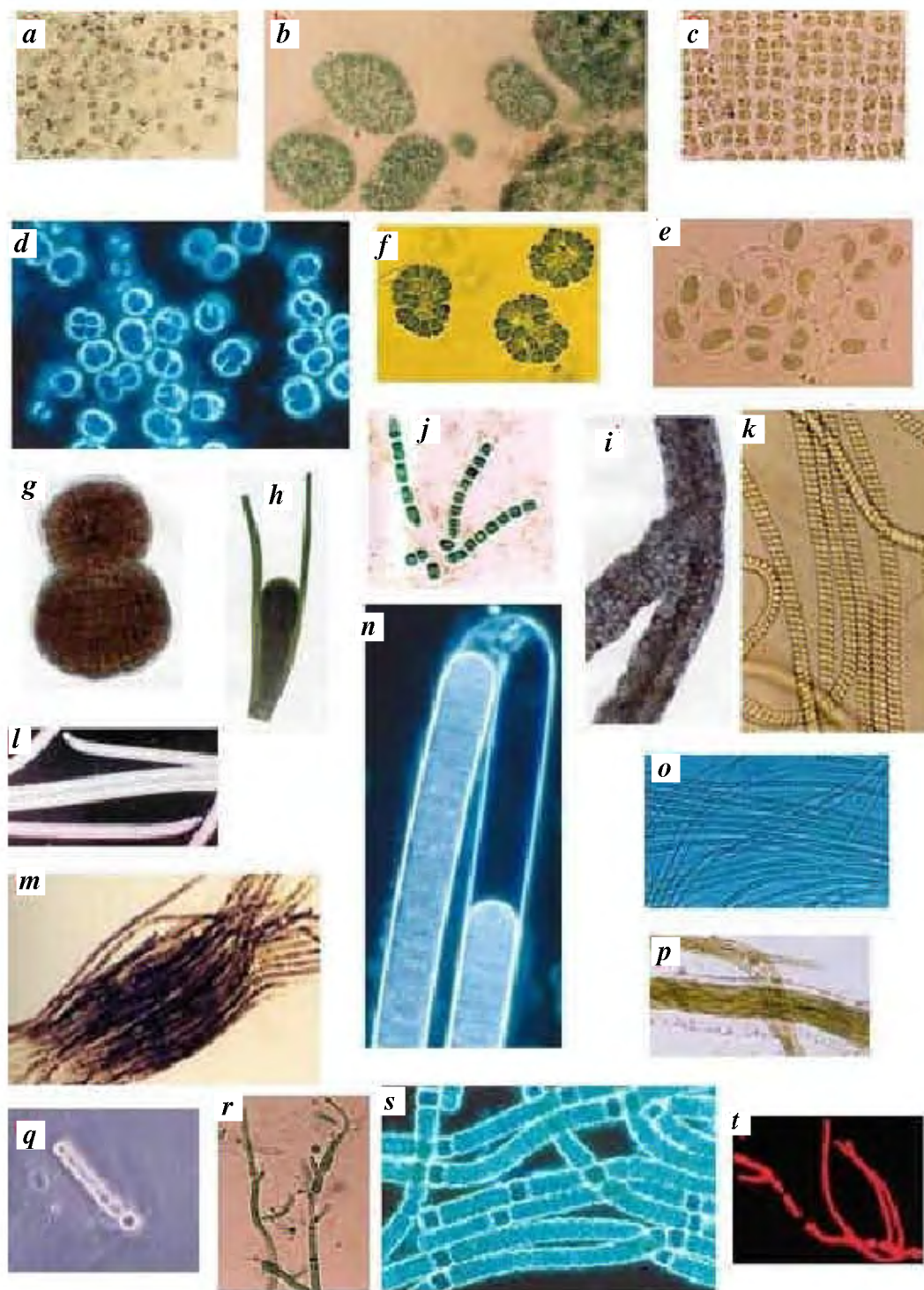


Figure 2a–t. *a*, Simple coccoid cyanobacterium *Synechocystis pevalekii*; *b*, *Microcystis pulverea* colonies with irregularly arranged cells in a common mucilaginous matrix; *c*, *Merismopedia glauca* – a tabular colony with regular cell arrangement; *d*, *Chroococcus turgidus* – cells with distinct individual mucilage sheath (dark field photomicrograph); *e*, *Gloeotheca rupestris* – cylindrical cells surrounded by a vesicular sheath; *f*, *Gomphosphaeria aponina* – radially arranged cordate cells in a hollow spherical mucilaginous matrix; *g*, *Myxosarcina concinna* – a sarcinoid colony with densely packed variously pressed cells; *h*, *Chamaesiphon sideriphilus* – exospore producing form showing base and apex differentiation; *i*, *Xenococcus acervatus* – many layered cells epiphytically growing on *Lyngbya majuscula*; *j*, *Stichosiphon sansibaricus* – rows of endospores in a common mucilage; *k*, *Spirulina subsalsa* – spirally twisted trichome; *l*, *Oscillatoria formosa* – simple trichomatous form (dark field photomicrograph); *m*, *Trichodesmium erythraeum* – many trichomes aggregated in a bundle; *n*, *Lyngbya hieronymusii* – a filamentous form with unlamellated sheath; *o*, *Phormidium valderianum* – a filamentous form showing granules on either side of the cross walls; *p*, *Microcoleus chthonoplasts* – many trichomes enclosed in a common sheath; *q*, *Richelia intracellularis* – a few celled filament with heterocyst on both ends (phase contrast photomicrograph); *r*, *Dichothrix bauriana* – a false branched heterocystous filament; *s*, *Nodularia spumigena* – heterocystous filamentous form (dark field photomicrograph); *t*, *Hapalosiphon welwitchii* – a heterocystous and true branched form (fluorescence photomicrograph).

Filamentous forms produce a row of cells, referred to as trichome. Trichomes may be simple, straight (Figure 2 *l*), as aggregated bundles (Figure 2 *m*), and or permanently spirally coiled (Figure 2 *k*). The trichome with the enclosing sheath is referred to as a filament (Figure 2 *n, o*); in some forms, trichomes are enclosed