Nodules had, although, been represented on drawing of legume roots since the 16th century (Dalechamps 1587) the inducing agent remained a mystery for the next 300 years. In 1679 Malpighi believed the nodules on Pisum sativum plants to be insect galls. Nodules were variously regarded as pathological growths caused by insect or fungal attack or as storage organs (deCandolle 1825; Woronin 1867; Trevisan 1851).

Pasteur's demonstrations of the role of microorganisms in disease provide the climate for discovering the true nature of nodules. Woronin (1867) noted bacteria like bodies in nodules which he believed, induced these diseased outgrowths. Ericksson (1873) found nodules on a dozen legume species and examined
sections of *Vicia faba* nodules in detail. The inner cortex of nodule contain dense cells containing vibrio like bodies and fine 'hyphal' strands crossing the empty outer cortical cells. Woronin's (1878) description of club root of crucifera caused by *Plesmodiophora brassicae* prompted suggestions that these strands were myxomycetes and the bacteroids the spores. Frank (1879) found hyphae and bacteroids in the small cell and thought these were budding off from hyphae.

Ward (1887) and Prazmowski (1890) studied the development of infection thread during nodule formation and described the infection thread as terminal hypha which ramified through the young nodule before budding off the bacteroids. Ward was unable to culture the organism, but from his and Frank's field observations believed the infecting organism to be ubiquitous. Two years previously Hellriegel and Wilfrath (1866-1888) demonstrated that legumes fix atmospheric nitrogen when nodulated by microorganism present in soil extracts. Beijerinck (1888) showed that such nodules were formed by bacteria which he isolated and named as *Bacillus radicicola*. Later he used it to reinoculate *Vicia faba* and thus, produced nodules artificially.

Prazmowski (1890) also succeeded in culturing and reinoculating the nodule organism from peas. He regarded the infection threat as a gelatinous tube
excreted by the bacteria for protection. By 1890
Frank had developed some definite views on the nature
of nodule organism(s), having cultured the nodule
bacteria of peas and beans, which he called *Rhizobium*
*leguminosarum*. He also obtained nodulation on host
plants after inoculations. He believed the infection
tube was formed by the plant cytoplasm and bacteria
to conduct the symbiotic organisms—the minute
cocoid form into the cortex of the root where they
were destined to grow. The infection thread is formed
by the normal cell wall constituents of the plant was
confirmed later by McCoy (1932) on histochemical studies.

Scientists like Spratt (1919), Jimbo (1927),
Thornton (1930), Carolli (1934), Thimann (1936), Fred
et al., (1932), Georgi and Beguin (1939), Wipfl and
Coops (1940), and Bond (1948) have worked on the formation of nodules, their morphology and development on
the roots in various legume hosts.

Anatomy and development of nodules on numerous
cultivated leguminous plants have been reported in
detail by Fred et al., (1932), Siderdorf (1938), Allen
and Allen (1940), Fraser (1942), and Bond (1948). The
descriptions of nodule formation on certain leguminous
tree and shrubs are given by Spratt (1919), and
Lechtova-Trnka (1931). They assumed that the nodules
on the roots of "woody species are different only in
minor degree from the nodules of herbaceous plants."
Yet the detailed reports of nodules on woody species are lacking.

Among the legumes which contain 431 genera with 8887 species (Morris, 1956), or 700 genera with 14,000 species (Tutin 1958), only about 8-12% of these have been examined for nodulation (Allen and Allen, 1961), and a much smaller percentage included the ineffective trials. Nodule formation is more common only present in the subfamilies Mimosoidae and Papilionoideae; and commonly absent in the sub-family Caesalpinoideas. Near about 70% of Caesalpinioid species lack nodules as reported by Allen and Allen (1961).

Kidby and Goodchild (1956) studied the form of nodules, which is determined primarily by the host but modified by the effectiveness of the associated Rhizobium. It varies a good deal in size and shape (Corby, 1971).

