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Comparative Study of Phosphate Solubilizing Bacteria from Paddy Field

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Abstract: ***Rice (Oryza sativa) is important grain providing one-fifth of caloric requirement of humans globally. Phosphorus is one of the major macronutrients required by plants. But, most of phosphorus present in soil is in insoluble forms and un-utilizable by the plants. Phosphate solubilizing bacteria (PSB) existing in rhizosphere solubilizes insoluble, in-organic and organic phosphorous forms thereby making soluble phosphate retrieved by the plant root from the soil environment. The present work is comparative study on phosphate solubilizing bacteria isolated from rhizosphere of rice plant and from non-rhizospheric soil (bulk soil) of the paddy field during flowering and harvest stage. Total number of PSB isolates obtained from rhizospheric soil is more as compared to bulk soil; 21 and 17 in flowering stage and 14 and 13 from harvest stage samples respectively. Out of total 38 isolates from flowering stage and 27 from harvest stage all have phosphate solubilizing ability in qualitative detection which was then quantified. It has been observed that potential for phosphate solubilization was higher among the isolates from harvest stage (282 µg/ml to 705 µg/ml) with reference to isolates from flowering stage (186 µg/ml to 645 µg/ml). Other traits of isolates aiding in nutrient acquisition by plant viz. nitrogen fixing ability and potassium solubilization were also determined.***

Keywords: Phosphate solubilizing bacteria, Rhizosphere, Flowering stage, Harvest stage, Nutrient acquisition

1.Introduction

Phosphorus, potassium and nitrogen are three major macronutrients required by plants. A large amount phosphorus existing in soil is insoluble type and not exploitable by the plants [1]. Additionally, phosphorus is applied to the soil in the form of phosphatic manure; a large portion of it is rapidly immobilized and thus become unavailable to plants [2]. The soil microbes, referred to as phosphate solubilizing microbes, having solubilizing ability dissociate these forms and make it available to the plants. Mineral phosphorus solubilization in the soil is probably due to secretion of organic acids, such as gluconic acid, 2-ketogluconic acid [3].

Rice (Oryza sativa) is the most important grain satisfying more than one-fifth of the caloric requirement of humans globally [4]. Cultivation of rice crop needs external application of fertilizers. At present, to increase rice production, chemical fertilizers and pesticides are applied in abundance in the paddy fields. This led to several environmental problems viz., deterioration of quality of soil and water and their health, resistant pathogens emergence and elimination of useful flora implicated in growth of paddy [5]. Thus, sustainable agriculture demands replacement of agrochemicals with the eco-friendly soil microbiota. In the same context, agricultural microbiologists are now a days interested in identifying soil bacteria that can solubilize mineral phosphate, thus aiding plant growth. Ashrafuzzaman M. *et al*., 2009 [6] have reported that ability to solubilize precipitated phosphates in soil and making it available to the plant can be one of the possible mechanisms of plant growth promotion by rhizobacteria under field conditions. Previous reports suggest that PSB are the most promising bacteria among the PGPR and thus can be used as biofertilizers for nutrient use efficiency and plant growth promotion [7].

2. Materials and Method

Sample Collection: Soil and rice plants were collected from Paddy fields at Vyara, District-Tapi, Gujarat, India, in *Kharif* season during flowering and harvest stage. The five rice plants were uprooted from the irrigated plot along with surrounding soil adherent to the roots from the four corners and one from the centre of the plot. The rice plants were kept in sterile polythene bags and taken to the laboratory and processed as composite sample.

Processing of Sample for isolation of Phosphate Solubilizing Bacteria: Using 1 ml supernatant from bulk/rhizospheric soil suspension, serial dilution was made up to 10^{-6}. 0.1ml of aliquot from last three dilutions were transferred and spread on Pikovskaya's agar medium. The plates were incubated at room temperature (30±2°C) for 48-72 hr. The isolated colonies were studied for their growth characteristics and sub-cultured on the respective media for acquiring pure culture of the isolates.

Qualitative Determination of Plant Growth Promoting Traits:

Phosphate solubilization: All isolates were screened for phosphate solubilization. A bacterial culture was inoculated as a line on Pikovskaya Agar medium [8] containing inorganic phosphate. The plates were then incubated at room temperature for 48-72 hrs and examined for zone of clearance.

Nitrogen fixing ability: It was checked as per method described by Gothwal *et* al., 2002 [9] using Nitrogen free malate medium containing bromothymol blue as an indicator. Change of colour of the medium surrounding the colony to blue indicates nitrogen fixing ability of bacteria.

