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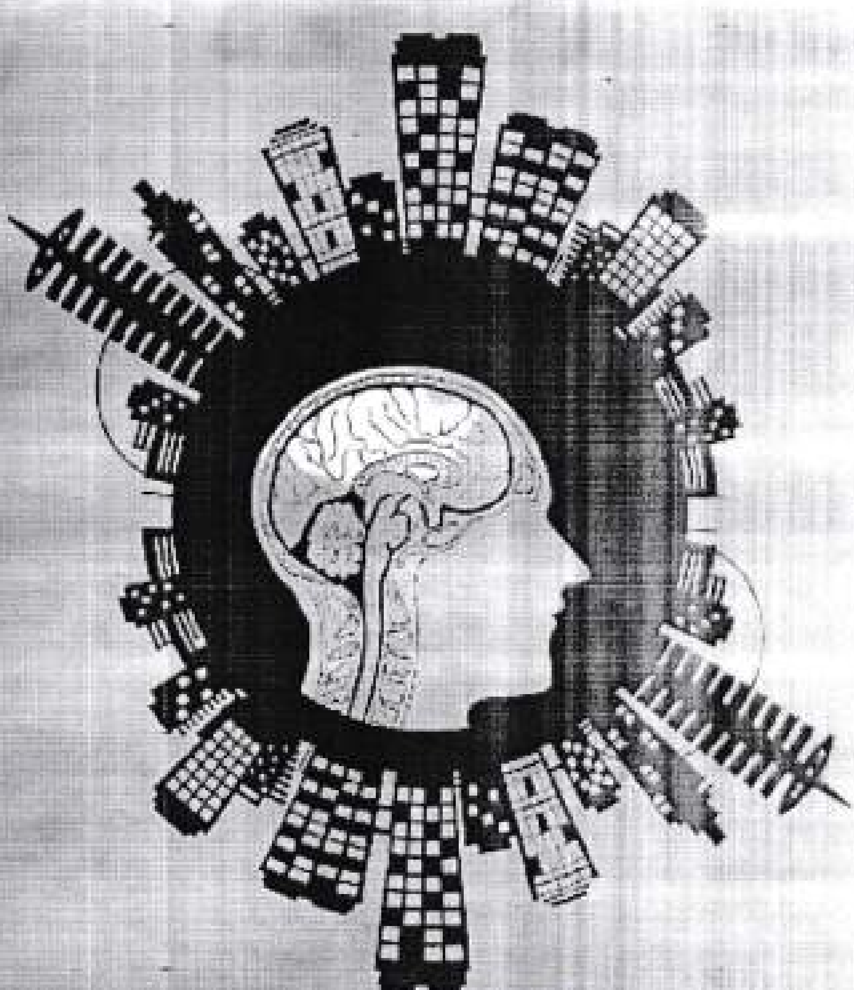

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IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani

ISOLATION AND SCREENING OF PHOSPHATE SOLUBILIZING BACTERIA (PSB) FOR THEIR POTENTIAL USE IN SUSTAINABLE AGRICULTURAL

Sanjay. M. Dalvi

Department Of Botany, Shri Guru Buddhiswami Mahavidyalaya, Purna (Jn.) – 431511

And

V. N. Kadam

Department Of Botany, Shri Guru Buddhiswami Mahavidyalaya, Purna (Jn.) – 431511

And

R. R. Rakh

Department Of Microbiology, Shri Guru Buddhiswami Mahavidyalaya, Purna (Jn.) – 431511

Abstract:

In this study soil samples from rhizospheric niches ofTur, Soya bean, Neem,and Bavanchya were collected in polythene bags and brought to the laboratory. All rhizospheric soil samplesscreened for phosphate solubilizing bacteria on Pikovskaya agar by serial dilution method. Among the soil samples screened, rhizospheric niches from the Soya bean shown highest phosphate solubilizing bacteria, 114 than the other rhizospheric soil sample.The rhizospheric niches of Tur, Neem and Bavanchya shown 47, 07, and 02 phosphate solubilizing bacteria.In total of 170 phosphate solubilizing bacterial isolates from different niches, 5 isolates, SMD16, SMD36, SMD37, SMD38 and SMD40 were found qualitativelyproduce more than 5 mm zone of solubilization on Pikovskaya's agar plates after 9 days incubation.Out of these 5 bacterial isolates, two bacterial isolates, namely SMD 36 and SMD 37 showed maximum P solubilization in PKV broth supplemented with tri – calcium phosphatequantitatively.

