CHAPTER I
INTRODUCTION

Azotobacter was isolated and described by Beijerinck in 1901 and has since then drawn the attention of microbiologists of many countries, owing to its great theoretical and practical importance. No other soil organism has been so extensively studied as Azotobacter, because of its nitrogen fixing ability.

One of the essential factors associated with soil fertility for plant growth is its nitrogen content. The study of the fixation of nitrogen and its fluctuation in soil has assumed considerable importance. The decline in soil nitrogen following practices of cultivation of agricultural crops and continuous disturbance of nitrogen equilibrium in natural soils can be prevented by taking various steps to maintain the nitrogen level in soil. The phenomenon of symbiotic nitrogen fixation in soil was first observed by Hellriegel and Wilfarth (1888) in the case of leguminous crops which were found to harbour nitrogen fixing organisms in the nodules of their roots. After Winogradsky (1895) discovered anaerobic nitrogen fixer Clostridium pasteurianum, Beijerinck (1901) isolated nitrogen fixing free-living organism, Azotobacter chroococcum. Azotobacter has been studied with regard to its contribution to soil nitrogen enrichment and adaptability under varying soil conditions.

Rice (Oryza sativa L.) is one of the most important food crops, and is grown in India year after year in the same soil. Among the various inputs responsible for increased yield of rice,
nitrogen is probably the most important. In studying the fixation of nitrogen in rice soil it should be remembered that these soils remain alternatively dry and water logged, according to seasons, year after year. In the water logged soils the conditions become distinctly unfavourable to *Azotobacter* and it is reasonable to assume that no great amount of nitrogen is fixed during the period. Some nitrogen fixation is possible particularly in the winter when the soil conditions are essentially suitable for the growth of *Azotobacter* and other N₂-fixing bacteria. The average soil temperature is suitably low and in the soil prevail aerobic conditions. Moreover, the organic matter in the form of roots and straw left in the field after harvest may be utilized as a source of energy by the N₂-fixing bacteria. The first part of the present work was taken up to estimate the population of *Azotobacter* in the paddy soils of Coimbatore under puddled and dry conditions. The morphological, physiological and biochemical differences of the different species of *Azotobacter* from the soil with particular reference to their N₂-fixing capacities were examined.

**Influence of plant roots on nitrogen fixation by Azotobacter:**

Dobereiner(1961) has reported 20 and 50 folds of increase in the number of *Beijerinckia* in the sugarcane rhizosphere and rhizoplane respectively.

Short gram-negative rods generally make up a larger proportion of rhizosphere microflora. These gram negative rods are mainly strains of *Pseudomonas* and *Azotobacter* (Alexander, 1961) and
evidence has been obtained that some of these organisms fix atmospheric nitrogen. Plant roots influence nitrogen fixation through rhizosphere effect. Vancura and Macura (1961) observed that organic acids and sugars which were present in the root exudation of wheat and barley could be used as energy substrates by *Azotobacter*, but the amino acids tended to inhibit the growth. Paul and Newton (1961) noted marked increase in nitrogen fixation by *Pseudomonas* spp. For the beneficial effect of bacterial inoculation introduced organism should be established in the rhizosphere of the crops. Alison *et al.* (1947 and Clark, 1940). They concluded that there is no preferential stimulation of nitrogen fixing bacteria in the rhizosphere.

Aiyer (1963) observed that establishment of *Azotobacter* in the paddy rhizosphere was facilitated by inoculation with blue-green alga *Tolypthrix tenue*. He also observed that at all stages of growth paddy rhizosphere supported a significant higher population of *Azotobacter*. Ogan (1979) observed that in the wild rice *Zizania aquatica* the bacteroids were more active on the surface soil and root surfaces. Another important observation was that rhizosphere of *Azotobacter* inoculated paddy crops supported significantly larger population of this organism than that of uninoculated paddy crops.

Okuda *et al.* (1961) showed that the photosynthetic nitrogen fixing organism *Rhodopseudomonas capsulatus* in association with *Azotobacter* fixed three times the amount of nitrogen fixed by
either of them alone. It was also observed by Okuda (1960) *Rhodopseudomonas capsulatus* supplies carbohydrates for the nutrition of *Azotobacter* and the latter organism supplied fatty acids for the nutrition of *Rhodopseudomonas*. This mutual beneficial effect may be responsible for fixing the atmospheric nitrogen in liquid medium, in quantities much higher than that fixed by any one of the organisms independently.

In tropics *Rhodopseudomonas* may be playing a vital role in nitrogen fixation and more so in association with *Azotobacter*. Taking this idea the rhizosphere effect of *Azotobacter* in association with *Rhodopseudomonas* on rice plant was determined in the second part of the present thesis.

**Plant response to Azotobacter as a fertilizer:**

With agricultural inputs particularly nitrogenous fertilizers becoming scarce and costlier, there is need for economizing and optimizing their use. One such possibility is through exploitation of microorganisms. *Azotobacter* being the widely known non-symbiotic nitrogen fixing bacterium, increased attention has been given to *Azotobacter chroococcum*, in different parts of the world to study its efficiency in fixing atmospheric nitrogen at the early stages of crop growth. Encouraging results are available to show that such studies may be useful in rice cultivation wherever soil nitrogen status is low and fertilizer nitrogen application is below optimal level. Very little investigation has been attempted in our country to find out the role of *Azotobacter* supplementing nitrogen nutrition, of rice crop. The third part of the study was initiated to fulfill the lacunae in this line.