

The Role of Cyanobacteria on Agriculture

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Abstract

Cyanobacteria (blue-green algae) are a diverse group of prokaryotes, having oxygenic Photo synthesis and the most successful and oldest life forms present on the planet earth and play an important role in maintenance and build up of soil fertility, consequently increasing plant growth and yield as a natural bio fertilizer (reduced input cost), nutrient cycling, N₂-fixation, bioavailability of phosphorus, water storage and movement, environmental protection and prevention of pollution and land degradation especially through reducing the use of agro-chemicals, and recycling of nutrients and restoration of soil fertility through reclamation.

Key words: Cyanobacteria, Agriculture, biofertilizer, biocontrol, nitrogen fixation

1. Introduction

Cyanobacteria (blue-green algae) are Gram-negative prokaryotes, perform oxygenic photosynthesis, and also fix atmospheric N₂, the most successful and oldest life forms present on the planet earth. They can easily survive the extreme environments such as hot springs, hyper-saline waters, freezing environments, and arid deserts ([Singh 2014](#)). They estimated about 150 genera and 2,000 species which ranging from unicellular, colonial and filamentous to branched filamentous forms (Boone and Castenholz 2001).

They are ubiquitous in their distribution and grow in both aquatic and terrestrial environments including ponds, lakes, water streams, rivers, and wetlands exhibiting wide range of temperature, salinity, water potential, p^H. Their wide spread distribution reflects abroad spectrum of physiological properties and tolerance to environmental stresses. (Singh *et al.* 2013a).

Increasing food demand has largely amplified the utilization of chemical fertilizers to achieve high and significant rise in crop yield. However, careless and over use of these fertilizers is leading to the pollution of both soil and water. Cyanobacteria offer an economically attractive and environment friendly alternative to chemical fertilizers which increase the soil productivity both directly and indirectly (Thatoi *et al.* 2013).

The application of Cyanobacteria in management of soil and environment includes the economic benefits (reduced input cost), nutrient cycling, N₂-fixation, bioavailability of phosphorus, water storage and movement, environmental protection and prevention of pollution and land degradation especially through reducing the use of agro-chemicals, and recycling of nutrients and restoration of soil fertility through reclamation ([Shukia *et al.* 2008](#)). Because of those abilities Cyanobacteria have positive effects on agricultures, especially for crop production which serve as human food.

2. The role of Cyanobacteria in agriculture

The world population increase from year to year continuously. In order to provide food to all population, the annual production of cereals need is increase. This target puts enormous pressure on agriculture sector to achieve the food security especially developing country including Ethiopia those economies extensively depend on agriculture. To achieve this target needs the utilization of agro chemicals, intensive tillage, and over irrigation, which have undoubtedly helped many developing countries to meet the food requirement of their people, but it leads environmental and health problems and increased cost of agricultural production. In order to solve these problem using of Cyanobacteria offer an economically attractive and environment friendly alternative to chemical fertilizers which increase the soil productivity both directly and indirectly (Singh *et al.* 2013a). Cyanobacteria play an important roles in improving crop production through different mechanisms including as bio fertilizers such as enhanced solubilization and mobility of nutrients, improving the physico-chemical conditions of soils, mineralization of simpler organic molecules such as amino acids for direct uptake and Stimulation of the plant growth due to their plant growth promoting attributes and as bio pest side (biological control agent). Soil is the habitat of some terrestrial Cyanobacteria species which are beneficial organisms for soil fertility by fixing atmospheric nitrogen (N), binding soil particles, helping to maintain moisture and preventing erosion. Increase of essential microelements in soil which are necessary for plant growth and plant ion uptake, increase of N content of the surface soil, as well as production of plant growth promoting substances such as phytohormones (Auxin, Gibberellins) and other plant growth regulator substances (PGRs) such as amino acids, sugars and vitamins are the most important factors that are suggested for plant growth stimulating effects of these microorganisms ([Shukia *et al.* 2008](#)).

