Biotechnology of Biofertilizers

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Alpha Science International Ltd
P.O. Box 4067, Pangbourne RG8 8UT, UK

ISBN 1-84265-099-8

Printed in India.

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Foreword

It is roughly estimated that the requirement of N would reach 140 million tonnes in 21st Century. At present, there is a gap of 10 million tonnes of plant nutrients between the removal by crops and supply through chemical fertilizers. It has been realised that in order to fill this gap, there is a need to have some alternate source of plant nutrients. Biofertilizers are important sources for supplementing plant nutrients. The role of biofertilizers in agricultural production assumed special significance in the context of both the cost and environmental impact of mineral fertilizers. Biofertilizers however cannot totally replace chemical fertilizers. For most crops, 15–20% nitrogen requirement can be met through biofertilizers. In addition to biological nitrogen fixation and phosphate solubilization, biofertilizers help to increase plant growth by secretion of growth promoting substances and improving soil properties by leaving organic residues. Biological fertilizers are renewable, cost effective and eco-friendly and they will play an important role in maintaining soil health for sustainable production.

The commercialisation of biofertilizers started in India in the year 1934 with the production and marketing of about less than a tonne in a year. India has now emerged as the largest biofertilizer producing country in the world. The available data indicate that the all India production capacity of biofertilizers is about 8115 tonnes as against the current production of around 5117 tonnes. According to an estimate of the potential for biofertilizer usage in India based on areas covered by different agricultural and horticultural crops, the requirement of biofertilizers in 2005 AD would be 2.5 lakhs tonnes. In spite of significant scientific developments, the process of biological nitrogen fixation is not fully exploited for the benefit of crop production. The beneficial microbial systems involved in nitrogen fixation are many times crop and region specific as well as soil specific in natural ecosystems. Modem molecular biology and biotechnology have made it possible to tailor the genetic potential of the desirable nitrogen-fixing systems suitable for the particular environment.

The book "Biotechnology of Biofertilizers" written by Prof. Dr. S. Kannaiyan covers the basic issues of biological nitrogen fixation and provides a thorough understanding of the processes involved in symbiotic nitrogen fixing system. The scope for extending this biological process to non-legumes is discussed. The potential benefits from the N_2 fixing symbiotic systems such as *Sesbania rostrata*, *Azolla*, free-living cyanobacteria to rice crop and associative symbiotic N_2 fixer *Azospirillum* to rainfed crops have been discussed in detail. The recent developments in biofertilizer technology such as immobilization of cyanobacteria for maximising ammonia production in rice soil ecosystem and endophytic nitrogen fixation in wheat have also been included which are considered as potential technologies for the future. The solubilization and mobilization of nutrients by phosphobacteria and VA mycorrhiza and their role as bioinoculants, *Acetobacter diazotrophicus* as a novel biofertilizer for sugarcane and the cycad-cyanobacterial symbiosis have been clearly elucidated.

I congratulate Prof Dr. S. Kannaiyan for his efforts in bringing out this valuable and timely publication. The information given in this book on BNF and Biofertilizer Technology will help to foster sustainable advances in crop productivity, without associated ecological harm. Prof. Dr. S. Kannaiyan deserves our gratitude for this labour of love.

PROF. M.S. SWAMINATHAN

Preface

The drastic use of chemical fertilizers in present agriculture system has become a key source for high crop yield. The developments in Agricultural Biotechnology has led to the shift from the use of traditional varieties to currently available nitrogen responsive improved varieties, besides hybrids and the latest varieties developed through tissue culture and plant genetic transformation. These fertilizer responsive high yielding varieties are playing important role in meeting out the food grain requirement for the growing population of the 21st century. The unbalanced use of fertilizers is polluting the environment at a faster rate, which has become the burning issue for the developed and developing countires.

The excessive reliance on chemical fertilizers is not a viable strategy in the long run because of the costs, both in domestic resource and foreign exchange, involved in setting up fertilizer plants and sustaining the production. In this context, there is an acute need to have some cheaper source of plant nutrients. The appropriate combinations of chemical fertilizers, organic manures, crop residues, compost and biofertilizers have become the today's need for sustainable agriculture. Biofertilizers are the important component of integrated nutrient management. They are cost effective, eco-friendly and renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural system.

Tremendous amount of progress in the Microbiology, Biochemistry and Genetics of Biological Nitrogen Fixation (BNF) and Biofertilizers Technology has been made in the last 30 years. Yet, we are still hoping for breakthroughs in the transfer of symbiotic nitrogen fixation process from legumes to non-legumes. Interestingly, the present day tools available in Genetic Engineering and Molecular Biology have made it possible to introduce choice attributes in nitrogen fixing microorganisms. It is also highly appropriate to manipulate the potential nitrogen fixing microorganisms by biotechnological means for exploiting their full potential as biofertilizers under low cost production technology in agriculture.

I edited this book with the objective of analysing the biotechnological approaches for the full exploitation and utilization of the biofertilizer technology for the benefit of human life and human welfare. I am very much grateful to all the leading National and International scientists, in the field of BNF and Biofertilizers Technology who have contributed chapters clearly elucidating the recent developments in various biofertilizers and suggesting the ways and means for deriving the maximum benefits from these potential biological systems. I wish to thank Dr. M. Chandrasekaran, Technical and Personal Officer and Dr. M. Chinnadurai, Planning and Monitoring Officer at the Office of the Vice-Chancellor, Dr. K. Kumar, Associate Professor (Agricultural Microbiology) and Ms. P. Yasotha, Research Fellow, *Azolla* Laboratory, TNAU, Coimbatore for their sincere involvement and help at various stages of the publication of this book. I am so thankful to Mr. B. Chokkalingam, Computer Operator, Computer Centre, TNAU, Coimbatore for his neat typing and excellent execution in the documentation of the manuscript and to Mr. V. Gopinath Rao, Personal Assistant to the Vice-Chancellor for his secretarial assistance. I profoundly thank M/s Narosa Publishing House, New Delhi for coming forward to bring this work to the limelight of the scientific community. I wish to place on record my special thanks to my wife Mrs. Banumathi Kannaiyan, my son Mr. K. Lenin and my daughter Miss K. Curie, who are always with me in all my academic and scientific endeavours.

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1. An Appraisal of Biofertilizers in India

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Introduction

About 100 million years ago, sometime in the Cretaceous period, Angiosperms (flowering plants) appeared on earth when soil became extensive with the formation of soil profiles. Due to the accumulation of dead plant biomass, saprophytic microorganisms flourished and rhizosphere associations followed. *Homo sapiens* (man) who evolved from his ancestors about half a million years ago lived hunting and gathering food by random harvest (about 8000 BC) until he learned the art of growing plants for food by clearing jungles and sowing seeds of the previous crop. Very soon he learned that the same soil cannot endlessly support plant growth. Two centuries ago, many conjectures were made regarding the chemical ingredients in soil which supported plant growth and they were set aside when J.B. Boussingault, a French agricultural scientist in 1834 revealed the important chemical constituents of both plants and soil and also proposed that leguminous plants fix nitrogen from the air. Subsequently, the discoveries of legume root nodules by Hellreigel in Germany in 1886 and the causative microorganism of such nodules as *Rhizobium* by Beijerinck heralded the dawn of soil microbiology as a distinct discipline of soil science. These fascinating findings and many references to them have been chronicled by Fred *et al.* (1932) in their classical monograph. Soil was no longer considered an inert medium supporting the growth of plants since later discoveries of Beijerinck, Winogradsky and others resulted in our understanding the diversity of beneficial soil microorganisms and their role in plant growth (Tables 1 and 2).

Table 1. Major types of beneficial interactions/associations between plants and soil microorganisms (Subba Rao, 1993)

Nature of interactions/associations	Examples of higher plants involved	Examples of microorganisms involved
Rhizosphere, Rhizoplane and Phyllosphere microflora	All plants with roots and leaves	Bacteria, fungi and *Actinomycetes*
Ectomycorrhizae	Forest trees—*Pinus*	Mostly basidiomycetous fungi—*Boletus, Lactarius, Armillaria*
Endomycorrhizae (VAM fungal association)	Certain orchids, cereals, grasses and legumes	*Rhizoctonia, Endogone* and *Glomus*
Root nodules of nodulating legumes	Soybean, gram, Lucerne etc.	*Rhizobium* spp. and *Bradyrhizobium* sp.
Root nodules of plants other than legumes	*Alnus, Myrica, Casuarina*	*Actinomycetous endophytes* (*Frankia*)
Leaf nodules	*Psychotria, Pavetta*	*Klebsiella*
Algal associations with higher plants	*Cycas, Zamia, Heterozamia, Gunnera scabra, Azolla*	*Anabaena, Nostoc*
Associative symbiosis	Grasses, *Sorghum* and millets	*Azospirillum*

Chemical fertilizers which contain NPK were known only several years after our knowledge on the role of soil microorganisms in soil fertility. Fritz Haber, a German Chemist successfully synthesized nitrogen and hydrogen into ammonia during the early years of World War I eventhough superphosphate as a fertilizer was produced by John Bennet Lawes and his associate J.H. Gilbert in 1840 at the Rothamsted Experimental Station in England. Since these discoveries, the World's population, food production and fertilizer consumption have increased gradually. In 1980, global population figures stood at 4374 millions and by 2000, the projected figures are 6253 millions. To guarantee food for all, the fertilizer consumption has to increase from 113 mt (in 1980) to 307 mt (in 2000), most of the deficit noticeably occurring in developing countries where infrastructural facilities for fertilizer production are poor. Many developing countries resort to importing chemical fertilizers from abroad which adds to the cost of food production, not to speak of the added burden on the exchequer to buy hard currency.

Table 2. Major microbiological processes in soil by free-living microorganisms which indirectly influence plant growth (Subba Rao, 1993)

Nature of microbial processes	Examples of microorganisms involved
Aerobic decomposition of organic matter (cellulose, lignin, chitin etc.)	*Trichoderma, Fomes, Armillaria, Achromobacter Nocardia, Streptomyces*
Anaerobic decomposition of organic matter	*Clostridium*, methane bacteria (*Methanobacter* and *Methanococcus*)
Non-symbiotic nitrogen fixation	*Anabaena, Azotobacter, Berijerinckia*
Nitrogen immobilization	Bacteria, fungi and actinomycetes
Nitrogen mineralization	*Pseudomonas, Bacillus, Serratia*
Nitrification	*Nitrosomonas, Nitrobacter*
Denitrification	*Pseudomonas, Achromobacter*
Phosphate solubilization	*Pseudomonas, Bacillus, Aspergillus*
Sulphur transformations	*Thiobacillus, Beggiatoa, Desulfovibrio*
Iron transformations	*Gallionella, Ferribacterium, Leptothrix*
Manganese transformations	*Aerobacter, Corynebacterium, Flavobacterium, Cladosporium*
Copper transformations	*Desulfovibrio, Clostridium, Escherichia*

Two basic questions raised by many agricultural scientists and technocrats are:

(i) Can developing countries continue to rely on chemical fertilizers (imported or local) since high yielding varieties of crops need heavy chemical inputs? (ii) If not, can organic farming which includes harnessing beneficial microorganisms meet partially the demands on chemical fertilizers? Many advocates of organic farming frame their arguments that chemical fertilizers destroy the structure of soil and hence application of organic fertilizers can recoup the loss of soil tilth. Others advocate a 'judicious combination' of chemical and organic inputs to meet the shortfall in chemical inputs. Such arguments are appealing but data on neither the quantitative replacement values at the field level between the two types of inputs nor the grain production ratios are lacking. The general belief, however, exists that organic farming is a 'good thing' and has to be practiced anyhow. Yet another related question is do we have enough organic matter or microbial inputs that can be made available on a large scale in the near future designed for intensive farming?

Be that as it may, let us examine what we have by way of microbial inputs in agriculture from both the fundamental as well as applied angles. The term 'Biofertilizer' is used only in India, while other countries prefer to use the term 'Microbial Inoculants'. Microbial inoculants are carrier-based preparations containing beneficial microorganisms in a viable state intended for seed or soil application and designed to improve soil fertility and help plant growth by increasing the number and biological activity of desired microorganisms in the root environment (Subba Rao, 1993).

Rhizobium inoculant for grain legumes and green manuring for rice

Rhizobium inoculant was first made in USA and commercialized by private enterprise in 1930s and the bizzare situation at that time has been chronicled by Fred *et al.* (1932). Initially, due to absence of efficient bradyrhizobial strains in soil, soybean inoculation at that time resulted in bumper crops but incessant inoculation during the last four decades by US farmers has resulted in the build up of a plethora of inefficient strains in soil whose replacement by efficient strains of bradyrhizobia has become an insurmountable problem. Today, inoculants for soybean rarely sell due to lack of response in increased grain yield. In fact, this inability of introduced efficient strains of Rhizobia to dislodge native inefficient strains, commonly referred to as the "competition problem" for nodule occupancy resulting in the lack of desired response is the prime problem faced by rhizobiologists. This problem appears to be compounded for Indian pulse crops nodulated by the promiscuous cowpea miscellany which enjoys free interchange among pulse crops to produce nodules on roots, many of them being ineffective ones. Therefore, the Indian rhizobiologist must reckon with this established fact so as to 'tailor' new inoculant strains capable of restricted host preference coupled with competitive ability. Unfortunately, no single scientist has been seized with this problem in India.

