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Full Length Research Paper

Optimisation of fermentation conditions of phosphate solubilising bacteria- a potential biofertilizer

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Abstract

Biofertilizers have become one of the most important component of integrated nutrient management for sustainable agriculture as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers. Phosphate solubilising bacteria are on the major source of biofertilizer as they have the ability to convert insoluble forms of phosphorus to an accessible form leading to increase in the phosphorus uptake by plants. The present investigation deals with optimisation of various parameters for mass production of Phosphate solubilising bacteria isolated from rhizosphere soil samples of Maize and Tomato plants. They were identified as *Bacillus megaterium* and *Pseudomonas aeruginosa*. Mass production was carried out at the optimised condition in submerged batch fermentation. These mass cultures can be used further as potential biofertilizer by packing in suitable carrier materials and added to rhizosphere soils directly or through application with the seeds.

Keywords: Rhizosphere, Phosphate solubilisation, Biofertilizer, *Bacillus megaterium, Pseudomonas aeruginosa*.

INTRODUCTION

Biofertilizers accelerate certain microbial processes in the soil or rhizosphere which augment the extent of availability of nutrients like nitrogen, phosphorus etc in a form easily assimilated by plants. Biofertilizers are low cost, renewable sources of plant nutrients which supplement chemical fertilizers and their use for agricultural improvement has been a topic of research for a number of years (Suslov *et al.,* 1982; Davinson, 1988; Kloepper, 1994; Glick, 1995). They can be used either for seed treatment or soil application.

Phosphorus is the second essential mineral nutrient for plant development and growth preceded by Nitrogen, making up about ~0.2% of plant dry weight. The concentration of soluble P in soil is usually very low, normally at levels of 1 ppm or less. The cell might take up several forms of phosphorus but the greatest part is absorbed in the forms of $HPO4^{2-}$ or $H2PO4^{-}$ (Beever *et al.,* 1980). Phosphorus solubilising bacteria play role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil phosphorus pools by solubilisation and mineralization (Hilda, 1999).

Several strains of organisms belonging to various genera such as *Aspergillus, Azolla, Azospirillum, Azotobacter, Alcaligenes, Arthrobacter, Acinetobacter, Bradyrhizobium, Bacillus, Burkholderia, Cyanobacteria, Enterobacter, Erwinia, endophytic diazotrophs, Flavobacterium, Pseudomonas, Rhizobium, Serratia* .etc grouped under 'Plant Growth Promoting Rhizobacteria' are presently being used as biofertilizers (Kannaiyan *et al.,* 2004).

PGPR exert a positive effect on plant growth by two ways, Direct and Indirect. Direct growth promotion can be through the synthesis of phyto hormones (Xie *et al.,* 1996), N_2 fixation (Christiansen, 1992), reduction of membrane potential of the roots (Bashan *et al.,* 1991),

synthesis of some enzymes that modulate the level of plant hormones (Glick *et al.,* 1998), as well as the solubilisation of inorganic phosphate and mineralization of organic phosphate, which makes phosphorous available to the plants (Krasilnikov, 1961; Gaur, 1972; Subba Rao, 1982). Indirect growth promotion is the decrease or prevention of deleterious effect of pathogenic microorganisms, mostly due to the synthesis of antibiotics (Sivan *et al.,* 1992) or siderophores (Leong, 1986) by the bacteria.

The objective of this study is to isolate and screen efficient strains of phosphate solubilising bacteria from the rhizospheric soils and optimise various growth parameters for their mass production in the fermentor.

MATERIALS AND METHODOLOGY

Isolation and identification of phosphate solubilising bacteria

The soil samples were collected from rhizospheric region of Maize and Tomato plants in rural parts of Bengaluru. Microorganisms mainly the bacterial strains were isolated by serial dilution of soil samples and an aliquot of 100µl from decimal dilutions were considered. The samples were spread onto the Pikovskaya's agar medium and incubated for 24 hours at room temperature for the detection of phosphate solubilising bacteria (Pikovskaya *et al.,* 1948). The prominent colonies showing a clear zone on the Pikovskaya's agar were selected and purified by repeated culturing on nutrient agar medium. The bacteria were identified and screened by their colony characteristics, Staining techniques, microscopic observations and biochemical characteristics and molecular characterization.

