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PGPR PRODUCTION STRATEGY FOR SUSTAINABLE FARMING POTENTIAL OF CYANOBACTERIA GLOEOCAPSA

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ABSTRACT

Background: Cyanobacteria also known as blue green algae are well known photosynthetic autotrophic prokaryotes. Cyanobacteria is a preferable alternative to chemical fertilisers for increasing soil productivity.

Methods: For the current study the cyanobacterial samples were isolated from various paddy fields from Nasik District. PGPR activity of isolated cyanobacterial strains was determined viz. production of indole acetic acid, siderophores, nitrogen fixation and solubilization of various metals like potassium, phosphate, calcium, magnesium, sulphur, zinc and manganese. The seed germination and pot culture studies of the isolated cyanobacterial strain Gloeocapsa-1 showed a high rate of germination in terms of root and shoot length.

Results: Out of 6 different cyanobacterial spp., Gleocapsa was selected. The strain 1 labelled gleocapsa showed higher PGPR activity which was determined by production ofindole acetic acid, siderophores, nitrogen fixation and Metal solubilization. The significant effect of cyanobacterial extracts on the growth of shoot length and root length was determined by Single factor ANOVA at 0.05 level of significant difference.

Keywords: Blue green algae, Cyanobacteria, Gloeocapsa, Indole acetic acid, PGPR activity, Siderophore

INTRODUCTION

Cyanobacteria are prokaryotic, oxygenic, mostly unicellular or filamentous organisms which carry out photosynthesis in presence of light. They grow abundantly in surroundings or environment wherein ample moisture and daylight are available such as soil, fresh or marine water sources **[1]**.

Cyanobacteria also known as blue-green algae are a valuable source of pigments. The pigments produced by blue green algae include phycocyanin along with β-carotene and Chlorophyll pigments **[2-5]**.

To enhance the productivity of soil, cyanobacteria is a better alternative than chemical fertilizers. Recently attention has been given to the study of cyanobacteria in paddy fields as rice (*Oryza sativa* L.), a major food for the majority of people in the world. Advantageous effects of cyanobacterial inoculation were reported for other crops also in addition to rice such as wheat, soyabean, oat, tomato, radish, cotton, sugarcane, maize, chili, bean, muskmelon and lettuce **[6]**.

In order to explore the distribution of these economically important microorganisms and their potential as a biofertilizer, we have isolated and identified heterocystous cyanobacteria from paddy soils of Nashik district.

2. MATERIALS AND METHOD:

2.1. Sample Collection:

Soil samples were obtained from several rice fields in Trimbakeshwar Taluka of Nashik District, Maharashtra, India, for the isolation of Cyanobacterial strains. The soil was collected in plastic bags from a 2-3cm depth beneath the soil surface and transported to the laboratory for further analysis.

2.2. Isolation and Screening of Cyanobacteria

The various quantities of the soil sample were obtained by dilution and inoculation in a sterile Erlenmeyer flask containing BG11 media. After inoculation, the flask was kept at room temperature for 15 days for enrichment under continuous dark and sunlight. After the cyanobacteria enrichment process, a loopful of the sample was streaked on BG-11 using a sterile brush.The plates were incubated at room temperature under window seals until significant growth was observed **[1]**.

2.3. Identification of Cyanobacteria

The colonies were examined under a microscope for identification using morphological features after noticeable growth on the plates. Wet mounts of the colonies were seen under low (10X) and high power (45X) objective lenses of a

compound light microscope for microscopic examination.

2.4. Detection of IAA

The cyanobacteria-enriched broth was centrifuged for 10 minutes at 10000 rpm. The pellet was discarded, and the supernatant was taken to be analysed further. With 1.0 M HCl, the supernatant was acidified to pH 2.8. Three times with ethyl acetate (1:3 v/v) were extracted from the acidified supernatant. The extracts were then vacuum evaporated.Ethyl acetate (1:3 v/v) was used to extract the acidified supernatant three times. Following that, the extracts were vacuum evaporated. The pH of the residual aqueous fraction was raised to 7.0 by adding 1 N NaOH and an equivalent volume of ethyl acetate. The organic phase was separated from the aqueous phase. In the organic phase, 1 mL Salkowasky's reagent was added and incubated for 30 minutes in the dark. The samples were then examined to see if they had changed colour **[7]**.

