ISOLATION AND CHARACTERIZATION OF ALKALINE PHOSPHATASE PRODUCING BACTERIA FROM MYLAVARAM SOILS, A.P., INDIA

V. Bhagya Lakshimi1, Dr. Shaheda Niloufer2

1FED, LBRCE, Mylavaram, bhagyavvrk@gmail.com
2 FED, LBRCE, Mylavaram, snw8481@gmail.com

ABSTRACT

Alkaline phosphatase producing bacteria were isolated and characterised from legume plant root zone from Mylavaram soils. Alkaline phosphatases are crucial in phosphate metabolism. The enzyme with its wide specificity and activity is potential in bioprocessing. Alkaline phosphatase is the most commonly used enzyme in immunoassays. Most of the microbial alkaline phosphatases are of significant application in diagnostic studies is obtained from bacteria. Alkaline phosphatase based biosensors play an essential role in environmental monitoring. Microbial alkaline phosphatases have a major application as biofertilizer. They are also useful for the evaluation of the soil quality and the perturbation occurring in agricultural fields. In this study Bacillus megaterium was isolated from rhizosphere soil sample from agricultural soils and production of alkaline phosphatase was carried out.

Key words: Alkaline phosphatases, bacteria, Mylavaram, rhizosphere, agriculture etc.

INTRODUCTION

Excessive use of chemical fertilizers added to soil and antimicrobial agents on plants are causing environmental damage (Kennedy et al. 2004). Several studies have been carried out to use microorganisms especially bacteria in crop improvement (Bhattacharyya and Jha, 2012; Ahemad and Kibret 2013). Along with Physico-chemical parameters, it is very essential to understand the possible roles of soil enzymes in order to maintain soil health and its fertility management in ecosystems. These enzymes, usually found in the soil, may have significant effects on soil biology, environmental management, growth and nutrient uptake in plants growing in ecosystems. Their activities may, however, be influenced by unknown cultural management practices either in a major or minor amount. Studies focusing the discovery of new enzymes from microbial diversity in the soil might be the most suitable practices that may positively influence their activities for improved plant growth as well as rendering the friendly biological environments in order to sustain other living beings. Phosphorus being macro nutrient is essential for growth and development of plants and involved in photosynthesis, energy transfer, signal transduction and respiration (Zaidi et al. 2010). Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crops. Phosphorus is an essential element for plant development and growth making up about 0.2 % of plant dry weight. Plants acquire P from soil solution as phosphate anions. In these forms, P is highly insoluble and unavailable to plants.

Alkaline Phosphatase enzyme hydrolyzes the phosphomonoesters from number of organic molecules like ribonucleotides, deoxyribonucleotides, proteins, alkaloids, phosphate esters and anhydrides of phosphoric acid (Holander 1971). Alkaline Phosphatase is a metalloenzyme (Mori et al., 1999) which shows its catalytic activity optima at alkaline pH (Rina et al. 2000). Strains from the genera Pseudomonas, Bacillus and Rhizobium are among the most powerful phosphate solubilizers (Rodriguez et al., 1999). Along with other soil quality parameters the role of alkaline phosphatase enzyme in solubilizing phosphate nutrient from soil was also studied at the five selected sampling stations. Mostly the bacterial strains like E.coli., Bacillus spp., Pseudomonas spp., Xanthomonas spp., Enterobacter spp., Acidovorans spp., Azotobacter spp. Arthrobacter spp. Acetobacter spp. Vibrio spp. etc., The fungi like Aspergillus spp., Chldosporium sp., Penicillium spp. Mucor spp., Fusarium spp., Curvularia spp., etc, are producing extra cellular alkaline phosphatase (Tigerstrom 1984; Prada et al.,1996).

Study Area:
Mylavaram is a village in Krishna district of the Indian state of Andhra Pradesh. It is located in Mylavaram mandal of Vijayawada revenue division. Samples of soil were collected from different fields of the leguminous plants in Mylavaram village of Krishna district of Andhra Pradesh.

Materials and methods:
Isolation of bacterial strain
Rhizosphere soil sample of legume plant was collected in a sterile container. The collected sample was serially diluted up to $10^7$ dilutions using sterile saline as a blank and the diluted samples were plated into the sterile nutrient agar plates using spread plate method. The plates were incubated at 37°C. The isolated colonies were further purified by streak plate method using sterile nutrient agar medium. The pure cultures were inoculated into sterile nutrient agar slants and nutrient broth for further use.

Screening for phosphatase activity
The isolated pure strains were screened for the production of extracellular phosphatase production using Hydroxyapatite (Soil extract agar) as a screening medium10. The pure cultures were streaked at the centre of the sterile Hydroxyapatite plates and the plates were incubated at 37°C for 24 hours. The observation was made to see the phosphate solubilization zone around the colony. Only positive and better zone formed was taken for further study. The CaCl$_2$ and KH$_2$PO$_4$ was sterilized separately and added before pouring into the plates. The pH was adjusted by sterile 1N sodium hydroxide solution before pouring into the plates. The soil extract was prepared by mixing 500g of soil in one litre of water and steaming for 20 minutes. The extract was filtered through normal filter paper.

Subculturing
The positive and better zone formed strain was sub cultured on luria agar plates. The pure cultures were retrieved every month and stored at 4°C.

Figure 1: Screening of bacterial culture

Figure 2: Identification of Halo zone

After screening for bacterial culture, the phosphate solubiling zone was found by performing plate assay in Hydroxyapatite medium. This Better zone obtained from the plate assay revealed the presence of phosphate solubilizer in isolated culture. The identification study showed that the isolated positive strain has been assigned as *Bacillus megaterium* (Identification was done by the department of Microbiology, Acharya Nagarjuna University, Guntur)

Conclusion
In this study *Bacillus megaterium* was isolated from rhizosphere soil sample from agricultural soils and production of alkaline phosphatase was carried out. The culture parameters like pH, substrates, temperatures, carbon sources, nitrogen sources and additives must be optimized for better production of alkaline phosphatase. Additionally growing the legume vegetables at least once in a season will help in increasing soil fertility as they have the capacity to fix atmospheric nitrogen through their root nodules. This reduces the use of chemical fertilisers like urea and ammonium nitrate.

References: