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RESEARCH ARTICLE

ISOLATION AND CHARACTERISATION OF PHOSPHATE SOLUBILISING BACTERIA (PSB) FROM THE POTATO GROWING SOILS OF GWALIOR

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ABSTRACT

Soil pH plays an important role in the survival of soil microbes. These effect the soil micro and macronutrients. Phosphorus (P) is a major growth macronutrient in the soil taken up by the plants for growth, development and yield of every crop. Phosphate solubilising bacteria (PSB) play role in phosphorus nutrition by enhancing its availability into rhizosphere by release from inorganic and organic bound sources in soil through phosphorus solubilisation and mineralisation. In the present study a comparative assessment has been made on the Pikovskaya agar medium (PVK) containing methyl red, methyl red orange and bromophenol blue. These are used for observing the differential phosphate solubilising activity of phosphate solubilising bacteria (PSB) in the potato growing soil. The study revealed higher number of PSB forms revealed in PVK containing methyl red followed simultaneously by PVK containing bromophenol blue and methyl red orange respectively.

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INTRODUCTION

Phosphorus (P) is a major growth macronutrient taken up from soil by plants. Its presence in the soil is an important factor for determining the growth and development and the yield of any crop. Phosphorus solubilising bacteria (PSB) play role in phosphorus nutrition by enhancing its availability into rhizosphere. The same in released from inorganic and organic sources, present in the soil, through solubilisation and mineralisation. Phosphorus contents of soil are typically high however, the available phosphate ions (Pi), or available forms of phosphorus for roots to absorb, are usually suboptimal. It is only the soluble forms of phosphate ions (Pi), like HPO_4^{2-} or H_2PO^{4-} which are absorbed. The crops such as potato, are P demanding and therefore, rely on the use of high amounts of chemical phosphate fertilisers. Alternatively, however, phosphate solubilising bacteria being a group of plant root associated PGPR, facilitate the hydrolysis of a wide range of phosphorus bound compounds in the soil and more so in the rhizosphere (Rodriguez and Fraga 1999). Plants acquire P from soil solution as phosphate anions. Phosphate anions however, are extremely reactive and may be immobilised through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} which depends on the particular property of a soil.

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In these forms, phosphate is highly insoluble and unavailable to plants. Ubiquitous presence of PSB with variation in their forms and population in different soils is now known. Their population depends on different soil properties both physical and chemical organic matter, bound P cum free P content and crop cultural activities (Kim *et al.*, 1998). Larger populations of PSB are reported present in agricultural and rangeland soils (Yahya and Azawi, 1998). The PSB increase the availability of soluble phosphate. These can enhance plant growth by either increasing the efficiency of biological nitrogen fixation or by besides enhancing P, the availability of other trace elements such as iron, zinc, etc. These may do this by production of plant growth-promoting regulators also (Kucey *et al*., 1989; Ponmurugan and Gopi, 2006). Some bacterial and fungal species, known as PSMs, assist plants in mobilisation of insoluble forms of phosphate. PSMs include various groups of microorganisms, which not only assimilate phosphorus from insoluble forms of phosphates, but also bring about availability of large portion of soluble phosphates which are released in such quantities which are in the quantities higher than the required. Hence, the use of PSM as biofertilisers for crop improvement is now known (Richardson, 2001; Khan *et al.,* 2007). Bacterial species which solubilise insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate, include bacterial genera *Pseudomonas*, *Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus,*

Aereobacter, Flavobacterium and Erwinia (Karpagam and Nagalakshmi, 2014). In this study, Pikovskaya agar medium (PVK) media included with different pH indicators were used for isolation to determine the presence of phosphate solubilising bacteria (PSB) in soils associated with the potato plants. The use of methyl red (MR) indicator in PVK medium Parikh and Jha, (2012) is used to characterise PSB, whereas, the use of bromophenol blue (BPB) has been employed Hu *et al.,* (2010) under *in vitro* conditions. BPB has also been employed to label phosphate solubilising fungi (PSF) under culture (Ejikeme and Uzoma, 2013). The results in the present study pertain to characterise PSB from both the rhizospheric and non-rhizospheric soils of potato in Gwalior. Using these different indicators in PVK, methyl red (MR) and bromophenol blue (BPB) rarely used indicator in mass characterisation of PSB and hardly ever used indicator methyl red orange (MRO).

