Azotobacter is one of the most important and well known free-living heterotrophic bacterium which plays a beneficial role in nitrogen fixation. Beijerinck in 1901 discovered the bacteria and after that many reports on the existence of the bacteria have appeared. An enormous number of papers has been published on the morphology, taxonomy, physiology, distribution in nature and biological nitrogen fixation of Azotobacter species. The mechanism and biochemistry of nitrogen fixation have been fully discussed by Wilson (1952), Mulder et al. (1974) and Stewart (1976).

1. Morphology of Azotobacter:

The size and form of Azotobacter cells vary depending on species, strain, age of culture and growth conditions. Prazmowski (1913) reported that the dark brown A.chroococcum can change into a colourless race similar to A.vinelandii and A.agilis a yellow race similar to A.beijerinckii. He therefore concluded that the various species described are merely one greatly variable species. To prove this, Omeliansky (1913) found that pigment formation by Azotobacter depends on the composition of the medium. This is not sufficient to deny the existence of the various species altogether, especially since the genus itself is very variable, and it is difficult to establish the limits of its systematic position.
*Azotobacter* is morphologically classified into five groups, by Lohnis and Smith (1923). They described the gonidangium in *Azotobacter*. The group variations are (a) Cells are oval or bluntly rod shaped measuring 2×4 μ in size subject to great variations and is largest in *A. agilis*; (b) Cells are spherical measuring 2–3 μ in diameter in short chains or clumps arising by shortening of rod shaped cells; (c) Spherical cells or smaller rod shaped cells sometimes less than 1 μ in diameter, arising in aging cultures or under special conditions of nutrition, a process which Winogradsky (1938) described as monocytosis; (d) Roughly spherical cells or resting cells with contracted cytoplasm and a double combined cell wall. Their formation according to Winogradsky (1938) is favoured by simple organic compounds like butanol as the source of carbon and may be permanently suppressed by cultivation in glucose or mannitol media. At least one species, *A. agilis* lacks cyst formation and probably for this reason also they are resistant to dessication, which is characteristic of other members of the genus. Lastly (e) Large irregularly swollen or filamentous cells. These characters played an important role on the discussion of pleomorphism by the authors.

Dondero and Zelle (1953) described a more complicated scheme of development of big cells of *A. agilis*. According to them, the cells either reproduced their own type or reverted
to the normal. These either remained constant or sometimes reverted to the production of big cells.

Azotobacter cells contain granular inclusions, particularly fat like bodies of unknown nature. Chromatic material stainable by Robinov's method, is certainly present at least at some stages of growth, and Bisset and Hale (1953) claim the existence of vesicular nucleus in *A. chroococcum*. Smith (1935) found a variety of *A. agilis* that appears exclusively as giant cells. The behaviour of the big cell when transferred to fresh media has been described somewhat differently.

The genus *Azotobacter* is described as gram negative. But *A. beijerinckii* and *A. vitreum* show certain number of gram positive cells. Most *Azotobacter* species possess flagella although only *A. agilis* and *A. vinelandii* show conspicuous motility. Motility is absent in *A. beijerinckii*.

2. Taxonomy of *Azotobacter*:

The problem of taxonomic position of *Azotobacter* arose immediately after its description by Beijerinck in 1901. According to Hoffer (Bergey's Manual of Determinative Bacteriology, 1944) the family *Azotobacteraceae* can be regarded as consisting of a single genus *Azotobacter*. The genus *Azotobacter* is characterized by the following characteristics: Cells of various sizes from rod shaped to coccoid, with slimy capsules,
some species form cysts, cells not in frequently contain
inclusions, gram negative, peritrichous, do not grow or grow
poorly in media containing protein - capable of oxidizing
various carbohydrates to $\text{CO}_2$ and water. The majority cannot
grow below pH 5. The optimum growth range is between pH
7-7.5. Generally fixes nitrogen non-symbiotically. Molyb-
denum is required for nitrogen fixation.

While *Azotobacter* is recognized as a genus, the
differentiation into species is not clear cut. Since
Beijerinck's description of *Azotobacter* (1901) eighteen species
have been described.

