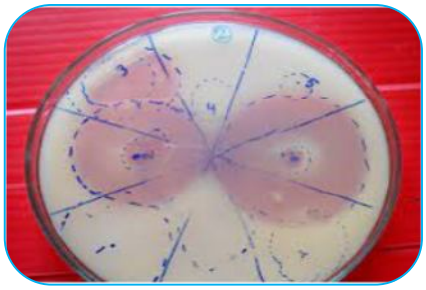
INDIAN STREAMS RESEARCH JOURNAL
ISSN NO : 2230-7850
IMPACT FACTOR : 5.1651 (UIF)
VOLUME - 12 | ISSUE - 9 | OCTOBER - 2022

COMPARATIVE STUDIES ON PHOSPHATE SOLUBILISING BACTERIA FROM DIFFERENT RHIZOSPHERIC SOIL"

Dr. Anand Mukundrao Pande
Microbiology Research Laboratory, R. A. College Washim 444505 (MS) India.

ABSTRACT



Phosphorus is one of the major limiting factors for crop production on many tropical and subtropical soils. It is therefore necessary to identify and incorporate efficient strains of phosphate solubilizing microorganisms in to cropping systems.The present investigation is being carried out with the aim of isolation and characterization of Phosphate solubilizing bacteria from different rhizosphere soil of viz:soybean, wheat, maize, chick pea and pigeon pea were collected from district Washim. Also a comparison of the bacterial isolates on the basis of their phosphate solubilizing bacteria abilities has performed.Hence the present study indicates the wheat at the hot spot and W-PSB3 as the most dynamic strain of choice for producing Phosphate Solubilization -inoculants.

KEYWORDS: PSB, Rhizospheric Soil, Soybean, Wheat, Maize, Chickpea

INTRODUCTION:

The world of agriculture in the last few eras has been intensely dependent on chemical fertilizers as source plant nutrients to meet the increasing demand for food. Conversely, in recent years environmentalist and agricultural scientists have realized that continued and persistent use of chemical fertilizers depletes the soil, and causes environmental pollution and unevenness in the soil microbial activity. Hence, increasing awareness is being built on the use of organics together with bio-fertilizers to sustain the soil fruitfulness and plant productivity.

Phosphorus is one of the major limiting factors for crop production on many tropical and subtropical soils. It is therefore necessary to identify and incorporate efficient strains of phosphate solubilizing microorganisms in to cropping systems (Fankemet al., 2006).Among the plant nutrients, next nitrogen the phosphorous is an important plant nutrient and serves important key function in the plant viz. it help in photosynthesis, nucleus formation and cell division etc. (Gaur, 1990).

The application of organic fertilizer has been practiced for more than thousand years in many countries since it provides essential nutrients to plants, improves soil structure, helps in the moisture retaining capacity in various soils and increases microbial activities (Chen et al., 2006). In developing countries like India the stress on agriculture is increasing day by day. The land under farming is decreasing and this has posed an extra burden on agriculture. Therefore the land available for agriculture should be utilized economically (Maheshwari, and Dubey2000).

Application of phosphate solubilizing bacteria (PSB) asbio inoculants can solubilize the fixed soil P and applied phosphates resulting in higher crop yields (Gull, Hafeez, Saleem, & Malik, 2004). PSB, therefore, find application in cropimprovement as enhancers of solubilization of re-precipitated

rhizospheric P (Shekharet al.,1999). Naturally occurring rhizospheric phosphorus-solubilizing microorganisms (PSM) have first been reported in 1903 (Khan et al., 2007).

Many fungi and bacteria are potential solubilizers of bounded phosphates in soil. Thus, they play an important role in soil by solubalizing phosphorus and making it available to plant (Rokadeet al., 1992). Microbial inoculants prepared from Rhizobium, Azotobacter, Azospirillumand Phosphate solubilizing microorganisms are known to increase the yield of crops (Pikovskyet al., 1985).

Indian farmers regularly practicing the use of phosphorus bio-inoculant and have frequently experienced the phosphorus solubilizing activity especially in Rhizosphere is very important. The use of native efficient phosphate solubilizing micro flora may be tried in its own soil for successive crops yield. Hence, the studies on isolation of phosphate solubilizing bacteria from appropriate rhizospheric source and their comparative evaluation of phosphate solubilizing potential with different rhizosphere soil of soybean, wheat, maize, pigeon pea, chickpea and measurement of phosphate solubilizing efficiency have been carried out.

