

# जैवउर्वरक सूचना पत्र

## BIOFERTILISER NEWSLETTER

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## From Editor's Desk

Esteemed Readers,

The past few decades have witnessed tremendous agricultural development and economic growth through the spread of high yielding crop varieties and hybrids. While the agricultural production have been very impressive particularly in addressing food security in the developing countries, the input intensive agriculture has been much dependent on greater use of chemical fertilizers, resulting in some undesirable effects on the environment and the overall sustainability of the farming systems. Accordingly, to address this global concern, the wider use of biofertilizers is gaining importance. Thus, the demand for technologies and products based on biological processes has been increasing steadily. Worldwide data for biofertilizer market are not available though the sale volume is estimated to be US\$ 3 billion. This is likely to increase further, as more area comes under organic farming. Currently, nearly 35 million hectares of land are cultivated organically in the globe which eventually increases the demand of biofertilizers. While some Asian countries like Japan, Chinese Taipei, Korea and India have made significant advances in the development and use of biofertilizers, their potential remains largely underutilized due to several reasons including the quality of the biofertilizer produced.

The potential of biofertilizer technology could be harnessed only when biofertilizers are produced as per Indian or International Standards in the factory. Although our National Standards are amended time to time to suit farmer's need but the microbial protocols to enumerate the microbial load in the product is far from satisfactory. Often, the total microbial counts are enumerated including living or dead microbes due to technological limitations. However, recently new biotechnological/molecular protocols are available which could be used for quality control of biofertilizers. This issue seeks to highlight the modern diagnostic techniques for microbial enumerations. Further, this issue unravels the developments of biofertilizer scenario in South Asian countries like China, Indonesia, Vietnam, Phillippines, Thailand, Malaysia and Korea.

This issue has also attempted to highlight the current knowledge on biofertilizers in the form of research notes, new reports, seminar news and book reviews.

Hope, this issue would meet the expectations of our wide readers.

Dr. R.N.Bisoyi  
Editor

# Improved Diagnostic Techniques for Quality Control of Biofertilizers

D.Balachander, S.Karthikeyan, K.Chendrayan and K.Kumar  
Department of Agricultural Microbiology, Tamil Nadu Agricultural University,  
Coimbatore- 641 003

## Abstract

*Biofertilizers are the preparations containing microbial cells applied through seed or soil to enhance the crop growth through nutrient transformations or by producing growth promoting substances. Even though, hundreds of bacteria and fungi are identified for supply of nutrients, only few have been commercially exploited as biofertilizers, which include Rhizobium, Azospirillum, Azotobacter, Gluconacetobacter, cyanobacteria (for N<sub>2</sub> fixation); Bacillus, Pseudomonas, Aspergillus, Penicillium (for P solubilization); Bacillus (K solubilization) and arbuscular mycorrhiza (for P mobilization). These organisms are commercially formulated as either liquid or carrier based and supplied for various crops. To ensure the quality and in turn the efficiency at field, although, appropriate quality assessment system is in place under Fertilizer (Control) Order but, present quality standards concentrate mainly on total microbial load and physico-chemical characteristics of finished product rather than their impact on improving productivity of target crops/plants. Poor efficiency of strains often makes poor performing bio-inoculants. Hence, to ensure the presence of desired effective strain there is a need to incorporate improved diagnostic biotechnological techniques in quality assurance system for biofertilizers.*

## Introduction

The success of biofertilization technology depends very much on the quality of the inoculant used, as the presence of adequate numbers of viable effective cells in the product is necessary to cause infection, to produce nodulation effectively and to create conditions in rhizosphere for increased nutrient mobilization through their biological activity. Quality is the key factor for the success of any product and is applicable for microbial inoculants also. Because farmers are unable to judge the quality of the inoculants at the time of purchase or even at use,

there is often less incentive for an inoculant producer to institute quality control programmes (Thompson, 1999). Unfortunately much of the inoculant produced in the world today is relatively of poor quality (Brockwell and Bottomley, 1995) and the regulation of inoculant producers either do not exist or are inadequately funded and implemented.

## Present quality standards

Bureau of Indian Standards (BIS) notified specifications for four biofertilizers long back for use of biofertilizer producers. Recently, in November, 2009, Govt. of India,

Ministry of Agriculture, Dept. of Agriculture & Co-operation, New Delhi amended and notified the specifications for testing the biofertilizers under Fertilizer Control Order, 1985. The same has been circulated to State Governments to ensure the production and availability of quality biofertilizers. For ensuring a greater control on the quality of biofertilizers, state Governments have been requested to ensure regular checking of the quality of biofertilizers produced / marketed in their States. Quality in inoculant is affected by variety of factors. These factors include incorporation of superior strain of the organism, the use of good quality carrier material, the survival of the organism in desired numbers in broth and carrier, free from excessive contamination, adequate shelf life, proper packing formulation that is easy and effective to supply, etc. Besides, physico-chemical and microbial attributes of product, efficiency of the strain and its potential for nutrient mobilization under field conditions is very important. The current quality control mechanism, although ensures the strength and purity of organisms in appropriate medium but do not fully address the requirement of strain's efficiency and its nutrient mobilization potential. Hence, it is essentially needed that the existing quality assessment systems are strengthened with modern biotechnological tools.

### **Biotechnological tools**

Since, few strains are used in commercial production of biofertilizers and conventional microbial protocols take long time to authenticate, advanced protocols of strain authentication are available. Serological techniques have been successfully

used in the quality control of inoculants. Serological methods like agglutination, fluorescent antibody technique (FA), Enzyme linked immunosorbent assay (ELISA) and gel immunodiffusion techniques, etc. are being used to authenticate the strains of *Rhizobium* (Somasegaran and Hoben, 1994). The importance of rapid identification techniques using biotechnological protocols in biofertilizer production units to test the efficacy of biofertilizer quality have been highlighted (Bisoyi, 2000). ELISA has been used as a strain-specific serological technique for the identification of *R. meliloti* in commercial alfalfa inoculants (Olsen *et al.*, 1989). The same technique has been applied successfully for enumerating *Rhizobium* sp. (peanut) in peat inoculant (Nambiar and Anjaiah, 1985). Serological methods have excellent potential in giving much quicker results for inoculant quality control if the necessary equipment, chemical and biological reagents are readily available and cheap. The membrane-filter immunofluorescence technique was evaluated for enumerating various rhizobial species in inoculants prepared with pre-sterilized peat with good results (Somasegaran, 1985). Compared to the ELISA and immunofluorescence techniques, which give total cell counts (viable and non-viable), the immunoblot procedure (Olsen and Rice, 1989) makes a template of the resultant colonies (growing on an agar plate) on circular nitrocellulose membranes before identification of the rhizobia from contaminants. In the immunoblot technique, the correlation between the immunoblot analysis and plant-infection test was high ( $r = 0.90$ ) for 16 commercial alfalfa inoculants (Olsen and Rice, 1989). Use of nucleic acid fluorescent stains in the identification of

*Rhizobium* was also attempted (Olsen and Rice, 1996).