Many approaches towards the construction of mutants of *Rhizobium* capable of increasing plant biomass or grain yield have been outlined from time to time (Maier and Triplett, 1966). Indeed many such strains have been constructed and US patents granted. However, only one instance of definite increased yield at the field level for soybean using *Bradyrhizobium japonicum* has been accepted (Williams and Phillips, 1983). Competitive ability of strains to ward off inefficient strains to form efficient nodules in large numbers is the key factor in *Rhizobium* biofertilizer technology. Research efforts to upgrade strains are negligible even though biotechnological approaches to improve them are not wanting. A feasible approach is the construction of strains capable of oxidizing H_2 evolved during N_2 fixation by an uptake hydrogenase system so that the electrons produced in this reaction are funneled through an efficient energy-conserving electron transport chain to the more important function of nitrogen reduction. Such efficient Hup^+ strains of *Bradyrhizobium japonicum* showed a 17 per cent yield increase over the Hup^- inefficient strains (Evans *et al.*, 1985) but the problem of competitiveness in soil and the stability of Hup^+ genes in bacteria still pose hurdles. Mass inoculation of Hup^+ strains year after year may dislodge inefficient strains and improve nodule occupancy of Hup^+ strains. This method has proved successful for soybean (Dunigan *et al.*, 1984; Martensson, 1990). Other possibilities to confer competitive ability to strains are identification of genetic loci involved bacterial cell surface characteristics for attachment, antibiotic production capable of warding off inefficient strains, increasing nodulation efficiency and restriction of host range (Maier and Triplett, 1966). If a legume can produce root nodules only by a 'designer' strain and not by native Rhizobia in soil, that would provide an ideal opportunity for mass inoculation with that particular strain affording ample opportunity for manufacturers of *Rhizobium* inoculants. The solution to the competition problem may not be as simple as that but the question certainly needs protracted attention by research workers.

Of the several green manure crops, the use of *Sesbania rostrata* nodulated by *Azorhizobium caulinodans* in rice cultivation has attracted the attention of many research workers beginning from Dreyfus and Dommergues (1981) and Dreyfus *et al.* (1985) more so because nodules are present both on roots as well as stem and nodulation is insensitive to the presence of combined nitrogen in soil (Rinaudo *et al.*, 1982). Species of stem nodulated *Aeschynomene* (Subba Rao *et al.*, 1980; IRRI, 1988) and non-stem nodulating *Neptunia* (Shaede, 1940; Subba Rao *et al.*, 1995) which occur in water logged areas of South India could also prove useful as indigenous green manure legume plants for harnessing in rice cultivation. The limiting factor in extending green manure practice at the farmers level lies in the fact that it is labour intensive requiring a separate piece of land unless intercropped with rice. *Azorhizobium* inoculation appears to improve nodulation (Kannaiyan, 1993) and both seed and the bacterial culture must be made available on time. The economics of green manuring in intensive rice cultivation is yet to be worked out. What appears more interesting is the biotechnological possibility of extending the stem nodulating habit to other legumes as well as cereals. Indeed, the work of Cocking *et al.* (1990) which demonstrated the ability of *Azorhizobium* to induce nodulation on rape and rice seedlings in the laboratory appears to be a stepping stone in rendering cereals susceptible to nodulation by nitrogen fixing bacteria.

