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Characterization of phosphate solubilizing bacteria isolated from paddy rhizosphere of Vidarbha region

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Abstract
In present investigation main aim to study morphology, biochemical characters and phosphate solubilising efficiency of phosphate solubilizing bacteria (PSB) from different soil samples were collected during June to October 2014. A total of forty-one (41) PSB were isolated from the eighty two (82) rhizospheric sample of rice plant of Vidarbha region, Maharashtra. Out of 41 isolates; 10 isolates show remarkable zone of solubilization. *In vitro* evaluation of phosphate solubilising ability of these ten (10) isolates were studied at department of plant pathology, Post Graduate Institute Dr. P.D.K.V., Akola during the June 2014 to May 2015. The zone of solubilization was studied on Pikovskaya's (PVK) agar and quantitative phosphate solubilization was carried out by Vanado- molybdate method. PSB9 showed the maximum phosphate solubilization of 10.17 mm in PVK's agar plates along with phosphate solubilizing activity (41.00 mg P_2O_5/100ml) in PVK's broth and pH of the medium decreased up to 3.80. However, the isolate PSB-3 shows the least solubilizing activity. Potent isolates show good phosphate solubilizing ability and thus were found potential further used as bio fertilizer agents.

Keywords: phosphate solubilizing bacteria, psuedomonas, rhizosphere, rice

Introduction
Rice is the staple food of more than half of the world's population; phosphorus is one of the important nutrients for plants which are low in soil. Plants absorb their required phosphorus from the soil solution in the form of phosphate anion (HPO_4^{2-} or H_2PO^{4-}). Of course, there are two form of mineral and organic phosphorus in large quantities and in the range of 400-1200 mg/kg in soil, but the concentration of soluble phosphate in soil is usually very low and in range of 1 mg/kg or lesser. So, there is no problem in most soils in terms of total amount of phosphorus, but its availability and accessibility is a problem. It is therefore necessary to identify and incorporate efficient strains of phosphate solubilizing microorganisms into cropping system (Fankem *et al.*, 2006) [6]. Most of it exists in soil insoluble metallic complex with iron and aluminium in acidic soil or with calcium carbonate in alkaline soil, as a result only a small fraction of phosphate is available for plant growth. Phosphate is highly insoluble and unavailable to plants in these forms (Maheswar and Sathiyavani, 2012) [11]. The insoluble phosphate, which is not directly available to plants or microorganisms, usually comprises 95-99 percent of the total phosphate (Hayman, 1975) [7]. A number of factors such as nature and content of clay, active sesquioxides, lime, pH and organic matter influence the availability of soil phosphate (Karunai *et al.*, 2011) [8]. Root development, stalk and stem strength, flower and seed formation, crop maturity and production, nitrogen fixation in legumes, crop quality and resistance to plant diseases are the attributes associated with phosphorus nutrition. The phosphate solubilizing microorganisms convert the insoluble phosphate into soluble forms by acidification, chelation and exchange reaction (Chen *et al.*, 2006) [4]. The solubilisation effect is generally due to the production of organic acids by these organisms (Ponmurugan and Gopi, 2006) [12]. Production of organic acids results in acidification of the microbial cell and its surrounding. These bacteria can play important role in supplement of phosphate to plants in a friendlier environmentally and in sustainable manner (Khan *et al.*, 2007) [9]. Due to the poor adaptability to change soil and agro climate condition, phosphate solubilizing microorganisms isolates elsewhere have not been very consistent in their performance everywhere (Alagawadi *et al.*, 1992) [1]. The aim of our investigation was to isolate the native phosphate solubilizing bacteria from rhizosphere paddy plant grown in vidarbha regions districts. The objective of

present investigation is morphological and biochemical characterization of PSB as well as find out efficient PSB isolates from paddy rhizosphere of Vidarbha region.

