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From Editors Desk

Dear Readers,

During last two decades, biofertilisers have come a long way and have acquired a status of main stream agricultural inputs. Demand is growing with every passing year and more and more production units are being established to meet growing demand.

With the introduction of liquid formulations, biofertilisers are going through a silent revolution. Black dirty looking, carrier based biofertilisers are being replaced with liquid inoculants. Increased access to improved microbial technology, new biotechnological protocols and incorporation of new instruments and machines are promising contamination free, high count inoculants, with larger shelf life and better tolerance of temperature during storage. With the introduction of tissue culture based production technology, now it has become possible to prepare, high spore count, low volume Arbuscular Mycorrhizal biofertilisers. Encouraged with the technology many AM biofertiliser units are being established.

Usefulness and superiority of liquid inoculants over carrier based formulation and Mass production of AM biofertilisers are the major attractions of this issue. Besides these permanent columns on Research notes and new reports and Book Reviews are there in their original format. I hope the entire gamut of information being presented in this issue will be useful for the readers.

Dr. R.N. Bisoyi
Editor

Liquid Inoculant Technology: A Boon to Pulse Production

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Introduction

Through BNF process significant amount of N is added to agricultural soils. Among the different BNF processes, legume-*Rhizobium* symbiosis is the most effective means of N addition to terrestrial ecosystem. The main objective of research in this area since the last hundred years is to harness the potential of this process so that nitrogen requirement of crop plants could be met at lesser cost. The effective use of this technology will not only result in increased crop productivity and crop quality but also reduce environmental pollution. Although basic knowledge of this process has increased markedly, benefits of this technology has not been fully realized at the field level (Shantharam and Mattoo, 1997).

In order to enhance the effectiveness of this process, extensive research investigations have been conducted for selection of efficient strains. Researchers have been successful in developing and identifying rhizobial strains with enhanced nodulation, expanded host range and tolerance to environmental stress. In spite of availability of efficient rhizobial strains for different crops and for different conditions, the performance under field conditions is yet to be satisfactory. This failure may be attributed to poor inoculant technology, which involves transfer of *Rhizobium* from a pure slant culture in laboratory to legume rhizosphere at field condition.

The success of legume inoculation is mainly dependent on the quality and characteristics carrier material. A good quality inoculant should be made of a superior carrier material. Smith (1992) has listed characters of an ideal carrier material. Peat was considered as best and in the absence of peat, lignite and coal were used (Dube *et al* 1980; Kandasamy & Prasad, 1971). The cost of solid carrier based inoculant production is high as it is labour and energy intensive process, involving processes such as mining, drying, milling, sieving and correcting pH (Somasegaran and Hoben, 1994).

The carrier based *Rhizobium* inoculants produced in India are generally lignite, coal or charcoal based. The major disadvantages associated with these carriers are shorter shelf life, poor quality, high contamination and unpredictable field performance. In order to reduce contamination, it is advised to use sterilized carrier material but complete sterilization is usually not achieved as these materials are heterogeneous. Further, the process of sterilization is cumbersome and not cost effective at the commercial level. Bioinoculant formulations of good quality are expected to have higher population of desired microorganisms without contamination for long period of storage.

Liquid inoculant formulation may provide solution to some of these problems associated with carrier based inoculants

and may enhance yield of treated plants markedly. Research to develop a suitable liquid inoculant formulation for *Rhizobium* sp. was pursued in this department and important findings are summarized here.

Liquid *Rhizobium* inoculants

Liquid formulations are not broth culture of rhizobia obtained from the fermentor or water suspension of the carrier based inoculant of rhizobia. They are special liquid formulations containing viable propagules of desired bacteria, components of nutrient media and certain chemicals primarily function as bacterial cell protectants. These cell protectants are chemical amendments which will promote cell survival in inoculant formulations during storage and after application to seed or soil. Further, these amendments offer protection to cells under extreme conditions such as high temperature, desiccation and presence of toxic compounds. Several polymers have been used as additives in liquid inoculant formulations because of their ability to limit heat transfer, rheological properties and water retention properties (Mugnier and Jung, 1985). Poly vinyl pyrrolidone (PVP) is one such polymer, which adsorbs bacterial toxins and protects enzyme systems as well as proteins against high temperature. Addition of PVP in the medium was known to protect both fast and slow growing *Rhizobium* (Bushby & Marshall, 1977). Studies by Vincent *et al* (1962) and Bushby & Marshall (1977) have showed that addition of maltose (9%) and montmorillonite clay could protect *Rhizobium* against high temperature and desiccation. Compared to clay, polymers that are soluble make preparation of inoculants and its application on to seeds simple. Polymers and other additives used in liquid inoculants should be selected based on their properties such as solubility in water, toxicity and chemical complexity (Deaker *et al*., 1994). Some of the polymers and chemicals which can be used as additives and protectants in liquid inoculants includes PVP, methyl cellulose, gum arabica, trehalose, glycerol, sodium alginate, poly ethylene glycol, poly vinyl alcohol and tapioca flour (Panlada *et al*., 2007). Liquid inoculant formulation of cowpea rhizobia prepared with PVP as an osmo-protectant has been observed to have higher shelf life than those without PVP amendment (Girisha *et al*., 2006).

Investigations conducted in this laboratory suggest that liquid formulations are better than carrier-based formulations as they support higher number of bacterial cells for a longer time (Fig.1). The solid carriers did not support higher number of rhizoibia possibly because they are heterogeneous in nature and do not have sufficient nutrients in easily available form. Further, the rate of loss of moisture from solid carriers is faster than liquid formulations. Liquid inoculants contain sufficient nutrients which permit the increase in population up to the level of thousand million cells per ml, which results in three to four fold increase in the numbers of viable bacteria compared to carrier based inoculants. Liquid formulations are easy to prepare as well as apply on to seeds. The number of bacterial cells surviving on legume seeds was noticed to be higher when they were treated with liquid inoculants.

An important advantage of liquid formulation is that, it is possible to achieve complete sterilization of medium. Further, sterilization of liquid medium is easier compared to solid carriers. It is possible to maintain liquid inoculants without contamination; hence, it would be easier to meet BIS standards. The BIS standards suggest

that at 10^{-5} dilution there should be no contaminants in inoculants formulation. Any contamination occurring during storage can be easily noticed with liquid inoculants which is not possible with solid carriers.

It is important to have more number of bacterial cells per seed, to overcome the competition from native *Rhizobium* and to offset the death of cells due to biotic and abiotic stresses. A strong correlation exists between the number of surviving cells on seeds and nodulation in legumes. The number of bacterial cells surviving on groundnut seeds were higher when liquid inoculant was used compared to carrier based inoculant (Fig. 2). The chemical constituents of liquid formulation permit more number of bacteria to survive on seeds, possibly by offering protection against many biotic and abiotic factors. One of the major advantages of using liquid *Rhizobium* inoculant is that it does not require any sticker material, unlike carrier based biofertilizer.

Carrier materials such as lignite and charcoal may not offer protection against stresses. Data presented in Fig. 3 clearly indicates that liquid formulation offers protection to rhizobial cells against high temperature. The duration between the preparation and utilization of biofertilizers is generally more under our conditions. Further, conditions during storage and transportation may not be congenial for bio-inoculants. For example, temperature in many parts of the country may reach up to 45^0C especially in *kharif*. In such conditions the quality of biofertilizers is adversely affected. Usually the shelf life of most microbial inoculants stipulated in India is six months from the date manufacture. Hence, there is a need to manufacture biofertilizers with longer shelf life. These studies suggest that the liquid *Rhizobium* inoculant can be stored without significant loss in viability for more than one year with high percentage of recovery of viable cells (Fig. 4). During the same time the loss of viable cells from the lignite based inoculant was significant. Further, liquid inoculants can be stored without loosing viability under high temperature conditions also. The special cell protectants added to liquid *Rhizobium* inoculants were found to protect bacterial cells against high temperature (> 40^0C).

