ISSN 0971-7390

BIOFERTILISER NEWSLETTER

Vol. 12 No. 1 July 2004

Contents

Biofertiliser Newsletter (BFNL) is a bi-annual publication under 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, extension news, results of field trials, information about recent events, research notes, new reports, new species, corporate news, news related to conferences, seminars etc and review of books are especially welcome. Opinion 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

Biofertilisers has made rapid strides during the last 15 years in the country. Total production and consumption has increased from a mere 900MT in 1989-90 to well above 12,000 MT in 2003-04. The journey which was started with *Rhizobium* is now being accompanied by large number of microbial formulations comprising of *Azotobacter*, *Azospirillum*, *Acetobacter*, Blue green algae, *Azolla*, VAM and various phosphate solubilizing microorganisms. With every passing year scientists are discovering new species and new formulations. Large numbers of endophytes and plant growth promoting bacteria (PGPR) have been identified with potential for plant growth promotion. Continuous strengthening of our knowledge to the understanding of Plant-Microbe interaction and their combined effect on plant growth promotion is opening new frontiers for the benefit of mankind. Increasing inclination of consumers in the recent years, towards organically grown crops and gradual shift of some farmers from conventional agriculture to organic agriculture has further underlined the scope and importance of these microbial systems.

Various systems which has been identified and put into commercial formulations by different agencies are being used by farmers. Their responses in terms of increase in crop yield vary from 0 – 25%. This inconsistency in response has been attributed to various factors. Among them poor microbial load, higher level of contamination and use of improper strains have been identified as three most important factors for their poor response. To take a stock of ground realities and to devise appropriate strategies for future, a National Conference on Quality Control of Biofertilisers was organized by the NBDC and Department of Agriculture and Cooperation, Govt. of India during 14-15 January, 2004. Participating scientists and industry representatives have identified the constraints and suggested various remedies. I hope that, even if half the recommendations of this forum are honestly implemented in real sense, the biofertiliser scenario can under go a sea change and can ensure the internationally acceptable quality to the farmers.

Biofertiliser Newsletter, which is dedicated to the dissemination of latest developments in the field of biofertilisers since last 11 years has reported numerous discoveries for the benefit of its readers. So far 11 volumes comprising of 22 issues have been published. First issue of the 12th edition is in your hands. I hope with this we have not only been able to give information on recent developments to our readers, but have also been able to give a platform to express the opinion of the eminent personalities associated with the science and application of biofertilisers. We strive to continue our endeavor with dedication, hard work and positive approach.

A.K. Yadav
Editor

Fly ash as alternative carrier substrate to economize the biofertiliser production

P. Sudhakar, J.K. Ghosh and S. Raje Urs
Central Sericulture Research and Training Institute
Central Silk Board, Berhampore – 742 101, West Bengal

Since the beginning of biofertiliser use at large scale, several carrier materials have been tried like FYM, compost, peat soil, coal, charcoal, cellulose powder, lignite, talc, bagasse, sedge peat, press mud, teak leaf meal, coconut shell powder etc. Tilak *et al* (1979) and Tilak and Subba Rao (1978) studied 17 indigenously available carriers for their ability to support the growth of *Azospirillum* and *Rhizobium* inoculants. They found charcoal alone, charcoal in combination with other materials as promising carrier. Lignite was also found to be reasonably good carrier. As a result charcoal and lignite have become most popular and widely used carrier materials, with charcoal as prominent carrier material in central and eastern states and lignite in western, south central and southern states. But as charcoal is increasingly becoming unavailable due to deforestation, poor quality and high cost and high transportation cost of lignite, need is being felt for their replacement with locally available low cost material.

An ideal carrier material should be inert and neutral in nature and capable of supporting appropriate microbial load during the entire period of shelf life at room temperature. Large quantities of fly ash, a residue of burnt coal (100 million tons/year, Sarma *et al* 2003) is being produced by 82 thermal power plants in the country which is not only a pollution hazard but is also difficult in safe disposal. National Flyash Mission supported by different funding agencies has initiated the efforts to find out alternative uses of fly ash. In agriculture, fly ash use is being promoted as carrier material in VA-mycorrhizal production (TERI, New Delhi) and as neutralizing manure in acidic soils (Srivastava and Chhonkar 2000, 2000a; Kumar *et al* 2001). Out of the three forms of fly ash the pond ash being easily available for free, neutral in pH and having the required characteristic features as that of charcoal bears great potential for use as a carrier in biofertiliser production. In the present study an attempt was made to study the usefulness of fly ash along with some other locally available low cost materials, either alone or in combination as carrier material in biofertiliser production.

Carrier substrates

Three low cost and indigenously available substrates such as peat soil (PS) collected from the bottom of a lake;

charcoal (CC) and Fly ash (FA) were selected for evaluation, individually and in combination with each other in a ratio of 1:1. All the carriers and their combinations were dried and finely powdered to pass through 100 mesh sieve. Six day old *Azotobacter* and phosphate solubilizing bacterial broth cultures with a population load of $>10^{10}$cell /ml were blended with these carriers. Quantity of the broth added, was adjusted to obtain a moisture level of 50% of total water holding capacity of the carriers. Prepared biofertiliser packets were stored at 25°C. Viability of added bacterial cells and other microbial contaminants were monitored from 0 to 180 days at monthly interval by serial dilution and standard plate count method on nutrient agar plates. Important physico-chemical characteristics of the bacterial broth blended carrier materials are given in Table 1. From moisture content point of view charcoal alone retained maximum moisture at 41.63% followed by CC+FA at 30.37% while fly ash alone retained minimum moisture at 16.56%.

Effect of sterilization on native contaminants of carrier substrates

Though all the materials selected for the study were inert, low cost and indigenous but they differed in their natural microbial flora. Peat soil was found to be possessing highest microbial load followed by charcoal and fly ash (Fig. 1). By exposure to UV-irradiation although, the microbial populations came down significantly in all the carriers but they could not be completely eliminated. Autoclaving was far more effective and eliminated the native fungal flora completely in charcoal and fly ash (Fig. 1).

Survival of Biofertiliser organisms and proliferation of contaminants

Population of *Azotobacter* and PSB's in different carriers (Table 2) showed decreasing trend over time. Initial population in all the variants of biofertilisers tested was above 10^{10} cells/ml, but they declined to 10^8 after 3 months of storage in individual carrier based biofertilisers. Though the decline in population was also observed in mixed carriers but they maintained the population of 10^8 till four months. Contaminants were found to be lowest in CC+FA. Level of contamination was higher in all peat soil formulations. Level of microbial contamination and moisture content in different carrier based biofertiliser packets after 120 days of storage are depicted in Fig. 2.

Table 1. Physico-chemical characteristics of different carrier based biofertiliser formulations.

Carrier	OC(%)	WHC(%)	pH	Broth used*	Moisture content
CC	21.60	114.2	7.2	600	41.63
FA	Trace	37.1	6.8	200	16.56
PS	41.65	66.2	6.5	250	23.71
CC+FA	28.95	75.6	7.0	400	30.37
CC+PS	55.25	90.2	7.2	400	32.62
FA+PS	39.50	51.6	7.0	400	28.92
* Fully grown bacterial broth in ml/kg of carrier					

Table 2. Survival of *Azotobacter* and PSB in different carrier based biofertilisers.

Carriers	Total viable count after days of storage (x 10^7/g)						
	0	30	60	90	120	150	180
Azotobacter							
CC	5620	560	316	31.6	1.8	0.89	0.56
FA	3160	320	100	5.60	1.8	0.03	0.03
PS	320	5620	316	31.6	5.6	0.56	0.31
CC+FA	5620	560	1000	31.6	5.6	3.98	0.31
CC+PS	10000	5620	562	56.2	17.8	0.79	0.56
FA+PS	5620	5620	1000	56.2	10.0	1.78	0.31
PSB							
CC	1770	5620	562	31.6	5.6	3.16	1.77
FA	1770	1770	100	10.0	3.2	0.18	0.03
PS	3160	3160	316	17.8	3.2	1.78	0.03
CC+FA	5620	5620	562	56.2	31.6	3.16	1.00
CC+PS	5620	5620	562	56.2	17.8	3.16	0.31
FA+PS	10000	5620	562	56.2	10.0	3.16	0.31

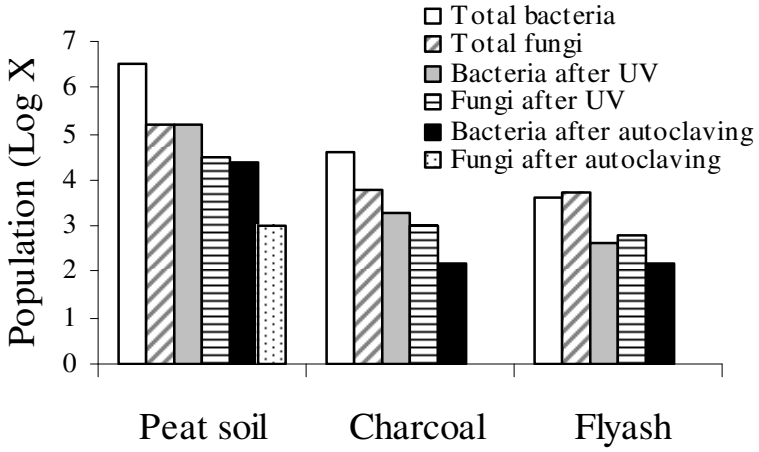

Fig. 1. Native microbial population in different carriers before and after UV/autoclave sterilization.

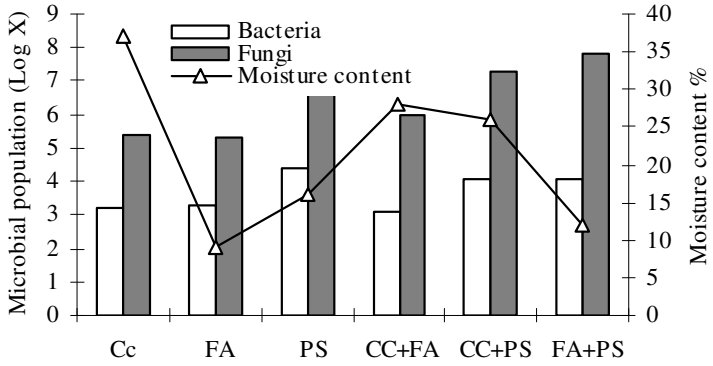

Fig. 2. Microbial contamination and moisture content in biofertiliser packets after 120 days of storage

Table 3. Cost of production with different carriers

Carriers	Cost of carrier* (Rs.)	Cost of bacterial broth** (Rs.)	Total cost (Rs.)	Reduction in cost over charcoal
CC	6000/-	7200/-	13,200/-	-
FA	500/-	2400/-	2900/-	78.03
PS	1050/-	3000/-	4050/-	69.03
CC+FA	3250/-	4800/-	8050/-	39.0
CC+PS	3525/-	4800/-	8325/-	36.9
FA+PS	325/-	4800/-	5125/-	61.2

* Total cost of carrier including transportation, pulverization etc.
** Cost of bacterial broth consumed per MT

On the basis of total viable count, contamination load and moisture content CC+FA combination was found to be most suitable alternative to charcoal alone.

Economics

All the carriers and their combinations were also evaluated on their economic aspects (Table 3). The economics was calculated on the basis of expenditure required for obtaining the base materials in usable form and the cost of bacterial broth required for preparing the biofertiliser packets. Analysis reveals that the cost of production with charcoal alone stands at Rs. 13,200/- per MT compared to Rs. 8050/- for CC+FA, which is the best available alternative, based on the present study. The use of fly ash as carrier material in biofertiliser production not only reduces the cost of production of biofertilisers by about 39% but also ensure the judicious utilization of potentially hazardous industrial waste.

Acknowledgements

The authors express their sincere thanks to the NTPC, Farakka, Murshidabad for providing the base material of fly ash for the study.

References

Sarma, J.S., Sharma, P.D. and Kumar, V. 2003 The use of fly ash in agriculture in India. In Proc. III *International Conf. on Flyash Utilization and Disposal*. 19-21 Feb. 2003. Published by Central Board of Irrigation and Power, New Delhi.

Srivastava, A. and Chhonkar, P.K. 2000 Effect of flyash on uptake of phosphorus, potassium and sulphur by sudan grass and oats grown on an acid soil. **J. Indian Soc. Soil Sci. 48** : 850-853

Srivastava, A. and Chhonkar, P.K. 2000a Influence of flyash on micronutrient availability and uptake by sudan grass and oats grown on coal mine spoils. . **J. Indian Soc. Soil Sci. 48** : 859-862

Tilak, K.V.B.R. and Subba Rao, N.S. 1978 Carriers for legume (*Rhizobium*) inoculants. **Fertilizer News. 23** : 25-28.