Azotobacter

Of the several species of *Azotobacter*, *A. chroococcum* happens to be the dominant inhabitant in arable soils capable of fixing N_2 (2–15 mg N_2 fixed/g of carbon source) in culture media (Mishustin and Shilnikova, 1969, 1971). The bacterium produces abundant slime which helps in soil aggregation. The numbers of *A. chroococcum* in Indian soils rarely exceeds 10^5/g soil due to lack of organic matter and the presence of antagonistic microorganisms in soil. The bacterium produces an anti-fungal antibiotic which inhibits the growth of several pathogenic fungi in the root region thereby preventing seedling mortality to a certain extent. Seed germination and plant stand are improved in plants upon inoculation with improved strains of *A. chroococcum* capable of elaborating growth substances (Shende *et al.*, 1977) eventhough quantitative assessment of these physiological attributes *in situ* in soil has not been made. In Russia, Mishustin and Shilnikova (1969) summed up many field experiments carried out between 1958 and 1960 using commercial preparations of 'Azotobakterin' and found that increase in yield due to inoculation varied from 7.0 to 12.0 per cent. Those experiments were followed up by many Indian scientists with Indian isolates of *A. chroococcum* (Sundara Rao *et al.*, 1963) who found that vegetables such as brinjal, tomato, cabbage and onion benefitted largely to variable extents from 2 to 50 per cent over uninoculated control. Later, Shende and Apte (1982) have showed that *A. chroococcum* inoculated *Sorghum*, maize and cotton plants showed increase from 9.3 to 71.7 per cent levels. At Indian Agricultural Research Institute, New Delhi (IARI), Shende (personal communication) maintains that there is host varietal dependence and internal colonization of roots by improved strains of *A. chroococcum*. It is the conviction of the author that much work remains to be done to design recombinant strains which have all the attributes mentioned above so that we have superior strains with high saprophytic ability coupled with early autolysis in soil so that fixed metabolities are released into the rhizosphere. Notwithstanding all these limitations, one of the best selling microbial product in India happens to be *A. chroococcum* because the farmers can see improvement in plant vigour in the early stages of growth, which however, begins to diminish to the level of uninoculated controls as the plants advance in age. Incidentally, India and probably Egypt are two countries which advocate the use of *Azotobacter*. However, there have been no attempts to monitor the fate of the added inoculant in the rhizosphere. Virtual absence of quality control measures provides room for many shady operators of this product in India.

Azospirillum

Azospirillum lipoferum and *A. brasilense* (*Spirillum lipoferum* in earlier literature) are primary inhabitants of soil, the rhizosphere and intercellular spaces of root cortex of graminaceous plants. Despite the fact that *Spirillum* was known since time of Beijerinck in 1925, it was the work of Dobereiner and Day (1975) that emphasized the nitrogen fixing ability of these bacteria in grasses. Isolates of these bacteria from Indian soils were made for the first time by Lakshmi Kumari *et al.* (1976) and tested for performance in pots by Subba Rao *et al.* (1979) and for their agronomic potential in the field under the BNF project of the ICAR

(Subba Rao, 1982–1986). Subsequently, the work at Tamil Nadu Agricultural University has shown the usefulness of these organisms in rice, sugarcane and oilseed crops. Presently, *Azospirillum* is being produced and marketed in southern India, but no quality control procedures are being followed and hence there exists the possibility of spurious manufacturers of these inoculants.

Apart from the nitrogen fixing ability of *Azospirillum*, the beneficial aspect of the organism lies in its ability to enhance the biomass of the root system, thereby affording greater surface area for absorption of native nutrients. Many other nitrogen-fixing azosprilla have now been described by the Brazilian workers such as *A. amazonense*, *A. halopraeferans* and *Herbaspirillum seropedicae* (Boddey and Dobereiner, 1988).

Cyanobacteria

Both free-living as well as symbiotic cyanobacteria (blue green algae) have been harnessed in rice cultivation in India. The pioneering work of De (1939) first demonstrated the role of BGA in rice cultivation which was followed up by Singh (1961) and Venkataraman (1972). A composite culture of BGA having heterocystous *Nostoc*, *Anabaena*, *Aulosira* etc. is given as primary inoculum in trays, polythene lined pots and later mass multiplied in the field for application as soil based flakes to the rice growing field at the rate of 10 kg/ha. The final product is not free from extraneous contaminants and not very often monitored for checking the presence of desired algal flora. Once so much publicized as a biofertilizer for the rice crop, it has not presently attracted the attention of rice growers all over India except pockets in the Southern States, notably Tamil Nadu. The benefits due to algalisation could be to the extent of 20–30 kg N/ha under ideal conditions but the labour oriented methodology for the preparation of BGA biofertilizer is in itself a limitation. Quality control measures are not usually followed except perhaps for random checking for the presence of desired species qualitatively. Recently, Kannaiyan (1996) reported that immobilized cyanobacteria in polyurethane foam and sugarcane waste with rice husk and soil as 1:1 material was found to be useful as a carrier based BGA inoculant.

Anabaena azollae is a nitrogen fixing symbiotic inhabitant of a tiny water fern *Azolla* and together form an organic input in rice cultivation. First demonstrated in Vietnam in 1957, the potentiality of *Azolla* has been recognized in the USA, Indonesia, Japan, The Philippines, China and India (Talley *et al.*, 1977; Singh, 1977; Kannaiyan, 1993). Incorporation of 10 t/ha *Azolla* biomass into the puddled rice field appears to be equivalent to a basal application of 25–30 kg N/ha, a practice which has been observed to be more beneficial than the dual culturing of *Azolla* with rice side by side in the same field to be incorporated later at the peak growth of the fern.

