Ministry of Education, Culture, Sports, Science and Technology (MEXT)
Forum for Nuclear Cooperation in Asia (FNCA) Biofertilizer Project

# FNCA Biofertilizer Newsletter

Issue No. 8
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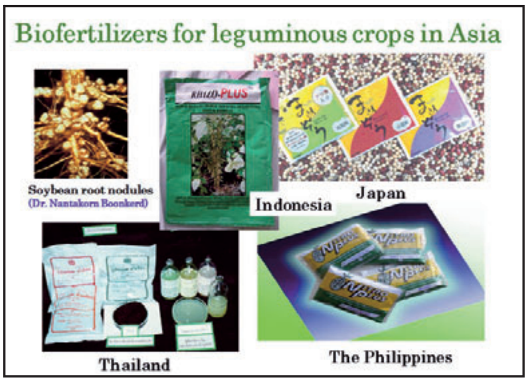
## Overview of FNCA Biofertilzer Project in 2009

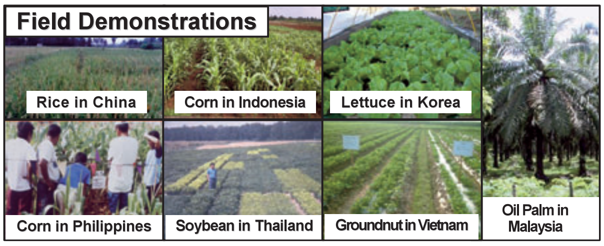
Tadashi Yokoyama, Tokyo University of Agriculture and Technology (TUAT)
Shotaro Ando, National Agriculture and Food Research Organization (NARO)

In order to supply food to increasing population in Asia, agricultural production should be increased and a large amount of fertilizer is required. Most of fertilizers are chemical fertilizers, however oil and natural gas are needed to produce chemical fertilizer and shortage of rock phosphate, which is a low material for phosphorus chemical fertilizer, is beginning a serious problem. Furthermore, improper use of chemical fertilizer and other agrochemicals damage agro-environment, for example nitrate pollution in ground water. Therefore, establishment of environmental friendly sustainable agriculture in Asia and reduction of agrochemical input are required. This biofertilizer 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.




We carried out the first phase of biofertilizer project from 2001 to 2006. Important outcomes from the first phase were as follows. We found universal carrier for biofertilizer in the Philippines and confirmed the effectiveness of biofertilizer to several crop productions by field experiments. Furthermore, we confirmed higher microbial activities in carriers sterilized by irradiation than by autoclave and also demonstrated that use of biofertilizer increased farmer's income by reducing the cost of chemical fertilizer application. Biofertilizer Manual, which was the outcome from the first phase, can be downloaded from the website of the FNCA.

Universal carrier for biofertilizer was found in the Philippines. **Clay loam soil + charcoal (3:1) are good combination of materials of carrier for various bacterial strains.**




In the second phase of this project, we are devoting our efforts to three objectives as follows. The first objective is development of multi-functional biofertilizer, which consists of multiple inoculants with promoting plant growth or inhibiting plant diseases. Concerning this development, several isolates inhibiting plant diseases have been discovered and China, Thai, Philippine, and Vietnam researchers are finding new combinations of inoculants, which show positive effect on plant growth in greenhouse and field experiments. The second objective is 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. The third objective is dissemination of radiation sterilization method of carrier using $^{60}$Co to improve quality of carrier for biofertilizer. Some private sectors in Indonesia and Malaysia have already started radiation sterilization of their carriers using $^{60}$Co .

In this news letter, we introduce activities of the FNCA biofertilizer project in 2009. Promoting understanding of biofertilizer, strengthening linkage between end-users of biofertilizer, farmers union, and fertilizer producers, and dissemination of biofertilizer are important to establish environmental friendly sustainable agriculture in Asia.

# TOPIC : Biofertilizer Research in Japan

## Safety and Quality Requirement of Bio-pesticides in Japan

**Masataka Aino,**
**Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries**

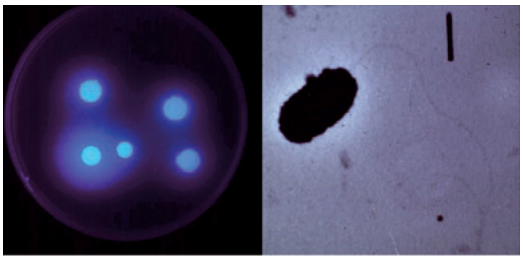
Bio-pesticides are considered to be a more environmentally sound agents than the chemical counterparts for controlling crop diseases.

Recently, the Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF) has revised the Agricultural Chemicals Regulation Law to minimize the intake of chemical residues through foods based upon the concept of Positive List System of Agricultural Chemical Residues in Foods. In addition to the direct adverse effect of pesticides on human health, scientists express a more serious concern of their effect on the surrounding ecosystem, and indirectly on human health.

In view of the potential risk of the chemical pesticides, biologically-based pesticides, such as microbial pesticides, become increasingly popular because of people's preference of organic-/bio-products over the chemical counterparts. However, all the microbes are not necessary to be safe when they are used as bio-pesticides; it is the minimum requirement, therefore, to confirm the safety of the bio-pesticides against men, animals and the surrounding ecosystem when registered to MAFF.

