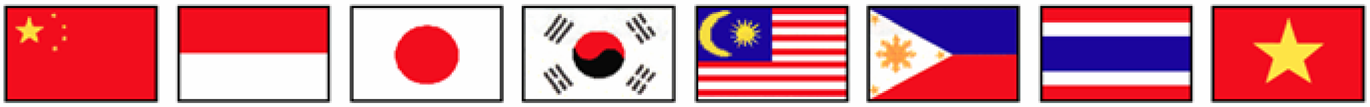

# FNCA Biofertilizer Newsletter

**Issue No. 5**
**July 2005**

## Message from Malaysia

*Dear Colleagues,*

I am honoured to be given the opportunity to coordinate inputs on biofertilizer research and biofertilizer industry in Malaysia in the present issue of the FNCA Biofertilizer Newsletter. There are many institutions in Malaysia that are involved in scientific research on biofertilizer microorganisms and biofertilizer inoculum productions, amongst them are: Universiti Pertanian Malaysia (UPM), Malaysian Agricultural Research and Development Institute (MARDI), Malaysian Rubber Board (MRB), Universiti Sains Malaysia (USM), Universiti Malaya (UM), Universiti Kebangsaan Malaysia (UKM) and Malaysian Institute for Nuclear Technology Research (MINT).

Of these, MRB, or Rubber Research Institute Malaysia (RRIM), as it was formerly known, began earliest in the 1970's in mass production of *Rhizobium* inoculum, for use on leguminous cover crops in rubber plantations. The production of the inoculum in latter years includes sterilization of coconut coir dust as carrier material, packed in sturdy and commercializable packets, at the MINTec-SINAGAMA gamma irradiation plant in MINT. Use of $^{15}N$ to quantify biological nitrogen fixation contribution in particular cropping systems and to evaluate efficiency of different $N_2$ fixing microorganisms, including *Bradyrhizobium* and *Azolla* was also noted, as recorded in publications by scientists from universities and research institutions in Malaysia since the 1980's. Application of nuclear technology in biofertilizer research and biofertilizer production is thus quite close to the hearts of many of us.

Private enterprises and companies have been actively involved in production and marketing of biofertilizer products; Malaysian Agri Hi-Tech Sdn. Bhd. has been producing arbuscular mycorrhizal fungi (AMF) inocula suitable for use in plant nurseries, oil palm plantations, fruit orchards, golf courses and landscape on a large scale.

With agriculture (food production, food safety) and biotechnology being in the limelight at present in the country, it is envisaged that the local biofertilizer industry will flourish, too, capitalizing on the fusion of biotechnology and other technologies including radiation technology.

This issue will highlight on AMF and the $N_2$ fixers. Many thanks to all contributors; it is also my personal wish that the information collated here will benefit all readers.

Happy reading.
Thank you and Warm Regards.

*Khairuddin Abdul Rahim*

FNCA Biofertilizer Project Leader, Malaysia

# USE OF $^{15}N$ ISOTOPE DILUTION TECHNIQUE IN FIELD EVALUATION OF $N_2$ FIXATION IN YOUNG OIL PALM INOCULATED WITH *BACILLUS SPHAERICUS* UPMB 10

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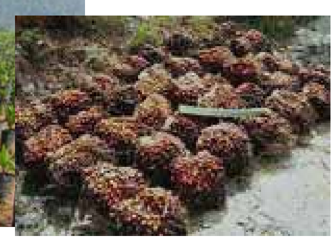
## Introduction

The $^{15}N$ isotope dilution technique has been extensively used in the quantification of biological $N_2$ fixation (BNF) in a crop-microorganism symbiotic system. An advantage of this technique is its accuracy in discriminating $N_2$ fixed from the atmosphere and N assimilated from the soil, employing the use an appropriate non-$N_2$ fixing plant as the reference crop (Danso, 1986; Barrie, 1991). Quantification of BNF by diazotrophic bacteria (e.g. *Azospirillum* spp. and *Bacillus* spp.) in association with non-leguminous crops is less common. However, BNF involving non-legumes and associative $N_2$ fixing microorganisms is becoming increasingly imperative in attempts to develop a sustainable agricultural system. The BNF process is envisaged to reduce dependency on N fertilizer. Since a huge portion of crop production goes to N fertilizer, a good BNF system can help reduce production cost, especially in the tropics where there is an dire need to increase food crop production (Cocking, 2000).

