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Contents

Biofertiliser Newsletter (BFNL) is a bi-annual publication under National Project of Organic Farming (Formerly National Project on Development and Use of Biofertilisers), Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India. BFNL is registered with Indian Scientific Documentation Centre. Scientific articles, short research notes, results of field trials, abstracts of selected Ph.D. theses having direct relevance to biofertiliser technology, information about recent events, new reports, new species, extension news, corporate news, news related to conferences, seminars etc and review of books are especially welcome. Opinions expressed in articles published in BFNL are those of the author(s) and should not be attributed to this centre. Acceptance of manuscripts for publication in BFNL shall automatically mean transfer of copyright to Biofertiliser Newsletter.

From Editor's Desk

Dear Readers,

Twentieth century has witnessed many technological revolutions in almost all spheres of our life. Agriculture is no exception and our scientist ensured the best utilization of knowledge to the benefit of mankind. Utilization of various microbial systems for harvesting the native nutrient mobilization potential in the form of biofertilisers was an important milestone in the last decade of the century. These microbial preparations are now important components of various crop's nutrient packages. With the introduction of modern organic farming approach various microbial systems with human intervention are expected to play much greater role, not only in nutrient management, but also in plant protection and soil fertility improvement. Since the basic philosophy of organic farming revolves around living soil, and living soil is possible only with the sufficiently high population of various microorganisms, this science need to be strengthened with better understanding of the plant-microbe, microbe-soil parameters, microbe-microbe and microbe-other soil organism's interactions.

Realizing the emerging prospects of organic farming and the likely role of microbial systems in organic farming, the scope and mandates of "National Project on Development and Use of Biofertilisers" are being redefined and enlarged to include the overall science of organic farming. Since October 2004, the National Project on Development and Use of Biofertilisers is being restructured and renamed as "National Project on Organic Farming". Under these revised mandates the existing National Biofertiliser Development Centre at Ghaziabad and its six Regional Biofertiliser Development Centres at Bhubaneshwar, Bangalore, Hisar, Jabalpur, Imphal and Nagpur are being renamed as National Centre of Organic Farming and Regional Centres of Organic Farming. All the earlier activities of National and Regional Biofertiliser Development Centres related to the development, quality control and promotion of biofertilisers will continue to be taken up by these centres.

Biofertiliser Newsletter, which is dedicated to the dissemination of latest developments in the field of biofertilisers since last 11 years, will continue to serve its readers with the same spirit, dedication and objectives. The National Centre of Organic Farming, Department of Agriculture and Cooperation, Ministry of Agriculture, Govt. of India, Ghaziabad, has taken over the responsibility of its publication from the 13th volume onwards. We hope to take Biofertiliser Newsletter to new information heights under this new setup.

The later half of the twentieth century was of input intensive green revolution. We hope that the twenty first century will be of knowledge intensive organic farming revolution marching on microbial wheels. I welcome you all in the new era of agriculture. From the entire family of Biofertilisers and organic farming, I wish our esteemed readers a very happy new year.

A.K. Yadav
Editor

Suitability of Three Solid Materials as Carrier of Multiple Biofertiliser Agents, their Survival under Three Storage Conditions and Effect on Rice

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सारांश

प्रस्तुत विवरण में तीन जैव उर्वरक जीवाणु, एजोस्पिरिलम एमेजोनेन्से (A-10), बेसिलस मैगाथिरियम (P-5) तथा स्यूडोमोनास पिकेटी (Psd-7) के एकाकी एवं मिश्रित कल्चर को विभिन्न वाहकों (चारकोल, कम्पोस्ट एवं चारकोल+कम्पोस्ट+कागज कारखाना-अवशिष्ट का मिश्रण) के साथ मिलाकर अलग-अलग तापक्रम पर भंडारित कर उनकी जैव उर्वरक रुप में उपयुक्तता जाँचने तथा उनके प्रयोग से धान की उत्पादकता पर पड़ने वाले प्रभाव का अध्ययन किया गया है। अध्ययन से प्राप्त परिणामों से ज्ञात होता है कि तीनों ही प्रकार के जीवाणु मिश्रित कल्चर में बिना एक दूसरे को प्रभावित किये अपनी वाँछित मात्रा बनाये रखने में सक्षम थे। मिश्रित कल्चर में प्रत्येक जीवाणु की कुल मात्रा उनके एकाकी कल्चर के लगभग समकक्ष पायी गयी । सभी एकाकी एवं मिश्रित कल्चर विभिन्न वाहकों के साथ यद्यपि १६४ दिन तक के भंडारण में वाँछित मात्रा में पाये गये परंतु उससे अधिक समय के भंडारण में उनकी कुल मात्रा में तेजी से गिरावट हुई । तीन विभिन्न भंडारण अवस्थाओं में से रेफ्रिजिरेटर में (७°से.) तथा साधारण तापक्रम पर कमरे में भंडारण करने पर लगभग समान परिणाम प्राप्त हुऐ। परंतु ऐसे पैकेट जो कि जमीन के अंदर बने गड्ढे में रखे गये थे और जिसमें कमरे के मुकाबले औसतन कम तापमान आँका गया, में अन्य दो अवस्थाओं के मुकाबले कम जीवाणु पाये गये। धान की फसल में इन जैव उर्वरक नमूनों की उपयोगिता एवं प्रभाविता जाँच में पाया गया कि तीनों जीवाणुओं का मिश्रित कल्चर मिश्रित वाहक के साथ सर्वाधिक प्रभावी था जिसके फलस्वरुप उनके उपचार से अनुपचारित के मुकाबले धान की २३ प्रतिशत अधिक उपज प्राप्त हुई। संक्षेप में इस अध्ययन से यह निष्कर्ष निकलता है कि जैव उर्वरकों में एक से अधिक प्रकार के जीवाणुओं के मिश्र कल्चर भी प्रयोग किये जा सकते हैं और संभवतः ऐसे मिश्रित कल्चर उन जीवाणुओं के एकाकी कल्चर से अधिक प्रभावी व क्रियाशील होंगे।

Introduction

Effectiveness of biofertiliser as a nutrient input in crop production depends on several factors, which include the efficiency of the microbial strains, their shelf life in carrier and soil and weather parameters during crop growth. Longer shelf life of microbial agents in the carrier is desirable so that in between the time of production and actual inoculation in farmer's field, particularly of those located in remote areas, the cells remain viable. Although, peat, charcoal or lignite powder are suitable as carrier of biofertiliser, there is a scope for development of better carrier to improve effectiveness of biofertiliser agents and for longer shelf life (Iswaran *et al* 1979, Abd-Alla and Omar 2001). Generally, biofertiliser formulations used to improve N and P nutrition of crops, contain single strain. In many instances, application of two inocula in separate preparation is shown to influence plant growth in synergistic manner (Subba *et al* 1985, Will and Sylvia 1990, Okon *et al* 1994, Savalgi *et al* 1994). However, delivery of dual/ multiple microbial inocula in a single carrier may be cost effective, but compatibility of the

microbial agents in the carrier needs to be evaluated. This is because the co-occurrence of the microbial agents may also result in antagonistic interactions (Abd-Alla and Omar 2001). Recently, we isolated and screened efficient strains of azospirilla, phosphate solubilizing bacteria and fluorescent pseudomonads from rice rhizosphere soil and some of these strains enhanced rice growth on application in field experiment (Thakuria *et al* 2004). From this collection, three efficient biofertiliser agents, namely, N_2-fixing *Azospirillum amazonense* A-10 MTCC 4716, P-solubilizing *Bacillus megaterium* P-5 MTCC 4714 and PGPR *Pseudomonas picketti* Psd-6 MTCC 4715 were used to develop a multiple microbial formulation in three carriers. The compatibility of individual agents was evaluated in terms of population in three carriers under three storage conditions over a period of eight months.

Materials and Methods

Three solid materials used as carriers of biofertiliser agents were 1) charcoal, 2) compost and 3) a mixture of charcoal, compost and lime sludge (paper mill waste). A large quantity of lime sludge (80,000 Mt/ annum) is generated in Nagaon paper mill, Jagiroad, Assam, which are available free of cost and therefore the possibility of its use in development of a suitable carrier was explored. The mix carrier contained lime sludge, charcoal and composts in 1:1:1 ratio. The ingredients were mixed uniformly. These were oven dried and sieved with 0.5 mm diameter sieve. The nutrient content and moisture holding capacity of the carriers are shown in Table 1.

Preparation of microbial culture and carrier: The three microbial agents, A-10, P-5 and Psd-6 were grown in Nfb broth supplemented with NH_4-N, Pikovskaya's and Kings B broth, respectively at 30±2 ^{0}C in an environmental shaker (ROTEK-LES). The population density of the microbial agents in their culture broths was determined by plate count technique. The sieved carrier materials were autoclaved twice at 121^{0}C and 15 lb/in^2 pressure for an hour at 24 hours interval. A known quantity of carrier was mixed with equal volume of the three culture broths to obtain 40% of moisture holding capacity (MHC) in each carrier. One hundred gram of this multiple microbial inocula were transferred to polyethylene bags and sealed. As the MHC of the three carriers was different, 40% MHC was obtained by adding sterile distilled water. In case of preparation with the single strain, one equal volume of its broth was mixed with two equal volume of sterile distilled water. In order to determine the population of the microbial agent by destructive sampling technique at five different time interval, all together 20 polyethylene packets prepared for each of single and multiple microbial inocula and stored under each storage condition.

Storage condition: The multiple microbial agent containing biofertiliser packets were stored in 3 different conditions, at ambient room temperature, under refrigerated condition (7^{0}C) and underground (in pit). A 30 cm deep, 25 cm width and 30 cm long pit was prepared under a tree and lined with polyethylene sheet. The biofertiliser packets were placed in the pit and top of the pit was covered with thatch. The temperature in the pit was expected to be less than ambient for which microbial agents were also expected to survive for longer period. The maximum and minimum temperature inside the pit was recorded at 7.00 AM and 2.30 PM, respectively and are shown in Fig 1. The population of microbial agents was determined by plate count technique at 0, 46, 97, 164 and 234 days after storage.

Table 1: Physico-chemical characteristics of three carriers

Type of carrier	MHC (%)	pH	Total N (%)	Total P (%)	Total K (%)
Compost	108	6.4	1.24	0.14	2.0
Charcoal	98	6.6	0.05	0.17	1.2
Mix carrier	84	7.8	0.95	0.15	3.9

Effect of multiple microbial inocula on rice: A micro-plot (3m x 2m) field experiment was conducted during Aug- Nov 2002 to determine the effect of carrier-based single and multiple microbial inocula on summer rice. There were 7 treatment combinations (Table 3). In all the treatments, N was reduced by 50% and in P-5 treated plots P was reduced by 50% and applied as rock phosphate (RP). Biofertiliser (freshly prepared) was applied to rice seedlings at the time of transplanting by slurry method (Thakuria *et al* 2004). The crop was harvested at 85 days after transplanting, threshed and grain yield recorded on air-dry weight basis.

Results and Discussion

The variation in initial population of three biofertiliser agents in the carrier was due to varying density of their cell in the respective broth within 48 hr of inoculation (Thakuria *et al* 2004). There was no appreciable difference in population of the A-10 and P-5 in biofertiliser packets containing single or multiple inocula over a period of 8 months (Table 2). However, the population of Psd-6 in single inoculum packets was slightly higher than their population in multiple inocula packets. This result suggests that the three beneficial bacteria of rice rhizosphere are compatible to each other in three carrier materials. These strains were cohabitants of rice rhizosphere. Earlier, Abd-Alla and Omar (2001) reported that depending upon the strain, N-fixing *Rhizobium leguminosarum* and P solubilizing *Aspergillus niger* exhibited synergistic or antagonistic interaction in peat or wheat bran carrier under different storage conditions. In this study, the population density of the three organisms over 164 days (5½ months) of storage was found to remain above the standard number required for solid carrier-based biofertiliser (Table 2) (Tripathi, 2004). The drop in population was conspicuous at 234 days after preparation of the biofertiliser but the extent of drop from initial value in the P-5 population was highest.