by a common sheath (Figure 2 *p*). Some filamentous species are characterized by true cell differentiation and form heterocysts which unlike vegetative cells, lack an oxygenic photosystem, possess extraordinarily thick cell wall, and lack biliprotein pigments and carboxysomes (Figure 2 *q, t*). These cells are considered as the sites of nitrogen fixation, provide vegetative cells with combined nitrogen and are not viable when disconnected from the trichome. Many heterocystous cyanobacteria also form a second cell type, an akinete, which can germinate when conditions are suitable for growth. Akinetes are common among freshwater forms but uncommon in marine habitat, probably because of the stability of the marine environment. Desiccated akinetes in soil samples have survived for 70 years¹¹. The filaments are either unbranched or may be branched with uniseriate or multiseriate arrangement of cells (Figure 2 *t*). Besides presence of true branching, false branches may occur in some forms (Figure 2 *r*).

Biodiversity of marine cyanobacteria

Of the total 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 oceans¹². India has a vast coastline of over 7500 km; in addition it has many lakes, ponds, puddles, backwater 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 conditions, suits their existence in a variety of intertidal habitats. Desikachary⁹ suggested that probably 20% of all known cyanobacteria occur in saline conditions and a majority of them are truly marine. However, little work has been done to understand the cyanobacterial biodiversity of marine environments of India^{13–15}.

There are several reports of smaller number of species from different parts of the world including brines¹⁶. Thajuddin and Subramanian^{6,17} 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 (lat. 15°45'N and long. 77°33'E to Cape Comorin lat. 8°5'N and long. 77°33'E) of Tamil Nadu and from Cape Comorin to Bhimunipattanam of Andhra Pradesh (lat. 17°55'N and long. 83°25'E) encompassing the regions such as the Arabian Sea, Indian Ocean, Palk Bay, Palk 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 salt pans.