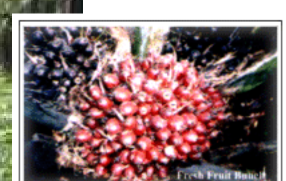
Exploitation of the BNF process and plant growth stimulation involving associative $N_2$ fixing bacteria on oil palm (*Elaeis guineensis*) can potentially make Malaysian palm oil industry more sustainable and environmental friendly (Dobereiner and Baldani, 1998; Shamsuddin *et al*., 1999). The palm oil industry plays a significant role in Malaysian economy through its contribution of about 4.3% (RM 14.5 billion) of the total GDP in 2000 (RM 341 billion) (Department of Statistics Malaysia, 2001). $N_2$-fixing *Bacillus sphaericus* UPMB 10 is a Gram-variable bacteria isolated locally from oil palm roots (Shamsuddin and Roslina, 1995). Results from nursery trials have shown UPMB 10 strain to be a potential biofertilizer for oil palm seedlings with a $N_2$ fixing capacity of up to 33% Ndfa (539 mg N plant$^{-1}$) in non-sterile soil (Amir, 2001). However, beneficial effects of the plant growth promoting rhizobacteria (PGPR) have not been demonstrated in increasing yield of young oil palms under field conditions. The present paper outlines the steps undertaken to conduct the field trial.

Oil palms at nursery stage



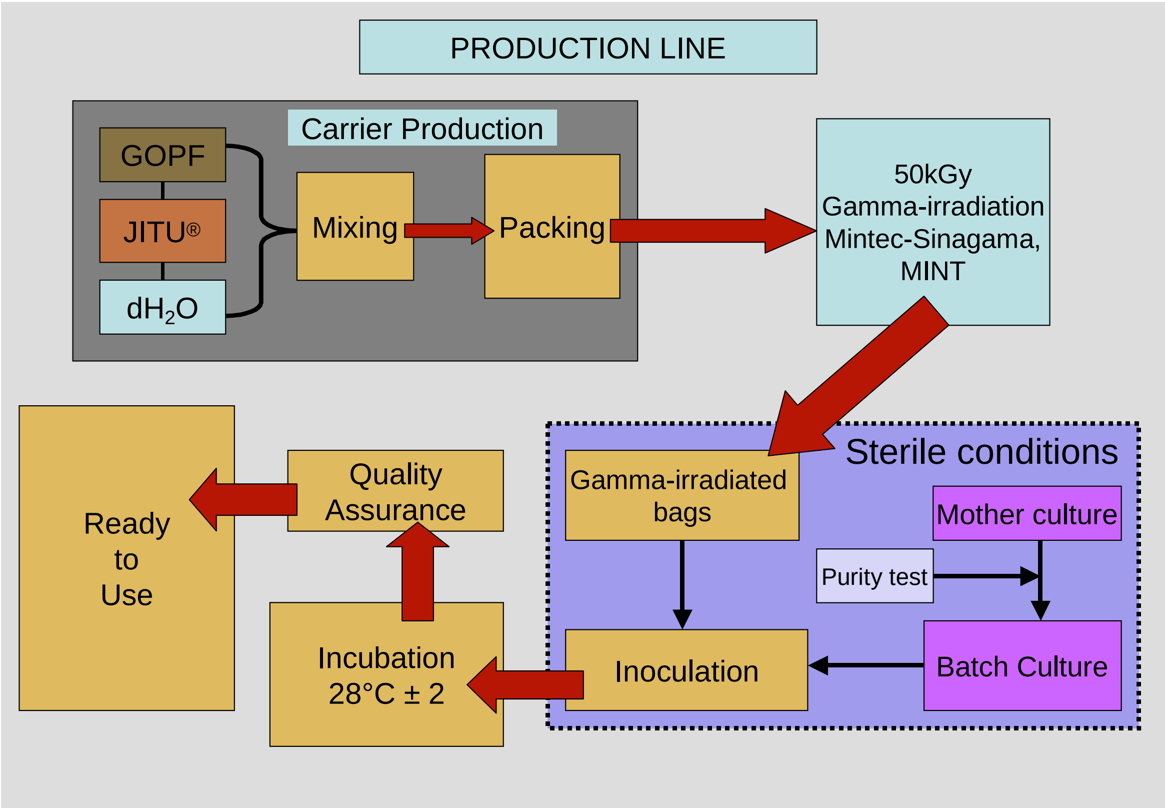
Bunches of oil palm fruits after harvest

Typical oil palm plantation



Oil palm fruits on trees

## Production of *Bacillus sphaericus* UPMB 10 inoculum




**Process flow for the production of the PGPR**




## Field application of $^{15}N$ dilution to young oil palm trees

The $^{15}N$ labelled ammonium sulphate enriched with 10% atom excess (a.e.) equivalent to 10 kg N $ha^{-1}$ will be dissolved in distilled water. The $^{15}N$ solution (1000 mL) will be applied to each isotopic-plot (one palm $m^{-2}$) inoculated with UPMB 10 and non-inoculated (reference plants) which is equivalent to 1 g N $plant^{-1}$ at planting.

