Research Paper

Volume : 4 | Issue : 5 | May 2015 • ISSN No 2277 - 8179

Effect of Nutrient Supplement on Growth And Nitrogenase Activity of Some Cyanobacterial Strains from semi-Arid Soils

Environmental Science

KEYWORDS : Cyanobacteria; semi-arid; nutrient supplement; nitrogenase.

R. Nisha	Department of Bioscience, Himachal Pradesh University, Summer Hill, Shimla. (HP),India
A. Kaushik	University school of environment management, Guru Gobind Singh IP University, New Delhi
C.P. Kaushik	Department of Environmental Science and engineering, G.J.U. Hissar

ABSTRACT

In the present investigation effect of various nutrient supplements iron, phosphate/potassium and exogenous nitrogen sources on growth and nitrogen fixing ability of cyanobacteria have been studied in terms of chlorophyll, carotenoid and nitrogenase activity. The response was found to be species specific to K+/PO43- and Fe3+ and nutrient saturation occurred at different concentration in term of photosynthetic pigment concentration. Amongst exogenous N-sources, nitrate alone had a stimulatory effect on chlorophyll of some strains while ammonium and amino acids tended to suppress the concentration of chlorophyll and other pigments. While exogenous nitrogen suppressed nitrogenase activity, the other nutrients significantly increased N2 fixing potential of the cyanobacteria.

Introduction

Cyanobacterial use as biofertilizer for soil reclamation and improved soil fertility is gaining importance for sustainable agriculture. These diazotrophs capable of fixing atmospheric nitrogen, reduce our dependence on synthetic nitrogen fertilizers, thereby serving as energy conserving and environmentally safe alternatives. Cyanobacteria are also reported to possess soil phosphate solubilizing enzymes (Davison and Pearson, 1996) thus providing phosphorus nutrition to the crop plants. Cyanobacteria, which are autotrophic microorganisms also add organic matter to the soils. In the arid and semi-arid regions, algal crusts constituted by cyanobacterial species have been recently found to play an important role in improving soil structure and stability and can grow and adapt in nearlly all the changing environment (Belnap and Gillette, 1998; Malam Issa et. al, 2001, Nisha et al.,2007, Mager and Thomas, 2011, Maqubela et al.,2009,2012). The soils in these regions are usually poor in organic matter with limited nitrogen and phosphorus (Nelson and Oades, 1998). In this context, it becomes important to examine whether cyanobacterial growth and its nitrogen fixing ability could be improved by altering nutrient concentration in these soils through amendments.

In the present investigation, growth and nitrogenase activity of some cyanobacterial strains isolated from the soils of in Hisar, Haryana, a semi-arid region were studied in response to supplement with essential nutrients viz. nitrogen, phosphorus, potassium and the micronutrient, iron.

Materials and Methods

1.1 Study area & algal isolation :

The cyanobacterial strains were isolated from aridisols of Hisar lying in Haryana (27°40'N, 30°56'S and 74°28'E, 70°35'W), Northern India. The geomorphic land form in the area is characterized by organically poor aeolian sand with some isolated or stabilized sand dunes.

Soil samples (0-15 cm) were collected and algal strains were isolated from fresh soil using serial dilution (10^{-2}) following standard culturing and purification techniques (Kaushik, 1987) using BG-11 N-free medium (Stainer et al., 1971). Cultures were incubated at 28 ± 3°C under continuous illumination of 3000 lux using cool white fluorescent lamps. They were examined microscopically for various structural features for identification (Desikachary, 1959). Unialgal cultures were maintained at 25°C ± 3°C for further studies.

Nutrient amendments:

In order to find out the optimal conditions for growth of the selected strains alteration in the concentration of some macro and micronutrients were made. Nutrient modifications of Fe^{3+} (4 ppm), K^+/PO_4^{3-} (40 ppm) were done in the culture medium using ferrous ammonium sulphate and K_2PO_4 respectively. The original concentrations in the medium (2 ppm Fe^{3+}; 20 ppm K^+/PO_4^{3-}) served as control.

