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Isolation, Biochemical Characterization and Production of Biofertilizer from *Bacillus megaterium*

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ABSTRACT- The isolation of phosphate solubilizing bacterial strains exhibiting high ability to solubilize soil phosphorus is a matter of great interest with high applicability. The use of phosphate solubilizing bacteria as inoculants simultaneously increases phosphate uptake by the plant and increase crop yield. Strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* species are among the most powerful phosphate solubilizers. In this present study different cultivated soil samples were investigated for the isolation of phosphate solubilizing bacteria by Pikovskayas agar media. We were found 2 bacterial strains SS1 and SS2 as a phosphate solubilizing bacteria. Both two bacteria are characterized by morphological and biochemical tests. The strain SS2 was confirmed as a *Bacillus megaterium*. Then *Bacillus megaterium* is used for the production of longer sustainable phosphate solbulizing biofertilizer. After an interval of 180 days it has $5x10^{13}$ CFU count.

Key-words- Phosphate solubilization; Soil bacteria; Plant-growth-promoting bacteria; *Rhizobacteria*; Phosphates; Biofertilizer

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INTRODUCTION

Bacillus megaterium was discovered and described [1] in 1884. *Bacillus megaterium* was used by Lwoff and Guttman in the studies that discovered lysogeny. *Bacillus megaterium* is one of the first bacteria's genome that has been fully coded. *Bacillus megaterium* is a biological fertilizer based on a selected strain of naturally-occurring beneficial eubacteria *Bacillus megaterium*. *Bacillus megaterium* is a gram positive, rod shaped, endospore forming bacteria. It is used as an effective soil inoculant. *Bacillus megaterium* have ability to solubilize phosphorus, which is good for plant. Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, transporting of the genetic traits [2].

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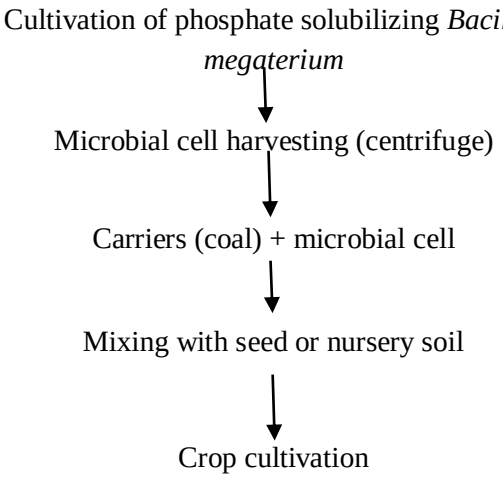
To achieve a high-yield in agriculture, it is necessary to apply phosphorus fertilizers that deliver the nutrients to plants [4]. However, the production of phosphorus fertilizer is a costly process that requires use of nonrenewable phosphate resource (phosphorite), mineral acids (sulfuric acid) and generates many environmental hazardous byproducts [5-6].

Phosphorus solubilizing microorganisms are ubiquitous in soils and could play an important role in supplying phosphorus to the plants [7]. The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorous in soil by Ahmad Ali Khan [8]. Microorganisms also solubilize sparingly soluble phosphates by decreasing the pH of the surrounding environment or acting on the calcium, iron, aluminum, and magnesium salts. In rice plantations, for example, a large amount of organic acids is generated, this increasing phosphorus availability to wheat [9]. *Bacillus megaterium* var. phosphaticum was used to create a bio-preparation called Phosphobucterin with the purpose of enhancing mineral phosphorus solubilization [10]. If phosphorus is present in the complex structures of the soil and, at the same time, readily decomposable carbon

sources, such as manure, are incorporated in the soil, phosphorus solubilization can be increased due to biological activity stimulation. This organic carbon increase may aid to complexing the soila luminum in acids, thus reducing the aluminum phosphate. Taking into account that the isolation of bacterial strains exhibiting high potential of soil phosphorus solubilization has been little studied in Romania, we believe it would be useful to approach this subject of great interest and practical applicability. Approximately 90% to 95% phosphorus formed in soil is present in insoluble forms [11], which are not utilized by plants, so phosphorus solubilizing bacteria play an important role to solubilize phosphorus, *P. vazquez* [12].

MATERIALS AND METHODS

This project is performed in Microbiology section of Chaperon Biotech Pvt. Ltd, Kanpur, India for duration of 8 Months. Isolation of *Bacillus megaterium* bacteria from soil sample was done on Pikovskayas agar media by dilution plate technique. Detection and estimation of the phosphate solublization ability of microorganisms have been possible using plate screening method. Phosphate solubilizers produce clearing zones around the microbial colonies in media. Insoluble mineral phosphates such as tricalcium phosphate or hydroxyapatite are contained in the media. The phosphate solubilizing bacteria is isolated for further identification by morphological & biochemical analysis. The morphological tests performed are Gram Staining & Endospore staining. The biochemical analyses performed were Catalase test, Starch hydrolysis test, Citrate hydrolysis test, Methyl red test, Vogous proskar test, Casein hydrolysis test, Indole test, Gelatine hydrolysis test, Urease hydrolysis test, Mannitol fermentation test, Carbohydrate fermentation test, and Salinity test etc. is shown in Table 1. The biofertilizer is prepared by immobilizing the bacterial cell and kept for 36 hours at 37^0C on rotatory shaker. After immobilization the bacterial culture is added to carrier i.e. charcoal and preserved for further use.