Potassium solubilization: Using Aleksandrove agar medium, potassium solubilization of the isolates was

checked. Zone of clearance around the bacterial growth is an indicative of potassium solubilisation [10].

Quantitative Determinations of Phosphate Solubilization:

Phosphate solubilization ability of the PSB isolates was determined by the method described by Olsen *et al.*, 1954 [11]. Briefly; the fresh bacterial cultures were inoculated in sterile 50 ml Pikovaskaya broth (in 100ml conical flask), and incubated at room temperature for 7 days. After incubation, cultures were centrifuged at 8000 rpm for 5 min. To the 5ml supernatant, 5ml Ammonium Molybdate reagent (15gm ammonium molybdate dissolved in 400 ml dist. water, added to it 342 ml conc. HCl and set final volume to 1 lit using dist. water) was added and after shaking solution well, 1ml working solution of chlorostanous acid (1 gm $SnCl_2.2H_2O$ in 25 ml Conc. HCl-stock solution; 0.5 ml of stock solution was diluted to 66 ml by adding distilled water to make working solution) was added. The total volume of reaction mixture was made to 25 ml by adding distilled water. Absorbance of resulting solution was checked at 660 nm. Quantitation of solubilized phosphate was determined by extrapolating the absorbance values of test solutions with standard graph prepared using varying concentrations of standard K_2HPO_4 and were expressed as µg/ml.

3. Results and Discussion

In total 38 isolates were obtained from rhizospheric and non-rhizospheric samples from flowering stage and 27 from harvest stage. Total number of PSB isolates obtained from rhizospheric soil is more as compared to bulk soil; 21 and 17 in flowering stage and 14 and 13 from harvest stage samples respectively. Reyes *et al.*, 2006 [12] have also reported that rhizospheric soil possesses higher concentrations of phosphates solubilizing bacteria (PSB) as compared to non-rhizosphere soil. Out of total 38 isolates from flowering stage and 27 from harvest stage all have phosphate solubilizing ability as determined using tri-calcium phosphate amended solid and liquid media for qualitative and quantitative determinations. It has been observed that potential for phosphate solubilization was higher among the isolates from harvest stage (282 µg/ml to 705 µg/ml) with reference to isolates from flowering stage (186 µg/ml to 645 µg/ml). Free-living phosphate solubilizing bacteria liberate inorganic phosphate (soluble and thus plant utilizable) from insoluble inorganic and organic phosphate compounds present in soil [13]. The phosphate solubilization by the isolates were categorized into three groups viz., low phosphate solubilizer (<200µg/ml), intermediate phosphate solubilizer (200-400µg/ml) and high phosphate solubilizer (>400µg/ml), according to the extent of inorganic phosphate production was recorded. Among 38 flowering isolates, 5% isolates can be categorized as low phosphate solubilizer, 45% as intermediate solubilizer and 50% as high phosphate solubilizers. Accordingly, among 27 harvest stage isolates none fell in first category while 15% were intermediate solubilizer and 85% were high phosphate solubilizer.

Nitrogen fixing ability and potassium solubilization of the PSB isolates was also evaluated. An array of plant developmental processes are regulated by internal signals and it depends on the sufficient supply of mineral nutrients from soil to plant through roots. Thus, the availability of nutrients is a major limiting factor for the plant growth in many environments of the world. Non-symbiotic nitrogen fixing bacteria can provide better solution to make available inert nitrogen in the form utilizable by plant [9]. In the present study 42 isolates form total 65 PSB isolates presented nitrogen fixing ability also.

Concentration of soluble potassium, similar to phosphate is usually very low, and the chief fraction of potassium and phosphate in soil are in the form of insoluble rocks and minerals deposits [14]. Microorganisms play a key role in the potassium and phosphate cycling in nature. Similarly, potassium and phosphate solubilizing bacteria in soil are central to the solubilization of potassium and phosphorous and making it utilizable in plant rhizosphere [15]. Potassium solubilizing isolates obtained in this study were 10 out of 38 PSB in flowering stage and 15 out of 27 PSB in harvest stage. Thus the total potassium solubilizing isolates were more in harvest as compared to flowering stage.

