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Research Article

ISOLATION OF POTENTIAL RHIZOSPHERIC *AZOTOBACTER* SP. FROM TRIBAL FIELD OF PATALKOT AREA

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ABSTRACT

Tribal community usually follows traditional farming, characterized by application of negligible inputs. At many places they usually practice monoculture and cultivate a crop year after year leading to the erosion of soil fertility. As part of resorting to inclusive development, scientific communities has responsibility to restore fertility of tribal's farm land for sustainable agriculture especially in view of recent revelation of growing adverse impact of climate change on food grain production. The objective of this study thus was to isolate indigenous isolates of *Azotobacter* from the rhizosphere of wheat and maize of tribal's farm land of Patalkot (District Chhindwara, MP). Subsequently, their effect vis-à-vis exotic *A. chroococcum* 5576 (positive control) and uninoculated plant (negative control) on growth and yield of little millet (*Panicum sumatrense*) under pot conditions studied. Potential bacterial isolates RGW4, HRM3 and HGW1 showed nitrogen fixation rate (mg) per gram of sugar consumption 19.04, 22.30, and 19.11, phosphate solubilization index 4.21, 3.05 and 2.38 and percentage production of siderophore 40.74, 41.10 and 11.26 respectively. Importantly, inoculation with RGW4, HRM3 and exotic Azo-5576 (positive control) showed enhanced grain yield by 19.77%, 17.79%, and 14.97% respectively and enhanced total biomass yield by 25.74%, 22.77% and 17.82% (Azo-557) respectively as compared to uninoculated plant (negative control).

Keywords: Little millet, *Panicum sumatrense, Azotobacter chroococcum*

1. INTRODUCTION

Millets, wheat and maize are the crops the tribal people cultivate in their farmland. Tribal agriculture however has general characteristics of low input uses and continuous practice of monoculture that lead to erosion of soil fertility. Other causes for low productivity of crops in the state are ascribed to poor soil depth and fertility, soil erosion, lack of awareness regarding scientific package of practices, inadequate input supply and lack of good quality seeds/Varieties [1, 2].

Little millets (*Panicum sumatrense*) colloquial names are *kutki or smai* and realizing the excellent nutritional composition of these grains they are now calls as nutrias grain or nutria cereals. Madhya Pradesh tribal area is a most important state for little millets cultivation and it is having tribal food nutrition security [1]. Recent hues and cries all over the world about climate change and its adverse impact on crop productivity has led to the initiation of research effort to mitigate climatic impact. Study on the soil microbial ecology and restoration of proper microbial community especially those directly benefitting the plants constitute some of the important efforts in this wake. Inclusion of tribal farmland in this effort is also important as this is of utmost importance for inclusive development. Isolation, identification, multiplication and addition of potential growth promoting microbes also called PGPR (plant growth promoting rhizobacteria) microbes to act as biofertilizers form the main research areas in this regard.

The biofertilizers have potential to transform nutritionally important growth element from unavailable to available form through biological mechanism [3]. The uses of inoculants as a bioferlizer to inhance plant growth and productivity offers better alternative to costly chemical fertilizers [4]. Few bacteria have the capacity to fix atmospheric nitrogen and solubilize unavailable form of phosphorus to solubilize form. Plant growth promoting bacteria special reference to *Azotobacter* species has this capacity to convert nitrogen and phosphorus in the soil [5, 6]. Several species of *Azotobacter* are able to produce

phytoharmones, siderophore and thus used for biocontrol purpose [7, 8].

Indigenous biofertilizer having ecological adaptation, are obviously considered to be efficient in performance as compared to exotic inoculums. It is also low cost alternative to chemical fertilizer [9]. The major goal was to select indigenous biofertilizer (*Azotobacter sp.*) with the greatest potential for plant growth promotion, and yield of tribal crop special reference to little millet.

2. MATERIALS AND METHODS

2.1. Soil sample collection

Rhizospheric soil samples of wheat and maize growing area were collected from the patalkot area of district Chhindwada, Madhyapradesh in the sterile polyethylene bags.

2.2. Procurement of PGPR (positive control)

Rhizobacterum *Azotobacter chroococcum* (NCIM No. 5576) was procured from National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India. Culture was maintained on Jensen medium at 30°C for further study.

