STUDY OF PHOSPHATE SOLUBILIZATION AND ANTIMICROBIAL ACTIVITY OF BACILLUS LICHENIFORMIS ISOLATED FROM RHIZOSPHERE OF CAJANUS CAJAN CULTIVATED IN MARATHWADA REGION

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Abstract: Rhizosphere is the region where soil and roots makes contact. It is subterranean habitat for microorganisms. The microbial growth is enhanced by nutritional substances released from plant tissues. The growth of plant is also influenced by the products of microbial metabolism. The inorganic phosphorus which is unavailable to plant is solubilized by bacteria and fungi. Biocontrol of plant pathogen is an alternative to chemical pesticides which causes environmental pollution and development of resistant strain. In the present work screening of phosphate solubilizing *Bacillus licheniformis* from rhizosphere of *Cajanus cajan* cultivated in Marathwada region was done by using pikovskaya's medium. About 70 different soil samples were collected from different rhizosphere region of red gram cultivated in Marathwada region. From this isolates of *Bacillus licheniformis* (PSBL) were isolated & discussed in this paper. Isolates of PSBL were identified depending upon their morphological, cultural characteristics & biochemical tests. The isolates of PSBL show diverse levels of phosphate solubilizing activity at different pH & temperature. The study of phosphate solubilizzation at different pH & temperature revealed that all isolates showed maximum phosphate solubilizzation at pH 8.0 & temperature 30ºC. Efficient isolates of PSBL were further tested for antimicrobial activity against *E. coli* NCIM2064, *Klebsiella pneumoniae* NCIM2719, *Salmonella typhimurium* NCIM623564, *Fusarium oxysporum* NCIM1281 and *Xanthomonas compestris* NCIM2956. It was observed that isolate PSBL 07 showed high PSE & antimicrobial activity; hence it could be exploited as biofertilizer and biocontrol agent.

Keywords: Antimicrobial activity, *Bacillus licheniformis*, phosphate solubilization, rhizosphere.

Introduction: *Cajanus cajan* L. (Red gram) is also known as pigeon pea (Arhar or tur in local language).It is one of the most extensively used pulses in India. Pigeon pea probably evolved in South Asia and appeared about 2000 BC in West Africa, which is considered a second major center of origin. It is a leguminous shrub that can attain height of 5 M. It is a major pulse crop of India. Among total pulses, the red gram accounts for 14.5% in area and 15.5% in productivity. Maharashtra is the largest producer with approximately 10.51 lakh hector area with average productivity of 6.03 Q/ha. Being important nitrogen fixing crop, it is widely grown for enriching the soil. Its deep penetrating roots helps in bringing nutrients from deeper layers of soil. The term rhizosphere was introduced by German scientist Hiltner. It is the region where soil and roots makes contact. It is a unique subterranean habitat for microorganisms [1]. The microbial growth is enhanced by nutritional substances released from plant tissues e.g. amino acids, vitamins and other nutrients. The growth of plant is also influenced by the products of microbial metabolism that are released into the soil [2]. The rhizosphere represents a tremendously complex biological system. It is a hotspot of microbial interaction. There are several beneficial microorganisms in the rhizosphere, which can improve soil quality, enhance crop production, protection, conserve natural resources and ultimately create more sustainable agricultural production and safe environment .Some microbes can produce metabolites that enter plant tissues and induce defense against other plant pathogen. Plant growth promoting rhizobacteria were first defined by Kloepper & Schroth [3]. Rhizobacteria may present a front line of defense for plants against pathogens against biotic and a biotic stresses. The nature and practice strategies for detection and characterizing systems for biological control of plant and soil born pathogen have been elucidated earlier [4]. They are becoming increasingly important component in the management of nutrients and harmful microorganisms in sustainable crop production. Phosphorus is an essential nutrient for plant. It is a major growth limiting nutrient & unlike the case for nitrogen there is no large atmospheric source for it [5].

Biocontrol of plant pathogen is an attractive alternative to chemical pesticides, which cause environmental pollution and development of resistant strain. The use of beneficial microorganisms (biopesticides) is considered as one of the most promising method for disease control. Most of the bacterial strains exploited as biopesticides belong to the genera *Agrobacterium, Pseudomonas & Bacillus* [6]. Biological control agents colonize the rhizosphere, the site requiring protection & leave no toxic residues, as opposed to chemicals. Today's main problem is the development of drug resistance among pathogens (both human & plants).So there is

continuous need of new antibiotics producing strains. In the present study screening of *Bacillus licheniformis* from rhizosphere soil of red gram cultivated in Mrathwada region was done. Then its phosphate solubilizing efficiency & antimicrobial activity was studied.

Materials & Methods:

Collection of rhizosphere soil sample: Soil samples were collected from rhizosphere of red gram cultivated in different regions of Aurangabad, Jalna, Latur, Osmanabad, Nanded, Parbhani, H ingoli and Beed district area in polypropylene bags & stored at low temperature for further study.

Isolation & identification of *Bacillus licheniformis*: The soil sample was serially diluted by serial dilution method. The dilutions from 10^{-2} to 10^{-7} were spread on nutrient agar medium (M001-Hi-Media).The plates were incubated at 30 ºC for 24-48 hrs. The isolates *Bacillus licheniformis* were identified by morphological, cultural & biochemical tests [7], [8].

Phosphate solubilization: The isolates of *Bacillus licheniformis* were spot inoculated on pikovskayaś agar (M520 Hi-media) plates containing: Glucose 10.0 gram, Tri calcium phosphate – 5.0 gm, $(NH_4)_2SO_4$- 0.50 gm, KCL-0.20 gm, $MgSO_4.7H_2O$- 0.10 gm, $MnSO_4$- Trace, $FeSO_4$- Trace, Yeast extract- 0.50 gm, Agar-15gm, in 1 liter of distilled water. The plates were incubated at 35 ºC for 4-7 days. After incubation phosphate solubilizing bacteria were detected by the appearance of transferent halo zone around its colony[9]. The zone diameter around the colony is measured and phosphate solubilizing efficiency was calculated by using following formula [10].

