Cyanobacteria as potential options for environmental sustainability – promises and challenges

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Abstract Cyanobacteria represent an ancient group of photosynthetic prokaryotes, whose ubiquity, metabolic flexibility and adaptive abilities have made them a subject of research worldwide. These structurally simple organisms combine in themselves interesting facets of plant and bacterial metabolism, which is amenable to genetic exploitation. Despite their globally recognized significance in the sustenance of fertility in rice based cropping systems, they have not been tapped for their extraordinary repertoire of activities, especially their beneficial role as biological agents in remediation and amelioration of soil and water environment and as sinks for greenhouse gases. The information available on these aspects and future lines of research for more efficient utilization of these microorganisms is presented.

Keywords amelioration · bioremediation · CO₂ · cyanobacteria · methane · soil fertility

Introduction

Cyanobacteria are cosmopolitan microorganisms, which play significant roles in diverse ecosystems. The favorable conditions provided by the rice fields for nitrogen fixation by these organisms leads to enhanced plant-available N in soil and yield improvement of rice [1, 2]. Therefore, the sustenance of productivity of rice fields has been attributed to their adaptation to the unique soil - water ecology; their widespread temporal and spatial distribution and diverse metabolic activities [3, 4]. Among the beneficial effects of cyanobacteria, excluding supplying nitrogen in rice fields, their influence on the physical, chemical and biological properties of the soil and soil water interface has immense significance [5–7]. Cyanobacteria liberate extracellular substances and modulate pH, temperature and redox activity, besides playing a role in the volatilization of ammonia and methane generation; therefore, are directly of indirectly implicated in the management and productivity of rice ecosystem [2, 8]. Cyanobacterial inoculation is also known to improve the stability of soil due to excretion of polysaccharides, lipids which aid in enhancing aggregation [7]. Their influence on other crops besides rice, e.g. wheat, tomato and pulse and vegetable crops is also documented [9–11].

The less investigated beneficial effects of cyanobacteria include curbing of ammonia volatilization, suppressing weeds, reducing methane emission, transformation of P, Fe, Mn, Zn Cu, pesticide degradation and reclamation of wastelands/degraded soil [2, 8, 12]. This compilation focuses on some of these metabolic activities of cyanobacteria and their applications from the agricultural and environmental perspective.

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Role of cyanobacteria in the mitigation of emission of greenhouse gases

Cyanobacteria in methane dynamics of rice fields

Rice fields represent one of the major anthropogenic sources of methane emission with the largest uncertainty. Precise global estimates regarding the spatio-temporal dynamics are difficult due to several factors including seasonal variability, site specific environmental variables, changing agronomic practices and rice cultivars. However, rice agriculture is considered one of the few sources of methane, where emission reduction through management is considered possible through concerted efforts [13]. One of the major mitigation options involves developing strategies which enhance the methane oxidation potential in the standing water in continuously flooded rice fields and the rhizosphere.

The photosynthetically generated O_2 is of crucial importance in the maintenance of redox potential and oxygenic conditions of soil despite frequent flooding. Laboratory simulation experiments were undertaken using soil cores from rice fields inoculated with cyanobacterial/ *Azolla* biofertilizers. The results revealed a rapid decrease in head-space concentrations of methane [8] in the soil cores from these treatments as compared to the treatments involving the use of chemical fertilizers. This can be attributed to the significant role of oxygen evolved by cyanobacteria during photosynthesis as oxygen donors to methylotrophs or direct enhancement of methane oxidation.

Cyanobacteria as CO_2 sinks

The CO_2 level in the atmosphere, which has been stabilized for 100, 000 years has been rising after the industrial

revolution, to a present level of 360ppm [14]. Geological predictions are that it may reach to a level of 480ppm by 2050. Various solutions have been proposed to mitigate the greenhouse effect of CO_2 emissions. One of the options currently being explored is the use of cyanobacteria as CO_2 sinks. Cyanobacteria are attractive candidates in this respect as they are fast growing and easier to manipulate in open ponds. They possess an essential biophysical mechanism (carbon concentrating mechanism, CCM), which concentrates CO_2 at the site of photosynthetic carboxylation [14]. In this respect the carbon concentrating mechanism (CCM) in cyanobacteria is similar to that found in C_4 land plants. This enables them to maintain high rates of CO_2 fixation, and also grow under low CO_2 [15, 16].