1. *A. chroococcum*, Beijerinck, 1901
2. *A. agilis*, Beijerinck, 1901
3. *A. vinelandii*, Lipman, 1903
4. *A. beijerinckii*, Lipman, 1904
5. *A. woodstonii*, Lipman, 1904
7. *A. hilgardii*, Lipman, 1909
8. *A. smyrnii*, Lipman and Burgess, 1915
9. *A. indicum*, Starkey and De, 1939
10. *A. unicapsulare*, Batshinkaya and Kondratijeva, 1941
11. *A. galophilum*, Sushkina, 1945
12. *A. nigricans*, Krasilnikov, 1949
14. A. avaxii, Panosjan, 1950
15. A. lactificogenus, Kaufmann and Toussaint, 1951.
16. A. insignis, Derx, 1950
17. A. macrocytogenes, Jenson, 1954

A critical review of these species permits the conclusion, that the majority of the bacteria cannot be considered as valid species. A. smyrnii and A. hilgardii differ only in cultural properties from A. vinelandii, while differing essentially from A. chroococcum. Therefore, they cannot be included in the species A. chroococcum (Bergey's manual 6th edition), but in the species A. vinelandii. The only difference between A. woodstonii and A. chroococcum is that the former has weak nitrogen fixing activity, and as this cannot serve as a taxonomic criterion this bacteria was included in A. chroococcum. A. vitreum described by Lohnis and Westermann (1909) was later included by Lohnis and Smith (1923) in the species A. agilis. Smith in 1935 reviewed the matter and recommended the inclusion of this bacterium in the species A. vinelandii. But Krasilnikov included this species under A. agilis. The relatively small size of A. vitreum makes it more related to A. vinelandii. Lohnis and Smith (1923) and Hoffer (Bergey's Manual, 6th Edition) included the bacterium A. beijerinckii, and A. chroococcum into one species. According to Khudyakova (1950) A. fluorescens is a variety of A. vinelandii.
A. avaxi differs from A. chroococcum in that, in agar supplemented with solenchak soil extract it forms egg shaped cells devoid of slimy capsule. Panosyan who isolated this bacterium himself noted that it is very closely related to A. chroococcum.

A. insignis has not been described in detail. It should apparently belong to the genus Pseudomonas. According to Batshinkaya and Kondratjeva (1941) the only difference between A. unicapsulare and A. chroococcum is in capsule formation. A. acidium may be regarded as an acid forming variety of A. chroococcum. Sushkina (1949) who first described A. galophilum later considered it a halophilic variety of A. chroococcum and not a new species. A. agilis and A. vinelandii also have many features in common; because of growth in synthetic media, supplemented with organic and inorganic nitrogen sources, formation of diffusable pigment and active movement. (Hoffer; Bergey's Manual 6th Edition, 1954) united them as a single species A. agilis. Other investigators are of the opinion that the differences merit the existence of separate species (Jenson, 1954, Kluyver and Van Reenan, 1933, Winogradsky, 1938). Johnstone and Fishbein (1956) investigated the pigments of A. agilis and A. vinelandii by means of fluorescent analysis and found that they differ from each other. Each of these species has distinct biochemical properties, which manifest in different ability to oxidize Krebs cycle intermediates. From
the foregoing account it is clear that they merit the constitution of two independent species.

Starkey and De (1939) isolated from Indian rice soil an organism which they named *A. indicum*. It differs morphologically from other *Azotobacter* species by smaller size of its cells which contain large fat inclusions, and physiologically by its slow growth and copious formation of terraceous slime, and particularly by its ability to grow and fix nitrogen over a pH range of 3.9. Kaufmann and Toussaint (1951) described another nitrogen fixing organism *A. lecticogenus*. According to description it resembles *A. indicum*, in its morphological characteristics, but it is motile. *A. macrocytogenes* described by Jenson (1954) is closely related to *A. indicum* in physiological and ecological properties differing from the later morphologically and cytologically.