2.1 Extracellular substances produced by Cyanobacteria

Cyanobacteria excrete a great number of substances that influence plant growth and development. The substances produced by Cyanobacteria include growth-promoting regulators, vitamins, amino acids, polypeptides and exopoly saccharides (Sood et al.2011). Those substances play great role in agriculture both directly and indirectly, for example substance growth-promoting regulators helps for obtained maximal growth as well as produce maximal yield such types of substance produced from Cyanobacteria consider as direct role and also exopoly saccharides produced by Cyanobacteria play great role in soil formation and protection from erosion. In opposite to these some substance produced by Cyanobacteria play indirect role in agriculture through reduction of other microorganisms that are not suitable for plant growth (Zaccaro 2000).

2.2 Nitrogen fixation by Cyanobacteria

Nitrogen is one of macro element and required in higher amount by plant and an essential constituent of proteins, nucleic acids, chlorophylls, enzymes, and other physiological substances in green plants and it account 79% from our atmosphere but most of the plant cannot utilize it and this lead to reduction of crop production (Malik et al. 2001; Cameron and Haynes 1986).

Nitrogen fixation is the process by which nitrogen in the atmosphere is converted into a more active form that can be used by plants. Many free-living blue-green algae (Cyanobacteria) fix atmospheric nitrogen through the process of biological nitrogen fixation (Prasanna et al.2013b) and since they are photosynthetic, they do not compete with crop plants and heterotrophic soil micro flora for carbon and energy. Even though, 79% the atmosphere is covered by nitrogen due to the triple bond presence in nitrogen molecule which give there stable nature plant cannot utilize it. By converting this unutilized form of nitrogen to utilized form Cyanobacteria play great role and this lead to increment of plant growth and yield production.

2.3 Cyanobacteria as bio fertilizer

Bio fertilizer is a substance which contains living microorganisms they can colonizes the rhizosphere or the interior of the plant and promotes growth, when applied to seed, plant surfaces or soil (Vessey, 2003). Bio fertilizer produced from Cyanobacteria increase agricultural production through promote plant growth by enhance the availability of nutrients to the plants, improve soil chemical and biological characteristics, synthesis of growth promoting substances, restore the soil's natural nutrient cycle and build soil organic matter, converts complex nutrients into simple nutrients for the availability of the plants, decrease soil salinity, preventing weeds growth ,increase phosphate in soil by excretion of organic acids, increase water holding capacity through their jelly structure and protect plants from soil born diseases (El-Habbasha *et al.* 2007; Yosefi *et al.*,2011 ;Song *et al.* 2005 Roger and Reynaud 1982 ; Saadatnia and Riahi 2009; Thajuddin and Subramanian 2005). The use of biofertilizer in preference to chemical fertilizers, biofertilizer offers ecologic and economical benefits by improve soil health and increase soil fertilities and decrease the use of agrochemical such as inorganic fertilizers, insect side, herb side etc and also utilization of biofertilizer can protect crops from soil born disease.

2.4 Plant growth substances produced by Cyanobacteria

Cyanobacteria produce a wide array of compounds like amino acids, auxins, gibberellins, cytokinins (Sood *et al.*2011). All these compounds increase the availability of nutrients and help the plants in taking up nutrients and it result better growth of plant and increase agricultural productivities (Mader *et al.* 2011). Cyanobacteria benefit plants by producing growth promoting regulators (the nature of which is said to resemble gibberellins and auxins), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phyto pathogen bio control and polymers, especially exo polysaccharides, that improve soil structure and exo enzyme activity (Zaccaro 2000). Moustafa and Omar (1990) reported that inoculation of tomatoes with a mixture inoculum of *Azospirillum lipoferum* and Cyanobacteria, formally called blue-green algae (a mixture of different cyanobacteria strains) and/or cyanobacteria alone as bio fertilizer led to increase significantly as improved the quality of tomato fruits. Also, Kotb *et al* (1990) showed that inoculation with *Azospirillum* and /oralgae gave significant positive differences for fresh weight of tomato fruits and plants dry weight when compared to the control plants without inoculation.

2.5 Phosphate uptake

Phosphorus (P) is the 11th most abundant element found in earth's crust and necessary for survival of life, as it is the main backbone of DNA, RNA, and ATP, the key components of a living cell. Phosphorus is a limiting nutrient for crop growth having no substitute and hence, food security worldwide depends on the P availability in soil for crop production (Cordell *et al.*, 2011).