2.3. Isolation of N_2 fixing bacteria

Nitrogen fixing bacteria were isolated from rhizosphere soil in nitrogen free medium (Jensen) by spared plate technique and maintained on same medium at 4°C for the future study. Purification of isolates was done by the streaking method on same nitrogen deficient medium [10].

2.4. Testing cyst forming ability

Cyst forming ability ofthe isolates was tested by the method of Socolofsky and Wyss [11, 12].

2.5. Morpho-physiological characterization of PGP bacteria

Morpho-physiological tests were carried out according to the manual of bacteriology [13]. Characteristic such cell shape, motility, pigmentation, gram reaction, catalase, oxidase, carbon source utilization (Sucrose, starch, dextrose, glucose and mannitol), urease, indole, citrate test, ammonia and HCN production were carried out.

2.6. Testing PGP (plant growth promoting) traits

2.6.1. Estimation of nitrogen fixation capacity

Nitrogen fixation ability of bacteria was tested applying semi microkjedhal method. Pure colony of *Azotobacter* isolates was grown in 50 ml of nitrogen free medium under control condition at 30°C for 12 days in ambient orbital incubator shaker (INSCIN ISI-096A). Nitrogen fixation efficiency of each bacterium was determined in the terms of total nitrogen fixed per gm sucrose consumption [14].

2.6.2. Phosphates solubilizing efficiency

Phosphates solubilizing efficiency was checked by the method of Pikovskaya [15] and solubilization index were calculated accordingly formula describe by Edi Premono [16].

Solubilizing index = Colony diameter + Halo zone diameter / Colony diameter

2.6.3. Estimation of siderophore production

Siderophore-producing ability was estimated by universal Chrome Azurol Sulphonate (CAS) assay qualitatively and quantitatively [17-19].

Quantitative estimation of siderophore was done by taking supernatant of 48 hours old bacterial culture [20].

Percentage siderophore unit (psu) = Ar-As/Ar x 100

Where; **Ar** = absorbance of reference (CAS solution and un inoculated broth), **As** = absorbance of sample (CAS solution and cell-free supernatant of sample).

2.7. Inoculation and treatments

Broth having CFU 10^9/ml served as inoculum was counted before the inculcation. Earthen pots of black polythene bags (2.5 kg capacity, 20 cm height and 15 cm wide) were filled with sterilized (autoclaved at 121°C) soil. Four types of treatment including no inoculation (negative control), inoculation with native RGW4 and HRM3 (experimental isolates) and *Azo* 5576 (positive control) were assessed for the growth of little millet under control condition. Bacterial suspension @ 0.1 ml per seed was inoculated around the seed at the time of sowing. Each experimental treatment was carried in randomized complete block design with three replications [21].

2.8. Growth condition and measurement

Plants were grown under the green house condition from July to September (temp. range from 25-28°C and RH 30-55%) for the period of 90 days. Pots were watered regularly to the field capacity to maintain soil moisture level. Pre and post harvesting growth parameters of plants such as plant height, stem diameter, no. of leaf and productive tillers, panicle length, dry weight of root,

total biomass and grain yield per plant were measured by conventional method.

2.9. Statistical analysis

Outcome of experiment data were statically analyzed by Mean ±Standard Deviation.

3. RESULT AND DISCUSSION

3.1. Isolation of PGPR bacteria

Three fast growing bacteria were selected on N_2 free medium and screened on the basis of their PGP trait. They were designated as RGM4, HRM3 and HGW1.

3.2. Morpho-physiological characterization

The three selected isolates were characterized morphologically and physiologically, the findings are given in table 2. Many of the characters they shared were similar, though a few variations were also observed. Since cyst formation is the most distinguishing character of *Azotobacter*, the isolates seemed most probably the species of *Azotobacter*.

3.3. PGP activity

All the three isolates exhibited N_2 fixing, phosphate solubilizing and siderophore forming activities. Plant growth promoting activity in *Azotobacter* sp. earlier reported [22]. Presence of ferredoxin, hydrogenase and an important enzyme nitrogenase required for nitrogen fixation was reported in *Azotobacter* [23].