The nature of shores in South India and Andaman and Nicobar and Lakshadweep Islands of India varied markedly in different regions. Except for the rocky spots of Kovalam, Vizhinyam Edeva, Varkale (Kerala), and Dona Paula (Goa), the shores of the Arabian Sea region (West Coast) are essentially sandy. The shores of the Bay of Bengal region are mostly sandy with few extensive rocky spots at Bhimanipattanam, Vishakapatnam, Pudumadaka (Andhra Pradesh), Kovalam and Mahabalipuram (Tamil Nadu) (Figure 1 *a*). The shores of the Palk Strait region exhibit greater variety. Along the great lagoons, the shores are clayey and particularly in Adhirampattinam and Muthupet area, swampy and inaccessible. The shore towards the Palk Bay from Palk Strait is sandy and rich in organic matter due to deposition of dense layers of sea grass over vast stretches. However, most of the Palk Bay has a sandy shore with scattered boulders and masses of coral rocks. The shores of the Gulf of Mannar region vary from place to place. On the South Coast of Mandapam, the shores are rocky due to extensive sand stones; in Rameshwaram Islands, the shore is slightly rocky in some areas but mostly sandy. About 6–10 km away from the Mandapam and Ervadi seashore there are several small islands, viz. Kurusadai, Shingle, Pulli, Pullivasal, Nallathannithevu, Upputhannithevu and Hare Island. A portion of the shore in these islands is made up of coral reefs. The shores towards Cape Comorin (Tiruchendur, Idinthakarai, etc.) are sandy with intermittent rocky spots, while at Cape Comorin it is partly rocky and partly sandy. The majority of the shores in Andaman (smooth, middle, north, little Andamans), Nicobar (Car Nicobar, Katchal island) and Lakshadweep Islands (Kavarathi, Agatti, Amini, Kadamath) are sandy with rocky and vast coral stretches in some areas.

Rocky shores

The type of substrata in the intertidal area influenced greatly the availability of benthic cyanobacterial biodiversity. Thajuddin and Subramanian⁶ 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 (Figure 1 *b*), as many as 145 species were observed which included both benthic and planktonic forms. Little¹⁸ and Renaunt *et al.*¹⁹ 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-developed cryptoendolith layer such as that

described by Moul²⁰, with *Oscillatoria nigroviridis*, *Lyngbya confervoides* and *Phormidium valderianum* at a depth of 6 mm inside the rock.

Sandy shores

On sandy shores, the cyanobacterial population was very poor due to rough tides, absence of substratum, and poor nutrient content of water⁶. In some areas, the stagnated sea water ponds and puddles showed rich populations of cyanobacteria in the form of thick mats (Figure 1 g, h), because these habitats remained undisturbed for relatively long periods. *Lyngbya confervoides*, *L. martansiana*, *Microcoleus chthonoplastes*, *M. acustissimus*, *Oscillatoria salina*, *O. tenuis*, *Spirulina subsala* sp. *labynithiformis*, *Pseudanabaena schemidlei* were predominant in these mats.

Backwater and estuarine areas

Backwater and mangrove 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 environments⁶. This is on account of rich organic muddy substratum, relatively stagnant shallow water conditions, sheltered nature (hence reduced water movement), and optimum salinity conditions (15–30 ppt). Thajuddin and Subramanian⁶ reported as many as 58 species of cyanobacteria belonging to 22 genera in backwaters and mangrove habitats of the southern east coast of India (Figure 1 f).

Salt pans

Thajuddin and Subramanian⁶ reported 50 species of 19 genera in salt pans with salinity of over 50 ppt (Figure 1 e). Non-heterocystous forms in general and the species belonging to the family Oscillatoriaceae in particular were dominant; and some could grow at even 340 ppt salinity. High sulfide content in hypersaline environment is toxic to heterocystous forms²¹. However, this contention was subsequently disputed and the prevailing anaerobic conditions in the dark in these environments were believed to exclude the heterocystous forms²². Hof and Frey²³ who studied the flora of salt waters, divided algae into physiological groups, halotolerant and halophilic. Halotolerant species are not able to grow at NaCl concentration above 3 M (175.5 ppt), e.g. *Calothrix scopularum*; other examples include salt-water forms such as, *Microcoleus chthonoplastes* and *Lyngbya aestuarii*. Halophilic species such as *Spirulina subsala* can grow at salt concentrations above 3 M and therefore occur commonly in salt pans. Feldmann²⁴ divided cyanobacteria into euryhaline and stenohaline forms – those living in brines as hypersaline and those in brackish water as hyposaline; *Microcoleus chthonoplastes* is a euryhaline representative.