The three carriers were ideal in maintaining the population of the biofertiliser agents although, a higher level of population was observed in the mixed carrier (Table 2). In fact, the mixed carrier was more effective in holding higher level of population. The nutrient content of this carrier was more and its pH was also slightly above neutrality (Table 1), which might have made it more suitable for the microorganisms. Lime sludge is a paper and pulp industry waste and its disposal is a problem. This material involves no cost except the cost of transportation, whereas purchase of charcoal and preparation of compost for use as carrier involves money. Our results show that the mixture of charcoal, compost and lime sludge is a cheap and efficient carrier for the three microbial agents and show a prospect of its use in manufacture of multiple microbial inocula. The other interesting aspect of this study was survival of the microbial inoculants under three storage conditions. As expected, the highest level of population was observed in different time interval (up to 8 months) under refrigerated condition irrespective of the carrier (Table 2).

Table 2: Population (log cfu/g) of A-10, P-5 and Psd-6 in single and multiple microbial inoculums in three carrier base during storage

Strain	Days of storage	Carrier											
		Charcoal				Compost				Mixed carrier			
			At room temp				At room temp				At room temp		
		At 7^{0}C	Single	Multiple	In pit	At 7^{0}C	Single	Multiple	In pit	At 7^{0}C	Single	Multiple	In pit
A-10	0	9.31	9.0	9.31	9.3	10.0	10.2	10.0	10.0	10.4	10.7	10.4	10.4
	40	8.18	8.0	7.9	7.0	8.4	8.9	8.4	7.6	8.53	8.51	8.5	8.5
	97	8.04	7.4	7.3	6.9	7.7	8.8	8.3	7.4	8.0	8.3	8.4	8.4
	164	7.48	6.7	7.0	5.7	7.4	8.0	7.1	6.5	7.0	7.0	7.3	6.9
	234	6.1	5.8	6.0	5.0	7.0	6.0	5.4	4.8	6.8	6.4	6.2	5.2
P-5	0	8.51	8.7	8.5	8.5	8.6	8.5	8.6	8.6	9.9	10.0	9.9	9.9
	40	8..0	8.1	7.7	7.3	8.0	7.2	7.8	7.4	9.8	8.6	8.7	8.0
	97	7.78	7.9	7.7	7.0	7.3	7.3	7.6	7.3	8.7	8.9	8.6	8.0
	164	7.7	6.8	6.7	5.1	7.3	7.0	6.4	6.7	8.0	7.8	7.7	6.8
	234	6.0	4.5	5.4	4.2	6.7	4.5	4.4	5.1	7.6	4.8	4.4	5.7
Psd-6	0	8.58	9.0	8.5	8.5	10.0	9.8	10.0	10.0	10.4	11.1	10.4	10.4
	40	7.95	7.9	7.8	7.0	8.5	8.4	8.4	7.7	9.0	9.1	8.8	8.0
	97	7.7	7.7	7.7	6.7	8.3	7.2	8.1	6.7	9.0	9.1	8.7	7.9
	164	7.48	6.4	6.3	6.0	7.3	7.0	7.0	6.2	8.3	9.2	8.4	7.1
	234	6.0	5.1	5.0	4.3	6.4	5.5	5.6	4.2	7.5	7.9	6.7	5.2

Drop in population was observed after 97 days in packets stored under room temperature and in pit. Under ambient room temperature and in pit, cell counts were reduced by 35.55 to 55.15% and 41.18 to 58.29%, respectively from their initial values. Prolonged shelf life of the biofertilisers is required from manufacturers, marketing and user's perspective. Farmers are generally advised to store biofertiliser packets in shade before use to prevent cell death. The survival of cells in carriers stored in pit was tested with a view that low temperature in pit will ensure survival of cells. However, contrary to our expectation, the cell number in pit was reduced after 164 days to greater extent than that in ambient room temperature. Overall the daily maximum and minimum temperature in the pit was lower than ambient room temperature (Fig 1). Up to 155 days, the difference in daily average max and min temperature in pit was less whereas for the period beyond 155 days, the difference in average daily max and min temperature was more than that of the preceding period. In room, difference in daily average max and min temperature in the two periods was not as striking as in the pit (Fig 1). It appears that a higher difference in mean daily maximum and minimum temperature is detrimental for survival of the biofertiliser agents. This result indicates that biofertiliser packets may be stored for 6 to 7 months period in room or pit. Considering the low max and min temperature in pit during summer month, it will be worthwhile to observe the cell survival by storing them in pit from the early part of summer (May) in a separate experiment. During winter months, biofertiliser packets can be stored under ambient room temperature.

Fig 1: Maximum and minimum temperature in room (A) and in pit (B) during 234 days of storage of biofertilisers (01.06.02 to 20.01.03). Straight line indicates the temperature under refrigerated condition (7^{o}C)

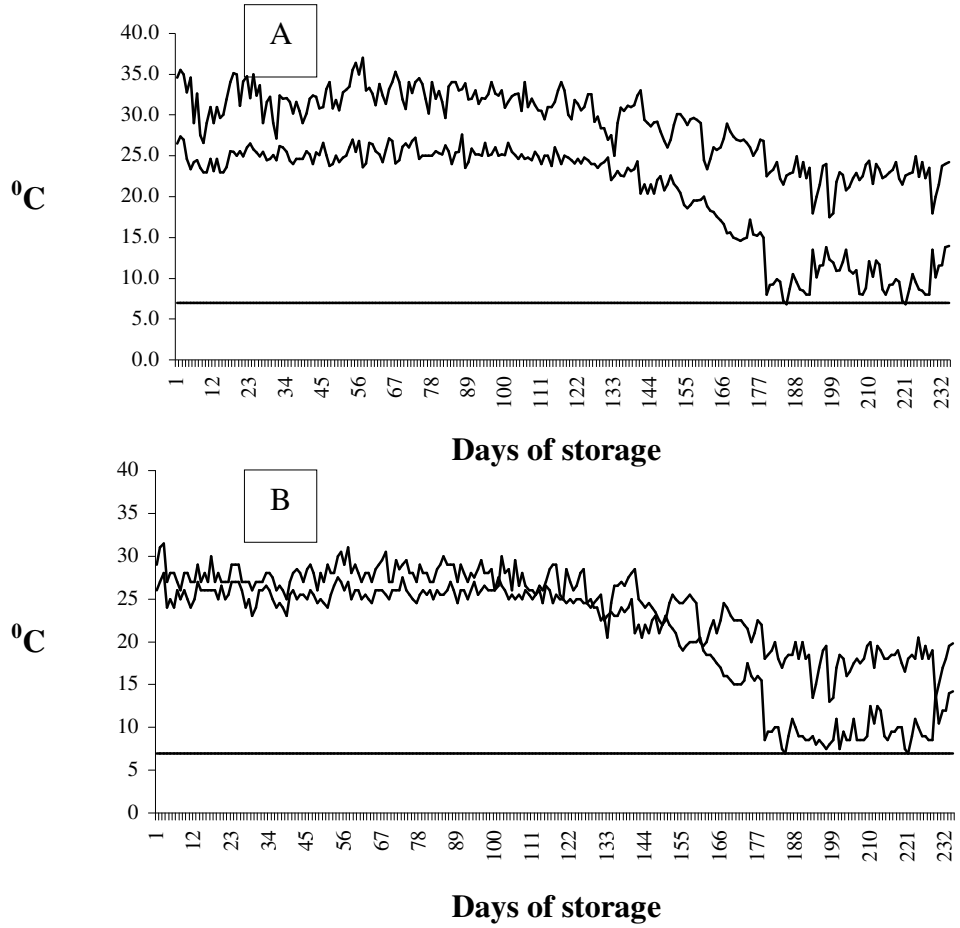

Table 3: Effect of three solid carrier-based single/ multiple microbial inocula on grain yield of summer rice, 2002

Treatment (NPK kg ha^{-1})	Grain Yield (q/ ha)	% increase over control
Control (no fertiliser, no biofertiliser)	22.40	-
A-10 in mixed carrier @ 1g /m^2 + NPK 20:20:20 kg ha^{-1}	25.27	12.81
P-5 in mixed carrier @ 1g /m^2 + NPK 20:10 (as RP):20 kg ha^{-1}	25.19	12.46
Psd-6 in mixed carrier @ 1g /m^2 + NPK 20:20:20 kg ha^{-1}	24.79	10.67
A-10 + P-5 + Psd-6 in mixed carrier @ 1g /m^2 + NPK 20:10 (as RP): 20 kg ha^{-1}	27.54	22.95
A-10 + P-5 + Psd-6 in charcoal carrier @ 1g /m^2 + N:P_2O_5: K_2O @ 20:10 (as RP): 20 kg /ha	26.77	19.51
A-10 + P-5 + Psd-6 in compost carrier @ 1g /m^2 + N:P_2O_5: K_2O @ 20:10 (as RP): 20 kg /ha	26.94	20.27
$LSD_{0.05}$	**2.19**	**-**

Effect of single and multiple microbial inocula on summer rice: The grain yield of summer rice without fertiliser and biofertiliser was poor. However, application of appropriate combination of fertiliser and single microbial inoculum in mixed carrier increased grain yield significantly (Table 3). Application of P at half the recommended dose as RP along with PSB inoculum singly was cost effective as the grain yield under this treatment was statistically at par with that obtained by application of A10 along with 20 kg P_2O_5/ha as SSP. Similarly, A10 alone in the mixed carrier was effective in reducing dose of fertiliser N by 50%.

The multiple microbial inocula in three carriers increased grain yield more than the single inoculum but the effect was statistically significant only in case of mixed carrier based multiple microbial inocula. However, the effect of the multiple microbial inocula in the three carriers on the summer rice was not statistically significant. Overall, this study has shown that mixed carrier based multiple microbial inocula is a superior formulation for survival of the biofertiliser agents longer and producing better effect on rice grain yield. Based on this study, large scale field testing of the product is recommended.

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Microbes are small wonders,
Let them play freely in the soil
They will take care of our soils for generations to come

Effect of *Rhizobium* and Phosphate Solubilizing Bacteria on the Yield and Nutrition of Pea (*Pisum sativum* L.)