Three types of exogenous N-sources i.e. amino acids, nitrate and ammonium nitrogen were used. Mixed amino acids (tyrosine, aspartic acid, isoleucine, proline, glycine, leucine, each 0.5 mM concentration), KNO_3 (10 mM) and NH_4Cl (3 mM) were used. The pH of the medium was maintained at 8.5 using buffer for better growth

2.3 Growth response :

Algal growth was studied at 5-d interval. Chlorophyll estimation was done by hot extraction method using methanol following Mackiney (1941). Carotenoid estimation was done following Jenson (1978). Nitrogenase activity was estimated using 15 day old algal cultures by Actylene Reduction Assay (ARA) technique, using GC (Chemito) equipped with Porapak R Column and Flame Ionization Detector (FID) detector following Hardy et al. (1973).

The data was statistically analysed to study the significance of differences due to amendments using Duncan's Multiple range test and t-test.

3 Results

3.1 Growth responce:

Supplementing the medium with 40 ppm potassium and phosphate did not have any significant effect on chlorophyll in most of the algal species. Except in case of *N. punctiforme* and *N. calcicola*, where there was a significant increase in chlorophyll content. Carotenoid content, was also found to increase significantly ($p < 0.05$), in these two species along with *N. spongiaeforme*. Thus, algal response to increased K^+/PO_4^{3-} was species specific and in most of the species growth was optimal at 20 ppm concentration (control), whereas in *N. calcicola* and *N. punctiforme* better growth was attained at 40 ppm concentration of K^+/PO_4^{3-}.

Addition of Fe^{3+} in the medium tended to favour chlorophyll biosynthesis in fifty percent of the algal species showing 30 to 150% increase while in others, there was no significant change. Carotenoid content tended to increase by 8 to 23% when further Fe^{3+} supplement was given. Statistically significant ($p < 0.005$) increase in chlorophyll and carotenoid concentration of the cyanobacterial species due to these essential and micronutrients are shown in Table 1 based on Ducan's multiple range test. The

response was found to be species specific.

Growth of the cyanobacterial strains in response to added nitrogen supplement varied with the type of N-source. In the presence of exogenous amino acids, there was a decline in chlorophyll concentration of the cyanobacterial species. In response to NO_3^-, there was 17 to 120% increase in chlorophyll concentration of half of the total species while ammonium NH_4^+-N was inhibitory for the growth of these species as evident from lower concentration of chlorophyll under this treatment as compared to control except in case of *N. calcicola*. Carotenoid concentration, on the other hand, tended to increase by 46 to 130% in the presence of amino acids and by 12 to 224% in the presence of nitrates. NH_4^+-N was however stimulating only in case of *N. punctiforme*, while other species showed a decline in pigment concentration (Table 2). It is thus evident that *N. calcicola* and *N. punctiforme* are two such species of *Nostoc* where growth is nutrient saturated at a relatively higher level.

3.2 Nitrogenase activity :

All the 6 species which are heterocystous in nature showed nitrogenase activity ranging from 0.31 to 5.16 n mole C_2H_4/mg dry weight/h in control. Supplementing the medium with double dose of K^+/PO_4^{3-} increased the nitrogenase activity by 2 to 10 times in most of the species. Iron supplement increased the nitrogenase activity by 2 to 6 times in most of the species except *A. doliolum* and *N. calcicola* where no effect was observed at higher Fe^{3+} concentration showing nitrogenase saturation at 2 ppm concentration of iron. All the exogeneous sources of nitrogen were inhibitory for the nitrogenase activity of these species and there was total suppression of nitogenase in the presence of NH_4^+-N where no nitrogenase activity was detectable (Table 3).

Discussion

The semi-arid soils from where the cyanobacterial strains have been isolated are not rich in organic matter. Their total Kjeldahl nitrogen content varied from 0.004 to 0.06%, available phosphorus from 0.003 to 0.005% and potassium 0.03 to 0.04% (results not shown). As supplement of PO_4^{3-} and K^+ tend to increase nitrogenase activity of these algae, use of phosphate and potassium fertilizers along with the algal biofertilizer would improve the nitrogen status of the soil and increase productivity. As concentration of chlorophyll in only two or three species increase in response to these amendments, we may expect less CO_2 fixation in most of the species barring a few species. Consistently higher nitrogenase activity in all the species suggests that greater N_2 fixation and relatively less CO_2 fixation may help narrow down the C : N ratio of the soil creating better soil conditions, if the K^+/PO_4^{3-} supplements are given.