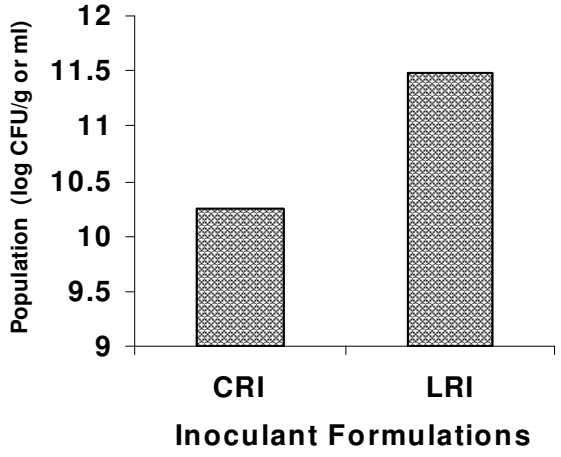

Fig. 1: Population of *Rhizobium* in Carrier and Liquid inoculant formulations
LRI : Liquid *Rhizobium* Inoculant, CRI : Carrier based *Rhizobium* Inoculant.

Liquid inoculant formulations are easy to apply and can be effectively integrated with mechanized farming. The amount of inoculant needed for seed inoculation is less, it can be simply sprayed on to seeds and there is no need of any sticker material unlike carrier-based inoculants. Liquid inoculants can easily be used in advanced seed drills, since it can be sprayed on to seeds as it passes through seed augar and dries before it get placed in to the seed bin on the planter.

The liquid inoculants could be produced by simple fermentation process with minimum labour, space and energy, as the culture from the fermentor is directly packed under aseptic conditions and stored. The cost of production of liquid formulation could be lesser than that of carrier formulation as the cost of processing and sterilization of solid carrier material is eliminated.

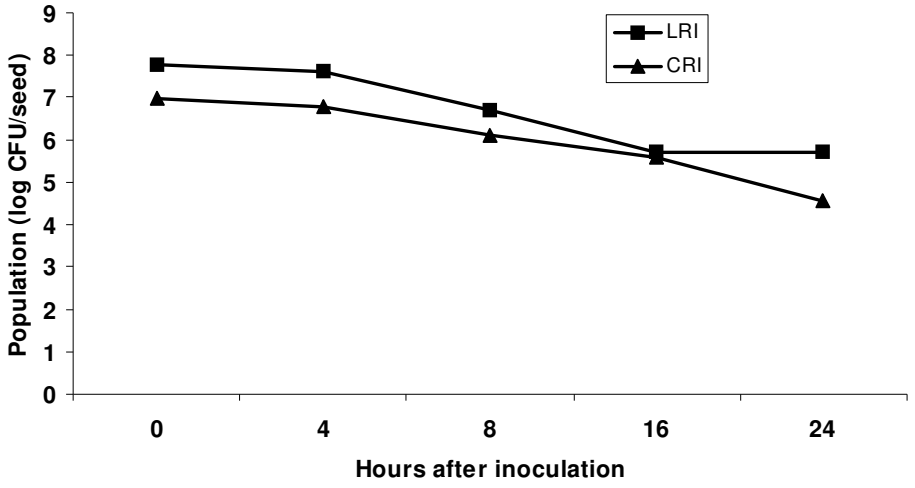

Fig. 2: Survival of *Bradyrhizobium* sp. (Arachis) on groundnut seeds.
LRI : Liquid *Rhizobium* Inoculant, CRI : Carrier based *Rhizobium* Inoculant
LRI and CRI were used at the rate of 4 ml and 20 grams per kg of seeds, respectively.

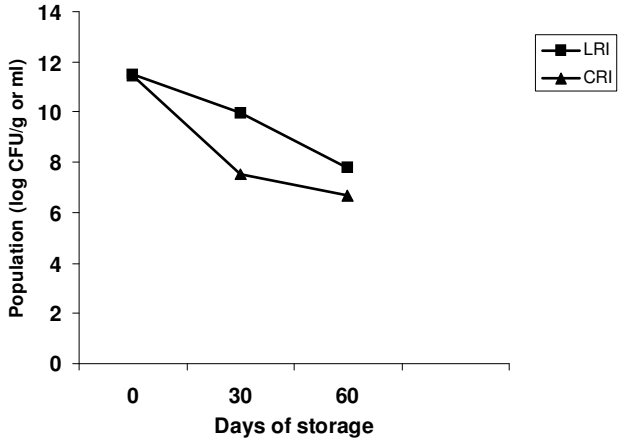

Fig. 3: Survival of *Bradyrhizobium* sp. (Arachis) stored at 48^0C.
LRI : Liquid *Rhizobium* Inoculant, CRI : Carrier based *Rhizobium* Inoculant

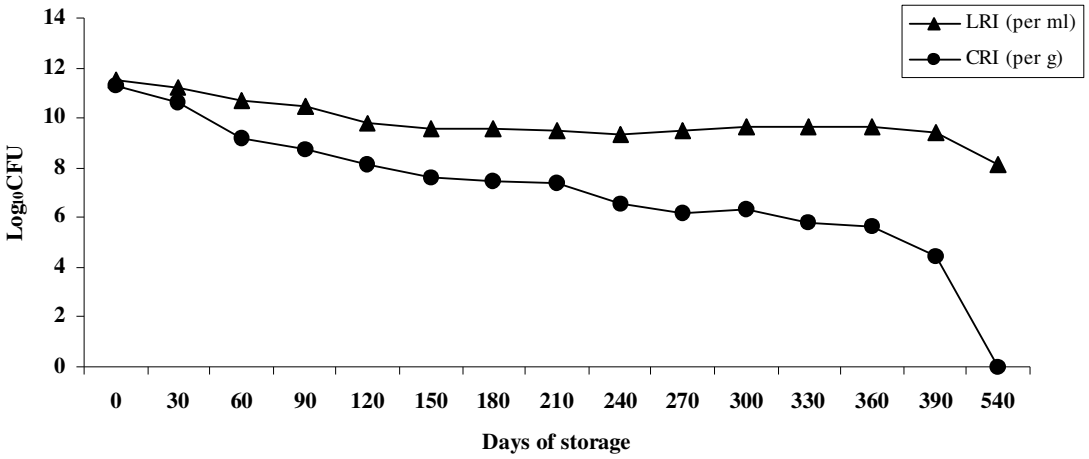

Fig. 4: Survival of *Bradyrhizobium* sp. (arachis) stored at room temperature. LRI : Liquid *Rhizobium* Inoculant, CRI : Carrier based *Rhizobium* Inoculant

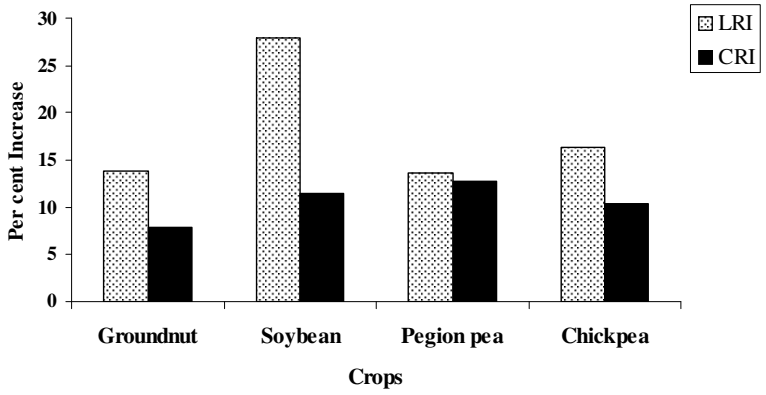

Fig. 5: Increase in grain yield of pulses due to inoculation with liquid *Rhizobium* inoculant in farm trials. LRI : Liquid *Rhizobium* Inoculant, CRI : Carrier based *Rhizobium* Inoculant

Field response of liquid *Rhizobium* inoculant formulation

The field efficacy of liquid inoculant formulation was tested in on-farm-trails (120) conducted on farmers' fields under different agro-climatic conditions (13 districts in 7 states) in India, for two successive years on four important pulse crops such as groundnut, pigeon pea, chickpea and soybean. There was increase in number of nodules, nodule dry weight, when plants were inoculated with the liquid inoculants compared to carrier based inoculants. Depending on legume species, increase in seed yield due to the application of liquid inoculants ranged from 4 to 25 per cent (Fig. 5). Among legumes soybeans responded markedly to inoculation with liquid inoculants.

This new technology appears to offer possibility to over come the constraints associated with conventional biofertilizers technology. The liquid inoculants are easy to use and have been noticed to enhance seed yield significantly in major pulse crops.

Therefore this technology could be extended to rhizobia of other pulses. This research project on liquid rhizobial inoculant was funded as NATP project and the possibility of extending the liquid inoculant technology to other important microbial inoculants such as *Azotobacter*, *Azospirillum* and Phosphate solubilizing bacteria is being explored in this department in a project funded by the Department of Biotechnology, New Delhi.

Acknowledgement: The funding from NATP, ICAR and DBT, New Delhi, to pursue research on liquid inoculant technology is gratefully acknowledged.

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MASS PRODUCTION OF ARBUSCULAR MYCORRHIZAL BIOFERTILIZER

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Introduction

Many microorganism form symbioses with plants that range from parasitic to mutualistic. Among this the most widespread mutualistic symbiosis is the arbuscular mycorrhizal association. Arbuscular mycorrhizal (AM) symbiosis occurs between the fungi of the Glomeromycota (Schubler *et al.,* 2001) and majority of terrestrial plants. The phycobiont correspond to 80% of plant species and this association involves an intimate relationship between plant roots and fungal hyphae. This mutualism is manifested in bidirectional nutrient exchange: the fungus is nourished by plant photosynthates and plant mineral nutrition particularly phosphate is enhanced by the fungus (Smith and Read, 1997). AM fungi are obligate biotrophs, depending on living root tissue for carbohydrate supply to complete their asexual life cycle.

Culturing of AMF

The obligate symbiotic nature of the fungus makes axenic cultivation an important challenge for both scientific and practical point of view. Inability to culture AM in the laboratory is the major limiting factor in their application in agriculture. Though AMF has very broad specificity towards plants including various agricultural horticultural and forestry plant species, but the ability to produce AM in bulk quantities is a major bottleneck. AM biofertilizer is currently recommended only for transplanted and nursery raised crops because of the difficulty in inoculum production as well as the bulk requirement of the inoculum. Various methods were developed for mass production of AM fungi world wide.

Tissue culture method

Tissue culture method was developed for production of AM spores in aseptic conditions. Mosse (1959) obtained germination of spores in sterile conditions. Many Endogonaceae germinate in a few days when they are placed on agar medium. However retarded growth of resultant hyphae occurs. For germination, the spores do not require a plant signal, even it can germinate in water. Following germination, the fungus uses triglyceride and glycon reserves in the spore to support growth and the hyphal germ tube extends from the spore. Latter the hyphal growth ceases, when the spore reserve are depleted. Moreover, because of the absence of the natural plant metabolic factors in the culture medium, hyphal growth retards. The plant extracts have been found to improve the hyphal growth. A range of organic substances viz., vitamins, and sulphur compounds has been found to promote hyphal growth to different extents (Hepper, 1984). The presence of roots or cell suspension is also stimulatory for hyphal development.

In vitro AM production

Since AM can not be grown on laboratory culture media, it is grown *in vitro* using root organ culture method as dual cultures. Root organ cultures

consist of excised roots that proliferate under axenic conditions on a synthetic nutrient media, supplemented with vitamins, minerals and carbohydrates. Continuous cultures have been obtained through transformation of roots by soil bacterium *Agrobacterium rhizogenes*. A single Petridish culture can generate several thousands of spores and meters of hyphae with in 4-5 months.

The desirable characteristic of the transformed roots is their ability to quickly form numerous lateral roots. These roots are better adopted to growth in culture than normal roots and they also survive longer periods without subculture. Transformed roots have the same synthetic capacity as roots of the plant from which they have been obtained. The rate of *in vitro* spore production was high in transformed roots and yield an average of 9500-10,000 spores / Petri dish after 5 months of dual culture. The root organ culture system supported extensive root colonization with many arbuscules, vesicles and more of intraradical mycelium and hence exhibited higher inoculum potential (Declerck *et al.,* 1996). The cost of *in vitro* inoculum may appear high compared to the cost of green house propagated one, but its use as starting inoculum is warranted for purity.

Aeroponic system of AM inoculum production

Colonised roots, spores and hyphae of the endophyte could be produced in aeroponic systems. Precolonized plants are needed for this system. In aeroponic system, a fine mist of defined nutrient solutions dispersed in air and roots of the host suspended in the chambers are bathed intermittently. This method of AM multiplication is free from any substrate and can be sheared which resulted in very high propagule numbers (Sylvia and Jarstfer, 1992).

Soil based inoculum production

Culturing AM fungi on plants growing in disinfected soil is the most commonly used technique for increasing number of AM propagules (Menge, 1984). Due to their obligate symbiotic status, large scale production requires control and optimization of both host growth and fungal development. The large scale inoculum production process entails the following stages.

Starter culture

Large scale production begins with a starter culture. The starter culture must be pure and it can be obtained originally from a single spore that can germinate and colonize the host roots. AM fungal strain can also be germinated from colonized root segments. The cultures can be obtained through subsequent pot culture using isolated spores or colonized root segments. A technical problem encountered here is the dormancy of AMF spores and decrease in germination rates.

Choice of host plant

The most important criteria required for the host plant is the high mycorrhizal potential (ie. Its capacity to be colonized by the AMF strain and to promote its growth and sporulation), a tolerance to growth under greenhouse conditions, and an extensive root system. Plants such as maize, sorghum and bahia grass have been suggested as suitable hosts for inoculum production (Douds *et al.,* 2000)

Optimum growth conditions

Pasteurized / steamed substrats are required to produce good quality inoculum. A well aerated substrate such as coarse texture sandy soil with vermiculite or perlite can be used. P application at higher levels, depress sporulation. Free application of N seems to increase the spore production in

several isolates of AM fungi (Douds and Scheck, 1990 a) except a few. Potassium, magnesium and a selection of micro element ratios may also affect inoculum development, especially when inert growing substrates are used. Other edaphic factors such as pH, soil temperature must also be controlled to optimize AMF propagation.

Soil less substrate based systems are also widely used for the multiplication using vermiculite, perlite like inert substrates. Primary advantage of using soil less substrates are for their purity and free from soil contaminants and also the substrate can hold sufficient water for plant growth and allow good aeration. Frequent additions of nutrient solutions and also the use of fertilizers in minimal quantities may help in the nutrient management of host roots. (Douds and Schenck, 1990a).

Inoculum from pot cultures may be stored for several months or years. However, for more reliable storage of a wide range of AM fungi, the fungi can be dried *in situ* with the host and then frozen at – 70ºC (Douds and Schenck, 1990b).

Improving the inoculum Quality in pot cultures

Though the better substrate and suitable host combination is arrived today for mass production of AM under pot culture, still the problem exists with production. It is mainly due to poor sporulation. Since spore production is less, the inoculum may contain only less spores, that warranted the bulk requirement of AM inoculum for applicaton. Unless the dose for application is reduced, this may not be recommended for all crops. Increasing sporulation in the substrate host system may pave way for the possibility of reducing AM inoculum dose to the field crops.

Given the obligate biotrophism of AM fungi it is logical that most of the interactions between this group of fungi and the rest of the soil microbiota take place during their symbiotic phase. It has been described that root colonization of AM fungi can be improved by the presence of certain microorganisms such as *Azospirillum , Rhizobium, Acetobacter, Pseudomonas* etc. Some microbes have been shown to induce specifically the formation of arbuscule while having little or no effect on the total percentage of root colonization However if these effects take place through a direct stimulation of AM fungi or via the stimulation of the root exudates production, mycorrhizal colonization can be increased significantly.

Considering these aspects in mind, a pot culture experiment was conducted recently in the department of Agricultural Microbiology, TNAU to enhance the spore load and AM colonization in the commercial inoculum being produced using vermiculite as substrate. This particular experiment was conducted using tomato (var. Co.3) as model host system.

The treatment includes

1. *Azospirillum*
2. *Azotobacter*
3. *Pseudomonas*
4. Phosphobacteria
5. PPFM (pink pigmented facultative methylotroph)
6. Control (AM alone)

All these treatments were imposed on AM inoculated tomato seedlings. Tomato seedlings were raised in plastic crates with the inoculation of AM fungi *(Glomus mosseae*) at the rate of 5 g kg soil^{-1} and planting was done after one month in pots. Again AM inoculation was done while planting in pots (containing 10 kg soil) Properties of the soil used for the

studies are described as follows. pH 6.6; EC.0.20dSm^{-1}; available nitrogen 300 kgha^{-1};available phosphorus12.0 kgha^{-1} and available potassium 280 kg ha^{-1}. *Azospirillum, Azotobacter, Pseudomonas,* Phosphobacter and PPFM were inoculated at the rate of 25 ml (containing 10^8 cells / ml) pot^{-1} while planting. Plant samples were taken after 45 and 90 days of planting. Growth biomass, AM fungal colonization in roots, spore load as well as rhizosphere population were estimated. Fruit yield was also recorded.

The results indicated that co culturing of PGPR organisms and AM fungi, in tomato significantly enhanced the growth and yield of plants. On 45th DAP, *Pseudomonas* inoculation registered 40% increase in root length, 25% increase in biomass over inoculation of AM alone. And at 90 DAP the increase was 48 and 53% respectively (Fig.1).

AM colonization was reported to be high in *Pseudomonas* inoculation (96-97%) which was followed by *Azotobacter* and PPFM. Increased AM colonization may occur through a direct stimulation of AM fungi or via, the stimulation of the root exudates production which is highly correlated to mycorrhizal colonization. Considerable increase in spore number was observed and it was about 33% and 68.8% higher over AM inoculation alone at 45 and 90 DAP respectively (Table1). PGPR not only accelerated the root growth but also enhanced the mycorrhizal colonization and stimulated the spore production (Bhowmik and Singh, 2004). Besides increasing spore load and AM colonization 6-11% increase in fruit yield was recorded with the inoculation of PGPR along with AM fungi (Data not presented).

Fig.1 Influence of AM fungi and PGPR inoculants on growth of tomato

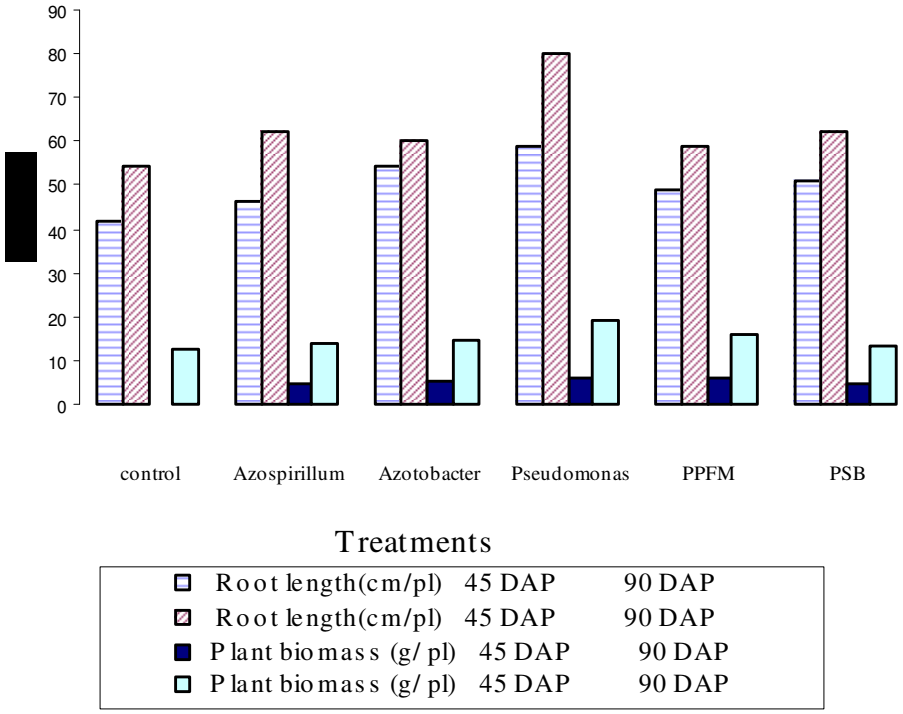

Table.1 Influence of Coinoculation of PGPR organisms on AM fungal population in Tomato

Treatments	AM Colonization (%)		Spore load (No. / 100 g)	
	45 DAP	90 DAP	45 DAP	90 DAP
Uninoculated control (AM alone)	80.5	82.0	43.6	46.5
Azospirillum	87.0	88.0	52.7	56.6
Azotobacter	91.2	92.0	56.5 (29.5*)	66.3 (42.6*)
Pseudomonas	96.0	97.0	58.0 (33.0*)	78.5 (68.8*)
PPFM	87.0	89.0	51.0	58.2
PSB	82.0	90.0	51.3	57.5
CD at 5 %	3.4	4.02	10.2	6.6

(*Percent increase over uninoculated control)
(All the treatments are uniformly given with AM inoculation)

Table.2 Effect of coinoculation of PGPR organisms on microbial population in the rhizosphere of tomato (cfu/g)

Treatments	*Azospirillum* $x10^6$(cfu/g)	*Azotobacter* $x10^4$(cfu/g)	*Pseudomonas* $x10^4$(cfu/g)	PPFM $x10^4$(cfu/g)	PSB x 10^4(cfu/g)
Un.control (AM alone)	0.47	9.0	13.0	1.0	7.5
Azospirillum	116.0	11.0	46.0	3.0	20.0
Azotobacter	2.20	14 x 10^6	28.0	3.0	33.5
Pseudomonas	0.70	15.0	99x10^6	2.0	19.0
PPFM	0.70	17.0	13.0	70.6x10^5	15.0
PSB	1.33	13.0	16.0	2.0	25x10^6

(All the treatments are uniformly given with AM inoculation)

Population of *Azospirillum, Azotobacter, Pseudomonas,* PPFM and PSB were recorded in all the treatments at 90 DAP. The results showed that survival of all the inoculated organisms was found satisfactory after, 3 months of inoculation especially *Pseudomonas* sp. population was in10^6-10^7 (99X10^6 cfu) at 90 DAP

(Table 2). It has been proposed that the fungus can increase the survival of free living bacteria in the rhizosphere probably by increasing the exudation ability of the plant or directly by creation of new microhabits due to the development of mycelial network.

Present results suggest that coinoculation of PGPR organism especially *Pseudomonas* while culturing AM fungus will increase the spore load as well as root colonization in the inoculum which in turn improve the quality of the commercial inoculum

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Research Notes and New Reports

Coimmobilization of *Azospirillum lipoferum* and *Bacillus megaterium* for Successful Phosphorus and Nitrogen Nutrition of Wheat Plants - The efficacy of strains of *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum* spp. in *in vitro* solubilization of Ca_3PO_4 was studied. *Pseudomonas fluorescens* and *Bacillus megaterium* strains were found to be most powerful phosphate solubilizers on Pikovskaya (PVK) plates and liquid medium. *Azospirillum lipoferum* strains showed weak zones of solubilization on the PVK plates. Phosphate solubilization by the tested organisms was accompanied with pH reduction of the culture medium. Maximum pH reduction was 2.8, 1.2 and 0.5 units for *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum lipoferum* strain 137, respectively. Alginate and agar immobilization of the tested bacteria or coimmobilization of *A. lipoferum* 137 and *B. megaterium* significantly enhanced phosphorus solubilization for four consecutive 4-day cycles. In a pot experiment, phosphorus mobilization in wheat (*Triticum aestivum* L. cv. Beni Swif 1) inoculated with *B. megaterium* or *A. lipoferum* 137 as single or mixed inocula (as free or alginate immobilized beads) was studied in presence of Ca_3PO_4. Wheat inoculated with mixed inocula exhibited high shoot dry weight, total nitrogen (N) yield and the shoot phosphorus content increased by 37 and 53 % compared to the plants inoculated with *A. lipoferum* and uninoculated ones, used as control, respectively. Maximum nitrogenase activity (measured by acetylene reduction assay) was observed in mixed inoculum treatment, and was increased by 500 and 32 % compared to uninoculated and *A. lipoferum* inoculated plants. Results demonstrate the beneficial influence of coinoculation of *A. lipoferum* and *B. megaterium* for providing balanced N and P nutrition of wheat plants.
(Source - *El-Komy* 2005 *Food Technol. Biotechnol. 43* (1) 19–27)

Effect of P-solubilizing *Azotobacter chroococcum* on N, P, K uptake in P-responsive wheat genotypes grown under greenhouse conditions - Natural and mutant strains of *A. chroococcum* were isolated from Indian soils. Their ability to dissolve phosphate and their phytohormone production were tested under *in vitro* conditions. In addition the effect of bacterial inoculation of *Azotobacter* on N, P, K uptake by three P responsive wheat genotypes (*Triticum aestivum* L.) under greenhouse conditions at five nutrient levels (Control, 90 kg N ha^{-1}, 90 kg N + 26 kg P ha^{-1}, 120 kg N ha^{-1} and 120 kg N + 26 kg P ha^{-1}) was studied. *In vitro* phosphate solubilization and growth hormone production by mutant strains was more than by the soil isolates. Inoculation of wheat varieties with the soil isolates and mutant strains of *A. chroococcum* showed greater NPK uptakes as compared with parent soil isolates. Mutant strains M15 and M37 were proved to be the most effective for all three wheat varieties with regard to NPK uptake as well as root biomass production under greenhouse conditions.
(Source – Narula et al 2000Journal of Plant Nutrition and Soil Science, Vol 163(4) 393-398)

Solubilization of inorganic phosphates and growth emergence of wheat as affected by *Azotobacter chroococcum* mutants - Phosphate-solubilizing strains of *A. chroococcum* isolated from the wheat rhizosphere

were evaluated for their ability to solubilize tricalcium phosphate (TCP), Mussoorie rock phosphate (MRP) and also for indole-acetic-acid (IAA) production. Strains were selected on the basis of the clearance zone on solid agar media of Pikovskaya and Jensen's media containing TCP, and phosphate solubilization in Jensen's liquid culture medium containing both TCP and MRP. Mutants of the best phosphate-solubilizing (TCP 1.52 7g ml^{-1} MRP 0.19 7g ml^{-1}), IAA-producing *A. chroococum* strain P-4, were developed and screened for P solubilization and phytohormone production. Five mutants solubilized more P (in the range of 1.5-1.7 7g/ml^{-1} of TCP and 0.19-0.22 7g ml^{-1} of MRP) than the parent strains. *In vitro* growth emergence studies of three wheat varieties, viz. C-306, WH-542 and HD-2009, showed better performance with phosphate-solubilizing mutants than with the parent strain.
(Source – Kumar and Narula 1999 Biology and Fertility of Soils 28(3) 301-305)

Characterization and screening of bacteria from rhizosphere of maize grown in Indonesian and Pakistani soils - Thirty rhizobacteria isolated from maize grown in Pakistani and Indonesian soils were evaluated for their morphological characteristics, nitrogen fixation, P-solubilization, indole acetic acid (IAA) and siderophores production. Nitrogenase activity was detected in nineteen isolates ranging from 21.8-3624 n moles C_2H_4 produced/h/mg protein. Most of the isolates produced IAA, ten were capable of siderophore production while four were P-solubilizers. Ultrastructural studies of *Pseudomonas* sp. F14 indicated characteristic rhizospheric colonization within 48 h that was observed to change considerably with the passage of time from few bacteria to micro colonies. Random amplified polymorphic DNA (RAPD) analysis of 30 bacterial strains using 30 oligonucleotide primers resulted in considerable level of genetic diversity, with genetic distance ranging from 2-16%. Indonesian isolates were found to be more diverse as compared to Pakistani isolates. The characterization and screening of rhizobacteria of maize rhizosphere has helped in selection of isolates F7, LS-1, 3.1.1.C, F2, F3 and F13 as superior strains for use as bioinoculant. Moreover isolate F14 identified, as *Pseudomonas fulgida* by partial 16S rRNA sequence analysis is a novel strain regarding its tremendous potentials for inoculum production to enhance the yield of maize. (© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim).
(Source – Naureen et al 2005, Journal of Basic Microbiology 45 (6) : 447-459).

Interactive Effect of Rhizotrophic Microorganisms on Growth, Yield, and Nutrient Uptake of Wheat - The interactive effect of rhizotrophic microorganisms on growth, yield, and nutrient uptake of wheat (*Triticum aestivum* L.) was determined in a pot experiment using sterilized soil deficient in available phosphorus (P). Positive effect on plant vigor, nutrient uptake, and yield in wheat plants was recorded in the treatment receiving mixed inoculum of nitrogen-fixing *Azotobacter chroococcum* + phosphate solubilizing microorganism (PSM) *Pseudomonas striata* + arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum*. The available P status of the soil improved significantly ($P \leq 0.5$) following triple inoculation with *A. chroococcum, P. striata*, and *G. fasciculatum*. The residual nitrogen (N) content of the soil did not change appreciably among the treatments. Addition of *Penicillium variable* to single- or double-inoculation treatments negatively affected the measured parameters. The population of *A. chroococcum*, PSM, percentage root

infection, and spore density of the AM fungus in inoculated treatments increased at 80 days of wheat growth. The present finding showed that rhizotrophic microorganisms can interact positively in promoting plant growth, as well as N and P uptake, of wheat plants, leading to improved yield.
(Source – Zaidi and Khan 2005 Journal of Plant Nutrition, 28 (12) : 2079-2092).

Improvement of Rice Productivity by Application of *Azotobacter* Inoculum - The beneficial effect of applying carrier based inoculum of one highly potent rhizospheric phosphate solubilizing and N_2 fixing strain of *Azotobacter* sp. R12 on two rice varieties was assessed. The strain solubilized about 61% rock phosphate in 3 days and produced 2-keto-gluconic acid as the active principle for solubilization. The rice varieties were medium grained MTU 7029 (Swarna massouri) of rainy season (Aman) and a fine grained IET 4094 (Khitish) for summer season (Boro) are described . The effect of bacteria was tested with the application of different doses of phosphate and nitrogen. The growth, nitrogen content, weight of thousand grains, chlorophyll content, and yield of the crops were remarkably increased with application of the strain. The strain R12 showed its best performance at zero level of P_2O_5 and N_2. In the presence of high level of P_2O_5 and N_2 beyond the recommended dose the performance of the isolate was not satisfactory. The strain was applied to the crop field by seed-inoculation, seedling-inoculation and twice subsequent application of carrier-based inoculum at the intervals of fifteen days.
(Source – Mandal et al 2006 Paper presented at The 18th World Congress of Soil Science (July 9-15, 2006).

Impact of the use of biofertilizers on cotton (*Gossypium hirsutum*) crop under irrigated agro-ecosystem - High nitrogen fixing, phosphate solubilizing, phytohormones producing isolates of *Azotobacter*, *Azospirillum*, *Acetobacter* and *Pseudomonas* were used as inoculants for cotton. Important cultures were selected on the basis of their effect on root/shoot length and chemotactic behaviour. Selected bioinoculants were earlier tested for their beneficial properties like nitrogen fixation (ARA), ammonia excretion, IAA production etc. These bio-inoculants were further tested for phosphate solubilization property. Various chosen strains were tested with Desi (HD 123) and American (H 1098) cotton under field conditions (as for wheat). Plant height and boll weight were determined at the time of harvesting whereas survival rate of inoculated bacteria was determined after 30, 80 and 130 days respectively. In the year 2000 - 01, on the basis of boll number and boll weight plant $^{-1}$ AVK 51 (36; 76.2 g plant $^{-1}$), HT 57 (27; 56.9 g plant $^{-1}$), AC18 (33; 61.5 g plant $^{-1}$), Ala 27 (36; 61.4 g plant $^{-1}$) and *Pseudomonas* (34; 71.3 g plant $^{-1}$) were identified as significant both for American and desi cotton varieties. Highest survival rate was observed with Mac 68 (33.4×10^5) followed by HT54 (31.5×10^5) after 30 days of sowing, which decreased after 80 days and remained constant up to 130 days. This trend was observed with all the cultures. Similar results were observed in 2001 - 02. 25 kg ha $^{-1}$ N saving was observed with *A. chroococcum* (AVK51) bioinoculant for cotton crop.
(Source – Narula et al 2005, Archives of Agronomy and Soil Science 51 (1) : 69 – 77)

Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilising bacteria and a mixed rhizobial culture - Isolation of phosphate solubilising bacterial strains was carried out from rhizosphere, roots

and nodules of chickpea, to study the viability for solubilisation of tri-calcium phosphate and the effect on growth of chickpea plants. The potential of isolated bacterial strains to solubilise phosphate was qualitatively evaluated by the measurement of a clear zone around the colonies. The diameter of this zone ranged from 21 to 83 mm. Phosphate solubilisation, by phosphate solubilising bacterial isolates, was quantified by spectrophotometry and was found to range from 65 to 130.5 µg/mL. The drop in pH ranged from 5.6 to 3.6. The plant growth, shoot phosphorus and nitrogen concentrations, nodulation efficiency and nitrogenase activity were significantly enhanced, showing the positive effect of phosphate solubilising bacteria inoculation. Phosphate solubilising bacterial strains CPS-2, CPS-3 and Ca-18 had the maximum positive effect on shoot length, shoot dry weight and nodulation of chickpea plants. Treatments inoculated with non-phosphate solubilising bacterial strains IFA_1 and IFA_2 showed the minimum values in all the parameters.
(Source – Gul et al 2004, Australian Journal of Experimental Agriculture 44(6) : 623-628).

Establishment of phosphate-solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under green house conditions - A pot experiment was conducted in the green house to investigate the establishment of phosphate solubilizing strains of *Azotobacter chroococcum*, including soil isolates and their mutants, in the rhizosphere and their effect on growth parameters and root biomass of three genetically divergent wheat cultivars (*Triticum aestivum* L.). Five fertilizer treatments were performed: Control, 90 kg N ha($^{-1}$), 90 kg N + 60 kg P2O5 ha($^{-1}$), 120 kg N ha($^{-1}$) and 120 kg N + 60 kg P2O5 ha($^{-1}$). Phosphate solubilizing and phytohormone producing parent soil isolates and mutant strains of *A. chroococcum* were isolated and selected by an enrichment method. *In vitro* phosphate solubilization and growth hormone production by mutant strains was increased compared with soil isolates. Seed inoculation of wheat varieties with P solubilizing and phytohormone producing *A. chroococcum* showed better response compared with controls. Mutant strains of *A. chroococcum* showed higher increase in grain (12.6%) and straw (11.4%) yield over control and their survival (12-14%) in the rhizosphere as compared to their parent soil isolate (P4). Mutant strain M37 performed better in all three varieties in terms of increase in grain yield (14.0%) and root biomass (11.4%) over control.
(Source – Kumar et al Microbiol Res. 2001;156(1):87-93).

Effect of P-solubilizing *Azotobacter chroococcum* on N, P, K uptake in P-responsive wheat genotypes grown under greenhouse conditions - Natural and mutant strains of *A. chroococcum* were isolated from Indian soils. Their ability to dissolve phosphate and their phytohormone production were tested under in vitro conditions. In addition the effect of bacterial inoculation of *Azotobacter* on N, P, K uptake by three P responsive wheat genotypes (*Triticum aestivum* L.) under greenhouse conditions at five nutrient levels (Control, 90 kg N ha[$^{-1}$], 90 kg N + 26 kg P ha[$^{-1}$], 120 kg N ha[$^{-1}$] and 120kg N + 26 kg P ha[$^{-1}$]) was studied. In vitro phosphate solubilization and growth hormone production by mutant strains was more than by the soil isolates. Inoculation of wheat varieties with the soil isolates and mutant strains of *A. chroococcum* showed greater NPK uptakes as compared with parent soil isolates. Mutant strains M15 and M37 were proved to be the most effective for all

three wheat varieties with regard to NPK uptake as well as root biomass production under greenhouse conditions. (Source – Narula et al 2000, Zeitschrift für Pflanzenernährung und Bodenkunde 163 (4) : 393-398).

Azide resistant mutants of *Acetobacter diazotrophicus* and *Azospirillum brasilense* increase yield and nitrogen content of cotton - Evolution of symbiotic plant-microbe interactions has provided mankind a powerful and environment-friendly means to increase yield of agricultural crops. Here, authors report that some azide resistant mutants of two microbial strains can significantly enhance the productivity of cotton varieties, as an attractive and cheap biological substitute of chemical fertilizers, for improved yield of an important cash crop, without any untoward impacts. Sodium azide resistant mutants were isolated from each strain of *Azospirillum brasilense* and *Acetobacter diazotrophicus* on different concentrations of sodium azide ranging from 5-60µg/ml. These azide resistant mutants were assessed for their performance on cotton (varieties H-117, HD-123) for various parameters. Inoculation of cotton seeds with mutants obtained better results than inoculation with their respective parental strains. Azide resistant mutants, when used as biofertilizers, showed increased plant height, early flowering, more yield, and high biomass and total nitrogen content. They also increased, in cotton genotypes, the indole acetic acid production and ammonia excretion due to high nitrogenase activity.
(Source – Sharma and Vasudeva 2005 Journal of Plant Interactions, Vol 1 (3) : 145-149).

Effect of phosphate solubilizing starains of *Azotobacter chroococcum* on yield traits and their survival in the rhizosphere of wheat genotypes under field conditions - A field experiment was carried out to investigate the establishment of phosphate-dissolving strains of *Azotobacter chroococcum*, including soil isolates (wild type) and their mutants, in the rhizosphere and their effect on the growth attributes and root biomass of three genetically divergent wheat cultivars (*Triticum aestivum* L.). Four fertilizer doses were applied : 90 kg N ha^{-1}, 90 kg N + 60 kg $P_2O_5ha^{-1}$, 120 kg N $ha^{-1}$and 120 kg N + 60 kg P2O5ha^{-1}, besides a control plot without fertilizers or bioinoculants. Phosphate-solubilizing and phytohormone-producing parent soil isolates and mutant strains of *A. chroococcum* were isolated and selected following the enrichment method. On an overall basis the mutant strains performed better than the soil isolates for *in vitro* phosphate solubilization (11–14%) and growth hormone production (11.35%). Seed inoculation of wheat varieties with phosphate-solubilizing and phytohormone-producing *A. chroococcum* showed a better response over the control. Mutant strains of *A. chroococcum* showed a higher increase in grain (15.30%) and straw (15.10%) yield over the control and better survival (12–14%) in the rhizosphere as compared to their parent soil isolate (P4). Mutant strain M15 performed better in all three varieties in terms of increase in grain yield (20.8%) and root biomass (20.6%) over the control.
(Source – Narula et al 2001 Acta Agronomica Hungarica, 49 (2) : 141 – 149).