Tilak, K.V.B.R., Lakshikumari, M. and Nautial C. 1979 Survival of *Azospirillum brasilense* in different carriers. **Curr. Sci. 48** : 412-413.

Efficacy of Biofertilisers Integrated with Chemical Fertilisers *in-vivo* in Soybean

S.U. Meshram, S.S. Pande, Arti S. Shanware, R.R. Kamdi and V.S. Tajane
Rajiv Gandhi Vikas Biotechnology Centre,
Nagpur University, Nagpur – 440 033.

Five main legume crops viz. Tur (*Cajanus cajan*), soybean (*Glycin max*), green gram (*Vigna radiata*), black gram (*Vigna mungo*) ground nut (*Arachis hypogea*) and chickpea (*Cicer arietinum*) are cultivated widely in Vidarbha region of Maharashtra. Among these five crops soybean is most important crop of the region. In spite of wide area coverage the productivity of these legumes are very low compared to national average. Poor productivity in this region is mainly attributed to poor availability of native rhizobia in soil due to harsh climatic conditions. To ensure optimum productivity and to ensure healthy return to the farmers it is essential that suitable packages be identified and developed under integrated nutrient management system with judicious utilization of biofertilisers and chemical fertilisers. Keeping this aim in view a field trial was conducted to study the efficacy of biofertilisers alone and in combination with chemical fertilisers.

Materials and Methods

A field experiment was conducted at village Talodhi (Balapur) Dist. Chandrapur for soybean crop (var. JS-335) during the year 2003-04 under the Department of Biotechnology, Govt of India, sponsored R & D project on "Biotechnology of Biofertilisers". The experiment was laid in randomized block design with 12 treatments and replicated thrice as per the details given in Table 1. Two *Rhizobium* strains, RS-1 and SB-119 were used in the study as biofertilisers and applied as seed treatment @ 200 gm/25 kg seed. FYM was applied basally at 10 tones/ha. Seed bacterization was done in morning hours immediately before sowing in the field itself. Entire phosphorus was applied basally while nitrogen was applied in two equal splits at the time of sowing and after 30 days of sowing. Regular cultural practices were followed as per the requirement and package of practices. Soil analysis parameters were studied as per the standard methods of Jackson (1967) and Piper (1967).

Results and Discussion

The experimental soil was loamy in texture, moderately well drained, shallow, non-calcareous, having conductivity 0.068 dsm^{-1}, pH 7.6, available N 169 kg/ha, P_2O_5 14.8 kg/ha and K_2O at 302 kg/ha. The organic carbon status of the soil was medium at 0.43%.

Table 1. Nodulation and grain yield of soybean as influenced by *Rhzobium japonicum* and PSB biofertilisers in combination with chemical fertilisers.

Treatments	No. of nodules/plant at		Dry wt of nodules at 60 DAS	Total grain yield Q/ha	% increase over control
	30DAS	60DAS			
Control	22.12	36.02	73.9	11.38	-
Rhizobium RS-1	33.12	80.3	130.8	14.18	24.82
Rhizobium SB-119	35.2	82.2	132.4	14.80	30.50
RS-1+15 kg N/ha	31.2	78.2	120.4	15.32	34.86
SB-119+15 kg N/ha	29.0	73.3	114.3	15.04	32.16
30 kg N/ha	27.6	68.6	98.41	13.58	19.33
PSB	30.2	77.3	108.2	12.92	13.53
PSB + RS-1	29.4	86.3	134.5	13.68	20.21
PSB+ SB-119	28.2	80.4	109.3	13.32	17.93
PSB+37 kg P_2O_5/ha	29.2	74.0	108.1	13.42	17.93
75 kg P_2O_5/ha	24.7	62.0	104.2	12.26	07.73
30 kg N + 75 kg P_2O_5/ha	26.2	69.0	104.2	12.83	12.74
SE (d)	1.61	7.01	6.23	0.46	-
CD at 5%	3.63	15.8	14.0	1.29	

Data obtained in total number of nodules and total nodular mass reveal that both the strains of *Rhizobium* (RS-1 and SB-119) under study significantly improved nodulation status under all treatments (Table 1). Combined inoculation of *Rhizobium* and PSB although, recorded less number of nodules during early growth, but the deficiency was overcome by 60 days of growth (Table 1).

Delay in nodule development due to dual inoculation may be due to mixed microbial load in the rhizosphere and lesser availability of root hairs to rhizobia in presence of PSBs. Among two *Rhizobium* strains under study (RS-1 and SB-119), both performed equally, when applied alone or in combination with PSB and chemical fertilisers. Interestingly the treatments provided with only chemical fertilisers and only PSB without *Rhizobium* also showed significant improvement in nodulation over control. But the total nodular mass was invariably much higher in treatments provided with *Rhizobium*. This indicates that in particular soil although, there was enough of native *Rhizobium*, but the strain was less effective and was able to establish symbiotic relationship to a certain extent only under high nutrient availability, while the introduced *Rhizobium* strains of RS-1 and SB-119 were highly infective and effective under all circumstances without or with chemical fertilisers. In terms of grain productivity, highest productivity was obtained in treatments where two *Rhizobium* strains were applied in combination with 15 kg N/ha followed by only *Rhizobium* inoculation. Combined inoculation of PSB + *Rhizobium* with both the strains were slightly less productive compared to only *Rhizobium* inoculation. PSB alone although, significantly increased the

productivity compared to control, but it was far less than the *Rhizobium* treatments.

From the above assessment it is evident that the application of chemical fertilisers was not only beneficial to the crop productivity, but was also promoting the native *Rhizobium* to establish symbiotic relationship. Introduced *Rhizobium* strains were highly infective and effective, resulting into significant increase in productivity. Hossein and Alexander (1984) and Dansu *et al* (1990) has also observed similar results in soybean with *Bradyrhizobium japonicum*. PSB inoculation alone and in combination with chemical P-fertilisers was also effective and was significantly contributing to productivity compared to control. These findings are in conformity of the observations made by Gaur (1990). Interestingly, combined application of *Rhizobium* and PSB as seed treatment could not provide any additional advantage over single inoculation. Possibilities should be explored to use them separately with *Rhizobium* as seed treatment and PSB as soil treatment. Judicious combination of chemical fertilisers and *Rhizobium* is best possible package to maximize the productivity.

Post harvest soil analysis (Table 2) suggests significant improvement in soil fertility due to the application of biofertilisers. It is evident from higher amounts of available N, P_2O_5 and K_2O in all the biofertiliser treated plots. Organic carbon was also found to be improved marginally. Latif *et al* (1992) had also found similar improvement in soil fertility due to the use of biofertilisers.

Table 2. Effect of integrated use of biofertilisers and chemical fertilisers on fertility status of soil after harvest of the soybean crop.

Treatments	pH	EC (dsm^{-1})	Organic C (%)	Available N (kg/ha)	Available P_2O_5(kg/ha)	Available K_2O (kg/ha)
Control	7.60	0.070	0.43	169	14.8	292
Rhizobium RS-1	7.53	0.072	0.52	202	15.5	308
Rhizobium SB-119	7.69	0.072	0.54	198	15.5	306
RS-1+15 kg N/ha	7.79	0.074	0.49	183	15.3	298
SB-119+15 kg N/ha	7.83	0.073	0.48	187	14.7	314
30 kg N/ha	7.43	0.069	0.40	180	14.6	310
PSB	7.76	0.072	0.47	178	17.6	302
PSB + RS-1	7.89	0.084	0.53	190	17.2	300
PSB+ SB-119	7.71	0.090	0.51	187	17.0	298
PSB+37 kg P_2O_5/ha	7.66	0.082	0.49	174	18.1	312
75 kg P_2O_5/ha	7.83	0.072	0.42	170	15.8	296
30 kg N + 75 kg P_2O_5/ha	7.48	0.068	0.42	176	15.2	298
SE (d)	NS	NS	0.076	3.14	1.20	4.05
CD at 5%	NS	NS	0.034	7.08	2.71	9.15

References

Dansu, S.K.A., Kapuya, J. and Hardarson, G. 1990 Nitrogen fixation by varying the methods of inoculating with *Bradyrhizobium japonicum*. **Plant Soil 125** : 81-86

Gaur, A.C. 1990 Phosphate Solubilizing Microorganisms as Biofertilisers. Omega Publishers, New Delhi.

Hossain, A.K.M. and Alexander, M. 1984 Enhancing soybean rhizosphere colonization by *Rhizobium japonicum*. **Appl. Environ. Microbiol. 48** : 448-472.

Jackson, M.L. 1967 Soil Chemical Analysis. Asia Publishing House, Bombay.

Latif, M.A., Mackenzie, M., Ali, G.R. and Fairs, A.F.I. 1992 Effects of legumes on soil physical quality in a maize crop. **Plant Soil. 140** : 15-23.

Piper, C.S. 1967 Soil and Plant Analysis. Asia Publishing House, Bombay.

Publications available with NBDC and RBDCs on sale/demand

1. Biofertiliser Newsletter – Biannual (Free circulation). (Back volumes since 1996 available with NBDC).
2. A Book on Biofertiliser for Extension Workers (1995) by P. Bhattacharyya & U.C. Mishra Cost Rs. 125/- (available with NBDC and all RBDCs)
3. जैव उर्वरक विस्तार प्रशिक्षण पुस्तिका (1997) द्वारा पी. भट्टाचार्या एवं यू.सी मिश्रा मूल्य रु. 125/- (available with NBDC and all RBDCs)
4. Three decades of Research in Biofertilisers and Organic Farming in North East India. (2001) by A.K. Yadav and S. Raychudhuri. (available with RBDC, 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 RBDC, Nagpur, Also available in CD)
7. Use and Development of Microbial inoculants (1999) by T. Singh, A.K. Yadav and S. Raychaudhuri. (available with RBDC, Imphal)
8. Catalogue on Biofertiliser's strains having detailed information on various promising biofertiliser strains being used in India (available with NBDC)
9. Biofertiliser Scenario of Andhra Pradesh – A Documentary Report. by P. Bhattacharyya. (available with RBDC, Nagpur)
10. Biofertilisers in Maharashtra and Goa – Documentary Report. by P. Bhattacharyya (available with RBDC, Nagpur)
11. 1050 Crop Demonstrations on Biofertilisers by M.R. Motsara and R.N. Bisoyi. (available with NBDC)
12. 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).
13. A Refresher Course Manual on Biofertilisers (2000) by T. Singh, T.K. Ghosh, M.K. Tyagi and J.S. Duhan, RBDC, Hisar (RBDC, Hisar)
14. Biofertiliser Situation in Orissa (1999), by K. Chandra *et al* RBDC, Bhubaneshwar (available with RBDC, Bangalore in CD)
15. Biofertiliser Vision 2000 (2000), by K. Chandra *et al* RBDC, Bhubaneshwar (available with RBDC, Bangalore in CD)

Biofertilisers in Cotton Based Cropping Systems

A.R Raju, B. Uma and D. Praveen Kumar[1]
Central Institute for Cotton Research,
P.B. No. 2 Shankarnagar, P.O. Nagpur – 440010
[1] HAI Warangal, Andhra Pradesh

In a sample survey conducted by Central Institute for Cotton Research (CICR), Nagpur in two different cotton growing districts of the country to assess the awareness and adoption of biofertiliser technology, it was found that, in Sirsa district of Haryana and Amravati district of Maharashtra, while level of awareness was among 8% and 28% of the farmers but the technology was actually adopted by only 2 and 17% of the farmers of these two districts respectively (Raju *et al* 2001 and Wasnik *et al* 2001). The higher adoption rate under rainfed conditions of Maharashtra was possibly due to the predominant soybean-gram/ wheat-cotton rotation where institutional agencies promoted seed inoculation in soybean and gram crops. Constant efforts were also made by CICR, RBDC, Nagpur and different public and private agencies engaged in institutional linkages with rural farmers (Pundareekshundu and Singh 1984, Bhattacharyya 1999). Concerted efforts taken up by Government institutions and private firms for compulsory seed inoculation in soybean, their distribution through *Panchayat Samitis* and along with seed bags by seed companies, helped farmers in trial, evaluation and adoption of this technology.

Cotton being high input demanding cash crop has attracted lot of attention from the promoters of such environment friendly technologies and large number of microbial strains belonging to N_2-fixing and P-solubilizing bacteria have been identified for increasing the productivity of cotton at low cost (Bhattacharyya 1999). Since last few years CICR, Nagpur has initiated intensive efforts in identification of proper microbial systems for use in cotton under Vidarbha conditions, which is famous for its high summer temperature, low moisture and low aerial humidity. Some strains of *Azotobacter chroococcum*, *Azospirillum brasilense* and a strain of *Acetobacter diazotrophicus* were found to be significantly improving the productivity of cotton. In this paper an attempt has been made to summarize the potential of different strains of such beneficial bacteria in improving the productive potential of cotton.