Optimisation of media

Media Optimization for mass culturing of both the isolates was carried out on Kings B- media. The medium was prepared with a pH set at 7.2. The media was sterilized at 121^{0}C for 15 minutes. After sterilization, the test cultures were inoculated and incubated at 37 °C for 5days at 250 rpm orbital shaker. Weight of the pellet was checked regularly at an interval of 24 hours by centrifuging 2ml of the culture broth.

Optimisation of pH

100ml of King's broth was prepared in four different 250ml Erlenmeyer flask with different pH (5-8). The media was sterilized at 121^{0}C for 15 minutes. After sterilization, the test cultures were inoculated and incubated at 37 °C for 5days at 250 rpm on orbital shaker. The pellet Weight was checked every day by centrifuging 2ml of the culture broth.

Optimisation of incubation temperature

100ml of King's broth was prepared in three different 250ml conical flask with the pH set to 7.2 and autoclaved at 121^{0}Cat 15 lbs for 15 minutes. After sterilization, the test cultures were inoculated and incubated at three different temperatures i.e., 28^{0}C, 33^{0}C, 37^{0}C and 39^{0}C for 5days at 250 rpm on orbital shaker. The pellet weight was checked regularly at an interval of 24 hours for a period of 5 days by centrifuging 2ml of the medium.

Optimisation of carbon source

100ml of King's broth was prepared in different 250ml Erlenmeyer flask with six different carbon sources viz., Glycerol, fructose, glucose, lactose, sucrose and starch at the concentration of 1.0% to the medium and the pH set to 7.2. The medium was sterilized at 121^{0}C for 15 minutes. After sterilization, the test cultures were inoculated and incubated at 33^{0}C and 37^{0}C for *Bacillus megaterium* and *Pseudomonas aeruginosa* respectively for 5days at 250 rpm on orbital shaker. The pellet Weight was checked for 5 days regularly at an interval of 24 hours.

Optimisation of nitrogen source

Effect of different nitrogen sources was checked on the growth of *Bacillus megaterium* and *Pseudomonas aeruginosa* using 1.0 % of the nitrogen source. 100ml of King's broth was prepared in different 250ml Erlenmeyer flask with six different nitrogen sources i.e., Tryptone, Peptone, Sodium nitrate, Potassium nitrate, Ammonium sulphate, Yeast extract. The pH of the medium was set to 7.2 and autoclaved. After, sterilisation the test cultures were inoculated and incubated at 33^{0}C for *Bacillus megaterium* and 37^{0}C for *Pseudomonas aeruginosa* for 5days at 250 rpm on orbital shaker. Weight of the cell pellet was checked every day for a period of 5 days.

Batch fermentation

1000ml of Kings B medium was prepared and sterilized at 121^{0}C for 15 minutes. After sterilization, the media was cooled and 3% inoculum of the isolated microorganism was added to the production media separately. All parameters like pH, temperature, rotational speed were set according to the optimized parameters. The turbidity of the media was checked regularly for every 4hrs until 48hrs for microbial growth using UV spectrophotometer

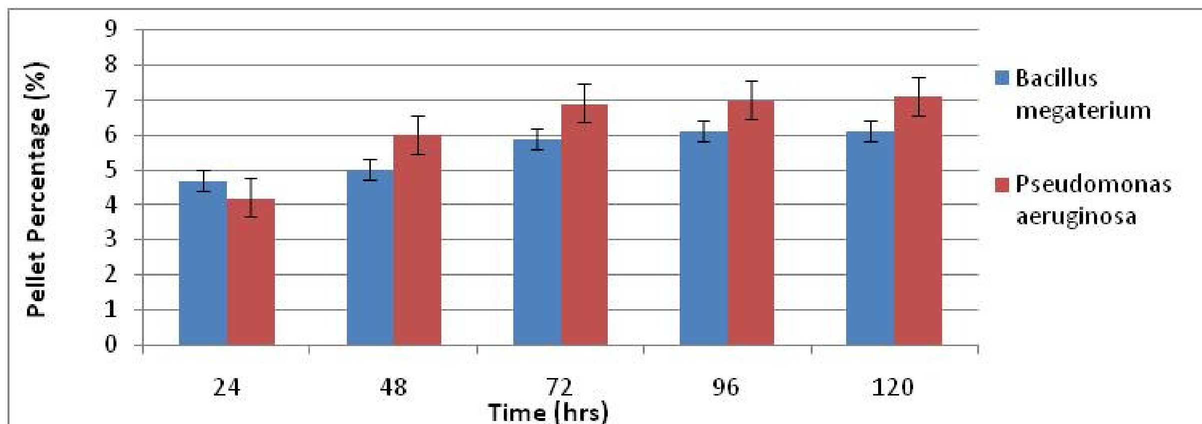

Figure 1. Optimisation of media for *Bacillus megaterium* and *Pseudomonas aeruginosa.*

