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Isolation, Identification and Characterization of Phosphate Solubilizing Bacteria found in Rhizospheres soil of coal mines landfills of Korea, Korba and Surajpur district, Chhattisgarh, India

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Abstract: Phosphorus is an essential element for plant development and growth. Low phosphate solubility is one of the most important factors limiting the plant growth in various soils. Many microorganisms can enhance phosphate solubility, but little is known about the magnitude of their phosphorus solubilizing ability.
Occurrence of a good number of phosphate solubilizing bacteria (PSB) were isolated, purified and identified from twenty eight different representative soil samples collected from rhizospheric soil of coal mines landfills of Korea, Korba, and Surajpur district of Chhattisgarh, India. The present study focuses on the Phosphate Solubilizing capacity of bacteria based on the formation of visible or halo zone on Pikovskaya media (PVK). The dominance of Pseudomonas syringae (5mm) as major Phosphate Solubilizers, along with Bacillus subtillis (4mm) followed by Pantoea dispersa (3mm), Bacillus circulans (3mm). Use of these PSB as bioinoculants will increase the available P in soil, help to minimize the phosphate fertilizer application, reduces environmental pollution and promotes sustainable agriculture. So far the application of PSB in most examined traits was better than chemical fertilizer.
Keywords: Phosphate solubilizing bacteria (PSB), phosphates, Pikovskaya media (PVK), Rhizospheric soil.

I. INTRODUCTION

Phosphorus is an essential element for plant development and growth. It functions as one of the major players in the process of photosynthesis, nutrient transport, energy transfer, early growth and root formation, cell division, DNA and RNA formation, seed development, flower blooms, improvement in plant strength and to tolerance for unfavorable environmental conditions [1]. Most of the soils are deficient in phosphorus containing small fraction (0.05%) of total phosphorus of which only 0.1% is available to plants [2]. It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth [3].

Phosphate solubilizing microorganisms (PSM) play a significant role in making phosphorus available to plants by bringing about favourable changes in soil reaction in the soil microenvironment leading to solubilization of inorganic phosphate sources. Some microorganisms associated with different pant rhizosphere are able to solubilise inorganic insoluble P salts [4] [5].

Use of these microorganisms as environment friendly biofertilizer helps to reduce the much expensive phosphatic fertilizers. Phosphorus biofertilizer could help increase availability of accumulated phosphate by solubilization efficiency of biological nitrogen fixation and increase availability of Fe, Zn etc., through production of plant growth promoting substances [6]. Phosphate solubilizing microorganisms are found in all types of soils but their number varies with soil climate as well as history [7]. This study aims to investigate and characterize various species of phosphorous solubilizing microorganisms found in coal mines landfills of Korea, Korba and Surajpur district Chhattisgarh, India.

II. MATERIALS AND METHODS

A. Collection Of Soil Samples

Rhizosphere soil sample were collected from randomly selected 07 locations from plant growing in coal mine landfill area. Total 28 Soil sample were air dried and used for isolation and enumeration of phosphate solubilizing bacteria (PSB).

B. Isolation of Phosphate Solubilizing Bacteria

The collected soil samples were serially diluted using sterile water blanks and plated on Pikovskaya's Agar medium. The plates were incubated at 37^0C for 2-4 days. After incubation the phosphate solubilizing microorganisms were selected based on the zone of clearing around the colonies. The isolated phosphate solubilizing bacteria were purified by repeated culturing and maintained on Nutrient Agar slants at 4^0C.

C. Identification of Phosphate Solubilizing Bacteria

The isolated bacterial strains were identified using standard biochemical tests as listed in the Bergey s Manual of Determinative Bacteriology [8]. The specific bacteria were isolated by a culture-dependent method and identified by their 16S rRNA sequences.

D. Utilization of Carbon Sources

The utilization of carbon sources by PSB isolates were estimated in LB broth. Filter sterilized carbon sources are inoculated aseptically into the sterile medium at 1 percent level. The PSB cultures were inoculated at the rate of 1.0 ml and incubated at room temperature. The growth was observed by the turbidity of the broth read at 560 nm.

E. Measurement of P Solubilization Zone

The PSB strains were inoculated in solid medium and incubated for 48 hours at 37 ºC. After the incubation period, the diameters of the P solubilization zone produced around the colonies were measured.