Agronomic potential of some selected microbial strains

Azotobacter chroococcum – *A. chroococcum* is known for its moderate N_2-fixing potential (25-30 kg N ha^{-1}), hormone and vitamin secretion, P-solubilization and for its anti-fungal

activities. Seed treatment with *A. chroococcum* (C_2 strain) in LRA 5166 and AKA 8401 cotton along with 50% of recommended fertiliser-N, improved seed cotton yield to the extent of 160-225 kg ha^{-1} (Bonde and Raju 1998 and Raju *et al* 1998). Application of organic manures @ 5 t ha^{-1} once in three years along with *A. chroococcum* inoculation, improved cotton seed yields equivalent to that of full recommended doses of fertilisers and brought yield stability in *G. hirustum* cotton on long term basis (Raju *et al* 2001 , Raju 2002). *A. chroococcum* Nagpur local isolate in hybrid cotton improved seed cotton yield by 372 kg ha^{-1} with only 50% of recommended doses of fertilisers, making it at par with full recommended doses of fertilisers. This strain was found to be superior over the commercially available analog resistant mutants of *A. chroococcum* Mac 27 and Mac 68 in vertisols (Raju *et al* 2001). *A. chroococcum* analog resistant mutants like E-12, Ala-27, heat tolerant mutants HT 54(i), wild isolates AC-1, AC-2 and a strain of *Pseudomonas* were found suitable for cotton based cropping systems. High temperature, low soil moisture and high synthetic N-fertiliser use are the major constraints that affect the potentiality of wild isolates, while metabolic analogs and heat resistant mutants were found to be effective under similar situations. Physiological potentiality of some selected strains of *Azotobacter* and their effect on yield improvement in cotton and wheat are given in Table 1.

Table 1. Physiological potentiality of different *Azotobacter* strains and their effect on yield improvement in cotton and wheat.

Strains	ARA n moles C_2H_4 hr^{-1} mg^{-1} protein	P-solubilization potential	NH_4 secretion µg ml^{-1}	IAA µg ml^{-1}	Cotton yield improvement (kg ha^{-1})			
					Rain-fed		Irrigated	
					G. arboreum	*G. hirsutum*	*G. hirsutum*	Wheat
Heat tolerant strains								
HT 54	889	0.908	22	5	57	147	-	217
HT 54(i)	548	0.823	21	6	115	187	184	407
HT54(ii)	298	0.818	14	3	80	212	-	173
HT 57	692	0.808	20	7	276	40	94	220
Analog resistant strains								
Mac 27	2184	0.947	63	Trace	309	185	-	297
Mac 68	482	0.922	43	4	221	79	-	260
ALA 27	613	0.871	15	5	86	269	283	350
MSX 9	613	0.871	21	9	138	218	-	393
E 12	734	-	-	-	120	153	211	323
Others								
IS 16	521	0.832	16	28	-	211	-	-
Ac-18	-	0.852	-	-	-	-	242	346
Ac-29	2046	0.852	16	-	111	200	52	197
Nagpur 2	2635	0.552	13	38	157	-	372	47

Azospirillum brasilense – *Azospirillum* is known for its tolerance towards higher concentrations of chemical N than A. chroococcum as it temporarily suspends its N_2-fixation and multiplies its population by using available synthetic N. Once the level of N reaches below a threshold level it switches over to atmospheric N-fixation. Although, it responds favourably to organic manures, but is not dependent on it's availability. The research work conducted at CICR,Nagpur on cotton has revealed that 225 kg ha^{-1} seed cotton yield improvement can be obtained by seed treatment with *Azospirillum brasilense* IARI-strain in LRA 5166 and AKA 8401 cotton (Raju *et al* 1998). Seed treatment with *A. brasilense* and application of organic manures at 5 t ha^{-1} once in three years along with 50% recommended doses of fertilisers improved seed cotton yield on par with that of full recommended dose of fertiliser-N and brought yield stability in *G. hirsutum*. Raju *et al* 2000, 2001 and Raju 2002 had observed that inoculation with *A. brasilense* Nagpur-local isolate in *G. arboreum* cotton with 50% of recommended fertiliser-N improved the productivity by 366 kg ha^{-1} and produced similar yield to that of full recommended fertilisers. Under irrigated conditions *A. brasilense* strain PH-2, FS and *Acetobacter* improved seed cotton yields and strain PH-2, TNAU and FS improved wheat yield significantly (Table 2).

Economics of biofertiliser use – Biofertilisers can play significant role in sustainable crop production under integrated nutrient management system. Due to low cost and consistent performers, the biofertilisers not only put less-burden on farm budget, but also provide nutrients slowly, which can not be lost by leaching under heavy rains. Under INM system approach CICR Nagpur has tried various microbial strains in cotton based cropping system with 75% of recommended fertilisers and correcting nutrient deficiencies of major and micronutrients in the mid season by foliar application. Results (Table 3) indicates that cost benefit ratio under such system is strongly in favour of the integrated use of biofertilisers with reduced doses of chemical fertilisers.

Table 2. Response of cotton and wheat to *Azospirillum, Acetobacter* and *Pseudomonas* strains during 1999-2003.

Microbial strains	Yield improvement in kg ha^{-1}			
	Rain-fed		Irrigated	
	G. arboreum	*G. hirsutum* hybrid	*G. hirsutum*	Wheat
Azospirillum strains				
FS	74	206	219	209
Nagpur	366	-	174	140
TNAU	-	-	108	342
RBDC	-	-	180	170
PH-2	-	-	354	336
Acetobacter 35-47	-	51	280	172
Pseudomonas	196	35	237	-

Table 3 Effect of bio-inoculants on seed cotton yield and economic indicators in hybrid cotton AHH 468.

Case Study - I

No.	Treatment	Seed cotton yield (kg ha^{-1})				Economic indicators B : C ratio			
		2000	2001	2002	Mean	2000	2001	2002	Mean
T1	RDF 90:45:45	1921	1751	1653	1775	4.12	3.67	3.41	3.73
T2	50% RDF	1430	1277	1446	1384	3.80	3.29	3.85	3.65
T3	T2+Mac 27	1418	1418	1872	1569	3.71	3.71	5.22	4.21
T4	T2+E-12	1520	1690	1401	1537	4.05	4.61	3.65	4.10
T5	T2+Mac 68	1511	1367	1510	1463	4.02	3.54	4.01	3.86
T6	T2+ HT 54	1436	1567	1590	1531	3.77	4.20	4.28	4.08
T7	T2+HT 541	1710	1553	1450	1571	4.68	4.16	3.81	4.22
T8	T2+HT 542	1726	1636	1376	1596	4.90	4.43	3.57	4.30
T9	T2+HT 57	1428	1468	1375	1424	3.74	3.87	3.57	3.73
T10	T2+MSX 9	1816	1466	1523	1602	5.03	3.87	4.06	4.32
	SED	267	Sig	237	Sig	-	-	-	Sig
	CD	NS	329	NS	174	-	-	-	0.59

Case Study - II

No.	Treatment	Seed cotton yield (kg ha^{-1})				Economic indicators B : C ratio			
		2000	2001	2002	Mean	2000	2001	2002	Mean
T1	RDF 90:45:45	1931	1748	1251	1643	4.15	3.66	2.34	3.38
T2	50% RDF	1676	1222	1137	1345	4.63	3.10	2.82	3.51
T3	T2+AC 1	2017	1616	1406	1680	5.70	4.37	3.67	4.58
T4	T2+AC 2	1841	1555	1332	1576	5.11	4.16	3.42	4.23
T5	T2+AC 29	1892	1575	1169	1545	5.28	4.23	2.88	4.13
T6	T2+ IS 16	1986	1568	1114	1556	5.59	4.21	2.70	4.17
T7	T2+Ala 27	1701	1754	1388	1614	4.65	4.82	3.61	4.36
T8	T2+FS	2017	1616	1020	1551	5.70	4.37	2.39	4.15
T9	T2+35-47	1690	1470	1027	1396	4.61	3.88	2.41	3.63
T10	T2+Ps	2034	1332	1375	1580	5.75	3.42	3.57	4.25
	SED	212	Sig	Sig	Sig	-	-	-	Sig
	CD	NS	409	152	132	-	-	-	0.80

Results of two such studies (Table 3) clearly indicate that while the cost to benefit ratio with 100% recommended doses of fertilisers stands at 3.73 to 3.38 the same with integrated use of biofertilisers with 50% recommended doses of fertilisers stands at 3.63 to 4.58 (Table 3). Bhattacharyya (1999) reported a net gain of Rs. 1940/- and Rs. 433.50 due to biofertiliser use in trials conducted by Gujrat Agricultural University and as per the assessment of Department of Biotechnology respectively.

References

Bhattacharyya, P. 1999 Role of biofertilisers in cotton. In *Handbook of Cotton in India*. Eds V. Sundarm,. *et al* Indian Soc. Cotton Improvement (ISCI), Mumbai. pp 159-166.

Bonde, W.C. and Raju, A.R., 1998 Effect of *Azotobacter chroococcum* on seed cotton yield of cotton var. LRA 5166. **J. Indian Soc. Soil Sci. 23** : 226-227.

Pundareekakshudu and Singh, J.V. 1984 Studies on the efficiency of *Azotobacter* on N utilization in rainfed cotton. Paper presented in Seminar on Soil Resources and Productivity Management. Indian Soc. Soil Sci., IARI, New Delhi. Dec. 7-10.

Raju, A.R., Monga, D., Meshram, M.K., Lakshminarayana, K., Wasnik, S.M. and Uma, B. 2001 Integrated nutrient management of cotton based cropping systems under irrigated and rain-fed agro-eco-systems. Paper presented at National Seminar on Organic Farming Systems- An eco-friendly approach for evergreen revolution. Jointly organized by P.D. Krishi Vidyapeeth and V.N. Smruti Pratisthan, Pusad at College of Agriculture, Nagpur. Feb 13-14.

Raju, A.R. 2002 Bio-inoculant technology for economic nutrient management in rain-fed cotton. II International Agronomy Congress, New Delhi, Nov. 26-29.

Raju, A.R., Meshram, M.K., Uma, B. and Suchitra, S. 2001 Bio-inoculant technology for eco-friendly and economic nutrient management of rainfed cotton in vertisols of central India. Souvenir, National Seminar on Sustainable Cotton Production Technology and Future Strategies. CICR, Nagpur. Sep. 10-11, p-81.

Raju, A.R., Meshram, M.K. and Chakraborti, M. 2000 Local isolates of bioinoculants in advance sown hybrid cotton. International Conf on Managing Natural Resources for Sustainable Agricultural Production in the 21st Century. New Delhi. Feb. 14-18. Extended Summaries Vol II : 661-662.

Raju, A.R., Meshram, M.K. and Chakraborti, M. 1998 Nitrogen fixing bio-inoculants for rainfed cotton. **Biofertiliser Newsletter 6(1)** : 15-18.

Wasnik, S.M., Bhaskar, K.S., Raju, A.R. and Barbde, N.P. 2001 Approaches for creating awareness among cotton growers for eco-friendly cotton cultivation practices. Paper presented at National Seminar on Organic Farming Systems- An eco-friendly approach for evergreen revolution. Jointly organized by P.D. Krishi Vidyapeeth and V.N. Smruti Pratisthan, Pusad at College of Agriculture, Nagpur. Feb 13-14.

What do nitrogen-fixing bacteria and lightning have in common? - Both can split dinitrogen. Dinitrogen, or N_2, makes up 79% of our atmosphere and is colorless, odorless, non-toxic and incredibly stable. These two systems are originators of life on this planet. Without nitrogen fixation, the whole cycle would come to a halt and life as we know it would be over.

Quality Control of Biofertilisers in India - Myths and Realities

S.Raychaudhuri
ICAR Research Complex for NEH Region, Manipur Centre
Lamphelpat, Imphal, Manipur-795004

Biofertiliser Technology

The wide-spread acceptance and long term adoption of any technology or product depend upon their ability to deliver consistent desired results up to the customer's satisfaction. The reliability of the technology is gauged by the performance of the product. Technologically, the biofertilisation or microbial inoculation is a proven one where large number of a single or multiple species of microorganisms having desired attributes, are deliberately introduced into agricultural systems to incite response. The product 'Biofertilisers' or 'Microbial inoculants' is the physical manifestation of the technology. Therefore, failure of any such product (biofertiliser or microbial inoculant) evokes doubts in the mind of the users on the credibility of the technology itself.

Limitations

Although, biofertilisation is a proven technology (Nutman 1976, Peoples *et al* 1995), it has it's own limitations in demonstrating significant response in highly input intensive agriculture and lacks dramatic exhibitionism. Moreover, it has it's limitation in delivering good in some special conditions like bacteriophage infested soil, unfavorable soil pH and temperature, imbalanced nutrients in soil, moisture stress in addition to faulty application method, or wrong choice in spite of no fault in the product. Unfortunately, some biofertiliser (BF) manufacturers take these limitations as an opportunity to technically shield their spurious products from customer's dissatisfaction, when their products fail to produce any result in the field. Then what should be the basis to assess the product quality? The customers stress on their field performance only. But technically speaking, that may be too harsh for the manufacturers and practically non-feasible proposition. That is why, guidelines are framed throughout the world setting some standard parameters for biofertiliser product in their respective country with an assumption that strict adherence to those parameters will invoke desirable response (Date 1969, Hiltbold *et al* 1980, Anonymous 1986).

India is one of the largest producer and consumer of biofertilisers in the world, yet its products often viewed with skepticism in and outside the country (Eaglesham 1989, Thompson 1999, Bagyaraj *et al* 2000, Bhattacharyya 2000). Even many questions are raised on the role of quality control agencies and the quality parameter itself, in which some are valid, genuine and practical but some are far fetched imagination of

ignorance. The whole scenario of Indian inoculants quality and their control is laced with it's equal shares of myths and realities.

Quality control scenario in India

There are more than 147 production facilities all over India producing more than 12,152 tones of different types of biofertilizer as per the estimates in 2003-04 (Dwivedi and Bhattacharyya 2004). But there is no statutory law or enforcing agency to control these products. However, the Bureau of Indian Standard (BIS) has published the standards for *Rhizobium* (IS-8268:2001), *Azotobacter* (IS-9138:2002), *Azospirillum* (IS-14806:2000) PSB (IS-14807: 2000).

But these are purely of voluntary in nature and are being regulated on firms and products who opted for BIS certification. Moreover, the BIS does not have any infrastructural facility of its own for sampling and testing of biofertiliser products (Kumar 1997).

In some cases manufacturers send their own samples to a few identified laboratories, voluntarily for getting the certificate of quality. In such cases the volunteered samples would receive utmost care of the manufacturers during production and pass the tests. But definitely those packets are not the true representatives of the whole lot/batch and do not reflect the actual quality. In fact, no statistically sound coordinated mechanism of sampling and testing for biofertilisers exist in India. Moreover, because of the product nature, there is practical difficulty in maintaining the confidentiality about the identity of the manufacturers from the actual testing personnel to avoid biasness or malpractice.

In absence of any quality control law (for biofertiliser) or enforcing agency, the job is left with the manufacturers themselves with the hope that, in a competitive market only good quality products survive. Under these circumstances, it is not surprising, that many questions are raised not only by the producers and users but also by the persons involved in quality control of biofertilisers. The "National Conference on Quality Control of Biofertilisers" during 14-15 January, 2004 has also discussed many of such relevant questions and put forward various proposals (Bhattacharyya and Dwivedi 2004). An attempt is made in the following sections to address some of the issues related to quality control parameters and BIS standards in light of the author's personal experience, gained during his long association in the field.

Some issues and views

'Microbial Inoculant' or 'Biofertiliser' ? - The term, 'Biofertiliser' suits more with the materials like compost, FYM, vermicompost etc. Microbial inoculant is a better term, although, in that too, the word 'Microbial' is superfluous. The term 'Inoculant' is enough. Prefixing the term 'inoculant' with rhizo, azoto etc. (e.g. Rhizo-inoculant) to specify the product will be better.

Nodulation test in quality testing parameter - Often, questions are raised on validity of nodulation test among the testing parameters for rhizobial packets, as nodulation test takes around 40 days

and the testing reports can not reach the users/customers before the packets are used in fields. However, as there is no better option to authenticate the effectiveness of the strain used, the test is to be retained. But the level of 50% increase in dry weight seems to be unrealistic and needs to be revised. Bhattacharyya and Dwivedi (2004) have also expressed similar opinion and has suggested to restrict the increase to only 25%.

Counting of rhizobia by plant infection count - Often questions are raised on the usefulness of serial dilution and plate count method for authentication of total viable count, as it only indicates the total rhizobial population irrespective of the fact that, the organisms present there have the infective potential or not. The evaluation of actual number of infective rhizobia is much more important than the mere plate count. This also becomes necessary, as most of our rhizobial packets pass through some period of high temperature, where the rhizobia are likely to loose their plasmid borne *nod*-genes. Therefore, in case of rhizobia MPN (most probable number) count by plant infection method can be more authentic and reliable over the routine plate count method.

Serological test is impractical - Many strains are used for Rhizobial biofertiliser production by the manufacturers and everyday new strains are added. Availability of specific antiserum to the quality testing personnel for each one is a problem. The production of new specific antibodies can take two months (Wright 1986). Moreover, the test has it's limitation, due to the change in growth or storage condition, antigen of an inoculum may also change leading to loss or variation in specificity of antibody (Tas *et al* 1995). In such a scenario, the responsibility of making available the specific antisera must lie either with the manufacturer (Bhattacharyya and Dwivedi 2004) or with the strain supplying agencies.

Why serological tests, when rabbit killing is not permitted? - Rabbits are not at all killed for antisera production. Accidental injury in handling of rabbits during injection, titer bleeding or blood collection by marginal ear vein puncture (Somasegaran and Hoben 1985) can be avoided easily with a little bit of care. Of course, animal welfare/comfort should be given due consideration.

No serological test for *Azotobacter/Azospirillum*, Why ? - Because, antiserum of a particular strain of *Azotobacter/Azospirillum* often gives positive agglutination reaction with other strains also.

Is it necessary to introduce immuno-fluorescence test to detect contaminants during fermentation – Contaminants are biggest hurdles in quality production of biofertilisers. Immunofluorescence test by using FITC conjugated specific antiserum provides an excellent tool for quick detection of contaminants during fermentation. This should be included in the guidelines of BIS specifications meant for producers. Strain supplying agencies can undertake the production and supply of such specific antiserums on actual cost basis and each manufacturer should be asked to carry out such tests with the help of

specified antiserum on routine basis. Bhattacharyya and Dwivedi (2004) have also stressed the need for inclusion of this test for detection of contaminants.

CFU/N-estimation/Pigmentation-which one is best for *Azotobacter* - The three parameters i.e. CFU (Colony Forming Units), N-estimation and pigmentation are indicators of three different traits of BF packets. The CFU is important, because it indicates the level of bacterial loads in packets. The N-estimation indicates the N-fixing potential of the strains used in the BF packets. However, the author feels that estimation of nitrogen (fixed in laboratory grown culture) of very small quantity, by 'Kjeldahl method' does not give satisfactory results. Because, cumulative percentage of error from each step involved in the method from weighing /measuring of wet sample to end point in titration is too high to ignore. Therefore the existing Kjeldahal method be replaced with some highly reliable and accurate method. Use of ARA (Acetylen reduction assay) should be considered for evaluation in place of N-estimation by Kjeldahl method. Pigmentation is the indicator of species nature (Tchan 1984). Thus, CFU coupled with pigmentation is reasonably reliable indicator for detection of *Azotobacter* in packets (rapid test).

For PSB, 10 mm clearing zone is unrealistic - The specified 10 mm clearing zone is not unrealistic. But the conditions/protocol must be specified in minute details for this parameter to test phosphate solubilizing biofertilisers. Because same strain can produce different sizes of clearing zones in different laboratories. A little change in media composition, temperature range, humidity (inside incubator), water quality (if not distilled), brand of chemicals (particularly, TCP and sugar), initial pH of media etc influence the acid secretion by the bacteria, thus, may change the size of clearing zone. Bhattacharyya and Dwivedi (2004) are also of similar views and suggested for necessary modification. In my opinion the clearing zone test should be replaced with total P-solubilization and estimation by spectrophotometer.

Biofertilisers can't be controlled by legal act - Because some people believe, it is the preparation of living organisms, thus, influenced by various biotic/abiotic factors and can't be controlled. The author feels otherwise. All the legal things on this earth are for human beings who are also living organisms. Nonliving things do not violate or manipulate. Thus, BF also needs legislation, not for the microorganisms used in it, but for those who are manufacturing it (Yadav and Raychaudhuri 2004, Bhattacharyya and Dwivedi 2004). The realistic modalities and statutory requirements need to be formulated properly, as it exists in many countries (Hiltbold *et al* 1980, Date 1969, Rennie 1993).

Liquid inoculums have no specification - There is no specified standards for liquid inoculum (L.I) products by BIS. Nowadays L.I. products are fast gaining popularity among producers/users because of it's cosmetic appearance, reduced volume, cleanliness and user friendliness (CRIDA 2003, Rupela *et al* 2004). This is a desirable trend because:

- The whole system of L.I production is to be sterile
- Easier for quality control agencies/laboratories to test.

How effective the superstrains/ supercultures are ? - These are mostly publicity gimmick, a part of marketing strategy. However, some of such super products may contain (externally added) growth promoting chemicals to ensure crop response which is totally against the philosophy of biofertilisation technology. The recommended tests under BIS guidelines do not include any test to detect such chemical/biochemical adulteration.

Prior approval should be compulsory for new BF product - Because consumers are not aware of their rights, consumer protection lobby is weak and violators are many. In this condition, approval of any new product before launching by a competent authority (Bhattacharyya and Dwivedi 2004) will put some sense of confidence among the users and will be encouraged to use biofertilisers. Moreover, there is widespread apprehension about genetically engineered organisms all over the world, which must be properly assessed before release in the nature.

Multistrain vs single strain BF - Multistrain BF products should not be encouraged unless the quality control enforcement comes into play with proper modification of the present BIS standards. Because such products give one more shot in the arms of spurious manufacturers.

There are many more questions and issues, but discussing all of them require many more pages which is beyond the scope of this article. However, most of the doubts and confusions are cropped up from two basic drawbacks in the production systems rather than the BIS's specifications which require appropriate attention.

Biofertilizers production technology

Most of the difficulties in testing the quality of biofertilisers as per the BIS specifications lies with the non-sterile system of production. The problem is further compounded with the BIS's contaminant-allowance of upto 10^5 g^{-1} carrier (Hegde 2002). Ascertaining the level of rhizobia in presence of high contaminant level is difficult by the conventional plate count methods (Tas Eva *et al* 1995). The nodulation test is also time consuming. The *Azotobacter/Azospirillum* packets (where selective media are used) can be tested with some degree of presumption with pigmentation test, pellicle formation, growth in N-free media fare rapidly, with microscopic aids but those are also not confirmatory. Under the circumstances if all the production units follow (by legislation or voluntarily) sterile system of production, automatically, the quality of the products shall improve and will be advantageous for the quality control agency for faster monitoring of finished products.

Source of strains

Biofertilizer units use variety of strains publicizing special attributes, whose history, actual performance and even source are unknown. Thus, the

consequence is frequent failure of the product in the field. The large number of strains pose difficulties for the quality control agencies to ascertain the level and identity of unknown claimed strains in the packets. To verify the authenticity of claimed strain becomes very difficult due to non availability of respective specific monoclonal antibody. However, serological authentication is also sometimes neither a practicable (Bhattacharyya and Dwivedi 2004) nor a full-proof method (Tas Eva *et a*l 1995). To overcome the difficulty it should be made mandatory for all the production units, to use properly tested and recommended strains. One neutral body must be entrusted with the responsibility to collect strains from all sources, cross-check the claim, catalogue, maintain and supply on demand. At the same time, BIS specification should include confirmation of strains by at least one of the modern biotechnological applications like RFLP, protein profile analysis, molecular finger printing or fatty acid methyl ester (FAME) profile.

Immediate measures

While developing the system of single source supply of properly documented / evaluated strains to the manufactures may take some time, but compulsory adoption of fully sterile system of production should not be delayed. National Conference on Quality Control of Biofertilisers in its recommendations has also listed immediate and long term initiatives (Bhattacharyya and Dwivedi 2004). In my opinion following measures need immediate attention and speedy implementation.

1. BF legislation is absolutely necessary, particularly when organic farming is growing to occupy a large place in Indian agriculture.
2. Proper mechanism needs to be developed for random sampling of biofertiliser packets at manufacturers, retailers and users end.
3. The responsibility of supplying the specific antiserum to the testing laboratory must be entrusted to the strain supplying agencies.
4. No multi-strain biofertiliser should be allowed.
5. The BIS standards need modification, particularly on contaminant allowance, authenticity of strains, testing parameters, and protocols for different formulations like liquid, granular etc.
6. Strain identity must be declared on packets along with its efficiency and expected total viable count.
7. Manufacturers can also print a quality guarantee on the lines of Australian manufacturers, where every inoculant packet is printed with "A sample packet of this batch of which this packet belongs, has been tested by AICRS and has been found to be of appropriate quality".

In recent years, with growing concern for health, environment and food quality, organic agriculture is growing very fast worldwide. India is also no exception. Biofertiliser being an important component of both the integrated and organic agriculture, the whole 'biofertiliser affair' needs to be properly organized. That will help in rebuilding the consumer's confidence on biofertiliser product and contribute to

the productivity of Indian agriculture, particularly, to the organic movement in India.

References

Anonymous 1986 Indian Standards for *Rhizobium* Inoculants. IS-8268, 1986.

Bagyaraj, D.J., A.N. Balakrishna and C.V. Suvarna 2000 Quality control and constraints in biofertiliser Technology. In *Current Status of Biofertiliser Technology and its Promotion in Karnataka, Tamilnadu, Kerala and Pondicherry*. Ed. R.N. Bisoyi, Publ. RBDC, Banglore. pp 60-63

Bhattacharyya, P. 2000 Status of Quality Control : Critical Review. In *Current Status of Biofertiliser Technology and its Promotion in Karnataka*, Tamilnadu, Kerala and Pondicherry. Ed. R.N. Bisoyi, Publ. RBDC, Banglore. pp 64-70

Bhattacharyya, P. and Vandana Dwivedi 2004 *Proc. Nat. Conf. on Quality Control of Biofertilisers*. 14-15 January, 2004. NBDC, Ghaziabad Publ. p-145

CRIDA 2003 Liquid *Rhizobium* Inoculant for improved shelf life. In Rainfed Agro Ecosytem (of NATP). Annual Report 2002-03. CRIDA, Santosh Nagar, Hyderabad. pp 60-61

Date, R.A. 1969 A decade of inoculant quality control in Australia. **J. Aust. Inst. Agric. Sci. 35** : 27-37.

Dwivedi, V and P. Bhattacharyya 2004 Production and consumption of biofertilisers in India : Current scenario. In *Proc. Nat. Conf. on Quality Control of Biofertilisers*. Eds P. Bhattacharyya and V. Dwivedi, 14-15 January, 2004. NBDC, Ghaziabad Publ. pp 22-33

Eaglesham, Allan, R. J. 1989 Global importance of *Rhizobium* as an inoculant. In *Microbial Inoculation of Crop Plants*. Eds. R. Campbell and R. M. Macdonald, IRL Press, Oxford, New Work, pp 29-48.

Hegde, S.V. 2002 Liquid biofertilizer in Indian agriculture. **Biofertilizer Newsletter 10(2)** : 17-22

Hiltbold, A.E., D.I. Thuflow and H.D. Skipper 1980 Evaluation of commercial soybean inoculants by various techniques. **Agron. J. 72** : 675-681.

Kumar, K. 1997 Standardization of *Rhizobium* inoculants. The Indian Scenario In *Extending Nitrogen Fixation Research to Farmer's Fields*. Eds. O.P. Rupela, C. Johansen and D.F. Herridge. ICRISAT, Pantancheru, India. pp 133-138.

Nutman, P.S. 1976 Field experiments on nitrogen fixation by nodulated legumes. In : *Symbiotic Nitrogen Fixation in Plants*. Ed. P.S. Nutman Cambridge Univ. Press, Cambridge.

Peoples, M.B., D.F. Herridge and J.K. Ladha 1995 Biological nitrogen fixation : an efficient source of nitrogen for sustainable agricultural production. **Plant Soil 98** : 183-194.

Rennie, R.J. and R.K. Hynes. 1993 Scientific and legislative quality control of legume inoculants for lentil and field pea**. J. Prod. Agric**. **6** : 569-574.

Rupela, O.P. 2004 Production and quality control of microbial

inoculants : Some perspectives. In *Proc. Nat. Conf. on Quality Control of Biofertilisers*. Eds. P. Bhattacharyya and V. Dwivedi, 14-15 January, 2004. NBDC, Ghaziabad Publ. pp 8-21

Somasegaran, P and H. J. Hoben 1985 *Handbook of Rhizobia. Methods in Legume-Rhizobia Technology*. Springer Verlag. pp 450.

Tas Eva, Aimo Saano, Petri Leinonen and Christina Lindstrom 1995 Identification of *Rhizobium* spp in peat-based inoculants by DNA hybridization and PCR and it's application in inoculant quality control. **Appl. Environ. Microbiol. 61(5)** : 1822-1827.

Tchan, Yao-Tseng 1984 Family II. Azotobacteraceae Pribram 1933, 5^{AL}. In Noel R. Kreig and John G Holt (eds) *Bergey's Manual of Systematic Bacteriology*, Vol 1.. Williams and Wilkins. pp. 227-234.

Thompson, J. A. 1999 An outsider's view of the legume inoculant production scene in India. In *Use and Development of Microbial Inoculants*. Eds. T Singh, , A. K. Yadav, and S. Raychaudhuri, NER Conf on Biofertilisers, 22-23 January, 2003, RBDC, Imphal Publ. pp 53-80

Wright , S. F., J. G. Foster and O. L. Bennett 1986 Production and use of monoclonal antibodies for identification of *Rhizobiam trifolii*. **Appl. Environ. Microbiol. 52** : 119-123.

Yadav, A.K. and S. Raychaudhuri 2004. Biofertilisers as agro inputs - Past present and future perspectives. In *Biotechnology in Sustainable and Organic Farming*. Eds. A.K. Yadav, S. Raychaudhuri and N.C. Talukdar. Shree Publishers and Distributor, New Delhi. pp 1-21.

OBITUARY

Dr. Mangesh Kumar Tyagi, Assistant Microbiologist at Regional Biofertiliser Development Centre, Imphal passed away on 19th May 2004 after a brief period of illness. The entire National Biofertiliser Development Centre and Regional Biofertiliser Development Centre family deeply condole the untimely demise of Dr. Tyagi and pray to the almightly for the peace of the departed soul. May the god give enough courage to Mrs. Alka Tyagi and her two kids to bear this irreparable loss.

Abstract Proceedings of National Conference on Quality Control of Biofertilisers

To assess the current quality control scenario in the country and to concretize the expert opinion on quality improvement, a two day's National Conference on "Quality Control of Biofertilisers" was organized by the National Biofertiliser Development Centre, Ghaziabad, during 14-15 January 2004. The conference was sponsored by the Department of Agriculture and Cooperation, Government of India and was attended by nearly two hundred participants from Ministry of Agriculture, Govt. of India, ICAR, DBT, BIS, NIC, State Agricultural Universities, Central/State Govt officers, Biofertiliser Production Units, International/National Research Institutions, Fertiliser Association of India, NGOs and some progressive farmers.

The conference was inaugurated by Shri Satish Chander, Joint Secretary (INM), Ministry of Agriculture, Govt. of India, New Delhi with Shri M.K.S. Sundaram, Special Secretary (Agril), Govt of U.P. as Guest of Honour. Dr. Y.D. Gaur, Scientist Emeritus, Div. of Microbiology, IARI New Delhi delivered keynote address entitled "*Biofertiliser in India: Strength and Technological Constraints*". The website of National Project on Development and Use of Biofertilisers and a exhibition of different biofertiliser products and publications was also inaugurated by Shri Satish Chander, J.S.(INM). To commemorate the occasion a Souvenir and a Book of Abstracts was released by Dr. J.S. Mann, Additional Commissioner (INM), Ministry of Agriculture and Shri H.K.L. Mehta, ADG, CPWD respectively.

Shri Satish Chander, JS (INM) in his inaugural address emphasized that he is worried for the future of biofertilisers because of its poor consumption and poor quality. He stressed that it is a challenge to regenerate the faith of farmers towards the use of biofertilisers and only the quality assurance can resolve this issue. He said that BIS specifications of *Rhizobium*, *Azotobacter*, *Azospirillum* and PSB are available but these are not sufficient to control the quality. There needs appropriate regulatory measures, for which it is necessary to examine all available acts like Fertiliser Control Order (FCO), BIS Act, Weights and Measures Act etc. This situation calls for a national dialogue to evolve an action plan which is acceptable to all concerns. He emphasized that a sincere cooperation and collaboration between the Industries and Government is necessary to uproot the menace from the trade. He also urged for upgrading the technology for biofertiliser production.

Different aspects of biofertiliser production, promotion and quality control were discussed in five technical sessions spread over two days. Details of presentations and deliberations made

under different technical sessions are as follows:

Technical session – I

1. Government Contribution on Production and Promotion of Biofertilisers in India. Dr. P. Bhattacharyya, Director, NBDC.
2. Production and quality control of microbial inoculants. Dr. O.P. Rupela, ICRISAT, Hyderabad.
3. Production and Consumption of Biofertilisers in India. Dr. Vandana Dwivedi, Regional Director, NBDC
4. Marketing Avenues for Biofertiliser – constraints and strategies. Dr. P.K. Awasthi, Marketing Director, KRIBHCO.
5. Challenges before Biofertiliser Industry. Dr. Alok Dwivedi, DGM, NAFED Biofertiliser, Indore.

Technical Session – II

1. Commercial Progress in Biofertiliser Research in India. Dr. B.D. Kaushik, Head, Microbiology Div., IARI, New Delhi.
2. New Trends in Biofertiliser Production Technology. Dr. R.N. Bisoyi, Regional Director, RBDC, Bhubaneshwar.
3. VA Mycorrhiza Production Technology and Quality Control. Dr. Alok Adholeya, TERI, New Delhi.
4. Potentialities of Liquid Biofertilisers in India. Sh. Ravi Kanitkar, K. Krishimitra, Pune.
5. Comparative Performance of Liquid and Carrier Based *Rhizobium* Biofertilisers in India. Dr. S.V. Hegde, Professor (Retd), UAS, Bangalore.
6. Quality Control of Biofertiliser with Reference to Liquid Formulations for Sustained Production. Dr. V.N. Tiwari, Assoc. Prof. CSAUA&T, Kanpur.

Technical Session III

1. Experience in testing the quality of biofertilisers in India. Dr. M.C. Kabi, Professor, BCKVV, Kalyani
2. Quality Control Package of Different Biofertilisers: Current and Future Perspectives. Dr. A.K. Yadav, Regional Director, RBDC, Nagpur.
3. Impact of Production Technology on Biofertiliser Quality. Dr. N.P. Shukla, Project Director, MPCST, Bhopal.
4. Formulation of Regulatory Mechanism for Ensuring Quality of Biofertilisers – A Big Challenge. Dr. N. Tripathy, Director, CFQC&TI, Faridabad.
5. Role of BIS on Biofertiliser Standards. Dr. Sudhir Rahi, BIS, New Delhi.

Technical Session IV

1. Need of Adequate Extension Work for Biofertiliser Promotion. Dr. Krishan Chandra, Regional Director, RBDC, Bangalore.
2. Training and Demonstration – Important Tools of Biofertiliser Promotion. Dr. D. Kumar, TES, NBDC
3. Impact of Biofertiliser Award on Motivation. Dr. B.C. Biswas, Addl. Director, FAI, New Delhi.

Technical Session V

This technical session was an interactive session conducted by Dr. D.L.N. Rao, Project Coordinator, AICRP-BNF, IISS, Bhopal and Dr. A.K. Yadav. 21 representatives of different production units, scientists and Director and Regional Director of NBDC expressed their views. Some of the

common views and concerns are as follows:

1. Production technology needs to be upgraded from unsterile to sterile.
2. Mushrooming growth of ill-equipped production units be stopped.
3. Biofertiliser production units should be compulsorily registered with some central body and the claims of production units should be supported with adequate field and laboratory data.
4. Every state should have testing laboratory
5. Test parameters should be simple.
6. Serological tests and supply of anti-serum should be the responsibility of strain supplying agency.
7. Subsidy should be withdrawn.
8. Improved fermenters should be brought in
9. Need of training on quality control
10. Needs full automation in production system
11. Publication of testing manual
12. Need of legal act either in the form of FCO or any other act.
13. Need for preparation of BIS specifications for liquid biofertilisers.

Plenary Session

The plenary session was chaired by Dr. N. Tripathy, Director, CFQC&TI and Dr. P. Bhattacharyya, Director, NBDC and was mainly devoted to the recommendations of five technical sessions. Exhaustive recommendations and proposed actions to be taken up by different agencies are being enumerated in subsequent paras.

In the valedictory address, Chairman Dr. N. Tripathy appreciated the efforts made by the organizers and complemented Dr. Bhattacharyya and his team for addressing the emerging issues on quality control of biofertilisers. He emphasized that Government is committed to improve the quality of biofertilisers which calls for coordinated efforts involving all the stakeholders. Dr. Bhattacharyya mentioned that "honesty" from all sides is the simple protocol for quality assurance. The conference ended with the vote of thanks from Dr. Vandana Dwivedi, Regional Director, NBDC, Ghaziabad.