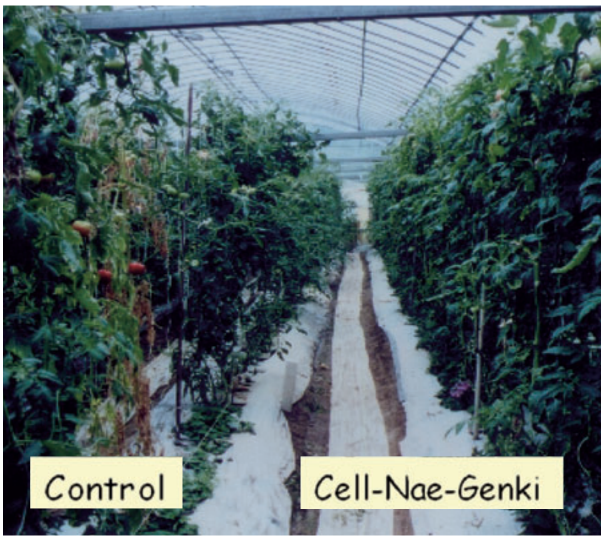
For the registration of bio-pesticides, MAFF requests a variety of relevant documents such as toxicology data of microbial against not only human and animals health (Acute oral toxicity, acute dermal toxicity, acute pulmonary toxicity, acute injection toxicity, primary eye irritation, dermal irritation, hypersensivity incidents), but also non-target organism and environmental toxicity (Toxicity against freshwater fish, avian, plant, non-target insect, honey bee, silkworm, soil microorganisms). In addition, analytical and residual data of the pesticides shall be attached.

As of 28 bio-pesticides have been registered to MAFF for a commercial use in Japan. Currently, the bio-pesticide production is only about 0.1% of the chemical counterparts. But, with an increase in their popularity among producers and consumers, from now onward, it is most likely to increase the reiterated bio-pesticides.



*ISR-agent : Pseudomonas fluorescens*


Bio-pesticide:Cell-Nae-genki




The suppression effect of Cell-Nae-Genki on tomato bacterial wilt under field condition

# Deep Placement of Lime Nitrogen or Coated Urea and Paper Pot Inoculation Method Promote Soybean Growth and Nitrogen Fixation

Takuji Ohyama, Niigata University

Average soybean yield in Japan is low (1.64t/ha: 2008) compared with the potential yield. Soybean plants use the N derived from three sources; $N_2$ fixation, soil N, and fertilizer N. A heavy supply of N fertilizer usually depresses nodule development and $N_2$ fixation activity, which sometimes results in the reduction of seed yield. We developed a new fertilization technique for soybean cultivation by deep placement (at 20 cm depth from soil surface) of slow release N fertilizers, coated urea and lime nitrogen (calcium cyanamide) at the rate of 100kgN/ha. In addition, paper pot inoculation method of rhizobia was examined. Figures show the results of the experiment in the first cropping of reclaimed field, where no rhizobia could be detected. Without inoculation and additional fertilizer (Control) plant growth was very poor and the seed yield is about 10g/plant. When rhizobia were inoculated in paper pot or seed inculation, plant growth was promoted and the seed yield increased to about 23 g/plant. When deep placement of coated urea or Lime nitrogen was supplied, seed yield was as much as 40 g/plant.

The treatments consistently promoted seed yield compared with conventional cultivation in the field with various type of soils. Fertilizer N was efficiently used to supplement N during seed filling stage without concomitant depression of nitrogen fixation and nodule growth. The result showed that deep placement of lime nitrogen or coated urea promoted nodulation and $N_2$ fixation in the upper root system at pod filling stage.

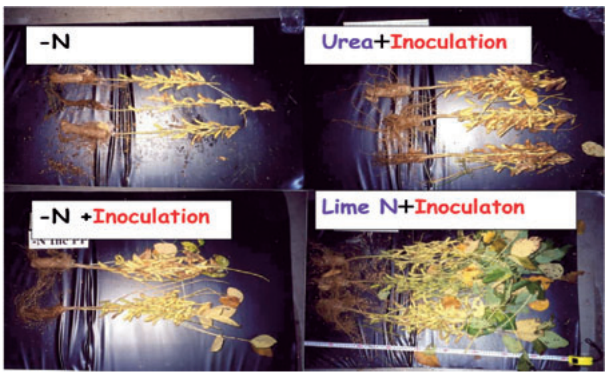
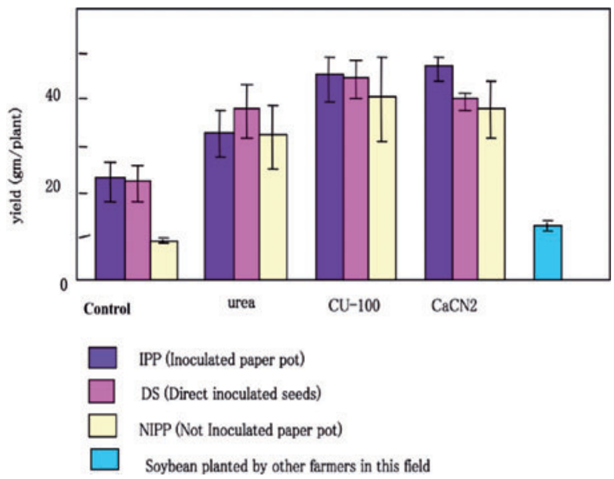



Photo of plant growth in the same field




The effect of deep placement of urea, coated urea (CU-100) or lime nitrogen ($CaCN_2$) and paper pot inoculation on the yield of soybeans cultivated in the first cropping of reclaimed field

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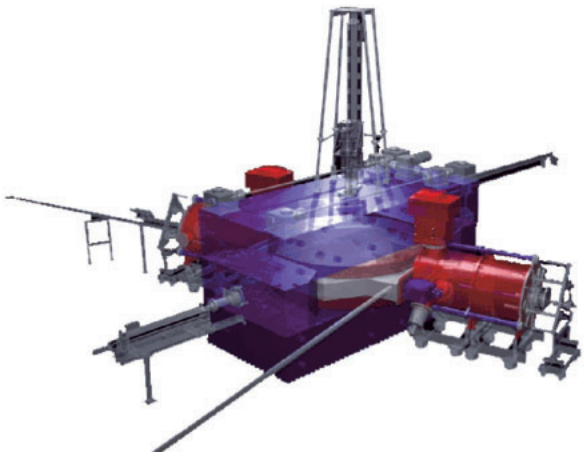
# Application Potentiality of Ion-Beam Mutation Breeding Technology in Biofertilizer / Biopesticide

Issay Narumi, Japan Atomic Energy Agency (JAEA)

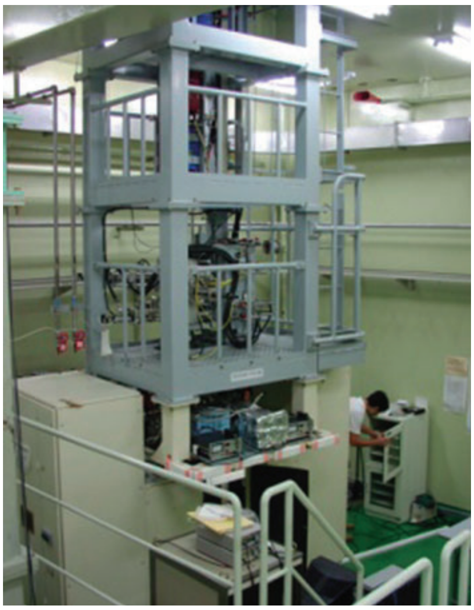
Mutation breeding is one of the valuable breeding techniques. Ionizing radiation, especially gamma-rays, has been used for several decades to produce new plant varieties. In addition, mutation-breeding technology using ion beam irradiation has undergone major developments in recent years, and has been applied to various bioresources such as ornamental plants, crops and industrial microbes. Generally, the biological effectiveness of ion beam irradiation is higher than that of gamma irradiation. In fact, it has been shown that ion beam mutagenesis has a high mutation frequency and a broad spectrum of mutation compared to gamma-ray mutagenesis in chrysanthemum. In another instance, it has been shown that ion beam irradiation can induce single base substitutions as well as large and complex alterations in *Aspergillus oryzae* genome.

Ion-beam mutation-breeding technology will be a promised way to produce biofertilizer/biopesticide inoculants for the next generation. Several collaborative studies have started to improve the phenotype of inoculants using the ion beam irradiation facility at Japan Atomic Energy Agency (JAEA). For example, the selection of environmental stress-tolerant mutants for *Bradyrhizobium japonicum,* the selection of board-host-range mutants for *Pseudomonas fluorescens,* and the selection of fungicide-resistant mutants for *Paecilomyces fumosoroseus* are planned for this year.

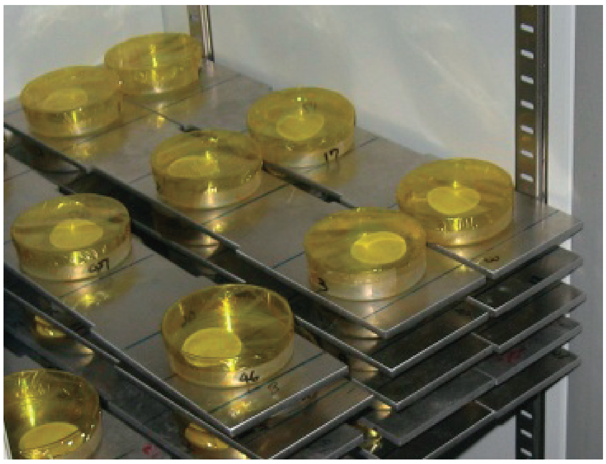
After creating the desired mutant by mutagenesis, it is needed to determine the genotypic difference between the mutant and its parental strain. Getting a clearer picture of genomic alteration can help us ensure the safety and traceability of inoculants. Next-generation DNA sequencer that can read the microbe genome in a short time will make a significant contribution in this regard.



Azimuthally varying field (AVF) cyclotron at JAEA-TIARA



Depth-controlled seed irradiation equipment at JAEA-TIARA (Takasaki Ion Accelerators for Advanced Radiation Application)



Microbe samples on membrane filter ready to irradiate

# Effect of Difference of Inoculation Method and Inoculum Density of *Bradyrhizobium japonicum* USDA110 on Production of Soybean ( *Glycine max* L. Merr.)

Takeo Yamakawa, Kyushu University

Root nodule bacteria build up symbiotic relationships with legume plants and induce the nodule that this organ fix $N_2$. On soybean (*Glycine max* L. Merr.), the inoculation method of useful *Bradyrhizobium* strains with high $N_2$ fixing efficiency on seed surface is popularized widely. But, occupation ration of useful strains inoculated on the seed coat is usually low because of their low competition against indigenous rhizobia. Therefore, several inoculation techniques to increase occupation of useful *Bradyrhizobium* starain USDA110 on soybean root were investigaed.

The object of this research was to clarify the effect of inoculation method and inoculum density of USDA110 on production of soybean. In 2006, five experimental plots were designed for no inoculation (NI), two seed coating inoculation at the density of $10^5$ (SI5) and $10^7$ cells seed$^{-1}$ (SI7), and two soil inoculation at the estimated density of $1.7\times10^3$ (PI7) and $1.7\times10^5$ cells g$^{-1}$ soil (PI9) . PI plot was plowed after sprinkling of peat moss dredged with USDA110l culture. The density of indigenous rhizobia in this field was about $1.4\times10^5$ cells g$^{-1}$ soil. Therefore, occupation ratio of serotype USDA110 and ARA (Acetylene Reduction Activity) of the nodule in SI5 plot were significantly higher than NI plot, and the occupation ratio of serotype USDA110 of the nodule in SI7 plot was significantly higher than NI plot from initial growth, and it was assumed that the fixed nitrogen was distributed in large amount to pod and seed, and from this result seed yield significantly increased compared with NI plot. In PI9 plot, many nodules of USDA110 were formed and seed yield significantly increased compared with NI plot, but ARA was significantly lower than NI plot at V6.4, so the reason why yield significantly increased was still unclear. In PI7 plot many nodules were formed and ARA was significantly higher than other plots but occupation of serotype USDA110 in the nodule was low and seed yield did not increase.

For seed coating inoculation, it was thought that the inoculation of $10^5$ cells seed$^{-1}$ was an effective inoculation rate since no further increase of the seed yield was observed by high density ($10^7$ cells seed$^{-1}$) amount. For previous soil inoculation, the result of PI9 plot indicated that the inoculation method using peat moss was effective and the inoculum density was enough much the same density of indigenous rhizobia.

**Table 1. Transition of nodule number in each growth stage effected by inoculation methods**

| Growth stage | Treatment | Superficical root | Tap root | | Lateral roots | | Total |
|---|---|---|---|---|---|---|---|
| | | | Upper | Lower | Upper | Lower | |
| V6.4 | NI | 13.7 | 32.7 | 24.3 | 39.7 b | 13.7 | 124.0 |
| | SI5 | 18.3 | 37.7 | 33.0 | 38.3 b | 16.3 | 143.7 |
| | SI7 | 17.3 | 33.0 | 18.7 | 42.7 b | 27.7 | 139.3 |
| | PI7 | 8.3 | 33.7 | 34.7 | 70.7ab | 25.3 | 160.7 |
| | PI9 | 16.0 | 21.0 | 24.0 | 90.7a | 18.3 | 170.0 |
| R5.7 | NI | 47.7 | 46.7 | 28.0 a | 214.3 | 35.3 | 372.0 |
| | SI5 | 55.0 | 30.3 | 24.7ab | 167.0 | 27.7 | 304.7 |
| | SI7 | 61.7 | 37.3 | 13.3 b | 211.3 | 34.0 | 357.7 |
| | PI7 | 31.3 | 32.0 | 15.7ab | 237.7 | 25.3 | 342.0 |
| | PI9 | 39.0 | 29.3 | 14.0ab | 183.3 | 27.0 | 292.7 |

Growth stage follwed by Fehr (1971). Mean followed by same and without letters within a column of each stage are not significantly different using LSD ($P<0.10$).

**Table 2. Occupation ratio of serotype USDA110 in nodule of each growth stage.**

| Growth stage | Treatment | Tap root | | | Lateral roots | | Total |
|---|---|---|---|---|---|---|---|
| | | | Upper | Lower | Upper | Lower | |
| V6.4 | NI | 12.5 | 15.0bc | 25.0ab | 20.5bc | 5.6 b | 17.3 b |
| | SI5 | 16.7 | 23.1bc | 29.7 b | 27.5 b | 12.5 b | 24.0 b |
| | SI7 | 0.0 | 70.3 a | 42.9 a | 34.8ab | 16.7 b | 327.3 a |
| | PI7 | 0.0 | 6.3 c | 9.1 b | 11.8 c | 5.0 b | 8.7 c |
| | PI9 | 6.7 | 27.8 b | 31.8ab | 46.3 a | 53.3 a | 38.7 a |
| R5.7 | NI | 10.0 b | 31.6 | 50.0 | 22.4 b | 35.7 | 26.0 b |
| | SI5 | 44.8 a | 38.5 | 23.1 | 45.7 a | 50.0 | 43.3 a |
| | SI7 | 47.8 a | 58.8 | 28.6 | 53.9 a | 28.6 | 50.0 a |
| | PI7 | 27.3ab | 25.0 | 40.0 | 25.0 b | 25.0 | 24.7 b |
| | PI9 | 50.0 a | 53.0 | 33.3 | 55.2 a | 54.0 | 53.3 a |

Growth stage follwed by Fehr (1971). Mean followed by same letters within a column of each stage are not significantly different using Chi square test (*P<0.05*).

**Table 3. Acetylene reduction activity (ARA: μmole $g^{-1}$ DW) at each growth stage**

| Treatment | Growth stage | |
|---|---|---|
| | V6.4 | R5.7 |
| NI | 48.0 a | 9.2 c |
| SI5 | 50.0 a | 16.3 b |
| SI7 | 39.1ab | 7.9 c |
| PI7 | 48.0 a | 28.4 a |
| PI9 | 33.3 b | 10.1 c |

Growth stage follwed by Fehr (1971). Mean followed by same letters within a column of each stage are not significantly different using LSD (*P<0.05*).

**Table 4. Seed yield and yield components of each treatment**

| Treatment | Pod number | Seed number $pod^{-1}$ | | 100 seed g DW | yield kg DW $10a^{-1}$ | Yield index |
|---|---|---|---|---|---|---|
| NI | 477.5 | 1.81 | 0.93 | 21.2 | 16.5.5 b | 0.57 |
| SI5 | 545.5 | 1.85 | 0.94 | 21.5 | 210.2 a | 0.58 |
| SI7 | 527.5 | 1.80 | 0.92 | 22.7 | 195.9 a | 0.55 |
| PI7 | 486.7 | 1.84 | 0.94 | 20.6 | 172.7 b | 0.57 |
| PI9 | 577.8 | 1.79 | 0.93 | 21.8 | 208.8 a | 056 |

Growth stage follwed by Fehr (1971). Mean followed by same and without letters within a column of each stage are not significantly different using LSD (*P<0.10*).