MATERIALS AND METHODS

Soil sample

The soil samples were collected from potato soils of Central Potato Research Station, (CPRS), Gwalior, M.P. state of India. The samples were stored at 4^0C in sterile polyethylene bags till study. Bacteria were isolated by serial dilution method. A series up to 10^{-7} dilution was prepared under aseptic conditions. 0.1ml aliquot was taken and spread on Nutrient agar (NA) medium containing: 5.0 g peptone, 1.5 g yeast extract, 1.5 g beef extract, 5.0 g NaCl and 20 g agar per liter of distilled water maintained at pH 7.2. Plates were incubated for 2 days at 28±1°C to observe the colonies of bacteria. Single colony were further, characterised on different Pikovskaya agar medium (PVK) medium containing, separately methyl red, methyl red orange and bromophenol blue as indicators for characterising their phosphate solubilising activity.

Isolation of PSB in different medium

a)Pikovskaya agar medium containing Methyl red

The Phosphate solubilisation potential of selected strains of bacteria was tested *in vitro* by spot inoculation of the bacterial isolates on the Pikovskaya agar medium containing Yeast extact (0.50 g), Ferrous sulphate (0.00001 g), Dextrose (10.0 g), trialcium phosphate (5.0 g), Ammonium sulphate (0.50 g), Potassium choloride (0.20 g), Magnesium sulphate (0.10 g), Manganese sulphate (0.0001 g), Agar (15.0 g) in 1000 ml of distilled water maintained at pH 7.2 (Pikovskaya, 1948) added with 1% methyl red (Parikh and Jha, 2012). Plates after 96 hr incubation at 28±1°C were observed for the development of clear zone around the specific colony.

b)Pikovskaya agar medium containing Methyl Red orange

The phosphate solubilisation bacteria were characterised by spot inoculation of the single bacterial isolate on the Pikovskaya agar medium (Pikovskaya, 1948) containing tricalcium phosphate pH 6.6 modified with 1% of methyl red orange. Plates after 96 hr incubation at 28±1°C were observed for the clear zone around the colony.

c)Pikovskaya agar medium containing Bromophenol blue

The phosphate solubilisation bacteria were characterised by spot inoculation of the single bacterial isolate on the Pikovskaya agar medium (Pikovskaya, 1948) containing tricalcium phosphate pH 6.0 with 1% of bromophenol blue (Gupta *et al.,* 1994). Plates after 96 hr incubation at 28±1°C were observed for the clear zone around the colony. The incubated colonies were observed visually and by magnification to study morphological characteristics for colour, colony shape, colony margins, and their elevation following Goenadi and Sugiarto, (2000). Bacterial slides were prepared for Gram staining. So as to differentiate the isolated bacteria into gram positive and gram negative groups (Aneja, 2007).

RESULTS

Isolation of PSB in different medium

In the present study 40 soil samples both from the rhizosphere and non-rhizosphere of potato were collected from different experimental sites.

Fig.1. Bacteria on Pikovskaya agar containing tricalcium phosphate with (A) methyl red (B) methyl red orange and (C) bromophenol blue

On nutrient agar nearly 110 colonies of various morphologies were observed. Out of these only 40 were seen to have characteristics of PSB and were accordingly labeled as PSB1 to PSB40 (Table 1). This was observed when serial dilutions of aliquot were spread on Nutrient agar (NA) and subsequently subjected to PVK medium characterisation using MR, MRO and BPB as indicators. PSB1 to PSB 40 were selected on the basis that these produced zones of clearance around the colony after 96 hr of incubation at 28±1°C, on PVK containing, methyl red, methyl red orange and bromophenol blue (Fig. 1). Higher number of 18 PSBs were found in the Pikovskaya agar containing methyl red followed by 13 on bromophenol blue and 9 on methyl red orange (Table 1 and Fig. 2)

