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In-vitro Phosphate Solubilization by Maize Rhizosphere Bacteria.

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ABSTRACT

In present study isolation of Phosphate Solubilizing Bacteria was carried out from rhizosphere soil sample of maize plants growing in different areas of Una (Himachal Pradesh, India) and Bhatinda (Punjab, India). A total of 22 bacterial isolates were obtained which were subjected to primary screening by plate assay and secondary screening under liquid culture conditions. On the basis of secondary screening two isolates viz. MCKB3 and MCKB5 were selected. The isolates were grown in different conditions to assess the impact of various cultural parameters on in vitro Phosphate solubilization. Dextrose and ammonium nitrate were found to be the best suitable Carbon source and Nitrogen source at pH 7. The incubation time was also varied and found that the activity of the isolates was maximum after 96 hours. When both the isolates was grown under optimized conditions, the Phosphate solubilization activity increased to 18.7 and 19.6 µg/ml for MCKB3 and MCKB5 respectively. The isolates were then tested morphologically and biochemically to partially characterize them and they showed similarity with the genera *Bacillus* (MCKB3) and *Pseudomonas* (MCKB5).

Keywords: Phosphate solubilization, bacteria, rhizosphere, maize, biofertilizer

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INTRODUCTION

Maize is one of the very widely cultivated cereal crops of world. According to an estimate it is cultivated in 8.7m ha (2010-11) in India (http://farmer.gov.in/imagedefault/pestanddiseasescrops/normalmaizeproductiontechnologies.pdf). The productivity of maize is dependent on many factors including the optimum level of Phosphorus. Phosphorus (P) is one of the major nutrients which plays a crucial role in the growth and metabolism of plants. Sub optimal levels of P has been found to result in reduction in leaf growth [1] as well as significantly lesser grain yield [2]. The total phosphorus content in most of surface soil is very low [3]. Hence the farmers have to supplement the soil with phosphatic fertilizers which adds to the cost and also is not favorable for the environment [4]. Also, many workers [5,6] have reported that as much as t 75 to 90% of added P fertilizer in agricultural soils is precipitated by iron, aluminum and calcium complexes present in soils and thus become insoluble and unavailable to plants.

One of the possible methods for dealing with such type of problem is with help of phosphate solubilizing microorganisms. These microbes secrete various organic and inorganic acids and chelating agents which convert the insoluble phosphate into bioavailable soluble form [7] Therefore, Phosphate solubilizing microorganisms can be used as inoculants to increase crop yield by solubilizing insoluble P in soils [8].

MATERIALS AND METHODS

Isolation of Phosphate solubilizing Bacteria

A total of four soil samples were collected from rhizosphere of maize plant (*Zea mays*). Two fields from Una (himachal Pradesh) at the distance of 1 km from each other, and two from Bathinda (Punjab) at distance of 2 km. The samples were then air-dried, powdered and mixed well. The soil samples were serial diluted 10-6.An inoculum of 0.1 ml of appropriate dilution was spread over plate having Pikovskaya's media of pH- 7.0[9]. The composition of Pikovskaya's medium was as follows (g/L): Glucose- 10, Tricalcium Phosphate- 5, Ammonium sulphate- 0.5, sodium chloride- 0.2, Magnesium sulphate- 0.1, Potassium chloride- 0.2, yeast extract- 0.5, Manganous sulphate- 0.002, Ferrous sulphate- 0.002. The bacterial isolates obtained were purified by streaking on fresh Pikovskaya's media & purified isolates were transferred on to slants and stored in refrigerated conditions at 4^{o}C.

Screening of bacterial isolates

All the purified phosphate solubilising bacterial strains obtained were spotted over Pikovskaya's media having insoluble phosphorus source (Tri-Calcium Phosphate). After incubation period zone of hydrolysis and colony diameter was measured. After incubation period, the phosphate solubilization efficiency (PSE) was calculated on the basis of colony size and zone of hydrolysis as per the given formula [7]:

$$PSE\ (in\ \%) = (Z-C)/C \times 100$$

(Z= Solubilization zone diameter;C = Diameter of bacterial colony)

All the bacterial isolates were further subjected to Secondary screening by evaluation of solubilisation of insoluble phosphorus into soluble form in Pikovsakya's broth under agitated conditions by the method described by Narveer *et al.* [10]. The quantity of solubilized phosphorus in the supernatant was assessed by John's [11] method. The best two isolates were used for subsequent experiments.

Optimization of conditions

The conditions for maximum phosphate solubilisation were optimized by varying the incubation period (24 to 120h), pH (3 to 9), carbon source (dextrose, starch, fructose and sucrose), nitrogen source (beef extract, peptone, tryptone, ammonium chloride, ammonium nitrate, ammonium sulphate). During optimization process one of the conditions was varied in each experiment keeping the other variables constant.

