Isolation, screening and biochemical characterizations of Phosphate Solubilizing bacteria from Agricultural soil of Latur Region, Maharashtra

F.S.Biradar[1] and V.S. Shembekar[2]

[1]Research Scholar, Department of Biotechnology,COCSIT, Latur.

[2]Professor, Department of Zoology R.S. College Latur.

Abstract-

Phosphorous is an indispensible macro-element required for plant Nutrition. Phosphorous is found in soils both in an organic form and inorganic (minerals) form and its solubility in soil is low. Plants obtain P from soil solution as phosphate anion. Phosphate solubilizing Bacteria play vital role by elevating its availability to plants through release from organic and inorganic soil P by mineralization and solubilization.Primary mechanism in soil for mineral phosphate solubilization is lowering of soil PH by microbial production of organic acids and mineralization of organic P by acid phosphatases. Use of PSB as a bioinoculants enhances P uptake. In the present work, the efforts have made to isolate the phosphate solubilizing from agricultural soil followed by its biochemical characterization. In this study, isolation, screening and biochemical characterization of 50 isolates of phosphate solubilizing bacteria (PSB) from different soil samples of Latur region were carried out. Phosphate solubilizing activities of all isolates were tested on Pikovskaya Agar medium (PVK) by analyzing the clear zone formation. Further study has made to identify PSB using biochemical characterization. This article incorporates crucial information about phosphate solubilizing Bacteria (PSB) found in Agricultural soil of Latur region of Maharashtra.

Keywords: soil phosphorous, mineralization, soil solution, clearzone, phosphate solubilization.

Introduction:-

Phosphorous (P) is one of the major growth limiting nutrient which affect the overall growth of plants by influencing various key metabolic process viz photosynthesis and respiration of plants, cell division and development energy transfer, synthesis and breakdown of carbohydrates. [1,2,7,8, 13, 14, 18, 20].

The maximum amount of soil phosphorus approximately 95-99% is present in the form of insoluble phosphate and therefore cannot be utilized by plants. Phosphate solubilizing Bacteria (PSB) make it available to plants by solubilizing insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, rock phosphate and hydroxyl apatite. Bacteria are more effective in solubilization of phosphorus than fungi [13]. Among the whole microbial population in soil phosphate solubilization, fungi constitute only 0.1 to 0.5% whereas PSB constitute 1 to 50% in phosphate solubilization potential [4, 13].

Bacterial strains pseudomonas, Bacillus, Rhizobium and Enterobacter along with penicilium and Aspergillums fungi are the most powerful phosphate solubilizer [19]. Bacillusmegaterium, B.circulans, B.Polymyxa, B.subtillis, B.Sircalmous, Pseudomonas striata and Enterobacter could be referred as most important strains. [16]

Occurrence

High proportion of PSB is concentrated in the rhizosphere and they are metabolically more active than from other sources. [17]

Usually one gram of fertile soil contain 10^1 to 10^{10}bacteria. Soil Bacteria are in bacilli, cocci or spiral shapes. Bacilli are common in soil whereas spirilli are rare in natural environments [3]. The PSB are ubiquitous and its population depends on different soil properties physical, chemical properties, organic matter and P content [9]. Larger population of PSB are found in agricultural and rangeland soils. [21]

Mechanism of P-solubilization

Some Bacterial strains have solubilization and mineralization potential for inorganic and organic P respectively [5]. Phosphate solubilizing Activity (PSA) is determined by the ability of bacteria to secret organic acids, hydroxyl and carboxyl groups of organic acids chelate cations (Ca, Al, Fe) and decrease the PH in basic soils [10]. P-solubilization ability of PSB has direct correlation with PH of the medium. Inorganic acids e.g. HCl can also solubilize phosphate but they are effective as compared to organic acids at the same PH [9].

Isolation, identification, biochemical characterization, growth and P-solubilizing activity of these bacteria have been extensively studied by many researchers. However, minuscule information is available regarding their isolation, characterization and identification of the strains dominating agricultural soil of Latur region of Maharashtra.

The objective of this study was to isolate identify and characterize phosphate solubilizing Bacteria from the Agricultural soil of Latur region, Maharashtra.

Materials and Methods

Collection of soil sample

The soil samples were collected using sterile tools from the rhizosphere (5'' - 6" depth) from the different areas of Latur region. All collected soil samples were stored in polythene bags and transported aseptically to Laboratory and stored at 4^0C prior to be analyzed.