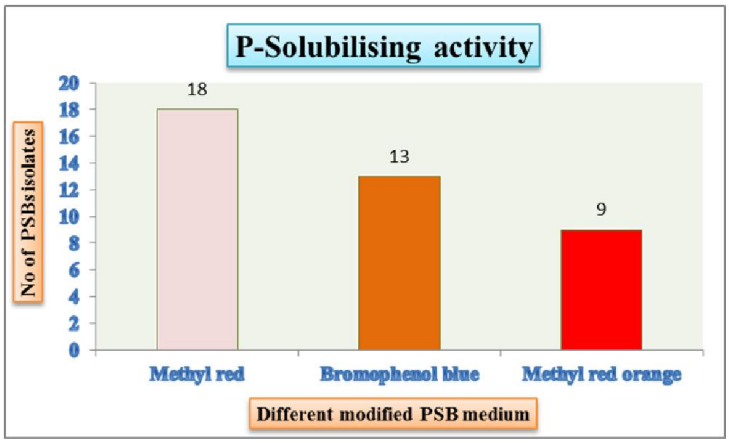

Fig. 2. Number of PSBs isolates found in different modified phosphate solubilising media

Table 1. Morphological Characteristics of bacterial isolates from potato soil

Isolates	Media type			Gram stain	Colony morphology		Experimental site
	MRO	MR	BPB		colour	shape	
PSB1	-	P	-	+ve	Peach	Small, round	D/1/2
PSB2	-	P	-	+ve	Off white	Shiny, oval	B/4/5
PSB3	-	P	-	+ve	Pink	Small, round	B/34/35
PSB4	-	P	-	+ve	Off white	Irregular	B/4/5
PSB5	-	P	-	+ve	Off white	Oval, Mucoid	D/1/2
PSB6	-	P	-	+ve	Off white	Irregular, shiny	B/34/35
PSB7	-	P	-	-ve	Light brown	oval	B/4/5
PSB8	-	P	-	+ve	Off white	irregular, shiny	B/34/35
PSB9	-	P	-	+ve	Off white	Irregular, Transparent	B/4/5
PSB10	-	P	-	+ve	Yellow	round, Mucoid	B/34/35
PSB11	-	P	-	+ve	Peach	Small, oval	B/4/5
PSB12	-	P	-	+ve	Off white	Irregular, Mucoid	B/4/5
PSB13	-	P	-	+ve	Off white with brown pigmentation	Round, flat	D/1/2
PSB14	-	P	-	+ve	Off white	Round, flat	B/34/35
PSB15	-	P	-	+ve	Off white	Irregular, shiny	B/34/35
PSB16	-	P	-	+ve	Off white	circular	B/4/5
PSB17	-	P	-	+ve	Off white	circular	B/34/35
PSB18	-	P	-	-ve	peach	Round, shiny	B/4/5
PSB19	P		-	+ve	yellow	Oval, flat	B/4/5
PSB20	P	-	-	-ve	Off white	Circular, flat	B/34/35
PSB21	P	-	-	+ve	Off white	irregular	B/4/5
PSB22		-	P	+ve	Off white	Circular, shiny	B/4/5
PSB23	-	-	P	+ve	Off white	circular	B/4/5
PSB24	-	-	P	+ve	purple	round	D/1/2
PSB25	-	-	P	+ve	Brown with pigmentation	oval	D/1/2
PSB26	-	-	P	+ve	Off white	Irregular, flat	D/1/2
PSB27	-	-	P	-ve	Yellow	Round, shiny	B/4/5
PSB28	-	-	P	+ve	Off white	Circular, flat	B/34/35
PSB29	-	-	P	+ve	Off white	Circular, flat	B/4/5
PSB30	-	-	P	-ve	Light peach	round	D/1/2
PSB31	-	-	P	-ve	Light yellow	Round, ,Transparent	B/34/35
PSB32	-	-	P	-ve	peach	circular	D/1/2
PSB33	-	-	P	+ve	Light yellow	round	B/34/35
PSB34	P	-	-	+ve	Off white	Circular, flat	D/1/2
PSB35	P	-	-	+ve	Off white	irregular, flat	B/4/5
PSB36	P	-	-	-ve	Off white	Circular, flat	B/34/35
PSB37	p	-	-	-ve	Off white	irregular, mucoid	D/1/2
PSB38	P	-	-	-ve	Off white	Circular, flat	B/34/35
PSB39	p	-	-	-ve	Off white with brown pigmentation	round	B/34/35
PSB40	-	-	P	-ve	Light yellow	Circular	B/34/35