Table 1: Types of sample and their designation

Designation	Farmer name	Sampling site	Host plant	Sample code
RGW4	Ratan singh	Ghatlinga	Wheat	RGW
HRM3	Hajari	Rated	Maize	HRM
HGW1	Harilal	Gudichatri	Wheat	HGW

Table 2: Morpho-physiological characters of potent isolates

Morpho-physiological traits	RGW4	HRM3	HGW1
Cell shape	Small rod	Medium rod	Avoid
Motility	+	+	-
Pigmentation	Brown	Green after aging brown	Brown
Gram reaction	-Ve	-Ve	-Ve
Cyst formation	+	+	+
Catalase	+	+	+
Oxidase	+	+	+
Urease	+	+	+
Indole	+	+	+
Sucrose	+	+	+
Starch	+	-	-
Dextrose	+	+	+
Glucose	+	+	+
Mannitol	+	+	+
Citrate	+	+	+
HCN	+	+	+
Ammonia	+	+	+

Table 3: Plant growth promontory characters of potent isolates (Mean±SD) n=3

PGP traits	RGW4	HRM3	HGW1
Nitrogen fixation efficiency	19.04±2.0	22.30±1.8	19.11±2.1
Phosphate solubilizing index (PSI)	4.21±0.08	3.05±0.27	2.38±0.10
Siderophore qualitative analysis	++	++	+
Siderophore quantitative analysis	40.74±0.62	41.10±0.36	11.26±0.42