RECOMMENDATIONS OF THE CONFERENCE

1. Instead of replacement to chemical fertilisers, biofertilisers should be promoted as an important component of integrated nutrient supply system.
2. Technical advancements achieved by different research and developmental institutions should be made available to the producers for upgradation of production technology.
3. The term biofertiliser should be defined as "*the product containing carrier based (solid or liquid) living microorganisms which are agriculturally useful in terms of N_2-fixation or P-solubilization etc to increase the productivity of the soil and/crops*". The product not conforming to this definition should not be named as biofertiliser.
4. Specifications and testing protocols should be simple and easy to adopt.
5. The number of strains should be restricted to 1-3 per region and the producers should use only the recommended strains.
6. Shortcomings and mistakes in BIS bulletins should be rectified immediately to avoid confusion.

7. Conference suggested some modifications in quality parameters of BIS specifications. Some of which are as follows:
   - Serological testing in the case of *Rhizobium* should be optional at the product testing stage. It should be made mandatory during authentication of strain at the level of strain supplier.
   - In *Rhizobium* the clause "50% increase in dry weight of treated plants" should be replaced with 25% increase in dry weight of treated plants.
   - Nodulation test should be retained in spite of the fact that it is time consuming.
   - Mesh size of the carrier for all biofertilisers should be uniform.
   - Immuno-fluorescence test can be added to detect contaminants.
   - 10 mm solubilization zone for PSB seems to be unrealistic and should be reduced to 5mm. Spectrophotometric measurement of P-solubilization should be made mandatory.
   - *Aspergillus* and other fungi which can be pathogenic should not be used as P-solubilizers in commercial production.
8. It was also resolved by majority of participants that:
   - There can be separate registration committees of central and state governments.
   - All manufacturers should be registered with DAC/NBDC
   - No new biofertiliser (new microorganism formulation) be allowed without the approval of DAC/NBDC.
   - For registration full details in respect of equipment, facilities, manpower, strains, field performance data etc should be provided to NBDC
   - If a sample fails the retesting should be allowed for testing the same batch in other reference laboratory.
   - NBDC/RBDCs and some state laboratories should be declared as reference laboratories.
   - In every state there should be at least one testing laboratory.
9. No biofertiliser production units be allowed to produce, unless they employ a qualified microbiologist.
10. Biofertiliser should be controlled legally by any act. The control system should be promotive instead of prohibitive.
11. BIS specifications for remaining biofertiliser should be prepared.
12. All production units should be inspected once a year by some competent Government official.
13. Liquid biofertilisers be encouraged and their total viable count should be kept at minimum 10^9/ml.
14. Multi-microorganism/strain biofertilisers should not be allowed unless their suitability is established scientifically.
15. New biofertiliser formulations should be got approved from NBDC and preparations such as –plant extracts, sea weed extracts/ growth regulators etc should not be considered as biofertilisers.
16. Uniform application methodology and publicity material should be brought out by NBDC in consultation with ICAR.
17. Packaging should be improved.

Corporate News

CSR&TI, Berhampore Developed Mulberry Specific Biofertilisers.

Central Sericulture Research and Training Institute has developed two mulberry (*Morus alba* L.) specific biofertilisers under the trade name (i) NITROFERT – *Azotobacter chroococcum* based N_2-fixing biofertiliser and (ii) PHOSPHOFERT – VA mycorrhizal biofertiliser. Both the biofertilisers are being produced on commercial scale since 1999-2000 at annual production capacity of 20-30 and 5-10 MT respectively per annum with a unit sale price of Rs. 25/- per Kg. By applying these biofertilisers at 20 Kg NITROFERT/ha/year and 75 Kg PHOSPHOFERT/ha/4 years along with 50% and 80% reduced levels of N (336 Kg/ha/year) and P (180 Kg/ha/year) respectively a sericulture farmer can achieve an economic gain of 53.3% under irrigated conditions and 45.2% under rainfed conditions. Reduction in fertiliser doses to the tune of 150 Kg N and 50 Kg P/ha/year is also possible under rainfed conditions by supplementing with NITROFERT at 10 Kg/ha/year and PHOSPHOFERT at 40 kg/ha/4 years without any compromise in leaf yield and quality. Up till now this Institute has successfully produced and marketed about 9.5 MT of NITROFERT and 7.1 MT of PHOSPHOFERT to the sericulture farmers. Further both the biofertilisers have also been tested with various other agricultural and vegetables viz. wheat, mustard, onion, potato, tomato, lady finger, cabbage and cauliflower etc. and have shown remarkable improvements in yield and quality.

For experimentation and use in different crops, please contact Director, Sericulture Research and Training Institute (CSR&TI), Central Silk Board, Ministry of Textiles, Govt. of India, Berhampore – 742 101, West Bengal.

(Source – CSR&TI, Berhampore, W. Bengal)

Vasant Dada Sugar Institute's Biofertilisers

Vasant Dada Sugar Institute (VSI) at Manjri (Bk), Distt. Pune, Maharashtra is a R&D Institute devoted to the service of the sugar industry and sugarcane growing farmers. Development and promotion of biofertilisers is one of their thrust areas. The institute has developed necessary technical know-how for the production of *Azotobacter, Azospirillum, Acetobacter, Rhizobium* and phosphate solubilizing biofertilisers. Institute is also producing specific microbial inoculants for the enrichment of biocompost and decomposing culture for composting the sugarcane trash and PMC + spent-wash. *Trichoderma* inoculant for biodegradation of cellulolytic waste is also being produced on demand. All these products are based on VSI technology and supplied to end users at reasonable service charges. VSI's infrastructure for biofertilisers production is of very high standard and is rated as the best in the western region of the country. The production capacity of

the VSI's biofertilizer production unit is 500 tonnes per annum.

The institute also produces very high quality vermicompost for enhancing soil fertility and productivity. The constant use of vermicompost helps to increase organic matter and water holding capacity of the soil. It also helps to buildup microbial population in soil.

Special features of the VSI's organic and biological products are:

- Properly lab tested
- Excellent result oriented
- With optimum efficiency and utility
- Residue free
- Assured quality
- Non-hazardous
- Pollution free
- Biodegradable and
- Swift and effective in action

Other services offered by the VSI are:

Technical know-how – Technical know-how is available for establishment of biofertilisers and vermicompost units.

Microbial analysis – Facilities for microbial analysis of FYM, compost, vermicompost, biofertilisers and soil are available

Supply of mother cultures – Pure and authentic mother cultures are available for production units.

Identification of cultures – VSI undertakes the identification and confirmation of fungal and bacterial cultures for use in different wings of agriculture.

Disease diagnosis – Disease diagnosis service is available for sugarcane growers with their remedial measures and recommendations.

Supply of earthworms – For production of vermicompost, authenticated species of highly effective earthworms are available.

For further details contact Head, Plant Pathology and Agricultural Microbiology, Vasant Dada Sugar Institute, Manjri (Bk), Tal. Haveli, Distt. Pune- 412 307, Maharashtra.

(Source – VSI, Manjri, Pune)

NEW REPORTS AND RESEARCH NEWS

Great Little wonder

Scientists from M.S. Swaminathan Research Foundation (MSSRF) have claimed to have discovered a bacterium which can function as *Rhizobium*-like microorganism for all crops. Moreover the bacterium can also solubilize fixed phosphorus. MSSRF scientist Dr. P. Loganathan says that the properties and characteristics of this bacterium are very ideal for its use as biofertilisers. The bacterium named *Swaminathania salitolerans* was isolated from *Porteresia coarctata* – a wild variety of rice. It was found in the Pichavaram mangrove forest, situated along the Tamilnadu coast. During experiments its inoculation helped a rice variety (*panni*) which is widely used in south India to grow in saline soils. The bacterium belongs to the family Acetobacteraceae under the group Alpha proteobacteria and is highly tolerant to salinity.

(Source - Down to Earth, February 15, 2004 p-27)

Microbial fuel cell that generates electricity

Researchers from US-based Pennsylvania State University have developed an electricity generator that is fuelled by microorganisms feeding on human waste. The device, the "Microbial Fuel cell" (MFC) uses specific types of bacteria under anaerobic conditions to degrade human waste and generate electricity. In any respiratory process the electron released during the reaction, eventually combined with oxygen molecules. By depriving these bacteria of oxygen, the power released in the form of electrons can be used to power an electric circuit. The MFC comprises a 15 cm long 'can' with a central cathode rod that is surrounded by a proton exchange membrane. Eight anode (long slender graphite rods) are arranged around the cathode. Bacteria cluster around the anodes and breakdown the organic waste as it is pumped in, releasing electrons and protons. With no oxygen to help mop up the electrons, the bacterial enzymes transfer them to the anodes, while the protons migrate to the central cathode. Molecules on proton exchange membrane encourage the protons to pass through the cathode. There they combine with oxygen from the air and electrons from the anodes to produce water. During the transfer of electrons from the cathode, a voltage is created, enabling the MFC to power an electric circuit. If scaled up, the system can produce 51 KW of power from the waste of one lakh people.

(Source – Down To Earth April 30, 2004.)

Colonization and growth promotion of non-legumes by *Rhizobium* bacteria.

Rhizobium leguminosarum bv. *trifoli* strain R39 isolated from red clover nodules, in field experiments on loamy sand, stimulated not only the growth of red clover but also the growth of maize, spring wheat, spring barley and oil radish. After seed inoculation, this strain colonized the rhizosphere of these plants and migrated to near by non-inoculated spontaneous weed plants. Following crop harvest, the *Rhizobium* strain was able to re-establish in the rhizosphere of plants

subsequently grown in the same field. Microbiological and electron microscopical investigations on maize and wheat showed that R39 had colonized the rhizoplane of the active growing roots (zone of emerging laterals and root tip mucigel). The production of phytoeffective metabolites by this strain may be important for the stimulation of root growh and nutrient uptake by plants.

(Source – G. Hoflich (1999) Microbial Biosystems : New frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Eds. C.R. Bell, M. Brylinsky and Johnson-Green, Atlantic Canada Society for Microbial Ecology, Halifax, Canada.)

A New Species of *Devosia* That Forms a Unique Nitrogen-Fixing Root-Nodule Symbiosis with the Aquatic Legume *Neptunia natans* (L.f.) Druce

Rhizobia are the common bacterial symbionts that form nitrogen-fixing root nodules in legumes. However, recently other bacteria have been shown to nodulate and fix nitrogen symbiotically with these plants. *Neptunia natans* is an aquatic legume indigenous to tropical and subtropical regions and in African soils is nodulated by *Allorhizobium undicola.* This legume develops an unusual root-nodule symbiosis on floating stems in aquatic environments through a unique infection process. On analysis of low-molecular-weight RNA and 16S ribosomal DNA (rDNA) sequence of similar fast-growing isolates from India that were previously used to define the developmental morphology of the unique infection process in this symbiosis with *N. natans* have established that they are phylogenetically located in the genus *Devosia*, not *Allorhizobium* or *Rhizobium*. The 16S rDNA sequences of these two *Neptunia*-nodulating *Devosia* strains differ from the only species currently described in that genus, *Devosia riboflavina.* From the same isolated colonies, authors located their *nodD* and *nifH* genes involved in nodulation and nitrogen fixation on a plasmid of approximately 170 kb. Sequence analysis showed that their *nodD* and *nifH* genes are most closely related to *nodD* and *nifH* of *Rhizobium tropici*, suggesting that this newly described *Neptunia*-nodulating *Devosia* species may have acquired these symbiotic genes by horizontal transfer.

(Source – Rivas *et al* 2002, Applied and Environmental Microbiology, November 2002, Vol. 68, No. 11 p. 5217-5222)

***Sinorhizobium americanus* sp. nov., a New *Sinorhizobium* Species Nodulating Native *Acacia* spp. in Mexico**

The sinorhizobia isolated from root nodules of *Acacia* species native of Mexico constitute a diverse group of bacteria on the basis of their metabolic enzyme electromorphs but share restriction patterns of the PCR products of 16S rRNA genes and a common 500 kb symbiotic plasmid. They are distinguished from other *Sinorhizobium* species by their levels of DNA-DNA hybridization and the sequence of 16S rRNA and *nifH* genes. *nolR* gene hybridization patterns were found useful to identify sinorhizobia and characterize species. A new species, *Sinorhizobium americanus*, is described and the type strain is CFNEI 156 from *Acacia acatlensis*.