P is the presence of clear zone of P utilisation; MRO, MR and BPB refer to Pikovskaya medium containing Methyl red orange, Methyl Red and Bromophenol blue respectively. Experimental sites constitute the blocks on the 400 aecr farm of CPRS, Gwalior (M.P.).

DISCUSSION

The presence of a specific bacterial population/s in a certain soil system is due to its survival cum adaptation potential. The factors responsible that effect the presence and growth of bacteria may be due to nutrient uptake, soil pH, moisture content, organic matter and enzyme activities of the organisms present. Estimation of PSB population from potato soil were studied with main focus on their phosphate solubilising capacity on single media with 3 different pH indicators. In present study PSBs grown on Pikovskaya agar medium (PVK) containing different pH indicators showed that the PVK containing methyl red had highest PSB forms growing followed by the presence of bromophenol blue and methyl red

orange indicators. Methyl red orange and bromophenol blue are sulphur containing indicators, and sulphur is known to be pH changer and also selectively antagonistic for the growth of some bacteria. The finding therefore, may suggest that the two are selectively inhibitory for certain PGPR. Parikh and Jha, (2012) using methyl red in PVK and bacterial colony showing clear zone around suggested that the zone is due to release of PO_4 from tri-calcium phosphate in the media. This they suggested was due to pH change of PVK in the cleared zone. This is what prompted here to use other pH indicators in PVK and ascertain the extent to which other pH indicators can replace methyl red in showing the presence of PSB by developing clear zones due to phosphate release. Perusal of studies seem to show that employing Methyl red, bromophenol blue and methyl red orange to ascertain PSB in PVK is very rare or hardly any report. Further, Chung *et al.,* (2005) have reported solubilising activity under liquid medium culturing conditions containing $Ca_3(PO_4)_2$, $AlPO_4$ and $FePO_4$. According to this study $Ca_3(PO_4)_2$, is more phosphate releasing substrate by PSB than $AlPO_4$ and $FePO_4$. Whereas, BPB in PVK has been employed to ascertain PSB *Pantoea stewartii* Hu *et al.,* (2010) and phosphate solubilising fungi Ejikeme and Uzoma, (2013) and others, the report for the use of MRO as PSB indicator in PVK are hardly available. The present study is basically a preliminary work to mark the diversity of PSB by utilising various pH indicators in PVK media of 3 different pH. 40 phosphate solubilising bacteria from potato soils from Gwalior experimental site are reported which are now under further study. Dawwam *et al*., (2013) and Malboobi *et al.,* (2009) also reported different strains of PSB from potato soil. However, the PSB here can tolerate extreme climates of Gwalior and hence can be subjected for their exploitation as biofertilisers under arid and semi-arid conditions. It is therefore suggested that whenever, PVK is employed to ascertain PSB, beside methyl red, MRO, BPB can also be used individually so that the other PSB which could otherwise be missed are also visualized, along with change in the pH of the PVK. Soil Microorganisms play a decisive role in maintaining the ecological balance by active participation in Carbon, Nitrogen, Sulphur and Phosphorous cycles in nature (Karpagam and Nagalakshmi, 2014). The identification and characterisation of these forms is under study and shall follow as a separate report elsewhere.

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