De Ley and Park (1968) on basis of the base pairing studies subdivided *Azotobacter* as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>(%G+C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. chroococcum</em></td>
<td>64.8 - 66.0</td>
</tr>
<tr>
<td><em>A. vinelandii</em></td>
<td>65.8 - 66.5</td>
</tr>
<tr>
<td><em>A. beijerinckii</em></td>
<td>66.2</td>
</tr>
<tr>
<td><em>A. naspali</em></td>
<td>63.2 - 64.6</td>
</tr>
<tr>
<td><em>Azotomonas macrocytogenes</em></td>
<td>58.2 - 58.6</td>
</tr>
<tr>
<td><em>Azotomonas insigne</em></td>
<td>56.9 - 57.9</td>
</tr>
<tr>
<td><em>Azotococcus agilis</em></td>
<td>52.5 - 53.5</td>
</tr>
</tbody>
</table>
Since *A. chroococcum*, *A. vinelandii* and *A. beijerinckii* had the same base ratios they were included in the genus *Azotobacter*. Species *macrocytogenes*, and *insigne* were included in the second genus *Azomonas*. The base pairing ratio being different for species *agilis* from the other two genera this was placed in the genus *Azotococcus*.

According to the latest *Sergey's Manual of Determinative Bacteriology 8th Edition* (Becking, 1974) only four species namely *A. chroococcum*, *A. beijerinckii*, *A. vinelandii* and *A. paspali* are recognized. The species *A. agilis*, *A. insigne* and *A. macrocytogenes* are now included in the genus *Azomonas* and *A. indicum* is now included in the genus *Beijerinckia* and is known as *Beijerinckia indica*.

3. **Physiology of Azotobacter**

There is a great variation in the capacity for nitrogen fixation by *Azotobacter*. When *Azotobacter* is grown in association with cellulose decomposing bacteria, nitrogen fixation is stimulated. *A. chroococcum* fixes more nitrogen when grown in association with a capsulated organism *Aerobacter aerogenes*. Members of granulated bacteria group were found capable of fixing nitrogen themselves; this power becoming very pronounced in the presence of *Azotobacter*. According to Gerlack and Vogel (1902), *A. chroococcum* is able to fix large quantities of atmospheric nitrogen when grown in pure culture in the
presence of salts of organic acids or sugars. When *A. vine-
landii* is contaminated by an aerobic spore former *Bacillus*
circulans, the former fixed considerably more nitrogen than
it did alone.

*Azotobacter* utilizes a number of hexoses (glucose),
pentoses, alcohols (mannitol) and salts of organic acids such
as malate, lactate, butyrates and succinates. Cellulose is
available to *Azotobacter* only when cellulose decomposing
bacteria are present but not directly (Pringsheim, 1909).
Ammonium salts are utilized more readily than nitrate as a
source of nitrogen. The organism can withstand 10% of MgSO₄.
*Azotobacter* resists quick drying and is sensitive to high
temperature; the cells are destroyed by heating for a few
minutes at 55°C (Stapp and Ruschmann, 1924).

4. Ecology of *Azotobacter* population:

Beijerinck and Van Delden (1902) demonstrated that
*Azotobacter* is of universal occurrence in the soil. It was
reported in most of the soils in Java, in all soils in India,
in half of Polish soils, 33% of the cultivated soils in Japan
and practically in all soils in China. Lohnis and Westermann
(1909) found twenty one different types. Lipman and Burgess
(1915) reported a number of forms in American soils. Nair
and Satyadas (1964) stated that the black soils had the highest population of *Azotobacter* followed by red alluvial and sandy soils. Verma *et al.* (1966) found that high clay content was not favourable for the growth of *Azotobacter*. Mishustin (1970) observed that in poor soils it has short span of life. Rangaswamy and Sadasivam (1964) found that surface soil of paddy fields harboured more *Azotobacter* than sub surface soil; organic matter phosphorus and calcium contents seem to influence the population, but N and K contents had little influence.

Iswaran and Abiswar Sen (1958) reported that the favourable pH range for *Azotobacter* is between 7.6 and 9 and below pH 5.8 it did not grow well. This was confirmed by Nair and Ramaswamy (1965). Ibrahim (1974) obtained negative correlation between *Azotobacter* population and soluble salt content of the soil. Mishustin (1954) observed that *Azotobacter* started to multiply at a soil relative humidity of about 25-40 per cent with an optimum at 60 per cent relative humidity. Malek (1971) reported that the organism was resistant to drought, but optimum activity was found to be 60 per cent water holding capacity. Ahrens (1972) noted that microbial counts decreased with progressive drying of the soil and the loss of soil moisture was more harmful than submergence, however the presence of calcium carbonates, increased the tolerance of the organism to submergence. Rao *et al.* (1973) observed a decrease in nitrogen fixation in upland soils.
Pathak and Shrikhande (1953) observed that a soil temperature of 40°C was found to be optimum for nitrogen fixation. Schmudt-Lorens and Rippel-Baltes (1957) reported that an oxygen pressure of 0.04 atm was optimal for rapid growth and efficient fixation of nitrogen. This was confirmed by Parker and Scutt (1960). The normal rate of Simazine stimulated the growth of Azotobacter population (Gaur et al., 1978). Results obtained by Hegazi and Ayoub (1979) show that organic carbon content, soluble salt content, the type of soil and the depth affected the population of Azotobacter.