(Source - Toledo *et al* 2003, Systematic and Applied Microbiology, January 2003, vol. 26, no. 1, pp. 54-64(11)

New Rhizobial species

Rhizobia a term popularly refers to all those symbiotic bacterial species which form N_2-fixing nodule with legumes and non-legumes. Initially the group was comprised of one genus *Rhizobium*, but now the group comprise of many genera and species broadly distributed in domain bacteria at distinct phylogenetic locations. In BFNL Vol 10 No. 2 (pp 26-30) Yadav and Raychaudhuri (2002), gave an exhaustive account of recent advances in phylogeny and taxonomy of Rhizobia. In this account all validly published species up to Dec. 2001 were included. After that few more species have been described and published. As per the current taxonomy of rhizobia updated till 9th June 2004, few more species have been added. These are as follows:

Genus – *Rhizobium*
Rhizobium indigoferae
Rhizobium loessense

Genus – Sinorhizobium
Sinorhizobium kummerowiae
Sinorhizobium morelense

Genus - *Bradyrhizobium*
Bradyrhizobium yuanmingense

Other Rhizobia
Methylobacterium nodulans
Burkholderia tuberum (STM678)
Burkholderia phymatum (STM815)
Ralstonia taiwanensis
Devosia neptuniae
Blastobacter denitrificans

(Source – List of bacterial names with standing in nomenclature (LSBN-2004), http://www.bacterio.cict.fr./ and http://www.rhizobia.co.nz/)

A new species of *Bradyrhizobium* proposed

Some varieties of *Beta vulgaris* cultivated in North Spain present great deformations that resemble the tumours produced by *Agrobacterium* species. During a study to try the isolation of the agent responsible for these deformations, authors of the present study isolated several endophytic slow-growing bacterial strains whose macroscopic morphology resemble that of *Bradyrhizobium* spp. These strains were not able to produce tumours in *Nicotiana tobacum* plants and according to the phylogenetic analysis of 16S rRNA, they are closely related to *Bradyrhizobium*. The phenotypic and molecular characteristics of these strain showed that they are different from all the species of *Bradyrhizobium* already described. Sequence analysis of the 16S rDNA intergenric spacer region indicated that these new strains form a homogenous group, related to *B. japonicum*, *B. liaoningense* and *B. yuamnigense*. The result of DNA-DNA hybridization confirmed that they are new species of *Bradyrhizobium* for which the authors have propose the name *Bradyrhizobium betae* sp. nov. Type strain is PL7HG1^T (LMG 21987^T, CECT 5829^T).

(Source Rivas *et al* Int. J. Syst. Evol. Microbiol., 2004, **54**, 1271-1275)

A new endophytic nitrogen-fixing bacterium *Pantoea* sp. isolated from sugarcane

Two N_2-fixing isolates, 9C and T2, obtained from surface-sterilized stems and roots, respectively, of sugarcane variety ML3-18 by the authors. Both isolates showed acetylene reduction and H_2 production in nitrogen-free media. Nitrogenase activity measured by H_2 production was about 15 times higher for isolate 9C than for T2 or for *Gluconacetobacter diazotrophicus* (PAL-5 standard strain, ATCC 49037). The *nifH* gene segment was amplified from both isolates using specific primers. Classification of both T2 and 9C was made on the basis of morphological, biochemical, PCR tests and 16S rDNA sequence analysis. Isolate 9C was identified as a *Pantoea* species from its 16S rDNA, but showed considerable differences in physiological properties from previously reported species of this genus. For example, 9C can be cultured over a wide range of temperature, pH and salt concentration, and showed high H_2 production (up to 67·7 nmol H_2 h^{-1} 10^{10} $cell^{-1}$). Isolate T2 was a strain of *Gluconacetobacter diazotrophicus.* This N_2-fixing endophyte, i.e. *Pantoea*, was able to produce H_2 and to grow in a wide range of conditions, may well be valuable for agriculture.

(Source Loiret *et al* Journal of Applied Microbiology, Online Early, accessed on 28 June 2004)

***Paenibacillus brasilensis* sp. nov., a novel nitrogen-fixing species isolated from the maize rhizosphere in Brazil**

Sixteen nitrogen-fixing strains isolated from the rhizosphere of maize planted in Cerrado soil, Brazil, which showed morphological and biochemical characteristics similar to the gas-forming *Paenibacillus* spp., were phenotypically and genetically characterized. Their identification as members of the genus *Paenibacillus* was confirmed by using specific primers based on the 16S rRNA gene. SDS-PAGE of whole-cell proteins, API 50CH, morphological and biochemical tests, amplified rDNA-restriction analysis (ARDRA), DNA-relatedness analyses, denaturing-gradient gel electrophoresis (DGGE) and 16S rRNA gene sequence determinations were performed to characterize the novel isolates and to compare them to strains of other nitrogen-fixing *Paenibacillus* spp. Phenotypic analyses showed that the 16 strains were very homogeneous and shared a high level of relatedness with *Paenibacillus polymyxa* and *Paenibacillus peoriae*. However, none of the novel isolates was able to ferment glycerol (positive test for *P. polymyxa*), L-arabinose or D-xylose (positive tests for *P. polymyxa* and *P. peoriae*) or utilize succinate (positive test for *P. peoriae*). Genetic approaches also indicated a high level of similarity among the novel isolates and *P. polymyxa* and *P. peoriae*, but the novel strains clearly could not be assigned to either of these two recognized species. On the basis of the features presented in this study, the 16 novel isolates were considered to represent members of a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus brasilensis* is proposed. The type strain is PB172(T) (=ATCC BAA-413(T)=DSM 14914(T)

(Source - von der Weid, *et al* 2002 Int. J. Syst Evol. Microbiol 52 : 2147-2153)

News on Conference, Seminar, Symposia, Fairs and Training Courses

6TH European Nitrogen Fixation Conference on Biological Nitrogen Fixation (BNF) is being organized at Toulouse, France during 24-27 July, 2004. The Organizing committee members consists of Dr. Julie Cullimore, Frans de Boruion, Jean Denarie, Thierry Huguest, Daniel Kahn and Andre Jrigalet. Further details can be down loaded from their webside http://www.toulouse.inra.fr/benfc

The 3rd International Nitrogen Conference is scheduled for 12-16 October, 2004 at Nanjing, China. The theme of the conference is "*Impacts of Population Growth and Economic Development on the Nitrogen Cycle: Consequences and Mitigation at Local, Regional and Global Scales*". For further details contact Dr. Zhengqin Xiong, Ms. Huilin Li, Ms Lili Zhu, P.O.Box 821, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China, Tel: +86-25-6881019, +86-25-6881028, Fax: +86-25-6881028, E_mail: n2004@ns.issas.ac.cn, Website: http://www.issas.ac.cn/

The 14th International Conference on Nitrogen Fixation will be held from October 28 to November 1, 2004 at Beijing International Convention Centre, Beijing, P.R. China. Contact persons – Prof. Yiping Wang, College of Life Sciences, Peking University, Beijing 100 871, P.R. China, Tel. 86-10- 6274 8490. Further details and updated information can be downloaded from http://ww.n2ix.com

22nd Latin-American Conference on Rhizobiology & 1st Brazilian Conference on Biological Nitrogen Fixation is scheduled for 13th to 15th September, 2004 at the Hotel Montanhês, situated in Miguel Pereira, 110 km from the city of Rio de Janeiro. The organizing committee comprise of Veronica Massena Reis (Presidente), Norma, Rumjaneck , Rosangela Stralioto , Sérgio Miana de Faria and Segundo Urquiaga. For further details contact Embrapa Agrobiologia, Área de Comunicação e Negócios, Tel: (0xx21) 2682-1500 r: 237 / 245, E-mail: sac@cnpab.embrapa.br

19th North American Symbiotic Nitrogen Fixation Conference – was scheduled for June 27 – July 1, 2004 at Montana State University, Bozeman, Montana. For further details contact Sabra Slade at 406-994-3083 0r sslade@montana.edu.

Agriculture International Exhibition (AGRI INTEX 2004) will be organized during 20-25 August, 2004 at Coimbatore, India. The Exhibition is being organized by the Coimbatore District Small Scale Industries Association (CODISSA) and supported by Tamil Nadu Agricultural University, Coimbatore. Further details can be down loaded from www.industrialtradefair.com and

www.agriintex.com. Email cointec.biofestival.com

New Bio Festival 2004, AgBio & You is proposed for 10-11 August, 2004, at Melbourne Convention Centre, Victoria, Australia. For further details log on to www.biofestival.com.

Agricultural Biotechnology International Conference (ABIC 2004) will be held during 12-15th September, 2004 at Cologne, Germany. For more details contact www.abic2004.org/index.html

Australian Society for Microbiologists Annual scientific meeting 2004 will be organized during 26 September to 1st October, 2004 at Sydney Superdome, Sydney, NSW, Australia. For more details log www.asm2004-org.

Com-Bio 2004 Annual scientific meeting of the Australian Society of Biochemistry and Molecular Biology will be organized during 26-30th September, 2004 at perth, WA-Australia. For more details see www.asbmb.org.au/combio2004-07-22

Bio Japan 2004 will be held during 28-30th September, 2004 at New Takanawa Prince Hotel, Shingawa, Tokyo, Japan. For more details see www.expo.nikkeibp.co.jp/biojapan/2004/eng./

Training Course on Bio and Organic inputs in Sugarcane cultivation – A two days National Training Programme on Bio and Organic Inputs was organized by Vasant Dada Sugar Institute, Bk., Manjri, Distt. Pune, India during 20-22nd July, 2004. The programme was attended by more than 20 participants from 7 different states. The programme was sponsored by the Ministry of Agriculture, Govt. of India.

Organic Agri Fair 2004. – Organic Agriculture Scientific Society for Integrated Services (OASIS), Coimbatore and Indian Society for Certification of Organic Products (ISCOP), Coimbatore jointly organized an Organic Agri Fair, 2004 on 12-13th June, 2004 at Dr. T.V. Sivanandha Memorial Hall No. 9/7, 10th Street, Tatabad, Coimbatore. During the fair a seminar on Organic Agriculture was inaugurated on 13.06.04 by Dr. Ramaswamy, Vice Chancellor, Tamil Nadu Agricultural University, Coimbatore. During the fair wide variety of organic and biological inputs and organically grown products were displayed. The fair and seminar was participated by large number of Scientists, Government officials and farmers.

(Information collected and compiled by RBDC Bangalore and RBDC, Nagpur)

Book Review

Proceedings of National Conference on Quality Control of Biofertilisers, 14-15 January, 2004 Edited by P. Bhattacharyya and Vandana Dwivedi, National Biofertiliser Development Centre, Ghaziabad, India. pp 154.

With an aim to provide an ideal forum to Government officials, scientists and entrepreneurs to interact on quality concerns on biofertilisers and suggest possible ways for effective implementation of quality protocols a National Conference on Quality Control of Biofertilisers was organized on 14 – 15 January, 2004 at NBDC, Ghaziabad. The present compilation is a collection of full length papers presented during the conference and contributed by the experts from Central/State Governments, research organizations and biofertiliser production units alongwith a summarized proceedings of the conference and recommendations.

19 chapters are pertaining to Government contribution, production, consumption, marketing avenues, commercialization, new trends, new formulations, quality control parameters, BIS standards, laboratory protocols for quality testing and formulation of regulatory mechanism for effective promotion of different biofertilisers. Subsequent three chapters are devoted to the resume of the National Conference, recommendations of the conference and technical programme of the National Conference.

Chapter on production and quality control of microbial inoculants provide an in-depth analysis on *Rhizobium* inoculant's production, production technology, carrier materials, liquid formulations, quality deterioration in transit and overtime and on regulations for promoting inoculant use. Chapter on "Current scenario on production and consumption of biofertilisers in India" presents a balanced view of total potential, current scenario of production, consumption and distribution of production units with their installed production capacity and actual production in different states of the union. Chapter on commercial progress of biofertiliser's research and new trends in biofertiliser production technology summarizes recent developments. Potentiality, usefulness and quality control of liquid inoculants have also been assessed and described in few chapters. Chapter on experience and testing the quality of biofertilisers presents a country wide real picture, assessed over 20 years on the quality scenario of *Rhizobium* and *Azotobacter* biofertilisers. In chapter entitled Quality control package of different biofertilisers, current scenario on quality control front, BIS standards vis-à-vis standards of other countries, strategies for successful application of quality control regime in India and emerging trends and future prospects have been discussed. Impact of production technology on biofertiliser quality and formulation of regulatory mechanism for ensuring quality of biofertilisers have been specifically described in detail.

In brief, it can be stated that the compilation provides excellent information not only on the current scenario and status of production, consumption and quality control of biofertilisers in the country but also provides an in-depth analysis of various factors leading to substandard quality and suggests various remedial measures to overcome the short comings. Special emphasis has been made on the improvement of product formulations, promotion of liquid inoculants, improvement in quality parameters, modification in BIS standards and up-gradation of production technology.