Genetic markers, probes and PCR based techniques are recently employed for strain identification. Several PCR-fingerprinting techniques have been developed to identify bacteria at the strain level. Those methods are applied with cultured bacterial cells avoiding DNA-extraction and combining convenient analysis with universal applicability and the potential of automation (Versalovich *et al.*, 1994). Now, oligonucleotide primers have been developed to study diazotrophic isolates and applied for PCR-fingerprinting (Smida *et al.*, 1996). The electrophoretically separated band patterns were highly reproducible and strain specific. A variety of phylogenetic oligonucleotide probes was developed for *Azospirillum* spp. (Kirchhof and Hartman, 1992), *Herbaspirillum* spp. (Baldani *et al.*, 1996), *Azoarcus* (Hurek *et al.*, 1993), *Acetobacter diazotrophicus* (Kirchhof *et al.*, 1997). New whole-cell binding 16S rRNA-directed oligonucleotide probes have been developed and are available for *in situ* localization studies of *Herbaspirillum* spp. and *Azospirillum* spp. (Hartmann *et al.*, 2000). Reports are available for the identification of lambda clones containing the *hupB* gene of *Anabaena* sp. using PCR technique (Gubili and Borthakur, 1996). Recently, strain specific identification of *Azospirillum* using *nifH-lacZ* fusions have been demonstrated in *Azospirillum*-wheat associations (Arsene, 1994; Pereg Gerk *et al.*, 2000). Recently, Department of Agril. Microbiology, Haryana Agricultural University, Hisar has developed biofertilizer quality testing kit (Personal communication). The marker tagged strains (*lacZ*

fusion) were enzymatically detected based on the colour development against dilution of the sample. Even though this test kit is quick, cheap and simple, the bio-safety regulations of Genetically Modified Organism restricts the use of these strains at the earliest. Hence there is an urgent need to develop some PCR-based quality test kits for specific strains of biofertilizers in India.

#### **Viable cell count by Fluorescent dyes**

DAPI is a DNA-specific probe which forms a fluorescent complex by attaching in the minor groove of A-T rich sequences of DNA (Kapuscinski, 1995) and a recommended dye for enumerating an active protozoa (Griffiths and Ritz, 1988). Later it was also used to monitor the microbiological quality of water (Saby *et al.*, 1997). Footprinting experiments at low binding ratios indicate that DAPI prevents the cleavage of DNA at AT sequences of 3 to 4 bp, implying that DAPI binds to sites with the 3 or 4 nearest-neighbor AT base pairs. DAPI also binds to poly[d(G-C)<sub>2</sub>], presumably in the major groove of the polynucleotide. Like DAPI, there are several fluorescent staining are available to view the viability of the bacteria.

Since then, dual staining with fluorescent dyes, as revealed by the LIVE/DEAD® BacLight™ Bacterial Viability Kit (Invitrogen), commonly used to determine the proportion of viable cells within a bacterial culture, has opened up new prospects in this field. Over 20 bacterial species have been successfully tested (Molecular Probes, 2004). The method is based on two fluorescent stains: SYTO9, which penetrates all bacterial cells

(optimum excitation and emission: 480 and 530 nm), and propidium iodide, PI, (optimum excitation and emission: 490 and 635 nm), which penetrates cells with damaged membranes. In dead cells, PI displaces SYTO9 from DNA due to higher affinity for nucleic acids. When both dyes are used in combination, viable cells fluoresce in green whereas dead or damaged cells fluoresce in red. This selective staining procedure has been successfully applied to the enumeration of viable and total bacteria, using membrane filtration and epifluorescence microscopy in drinking water (Boulos et al., 1999), lake or deep sea sediments (Haglund et al., 2003; Queric et al., 2004), hypersaline environments for extremophilic archaea living in a wide range of pH (Leuko et al., 2004) and probiotic preparations (Alakomi et al., 2005). This staining procedure is also used for measuring viable cell concentrations in soil (Pascaud et al., 2009). In future quality analysis programmes of bioinoculants, one of these fluorescent-based dyes could take an important role in counting the number of cells in the inoculant rather using conventional plate count methods.

### Conclusion

Biofertilizers have shown great potential as a supplementary, renewable and environmental friendly source of plant nutrients and are an important component of INM and IPNS. Keeping in view of the changing scenario of production systems and environmental concerns associated with chemical fertilizers, it is necessary that biofertilizers play a more significant role in production systems and maintain ecological

equilibrium and sustainability as well. What is most needed is a business driven approach that delivers commercially feasible results which attracts the attention of business houses and policy makers (Adholeya and Pant, 2007).

Biofertilizer quality control is the key issue to enhance its contribution to Integrated Nutrient Management as well as to reduce the environmental issues of using chemical fertilizers. Higher the quality in turn will reflect the crop effect, and there by usage will increase among the farmers. To address and strictly monitor the quality issue, the modern biotechnological tools will be the best choice. There are several PCR based molecular techniques available to detect the specific group or species of microorganisms in natural or artificial ecosystems as well as clinical, food and environmental samples. These techniques are used as diagnostic kit for detection of pathogenic microorganisms in human, animal and food materials. The same technique can be modified and applied to detect particular strain and cell viability in the commercially prepared inoculant packs. As any other diagnosis, biotechnological approach of quality standard would certainly increase the quality of the biofertilizer product which ultimately contributes to increased agricultural productivity.

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# Current Biofertilizer Scenario in South Asian Countries

R.N.Bisoyi

Regional Centre of Organic Farming  
A-153, Sahid Nagar, Bhubaneswar

## Introduction

In order to supply food to increasing population in Asia, agricultural production needs to be increased and to achieve desired production levels increased supply of nutrients coupled with sustained soil health is very much essential. Declining chemical nutrient availability, increasing cost and their effect on soil health and environment has created a global alarm and emphasized the need for introduction of renewable and sustainable systems of nutrient mobilization. Use of biofertilizers has been found to be the most effective in furthering the goal. To promote the use of biofertilizers and to ensure steady technological support for this environment friendly technology a trans-national Asia Specific project entitled "Forum for Nuclear Co-operation in Asia Biofertilizer Project (FNCA-BP) was launched during 2001. This project aims to reduce the amount of chemical fertilizer input without decreasing yield of crops, by using function of beneficial microorganisms in biofertilizer, which increase availability of plant nutrients from soil.

## FNCA Biofertilizer Project

During the first phase of project (2001 to 2006) efforts were mainly on development of universal carrier for biofertilizer in the Philippines and confirmation of effectiveness of biofertilizer to several crop production

systems by field experiments. Project results further emphasized that higher microbial activities can be achieved in carriers sterilized by irradiation than by autoclaving and also demonstrated that use of biofertilizer increased farmers income by reducing the cost of chemical fertilizer application.

In the second phase of project, efforts were made to achieve:

- a. Development of multi-functional biofertilizer, which consists of multiple inoculants with promoting plant growth or inhibiting plant diseases. Several isolates inhibiting plant diseases have been discovered in China, Thailand, The Philippines, and Vietnam. Researchers are concentrating on developing new combinations of inoculants, which show positive effect on plant growth in greenhouse and field experiments.
- b. Improvement of inoculants by radiation based microbial mutation breeding in order to keep high quality of inoculants under tropical conditions. Japanese and Korean researchers started radiation-based microbial mutation breeding to obtain strains, which are tolerant to high temperature and drought stresses. Several promising stains were successfully obtained in 2009.
- c. Dissemination of radiation sterilization method of carrier

using  $^{60}\text{Co}$  to improve quality of carrier for biofertilizer. Some private sector producers in Indonesia and Malaysia have already started radiation sterilization of their carriers using  $^{60}\text{Co}$  (Yokoyam & Ando, 2010).

### **Biofertilizer scenario in China**

In China, the Studies on biofertilizers started from early 50s of 20th century. A range of different biofertilizers have been developed from single strain inoculant to multi-functional products, which can promote growth of crops, provide NPK nutrients, prevent soil-borne diseases and improve soil quality. By the year 2008, about 500 biofertilizer manufacturers were producing 11 classes of biofertilizers covering 700 registered products including rhizobia, associative Nitrogen-fixation bacteria, P-solubilizing strains, P-decomposing bacteria, silicate bacteria, pesticide & herbicide degrading bacteria and PGPR. The total production was over 5200 ton/year.

### **Achievement of research Group on biofertilizers:**

For exploiting new microbial bio-resources for biofertilizers, for the first time in China a study was initiated for screening of high efficiency strains. From 1996 to 2005, scientists isolated hundreds of rhizobia, P-solubilizing microbes, and pesticides & herbicides degrading microbes. Compared with earlier strains, the new isolated strains exhibited higher efficiency in P-solubilization, pesticides & herbicides degradation and N-fixation in soil. (Bingquan, 2010)

### **Biofertilizer scenario in Indonesia**

Significant adverse impacts in Indonesian soils were noticed due to

continuous and irrational application of agrochemicals for agriculture and avoidance of using organic fertilizer since the years of seventies. Soil degradation, environmental pollution, leveling-off and decrease in land productivity are among the important negative impacts of long application of chemical fertilizer. In the last few years, tremendous increase in price of chemical fertilizer has stimulated scientists, Governments and farmers to seek for alternatives to chemical fertilizer i.e. organic fertilizers, bio-fertilizers and bio-organic fertilizers (organic fertilizers enriched with beneficial soil microbes). Organic fertilizers and bio-fertilizers are locally available, cheaper and environmentally friendly. Bio-fertilizers have been shown to benefit many crops such as food crops, horticulture as well as for plantation crops. Initially biofertilizer were used for certain crops such as *Rhizobium* for certain legume such as soybean and mycorrhizae for pine trees, but nowadays, many beneficial soil microbes are being used as inoculants, such as nitrogen fixing bacteria (*Rhizobium*, *Azospirillum*, *Azotobacter*), phosphate solubilizing microbes (bacteria and fungi), mycorrhizae, plant growth promoting rhizobacteria, antagonist, endophytic fungi, and organic matter degrader or decomposer. Indonesian Government strongly support the use of organic fertilizer and bio-fertilizer with or without chemical fertilizer. It is also a common practice in Indonesia to improve the quality of organic fertilizers by enriching them with beneficial soil microbes and such products are known as bio-organic fertilizers. Beneficial soil microbes applied are mostly nitrogen fixing bacteria such as *Azotobacter* and

*Azospirillum* and phosphate solubilizing microbes. Biofertilizers and bio-organic fertilizers are produced by farmers, small, medium and large scale private companies and Indonesian Government. In 2009, in order to support promotion of bio-organic fertilizer, Government provided as much as 400,000 tons of bio-organic fertilizers. This bioorganic fertilizer was distributed freely and subsidized to the farmers. Other significant and important Government support in promoting good quality bio-fertilizer and bio-organic fertilizer in Indonesia was, that Ministry of Agriculture imposed Ministry Act/Decree related to the criteria and requirement for organic fertilizer and bio-fertilizer as well as for bio-organic fertilizer (Ministry Decree No. 28/Permentan/SR.130/2009). The main purpose of this Ministry Decree was to ensure that the organic, bio-fertilizer and bio-organic fertilizers produced are of good quality. Only registered fertilizers and fertilizers which passed quality and effectiveness tests were granted Marketing Certificate by Ministry of Agriculture and allowed for sale their product in the free market in Indonesia. In 2009, as much as 271 products of organic and bio-organic fertilizers and 36 bio-fertilizers were awarded with such Certificate by Ministry of Agriculture (Anas, 2010).

#### **Biofertilizer scenario in Korea**

In Korea, at the moment, mutant induction for the improved function of the biofertilizer is being carrying out for nitrogen fixation, nutrient solubilization, antifungal activity, phytohormone production, environmental tolerance, etc. Research for field experiments and price reduction for irradiation of the

carrier for biofertilizer is being followed. So far there are only 2 commercial irradiation facilities. One is for the sterilization of medical supplies (SOYA) and the other is for the sterilization of 20 authorized items such as spices, etc. (GREENPIA). It is quite difficult economically for small and medium industry to transport and irradiate carrier because the facility is located at Keonggi province. There are about 60 small and medium producers, producing various inoculants. Agricultural Ministry provides partly price reduction during the delivery from farmers union to farmer in a manner of subsidy (Young-Keun, 2010).

#### **Biofertilizer scenario in Malaysia**

Biofertilizer are being increasingly accepted by the agricultural community in Malaysia. The notion that biofertilizer is the same as organic fertilizer or compost is diminishing as more farmers and the plantation industry are now aware that biofertilizer refers to the functional living microorganisms, and not to the organic matter (including composts) which is used as the carrier in many biofertilizer products. The incorporation of these two main components leads to coinage of the term bioorganic fertilizer. Research on new biofertilizer microorganisms, either involving single strain multifunctional microorganisms or on consortia of microorganisms with each member imparting a specific function (e.g. N<sub>2</sub> fixing, phosphate solubilising, potassium solubilising, plant growth promoting) flourish in academic institutions (especially at University Putra Malaysia), research institutions (including MARDI and Malaysian Nuclear Agency) and local microbe-based companies. Mass

production of biofertilizers and bioorganic fertilizers requires quality control of the functional microorganisms as well as the carriers or substrates. The right carriers or substrates contribute to the ease of application of the biofertilizer products by the farmers or plantation workers onto the crops. Amongst the various requirements for a biofertilizer product, most important is the presence of pure inoculum in a sterilized carrier or substrate. The current practice of most biofertilizer companies to sterilize their carriers using autoclave may need to be reviewed in terms of sterilization efficiency and cost. Sterilization of biofertilizer carriers by gamma irradiation is an attractive option, especially in terms of bulk sterilization in pre-packed containers and speed of sterilization. Evaluation on cost of irradiation and transportation to and from the irradiation centre need to be considered by the company. To date the Malaysian Agri Hi-Tech Sdn. Bhd., a microbial-product based company has sent vermiculite medium and product containers for gamma irradiation at the Malaysian Nuclear Agency (Nuclear Malaysia) MINTec-SINAGAMA Irradiation Plant, as part of the production process. Indications are there, that the practice will be adopted by other biofertilizer companies in the near future. It is only realistic that the products and technology developed at the universities and research institutions is transferred to companies and adapted for commercialization purposes. Efforts have been made for this purpose so that potentially good products could be marketed more efficiently. Currently, several plantation industries are also receptive to the establishment of their

own biofertilizer plants at site (Rahim, 2010).

### **Biofertilizer scenario in The Philippines**

The National Institute of Molecular Biology and Biotechnology (BIOTECH) formerly known as the National Institutes of Biotechnology and Applied Microbiology was established by the University of the Philippines (UP) Board of Regents as the national center of excellence for research in microbiology, genetics, chemistry and engineering based at University of the Philippines Los Baños (UPLB). BIOTECH has achieved its goal in harnessing new technological development and scientific breakthroughs in these fields: in the generation of energy from renewable sources, in the further improvement of crop, livestock and forest production and utilization, as well as in the preservation of environment. Through the years, BIOTECH has proved to be worthy of being a national center of excellence. It has developed cost-effective products making use of locally available materials, and technologies which are environment-friendly. UPLB through BIOTECH provided its resources in the development of alternative fertilizer technologies that enhance and sustain crop production. As a result, BIOTECH was able to develop microbial-based fertilizers that are safe to use and demonstrated to be giving socioeconomic benefits to intended clients. One of these products is **Bio N**. The microorganism isolated from the roots of talahib, formulated and packaged, has now a registered brand name, **Bio N**. To make it readily available to the farmers, **Bio N** is now being produced in sixty eight (68) mixing

plants all over the Philippines. This was made possible with the support of the Department of Agriculture and other partner agencies (Anarna, 2010).