Phosphate solubilization efficiency = Solubilization diameter ÷Growth factor ×100 (1)

Detection of antimicrobial activity: The antimicrobial activity of isolates of PSBL was determined by using agar diffusion (well) method. The broth culture of isolates of PSBL was centrifuged. Then supernatant was loaded into the well bored and test organism seeded agar plates. For bacteria nutrient agar & for fungi sabouradś agar was used. The test organisms used were *E. coli* NCIM2064, *Salmonella typhimurium* NCIM623564, *Klebsiella pneumoniae* NCIM2719, *Xanthomonas compestris* NCIM2956 & *Fusarium oxysporum* NCIM1281. The plates were kept in freeze for 30 minutes and incubated at 30 ºC for 24 hours for bacteria & 25 ºC for 3-4days for fungi. The diameter of zone of inhibition was measured & noted in the table.

Optimization of pH and temperature: The phosphate solubilizzation was studied at different pH (4,5,6,7,8,& 9) and at different temperature (20,25,30,35,40&45ºC) in triplicate by using pikovskayaś medium and results were recorded.

Result & Discussion: Soil samples were collected from different rhizosphere region of red gram cultivated in Marathwada region & from this isolates of *Bacillus licheniformis* were isolated and identified. *Bacillus licheniformis* was confirmed on the basis of morphological, cultural & biochemical characters. On the nutrient agar after 24-48 hrs at 30 ºC, the young colonies were pale, opaque, flat, irregularly shaped and smooth. Isolates of *Bacillus licheniformis* were Gram positive, motile, rod shaped bacteria. They are Indole –ve, methyl red +ve, vogeus propskauer +ve, citrate +ve, catalase +ve, oxidase –ve, urease +ve, nitrate reduction +ve, starch hydrolysis +ve, Glucose +ve, xylose +ve, mannose +ve, arabinose +ve. Isolates of *Bacillus licheniformis* were subjected for detection of phosphate solubilization activity. Phosphate solubilizing efficiency was determined by using formula (Eq.1) & noted (Table 1).

Table 1 Phosphate solubilizzation efficiency of isolates of *Bacillus licheniformis* at pH 8 & temp 30 ºC

Sr. no	isolates of *B. licheniformis*	Phosphate solubilizzation Efficiency
1	PSBL01	220
2	PSBL02	233
3	PSBL03	300
4	PSBL04	200
5	PSBL05	210
6	PSBL06	333
7	PSBL07	374
8	PSBL08	300
9	PSBL09	250
10	PSBL10	185

The isolates of phosphate solubilizing *Bacillus licheniformis* (PSBL) were subjected for study of antimicrobial activity against *E. coli* NCIM2064, *Salmonella typhimurium* NCIM623564, *Klebsiella pneumoniae* NCIM2719, *Xanthomonas compestris* NCIM2956 & *Fusarium oxysporum* NCIM1281. Zone diameter was measured & noted (Table 2).

Table 2 Antimicrobial activity of isolates of phosphate solubilizing *Bacillus licheniformis*

Sr. No	Isolates of *B. licheniformis*	*E. coli* NCIM 2064	*Salmonella typhimurium* NCIM 623564	*Klebsiella pneumoniae* NCIM 2719	*Xanthomonas compestris* NCIM 2956	*Fusarium oxysporum* NCIM 1281
1	PSBL01	-	12	12	14	13
2	PSBL02	16	13	10	11	14
3	PSBL03	14	10	13	-	12
4	PSBL04	10	12	-	13	15
5	PSBL05	17	15	12	11	-
6	PSBL06	12	13	10	10	11
7	PSBL07	16	15	14	17	
8	PSBL08	14	11	11	10	13
9	PSBL09	13	14	12	-	15
10	PSBL10	18	-	10	13	14

Value represents diameter of zone of inhibition (mm).

Bergey's manual of systematic bacteriology was used for identification of *Bacillus licheniformis*. Phosphate solubilization was confirmed by observation of clear zone around the colony on Pikovskaya's agar plate. The zone is formed due to microorganism which cleaves phosphate molecules present in the medium. The study of phosphate solubilizzation at different pH & temperature revealed that all isolates of *Bacillus licheniformis* showed maximum phosphate solubilizzation at pH 8.0 & temperature 30ºC. It was observed that isolate PSBL 07 showed high PSE & antimicrobial activity against *E .coli* NCIM2064, *Salmonella typhimurium* NCIM 623564, *Klebsiella pneumoniae* NCIM2719, *Xanthomonas compestris* NCIM2956 & *Fusarium oxysporum* NCIM1281, hence it could be exploited as biofertilizer and biocontrol agent. Continuous and indiscriminate use of chemicals leads to development of resistant strain towards pesticides. Chemical pesticides also adversely affect the useful microorganisms in the soil. Bio-control of plant pathogen is an attractive alternative to chemical pesticides which causes environmental pollution & development of resistant strain.

Phosphate solubilization takes place through different microbial processes. Phosphate solubilizing bacteria are being used as biofertilizer since 1950s [11]-[13]. Bacillus licheniformis found more effective against *Fusarium oxysporum* [14]. Most of the genus Bacillus is known to produce several important peptide antibiotics like Bacitracin, Polymyxin, Gramicidin, Tyrosidine & Bacilysin. The compounds like Bacillomycin, Mycobacillin & Fungistatin obtained from Bacillus species are effective against molds and yeasts [15].

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