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

FNCA Biofertilizer Newsletter

Issue No. 9
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Overview of FNCA Biofertilizer Project 2010

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Establishment of environmental friendly sustainable agriculture and reduction of agrochemical input are required in Asia. This FNCA 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 and the Biofertilizer Manual, which can be downloaded from the website of the FNCA, was published as one of the outcomes of the project. In the second phase of this project from 2007, we are devoting our efforts to three objectives as follows. The first one is development of multi-functional biofertilizer, which consists of multiple inoculants with promoting plant growth or inhibiting plant diseases. The second one is improvement of inoculants by radiation-based microbial mutation breeding in order to keep high quality of inoculants under tropical conditions. The third one is dissemination of radiation sterilization method of carrier using Co-60 to improve quality of carrier for biofertilizer.

In this news letter, we introduce activities of the FNCA Biofertilizer Project in 2010. At first, we would like to introduce the FNCA 2010 workshop on Biofertilizer Project, which was held in Manila, the Philippines, Nov. 8-12, 2010, joingly with the workshop on Mutation Breeding Project. A total of 54 experts and staffs from Bangladesh, China, Indonesia, Japan, Malaysia, The Philippines, Thailand, Vietnam, and RCA/IAEA participated in this workshop.

At the workshop on Biofertilizer Project, summary report was presented by each participationg country and five topics were discussed. The first topic was "application of radiation sterilization of carriers" and it was reported that autoclave-sterilization of carriers showed a deleterious effect on survival of *Azospirillum*, whereas gamma-irradiation did not. The second topic was "development of FNCA Manual for Quality Standard of Biofertilizer" and future plan was concluded that FNCA Biofertilizer Quality Standard Manual would be edited based on the Quality Standard manual of biofertilizer of Thailand. The third topic was "development of multi-functional biofertilizer" and different kinds of works on the development of multi-functional biofertilizers that have multi-effects on the growth, nutrition uptake of plant as well as reduction of plant disease were reported. Then, the forth topic, "commercial application of biofertilizer", and the fifth topic, "application of biofertilizer with plant growth promoter of oligo-chitosan" were discussed. The special presentation was also given by Dr. Mercedes Umali Garcia, University of Philippine, Los Banos (UPLB) on development and promotion of Bio-N, biofertilizer with *Azospirillum*, and its great success story.

Participants visited the Bio-N laboratory in the National Institute of Molecular Biology and Biotechnology (BIOTECH) of UPLB. They observed and discussed about process flow on Bio-N production, especially on carrier preparation.

Then, they visited Bio-N test field and got an explanation that the farmer used Bio-N to reduce usage of chemical fertilizer and got a good result for yield of corn.

On November 12th, FNCA 2010 Meeting on Technology Transfer from Research to Commercial Application was held. Approach for technology transfer in several countries, current status and challenges that nuclear research institutions faced in transferring research results from the laboratory to the market place were discussed.

Participants of workshops on Mutation Breeding and Biofertilizer Project

Carrier Production of Bio-N,
Biofertilizer in the Philippines

A scene of Biofertilizer Project Wroskhop

The Effect of Gamma-Sterilization of Biofertilizer's Carrier on the Density of Rhizobial Inoculants

Kouhei Tejima, Issay Narumi, Japan Atomic Energy Agency (JAEA)

Nitrogen fixation through the legume-*Rhizobium* symbiosis is important for legume production. To utilize symbiotic nitrogen fixation for agricultural production more effectively, it is necessary to develop advanced rhizobial biofertilizers that keep the amount of viable inoculants for a definite period of time. The inoculants survival in the biofertilizer could be affected by the physical and chemical properties of carrier materials and by the competition with native microbes in the carrier. Gamma-irradiation is expected to sterilize the carrier materials without changes in physical and chemical properties. In an effort to demonstrate the effect of gamma-sterilization, the survivals of inoculants were monitored to assess the shelf life of biofertilizer.

