The Bioscan 9(1): 143-150, 2014
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

OPTIMIZATION OF BEST CULTURAL CONDITIONS FOR HIGH PRODUCTION OF PHOSPHATE SOLUBILIZING ACTIVITY BY FLUORESCENT *PSEUDOMONAS* ISOLATED FROM NORMAL AND REPLANT SITES OF APPLE AND PEAR

DEEPSHIKHA THAKUR*, MOHINDER KAUR AND VINEET SHYAM
Department of Basic Science,
Dr. Y. S Parmar University of Horticulture and Forestry, Nauni, Solan - 173 230 (H. P), INDIA
e-mail: deepshikhathakur86@gmail.com

KEYWORDS
Optimization
Cultural conditions
Fluorescent
pseudomonas
Phosphorous

Received on :
12.09.2013

Accepted on :
21.01.2014

***Corresponding author**

ABSTRACT
Bacteria belonging to *Pseudomonas* genera are known to be efficient phosphate solubilizers. As the physiological and nutritional requirement of an organism is genetically predetermined, it is important to provide the appropriate nutrient sources and proper environment for optimal production of activity. So, in our study optimization and standardization of the cultural conditions for the optimum production of phosphate solubilizing activity has been done. From the results it is concluded that out of four media i.e. PVK, NBRI-P, NBRI-Y and Kings, the maximum average of P-solubilizing activity by fluorescent *Pseudomonas* strains isolated from the normal and replant sites of apple and pear was observed in NBRI-P at 48 h, with the maximum release of available phosphate i.e. 606 μg/ml. Maximum average growth along with maximum P-solubilizing activity of all the isolates was obtained at 28 ^{o}C and pH 7, with the maximum release of available phosphate i.e. 845 and 788 μg/ml respectively. The optimum concentration of TCP for maximum P-solubilizing activity is 5 g/l in NBRI-P medium with the maximum release of available phosphate i.e. 756 μg/ml.

INTRODUCTION

Cultural conditions play an important role in cellular growth and also in production of biological activities by microorganisms (Kotake *et al*., 1992). Understanding which environmental factors are important and how these influences the production of secondary metabolic activities is important. Jha *et al*., (1992) found that biological activity and composition of soil microbes are generally affected by many factors including physico-chemical properties of the soil, temperature and vegetation. Microorganisms require carbon, nitrogen, phosphorus, sulphur and other growth factors. They are sensitive to temperature, pH, oxygen/carbon dioxide in their environment (Jenning, 1995). The challenge is faced to provide the organisms with conditions that allow expression of secondary metabolites and accumulations of unusual metabolites (Bushell, 1989 and Demain, 1992). A number of media generally employed for expression of secondary metabolism of microorganisms and initial evaluation of media are usually made. Selection of media is complex since the possible variations are so large. Simple media works very well as broth and agar and this has been validated many times with novel bioactive compounds being produced (Jenning, 1995). So, the development of media, which increase the production of bioactive compounds, is very important. By using this approach, chances of finding novel compounds increased and could be worth investigating for microorganism's secondary metabolism. Although a good growth may occur in many media but secondary metabolites may only be produced in a specific medium (Bentley and Keil, 1962). Sometimes a given organism may produce one metabolite on one medium and a totally different one on another medium (Oxford *et al*., 1935).

Phosphorus in soils is immobilized or becomes less soluble either by absorption, chemical precipitation or by both processes (Tilak *et al*, 2005). P solubilizing microorganisms brings about mobilization of insoluble phosphates in the soil and increase plant growth under conditions of poor phosphorus availability (Tripura, 2007). Bacteria belonging to *Pseudomonas* genera are known to be efficient phosphate solubilizers (Gulati *et al*., 2007). The production of P solubilizing activity has been found to be highly dependent on the cultural conditions. Each species or a strain has a characteristic minimum, optimum and maximum temperature. The optimal temperature for growth may not be that best suited to product formation especially where the product is predominantly non growth associated as in the case of many secondary metabolites (Woodruff, 1961). The conducted experiment was focused on the optimization of a suitable growth conditions for five selected *Pseudomonas* isolates i.e. An-1-Naga, An-3-Kho, Ar-1-kho, Pn-2-Kho and Pn-2-Panch that could induce higher levels of growth and phosphate solubilization.