Phosphorus is the second major plant nutrient after nitrogen in terms of quantitative requirements for crop plants. The problem of P management in soil is highly intricate, as the applied phosphate through fertilizers is

often fixed and becomes unavailable to the crops. In organic matter rich soils, P availability is due to excretions of enzymes or acidic metabolites produced by microorganisms including Cyanobacteria (Rogers *et al.*, 1991).

Phosphate, an essential mineral for plant growth and development, is the limiting nutrient for biomass production in natural ecosystem. It is often added to the soil in the form of phosphatic fertilizers, however, plants utilize only a small amount of this nutrient and major part of phosphate is rapidly converted to insoluble complexes in the soil which plants cannot utilize. Soil microorganisms are effective in releasing phosphate from inorganic and organic pools of total soil P through solubilization and mineralization (Chen *et al.* 2006). In this processes of conversion of unavailable form to available from Cyanobacteria play a role by excretions of enzymes or acidic metabolites.

Cyanobacteria, like P solubilizing bacteria, have the ability to solubilize insoluble $(Ca)_3(PO_4)_2$ (tricalcium diphosphate); $FePO_4$ (ferric orthophosphate); $AlPO_4$ (aluminum phosphate) and $(Ca_5(PO_4)_3(OH))$ (hydroxyl apatite) present in soils and sediments (Vaishampayan *et al.* 2001). Cyanobacteria enhanced decomposition and mineralization of phosphate and transformed it into easily available soluble organic phosphates/orthophosphates more over application of Cyanobacteria in crop fields plays an important role in mobilization of inorganic phosphates by means of extracellular phosphates and excretion of organic acids.

2.6 Degradation of agrochemicals

Modern agriculture is intensively depended on use of agrochemicals (herbicides, insecticides, Fungicides etc). This is practiced to increase the global food production by killing crop pests but at the same time, it has started polluting the environment. Biological degradation/removal of these chemicals involving several microorganisms including Cyanobacteria has been well established (Subashchandrabose *et al.* 2013). Cyanobacterial species like *Nostoc linckia*, *N. muscorum*, *Oscillatoria animalis* and *Phormidium foveolarum* degraded methyl parathion, an organo phosphorus insecticide (Fioravante *et al.* 2010).

2.7 Cyanobacteria in restoration of soil fertility

Cyanobacteria supplementation enhanced soil quality by conserving organic matter, nitrogen, phosphorus and moisture in soil. The organic matter of decomposed Cyanobacteria get mixed in the soil and acts as binding mucilaginous agent for soil and results in enhancing the humus content and suitability for growth of other plants (Maqubela *et al.* 2009). Natural and anthropogenic disturbances degrade the biological soil crusts and full recovery of such crusts may take decades under natural conditions. However, Cyanobacterial inoculation greatly speeds up the recovery process, and such biological crusts occurring in semiarid and arid regions of the world play an important role in maintaining and restoring the ecosystem of the area (Wang *et al.* 2009).

Cyanobacteria produce extracellular polymeric substances (EPS) which improve the water-holding capacity of soil and prevent erosion so the combinations of Cyanobacterial strains (*Anabaena doliolum*, *A. torulosa*, *Nostoc carneum*, *N. piscinale*, *Oscillatoria*, *Plectonema*, *Schizothrix* etc.) enhanced soil microbial biomass, carbon, nitrogen and humus content thus retaining moisture and helping in soil formation (Prasanna *et al.* 2013b).

2.8 Cyanobacteria as Bio-control agents

As all living organisms, plants must face infections and diseases following the attacks of a mass of plant pathogens and pests from animal, microbial or viral origin. These diseases can be minor causing solely a reduction of plant-growth capacities or can be at the origin of much more severe damage leading to plant death in the worst case. Plant diseases are responsible for the loss of at least 10% of global food production, representing a threat to food security (Strange & Scott, 2005). To prevent or control these diseases, producers have become increasingly dependent on agrochemicals, especially over the past few decades, as agricultural production has intensified. However, despite the great effectiveness and ease of utilization of these products, their use or misuse has caused many problems including significant pollution of soils and ground water reservoirs, accumulation of undesirable chemical residues in the food chain, emergence of fungicide-resistant strains of pathogens, not to mention health concerns for growers (Strange & Scott, 2005).

