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REVIEW PAPER

Cyanobacteria: A new horizon for organic fertilizer in rice fields

*ANJULA CHAUDHARY AND ABHA VASHISTHA

School of Biosciences, Institute of Management Studies
Ghaziabad-201013, Uttar Pradesh, India.
email id: anjula.chaudhary@imsuc.ac.in

ABSTRACT

Rice is the staple food for the world's population as it full fills the calorie demands of major part of population. Cyanobacteria are one of the major elements in nitrogen fixing flora. These (blue green algae) are capable of fixing atmospheric nitrogen and convert it into an available form of ammonium required for plant growth. Dominant nitrogen-fixer blue-green algae are *Anabaena, Nostoc, Aulosira, Calothrix, Plectonema* etc. These are one of the major components of the nitrogen fixing biomass in rice fields. The importance of cyanobacteria in agriculture is their ability to fix nitrogen as it is one of major requirement ofcrops. Biofertilizers are cost effective, eco-friendly and easier to use so are these good friend of farmers.

Keywords: *Cyanobacteria, Biofertilizers, Nitrogen fixation, Rice field, Inoculants, Soil fertility and Biofilm.*

INTRODUCTION

Rice (*Oryza sativa* L.) is the world's most important food crop and a primary source of food for more than half of the population. Rice is a grain consumed exclusively by humans, supplying 20% of daily calories for world population. The world's rice demand is increasing day by day and it is estimated that by 2020 it will be 118.93 million tones. Rice can be grown in various agroclimatic conditions like rainfed low land areas, rainfed upland areas, flood prone and irrigated areas. During ancient times the soil nutrients were in plenty amount but now a days due to use of advanced technologies in agriculture, the productivity gets increased but todays concern is about the use of excessive amount of chemical fertilizers in soil and their adverse effects on environment.Extensive crop production requires the use of nitrogenous fertilizers. However, fertilizers are in short supply and quite expensive in developing countries. Also, the continuous use of chemical fertilizers causes the ecological and biochemical imbalance in the rice field (Roger and Kulasooriya, 1980).The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion and acidification of water. These problems can be tackled by the use of biofertilizers (Choudhury and Kennedy 2005, Rai 2006). To overcome this problem biofertilizers are gaining large interest.The first ever production of cyanobacterial biofertilizer in India is reported to be in 1972 at IARI, New Delhi.

A **Biofertilizer** is a substance which contains living microorganisms which, when applied to seed, plant surface, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.Cyanobacteria gets its common name from the blue-green pigment, phycocyanin, which along with chlorophyll *a* gives cyanobacteria a blue-green appearance. Phycocyanin is a protein that functions as the photosynthetic pigment in photosystem II, whereas in plants chlorophyll *b* is the pigment in photosystem II (Clark, 1998).

Cyanobacteria are a diverse group of prokaryotes which resemble gram negative bacteria in their structure and possessing oxygen evolving photosynthetic system (Prabina *et al*., 2004).They comprise about 150 genera and 2,000 species ranging from unicellular, colonial, filamentous to branched filamentous forms and are divided into 5 subsections i.e. *Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales* (Boone and Castenholz, 2001). They are ubiquitous in their distribution and grow in all sorts of aquatic and terrestrial environments exhibiting wide range of temperature, salinity, water potential, pH and irradiance.The main feature of cyanobacteria is their association with vascular/non-vascular plants and production of growth-promoting substances (Nanjappan Karthikeyan *et al*., 2007). Non-heterocystous cyanobacteria can fix N_2 and convert it into an available form of ammonia required for the plant growth.The enzyme complex responsible for this nitrogen fixation is called nitrogenase (Bold, 1985). Nitrogen fixing cyanobacteria are unique as they are able to assimilate both carbon and nitrogen. Efficient nitrogen fixing strain like *Nostoc linkia, Anabaena variabilis, Aulosira fertilisima, Calothrix sp., Tolypothrix sp, and Scytonema sp.* were identified from various agro-ecological regions and utilized for rice production (Prasad and Prasad, 2001). Algalization of soil with living di-nitrogen fixing cyanobacteria has become a common practice in tropical countries for many years (Venkatraman *et al*., 1974). Cyanobacteria play a spectrum of roles in the field of biofertilizer, energy production, human food, animal feed, polysaccharides, biochemicaland pharmaceuticals. Along with nitrogen fixation, cyanobacteria have desirable impact on the soil properties. Cyanobacteria are known to macro-aggregation and result in improving resistance to soil erosion because as primary producers, they contribute to the enrichment of soil with soil organic matter (SOM) and improvement in biological activity. Improvement in the aggregation of cyanobacteria inoculated soils which could be related to increasing in soil carbon and extra-cellular polymorphic substance (EPS) that cause change in the soil micro-morphological characteristics of the aggregates. Significant contribution of cyanobacterial biomass enhanced microbial activity and plant growth promotion, emphasizing their significance in sustainable management of rice ecosystem.