5. Azotobacter population in rhizosphere:

Hiltner (1904) introduced the concept of the 'Rhizosphere' to express the zone of increased microbiological activity immediately around the root of higher plants. After Lipman and Starkey (1935) found the abundance and increased activity of the microorganism in the root zone more attention was paid to Azotobacter species because of their capacity to fix nitrogen and synthesize growth promoting substances. The possibility of bringing about nitrogen fixation and increasing crop yield through inoculation of soil with free-living bacteria was attempted by Voorhees and Lipman in 1907. As plants and microorganisms are closely associated the inoculated bacteria should adopt itself in the zone of root system of the plants. Wide divergence of opinion exists in literature as to
the relative preponderance of *Azotobacter* species in the rhizosphere and in the non-rhizosphere soil. The results depend on many other factors such as soil moisture, nature and species of the crop, fertility status of the soil and the nature of other microflora present in the soil and in the rhizosphere.

Different methods were employed to collect the rhizosphere samples by different workers. Starkey (1929) preferred to use scrapings from the root for microbiological work on rhizosphere. Clark and Mitchell (1940) have occasionally employed root surface scrapings. But other workers Thom and Humfeld (1932), Clark and Thom (1939), Clark (1948) have used samples containing whole root with adhering soil. Low and Webley (1958) described a different method where selected plants were uprooted carefully with roots and the plant roots were cut off. The entire root system with the adhering soil was then immersed in sterile water. Serial dilutions were made from this solution and the content in the flask were evaporated to get the dry weight of the rhizosphere soil.

Results obtained by Allison et al. (1947) and Clark (1948) showed that there was little or no preferential stimulation of the nitrogen fixing bacteria in the soil. Brown et al. (1962) in their studies of the distribution of *Azotobacter* in the rhizosphere of some crop plants observed very little
rhizosphere effect and found that frequently *Azotobacter* was suppressed. However, the same authors (Brown et al. 1964) studied the *Azotobacter* population in the rhizosphere of the inoculated crop and found very high population both in the rhizosphere and non-rhizosphere soil. Montifel et al. (1950) observed that the rhizosphere of the oak favoured the growth of *Azotobacter*. Daste (1950) reported active multiplication of *Azotobacter* in the rhizosphere of *Triticum sphaerium*, followed by progressive reduction in their numbers suggesting an inhibitory action of roots. Meshkov (1950) observed that the rhizosphere solution from peas stimulated the development of not only *Azotobacter* but also other soil bacteria much more than that from corn. Vainova and Raikova (1954) studying the distribution of *Azotobacter* in the soils of Bulgaria observed that the *Azotobacter* population in relation to many cultivated plants was higher in the rhizosphere than that of soil outside the rhizosphere.

Parker (1951) reported significant amount of nitrogen being fixed by non-symbiotic nitrogen fixing bacteria developing in the rhizosphere of non-leguminous plants utilizing the organic matter coming from the roots as energy source.

Rovira et al. (1971) pointed out that rhizosphere nitrogen fixing bacteria are heterotrophic microorganisms and they depend upon the host plants for their supply of energy and
carbon. In the rhizosphere the energy yielding components come from three different sources one of which is the root exudates. Balandreau et al. (1978) also observed that in their studies on the detection of preferential sites of bacterial colonization root mucigel has attracted much attention because it serves as a substrate for microorganisms. Dobereiner (1974) found that the colonization of *Azotobacter* when it occurred was reportedly limited to the rhizosphere soil, while a few cells were found on the root surface probably due to the activity of concentrated exudates of many plant species.

