GENERAL INTRODUCTION

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Microorganisms including bacteria, fungi, algae and actinomycetes play important roles in the improvement of plant productivity. Many of these microorganisms fix atmospheric nitrogen, either symbiotically or in free-living condition and enrich the soil. Some solubilize insoluble phosphates in soil, thereby, making it available to crops. Others, provide important services to the plants by producing growth hormones and many other important metabolites.

The fast depletion of fossil fuel resources, increasing costs of chemical fertilizers and the environmental pollution caused through the use of these fertilizers calls for more attention to the use of microorganisms, with the aim of increasing the availability of nutrients to plants. Crop productivity is expected to be improved by application of microorganisms with desired features to the rhizosphere in the field.

A group of soil bacteria, collectively known as root-nodule bacteria infect the roots of leguminous plants and incite morphologically defined nodules on them. Within the nodule the bacteria differentiate into irregularly shaped bacteroids and fix atmospheric nitrogen. These bacteria were grouped under the genus Rhizobium in the eighth edition of Bergey's Manual of Determinative Bacteriology (Jordan and Allen, 1974). These are aerobic, gram negative rods, occurring singly or in pairs ; non-spore forming, often, with prominent granules of poly-β-hydroxybutyrate ; generally motile when young, having peritrichous, polar or subpolar flagella. Temperature requirement for optimum growth of these bacteria lies between 25°C to 30°C when grown on medium notably yeast extract mannitol agar. Recently, the root-nodule bacteria on the basis of their physiological (Graham, 1964 ; Moffett and Colwell, 1968 ; Norris, 1965 ; 't Mannetje, 1967), biochemical (Martinez De-Drets and Arias, 1972 ; Skotnicki and Rolfe, 1977) and genetic (Herberlein et al., 1967 ; Gibbins and Gregory, 1972 ; De Smedt and De Ley, 1977 ; Crow et al., 1981) characters have been taxonomically separated under two generic heads, Rhizobium and Bradyrhizobium (Jordan, 1984). The members of the genus Rhizobium are relatively fast-growing having mean generation time of 2 to 4 hours, and with a colony diameter of about 2 - 4 mm. These bacteria can use pentoses, hexoses or disaccharides as carbon sources while the members of the genus Bradyrhizobium are slow-growing ones having mean generation time of about 6 to 8 hours. The colonies of Bradyrhizobium generally do not exceed 1 mm in diameter and the members are unable to utilize disaccharides and certain organic acids of the TCA cycle as carbon sources. Strains of Rhizobium are characterized by their lowering of medium pH possibly due to production of extracellular organic acid while the strains of Bradyrhizobium meet an alkaline end point in yeast extract mannitol medium.

The root-nodule bacteria while inside the nodule, receive, a steady supply of nutrients from the host plant. In return the host plant receives a continuous supply of reduced nitrogen, biologically fixed from the atmosphere by the bacterial symbiont. The establishment of root-nodule bacteria-legume symbiosis is a complex development process which is not fully understood yet. However, the process is repressed by the presence of fixed nitrogen, such as, nitrate. Nitrate also serves as a potential source of nitrogen for plants and many bacteria. Besides nitrogen fixation, root-nodule bacteria possess an alternative route of nitrogen assimilation through assimilatory nitrate reduction. In this process nitrite is the intermediate product of nitrate reduction and a key intermediate in the reduction of nitrate to NH_4^+. Another pathway of nitrate reduction which is a dissimilatory type where nitrate appears to support bacterial growth by serving as terminal electron acceptor and, thereby, causing the generation of much needed ATP during anaerobic condition. In many instances the process results into disappearance of nitrate from soil by production of NO, N_2O or N_2 and in sum is known as denitrification. Dissimilatory nitrate reduction is a cascade of anaerobic respiration where nitrate is utilized as the terminal electron acceptor in lieu of oxygen.