As carrier materials, three kinds of Japanese typical soil (cultivated topsoil of Andosol, Gray Paddy soil and forest subsoil of the Kanto Loam Formation) and Japanese peat soil "Keto-tsuchi") were prepared. To make soil-based artificial carriers, each Japanese typical soil was mixed with charcoal powder (3:1). In order to optimize gamma-ray dose to sterilize soils, the survival rate of native microbes in the prepared soils were determined. As a result, 50 kGy was determined to be suitable to sterilize soils (Fig. 1).

Following the sterilization of the carrier materials by 50 kGy of gamma-irradiation or autoclaving at 121°C for 40 min, water suspension of *Bradyrhizobium japonicum* strain USDA110 was inoculated. Additionally, biofertilizers that were consisted of the inoculants and one of the non-sterilized carriers were prepared as controls. The initial density of inoculants in the biofertilizer was adjusted to 6.6×10^{7} cells g^{-1}. The biofertilizer was packed in polyethylene bags and stored for 6 months in 4°C or 30°C. After storage, viable inoculants in the biofertilizer were enumerated by the dilution plate method. The number of inoculants in biofertilizers made from non-sterilized carrier was monitored by PCR that amplifies the *nod*D-*nod*Y region of *B. japonicum*.

The inoculants density after 6-month storage was greater than the initial density in biofertilizers made from sterilized carriers, while that was significantly decreased in biofertilizers made from non-sterilized carriers (Fig. 2). Gamma-sterilization was superior to autoclave-sterilization in enhancing the survival of inoculants. Because of the stability of supply, the high sterilization effect with lower radiation doses, and the high performance in maintaining the inoculants density, "Keto-tsuchi" was selected as those most suitable for a biofertilizer's carrier.

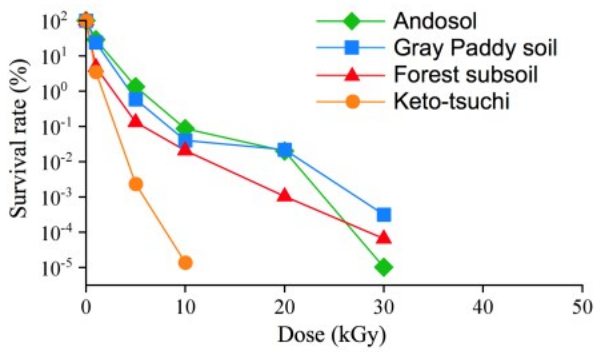


Fig. 1
Survival rates of native microbes in the different kinds of soil after gamma-irradiation. Survival microbes were not detected after 50 kGy of irradiation.

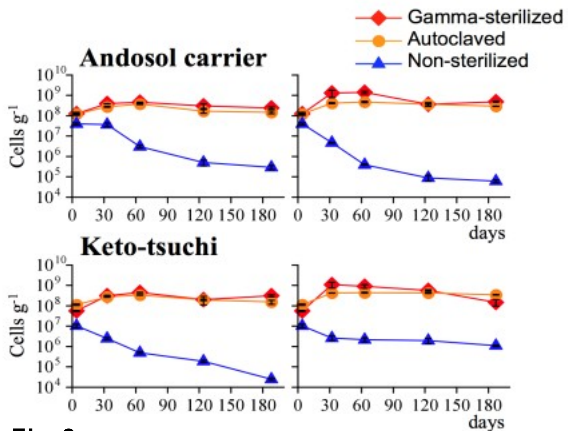


Fig. 2
Survival of rhizobial inoculants in biofertilizer stored at 4°C (left) and 30°C (right). Error bars indicate ±SE around means.

Country News

Biofertilizer Activities in Bangladesh

Md. Saidul Islam, Bangladesh Atomic Energy Commission (BAEC)

Biofertilizer is becoming very popular in Bangladesh compared to chemical fertilizer for its easy availability andecofriendly nature to the environment. The following organizations are involved in biofertilizer activities in the country: Bangladesh Institute of Nuclear Agriculture, National Institute of Biotechnology, Bangladesh Agricultural University, Bangladesh Agricultural Research Institute etc.