Strzelczyk (1961) observed that the growth of *Azotobacter* depended on the species of the plants and the stage of growth. While radish, lupin and barley stimulated the growth of *Azotobacter*, wheat, maize and millet had weak effect and onion inhibited the growth. The stimulatory effect was highest during flowering.

Vancura and Macura (1952) on the other hand observed the increased population of *Azotobacter* in the oats, barley and wheat. Darzneik (1960) reported an increased population of *Azotobacter* in the rhizosphere of many plants during the early stages of their growth. At the end of the vegetative period the number of *Azotobacter* cells in the rhizosphere decreased and equalled that in the soil outside the rhizosphere.
According to Brown et al. (1962) naturally occurring Azotobacter were few in the rhizosphere and any increase in number in the zone was small and depended upon the plant species, age of the plant and soil type and there was none on the root surface. Inoculation with rhizosphere resulted in good establishment of number in the rhizosphere and the number was maintained throughout the plant life. Rovira (1963) reported that in the same culture wheat showed a significant positive response to inoculation with Azotobacter and Clostridium and maize and tomato were unaffected by the inoculation. Patel (1969) also observed that inoculated plants had significantly larger population in the rhizosphere than in uninoculated control.

According to the recent reports the rhizosphere effect increases with the age of the plant and reaches a maximum when the plant is in the vegetative phase (Purushothaman et al., 1976 and Sivappa Shetty et al., 1976). Maximum population was noticed at 30 and 40 days of sowing in wheat and tomato and in the later the population declined with age. Nair et al. (1972) recorded maximum Azotobacter population at tillering phase in rice. The enhanced population during the vegetative growth phase appear to be related to the intense photosynthetic production and its excretion, and also to probable increased root sloughing in rhizosphere (Dobereiner and
Compelo, 1971 and Dommergues et al., 1973). Maximum build up of *Azotobacter* population in rice soil, in rhizosphere and rhizoplane was noticed at six weeks of growth i.e., during the active vegetative growth period (Sivappa Shetty, 1976). While report of Lehri and Tiwari (1976) revealed optimum colonization during preflowering stage in rice, Purushothaman et al. (1976) recorded optimum colonizing tendency at different stages for different rice varieties. For the popular variety IR.20 maximum population was recorded at tillering stage and later it decreased with age.

Mishustin and Marenko (1965) observed that the positive effect of *Azotobacter* on agricultural plants was manifested only in soil containing sufficient organic matter in addition to P₂O₅ and calcium under suitable conditions. They observed that yield of crop plants like maize and wheat can be increased up to 20 per cent through *Azotobacter* inoculation.

Application of heavy doses of nitrogen in the form of ammonium sulphate decreased the population by about 50 per cent both in the rhizosphere and non-rhizosphere soils of rice and ragi (Bagyaraj and Bhat, 1968; Emmimath and Rangasami, 1971). But application of 40 kg/ha of phosphate and 20 kg/ha of potassium without nitrogen brought about a significant increase in *Azotobacter* population. Soric et al. (1971) reported that nitrate form of nitrogen had greater
influence on the dynamics of Azotobacter activity in the soil than ammonium sulphate. However, he noted the negative effect of higher doses of nitrogenous fertilizers notably ammonium sulphate. Similar results were obtained by Venkata Rao et al. (1972). Balasubramanian et al. (1972) recorded higher population by application of PK, NPK and farm yard manure, while Thakre and Saxena (1972) reported that combination of phosphate at 80 kg/ha and potash at 40 kg/ha increased the population of Azotobacter over the application of phosphate alone. Lehri and Tiwari (1976) studied the rhizosphere microflora of rice crop inoculated with Azotobacter under varying nitrogen doses and observed maximum population at 60 kg/ha N rather than at 120, 30 or 0 kg/ha.

Foliar spray of chemicals have been reported to alter root exudates of plants and in turn the rhizosphere microflora. Kandasamy and Rangaswami (1967) reported that any change on the quality and quantity of root exudates would in turn affect the rhizosphere microflora. Foliar spray of ammonium sulphate decreased the rhizosphere population much more than disodium hydrogen phosphate or potassium chloride (Bagyaraj and Rangaswami, 1972).