MATERIALS AND METHODS

Effect of different media

Effect of best media on the production phosphate solubilizing activity was studied by growing *Pseudomonas* isolates in each media broth i.e. Nutrient agar, Kings B, National Botanical Research Institute-P (NBRI-P), National Botanical Research Institute-Y (NBRI-Y) (Nautiyal, 1999) and Pikovskaya's (PVK) (Pikovskaya's 1948). In each case 0.5 ml of inoculum of overnight grown (18 h) culture of bacteria was used to inoculate 100 ml of each media in a 250 ml Erlenmeyer flask. Flasks were incubated at 28 $\pm$ 2 °C under shake conditions (100 rpm) for 48 h. Cultures were centrifuged at 10,000 rpm for 20 min at 4 °C and supernatants were separated and stored at 4°C in small aliquots (5 ml). Phosphate solubilizing activity was assayed by well plate assay and spectrophotometric method. Activity was expressed in terms of mm diameter of pinkish/orange zone produced around the well by 100 μl of cell free culture supernatant on the media plate. Colorimetric estimation of phosphate solubilizing activity was done by using spectrophotometric assay at 660 nm. Growth was observed as absorbance at 540 nm. The cell free supernatant after production of P solubilizing activity was also analysed for pH. The medium that gave best results was used for further experiments.

Effect of incubation time

Effect of incubation time on the production of phosphate solubilizing activity was studied by growing the *Pseudomonas* isolates for different time intervals (24 h, 48 h, 72 h and 96 h). Phosphate solubilizing activity was assayed by well plate assay method. Plates were incubated at 282 ºC for 48 h and were observed for yellow zone produced around the well (7 mm) by 100 μl of cell free culture supernatant. Colorimetric estimation of phosphate solubilizing activity was done by using quantitative assay (Dickman and Bray's, 1940; Bray and Kurtz, 1945; and Olsen *et al*., 1954) at 660 nm. The supernatant was analysed for pH and growth as absorbance at 540 nm. The incubation time that gave best result was used for further experiments.

Effect of different incubation temperature

The effect of different temperature of incubation i.e. 4 °C, 28 °C, 37 °C and 50 °C on the P-solubilizing activities was studied by using the best-selected NBRI-P medium. Phosphate solubilizing activity was assayed by both well plate assay and liquid assay method as mentioned in the previous section. The cell free culture supernatant was also analysed for pH and growth at 540 nm. The incubation temperature that gave best result was used for further experiments.

Effect of pH

Effect of different pH i.e. 5, 6, 7 and 8 on the production of phosphate solubilizing activity was studied using the NBRI-P medium of different pH and all other parameters were kept constant i.e. temperature (28 °C), incubation time (48 h), shake condition (100 rpm). Phosphate solubilizing activity was assayed by well plate assay method and liquid assay method as mentioned in the previous section. The cell free supernatant was also analysed for pH and growth at 540 nm. The incubation pH that gave best result was optimized and used for further experiments.

Selection of optimum inorganic phosphate concentration of best media for the production of phosphate solubilizing activity

Effect of different inorganic phosphate concentrations on the P-solubilizing activity was studied using the different concentrations of tricalcium phosphate in the NBRI-P medium. Phosphate solubilizing activity was assayed by well plate assay method and liquid assay method as mentioned in the previous sections. The cell free culture supernatant was also analysed for pH and growth at 540 nm. The concentration of TCP that gave best result was used for mass production.

RESULTS

Effect of growth media at different time intervals

The effect of different media on P-solubilizing activity produced by *Pseudomonas* sp. at 24, 48, 72 and 96 h (Table 1, 2, 3 and 4) revealed that the maximum average P-solubilizing activity in quantitative measures was observed in NBRI-P. The maximum P-solubilization in terms of qualitative measures was observed in PVK medium by An-1-Naga (40 mm) at all the intervals followed by NBRI-P media. The maximum release of available phosphate at 24 h was observed by An-1-naga in NBRI-P media i.e. 606 μg/mL; at 48 h in NBRI-P media by Pn-2-Panch i.e. 899 μg/mL; at 72 h by An-1-Naga i.e. 878 μg/mL in NBRI-Y media; at 96 h by Pn-2-Panch i.e. 1178 μg/mL in NBRI-P media. The minimum P-solubilization was observed in King's media but it came out to be the best media for maximum growth in terms of optical density. All the *Pseudomonas* isolates were statistically different from each other. The interaction study revealed that as the incubation period increased gradually the cell density also increased.

From the results it could be observed that the efficiency of a medium for supporting P-solubilizing activity production in terms of mm diameter was of following order: PVK > NBRI-P > NBRI-Y > Kings, whereas in terms of available Phosphorus (Pi) the order was found out to be NBRI-P > NBRI-Y > PVK > Kings. Thus, out of four media, the maximum average of P-solubilizing activity was observed in NBRI-P media at 48 h incubation time. The interaction study revealed that the difference in activity was significant. Thus, on the basis of results obtained, NBRI-P media and 48 h incubation was used for further studies.