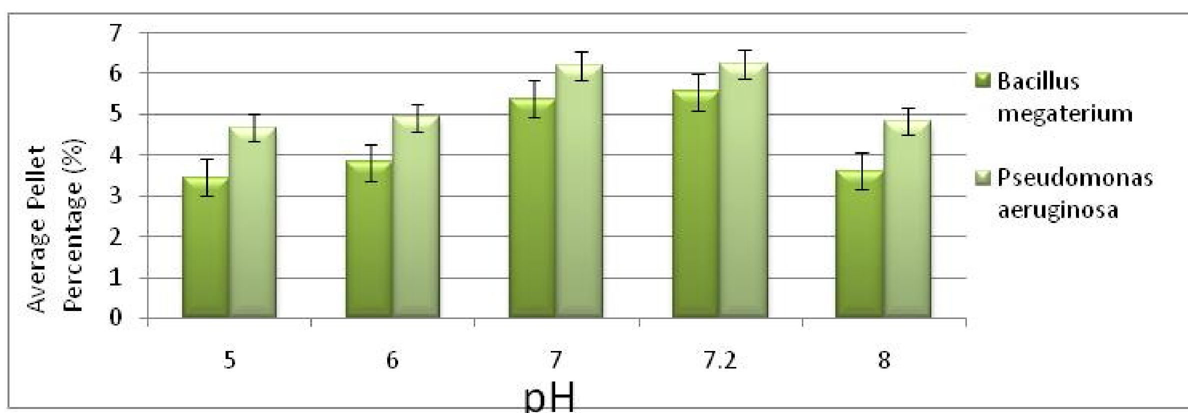

Figure 2. Optimisation of pH for *Bacillus megaterium* and *Pseudomonas aeruginosa.*

at 660nm.

RESULTS

The Bacterial colonies were obtained from the rhizosphere soil collected from the Maize and Tomato plants on Pikovskaya's media specific for phosphate solubilising bacteria which were identified as *Bacillus megaterium* and *Pseudomonas aeruginosa* using standard microbiological techniques and Molecular characterization by 16s RNA studies and sequencing.

Similarly phosphate solubilising bacteria mainly *Bacillus* species (Rajarathinam *et al.,* 1995; Bhattacharya *et al.,* 1998; Kole *et al.,* 1998) and *Pseudomonas* species (Nair *et al.,* 1977; Gupta *et al.,* 1998; Pal *et al.,* 2000) have been isolated from rhizosphere soil of different plants.

The pellet percentage which represents the growth percentage was calculated for both isolates grown in standard Kings B media at 37^0C and initial pH set at 7.2. The result showed that the maximum cell pellet was seen during the fourth day from inoculation and the average pellet percentage of *Bacillus megaterium* and *Pseudomonas aeruginosa* was found to be 5.56 and 6.24 respectively (Figure 1).

The optimisation of the pH was carried out to check the maximum growth of the phosphate solubilising bacteria at different pH value, where in both *Bacillus megaterium* and *Pseudomonas aeruginosa* proved to grow luxuriantly at a pH value of 7.2 and the average pellet percentage 5.56 and 6.24 respectively (Figure 2).

The optimisation of incubation temperature plays a major role in the growth of the microorganism as the microorganisms are metabolically active at optimum temperature. In the present study the optimisation temperature of the isolates *Bacillus megaterium* and *Pseudomonas aeruginosa* was carried out where in the isolates showed maximum growth at the incubation temperature of 33^0C and 37^0C respectively (Figure 3).

