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Isolation and Screening of Potassium and Phosphate Solubilising Bacteria in Stressed Soils of different regions of Madhya Pradesh for Production of Biofertilizers

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ABSTRACT

Microorganisms play a vital role in the environment for the biotransformation, bio-geochemical cycling and metal and mineral transformation. Microbial mineral solubilisation is an important process as it helps in the transformation of insoluble form of minerals to the soluble forms and therefore can be used as potent biofertilizers. Phosphorus and potassium are two of the most important minerals needed by plants for their proper growth and development but most of it is found in the bound form in soil and thus cannot be utilized by the plants leading to deficiencies. The main objective of this work was to isolate and screen out such PSBs and KSBs from different industrial areas in Madhya Pradesh on the basis of their solubilisation potential. Six different strains were isolated from different regions of Madhya Pradesh which included Richai, Kamore, Maneri Singrouli, Suhagpur and Amarkantak. Their morphological and biochemical characterizations were done and the K-ratio (Khandeparkar's ratio) i.e, the solubilisation index was calculated to check the zone activity. The isolates from Richai gave the best results.

Keywords:

Biotransformation, solubilisation index, biofertilizers, organic matter, adaptation, enrichment, screening.

INTRODUCTION

Microorganisms are involved in a range of processes that affect the transformation of soil phosphorus and potassium, and thus form the integral part of the biogeochemical cycling of nutrients. Inorganic P and K occur in the soil mostly as mineral complexes most of them appearing after the excessive use of chemical fertilisers, these insoluble precipitated forms cannot be absorbed by plants (Renegel and Marschner 2005). Organic matter is also an important source of these immobilized minerals that accounts for 20-80% of P in the soil (Richardson 1994). Potassium although abundantly present in the soils but is mostly found bound with other minerals like silicates and therefore is unavailable, thus only 0.1 - 2.0% of P and K are available to plants. This is the reason why the phosphate solubilising bacteria and potassium solubilising bacteria play such an important role in mineralization. These microorganisms play a vital role in making available insoluble forms of potassium by

mineralization. They solubilise potassium from insoluble forms like mica, feldspar and others by producing organic acids, siderophores and also capsular polysaccharides. Phosphorus and Potassium uptake of plants can be increased by using PSBs and KSBs as bio-inoculants.

MATERIALS AND METHODS

Study sites and sampling

The state of Madhya Pradesh is richly endowed with mineral wealth; coal, limestone, manganese, bauxite, copper-ore, dolomite, pyrophyllite are few of the minerals found in different districts of M.P. Some of the microbes like bacteria fungi and certain VAM (Vesicular Arbuscular Mycorhizzae) have the ability to solubilise minerals and make them available to the plant species, these microorganisms are known as PGPRs (Plant Growth Promoting Microorganisms)

Sampling: For this study, such sites were chosen which had mineral or ceramic industries as the probability of finding the PSBs and KSBs is higher in such stressed soils.

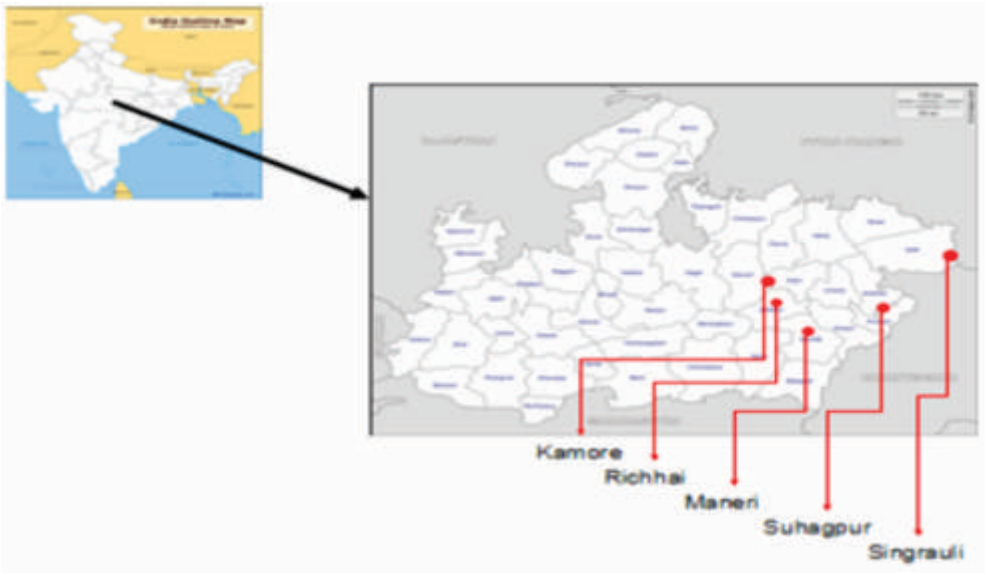

Fig 1. Sampling sites (different districts of Madhya Pradesh)

The soil samples were collected from the different industrially rich areas of Madhya Pradesh with ceramic and mineral industries like Richai, Kamore, Singrouli and Amarkantak. The soil samples were collected from the rhizospheric region of the plant species present in these areas in zip lock bags and were brought to the laboratory for further processing.

Isolation of PSBs and KSBs: For the isolation of Phosphate solubilising bacteria, the soil samples were serially diluted up to 10^{-7} and plated (Jacksons,1973) out on Pikosvskya's medium (Pikosvskya 1948) which is the specific growth medium for phosphate solubilising microbes. After 48 hours of incubation at 32^{0}C, the prominent colonies of bacteria were isolated and maintained as pure cultures. For the isolation of Potassium solubilising bacteria the adaption and enrichment method was applied.

Adaptation: The soil samples were sieved properly and the insoluble source of K (mica powder) was mixed in 1:1 ratio and these soil samples were incubated for 7-10 days.