The remarkable adaptability of cyanobacteria to salinity is well documented. *Phormidium valderianum* BDU 30501 was shown to grow at salinity reaching 100 ppt. Thajuddin and Subramanian⁶ reported that as many as 75 of the species which were originally reported from freshwater sources by Geitler⁸ and Desikachary⁹, were also marine. It is therefore difficult to strictly segregate cyanobacteria into marine and freshwater species which is possible with other algal forms.

A bundle forming marine cyanobacterium *Trichodesmium erythraeum*, forms massive blooms in many parts of tropical and subtropical waters^{26–29}. Thajuddin²⁸ and Thajuddin and Subramanian^{6,30} studied the ecobiology of plankton in general and *Trichodesmium erythraeum* in particular in the area between Mandapam, Rameswaram and Kurusadai Islands (Gulf of Mannar). *Trichodesmium* generally constitutes a minor component in the marine plankton. But in certain seasons, there was a sudden rise in *Trichodesmium*, forming major blooms, followed by a steep fall in population (Figure 1 c and Figure 2 m). There was a good positive correlation between the temperature, salinity and the levels of chloride, ammonia, inorganic phosphate and total phosphorus and iron during the period of study (1988, 1989); typical negative correlation existed between wind speed, rain fall, sea level pressure and dissolved oxygen. Amongst various factors that influence the blooms of *Trichodesmium*, ammonia was more a consequence than a cause of the bloom, because of the high rates of hydrogen fixation observed in this organism³⁰. As many as six species of cyanobacteria namely, *Ammeatoidea normanii*, *Dichothrix spiralis*, *Oscillatoria borneti*, *Pseudoanachobroa fluminensis*, *Radaisia violacea* and *Siphononema polonicum*, were reported for the first time from Indian coasts^{14,31}.

Biodiversity of freshwater cyanobacteria

Cyanobacteria, belonging to the order Chroococcales, and families Oscillatoriaceae and Nostacaceae occur ordinarily as planktonic forms. Several species grow in abundance and colour the entire body of water, forming the so-called water-blooms. Huber-Pestalozzi (cited by Desikachary⁹) lists as many as 41 genera of 251 species of cyanobacteria that occur as freshwater planktons. *Microcystis* is one of the dominant organisms that is associated with almost permanent blooms³² in tropical freshwaters that are exposed to constant sunshine, warmth and nutrients like phosphate, silicate, nitrates, CO₂ and lime. The source of nitrogen for freshwaters includes drainage from the surrounding areas, precipitation bringing in dissolved free ammonia from atmosphere and autochthonous release of nitrates from the dead populations. Nitrates form a preferred non-toxic source of combined nitrogen for most cyanobacteria and are produced as a result of high rate of nitrification under waterlogged conditions³³. 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. Gas vacuole containing cyanobacteria form dense growth on the water surface in ponds, reservoirs and lakes and cause serious nuisance because of visual appearance, production of toxins³⁴ and unpleasant odour produced by substances such as geosmin³⁵.

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 m³⁶. Huber-Pestalozzi³⁷ has listed species of genera, *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Arthrospira*, *Coelosphaerium*, *Gloeotrichia*, *Lyngbya*, *Microcystis*, *Nostoc*, *Nodularia*, *Oscillatoria*, *Spirulina gomontina* and *Lyngbya* 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 aeruginosa*, *Microcystis flos-aquae*, *Gloeotrichia echinulata* and several species of *Anabaena*³⁸.

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^{39–41}. Waterlogged rice field is an ideal habitat for these cyanobacteria which are capable of nitrogen fixation⁴⁸. These include, species of *Anabaena*, *Aulosira*, *Calothrix*, *Cylindrospermum*, *Gloeocapsa*, *Nostoc*, *Rivularia*, *Scytonema* and *Tolypothrix*⁴². N₂-fixing cyanobacteria have also been reported in sugarcane and maize fields in India⁴³. Besides nitrogen fixation by heterocystous and few non-heterocystous 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 cyanobacteria constitute about 15% of the total algal flora in the tropics and about 2% in the temperate climate.