Materials and Methods

Collection of soil sample and Isolation of phosphate solubilising bacteria

Forty one Soil samples were collected from the rhizosphere soil at 25-30 days (at nursery stage) and 55-65 days (at pre flowering stage) old paddy plants from different areas of major paddy growing regions of Vidarbha *viz*., Gadchiroli, Chandrapur, Gondia and Bhandara districts. Soil samples were drawn from 5-25 cm depth of rhizosphere region, air dried in laboratory at room temperature and then used for isolation. One gram of soil from each sample was suspended in 9 ml blank sterile distilled water and serially diluted up to 10^{-6}. The dilutions were plated on Pikovskaya's (PVK) agar medium in order to isolate the PSB. Colonies forming a halo zone were re-transferred to PVK agar medium for maintaining the purity of the culture. The bacterial cultures were stored at 40^0 C and regularly transferred on nutrient agar slants during study (Sanjotha *et al*., 2011) [14].

Biochemical Studies

Biochemical tests *viz*., starch hydrolysis, H_2S production, gelatinase test, citrate utilization, catalyse activity, nitrate reduction, Indole Acetic Acid production, Urease Test and Gram's reaction were carried out for biochemical confirmation of isolates. (Shinde, 2003) [15].

Results and Discussion

Total forty-one (41) phosphate solubilizing bacteria were isolated from the eighty two (82) rhizospheric sample of rice plant of Vidarbha region, Maharashtra. Among them, 10 (Ten) isolates show remarkable zone of solubilisation and these selected for morphological and biochemical study. Studies on clear zone formation by selected PSB isolates around their colony on Pikovskaya's agar medium were also carried out in present investigation.

The data presented in Table 1 (Plate 3, 4 and 5) shows that, all ten isolates were Gram -ve, rod shaped, colonies were irregular with white to dull yellow pigmentation. All isolates showed H_2S production test negative except PSB-3. PSB-2 and PSB-5 shows citrate utilization negative reaction whereas all other isolates were positive to citrate utilization. All isolates were found efficient to liquefy gelatine and positive to catalyse activity. The result obtained that PSB-1, PSB-5 and PSB-6 isolates showed nitrate reduction test positive. However among all PSB isolates, five isolates *i.e.* PSB-1, PSB-2, PSB-3, PSB-4 and PSB-8 were able to produce Indole Acetic Acid and the only three isolates *viz*., PSB-5, PSB-6 and PSB-8 showed negative reaction to urease test. All isolates shows Starch hydrolysis negative whereas highest colony forming unit observe in PSB-10 (16.6 cfu$\times 10^{-7}$/g soil). According to above mentioned results of morphology and biochemical tests all ten isolates were probably identified as genus *Pseudomonas*. Present findings are in agreement with the finding of Shinde (2003) [15] who reported ability of ten *P. fluorescens* isolates to produce H_2S gas, hydrolyse starch, liquefy the gelatin and auxin production (IAA).

The data from Table 2 revealed that, among all bacterial isolates maximum halo (clear) zone (Plate No.6) was observed in PSB-4 on 2nd, 3rd and 4th day of incubation *i.e.* 8.84 mm 9.17 mm and 10.17 mm respectively. While minimum halo zone was recorded in PSB-3 during all three observations (2nd, 3rd and 4th day of incubation) *i.e.* 2.60 mm, 2.67 mm and 3.33 mm respectively. From the results it is concluded that PSB-4 was efficient phosphate solubilizer and PSB-3 was less efficient phosphate solubilizer as compared to all other PSB isolates of the region. The results were concurrence with the findings of Rashid *et al*. (2004) [13], Alam *et al*. (2002) [2] and Balamurugan *et al.* (2010) [3].

The data presented in Table 3 shows that qualitative estimation of PSB isolates on 8th and 15thday of incubation PSB isolates on PVK's broth with intial pH level was 7. PSB produce organic acid which decreased the pH of the medium and solubilized Tricalcium phosphate in broth medium. Among all PSB isolates maximum reduction in pH on 8th day was recorded in PSB-9 *i.e*. 3.80 with 41.00 mg P_2O_5/100ml solubilization. and minimum reduction in pH was recorded in PSB-1 *i.e*. 4.80 pH with 16.75 mg P_2O_5 /100ml as compare to control (6.90 pH) and 0.00 mg P_2O_5 /100 ml. Results recorded on 15th day shows maximum P_2O_5 solubilization by PSB-9 *i.e.* 52.50 mg P_2O_5/100 ml with second maximum reduction in pH (3.60). Similar results were reported by Chen *et al*. (2006) [5], who isolated 36 strain of phosphate solubilizing bacteria which solubilized tricalcium phosphate 1.5 to 519.7 mg/l with decreasing the pH of the medium.

