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PHOSPHATE SOLUBILISING CYANOBACTERIA AND THEIR ROLE AS BIOFERTILIZER

Biological Science

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ABSTRACT

BACKGROUND: Phosphate solubilising cyanobacteria are capable of hydrolyzing organic and inorganic phosphorus compounds to soluble phosphorus that can be easily assimilated by plants. Phosphorus is a macronutrient necessary for key metabolic processes. The availability of phosphorus for plant uptake is only 0.1% in the soil. Consequently, the demand for the use of phosphatic fertilizers for plant growth has greatly increased.
AIM: Investigation of Phosphate solubilising cyanobacteria
OBJECTIVE: To study the alkaline phosphatase activity of cyanobacteria
METHODS: Growth of heterocystous cyanobacteria in BG11 medium was studied under phosphate starvation condition for the assessment of nitrogen fixation and phosphate solubilisation activities.
RESULTS: All the four cyanobacterial isolates showed positive test for phosphate solubilising and nitrogen fixation activities.
CONCLUSION: The phosphate solubilising cyanobacteria may be used as phosphatic biofertiliser in agricultural fields.

KEYWORDS

Cyanobacteria, Phosphate solubilisation, Nitrogen fixation, Alkaline phosphatase

INTRODUCTION

Phosphate solubilising cyanobacteria are beneficial oxygenic photosynthesiser prokaryotes capable of hydrolyzing organic and inorganic phosphorus compounds to soluble phosphorus that can be easily assimilated by plants. Phosphorus is a macronutrient necessary for key metabolic processes in plants such as cell division, energy generation, membrane integrity, signal transduction, photosynthesis, and biosynthesis of macromolecules, respiration and fixation of nitrogen[1, 2]. Plants absorb phosphorus in the form of orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}). In top soil phosphorus accounts for 50 to 3000 mg/Kg of soil. But, due to precipitation with cations in soil, immobilization, adsorption and interconversion to organic form the availability of phosphorus for plant uptake is only 0.1%[3, 5]. Consequently, the demand for the use of phosphatic fertilizers for plant growth has greatly increased. However, the phosphatic fertilizers contain a variety of heavy metals which accumulate in soil and cause a detrimental effect on soil fertility, eutrophication of water bodies and a widening carbon footprint[6,7]. The phosphate solubilising cyanobacteria are bioinoculants that are promising substitutes for agrochemical. The PSC adopt different strategies to solubilise insoluble phosphorus to soluble form and can reduce the chemical phosphate fertilizer input in agricultural land[8].

A number of cyanobacterial species are known to produce extracellular phosphate solubilising enzymes (phosphatases) viz. *Anabaena* ARM310[9], *Anabaena variabilis*, *Westiellopsis prilifica*[10], *Calothrix braunii*, *Nostoc* sp. *Scytomema* sp.[11] etc. The present study has been undertaken to evaluate the extracellular phosphate solubilising activity of heterocystous cyanobacteria viz. *Nostoc muscorum, Nostoc calcicola, Anabaena oryzae* and *Scytonema varium.*

MATERIALS AND METHODS

The cyanobacterial flora viz. *Nostoc muscorum, Nostoc calcicola, Anabaena oryzae* and *Scytonema varium* were isolated from rice grown shallow water and identified by relevant monograph[12]. The cyanobacterial isolates were maintained in pure culture in BG11 medium in a growth chamber under12/12hL/D cycle at 25±2°C and 1500 lux light intensity using fluorescent lamps.

For maintaining phosphate starvation cyanobacterial filaments were sub cultured in BG11 medium containing combined nitrogen but without dipotassium hydrogen phosphate (K_2HPO_4). Potassium chloride was added to maintain the availability of potassium in the medium. Thirty days old cultures were used as inocula for further experiments. Tricalcium phosphate [$Ca_3(P_{O4})_2$] was added at a concentration of 1 mg/ml (w/v) in each of the culture of medium.

Pre-weighed phosphate cyanobacterial starved cells of each of the four isolates were inoculated and the growth conditions were recorded as follows:
(1) CN^+SP^-: BG11 medium containing combined nitrogen but without dipotassium hydrogen phosphate.

(2) CN^+ SP^+: BG11 medium containing both combined nitrogen and dipotassium hydrogen phosphate.

(3) CN^+ ISP^+: BG11 medium containing both combined nitrogen and insoluble tricalcium phosphate.

(4) CN^- SP^-: BG11 medium without combined nitrogen and dipotassium hydrogen phosphate.

(5) CN^- S^{P+}: BG11 medium without combined nitrogen but with dipotassium hydrogen phosphate.

(6) CN^- ISP^+: BG11 medium without combined nitrogen but with insoluble tricalcium phosphate.

The growth of cyanobacterial isolates under these subculture conditions was recorded after thirty days. The growth of cyanobacterial isolates was estimated in terms of chlorophyll-a by the method of Mackiney[13].