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Partial Characterization of selected isolates

Selected strains were characterized for various morphological and biochemical characteristics according to Bergey's manual [12]

RESULTS AND DISCUSSIONS

Phosphate solubilizing bacteria (PSB) form a major group of bacteria which play a crucial role in the Phosphorus nutrition of plants. In the present investigation a total of 22 bacterial isolates were obtained from the four different maize rhizosphere samples. From the sample A taken from Una (Himachal Pradesh), seven isolates were obtained. Sample B and C were taken from Bathinda (Punjab). From these five isolates were obtained from these samples. Five isolates were obtained from Sample D taken from Una. The population of the bacteria is more in the rhizosphere as compared to bulk soil. Olivera *et al*. [13] conducted a study on the isolates obtained from the rhizosphere of the maize and found the bacterial isolates B17 and B5, which were reported to belong to *Bacillus* sp. and *Burkholdria* sp., were the most effective phosphate solubilizers. Similarly PSB's have been isolated from rhizospheres of several different plants like tomato, groundnut, maize, soyabean, mungbean, potato, mustard, wheat etc. [14-17, 7]

All the twenty two isolates were subjected to primary screening by measuring the Phosphate Solubilization Efficiency (PSE%) on the basis of zone of Phosphate solubilization. Maximum PSE of 83 % was found in case of MCKB3 while MAKU3 showed 7% PSE. In case of many isolates like MAKU 5, MDKU 2 etc. no zone of phosphate solubilization was observed.

Secondary screening was done after primary screening because some of the isolates did not show clear zone formation but may be having the ability to solubilize a good amount of phosphorous in liquid medium. In case of MCKB2 the isolate did not show the formation of clear zone but the amount of phosphorous solubilized by it in liquid medium was 12.6µg/ml and the isolate MDKU3 has PSE of 66% but solubilized only 7.3µg/ml of phosphorous in liquid medium. Many other workers have also questioned the reliability of primary screening on solid media plates [18,19].

The two isolates MCKB3 and MCKB5 that showed maximum phosphorous solubilization during secondary screening were utilized for further study. The conditions in which maximum phosphorous solubilization can takes place by the isolates were optimized. Incubation time was the first parameter and in the case of both the isolates incubation time of 4 days (96 hours) was best at which maximum phosphorous solubilization was observed that was upto 15.3µg/ml and 16.7µg/ml respectively (Fig. 1). Narveer *et al*. [17] have also reported maximum phosphate solubilization in case of *Bacillus* sp. NPSBS 3.2.2 after 96 hours of incubation. Many workers [20,21] have reported 72 hours of incubation to be most suitable while others have reported a time period of upto 10 to 15 days [22,23].

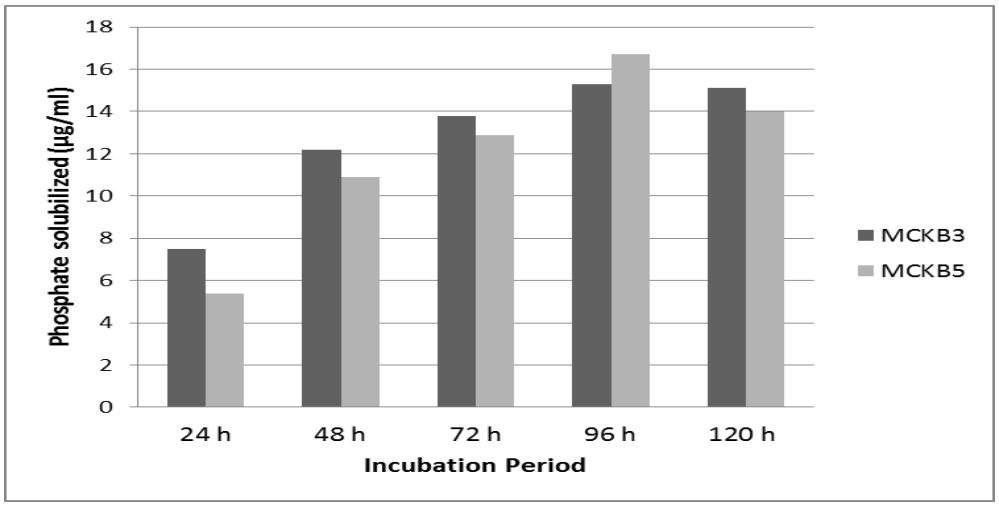

Figure 1: Effect of Incubation period on phosphate solubilizing efficiency (µg/ml) of isolates MCKB3 and MCKB5

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When the amount of phosphorous solubilized under shaking and non shaking conditions was compared, it was found that former was better than later in case of both the isolates. 15.6 µg/ml solubilization was observed in non-shaking conditions while and 16.9µg/ml under shaking condition in case of MCKB5. Similar trend was also observed case of the isolate MCKB3 also. respectively. Proper aeration, mixing and increased availability of the dissolved oxygen, nutrients, calcium phosphate (insoluble Phosphate) can lead to increase in phosphorous solubilization [24].