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सारांश

राइजोबियम व पी.एस.बी. जैव उर्वरकों के मटर की फसल में अलग-अलग एवं संयुक्त प्रयोग को दो नत्रजन स्तर एवं चार स्फुर स्तरों के साथ जाँचा गया । राइजोबियम स्ट्रेन पीआर६३०० तथा बैसिलस पौलिमिक्सा स्ट्रेन बीपी४११ को विभिन्न-उपचारों में अलग-अलग, प्रयोग के समय एक साथ मिलाकर तथा उनको मिश्रित कल्चर के रुप में प्रयोग किया। स्फुरद सिंगल सुपर फास्फेट तथा मसूरी रॉक फास्फेट रुप में ३० एवं ६० किलो P_2O_5 प्रति है. की दर से तथा नत्रजन शून्य व १० कि. प्रति हैै. की दर पर प्रयोग किये गये । पूरे परीक्षण में कुल मिलाकर ४० उपचार रखे गये।

सर्वाधिक अन्न व भूसे की प्राप्ति राइजोबियम व पी.एस.बी. के सयुंक्त प्रयोग से प्राप्त हुई जो कि उनके अलग-अलग प्रयोग के मुकाबले बहुत अधिक थी । सिंगल सुपर फास्फेट ३० किलो व ६० किलो तथा मसूरी रॉक फास्फेट ३० किलो का प्रयोग धान्य एवं भूसा उत्पादन में समान रुप से प्रभावी पाया गया जबकि मसूरी रॉक फास्फेट का अधिक प्रयोग (MRP_{60}) हानिकारक सिध्द हुआ । सर्वाधिक धान्य (१७.३ क्वि. प्रति है.) तथा भूसा (४१ क्विं/है) उत्पादन शून्य नत्रजन स्तर पर राइजोबियम+पी.एस.बी. तथा सिंगल सुपरफास्फेट-३० के संयुक्त प्रयोग से प्राप्त हुआ । नत्रजन एवं स्फुरद संग्रहण मे भी मिलते जुलते परिणाम प्राप्त हुऐ तथा सर्वाधिक नत्रजन (१४७ कि/है) राइजोबियम+पी.एस.बी+सिंगलसुपर फास्फेट-३० उपचार में तथा सर्वाधिक स्फुरद (१६.६ कि/है) संग्रहण मिश्रित कल्चर+मसूरी रॉक फास्फेट-३० उपचार में पाया गया राइजोबियम व पी.एस.बी. का संयुक्त प्रयोग तथा उनके मिश्र कल्चर का प्रयोग सभी नत्रजन एवं स्फुरद स्तरों पर उनके अलग-अलग प्रयोग के मुकाबले हर मामले में अधिक प्रभावी पाया गया ।

Introduction

Pea is the most important pulse crop after gram, being used as a source of protein in the country. The growth and productivity of pea is largely affected by the availability of *Rhizobium* at the time of seed germination. *Rhizobium* inoculation as biofertiliser is reported to increase grain and straw yield of pea (Singh and Singh 1992). However the efficiency of this symbiotic relationship depends upon the availability of various nutrients among which phosphorus plays very important role. Several phosphate solubilizing bacteria and fungi possess the ability to solubilize insoluble phosphorus present in the soil (Gaur 1985). PSM inoculation alone is reported to increase the grain yield of gram (Tomar *et al* 1993). The positive interaction of *Rhizobium* and PSM as co-inoculants has also been observed in many situations. Dudeja *et al* (1981) reported the increase in grain yield and nutrient uptake in gram by *Rhizobium* and PSM co-inoculation. The dual inoculation of phosphate solubilizing bacteria with *Bradyrhizobium japonicum* increased the biological N_2-fixation and N and P content in soybean (Pant *et al* 1996, Dubey 1996).

As on now, both *Rhizobium* and phosphate solubilizing biofertilisers are prepared separately which involve high cost of production. It also creates confusion among the farmers at the time of their use. In spite of publishing some reports on synergistic effect between *Rhizobium* and phosphate solubilizers no systematic research has been carried out on the interaction between them. The present investigation was undertaken to evaluate the effect of *Rhizobium* and phosphate solubilizing bacteria interaction on the yield and nutrient uptake of pea.

Materials and Methods

The field experiment soil was typically loam having pH 7.85 , EC 0.52 dsm^{-1}, organic carbon 0.53% , CEC 11.5 Cmol (p+) kg^{-1} , available N 234.0 kg ha^{-1}, total N 0.062%, available P_2O_5 18.55kg ha^{-1} and available K_2O 380 kg ha^{-1}. Thirty two treatment combinations consisting two levels of N viz: 0, 10 kg ha^{-1}, two levels of P viz 30 and 60 kg P_2O_5 ha^{-1} applied through single super phosphate (SSP) and Mussorie rock phosphate (MRP) and four biofertilisers viz *Rhizobium leguminosarum* strain, PR6300 (*Rhizobium* alone), *Bacillus polymyxa* strain BP411 (PSB alone), combined application of *Rhizobium*+PSB and mixed culture of *Rhizobium* and PSB . In individual culture of *Rhizobium* and PSB culture packets prepared separately and used individually as seed treatment. In combined application one packet each of *Rhizobium* and PSB were mixed together and applied as seed treatment. In mixed culture, *Rhizobium* and PSB were inoculated in Pikovskaya medium, grown together and prepared into one single packet (Tyagi 1998). The experiment was laid out in factorial randomized block design with three replications. The Mussorie rock phosphate used in this experiment was sedimentary in origin, having pH 7.95, EC 0.93 dsm^{-1}, $CaCO_3$ 20.25 %, organic carbon 1.15% nitrogen 0.01%, total P 9.2 % citric acid soluble P 1.23 %, total S 3.2 % and total K 0.13 %. Nitrogen applied through urea, phosphorus as per treatment, potash as muriate of potash @ 40 kg K_2O ha^{-1} and 25 kg Zinc sulphate were drilled at the time of sowing. The post harvest data on grain and straw yields were collected after harvest of crop. Total N and P were determined by Micro–Kjeldahl and vanado-molybdo-phosphoric acid method (Jackson 1973). The soil samples were collected before the start of experiment and after the harvest of crop and analyzed for various determinations. Available P was determined by extracting 0.5 N $NaHCO_3$ (pH 8.5) as described by Olson *et al* (1954). The solubilization or fixation of applied and native phosphorus, relative agronomic effectiveness (RAE) and Harvest Index (HI) were calculated by using following formulae:

Solubilization / Fixation of applied and soil P (P_2O_5 kg ha^{-1}) = (Total P uptake + post harvest available P) – (Initial status of P + applied P)

RAE =Yield response due to MRP x 100

$$HI = \frac{\text{Grain yield}}{\text{Total dry matter yield}} \times 100$$

Results and Discussion

Grain and straw yield - The grain and straw yield of pea did not respond to N application @ 10 kg ha^{-1} (Table 1). The maximum 15.6 and 37.7 q ha^{-1} grain and straw yield were observed with SSP_{60} treatment at N_0 level which were found at par with SSP_{30} and MRP_{30} level of P. The incorporation of MRP_{60} significantly reduced the yield and the treatment was significantly inferior to the other treatments. Mussorie rock phosphate contains 20% free $CaCO_3$ which re-precipitated the solubilized P resulting in reduction of available P in soil

when applied in higher doses (Singh *et al* 1982). Among the various biofertiliser treatments, the maximum grain and straw yields were recorded with the combined use of *Rhizobium* + PSB which was found at par with mixed culture of *Rhizobium* and PSB and significantly higher over their individual inoculation. The increase in grain and straw yield were mainly due to available N and P to the plants by *Rhizobium* and PSB. The beneficial effect of applied P on pea and other legumes were also reported by Tiwari *et al* (1989). Similarly the positive effect of *Rhizobium* and PSB has also been reported by Alagawadi and Gunasekaran (1996).

Total N and P uptake - Both N and P uptake (Table 2) was found to be significantly higher in *Rhizobium* + PSB and mixed culture treatments over their individual treatments. Application of N (10 kg ha^{-1}) had no positive impact on total N and P uptake; on the contrary the application of N slightly reduced the total N and P uptake compared to their corresponding treatments with no N-application. Highest N uptake was recorded in *Rhizobium* + PSB treatment at N_0 level with SSP_{30} followed by at SSP_{60} and MRP_{30}. In mixed culture treatment highest N uptake was observed with SSP_{60} followed by SSP_{30} and MRP_{30}. Highest P-uptake at 16.6 kg ha^{-1} was recorded at N_0 levels with mixed culture at MRP_{30} followed by *Rhizobium* + PSB with MRP_{30} and SSP_{30}. The superiority of combined application of *Rhizobium* and PSB on higher nutrient uptake had also been reported by Alagawadi and Gaur (1988), Dudeja *et al* (1981) and Srivastava and Ahlawat (1995).

Table 1. Interaction effect of *Rhizobium*, PSB and P-sources on the grain and straw yield of pea.

P-source	*Rhizobium* alone		PSB alone		*Rhizobium*+PSB		Mixed culture		Mean	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
No nitrogen										
SSP_{30}	14.11	36.9	13.4	34.5	17.1	40.0	16.8	39.5	15.4	37.7
SSP_{60}	14.79	37.4	13.4	35.7	17.3	41.0	17.0	40.9	15.6	37.7
MRP_{30}	13.10	36.6	12.9	33.3	16.9	40.0	16.2	39.4	14.8	37.3
MRP_{60}	09.50	33.9	11.0	31.4	10.0	36.6	10.6	33.9	10.3	33.5
Mean	12.91	36.2	12.7	33.7	15.3	38.9	15.1	38.4	14.0	36.8
Nitrogen @ 10 kg ha^{-1}										
SSP_{30}	11.7	33.7	13.0	32.8	16.6	35.4	16.0	34.7	14.3	34.2
SSP_{60}	14.0	33.8	13.9	31.1	16.3	36.9	16.3	35.1	15.1	34.2
MRP_{30}	13.0	34.0	13.9	32.4	16.3	35.0	16.2	36.9	14.9	34.6
MRP_{60}	13.1	31.1	11.8	30.8	12.8	31.7	11.9	32.3	12.4	31.5
Mean	12.9	33.2	13.2	31.8	15.5	34.8	15.1	34.8	14.2	33.6
Phosphorus x Biofertiliser										
SSP_{30}	13.0	35.3	13.2	33.6	16.9	37.7	16.4	37.1	14.9	35.9
SSP_{60}	14.4	35.6	13.7	33.4	16.8	38.9	16.6	37.9	15.0	36.5
MRP_{30}	13.1	35.6	13.5	32.8	16.6	37.6	16.2	38.1	14.9	35.9
MRP_{60}	11.3	32.5	11.4	31.1	11.4	31.1	11.3	33.1	11.4	32.5
Mean	12.9	34.7	12.9	32.7	15.4	36.8	15.1	35.6	-	-
CD (p=0.05) for grain and straw respectively – N levels NS, 1.0; P-levels 0.7, 2.1; Biofertiliser 0.7, 2.1; NxP 1.0, 2.8; PxB 1.5, 4.2; NxB 1.0, 2.8 and NxPxB 2.0, 5.4. (NS = Non significant)										

Table 2. Interaction effect of *Rhizobium*, PSB and P-sources on total N and P uptake (kg ha^{-1}) by pea crop.

P-source	*Rhizobium* alone		PSB alone		*Rhizobium*+PSB		Mixed culture		Mean	
	N-uptake	P-uptake	N-uptake	P-uptake	N-uptake	P-uptake	N-uptake	P-uptake	N-uptake	P-uptake
No nitrogen										
SSP_{30}	114.3	13.5	104.6	12.2	147.0	16.1	136.3	14.7	125.5	14.1
SSP_{60}	112.3	13.9	101.7	13.6	145.1	14.5	141.0	15.2	125.3	14.3
MRP_{30}	108.4	12.3	99.7	12.6	145.0	16.2	134.9	16.6	122.0	14.4
MRP_{60}	85.7	11.6	86.0	11.6	104.4	12.6	101.8	12.4	94.4	12.1
Mean	105.5	12.8	98.0	12.5	135.4	14.9	128.5	14.7	116.8	13.7
Nitrogen @ 10 kg ha^{-1}										
SSP_{30}	99.2	11.6	98.5	11.8	134.4	15.0	126.2	14.1	114.6	13.1
SSP_{60}	105.8	13.1	94.5	12.2	136.6	16.3	129.6	14.8	116.0	14.1
MRP_{30}	104.0	12.4	98.5	12.2	128.3	14.6	132.5	14.6	115.9	13.4
MRP_{60}	94.2	12.2	84.1	10.6	107.8	12.1	100.8	10.9	96.6	11.4
Mean	100.8	12.3	93.9	11.7	126.7	14.5	122.2	13.6	110.8	13.0
Phosphorus x Biofertiliser										
SSP_{30}	106.8	12.6	101.6	11.9	140.7	15.6	131.3	14.4	120.1	13.6
SSP_{60}	109.8	13.5	98.1	12.9	140.9	15.4	135.3	14.9	120.7	14.2
MRP_{30}	106.2	12.4	99.1	12.4	136.7	15.4	133.7	15.5	118.9	13.9
MRP_{60}	89.9	11.9	85.1	11.1	105.9	12.4	101.3	11.6	95.6	11.7
Mean	103.2	12.6	95.1	12.1	131.1	14.7	125.4	14.1	-	-
CD (p=0.05) for grain and straw respectively – N levels NS, 1.0; P-levels 0.7, 2.1; Biofertiliser 0.7, 2.1; NxP 1.0, 2.8; PxB 1.5, 4.2; NxB 1.0, 2.8 and NxPxB 2.0, 5.4. (NS = Non significant)										

Solubilization and fixation of applied and native P - A small amount of native P was solubilized with SSP_{30} and all biofertiliser treatments with maximum solubilization by combined application of *Rhizobium* + PSB followed by mixed culture (Table 3). Increase in dose of SSP_{30} to SSP_{60} reversed the reaction and there was net fixation, with highest fixation in individual biofertiliser treatments at N_{10} level. This was mainly due to excess application of water soluble P than the crop requirement and alkaline calcareous nature of the soil. However, in case of Mussorie rock phosphate the solubilization was found to be very high compared to single super phosphate with highest solubilization at MRP_{30}. The data further showed almost similar values of solubilization in combined and mixed culture of *Rhizobium* and PSB. Both these treatments had very high values over their individual inoculations.