Key Word: Rhizospheric Soil, Pikovskaya Agar,Phosphate Solubilizing bacteria.

1.0 Introduction:

Phosphorus is an essential macronutrient for growth and developmentof plants involved in important metabolic pathways likephotosynthesis, biological oxidation, nutrient uptake and cell division(Illmer and Schinner 1992, Gupta *et al.,* 2012). Worldwide soils are supplementedwith inorganic P as chemical fertilizers to support crop production,but repeated use of fertilizers deteriorates soil quality (Tewari *et al.*,2004).The present scenario is shifting towards a more sustainable agriculture by using Phosphate Solubilizing Bacteria.

Natural solubilization of mineral phosphates is an important mechanism exhibited by different microorganisms, knownas phosphate solubilizing microorganisms (PSM). Bacteria arethe predominant microorganisms that solubilize mineralphosphate in nature, as compared to other microorganisms (Yin, 1988, Paul and Sinha, 2017).Phosphate solubilizing bacteria (PSB) play an importantrole in biogeochemical phosphorus cycling in both terrestrial and aquatic environments (Das *et al.* 2007).

Application of phosphate-solubilizing bacteria increases soil fertility due to their ability to convert insoluble P to soluble P by releasing organic acids, chelation and ion exchange [Omar (1998), Narula et al.,(2000), Whitelaw,(2000)].The important genera of P-solubilizing bacteria include *Achromobacter, Aerobacter, Alcaligenes, Azotobacter, Bacillus,Escherichia, Pseudomonas, Serratia* and *Xanthomonas* (Li, 1981; Datta *et al.*, 1982; Venkateswarlu *et al.*, 1984; Gaur, 1990), *Azospirillum* (Seshadri *et al.*, 2000).

The present investigation mainly focuses on the isolation and screening for high Phosphate Solubilizing Bacteria from rhizospheric niches of different plants, qualitative and quantitative estimation of phosphate solubilizing efficiency.

2.0 Materials and Methods:

2.1 Chemicals:

All the chemicals used during the study were procured from M/S Hi-media, Mumbai, Glaxo Ltd., Mumbai, Sigma Aldrich, USA, unless and otherwise specified in the text. Analytical/Guaranteed (AR/GR) grade chemicals and double glass-distilled water was used.

2.2 Collection of Soil sample from Rhizospheric Niches:

Soil samples were collected from the rhizospheric niches of four crop plants viz., *Tur, Soya bean, Neem, and Bavanchya* grown in the farmer field (Photo Plate 2.0), near the Purna City. For this purpose, the plants were uprooted carefully, shoots were cut off and roots along with rhizosphere soils were brought to the laboratory in polythene bags. The soil samples were processed immediately or stored at 4 – 8 °C for the isolation of Phosphate solubilizing microorganisms.

Photo plate 2.0: Collection of Soil sample from different Rhizospheric niches of plants

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2.3 Isolation of Phosphate Solubilizing Bacteria (PSB):

Phosphate Solubilizing Bacteria (PSB) was isolated from the rhizospheric soil samples by dilution plate technique using Pikovskaya's medium (Pikovskaya 1948) containing tri-calcium phosphate (TCP) (Gupta *et al.*, 2012, Kaur, 2014). One gram each of the soil sample was transferred to 9 ml sterile dilution blank under aseptic conditions and serial dilutions were made. Appropriate soil dilutions were plated on Pikovskaya's agar medium by spread plate technique and incubated at 30 ± 1 °C for 2-3 days. The colonies forming halo zone of clearance (Pikovskaya's medium) around them were counted as P - Solubilizers. All the bacterial colonies exhibiting halo zones were selected, purified and maintained on nutrient agar slants for further studies.