**a. Labelling technique**

Soil labelling will be carried by incorporating the $^{15}N$ labelled $(NH_4)_2SO_4$ into the soil at planting time by uniformly spraying the fertilizer solution within $1m^2$ of the isotopic-plot. $^{15}N$ is applied only once during the study period of 1 year.

**b. Plant sampling**

Samples will be obtained by destructive harvest of the oil palm trees one-year after planting. Each palm will be sectioned into leaves, stem and roots. A few oil palm trees will be destructively sampled at start of experiment to determine initial N content.

## Inoculation of UPMB 10 on the oil palms

Each $N_2$-fixing isotopic-plot of young oil palm will receive 3 kg of the PGPR inoculum. The inoculum is thoroughly mixed with planting soil and applied under and around the oil palm seedling at planting. Subsequent application will be spread uniformly within the weeded circle. Each isotopic-plot will be inoculated with respective inoculum treatments at planting and at four monthly intervals (3 kg inoculum/isotopic-plot/application). The uninoculated control treatment plots will receive inoculum carrier and sterilized (γ-irradiated, 50 kGy) inoculum.

## Conclusion

It is envisaged that this isotopic dilution technique could elucidate quantification of $N_2$ fixed by *B. sphaericus* UPMB 10 in association with young oil palms under field situations.

**＜References＞**

Amir, H.G. 2001. Nitrogen fixation and plant growth enhancement by beneficial rhizobacteria in association with oil palm seedlings. Ph.D. thesis. Universiti Putra Malaysia. 155 pp.

Barrie, A. 1991. New methodologies in stable isotope analysis. In: Anon., Stable Isotope in Plant Nutrition, Soil Fertility and Environmental Studies. IAEA Publication, Vienna, pp. 3-25.

Cocking, E.C. (2000). Helping plants get more nitrogen from the air. *European Review* 8 (2): 193-200.

Danso, S.K.A. 1986. Review: Estimation of $N_2$-fixation by isotope dilution: an appraisal of technique involving $^{15}N$ enrichment and their application – comments. *Soil. Biol. Biochem*. 18: 234-244.

Department of Statistics Malaysia (2001).

Dobereiner, J. and Baldani, V.L.D. (1998). Biological nitrogen fixation by endophytic diazotrophs in non-legume crops in the tropics. In *Nitrogen fixation with Non-legumes,* ed. K.A. Malik, M.S. Mirza and J.K. Ladha, pp 3-7. Kluwer Academic publisher, Great Britain.

Shamsuddin, Z.H. and Roslina, A.W. (1995). Isolation of *Azospirillum* from oil palm roots. *18th Microbiology Symposium*, Kuching, Sarawak.

Shamsuddin, Z.H., Amir, H.G., Roslina, A.W., Halimi, M.S., Nazalan, N., Ramlan, M.F. and Marziah (1999). Growth of oil palm seedlings inoculated with $N_2$ fixing *Azospirillum* and rhizobacteria. In *Colloqium on advances in oil palm research under IRPA-funded programmes in 7th Malaysia plan*. PORIM Kuala Lumpur.

# COMMERCIAL POTENTIAL OF AMF APPLICATION IN THE AGRICULTURE AND SOIL REHABILITATION AND CONSERVATION SECTOR

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## What is AMF?

Arbuscular mycorrhizal Fungi (AMF) is a distinct group of fungi that form obligate symbiotic relationships with the roots of flowering plants. Today, mycologists and plant researchers are typically well versed in the function of arbuscular mycorrhizas and their consequences for nutrient cycling and plant productivity. AMF may have been described as early as 1842 (Nageli 1842), but most of Nageli's drawings only remotely resemble the arbuscular mycorrhiza. Rayner (1926-1927) and Trappe and Berch (1985) cited other early observations of the symbiosis during the period 1875-1895. Most surveys of host plants and sophisticated anatomical descriptions of what are most certainly arbuscular mycorrhizas are given by Schlicht (1889), Dangeard (1896), Petri (1903), Jones (1924) and Lohman (1927). Schlicht (1889) had already observed the basic anatomical relationships between host and fungal tissues. Janse (1897) called the interamatrical spores "vesicles" and determined that other structures, named "arbuscules" by Gallaud (1905), were located in the inner cortex. Gallaud (1905) made very accurate observations of the arbuscule is enirely surrounded by a host membrane, which was later confirmed by Cox and Sanders (1974) using Transmission Electrom Microscopy (TEM).

Frank (1885) gave the name "mycorrhiza" to the peculiar association between plant roots and ectomycorrhizal fungi. A thorough discussion of the derivation of the word "mycorrhiza", including the incorporation of the secondr is given by Kelly (1931, 1950). In another publication, Frank (1887) recognized a distinction between ectotrophic and endotrophic mycorrhizas, which included at the time only ericaceous and orchid mycorrhiza. The name for the arbuscular mycorrhizal symbiosis has changed through the years.