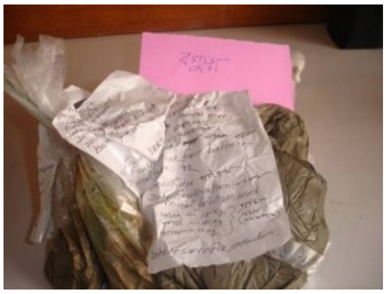
A biofertilizer demonstration field of soybean treated with and without inoculum

◆Status of biofertilizer development in Bangladesh◆

1. Rhizobial biofertilizer for pulse and oil seed legume (lentil, chickpea, mungbean, cowpea, black gram, groundnut and soybean): Intensively studied and best developed in Bangladesh and is a mature technology.
2. Azolla for wet land boro rice: Mature technology (not in practice).
3. Phosphorus solubilizing bacteria (PSB): On-farm evaluation going on.
4. *Azospirillun* / *Azotobacter* / blue green algae: Evaluating at field condition.
5. Mycorrhiza: Evaluating at pot and field condition.

A rice field of Dhamkuri village, Nowgaon District

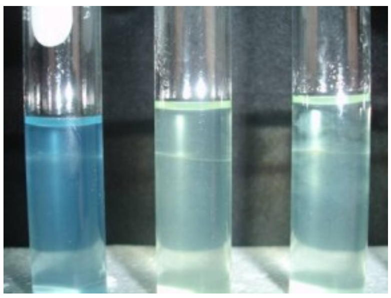
Sample collection in poly bag



Collected Sample in Laboratory

Sample preparation

Weighing of rice root



Bacteria in semi solid medium

Study on High Effective Phosphate-solubilizing and Multifunctional Biofertilizers in China

Fan Bingquan, Chinese Academy of Agricultural Sciences (CAAS)

1. Recent research of biofertilizer in our group

In order to increase effects of P-solubilizing biofertilizers on plant yield, our studies are focusing on the optimum combination of P-solubilizing strains with various soils and crop plants in China. The test crops are soybean, paddy rice, wheat and corn, the test soils are Chao soil, Drab soil, ALBISOL, Black Soil, and Dark Brown Chernozemic Soil. Phosphate-solubilizing strains are P8, Pn1, A6, P21，P24-B1，P40-2，P36-1，P40-1，P41-3A，P31，10243，P2-1A，10245，11107, RN5, *Pantoea ananatis* and *Bacillus subtilis.* Thirty optimum combinations of phosphate-solubilizing strains with 5 types of soils and 4 crop species in North China and North-Eastern China are listed in Table 1.

The result showed that there exists significant suitability among P-solubilizing microbes with crops and soils. Strain P36-1 is suitable for wheat on Chao soil; strain P21 is suitable for wheat on Drab soil; strain P36-1 and P40-1 are suitable for corn on Chao soil; strain P2-1A is suitable for corn on Drab soil; strain 10245 and P41-3A are suitable for corn on ALBISOL; strain P40-2 is suitable for corn on Black soil; strain P30-1 is suitable for corn on Dark Brown Chernozemic soil; strain P2-1A+RN5 is suitable for soybean on Black soil; and strain 11107+RN5 is suitable for soybean on Drab soil; *Pantoea ananatis* is suitable for soybean on Dark Brown Chernozemic soil; and *Bacillus subtilis* is suitable for Paddy rice on Dark Brown Chernozemic soil.

2. Development of multifuctional Biofertilizers in China

Some multifunctional biofertilizers are developed. The test strains are (1) *Klebsiella pneunoniaee*; (2) *Penicillium oxalicum* P8; (3) *Bacillus mucilaginosus*; (4) *Trichederma longbrachiatum;* (5) *Trichoderma onigii*; (6) *Trichoderma citrinoviride*; (7) *Bacillus mucilaginosus*; (8) *B. megaterium* (Ba5); (9) *B. megaterium* (ATCC14581); (10) *B. circulans*. Test crops are soybean, corn and rice. Test soils are Chao soil and Dark Brown Chernozemic soil. We have gotten 10 kinds of high effective multifunctional biofertilizers (**Table 2**).