Cyanobacteria increase the fertility of soil and consequently the yield of rice and other crops by:

a. Increase in soil pores due to its filamentous structure and production of adhesive substances.

b. Excretion of growth-promoting substances such as hormones (auxin and gibberellin), vitamins and amino acids (Roger and Reynaud 1982, Rodriguez *et al.,* 2006).

c. Increase in water holding capacity through their jelly structure (Roger and Reynaud, 1982).

d. Increase in soil biomass after their death and decomposition (Saadatnia and Riahi, 2009)

Important nitrogen fixing Cyanobacteria

Unicellular	Filamentous	
	Non-heterocystous	Heterocystous
Aphanothece, Chroococcidiopsis, Dermocarpa, Gloeocapsa, Myxosarcina, Pleurocapsa, Synechococcus, Xenococcus	*Lyngbya, Microcoleus chthonoplastes, Myxosarcina, Oscillatoria, Plectonema boryanum, Pseudoanabaena, Schizothrix, Trichodesmium*	*Anabaena, Anabaenopsis, Aulosira,Calothrix,Camptylonema, Chlorogloea,Chlorogloeopsis, Cylindrospermum, Fischerella, Gloeotrichia, Haplosiphon, Mastigocladus, Nodularia, Nostoc, Nostochopsis, Rivularia,Scytonema,,Stigonema, Tolypothrix,Westiella, Westiellopsis*

(Vaishampayan *et al.,*2001, Pereira *et al.,*2008, Rana *et al.,*2012, Prasanna *et al.,*2013)

Cyanobacteria are well adapted for a variety of environments and they are used as inoculants to boost up the crop productivity especially in rice. Rhizobacteria are also beneficial for growth of certain plants and are known as plant growth promoting rhizobacteria (PGPR) and was first described by Kloepper and Schroth. PGPR affect the plant growth by various mechanisms like:

a. Fixation of atmospheric nitrogen that is transferred to the plant

b. To prevent the harmful effect of phytopathogenic organisms to rhizosphere

c. Secondary metabolite production

d. Production of siderophores that chelate iron and make it available to the plant root

e. Solubilization of minerals such as phosphorus

PGPR has a range of reported properties including nitrogen fixation, phosphorous solubilization, production of antibiotic and cytokinin and increase root and shoot growth of crops.

Effects of cyanobacterial fertilizer on rice fields

For proper cultivation and production of rice, management of various nutrients particularly nitrogen is important for the growth, development and higher yield of rice plants large amount of nitrogen is required and the main disadvantage is that nitrogen is absent from mineral constitution of soil. Rice consumes about 16-17 Kg N for the production of each tone of rough rice including straw. Rice field ecosystem provides an environment favourable for the growth of cyanobacteria with respect to their requirement for light, water, temperature, humidity and nutrient availability. Biofilm based biofertilisers are also in trend to improve crop productivity. Fritsch (1907) initially observed the abundance of cyanobacteria in rice fields. The main impact of cyanobacterial fertilizer on rice field is enhanced grain yield which remarkable effect is also seen on other parameters like nitrogen content and number of tillers, number off filled panicles etc. Cyanobacterial populations are dormant as spores in the dry season. Germination of spores takes place when the rice paddy fields are in waterlogged conditions. They grow together with the rice plants and form cyanobacterial patches. Surveys were conducted on rice fields all over the world and it was suggested that *Anabaena* and *Nostoc* spp. are quite ubiquitous in rice fields.