## AMF benefits

The mycorrhiza colonized plant is better nourished and better adapted to its environment. It obtains increased protection against environmental stresses (Sylvia and Williams, 1992), including drought (Subramanian et al 1995) cold (Charest et al 1993) Paradis et al 1995), Salinity (Davis and Young, 1985) and pollution (Leyval et al 1994; Shetty et al 1995). In addition, symbiosis tends to reduce the incidence of root diseases and minimizes the harmful effect of certain pathogenic agents (Dehne, 1982). By and large, the growth and health of colonized plants is improved. At the same time they obtain increased protection against environmental conditions detrimental to their survival.

AMF biotechnology in agriculture can be gradually integrated with conventional agricultural practices. Over the medium term, it is quite possible to restore soil quality and improve soil sustainability (Betlenfalvay and Linderman. 1992). It has been shown that appropriate management of mycorrhizae in agriculture allows a substantial reduction in the use of chemicals, thus lessening the level of pollution of surface waters, reducing farm labor and costs of production, while maintaining yields at their highest levels. The advantages and benefits of adopting mycorrhizae in agriculture, allows us to better visualize the scope of this phenomenon at the crop level and in turn, the impact of its long term adoption on the quality of life.

General advantages and benefits of adopting AMF in agriculture system

| Field | Advantages | Benefits |
|---|---|---|
| Agriculture | Agrosystem stability<br><br>Plant production<br><br><br><br><br>Stress resistance<br><br><br>Resistance to pathogens<br><br><br><br><br><br>Value added to product | Improved plant roots, soil microflora and the abiotic geochemical soil matrix.<br>Increase shoot and root biomass<br><br>Drought, cold and pollution<br><br>Crop protection, reduced use of pesticides, fungicides, improvement in animal, plant, human health and product quality<br><br>Increased synthesis of primary and secondary metabolites |
| Plant physiology | Improvement in nutrition<br><br><br>Carbohydrate level<br><br><br>Tolerance to water stress<br><br><br><br><br>Resistance to low temperatures | Reduced chemical fertilizers (10-30%)<br><br>Improved photosynthetic activity<br><br>Cultivation of arid soils or soils unfit for agriculture and altered hormonal balance<br><br>Diversity of crops in inhospitable areas |
| Plant morphology | Transformation of root system and root architecture | Adaptation to stress, increased resistance to erosion, fixation of soils, increased numerous and branched roots, root apices and improved production with certain root crops |

| | | |
|---|---|---|
| Plant community | Microorganism diversity in the subsoil | Reestablishment of soil microflora and improvement of soil quality |
| | Habitat restoration | Stabilize the biogeochemical cycling |
| | Survival of partners | Improvement in yields, better acclimatization at transplanting and diversity of plant cover |
| Micropropagation plants | High value plants | Uniform size, better growth, shorten nursery period |

The benefits of AM fungi are not only limited to improved uptake of nutrients and water from soil. In recent years, it has been shown that these fungi interact with other soil microorganisms with same special positive attitude. For example, it has been shown that the conversions of atmospheric nitrogen from a gas into a form of fertilizer nitrogen (biological nitrogen fixation) by soil bacteria can be greatly enhanced by the presence of AMF (Camel et al 1991; Finlay and Sonderstrom, 1992; Jakobsen and Rosendahl, 1990). The myriad of interactions that can occur in the soil around roots with AMF is unlimited, but recent results indicate that the microbial associations with AMF play significant roles in the benefits observed by inoculation with AMF fungi. Establishing the mycorrhiza "team" on plant roots early in early stages of growth cycle is clearly the right approach for exploiting these remarkable fungi in agriculture. Inoculation with mycorrhizal fungi and their microbial associates can maximize crop yields and quality while minimizing applications of chemical fertilizers and pesticides that can be harmful to people and the environment.

## Biological and biochemical mechanism of AMF

Arbuscular mycorrhizal fungi establish a network of microscopic hyphal filaments in the soil. When these filaments come in contact with a young root, they thread their way between the cortical cells and quickly propagate, forming intracellular arbuscules and in some cases, intercellular vesicles. These arbuscular fungi are so called because of the tree-like structures that are differentiated in the roots. Spores are also differentiated in the soil and roots. They act as reserve, propagation organs and as a reference structure for species identification. Thus far we have no serious clues to the sexuality of AMF. Their association with the Zygomycetes is based on the similarity of their spores to the spores of other known representatives of this class.

The close association created between the plant and fungus through the hyphal network allows the exchange of nutrients for the survival and growth of the two partners. First, the wide dispersal of the fungus in the soil through its large filament network gives the fungus access to a much larger volume of soil and higher area for obsorption than the root system itself. The fungal hyphal act more or less as a pump, supplying the root with a supplement of water and mineral salts to which it normally would not have access. In return, the fungus receives metabolized nutrients from the plant that is unable to synthesize by itself, such as sugars, amino acids and secondary metabolites.