Selection of optimum incubation temperature for the production of phosphate solubilizing activity

The maximum average phosphate solubilizing activity along with maximum average growth was recorded at 28 ºC (Table 5) for all the *pseudomonas* isolates. Maximum production of phosphate solubilizing activity in qualitative measures was observed in Pn-2-kho (20 mm) at 28 ºC and maximum release of available phosphate was observed in An-1-naga (845 μg/ml) at 28 ºC. There was a corresponding decrease in the pH of the culture medium. Thus, the optimum incubation temperature of 28 ºC was used for further experiments. Almost

Table 1: Effect of different media and incubation time on the production of phosphate solubilizing activity by selected isolates at 24 h

Medium	Pseudomonas*Isolates*	*pH*[4]	*Growth*[1] A_{540}	*Phosphate solubilizing activity* Plate assay yellow zone (mm dia)[2]	Liquid assay concentration (μg/mL)
PVK	AN-1-Naga	6.0	0.44	40	489
	AN-3-Kho	6.5	0.46	39	581
	PN-2-Panch	6.4	0.56	35	442
	PN-2-Kho	7.2	0.65	39	446
	Ar-1-Kho	7.0	0.66	36	378
NBRI-P	AN-1-Naga	6.8	0.45	39	606
	AN-3-Kho	6.7	0.34	39	410
	PN-2-Panch	6.6	0.56	17	167
	PN-2-Kho	6.4	0.44	16	133
	Ar-1-Kho	6.0	0.35	18	399
NBRI-Y	AN-1-Naga	6.7	0.35	20	585
	AN-3-Kho	6.4	0.34	17	292
	PN-2-Panch	6.8	0.58	19	253
	PN-2-Kho	6.7	0.54	21	339
	Ar-1-Kho	6.5	0.65	16	357
King's	AN-1-Naga	6.6	0.54	-	521
	AN-3-Kho	7.2	0.59	-	374
	PN-2-Panch	6.8	0.62	-	364
	PN-2-Kho	6.3	0.82	-	332
	Ar-1-Kho	7.5	0.32		185
	$CD_{0.05}$ (T)	0.10	0.01	0.63	1.06
	$CD_{0.05}$ (I)	0.11	0.01	0.71	1.18
	$CD_{0.05}$ (T´I)	0.22	0.02	1.41	2.37

Table 2: Effect of different media and incubation time on the production of phosphate solubilizing activity by selected isolates at 48 h

Medium	*Pseudomonas*Isolates	pH[4]	Growth[1] A_{540}	Phosphate solubilizing activity Plate assay yellow zone (mm dia)[2]	Liquid assay concentration (μg/mL)
PVK	AN-1-Naga	6.0	0.76	40	528
	AN-3-Kho	5.9	0.77	40	771
	PN-2-Panch	5.8	0.76	37	603
	PN-2-Kho	5.8	0.65	39	489
	Ar-1-Kho	5.4	0.72	37	456
NBRI-P	AN-1-Naga	6.2	0.53	20	788
	AN-3-Kho	5.9	0.45	18	710
	PN-2-Panch	6.0	0.56	18	899
	PN-2-Kho	6.0	0.65	19	706
	Ar-1-Kho	6.1	0.68	19	703
NBRI-Y	AN-1-Naga	6.3	0.54	21	856
	AN-3-Kho	5.9	0.56	23	721
	PN-2-Panch	5.7	0.60	22	428
	PN-2-Kho	5.5	0.59	21	881
	Ar-1-Kho	6.0	0.66	19	492
King's	AN-1-Naga	6.2	0.66	-	535
	AN-3-Kho	5.8	0.67	-	365
	PN-2-Panch	5.9	0.70	-	539
	PN-2-Kho	5.7	0.88	-	492
	Ar-1-Kho	5.2	0.45	-	381
	$CD_{0.05}$ (T)	0.08	0.01	0.70	1.13
	$CD_{0.05}$ (I)	0.09	0.01	0.78	1.26
	$CD_{0.05}$ (T´I)	0.18	0.02	1.5	2.53

*P-solubilizing activity expressed in terms of mm diameter of pinkish/yellow zone around the well on different media at 28ºC at 24 h

**Phosphate solubilizing activity expressed in terms of tricalcium phosphate solubilization, which in turn represents μg/ml of available orthophosphate as calibrated from the standard curve of KH_2PO_4 (0-10 μg/mL)

all the *pseudomonas* isolates were statistically significant.

Selection of pH of best media for the production of phosphate solubilizing activity

The optimum pH of the medium for the production of phosphate solubilizing activity by *pseudomonas* isolates was determined by using NBRI-P media of different pH ranging from 5 to 8 at 28 ºC. The results (Table 6) showed that the optimum pH for the production of phosphate solubilizing activity was pH 7. The maximum average phosphate solubilizing activity in terms of mm diameter was observed in Pn-2-panch (24 mm) at pH 7 and maximum release of available phosphate was observed in An-1-naga (788 μg/ml) at pH 7. The results revealed that the average growth obtained was maximum at pH 7. Almost all the *pseudomonas* isolates were statistically significant. Results depicted that almost all

Table 3: Effect of different media and incubation time on the production of phosphate solubilizing activity by selected isolates at 72 h