Since Azotobacter is a heterotrophic organism it depends upon the exogenous supply of combined carbon source.
Krishnamurthy and Ravikumar (1973) found that Azotobacter population was more in the top 7.5 cm soil layer applied with cattle manure than with no manure in the plot. Sundara Rao and Venkatram (1963) and Bakale et al. (1976) found increased population of Azotobacter by application of farm yard manure.

6. Nitrogen fixation:

Biological nitrogen fixation is a process by which nitrogen of the atmosphere is reduced to ammonia. Biological nitrogen fixation is carried out both by free-living non-symbiotic and symbiotic microorganisms. It was Winogradsky (1895) who isolated in pure culture a nitrogen fixing organism from the soil. Winogradsky (1895) found that inoculation of sugar nutrient with soil under aerobic condition resulted in fermentation of sugar, acetic acid, butyric acid, CO₂ and hydrogen and in fixation of atmospheric nitrogen. In 1901 Beijerinck isolated two more nitrogen fixing bacteria, Azotobacter chroococcum and Azotobacter agilis. Phosphorus compounds increased nitrogen fixation due to their effect on metabolism of Azotobacter cells. In vitro experiment on nitrogen fixation have reported that the efficiency of fixation is maximum on the second and third day of growth, which on the eighth day fell to an insignificant minimum, thus showing the process is economical only in the beginning. Jenson et al. (1940) have however emphasized that Azotobacter does not play a big role in nitrogen fixation, in soil.
The efficiency of *Azotobacter* as nitrogen fixer has been studied under different conditions and it has been established that the efficiency dies out with time (Yoshida et al., 1973).

Most of the nitrogen fixed by *Azotobacter* is present as cell substance, although nitrogenous compounds are usually excreted during active growth. Roberg (1936) and Horner and Burk (1939) estimated the extracellular nitrogen to be 10 to 25% of the total in young culture. The amount increases considerably when the medium is depleted of energy material and autolysis takes place. It also varies in different species and is influenced by the composition of the medium. Borlets (1939) estimated 45% of extracellular nitrogen in 4 days cultures of *A. vinelandii* in a medium of low iron content.

Krasilnikov (1940) found that inoculation of *A. chroococcum* fixed 63 to 90 lb N per acre in legumes during vegetative period. Bershova (1954) claimed that inoculation increased the nitrogen status in the rhizosphere soil, while Virtanen and Fincola (1954) failed to confirm the findings. According to Garbosky (1956) the soil treated with *A. chroococcum* contained 6 to 20 lb of N per acre. Sundara Rao and Iswaran (1959) reported that the amount of nitrogen fixed was maximum at the end of the third week and gradually decreased thereafter. In 1963 he reported that inoculation increased the N and P uptake in wheat. Rangaswami and Venkatesan (1966) observed that *A. chroococcum* fixed 1.69 mg
of N per ml of culture. In 1969 Krishnarajan estimated that *A.chroococcum* fixed 1.70 mg of N per ml of culture. Nair *et al.* 1972 reported that it had fixed 14 to 20 kg nitrogen per hectare in rice rhizosphere. Puri (1972) estimated that it could fix as much as 30 to 50 kg N/ha during single summer season. *A.chroococcum* was found to fix about 20 kg N/ha in marine alluvial soils of Thailand (Tangcham *et al.*, 1974). In clay loam soil nitrogenase activity was inhibited due to the adsorption of the organism to the clay soil and thereby inhibited the biological nitrogen fixation (Purushothaman *et al.*, 1979).

Shende *et al.* (1975) showed that the organism could fix nitrogen as much as 9.0 to 17.2 mg per g of sucrose consumed. Purushothaman *et al.* (1976) observed more nitrogen fixation in rice rhizosphere than in the field soil.