Cyanobacteria of 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 subsalsa* are found in these mats. Thajuddin and coworkers^{13,29} 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 Pudukkottai District, Tamil Nadu, 18 species exhibited varying degree of salinity tolerance (45–90 ppt)¹³.

Cyanobacteria have been reported from thermal waters all over the world. Setchell⁴⁴ suggested the upper limit of cyanobacteria as 65°C to 68°C and Lemmermann⁴⁵ described it as 69°C. *Mastigocoleus laminosus*, *Phormidium 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. Taylor⁴⁶ reported *Calothrix* and *Rivularia* as common cyanobacteria inhabiting marine Arctic areas 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 nonmarine algae are present in Antarctica. The flora is dominated by species of *Anabaena*, *Aphanocapsa*, *Calothrix*, *Chroococcidiopsis*, *Gloeocapsa*, *Lyngbya*, *Mastigocladus*, *Microchaete*, *Microcoleus*, *Oscillatoria*, *Phormidium*, *Plectonema*, *Pseudoanabaena*, *Nodularia*, *Nostoc*, *Schizothrix*, *Scytonema*, *Stigonema*, *Synechococcus* and *Tolypothrix*⁴⁷.

Many extremophiles have evolved to grow best at extremes of pH. Cyanobacteria are indeed present in acid lakes (pH 4.1–5) and have even found to dominate at low pH⁴⁸. 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) in acid lakes in Germany⁴⁹. Extreme alkaliphiles 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 alkaliphilic cyanobacteria were reported to grow under alkaline conditions⁵⁰. Very often, soda lakes are monospecific, inhabited by *Spirulina platensis* which serves as human food (single cell protein) of high nutritional value⁵¹.

Cyanobacteria are often exposed to heavy metal pollution due to the disposal of industrial and domestic wastes into waterways. Cyanobacteria growing in metal-polluted environments display the ability to tolerate high concentration of toxic metals, Cu, Cd, and Zn⁵². Unicellular marine cyanobacterium *Synechococcus* PRIMN I and *Synechococcus* UTEX 625 and *Synechococcus* TX 20 (freshwater strain) are capable of metallothionin synthesis (a low molecular weight metal-binding protein) on exposure to heavy metals⁵³. The metallothionin III or phytochelatin binds metals to form phytochelatin-metal complex. This complex can sequester metals from the cytosol and detoxify or safely transfer them into the vacuole. A part of the metals can also be compartmentalized by proteins, nucleic acids and enzymes. Metal from such a pool may be used by apoenzymes and proteins for metabolic processes⁵⁴.

Cyanobacterial associations

Though cyanobacteria are capable of independent existence, they form symbiosis with major plant and animal groups: algae, fungi (lichens), bryophytes, pteridophytes, gymnosperms, angiosperms⁷ and animals⁵⁵. The most common cyanobacterium found in symbiosis with plants belong to the filamentous heterocystous genus *Nostoc*⁵⁶. Usually, the proportion of heterocysts to vegetative cells is higher in symbiotic forms compared to free-living cyanobacteria and this is determined by the nitrogen status of the environment⁵⁷.

A number of cyanobacteria live endophytically in other algae. The small filamentous cyanobacterium *Richelia intracellularis* (Figure 2q) lives endophytically in cells of the marine diatom *Rhizosolenia* spp., whereas *Nostoc symbioticum* occurs in *Geosiphon pyriformis*, a siphonous green alga. Species of *Calothrix*, *Cyanodictyon*, *Lyngbya* and *Phormidium* have been reported from the mucilage of other algae. *Rhopalodia* is a freshwater centric diatom in which unicellular cyanosymbiont appears intracellularly as inclusion bodies⁵⁷.