Role of K^+ has also been reported in photosynthetic recovery of some cyanobacteria after dehydration (Qui and Gao, 1999). Supplementing K^+ would have added advantage under semi-arid condition as this element is also reported to regulate osmotic balance in the alga through ion regulation (Apte, 2001), and in maintenance of intracellular pH (Erdman and Hagemann, 2001), which collectively would be useful for the growth of these cyanobacteria under the water stress condition of semiarid tropics.

Phosphate fertilization has been reported to dramatically increase nitrogen fixation in microbial mats (Stal, 2000). De Nobel *et al.* (1997) also stressed that phosphate concentration determines the optimal activity of nitrogenase. In the semi-arid soils with relatively low phosphorus content, supplementing phosphates would increase N_2-fixation.

Since ferric ions act as a cofactor in nitrogenase enzyme, it was observed that in most of the present algal species substantial increase of 2-6 times in nitrogenase activity occurred at 4 ppm concentration of Fe^{3+} ions as compared to that at 2 ppm (control medium). Iron acts as electron carrier or as electron donor in a number of processes including N_2-fixation. In natural microbial mats a layer of oxidized iron in Fe^{3+} form has been observed (Ehrenreich and Widdel, 1994) which may act as a barrier between aerobic and anaerobic parts of the soil sub-system to protect the nitrogenase enzyme from oxygen. Ferric ions have also been reported to provide a UV screen to the cyanobacteria (Pierson and Olson, 1989) which may be of special significance in the semi-arid region where there is exposure to high intensity solar radiations containing UV-radiations.

All the cyanobacterial species in the present study could grow in nitrogen-free medium serving as control, since they are heterocystous forms and could fix atmospheric N_2. In the presence of external source of nitrogen as nitrate, chlorophyll content increased in some strains but it decreased in the presence of ammonium ions. Any form of exogenous nitrogen, particularly NH_4^+-N was inhibitory for nitrogenase enzyme. Ammonia lowers the supply of reductant and/or energy (Ohmori and Hattori, 1974), explaining its inhibitory effect on nitrogenase. Although externally supplied amino acids have been reported to be metabolized by algae and act as osmoprotectants (Reddy *et al.*, 1989), yet they were found to have an inhibitory effect on nitrogenase activity of the cyanobacteria.

It becomes evident from the present study that while using cyanobacterial species in the semi-arid soils as biofertilizers phosphate and potassium should be supplemented in the form of inorganic fertilizers to boost growth and N_2 fixing potential of the cyanobacteria. In the semi-arid soils where the upper profile is usually deficient in iron, supplement of this micronutrient would be useful to promote cyanobacterial growth and nitrogenase activity.

The present study shows that growth and nitrogen fixing potential of cyanobacteria can be improved by careful management of various macro and micro nutrients for successfully exploiting them as biofertilizers in semi-arid soils.

Acknowledgements

Financial assistance from the University Grants Commission, New Delhi, India in the form of a Major research project and fellowship to Rani Nisha is gratefully acknowledged.

Table 1. Effect of nutrient supplement on pigment concentration of indigenous heterocystous cyanobacteria

Species	Chlorophyll (µg/ml)			Carotenoids (µg/ml)		
	Control	PPS	IS	Control	PPS	IS
N. linckia	5.17^a (100)	5.26^b (102)	5.25^b (98)	0.013^a (100)	0.014^a (108)	0.016^b (126.15)
N. spongiaeforme	7.66^a (100)	6.25^b (81.6)	6.22^b (81)	0.018^a (100)	0.026^b (148)	0.017^a (94)
A. doliolum	3.05^a (100)	3.03^a (99)	3.03^a (99)	0.012^a (100)	0.011^a (92)	0.014^a (116.6)
N. ellipsosporum	3.28^a (100)	3.76^b (114.14)	7.9^c (241)	0.012^a (100)	0.011^a (92)	0.014^a (116.6)
N. punctiforme	4.26^a (100)	5.94^b (139)	5.6^b (131)	0.008^a (100)	0.013^b (162.5)	0.008^a (109)
N. calcicola	1.85^a (100)	4.52^b (244)	4.64^b (251)	0.006^a (100)	0.007^b (117)	0.006^a (100)

Parameter values in response to supplements of Phosphate/ Potassium (PPS) and Iron (IS) followed by same superscript in rows (a, b, c etc) do not vary significantly ($p < 0.05$) from each other based on Duncan's Multiple Range Test. All others are significantly different ($p < 0.01$). Values in parentheses represent

Volume : 4 | Issue : 5 | May 2015 • ISSN No 2277 - 8179

relative values.