MATERIALS AND METHODS

Collection of Soil Samples:

For present study, the ten different rhizospheric soil samples were collected from viz; soybean, wheat, maize, chickpea, and pigeon pea crops plants were collected from different locations nearby Washim city.For the collection and preservation of samples, each sample was collected from 15 cm depth and it was packed in sterile polythene bag and labelled properly. The collected soil samples were enriched in nutrient broth and incubated for 24 hrs. at 370C .all enriched sample were further sub cultured on nutrient agar plates adopting streak plate method. The well isolated colonies were again sub culture on nutrient agar slant. Bacterial isolates were identified on the basis of conventional method according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Screening of Phosphate Solubilising Bacteria

The screening of phosphorus solubilising bacteria has been done using Pikovskaya's medium.Colonies showing halo zones were picked and purified by 5 times subculture method on Pikovskaya's (PVK) agar medium for characterization. (Goenadiet al., 1999).

Characterization of selected Phosphate Solubilising Bacteria

The selected bacterial isolates will be characterized on the basis of various morphology and biochemical, characterized as per Bergey's manual of systematic bacteriology (John G.Holt 1994).

Screening for Phosphate Solubilization Activity

The Phosphate Solubilization Efficiency (PSE) is the ratio of total diameter .i.e. clearance zone including bacterial growth and the colony diameter. This is determined by calculating the Phosphate Solubilization index (PSI) adopting following formula. The observations were recorded in triplicate and the results are expresses in terms of mean. (Karpagam and Nagalakshmi 2014)

$$\text{PSI} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\textit{Colony Diameter}}$$

RESULTS AND DISCUSSION

Isolation of Rhizobacteria from Rhizospheric Soil

Present investigation. A total of 107 different types of bacterial isolates (Table-1) were obtained from all the five the rhizosphere soil sample. Out which only 13 (12.14) per cent isolates showed the zone of clearance around the colony. The isolates with the zone of clearance were consider as p-solubilising bacteria and were named after the type of rhizosphere as S-PSB1,S-PSB2 ,W-PSB1,W-

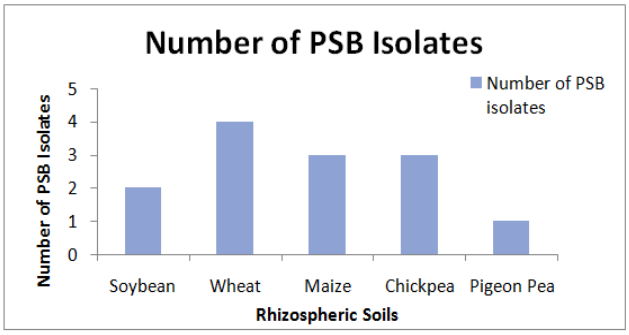
PSB2,W-PSB3,W-PSB4,M-PSB1,M-PSB2,M-PSB3 C-PSB1,C-PSB2,C-PSB 3, P-PSB1. From the result it was observed that, the maximum number of PSB i.e. 04 (19.04) Percent was obtained from soil sample of wheat rhizosphere in which total 21Number of dissimilar Bacterial isolates were recovered. Followed by wheat the the maximum number of PSB i.e. 03 (13.63) percent was obtained from soil sample of chick pea rhizosphere in which total 22 Number of dissimilar Bacterial isolates were recovered. In case of Maize the number of PSB i.e. 03 (13.04) percent was obtained from soil sample of wheat rhizosphere in which total 23 Number of dissimilar Bacterial isolates were recovered. In case of Soybean the number of PSB i.e. 02 (10.52) percent was obtained from soil sample of wheat rhizosphere in which total 19Number of dissimilar Bacterial isolates were recovered. Whereas the minimum number of PSB i.e. 01 (4.54) percent was obtained from soil sample of Pigeon Pea rhizosphere in which total 22 Number of dissimilar Bacterial isolates were recovered.

Table 1: Population Dynamics of Phosphate Solubilising Bacteria in Different Rhizosphere

Sr.No.	Rhizosphere Type	Number of dissimilar Bacterial isolates	Number of PSB isolates	Strains Code Number
1.	Soybean	**19**	**02 (10.52)**	S-PSB1 S-PSB2
2.	Wheat	21	04 **(19.04)**	W-PSB1 W-PSB2 W-PSB3 W-PSB4
3.	Maize	**23**	**03 (13.04)**	M-PSB1 M-PSB2 M-PSB3
4.	Chickpea	**22**	**03 (13.63)**	C-PSB1 C-PSB2 C-PSB3
5.	Pigeon Pea	**22**	**01 (04.54)**	P-PSB1
6.	**TOTAL**	**107**	**13 (12.14)**	-