2.5. Detection of Siderophores:

The enriched cyanobacterial broth was centrifuged at 10000 rpm for 10 min at 4^0C. With 1.0 N HCl, the supernatant was adjusted to an acidic pH. Following that, an equivalent volume of ethyl acetate was added, and the solution was vigorously stirred due to which the two layers separated out. After mixing 0.5 ml of extract from the top layer with 0.5 ml of CAS reagent, the colour change from blue to pink was examined **[8]**.

2.6. Pigment Extraction:

1ml sample of the cyanobacterial culture suspension was obtained from the enriched broth. The cyanobacterial culture sample was centrifuged at 15,000 rpm for 7 minutes at room temperature, and the supernatant was discarded. The procedure was repeated 2 time to obtain the measurable quantity of sample. 1ml acetone was added to the pellet, and the sample was homogenised using a vortex (2000rpm for 2 minutes).To extract the pigment from the cells, the samples were covered in aluminium foil and incubated at 4°C for 24 hours. After an overnight incubation period, the sample was centrifuged at 15,000 rpm for 7 minutes, yielding a bluish-purple pellet with no green colour. The spectrophotometer was calibrated using acetone as a blank. The extract was centrifuged for 10 minutes at 10000 rpm, and the supernatant was spectrometrically measured at 665 nm against acetone **[9]**.

2.7. Estimation of Nitrogen:

For detecting nitrogen, Nessler's Reagent is utilized. At 40°C, a known volume of homogenous cyanobacterial culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was collected, 2-3 drops of nessler's reagent were added, and the

mixture was incubated for 10 minutes at room temperature. 2-3 drops of TCA reagent are added to this mixture, and the result is measured at 425nm **[10]**.

2. 8. Solubilization of Different Metals:

2.8.1. Phosphate Solubilization

Next to nitrogen, phosphorus is the most significant essential element in plant nutrition (N). Plant growth-promoting rhizobacteria in the soil use a variety of techniques to make use of inaccessible forms of phosphorus, hence assisting in the availability of phosphorus for plants to absorb. Modified Pikovskaya's agar plates were produced and test culture was spot injected on them.The test culture was incubated for 48 to 72 hours at 280°C. After incubation, the plates were examined for the zone of phosphorus solubilization clearing. The clearing zone and growth zone of the culture organism were calculated **[11]**.

2.8.2. Potassium Solubilization:

The third most important macronutrient for plant growth is potassium (K). Rhizobacteria that promote plant growth can solubilize potassium rock by producing and secreting organic acid. The modified Aleksandra agar plates were produced and test culture was spot injected on them. The plates were inoculated for 48-72 hours at 280°C.The zone of clearance of potassium solubilization were checked **[12]**.

2.8.3. Calcium solubilization:

Calcium is involved in the activation of some plant enzymes and affects the transport of other nutrients into the plant. Stunting is caused by a calcium shortage. Photosynthesis and plant structure are both aided by this nutrient. Inadequate calcium can also cause blossom end rot. The test culture was spot inoculated on modified Yeast extract calcium carbonate agar plates. The plates were incubated for 48-72 hours at 280°C. The plates were then examined for the zone of calcium solubilisation **[13]**.

2.8.2. Magnesium solubilization:

Magnesium, like potassium, is particularly mobile in plants, and when deficient, it is translocated from older to younger tissues, causing symptoms to emerge initially on the oldest tissues and then spread to younger tissues. The test culture was spot injected on modified Yeast dextrose magnesium carbonate agar plates. These plates were incubated for 48-72 hours at 280°C.Magnesium is a component of the chlorophyll molecule, which is essential for plant nourishment. It is tightly related to energy-supplying phosphorus compounds and serves as a transporter and potent activator in a range of enzyme operations **[14]**.

2.8.3. Sulphur Solubilization:

Sulphur is a key component of chloroplast formation and function and is found in

various amino acids (particularly cysteine and methionine) and vitamins. It's found in the iron-sulphur complexes of photosynthesis' electron transport chains. It's required for legumes to fix N2 and convert nitrate to amino acids, which are then converted to protein. The test culture was spot injected on the modified thiosulphate medium plates. After that, the plates were incubated at 280°C for 48-72 hours, and the zone of sulphur solubilization clearing was examined. [15]

2.8.5. Zinc Solubilization:

Zinc is a chemical element that is chemically similar to magnesium. Both elements have just one normal oxidation state (+2), and the ions Zn2+ and Mg2+ are similar in size. The test culture was spot inoculated on the modified Bunt and Rovira agar plates. After that, the plates were incubated at 280°C for 48-72 hours and tested for a zone of zinc solubilization clearance **[16]**.