Relative Agronomic Effectiveness (RAE) & Harvest Index (HI) - It is evident from the data that the mean values of RAE were obtained 99.9 percent in MRP_{30} and 74.9 percent in MRP_{60} treatment (Table 4). This indicates that MRP_{30} inoculated with *Rhizobium* and PSB was as good as SSP_{30} while MRP_{60} proved inferior. Among different biofertilisers, both mixed and single cultures had slightly lower values than their single inoculation at MRP_{30} level, whereas in case of MRP_{60} the differences were observed slightly on higher side. Almost similar values of harvest index were also observed in treatment with SSP_{30}, SSP_{60} and MRP_{30}.

Table 3. Interaction effects of *Rhizobium*, PSB, and P-sources on the solubilization/fixation of native and applied P during the growth of pea crop.

P-sources	*Rhizobium* alone	PSB alone	*Rhizobium*+PSB	Mixed culture	Mean
No nitrogen					
SSP_{30}	4.24	3.94	13.4	9.7	7.8
SSP_{60}	-23.6	-22.6	-20.1	-18.4	-21.2
MRP_{30}	29.9	34.2	42.2	43.2	37.4
MRP_{60}	30.0	32.1	34.2	34.4	32.7
Nitrogen @ 10 kg ha^{-1}					
SSP_{30}	0.7	2.5	10.5	8.3	5.4
SSP_{60}	-24.8	-25.7	-15.7	-20.5	21.7
MRP_{30}	30.8	31.8	35.2	36.7	33.6
MRP_{60}	32.1	29,.9	33.4	29.5	31.2
Overall mean	22.1	22.5	23.7	23.7	23.0

Table 4. Interaction effect of *Rhizobium*, PSB, and P-sources on RAE and Harvest Index

P-sources	*Rhizobium* alone	PSB alone	*Rhizobium*+PSB	Mixed culture	Mean
Relative Agronomic Efficiency (RAE %)					
MRP_{30}/SSP_{30}	100.5	102.1	98.4	98.7	99.9
MRP_{60}/SSP_{60}	78.5	83.6	67.8	67.8	74.9
Mean	90.8	92.9	83.1	83.4	-
Harvest Index					
SSP_{30}	26.9	28.2	30.9	30.6	29.2
SSP_{60}	28.7	29.0	34.2	30.5	30.6
MRP_{30}	26.6	29.1	30.2	29.8	29.0
MRP_{60}	25.7	26.9	25.6	25.4	25.9
Mean	27.0	28.3	30.2	29.1	-

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Can Mushrooms fix atmospheric nitrogen?

It is generally reported that fungi like *Pleurotus* spp can fix atmospheric nitrogen. The way they do it is still not clear. Two researchers from Sri Lanka hypothesized that only associations of fungi and diazotrophs can fix nitrogen. It was tested *in-vitro*. *Pleurotus ostreatus* was inoculated with a bradyrhizobial strain nodulating soybean and *P. ostreatus* with no inoculation was maintained as control. At maximum mycelial colonization by the bradyrhizobial strain and biofilm formation they were subjected to ARA assay. While distinct nitrogenase activity was observed in inoculated mycelial biofilm, the same was missing when the fungal mycelium or bradyrhizobial strain were alone. A significant reduction in mycelial dry weight and a significant increase in nitrogen concentration were observed in the inoculated cultures compared to control. The mycelial weight reduction could be attributed to carbon-transfer from the fungus to the bradyrhizobia, because of high carbon cost of biological nitrogen fixation. The study clearly demonstrates that mushrooms alone can not fix nitrogen, but when they are in association with diazotrophs; diazotrophs fix the nitrogen on their expanse. Such studies have implications for future identification of as yet unidentified N_2 fixing systems occurring in the environment.

(Source – Jayasinghearachchi and Seneviratne 2004, Indian Acad. Sci. 29(3) : 293-296)

Response of French Bean Cultivars to *Rhizobium* at Four Locations in Temperate Region

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सारांश

फ्रैन्चबीन फसल की चार प्रजातियों (कन्टेन्डर, प्रीमियर, एसवीएम-१ तथा केन्टुकी वन्डर) के बीजों को राइजोबियम जैव उर्वरक से उपचारित कर चार अलग-अलग स्थानों पर बोया गया और उनकी राइजोबियम ग्रंथियों, कुल पौध शुष्क भार एवं उपज की जाँच की गई। बुवाई के ३१ दिन बाद जाँच करने पर जहाँ केन्टुकी वन्डर प्रजाति में चार में से तीन स्थानों पर सर्वाधिक राइजोबियम ग्रंथियाँ पायी गईं वहीं एस.वी.एम-१ प्रजाति में ५१ दिन पश्चात् चार में से दो स्थानों पर सर्वाधिक ग्रंथियाँ देखी गईं। पौध शुष्क भार एवं कुल बीज उत्पादन विभिन्न प्रजातियों में अलग-अलग स्थानों पर अलग-अलग रहा। ऐसा शायद उनके प्रजाति गुण एवं उस स्थान विशेष की भौतिक रासायनिक एवं वातावरणीय विभिन्नताओं के कारण संभव है ।

Introduction

The usefulness of nodule forming bacteria (*Rhizobium phaseoli*) and French-bean (*Phaseolus vulgaris*) association have long been recognized due to their ability to trap elemental nitrogen and transform it into usable organic form for the increased productivity of the host plant. The efficiency of this symbiotic association is influenced by various soil and environmental factors such as soil air, pH, texture, structure, moisture and soil-microflora of that particular location (Prayitno *et al* 1999). Besides physico-chemical characters, different rhizobial strains also behave differently to different cultivars, soil, temperature and soil chemicals. Present study was an attempt to analyze the comparative potential of *Rhizobium*-French bean association with four French bean cultivars at four different locations, keeping single *Rhizobium* strain as constant in all the treatments.

Materials and Methods

Four varieties of French bean viz: Contender, Premier, SVM-1 and Kentucky wonder were taken as varietal variants. Commonly used rhizobial inoculant was used to treat the seeds @ 200 gm inoculant per 10 kg of seeds as per the recommended method of seed treatment. Seeds were sown in net plot of 4.5 m^2 with three replications each at four different locations, few kilometers apart from each other at 1446 to 1650 ft. above MSL. The distance of 10 x 45 cm was maintained by thinning, 3 weeks after sowing. For nodular count and total dry weight measurements three plants per plot with intact root system were uprooted at 31 and 51 days after sowing (DAS) and shook gently in stagnant water to remove soil. After nodular count plants were dried in an oven at 80^{o}C and weighed. Crop was harvested after maturity and threshed after sun drying.

Results and Discussion

Four French bean cultivars inoculated in this study at four locations responded significantly for the appearance of number of nodules on their roots (Table 1). The highest number of nodules per plant in early stages of growth (31 DAS) was recorded in Kentucky wonder at 3 out of four locations, where as SVM-1 nodulated profusely at second stage of observation (51 DAS). This must be due to specificity of French bean varieties and strain of rhizobia (Chui and Nador 1984). A significant increase in number of nodules per plant from 10.68 to 22.69 was observed as crop advanced from 31 days to 51 days of growth, however, a reduction in number of nodules in Kentucky wonder at 3 out of 4 locations and SVM-1 and Premier at one location was evident which might be due to the soil and environmental factors as also reported by Leon *et al* (1987).

Increase in shoot weight over time and at different locations showed different trends in different varieties (Table 2). While var. Contender and Premier showed increase in dry weight after 31 to 51 days and decrease thereafter at all the four locations, the var. SVM-1 and Kentucky wonder showed consistent increase in dry weight till harvest at three out of four locations. Such variations can be attributed to varietal specificity and differences in physico-chemical characteristics of 4 locations. Reduction in dry weight in two varieties at harvest may be due to the cessation of growth in a short duration crop and abscission of leaves from the shoot (Quintero *et al* 1983). Seed yields recorded at location 1 and 4 (Table 3) also showed wide variations, indicating the interaction of not only *Rhizobium*-French bean cultivar association but also of the factors other than varieties on this symbiotic association.

Table 1. Nodulation in four varieties of French bean at four locations

Varieties	Number of nodules after days of sowing									
	Location 1		Location 2		Location 3		Location 4		Mean	
	31	51	31	51	31	51	31	51	31	51
Contender	26.8	46.3	1.1	4.0	3.9	9.7	4.15	4.7	9.0	16.2
SVM-1	31.1	75.1	5.7	9.4	5.7	69.4	7.4	6.3	12.5	40.1
Premier	20.3	43.0	1.6	2.8	1.2	1.9	6.7	4.9	7.4	13.1
K. wonder	33.1	68.4	6.0	4.5	5.1	4.7	10.5	7.5	13.7	21.3
SEm±	**4.20**	**11.44**	tc<t	tc<t	tc<t	tc<t	2.59	4.78	-	-
L.S.D.	8.53	23.22					5.26			
Bold figures - Significantly higher at 5%, tc = calculated t value, t = table value										

Table 2. Shoot dry matter (gm) per plant as recorded at four locations at three growth stages (31DAS, 51DAS and at harvest)

Varieties	Location 1			Location 2			Location 3			Location 4		
	31	51	Har	31	51	Har	31	51	Har	31	51	Har
Contender	2.80	8.91	5.54	1.36	7.00	4.38	1.67	8.36	3.15	1.56	7.48	5.69
SVM-1	2.80	6.17	11.4	1.24	7.43	9.69	1.39	5.58	5.53	1.22	5.89	6.99
Premier	3.48	10.4	7.20	1.74	7.85	7.38	1.18	8.19	4.39	1.82	7.23	7.01
K. wonder	2.67	5.48	11.7	1.48	6.78	12.6	1.49	8.33	7.03	1.54	6.53	6.73
SEm±	1.58	2.62	0.58	tc<t	tc<t	tc<t	tc<t	tc<t	tc<t	0.39	2.98	0.71
L.S.D.	**3.21**	**5.31**	**1.18**							0.79	NS	NS
* Significantly higher at 5%, tc = calculated t value, t = table value, Har = Harvest												

Table 3. Log transformed values of seed yield obtained from 120 plants per plot of 4.5 m^2 at 2 locations

Variety	Location 1	Location 4
Contender	415.9	794.3
SVM-1	1377.2	988.5
Premier	679.2	1086.4
K. wonder	1475.7	870.9
SEm±	1.11*	1.07*
L.S.D.	1.25	1.16

* Significantly higher at 5%

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FNCA Biofertiliser Project and FNCA Biofertiliser Newsletter

Forum for Nuclear Cooperation in Asia (FNCA) at Asia Cooperation Centre, Japan Industrial Forum Inc (JAIF) is running a specific project on biofertilisers. The project is being run in eight participating countries, namely China, Indonesia, Korea, Malaysia, The Philippines, Thailand, Japan and Vietnam. The FNCA Biofertiliser Project aims to improve and disseminate biofertiliser technology to increase the yields of grain legumes and other crops which are important and feed in Asia, to enhance environment friendly sustainable farming practices by reducing the amount of chemical fertiliser application. Under FNCA Biofertiliser Project a **Who's Who in Biofertilisers** has been constructed for exchanging information among researchers, producers, technicians, farmers, students and people who are interested in biofertilisers. To become a member of the Who's Who register at http://www.fnca.jp/english.