The results showed that the combination of *Azotobacter chroococcumm*, *Penicillium oxalicum* P8, *Bacillus mucilaginosus* with rice plant in Dark Brown Chernozemic soil achieved a high biomass yield. The combination of *P. oxalicum* P8, *T. longbrachiatum, T. citrinoviride,* and *T. konigii* with soybean in Chao soil got a highest biomass yield. The combination of *P. oxalicum* P8, *T. longbrachiatum, T. citrinoviride,* and *T. onigii* with soybean in Dark Brown Chernozemic soil was the best one. The combination of *K. pneunoniaee, P. oxalicum* P8, *B. mucilaginosus , T. longbrachiatum, T. citrinoviride,* and *T.* konigii with corn in Chao soil had a marked effect on corn biomass.

Interesting topics of biofertilizer in China are (1) to develop P-solubilizing biofertilizer; (2) to develop nitrogen-tolerant N-fixing biofertilizer; (3) to develop plant growth-promoting biofertilizer; (4) to develop antagonistic biofertilizer; and (5) to develop silicate-dissolving biofertilizer.

Table 1. Optimum combination of high effective P-solubilizing microbe with various soils and crops

Crop	Soil type	Strain	Fresh Wt(g)	Dry Wt(g)
wheat	Chao soil	P30-1 P36-1	5.5 5.6	0.6 0.7
wheat	Drab soil	P21 P24-B1 P40-2	6.1 5.6 5.3	0.8 0.7 0.7
Corn	Chao soil	P36-1 P40-1 P41-3A P31 10243	15.6 15.6 15.8 15.6 15.9	1.38 1.36 1.3 1.3 1.3
Corn	Drab Soil	P2-1A 10245 P41-3A P31	15.0 13.0 12.8 12.8	1.3 1.1 1.2 1.1
Corn	ALBISOL	10245 P41-3A	11.1 11.1	1.1 1.1
Corn	Black Soil	P30-1 P41-3A P40-2	8.5 8.2 8.7	0.8 0.8 0.9
Corn	Dark Brown Chernozemic Soil	P30-1 10245 P21 10243	8.2 7.7 7.5 7.4	0.9 0.8 0.8 0.9
Soybean	Black soil	P2-1A+RN5 P41-3A+RN5	12.6 9.5	1.8 1.3
Soybean	Drab Soil	P41-3A+RN5 P40-1+RN5 11107+RN5	10.4 10.3 12.7	1.4 1.5 1.8
Soybean	Dark Brown Chernozemic soil	*Pantoea ananatis*	6.74	0.60
Paddy rice	Dark Brown Chernozemic soil	*Bacillus subtilis*	6.31	1.27

Table 2. Effects of high effective multifuctional biofertilizers in different soils and crops

Strain combinations	Crop	Soil type	Dry Wt (g)	Fresh Wt (g)
K. pneunoniaee, T. longbrachiatum, Trichoderma.konigii,T. citrinoviride	soybean	Chao soil	3.37	17.0
B. mucilaginosus, T. longbrachiatum, T. citrinoviride, T konigii	soybean	Chao soil	3.34	18.5
P. oxalicum P8, *T. longbrachiatum, T. citrinoviride, T. konigii*	soybean	Chao soil	4.49	20.0
K. pneunoniaee, P. oxalicum P8, *B.mucilaginosus , T. longbrachiatum, T. citrinoviride, T. konigii*	soybean	Chao soil	3.34	18.75
B. mucilaginosus, T. longbrachiatum, T. citrinoviride, T. konigii	soybean	Dark Brown Chernozemic soil	4.18	18.5
P. oxalicum P8, T. longbrachiatum, T. citrinoviride, T. konigii	soybean	Dark Brown Chernozemic soil	4.49	20.00
K. pneunoniaee, P. oxalicum P8, *B.mucilaginosus , T. longbrachiatum, T. citrinoviride, T. konigii*	corn	Chao soil	0.67	4.53
P. oxalicum P8, *T. longbrachiatum, T. citrinoviride, T.konigii*	corn	Dark Brown Chernozemic soil	0.53	4.31
Azotobacter chroococcumm+chem, Penicillium oxalicum P8, *Bacillus mucilaginosus*	rice	Dark Brown Chernozemic soil	3.31	17.5
Trichederma longbrachiatum, Trichoderma onigii, Trichoderma citrinoviride	rice	Dark Brown Chernozemic soil	2.87	15.3