The following evidence obtained from research to date as biological control by AMF

1. Colonization by AMF may offer protection against soil-borne pathogens (Davis and Menge, 1980; Dehne, 1982; Krishna and Bagyaraj, 1983).

2. Infection of roots by nematodes may be reduced when they are colonised by AMF ( Sitaramaiah and Sikora 1982; Cooper and Grandisum 1987)

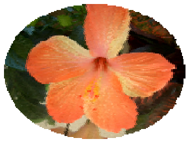
3. Increased nurient concentrations in the leaves of AMF colonised plants may lead to enhanced development but not increased incidence of foliar pathogens (Graham and Menge 1982).

## Agricultural and land reclamation potential

Research work on the potential value of Arbusculr mycorrhizal fungi in agriculture and land reclamation followed from the findings in the 1950s,1960s and 1970s that they could substantially increase P uptake and plant growth under certain circumstances. However, the increasing number of observations that such fungi already exist in most agricultural soils led some to conclude that there would be little value in inoculation (Menge 1985). Khan (1972) may have been among the first to demonstrate that such a practice could be beneficial in some circumstances, but it would frequently prove to be uneconomic because of the large cost of inoculum production relative to the cost of phosphate fertilizer (Menge 1985) However, the practicality of inoculation soils that are inherently low in inoculumpotential such as sterile citrus nursery beds, sterile potting media or soils that are highly disturbed may be greater. For example, the revegetation of disturbed lands, and the course of plant succession in such environments may be strongly influenced by inoculation with mycorrhizal fungi Much of the pertient literature on use of arbuscular mycorrhizal fungi in land reclamation was summarized in a publication edited by Williams and Allen (1984).

As a matter of fact colonized plants are better able to obtain their nourishment in the soil and resist environmental stresses gives fungal symbionts a biofertilizing and crop protection role. In agriculture, the increased uptake of soil minerals by colonized plants means that it is possible to consider substantially reducing applications of fertilizers and pesticides and at the same time obtain equivalent or even higher crop yields (Abbott and Robson, 1991). Through appropriate management of mycorrhizae in agriculture, it is also possible to maintain soil quality and sustainability while protecting the environment over the long term and reducing costs of production.

## Future perspectives

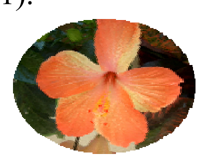


Future needs are of two major kinds. The mechanism by which AMF influence root system needs to be understood at a basic level so that root system design and modification can be used as a management tool, especially within intensive horticultural systems. In addition the use of AMF within sustainable systems needs to be better understood in relation to nutrient cycling.

It is important that AMF should not be seen as the single and final solution for the control of plant pathogens, chemical fertilizers, pesticides and insecticides but as part of an integrated approach. In ‘ open systems’ management is likely to be the way forward, with practices shaped by research aimed at a greater understanding of the role of AMF in plant-soil systems. However, in ‘closed systems’ inoculation is likely to be the way forward in many cases. For many years difficulties associated with inoculum production have prevented large-scale use. However, inoculum is now available commercially throughout Western Europe, Japan, USA, Malaysia and South America. It is certain that only by adopting these approaches is it likely that the benefits of AMF to agricultural production will be optimized, leading to a reduction in chemical inputs and increased sustainability.

Malaysian government has promoted Malaysia to be a garden country. The importance of landscape is apparent in the recent and fast development of Malaysian highway projects. However, the development of these areas have often been characterised by poor establishment of plants due to the disturbed and compacted soils found along highways. In this study the application of commercially available AMF inoculum (MycoGold™) and organic matter (MycoOrganic™) in Malaysian high way landscape plants are UPM Interchange (PLUS) and Sepang Toll Plaza (ELITE) to improve root growth. In UPM Interchange the soil pH ranged from 4.6-6.5 and bulk density is 5.2g/ $cm^{-3}$. Total of 7 trees, one shrub and 7 cover crops were treated with MycoGold™ and MycoOrganic™. All plant roots had colonized with AM fungi. The highest per cent infection (70 %) and spore count (227/10g soil) were recorded in *Acacia mangium* roots and rhizosphere soils. In Sepang Toll Plaza soil pH ranged between 5.2-6.8 and bulk density was about 4.8 g/ $cm^{-3}$. Mycorrhizal infection was noted on all treated plants. Out of 6 plants the highest percentage of infection (35%) and spore count (20/10g soil) were recorded in Duranta *repen 'gold'*. Twelve AM fungal spores were isolated from both rhizosphere soil samples. Out of 12, nine species from Commercial product and 3 from native spores. After application of commercial AMF inoculum and organic matter in landscape plants, we observed the fast recovery of cover crops and trees are as evidenced by the high production of new leaflets. These preliminary results are indicates that AMF inoculation can be profitably exploited for Malaysian high way landscape plant development. (Raja et al 2001).