Under FNCA Biofertiliser Project a FNCA Biofertiliser Newsletter is also being published, which is available to members on line at http://www.fnca.jp/english/bf/e-newsletter. So far four volumes of this news letter have been released and all the volumes are available on line. Any information by registered members can be sent to the network on bf-info@iijnet.or.jp. For further details contact FNCA secretariat at bf-info@fnca.jp.

Seminar Conferences, Symposia, Workshop, Fairs and Training Programmes

National Symposium on Modern Biological Sciences (*SyMBios-04*) – is scheduled for 8-9 October 2004, at Department of Bioscience and Research, SNMV, College of Arts and Science, Malumachampatti, Coimbatore – 641 021, Tamil Nadu. The technical sessions will highlight the crucial issues in life sciences, covering: Microbial and fermentation technology, Agricultural and environmental biology, Immunology, Enzymology, Endocrinology, Biodiversity and Genetic Engineering. For further details contact Symposium Committee, SyMBios – 04, SNMV College of Arts and Science, Coimbatore – 641 021, Phone 0422-2610403, 2610894. Email – symbios04@yahoo.com.

MICROTECH-2K4 - 45th Annual Conference of Association of Microbiologists of India is scheduled for 23-25 November, 2004 at Dairy Microbiology Division, National Dairy Research Institute (Indian Council of Agricultural Research), Karnal-132 001, Haryana. The theme of the conference is "Microbial Technology - Targeting Microbes for Global welfare". Various topics to be covered under the theme are as follows:

- Microbial genetics, molecular biology and bioinformatics
- Agricultural microbiology: Plant-microbe interaction
- Food and dairy microbiology: Health promoting food
- Industrial and applied microbiology: Commercialization
- Virology, veterinary and rumen microbiology
- Ecology: Microbes and environment
- Microbial diversity and modern approaches to identification
- Medical and pharmaceutical microbiology

Each session will have invited lectures on contemporary topics by experts. An exhibition of scientific instruments, lab ware and literature is also being arranged by the organizers during the conference. For further detail contact Dr. Kishan Singh, Organizing Secretary, Dairy Microbiology Division, National Dairy Research Institute, Karnal – 132 001, Haryana, India. Phone 0184-2259199 (O), 0184-2204567®, Fax 0184-2250042. Email ksndri@rediffmail.com

26th Annual Conference and Symposium of Indian Society of Mycology and Plant Pathology is scheduled for 7-9 October 2004 at Department of Botany, Goa University, Goa - 403 206. The theme of the conference is "Advances in Fungal Diversity and Host Pathogen Interactions". The three day symposium will consists of: Oral and poster sessions, P.P. Singh Memorial Pesticide India award competition, Smt. Guman Devi Verma memorial best women scientist award competition, P.R. Verma award competition for students, Prof V.P. Bhide memorial award lecture and N. Prasad memorial lecture. Besides other sessions there will be a special session on Mycorrhizae-plant interaction and productivity. For further details contact Dr. B.F. Rodrigues, Organizing Secretary, 26th Annual Conference and Symposium of ISMPP, UGC-SAP Department of Botany, Goa University, Teleigao Plateau, Goa-403 206. Phone 0832-2451345-48 Extension-254/354. Email felinov2001@yahoo.co.uk and felinov@rediffmail.com

XIII Southern Regional Conference on Microbial Inoculants is scheduled for 3-5 December 2004 at Department of Microbiology, College of Agriculture, Bijapur–586 101, Karnataka. The theme of the conference is "*MICROBES* : *Wheels of Organic farming*". The conference includes lead lectures from eminent microbiologists, presentations from both senior and young scientists and poster presentations on Nitrogen fixation, P-solubilization, Plant Growth Promoting Rhizobacteria, Organic matter decomposition, Organic farming, Biocontrol and Bioremediation, Microbial interactions, Mass production techniques, Quality control and Molecular farming. For further details contact Dr. A.B. Patil, Organizing Secretary, XIII Southern Regional Conference on Microbial Inoculants, Department of Agricultural Microbiology, College of Agriculture, Bijapur-586 101, Karnataka. Phone 08352-267168, 267458, 267378, Email ashokrajbpatil56@rediffmail.com.

Interactive Workshop on Biofertilisers – is scheduled for 5 – 6 November 2004 at Indian Agricultural Research Institute, New Delhi. In the recent years various developments has taken place in the field of serology, molecular markers, enhancement in efficiency of organisms for specific traits through genetic manipulation and modernization of biofertiliser production technology. But in the view of the organizers the adoption and large scale use of biofertilisers is still a distant dream. There may be several physical, environmental, technological, infrastructural, market related or human resource development related constraints, which in one way or the other limiting the rapid proliferation of this environment friendly technology. To identify the constraints and to concretize the expert opinion, it is proposed to organize this workshop. The workshop is being organized under "National Agricultural Technology Project (NATP)", Team of Excellence on Human Resource Development in Biofertilisers. The workshop is sponsored by Indian Council of Agricultural Research, New Delhi and is being organized jointly by the Division of Microbiology, IARI and Centre for Conservation and Utilization of Blue Green Algae, IARI, New Delhi. Dr B.D. Kaushik, Head Division of Microbiology, IARI is the Chairman and Dr. Sunil Pabbi of Centre for Conservation and Utilization of BGA is Organizing Secretary. Last date for submission of papers is 21.10.2004 and last date for communication of acceptance is 25.10.2004. Further details can be obtained from Dr. Pabbi, Organizing Secretary, Centre for Conservation and Utilization of BGA, IARI, New Delhi – 110 012, Phone 011-25848431, Fax 011-25846420, 25741648. Email sunilpabbi@lycos.com.

National Workshop on Phosphate Rich Organic Manure – is scheduled for 28th December, 2004 at Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan. The present workshop is forth in the series. It will provide a platform for the interaction of ideas among different scientists from various parts of the country, especially on the technology and engineering aspects involved in large scale manure making. Following main aspects will be discussed:

- Role of various microorganisms in decaying organic matter and in dissolving rock phosphate.
- Cost of production of manure by aerobic and anaerobic methods including capital cost, operating cost, machinery required and size of plant for commercial operations.
- Biogas production and refining.
- Results of field trials of PROM.

The workshop is being organized by the PROM Society and sponsored by Rajasthan State Mines and Minerals Limited, Udaipur. For further details contact Prof. M.S. Shekhawat, Chairman, Organizing Committee, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan.

11th Congress of African Association for Biological Nitrogen Fixation (AABNF 2004) – is scheduled for 22-27 November,2004 at Laboratoire de Microbiologie, Centre de Recherche de Bel-Air, B.P. 1386, Dakar, Senegal. The theme of the congress is "Impact of Biological Nitrogen Fixation on Agricultural Development in Africa". For further details contact Ibrahima Ndoye, Chairman, National Organizing Committee, Laboratoire de Microbiologie, Centre de Recherche de Bel-Air, B.P. 1386, Dakar, Senegal. Fax. +(221) 832 16 75, Email aabnf2004@ird.sn, Web http://www.ird.sn/aabnf2004/

105th Annual Meeting of American Society of Microbiology (ASM) – is scheduled for 5-9 June 2005. For further details contact "Meetings Department, ASM, 1325 Massachusetts Avenue, N.W., Washington D.C. 20005-4171. Fax +202-942-9340, Email MeetingsInfo@asmusa.org.

Second Global Conference on Plant Pathology – is scheduled for 25-29 November 2005 at Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur – 313 001, India. The conference is being organized jointly by the Indian Society of Mycology and Plant Pathology and Maharana Pratap University of Agriculture and Technology, Udaipur, India. The theme of the conference is "**Plant Health and Global Wealth**". The official language of the conference is English. There will be oral and poster presentations. Oral presentations will be by invitations from respective technical session conveners. Contributed papers will be presented as posters. The dead line for receipt of abstracts is March 31, 2005. Last date for registration is October 15, 2005. For further details contact Dr. H.N. Gour, Secretary ISMPP, Conference Secretariat, Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur – 313 001, India. Tel. 0294-2413612, 2465325, Fax – 094-2420447, 2470682. Email – gour_hn@sancharnet.in.

EM European Conference, Amsterdam-2004 – was convened during 18-20 September 2004 at De Meervaart Amsterdam. Meer en Vaart. 300 1068, LE, Amsterdam. The conference was aimed at discussing the role of Effective Microorganisms with the aim of improving the environment on a multitude of levels. Most of the presentations were from the scientists and researchers of the European Union. Conference was concluded with the panel discussion and framing of recommendations. Representatives of more than ten EU countries presented the state of EM technology in their respective countries and in some cases they showed the application of EM in some crops and plants. Last day of the conference was for field trips to companies, organizations and firms which have integrated the EM technology in their work. The organizing committee chairman was Jan F. Hoekstron.

Rhizobium whispers to legumes
Give me shelter and food
I promise to ensure you steady supply of nitrogen

Research Notes and New Reports

Study on A Nitrogen-Fixing *Pantoea agglomeras* Isolated from the Root of Sugar Cane - A strain of nitrogen-fixing bacteria, W11, isolated from the root of sugar cane has been identified as *Pantoea agglomeras* using an auto-identification system for bacterium based on 96 biochemical test data. W11 has the properties of tolerance to acid(pH3.5) and high sucrose concentration (30%), appreciable amount of acid was formed and reduced the acidity of medium to pH of 4.41, the nitrogenase activity was inhibited in presence of 3.1nmol NH_4NO_3 or 58.5nmol $NaNO_3$. The optimum growth and maximum nitrogenase activity of W11 occurs at the medium containing 10% sucrose and 0.25g/l sodium glutamate, pH5.5, 28-30^oC. Its behaviour of tolerance to acid and high sucrose concentration, acid production and the use of carbon substrates are similar to that of *Acetobacter diazotrophicus*. The strain W11 was inferred to have a nitrogen-fixing relationship associated with the root of sugar cane.
(Source - LI Xinghong, LI Dachei, LI Wei, PENG Lie Department of Technical Physics, Peking University, Beijing, 100871)

Symbiotic Bacteria On Some Plants Use Light For Energy - Certain leguminous plants that live in rice fields have the exceptional feature of forming nitrogen-fixing nodules on their stem surfaces (*Aeschynomene* sp in symbiosis with *Bradyrhizobium*). These contain symbiotic bacteria capable of using light as an energy source. Although it had been suspected that bacterial photosynthesis played a role in this highly particular nitrogen-fixing symbiosis, clear evidence of this had never been presented. The work of the IRD (Institute de recherché pour le dévelopement, ex-ORSTOM) Research Unit "Tropical and Mediterranean Symbioses" at Montpellier, France, has recently achieved the first demonstration of the process.

The researchers first isolated and sequenced the four genes coding for the major proteins of the bacteria's photosynthetic apparatus. Subsequently, by genetic transformation, they created a photosynthetic mutant of *Bradyrhizobium* whose photosynthetic unit, or reaction center, was deactivated. They inoculated the mutant strain into roots and stems of *Aeschynomene sensitiva*. These photosynthesis-negative plants were still capable of inducing nodules and fixing nitrogen, but on stems, this ability was severely reduced. The number of stem nodules had halved and so had their nitrogen-fixing capacity; the plant's growth had been retarded to a similar degree. This experiment demonstrates clearly and for the first time that bacterial photosynthesis plays an essential role in nodulation and nitrogen fixation. How does this bacterial photosynthetic activity function in the processes of nitrogen-fixing symbiosis? The scientists involved put forward two hypotheses:

- Firstly, the *Bradyrhizobium* bacteria, thanks to their ability to photosynthesize, could survive at the stem surface devoid of nutrient elements. This absence would be counter balanced by photosynthesis which provides the chemical and biochemical energy necessary for the bacteria's growth. In addition, this energy would be used by *Rhizobium* to infect the cells in the stem and fix nitrogen in the nodule thus generated. The studies have shown in stem nodules an extremely strong expression of the genes which govern the photosynthetic apparatus.

- Secondly, the researchers do not rule out that the energy that bacterial photosynthesis produces represents a saving for the leguminous plant in terms of energy, it must supply for symbiosis to function. The plant can use its own photosynthates and the resulting energy for its growth.

Sequencing of the genes that control *Bradyrhizobium* photosynthetic activity has furthermore allowed their phylogenetic history to be traced. It appears that this activity is an ancestral character of all the *Bradyrhizobium* bacteria, one which has been lost by most soil-dwelling *Bradyrhizobium* species which infect only the roots because it lost its utility in an environment devoid of light. Nevertheless, the property has been conserved by species which attach themselves to stems of *Aeschynomene* because it constituted a selective advantage.

Beyond the interest of the results for fundamental science, promising prospects have been opened up for improving the productivity of rice cultivation. Owing to the symbiosis with the *Bradyrhizobium*, the *Aeschynomene* species which grow among the rice plantations in West Africa constitute a natural plant fertiliser. On decomposition, these leguminous aquatic plants fertilise the rice-field soil by way of their input of nitrogen compounds.

Recent researches have also shown that these photosynthetic bacteria are capable of generating massive colonies on the roots ("floating" nodal roots exposed to the light) of a wild rice, *Oryza breviligulata*, without forming a nodule. These bacteria were inoculated into several crop species (*O. sativa, O. glaberrima*) in the greenhouse and, in conjunction with the Guinea Institute of Agronomic research (IRAG), in a mangrove rice field in that country. The inoculation turned out to be highly beneficial to rice growth. Inoculated plantations showed a more developed rooting system (55% more in length than in control plantations) and wider stems (by 25%). These results should lead to some important applications both in Guinea and in other tropical rice farming regions.
(Source – unisci.com/stories20011)

Induction of Root Nodules and Genetic Variability for Paranodulation in Rainfed Upland Rice - There are several reports on induction of nodule-like structures designated as paranodules on the roots of wheat, rice, maize and barley. Interestingly, these paranodules have been shown to be colonized by bacteria, and also that, the colonizers fix nitrogen within these paranodules. The present investigation was carried out by the authors to determine the genetic variability for 2,4-D induced paranodulation in rain-fed upland rice germplasm. Seeds of 41 rain-fed upland rice genotypes were germinated and after a week, transferred to culture tubes containing Yoshida's rice culture medium, with varying concentrations of 2,4-D (0.0, 0.5, 1.0 and 1.5 ppm). After 35 days, the number of paranodules per seedling was recorded. Paranodules were distinguished from calli by their rounded, dome-like appearance and no-further irregular growth. Out of the 41 rice varieties studied only 2, did not paranodulate, this suggests that, the induction of paranodules may be achieved in most of the rice genotypes, but the degree of induction may vary greatly. When genetic variability for 2,4-D induced paranodules was investigated, significant variation for 2,4-D induced paranodulation was traceable to genotype, 2,4-D and genotype-2,4-D interaction effects. The mean number of paranodules per seedling varied significantly with the concentration of 2,4-D. In spite of moderate heritability

for the trait (60%), due to the existence of a high level of genotypic coefficient of variation (135%), very high level of genetic advance as percentage over mean (215%) has been predicted, indicating good potential for selection of genotypes with higher paranodulation, from the rice germplasm screened. Authors stressed that, once efficient paranodulation, followed by colonization with nitrogen fixing bacteria, and effective N_2-fixation by the bacteria have been accomplished, the utility of paranodulation would hinge upon its agronomic suitability, which remains to be established yet.
(Source - Girish M. Kalagudi and V. V. Shenoy Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad 580 005, India)

Nitrogen-Fixing Crops Might Be Produced More Easily – In nature there are large number of plants having symbiotic association with VAM and rhizobia both. Out of these two, while VAM-plant symbiosis is very ancient (as is evident from fossils records of early land plants), the *Rhizobium*-plant association is a recent development. Recent studies by Dr. Martin Parniske have revealed that plant-*Rhizobium* association actually uses some of the same genes that control the very common mycorrhizal association of plant roots with fungi. This suggests that, the evolution of nitrogen-fixing symbiosis used some of the genes that were controlling the plant-fungal partnerships which are widespread in the plant kingdom. In other words the part of genetic blueprint needed to establish a symbiotic relationship with nitrogen-fixing bacteria is present in all major plant types, including important crop species such as wheat and rice. Consequently, relatively few genetic changes might enable breeders to produce a wide range of plants that can establish symbiotic relationships with nitrogen fixing bacteria, and perhaps manufacture their own nitrogen fertiliser.

To prove their point the authors studied Lotus plants, which were unable to form a symbiosis, either with mycorrhizal fungi or with nitrogen-fixing bacteria, because of a gene mutation, in comparison with normal plants that could form both kinds of partnership. In the mutant lines, the relationships failed in their early stages. Analysis at the DNA level enabled the scientists to find the gene involved, so called "SYMRK" (symbiosis receptor-like kinase). This gene produces a molecule that is an essential early link in the chain of events that enables the Lotus plant to recognize, and respond to, mycorrhizal fungi and nitrogen-fixing bacteria living in the soil around its roots. The chemical structure of the SYMRK molecule suggests it may itself be the receptor that recognizes and binds to molecules specifically produced by mycorrhizal fungi and nitrogen-fixing bacteria. Although, more research is required, but the researchers think it likely that the SYMRK molecule sits in the outer membrane of the cells of the plant's roots where it is able to bind to chemicals produced by potential fungal and bacterial partners. The binding process changes the structure of the SYMRK molecule and triggers a cascade of reactions that activate genes involved in establishing a successful symbiosis
(Source - Nature 417 : 27 Jun 2002, 959-962)

Effects of Elevated CO_2 on Nitrogen Fixation in Wetland Plants - A C_3 sedge (*Scirpus olneyi*) and C_4 grass (S*partina patens*) common to the wetlands of Chesapeake Bay were grown in open-top chambers receiving atmospheric CO_2 concentrations of 360 and 660 ppm to study the effects of elevated CO_2 on nitrogenase

activity and nitrogen fixation. Atmospheric CO_2 enrichment increased nitrogenase activity by 35 and 13% in the C_3 and C_4 species, respectively; and these stimulations led to increases in nitrogen incorporation of 73 and 23%, respectively, in the C_3 and C_4 species. These differential responses of C_3 and C_4 plants to elevated CO_2 were explained by the authors as being "in rough proportion to the relative effect of elevated CO_2 on canopy photosynthesis measured throughout the day". On another note, the authors determined that elevated CO_2 significantly stimulated nitrogenase activity in nonsymbiotic nitrogen fixing microbes that existed in plant-free soil sediments. The authors further contend that if CO_2 concentration of the air continues to rise, it is likely that photosynthetic rates will increase in these C_3 and C_4 wetland species, thus providing greater resources to support enhanced nitrogen-fixation by symbiotic microbial organisms associated with these plants. Moreover, increase in the air CO_2 content should also cause "an increase in the N_2-fixing activity of free-living microorganisms in the marsh ecosystem". Thus, elevated CO_2 is likely to increase the availability of nitrogen to plants in wetlands and all ecosystems where nitrogen-fixing organisms exist.
(Source - Dakora and Drake 2000 Plant, Cell and Environment **23**: 943-953)

Classification of Rhizobia Based on *nodC* and *nifH* Gene Analysis Reveals a Close Phylogenetic Relationship Among *Phaseolus vulgaris* Symbionts - The *nodC* and *nifH* genes were characterized in a collection of 83 rhizobial strains which represented 23 recognized species distributed in the genera *Rhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Bradyrhizobium*, as well as unclassified rhizobia from various host legumes. Conserved primers were designed from available nucleotide sequences and were able to amplify *nodC* and *nifH* fragments of about 930 bp and 780 bp, respectively, from most of the strains investigated. RFLP analysis of the PCR products resulted in a classification of these rhizobia which was in general well-correlated with their known host range and independent of their taxonomic status. The *nodC* and *nifH* fragments were sequenced for representative strains belonging to different genera and species, most of which originated from *Phaseolus vulgaris* nodules. Phylogenetic trees were constructed and revealed close relationships among symbiotic genes of the *Phaseolus* symbionts, irrespective of their 16S-rDNA-based classification. The *nodC* and *nifH* phylogenies were generally similar, but cases of incongruence were detected, suggesting that genetic rearrangements have occurred in the course of evolution. The results support the view that lateral genetic transfer across rhizobial species and, in some instances, across *Rhizobium* and *Sinorhizobium* genera plays a role in diversification and in structuring the natural populations of rhizobia
(Source – Laguerre *et al* 2001 Microbiology **147**, 981-993)

Rhizobia as a Biological Control Agent Against Soil Borne Plant Pathogenic Fungi - Rhizobia promote the growth of plants either directly through N_2 fixation, supply of nutrients, synthesis of phytohormones and solubilization of minerals, or indirectly as a biocontrol agent by inhibiting the growth of pathogens. The biocontrol effect of rhizobia is due to the secretion of secondary metabolites such as antibiotics and HCN. Siderophore production in iron stress conditions provides rhizobia an added advantage, resulting in exclusion of pathogens due to iron starvation.
(Source – Deshwal *et al* Indian J. Experimental Biol. 41 : 1165-1183)

Coral's Symbiotic Bacteria Fluoresce and Fix Nitrogen - Coral harbors numerous symbiotic organisms. For example, dinoflagellate algae live in the coral's gastrodermal cells, supplying the animal with carbohydrates. Scientists have known that bacteria exist inside some coral species, but their functions have been largely unknown. Cyanobacteria fix nitrogen on coral reefs and so have been thought to do the same inside living coral. Recent research shows that it is indeed the case. The cyanobacteria express nitrogenase, which converts the seawater's N_2, which the coral can't use, into organic forms that it can use. The researchers also made clear the source of the Great Star's daytime orange glow. They note that the fluorescence spectrum of phycoerythrin is similar to that of proteins responsible for much of the fluorescence seen in different coral species. In addition to its symbiosis with the coral, the cyanobacteria also may have a relationship with the dinoflagellates, the latter supplying the cyanobacteria with glycerol.
(Source – Chemical and Engineering News Vol 82(33) p 7)

16S Ribosomal DNA Characterization of Nitrogen-Fixing Bacteria Isolated from Banana (*Musa* spp.) and Pineapple (*Ananas comosus* (L.) Merril) - Nitrogen-fixing bacteria isolated from banana (*Musa* spp.) and pineapple (*Ananas comosus* (L.) Merril) were characterized by amplified 16S ribosomal DNA restriction analysis and 16S rRNA sequence analysis. *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*, *Burkholderia brasilense,* and *Burkholderia tropicalis* were identified. Eight other types were placed in close proximity to these genera and other alpha and beta Proteobacteria.
(Source – Cruz *et al* 2001 Applied and Environmental Microbiology, 67(5) : 2375-2379)