2.4 Estimation of Phosphate Solubilization Efficiency:

The phosphate solubilization efficiency of the bacterial colonies exhibiting strong halo zone was carried out qualitatively and quantitatively.

2.4.1. Qualitative Estimation of Phosphate Solubilization Efficiency:

Pure cultures of phosphate solubilizing bacteria were spot inoculated on the plates containing Pikovskaya's medium (Jackson 1973, Katoch, 2003 and Kaur 2012). The plates were incubated at 28±1°C and halo zone around colonies were recorded at regular interval upto 10 days. The abilities of the isolated phosphate solubilizing bacterium to solubilize TCP on Pikovskaya's agar media were determined in terms of solubilization index (SI). Phosphate solubilization index was calculated by measuring the colony diameter and the halo zone diameter and the colony diameter, using the following formula of Edi-Premono *et al.*, (1996).

$$\text{Phosphate Solubilization Index (SI)} = \frac{(\text{Colony diameter} + \text{Halo zone diameter})}{\text{Colony diameter}}$$

2.4.2. Quantitative Estimation of Phosphate Solubilizing Efficiency:

The quantitative estimation of solubilized Phosphate by bacterial isolates was done by Chloromolybdic Acid Method (Jackson, 1973).

2.4.2.1. Chloromolybdic acid Method:

The phosphate solubilizing activity of the selected bacterial isolates as citrate soluble P was determined quantitatively in liquid medium following Chloromolybdic Acid Method (Kaur, 2014, Jackson, 1973).

2.4.2.1.1. Chloromolybdic Acid:

15 gm Ammonium molybdate was dissolved in about 400 ml of distilled water, filtered and then 400 ml of 10 N HCl was added slowly with rapid stirring. Volume was made to 1000 ml with distilled water and stored in amber glass bottle.

2.4.2.1.2. Chlorostannous Acid:

Stock solution:

$SnCl_2.2H_2O$	10 gm
Con. HCl	25.0ml

$SnCl_2$ crystals were dissolved in Con. HCl and solution was kept in glass bottle under airtight stopper.

Working Solution:

Fresh working solution was prepared by adding 1.0 ml of the above solution to 132.0 ml of distilled water.

2.4.2.1.3. Quantitative Estimation P Solubilization by Chloromolybdic Acid Method:

The phosphate solubilizing bacteria were grown in 50 ml nutrient broth for 24 hr. at 30 °C in incubator shaker. 0.1 ml of each phosphate solubilizing bacteria was aseptically transferred to 100 ml PKV broth contained in 250 ml conical flask. The flasks were incubated 30 °C in a rotary shaker at 130 rpm. Five ml of culture was taken out in sterile condition at regular interval of 2 days from third day onward and centrifuged at 10000 rpm for 10 min. Then 500 µl of each supernatant was transferred to 50 ml volumetric flask. This was followed by addition of 10.0 ml Chloromolybdic acid. The content of the flask was diluted to 40.0 ml with distilled water. Then 1 ml of Chlorostannous acid was added. After mixing, the volume was made up to 50.0 ml with distilled water. The blue colour intensity of the solution was measured at 600 nm. The soluble P was estimated from standard curve of KH_2PO_4 (100 ppm) drawn against O.D. 600 nm.

3.0 Result and Discussion:

Natural solubilization of mineral phosphates is an important phenomenon displayed by different microorganisms, known as *phosphate solubilizing microorganisms (PSM)*. Bacteria are the principal microorganisms that solubilize mineral phosphate in nature, as compared to other microorganisms (Yin, 1988). *Phosphate solubilizing bacteria (PSB)* play an important role in biogeochemical phosphorus cycling in both terrestrial and aquatic environments (Das *et al.*, 2007).