Mutant Induction of Mineral Phosphate Solubilizing *Pantoea* by Radiation

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

Three mineral phosphate solubilizing bacteria (PSB) were isolated from common bean rhizosphere and dry field soil. 16S rDNA analysis indicated that the isolate P2 and P3 are closely related to *Pantoea dispersa* while isolate P4 is *Pantoea terrea*. Isolates P2 and P3 exhibited stronger mineral phosphate solubilizing (MPS) activity than that of isolate P4. Unlike P2 and P3, prolonged incubation of P4 in Pikovskaya (PVK) and National Botanical Research Institute's Phosphate (NBRIP) liquid growth media exhibited poor P solubilization as well as increase in pH towards neutral. Production of gluconic acid through direct oxidation pathway of Glucose (DOPG) by these P solubilizing isolates was confirmed by reverse phase HPLC. Specific activity of Glucose dehydrogenase and gluconic acid production by isolate P4 was significantly higher than that of P2 and P3. However, high P solubilization potential of P2 and P3 indicated the possible involvement of other P solubilizing mechanisms. Mutant clone libraries for phosphate solubilizing strains were created by gamma radiation induced mutagenesis and screened for modified MPS activity. Mutant P2-M1 recorded the highest P-solubilizing potential among any other wild or mutant clones by releasing 504.21 μgml^{-1} of phosphorous i.e. 35% higher than its wild type by the end of day 5. A comparative evaluation of TCP solubilization by wild type isolates of *Pantoea* and their mutants, led to select three MPS mutant clones such as P2-M1, P3-M2 and P3-M4 with a potential to release > 471.67 μgml^{-1} of phosphorous from TCP. We propose that mutants like P2-M1, P3-M2 and P3-M4 with enhanced MPS activity could be deployed for biofertilization.

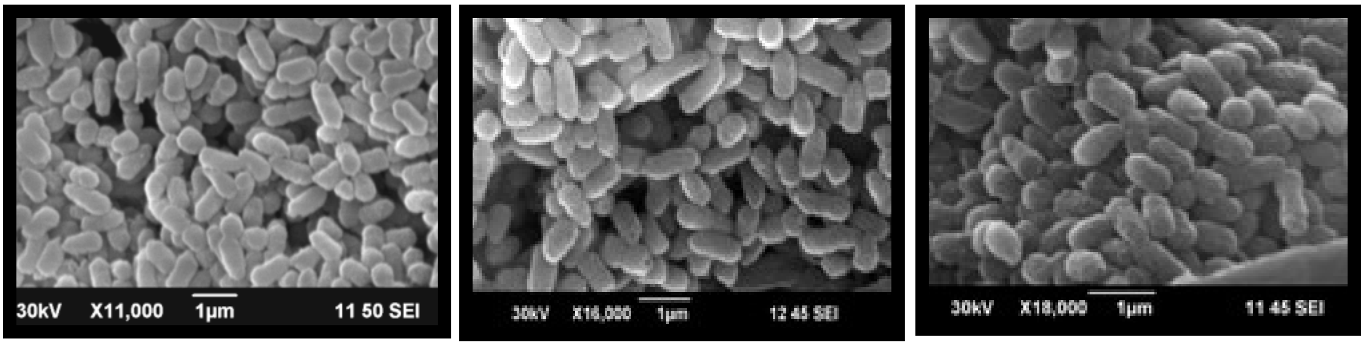
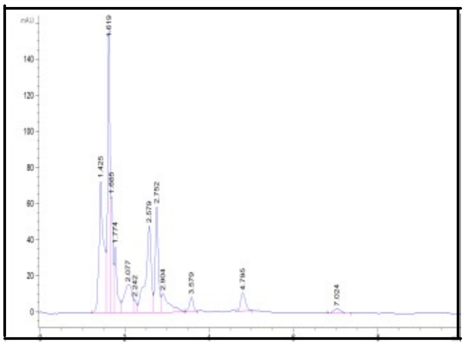
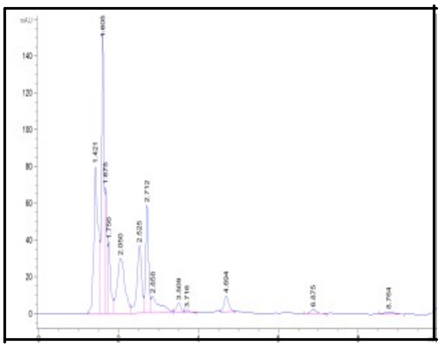


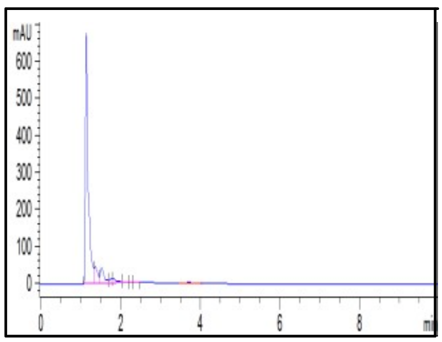
Fig. 1. Scanning electron micrographs of *Pantoea* strains (a) P2, (b) P3 and (c) P4.



***Pantoea dispersa* strain P2**



***Pantoea dispersa* strain P3**



***Pantoea terrea* strain P4**

Fig. 2. HPLC detection of gluconic acid production by *Pantoea* isolates

Bright Outlook for Biofertilizer in Malaysia

Khairuddin Abdul Rahim, Malaysian Nuclear Agency (Nuclear Malaysia)

Looking at the increasing entries of innovative biofertilizer and bioorganic fertilizer products at innovation expositions and agricultural exhibitions in the country for the past few years, one would feel the breath of fresh air in terms of the acceptability of such products for the Malaysian agricultural industry. It is also heartening to note that the giant plantation industries of oil palm and other commodity crops like rubber and rice are taking biofertilizer and bioorganic fertilizer seriously, although they have been using chemical fertilizers for increased crop productivity since the sixties. Chemical fertilizers and other agrochemicals have been ingrained in the country's agricultural programs, and this include in the provision of fertilizer subsidies for smallholders and individual farmers of selected industrial crops.

With increasing awareness on food and environmental safety from injudicious use of chemical fertilizers and other agrochemical inputs, notwithstanding the increasing prices of petroleum-based fertilizers and other chemicals, the time has come for the bioorganics and the functional indigenous microorganisms to come into the picture, with their own roles in crop productivity, environmental safety and general societal wellbeing. The platforms of Green Technology and Waste-to-Wealth (W2W) are now available for the advancement of biofertilizers and other natural products through new industrial initiatives. Agricultural wastes from large oil palm plantations, in the forms of empty fruit bunches (EFB) after the oils and oleo chemicals have been pressed out of them, previously an almost unwanted and problematic organic mountains, and the often foul-smelling fermenting palm oil mill effluent (POME) in vast, space-consuming ponds, are now being treasured. They are now the bases for new high-value products, including as carriers and ingredients in bioorganic fertilizer formulations. The plantations are now scrambling to build their own institu biofertilizer and bioorganic fertilizer plants.

The use of nuclear technology, particularly in the form of gamma irradiation sterilization of media and substrates for biofertilizer products has been adopted by several companies and enterprises. The factors of sterilization time for bulk samples in pre-packed bags or containers and energy cost make gamma sterilization attractive. However, the factors of logistics, including escalating transportation cost have to be considered, too. The available gamma irradiation facilities in Malaysia may not be able to cope with increasing demand for sterilization services in the future.

On the success of from laboratory to market, we are pleased to note that Nuclear Malaysia has undertaken to supply fresh cultures of one of her biofertilizer microorganism strains for an agreed period at an agreed fee, to Malaysian Agri Hi-Tech, an enterprising and modern agricultural bioproduct company, which also utilizes Nuclear Malaysia's gamma irradiation facility as part of their production. This shows there is hope for R&D to Technology Transfer Commercialization in the biofertilizer arena.

Best wishes to all!

Development of Multi-functional Biofertilizer in Thailand

Sompong Meunchang and Achara Nuntagij, Department of Agriculture (DOA)

The increased crop yields are widely observed by the biological N_2–fixing, by the biological P and K solubilization, and by the biological antagonistic associations, even thought by increasing capability of plant nutritional adsorption. These multimode and natural functions of microorganisms are able to promote plant growth and yield production.