Lichens are symbioses of fungi (ascomycetes and basidiomycetes) with green algae or cyanobacteria. The most common cyanobacteria found in lichens are species of *Calothrix*, *Fischerella*, *Gloeotheca*, *Nostoc* and *Scytonema*⁵⁸. The vegetative body of a lichen is termed thallus and is composed of two organisms, a fungal component (mycobiont) and a green alga or cyanobacterium (phycobiont). Of a total of 18,000 species of lichens, about 8% are composed of nitrogen-fixing cyanobacteria (cyanolichens). The structure of the lichens thallus varies from *Collema*, where the fungal hyphae and cyanobacterial filaments intermingle through the thallus, to *Peltigera canina*, where the cyanobacteria are confined to the layer beneath the upper cortex of the fungal hyphae⁵⁹.

A number of bryophyte taxa are known to harbour symbiotic cyanobacteria which usually live intercellularly and belong to the genus *Nostoc*⁶⁰. The cyanobionts, *N. calcicola* and *N. sphaericum* occupy mucilage filled cavities on the ventral side of the gametophyte of the bryophytes, *Aneura*, *Anthoceros*, *Blasia*, *Cavicularia*, *Diplolaena*, *Notiothylus*, *Pellia*, *Riccardia*, *Riccia* and *Sphagnum*. The cyanosymbionts presumably can penetrate through 'stomata' or special pores. Among pteridophytes, by far the best studied cyanom is that of *Azolla* spp. with *Anabaena azollae*^{61,62}. *Azolla* is a genus of heterosporous aquatic fern that grows on the surface of freshwater ponds, lakes, streams or irrigation channels. *A. azollae* infects the fronds of the fern at an early stage of plant development and becomes enclosed in a pocket within the frond. The cyanobiont normally remains together with the plant through successive cycles of vegetative and sexual reproduction. Due to high rates of N₂ fixation and biomass production, *Azolla* is an effective green manure for flooded crops and has been used as a biofertilizer in rice-growing countries for centuries.

As many as 150 species in nine genera belonging to the family Cycadaceae of Gymnosperms, which produce coralloid roots, contain nodule-like structures that are inhabited by heterocystous cyanobacteria belonging to *Anabaena* or *Nostoc*⁶³. The cyanobiont infects coralloid roots, via mucilaginous spaces in the tips, and is usually located intercellularly in *Ceratozamia*, *Cycas*, *Dioon*, *Encephalartos* and *Zamia*. In *Macrozamia communis*, both intercellular and intracellular localization occur in the extra coralloid-root area⁶⁴.

In Angiosperms, the best-known symbiotic cyanobacterial association is formed between *Gunnera* sp. and *N. punctiforme*, where the cyanobiont is located intracellularly in special stem nodules⁶⁵. These nodules develop from secretory glands that produce mucilage which helps the cyanobiont invade the glands. The cyanobiont penetrates intercellularly through gland tissue, and then at the base of the gland enters into thin walled meristematic cells by a special mechanism which results in the formation of a host-plasmalemma-derived membrane around the intercellular cyanobacteria⁶⁵. Other less intensively studied angiosperm-cyanobacterial associations that are located in root nodules⁶⁶ are reported from *Trifolium alexandrinum* and *N. punctiforme* and from *Lemna triscula* that harbour cyanobacteria of different taxa⁶⁷.

Symbiotic cyanobacteria have been reported in a large variety of marine sponges in which the unicellular cyanobacterium, *Aphanocapsa* sp. is situated both intercellularly and intracellularly in host vacuoles⁶⁸; marine echiuroid worms such as *Bonellia fuliginosa* and *Ikedosoma gego-shinense*, harbour cyanobiont intracellularly in special cells of the epidermal tissue⁶⁸. In green algae *Oedogonium oogonia* and *Codium bursa*, different species of filamentous cyanobacteria have been reported⁶⁹.

Potential applications in biotechnology

Cyanobacteria are one of the potential organisms, which are useful to mankind in various ways. Cyanobacteria constitute a vast potential resource in varied applications such as mariculture, food, feed, fuel, fertilizer, medicine, industry and in combating pollution⁷⁰⁻⁷⁵.

