Eco. Env. & Cons. 30 (February Suppl. Issue) : 2024; pp. (S168-S170)

ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i02s.033

Production of Phyto hormone from Phosphate solubilising soil isolates

Abhay Ghatage*, Pallavi Tandulwadkar, Swati Yadav and G.R. Pathade

Krishna Institute of Allied Sciences, Krishna Institute of Medical Science Deemed To Be University, Karad 415539, M.S., India

(Received 18 June, 2023; Accepted 21 August, 2023)

ABSTRACT

The transformation of insoluble phosphorous into soluble form is carried out by a number of microbes present in the soil. Phosphorous is essential for growth and productivity of plants in terms of cell division, photosynthesis and development of good root system and utilization of carbohydrates. Phosphorous deficiency results in the leaves turning brown accompanied by small leaves, weak stem and slow development. The large fraction of soil microbes can dissolve insoluble phosphorous in the soil and make them available to plants for its growth and development. In the present work we isolated Phosphate solubilising bacteria from rhizospheric soil from Betel Wine plant and promising strain were subjected for phytohormone (IAA) production.

Key words: *Phosphate, IAA, PGPR*

Introduction

A large fraction of soil microbes can dissolve insoluble inorganic phosphates present in the soil & make them available to the plants (Fankem *et al*., 2003). Assimilation of phosphate from organic compounds by plants and microorganisms take place through the enzyme "phosphate" which is present in a wide variety of soil microorganisms. Plants can absorb phosphate only in soluble form is carried out by a number of microbes present in the soil. Rhizospheric phosphate solubilising bacteria are capable of solubilising insoluble phosphates into soluble organic forms (Kundu *et al*., 2009). Plant Growth Promoting Rhizobacteria (PGPRB) is considered to promote plant growth directly or indirectly. PGPRB can exhibit a variety of characteristics responsible for influencing plant growth (Rodriguez *et al*., 1999). The common traits include production of plant growth regulators like auxins, gibberellins, n ethylene etc. Indole acetic acid is one of the most physiologically active auxins. Plant morphogenic effects may also be a result of different ratios of plant hormones produced by roots as well as by rhizosphere bacteria.

Materials and Methods

Media and cultural conditions

For isolation rhizospheric soil was collected from Betek Wine plant Vadgaon-haveli, satara, Maharashtra. Soil samples were collected from the selected sites at a depth of 15 cm from 3 different points within area. The Pikovaskayas agar medium (PVK) and temperature 27-30 ^{0}C was found to be as selective for the isolation of phosphate solubilising bacteria. For isolation serial dilution and spread method was used. Morphological and biochemical characterization was carried out.

Estimation of phosphorus solubilization

The phosphate solubilization activity was carried out as per method of (Fankem *et al*., 2006)

The phosphorus solubilization on solid agar medium was measured in terms of solubilization efficiency (SE): (%) = (Z-C)/ C×100

Where, Z is solubilization zone

C is colony diameter.

Estimation of phytohormone IAA produced by PSB

Bio-assay for IAA

IAA was determined in vitro all the test strains were screened for IAA production. Briefly, test bacterial culture was inoculated in the Pikovaskayas broth with tryptophan (0.1 g/l) or without tryptophan incubated at30^{0}C. Cultures were centrifuged at 3000 rpm for 30 min. Two milliters of the supernatant was mixed 2 drops of orthophosphoric acid and 4 ml of reagent Salkowaski (50 ml 35% perchloric acid; 1 ml 0.5 $FeCl_3$).

Results and Discussion

Results of cultural and morphological characteristics

Isolates were studied for their identification, colony characterization.

The colony characteristics of the isolate are as per table

The biochemical characteristics

The biochemical characteristics of isolates were as shown in following Table 3.

Ten isolates were designated as PSB1, PSB2, PSB3, PSB4, PSB5, PSB6, PSB7, PSB8, PSB9, PSB10. Amoungthese 1 isolates 3 were selected according to phosphate solubilization. From the above results of morphological & cultural characteristics with reference to Bergeys manual of systematic Bacteriology Volume-I and II, the organisms were identified as *Bacillus subtilis* (PSB1), *Pseudomonas aeruginosa* (PSB2), *Bacillus megaterium* (PSB3).