Enrichment: 250 ml of Enrichment broth (P. Sugumaran and B. Janartham, 2007) was prepared and all the adapted soil samples were added to the enrichment broth. These flasks were kept on the shaker for 10 days with constant stirring at 120 rpm to enhance the microbial activity. After 10 days these samples were serially diluted up to 10^{-7} and were plated out on Alexandrov's medium (Aleksandrov et al. 1967). Alexandrov's medium is a selective medium used for the isolation of KSBs. Mica powder was used in

the media as the insoluble source of K. After 72 hours of incubation various colonies of KSBs were obtained.

Screening: Multiple colonies of PSBs and KSBs were isolated from all the regions, but they were screened out on the basis of their ability to solubilise Phosphorus and Potassium from the insoluble source of P (Tricalcium phosphate) and K (Mica powder) respectively in the medium by showing a clear zone formation on Pikosvaskya's medium for PSBs and Alexandrov's medium for KSBs. The National Botanical Research Institute medium or the NBRI medium (Nautiyal 1999) was also used to check for the zone activity. The isolated strains of PSBs and KSBs were spot inoculated on the plates containing Pikosvaskya's medium/NBRI medium and Alexandrov's medium respectively (Fig 2 & Fig 3). The solubility index was calculated by using the Khandeparkar's ratio (K-ratio) (Prajapati 2012)

Fig 2: Clear zone formation by PSB on NBRI medium

Fig 3: Clear zone formation by KSB on Alekzandrov's medium

K ratio = clear zone/colony diameter

Greater the K ratio more efficient the strain in solubilising P and K.

The solubilising index was also calculated for all the isolates by using the following formula (Edi-Premono et al. 1996).

$$\text{Solubilising index} = \frac{\text{colony diameter + clearing zone}}{\text{Colony diameter}}$$

The screened colonies were selected, isolated and maintained as pure cultures for further processing. The morphological and biochemical characteristics of the isolated PSBs and KSBs were studied (Table 2).

RESULT AND DISCUSSION

The results for screening of isolates (PSBs and KSBs) from different regions and their solubilisation potential was calculated for solubilisation of TCP (tricalcium phosphate) and Mica powder respectively. It was found that the isolates from Richai region PR_4 and KR_4 for PSB and KSB respectively gave the K-ratio of 0.714 and the solubility index of 1.7 and K-ratio of 0.52 and solubility index of 2.42 which showed the best solubilising potential out of all the other isolates from different regions (Table 1)

DISCUSSION

The isolates both PSBs and KSBs isolated from the Richai region were showing the best solubilisation results followed by Kamore, Singrouli, Maneri and Amarkantak respectively. The best 6 isolates were chosen for further biochemical analysis and morphology of these isolates was studied (Table 2). Further these isolates can be subjected to molecular identification (16S rRNA) to identify these strains.

CONCLUSION

Phosphorus and potassium are the most important mineral elements needed by the plants for their proper growth and metabolism, unfortunately the tropical soils are deficient in them thus microorganisms having the ability to

Table 1. Screening of isolates (PSBs and KSBs) for solubilisation of TCP (Tricalcium phosphate) and Mica respectively from the different sampling sites around Madhya Pradesh

Isolates	Sampling sites													
PSBs	Richai				Kamore				Maneri		Amarkantak		Singrouli	
Isolates	PR1	PR2	PR3	PR4	PK1	PK2	PK3	PK4	PM1	PM2	PA1	PA2	PS1	PS2
K-ratio [1]	0.5	-	0.6	0.714	0.4	0.06	-	-	0.2	0.2	0.1	0.1	0.5	0.2
Solubility Index [2]	1.5	-	1.6	1.7	1.4	1.05	1.0	1.0	1.2	1.2	1.1	1.1	1.5	1.2
KSBs	Richai				Kamore				Maneri		Amarkantak		Singrouli	
Isolates	KR1	KR2	KR3	KR4	KK1	KK2	KK3	KK4	KM1	KM2	KA1	KA2	KS1	KS2
K-ratio [1]	0.4	-	0.06	0.52	0.3	0.04	-	-	0.2	0.4	0.2	0.1	0.72	0.5
Solubility index [2]	1.4	1.0	1.06	2.42	1.3	1.54	1.0	1.0	1.2	1.4	1.2	1.1	1.5	1.5

1. K ratio = clear zone/colony diameter;
2. Solubilising index = $\frac{\text{colony diameter + clearing zon}}{}$

Table 2. Morphological and biochemical charecterisation PSB and KSB

ISOLATES	GRAM STAINING	MORPHOLOGICAL CHAR	CATALASE	OXIDASE	IMVIC[1]	SH 2	CH 3	CU 4	GA 5	UA 6
P1	Gram + rods	Creamish white,waxy appearance	-	+	-	+	+	+	+	+
P2	Gram + rods	Transparent smooth colonies	+	+	-	+	+	+	+	+
P3	Gram - cocci	Cream rough colonies	+	-	-	-	+	-	+	+
K1	Gram + rods	Transparent colonies	+	-	-	+	+	+	+	-
K2	Gram + rods	Rough opaque white colonies	-	+	-	+		+	+	+
K3	Gram - rods	Transparent smooth colonies	+	+	-	-		+	+	+

1-IMVIC- Indole, Methyl-Red ,Vogus-Proskeur, 2-SH-StarchHydrolysis, 3-CH-Cellulose Hydrolysis, 4-CU-Citrate utilization, 5-GA-Gelatinase activity, 6-UA-Urease activity

mineralise these elements can be used as biofertilisers in such soils without having any side effects as caused by the application of chemical fertilisers like environmental pollution, biological magnification etc.

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