## Conclusions



Beneficial effects of mycorrhizas including plant growth are well established in controlled conditions and field trials. Nevertheless, although frequently reported as mycorrhizal effects, it should not be excluded that other microorganisms may also be involved in such positive interactions. Mycorrhizas are commonly defined as plant-fungus associations; in functional terms however they may better be regarded as a value regulating plant-soil interactions, possibly increasing carbon sink to roots and carbon efflux to soil (Garbaye, 1991). Their management in consideration of other soil microorganisms is a challenge and an opportunity for sustainable plant-soil systems (Staley et al 1992).

Further research is necessary, both at a basic and applied level, i.e., regarding the maintenance of inoculated organisms in the field. It is worth noting, however, that the number of field experiments is increasing every year. It is therefore to be hoped that, not withstanding the difficulties, and even some failures and contradictions, this body of experience will soon allow a wider utilization of mycorrhizas and associated organisms in plant generations.

***Acknowledgement***

***The Authors would like to express our appreciated to the Staffs, Management and Shareholders of Malaysian Agri Hi-Tech Sdn. Bhd. for their continuous supports in the promotion of AMF towards Sustainable Agriculture.***

**＜References＞**

Abbott, L.K. and A.D. Robson. (1991). The effect of root density, inoculum placement and infectivity of inoculum on the development of vesicular-arbuscular mycorrhizas. New Phytologist. 97: 285-299.

Betlenfalvay, G.J. and R.G. Linderman. 1992. Mycorrhizae in Sustainable Agricultrue, American Society of Agronomy Specail publication 54, American Society of Agronomy, Madison.

Camel, S.B. M.G.Reyes-Solis., R. Ferrera-Cerrat, R.L. Franson, M.S. Brown, and G.J. Bethlenfalvay. (1991). Growth of vesicular-arbuscular mycorrhizal mycelium through bulk soil. Soil Science Society American Journal 55: 389-393.

Charest, C. Y. Dalpe and A. Brown. 1993. The effect of vesicular-arbuscular mycorrhizae and chilling on two hybrids of *Zea mays* L. Mycorrhizae. 4: 89-92.

Cooper, K.M. and G.S. Grandison. 1987. Effects of vesicular-arbuscular mycorrhizal fungi on infection of tamarillo (*Cyphomandra betacea*) by *Melodogyne incognita* in fumigated soil. Plant Disease.71: 1101-1106.

Cox, G.G. and F.E.T. Sandrs. 1974. Ultrasturcture of the host-fungus interface in a vesicular-arbuscular mycorrhiza. New Phytologist. 73: 371-378.

Davis, E.A. and J. L. Young. (1985). Endomycorrhizal colonization of glass-house grown wheat as influenced by fertilizer salts when banded or soil-mixed. Canadian Journal of Botany. 63: 1199-1203.

Davis, R.M. and J.A. Menge. 1980. Influence of *Glomus fasciculatus* and soil phosphorous on phytopthora root rot of citrus. Phytopathology. 70: 447-452.

Dehne, H.W. (1982). Interaction between vesicular arbuscular mycorrhizal fungi and plant pathogens. Phytopathology. 72: 1115-1119.

Finlay, R.D. and B. Sonderstrom. (1992). Mycorrhiza and carbon flow to the soil. In: Mycorrhizal functioning. Allen, M.F. Ed., Chapman and Hall, New York, 134-160.

Frank, A.B. (1885). Ueber die aufWurzelsymbiose beruhende Ernahrung gewisser Baume durch unterirdische Pilze, Ber. Dtsch. Bot. Ges. 3: 128-145.

Frank, A.B. 1887. Ueber neue. Myorrhiza-formen. Ber Dtsch Bot Ges. 5: 395-409.

Gallaud, J. 1905. Etude sur les mycorrhizes endotrophes, Rev. Gen Bot. 17: 5-48.

Garbaye J. (1991). Biological interaction in the mycorrhizosphere. Experentia 47: 370-375.

Graham, J.H. and J.A. Menge. 1982. Influence of vesicular-arbuscular mycorrhizal and soil phosphorous on tale-all disease of wheat. Phytopathology. 72: 95-98.

Jackobsen, I. and L. Rosendahl. (1990). Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytologists 115: 77-83.

Janse J.M. 1897. Les endophytes radicaux de quelques plantes Javanaises. Ann Jardin Bot Buitenzorg. 14: 53-201.

Jones, F.R. 1924. A mycorrhizal fungus in the roots of legumes and some other plants. Journal of Agriculture Research 29: 54-60.

Kelley, A.P. 1931. The concept of mycorrhiza. Mycorlogia. 23: 147-151.

Kelley, A.P. 1950. Mycotrophy in plants. Chronica Botanica, Waltham, Mass.

Khan, A.G. 1972. The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals. I. Effects on maize growth. New Phytologist. 71: 613-619.

Krishna, K.R. and D.J. Bagyaraj, 1983. Interaction between *Glomus fasciculatum* and *Sclerutium rolfissi* in peanut. Canadian Journal of Botany. 61: 2349-2351.

Leyval, C., I.I. Weissenhorn, A. Glashoff and J. Berthelin. (1994). Influence of heavy metal on germination of arbuscular-mycorrhizal fungal spores in soils. Acta Botanica Gallica 141: 523-528.

Lohman, M.L. 1927. Occurrence of mycorrhiza in Iowa forest plants. University of Iowa Studies in Natural History 11;33-58.

Menge, J.A. 1985. Developing widescale VA mycorrhizal inoculations: is it practical or necessary? In: Molina R. (ed) Proceedings of the 6th North American Conference on Mycorrhizae. Forest Research Laboratory, Oregon State University, Corvallis, Ore., pp.80-92.

Nageli C., 1842. Pilze im Innern von Zellen. Linnaea 16: 278-285.

Petri, L. 1903. Ricerche sul signifacto morfologica del prosporoidi (sporangioli di Janse) nelle micorrize endotrrofiche. Nuovo G Bot Ital. 10: 541.

Raja, P., J. Herdhawati, C.A. Nazam[1] and I. Zubaidah. 2001. Rehabilitation of Malaysian highway landscape plants using commercial arbuscular mycorrhizal fungi. ICOM3. Diversity and Integration in Mycorrhizas. Adelaide, Australia

Rayner, M.C. 1926-1927. Mycorrhiza. New Phytologist. 25: 1-50.

Schlichet A. 1889. Beitrag zur Kenntniss der Verbreitung und Bedeutung der Mycorhizen. Landwirtschaftiche Jahrbucher. 18: 478-506.

Shetty, K.G., B.A.D. Herick and A.P. Schwab. (1995). Effects of mycorrhizae fertilizer amendments on zinc tolerance of plants. Environmental Pollution. 88: 307-314.

Sitaramaiah, K. and R.A. Sikora. 1982. Effect of the mycorrhizal fungus *Glomus fasciculatus* on the host parasite relationship of *Rotylenchus reniformis* in tomato. Nematology. 28: 412-419.

Staley, T.E., E.G. Lawrence and E.L. Nance. (1992). Influence of a plant growth promoting pseudomonad and vesicular arbuscular mycorrhizal fungus on alfalfa and birdsfoot trefoil growth and nodulation. Biology Fertility Soils. 14: 175-180.

Subramanian, K.S., C. Charest, L.M. Dwyer and R.I. Hamilton. (1995). Arbuscular mycorrhizae and water relations in maize under drought stress at tasseling. New Phytologist. 129: 643-650.

Sylvia, D.M. and S.E. Williams. (1992). Vesicular arbuscular mycorrhizae and environmental stress. In: Mycorrhizae in Sustainable Agriculture. Bethlenfalvay, G.J. and Linderman, R.G. Eds., ASA Special Publication 54: Madison, Wisconsin. 101-124.

Trappe, J.M., S.M. Berch. 1985. The prehistory of mycorrhizae: A.B. Frank's predessors. In: Proceedings of the 6th North American conference on mycorrhizae. Forest Research Laboraory, Oregon State University, Corvallis, Ore. pp. 2-11.

## A Few Photographs

**Conducting $^{15}N$-aided biofertilizer field trials on rice at MADA, Kedah.**

**Preparation of biofertilizer products for pot trials, MINT.**

**Analysis of N-15 using the emission spectrometer at MINT**

# N-FIXING MICROBES

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In order to achieve the goal of using indigenous microbes for local applications, research works to look into the availability of N-fixing microbes in local soil and water resources has been carried out. The samples include soil samples obtained from the paddy planting plots, compost samples, water samples etc. Potential N-fixers were isolated based on their abilities to survive and multiply under nitrogen-deficient condition.

The microbes were isolated and characterized based on microbiological and molecular approaches. Among the positive isolates were shown in figure below. Most of the isolates were identified to be members of the genus *Bacillus*. It was therefore hypothesized that *Bacillus* could be one of the major contributing groups forming the N-fixing reservoir in the local soil and water resources.