Both the methods are labour intensive and furthermore, vegetatively propagated *Azolla* has to be carefully nurtured during the winter months in nurseries to be used as seed material during the rice growing seasons. Mature sporocarps can also be used as inoculum and can minimize the bulk needed for the dual culturing method (Kannaiyan and Shanmugasundaram, 1992) but autolysis of fronds is a prerequisite for sporelings to emerge and perpetuate the life cycle. Careful monitoring for pests under restricted temperature regimes for obtaining better green biomass of the fern and maintaining the desired heterocyst frequency in the algal endosymbiont are vital factors in the proper establishment and utilization of the fern in the rice field and all these factors put together are deterrents for large scale adoption by farmers.

Phosphate solubilizing microorganisms

Several soil bacteria and fungi, notably species of *Pseudomonas*, *Bacillus*, *Penicillium*, *Aspergillus* etc. secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphates in soil (Garretsen, 1948; Sundara Rao and Sinha, 1963; Gaur and Ostwal, 1972). Increased yields of wheat and potato were demonstrated due to inoculation of peat based cultures of *Bacillus polymyxa* and *Pseudomonas striata*. Currently, phosphate solubilizers are manufactured by agricultural universities and some private enterprises and sold to farmers through governmental agencies. These appears to be no check on either the quality of the inoculants marketed in India or the establishment of the desired organisms in the rhizosphere.

VAM fungi

The transfer of nutrients mainly phosphorus and also zinc and sulphur from the soil *milleu* to the cells of the root cortex is mediated by intracellular obligate fungal endosymbionts of the genera *Glomus*, *Gigaspora*, *Acaulospora*, *Sclerocysts* and *Endogone* which possess vesicles for storage of nutrients and arbuscles for funneling these nutrients into the root system (Mosse and Tinker, 1975; Powell and Bagyaraj, 1984). By far, the commonest genus appears to be *Glomus*, which has several species distributed in soil. Availability of VAM (Vesicular Arbuscular Mycorrhiza) fungi in pure culture is an impediment in large scale production despite the fact that beneficial effects of VAM fungal inoculation to plants have been repeatedly shown under experimental conditions in the laboratory especially in conjunction with other nitrogen fixers.

Conclusion

While *Rhizobium* inoculants have been used for forage and fodder crops in Australia where established quality control methods exist, the use of such products is diminishing in the USA especially for soybean which was one of the dominant crops requiring rhizobial inoculation initially. The situation obtainable at present in India is reminiscent of that which existed in the USA in the first two decades of this century where quality control standards were non-existent. There is no doubt a set of standards in India formulated by the ISI for *Rhizobium* inoculants which is rarely used or enforced. Secondly, the performance of these products is often taken for granted under the pretext that 'inoculation is an insurgence against legume crop failures'.

No other country in the world except India which is currently producing and using P solubilizers, *Azotobacter*, *Azospirillum* and cyanobacterial products. The emphasis appears to be mainly on the non-availability of costly chemical fertilizers and 'judicial use of both chemical as well as organic inputs' in general rather than establishing guidelines for individual crops through carefully conducted field experiments. These inoculants have no specificity for crops and therefore are being used for an array of crops without feed back on whether they have established in desired numbers in the rhizosphere. This lack of specificity coupled with bulk sales mostly to governmental nodal agencies who in turn, supply to the cultivators often at cost or subsidized price are inherent drawbacks because no information on the success of inoculation with proper uninoculated controls is made available by the users. On the other hand, sale of biofertilizers from seed centres of retailers appears to be minimal and the true yardstick of success ought to be that the same customer voluntarily comes back to buy inoculants without the use of pressure tactics.

Basic research to improve strains is not being undertaken even in advanced agricultural institutions and the heavy financing by the Department of Biotechnology (DBT) in India in this sector has not paid any dividends. On the contrary, Microbiologists in agricultural universities seem to be content in field testing cultures borrowed or isolated at random. Currently, there exists no authoritative repository of agriculturally useful microorganisms in the country, which is a vital drawback in furthering sustained fundamental research. Furthermore, the need of the hour is to clearly establish whether the currently marketed microorganism or products have the seal of approval from a Central Quality Control Authority or four decentralized branches in India managed by qualified scientists with well equipped laboratories.