Medium	*Pseudomonas*Isolates	pH[4]	Growth[1]A_{540}	Phosphate solubilizing activity Plate assay yellow zone (mm dia)[2]	Liquid assay Concentration (μg/mL)
PVK	AN-1-Naga	4.9	0.78	40	535
	AN-3-Kho	5.0	0.79	39	556
	PN-2-Panch	5.4	0.66	36	546
	PN-2-Kho	5.0	0.72	35	378
	Ar-1-Kho	5.9	0.73	38	371
NBRI-P	AN-1-Naga	5.0	0.67	20	556
	AN-3-Kho	4.9	0.78	21	385
	PN-2-Panch	4.8	0.77	18	856
	PN-2-Kho	5.6	0.63	19	714
	Ar-1-Kho	5.8	0.69	19	371
NBRI-Y	AN-1-Naga	5.0	0.67	20	878
	AN-3-Kho	4.9	0.76	19	378
	PN-2-Panch	5.1	0.71	22	481
	PN-2-Kho	5.5	0.66	21	580
	Ar-1-Kho	6.0	0.68	19	510
King's	AN-1-Naga	3.8	0.77	-	528
	AN-3-Kho	4.2	0.79	-	456
	PN-2-Panch	4.0	0.80	-	553
	PN-2-Kho	3.5	0.88	-	510
	Ar-1-Kho	4.3	0.56	-	396
	$CD_{0.05}$ (T)	0.37	0.01	0.75	1.09
	$CD_{0.05}$ (I)	0.42	0.01	0.84	1.21
	$CD_{0.05}$ (T´I)	0.82	0.02	1.67	2.42

Table 4: Effect of different media and incubation time on production of phosphate solubilizing activity by selected isolates at 96 hrs

Medium	*Pseudomonas*Isolates	pH[4]	Growth[1]A_{540}	Phosphate solubilizing activity Plate assay yellow zone (mm dia)[2]	Liquid assay concentration (μg/mL)
PVK	AN-1-Naga	4.3	0.76	40	394
	AN-3-Kho	5.0	0.86	35	531
	PN-2-Panch	4.9	0.80	37	357
	PN-2-Kho	4.8	0.82	32	553
	Ar-1-Kho	4.6	0.78	37	524
NBRI-P	AN-1-Naga	5.1	0.76	18	531
	AN-3-Kho	4.7	0.79	19	378
	PN-2-Panch	4.3	0.80	17	1178
	PN-2-Kho	4.2	0.81	18	753
	Ar-1-Kho	4.0	0.79	16	210
NBRI-Y	AN-1-Naga	4.3	0.80	19	446
	AN-3-Kho	4.0	0.81	18	535
	PN-2-Panch	5.0	0.79	22	296
	PN-2-Kho	5.1	0.78	20	531
	Ar-1-Kho	5.5	0.85	17	285
King's	AN-1-Naga	4.0	0.88	-	296
	AN-3-Kho	4.0	0.78		274
	PN-2-Panch	3.9	0.83		399
	PN-2-Kho	3.6	0.84		456
	Ar-1-Kho	3.9	0.80		60
	$CD_{0.05}$ (T)	0.24	0.01	0.54	0.87
	$CD_{0.05}$ (I)	0.27	0.01	0.61	0.98
	$CD_{0.05}$ (T´I)	0.54	0.02	1.21	1.81

[1]Growth in terms of optical density at 540nm on the NBRI-P media,
[2]P-Solubilizing activity expressed in terms of mm diameter of pinkish/yellow zone around the well on different media at 28ºC for 48h.
[3]Phosphate solubilizing activity expressed in terms of tricalcium phosphate solubilization, which in turn represents μg/ml of available orthophosphate as calibrated from the standard curve of KH_2PO_4 (0-10 μg/mL).
[4]pH of the culture medium at the end of experiment

pseudomonas isolates differ statistically and significantly.

Selection of optimum tricalcium phosphate concentration of best media for the production of phosphate solubilizing activity

The optimum tricalcium phosphate (TCP) concentration of best media for the production of average growth and phosphate solubilizing activity was determined by using different concentrations of TCP i.e. 1, 3, 5 and 7 g. The results (Table 7) showed that the optimum concentration of TCP for the production of phosphate solubilizing activity is 5 g. Maximum average P-solubilizing activity in terms of mm diameter was shown by An-3-kho and Pn-2-kho (18 mm) and maximum release of available phosphate was observed in An-3-kho (756

Table 5: Effect of temperature on the growth and solubilization of Tricalcium Phosphate by selected fluorescent *Pseudomonas sp.* from rhizosphere of apple and pear in NBRI-P medium at different temperatures for 48 h under shake conditions (90 rpm)