## Country News

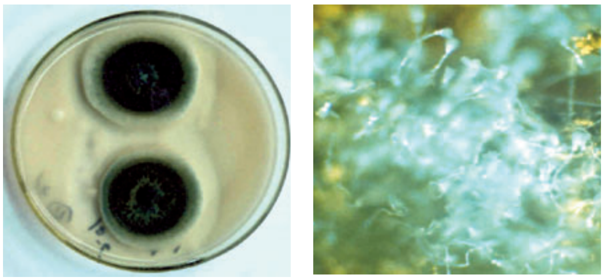
# Biofertilizer and Its Application in China

Fan Bingquan, Chinese Academy of Agricultural Sciences (CAAS)

In China, the Study of biofertilizers has started from 50s of 20th century. At present, biofertilizers have been developed from single strain inoculant into multi-functional products, which can promote growth of crops obviously, provide N-P-K nuitrients, prevente soil-born diseases and improve soil quality. By the year of 2008, about 500 enterprises manufacture biofertilizers in China, 11 kinds of products and registed products are about 700 including rhizobia, associate Nitrogen-fixation bacteria, P-solubilzing strains, P-decomposing bacteria, silicate bacteria, pesticide & herbcide degradation, PGPR. The total production is over 5200 ton/y.

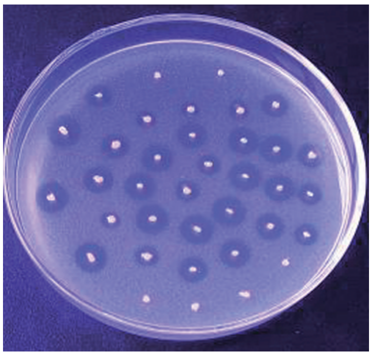
**Achievement of our research Group on biofertilizer:**

(1) exploiting new microbial bio-resources for biofertilizers

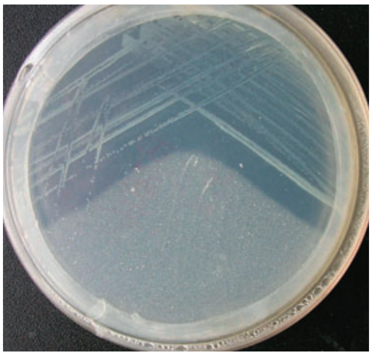


Left : *Penicillium oxalicum* on Pikovkaya medium
Right : GFP gene-labelled *Penicillium oxalicum* P8 strain

For the first time in China, we executed screening of high efficient strains in country-wide. From 1996 to 2005, we isolated hundreds of P-solubilizing microbes, pestcides & herbicides degrading microbes and rhizobium. Compared with literated strains, the new isolated strains have exhibited more efficiency on P-solublization, pestcides & herbcides degradation and N-fixation in soil, and besides, P-sulobilizing strains such as P8(*Penicillium oxalicum*), Pn1(*Penicillium oxalicum*), P21 (*Erwinia herbicola* var. *ananas*) and P40 (*Citrobacter freudii*) are first isolated in China.



BHC-A
(*Sphingomonas* sp.)
for HCH degradation



BTAH1
(*Exiguobacterium* sp.)
for atrazine

(2) We studied the suitability of selected strains with host crops in various types of soils.

In regions of food-stuff production, pestcides highly contaminated, legumina planting area, we have systematically investigated suitable strains of P-solubiling microbes, pestcides & herbcides degrading bacteria and rhizobium which are suitable in these regions.

**Table 1. Suitable P-solubilizing strains with host crops and soils**

| Host plant | Tested Soil | Increase Rate (%) |
|---|---|---|
| wheat | Chao soil;<br>Brown soil | 8.8%～14.8% |
| corn | Chao soil;<br>Brown soil, Red soil | 6.42%～28.1% |
| paddy | Chest-calcium soil,<br>Red soil | 8.59% |
| fruit tree | Chao soil,<br>Chest-calcium soil | 10% |

**Table 2. Suitable pestcides degrading strains with host plants and soils**

| Host plant | Tested Soil | Pestcides degradtion Rate (%) |
|---|---|---|
| wheat | Chao soil;<br>Brown soil | >95% atrazine (after 15d) |
| corn | Chao soil;<br>Brown soil, Red soil | >95% atrazine (after 15d) |
| paddy | Chest-calcium soil,<br>Red soil, Rice soil | 96.9% Dichlorvos & Trichlorfon(after 7d) |
| Vegetable | Brown soil | >95%pesticides |
| Tea tree | Red soil | >85% pesticides |

**Table 3. Suitable rhizobium with its host colver and soybean**

| Host plant | Tested soil | Weight increase of dry grass(%) | Protain increase(%) |
|---|---|---|---|
| Alfalfa, vector | Black soil | 10~50% | |
| Debao, vector | Black soil | 18% | |
| Zhongmu I,cevtor | Chao soil | 58% | |
| Aohan, vector | Chao soil | 21%~29% | |
| Xinmu , vector | Chao soil | 11.9% | 19.63% |
| Beimu, vector | Chao soil | | 13.59% |
| 83482 | Chao soil | 113.7% | 15.63% |
| soybean | Black soil | 8.5%~19.4% | |

Up to now, we have successful developed 5 species of high efficient P-solubiling biofertilizer, 6 species rhizobium, and 5 species pestcides degrading biofertilizer, and biofertilizer application techniques for farmer use.