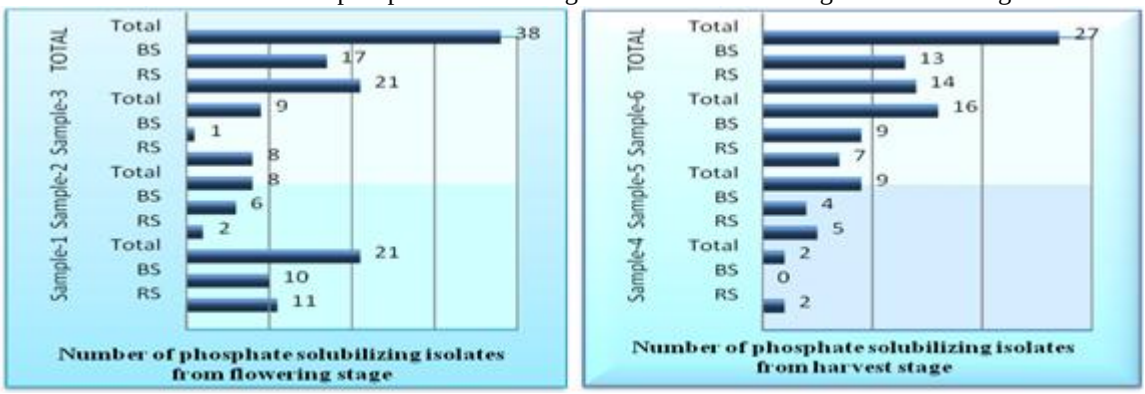
4. Observation tables and Charts

Table 1: Number of phosphate solubilizing isolates from flowering and harvest stage

FLOWERING STAGE ISOLATES												
Isolation Medium	Sample-1			Sample-2			Sample-3			TOTAL		
	RS	BS	Total	RS	BS	Total	RS	BS	Total	RS	BS	Total
Pikovskaya Agar	11	10	21	2	6	8	8	1	9	21	17	38
HARVEST STAGE ISOLATES												
Isolation Medium	Sample-4			Sample-5			Sample-6			TOTAL		
	RS	BS	Total	RS	BS	Total	RS	BS	Total	RS	BS	Total
Pikovskaya Agar	2		2	5	4	9	7	9	16	14	13	27

RS-Rhizosphere soil, BS-Bulk soil

Chart 1: Number of phosphate solubilizing isolates from flowering and harvest stage:



BS= Non-rhizospheric soil, RS= Rhizospheric soil

Table 2: Solubilization of TCP by isolates from flowering and harvest stage.

Phosphate Solubilization by isolates from Flowering stage				**Phosphate Solubilization by isolates from Harvest stage**			
Isolate No.	*Conc. of soluble Phosphate in µg/ml*	*Isolate No.*	*Conc. of soluble Phosphate in µg/ml*	*Isolate No.*	*Conc. of soluble Phosphate in µg/ml*	*Isolate No.*	*Conc. of soluble Phosphate in µg/ml*
PK-1	186.00	PK-20	574.00	PK-1	453.00	PK-20	531.00
PK-2	275.00	PK-21	599.00	PK-2	352.00	PK-21	506.00
PK-3	304.00	PK-22	382.00	PK-3	282.00	PK-22	487.00
PK-4	334.00	PK-23	509.00	PK-4	505.00	PK-23	670.00
PK-5	260.00	PK-24	505.00	PK-5	282.00	PK-24	609.00
PK-6	239.00	PK-25	522.00	PK-6	575.00	PK-25	649.00
PK-7	258.00	PK-26	642.00	PK-7	473.00	PK-26	677.00
PK-8	289.00	PK-27	349.00	PK-8	705.00	PK-27	381.00
PK-9	357.50	PK-28	645.00	PK-9	531.00		
PK-10	211.00	PK-29	195.00	PK-10	616.00		
PK-11	552.00	PK-30	618.00	PK-11	547.00		
PK-12	304.00	PK-31	415.00	PK-12	569.00		
PK-13	406.00	PK-32	296.00	PK-13	616.00		
PK-14	416.00	PK-33	530.00	PK-14	530.00		
PK-15	514.00	PK-34	455.00	PK-15	629.00		
PK-16	445.00	PK-35	267.00	PK-16	483.00		
PK-17	293.00	PK-36	570.00	PK-17	571.00		
PK-18	497.00	PK-37	235.00	PK-18	580.00		
PK-19	408.00	PK-38	277.00	PK-19	571.00		

Chart 2: Percentage distribution of Phosphate solubilizing isolates as low, intermediate and high phosphate solubilizers.