Carbon is one of the primary source of nutrition for the metabolic activity and growth of the microorganism. Thus, the present investigation deals with the analysis of different carbon source suitable for the phosphate solubilising bacteria as these bacteria play a major role as biofertilizers. The *Bacillus megaterium* isolate showed

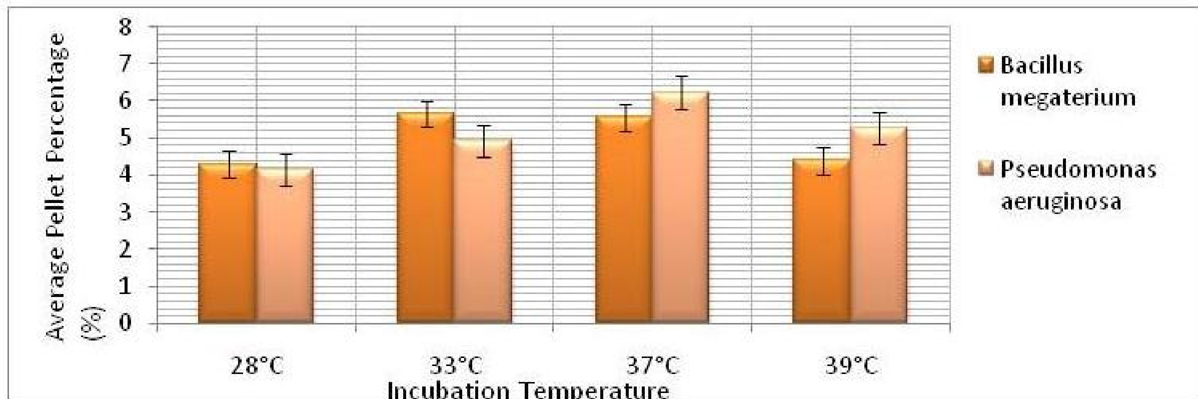

Figure 3. Optimisation of Incubation Temperature for *Bacillus megaterium* and *Pseudomonas aeruginosa.*

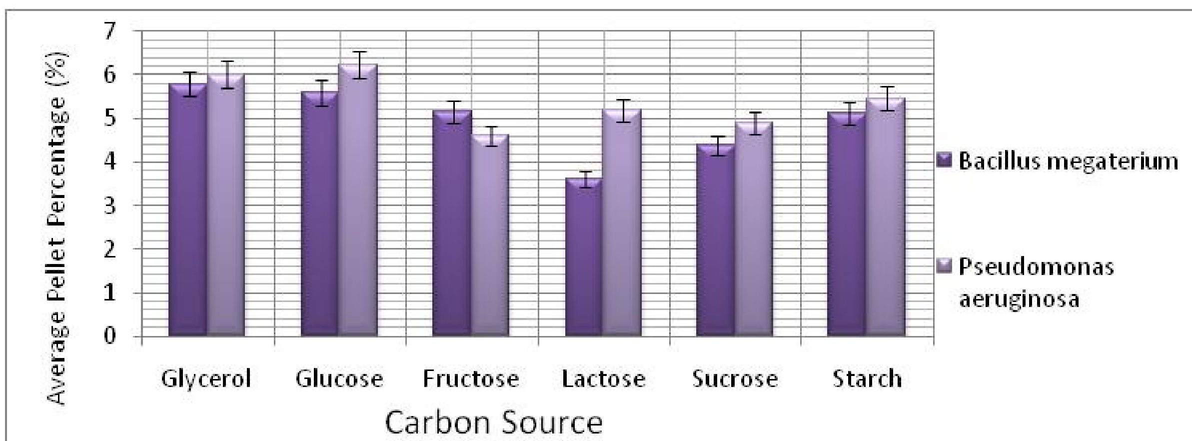

Figure 4. Optimisation of Carbon Source for *Bacillus megaterium* and *Pseudomonas aeruginosa.*