2.8.6. Manganese Solubilization:

Manganese is required for photosynthesis, as well as the formation of chloroplasts. Manganese insufficiency can cause colour changes in the leaves, such as discoloured patches. The test culture was spot inoculated on modified nutritional agar supplemented with MnO2 plates, which were then incubated at 280C for 48-72 hours and evaluated for zone of Manganese solubilization clearing **[17]**.

3. Seed germination: For the seed germination assay, wheat and rice were used. The seeds were surface sterilised for 1 minute with 0.01% HgCl2 and then thoroughly rinsed 3-4 times with distilled water to eliminate any disinfectant residue. These seeds were immersed in cyanobacterial extract overnight. After that, the seeds were placed in a petri dish with wet filter paper and allowed to sit at room temperature for 24 to 48 hours **[18]**.

4. Pot Culture:

Under in vitro settings, the capacity of the isolate of cyanobacteria to promote plant development was assessed using soil in pot culture. Rice and wheat seeds were procured, sown, and seedlings were cultivated for 15 days for the pot culture test. For the control, no fertiliser was used, but cyanobacterial extracts were sprayed on the soil of the test plants.After a 24-hour period, the seedlings were watered on a regular basis. Both the control and test plants' height and root length were measured after 15 days **[19]**.

5. Statistical analysis: An analysis was performed with single factor ANOVA and significant differences were surveyed at 0.05 level **[19]**.

3. RESULTS AND DISCUSSION

3.1 Isolation and screening of cyanobacteria

After 10-15 days incubation in BG11 medium inoculated with paddy field soil developed a green colour growth of cyanobacteria.

3.2 Identification of cyanobacteria

6 isolates were obtained from inoculated soil samples in BG11 media and further identified by microscopic examination. The isolate identified as *Gleocapsa* spp. was chosen for further investigation **(Table 1)**.

3.3. Detection of IAA

Salkowasky's reagent was used to detect IAA production. Color changes from yellow to pink have been recorded. Within a minute, colour development was observed at the highest IAA concentration, and it proceeded to intensify for the next 30 minutes. As a result, optical density was determined after 30 and 120 minutes **(Table 2) [7]**.

3.4. Detection of Siderophore

CAS reagent was used to detect siderophore synthesis. Color transitions from blue to pink were noted. The generation of siderophore in Gloeocapsa strains was evaluated for 6 days of incubation, and all Gloeocapsa strains produced positive results using the CAS assay method, as shown in **Table 3 [8]**.

3.5. Pigment extraction

At 625 nm, the chlorophyll concentration of Gloeocapsa strains was measured spectrophotometrically. **Table 4** demonstrates that the isolated cyanobacteria Gloeocaopa-1 strain has the highest chlorophyll concentration of all the cyanobacterial species **[9]**.

3.6. Detection of nitrogen: At a wavelength of 425nm, nitrogen was detected spectrophotometrically. The Nitrogen Fixation test yielded positive findings for the isolated cyanobacterial strains, as shown in **Table 5**. The Gloeocapsa-1 strain had the highest rate of nitrogen fixation, while the Gloeocapsa-2 strain had the lowest rate **[10]**.

3.7. Solubilization of different Metals

The ability of Gloeocapsa to solubilize various metals was assessed, and the results are shown in **Table 6**.

3.7.1. Phosphate: the Gloeocapsa strains had zones of solubilization. The Phosphate Solubilization test yielded positive findings for the isolated isolates. When compared to Gloeocapsa 2, the Gloeocapsa-1 strain had the largest hydrolysis zone **[11]**.

3.7.2. Potassium

On Aleksandrov's agar plates, the isolated Gleocapsa strains had the largest zone of potassium hydrolysis. The Gloeocapsa-1 strain had the larger zone of hydrolysis, whereas the Glpocapsa-2 strain had the smaller zone of potassium hydrolysis **[12]**.