The effectively functioning nodule have a relatively large red region due to leghaemoglobin, it may be masked by the dark pigmentation of the central nodule tissue itself (Cloonan 1963; Löbereiner 1965).

In detail organization of legume root nodule has been well studied by Allen and Allen (1940). According to them the nodule is generally exogenous to the root pericycle but in some cases e.g. Arachis hypogea it appears to be truly endogenous. Allen and
Allen in 1958 recognised four main zones in the nodules i.e., nodule cortex; meristematic zone; vascular system; and bacteroidal zone. Even though there are many papers describing the microorganisms in the central tissues of legume root nodules by Lachmann (1858), Woronin (1866, 1867), Frank (1879), and Ward (1887), Brunchorst (1889), was the first to use the term 'bacteroid'. He considered these as well organized protein bodies resembling bacteria. These forms (bacteroids) have been extensively discussed by Fred et al., (1932), Jordan (1962), and Jordan and Coulter (1965). They also called the swollen forms of Rhizobia in culture as bacteroids.

Chen and Thornton (1940) reported that the effective nodules have a much greater volume of bacteroidal zone than the ineffective ones, and they function for nitrogen fixation for longer times.

Norris in 1958 studying the characters of rhizobia observed that few strains are pink in colour while there are other strains which characteristically produced white or cream coloured colonies on yeast extract mannitol agar medium.

Fred et al., (1932), and Norris (1965), used the criterion of acid and alkali production under standard conditions to divide rhizobial strains into two major groups, i.e., fast growers and slow growers (corresponding with the rate of growth).
Vincent (1962a) reported that the 'fast growers' have a mean generation time of 2-4 hrs from relatively large colonies in 3-5 days which generally produce a large amount of free flowing gum. The 'slow growers' on the other hand have mean generation time of 6-8 hrs, yield colonies in 7-10 days and produce less gum which is dense and sticky.

Vincent (1970) suggested an experiment for measuring the bacterial growth by turbidity method which are cultivated in glass wares having matched optical path ways. By this method total amount of cell substance, number of cells for a particular organism could be obtained. Munevar Fernand and Arthur G. Hollum II (1981) studied the growth responses of Rhizobium japonicum strains at different temperatures measuring the growth change in optical density over time, they found that the maximum survival of strains was up to 33.7 - 48.7°C temperature. Donso et al., (1973), Pant and Gangwar (1984), Siddiqui et al., (1984) and Prasuna and Ali(1985) have estimated growth rates and patterns in several isolates of Rhizobium using these methods.

Holland (1962, 1965), Van Schreevan (1964), Chonkar and Subba Rao (1966), Holland and Parker (1965) and Chatel et al., (1969) have extensively studied the attribution of microbial antagonism (of bacteria, fungi and actinomycetes) in the rhizosphere, which might prevent adequate colonisation of Rhizobium on the legume roots, thereby,
indirectly preventing the nodule formation.

Some rhizobia responded differently in being more sensitive than others to actinomycetes and antibiotics (Robinson 1945; Anderson 1957 and Van Schreven, 1964) but the relative sensitivity of slow and fast growing strains could not become clear (Graham 1963a).

Josey et al. (1979), Antoun et al. (1982), Stein Michele et al. (1993), and Bromfield (1993) worked mainly to identify *Rhizobium* strains depending on their sensitivity to antibiotic concentration gradients and or by using intrinsic antibiotic resistance character.

Cole and Elkan (1979) studied the multiple resistance in *Rhizobium japonicum*, Gupta et al. (1982), on *Rhizobium* isolates from mung bean, Rai (1983), on the resistance of *Rhizobium* from *Cicer arietinum* against streptomycin, Hagedorn (1979), on relation of antibiotic resistance to effectiveness in *Rhizobium trifolii* populations, Bushky (1993), on the change in number of *Rhizobium* population isolated from *Vigna mungo* against antibiotic resistance, Sinclair et al., (1984), investigated intrinsic antibiotic resistance in relation to colony morphology in three *Vigna unguiculata* rhizobia, and Dakora (1985) also used intrinsic antibiotic resistance for characterization and identification of 83 isolates from nodules of *Vigna unguiculata*. and
Phaseolus vulgaris. The above scientists used the known antibiotics to measure the variation in resistance of rhizobial strains and differentiated them into separate strains on this basis.