Bio pesticides, which are used to suppress pathogen populations, are living organisms or natural products derived from these organisms. They can be divided into four main groups: microorganisms (microbial pesticides), other organisms (nematodes, insects...) used to control pests, natural substances that are derived from living organisms (biochemical pesticides) and plant-incorporated protectants (genetically modified plants) (Thakore, 2006). Biopesticides show several advantages when compared to chemical products. They decompose more quickly in the environment and are generally less toxic towards non-target species (Thakore, 2006). Additionally, their modes of actions are usually distinct from those of conventional pesticides.

In modern agricultural practices, the conventional pest control methods have not been found quite efficient due to survival of the reproductive structures of pathogens in the soil. Additionally, chemical pesticide agents inhibit the growth and development of crop plants (Manjunath *et al.* 2010). Various antagonistic microorganisms have been identified for several plant pathogenic microbes and among these, cyanobacteria play a major and

efficient role as antagonistic agents against several pathogenic microbes, insects and weeds (Prasanna *et al.* 2013a).

Cyanobacteria/bacteria amended compost and compost tea preparations were most effective for suppressing diseases caused by plant pathogenic fungi *Fusarium oxysporum*, *Pythium debaryanum*, *Pythium aphanidermatum* and *Rhizoctoniasolani* in tomato (Chaudhary *et al.* 2013). An aqueous extract/extracellular products from nitrogen fixing cyan bacterium *Nostoc muscorum* were efficient in controlling the growth of the plant pathogens *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. Recently, Natarajan *et al.* (2012) has identified two novel fungicidal compounds as benzoic acid derivative and majuscule amide C from *Calothrix elenkinii* and *Anabaena laxa* respectively. Chitosanase homologues and micro cystin like compounds with fungicidal activity have also been identified and characterized in *Anabaena laxa*, *A. iyengarii* and *A. fertilissima* (Gupta *et al.* 2012). The Cyanobacterial species *Calothrix elenkinii* produced fungicidal compounds against damping-off disease in tomato, chilli and brinjal (Manjunath *et al.* 2010). Cyanobacterial species (*Anabaena variabilis* RPAN59 and *A. laxa* RPAN8) helped in improving growth of *Fusarium* wilt challenged tomato plants following amendment in compost-vermiculite under controlled conditions (Prasanna *et al.* 2013a).

2.9 Cyanobacteria in soil physical properties improvement

Cyanobacteria are known to excrete extra cellularly a number of compounds like polysaccharides, peptides and lipids during their growth in soil. These compounds diffuse around soil particles, glue and hold them together in the form of micro aggregates. Besides these compounds, polysaccharides are made of fibres, which can also entangle clay particles and form clusters. These clusters or micro aggregates, in turn, grow and take the shape of macro aggregates and subsequently of larger soil aggregates. The interwoven nature of growing algal filaments may also help in binding the soil particles along with the organic C added through algal biomass (Rogers *et al.*, 1991).

3. Conclusion

Generally Cyanobacteria play great role in agricultural productivities through both directly and indirectly. As we mentioned Cyanobacteria have various mechanisms to improve agricultural production and using them as biofertilizer give a healthy environment and reduce agricultural input cost especially in developing countries include our country Ethiopia. However, using microbes in agriculture well not understood especially in developing countries include our country Ethiopia. So, this paper may be a clue for such countries.