Flow chart of Production of Biofertilizer from *Bacillus Megaterium*

Table 1: Biochemical Characterization of samples

S. No.	Biochemical test	Sample 1 (SS1)	Sample 2 (SS2)
1.	Catalase test	(+)ve	(+)ve
2.	Starch hydrolysis test	(+)ve	(-)ve
3.	Citrate hydrolysis test	(-)ve	(+)ve
4.	Methyl red test	(-)ve	(-)ve
5.	Vogous proskauer test	(+)ve	(-)ve
6.	Casein hydrolysis test	(-)ve	(+)ve
7.	Indole test	(-)ve	(-)ve
8.	Gelatine hydrolysis test	(-)ve	(-)ve
9.	Urease hydrolysis test	(-)ve	(-)ve
10.	Mannitol fermentation test	(+)ve	(+)ve
11.	Carbohydrate fermentation test	(-)ve	(-)ve
12.	Salinity test	(+)ve	(+)ve

RESULTS

The bacterial cultures were isolated from soil sample collected from IPR and Mandhana, Kanpur, India. The bacterial samples were identified by morphological and biochemical tests for the presence of phosphate solbulizing bacteria then all the isolates were subjected to various tes-tes for confirming their identity. All the check isolated and standard strains formed completely white, round, smooth and shiny colonies. During microscopic observation all the isolates were found to be gram positive and rods shape. Presence of endospores was confirmed by endospore staining and all the isolated bacteria were subjected to various biochemical tests for the confirmation of their identity. Bacterial samples (SS1& SS2) shows clear zone into the Pikovskayas agar media, so it is confirmed that bacterial sample (SS2) is *Bacillus megaterium* organism. Test is positive so that immobilized bacterial culture is added in to carrier i.e. charcoal. The cell count of *Bacillus megaterium* is determined by colony counting in an interval of 1 to 180 Days .e. mentioned in Table 2.

Fig. 1: Serial dilution plate on Pikovskayas agar media

Table 2: Biofertilizer cell count at different time interval

Time interval (Days)	Per gram of biofertilizer contains Bacterial Cell count (CFU)
0	11×10^{13}
7	10.9×10^{13}
30	10.5×10^{13}
60	8×10^{13}
180	5×10^{13}

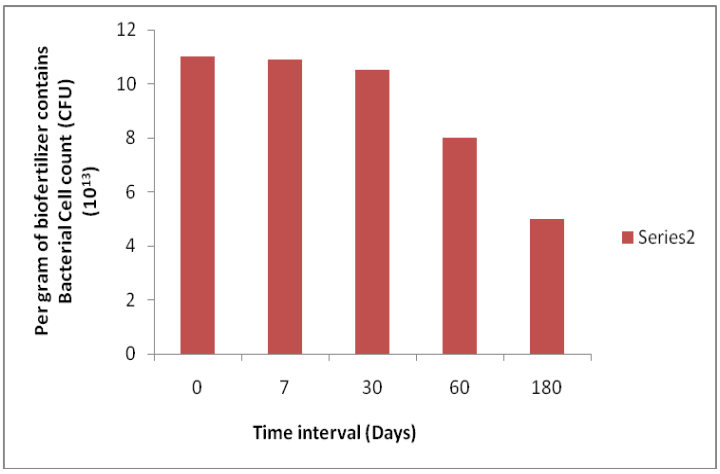


Fig. 2: Biofertilizer cell count at different time interval

DISCUSSION

The phosphatic fertilizer in current use requires a greater input that cannot be afforded by the farmers of the developing nations. Approximately 90% to 95% phosphorus formed in soil is present in insoluble forms that is not available for crop.[7] Microbiologists and soil scientists thus have a responsibility to society to find ways and means of making phosphorus available to crops, an economically efficient substitute for fertilization of crops. The solubilization of phosphatic compounds by naturally abundant PSM is very common under in vitro conditions [9]. Since most soils are deficient in plant-available phosphorus and chemical fertilizers are not cost-effective, there is interest in using rhizosphere competent bacteria (RCB) or soil microorganisms endowed with phosphate-solubilizing ability as inoculants to mobilize phosphate from poorly available sources in soil [10]. Although potential clearly exists for developing such inoculants, their widespread application remains limited by a poor understanding of microbial ecology and population dynamics in soil, and by inconsistent performance over a range of environments. Furthermore, promotion of growth of agronomically important plants, as a consequence of microbial inoculation, may not necessarily be associated with characteristics such as phosphate solubilization, which are manifest under laboratory conditions. Further, in order to ensure food security in developing countries, there is an urgent need for the sustainable intensification of agricultural production systems towards supporting productivity grains and income generation. In this context, novel, genetically-modified soil and region specific PSM and technologies for their ultimate transfer to the fields have to be developed, pilot-tested and transferred to farmers in a relatively short time [11]. In the present study many bacteria are isolated from soil among them two are predicted as *B. megaterium* by colony morphology. Then these strain SS2 is identified as *B. megaterium* by biochemical test. Then *B. megaterium* is use for the production of solid biofertilizer.

CONCLUSIONS

The present study deals with the production of phosphate dissolving *B. megaterium* as biofertilizer. It shows that strain SS2 was confirmed as a *Bacillus megaterium* species. It reviles that the *B. megaterium* has the ability to solubilize the free phosphate. Charcoal is used as carrier for the production of biofertilizer and it has the ability to give proper nutritional supply to the bacteria to carry on its life. The Cell count in the biofertilizer is also adequate after 6 months i.e $5x10^{13}$ CFU. Since the cell count is so good after 6 months than *B. megaterium* with charcoal can be used commercially.

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