Table 3. Result for the biochemical characteristics of the isolate

Test	PSB 1	PSB 2	PSB 3
Starch hydrolysis	+	-	+
Gelatin hydrolysis	+	+	+
Urease production	+	-	+
Oxidase production	-	+	-
Catalase production	+	+	+
Glucose production	+	+	+
Sucrose production	+	+	+
Lactose production	-	-	+
Mannitol production	-	-	+
Indole production	+	-	+
Methyl red test	-	-	+
VogesProskauer	+	-	+
Citrate utilization	+	-	+

Table 4. Solubilization efficiency of the isolated organism

Organism name	Colony diameter	Zone diameter	SE (%)
Bacillus subtilis	3 mm	5 mm	66%
Pseudomonas aeruginosa	5 mm	8 mm	60%
Bacillus megaterium	4 mm	7 mm	75%

Phosphate solubilization efficiency

Calculations

Solubilization efficiency (SE): (%) = (Z-C)/C*100

Where, Z is solubilization zone

C is colony diameter

Results for estimation of IAA

The production of IAA was detected by the forma-

Table 1. Colony characteristics of the isolates

Isolate Name	Size	Shape	Colour	Margin	Opacity	Elevation	Consistency
PSB 1	2mm	Circular	White	Irregular	Opaque	Convex	Moist
PSB 2	3mm	Circular	yellowish	Irregular	Opaque	Flat	Moist
PSB 3	1mm	Circular	White	Irregular	Opaque	Flat	Moist

Table 2. Gram nature, motility and spore properties of the isolates

Isolate name	Gram nature	Motility	Endospore staining
PSB 1	Gram positive rods	Motile	Pink coloured spores
PSB 2	Gram negative rods	Motile	No spores

Table 5. IAA production by isolates

Amount of standard solution(µg/mL)	O.D at 540nm	IAA (µg/mL)
0	0.0	-
200	0.08	-
400	0.10	-
600	0.12	-
800	0.15	-
1000	0.19	-
1200	0.22	-
1400	0.25	-
1600	0.28	-
Isolate PSB 1	0.23	1200
Isoalte PSB 2	0.09	340
Isoalte PSB 3	0.16	870

tion of pink colour. All the isolates were capable of developing pink colour, which showed the production of IAA.

Conclusion

During the course of investigation three bacterial isolates capable of solubilising phosphates were obtained by serial dilution and spread plate technique using Pikovaskayas medium. These three isolates were designated as PSB 1, PSB 2& PSB 3. These isolates further characterized with respect to their morphological, cultural and staining properties and then screened for their phosphate solubilising potential and it was found that PSB 1 was identified as *Bacillus subtilis*, Isolate PSB 2 was identified as *Pseudomonas aeruginosa* and Isolate PSB 3 was identified as *Bacillus megaterium*. The isolates were investigated for their ability to produce IAA by Salkowski method and were found to produce IAA of different concentrations. The isolate PSB 1 *Bacillus subtilis* showed the highest production of IAA, i.e. 1200 µg/ml.

Acknowledgement

The Authors express their science thanks to Krishna Vishwa Vidyapeeth formerly known as Krishna Institute of Medical Sciences Deemed To Be University, Karad, India for providing facilities

Conflict of Interest

There is no conflict of interest between authors & co-authors.

References

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A. and Young, C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology*. 34(1): 33–41.

Dadhich, L.K. and Gupta, R.K. 2000. Effect of phosphate solubilizing bacteria and phosphorus on growth pattern of clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.]. *Annals Biol.* 17: 107–110.

Gupta, S.C. 2004. Response of gram (*Cicer arietinum*) to types and methods of microbial inoculation. *Indain J Agric Sci.* 74: 73–75.

Fankem, H., Nwaga, D. and Deubel, A. 2006. Occurance and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeisguineensis*) rhizosphere in Cameroon. *Afr J Biotech*. 5: 2450-2460.

Kundu, B.S. and Nehra, K.Yadav, 2009. Methods of studying soil microbial diversity. *J Miecrbiol.* 49: 120-127

Rasipour, L. and Aliasgharzadeh, N. 2007. Interactive effect of phosphate solubilizing bacteria and *Bradyrhizobium japonicum* on growth, nodule indices and some nutrient uptake of soybean. *J. Sci. and Technol. Agric. and Natur. Resour.* 11 40(A): 64

Rodriguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv.* 17: 319-339.