# Biofertilizer in Indonesia

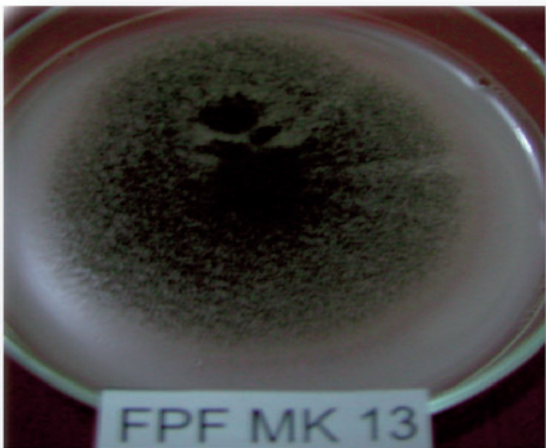
**Iswandi Anas,**
**National Nuclear Energy Agency (BATAN) & Bogor Agricultural University (IPB)**

Significant impact of continues and irrational application of agrochemicals for agriculture and avoidance of using of organic fertilizer since the years of seventies have been noticed significantly in Indonesia. Soil degradation, environmental pollution, leveling-off and decrease in land productivity are among the 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 alternative fertilizers 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 for many crops such as food crops, horticulture as well as for plantation crops.

Bio-fertilizer was only used for certain crops such as Rhizobium for a certain legume such as soybean and mycorrhizae for pine trees. Nowadays, some beneficial soil microbes have been used as inoculants, such as nitrogen fixing bacteria (*Rhizobium, Azospirillum, Azotobacter*), phosphate solubilizing microbes (bacteria and fungi), mycorrizae, plant growth promoting rhizobacteria, antagonist, endophytic fungi, and organic matter degrader or decomposer. Indonesian Government in combination c/q Ministry of Agriculture strongly support the use of organic fertilizer and bio-fertilizer with or without chemical fertilizer. It is also a common practice in Indonesia recently that organic fertilizer is improved its quality by enriched organic fertilizer with beneficial soil microbes known as bio-organic fertilizers. Beneficial soil microbes applied are mostly nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum* and phosphate solubilizing microbes (*Pseudomonas putida, Aspergillus* and *Penicellium* Figure 1.) and antagonist to plant pathogen such as *Trichoderma harzianum* or *T. viridae.* Bio-fertilizers 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 bio-organic fertilizers was distributed freely and subsidized to the farmers.

Figure 1.

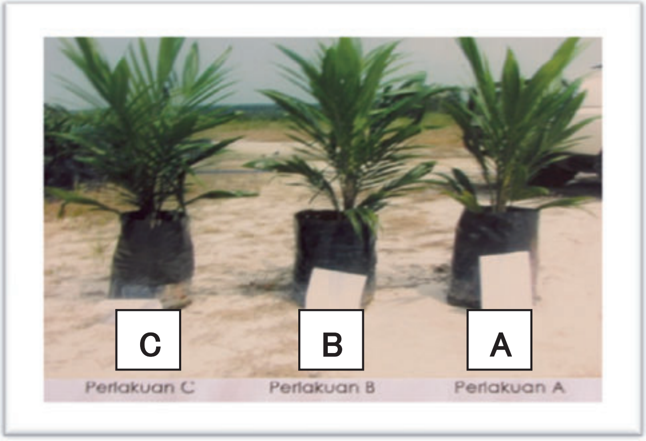
Phosphate solubilizing microbes
(bacteria)




Phosphate solubilizing microbes
(fungus)

Other significant and important government support in promoting good quality of bio-fertilizer and bio-organic fertilizer in Indonesia that Ministry of Agriculture has recently 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 is to ensure the organic, bio-fertilizer and bio-organic fertilizers produced are good quality. Only registered fertilizers and fertilizers which passed quality and effectiveness tests granted Marketing Certificate by Ministry of Agriculture can sell their product to the free market in Indonesia. In 2009, as much as 271 products of organic and bio-organic fertilizers and 36 bio-fertilizers have been awarded Certificate by Ministry of Agriculture.

Bio-fertilizer and bio-organic fertilizers with and without combination with chemical fertilizers have been shown to increase significantly yield of several crops such as rice, corn, soybean, oil palm (Fig. 2) and seedling of Acacia cracicarpa (Fig. 3)




A = 100% NPK

B = 50% NPK + 150 g BIOST

C = 50% NPK + 100 g BIOST

Figure2. Bio-organic fertilizer (BIOST) reduced the amount of chemical fertilizer by 50% and reduced fertilizer cost by 27% for seedlings and 24% for young oil palm trees (P.T. Sitosu Agro Cemerlang 2008) Azotobacter and Phosphate solubilizing were produced by Prof. Dr Iswandi Anas, IPB)

Figure 3. Ability of Rhizobium and phosphate solubilizing microbes to improve *Acacia crassicarpa* seedling (left), good and strong roots development with inoculation (center) and without inoculation (right)

# Current Biofertilizer Research in Korea

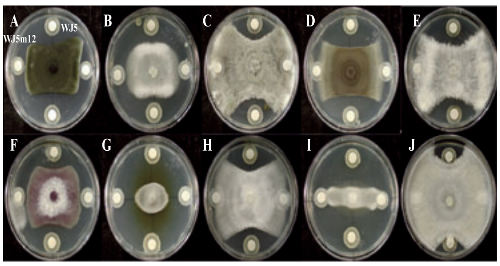
Lee Young-Keun, Korea Atomic Energy Research Institute (KAERI)

In Korea, at the moment, mutant induction for the improved function of the biofertilizer is carrying out (nitrogen fixation, nutrient solubilization, antifungal activity, phytohormone production, environmental tolerance, etc.). Researches of field experiments and price reduction of irradiation for the carrier of biofertilizer will be followed. We have only 2 commercial irradiation facilities. One is for the sterilization of medical supplies (SOYA) and the other is for the sterilization of the 20 authorized items such as spices, etc. (GREENPIA). It is quite difficult economically for small and medium industry to transport and irradiate carrier because this is located at Keong-gi province.