3.7.3. Calcium

The Calcium Solubilization test yielded positive findings for the isolated Gloeocapsa strains. When compared to the Gloeocapsa -2 strain, the Gloeocapsa -1 strain had the larger hydrolysis zone **[13]**.

3.7.4. Magnesium : The magnesium solubilization test yielded positive findings for the isolated Gloeocapsa strains. In comparison to the Gloeocapsa -2 strain, the Gloeocapsa -1 strain has the largest hydrolysis zone **[14]**.

3.7.5. Sulphur

On the thiosulphate medium agar plates, all of the isolated cyanobacterial strains displayed a zone of Sulphur Solubilization. The large hydrolysis zone was found in both Gloeocapsa strains **[15]**.

3.7.6. Zinc

On bunt and rovira agar plates, isolated Gloeocapsa strains displayed a zone of zinc solubilization. The Gloeocapsa-1 strain had the most hydrolysis zone, whereas the Glpocapsa-2 strain had the least zinc solubilization zone **[16]**.

3.7.7 Manganese

The Gloeocapsa -1 strain showed highest zone of hydrolysis **[17]**.

4. SEED GERMINATION

Gloeocapsa-1strain demonstrated a high rate of germination after 3 days of incubation when compared to the control. The germination rate of all cyanobacterial trials demonstrated that inoculating rice and wheat seeds with PGPR considerably improves seed germination and seedling vigour. With cyanobacterial strains, the rate of enhancement can vary. Gloeocapsa-1 has a good seed germination rate **(Table 6) [18]**.

5. Pot culture

The shoot length and root length of both the control and test plants were measured after 15 and 20 days to produce rice and wheat, respectively, and were found to be as shown in **Table 8 [19]**.

6. Statistical analysis

A single factor ANOVA was conducted at a significant difference level and a 0.05 level was surveyed. We may conclude that cyanobacterial extracts have a substantial effect on shoot and root growth because the p-value is <0.05. **(Table 8) [19]**.

Table 1: Shows the different species of cyanobacterial strains that were isolated

Sr. no	Isolate no	Microscopic observation	organism
1.	A	Soft, green gelatinous, membranous, Short, straight, parallel, filamentous and heterocyst presents.	*Anabaena*
2.	B	Vesicular, lobed, gelatinous, long, basal cells broad, filamentous and heterocystous	*Rivularia*
3.	C	Single,yellowish brown, filamentous, irregularly curved, bulbous at the base and heterocystous	*Calothrix*
4.	D	Spherical, greenish yellow, clustered together	*Anacystis*
6.	F	Colonial cells flattened along lines of division, embedded in dense mucilage formed by concentric layers of sheath each corresponding to a round of division.	Gleocapsa

Table 2: The production of IAA from Various Cyanobacterial Strains

Cyanobacterial strain	Observation	Result
Gloeocapsa-1	Color changes yellow to pink	Positive
Gloeocapsa-2	Color changes yellow to pink	Positive
Control	No color changes	-

Table 3: the production of Siderophore from various cyanobacterial strains

Cyanobacterial strain	Observation	Result
Gloeocapsa-1	Colour changes blue to yellow	Positive
Gloeocapsa-2	Colour changes blue to pink	Positive
Control	No colour changes	-

Table 4: The chlorophyll content of cyanobacterial Strains

Cyanobacterial strains	Absorbance (665nm)	Chlorophyll content (µg/ml)
Gloeocapsa-1	3.000	41.7
Gloeocapsa-2	2.386	33.16
Control	0.000	0000

Table: 5: The Nitrogen fixation by Gloeocapsa strains

Cyanobacterial strain	Absorbance (425)	Nitrogen conc.(mM)
Gloeocapsa-1	0.208	0.08
Gloeocapsa-2	0.164	0.05
Control	0.000	000

Table 6: Solubilization of different metals by Gloeocapsa strains

Metal	Cyanobacterial strains	
	Gloeocapsa-1	Gloeocapsa -2
Phosphate	+++	++
Potassium	+++	+
Calcium	+++	++
Magnesium	+++	++
Sulphur	+++	+
Zinc	+++	+
Manganese	+++	++

Table 7: Seed germination percentage

Species Name	Name of seed	Total number of seed	Number of germinated seed	Percentage of seed germination (%)
Gloeocapsa:1	Rice	10	10	100
	Wheat	10	10	100
Gloeocapsa:2	Rice	10	10	100
	Wheat	10	9	90
Control	Rice	10	8	80
	Wheat	10	7	70