The pH of medium was also varied to determine the optimum pH of the isolates and best results were obtained at pH 7. On changing the pH in either direction i.e. any increase or decrease in medium pH adversely impacted the phosphate solubilization activity of both the isolates (Fig. 2). Similar results were obtained by the scientists Sahu *et al*.[22] ; Chen *et al*. [25], Kuntia *et al.* [17] where pH 7 was optimum.

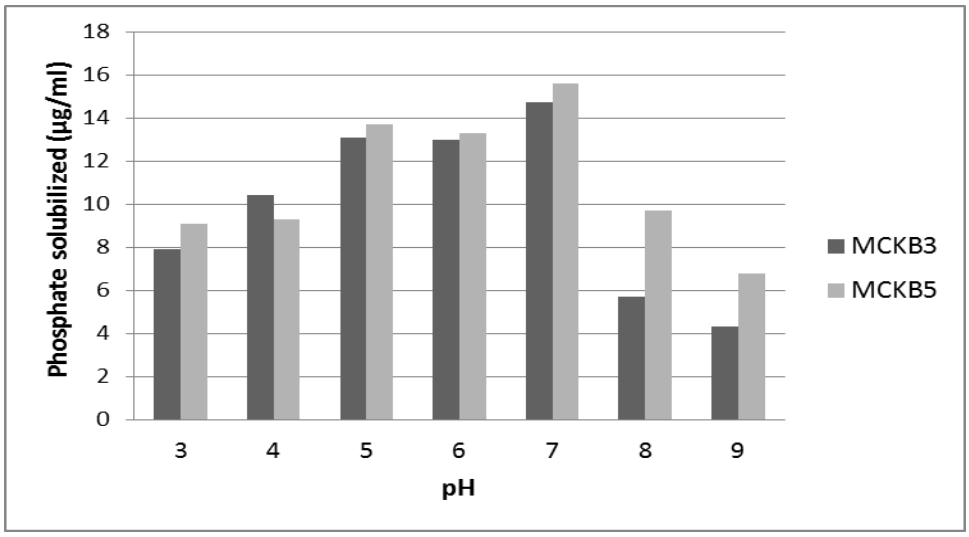

Figure 2: Effect of pH on phosphate solubilizing efficiency (µg/ml) of isolates MCKB3 and MCKB5

Various nutritional factors play a significant role in growth development & solubilization property of bacteria. So, different carbon and nitrogen sources were assessed for their impact on P solubilization efficiency of isolates. Out of the carbon sources which were tested, dextrose proved to be the best carbon source for both the isolates MCKB3 & MCKB5 (Fig. 3). However, the other Carbon sources also resulted in good level of phosphate solubilization. Glucose has been reported to be best carbon source by many workers *viz*. Pandey *et al.* [26]; Patel *et al.*[27]; Balamurugan *et al.* [19].

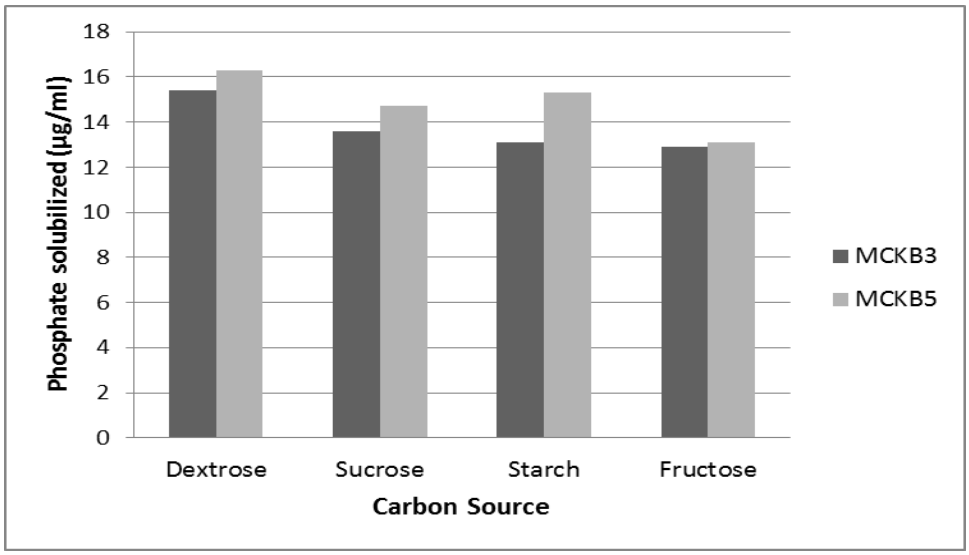

Figure 3: Effect of Carbon source on phosphate solubilizing efficiency (µg/ml) of isolates