The multi-functional biofertilizer might be developed from some microbes that have multi-mechanism. Some N_2-fixing bacteria can increase soil N utilization, increase solubilization efficiency of fixed soil P or phosphate rock P, and increase efficiency of use of fixed clay soil K or potassium rock. The product might be produced from single strain, multi-strains or multi-genus co-inoculation.

General procedure of developing multi-functional biofertilizer production is as follows.

1) Selection or screening of effective microbes. We must approve the special ability in laboratory, in pot or green house experiment and in field trial.
2) In case of co-inoculation, we have to ensure the impact of co-inoculation, as if strains do not inhibit or damage each other. Not only loss of population but also the point of activity should be investigated, too.
3) Proper carriers should be discovered such as peat soil, charcoal, compost and other materials. They might be sterilized by autoclave or irradiation.
4) The distribution or commercialization is also should be approved.
5) The utilization on each planting system should be also considered after farmers' acceptance.

In Thailand, multifunctional biofertilizer with co-inoculants of *Azospirillum* and *Bradyrhizobium* for leguminous plants are developing. At first, to select effective strains, we screened *Azospirillum* isolates respond with *Bradyrhizobium* specific for vegetable soybean. We tried pot experiment inoculated with liquid culture of each isolates, and compared *Azospirillum* inoculation, *Bradyrhizobium* inoculation, and co-inoculation (Fig. 1 and 2). To confirm the effectiveness of co-inoculation, we repeated in field experiments. Finally, we designed small scale production in 100 g bag with sterilized carriers by autoclaving or grammar irradiation and estimated that inoculants could survive for one year.

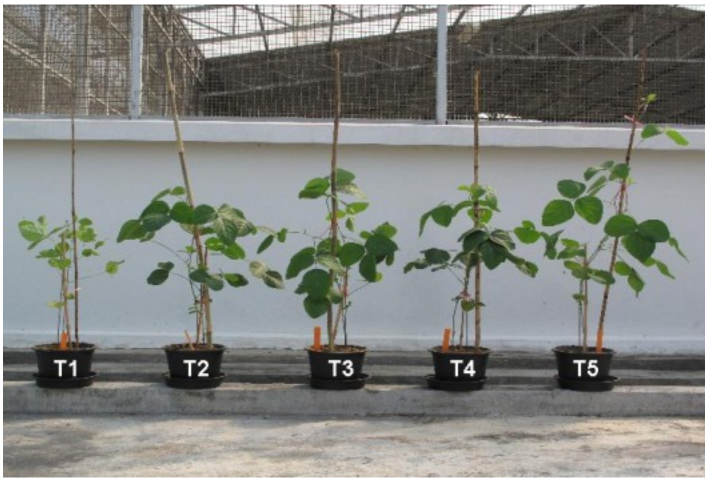


Fig. 1 Different growth of vegetable soy bean after inoculation multifunctional biofertilizer; T1 control, T2 NPK, T3 *Bradyrhizobium*+PK, T4 *Azospirillum*+PK T5 *Bradyrhizobium*+ *Azospirillum*+PK