F. Measurement of pH and Titrable acidity

PSB strains were isolated from the solubilization zone production, also known as the halo and were grown in LB broth and inoculated 1 ml to Pikovskaya's broth. After incubation period the pH was measured at different period of growth. The organic acid produced by PSB strain was estimated in terms of total titrable acidity of the culture filtrate [9]. Initially culture filtrates were centrifuged at 1000 rpm for 10 minutes. Five milliliter of supernatant was added with a few drops of Phenolphthalein indicator and titrated against 0.1N NaOH. The titrable acidity was expressed as ml of 0.1N NaOH consumed per 5.0 ml of culture filtrate.

III. RESULTS

A. Collection Of Soil Samples

Total 28 Rhizosphere soil sample were randomly collected from plant growing in mine landfills of Korea, Korba and Surajpur districts, Chhattisgarh, India (table-1). District Korea is divided into 2 blocks: Baikunthpur, Chirmiri. District Korba is divided into 3 blocks Kusmunda, Dipka and Gevra. Last Surajpur District is divided into 2 blocks Bhatgaon, Bishrampur.

Table 1

Sample collected from plant growing in mine landfills of Korea, Korba and Surajpur districts, Chhattisgarh, India

S.No	District	Coalfields	No. of samples
1	Korea, Chhattisgarh	Chirmiri Area	04
		Baikunthpur Area	04
2	Korba, Chhattisgarh	Kusmunda	04
		Dipka	04
		Gevra	04
3	Surajpur, Chhattisgarh	Bhatgaon	04
		Bishrampur	04
Total			28

B. Characterization Phosphate Solubilizing Bacteria

Stands for identification criteria of Phosphate Solubilizing Bacteria (PSB) Mainly 4 different kinds of PSB isolated code was PSB 2, PSB 5, PSB 8, and PSB 9 (Table-2).

Table 2
Characterization Phosphate Solubilizing Bacteria (PSB)

S.No	Characteristics	PSB strains Code No			
		PSB 2	PSB 5	PSB 8	PSB 9
1	Gram staining	+	+	-	-
2	Shape of cell	Rod	Rod	Rod	Rod
3	Motility	+	+	+	-
4	Spore	+	+	-	-
5	H_2S production	+	-	-	-
6	Indole Test	-	-	-	+
7	Methyl Red test	-	-		-
8	Voges proskauer test	+	-	+	-
9	Citrate utilization test	+	+	+	+
10	Urease test	-	-	-	-
11	Catalase test	+	+	+	+
12	Oxidase test	-	+	-	-

C. Utilization of Carbon Source

All PSB strains utilized for various carbon sources. The preferred carbon source varied from strain to strain. Most of the strains preferred glucose and fructose as carbon source (Table-3).

Table 3
Utilization of carbon source by PSB strains

S.No	Carbon Sources	PSB strains Code No.			
		PSB 2	PSB 5	PSB 8	PSB 9
1	Arabinose	+	-	+	-
2	Xylose	+	-	+	-
3	Sucrose	+	-	+	-
4	Lactose	-	-	-	+
5	Glucose	+	+	+	+
6	Fructose	+	+	+	-

D. Identification of PSB Strains

Based on the biochemical and morphological tests, PSB were identified at genus level. The specific bacteria were isolated by a culture-dependent method and identified by their 16S rRNA sequences. PSB 2 was identified as *Bacillus Subtillis*, PSB 5 as *Bacillus Circulans*, PSB 8 as *Pantoea dispersa* and PSB 9 as *Pseudomonas syringae* (Table 4).

Table 4
Identification of PSB strains

PSB Strains	*Identified PSB strains*
PSB2	*Bacillus subtillis*
PSB5	*Bacillus circulans*
PSB8	*Pantoea dispersa*
PSB9	*Pseudomonas syringae*

E. Measurement of P Solubilization zone and Titrable Acidity

The clear or halo zone was formed due to the solubilization of insoluble phosphates by acidification of association of either proton extrusion or organic acid secretion [10].