The pioneering studies of Kaplan *et al.* [17] using *Anabaena variabilis*, demonstrated that cyanobacteria were capable of accumulating inorganic carbon, ($C_i = CO_2 + HCO_3^- + CO_3^{2-}$) to such an extent that intracellular concentration could be 1000 folds more than the external environment. Since then DIC (Dissolved Inorganic Carbon) accumulation has been demonstrated in many cyanobacterial strains e.g. *Cocochloris peniocyctis* [18], *Anacystis nidulans* [19], *Chlorogloeopsis sp.* ATCC27193 [20], *Nostoc calcicola* [21] etc. The CCM raises the CO_2 concentration around the active site of ribulose biphosphate carboxylase/ oxygenase (Rubisco). The enzyme uses CO_2 as the substrate but it does so with very poor efficiency. This system has the effect of increasing the photosynthetic affinity for CO_2 / HCO_3^- well above the relatively poor affinity of cyanobacterial Rubisco for CO_2 [16]. CCM in cyanobacteria has been shown to consist of two basic components- (1) C_i transport systems and (2) Rubisco containing carboxysomes [15]. Based on the type of Rubisco present, carboxysome in cyanobacteria has been divided into two categories: α and β . Proteins of α carboxysome share homologies with chemoautotrophic proteobacteria while those of β carboxysomes are confined to cyanobacteria. Experimental evidences suggest that

Table 1 Inorganic carbon transport systems in cyanobacteria based on Badger *et al.* [22]

Ci Transporters	Category	Carbon species	Presence of Na^+	Cyanobacterial strains	Operon
BCT ₁	β cyanobacteria	HCO_3^-	Na-independent	<i>Synechococcus</i> PCC7942	Inducible, encoded by <i>cmpABCD</i>
Sbt _A	α and β cyanobacteria	HCO_3^-	Na -dependent	<i>Synechocystis</i> PCC6803	Na extrusion pump encoded by <i>SbtA</i>
BicA	α and β cyanobacteria	HCO_3^-	Na- dependent	<i>Synechococcus</i> 7002 <i>Synechococcus</i> WH8102 <i>Synechococcus</i> PCC6803	Inducible/Constitutive encoded by <i>BicA</i>
NDH-I ₃	β cyanobacteria	CO_2	Na -independent	<i>Synechococcus</i> 7002	Constitutive encoded by <i>ndhF₃₃/ndhD₃₃/chpY</i>
NDH-I ₄	β cyanobacteria	CO_2	Na -independent	<i>Gloeobacter</i> sp.	Inducible encoded by <i>ndhF₄₃/ndhD₄₃/chpX</i>

there are at least five distinct modes of active C_i uptake in cyanobacteria (Table 1) [22]. Irrespective of the nature of the inorganic carbon source, bicarbonate (HCO_3^-) is the ultimate species delivered to cytosol, which further diffuses to carboxysome, a proteinaceous body, containing most of the cellular Rubisco [15]. The accumulated bicarbonate is wasteful unless it is converted to CO_2 with the help of enzyme carbonic anhydrase located in carboxysome [23]. It is a metallozyme, which catalyzes the inter-conversion of HCO_3^- and CO_2 , a reaction which occurs spontaneously but too slowly to meet the physiological demands of a cell. It is an ancient enzyme widespread in archaea and bacteria domains [16]. Biological fixation of CO_2 through photosynthesis seems to be the easily manageable and cheap way to combat the inevitable danger of increasing concentration of CO_2 in the air.