Harvested cells from each treatment were suspended in 1 ml of distilled water. 4 ml of methanol was then added and incubated in a water bath at 60°C for 30 min. The cultures were then centrifuged for 10 min and the absorbance of the supernatant was recorded at 663 nm.

Phosphate solubilisation activity of each of the cyanobacterial isolates was assayed in terms of extracellular phosphatase activity in the culture filtrates. The activity of phosphatase was assayed on thirty days of growth. Alkaline phosphatase activity in the culture filtrates was assayed following the method of Freeland[14]. The alkaline phosphatase assay mixture contained 1·5 ml of 0·4 M Tris-HCl buffer (pH 8·5), 0·1 ml of 16·6 mM Phenolphthalein diphosphate and 1·4 ml of culture filtrate from the inoculated flasks, in a total volume of 3 ml. Tubes containing the assay mixture were kept for 30 min in dark at room temperature. After 30 min, the reaction was terminated by adding 0·1 ml of 1 M dipotassium hydrogen phosphate. The absorbance of the supernatant was measured at 540 nm. Uninoculated culture medium in a total volume of 3 ml of the assay mixture was used as blank. The amount of Phenolphthalein released was measured from the Phenolphthalein standard graph.

The enzyme protein in the culture filtrate was estimated following the method of Lowry *et al*[15] using bovine serum albumin as the standard,

and the alkalaine phosphatase activity was expressed as µg of Phenolphthalein liberated per µg protein per min.

The nitrogenase activity of each of the starved cyanobacterial isolates was assayed by acetylene reduction technique of Stewart *et al*[16] i.e. the ability of cyanobacterial nitrogenase complex to reduce acetylene (C_2H_2) to ethylene (C_2H_4) Thirty days old cultures were used for this purpose. Approximately 1g of air-dried cyanobacterial isolates, obtained from free living cultures was dispensed into 25ml glass vials. Sterilized distilled water was added to give a total volume of 7.5ml. The vials were sealed with screw caps fitted with silicon rubber septa. Acetylene generated from calcium carbide was injected into the vials, giving a gas atmosphere in the vials of air plus 10% acetylene.

All samples were set up in duplicate sets of three each for light and dark incubations; the latter for assessing dark nitrogen fixation. After incubation for specific duration in light or dark according to the requirement of the experiment, 100µl of gas phase was withdrawn using a gastight Hamilton syringe and analyzed for ethylene on AIMIL- Nucon 5765 model gas chromatograph with FID detector fitted with Porapak- T SS column (80 100 mesh; carrier gas nitrogen, 30ml/min; column temperature, 100°C; injector temperature, 100°C; detector temperature, 120°C). Acetylene reduction activity was expressed as n mol ethylene /g air dried material/h[17]. Nitrogenase activity was expressed in terms of nmol of ethylene formed per mg protein per hour.

Phosphate-solubilization activity of cyanobacterial isolates was conducted qualitatively by plating the cyanobacteria in agar BG-11 medium supplemented with precipitated tricalcium phosphate. The presence of clear zone around the cyanobacterial colonies after overnight incubation indicated the phosphate solubilisation activity of cyanobacteria.

The results obtained have been presented in Table-1, 2 and 3

Table-1: Production of Chlorophyll-a, Nitrogenase and Phosphorus solubilisation activity under normal culture condition in BG11 medium after 30 days of incubation

Cyanobacterial isolates	Chlorophyll -a (µg/ml)	Nitrogenase (n moles C_2H_4produced/ hr/mg dry wt)	P-solubilisation activity
Nostoc muscurum	0.456	3.2	+
Nostoc calcicola	0.465	3.5	+
Anabaena oryzae	0.470	5.5	+
Scytonema varium	0.462	5.4	+

Table-2: Growth of cyanobacterial isolates in terms of µg Chl-a/ml after 30 days of incubation under different culture conditions

Cyanobacterial isolates	Culture conditions					
	CN^+ SP^-	CN^+ SP^+	CN^+ ISP^+	CN^- SP^-	CN^- SP+	CN^- ISP^+
Nostoc muscurum	200.55	650.55	375.75	275.35	1750.75	1875.65
Nostoc calcicola	210.45	675.45	385.65	290.55	1765.85	1885.35
Anabaena oryzae	225.75	715.65	410.75	315.35	1870.75	1925.25
Scytonema varium	205.45	665.35	370.15	310.25	1655.35	17.65.25

CN^+: Medium with combined nitrogen; CN^-: medium without combined nitrogen; ISP^+: medium with tricalcium phosphate; SP^+: medium with dipotassium hydrogen phosphate; SP^-: medium without dipotassium hydrogen phosphate

Table-3: Alkaline phosphatase activity in terms of µg of phenolphthalein/µg protein/min of cyanobacterial isolates after 30 days of incubation under different culture conditions