Table 1: Morphological and biochemical characterization of the selected bacterial strains.

Sr. No	Isolate.	Colony morphology	Gram reaction	Cell shape	Biochemical characteristics*								Probable genus	cfu$\times 10^{-7}$/g soil
					1	2	3	4	5	6	7	8		
1	PSB-1	White circular	-ve	Rod	-	+	+	+	-	+	+	-	*Pseudomonas*	8.3
2	PSB-2	White circular	-ve	Rod	-	-	+	+	+	+	+	-	*Pseudomonas*	7.5
3	PSB-3	Dull yellow	-ve	Rod	+	+	+	+	+	+	+	-	*Pseudomonas*	8.0
4	PSB-4	Yellow	-ve	Rod	-	+	+	+	+	+	+	-	*Pseudomonas*	6.0
5	PSB-5	Dull yellow	-ve	Rod	-	-	+	+	-	-	-	-	*Pseudomonas*	3.7
6	PSB-6	Light yellow	-ve	Rod	-	+	+	+	-	-	-	-	*Pseudomonas*	5.2
7	PSB-7	Creamy round	-ve	Rod	-	+	-	+	+	-	+	-	*Pseudomonas*	5.8
8	PSB-8	Dull yellow	-ve	Rod	-	+	+	+	+	+	-	-	*Pseudomonas*	9.6
9	PSB-9	Dull yellow	-ve	Rod	-	+	+	+	+	+	+	-	*Pseudomonas*	9.0
10	PSB-10	white	-ve	Rod	-	+	+	+	+	-	+	-	*Pseudomonas*	16.6

*: Biochemical characteristics- 1. H_2S production, 2.Citrate utilization, 3.Catalyse test, 4.Gelatinase test, 5.Nitrate reduction test, 6.Indole production, 7. Urease test, 8.Starch hydrolysis

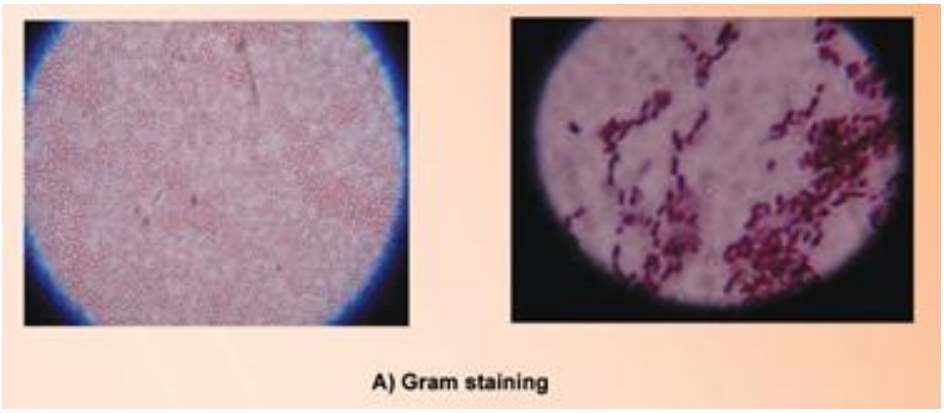
Table 2: Quantitative estimation of PSB by measurement of clear zone formation around their colony on pikovskaya's agar medium.