We induced mineral phosphate solubilizing mutants (6 *from Pantoea dispersa* P2, 6 from *Pantoea dispersa* P3, and 6 from *Pantoea terrea* P4) and antifungal metabolite producing mutants (2 from *Paenibacillus lentimorbus* and 1 from *Bacillus* sp. HKA-17) by using gamma ray radiation. *P. lentimorbus* WJ5a17 (mutant) was inoculated with zeolite (diluent), CMC (sticker), Congo red (UV protectant), and starch (nutrient). It was effective at the extent of 66.2% of Propineb against *Colletotrichum* spp. in red pepper, 84.4% and 73.4% of Diclomezine against *Fusarium oxysporum* and *Rhizoctonia solani,* respectively, in Chinese cabbage, and 90.8% of Flukioxonil against *Sclerotinia sclerotiorum* and 66.2% of Etridiazole against *Pythium ultimum* in raddish.

It will be possible for the industry which have irradiator to gather, transport, irradiate, and distribute the carrier of biofertilizer free of charge to the farmers union. Because there are about 60 small and medium producers domestically. Agricultural ministry can provide partly price reduction during the delivery from farmers union to farmer as a manner of subsidy. For the time being, we don't have that much budgets to do international co-works, but our government is expected to create and increase budgets because 'green growth' has been adopted as a national policy from 2008.

Mutants of *Pantoea* sp. with enhanced as well as defective mineral phosphate solubilizing activity



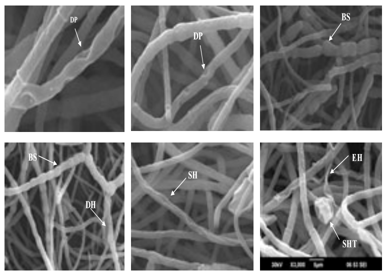

Antigungal spectra of *Paenibacillus lentimorbus* WJ5 and mutant against plant pathogenic fungi
A. *Alternaria alternate*; B. *Botrytis cinerea*;
C. *Botryoshaeria dothidea*; D. *Colletotrichium gloeospriodes*;
E. *Phythium ultimum*; F. *Fusarium oxysporum*;
G. *Alternaria solani*; H. *Phytophthora capsici*;
I. *Pyricularia grisea*; J. *Sclerotinia sclerotiorum*


Control


propinebu

Antifungal agent (*P. lentimorbus* WJ5a17), X100 diluted

Antifungal agent (*P. lentimorbus* WJ5a17), X100 diluted




Effect of antifungal metabolite on hyphal morphology
DP: depression, BS: bulging and swelling,
DH: degradation of hyphae, SH: shriveled hyphae,
EH: empted hyphae, SHT: swelling of hyphal tip

# News on Biofertilizer Projects in Malaysia

**Khairuddin Abdul Rahim, Malaysian Nuclear Agency (Nuclear Malaysia)**

Biofertilizer has been increasingly been 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. $N_2$ fixing, phosphate solubilising, potassium solubilising, plant growth promoting) flourish in academic institutions (especially at Universiti Putra Malaysia), research institutions (including MARDI and Malaysian Nuclear Agency) and the local microbe-based companies. The present environment is encouraging, with increasing awareness on 'green technologies' and 'green products' amongst the producers, and also to the new market pull for 'green agriculture' and 'organic farming', and the need for less dependency on agrochemicals in agricultural production.

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 requirement for a biofertilizer product is the presence of a pure inoculums in a sterilised carrier or substrate. The current practice of most biofertilizer companies to sterilise their carriers using the autoclave may need to be reviewed in terms of sterilisation efficiency and cost. Sterilisation of biofertilizer carriers by gamma irradiation is an attractive option, especially in terms of bulk sterilisation in pre-packed containers, speed of sterilisation. 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. There is good potential that the practice is 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 be transferred to companies and adapted for commercialisation 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 or bioorganic fertilizer plants at site.

With these new developments there is an urgent need for quality standards to be established for biofertilizer, bioorganic fertilizer, in addition to organic and chemical fertilizers. The recent inclusion for biofertilizer, to be discussed in the standards and technical committee for fertilizers, shows that we are in the right direction.

Bioorganic fertilizer produced by Nuclear Malaysia

FNCA Workshop on Biofertilizer, KL, Feb 2008

Signing of NDA with Humus Organic Sdn. Bhd.

Exhibition and Promotion

Innovation Competition

Highlights of Biofertilizer Project

# Current Biofertilizer Research in the Philippines

Julieta A. Anarna, University of Philippines Los Baños (UPLB)

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 the 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 socio-economic 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.

***Bio N*** complements/supplements the use of inorganic fertilizers. It replaces at least half of the required chemical inputs while increasing the yields. As indicated in Table 1, an amount of Two Thousand Pesos (PhP 2000.00 or USD 43.38) is saved per hectare per cropping for a 50:50 ratio of urea (46-0-0) and ***Bio N.*** This exemplifies ***Bio N***'s contribution to poverty alleviation.

Users of ***Bio N*** all over the country vouch for the efficacy of the microbial fertilizer in increasing the yield of crops even with reduced inputs of inorganic fertilizer.