Food and feed

Algal protein either as a supplement or as an alternative source has received worldwide attention. Some strains of *Anabaena* and *Nostoc* are consumed as human food in Chile, Mexico, Peru and Philippines. *N. commune* with high amount of fibre and moderate protein is of potential use as a new dietary fibre source and can play an important physiological and nutritional role in human diet⁷⁶. *Spirulina* is used as food supplement because of its excellent nutrient composition and digestibility. It has high protein content

(60–70%), 20% carbohydrate, 5% lipids, 7% minerals and 6% moisture. It is also a rich source of beta-carotene, thiamine and riboflavin and is one of the richest sources of vitamin B₁₂. It is commercially available in the market in the form of powder, granules or flakes and as tablets and capsules.

A large number of marine nitrogen-fixing cyanobacteria have been tested for their nutritional value with the hybrid *Tilapia* fish fry; a majority were acceptable as single ingredient feeds. Very high growth rates of *Tilapia* fish using marine cyanobacteria with in-door and out-door cultures have been reported⁷⁷. In our laboratory, the marine cyanobacterium *Phormidium valderianum* BDU 30501 was shown to serve as a complete aquaculture feed source, based on the nutritional qualities and non-toxic nature with animal model experiments. A technology for mass cultivation of this strain and production of pellet feed was developed and transferred to M/S ABL Biotechnologies, Chennai, who would commercially produce and market the marine cyanobacterial feed.

Fine chemicals

A variety of fine chemicals such as pigments, vitamins and enzymes with varied applications can be obtained on a commercially viable scale from cyanobacteria. A number of cyanobacteria are rich in vitamins and many can excrete them into the surrounding environment⁷⁸. Some marine cyanobacteria are potential source for large scale production of vitamins of commercial interest such as vitamins of the B-complex group and vitamin E⁷⁸.

The carotenoids and phycobiliproteins, characteristic of cyanobacteria have high commercial value. They are used as natural food colourants, as food additives to enhance the colour of the flesh of Salmonid fish and to improve the health and fertility of cattle⁷⁹. Feed grade *Phormidium valderianum* is an excellent source of phycocyanin, a blue natural colorant useful as a phycofluor in diagnostics; a technology for its inexpensive production has been transferred to M/S ABL Biotechnologies, Chennai.

Cyanobacteria being photoautotrophs have the ability to photosynthetically transform simple, labelled compounds such as ¹⁴CO₂, ¹³CO₂, ³H₂O, ¹⁵NO₃ into complex organic compounds. Isotopically labelled cyanobacterial metabolites such as sugars, lipids and amino acids are commercially available⁸⁰.

Cyanobacteria secrete enzymes that can be exploited commercially. In our laboratory, marine cyanobacteria have been used in large-scale production of enzymes such as beta lactamase, protease and lipase⁸¹ and the technology for beta lactamase has already been transferred to M/S ABL Biotechnologies, Chennai. Several common and unique sequence-specific endonucleases are known from *Anabaena cylindrica* (Acy I), *Anabaena flos-aquae* (Afl I & Afl III), *Anabaena variabilis* (AvaI & AvaII), *Anabaena*

variabilis UW (AvrII), *Microcoleus* sp. UFE 2220 (MstII), *Nostoc* sp. PCC 7524 (Nsp C I), which can be marketed at low cost since relative biomass production of cyanobacteria is much less expensive than bacteria⁸². Cyanobacterial isolates with capacity to mineralize organic phosphorus have been reported with alkaline phosphatase activity⁸³. Enzymes such as chitinase, L-asparaginase, L-glutaminase, amylase, protease, lipase, cellulase, urease and superoxide dismutase have been reported from cyanobacteria⁸⁴. Photo-production of ammonia and amino acids by cyanobacteria has also been described⁸⁵.

Analysis of extracellular growth-promoting substances liberated by *N. muscorum* and *Hapalosiphon fontinalis* was found to contain amino acids like serine, arginine, glycine, aspartic acid, threonine, glutamic acid, cystine, proline, valine, ornithine, lysine, histidine and iso-leucine⁸⁶. In addition, cyanobacteria are a rich source of several polyols, polysaccharides, lipids, fatty acids, halogenated compounds, etc. with varied properties employable as flocculants, surfactants and others⁸⁷.