#### **Biofertilizer scenario in Thailand**

Soil Microbiology Research Group, a section of the Soil Science Division, Agricultural Production Science Research and Development Office, Department of Agriculture, Ministry of Agriculture and Cooperatives, takes responsibilities of research concerning the production and utilization of Biofertilizers to promote growth yield and quality of crops and trees, and also to conserve environment condition equilibrium. Research and development of the group is considerably divided into four biofertilizers such as Rhizobium biofertilizer, PGPR biofertilizer, Mycorrhiza biofertilizer and Phosphate solubilizing biofertilizer (Nuntagij, 2010).

#### **Biofertilizer scenario in Vietnam**

Biofertilizer research in Vietnam aims at, development and application of multifunctional biofertilizer to increase crop yield and quality, save the mineral fertilizers and improve the soil health. Some of the major activities under the programme includes:

- ✓ Selection of microorganism having the activity related to plant nutrition, plant growth promoting substrate, plant and soil health.
- ✓ Combination of different selected microorganisms.
- ✓ Selection of carrier materials and utilization including sterilization methods.
- ✓ Evaluation of the benefit of microorganism to plant and soil health.

- ✓ Production and application of multifunctional biofertilizer.
- ✓ Promoting biofertilizer use.

These comprehensive reviews indicate the current scenario of biofertilizer developmental programmes carried out in South Asian countries which indicate their commitment to introduce biofertilizers as a source of biological nutrition for increasing agricultural productivity.

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## Research Notes and New Reports

### **Effect of inorganic and biofertilizers**

**on chilli:** An experiment was carried out at Horticulture Research Station, Mondouri, Bidhan Chandra Krishi Viswavidyalaya, West Bengal during 2007-08, to find out the efficacy of biofertilizers (Azospirillum and phosphate solubilizing bacteria) with different levels of inorganics on growth and yield of chilli (Arka Lohit and Arka Suphal). The experiment was laid out in Factorial RBD with two replications. Three levels (25%, 50% and 75%) of both inorganic N and P were included along with full dose of K and biofertilizer. There were altogether 12 treatment combinations. The biofertilizers were applied twice @15g/3 sq m during 30 and 60 days after transplanting (DAT) but inorganics were applied at 45 and 75 DAT. The results revealed that plants under treatment  $N_{75\%} P_{75\%} K_{100} +$  biofertilizers and  $N_{75\%} P_{75\%} K_{100} +$  biofertilizer recorded the maximum growth and yield in Arka Lohit and Arka Suphal respectively, indicating there is a chance of saving 25% both inorganic N and P in Arka Lohit and 25% N and 50% P in Arka Suphal through biofertilizer. (Source - Khan and Chattopadhyay 2009, J. Crop and Weed 5(1) PP 195-200)

### **Effect of biofertilizers on growth, yield and quality of onion cv. Sukhsagar;**

A field experiment was carried out during the winter season of two consecutive years 2006-07 and 2007-08 to study the effect of six combinations of biofertilizers and two chemical fertilizers on onion cv. Sukhsagar. The treatments were *Azotobacter*+PSB, *Azotobacter*+VAM, *Azotobacter*+ *Azospirillum*, *Azospirillum* +PSB, *Azospirillum* +VAM, PSB+VAM,

NPK 100%, NPK 50% and control. The height of the plant was maximum (43.46cm) with the application of *Azotobacter*+VAM. No. of leaves, no. of inflorescence/plot and bulb diameter were maximum with *Azotobacter* +*Azospirillum*. *Azotobacter* + *Azospirillum* and NPK 100% gave maximum length of bulbs (6.03cm). The maximum number of scale per bulb (9.81) was counted from NPK 50%. The plants raised under NPK 100% produced the maximum bulb weight 67.45 g. TSS % was found maximum (12.29%) from NPK 100% but the highest reducing sugar (1.420%) and starch percentage (6.27%) were noted from NPK 50%. The total loss of weight (%) up to 60 days, was found minimum (11.5%) from *Azotobacter*+PSB followed by *Azotobacter* + *Azospirillum* (14.32%). It is therefore, concluded that *Azotobacter* + *Azospirillum* combination is the best for onion as compared to others so far as the sustainability in production and environmental consideration are concerned. (Source - Ghanti and Sharangi, 2009, J. Crop and Weed 5(1) 137-130)

### **Development and use of *Azospirillum* co-aggregates using certain cationic ions and its bioinoculation effect on rice growth and yield**

- A study was conducted in the Department of Agricultural Microbiology, Faculty of Agriculture, University of Annamalai, India during 2006. *Azospirillum brasilense* MTCC (microbial type cultural collection)-125, obtained from Institute of Microbial Technology, Chandigarh, India were studied for co-aggregation with other plant growth promoting rhizobacteria, such as

*Azotobacter chroococcum* -2805, *Azorhizobium caulinodans* ORS (collection de O.R.S.T.O.M., Senegal)-571, *Bacillus megaterium* MTCC-3353 and *Pseudomonas fluorescens* MTCC-4828. The co-aggregation efficiency was found to be enhanced by adding certain cationic ions, such as calcium chloride, aluminium sulphate, magnesium sulphate, ferric chloride and sodium sulphate. Combination of *Azospirillum brasilense* with *Azotobacter chroococcum* using calcium chloride at a concentration of 1.6mM augmented the highest co-aggregation percentage and floc yield. *Azospirillum* co-aggregates exhibited higher survival in seed surface and spermosphere as compared to log phase PGPR cells, different combinations of *Azospirillum* co-aggregates were also studied for phyto-stimulatory effect, such as plant height, plant dry weight, plant nitrogen content, number of panicles, number of productive tillers and grain yield in rice (cv. ADT-43) grown under pot culture condition. It was found that combination of *Azospirillum brasilense* and *Azotobacter chroococcum* proved superior in positively augmenting growth and yield of rice crop. (Source-Guruswamy et al 2009 J Agricultural Research (Lahore) 47(2):107-119)

#### **Growth, yield and quality of sweet pepper (*Capsicum annuum* L.) as influenced by the application of VAM:**

- A field experiment was conducted in Uttaranchal, India, to assess the efficacy of VAM at two fertility levels on growth, yield and quality of sweet pepper during 2006-07. Application of mycoplex (a mycorrhizal biofertilizer) at 250 kg per hectare with 100% recommended dose of fertilizers gave the highest total leaf area per plant (2538.7 cm<sup>2</sup>), shoot fresh weight (210.2 g), shoot dry weight (74.7 g), root fresh weight (30.7 g), root dry weight (28.1 g), plant height (56.8 g), number of primary branches (4.67),

number of fruits per plant (5.37) and quality characters viz., average fruit length (10.8cm), fruit diameter (8.9 cm), fruit weight (50.8 g), ascorbic acid (9120mg/100g fresh weight), vitamin A (826 IU) and protein content (1.20%). The maximum (109.12 q/ha) and minimum (32.48 q/ha) fruit yield was observed with treatment T<sub>4</sub> having mycoplex at 250 kg/ha +100RDF and T<sub>11</sub> (no application of fertilizers and VAM), respectively (Source - Raghav and Kamal 2009 Environment and Ecology 27(3) : 1095-1097).