Sr. No.	Isolates	2nd day of Inoculation			3rd day of inoculation			4th day of inoculation		
		TZ (mm)	CD (mm)	CZ (mm)	TZ (mm)	CD (mm)	CZ (mm)	TZ (mm)	CD (mm)	CZ (mm)
1	PSB-1	6.30	2.60	3.70	7.00	3.60	3.40	8.30	4.16	4.14
2	PSB-2	12.67	4.33	8.34	14.00	5.00	9.00	17.00	7.33	9.67
3	PSB-3	8.67	6.00	2.60	9.60	7.00	2.67	11.66	8.33	3.33
4	PSB-4	11.00	2.16	8.84	12.00	2.83	9.17	14.50	4.33	10.17
5	PSB-5	9.16	2.33	6.83	11.33	3.66	7.67	13.50	4.16	9.34
6	PSB-6	8.77	4.00	4.77	10.83	5.17	5.66	12.00	6.00	6.00
7	PSB-7	8.70	3.30	5.40	10.33	4.16	6.17	13.00	4.33	8.67
8	PSB-8	10.33	4.00	6.33	12.00	6.00	8.00	14.33	5.50	8.83
9	PSB-9	15.00	6.83	8.17	17.00	9.16	7.84	20.33	9.33	10.00
10	PSB-10	11.00	4.83	6.17	12.50	5.50	7.00	15.00	8.00	7.66
	'F'- Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	S. E. (m)±	0.41	0.20	0.23	0.45	0.24	0.23	0.38	0.23	0.22
	C.D.(p=0.01)	1.64	0.79	0.95	1.84	0.96	0.93	1.52	0.92	0.89

TZ = Total Zone, CD = Colony Diameter CZ = Clear (halo) Zone

Table 3: Qualitative estimation of phosphate solubilizing bacteria.

Sr. No.	P solubilizing bacterial Isolates	8th day of incubation		15 th day of incubation	
		pH	mg P_2O_5 / 100ml	pH	mg P_2O_5 / 100ml
1	PSB- 1	4.80	16.75	4.60	18.90
2	PSB- 2	4.10	36.30	3.55	47.00
3	PSB-3	4.57	22.00	3.90	28.00
4	PSB-4	4.00	38.00	3.60	48.00
5	PSB-5	4.40	26.50	3.80	35.00
6	PSB-6	4.20	15.70	3.55	20.30
7	PSB-7	4.30	30.00	4.00	37.60
8	PSB-8	4.66	23.08	4.10	30.90
9	PSB-9	3.80	41.00	3.60	52.50
10	PSB-10	4.68	23.00	4.00	30.90
	control	6.90	0.00	6.80	2.00
	'F'- Test	Sig	Sig	Sig	Sig
	S. E. (m)±	0.038	0.37	1.28	4.11
	C.D.(p=0.01)	1.49	1.46	0.14	16.37