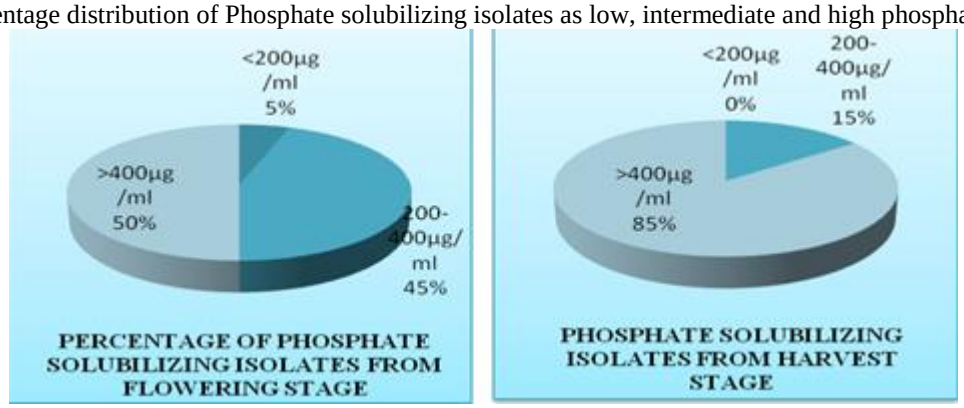


Table 3: Number of phosphate solubilizing isolates from flowering and harvest stage showing various nutrient acquisition traits

FLOWERING ISOLATES				
Sr. No.	Total No. of Isolates	Phosphate Solubilization	Nitrogen Fixing ability	Potassium Solubilization
Sample-1	21	21	15	5
Sample-2	8	8	2	4
Sample-3	9	9	6	1
TOTAL	38	38	23	10
HARVEST ISOLATES				
Sample-4	2	2	1	0
Sample-5	9	9	6	5
Sample-6	16	16	12	10
TOTAL	27	27	19	15

Chart 3: Nutrient acquisition traits of PSB isolates from flowering stage

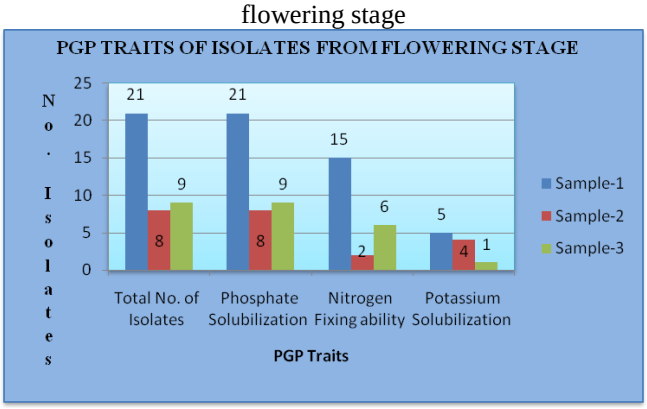
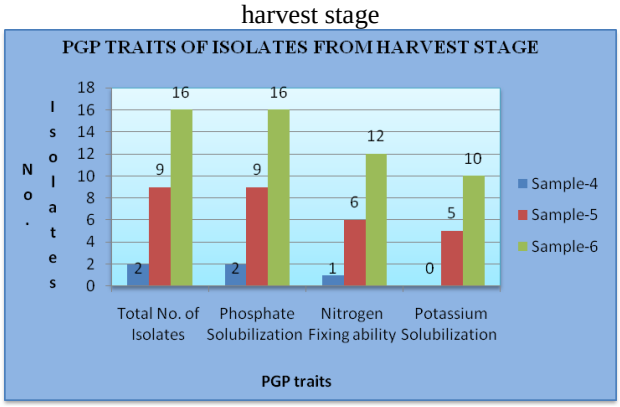


Chart 4: Nutrient acquisition traits of PSB isolates from harvest stage



5. Conclusion

Considering the worldwide importance of rice crop, application of phosphate solubilizers having other desirable traits can be an attractive approach to determine the nutrient management in eco-friendly and cost effective manner for sustainable agriculture. Duarah *et al.*, (2011) [7] have reported that treatments with PSB alone or in the form of consortia (of compatible strains) with or without the application of NPK chemicals externally improves germination index for rice and also vigour index and harvest index. They concluded that the uptake of NPK by plant and nutrient management can be improved by the use of PSB as biofertilizer in rice cultivation. In the present study, the phosphate solubilizing isolates also expressed nitrogen fixing ability and potassium solubilization which indicates their probable usefulness as biofertilizer. The findings of the present work are consistent with observations of Duarah *et al.*, (2011) [7]. Further experimentation is needed to check the most promising isolates among these for their potential as biofertilizer by the field experiments.

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