Nitrogen Fixing Bacteria Enhanced Bioremediation of a Crude Oil Polluted Soil - The use of nitrogen fixing bacteria to enhance bioremediation of a crude oil polluted soil was investigated in a 56-day study period. Soil pH ranged between 5.3 - 6.8. Soil moisture content ranged between 10-30%. Counts of heterotrophic bacteria ranged between 4.5 x 10^6 - 6.0 x 10^7cfu/g. Counts of hydrocarbon-utilizing bacteria ranged between 2.5 x 10^6 - 2.0 x 10^7 cfu/g. Levels of total organic carbon, nitrogen, phosphorus and hydrocarbons decreased with time during the study period except in the control . The highest percentage loss of crude oil (84%) was recorded in plots, which contained seeds of *Phaseolus vulgaris* (White beans) and 5g slurry of *Bacillus polymyxa*. Plots in which 5g slurry of *Anacystis* (*Chroococcus)* sp., 5g slurries each of *Azotobacter* sp., *Bacillus polymyxa* and *Anacystis* (*Chroococcus*) sp. were applied, recorded 56% and 80% losses of crude oil respectively. Plots in which 5g slurry of *Azotobacter* sp., 5g slurries each of *Azotobacter* sp. and *Bacillus polymyxa* were applied, recorded 80% loss of crude oil. The fertiliser (NPK 15:15:15) treated plot showed 64% loss of crude. The plot containing fertiliser (NPK 15:15:15) with seeds of *Phaseolus vulgaris*, and the plot containing fertiliser NPK 15:15:15 with 5g slurry of *Anacystis* (*Chroococcus*) sp showed 64% and 72% losses of crude oil respectively. The plot in which all treatment options were applied showed 56% loss of crude oil. The plot in which tilling alone was employed showed 72% loss of crude oil. The control recorded the least percentage loss of crude oil (24%) at the end of the study period. Results indicate that at 0.05 (95%) level of significance, there is a significant difference between nutrient level arising from treatment option and hydrocarbon level in soil samples. Thus the

% losses of crude oil recorded in treatment plots were due to treatment options applied (at 95% level of significance). The results indicated a higher bioremediation rate when nitrogen-fixing bacteria were used instead of when inorganic nutrient (fertiliser) was applied.
(Source - Odokuma and Inor 2002 Global Journal of Pure and Applied Sciences: 8(4): 455-470)

Use of Bacteria of the Genus *Azotobacter* for Bioremediation of Oil-Contaminated Soils - The rate of self-purification of oil-contaminated soil increases after introduction of bacteria of the genus *Azotobacter*. The bacteria can assimilate oil hydrocarbons as the sole source of carbon and energy, both in the presence of fixed nitrogen and during nitrogen fixation. The species *Azotobacter chroococcum* activates growth of hydrocarbon-oxidizing bacteria present in Devoroil.
(Source – Gradova *et al* 2003, Applied Biochem. Microbiol 39(3) : 279-281)

Nitrogen-Fixing Bacteria Capable of Utilizing Kerosene Hydrocarbons as a Sole Carbon Source - Several nitrogen fixing consortia (NFC) were isolated from kerosene contaminated soil, where *Phaseolus vulgaris* plants were being cultivated. The capability of these consortia for kerosene hydrocarbons removal was investigated and demonstrated. The NFC cultivated under aerobic conditions, and kerosene as sole carbon source, effected a maximum of 75% of reduction of the total kerosene hydrocarbons. Also, from experiments conducted to evaluate their atmospheric nitrogen fixing capability, all consortia showed nitrogenase activity: from 4 to 183 nmol N_2/3E09 bacteria/day. To the author's knowledge, this is the first report that shows a group of bacteria with the dual characteristic of fixing atmospheric nitrogen and capability to use kerosene hydrocarbons as a sole carbon source.
(Source - Pérez-Vargas *et al* 2000 Water Science & Technology Vol 42 No 5-6 pp 407–410)

Free-living heterotrophic Nitrogen-Fixing Bacteria isolated from fuel contaminated Antarctic soils – Five bacterial isolates closely resembling the species of *Azospirillum* and *Pseudomonas* were isolated from fuel contaminated Antarctic soils, fixed nitrogen in the dark heterotrophically and non-symbiotically. Two isolates utilized jet fuel vapours and volatile hydrocarbons for growth but not in N-deficient medium. In the researcher's opinion, bacteria such as these may contribute to *in-situ* biodegradation of hydrocarbons in Antarctic soil.
(Source – Eckford *et al* 2002 Applied and Environmental Microbiol. 68(10) : 5181-5185)

Cloning of a Mineral Phosphate-Solubilizing Gene from *Pseudomonas cepacia* - Authors have recently shown that the ability of some gram-negative bacteria to dissolve poorly soluble calcium phosphates (Mps+ phenotype) is the result of periplasmic oxidation of glucose to gluconic acid via the quinoprotein glucose dehydrogenase (GDH), a component of the direct oxidation pathway. *Escherichia coli* K-12 derivatives synthesize apo-GDH but not the cofactor pyrroloquinoline-quinone (PQQ) essential for formation of the holoenzyme. Therefore, in the absence of exogenous PQQ, these strains do not produce gluconic acid and are Mps-. Evidence is presented to show that expression of a single 396-base *Pseudomonas cepacia* open reading frame (designated gabY) in *E. coli* JM109 (a K-12 derivative) was sufficient to induce the Mps+ phenotype and production of gluconic

acid. We present the nucleotide sequence of this open reading frame which coded for a protein (GabY) with a deduced M(r) of 14,235. Coupled transcription-translation of a plasmid (pSLY4 or pGAB1) carrying gabY resulted in production of a protein with an M(r) of 14,750. Disruption of the open reading frame of gabY via site-directed mutagenesis changed the phenotype to Mps- and eliminated gluconic acid production. The deduced amino acid sequence of gabY has no apparent homology with those of previously cloned direct oxidation pathway genes but does share regions highly homologous with the histidine permease system membrane-bound protein HisQ as well as other proteins in this family. In the presence of 1 microM exogenous PQQ, both JM109(pSLY4) and JM109(pGAB1) produced 10 times as much gluconic acid as was seen with either the plasmid or exogenous PQQ alone.
(Source - Babu-Khan *et al* 1995 Appl. Environ. Microbiol., 61(3) : 972-978)

Anaerobic Nitrogen-fixing Consortia Isolated from Graminaceous Plants – A group of scientists belonging to Japan, Philippines, Thailand and Myanmar has reported the existence of anaerobic nitrogen fixing consortia (ANFICOs) consisting of N_2-fixing clostridia and diverse non-diazotrophic bacteria in non-leguminous plants. A major feature of these ANFICOs was that the nitrogen fixation by the anaerobic clostridia was supported by the elimination of oxygen by the accompanying bacteria in the culture. In various studies with these ANFICOs, non-diazotrophic bacteria specifically induced nitrogen-fixation of the clostridia in the cultures. ANFICOs are wide spread in wild rice species and pioneer plants which are able to grow in unfavourable locations. These results also indicate that clostridia are naturally occurring endophytes in graminaceous plants and that clostridial nitrogen-fixation arises in association with non-diazotrophic endophytes.
(Source – Minamisawa *et al* 2004 Applied and Environmental Microbiol. 70(5) : 3096-3102)

Activity and Survival of Spray-Dried *Beijerinckia* sp. Microencapsulated in Different Carbohydrates - Authors examined the possibility of preserving *Beijerinckia* cultures by encapsulation using a spray drier, for use in biotechnological processes in the production of biopolymers. An adequate choice of the wall (coating) material is one of the factors that determine the degree of cell survival and the maintenance of fermentative activity in the encapsulated inoculum. Malt dextrin, dehydrated glucose syrups, modified starch, and acacia (gum Arabic) were used as wall materials. The results showed that spray-dried *Beijerinckia* encapsulated in malt dextrin, stored for 2 months, and inoculated into sterile medium for rehydration, presented the greatest stability with respect to fermentative activity, but the glucose-encapsulated cells showed the highest percentage of viability during spray drying and during the storage period
(Source – Boza *et al* 2003 Applied Biochemistry and Biotechnology 111(2) : 113-128)

Towards Growth of Arbuscular Mycorrhizal Fungi Independent of a Plant Host - When surface-sterilized spores of the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* Sy167 were germinated on agar plates in the slightly modified minimum mineral medium described by G. Bécard and J. A. Fortin (New Phytol. 108:211-218, 1988), slime-forming bacteria, identified as *Paenibacillus validus*, frequently grew up. These bacteria were able to support growth of the fungus

on the agar plates. In the presence of *P. validus*, hyphae branched profusely and formed coiled structures. These were much more densely packed than the so-called arbuscule-like structures which are formed by AMF grown in coculture with carrot roots transformed with T-DNA from *Agrobacterium rhizogenes*. The presence of *P. validus* alone also enabled *G. intraradices* to form new spores, mainly at the densely packed hyphal coils. The new spores were not as abundant as and were smaller than those formed by AMF in the monoxenic culture with carrot root tissues, but they also contained lipid droplets and a large number of nuclei. In these experiments *P. validus* could not be replaced by bacteria such as *Escherichia coli* K-12 or *Azospirillum brasilense* Sp7. Although no conditions under which the daughter spores regerminate and colonize plants have been found yet, and no factor(s) from *P. validus* which stimulates fungal growth has been identified, the present findings might be a significant step forward toward growth of AMF independent of any plant host.
(Source – Hildebrandt *et al* 2002 Applied and Environmental Microbiology, 68 (4) : 1919-1924)

Crop-Specific Endophytic Colonization by a Novel, Salt-Tolerant, N_2-Fixing and Phosphate-Solubilizing *Gluconacetobacter* sp. from Wild Rice - A novel salt-tolerant, N_2-fixing and phosphate-solubilizing, *Gluconacetobacter* sp. (PA12) tagged with *gus*A gene, colonized *Porteresia coarctata* (wild rice) and Pokkali (salt-tolerant variety) more intensively when compared to Ponni (salt-sensitive variety). This was confirmed using a colony-counting method.
(Source – Loganathan and Nair 2003, Biotechnology Letter, 25(6) : 497-501)

Application of *Azotobacter* Enhances Pond Productivity and Fish Biomass in Still Water Ponds - Two strains (derepressed-nitrogen fixing, Mac-27 and phosphate solubilizing, PS-21) of *Azotobacter chroococcum* were inoculated in fish culture ponds, singly and in combination with inorganic fertilisers (urea, single super phosphate–SSP). Physico-chemical parameters of pond waters, plankton production and fish biomass were studied. Inoculation of *A. chroococcum* (Mac-27) enhanced nitrogenase activity and rate of nitrogen fixation. A slight reduction in nitrogen fixation and nitrogenase activity was noticed when urea at 96 kg ha^{-1} y^{-1} was mixed with the biofertiliser (Mac-27). Inoculation of PS-21 enhanced phosphate solubilization, but Kjeldahl-nitrogen concentration values remained low in comparison with controls. On the other hand, inoculation with *Azotobacter* (either strain) enhanced the accumulation of ammonium-N, nitrite-N and nitrate-N. A significant ($p < 0.05$) reduction in dissolved oxygen (DO) concentration also took place when *Azotobacter* (both Mac-27 and PS-21) was inoculated in fish ponds. However, when used along with inorganic fertilisers, the reduction was not significant. The pH values were only slightly lowered when the phosphate-solubilizing strain (PS-21) of *Azotobacter* was inoculated. Inoculation of biofertiliser enhanced plankton production, net primary productivity and fish biomass. However, highest values in most of these parameters were noticed only in ponds that were treated with the higher doses of inorganic fertilisers (urea 192 kg and SSP 1500 kg ha^{-1} y^{-1}).
(Source – Garg *et al* 1998, Aquaculture International 6(3) : 219-231)

BOOK REVIEWS

Organic Food Production in India – Status, Strategy and Scope. (2004) By P. Bhattacharyya, Agrobios (India), Jodhpur – 342 003. p-182 Price Rs. 495/- US $ 33.00 (ISBN 81-7754-228-1).