3.1 Isolation of Phosphate Solubilizing Bacteria (PSB):

In present investigation 170 phosphate solubilizing bacteria were isolated from different rhizospheric niches of healthy plants by using serial dilution method on Pikovskaya's (PKV) agar plates (Photo Plate 3.0). Out of 170, 114 phosphate solubilizing bacteria (PSB) were isolated on Pikovskaya Agar from the Soya bean rhizospheric niches, by using dilution technique, which were fare greater than the other rhizospheric niches sample. Similarly, from the rhizospheric niches of Tur, Neem and Bavanchya 47, 07, and 02 phosphate solubilizing bacteria were isolated (Table 3.0). Use of Pikovskaya's agar medium for isolation of Phosphate Solubilizing Bacteria (PSB) was a simple way to detect PSB through formation of halo zone on agar plate containing tri – calcium phosphate as a sole Phosphorous source (Kaur, 2014). These rhizospheric isolates were tentatively named as SMD 1 to SMD 170.

Table 3.0: Isolated PSB from different rhizospheric niches with their Phosphate Solubilization Index

Tentative Name of *SMD*	Phosphate Solubilization Index	Tentative Name of *SMD*	Phosphate Solubilization Index	Tentative Name of *SMD*	Phosphate Solubilization Index
SMD 1	1	SMD 64	2	SMD 127	0
SMD 2	2	SMD 65	2	SMD 128	2
SMD 3	2	SMD 66	2	SMD 129	2

SMD 4	1	SMD 67	2	SMD 130	1
SMD 5	1	SMD 68	1	SMD 131	1
SMD 6	**5**	SMD 69	1	SMD 132	1
SMD 7	1	SMD 70	2	SMD 133	2
SMD 8	1	SMD 71	2	SMD 134	2
SMD 9	1	SMD 72	2	SMD 135	0
SMD 10	1	SMD 73	2	SMD 136	0
SMD 11	2	SMD 74	2	SMD 137	1
SMD 12	2	SMD 75	2	SMD 138	1
SMD 13	2	SMD 76	2	SMD 139	1
SMD 14	2	SMD 77	1	SMD 140	1
SMD 15	3	SMD 78	1	SMD 141	0
SMD 16	4	SMD 79	1	SMD 142	0
SMD 17	1	SMD 80	1	SMD 143	0
SMD 18	4	SMD 81	2	SMD 144	0
SMD 19	4	SMD 82	1	SMD 145	0
SMD 20	4	SMD 83	1	SMD 146	0
SMD 21	1	SMD 84	1	SMD 147	0
SMD 22	**5**	SMD 85	2	SMD 148	0
SMD 23	1	SMD 86	2	SMD 149	0
SMD 24	1	SMD 87	2	SMD 150	0
SMD 25	1	SMD 88	1	SMD 151	0
SMD 26	4	SMD 89	2	SMD 152	1
SMD 27	2	SMD 90	2	SMD 153	1
SMD 28	**5**	SMD 91	2	SMD 154	1
SMD 29	4	SMD 92	2	SMD 155	1
SMD 30	**5**	SMD 93	2	SMD 156	1
SMD 31	4	SMD 94	1	SMD 157	1
SMD 32	2	SMD 95	1	SMD 158	2
SMD 33	4	SMD 96	2	SMD 159	2
SMD 34	4	SMD 97	2	SMD 160	2
SMD 35	2	SMD 98	1	SMD 161	1
SMD 36	**5**	SMD 99	1	SMD 162	2
SMD 37	**5**	SMD 100	1	SMD 163	2
SMD 38	**5**	SMD 101	0	SMD 164	1
SMD 39	4	SMD 102	2	SMD 165	2
SMD 40	**5**	SMD 103	0	SMD 166	2

SMD 41	4	SMD 104	1	SMD 167	2
SMD 42	2	SMD 105	0	SMD 168	1
SMD 43	1	SMD 106	2	SMD 169	2
SMD 44	2	SMD 107	1	SMD 170	0
SMD 45	1	SMD 108	2		
SMD 46	1	SMD 109	1		
SMD 47	1	SMD 110	0		
SMD 48	1	SMD 111	2		
SMD 49	1	SMD 112	2		
SMD 50	2	SMD 113	0		
SMD 51	1	SMD 114	1		
SMD 52	1	SMD 115	1		
SMD 53	4	SMD 116	2		
SMD 54	1	SMD 117	2		
SMD 55	1	SMD 118	0		
SMD 56	1	SMD 119	1		
SMD 57	4	SMD 120	2		
SMD 58	1	SMD 121	2		
SMD 59	1	SMD 122	2		
SMD 60	2	SMD 123	2		
SMD 61	2	SMD 124	0		
SMD 62	1	SMD 125	1		
SMD 63	2	SMD 126	1		