Isolation of PSB

Isolation of PSB was carried out by suspending 1g rhizospheric soil in 10 ml of Distilled water, the soil samples were serially diluted upto 10^{-6} dilution, plated on petridishes by spread plate technique and incubated at 30^0C for 7 days. At the end of incubation, PSB colonies were identified from the clear zone around the bacterial colony**(Shown in PhotoplateNo.1,2 and 3)** Colonies showing clear zone around them were considered as PSB. Single colonies with clear zone appearing on Pikovskayas Agar plates containing tricalcium phosphate were transferred in liquid Pikovskayas broth andsubcultured on Pikovskayas (PVK) Agar plates **(Photoplate No.4) or** slants for further study.

Identification and Characterization of PSB

The Bacteria were identified by morphological characteristics viz- different shapes, staining methods (Gram staining) and various biochemical tests such as Motility test, indole test, Methyl Red test, Vogues proskauer test, Casein hydrolysis test, Citrate hydrolysis, catalase test, oxidase test, starch hydrolysis, Gelatin hydrolysis test, Urease test, Mannitol fermentation test, Lactose fermentation test, sucrose fermentation test, H_2S production test, Nitrate Reduction test. Biochemical characterization and identification of Rhizobaterial spp. have been done by following the Bergey's Manual for bacteriology methods systematic.

The biochemical tests were performed according to microbiology presscott- Harely- 5th edition.

Table 1: Morphological Characteristics of Phosphate Solubilizing Bacteria (PSB)

Isolates (PSB)	Colony Morphology	Gram's Staining Reaction	Isolates (PSB)	Colony Morphology	Gram's Staining Reaction
LPsb-1	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-16**	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods
LPsb-2	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-17**	Round, moderate sized, Cream colored.	GPB Rods
LPsb-3	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-18**	Convex,Round,Shiny, Smooth, Whitish.	GPB Rods
LPsb-4	Large, flat, brown colored colonies	GPB Rods	**LPsb-19**	White, transluscent, circular in shape	GNB Rods
LPsb-5	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-20**	White, transluscent, circular in shape	GNB Rods
LPsb-6	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-21**	White, transluscent, circular in shape	GNB Rods
LPsb-7	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-22**	White, transluscent, circular in shape	GNB Rods
LPsb-8	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-23**	White, transluscent, circular in shape	GNB Rods
LPsb-9	Flat, Large sized, brown in color.	GPB Rods	**LPsb-24**	Round, moderate sized, Cream colored.	GPB Rods
LPsb-10	Round, Shiny, Smooth, Cream colored	GPB Rods	**LPsb-25**	Round, small sized, Cream colored.	GPB Rods
LPsb-11	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-26**	Flat, Large sized, brown in color.	GPB Rods
LPsb-12	Convex, Round, Shiny,	GPB	**LPsb-27**	Flat, irregular,smooth,	GNB

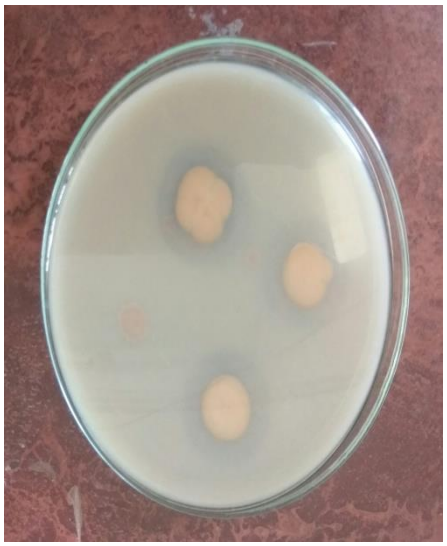
	Smooth, Whitish.	Rods		Pale brown colored.	Rods
LPsb-13	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-28**	Flat, irregular,smooth, Pale brown colored.	GNB Rods
LPsb-14	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-29**	White, transluscent, circular in shape.	GNB Rods
LPsb-15	Convex, Round, Shiny, Smooth, Whitish.	GPB Rods	**LPsb-30**	round, transluscent, white in color.	GNB Rods

GPB-Gram Positive Bacteria; GNB-Gram Negative Bacteria

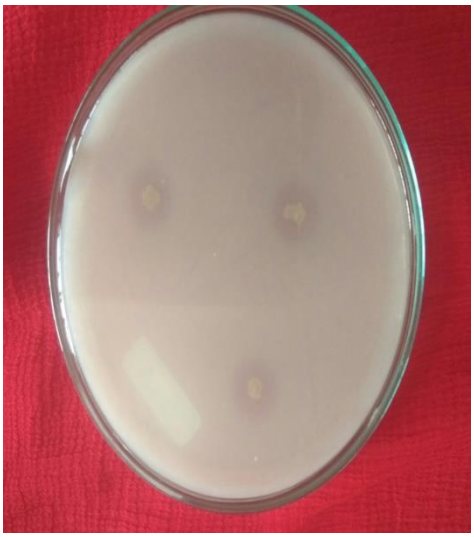
Table 2: Biochemical Characterization of Isolated Phosphate Solubilizing Bacteria According to Bergey's Manual (1981)