Not always the microbial association decreases nodulation but some fungi and actinomycetes occasionally even stimulate rhizobial growth. This statement was supported by Katting and Louw (1966), Sethi, Subba Rao (1950), Lim (1961), Anderson (1957), and Pacovsky et al. (1986). The endotrophic mycorrhizal association perhaps increases the nutritional status of root, than to counteract with Rhizobium on infection process, and helps in more nodule formation and nitrogen fixation particularly in soils with low phosphate.

The ability of Rhizobium to fix dinitrogen symbiotically is now known to be a factor of its nif gene expression, which synthesises the enzyme nitrogenase. This enzyme is the most versatile reducing catalyst which catalyses the reaction where dinitrogen is converted to ammonia (Hardy and Burns, 1968; Hardy and Knight, 1968). The term nitrogenase is used to denote the enzyme system that catalyses the ATP dependent reduction of dinitrogen. This nitrogenase contains two proteins; Fe protein, also called azo-ferredoxin, and Mo-Fe protein called molybdo-ferredoxin. The two proteins are needed for dinitrogen conversion to ammonia.
Much work has been done for estimating the nitrogenase activity in different isolates of *Azobacter*. In the laboratory, nitrogen fixation by living organisms has been measured by Kjeldahl analysis (Burris and Wilson 1957), 15 N-enrichment assay by mass spectrometry (Burris and Wilson, 1957), 13 N incorporation assayed by radio active counting by Campbell et al., (1967); and Nicholas et al., (1961). N₂ fixation by nitrogenase in cell free extracts have been measured by 15 N enrichment (Carnahan et al., 1960) by colorimetric analysis of ammonia (Dilworth et al., 1965), and N₂-H₂ uptake (Mortenson, 1964) or by H₂ evolution (Bullen et al., 1965), assayed manometrically.

Of these procedures only Kjeldahl analysis has been used to some appreciable extent for estimating nitrogen fixation in field samples (Stewart, 1966).

Dilworth (1966), found that acetylene reduced to ethylene in a reaction is analogous to reduction of (N₂ to H₂) dinitrogen to ammonia. The application of this reaction to a sensitive assay procedure for N₂-fixing activity was proposed by Hardy and Knight (1967).

Subsequently Koch, Evans and Russell (1967), Silver (1967), Sloger and Silver (1967), and Stewart et al., (1967), have successfully employed C₂H₂ reduction coupled with ethylene detection by H₂ flame ionization as an assay for nitrogenase activity. Hardy and
Knight (1967), believed that the acetylene-ethylene assay of nitrogen fixation represents one of the most important developments in $N_2$ fixation research.

Number of scientists worked on the capacity of (nitrogen fixation) nitrogenase activity of *Rhizobium* strains in various respects. Hess et al., (1981) observed that when grown alone, neither *Petunia* non-legume nor *Rhizobia* could express nitrogenase activity but were able to increase nitrogenase activity under symbiotic conditions. *Petunia* tissues proved superior to wheat in this respect. Rice et al., (1981) found that phenolic compounds from decomposing rice straw inhibited nitrogenase activity in three *Rhizobium* strains tested. Smith et al., (1982) observed significant variations among clover plants inoculated with a single *Rhizobial* strain in acetylene reduction. Van Brussel et al., (1979) performed the experiments to induce nitrogenase activity in symbiotically cultured rhizobia and bacteroids, revealed no differences by freeze-etching technique. Kitamura et al., (1981) studied the relative efficiency computed as:

$$ RE = \left( 1 - \frac{H_2 \text{ evolution in air}}{C_2H_2 \text{ reduction}} \right) \times 100 \% $$

in *Macroptilium atropurpureum* plants inoculated with five *Rhizobium* strains and found RE to be 80-90%.
Skotnicki et al., (1979) demonstrated nitrogenase activity in *Rhizobium* strains resistant to streptomycin and obtained positive results. Ilkhan et al., (1980) worked on 48 genotypes of *Arachis* and *Rhizobial* strains used for inoculation. The *Rhizobial* strains were able to compete with the natural rhizobia in the soil, formed nodules in all the genotypes and showed higher nitrogenase activity within the nodules of *Florigant* variety of *Arachis*. Barula et al., (1981) studied $N_2$ fixing ($C_2H_4$) activities in three *Rhizobium japonicum* cultures and 13 varieties of soybeans. The results showed the usefulness of acetylene reduction assay in evaluating the rhizobial cultures and the varieties in terms of $N_2$ fixing abilities. Aeo, V. Ranga (1983) tried nitrogenase assay of cultured *Rhizobia* using various nitrogenous compounds which depressed nitrogenase activity. Mostly ammonia depressed nitrogenase activity as compared to other N compounds. Sadykov et al., (1933) determined that nitrogenase activity of nodule bacteria were nearly two times low under lab. conditions compared to field conditions. Ludwig (1934) studied on *Rhizobium* free-living nitrogen fixation and observed its occurrence in specialised non growing cells.

Several workers studied the nitrogen fixing capacity ($C_2H_2$ reduction) in the nodules of different legumes in various aspects and concluded that temperature played an important role in limiting the acetylene
reducing activity in clovers as compared to light effect (Bockart et al., 1980), acetylene reducing activity was gradually increased up to 50th day and lessened onwards up to 80th day in woody species *Robinia pseudoacacia* (Moiroud et al., 1981). There is an increase in nodulation, seed yield and \( \ce{C_2H_2} \) reduction in forage legumes with the application of 50 mg P and 100 mg K/kg soil (Lynd et al., 1981). Dipankar (1982) studied the acetylene reduction activity in cowpea, peanuts and siratro plants nodulated by six different strains of *Rhizobium* in which peanuts proved efficient by showing several fold higher activity than other two host species. Johnson (1981), was able to study *Medicago sativa* plants which were capable of active nitrogen fixation in favourable environments, as well as under high water stress, i.e., in dry soils also.

Efficiency of the *Rhizobium* for higher \( \text{N}_2 \)-fixation is related with its capacity to recycle hydrogen released in the process. Some strains are able to do this because of the presence of yet another enzyme called the uptake hydrogenase. This was first reported in the pea system in the year 1941, and Dixon actually located hydrogenase activity in pea root nodule bacteroids later in 1968. In the year 1972 he proposed that hydrogen oxidation in the legume nodules is involved in preventing oxygen damage to nitrogenase but no experimental evidence was presented then.
Albrecht et al., (1979) and Emerich et al., (1979) observed that the uptake hydrogenase in legume nodules recycles the hydrogen produced by nitrogenase as biproduct and thus conserves metabolic energy.

Manus et al., (1979), and Lepo et al., (1980), observed that like nitrogenase the recycling hydrogenase is also under the genetic control of the bacterium and a few Hup(+) strains are able to grow autotrophically in hydrogen atmosphere. Subsequently the enzyme hydrogenase was purified and characterized from several sources including *Clostridium pasteurianum* by Fortenson, (1974), and Adams, (1981).

The *Rhizobium* strains Hup(+) or Hup(−) were studied by many researchers. Carter, (1978), surveyed and reported that 21% of *Rhizobium japonicum* strains were uptake hydrogenase positive, Ruiz-Argueso et al., (1981), reported 13% and Lim, (1981), 25% of *Rhizobium japonicum* to be Hup(+) strains. Most of the cowpea strains of *Rhizobium* examined were Hup(+) (Schubert et al., 1977). No strain of *R. meliloti* has been identified as Hup(+) by Ruiz-Argueso et al., (1979).

Dart and Bay (1971) studied the temperature effect raising the temperature from 20° to 40°C which approximately doubled the rate of H2 evolution from nodules i.e., there is a loss of H2 from nodules. Suzuki and Maruyama (1979) reported that two hydrogenase
activities measured by \( \text{H}_2 \) uptake were detected in the bacteroids from soybean and lupin nodules.

Both Lepo et al., (1981), and Maier et al., (1978) have used the capability for chemosynthetic growth as a key method in selection of Hup\(^{-} \) mutants and Hup\(^{+} \) revertants. Maier and Campbell (1978), used the capacity to reduce triphenyl tetrazolium chloride as an effective procedure for differentiating wild type Hup\(^{+} \) and Hup\(^{-} \) \textit{Rhizobium japonicum}, while Cantrell (1982) have shown that the capacity for reduction of this dye is not a reliable method for screening for Hup\(^{-} \) point mutants.

Dixon (1968), Emerich et al., (1980), Arima (1981), Drevon et al., (1982) and Rainbird et al., (1983) have shown that the rates of \( \text{CO}_2 \) evolution were substantially decreased in \textit{Rhizobium} bacteroids in nodules formed by Hup\(^{+} \) strains. This indicates that \( \text{H}_2 \) oxidising strains of \textit{Rhizobium} can utilize \( \text{H}_2 \) as an energy source and as a consequence, conserve more of their carbohydrate supply than Hup\(^{-} \) strains (Layzell and Rainbird, 1979).

Albrecht et al., (1979) observed significant increase in the amount of \( \text{N}_2 \) fixed, and increase in the yield when the soybeans were inoculated with \textit{Rhizobium japonicum} strains having Hup\(^{+} \) system. Hanus et al., (1979) were capable of growing \textit{Rhizobium}
Rhizobium japonicum Hup(+) strains autotrophically by supplying \( \text{N}_2 \) gas as energy source. Faier et al., (1979) studied the effect of various factors that regulate the expression of an \( \text{N}_2 \) uptake system in free living cultures of Rhizobium japonicum. They observed that carbon, \( \text{KNO}_3 \), \( \text{NH}_4 \text{Cl} \) all depressed Hydrogenase formation but none of them inhibited the hydrogenase activity. Zablotowicz et al., (1980) from their experiments recommended that Rhizobium strains selected for inoculation purposes should contain \( \text{N}_2 \) oxidizing capability as one of their desired characteristics. Ruiz-Argüeso et al., (1981) described a screening procedure for the selection of Rhizobium strains producing high energy efficient nodules, based on a test of their ability to induce uptake hydrogenase in symbiotic conditions.

Nelson et al., (1982), concluded that the main function of uptake hydrogenase in Rhizobium leguminosarum appears to be in the protection of nitrogenase from O\(_2\) damage. Nelson Louise. (1983), observed no difference in the plant dry weight, \( \text{N}_2 \) content and nitrogenase activity in pea plants inoculated with Hup(+) and Hup(-) strains. Haugland et al., (1983), devised a rapid colony screening method using methylene blue dye for identifying hydrogenase activity in Rhizobium japonicum. Lopez et al., (1983), examined the relative efficiency of \( \text{N}_2 \) fixation by nodules and the uptake hydrogenase
activity of bacteroids of $\text{Hup}^\text{(+)}$ \textit{Rhizobium japonicum} and \textit{Rhizobium leguminosarum}. They found in different legume hosts there was a strain dependent host effect on the expression of uptake hydrogenase. Rainbird et al., (1983) studied the significance of hydrogen evolution in the carbon and nitrogen economy of nodulated cowpea which showed a significant difference in the symbiosis but not on seed yield, total dry matter production and nitrogen fixation. Dadarwal et al., (1982-85) made extensive biochemical and genetical studies of hydrogenase in \textit{Rhizobium} of cowpea miscellany group hosts. Salminen (1984) studied the role of uptake hydrogenase in providing reductant for nitrogenase in \textit{Rhizobium leguminosarum} bacteroids.

Several attempts have been made to determine $\text{N}_2$-fixing efficiency on cross inoculation using various factors. They obtained significant results in the cross inoculation by different \textit{Rhizobium} strains treated against homologous and heterologous legume crop plants, even though there is much variation in respect of effectivity, infectivity, nodulation capacity, crop yield, total dry matter production and nitrogen fixation under field and laboratory conditions. (Abdel et al., (1979), Roughley et al., (1980), Mahmoud (1980), Brockwell et al.,(1980), Dadarwal et al., (1981), Idris et al., (1981), Bevanur et al., (1981), Hagerdon and Coldwell, (1981), Iruthayathas et al., (1981), Heyser Harold et al.,(1982),

While other workers in this field like Summerfield et al., (1981), Materon and Hagedorn, (1981), McLoughlin and Duncan, (1981), Lowther and Bonish, (1981), Bhuvaneswari (1981), Bonish (1980), Zurkowski Witold, (1982), Diatloff et al., (1983) observed no significant results but obtained poor results from cross inoculations such as reduction or no increase in the efficiency, crop yield, nitrogen content and nitrogenase activity.

The practical aspect of biological nitrogen fixation symbiotically has been to ensure availability of Rhizobium for nodulation in the legume crop host. Hence production of bacterial culture in suitable form for field application was taken up as an industry. Many researches were undertaken in this respect.

be used as the carriers for survival and maintenance of *Rhizobium* for longer periods. The oil and peat bases were superior for storing and applying bacteria in the field. This applied dimension is of immense significance.

Much work has been done in *Rhizobium* and the symbiotic nitrogen fixation aspects in India too. Some leading works are reviewed here.

Arora, (1954, 1956), studied the morphology and development of root and stem nodules of *Aeschynomene indica* and root nodules of *Crotalaria juncea*.

Narayan (1963, 1964), also contributed his work in this respect, he worked on the structure of nodules in *Vigna unguiculata* and in some legumes. In 1967 Sethi studied the antagonistic and association effects of soil fungi on *Rhizobium* utilization of combined nitrogen by *Rhizobium* species from some cultivated legumes in relation to their effectiveness has been observed by Gupta and Sen, (1969).

In 1970's progressive work has been carried out on *Rhizobium* and its symbiotic association with legumes. Kapil and Kapil, (1971), provided details on the origin, structure and antigeny of *Cajanus cajan* root nodules. Sethi and Subba Rao, (1972), observed the effect of fusanic acid on *Rhizobium* species. Bose and Venkataraman in the year 1972, reported that ultraviolet radiations induced mutations in *Rhizobium leucomum*arum. Kaushik
et al., (1973), studied the serological properties of
rhizobium mutants, while Dadarwal and Sen, (1974),
presented their work on varietal specificity for
rhizobium serotypes in relation to quantitative
characters like nodulation and crop yield. In 1977
Dadarwal et al., carried out experiments to study
compatibility, efficiency and nitrogen fixation by
rhizobium on chick pea. Shukla and Dwivedi, (1976),
observed that during the root hair invasion in
Trifolium alexandrium by the homologous and heterologous
strains of rhizobium, the root hairs were curled and
deformed. Sethi and Bhattacharya (1978), performed
experiments on biochemical aspects of six rhizobium
trifolii strains, to know their efficiency. (They found
that there is difference in utilization of asparagine
by the strains, none of them reduced triphenyl tetra-
zolium chloride and five of them reduced potassium
nitrate). Dadarwal et al., (1979), reported antigenic
characteristics and efficiency of green gram rhizobia,
and in the same year they also studied on the effect-
vity of rhizobium in heterologous hosts. From the
experiments they found that rhizobium strains showed
(less effectivity) limitations in nitrogen fixing
ability in heterologous hosts inoculated.

Singh et al., (1980) worked on the nitrogen
fixation by nitrate reductase deficient mutants of
rhizobium japonicum. Dadarwal, (1980), studied about
the host bacterium factors involved in legume symbiosis, while Neeru Narula et al., (1980), studied on the field evaluation of *Rhizobium japonicum* and soybean varieties by acetylene reduction method.

Dadarwal and Kundu, (1981), mentioned about the symbiotic and cultural properties of a *Vigna rhizobium*, having nitrogenase linked hydrogenase. Kundu et al., (1981), carried out investigations for induction of nitrogenase in mung bean *Rhizobium* under cultural conditions. Dadarwal et al., (1981) studied *in vitro* and *in vivo* nitrogenase activity of *Rhizobium* mutants, and their symbiotic effectivity. They found the mutants of *Rhizobium* to be less effective, with decreased nitrogenase activity than the parent strain. Dahiya and Khuran, (1981) described a better technique with 'chillum' jar, for screening of *Rhizobia* under summer conditions. They observed that pigeon pea rhizobia formed nodules well under 'chillum' jar conditions, this was probably due to lowering the temperature in summer by rapid evaporation from the outer surface of 'chillum' jar assembly. Venkateswarulu et al., (1981), studied on nodulation and nitrogen uptake patterns of selected grain legumes in desert soil. Vaishya and Gajendragadkar (1982), found significant difference in nodule number among the genotypes of *Vigna mungo* inoculated with *Rhizobium* and the grain yield is increased due to inoculation. Chhal and Rewari, (1982),
studied the relation of chlorophyll content and nitrogen fixation in *Lens esculenta* nodulated by different strains of *Rhizobium leguminosarum*. Shah and Gopal Rao, (1982), studied the initiation and development of root nodules in 7 species and structure of nodule in 4 species of tribe Trifoliae. They observed that origin of nodules in the investigated taxa to be exogenous, the nodules comprised all the four zones, and bacteroid zone is composed of both infected and uninfected cells.

Horal and Konde (1983) mainly contributed their work to study the effects of nodulation and dry matter of chick pea (*Cicer arietinum*). Ghai et al., (1983) observed the effect of UV radiation, which induced changes in antibiotic markers and in chemical composition of water soluble polysaccharides of nodulation ability of *Rhizobium trifolii*. Verma and Dadarwal, (1983), worked on chick pea mutants, for their effectivity and antibiotic resistance competence and found majority of antibiotic resistant mutants to be inferior to parent strain in infectivity. The streptomycin resistant mutants however are stable and highly competitive with native rhizobia.

Panh and Gangwar, (1984), studied on the properties of different *Rhizobium* isolates like growth behaviour, reduction in pH in the culture, polysaccharide production, glucose consumption and nitrate
reductase activity in relation to the nitrogen fixing ability in *Trifolium alexandrium*. Siddiqui *et al.*, (1934) worked on six *Rhizobium leguminosarum* strains and observed that growth of all six strains increased by UV radiation which also stimulated glutamine synthetase activity, but inhibited nitrate reduction. Gupta *et al.*, (1934) published their data on pH associated acid production and nitrate reductase activity in *Rhizobium*. Gaur and Sen, (1934), studied on surface and internal cell antigens of *Cicerarietinum* rhizobia and on their reisolates after plant passage. Rai and Prasad (1934) worked on the growth and symbiotic nitrogen fixation by *Vigna radiata* under stress conditions.