The role of microorganisms in solubilizing insoluble phosphates in soil and making it available to plant is well known (Kundu and Gaur, 1981). Phosphate solubilizing microorganisms include several Bacteria, Fungi, Actinomycetes, Yeast and Cyanobacteria (Gerretsen, 1948; Banik and Dey 1982 and Illmer and Schinner 1992). The phosphate solubilizing microorganisms can be isolated from different sources such as soil (Gupta *et al.*, 1986; Kapoor *et al.*, 1989), rhizosphere (Sardina*et al.*, 1986; Singh and Kapoor, 1994), root nodules (Suranga and Kumar, 1993), compost (Gupta *et al.*, 1993), and rock phosphates (Gaur *et al.*, 1973). These reports support the fact that phosphate solubilizing bacteria can be isolated from rhizospheric niches.

In this study, from rhizospheric niches of soya bean greater amount of phosphate solubilizing bacteria were isolated on Pikovskaya's agar plate. Kaur (2014) isolated 1270 bacteria was isolated on Pikovskaya's agar plate by serial dilution method at 10 -5 dilutions. Out of these 1270 bacterial isolates only 169 bacteria isolates were observed to be formed a halo zone around the colonies. Of 170

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bacterial isolates, only 8 bacterial isolates designated as SMD6, SMD22, SMD28, SMD30, SMD36, SMD37, SMD38 and SMD40 displayed greater efficacy of solubilizing the phosphate.