Pseudomonas isolates	Growth and solubilization of Phosphate																			
	A_{540}[1]					Zone (mm dia)[2]					Pi (μg/mL)[3]					pH[4]				
	Temperature (°C)				Mean	Temperature (°C)				Mean	Temperature (°C)				Mean	Temperature (°C)				Mean
	4	28	37	50		4	28	37	50		4	28	37	50		4	28	37	50	
AN-1-Naga	0	0.65	0.45	0	0.27	0	16	14	0	7.50	35	845	321	157	339.50	7.0	6.0	6.4	7.0	6.60
AN-3-Kho	0.32	0.60	0.43	0.14	0.37	14	14	12	0	10.00	1.5	285	498	66	212.60	6.5	6.3	6.5	6.9	6.55
PN-2-Panch	0	0.54	0.56	0.19	0.32	0	18	14	0	8.00	107	578	290	139	278.50	6.9	6.2	6.2	6.9	6.55
PN-2-Kho	0.40	0.64	0.50	0.01	1.82	14	20	14	10	14.50	144	745	123	48	265.00	6.2	5.9	6.2	7.0	6.32
Ar-1-Kho	0	0.57	0.42	0	0.24	0	18	0	0	4.50	74	371	212	121	194.30	6.9	6.0	6.9	7.0	6.70
Mean	0.14	1.75	0.47	.068		5.6	17.20	10.80	2.00		72.30	564.80	288.80	106.10		6.70	6.08	6.44	6.96	
$CD_{0.05}$	T				0.02					1.01					1.11					0.083
	I				0.01					0.90					0.99					0.073
	TxI				0.04					2.02					2.22					0.163

[1]Growth in terms of optical density at 540nm on the NBRI-P media.
[2]P-Solubilizing activity expressed in terms of mm diameter of pinkish/yellow zone around the well on different media at 28°C for 48h
[3]Phosphate solubilizing activity expressed in terms of tricalcium phosphate solubilization which in turn represents μg/ml of available orthophosphate as calibrated from the standard curve of KH_2PO_4 (0-10 μg/mL).
[4] pH of the culture medium at the end of experiment

Table 6: Effect of pH on the growth and solubilization of Tricalcium Phosphate by selected fluorescent *Pseudomonas sp.* from rhizosphere of apple and pear in NBRI-P medium at 28°C for 48 h under shake conditions (90 rpm)

Pseudomonas isolates	Growth and solubilization of Phosphate																			
	A_{540}[1]					Zone (mm dia)[2]					Pi (μg/mL)[3]					pH[4]				
	pH				Mean	pH)				Mean	pH				Mean	pH				Mean
	5	6	7	8		5	6	7	8		5	6	7	8		5	6	7	8	
AN-1-Naga	0.04	0.15	0.60	0.42	0.32	15	18	19	20	18.00	112	514	788	185	399.70	6.9	6.5	6.0	6.7	6.52
AN-3-Kho	0.05	0.21	0.62	0.54	0.35	12	16	20	16	16.00	174	355	556	174	314.70	6.8	6.2	6.2	6.2	6.35
PN-2-Panch	0.02	0.19	0.59	0.54	0.33	14	18	24	14	17.50	128	364	720	367	394.70	7.0	6.1	6.1	6.5	6.59
PN-2-Kho	0.06	0.22	0.67	0.43	0.34	14	16	22	15	16.75	185	367	555	292	349.70	6.1	5.9	5.9	6.8	6.25
Ar-1-Kho	0.01	0.15	0.58	0.50	0.31	12	15	18	16	15.25	149	307	385	217	264.50	7.0	6.6	6.2	6.0	6.45
Mean	0.036	0.18	0.61	0.48		13.40	16.60	20.60	16.20		149.60	381.40	600.80	247.00		6.82	6.32	6.14	6.44	
$CD_{0.05}$	T				0.010					1.41					1.34					0.174
	I				0.009					1.26					1.20					0.155
	TxI				0. 200					2.83					1.87					0.347

[1]Growth in terms of optical density at 540nm on the NBRI-P media.
[2] P-Solubilizing activity expressed in terms of mm diameter of pinkish/yellow zone around the well on different media at 28°C for 48h
[3]Phosphate solubilizing activity expressed in terms of tricalcium phosphate solubilization which in turn represents μg/mL of available orthophosphate as calibrated from the standard curve of KH_2PO_4 (0-10 μg/mL).
[4] pH of the culture medium at the end of experiment