#### **Synergistic effect of *Rhizobium*, PSB and PGPR on nodulation and grain yield of mungbean:**

- Field studies were conducted in Rajasthan, India, during the 2003, 2005 and 2006 kharif seasons, to determine the synergistic effect of the presowing inoculation of mung bean seeds with different inoculants (*Rhizobium*, PGPR and PSB) alone or in combination, on the nodulation and grain yield of mung beans. Results showed that the presowing inoculation of mung bean seeds with different inoculants significantly increased the nodulation and grain yield over the uninoculated control. Nodulation and grain yield was highest when seeds were inoculated with *Rhizobium*+PGPR + PSB, followed by *Rhizobium* +PGPR and *Rhizobium* alone. In pooled analysis, the combined inoculation of mung bean seeds with *Rhizobium* + PGPR +PSB gave significantly highest numbers of nodules/plant (21.0), dry weight of nodules/plant (87.66mg) and grain yield (12.94q/ha). It was at par with *Rhizobium* +PGPR with grain yield of 12.14q/ha. (Source - Bansal 2009 Journal of Food Legumes 22(1) : 37-39)

#### **Development of plant growth promoting rhizosphere microflora as inoculants for walnut (*Juglans regia* L.) - Rhizosphere microbial composition**

of walnut plants (Cultivar SKAU-W-0035) was studied to select the predominant and efficient isolates of plant growth promoting rhizobacteria as microbial inoculants, Rhizosphere of walnut was found to be inhabited by bacteria, fungi and actinomycetes with maximum population in June and minimum in December. The most predominant microorganisms in the walnut rhizosphere were species of *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Aspergillus* and *Penicillium*. Three isolates of each predominant bacterium were screened for plant growth promoting activities. *Azotobacter* (AZB III), *Azospirillum* (AZS II), *Bacillus* (BAC I) and *Pseudomonas* (PS II) were found to be efficient isolates owing to their ability to produce activity. Best growth of all three selected isolates was recorded in nutrient broth at pH 7 and temperature of 30 °C. The selected isolates were mass-multiplied in nutrient broth for 72hrs in pilot scale fermentors and mixed with various solid carriers. Shelf life of the cultures was determined by counting the population at 2, 3 and 6 month interval. Lignite was found to be the best carrier for *Azotobacter*, *Azospirillum* and *Pseudomonas*, whereas peat was most suitable carrier for *Bacillus* (Source - Dar et al 2009 Indian Forester (2009)135(7) : 943-953)

**Conversion of cassava wastes for biofertilizer production using phosphate solubilizing fungi-** - Two fungi characterized as *Aspergillus fumigatus* and *Aspergillus niger*, isolated from decaying cassava peels were used to convert cassava wastes by the semi-solid fermentation technique to phosphate biofertilizer. The isolates solubilized  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{AlPO}_4$  and  $\text{FePO}_4$  in liquid Pikovskaya medium, a process that was accompanied by acid production. Medium for the SSF fermentation was composed of 1% raw cassava starch and 3% poultry

droppings as nutrients and 96% ground (0.5-1.5 mm) dried cassava peels as carrier material. During the 14 days fermentation, both test organisms increased in biomass in this medium as indicated by increases in phosphatase activity and drop in pH. Ground cassava peels satisfied many properties required of carrier material particularly in respect of the organisms under study. Biofertilizer produced using *A. niger* significantly ( $p < .05$ ) improved the growth of pigeon pea [*Cajanus cajan* (L.) Millsp.] in pot experiments but product made with *A. fumigatus* did not yield desired results. (Source - Ogbo, 2010 Bioresource Technology 101(11) : 4120-4124)

**Role of some effective microorganisms in improving soil properties and productivity of peanut under North Sinai conditions** -

About 77 different microbial isolates (24 *Azotobacter*, 14 *Bacillus*, 9 *Pseudomonas*, 14 Actinomycetes and 16 Fungi), isolated from different plant rhizosphere and compost from different localities in 2 Egyptian governorates. The ability of microbial isolates in  $\text{N}_2$ -fixation, production of phytohormone, phosphate solubilization, antimicrobial (antibacterial and antifungal) and enzyme production were tested. The most powerful effective isolates were selected and identified being *Azotobacter chroococcum*, *Bacillus megatherium*, *Pseudomonas fluorescence*, *Streptomyces fulvissimus*, *Aspergillus candidus*, *Lactobacillus lactis* and *Saccharomyces cerevesiae*. Selected effective microorganism showed high compatibility when mixed together. *Azotobacter chroococcum* recorded the highest values of carbohydrates and microbial gum production. Two field experiments for Peanut were carried out in El-Sheikh Zowaied experimental station - El-Arish-North Sinai-DRC, Cairo, Egypt. Soil used was sandy,

received 1% chicken manure as organic matter and supplemented with the half dose of inorganic nitrogen, to evaluate the effect of employment of some effective microorganisms in improving sandy soil properties and productivity of peanut yield. Physical properties of soil (Hydraulic conductivity, Bulk density and aggregation) and chemical properties were improved by the product of organic matter decomposition during growth season, microbial gums and root growth promoting substances enhanced soil aggregation process, subsequently soil penetrability resistance decrease. The net result was less cohesion relation to adhesion forces between soil particles. Inoculation of Peanut plant for two seasons with mixture of selected effective microorganisms significantly increased: total microbial counts, CO<sub>2</sub> evolution, PDB, Actinomycetes and Fungi. Growth parameters of Peanut (shoot length, root length, shoot fresh and dry weight, root fresh and dry weight, chlorophyll content, number of leaves), yield parameters, mineral content (NPK) of Peanut in soil rhizosphere and in plant also, increased by inoculation. The highest effective of soil microorganisms treatment in improving sandy soil (El-Sheikh Zowaied) properties (physical and chemical) and productivity of Peanut plant were by amending soil combined treatment with organic matter, half dose of mineral fertilizers and inoculation with the five selected microbes as seed+soil+foliar, enhancing the highest pod yield Kg/fed of peanut was recorded with triple application of selected effective microorganisms being 832 and 842 Kg/fed at first and second season respectively. (Source – Arafa et al 2010 Research J Agriculture and Biological Sciences 6(3) : 228-246)

**Two-stage fermentation process for alginate production by *Azotobacter vinelandii* mutant altered in poly- beta**

**-hydroxybutyrate (PHB) synthesis -** A two-stage fermentation strategy, based on batch cultures conducted first under non-oxygen-limited conditions, and later under oxygen-limited conditions, was used to improve alginate production by *Azotobacter vinelandii* (AT6), a strain impaired in poly- beta -hydroxybutyrate (PHB) production. The use of sucrose as carbon source, as well as a high oxygen concentration (10%), allowed to obtain a maximum biomass concentration of 7.5 g l<sup>-1</sup> in the first stage of cultivation. In the second stage, the cultures were limited by oxygen (oxygen close to 0%) and fed with a sucrose solution at high concentration. Under those conditions, the growth rate decreased considerably and the cells used the carbon source mainly for alginate biosynthesis, obtaining a maximum concentration of 9.5 g l<sup>-1</sup>, after 50 h of cultivation. Alginate concentration obtained from the AT6 strain was two times higher than that obtained using the wild-type strain (ATCC 9046) and was the highest reported in the literature. However, the mean molecular mass of the alginate produced in the second stage of the process by the mutant AT6 was lower (400 kDa) than the polymer molecular mass obtained from the cultures developed with the parental strain (950 kDa). The use of a mutant of *A. vinelandii* impaired in the PHB production in combination with a two-stage fermentation process could be a feasible strategy for the production of alginate at industrial level (Source - Mejia et al 2010, J. Applied Microbiology 108 (1) : 55-61)

**Multifunctional properties of phosphate solubilizing microorganisms grown on agro-industrial wastes in fermentation and in soil conditions-** One of the most studied approaches in solubilization of insoluble phosphates is the biological treatment of rock phosphates. In recent

years, various techniques for rock phosphate solubilization have been proposed, with increasing emphasis on application of P-solubilizing microorganisms. The P-solubilizing activity is determined by the microbial biochemical ability to produce and release metabolites with metal-chelating functions. In a number of studies, it has been shown that agro-industrial wastes can be efficiently used as substrates in solubilization of phosphate rocks. These processes were carried out employing various technologies including solid-state and submerged fermentations including immobilized cells. This review paper deals critically with several novel trends in exploring various properties of the above microbial/agro-wastes/rock phosphate systems. The major idea is to describe how a single P-solubilizing microorganism manifests wide range of metabolic abilities in different environments. In fermentation conditions, P-solubilizing microorganisms were found to produce various enzymes, siderophores, and plant hormones. Further introduction of the resulting biotechnological products into soil-plant systems resulted in significantly higher plant growth, enhanced soil properties, and biological (including biocontrol) activity. Application of these bio-products in bioremediation of disturbed (heavy metal contaminated and desertified) soils is based on another important part of their multifunctional properties. (Source – Vassileva et al 2010, Applied Microbiology and Biotechnology 85(5) : 1287-1299).

**Earthworm casts as an alternate carrier material for biofertilizers: assessment of endurance and viability of *Azotobacter chroococcum*, *Bacillus megaterium* and *Rhizobium leguminosarum*** - Vermicast was used with lignite in different combinations (0:1, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1 and 1:0) as

carrier substrate for biofertilizers (*Azotobacter chroococcum*, *Bacillus megaterium* and *Rhizobium leguminosarum*). The viability count of biofertilizer organisms in stored carrier material was individually carried out once in 15 days for a total period of ten months. More than  $1 \times 10^7$  g<sup>-1</sup> viable cells of *A. chroococcum*, *B. megaterium* and *R. leguminosarum* were observed in 4:1, 5:1, 6:1 and 1:0 combination of carrier materials (vermicast:lignite) at the end of 10th month. In case of lignite carrier material, no viable cells were observed in  $10^7$  g<sup>-1</sup> at the end of 6th month for *A. chroococcum* and *R. leguminosarum* and 5th month for *B. megaterium*. The correlation of viable cells of the biofertilizers was negative with reference to incubation period. The increase of vermicast proportion in carrier materials showed increase in the survival rate. The results of the present study suggest that the vermicasts can be used as an alternate carrier material for *A. chroococcum*, *B. megaterium* and *R. leguminosarum*. (Source – Sekar and Karmegam 2010 Scientia Horticulturae 124(2) : 286-289).

#### **Evaluation of synergistic effects of bacterial and cyanobacterial strains as biofertilizers for wheat**

- An investigation was undertaken to screen, select and evaluate a set of bacterial and cyanobacterial isolates from the wheat rhizosphere for their role as biofertilizers in wheat. From an initial set of 23 cyanobacterial strains and 110 bacterial isolates from wheat rhizospheric soil, 3 bacterial and 3 cyanobacterial strains were selected based on their plant growth promoting potential under laboratory and controlled greenhouse conditions. *In-vitro* compatibility studies revealed positive interactions among the six strains. Pot experiments were conducted with wheat variety HD 2687, with a total of 51 treatments, along with recommended fertilizer controls. Various

combinations of the selected set of three bacterial (PW1, PW5 and PW7) and three cyanobacterial isolates (CW1, CW2 and CW3) were used along with 1/3 N and full dose of P and K fertilizers. Significant enhancement in the soil microbiological (Dehydrogenase activity, FDA hydrolase, Alkaline phosphatase and microbial biomass) and plant growth/yield parameters were recorded. Observations revealed a two-fold increase in panicle weight in selected combinations (PW1+PW7+CW3; PW1+CW1+CW2/CW1+CW3; CW2+CW3), as compared to control treatment involving full dose of chemical fertilizers. Such combinations, which also provided N savings of 40–80 kg N/ha are being further evaluated in field experiments. This study for the first time illustrated the positive and dynamic interactions among bacterial and cyanobacterial strains and their promise in integrated nutrient management of wheat crop. (Source - Nain et al Plant and Soil 331, (1-2) : 217-230).

**Effect of *Burkholderia* sp. KCTC 11096BP on some physiochemical attributes of cucumber** - Plant growth promoting rhizobacteria (PGPR) has been a focus of research for its potential as an eco-friendly alternative to chemical fertilizers in the agriculture industry. In current study, the effect of culture suspension (CS) of a novel gibberellins (GAs) producing bacterial strain *Burkholderia* sp. KCTC 11096BP, was observed on shoot length, shoot fresh and dry biomass, root fresh and dry biomass, chlorophyll contents, endogenous bioactive GAs ( $GA_1$  and  $GA_4$ ) and their immediate precursors, abscisic acid (ABA), soluble sugar contents and crude protein contents of cucumber (*Cucumis sativus* L.). It was found that growth attributes of cucumber were significantly promoted by the application of CS of *Burkholderia* sp. KCTC 11096BP. The quantity of

$GA_1$  and  $GA_4$  and their immediate precursors  $GA_{20}$  and  $GA_9$  respectively, were also significantly promoted as compared to their respective controls. Contrary to GAs, the quantity of endogenous free ABA in cucumber leaves was much lower in bacterial CS treated plants. Soluble sugar contents and crude protein contents of cucumber leaves were also significantly higher in bacterial CS treatments as compared to control. It was concluded that *Burkholderia* sp. KCTC 11096BP can be used as an eco-friendly bio-fertilizer in our farming systems. (Source – Kang et al 2010, European Journal of Soil Biology 46(3-4) : 264-268).

### **Role of Microbial Biofertilizers in the Development of a Sustainable Agriculture in the Tropics**

The new social and economic world order has created new challenges for the development of agriculture in the tropics. Functional microbial biofertilizers have been used in some tropical countries for more than half a century in both small and large farms. Biological nitrogen fixation, plant growth promotion, phosphorus solubilization, and translocation to host plants are the major benefits of biofertilizer use, observed or claimed by researchers and product developers. However, a major constraint for the further development of the microbial biofertilizer industry is the demonstration of consistent field effects of the marketed products. The article discusses the main issues related to the functional characterization and promotion of microbial biofertilizer in tropical countries, and the potential of these biofertilizers as tools for small- and large-scale sustainable crop production. (Source - Uribe et al 2010 Soil Biology 21 : 235-250).