The book entitled "Organic Food Production in India – Status, Strategy and Scope" provides comprehensive information on the present status, strategies needed and scope of organic food production in India. The contents spread over 8 chapters have been clubbed in three sections. While first section "Status" deals with the need for food security, emerging threats by the introduction of chemical input based agriculture and the emergence of organic farming movement, the second section "Strategy" comprehensively summarizes the importance and potential of different organic and biological inputs for nutrient management and plant protection and organic food production strategies for important crops. This section also deals with the Accreditation, Certification and Inspection processes for organic production and briefly describes National Programme on Organic Production and National Standards *vis-à-vis* standards of other countries. Status of organic market development from global, Asian and Indian perspectives and steps needed for creation of appropriate domestic organic market in India have also been summarized. The third section "Scope" mainly deals with the future prospects and strategies for organic food production in India. In brief the book is an excellent compilation of the past present and future prospects and strategies, covering all important aspects of this emerging subject. The summarized details and the recommendations are likely to give an impetus to the gradually but firmly growing organic farming movement in India. (AKY)

Bioinoculants for Sustainable Agriculture and Forestry (2002) edited by S.M. Reddy, S. Ram Reddy, M.A. Singarachary and S. Girisham. Jodhpur Scientific Publishers, p-221 (ISBN 81-7233-307-2).

The book forms the proceedings of National Symposium held during Feb 16-18, 2001 and covers different aspects of biofertilisers and bio-control agents. The contributions are from the leading microbiologists with proven expertise in their respective fields. The book presents a holistic picture of role of microorganisms in plant growth promotion and plant protection. Role of different biofertilisers in integrated nutrient management has been highlighted in specific detail. The prospects and role of transgenic microbial inoculants for sustainable agriculture has also been emphasized. The role of ectomycorrhizae in heavy metal tolerance and in reclaiming wastelands has been described as a special feature in the book.

Structure and importance of VAM fungi in growth and development of medicinal plants, tomato, sugarcane and millets have been specially emphasized by different workers. A critical review on actinorrhizae has also been included. *Azospirillum* plant association in stress tolerance has been specifically elaborated. Need of cyanobacterial biofertilisers in sustainable rice production has also been emphasized by some workers. Phosphate solubilizing and some other microorganisms which play active role in increasing plant productivity have also been discussed in two different chapters. Management of pests through microbial pathogens including viruses, and VAM fungi has found special mention from some eminent scientists. Management of mites through fungi formed the last two chapters. (AKY)

Biofertilizer – *Frankia* (2002) By Laxmi Lal, D.K. Publishers and Distributors, New Delhi (ISBN : 81-85680-61-2)
The book entitled "Biofertilizer-*Frankia* provides comprehensive information on *Frankia*-Actinorrhizal tree symbiotic associationship with an aim to harvest the potential of this system in improving forest productivity. The book consists of 12 chapters. Out of these, first four chapters deal with the historical account of the science of this association and essential microbiological aspects of the microsymbiont. Subsequent four chapters deal with the physiology and nitrogen fixation potential of the *Frankia*-tree symbiotic association. Last four chapters relate to the potentiality and salient features of the association and on future research needs. In brief the book is an excellent compilation of the present status of the knowledge available on this little understood symbiotic relationship, which offers tremendous potentialities for the future. (AKY)

Biofertilizer Technology (2004) by R.A. Sharma, S.R. Maloo, K.L. Totawat and L.L. Somani. D.K. Publishers and Distributors, New Delhi. p-376 (ISBN : 81-85680-90-6).
The book "Biofertilizer Technology" mainly deals with the prospects and potentialities of *Azolla* and Blue-green Algal biofertilisers in submerged paddy. Although *Azolla* and BGA biofertilisers have long been identified as potential source of biologically fixed nitrogen, but their actual role in nutrient mobilization and in delivering the benefits at farmers fields have always been under question. The book discusses in details about their potential and suggests various measures to overcome the applicational constraints for their increased and effective use in paddy cultivation in the days to come. (AKY)

Biotechnology of Biofertilizers 2003, edited by S. Kannaiyan, Kluwer Academic Publishers. p- 375 Price $ 153, (ISBN–140200219) - Biotechnology of Biofertilizers provides a thorough understanding of different processes in symbiotic nitrogen fixing systems and the possibilities of extending these agronomically potential and significant processes to non-legumes. The book has 28 chapters, dealing mainly with three important issues of this science (i) First issue deals with the potential benefits from the N_2 fixing symbiotic systems such as *Sesbania rostrata, Azolla*, and free-living cyanobacteria to rice crop and associative symbiotic N_2 fixer *Azospirillum* to rainfed crops. (ii) Second issue relates to the immobilization of cyanobacteria in a solid matrix such as polyurethane foam for maximizing ammonia production in rice fields and endophytic nitrogen fixation in wheat, which have also been included as they are considered as potential technologies for the future. (iii) Finally the solubilization and mobilization of nutrients by phosphobacteria and VA mycorrhiza and their role as bioinoculants, *Acetobacter diazotrophicus* as a novel biofertiliser for sugarcane and the cycad-cyanobacterial symbiosis have been clearly elucidated. (AKY)

Nitrogen Fixation from Molecules to Crop Productivity. 2002 (Proceedings of the 12th International Congress on Nitrogen Fixation. Edited by F.O. Pedrosa, M. Hungria, G. Yates and W.E. Newton. Kluwer Academic Publishers (ISBN -306-47615-0) – This book brings together the diverse disciplines that study nitrogen fixation and describes the most recent advances made in various field. Chemists are now studying FeMoco, the active site of nitrogenase in non-protein surroundings and the crystal structure of the

enzyme has been refined to 1.6 angstroms. Recent advances in the complex regulation of nitrogen metabolism and nitrogen fixation gene expression in the free living associative, endophytic, symbiotic and photosynthetic diazotrophs are detailed as well as the factors involved in the nodulation process and nodule metabolism in legumes. In recent years molecular techniques have expanded phylogenetic studies and genome sequencing. Extensive studies on biological nitrogen fixation in sustainable agriculture, particularly in the tropics, environmental stress on plants and microbes, rhizobial strain selection, methods of soil reclamation and newly discovered plant bacterial associations are described. Finally the possible avenues of nitrogen fixation research in the coming century, including the expression of nitrogen fixation genes and the establishment of nitrogenase function in plant organelles, the prospects of developing nitrogen fixation in rice and the development of resistant transgenic legumes are explored. All these developments were discussed at the 21st International Congress on Nitrogen Fixation held at Foz do Iguassu, Parana state, Brazil and are covered in these proceedings. (AKY)

Do You Know?
Humans have doubled the rate of nitrogen fixation in last three decades

Industrial nitrogen fixation has greatly increased the amount of nitrogen cycling between the living world and the soil, water and atmosphere. Total nitrogen fixation over entire earth surface, which was approximately 150 million MT (mMT) before the advent of man made industrial nitrogen fixation, now stand at 240 mMT, out of which human contribution is 90 mMT. Over the last two decades industrial nitrogen fixation has increased from 45 to 90 mMT.

The human driven global change is having serious impacts on ecosystems around the world. Some of the negative impacts, which have been identified with certainty include:

- Increased global concentrations of various oxides of nitrogen
- Losses of soil nutrients such as calcium and phosphorus, that are essential for long term soil fertility.
- Substantial acidification of soils and of the waters of streams, lakes and rivers.
- Greatly increased transport of nitrogen by rivers into estuaries and coastal waters, where it has become major pollutant.
- Accelerated losses of biological diversity, especially among plants adapted to low nitrogen soils and subsequently the animals and microbes that depend upon them.
- Caused changes in the animal and plant life and ecological processes of estuarine and near-shore ecosystems and contributed to long term decline in coastal marine fisheries.

(Source - www.stanford.edu/dept/news)

Publications available with NCOF and RCOFs on sale/demand

1. Biofertiliser Newsletter – Biannual (Free circulation). (Back volumes since 1996 available with NCOF).
2. A Book on Biofertiliser for Extension Workers (1995) by P. Bhattacharyya & U.C. Mishra Cost Rs. 125/- (available with NCOF and all RCOF's)
3. जैव उर्वरक विस्तार प्रशिक्षण पुस्तिका (1997) द्वारा पी. भट्टाचार्या एवं यू.सी मिश्रा मूल्य रु. 125/- (available with NCOF and all RCOF's)
4. Three decades of Research in Biofertilisers and Organic Farming in North East India. (2001) by A.K. Yadav and S. Raychaudhuri. (available with RCOF, Nagpur, Also available in CD)
5. Souvenir I and II NER Conference on Biofertilisers (1999 and 2001) by A.K. Yadav and S. Raychaudhuri (available in CD only)
6. Introducing Biofertilisers in the North Eastern Region – A manual for Extension Workers. (1999) by A.K. Yadav and S. Raychaudhuri. (available with RCOF, Nagpur, Also available in CD)
7. Use and Development of Microbial inoculants (1999) by T. Singh, A.K. Yadav and S. Raychaudhuri. (available with RCOF, Imphal)
8. Organic Farming –A Ray of Hope for Indian Farmers. (2004) By A. K. Yadav and Sarita Mowade. Edited by P. Bhattacharyya, Pub. V.B. Foundation Pune. (Available with R.C.O.F. Nagpur)
9. सेंद्रिय शेती - उज्ज्वल भविष्याचा किरण (२००४) ए. के. यादव व सरिता मोवाडे, संपादन डा. पी भट्टाचार्या, क्षेत्रीय जैव उर्वरक विकास केन्द्र, नागपुर (available with RCOF, Nagpur)
10. Proceedings of National Conference on Quality Control of Biofertilisers (2004) Edited by P. Bhattacharyya and V. Dwivedi. Publ. NBDC, Ghaziabad. (available with NCOF, Ghaziabad)
11. Catalogue on Biofertiliser's strains having detailed information on various promising biofertiliser strains being used in India (available with NCOF)
12. Biofertiliser Scenario of Andhra Pradesh – A Documentary Report. by P. Bhattacharyya. (available with RCOF, Nagpur)
13. Biofertilisers in Maharashtra and Goa – Documentary Report. by P. Bhattacharyya (available with RCOF, Nagpur)
14. 1050 Crop Demonstrations on Biofertilisers by M.R. Motsara and R.N. Bisoyi. (available with NCOF)
15. Recent Advances in Biofertiliser Technology (2001) by A.K. Yadav, M.R. Motsara and S. Raychaudhuri. Published by SPURT, New Delhi Cost Rs. 1100/- (available with publisher).
16. A Refresher Course Manual on Biofertilisers (2000) by T. Singh, T.K. Ghosh, M.K. Tyagi and J.S. Duhan, RBDC, Hisar (RCOF, Hisar)
17. Biofertiliser Situation in Orissa (1999), by K. Chandra *et al* RBDC, Bhubaneshwar (available with RCOF, Bangalore in CD)
18. Biofertiliser Vision 2000 (2000), by K. Chandra *et al* RBDC, Bhubaneshwar (available with RCOF, Bangalore in CD)
19. Biotechnology in Sustainable and Organic Farming-Scope and Potential, (2004) Edited by A.K.Yadav, S. Raychaudhuri and N.C. Talukdar. Shree Publisher and Distributors, New Delhi. Cost Rs.1500/-(Available with publisher)
20. Potentialities of Organic Farming in India (2003) by R.N. Bisoyi, RBDC, Bangalore (Available with R.C.O.F. Bangalore).