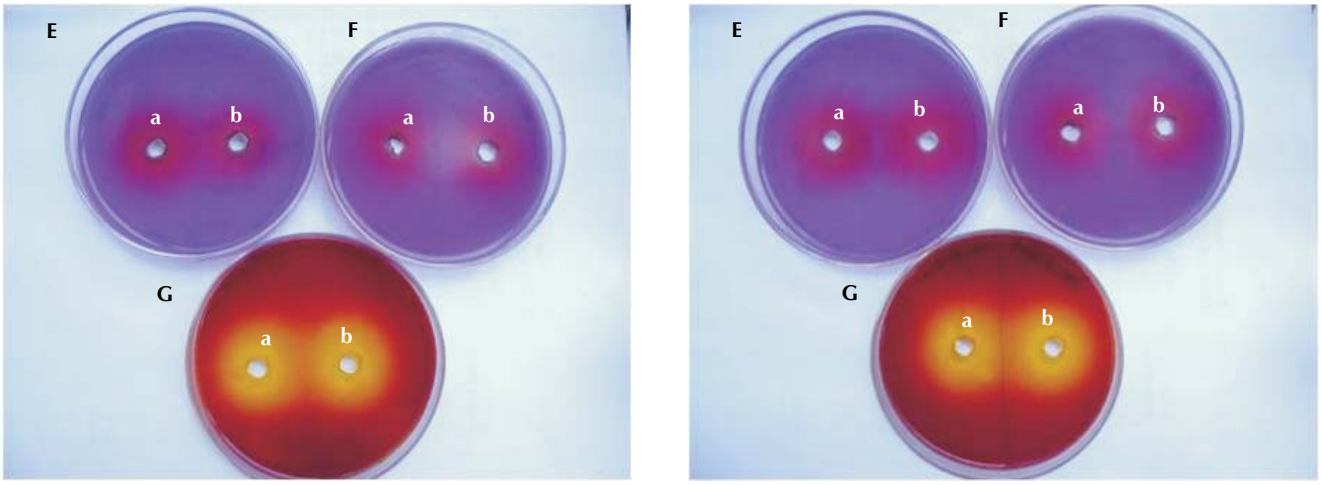

Fig1: Effect of different media on the production of phosphate solubilizing activity by fluorescent *Pseudomonas* isolates An-1-Naga a), An-3 -Kho b) Pn 2 Panch c) Pn 2 Kho d) on three different media NBRI-P (E) NBRI-Y (F) Pikovskaya's (G)

*μ*g/ml) at TCP concentration of 5g of the medium. The average growth obtained was also maximum at medium supplemented with TCP concentration 5 g/l. There was a corresponding decrease in the pH of the culture medium. So, the 5 g TCP concentration of the medium is considered best for growth and production of phosphate solubilizing activity. Results depicted that almost all *pseudomonas* isolates differ statistically and significantly.

DISCUSSION

In the present study, out of four medium, i.e. Pikovskaya's, NBRI-P, NBRI-Y, and Kings media, the best media for the production of P solubilizing activity was found out to be (NBRI-P), which is supported by Nautiyal (1999), who conducted the experiment with three medium PVK, NBRI-Y and NBRI-P. NBRI-P media has been used by Panhwar *et al*. (2012) for the isolation of P solubilizing bacteria from aerobic rice, who also concluded that highest P solubilizing activity was found in NBRI-P media. Lynn *et al*., (2013) also used NBRI-P media for characterization of P solubilizing strains from tomato.

All microbial activity is sensitive to environmental temperature. Each species or a strain has a characteristic minimum, optimum and maximum temperature. The optimal temperature for growth may not be that best suited to product formation especially where the product is predominantly non growth associated as in the case of many secondary metabolites (Woodruff, 1961). So, we have studied the effect of varying temperature i.e. 4 ºC, 28 ºC, 37 ºC and 50 ºC on the growth and production of P-solubilizing activity at 48 h. The optimum incubation time for the growth and P-solubilizing activity was found to be 48 h which is at par with 72 hours incubation time. Jena (2013) also concluded the incubation time of 72 h best for the production of maximum P-solubilizing activity of the *Pseudomonas* isolates.

Each microorganism has its own specific minimum, optimum and maximum temperature. P-solubilizing activity production has been found to be dependent on the temperature. In our study, the maximum P solubilization activity was produced at 28 ºC by *Pseudomonas* isolates. However, decline in yield of these activities was observed above and below 28 ºC. This behavior of all the *Pseudomonas* isolates is similar to usual response of mesophilic organisms where metabolic activities get slow down below and above the optimum temperature. This suggests that organisms are mesophilic in nature. Our results collaborates with (Mishra *et al*., 2009) who reported that *Pseudomonas lurida* grew at temperatures ranging from 4 to 30 °C, with a growth optimum at 28 °C. Also Behrendt *et al*., (2007) reported that for *Pseudomonas lurida*, the optimal growth temperature is 21 °C. Dileep Kumar (1998) has also reported optimum temperature of *Pseudomonas* at 25-30 ºC. Whereas, Illmer and Schinner (1992) has reported 30 ºC as optimum solubilization temperature for *Pseudomonas* sp.

The extracellular pH has a strong influence on the pathways of metabolism and product generation by microorganism. The optimum pH for growth rate may be different from that for growth yield and entirely different from the optimum for product formation. In our study, pH 7 is found to be best for the growth as well as P solubilizing activity which is also in collaboration with Yadav, (2013) who concluded pH 7.5 and Jena, (2013) who concluded pH 7.0 as optimum for the P solubilizing activity of the *Pseudomonas* isolates.

