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Role of Different Carbon Sources in Phosphate Solubilization

Microbiology

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ABSTRACT

Phosphate is an essential nutrient for the growth of plants and is supplemented to crops in the form of chemical fertilizers. In past decade tremendous studies on phosphate solubilizing organisms has been carried out. These organisms help in solubilizing the fixed phosphorus in soil and render it available to plants. The growth and efficiency of these organisms in turn is affected by the type of carbon sources. In the present study on Citrobacter freundii, efforts have been made to identify the role of various carbon sources in phosphate solubilization. Appropriate carbonaceous substrates exert a positive effect on phosphate solubilization. These phosphate solubilizing microorganisms are heterotrophs and solubilize insoluble phosphate by secreting organic acids. Therefore, the role of carbon sources is of utmost significance.

Introduction

The solubilization of rock phosphate using diverse types of C sources has been studied by several workers. Rose (1957) showed that glucose or xylose were the best energy sources for fungi in liquid medium, whereas Katznelson and Bose (1959) reported that either yeast extract or soil extract were essential for the proper growth of phosphate solubilizing organisms in liquid or solid medium. They observed that rock phosphate solubilizing bacteria, yeast and fungi utilized a variety of C compounds as energy sources, but the amount of phosphate solubilized varied significantly with different sources of energy. Gaur and Gaind (1983) reported that during 15 days of incubation in liquid medium, maximum rock phosphate solubilization by *A. awamori* was obtained with sucrose or mannitol as compared to glucose. Gaur and Sachar (1980) reported that, by increasing the concentration of glucose from 1 to 3 percent in the medium, the rate and the quantity of rock phosphate solubilized by *Aspergillus awamori* increased, and also greater acidity was produced by glucose at the higher concentration, which is an important factor in phosphate dissolution. Thus it is evident from different studies by various workers that the form of available carbon greatly affects phosphate solubilization. Under cultural conditions it has been observed that bacteria are more active in presence of hexoses and pentoses in the medium whereas fungi are equally effective in the presence of hexoses, pentoses as well as disaccharides. Carbon substrates, in soil are available in limited concentration for microbial growth; hence the organisms grow in close vicinity of roots in plant rhizosphere than in non- rhizosphere soil. The plant root exudates provide readily metabolizable carbon and nitrogen compounds for the growth of the heterotrophic forms and because of that the bacteria and fungi form an associative symbiosis with the root system to get substrates from the plant roots and provide mineral which normally could not be absorbed by the roots including phosphorus. Sperber (1958) observed dissolution of hydroxy apatite by bacteria, fungi and actinomycetes isolated from rhizosphere soil of leguminous plants. Similar observations with regard to higher populations of bacteria and fungi dissolving insoluble phosphates in rhizosphere as compared to non- rhizosphere have been made in wheat and maize (Ketznelsen,1959), oats (Louw and Webley,1959), soybean, groundnut , black gram and green gram (Bardiya and Gaur,1972), rye grass and wheat (Molla *et al*,1984).

Materials and Methods

Pikovskaya's media was used to study phosphate solubilization. Two types of media were prepared, one with Tri Calcium Phosphate (TCP) and other with Udaipur rock phosphate (URP), in concentration equivalent to 50 mg% P_2O_5 respectively. These media were further modified as follows.

The glucose in the media was replaced by 1% (w/v) of sucrose, fructose, maltose, lactose, galactose, xylose, mannose, mannitol and glycerol individually. These media were used to study the effect of C sources on phosphate solubilization.

Two sets (one with TCP and one with RP) of Erlenmeyer conical flasks (250 ml) containing 100 ml Pikovskaya's and modified Pikoskaya's broth (containing different carbon sources) were inoculated aseptically with 1.0 ml of inoculum in each. The inoculated flasks were incubated at 30°C ± 0.2 °C under static condition and shaken at 12 hr intervals up to 7 and 15 days for TCP and RP respectively. The medium was removed aseptically from the flask at periodic intervals to determine the content of water soluble P and change in the pH of the medium. Uninoculated flasks from each set were kept as control.

Inorganic phosphorus in the supernatant was estimated by chlorostannous reduced molybdophosphoric acid blue method described by Jackson (1973). The pH of the medium was measured by digital pH meter (Eutech cyberscan).

Results and Discussion

10 different C sources were considered as energy source for the organisms. These included monosaccharide sugars like hexoses- glucose, fructose, mannose, galactose and pentose sugar xylose, diasaccharides like sucrose, lactose, maltose and sugar alcohols like mannitol, glycerol. In presence of glucose the organism shows maximum solubilization of P, when TCP is used, followed by galactose and then sucrose. Complete order from maximum to lowest is as follows: Glucose> galactose> maltose> sucrose> fructose> lactose> mannose> xylose> mannitol> glycerol. Thus, simple sugars are preferred more as compared to sugar alcohols. For URP solubilization of P occurs in following order:-

Glucose > sucrose > fructose > galactose > mannitol > mannose = maltose > glycerol > xylose > lactose. Glucose is the most favoured C source for max solubilization. Similar results have been reported by Joshi *et al* (2012) for Aspergillus.