Table 2. Effect of exogenous Nitrogen supplement on pigment concentration of indigenous heterocystous cyanobacteria

Species	Chlorophyll (µg/ml)				Carotenoids (µg/ml)			
	Control	AS	NS	AAS	Control	AS	NS	AAS
N. linckia	5.17^{b} (100)	4.5^{c} (79)	6.79^{a} (119)	2.72^{d} (48)	0.013^{c} (100)	0.028^{b} (215.4)	0.0415^{a} (323.07)	0.0277^{b} (215.4)
N. spongiaeforme	7.66^{b} (100)	11.9^{a} (155)	8.9^{c} (116)	-	0.018^{c} (100)	-	0.035^{a} (194.00)	0.0275^{b} (152.7)
A. doliolum	3.05^{b} (100)	1.97^{c} (65)	6.64^{a} (218)	2.31^{bc} (76)	0.012^{b} (100)	0.0066^{c} (58)	0.0134^{b} (112)	0.0176^{a} (146)
N. ellipsosporum	3.28^{a} (100)	2.28^{b} (70)	1.2^{c} (37)	0.63^{d} (19)	0.012^{c} (100)	0.0088^{d} (73)	0.0202^{a} (168)	0.0191^{b} (158)
N. punctiforme	4.26^{a} (100)	2.05^{c} (48)	4.09^{b} (96)	0.30^{d} (7)	0.008^{c} (100)	0.0195^{b} (224)	0.034^{a} (425)	0.0183^{b} (229)
N. calcicola	1.85^{c} (100)	4.68^{a} (253)	4.09^{b} (221)	-	0.0063^{b} (100)	0.0035^{c} (58)	0.016^{a} (227)	-

Parameter values in response to Amino acid supplement (AAS), Nitrate supplement (NS) and Ammonium supplement (AS). Values followed by same superscript (a, b, c etc) do not vary significantly ($p < 0.05$) from each other based on Duncan's Multiple Range Test. All others are significantly different ($p < 0.01$). Values in parentheses represent relative values.

Table 3. Effect of different nutrient supplement on nitrogenase activity of indigenous heterocystous cyanobacteria

Species	Nitrogenase activity (nmoles C_2H_4/mg dry weight/h)					
	Control	PPS	IS	AAS	NS	AS
N. linckia	0.31^{a} (100)	3.48^{b} (1122)	3.15^{b} (1022)	ND -	ND -	ND -
N. spongiaeforme	1.07^{a} (100)	9.15^{b} (855)	6.21^{c} (580)	0.21^{d} (196)	0.31^{d} (29)	ND -
A. doliolum	1.72^{a} (100)	4.68^{c} (272)	1.72^{a} (100)	1.28^{b} (74.4)	0.07^{d} (4.07)	ND -
N. ellipsosporum	2.3^{a} (100)	4.42^{b} (192)	6.14^{c} (267)	0.18^{d} (7.8)	0.15^{d} (6.52)	ND -
N. punctiforme	2.64^{a} (100)	7.86^{b} (298)	7.86^{b} (298)	0.34^{c} (12.87)	0.43^{c} (16.3)	ND -
N. calcicola	5.16^{a} (100)	5.23^{a} (102.6)	5.16^{a} (100)	ND -	0.26^{b} (5.04)	ND -

Parameter values in response to supplement of Phosphate/potassium (PPS), Iron (IS), Amino acid (AAS), Nitrate supplement (NS) and Ammonia supplement (AS) followed by same superscript in a rows (a, b, c etc) do not vary significantly ($p < 0.05$) from each other based on Duncan's Multiple Range Test. All others are significantly different ($p < 0.01$). Values in parentheses represent relative values.