A) Gram staining

B) H_2S production test

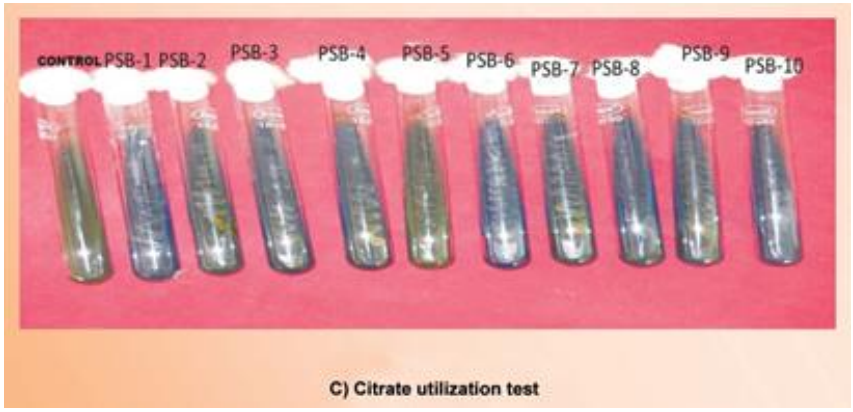


Plate 3: Biochemical characterization of bacterial isolates

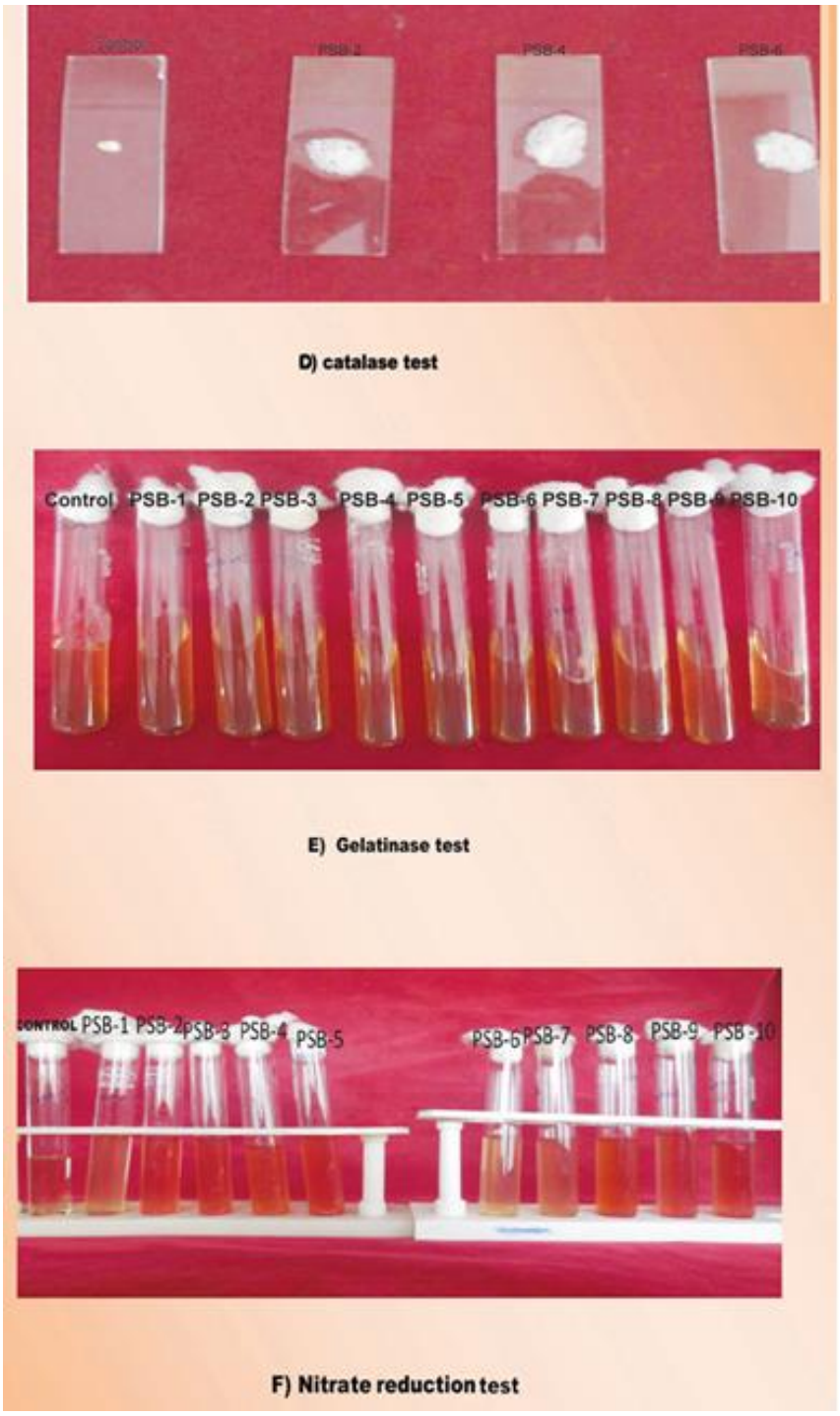


Plate 4: Biochemical characterization of bacterial isolates

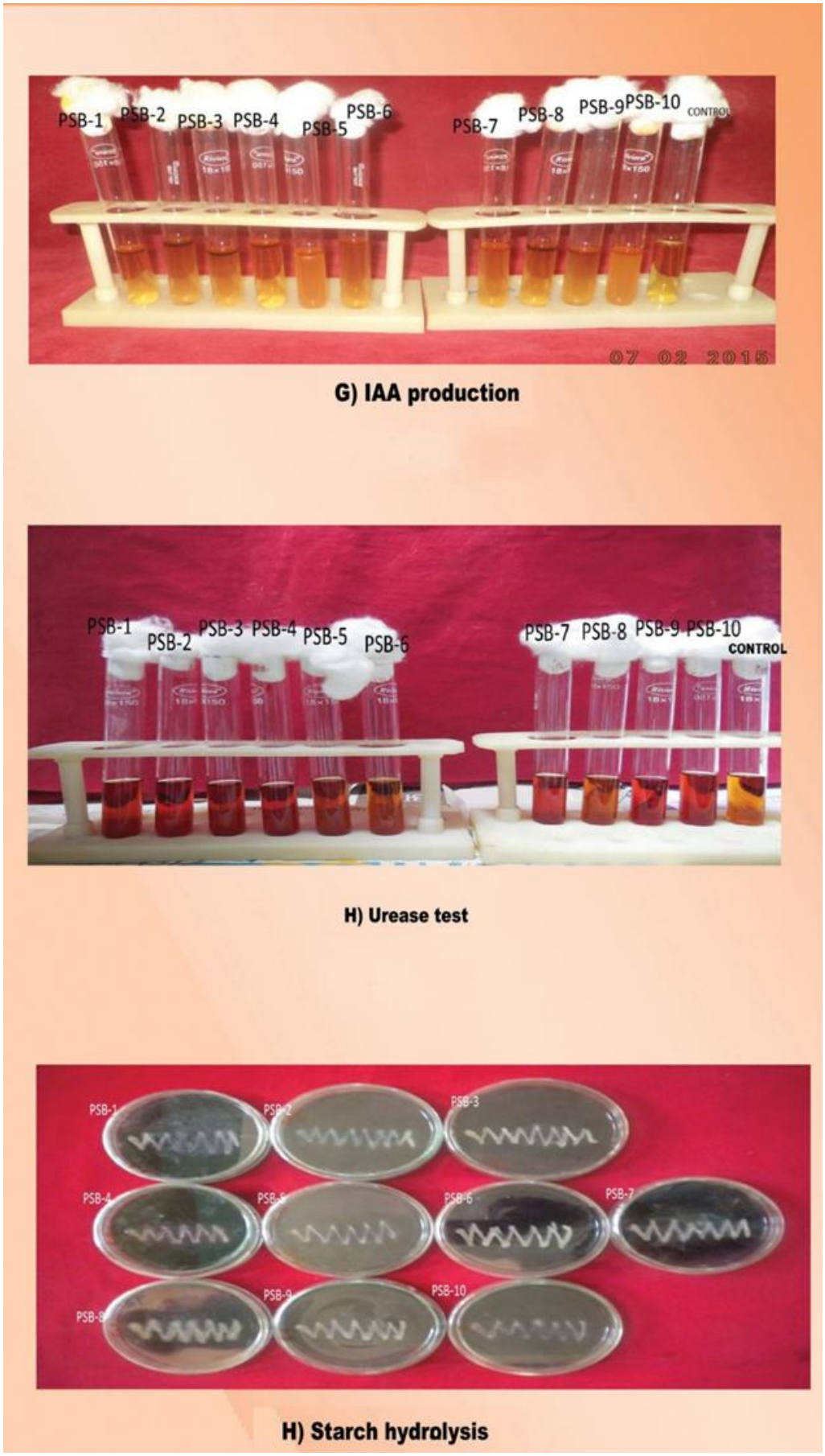