Dadarwal (1985) noticed the failure of effective rhizobia to form nodules on heterologous hosts, he therefore laid greater emphasis in studying mainly the physiological interactions of bacterium with the host legume leading to effective symbiosis. Jayachandran *et al.*, (1985), concluded from their field experiments that uptake hydrogenase positive strains of *Rhizobium* species conserved plant energy, more effectively than Hup(-) strains and thereby increased the crop yield in groundnuts. Dogra (1985) worked on the regulation of uptake hydrogenase in *Rhizobium* species *Dhaincha* (Sesbania) he showed that all the carbon substrates tested in the medium against hydrogenase repressed the enzyme activity. Balasubhramaniam, (1985), undertook
experimentation to obtain improved or superior *Rhizobium* strains by genetic engineering techniques for better symbiotic associations. Pant and Gangwar, (1985), reported that the *Rhizobium trifolii* isolates were highly effective on *Trifolium alexandrium*, they found the dry weight of nodules was correlated with the efficiency but the nodule number did not do so. Jayaraman et al., (1985), undertook a detailed survey to investigate nodulation in tree legumes like *Acacia*, *Albizia*, *Calliandra*, *Dalbergia*, *Desmanthus*, *Glyricidia*, *Indigofera*, *Leucaena* and *Enteurolobium* and found all plants to be well nodulated in the Nilgiri region. Shahi and Jayashankar (1985) discussed the importance of legumes in green manuring with regard to their contribution to plant nitrogen, soil organic matter and improvement in soil physio-chemical properties. Green manure according to them reduced the fertilizer cost to the farmers. Tilak (1985) studied the interactions of vesicular arbuscular mycorrhiza on symbiosis. The mycorrhizal association improved growth and yield in chick pea and pigeon pea. Pareek et al., (1985) from their experiments concluded that best soil temperature for nodulation, plant growth nitrogenase activity and nitrogen uptake was in a range of 21 - 22 ± 2°C. Lalita Batra (1985) studied the effect of soil salinity on nodulation and nitrogen fixation in four forage legumes. Rangarajan and

Much work was done on *Rhizobium* and its symbiotic association with legumes in 1985 also. Some of the major studies are, Isolation of temperature-tolerance strain of *Rhizobium* and its survival in inoculant carrier Jain et al., (1986), Hydrogen metabolism and nitrogen fixation in mung bean (*Vigna radiata*) plants inoculated with Hup(+) and Hup(−) *Rhizobium* strains, Timaiah and Lodha (1985), some physiological studies on *Rhizobium meliloti* and *Rhizobium* species cowpea, Ajay Kumar, (1986), studies on initial events during nodulation of legumes by *Rhizobium* Keena Vora, (1986), chemical characteristics of *Rhizobium japonicum* extracellular polysaccharides, Raj Gopal and Vittal Rai, (1986), Effect of
Roy, Subba Rao and Sharma, (1987), tested Cajanus rhizobia and *Rhizobium japonicum* for cross infectivity on heterologous hosts viz., soybean, lucerne, Bengal gram, pea, lentil, berseem, cowpea and green gram. None of the 16 strains of *Cajanus* rhizobia nodulated above legumes. *Rhizobium japonicum* strains selectively nodulated five cultivars of pigeon pea indicating high promiscuity. Roy et al., (1987), studied on population density of cowpea *Rhizobium* from six different locations of Delhi. They recorded highest population of 113 cells/g soil (estimated by soil dilution plant infection technique) in Alipur soils. Kaza et al., (1987) studied the compatibility of 4 *Rhizobium japonicum* strains in the field against three cultivars of soybeans. The four strains tested were found compatible with one of the cultivar and produced highest mean grain yield.

Mishra et al., (1987) conducted an experiment to evaluate the effect of different carrier based inoculants of *Rhizobium japonicum* on grain and straw yield of soybean. The carrier mixture lacaud + charcoal (1 : 1) proved to be most suitable carrier for *Rhizobium japonicum* as it caused significant increase in grain and straw yield. Sharma and Sen, (1987), studied serological characterization of *Rhizobial* strains. Nine *Rhizobial* strains isolated from wild legumes were serologically
characterized through agglutination reaction and precipitation band formation in agar-gel with seven antisera. Based on the reaction with antisera the 9 strains were placed under five groups. Sharma and Sen, (1987), studied on the biochemical characters of _Rhizobium_ strains from wild legumes. The nine strains were grouped into two based on changes in nine sugars tested. The first group showed rapid growth with production of acid and the second group showed slow growth with alkali production. The same scientists also studied the infectivity of these nine strains against seven hosts, and placed these strains into three groups.