Table 5
Measurement of phosphate solubilization zone and Titrable acidity

PSB Strain	***Incubation time (hour)***	***Solubilization zone (mm)***	***Organic acid (0.1N NaOH consumed)***
PSB2	48	4	9.2
PSB5	48	3	6.8
PSB8	48	3	7.1
PSB9	48	5	11.5

IV. CONCLUSIONS

This study reveals that wide varieties of phosphate solubilizing bacteria are present in rhizospheric soil of coal mines landfills area of Korea, Korba and Surajpur district Chhattisgarh India. Result showed *Pseudomonas syringae* (5mm) has greater phosphate solubilization efficiency along with *Bacillus subtillis* (4mm) followed by *Pantoea dispersa* (3mm), *Bacillus circulans* (3mm). Use of these PSB as bioinoculants will increase the available P in soil, help to minimize the phosphate fertilizer application, reduces environmental pollution and promotes sustainable agriculture. So far the application of PSB in most examined traits was better than chemical fertilizer.

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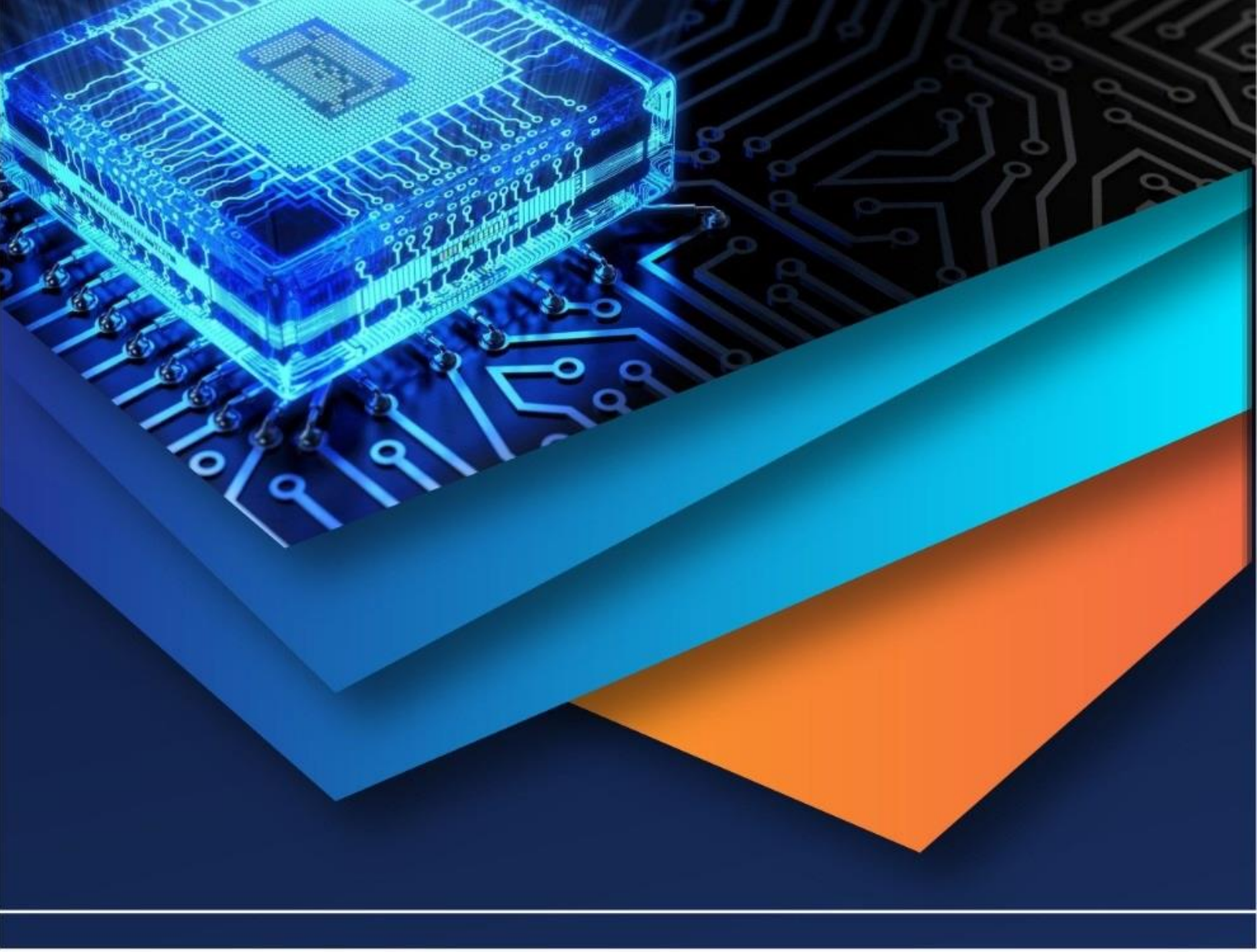

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