Several studies on the factors influencing the nitrogen fixation of *Azotobacter* have been made. Waksman (1952) and Ebert (1959) found that amide, ammonical and nitrate nitrogen strongly depressed fixation presumably due to the preferential uptake of applied nitrogen compounds. Sundara Rao and Iswaran (1959) found that the amount of nitrogen fixed from the atmosphere decreased when the concentration of nitrate nitrogen was increased. Nita and Vintu (1964) observed that nitrogen combined with phosphorus decreased fixation of nitrogen but mineral and organic fertilizers stimulated the *Azotobacter*
population and nitrogen fixation. Mineral nitrogen had a negative influence on the fixation of nitrogen by Azotobacter. Venkata Rao et al. (1972) reported that application of high doses of ammonium sulphate decreased nitrogen fixation. Narayanaswamy (1976) observed that in rice fields where there was little nitrogen, Azotobacter was able to fix appreciable quantity of nitrogen. Nagarajan (1976) reported that when nitrogen was supplied in excess of 43.75 kg/ha, the beneficial effects due to Azotobacter activity was less pronounced. Jagnow (1982) pointed out that pesticides and environmental chemicals inhibit nitrogen fixation. Harmful side effects of herbicides on Azotobacter was noted only in high doses.

The Azotobacter inoculation on plant growth and soil nitrogen was studied by Monib et al. (1979). Seed bacterization was done on Hordeum vulgare. The rate of colonization in root region was much higher when soils initially harboured low Azotobacter densities. Seed inoculation with Azotobacter in sand cultures and its effects on nitrogen balance was studied by Abd-El-Malek et al. (1979) on barley grains. In nitrogen deficient sand, seed inoculation increased plant length, dry weight and nitrogen content in addition to a significant increase in soil nitrogen.

7. Time and method of inoculation of Azotobacter in rice:

Many workers have reported that seed inoculation with Azotobacter increased the growth of rice (Rangaswami and
Mahadeviah, 1963; Rangaswami and Venkatesan, 1964; Neelakantan and Rangaswami, 1965 and Krishnarajan, 1969). Nagarajan observed that there was no significant difference between seed and soil and seedling and soil application of Azotobacter to rice. Seeds, seedling and soil application was found to be quite good methods of Azotobacter inoculation for rice (Lehri and Tiwari, 1976; Patil et al., 1976 and Rangarajan and Muthukrishnan, 1976). Oblisami et al. (1976) compared different methods of Azotobacter inoculation in rice and found that seeds (400g/ha), seedling (one kg/ha) and soil (two kg/ha) application at the time of planting was the best method of inoculation. From the above review it is seen that seeds, seedling and soil inoculations of Azotobacter are satisfactory methods of inoculation of rice.

8. Effect of Azotobacter on growth and yield components of rice:

Rangaswami and Mahadeviah (1963) studied the bacterization of rice seeds with Azotobacter and reported the possibility of obtaining robust and healthier seedlings. Rangaswami and Venkatesan (1964) concluded that cultures of Azotobacter, when added to rice field, brought favourable effects on plant growth. Brown et al. (1964) noticed that the Azotobacter on the root surface could produce growth substances which greatly modified the growth of the plant. Gopalakrishnamurthy et al. (1967) reported that when rice seeds were treated with Azotobacter, dry weight and nitrogen and phosphorus content of the plant
increased. Veeraraju and Mahadevan (1967) opined that inoculating rice seeds with \textit{Azotobacter} enhanced soil fertility and improved the growth of the plant. This was later confirmed by Krishnarajan (1969).

Mishustin (1970) suggested that \textit{Azotobacter} synthesized complex, biologically active compounds such as nicotinic acid, pentothenic acid, pyridoxin, biotin, heteroauxin, gibberellin and other compounds which stimulated germination and accelerated plant growth. Badgire and Bindu (1976) observed increase in plant height, number of tillers and number of leaves in rice due to seed inoculation of \textit{Azotobacter}. He also recorded significant increase in dry matter yields. Nagarajan (1976) also recorded significant increase in height, dry matter yield, and nitrogen content in rice varieties, Ponni, ADT.31 and IR.20. Similarly the inoculation with \textit{Azotobacter} increased the number of grains per panicle, panicle length, panicle weight and 1000 grain weight. Rangarajan and Muthukrishnan (1976) observed that in nursery stage \textit{Azotobacter} inoculation significantly increased the height, number of leaves, length and width of leaves and dry matter of seedlings of rice variety Kannaki over control. Combined application of \textit{Azotobacter} and organic manures increased the dry matter production at all stages of crop growth, while it significantly increased the height at tillering and panicle initiation stages. They also recorded favourable influence on other yield components of rice viz.,
productive tillers, length of panicle, number of grains per panicle and weight of 1000 grain compared to control.