The initiation of nodulation and nitrogen fixation by root-nodule bacteria is adversely affected by the presence of nitrate or ammonia in the soil. Fixed nitrogen exerts a strong repression also on the biosynthesis of nitrogenase enzyme. The presence of nitrogen fertilizers in the soil, as such, often becomes detrimental to the establishment of an effective nitrogen-fixing symbiosis. Furthermore, some strains of root-nodule bacteria under appropriate condition denitrify nitrate to the gaseous nitrogen oxides or to dinitrogen causing a net loss of nitrogen to the atmosphere from the soil. Denitrification also is a potential threat to the environment for it may result in the formation of nitric acid. Nitrogen fertilizer is the most important factor that limits plant productivity, as such provision of adequate supply of nitrogen to the soil is important for crop yield. The root-nodule bacteria-legume symbiotic system is, by and large, the most important diazotrophic systems in the world agriculture and about 80% of biological nitrogen fixation is indebted to symbiotic forms of life. However, biologically fixed nitrogen alone, as yet, cannot realize the full potential of the crop plants specially in view of its inherent biological limitations. In many instances conjugative use of chemical fertilizers along with bio-fertilizer components gives incremental benefits. Application of nitrogenous chemical fertilizers such as nitrate or ammonia, on the other hand, affecting the process of nodulation or inhibiting the functioning of already formed nodules by root-nodule bacteria results in reduced nitrogen assimilation efficiency. Therefore, it is essential to improve nitrogen assimilation efficiency of legumes by attempting to select or bioengineer "nitrogen tolerant" strains of root-nodule bacteria to confer maximum benefit to the host crop in the presence of varying residual fixed nitrogen. To meet the objective it is necessary to gain insight into nitrogen metabolism and the genetic capabilities of root-nodule bacteria, rhizobia and bradyrhizobia.

Next to nitrogen, phosphorus is the major plant nutrient and application of phosphatic fertilizers is essential for optimum crop yield. This is especially true for Indian fields since 98% of Indian soils contain insufficient amount of available phosphorus for plant growth. Were it not for soil microorganisms which play a significant role in mobilizing phosphate for use of plants, crop yield would be even at substantially decreased level.

Input of phosphate fertilizers to soil also helps nitrogen fixation and improved nodulation, dry matter yield and seed yield of legumes. Because of the fact that most of the phosphate in soil remain in the insoluble form and are not available to plants and most of the applied phosphate fertilizer is readily converted to insoluble form soon after application, responses of plants to phosphate application to soil are generally low. Clearly, increasing the solubility of soil phosphate _in situ_, preferably by microbial means would be a logical step towards meeting the yield potential of crop plants. The genetic properties of root-nodule bacteria to fix dinitrogen in the symbiotic association with legumes is now well established. Despite the super abundance of nitrogen as N_2 in the atmosphere and fixation of atmospheric N_2 by microorganisms into ammonia, nitrogen is limiting in soil. One of the reason is the denitrification process which causes loss of soil nitrogen. Denitrification causes loss of 30% nitrogen applied as chemical fertilizers all over the world. At the same time fixed nitrogen hinders the process of nodulation and nitrogen fixation. On the other hand, input of phosphate fertilizer helps to increase the level of nodulation and nitrogen fixation. Taking these facts together into consideration, it was our endeavour to study the capability, if any, of the strains of root-nodule bacteria to solubilize insoluble phosphate. We also surveyed a limited number of Rhizobium strains for their physiological capability to assimilate and dissimilate nitrate nitrogen. It was hoped that the strains of root-nodule bacteria having the complementary capability of phosphate solubilization, if any, with nitrogen fixation could profitably be exploited also as phosphate solubilizing biofertilizer to improve crop productivity. At present, the large demand for Rhizobium inoculum is encouraging research into the basic biology of Rhizobium-legume interactions. It is the expectation that inoculum of selected strains of Rhizobium will result in significant increase in crop yields. Whether the extent of the improvements that is expected will really be met under field conditions is still to be established. The present investigation tests the genetic and physiological capabilities of root-nodule bacteria in respect of phosphate solubilization potential and nitrate assimilation with a view to exploit these bacteria as biofertilizers. As such the investigation has been divided under two sections : (i) Solubilization of insoluble phosphate by root-nodule bacteria and (ii) Nitrate reductase activity of *Rhizobium meliloti*.

The long term objective of the present investigation is to identify and/or develop strains having high nitrogen fixation and phosphate solubilizing efficiency with decreased capacity of denitrification. The strains are expected to be used for inoculation of seeds for improved production of legumes. The succeeding crops, it is hoped, will also benefit from the residual nitrogen and the phosphate solubilization capacity of the resident strain in the soil.