Cyanobacterial isolates	Culture conditions					
	CN^+ SP^-	CN^+ SP^+	CN^+ ISP^+	CN^- SP^-	CN^- SP+	CN^- ISP^+
Nostoc muscurum	3.5	4.5	3.7	5.5	4.6	3.2
Nostoc calcicola	3.7	4.6	3.5	5.8	4.7	3.1
Anabaena oryzae	4.2	5.7	4.1	6.2	5.1	4.3
Scytonema varium	3.4	4.3	3.4	4.6	4.0	3.0

RESULTS AND DISCUSSION

The growth of the present four cyanobacterial isolates was measured in terms of chlorophyll-a content. From the result (Table-1) it is evident that the chl-a content of the present cyanobacterial isolates viz., *Nostoc muscurum*, *N. calcicola*, *Anabaena oryzae* and *Scytonema varium* was 0.456, 0.465, 0.470 µg/ml respectively under normal culture conditions after 30 days of incubation. The Nitrogenase content in the present cyanobacterial isolates viz., *Nostoc muscurum, N. calcicola*, *Anabaena oryzae* and *Scytonema varium* was 3.2, 3.45, 5.5 and 5.4 n moles C_2H_4 produced/h/mg dry wt. All the four cyanobacterial isolates showed positive test for phosphate solubilising activity. Among these isolates *Anabaena oryzae* showed maximum chl-a content (0.47µg/ml) and nitrogenase activity (5.5 n moles C_2H_4 produced/h/mg dry wt.).

In the presence of soluble or insoluble phosphates the growth was greater than in the absence of phosphates. The growth observed in the present investigation indicates that the present cyanobacterial isolates could utilise extracellular insoluble phosphates under both CN^+ and CN^- conditions (Table-2). The growth of cyanobacterial isolates was minimumal under culture condition of CN^+SP^-. Under such culture condition the chlorophyll-a content of *Nostoc muscurum*, *N. calcicola, Anabaena oryzae* and *Scytonema varium* was 200.55, 210.45, 225.75 and 205.45 µg/ml respectively after 30 days of incubation. A slightly high growth was noticed under culture conditions of CN^+ISP^+ and CN^-SP^-. Under culture condition of CN^+ SP^+ the growth of present cyanobacterial isolates was moderate. The highest growth rate was achieved under culture condition of CN^-ISP^+. Under such condition the chlorophyll-a content of *Nostoc muscurum, N. calcicola, Anabaena oryzae* and *Scytonema varium* was 1875.65, 1885.35, 1925.25 and 1765.25 µg/ml (Table-2). The present findings gain support from the work of Bose *et al*[18] who have also observed a more or less similar results in *Anabaena, Nostoc, Tolypothrix, Aulosira* and *Anacystis.* These cyanobacterial isolates have been shown to solubilise extracellular insoluble phosphates.

Under conditions of phosphate starvation cyanobacteria are known to show increased levels of intracellular and cell surface alkaline phosphatase activity to solubilize polyphosphates[19]. A shift in the pH was noticed towards alkalinity in all the inoculated treatments. This indicated the involvement of alkaline phosphatases in solubilization of extracellular phosphates. The alkaline phosphatase activity of *Nostoc muscurum, N. calcicola, Anabaena oryzae* and *Scytonema varium* was 3.5, 3.7, 4.2 and 3.4 µg of phenolphthalein/µg protein/min respectively under the culture condition of CN^+ SP^- after 30 days of incubation. A more or less situation was observed under the culture conditions of CN^+ISP^+ and CN^-ISP^+. The alkaline phosphate activity was high under culture conditions of CN^+ SP^+, CN^- SP^- and CN^- SP^+. Under culture condition of CN^- SP^- the alkaline phosphatase activity of *Nostoc muscurum, N. calcicola, Anabaena oryzae* and *Scytonema varium* activity was high, 5.5, 5.8, 6.2 and 4.6 µg of phenolphthalein/µg protein/ml respectively after 30 days of incubation (Table-3). The present findings are in accordance with the work of Anand Mohan and Baidyanath Kumar[20] who have observed the phosphate solubilisation capacity of these cyanobacterial isolates.

N_2 fixing ability of these heterocystous cyanobacteria can increase the content of easy available N or the ammonium content of algae extracts. The greater nitrogen fixing capacity influences greater improvement of plant growth[21-23]. The present investigation revealed that the presence of some growth-promoting substances viz. nitrogenase and P-solubilization that may be responsible for the beneficial effect on plant growth parameters. The results showed that P-solubilisation and nitrogen fixation in heterocystous cyanobacteria might be responsible for the enhanced growth of plants[24-27].

CONCLUSION:

It can be concluded that the cyanobacterial isolates seem to be an ideal choice for the preparation of cyanobacterial inoculant because of its ability to grow well and fix atmospheric nitrogen in the absence of combined nitrogen and soluble phosphates. The phosphate solubilising cyanobacteria can be used as substitutes for agrochemical. The phosphate solubilising cyanobacteria adopt different strategies to solubilise insoluble phosphorus to soluble form and can reduce the chemical phosphate fertilizer input in agricultural land.

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