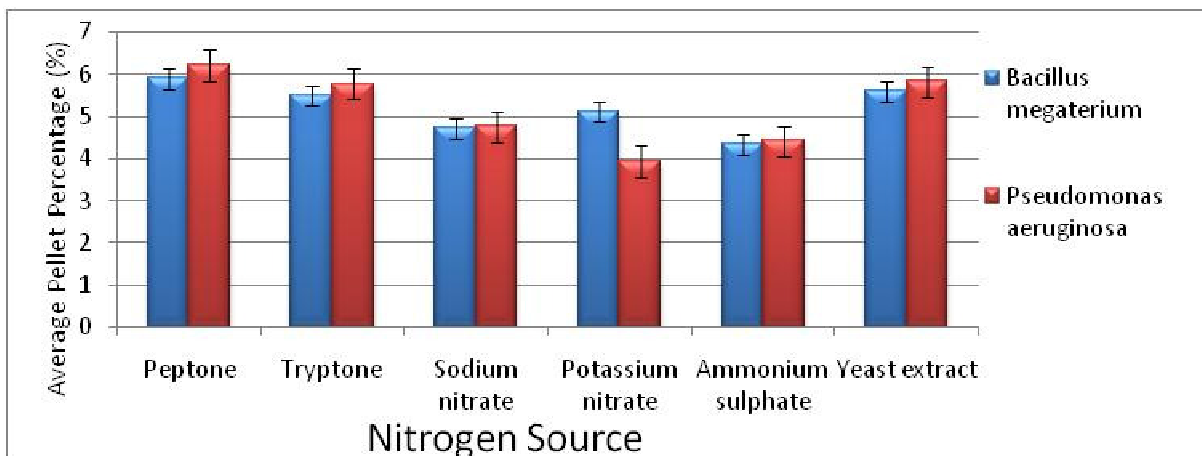

Figure 5. Optimisation of Nitrogen Source for *Bacillus megaterium* and *Pseudomonas aeruginosa.*

maximum growth with glycerol and *Pseudomonas aeruginosa* with glucose as a primary source of carbon in the media (Figure 4).

In the present investigation, the isolates *Bacillus megaterium* and *Pseudomonas aeruginosa* were grown with different nitrogen source to check their effect on the growth of the microorgansisms and it was found that both *Bacillus megaterium* and *Pseudomonas aeruginosa*

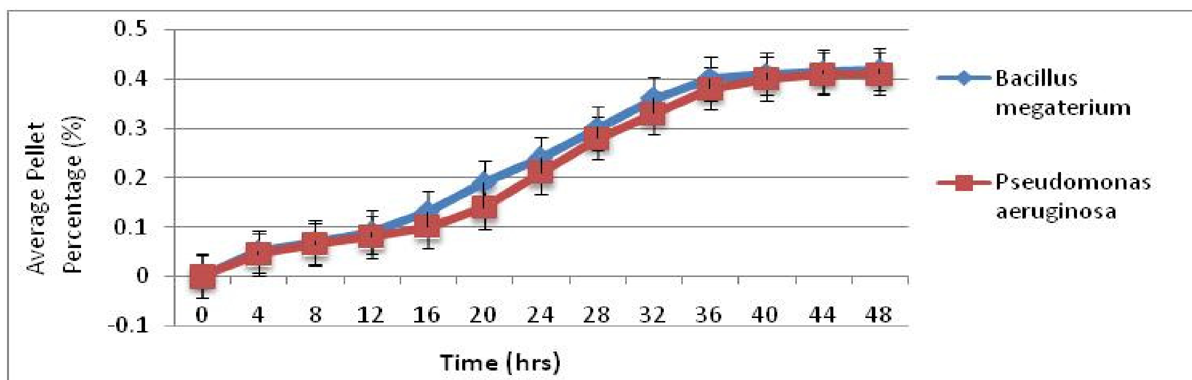

Figure 6. Growth curve of *Bacillus megaterium* and *Pseudomonas aeruginosa* under optimised conditions.

showed maximum growth with peptone with an average pellet percentage of 5.9 and 6.2 respectively (Figure 5).

The batch fermentation was carried out by adjusting all the optimised parameters and the turbidity readings inferred that the initial phase from ~0-10hrs after inoculation showed slight increase suggesting the Lag phase. Exponential growth pattern was observed from ~10- 40hrs i.e., the Log phase and then onwards a plateau observed suggesting a stationery phase. Thus, the present work suggests the optimum condition of the media for the commercial manufacturing of the biofertilizer (Figure 6).

DISCUSSION

In the present investigation both isolates were able to grow at different physiological parameters and utilize different carbon and nitrogen sources. However to conclude *Bacillus megaterium* grows best at pH 7.2, at 33^0C and when supplemented with Glycerol and Peptone as Carbon and Nitrogen source respectively and *Pseudomonas aeruginosa* grows best at pH 7.2, at 37^0C, Glucose as carbon and Peptone as nitrogen source.

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