(The book is a publication of NBDC, Ghaziabad and is available free of cost on demand to research institutions, libraries, Central/State Govt. Departments and laboratories, scientists associated with the science and application of biofertilisers and to biofertiliser production units). **(AKY)**

Biotechnology in Sustainable and Organic Farming – Scope and Potential, Edited by A.K. Yadav, S. Raychaudhuri and N.C. Talukdar. Shree Publishers and Distributors, 20 Ansari Road, Daryaganj, New Delhi-110 002. Cost Rs. 1,500/-, pp 339. Biotechnological harvesting of microbial potential in nutrient mobilization and plant protection is fast coming up as one of the widely acclaimed technology, suiting to both the requirements of optimum production and environment sustainability. Use of microbial systems for nutrient mobilization, popularly known as biofertilisers are getting popular day by day and new and new systems are being introduced to meet our requirement of different crops and under different cropping systems. In spite of great volume of literature on different aspects of this biotechnology in various national and international journals and in some books, the editors felt the paucity of review on specified topics and organisms in one volume. The book "*Biotechnology in Sustainable and Organic Farming – Scope and Potential*" has two sections. The first section consists of review articles (chapter 1 to 12) contributed by the eminent Indian scientists having considerable national and international reputation with many years of research, teaching and extension experience in the field. In these review articles an attempt has been made to give up-to-date information on various aspects of microbes and microbial systems being exploited for crop production. These articles include compilation of research on bacterial systematics, biology, biochemistry and biotechnology of plant-soil-microbe system. It also includes the present status of inoculant industry, it's statistics, problems and scope.

The second section (chapter 13 to 39) comprised of research papers presented by the scientists from various academic institutions, research organizations and technology disseminating institutions in the II NER Conference on Biofertilisers. The conference held during 22-24 January, 2001 at Assam Agricultural University, Jorhat, Assam was sponsored by the Deptt. of Biotechnology, Govt of India, New Delhi and Soil Conservation Society of India, New Delhi. The Conference was jointly organized by the Regional Biofertiliser Development Centre, Imphal and Assam Agricultural University, Jorhat, Assam. **(PB)**

Biofertiliser production scenario during the year 2003-04

(Based on the information submitted by production units to NBDC)

S.No.	States	Total No. of units	Installed production capacity	Actual production	Capacity utilization %
1.	Andhra Pradesh	10	1500	1120.58	74.7
2.	Arunachal Pradesh	1	75	-	0.00
3.	Assam	5	375	248.52	66.27
4.	Bihar	3	300	NA	NA
5.	Delhi	3	151	-	0.00
6.	Gujrat	5	900	473.38	52.65
7.	Haryana	2	99	7.10	7.17
8.	Himachal Pradesh	1	75	5.27	7.02
9.	Jharkhand	2	75	5.40	7.2
10	Karnataka	19	2210	848.58	38.39
11.	Kerala	6	425	370.68	87.21
12.	Madhya Pradesh	12	1475	632.86	42.9
13.	Maharashtra	37	3218	2189.73	68.04
14.	Meghalaya	1	75	-	-
15.	Mizoram	1	75	-	-
16.	Nagaland	1	150	8.12	5.41
17.	Orissa	10	600	61.36	10.22
18.	Pondicherry	3	150	80.32	53.54
19	Punjab	1	2	-	-
20	Rajasthan	3	750	381.79	50.90
21.	Tamilnadu	30	4575	4074.02	89.04
22.	Uttar Pradesh	8	375	149.48	39.86
23.	West Bengal	14	715	235.61	32.95
	Total	178	18,345	10,892.8	59.38

List of Biofertiliser Production Units in India

Arunachal Pradesh
Jolang Multipurpopse Cooperative Soc.
Vil. Jolang, Itanagar

Assam
Biofertiliser Laboratory
Assam Agricultural University
Jorhat

Assam Agro Industries Corpn. Ltd.
Khanapara, Guwahati

Hindustan Fertiliser Corpn. Ltd.
Namrup, Distt Dibrugarh

North East Green Tech Pvt. Ltd,
Bamunimaidan, Guwahati

Andhra Pradesh
Shakti Biotech Pvt. Ltd.
Vijayawada

Krishna Agro Bioproducts
Nacharam, Hyderabad

Bacterial Culture Production Lab.
Regional Soil Testing Laboratory
Rajendra Nagar, Hyderabad

K.N. Biotech
Hyderabad

Madras Fertilisers Ltd
Kondapally

Bihar
Hindustan Fertiliser Corpn. Ltd.
Begusarai

Pyrites, Phosphates and Chemicals Ltd
Rohta

Sone Command Development Agency,
Patna

Delhi
Myodelphia Chemicals Corpn. Pvt. Ltd.
Delhi

Microbiology Division
Indian Agricultural Research Institute,
New Delhi

IFFCO
New Delhi

Gujrat
Gujrat Agricultural University
National Agricultural Research Project,
Anand

Gujrat State Cooperative Marketing Fed.
Ltd., Ahmedabad

Gujrat State Fertilisers and Chemicals
Ltd., Vadodra

Krishak Bharti Cooperative Ltd.
Kribhco Nagar, Surat

Haryana
Ganpati Bio-Organic Ltd.
Jind

Biofertiliser Laboratory
CCS Haryana Agricultural University
Hisar

Himachal Pradesh
Central Laboratory
Shimla

Jharkhand
Birsa Agriculture University
Ranchi

Karnataka
Rhizobium Culture Production Lab.
Bangalore

Karnataka Compost Dev. Corpn. Ltd.
Bangalore

University of Agricultural Sciences
GKVK, Campus,
Bangalore

Sonwalkar Industries
Belgaum

Kadur Agro
Bangalore

Karnataka Biofertilisers
Bijapur

Hellaries Industries Ltd.
Bangalore

Terra Ferma Biotech
Bangalore

Multiplex Biotech Pvt. Ltd.
Bangalore

Madras Fertilisers Ltd
Bangalore

University of Agricultural Sciences,
Dharwad

Varanasi Research Foundation,
Bangalore

Rhizobium Culture Laboratory
Dharwad

Rhizobium Culture Production Lab.
Hebbal, Bangalore

West Coast Herbochem Ltd.
Bangalore

Karnataka Agro Industries Corpn. Ltd.
Bangalore

Samrath Biotech Ltd.
Hubli

Kerala
State Biofertiliser Lab.
Thiruvananthpuram

Agro Biotech Research Centre
Kottayam

Soil Testing Laboratory
Pattambi, Palakkad

The Fertilisers and Chemicals
Travancore
Kochi

Madhya Pradesh
J.N.Krishi Vishva Vidyalaya
Jabalpur

Hindistan Fertiliser Corpn. Ltd.
Gwalior

NAFED Biofertiliser
Indore

Narmada Biotech Ltd.
Barwaha

National Fertilisers Ltd.
Indore

The M.P. State Agro Industries Dev.
Corpn., Bhopal

Samridhi Bioculture Pvt. Ltd.
Indore

M.P. State Cooperative Oil Seed
Growers Fed. Ltd., Dhar

Hindustan Fertiliser Corpn. Ltd.
Bhopal

Trupti Biotech Laboratory
Balaghat

Maharashtra
Mauli Biotech
Pune

Vikash Kruti Sansthan Kendra
Latur

Javeri Agro Industries Corpn. Ltd.
Amravati

M.S. Industries
Amravati

Nav Maharashtra Chakan Oil Mill Ltd.
Sangli

Jain Biotech
Nagpur

Bio Agro Fertilizers
Pune

Niku Bio Research Lab.
Pune

Microplex Biotech
Wardha

Kumar Krishi Mitra Bio Products Ltd.
Pune

Agriculture Bacteriology
College of Agriculture
MPKV, Pune

MITCON Biotech
Pune

Chaudhury Agrotech
Jalakheda, Nagpur

Vaibhav Lakshmi Biocontrol
Laboratory, Wardha

Aviskar Biofarm Pvt. Ltd.
Ahmednagar

Arun Biofertilisers
Kolhapur

Environmental Protection Research
Foundation, Sangli

Nirmal Seeds Pvt. Ltd
Pachora

Nomain Agri Bio Pvt. Ltd.
Pune

Nilayam Biofertiliser Prod. Unit
Wardha

Kisan Agro Chem
Nanded

K-Ferts Ltd.
Nanded

Om Agro
Yavatmal

Jivan Jyoti
Parbhani

Sainath Agrovet Ind. Pvt. Ltd.
Kopergaon

Rashtriya Chemical and fertiliser Ltd.
Mumbai

Ankur Seeds Pvt. Ltd.
Nagpur

Institute of Natural Organic Agriculture
Pune

Sahakar Maharshi Shankar Rao Mohite Patil S.K., Solapur

Vasant Dada Sugar Institute
Pune

Maharashtra Research and Dev. Centre
Solapur

Samridhi Agrotech.
Pune

BAIF Dev. Research Foundation
Pune

Maharashtra Biofertilisers India Pvt. Ltd.
Latur

Meghalaya
Department of Agriculture
Tura

Mizoram
Biofertiliser Prod. Unit
Deptt of Agriculture
Aizwal

Nagaland
Biofertiliser Production Centre
Deptt of Agriculture
Midzephema

Orissa
East Coast Biofertiliser
Bhubaneshwar

Bacterial Inoculant Laboratory
Deptt of Agriculture
Bhubaneshwar

Orissa Agro Industries Corpn. Ltd.
Bhubaneshwar

Orissa Agro Industries Corpn. Ltd.
Sambalpur

Orissa Agro Industries Corpn. Ltd.
Rayagada

Sahoo Biofert.
Ganjam

Baba Biofert.
Puri

Dewborn Chemical
Bhubaneshwar

Hindustan Fertiliser Corpn. Ltd.
Cuttack

Pondicherry
Pondicherry Agro Service and Industries Corpn.
Pondicherry

Green Tech Horticulture Consortium Assoc.
Pondicherry

Punjab
Biofertiliser Production Unit
Ludhiana

Rajasthan
State Farms Corpn. Ltd.
Suratgarh

Rhizobia Scheme
Deptt of Agriculture
Durgapura, Jaipur

NAFED Biofertiliser
Bharatpur

Jaipur Biofertilisers
Hirapura Industrial Estate, Jaipur

Tamil Nadu
Madras Fertilisers Ltd
Jigani

Elbitech Innovation Ltd
Chennai

Biofertiliser Production Unit
Trichy

Biofertiliser Production Unit
Cuddalore

Biofertiliser Production Unit
Kudumiamalai

Biofertiliser Production Unit
Salem

Biofertiliser Production Unit
Ramnathpuram

Biofertiliser Production Unit
Tanjavur

Krishi Care Bio Inputs
Porur, Chennai

Madras Fertilisers Ltd.
Chennai

Main Bio Control Research Lab.
Chengalpattu

Southern Petrochemical Industries Corpn.
Chennai

Esvin Advanced Technologies Ltd.
Chennai

Deptt of Agricultural Microbiology
T.N. Agricultural University
Coimbatore

Deptt of Agricultural Microbiology
T.N. Agricultural University
Madurai

T. Stanes and Company Ltd.
Coimbatore

Darshini Bio Lab.
Tiruvananthpur

Regional Research Laboratory
TNAU, Paiyur

Monarch Biofertilisers and Research Centre
Chennai

Laxmi Biotech
Cuddalore

Mary Green Agro Tech Pvt. Ltd.
Chennai

Modern Nursery Div.
Forest Department
Dharampuri

The SIMA Cotton Research and Dev. Assoc.
Coimbatore

Uttar Pradesh
Kanhaiya Agro Tech Pvt. Ltd.
Mathura

IFFCO
Phulpur, Allahabad

Biofertiliser Production Unit
Deptt of Agriculture
Etah

Biofertiliser Production Unit
Deptt of Agriculture
Behraich

Biofertiliser Production Unit
Deptt of Agriculture
Orai

Regional Agriculture Testing and Demonstration
Meerut

KRIBHCO
Varanasi

Dhariwal Biotech.
Ghaziabad

West Bengal
Nodule Research Laboratory
B.C.K. V.V.
Mohanpur

Vivekanand Institute of Biotechnology
Neempeeth, 24 Parganas

West Bengal Forest Dev. Corpn. Ltd.
Kolkata

Eastern Enterprises
Kolkata

Microbac India
North 24 Parganas

Excel Biotech Pvt. Ltd.
Kolkata

Green Tech Farms and Agrovet Pvt. Ltd
Kolkata

Hindustan Fertiliser Corpn. Ltd.
Durgapur

Nitrofix Laboratories
Kolkata

Amit Biotech
Kolkata