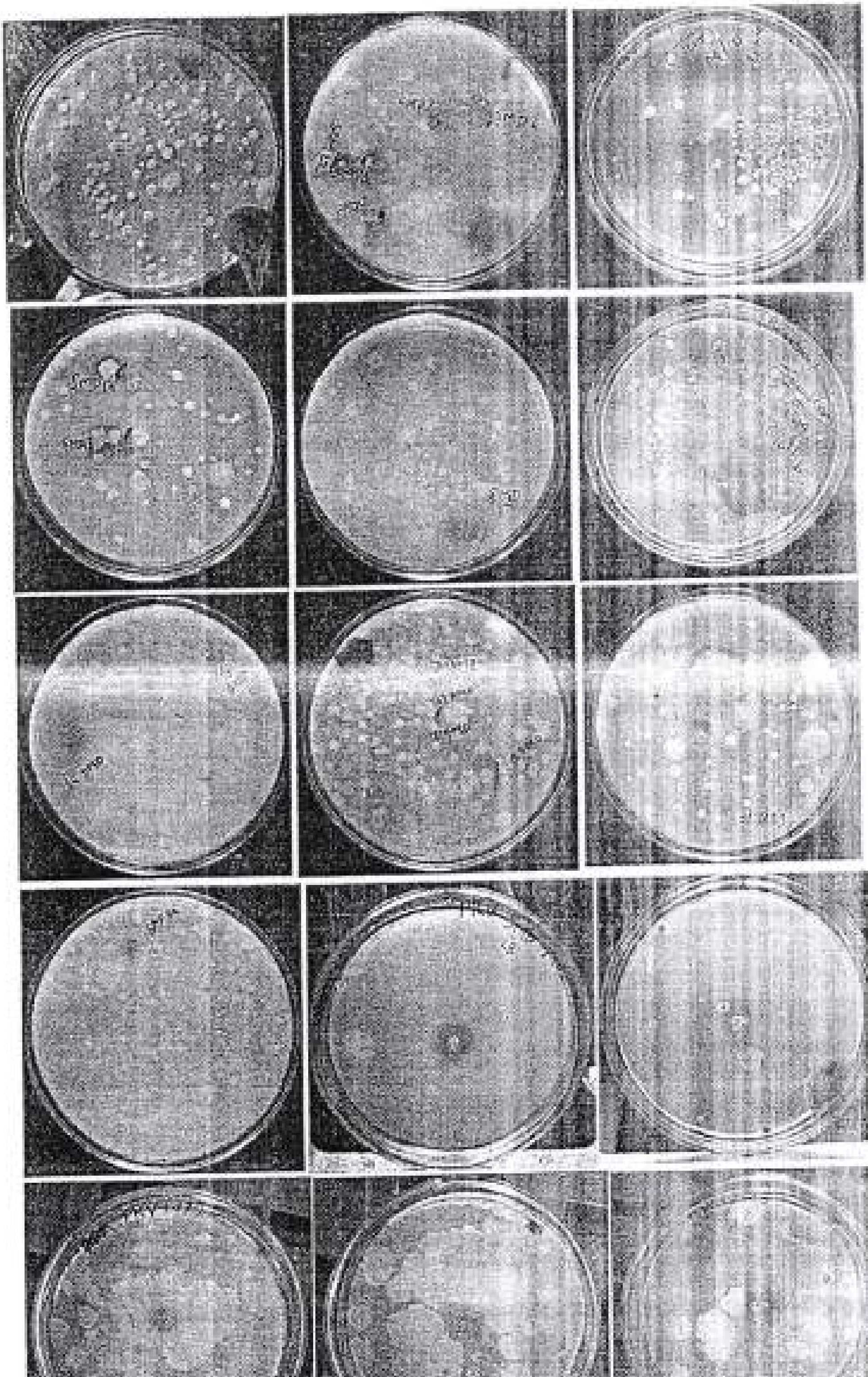

Photo Plate 3.0: Isolation of Phosphate Solubilizing Bacteria (PSB) with clear halo zone

3.2 Estimation of Phosphate Solubilization Efficiency:

The phosphate solubilization efficiency of the bacterial colonies exhibiting strong halo zone was carried out qualitatively and quantitatively.

3.2.1. Qualitative Estimation of Phosphate Solubilization Efficiency:

Qualitative screening of selected phosphate solubilizing bacterial isolates was completed by the method of Katoch, (2003) and Kaur (2012) revealed variations in phosphate solubilization efficiency. In total of 170 phosphate solubilizing bacterial isolates from different niches, 5 isolates, SMD16, SMD36, SMD37, SMD38 and SMD40 were found to be fare more than 5 mm zone of solubilization on Pikovskaya's agar plates after 9 days incubation (Photo plate 3.1). The Phosphate solubilization activity of these isolates of PKV agar plates was ranged between 2.0 to 5.0 (Table 3.1). Kaur (2014) stated that 169 phosphate solubilizing bacteria isolated from different rhizospheric niches revealed phosphate solubilization index in range between 1.36 to 3.17. Our results were somehow like results of Kaur (2014).

Table 3.1: Phosphate Solubilization Index of Selected Rhizospheric Isolates after 9 days of incubation

Rhizospheric Isolates	Diameter of Colony + Halo zone (mm)	Diameter of Colony (mm)	Diameter Halo zone (mm)	Phosphate Solubilization Index
SMD 16	10	5	5	2.00
SMD 36	10	3	7	3.33
SMD37	12	3	9	4.0
SMD38	9	3	6	3.0
SMD40	9	4	5	5.0

Similar kind of results were also recorded by Katoch (2012) where maximum zone of P solubilization in isolates designates as FB1-PB1, SB1-PB1, CP1-PB1, DH2-PB1, KT14-PB1, AM8-PB1 and PT12-PB1 was observed on 8th day of incubation, whereas in isolates FB2-PB2, PEA-PB1, RM2-PB1, CP2-PB4, WC2-PB1 and WSP1-PB1 on 9th day of incubation. Nine isolates showed maximum zone of solubilization on 10th day, 2 isolates (WSP2-PB2 and PT13-PB1) on 7th day and one isolate (SP2-PB1) on 6th day of incubation period.