Pharmaceuticals

Cyanobacteria in general and marine forms in particular are one of the richest sources of known and novel bioactive compounds including toxins with wide pharmaceutical applications⁸⁸. Gustafson *et al.*⁷³ reported anti-HIV activity of marine cyanobacterial compounds from *Lyngbya lagerheimii* and *Phormidium tenue*. A massive programme of screening of extracts from the large culture collection of marine cyanobacteria in our research facility for anti-viral, anti-bacterial, anti-fungal and immuno-modulatory activities has resulted in recovery of a compound from marine *Oscillatoria laete-virians* BDU 20801 that shows anti-candida activity⁸⁹. An immunopotentiating compound with male anti-fertility, without being toxic to other systems in a mice model, was found in the extracts of *Oscillatoria willei* BDU 130511 (ref. 89). Medically important gamma linolenic acid (GLA) is relatively rich in cyanobacteria namely *Spirulina platensis* and *Arthrospira* sp. which is easily converted into arachidonic acid in the human body and arachidonic acid into prostaglandin E₂ (ref. 90). Prostaglandin E₂ has lowering action on blood pressure and the contracting function of smooth muscle and thus plays an important role in lipid metabolism.

Biofertilizer

De⁹¹ attributed inherent fertility of tropical rice field soils to the activity of N₂-fixing cyanobacteria. A variety of cyanobacterial strains colonize rice fields wherein heterocystous species are capable of fixing atmospheric nitrogen. However, several non-heterocystous cyanobacteria are able to fix atmospheric nitrogen under microaerophilic conditions. *In situ* estimations using acetylene reduction technique have

shown an addition of 18–15 kg N ha⁻¹ yr⁻¹ due to the activity of diazotrophic cyanobacteria⁹². The role of N₂-fixing cyanobacteria in maintenance of the fertility of rice fields has been well substantiated and documented all over the world. In India alone, the beneficial effects of cyanobacteria on yield of many rice varieties have been demonstrated in a number of field locations⁹³. Beneficial effects of cyanobacterial inoculation have also been reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce^{94,95}. The importance of *Azolla* as an organic fertilizer in rice cultivation is well appreciated and practised in several countries. The cyanobacterial symbiont *Anabaena-azollae* fixes atmospheric nitrogen estimated between 120 and 312 kg N₂ per hectare. *Azolla* supplies 150–300 tons per hectare per year of green manure, which supports growth of soil microorganisms including heterotrophic N₂ fixers⁹⁶.

The use of algae and cyanobacteria in waste treatment is beneficial in different ways since they can bring about oxygenation and mineralization, in addition to serving as food source for aquatic species. Using the marine cyanobacteria *Oscillatoria* sp. BDU 10742, *Aphanocapsa* sp. BDU 16 and a halophilic bacterium *Halobacterium* US 101, Uma and Subramanian⁹⁷ treated ossein factory effluent which resulted in reduced calcium and chloride levels and enabled 100% survival and multiplication of *Tilapia* fish with only cyanobacteria as feed source. Shashireka *et al.*⁹⁸ found that *Phormidium valderianum* BDU 30501 was able to tolerate and grow at a phenol concentration of 50 mg/l and removed 38 mg/l within a retention period of seven days. This result opens up the possibility of treating a variety of phenol containing effluents. The organism was also effective in optimal sorption/desorption of heavy metal ions (Cd²⁺, Co²⁺)⁹⁹. Another marine cyanobacterium, *Oscillatoria boryana* BDU 92181 was effective in degradation and metabolization of melanoidin pigment which is abundant in distillery effluents¹⁰⁰. Studies at the National Facility for Marine Cyanobacteria (NFMC) have identified suitable cyanobacteria for treating a number of noxious effluents containing organophosphorus pesticides, detergents, antibiotics and other molecules⁷⁵, as also for degradation of solid wastes like coir pith by their lignolytic action¹⁰¹.

Cyanobacteria have long been considered either as organisms of academic curiosity or as organisms of nuisance value. Pioneering work of the last decades has raised the status of these microbes to a level where they are being viewed with favour in biotechnologically relevant spheres. Therefore, in tropical countries like India, it is essential not only to understand and preserve the biodiversity of cyanobacteria in yet unexplored habitats but also to gainfully exploit it for various applications including pollution abatement.

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