In the present study, fall in pH in liquid culture accompanied phosphate solubilization is noted which may be due to the production of organic acids. But no correlation could be established between acidic pH and quantity of inorganic phosphorus liberated. Similar observations were also observed by other workers (Pallavi and Gupta, 2013; Goenadi *et al*., 2000; Kundu and Gera, 2002), who could not correlate the quantity of P- solubilized and the decreased pH of the medium. Probably this may be due to the reason that solubilization depends not only on the pH and acid concentration but also on the structure and type of organic molecule (Johnston, 1952). In a study by Fankem *et al*., (2006) there was a decrease in pH which was not strictly proportional to the amount of P solubilized. In our study, the pH was found to decline from 7.00 (control) to minimum 5.9. In another study by Pandey *et al*., (2006) the pH of the broth was found to decline. from 6.00

(control) to 4.11, 3.91, 3.73 and 3.81 at temperatures 4, 9, 21 and 28 °C respectively.

The results in indicated that out of four tricalcium phosphate concentrations i.e. 1, 3, 5, 7 tested, the tricalcium phosphate concentration of 5 g/litre of the NBRI-P medium was found to be the optimum concentration for production of phosphate solubilizing activity of the *Pseudomonas* isolate. The same amount of TCP was also used by other workers in their studies (Lynn *et al*, 2013; Sundara Rao and Sinha, 1962; Dave and Patel, 1999 and Kundu *et al*., 2002). Jayadi, *et al*., (2013) substituted the TCP in the NBRIP with rock phosphate. It has been reported that rock phosphates, aluminium phosphates and iron phosphates are less solubilized as compared to tricalcium phosphate (Banik and Dey, 1982). Similar results were obtained by Kundu and Gera, (2002) and Dave and Patel (1999) while comparing the solubilization of various insoluble inorganic phosphates by *Pseudomonas* isolates. They observed that the trend of solubilization of different P sources is as follows: Bone meal > TCP >DCP > Iron phosphate >Senegal rock phosphate > aluminium phosphate.

REFERENCES

Banik S. and Dey B. K. **1982**. Available phosphate content of an alluvial soil as influenced by the inoculation of some isolated phosphate slolubilizing microorganisms. *Plant and Soil*. **69:** 353-364.

Behrendt, U., Ulrich A., Schumann, P., Meyer G. M. and Sproer, C**.** 2007.***Pseudomonas lurida* sp. nov, a fluorescent sp. associated with the phyllosphere of grasses. *International Journal Syst. Evol. Microbiol*.** 57: **979-985.**

Bentley, R. and Keil, J. G. **1962.** *J. Biol. Chem*. pp. 237-867.

Bray, R. H. and Kurtz, L. T. **1945**. Determination of total organic available forms of phosphorus in soil. *Soil Science.* **23:** 343-353.

Bushell ,M. E. **1989**. The process physiology of secondary metabolite production. In: microbial products: new approaches. (Eds. Baunberg, L. S Hunter and P. M Rodes) Cambridge University Press, Cambridge.

Dave, Arti and Patel 1999. Inorganic phosphate solubilizing soil *Pseudomonas*. *Indian Journal of Microbiology*. **39:** 161-164.

Demain, A. L. **1992**. Regulation of secondary metabolism. In: biotechnology of filamentous fungi: technology and products (Eds. D. B. Finklestein and C. Ball). Butterworth- Heinemann, Boston.

Dickman, S. R. and Bray, R. H. **1940**. Colorimetric determination of phosphate. Indus and Engineering chemical analytical edition **12:** 660-665.

Dileep Kumar, B. S. **1998**. Disease suppression and crop improvement through fluorescent *Pseudomonas* isolated from cultivated soil. *World Journal of Microbiology and Biotechnology*. **14:** 375-471.

Fankem, H., Nwaga, D., Deubel, A., Dieng, L., Merbach, W. and Etoa, F. X. **2006.** Occurence and functioning of phosphate solubilizing microorganisms from oil palm tree rhizosphere in cameroon. *African Journal of Biotechnology*. **5(24):** 2450-2460.

Goenadi, D. H., Siswanto and Sugiarto, Y. 2000. **Bioactivation of poorly soluble phosphate rocks with phosphorus solubilizing fungus. *Soil Science Society of America Journal.*** 64: **927-932.**

Gulati, A., Rahi, P. and Vyas P. 2007. Characterization of phosphate solubilization *fluorescent pseudomonas* from the rhizosphere of seabuckthorn growing in the cold desert of Himalayas. *Current Microbiology*. **56:** 73-79.

Illmer, P. and Schinner, F. 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soil. *Soil Biology and Biochemistry*. **27(3):** 257-263.