**Table 1. Comparative cost analysis (PhP) per hectare /cropping between chemical fertilizer and *Bio N* for rice and corn**

| Fertilizer | Quantity | Unit Cost (PhP) | Total Cost |
|---|---|---|---|
| UREA (46-0-0) | 2 bags | 1,150.00 (USD 25) | 2,300.00 (USD 50) |
| ***Bio N*** | 5 packets | 60.00 (USD 1.30) | 300.00 (USD 6.52) |
| Cost of 4 bags of UREA (usual rate) | | | 4,600.00 (USD 100) |
| Cost of UREA + ***Bio N*** | | | 2,600.00 (USD 56.52) |
| Savings per hectare | | | 2,000.00 (USD 43.38) |

**Field Trials**

**Table 2. Summary table on productive tillers, straw yield and grain yield of rice subjected to different treatments**

| Treatment | Plant Height at 60 DAP | # Productive Tillers 60 DAP | Straw Yield (Kg) | Grain Yield (Tons/ha) |
|---|---|---|---|---|
| **T1 = Control** | 102.30 c | 17 c | 5.0 c | 3.44 d |
| **T2 = RR** | 130.40 **a** | 20 **a** | 15.5 **a** | 6.19 b |
| **T3 = ½ RR** | 117.70 ab | 18 bc | 7.75 bc | 4.56 c |
| **T4 = Bio-N alone** | | 17 c | 9.50 b | 5.53 b |
| **T5 = ½ RR + Bio-N** | 123.20 **a** | 18 bc | 13.50 **a** | 7.50 **a** |
| **T6 = RR + Bio-N** | 128.60 **a** | 19 ab | 16.00 **a** | 6.88 **a** |

DAP – days after planting, RR – recommended rate

Values followed by the same letters within columns are not significantly different.

**Table 3. Yield of rice cv. RC 18 as affected by chemical fertilizer and BIO-N inoculation**

| Treatment | Fertilizer | Yield (kg./16sq.m) |
|---|---|---|
| **Recommended Rate** | 120-60-60 (4 bags of 46-0-0, 5 bags 0-18-0, 3 bags 0-0-60) | 6.65a |
| **Recommended Rate + BIO-N** | 120-60-60 (4 bags of 46-0-0, 5 bags 0-18-0, 3 bag 0-0-60, +5 packets BIO-N) | 5.88a |
| **½ Recommended Rate + BIO-N** | 60-60-60 (2 bags of 46-0-0, 5 bags 0-18-0, 3 bag 0-0-60, + 5 packets BIO-N) | 6.33a |

**CORN :**

**Table 4. Weight of husked corn ears of inoculated and uninoculated corn grown in an alley cropping with *Gliricidia* as the hedge crop**

| Treatment | Weight (Kg/ha) | | % increase Inoc./Uninoc. | Mean |
|---|---|---|---|---|
| | Uninoculated | Inoculated (with ***Bio N***) | | |
| **Unfertilized control** | 4, 209<br>- | 6, 648<br>- | **58.00** | 5, 338<br>- |
| **Full Recom. Rate (FRR) 150 kgN/ha** | 5, 544 **(31.72)** | 6, 057<br>- | **9.25** | 5, 801 **(8.67)** |
| **½ FRR** | 5, 852 **(39.03)** | 6, 160 | **5.26** | 6, 006 **(11.78)** |
| **MEAN** | 5, 502 | 6, 228 | 13.20 | |
| **C.V. (%)** | 14.56 | | | |

**Table5. Interaction effect of NPK levels and *BIO-N* on total yield of tomato cv. Diamante Biotech Demo site, DS 2008**

| Treatment | Yield (TONS/HA) |
|---|---|
| **T1 = CONTROL** | 3.8 |
| **T2 = FRR** | 11.5 |
| **T3 = ½ RR** | 8.5 |
| **T4 = ½ RR + Bio-N** | 12.5 |
| **T5 = Bio-n alone** | 3.2 |
| **T6 = RR+ BIO-N** | 8.3 |

# Current Biofertilizer Research in Thailand

Achara Nuntagij, Department of Agriculture (DOA)

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 on 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 kinds of biofertilizers such as Rhizobium biofertilizer, PGPR biofertilizer, Mycorrhiza biofertilizer and Phosphate solubilizing biofertilizer.

**Rhizobium biofertilizer**

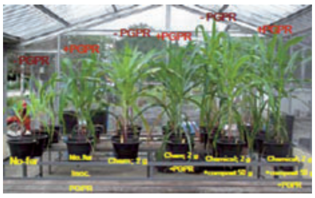



**Mycorrhiza biofertilizer**

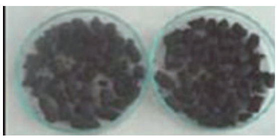


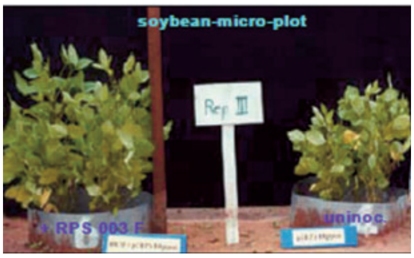

**PGPRI biofertilizer**



**Phosphate Solubilizing biofertilizer**



# Biofertilizer research and development in Vietnam

**Pham Van Toan, Ministry of Agriculture and Rural Development (MARD)**

**Aim: Research, development and** application of multifunctional biofertilizer to increase crop yield and quality, save the mineral fertilizers and improve the soil healths.

**Activities:**

1. Selection of microorganism having the activity related to plant nutritions, plant growth promoting substrate, plant and soil health.
2. Combination of different selected microorganisms
3. Selection of carrier materials and utilize including sterilization methods
4. Evaluation of the benefit of microorganism to plant and soil health
5. Production and application of multifunctional biofertilizer
6. Promoting the expand of biofertilizer use



Laboratory study



Inoculant production



Field testing



Farmers training and field visit

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