A biofilm is an aggregate of microorganisms in which cells adhere to each other or to a living or non-living surface. The use of biofilms has also been proposed as possible means to produce effective plant inoculum (Seneviratne *et al.,* 2008). Three major types of biofilms can occur in the soil: bacterial (including *Actinomycetes*), fungal, and fungal-bacterial biofilms. The biofilm fertilizers improve nitrogen fixing symbiosis in legumes, and could contribute directly to soil Nitrogen fertility in long term. Cyanobacteria play an important role in maintenance and building soil fertility, consequently increasing rice growth and yield as a natural biofertilizer (Song *et al.,* 2005). All dominant BGA in rice fields are nitrogen fixing, give the indication how rice has been grown continuously for many centuries without the addition of fertilizers (Watanbe, 1987).Cyanobacteria benefits in rice plants by producing growth-promoting substances followed by increasing the availability of P by excretion of organic acids was also exploited in the prevention of soil erosion process (Kumar & Rao, 2012). In a study conducted by Saadantia and Riahi (2009) the germination of rice seeds treated with cyanobacteria was faster than control and also there was an increase of 53% in plant height; 66% in roots length; 58% in fresh leaf and stem weight; 80% in fresh root weight; 125% in dry leaf and stem weight; 150% in dry root weight; 20% in soil moisture; 28% in soil porosity and a decrease of 9.8% in soil bulk density and 4.8% in soil particle density. There were significant differences ($P < 0.05$) in pot treated with BGA as compared with control. Mishra and Pabbi (2004) developed and transferred, a rural oriented algal biofertilizer technology for rice, to the farmers resulting in low cost production of crop without compromising yield and soil health.

Growth promoting substances production

Cyanobacteria are well known as biofertilzers as they help to fix nitrogen but they also enhance the crop productivity by producing some growth promoting substances. Growth promotory substances were reported to be like amino acids, auxins, gibberellins, cytokinins (Sood *et al.,* 2011). These growth promoting substances are reported to enhance the growth by making the nutrients available to plants by elevating their uptake by plants.

Associative Biological Nitrogen Fixation through cyanobacteria in rice

Biological nitrogen fixation is carried out by some prokaryotes including cyanobacteria, these nitrogen fixing prokaryotes are mainly found in soil in free living form. They fix the nitrogen without the direct interaction with other organisms. The examples in this category include *Azotobacter, Bacillus*, *Clostridium and Klebsiella*. Free living prokaryotes often behave as anaerobes during nitrogen fixation. They contribute to a very less amount of global nitrogen fixation due to scarcity of appropriate carbon sources (Wagner, 2012).

Associative types of microorganisms remain in close association with the rhizosphere region of members of family Poaceae (Rice, wheat, corn, oats, barley etc.). These bacteria fix an appreciable amount of nitrogen. Associative nitrogen fixation can supply 20–25% of total nitrogen requirements in rice and maize (Montanez *et al*., 2012). The most important examples exhibiting the associative nitrogen fixation is the species of *Azospirillum* (Saikia and Jain, 2007). In rice, associative nitrogen fixation supplied 20–25% N of the total need, as reported in one of the studies conducted at IRRI, Philippines (Ladha, 1987). Rice seedlings when inoculated with some strains of *Burkholderia spp*. isolated from rice plants contributed a relatively higher level of N to rice via BNF (Baldani, 2000).

Rhizobia were found to have the ability to attach them to rice root hairs, elicit deformation of rice root hairs and to form nodule-like structures. Rhizobia could colonize intercellularly, multiply and migrate inside the growing lateral roots, the stimulation of lateral root development and colonization of lateral root cracks and xylem of rice roots by A. caulinodans (ORS 571) has been reported (Gopalaswamy, 2000). In addition to forming nodules after crack entry invasion of emerging lateral roots, it is able to fix nitrogen in the free-living state up to 3% (v/v) oxygen and without differentiation into bacteriods (Kitts, 1994).

Azoarcus is capable of invading roots of the original hosts as well as rice plants, infecting the cortex region and is capable of expressing nitrogenase genes in the aerenchyma of rice roots.

Increase in yield of ricewas observed by (Kaushik, 2013) and also saving of fertilizer nitrogen, cyanobacterial inoculation improves the physico-chemical properties of soil, gradual build-up of residual soil nitrogen and carbon, improvement in soil pH and electrical conductivity. The grain quality interms of protein content was improved. Our recent studies have shown that the cyanobacteria (*Nostoc* and *Anabaena*) were capable of forming associations with wheat roots grown in liquid culture. Probably, such cyanobacteria contribute nitrogen and growth promoting substances to plants in the rhizosphere.

Conclusion and future prospects:

The history of cyanobacterial fertilizers lasts from 1960s and onwards, efforts have been made to use cyanobacteria as a potential biofertilizer. Use of cyanobacterial fertilizers will not only help fertilizers economically but also would help to protect the environment from the use of chemical fertilizers. Cyanobacterial species with higher rate of heterocyst formation will be more potent in nitrogen fixation. Further, colonization ability of cyanobacteria their diversity, interaction with other organisms, their applicability as biofertilizers, their formulations should be studied in order to use them in sustainable agricultural practices. Some molecular biology techniques will help to enhance their abilities and potential to be used as biofertilizers, some genetic changes may also include one or more important trait to improve growth promotion.

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