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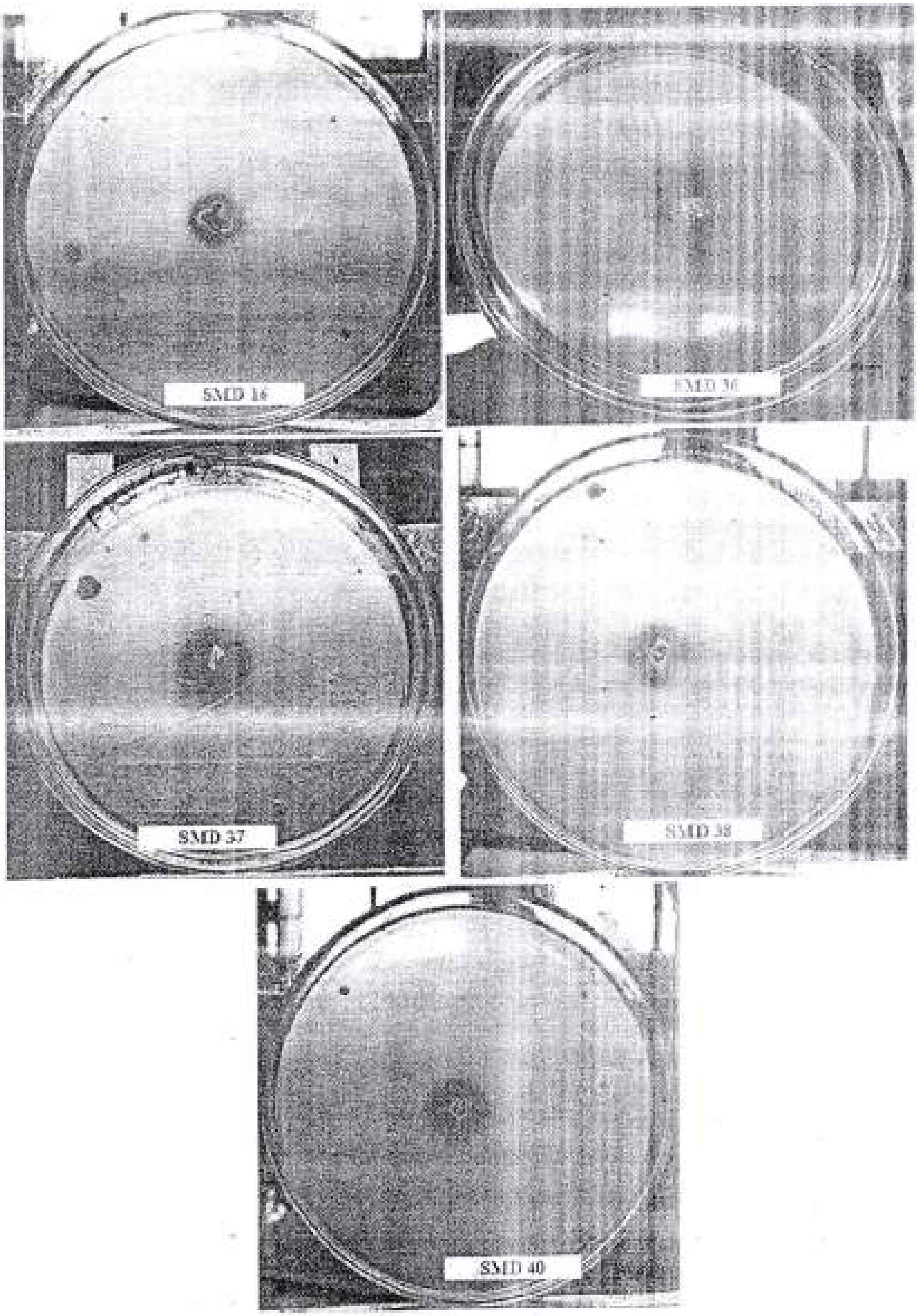

Photo Plate 3.1:Phosphate Solubilization Index of Selected Rhizospheric Isolates(Qualitative Estimation)

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