Plate 5: Biochemical characterization of bacterial isolates

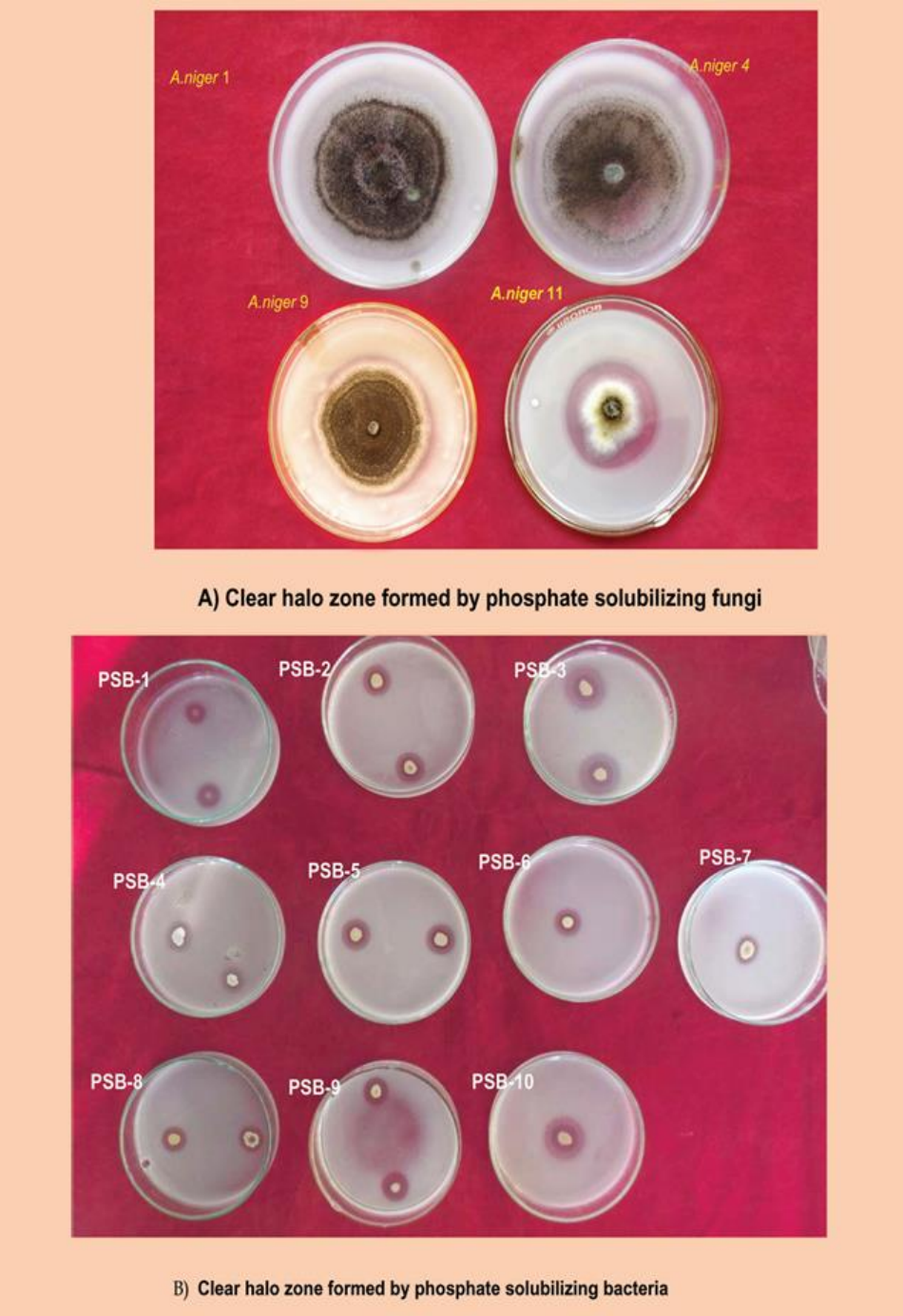


Plate 6: Clear halo zone formed by phosphate solubilizing bacteria

Conclusion

Paddy rhizospheric soils are rich in phosphate solubilizing bacteria. The isolate *Pseudomonas* strains on the basis of qualitative and quantitative estimation of phosphate solubilization showed maximum phosphate solubilizing activity. This strain can be used in the field as efficient bio fertilizers. Bio fertilizers are eco-friendly, free from hazardous chemicals, possess no detrimental health effects and are cost effective.

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