Isolates (PSB)	IN	MR	VP	Cit	Cat	Oxi	St	Gel	UR	MN	Lac	Suc	H_2S	NR
LPsb-1	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-2	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-3	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-4	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-5	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-6	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-7	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-8	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-9	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb10	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-11	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-12	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-13	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-14	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-15	-	-	+	-	+	-	+	+	-	+	+	+	-	+
LPsb-16	-	-	+	-	+	-	+	+	-	+	+	+	-	+
LPsb-17	-	-	+	-	+	-	+	+	-	+	+	+	-	+
LPsb-18	-	-	+	-	+	-	+	+	-	+	+	+	-	+
LPsb-19	+	+	+	+	+	+	+	-	-	+	+	+	+	+
LPsb-20	+	+	+	+	+	+	+	-	-	+	+	+	+	+
LPsb-21	+	+	+	+	+	+	+	-	-	+	+	+	+	+
LPsb-22	+	+	+	+	+	+	+	-	-	+	+	+	+	+
LPsb-23	+	+	+	+	+	+	+	-	-	+	+	+	+	+
LPsb-24	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-25	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-26	-	-	+	-	+	+	+	+	-	+	+	+	-	+
LPsb-27	-	-	-	-	+	+	+	-	-	+	-	+	+	+
LPsb-28	-	-	-	-	+	+	+	-	-	+	-	+	+	+
LPsb-29	+	+	+	+	+	+	+	-	-	+	+	+	+	+
LPsb-30	+	+	+	+	+	+	+	-	-	+	+	+	+	+

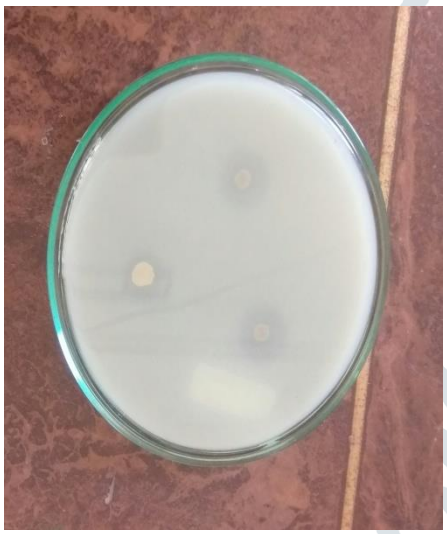
IN- Indole Test; **MR**- Methyl Red; **VP**- Vogues Proskauer; **Cit**- Citrate Utilization; **Cat**- Catalase; Oxi- Oxidase; **St**- Starch Hydrolysis; **Gel**- Gelatin Hydrolysis; **UR**- Urease; **MN**- Mannitol Utilization;**Lac**- Lactose Ferementation; **Suc**- Sucrose Fermentation; **H_2S**- H_2S production; **NR**- Nitrate Reductase



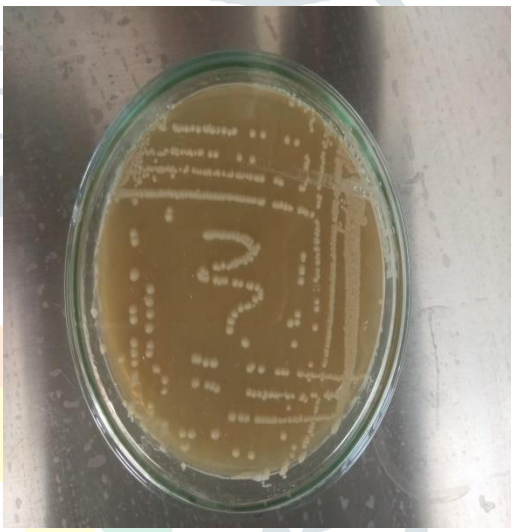
Photoplate No.1



Photoplate No.2



Photoplate No.3



Photoplate No.4

Results and Discussion

The Rhizobacterial cultures were isolated from soil samples collected from Agricultural areas of Latur region, Maharashtra State of India. Totally 50 isolates were screened for their phosphate solubilizing ability, of these 30 isolates showed clear zone on pikovskayas media containing Tricalcium Phosphate. The bacterial colonies showing clear halo zones around it, were considered as phosphate solubilizing bacteria **(Shown in PhotoplateNo.1,2 and 3)**

The bacterial isolates showing clear zone formation were further characterized by morphological test- Gram's staining and a series of Biochemical reactions.