Table 8: The shoot and roots length measurement of Test and Control

	Rice				Wheat			
	Shoot		Root		Shoot		Root	
	Control	Test	Control	Test	Control	Test	Control	Test
1	29.1	30.1	10.7	13.5	13.1	15.5	2.7	3.3
2	28.1	30.2	9.6	10.7	10.8	12.5	1.9	2.6
3	27.2	29.2	8.5	9.8	17.3	19.8	2.9	3.5
4	21.5	23.5	5.3	8.1	14.9	15.3	1.8	2.5
5	22.4	25.2	7.5	9.5	13.4	16.1	3.6	4.1
6	25.8	29.8	5.1	7.8	15.3	17.1	2.6	3.1
7	26.6	29.2	4.9	7.8	15.1	17.4	1.7	2.5
8	24.2	27.5	5.7	8.5	15.8	18.6	2.5	3.1
9	24.5	28.2	4.8	6.1	15.2	18.2	2.9	3.6
10	21.8	25.5	4.1	6.9	11.1	14.3	1.8	2.4

Wheat shoot						
ANOVA						

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	**78.508**	**9**	**8.723111**	**3.010045**	**0.050506**	**3.020383**
Within Groups	**28.98**	**10**	**2.898**			
Total	**107.488**	**19**				

***significant difference at 0.05 level ($P < 0.05$)**

Wheat root						
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	**6.3845**	**9**	**0.709389**	**3.503155**	**0.03185**	**3.020383**
Within Groups	**2.025**	**10**	**0.2025**			
Total	**8.4095**	**19**				

***significant difference at 0.05 level ($P < 0.05$)**

Rice shoot						
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	**110.052**	**9**	**12.228**	**2.97229**	**0.052403**	**3.020383**
Within Groups	**41.14**	**10**	**4.114**			
Total	**151.192**	**19**				

***significant difference at 0.05 level ($P < 0.05$)**

Rice root						
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	**85.4645**	**9**	**9.496056**	**3.412778**	**0.034562**	**3.020383**
Within Groups	**27.825**	**10**	**2.7825**			
Total	**113.2895**	**19**				

***significant difference at 0.05 level ($P < 0.05$)**

CONCLUSION

Cyanobacteria are comparable to algae and higher plants in that they are oxygenic, photosynthetic, and prokaryotic. They create oxygen and fix carbon from carbon dioxide using sunlight as their energy source and water as their reductant. Cyanobacteria excrete a variety of compounds that have an impact on plant growth and development. These bacteria have been shown to assist plants by creating vitamins, amino acids, polypeptides, antimicrobial compounds, and polysaccharides, which increase soil structure and fertility.Hormones, rather than chemical fertilisers, are now used as growth regulators for better plant growth.As a result, the goal of this study was to extract hormones from cyanobacteria and analyse their effects on plants. The cyanobacterial samples were taken from rice fields in the Trimbakeshwar Nashik District of Maharashtra, India. From the collected sample, the cyanobacterial species was separated. For 7 to 15 days, cyanobacterial cultures were kept in BG-11 medium at 25 ± 2°C. For identification and characterisation, the supernatant is used as a suspension. Wet mount method was used for identification and characterisation. Salkowsky's reagent was used to detect IAA from cyanobacteria, and all isolated cyanobacterial strains produced indole acetic acid. The CAS assay is used to detect siderophores produced by cyanobacteria; all

cyanobacterial strains produce siderophores. The nitrogen was detected via nesslerization, and the Gloeocapsa demonstrated the greatest capability for nitrogen fixation. Gloecocapsa's chlorophyll concentration is measured using acetone, and the Gloecocapsa has a high chlorophyll concentration. When isolated from various paddy fields, all three types of Cyanobacterial strains showed the strongest potential to solubilize potassium, phosphate, calcium, magnesium, sulphur, zinc, and manganese. The cyanobacterial extract was given directly to the seed in the seed germination assays, and the Gloeocapsa -1 demonstrated a high rate of germination. For further study, the root and shoot lengths are measured. The cyanobacterial isolates demonstrated a high rate of germination in root length and shoot length parameters in a pot culture experiment in which cyanobacterial extract was directly put on the experimental tray, indicating that cyanobacteria might be employed as a biofertilizer for crops.

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