An efficient microbiological growth medium for screening phosphate solubilizing microorganisms - A novel defined microbiological growth medium, National Botanical Research Institute's phosphate growth medium (NBRIP), which is more efficient than Pikovskaya medium (PVK), was developed for screening phosphate solubilizing

microorganisms. In plate assay the efficiency of NBRIP was comparable to PVK; however, in broth assay NBRIP consistently demonstrated about 3-fold higher efficiency compared to PVK. The results indicated that the criterion for isolation of phosphate solubilizers based on the formation of visible halo/zone on agar plates is not a reliable technique, as many isolates which did not show any clear zone on agar plates solubilized insoluble inorganic phosphates in liquid medium. It may be concluded that soil microbes should be screened in NBRIP broth assay for the identification of the most efficient phosphate solubilizers.
(Source - FEMS Microbiol Lett. 1999;170(1) : 265-70, Patented composition, available online at http://www.freepatentsonline.com/6638730.html)

Effect of the herbicides oxadiazon and oxyfluorfen on phosphates solubilizing microorganisms and their persistence in rice fields - A field experiment has been conducted with two herbicides viz. oxadiazon [5-terbutyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2-one] and oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenyl)-4-(trifluoromethyl) benzene] at rates of 0.4 and 0.12 kg a.i. ha^{-1}, respectively, to investigate their effect on the growth and activities of phosphate solubilizing microorganisms in relation to availability of phosphorus as well as persistence of the herbicides in the rhizosphere soil of wetland rice (*Oryza sativa* L. variety IR-36). Application of herbicides stimulated the population and activities of phosphate solubilizing microorganisms and also the availability of phosphorus in the rhizosphere soil. Oxyfluorfen provided greater microbial stimulation than oxadiazon. Dissipation of oxyfluorfen and oxadiazon followed first order reaction kinetics with half-life ($T_{1/2}$) of 8.8 and 12 days, respectively. Sixty days after application 0.5% and 3% of the applied oxadiazon and oxyfluorfen residues persisted, respectively, in the rhizosphere soil of rice.
(Source Das et al 2003, Chemosphere, Vol. 53 (3), pp. 217-221)

Endophytic Colonization of Rice by a Diazotrophic Strain of *Serratia marcescens* - Six closely related N_2-fixing bacterial strains were isolated from surface-sterilized roots and stems of four different rice varieties. The strains were identified as *Serratia marcescens* by 16S rRNA gene analysis. One strain, IRBG500, chosen for further analysis showed acetylene reduction activity (ARA) only when inoculated into media containing low levels of fixed nitrogen (yeast extract). Diazotrophy of IRBG500 was confirmed by measurement of $^{15}N_2$ incorporation and by sequence analysis of the PCR-amplified fragment of *nifH*. To examine its interaction with rice, strain IRBG500 was marked with *gusA* fused to a constitutive promoter, and the marked strain was inoculated onto rice seedlings under axenic conditions. At 3 days after inoculation, the roots showed blue staining, which was most intense at the points of lateral root emergence and at the root tip. At 6 days, the blue precipitate also appeared in the leaves and stems. More detailed studies using light and transmission electron microscopy combined with immunogold labeling confirmed that IRBG500 was endophytically established within roots, stems, and leaves. Large numbers of bacteria were observed within intercellular spaces, senescing root cortical cells, aerenchyma, and xylem vessels. They were not observed within intact host cells. Inoculation of IRBG500 resulted in a significant increase in root length and root dry weight but not in total N content of rice variety IR72. The inoculated plants showed ARA, but only when external carbon (e.g., malate, succinate, or sucrose) was added to the rooting medium.

(Source - Prasad et al Journal of Bacteriology, April 2001, p. 2634-2645, Vol. 183, No. 8)

Endophytic colonization ability of two deep-water rice endophytes, *Pantoea* sp. and *Ochrobactrum* sp. using green fluorescent protein reporter - Colonization ability of the two endophytic bacteria, isolated from surface sterilized seeds of Jaisurya variety of deep-water rice viz., *Pantoea* sp. and *Ochrobactrum* sp., was compared after genetically tagging them with a constitutively expressing green fluorescent protein gene (*gfp*). Confocal laser scanning microscopy (CLSM) of hydroponically grown seedlings of Jaisurya rice, inoculated with *gfp*-tagged endophytes, revealed that both *Pantoea* sp. and *Ochrobactrum* sp. colonized the intercellular spaces in the root cortex when inoculated separately. Colonization by *gfp*-tagged *Ochrobactrum* sp. was severely inhibited when co-inoculated with an equal number (10^5 c.f.u. ml^{-1}) of wild type *Pantoea* sp., but the converse was not true. *Pantoea* sp. was a more aggressive endophytic colonizer of its host than *Ochrobactrum* sp. The potential of using GFP reporter and CLSM as tools in evaluating competitive ability of colonization among endophytes is herewith demonstrated.
(Source – Verma et al 2004, Bitechnology Letters 26(5) : 425 – 429).

Nitrogen Source Effect on *Gluconacetobacter diazotrophicus* Colonization of Sugarcane (*Saccharum* Spp.) - Biological nitrogen fixation (BNF) in sugarcane is considered one of the principal reasons for the success of the Brazilian Ethanol Program (PRO-ALCOOL) for motor car fuel. The BNF influences positively the energy balance of sugarcane crops for alcohol production. *Gluconacetobacter diazotrophicus* is a nitrogen-fixing bacterium associated with sugarcane, and is particularly abundant and active in the early stages after germination. The objective of this work was to evaluate the effect of the addition of increasing amounts of two sources of mineral nitrogen (ammonium sulphate and calcium nitrate) on the population of *G. diazotrophicus* and also to evaluate its effect on nitrogenase (acetylene reduction) activity and accumulation of N by two sugarcane hybrids, SP 701143 and SP 792312. The results showed that both varieties differed in the form of nitrogen they prefer to uptake from the soil. The variety SP 701143 preferred ammonium sulphate, whilst the variety SP 792312 preferred N from calcium nitrate. In both varieties, the addition of increased doses of ammonium and nitrate inhibited the population of *G. diazotrophicus* but in the variety SP 701143 the inhibition was more pronounced in the presence of calcium nitrate. The acetylene reduction activity was inhibited in both varieties, especially in variety SP 792312 in the presence of either of the two nitrogen sources.
(Source – Medeiros et al 2006, Plant and Soil 279 : 141 – 152)

Inoculation Effects of *Pseudomonas putida*, *Gluconacetobacter azotocaptans*, and *Azospirillum lipoferum* on Corn Plant Growth Under Greenhouse Conditions - Alcohol production from corn is gaining importance in Ontario, Canada, and elsewhere. A major cost of corn production is the cost of chemical fertilizers and these continue to increase in price. The competitiveness of alcohol with fossil fuels depends on access to low-cost corn that allows growers to earn a sustainable income. In this study authors set out to determine if they can identify root-associated microorganisms from Ontario-grown corn that can enhance the nutrient flow to corn roots, directly or indirectly, and help minimize the use of extraneous fertilizer. Bacteria

were isolated from corn rhizosphere and screened for their capacity to enhance corn growth. The bacteria were examined for their ability to fix nitrogen, solubilize phosphate, and produce indole acetic acid (IAA) and antifungal substances on potato dextrose agar. Bacterial suspensions were applied to pregerminated seed of four corn varieties (39D82, 39H84, 39M27, and 39T68) planted in sterilized sand and unsterilized cornfield soil. The plants were grown under greenhouse conditions for 30 days. Three isolates were identified as having growth-promoting effect. These bacteria were identified as to species by biochemical tests, fatty acid profiles, and 16S rDNA sequence analysis. Corn rhizosphere isolates, *Gluconacetobacter azotocaptans* DS1, *Pseudomonas putida* CQ179, and *Azospirillum lipoferum* N7, provided significant plant growth promotion expressed as increased root/shoot weight when compared to uninoculated plants, in sand and/or soil. All strains except *P. putida* CQ179 were capable of nitrogen fixation and IAA production. *Azospirillum brasilense*, however, produced significantly more IAA than the other isolates. Although several of the strains were also able to solubilize phosphate and produce metabolites inhibitory to various fungal pathogens, these properties are not considered as contributing to growth promotion under the conditions used in this study. These bacteria will undergo field tests for their effect on corn growth.
(Source - Mehnaz and Lazarovits 2006, Microbial Ecology 51 (3) 326 – 335).