The morphological and biochemical characteristics of these Bacterial isolates were shown in Table 1 and 2. The results of Morphological and Biochemical tests showed that maximum number of Bacterial isolates could be of Bacillus Spp and few no. of isolates could be Rhizobium and pseudomonas.

Conclusion

It was concluded from the present study that- Bacillus spp, Rhizobium Spp and Pseudomonas Spp. are found commonly. However Bacillus SPP would be found as prevalent PSB in Agricultural soil of Latur region of Maharashtra state.

Acknowledgement

Authors are thankful to UGC-MANF for providing financial support, Department of Biotechnology COCSIT Latur and Department of Zoology, R.S. College Latur for providing Laboratory facilities.

References

[1] Ahemad M., Khan M.S., Effect of fungicides on plant growth promoting activities of phosphate solubilizing pseudomonas putida isolated from mustard (Brassica Campestris) rhizosphere chemosphere 86:945-950 (2012).
[2] Ahemad M. Zaidi A., Khan M-S and oves M, Biological importance of phosphorous and phosphate solubilizing microbes for crop improvement. Nova, New York, PP 1-4 (2009)
[3] A. Khan, G. Jillani, M.S. Akhtar, S.M.S. Naqvi2, M. Rasheed3. Phosporous solubilizing Bacteria: Occurrence, mechanism and their Role in Crop Production Published in J. agricfiol Sci. (1) : 48-58 (2009).
[4] Chen, Y. P., P.D. Rekha, A.B. Arunshen, W.A. Lai and C.C. Young 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol 34:33-41.
[5] Hilda, R. and R. Fraga 2000 Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech Adv. 17:319-359.
[6] Himedia Laboratories.com- Pikovskayas Media.
[7] Khan M.S. Ahmad E Zaidi A and oves M, Foundational aspect of phosphate- solubilizing bacteria; importance in crop production.In :Maheshwari DK et al (eds) Bacteria in agrobiology crop productionity springer. Berlin PP 237-265 (2013).
[8] Khan M.S., Zaidi A. Wani P.A., Ahemad M and oves M, Functional diversity among Plant growth promoting rhizobacteria, In: Khan M.S. Zaidi A, Musarat J (eds) Microbial Strategies for Crop improvement Springer, Berlin, PP 105-132 (2009)
[9] Kim, K. Y. D. Jordan and G.A. Mcdonald 1998 Effect of Phospate- Solubilizeng bacteria an vesicular-arbuscularmycorhizpe on tomato growth and soil microbial activity. Biol-fert soils 26:79-87
[10] KPomblekou, K. and M.A. Tabatabai 1994, Effect of organic acids on release of phosphorus from phosphate rocks soil Sci. 158:442-453.
[11] Kucey, R. M. N., H. H. Janzen and M. E. Legget 1989. Microbial mediated increases in plant available phosphorous Adv. Agron. 42:199:228.
[12] Presscott, Harley (2002) Laboratory Exercises in microbiology – fifth edition. The macgrow Hill company.
[13] Sanjotha G &SudhirManawadi, Volume2, Issue2, 2016 PP 1-6. Isolation, screening and characterization of phosphate solubilizing Bacteria from karwar costal region.IJRSMB- International Journal of Research Studies in Microbiology and Biotechnology.
[14] Shenoy V.V. and Kalagudi G.M., Enhancing plant phosphorous use efficiency for sustainable cropping, Biotechnol Adv. 23:501-513 (2005).
[15] Steveson, F.J. 2005 Cycles of soil; Carbon, Nitrogen, Phosphorus, Sulfur, micronutrient John wiley and sons, New York.
[16] Subbarao N.S. 1988 phosphate solubilizing micro-organism in :Biofertilizer in agriculture and forestry regional biofert Dev. Centre, Hissar, India PP. 133-142.
[17] Vazquez P. G. Holguim, M. Punete, A. E. cortes and Y. Basham 2000. Phosphate solubilizing micro-organisms associated with the rhizosphere of mangroves in a semi arid coastal lagoon. Biol-Fert Soils 30:460-468.
[18] Wang X, Wang, Tian J, Lim BL, Yan x and LiqoH, Overexpressing At PAP 15 enhances phosphorous efficiency in soyabean, plant physiol 151:233-240 (2009).
[19] Whitelaw M. A. 2000 Growth Promotion of plants inoculated with phosphate solubilizing fungi. Adv. Agrom 69:99-151.
[20] www.smart-fertilizer.com.
[21] Yahya, A. and S. K. A. Azawi, 1998 occurrence of phosphate solubilizing bacteria in some Iranian soils. Plant soil 117:135:141.