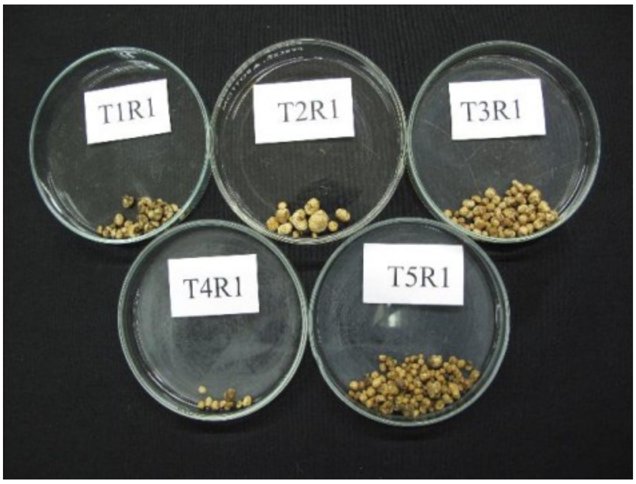


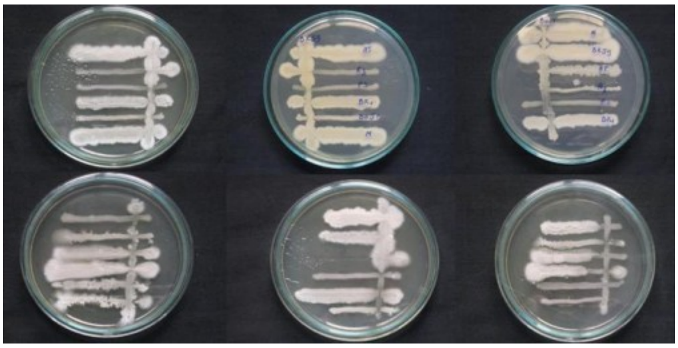
Fig. 2 Nodulation of vegetable soy bean after inoculation multifunctional biofertilizer; T1 control, T2 NPK, T3 *Bradyrhizobium*+PK, T4 *Azospirillum*+PK T5 *Bradyrhizobium*+ *Azospirillum*+PK

FNCA Biofertilizer Project
Biofertilizer Research and Development in Vietnam

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

◆ **Interesting Topic of Biofertilizer**◆

1. Biofertilizer containing N-Fixing microorganisms
2. Biofertilizer containing P. Solubilizing microorganisms
3. Biofertilizer consist of K. Solubilizing microorganisms
4. Biofertilizer containing PGPR microorganisms
5. Biofertilizer containing Mycorhiza
6. Biofertilizer containing microorganisms, which are antagonistic to root diseasse pathogens
7. Organic fertilizer containing benifit microbes like N-fixing, P,K-Solubilizing and PGPR
8. Biofertilizer containing microbes, which improve soil properties like water holding capacity



Screening of microorganisms using in biofertilizer production

Production of organic biofertilizer

Application of biofertilizer

Topic of Japan & Thailand

Massage from Thai Researcher Lives in Japan

Kunlayakorn Prongjunthuek, Department of Agriculture (DOA)

My name is Kunlayakorn Prongjunthuek, I come from Thailand. I work at Soil Microbiology Research Group, Soil Science Division, Department of Agriculture. My research field is about PGPR bio-fertilizer (PGPR = Plant Growth Promoting Rhizo Bacteria). I am participate in MEXT Nuclear Researchers Exchange Program Fellows in 2010.

My research theme is Indonesia, Japan and Thailand Azospirillum sp. phylogenetic relation and Mutation breeding of Azospirillum sp. using ion-beam facility in term of long self life in carrier. In screening I studied for IAA production ability and DNA isolation. After that I choose some and send to JAEA for irradiation. My research is in the level of intensity of radiation used in the screening for the mutation of microbes. This now is in the summary.

I used to dream that I could be here – Japan the land of civilization, cultures and unique language which people in the world dream of visiting this wonderful land once in their lives. Now I have been here for one year working as an exchange researcher in the Tokyo University of Agriculture and Technology (TUAT), Fuchu, Tokyo. This university is a famous university for agriculture and engineering.

As soon as I stepped on this place, I saw an enormous farm, greenhouse experiments that are lined up, a great number of trees along the path, and high buildings surrounding with various trees. I could feel of the pleasant and peaceful atmosphere. These are my great impression and they make the university worth being the University of Agriculture. It is unbelievable that big city like Tokyo has the university with a great number of big trees. It can enounce that ones who love trees will love this university like I do.

I do not only impress in the atmosphere of this university but also in a lot of various persons, my Lab mate, professional who is very kind and warm, and my friend both Japanese and other overseas. They all are lovely and helpful. They also advice and teach me everything I do not know, especially Japanese cultures and tradition. We did many activities of the lab together including selling things in booth in the traditional fair of the university. These help me not to feel lonely while living in foreign country. I love my professor and my friend very much.

Apart from what I said above. I would like to say that living in this university is so simple and convenient that it becomes my very impressing thing that cannot described. I promise that I am going to visit you again TUAT, Fuchu, Tokyo.

Ms. Prongjunthuek with Members of Tokyo University of Agriculture and Technology

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