Jena, S. K. and Chandi, C. R. 2013. Optimization of culture conditions of phosphate solubilizing activity of bacterial sp. isolated from Similipal biosphere reserve in solid-state cultivation by response surface methodology. *International Journal of Current Microbiolology and Applied Sciences*. **2(5):** 47-59.

Jayadi, M., Baharuddin and Ibrahim, B. 2013. In vitro selection of rock phosphate solubility by microorganism from Ultisols in South Sulawesi. *Indonesia American Journal of Agriculture and Forestry*. **1(4):** 68-73.

Jennings, D. H. 1995. The physiology of fungal nutrition. Cambridge Univ. Press, Cambridge.

Jha, D. K., Sharma, G. D. and Mishara, R. R. 1992. Ecology of soil microflora and mycorrhizal symbionts. *Biological Fertility of Soils*. **12:** 272-278.

Johnston, H. W. 1952. The solubilization of phosphate I. The action of various organic compounds on dicalcium and tricalcium phosphates. *MZJ Sci. Technol*. **23:** 436-446.

Kotake, C., Yamaski, T., Moriyama, T., Shinoda, M., Komiyana, N. and Furumai, T. 1992. *Journal of Antibiotic*. **45:** 1442-1450.

Kundu, B. S and Gera, R. 2002. Host specificity of phosphate solubilizing bacteria. *Indian Journal of Microbiology*. **42:** 19-21.

Lynn, T. M., Win, H. S., Kyaw, E. P., Latt, Z. K., and Yu, S. S. 2013. Characterization of phosphate solubilizing and potassium decomposing strains and study on their effects on tomato cultivation. *International Journal of Innovation and Applied Studies*. **3(4):** 959-966.

Mishra, P. K., Mishra, S., Bisht, S. C., Selvakumar, G., Kundu, S., Bisht, J. K. and Gupta, H. S. 2009. Isolation molecular characterization and growth promotiona activities of cold tolerant bacterium *Pseudomonas* sp. NARs9 (MTCC9002) from the Indian Himalayas. *Biol. Res*. **44:** 305-313.

Nautiyal, C. S. 1999. An efficient microbiological growth medium for screening phosphate solublizing microorganisms. *FEMS Microbiology Letters*. **170:** 265-270.

Olsen, S. R., Cole, C. V., Whatanable, F. S. and Dean, L. A. 1954. Estimation of available phosphorus by extraction with sodium bicarbonate. US Department of Agriculture. p. 939.

Oxford, A. E., Raistrid, H. and Simorart, P. **1935**. Studies on the biochemistry of microorganisms: Fulvic acid, a new yellow crystalline pigment, a metabolic product of *P. griseo-fulvum* Dierckx, *P. flexuosum* Dale and *P. brefeldianum* Dodge. *Biochem. J.* **29(5):** 1102-1115.

Pallavi, K. P. and Gupta, P. C. 2013. Effect of different carbon and nitrogen sources on solubilization of insoluble inorganic phosphate by psychrotolerant bacterial strains. *The Bioscan*. **8(4):** 1299-1302.

Pandey, A., Trivedi, P., Kumar, B. and Palni, L. M. 2006. Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas Putida* (BO) isolated from a subalpine location in the Indian Central Himalaya. *Current Microbiology*. **53(2):** 102-107.

Panhwarl, Q. A., Othman, R., Rahman, Z. A., Meon, S. and Ismail, M. R. 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. *African Journal of Biotechnology*. **11(11):** 2711-2719.

Pikovsakaya, R. E. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologia*. **17:** 362-370.

Sundara Rao, W. V. P. and Sinha, M. K. 1962. Phosphate dissolving microorganisms in the soil and rhizosphere. *Indian Journal of Agricultural Science*. **33(4):** 272-278.

Tilak, K. V., Ranganayaki, K. K., Pal, K. K., De, R., Saxena, A. K., Nautiyal, C. S., Mittal, S., Tripathi, A. K. and Johri, B. N. 2005.

Diversity of plant growth and soil health supporting bacteria. *Current Science*. **89(1):** 136-150.

Tripura, C., Sashidhar, B. and Podile, A. R. 2007. Ethyl methanesulfonate mutagenesis enhanced mineral phosphate solubiization by groundnut-associated *Serratia marscenes* GPS-5. *Current Microbiology*. **54:** 79-84.

Woodruff, H. B. 1961. Antibiotic production as an expression of environment. In: microbial reaction to environment (Eds. G. G. Meynell and H. Gooder). Cambridge University Press. pp. 317-342.

Yadav, H., Gothwal, R. K., Nigam, V. K. and Roy, S. 2013. Optimization of culture conditions for phosphate solubilization by a thermo-tolerant phosphate-solubilizing bacterium *Brevibacillus* sp. BISR-HY65 isolated from phosphate mines. *Biocatalysis and Agricultural Biotechnology*. **2(3):** 217-225.