4. REFERENCE

- Boone, D., Castenholz, R. (2001), "The *Archaea* and the deeply branching and photo trophic bacteria", In: Garrity GM (ed) *Bergey's manual of systematic bacteriology*, 2nd Edn. Springer-Verlag, NewYork, pp 33-38.
- Cameron, K., Haynes, R. (1986), "Retention and movement of nitrogen in soils", In: Haynes R. J. (ed.), *Mineral Nitrogen in the Plant-Soil System*, Academic Press, Orlando. P.166-241.
- Drobniewski, F. (1993), "*Bacillus cereus* and related species", *Clinical Microbiology Reviews*, **6**(4), 324–338.
- Chaudhary, V., Prasanna, R. and Bhatnagar, A. (2013), "Influence of phosphorus and pH on the fungicidal potential of *Anabaena* strains", *J Basic Microbiol*, **53**(3),201-213.
- Chen, YP., Rekha, PD., Arun, AB., Shen, FT., Lai, WA. and Young CC. (2006) "Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities", *Appl Soil Ecol* **34**, 33-41.
- Cordell, D., Rosemarin, A., Schröder, J. J., and Smit, A. L. (2011), "Towards global phosphorus security: a systems framework for phosphorus recovery and reuse options", *Chemosphere*, **84**, 747–758.
- El-Habbasha, S.F., Hozayn, M., Khalafallah, M.A. (2007), "Integration effect between phosphorus levels and biofertilizers on quality and quantity yield of faba bean (*Vicia faba* L.) in newly cultivated sandy soils," *Research Journal of Agriculture and Biological Science*, **3**(6), 966-971.
- Fioravante, I.A., Barbosa, F.A.R., Augustic, R., Magalhães, S.M. S. (2010), "Removal of methyl parathion by cyanobacteria *Microcystis novacekii* under culture conditions", *J Environmonit*, **12**, 1302
- Gupta, V., Prasanna, R., Srivastava, A.K., Sharma, J. (2012), "Purification and characterization of a novel antifungal endo-type chitosanase from *Anabaena fertilissima*," *Ann Microbiol*, **62**, 1089-1092.
- Kotb, S. I., Hashem, F.M., Montasser, M.S. (1990), "Utilization of bio fertilizer compensates for yield reduction of vaccinated tomato plants, 8th intentional congress on nitrogen fixation. May 20- 26, 1990, knox Ville. U.S.A.
- Mader, P., Kaiser, F., Adholeya, A., Singh, R., Uppal, H.S., Sharma, A.K., Srivastava, R., Sahai, V., Aragno, M., Wiemken, A., Johri, B.N., Iried, P.M. (2011), "Inoculation of root microorganisms for sustainable wheat rice and wheat black gram rotations in India," *Soil Bi o ch em*, **43**, 609 - 619 .
- Malik, F.R., Ahmed, S., Rizki, Y.M. (2001), "Role of Blue Green Algae in Paddy Crop Pakistan," *Journal of Biological Sciences*, **4**, 1217–1220.