Role of cyanobacteria in the reclamation of 'usar' land

The independence for C and N nutrition, in many of the cyanobacterial genera, imparts them with a capability to grow in nutritionally poor and denuded areas. However, the role of cyanobacteria in reclamation of inhospitable habitats and remediating potential of polluted waters in comparatively a less investigated area. Although basic studies related to the role of sodium/ salinity stress on the effect of different metals on growth and photosynthesis and field related studies on nitrogen fixation in rice are available [24–27], their utilization as bioameliorating/ bioremediating agents is less documented.

The 'usar' lands of North-Western India represent the most unproductive soil types which are reclaimed using mechanical process such as flushing with ample supplies of H_2O and removing dissolved salts or application of gypsum to modify chemical structure of soil [28]. However, the gradual sodiumization, and impermeable nature of these soils, coupled with high pH, has made such practices economically non viable. The usar soils are of two major types-saline (solonchak) and alkaline (solonetz). Saline soils/ salt affected soils occupy more than 7 Mha of land in India. A number of cyanobacteria are known to be tolerant to sodium and salinity stress [29] and the role of excessive production of extracellular polysaccharides [30] often has been implicated. Laboratory and field level studies have attributed the salinity/alkalinity tolerance of cyanobacteria to the accumulation of inorganic ions, organic compounds (sugars, polyols, quarternary amines) and osmoregulators [31, 32].

One of the earliest examples of the use of the reclamative potential is related to the report of Reh commission (1910–

1912), which investigated the documentation causes for the deterioration of land in the state of Uttar Pradesh (India) and the general aridity of the land was attributed to the increased salinity as a result of canal irrigation. An interesting observation [33] was made regarding the presence of thick stratum of cyanobacteria on the surface of soil during the rainy season (July-September) and retreating monsoon (December- January) in these inhospitable habitat, indicative of the pioneering ability of these organisms. Kaushik and his group [29, 31, 34, 45, 36] observed that enrichment of such soils with native isolates, over a period of time improved the soil quality and making it arable by bringing about a decrease in pH, exchangeable sodium, Na/Ca and overall increase in N, P, organic matter and water holding capacity of soil. This ultimately lowers the sodium adsorption ratio, which is an index of alkalinity and improves the hydraulic conductivity of sodic soils. These results compared favorably to the use of chemical amendments such as gypsum in such soils [36]

However, there exists a need to develop viable, low cost rural technologies for on site use by farmers, using alkalophilic and saline tolerant strains.

Bioremediation of polluted environments

The Earth environment is a result of complex interactions among the sun, the atmosphere, the oceans the land and the living organisms. In the absence of human intervention, the interaction between biota and atmosphere approaches a steady state. But man has consistently modified the environment and in quest for more comfort overexploited the nature. Rapid industrialization and urbanization has substantially enhanced the degradation of our environment by polluting two basic amenities of life- air and water.

Treating water bodies as sinks for wastes and indiscriminate use of water in industrial and agricultural activities has affected the quality and quantity of drinking water. Conventional treatment methods not only require relatively large amount of space and produce large amount of wet bulky sludge but there is also a problem of safe disposal of material. With the advances in biotechnology, bioremediation has become one of the major developing fields of environmental restoration, utilizing microorganisms to reduce the concentration and toxicity of various chemical pollutants such as heavy metals, dyes pesticides etc. Biological treatment especially using algae, for treatment of water bodies has manifold advantages over conventional methods, as algae are abundant in aquatic environment and known to accumulate high levels of metal/pollutant; therefore, the process involved is relatively cheap and environment friendly. Also,

they can be integrated with other processes e.g Aquaculture/ pisciculture, feed production, soil conditioner production, fine chemical production and recovery of metals.