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In the present research work ammonium nitrate proved to be the best nitrogen source with Phosphate Solubilization Efficiency of 15.6 µg/ml followed by ammonium sulphate (14.7 µg/ml) , ammonium chloride (13.3 µg/ml) in case of MCKB3. For MCKB5 the pattern was ammonium nitrate (16.9 µg/ml), ammonium sulphate (15.6 µg/ml), ammonium chloride (12.8 µg/ml). With organic nitrogen sources maximum phosphorous solubilization was shown when peptone was used as nitrogen source, however in case of MCKB3 the maximum phosphorous solubilization was shown with tryptone (Fig 4) Ammonium nitrate was reported as best carbon source by Nautiyal [28]. Several workers [29,30] have found the Ammonium ion to be a good source Nitrogen for microbial phosphate solubilization.

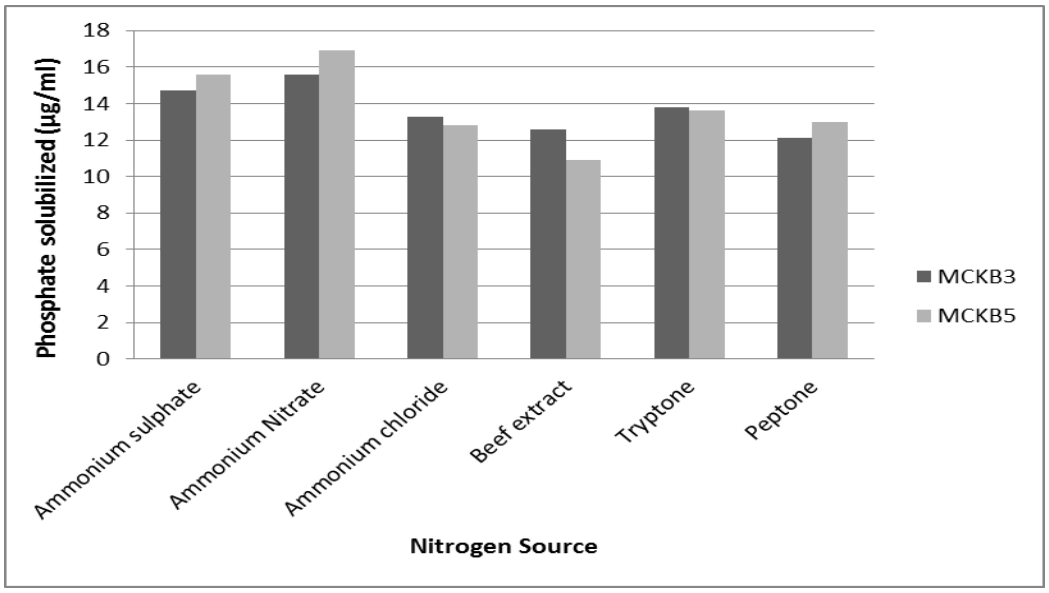

Figure 4: Effect of various Nitrogen sources on phosphate solubilizing efficiency (µg/ml) of isolates MCKB3 and MCKB5

It was found that the amount of phosphorous solubilized by the two isolate was increased when observed under all the optimized conditions of pH, incubation time, carbon source, nitrogen source and agitation. The isolate MCKB3 solubilized 19.4µg/ml of phosphorous and the isolate MCKB5 solubilized 21.2µg/ml of phosphorous under the optimized conditions.

Isolate MCKB3 was found to be gram negative, non-spore forming, rod shaped, motile, Indole negative, methyl red negative, Voges-Proskauer negative, Catalase positive, urease positive, citrate positive, glucose and lactose non-fermenting. While the isolate MCKB5 was gram positive, spore forming, non-acid fast, rod shaped, methyl red positive, voges-proskauer negative, Indole negative, Catalase positive, urease positive, citrate positive, fermentation test was positive. So by analyzing the results it seems that MCKB3 belongs to genus *Pseudomonas* and MCKB5 belong to the genus *Bacillus.* Thus the two isolates obtained from this study may be checked for their phospahte solubilization efficiency in soil to utilize them as biofertilizers.

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