Values in parenthesis are Percent population of PSB

Fig 1: Population Dynamics of Phosphate Solubilising Bacteria in Different Rhizosphere



Characterization of selected isolated Phosphate solubilising bacterial isolates

The PSB isolates were further characterized by a series of biochemical reactions (Table 2). The microscopic analysis revealed that out of 13 isolated PS bacteria only two isolates were found to be Gram positive whereas all other isolates were found to be Gram negative in nature and thus it can be stated that Gram negative bacteria dominated the rhizosphere of washim soils in the studies .Further biochemical test shows greater similarity of the PSB isolates with Bacillus , Azotobacter , Rhizobium and Pseudomonas species respectively Pseudomonas species are free living and most abundant in soil and can be cultured with ease in vitro and thus are more frequently encountered.

Table 2: Morphology and Cultural Characteristics of isolated Phosphate solubilising bacteria

Characters	PSB-1	PSB -2	PSB -3	PSB-4	PSB-5	PSB-6	PSB-7	PSB-8	PSB-9	PSB-10	PSB-11	PSB-12	PSB-13
Shape	Irregular	Round	Regular	Round	Round	Round	Irregular	Regular	Regular	Round	Round	Round	Round
Size	2-5mm	2-3mm	1-2mm	2-3mm	2-3mm	2-3mm	2-5mm	1-2mm	1-2mm	2-3mm	2-3mm	2-3mm	2-3mm
Elevation	Flat	Slimy	Convex	flat	Slimy	flat	Flat	Convex	Convex	Slimy	Slimy	Slimy	Slimy
Surface	Rough	Flat	Smooth	slimy	Flat	slimy	Rough	Smooth	Smooth	Flat	Flat	Flat	Flat
Margin	Entire	Regular	Entire	Regular	Regular	Regular	Entire	Entire	Entire	Regular	Regular	Regular	Regular
Colour	Whitish	Cream	brown	brown	Cream	brown	Whitish	brown	brown	Cream	Cream	Cream	Cream
Pigmentation	Yellowish	-	+	-	-	-	Yellowish	+	+	-	-	-	-
Morphological characters													
Gram Reaction	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
Cell Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Biochemical characters													
Glucose	+	+	+	-	+	-	+	+	+	+	+	+	+
Sucrose	-	-	+	+	-	+	-	+	+	-	-	-	-
Lactose	+	+	+	-	+	-	+	+	+	+	+	+	+
Maltose	+	+	+	-	+	-	+	+	+	+	+	+	+
Indole	-	-	+	+	-	+	-	+	+	-	-	-	-
Methyl Red	-	-	+	+	-	+	-	+	+	-	-	-	-
VogesPrausker	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	-	+	-	+	+	+	+	+	+	+
Catalase	+	-	+	+	-	+	+	+	+	-	-	-	-

Oxidase	-	-	-	+	-	+	-	-	-	-	-	-	-
Nitrate	+	-	+	+	-	+	+	+	+	-	-	-	-
Starch Hydrolysis	+	-	+	-	-	-	+	+	+	-	-	-	-
Gelatin Liquefaction	+	-		-	-	-	+			-	-	-	-
Casein Hydrolysis	+	-		-	-	-	+			-	-	-	-
H_2S	-	-	-	+	-	+	-	-	-	-	-	-	-
Possible Species	*Bacillus species.*	*Pseudomonas species*	*Azotobacter species*	*Rhizobium species*	*Pseudomonas species*	*Rhizobium species*	*Bacillus species.*	*Azotobacter species*	*Azotobacter species*	*Pseudomonas species*	*Pseudomonas species*	*Pseudomonas species*	*Pseudomonas species*

+: Positive; -: Negative

Screening for Phosphate Solubilization Efficiency

All the 13 selected isolates were found to be phosphate solubilizers showing clear zone around their colonies. The PSI measurement was shown in the (Table 3 and graphically represented in Fig 2).From the result it was observed that Among 13 bacterial isolates the Phosphate Solubilization Index (PSI) was ranged from 1.04 - 2.23 .The maximum SI i.e. (2.23) for phosphate was observed in case of soil isolate W-PSB3 isolated from wheat rhizosphere. Whereas the minimum SI i.e. (1.04) for phosphate was observed in case of soil isolate C-PSB3 isolated from cheek pea rhizosphere.

It was also observed that the isolate number S-PSB2 isolated from soybean rhizosphere W-PSB1,W-PSB2,W-PSB3,W-PSB4 isolated from wheat rhizosphere respectively , and M-PSB2 isolated from maze rhizosphere were found to be at higher side for their Phosphate Solubilization Index and ranged from 2.08 to 2.23 and hence consider as efficient strains .