ND – Not Detectable

REFERENCE

Apte, S.K. (2001). Coping with salinity/water stress: cyanobacteria show the way. Proceedings of Indian National Science Academy (PINSA), 5, 285-310. | Belnap, J. and D. A. Gillette. (1998). Vulnerability of desert biological soil crusts to wind erosion : the influence of crust development, soil texture and disturbances. Journal of arid environment, 39, 133-142 | Davison, I.R. and G.A. Pearson.(1996). Stress tolerance in intertidal seaweeds. Journal of Phycology, 32, 197-211. | De Nobel, W.T., J.L. Snoep, H.V. Westerhoff and L. R. Mur. (1997). Interaction of nitrogen fixation and phosphorus limitation in Aphanizomenon flos-aquae (cyanophyceae). Journal of Phycology, 33, 794-799. | Desikachary, T.V. (1959). In: Cyanophyta. Indian Council of Agricultural Research, New Delhi. p. 686. | Ehrenreich, A. and F. Widdel. (1994). Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. Applied Environment Microbiology, 60, 4517- 4526. | Erdmann, N. and M. Hagemann.(2001). Salt acclimation of algae and cyanobacteria: a comparison. In: Algal Adaptation to Environmental Stresses (Eds. L.C. Rai, J.P. Gaur). Springer Publication, pp. 323-361. | Hardy, R.W.F., R.C. Burns and R.D. Holsten. (1973). Application of the acetylene ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry, 5, 47-81. | Jensen, A. (1978). Chlorophyll and carotenoids. In: Handbook of Phycological Methods, Physiological and Biochemical Methods (Eds. J.A. Hellebust, J.S. Craige). Cambridge University Press, Cambridge, pp 59-70. | Kaushik, B.D. (1987). Laboratory Methods for Blue Green Algae. Associated Publishing Company, New Delhi. p. 171. | Malam Issa, O., Y. Le Bissonnais, C. Defarge, and J. Trichet. (2001). Role of a cyanobacterial cover on structural stability of sandy soils in the sahelian part of western niger. Geoderma, 101, 15-30. | Maqubela, M.P., P.N.S.Mnkeni, O.Malam Issa, M.T.Pardo, L.P. D'Acqui. (2009). Nostoc cyanobacterial inoculation in South African soils enhances soil structure, fertility, and maize growth. Plant Soil, 315: 79-92. | Maqubela, M.P., P. Muchaonyerwa, and P.N.S Mnkeni. (2012). Inoculation effects of two South African cyanobacteria strains on aggregate stability of a silt loam soil. African Journal of Biotechnology Vol. 11(47), pp. 10726-10735. | McKiney, G. (1941). Adsorption of light by chlorophyll solutions. Journal of Biology and Chemistry, 140, 315-322. | Nelson, P.N., and S.M. Oades. (1998). Organic matter, Sodicity and soil structure. In: Sodic soils, properties, management and environmental consequences (Eds. M. E. Sumner, R. Naidu). Oxford university press, New York. | Nisha R, A. Kaushik, C.P. Kaushik. (2007). Effect of indigenous cyanobacterial application on structural stability and productivity of an organically poor semi-arid soil. Geoderma. 138: 49-56. | Ohmori, M.and A. Hattori. (1974). Effect of ammonia on nitrogen fixation by the blue green algae Anabaena cylindrica. Plant Cell Physiology, 15, 131-142. | Pierson, B.K. and J. M.Olson. (1989). Evolution of Photosynthesis in anoxygenic photosynthetic prokaryotes. In: Microbial Mats. Physiological Ecology of Benthic Microbiol. Communities (Eds. Cohen, Y. and Rosenberg, E.). ASM, Washington. pp. 402-427. | Qiu, B. and K. Gao. (1999). Dried population of Nostoc flagelliforme (cyanophyceae) require exogenous nutrients for their photosynthetic recovery. Journal of Applied. Phycology, 11, 535-541. | Reddy, B.R., S.K. Apte and J. Thomas. (1989). Enhancement of cyanobacterial salt tolerance by combined nitrogen. Plant Physiol, 89, 204-210. | Stainer, R.Y., R. Kunisawa, M. Mandel and G. Cohen-Bazire. (1971). Purification and properties of unicellular blue-green algae (Order chroococcales). Bacteriol Review, 35, 171-205 | Stal, L. J.(2000). Cyanobacterial Mats and Stromatolites. In: The Ecology of Cyanobacteria (Eds. Whitton, B.A., Potts). Kluwer Academic Publishers, pp. 61-120. |