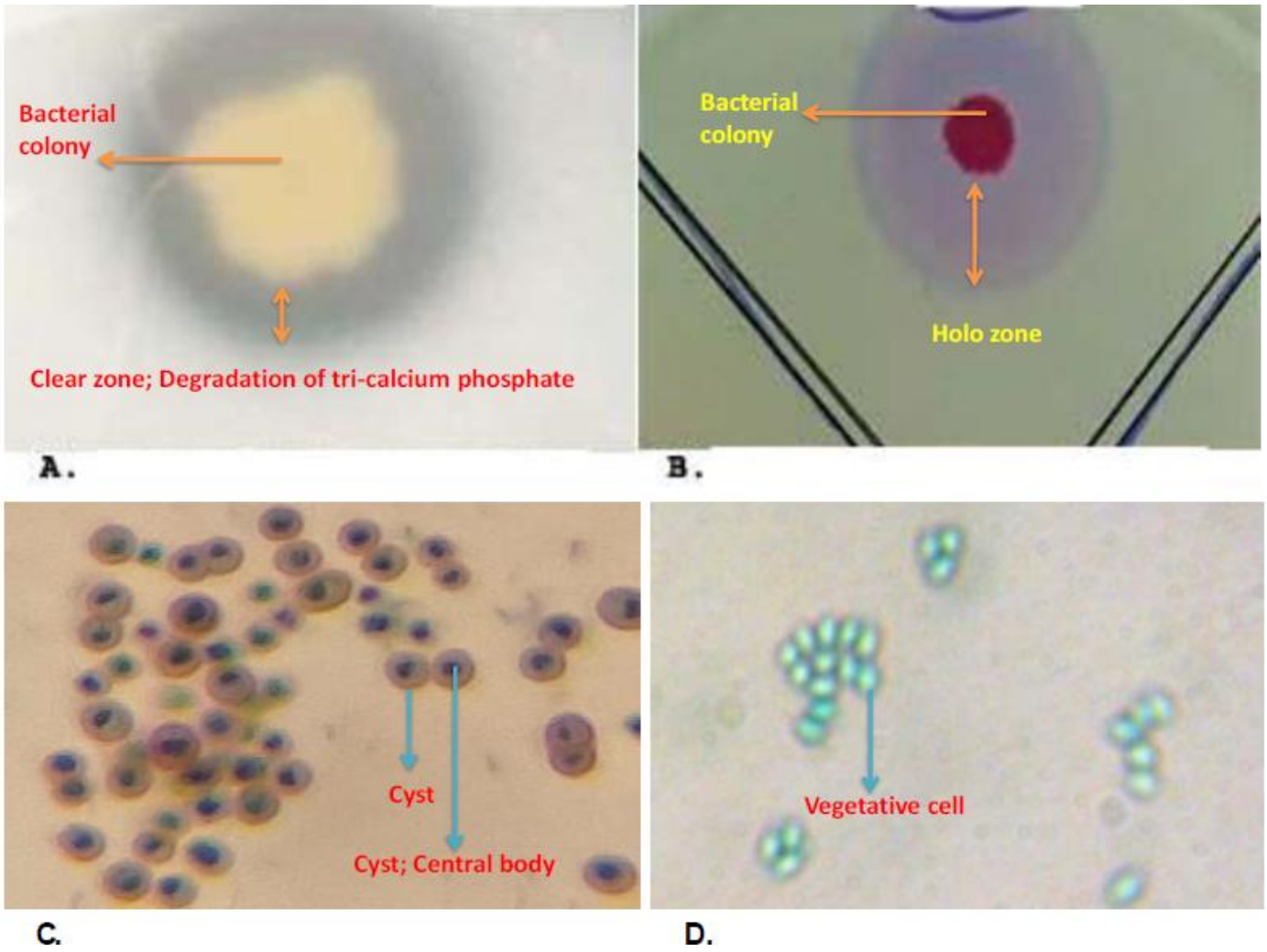

Fig. 1: Isolate RGW4 showing A. Phosphate solubilization, B. Siderophore formation, C. Cyst formation (100X), D. Vegetative cell (100X)

3.4. Growth observation

Pre harvesting data indicated that plant inoculated with rhizobacteria (RGW4, HRM3 and *Azo* 5576) showed increased number of leaf, reproductive tillers, stem diameter and length of panicle over uninoculated plant. The highest panicle length (12.27 cm) and number of reproductive tiller (4.23) was observed in plant inoculated with RGW4. Post harvesting data showed that all inoculation enhanced, root dry weight, total biomass and seed weight per plant in comparison to negative control. The highest root dry weight (0.158 gm) was observed in plant inoculated with HRM3 (Table 4).

Positive effect of indigenous *Azotobacter* on plant growth and grain yield of little millet was observed with respect to different growth attributes. The result indicated that inoculation with RGW4, HRM3 and exotic *Azo* 5576 (positive control) showed enhancement in the grain yield by 19.77%, 17.79%, and 14.97% respectively and enhanced total biomass yield by 25.74%, 22.77% and 17.82% respectively as compared to uninoculated plant (negative control). The result also indicated that indigenous isolates were more effective in enhancing grain yield as compared to the exotic strain (positive control). Earlier studies have also reported native strain to be potentially better to multiply in the region facing stress condition as compared to exotic ones [8]. Studies have also showed that application of *Azotobacter* to crop field increased 15 to 35% grain yield through positive effect on seed germination, resistance of seedlings to stress conditions in case of crop plants, and nitrogen fixation and production of growth promoting substances in case of bacterium [24, 25].

Table 4: Pot trial pre (stage of 85 days) and post (after 40 days) harvesting parameter

Treatments Growth traits studied	T0	T1	T2	T3
Height of plant (cm)	64.6±3.50	58.5±4.20	69.9±3.70	74.5±4.16
Stem diameter (mm)	2.30±0.18	2.74±0.40	2.98±0.38	2.84±0.25
No. of leaf/plant	11.70±1.22	14.30±2.10	15.20±1.70	17.05±2.30
No. of productive tiller/plant	3.55±0.65	4.14±0.67	4.23±0.54	4.18±0.71
Panicle length (cm)	11.37±1.61	11.42±1.22	12.27±1.05	12.11±1.25
Root dry weight (gm)	0.063±0.01	0.097±0.02	0.106±0.01	0.158±0.02
Total biomass of plant (gm)	1.01± 0.15	1.19± 0.19	1.27±0.21	1.24±0.12
Seed weight/plant (gm)	0.354±0.05	0.407±0.12	0.424±0.06	0.417±0.14

(Mean±SD) n=3 where; T0- Negative control, T1- Positive control (Azo 5576), T2-RGW4, T3-HRM3

4. CONCLUSION

The locally adapted isolates of *Azotobacter* thus have more positive impact on plant growth (little millet) and yield as compared to exotic isolate and control. The application of native inoculants thus is not only more efficient but also is cost effective. The results have far reaching impact on the tribal food security especially in view of current climate change related threat to food security and also associable with country's priority of application of science for inclusive growth.

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