### **Evaluation of *Ochrobactrum anthropi* TRS-2 and its talc based formulation for enhancement of**

**growth of tea plants and management of brown root rot disease**

The study was aimed to evaluate *Ochrobactrum anthropi* TRS-2 isolated from tea rhizosphere and its talc based formulation for growth promotion and management of brown root rot disease of tea. *Ochrobactrum anthropi* TRS-2, isolated from tea rhizosphere could solubilize phosphate, produce siderophore and IAA in vitro and also exhibited antifungal activity against six test pathogens. Application of an aqueous suspension of *O. anthropi* to the rhizosphere of nursery grown tea seedlings of five varieties of tea (TV-18, T-17, HV-39, S-449, UP-3 and) led to enhanced growth of the treated plants, as evidenced by increase in height, in the number of shoots and number of leaves per shoot. Treatment with *O. anthropi* also decreased brown root rot of tea, caused by *Phellinus noxius*. Multifold increase in activities of chitinase,  $\beta$ -1,3-glucanase, peroxidase and phenylalanine ammonia lyase in tea plants was observed on application of *O. anthropi* to soil followed by inoculation with *P. noxius*. A concomitant increase in accumulation of phenolics was also obtained. Further, talc based formulation of *O. anthropi* was prepared and its survival determined every month up to a period of 12 months. *Ochrobactrum anthropi* could survive in the formulation up to a period of 9 months with a concentration of  $7.0 \log_{10} \text{CFU g}^{-1}$ , after which there was a decline. Talc formulation was as effective as aqueous suspensions in both plant growth promotion and disease suppression. *Ochrobactrum anthropi*, either in aqueous suspension or as talc formulation induced growth of tea plants and suppressed brown root rot disease. It induced defense responses in tea plants.

*Ochrobactrum anthropi* and its talc based formulation can be considered as

an addition to available plant growth promoting rhizobacteria (PGPR) currently is being used for field application. The present study offers a scope of utilizing this bacterium for growth promotion and disease management which would help in reduction of the use of chemicals in tea plantations. (Source - U. Chakraborty, et al Journal of Applied Microbiology, 107(2) : 625-6340

**Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against *Fusarium* wilt of tomato**

*Pseudomonas fluorescens* strain Pf1 is studied as an effective biocontrol agent for the management of plant diseases and plant growth-promoting bacteria. Previous findings from our research group demonstrated that talc-based *P. fluorescens* Pf1 formulation effectively reduced several plant diseases in addition to promoting plant growth. The modernization of agro-techniques necessitates the development of a new formulation where liquid inoculants can play a significant role. Different chemicals such as trehalose, polyvinylpyrrolidone and glycerol were tested for the development of liquid formulation. Among these, glycerol amendment maintained the greater population level of *P. fluorescens* Pf1 up to 6 months of storage. Further, a study was conducted to standardize the dose of liquid-based formulation of Pf1 for seed treatment and seedling dip. An application of  $10 \text{ ml kg}^{-1}$  of seeds and  $150 \text{ ml ha}^{-1}$  of seedlings was found to be optimum for seed treatment and seedling root dip, respectively. The growth-promoting and antagonistic activities of Pf1 cultures of different ages were found to be greater up to 180 days of storage without much loss in viability of cells. The combination of seed treatment, seedling dip and soil drenching of liquid formulation recorded the minimum

disease incidence of *Fusarium* wilt on tomato under glasshouse (17.33%) and field (4.81%) conditions. In addition, the liquid formulation increased the tomato fruit yield compared to untreated control under glasshouse and field conditions. Thus, this study offered successful technology for development of a liquid-based bioformulation *P. fluorescens* Pf1. (Source - Manikandan et al Biological Control, 54 (2) : 83-89).

***Rhizobium vignae* sp. nov., a symbiotic bacterium isolated from multiple legume species grown in China**

- A group of rhizobial strains isolated from nodules of multiple legume species grown in different geographic regions of China had identical 16S rRNA genes which formed a subclade in *Rhizobium* together with *R. galegae*, *R. huautlense* and *R. alkasoli*, showing 99.8% sequence similarities with them. The DNA relatedness between the representative strain CCBAU 05176T and *R. galegae* ATCC 43677T, *R. huautlense* S02T and *R. alkasoli* CCBAU 01393T were 22.6%, 8.9% and 15.9%, respectively. The strains were also distinguishable from recognized *Rhizobium* species by other polyphasic taxonomic methods including PCR-based restriction fragment length polymorphism analysis (RFLP) of 16S-23S intergenic spacer (IGS), phenotypic and physiological tests, sequence comparison of housekeeping genes, and cellular fatty acids profiles. Therefore, authors proposed this group as a novel species, *Rhizobium vignae* sp. nov., and CCBAU 05176T (=HAMBI 3039T=LMG 25447T) was designated as the type strain. (Da Vei et al 2010, Int J Syst Evol Microbiol 10.1099/ijs.0.023143-0)

***Rhizobium alkalisolii* sp. nov., isolated from the legume *Caragana intermedia* growing in saline-alkaline**

**soils** - Three rhizobial strains isolated from nodules of *Caragana intermedia* grown in saline-alkaline soils in the north of China had identical 16S rRNA genes which showed 99.7% and 99.5% sequence similarities with those of *Rhizobium huautlense* SO2<sup>T</sup> and *R. galegae* USDA 4128<sup>T</sup>, respectively. Phylogenies of the housekeeping genes *atpD*, *recA* and *glnII* confirmed their distinct position differing from the known *Rhizobium* species. SDS-PAGE of whole cell soluble protein and a series of phenotypic and physiological tests allowed to differentiate the novel group from all closely related known *Rhizobium* species. The DNA relatedness between the representative strain CCBAU 01393<sup>T</sup> and *R. huautlense* SO2<sup>T</sup> and *R. galegae* USDA 4128<sup>T</sup> were 34.9 and 20.5 %, respectively. Therefore, authors propose this group as a novel species, *Rhizobium alkalisolii* sp. nov. and CCBAU 01393<sup>T</sup> (=LMG 24763<sup>T</sup>=HAMBI 3051<sup>T</sup>) is designated as the type strain. This strain could form effective nodules on *Caragana microphylla*, *Phaseolus vulgaris* and *Vigna radiata*. (Source - Lu et al 2009, International Journal of Systematic and Evolutionary Microbiology;59 : 3006.

***Rhizobium alamii* sp. nov., an exopolysaccharide-producing species isolated from legume and non-legume rhizospheres**

- A group of exopolysaccharide-producing bacteria was isolated from the root environment of *Arabidopsis thaliana*. The genetic diversity revealed by REP-PCR fingerprinting indicated that the isolates correspond to different strains. 16S rRNA gene sequence analysis showed that the isolates are closely related to the strains *Rhizobium* sp. YAS34 and USDA 1920, respectively isolated from sunflower roots and *Medicago ruthenica* nodules. These bacteria belong to the *Rhizobium* lineage of the Alphaproteobacteria, and the closest

known species was *Rhizobium sllae*. DNA–DNA hybridization experiments and biochemical analysis demonstrated that the nine strains isolated from *A. thaliana* and *Rhizobium* strains YAS34 and USDA 1920 constitute a novel species within the genus *Rhizobium*, for which the name *Rhizobium alamii* sp. nov. is proposed. The type strain is GBV016<sup>T</sup> (=CFBP 7146<sup>T</sup> =LMG 24466<sup>T</sup>). (Source - Berge et al 2009, Int J Syst Evol Microbiol **59** : 367-372;

#### **Effect of Root Inoculation with Plant Growth Promoting Rhizobacteria (PGPR) on Plant Growth, Alkaloid content and Nutrient Control of *Catharanthus Roseus***

-The effect of plant growth promoting rhizobacteria such as *Azotobacter*, *Bacillus* and *Pseudomonas* was tested separately or in combination in *Catharanthus roseus* for two consecutive years (2005 and 2006). The combinations of above mentioned PGPR strains significantly increased plant height, root length, root girth and alkaloid content in *C. roseus* in comparison to the control. In addition, all nutrient contents (N, P, K, Ca and Mg) were also significantly increased as compared to the control. The maximum N, P, K content was obtained from the combination of PGPR treatment. The results of this study suggest that PGPR applied in combination have the potential to increase the plant growth, alkaloid content and nutrient content of *C. roseus*. (Source - Karthikeyan et al 2010, Nat. Croat. Vol. 19(1) : 205-212