Effect of increasing concentration of glucose

Five different flasks having same basal media except that the concentration of glucose was increased from standard 1gm % were inoculated with *C. freundii* and incubated under static conditions for 7 days for TCP and 15 days for URP.

The results obtained show maximum solubilization in broth low in C concentration. It seems that suboptimal conditions for growth and biomass may be optimal for the production of metabolites which may be responsible for P solubilization. However, minimum threshold levels of C must be present to fuel the process of P solubilization.

Effect of CSL on phosphate solubilization

CSL is a cheaper source of sucrose and can be used as an alternative to glucose in the Pikovskaya's medium. The effect of three different concentrations of CSL on phosphate solubilizing activity was observed as mentioned in Table 3, the concentration of 3 % proved to be best for optimum solubilization of phosphate.

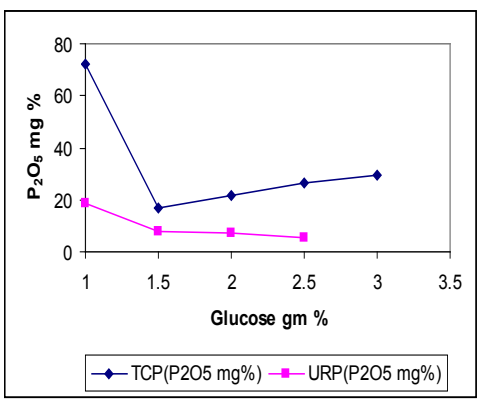
In this study the monosaccharides proved superior than disac-

charides, polysaccharides and sugar alcohols for RP solubilization while all the monosaccharides and two disaccharides, sucrose and maltose, proved best for TCP solubilization. Among all the monosaccharides hexoses were better than pentoses for both TCP and RP solubilization except mannose for TCP and fructose for RP.

Effect of increasing concentration of glucose

Five different flasks having same basal media except that the concentration of glucose was increased from standard 1gm % as shown in figure 6, were inoculated with *C. freundii* and incubated under static conditions for 7 days for TCP and 15 days for URP.

Figure 1
Effect of increasing concentration of glucose



The results obtained show maximum solubilization in broth low in C concentration. This is in contrast with the results obtained by Gaur and Sachar (1980) that showed that increasing the concentration of glucose from 1-3 percent the amount of RP solubilized by *A. awamori* increased. However, results are in agreement with findigs of Dave and Patel (1999) where 1 gm% was optimum for maximum solubilization. It seems that suboptimal conditions for growth and biomass may be optimal for the production of metabolites which may be responsible for P solubilization. However, minimum threshold levels of C must be present to fuel the process of P solubilization.

CSL is one of the basic raw materials in microbial industry. It is the water extract by- product resulting from the steeping of corn starch, gluten and other corn product. The used steep waters are concentrated to approximately 50 % solids and this is known as CSL. It is used in the commercial manufacture of feed stuffs and as a medium adjunct in the fermentation industries. CSL is a cheaper source of sucrose and can be used as an alternative to glucose in the Pikovskaya's medium. Its effect on phosphate solubilization was studied. 3 % concentration proved to be best for optimum solubilization of phosphate. In order to study this effect the Pikovskaya broth was modified by replacing 1% glucose with 1%, 2% and 3% CSL in three different flasks individually. Maximum phosphate solubilization up to 13.12 ±0.19 with TCP and 7.89 ± 0.19 was observed at 3% concentration of CSL.

In most of the cultivated lands the organic matter is low and therefore availability of phosphorus from mineralization process is also low. A large number of microorganisms including bacteria, fungi and actinomycetes are known to produce acidic metabolites which by change of soil pH or by direct chelation of metal cations, release fixed or insoluble phosphorus in available form. Such releases are more pronounced in plant rhizospheres where readily metabolizable carbon compounds are released by plant roots.

The plant root exudates provide readily metabolizable carbon compounds for the growth of the heterotrophic forms and because of that the bacteria and fungi form an associative symbiosis with the root system to get substrates from the plant roots and provide mineral which normally could not be absorbed by the roots including phosphorus. As already discussed the C source is an important parameter for production of organic acids. In the present study it was reported that monosaccharide sugars are preferred over disaccharides and sugar alcohols for better phosphate solubilizing activity. When the effect of various concentration of most preferred glucose on phosphate solubilizing activity was studied it was found that the organism brings about highest solubilization at low concentration of carbon.

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