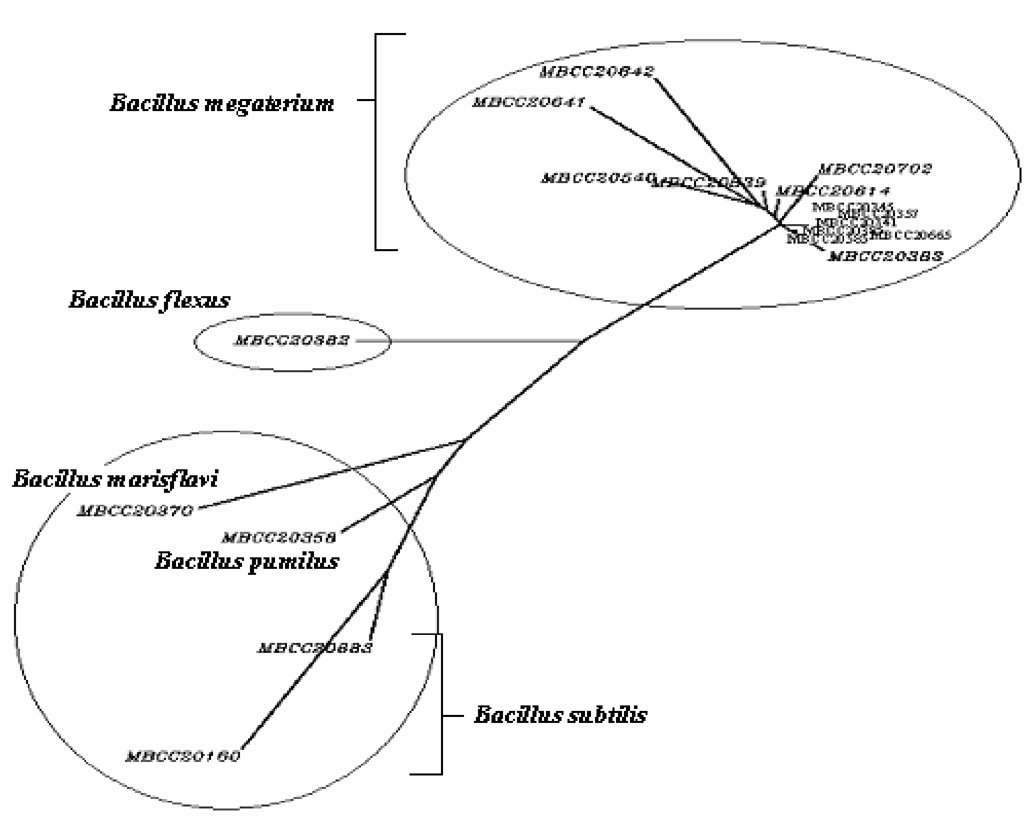
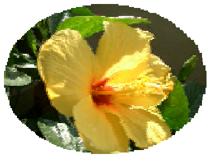



Diagram shows phylogeny of representative nitrogen-fixing isolates. MBCC represents MINT Bacteria Culture Collection. Isolates were identified based on 16S rRNA gene sequences. Data were presented at the 14th International Congress on Nitrogen Fixation, Beijing, China (Liew *et al.*, 2004).

**Reference**
Liew, Woan Ying Pauline, Jong, Bor Chyan and Abdul Rahim, Khairuddin (2004) A Study on Microbial Nitrogen Fixation in Malaysia. In *Proc. 14th International Congress on Nitrogen Fixation*, Beijing, China, 27 Oct – 1 Nov 2004.



## Project Leader of Malaysia

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### Research Interest

- Biofertilizer: arbuscular mycorrhizal fungi; symbiotic $N_2$-fixing microorganisms; plant growth promoting rhizomorphs.
- Bioprocessing: microbiology and microbial processing of *Aquilaria* oleoresin.
- Soil bioremediation: oil sludge; ex-mining land.
- Use of isotopic tracers (e.g. $^{15}N$, $^{32}P$) and other nuclear technology in soil fertility and plant nutrition studies (sago, oil palm, *Arachis hypogaea*, *Acacia, Hevea, Coffea, Theobroma cacao*, *Lansium*, leguminous cover crops).

### Present Responsibilities

1. Project Leader, Malaysia, for Forum for Nuclear Application in Asia (FNCA), Biofertilizer Research.
2. Project Leader, Microbiology and Microbial Processing of *Aquilaria* Oleoresin.
3. Project Leader, Bioremediation of soils contaminated with potentially toxic elements using effective microorganisms and plants (IRPA EAR 08-01-03-0005-EA0001)
4. Project Leader, Production of Biofertilizer Microorganisms and Organic Fertilizer of Agro By-Products.
5. Project Leader, Nitrogen Management in Rice (MoU MINT-MADA).
6. Team Member, Groundwater Microbiology of Malaysian Municipal and Industrial Landfill Sites, under Project on Landfill and Modelling of Pollutant Transport in Groundwater from Municipal and Industrial Disposal Sites (IRPA 08-01-03-0007 EA 0001).
7. Team member:, Studies on Ethnobotany of the Flora of Hutan Belum..

## ~Information~


FNCA Web Site: http://www.fnca.jp/english/

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FNCA Biofertilizer News Letter No. 5

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