A new nitrogen-fixing *Clostridium* species from a high Arctic ecosystem – A hitherto undescribed species of yellow-pigmented, Gram-negative *Clostridium* sp., possessing nitrogenase activity, has been isolated from a number of sampling sites on the Truelove Lowland of Devon Island in the Canadian high Arctic. This bacterium, tentatively designated *Clostridium arcticum* sp. nov., accounted for 19% of all isolates recovered which were capable of anaerobic nitrogen fixation.
(Source - Jordan DC and McNicol PJ, Can J Microbiol. 1979 Aug;25(8):947-8)

Efficacy of New Inexpensive Cyanobacterial Biofertilizer Including its Shelf-life - Four different carrier based (Neem, tobacco, Bel and rice straw) inoculants were studied in combination with four levels of chemical N for their field response on rice. All combinations under study significantly increased grain and straw yield of rice either alone or in combination with chemical fertilizer. A saving of 25 kg N ha^{-1} can be attained through cyanobacterial fertilization. Tobacco waste-based cyanobacterial biofertilizer was best in performance. Cyanobacterial acetylene reducing activity *in vivo* varied from 144 to 255 µmol C_2H_4 m^{-2} h^{-1} in different treatments, being highest for tobacco-based cyanobacterial biofertilizer integrated with 50% chemical N. The nutrient balance for total N, available N, total P and available P was found positive in biofertilizer- and chemical fertilizer-treated plots. The total and available K showed negative balance in all the treatments. The shelf-life of cyanobacterial biofertilizer can be augmented by selecting translucent packing material, dry mixing and paddy straw as a carrier. Dry mixing and a mixing ratio of 50:50 (carrier:cyanobacteria) gave better inoculum loading and shelf-life.
(Source – Jha and Prasad 2006, World J. Microbiology and Biotechnology Vol 22(1), 73-79)

Book Review

Molecular Basis of Symbiosis, Edited by Jörg Overmann Springer 2006 Hardcover XIV, 310 p. 60 illus., 5 in colour., ISBN 3540282106 Price £100.00 - In symbiotic associations, co-evolution of the partner organisms has led to specific mechanisms of signal exchange and reciprocal regulation, and resulted in novel physiological capabilities of the association as compared to those of the individual partners. Symbiosis research has recently entered an exciting era because molecular biology techniques are available for studying partner organisms in association and in a culture-independent manner. It is the goal of this book to contribute towards a broader perspective and an understanding of the function of symbiotic systems. 14 different model systems have been chosen, comprising well known symbioses as well as novel experimental systems which have only recently become amenable to experimental manipulation.

Microbial Root Endophytes Edited by Schulz, Barbara J.E.; Boyle, Christine J.C.; Sieber, Thomas N. Springer 2006, Hardcover XX, 367 p., 29 illus., 4 in colour, ISBN 3540335250 Price £115.00 - Plant roots may not only be colonized by mycorrhizal fungi, but also by a myriad of bacterial and fungal root endophytes that are usually not considered by the investigators of classic symbioses. This is the first book dedicated to the interactions of non-mycorrhizal microbial endophytes with plant roots. The book deals with diversity, life history strategies, interactions, applications in agriculture and forestry, methods for isolation, cultivation, and both conventional and molecular methods for identification and detection of these endophytes. The comprehensive reviews demonstrate the high diversity of interactions and will provoke further studies to better understand the mechanisms which determine whether a plant-microbial interaction remains asymptomatic, leads to disease or to a mutualistic interaction. The book is vey useful to students and scientists in botany, ecology, agriculture, forestry, microbiology, and soil biology.

Arbuscular Mycorrhizas: Physiology and Function. By David D. Douds, Yoram Kapulnik , 2000 Springer, 372 pages, ISBN 0792364449 - This book provides a structured compilation of reviews of what is known about arbuscular mycorrhizal symbiosis. Topics were selected to cover major steps in the life cycle of AM fungi (germination, signaling/recognition prior to colonization, and host regulation of colonization). Aspects of the physiological interaction within the root for which there has been exciting recent progress (regulation of host defenses, bidirectional transport of nutrients, modification of gene expression, and carbon metabolism) are then discussed. The focus then widens to effects of the mycorrhiza upon the whole plant (nutrition, water relations, reproduction, and resistance to disease), and expands further to essential aspects of the role of AM fungi in soil ecology (soil aggregation, and interactions with other soil microbes). Leaders in the field present critical reviews and point toward areas for future research. Therefore, this volume should be helpful for both researchers in the field and those interested in a thorough knowledge of this important symbiosis.

***In Vitro* Culture of Mycorrhizas, Edited by Declerck, Stéphane; Strullu, Désiré-Georges; Fortin, J. André**

Springer 2005 388 pp ISBN 3540240276 Price £100.00 - The technique of *in vitro* cultivation of root organs has been developed over the past few decades and opens up new ways of studying plant-fungi associations. It is a technical breakthrough, especially for the investigation of the ubiquitous arbuscular mycorrhizal fungi, since these obligate symbionts rely on plant tissue. This is the first book describing this unique *in vitro* cultivation, which has markedly improved our general understanding of symbiosis. Presented by an international group of authors, including pioneers of this technique, it should encourage researchers to apply the method in further new studies on mycorrhizal fungi and plant-fungi interactions. Various biological aspects such as the physiology, biochemistry, biodiversity, and life cycles of fungi as well as the effects of symbiosis on plant growth and development are described, including large-scale fungus production for biotechnological use. Detailed protocols allow the immediate application of the method to culture mycorrhizal fungi in vitro.

Mycorrhizal Technology in Agriculture: From Genes to Bioproducts By Silvio Gianinazzi 2002, Published by Birkhäuser, 296 pages, ISBN 3764364858 Price £ 65.00 - Arbuscular Mycorrhiza (AM) is the most common mycorrhizal type involved in agricultural systems. AM is the most widespread plant root symbiosis, and the fungi involved (Glomales) are known to promote plant growth and health by acting as biofertilizers, bioprotectors and bioregulators. The main aim of this book is to provide readers with theoretical and applied knowledge essential for the use of AM fungi in improving plant health and fitness, production of high quality food and in conservation of natural resources. The different chapters target understanding the role of AM fungi in sustainable crop production, discussing ways to improve biological equilibria between microorganisms in mycorrhizosphere, analysing genetic, physiological, cellular and molecular basis of AM functioning and establishing technologies for inoculum production, according to the regulatory guidelines for application.

Biofertilizers in Sustainable Agriculture/A.C. Gaur. New Delhi, Indian Council of Agricultural Research, 2006, viii, 288 p., Price $30. ISBN 81-7164-060-5 - "Biofertilizers or microbial inoculants include biologically active and efficient bacteria, actinomycetes, Azolla, mycorrhizae etc. that can provide plant nutrients viz. nitrogen and phosphorus at cheaper cost when compared with chemical fertilizers. Their integrated uses thus can cause a reduction in application of chemical fertilizers and pesticides and overall cost of cultivation of crops. The monograph deals with various aspects of biofertilizers and manures, and technologies for their production and use. The biofertilizers viz. *Rhizobium*, *Azotobacter, Azospirillum*, blue-green algae, *Frankia*, Mycorrhizae, *Rhizobium* sp. and mineral phosphate solubilising microorganisms viz. *Pseudomonas, Bacillus, Apergillus, Penicillium* and others have been discussed in different chapters. The methods for production of enriched manures, factors affecting composting, role of cellulytic and lignolytic micro-organisms, methods of vermicomposting and standards for maturity of compost have been discussed in detail. The book is useful for the farmers and NGOs, interested in organic farming and will serve the purpose of both biologists and farm scientists, who are interested in using renewable and alternative sources of energy as organic farming inputs."