Major metal pollutants which are commonly found in industrial wastes are Cu, Zn, Ni, Co, Pb, Cr, Cd [37, 38]. Microbial biomass can passively bind large amounts of metal(s), a phenomenon commonly referred to as bio-sorption [39]. Among the photoautotrophs, cyanobacteria are relatively more tolerant to heavy metals [40]. Verma and Singh [41] reported Cu uptake in a diazotrophic cyanobacterium *Nostoc calcicola* to be biphasic. During the first 10 min there was a rapid binding of cations to the cell wall, followed by subsequent metabolism dependent intracellular uptake for at least 1h. The metal uptake was observed to be dependent on various nutritional factors and the tolerance to the intracellular detoxification mechanism [42]. The uptake of metal ions (Cu, Pb, Zn, Ni, Cd and Cr) in *Spirulina platensis* was accompanied by liberation of protons suggesting an ion exchange mechanism [43]. Similar results were obtained by Ahuja *et al.* [44] in *Oscillatoria angustissima*, where copper and zinc binding was accompanied by release of magnesium ions. Rai *et al.* [45] demonstrated the bisorption potential of *Microcystis* sp. in removal of Ni and Cd. They further showed that alginate immobilized cells of the cyanobacterium can be used for removal of Cu by adjusting the flow rate, initial metal concentration and biomass density. Application of immobilized biomass has many advantages over the suspended biomass: these include the easy regeneration and reuse of immobilized biomass, easy separation of cells and effluent, minimal clogging and high biomass loading. The basic mechanism of metal uptake in cyanobacterium involves two basic phases. Firstly, a passive rapid phase in which ions bind to cell wall followed by a slower and metabolically dependent uptake in cytosol [48]. The carboxyl group on algal biomass has been found to be responsible for the binding to various ions [47]. An unicellular cyanobacterium *Synechococcus* sp. had been showed to have the ability of binding to metal ion e.g. Cu (11.3 mg/g biomass), Pb (30.4 mg/g biomass), Ni (3.2 mg/g biomass), Cd ((7.2 mg/g biomass) [48].

Many microorganisms synthesize intracellular metal binding proteins called metallothioneins (MTs), which are low molecular weight proteins or polypeptide (6000-8000 amu) These “MTs” are rich in cys residue and bind to metal ion in metal thiolate cluster. The characteristic organization of cys residue mediates the metal binding property [48]. Another mechanism involves the partitioning of metal ion between exopolymer sheath and cell wall [48].

The extensive use of textile dyes has also become an area of great concern because of their carcinogenic, muta-

genic and toxic nature, besides their low biodegradability leading to environmental pollution. Species of *Oscillatoria* have been reported to decolour dye from wastewater [49, 50]. Jinqi and Houtian [50] suggested that algae can utilize Erichrome blue SE and Black T as its sole source of carbon and nitrogen and that the process of azo compound degradation is related to the physiological metabolism of the algae. Many species of *Oscillatoria* have been reported to decolour dye in waste water [51]. Shah *et al.* [52] designed a laboratory scale reactor to integrate the hydrogen production and dye removal capability of a marine cyanobacterium. Cyanobacterial strains isolated from sites polluted by industrial textile effluents have been reported to degrade more than 55% of the synthetic dyes present in the samples. *Phormidium valderianum* [53], a cosmopolitan cyanobacterium, has been reported to remove more than 90% of textile dyes from solution in pH range higher than 11, besides simultaneously producing hydrogen [53]. Therefore, algae can play a major role in stabilization of ponds by not only enhancing oxygen production/level but also directly in degradation of azo dyes.

Cyanobacteria are known to commonly for blooms in water bodies and play a major role in eutrophication; however, they have not been adequately exploited for their nutrient scavenging role in polluted systems. In a study carried out at IARI, the application of selected green algal and cyanobacterial strains scavenge N and P from sewage effluents and also improved the yields of rice crop fertigated with sewage effluents [54].

Outlook for the future

Cyanobacteria are excellent model systems which can provide the biotechnologist with novel genes and biomolecules having diverse uses in agriculture, industry and environmental sustainability. Concerted efforts need to be undertaken to relate ecological behaviour *in situ* of these organisms to physiological/biochemical processes and the molecular basis of their regulation. Such biological systems also hold promise as efficient contraptions for harnessing greenhouse gases such as CO₂ and methane; therefore, need to be investigated in depth using molecular tools to identify the presence of related genes and their regulation.

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