Whereas all the other isolated p- solubilizing bacterial isolates under study showed their Phosphate Solubilization Index at lower side i.e. bellow 2 and ranged from 1.04 to 1.09.

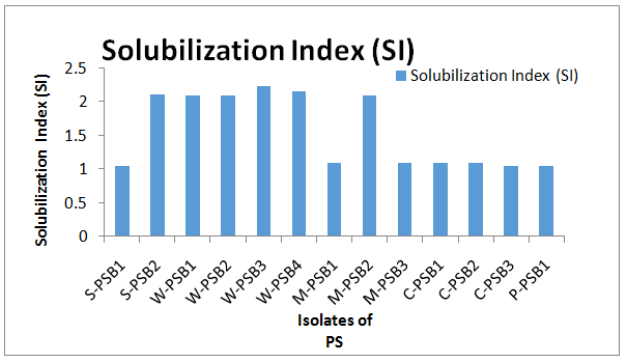
Hence the present study indicates the wheat at the hot spot and W-PSB3 as the most dynamic strain of choice for producing Phosphate Solubilization -inoculants.

Table 3: Solubilizing Index of Isolated Phosphate -Solubilization Bacteria

S.No	Isolates of PS	Colony Measurement (cm)	Zone Measurement (cm)	Solubilization Index (SI)*
1.	S-PSB1	2.0	0.1	1.05
2.	S-PSB2	1.9	2.1	2.10
3.	W-PSB1	1.2	1.3	2.08
4.	W-PSB2	1.1	1.2	2.09
5.	W-PSB3	1.3	1.6	2.23
6.	W-PSB4	2.0	2.3	2.15
7.	M-PSB1	1.1	0.1	1.09
8.	M-PSB2	1.2	1.3	2.08
9.	M-PSB3	1.1	0.1	1.09
10.	C-PSB1	2.1	0.2	1.09
11.	C-PSB2	2.2	0.2	1.09
12.	C-PSB3	2.1	0.1	1.04
13.	P-PSB1	2.0	0.1	1.05

*SI= (Colony diameter + Halo zone)/colony diameter

Fig 2: Solubilizing Index of Isolated Phosphate -Solubilization Bacteria



Conclusion

The present study enlightened the wheat rhizosphere as the most suitable resource for the isolation of efficient PSB in case of washim soils. Whereas the selected isolatesW-PSB3was found to be Pseudomonas species. It is the free living and most abundant in soil and can be cultured with ease in vitro and thus are more frequently encountered therefore this strain can be used priortisely for the development of P- inoculants and may prove to be potential applicants for being used as bio fertilizers.

References

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.

Fankem H, Nwaga D, Deube A, Dieng L, Merbach W, Etoa FX .(2006). Occurrence and Function of phosphate solubilizing microorganisms from oil palm tree (Elaeisguineensis) rhizosphere in Cameroon. Afr J Biotechnol5:2450–2460.

Gaur, A.C. 1990.Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific Publishers, New Delhi, India, 57(61): 114.

Goenadi , DH Pasaribu, R.A.; Isroi ;Hartono, H.; Misman,R. (1999): Phosphate solubilizing fungi isolated from tropical forest soil . Menara Perkebunan 67:1,40-51;31 ref.

Gull, M., F.Y. Hafeez., M. Saleem., and K. A. Malik: (2004)Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. Aus. J. Exp. Agric,; 44, 623-628.

Holt J. A., N. R. Kreig, P. H. A. Sneath (1994). Bergey's Manual of determinative Bacteriology. Baltimore, MD.

Karpagam,T and Nagalakshami, P .K., (2014). Isolation and Characterrization of Phosphate solubilizing Microbes from Agricultural soil.International Journal of current Microbiology Applied Science 3(3):601-614.

Khan, M. S., A. Zaidi and P. A. Wani.(2007). Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review.Agron. Sustain. Dev. 27:29-43.

Maheshwari, D.K.; R.C. Dubey; Prasad, G. and Navneet. (Eds.) (2000). Microbiology: Agriculture, Industry & Environment. Bishen Singh Mahendra Pal Singh, Dehra Dun, p. 269.

Rokade, S.M. and P.L. Patil.(1992). Phosphate solubilizing microorganism.A Review. I.J. Maharashtra Agric. Univ., 17: 456 - 466.

ShekharNautiyal,C.(1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms.FEMS Microbiology Letters.170 .265-270.