- Manjunath, M., Prasanna, R., Lata, P.D., Singh, R., Kumar, A., Jaggi, S. and Kaushik, B.D. (2010), "Bio control potential of cyanobacterial metabolites against damping off disease caused by *Pythium aphanidermatum* in solanaceous vegetables", *Arch Phytopathol PlantProt*, **43**,666- 677.
- Maqubela, M.P., Mnkeni, P.N., Issa, M.O., Pardo, M.T. and D'Acqui, L.P. (2009), " *Nostoc* cyanobacterial inoculation in South African agricultural soils enhances soil structure, fertility and maize growth", *Plant Soil*, **315**,79-92.
- Natarajan, C., Prasanna, R., Gupta, V., Dureja, P. and Nain, L. (2012), " Dissecting the fungicidal activity of *Calothrix elenkinii* using chemical analyses and microscopy", *Appl Biochem Microbiol*, **48**,51-57.
- Moustafa, S. S. and Omar, M.A. (1990), "Effect of bio fertilizers as an inoculant on yield and quality of tomato Egypt", *J. Appl. Sci*, **5**, 209-226.
- Prasanna, R., Chaudhary, V., Gupta, V., Babu, S., Kumar, A., Singh, R., Shivay, Y.S. and Nain, L. (2013a), " Cyanobacteria mediated plant growth promotion and bioprotection against *Fusarium* wilt in tomato", *Eur J Plant Pathol*, **136**,337-353 ..
- Prasanna, R., Sharma, E., Sharma, P., Kumar, A., Kumar, R., Gupta, V., Pal, R.K., Shivay, Y.S. and Nain, L. (2013b), " Soil fertility and establishment potential of inoculated cyanobacteria in rice crop grown under non flooded conditions", *Paddy Water Environ*, **11**,175-183.
- Roger, P.A., Reynaud, P.A. (1982) , "Free-living Blue-green Algae in Tropical Soils", Martinus Nijhoff Publisher, La Hague.
- Rogers, S.L., Cook, K.A. and Burns, R.G. (1991) , " *Advances in Soil Organic Matter Research*"; *the Impact on Agriculture and the Environment*, Royal Society of Chemistry, Cambridge, pp 175-184.
- Saadatnia and Riahi (2009), "Cyanobacteria from paddy fields in Iran as a bio fertilizer in rice plants" , *Plant Soil and Environment*, **55**(5) 207-212.
- Sharma, R., Khokhar, M.K., Jat, R.L., Khandelwal, S.K.(2012) , "Role of algae and cyanobacteria in sustainable agriculture System", *Wudpecker Journal of Agricultural Research*, **1**(9), 381 – 388.
- Shashirekha, S., Uma, L., Subramanian, G. (1997), " Phenol degradation by the marine cyanobacterium *Phormidium valderianum* BDU-30501", *J. Ind. Microbiol. Biotechnol*, **19** 130–133.
- Singh, J. S., Pandey, V. C., Singh, D. P. (2011a), "Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development", *Agric Ecosyst. Environ*, **140**, 339–353.
- Singh, H., Ahluwalia, A.S., and Khattar, J.I.S. (2013a) , "Induction of sporulation by different nitrogen sources in *Anabaena naviculoides*, a diazotrophic strain capable of colonizing paddy field soil of Punjab (India)", *Vegetos* **26**(1), 283-292.
- Singh, J. S. (2014), "Cyanobacteria: a vital bio-agent in eco-restoration of degraded lands and sustainable agriculture", *Climate Change Environ. Sustain*, **2**' 133–137.
- Strange, R.N., Scott, P.R. (2005), " Plant disease: A threat to global food security", *Annual Review of Phytopathology*, **43**, 83-116.
- Song, T., Martensson, L., Eriksson, T., Zheng, W., Rasmussen, U. (2005), " Biodiversity and seasonal variation of the Cyanobacterial assemblage in a rice paddy field in Fujian, China", *The Federation of European Microbiology Societies*, **54**, 131–140.
- Sood, A., Singh, P.K., Kumar, A., Singh, R., Prasanna, R. (2011), " Growth and biochemical characterization of associations between cyanobionts and wheat seedlings in co-culturing experiments", *Biologia*, **66**(1), 10 4-1 10 .
- Subashchandrabose, S.R., Ramakrishnan, B., Megharaj, M., Venkateswarlu, K., Naidu, R. (2013), " Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation", *Environ Int*, **51**,59-72.
- Thakore, Y. (2006), "The bio pesticide market for global agricultural use", *Industrial Biotechnology*, **2**, 194-208.
- Thatoi, H., Behera, B.C., Mishra, R.R., Dutta, S.K. (2013), "Biodiversity and biotechnological potential of micro organisms from mangrove ecosystems", *Ann Microbiol* **63**, 1-19.
- Thajuddin, N., Subramanian, G. (2005), " Cyanobacterial biodiversity and potential applications in biotechnology", *Current Science*, **89**, 47–57.
- Vaishanrpayan, A., Sinha, R.P., Hader, D.P., Dey, T., Gupta, A.K., Bhan, U., Rao, A.L. (2001), " Cyanobacterial bio fertilizers in rice agriculture", *Bot Rev*, **67**,453-516.
- Vessey, J.K. (2003), " Plant growth promoting rhizobacteria as bio fertilizers", *Plant Soil*, **255**, 571–586.
- Wang, W., Liu, Y., Li, D., Hu, C. and Rao, B. (2009), " Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area", *Soil Biol Biochem*, **41**,926-929.
- Yosefi, K., Galavi, M., Ramrodi, M., Mousavi, S.R. (2011), " Effect of bio-phosphate and chemical phosphorus fertilizer accompanied with micronutrient foliar application on growth, yield and yield components of maize (Single Cross 704)", *Australian Journal of Crop Sciences*, **5**(2), 175-180.
- Zaccaro, M. C. (2000), Plant Growth Promoting Cyanobacteria. The Fifth International Plant Growth Promoting Rhizobacteria (PGPR) Workshop, Buenos Aires, Argentina. May 5-6,2000, pp. 5-12.