#### **Developing Beneficial Microbial Biofilms on Roots of Non legumes: A**

#### **Novel Biofertilizing Technique**

- Biofilms are often complex communities of multiple microbial species and remain attached to surfaces or with interfaces. Such beneficial biofilms can be developed *in-vitro* and be used as biofertilizers (biofilmed biofertilizers , BBs) and biocontrolling agents for nonlegumes , when applied at high cell densities. This chapter describes research studies conducted so far in this field with special attention into development of biofilms of N<sub>2</sub>-fixing bacteria and P-solubilizing fungi. When these two distinct microbes were cocultured in vitro, the bacteria colonized fungal mycelia to form the biofilms. The biofilms showed higher rates of biological nitrogen fixation and organic acid production, which was directly proportional to the synthesis of indoleacetic acid-like substances, than microbes when used alone. The plant growth-promoting effects of such BBs were evaluated using rice (*Oryza sativa*), tea (*Camellia sinensis*), wheat (*Triticum aestivum*), and anthurium (*Anthurium andraeanum*). The biofilms formed nodule-like structures or “pseudonodules” on roots of such plants. For rice and tea, the results showed that recommended chemical fertilizers may be reduced by about 50% while applying BBs. Since this field of research is in its infancy, both laboratory and field experiments are required to fully explore the potential of this emerging biotechnological approach in the future. (Source – Seneviratne et al 2009 In Microbial Strategies for Crop Improvement, Edited by Mohammad Sagir Khan et al, Published Springer, pp 51-62)

## Seminar/ Conferences/Workshops

**National Seminar on “Vermitechnology, use of biofertilizers and Solid Waste Recycling for Sustainable Rural Development”** - In the recent years, all over the world, scientists have realized the value of earthworms in the production of organic manure. In this context a two days seminar was organized by Gandhigram Rural Institute, Gandhigram during 23-24 March, 2010 to expose the scientific fraternity and extension workers to the expertise of eminent personalities in the area of vermitechnology, use of biofertilizers and solid waste recycling for sustainable rural development. Major objectives of the seminar were: (1) To review existing knowledge on vermitechnology, (2) To discuss problems and prospects of organic solid waste recycling, (3) To create awareness on use of biofertilizers and plant growth regulators for enhancing the quality of vermicompost, (4) To provide opportunities for researchers to discuss about their research findings in the relevant field and (5) To document the organic waste management for sustainable rural development.

**Conference on Large Scale Introduction of Biofertilizer.** The conference was organized by Department of Agriculture, Govt. of Manipur, Imphal on 19<sup>th</sup> June, 2010. The agriculture Director L. Palendro, Agriculture Commissioner, Letkhogin Haokip addressed the occasion wherein biofertilizers were introduced in 10,000 hectares of paddy fields under Rastriya Krishi Vikas Yojana at the rate of 4 kg per hectare. It was declared that farmers District Level Officers would be given due training on biofertilizers and

farmers will be provided biofertilizers free of cost.

**Expert Consultation on Biofertilizers for Sustainable agriculture: 27-29 October, 2009-** Asia-Pacific Association of Agricultural Research Institutions (APAARI) in collaboration with council of Agriculture, Taipei organized the Expert Consultation at Taiwan Agricultural Research Institute, Taichung from 27 to 29 October, 2009. Besides all APAARI members, experts on biofertilizers from CG Centers and other international organization, representatives from industry, civil society and farmer organizations participated in the Expert Consultation. The meeting addressed: (i) Review the current status of research, development and use of biofertilizers in agriculture at the regional level; (ii) Develop consensus on issues of quality control, regulatory management, commercialization and marketing; (iii) Identify the role of public and private sector organizations and public-private participation in promoting use of biofertilizers in agriculture; (iv) Promote stewardship, public awareness and stakeholders' participation; and (v) Highlight technological and policy issues and areas of regional cooperation. It was concluded that biofertilizers have high potentialities to maintain sustainable agriculture in the Asia-Pacific.

**21<sup>st</sup> North American Symbiotic Nitrogen Fixation Conference: 13-18 June 2010** - The 21st North American Symbiotic Nitrogen Fixation Conference is scheduled for 13-18 June 2010 at the University of Missouri-Columbia in cooperation with the MU Conference Office

## Book Reviews

**Microbial Strategies for Crop Improvement by Khan, Mohammad Saghir; Zaidi, Almas; Musarrat, Javed, Published by Springer 2009, pp-359 ISBN 9783642019784 £135.00** -

This book presents the multidisciplinary nature and the many fascinating aspects of microbiological approaches for crop improvement in both conventional and stressed soils where quality and safety are the key concerns. The major goal is to provide a cross-section of the latest accomplishments and envisaged future directions in these areas. It gives a holistic view of the basic concepts and practical utility of microbes and thus presents an all-inclusive contemporary treatise on strategic aspects of the diverse microbial communities providing solutions to various customary agronomic problems. This book benefits people working in the area of agronomy, biotechnology, environmental biology, microbiology, plant physiology, plant protection and soil science. The contributions by eminent academicians and professionals ensure a good equilibrium between theory and practice without compromising the basic conceptual framework of the concerned subject. This book is highly useful for researchers, educational Institutions and libraries(RNB).

**Soil Microbiology by Tanuja Singh, S. S. Purohit & Pradeep Parihar, Published by Agrobios (India), ISBN: 9788177543902, 2010, Pages: 487, \$30:**

Soil microbiology focuses on the soil viruses, bacteria, actinomycetes, fungi, algae and protozoa, but it has traditionally also included investigations of the soil animals such as the nematodes, mites, and other microarthropods. Modern soil microbiology represents an integration of

microbiology with the concepts of soil science, chemistry, and ecology to understand the functions of microorganisms in the soil environment. The surface layers of soil contain the highest numbers and variety of microorganisms, because these layers receive the largest amounts of potential food sources from plants and animals. The soil biota form a belowground system based on the energy and nutrients that they receive from the decomposition of plant and animal tissues. The book entitled "Soil Microbiology" presents overall information to the readers about soil, soil microflora and their activities in chapters like, The Soil, Soil ecosystem and soil microbes, Taxonomy of Microorganisms, Microbial Biodiversity, The Natural Biological Capital of the Earth, Growth patterns of mixed population, Bacteria, Archaeobacteria and Actinomycetes, Actinoplanetes, Fungi, Algae, Nematodes, Protozoa in soil, The Rhizosphere, Chemical Interactions in Soil of biological origin, The Biofilm,, Soil microbes and Nitrogen fixation, Carbon Cycle and Microbes, Sulphur Cycle and Microbes, Iron cycle, Microbial Inoculants for Nitrogen Fixation, Rhizobium Biofertilizer, Application of Microbial Biofertilizers in Field Crops, Production of Rhizobium Biofertilizer, Azospirillum Biofertilizer, Azotobacter Biofertilizer, Blue Green Algae and Azolla as Biofertilizer, Estimation of Nitrogen Fixation, Vesicular-Arbuscular Mycorrhizae (VAM), The Cyclic System of Nutrient Management, Laboratory Culture of Microbial Biofertilizers, Mass Production of Biofertilizers, and quality control in bioinoculants. This book is useful to academicians, students, researchers as well as biofertilizer manufacturers (RNB).

