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

FNCA Biofertilizer Newsletter

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Overview of FNCA Biofertilizer Project 2012

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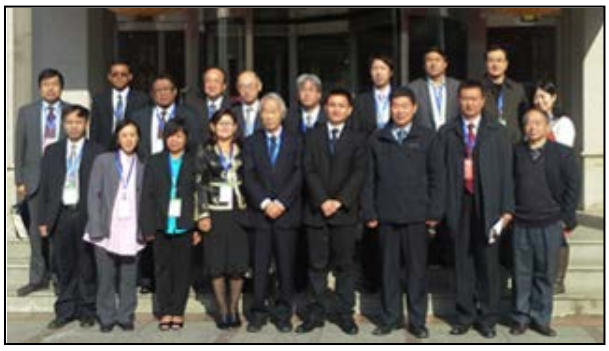
In order to promote environmental friendly sustainable agriculture in Asia, FNCA Biofertilizer Project aims to develop biofertilizers with radiation sterilization technology, using benefical microorgamisms, which increase the yields of crops while reducing the environmental burden of excessive use of chemical fertilizers.

At the first phase of this project from 2001 to 2006, FNCA Biofertilize Manual was published, which gives information and experiences of biofertilizer use in Asian countries, including their effectiveness, efficient production processes, storage and application on different crops, as an important outcome.

At the second phase from 2007 to 2011, main objectives are developing multifunctional biofertilizer having both function of plant growth promoting and resistance against plant pathogens, and dissemination of radiation sterilization method of carrier using ^{60}Co to improve quality of carrier for biofertilizers.

From 2012, new stage of second phase was started to enhance above 2 objectives and it is also focused on synergy effect between biofertilizer and irradiated oligochitosan as a new theme.

FNCA 2012 Workshop on Bifertilizer Project was held on November 6th - 9th in Beijng, China and 13 participants from 8 countries participated.

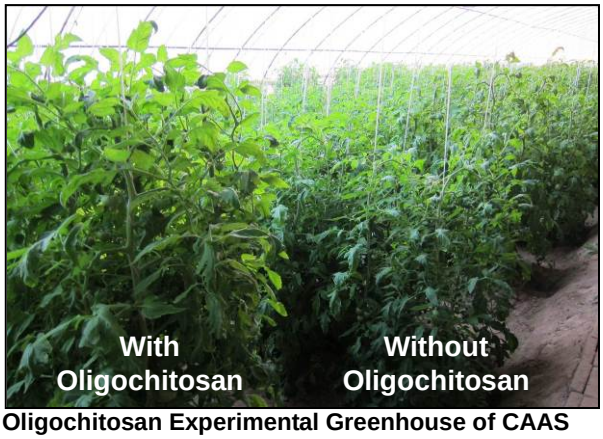


Participants of the Workshop

At the workshop, summaries of research activities in 2012 were reported by the participating countries. In addition, a discussion was held on project acitivity for 2013 - 2016, and agreements were made on application of radiation sterilization of carriers and challenges, development of multi-functional biofertilizer, development of FNCA guideline for biofertilizer quality analysis, and evaluation and activity plan of synergy effect between biofertilizer and irradiated oligochitosan.

For application of radiation sterilization of carriers, it was confirmed that the Philippines started to use gamma irradiation sterilization for commercial production of biofertilizer in October 2012. Further efforts to enhance the use of gamma sterilization of carrier for commercial application will be done by other countries.

The participants visited laboratories of Chinese Academy of Agricultural Sciences (CAAS) and research base on Daxing District of Beijing. They saw positive effect for oligochitosan experiment on strawberry and tomato in greenhouse. Then they visited a biofertilizer company located in Tongzhou District of Beijing and got an introduction of its products including biofertilizer.



Oligochitosan Experimental Greenhouse of CAAS

Development of Biofertilizers for Sustainable Agriculture in China

Fan Bingquan, Chinese Academy of Agricultural Sciences (CAAS)

We have done some research activities, first we isolated high effective P-solubilizing, N-fixing, antagonistic and herbicides-degrading microbe; secondly, we have done broth and pot experiments to evaluate the abilities of P solubilization, N fixation.

1. Isolation of high effective strains

1.1 Isolation of P-sulubilizing microbe

Eight P-sulubilizing fungi were isolated from arable soils in North China, they are Px1, P47, P41, P439, PS6, 2GW-5, K91 and Psf-1. Six P-sulubilizing Bacteria were isolated which are P21-3a, JP3-12, H16-6, Y5-2, Rong1 and CC2d.

1.2 Isolation of N-fixing Bactria

Eleven associated N-fixation bacteria were obtained from various soils. The strains are H29, H8, Jing5, G-N, GDN, K7, YM3, Xin2, Gl-1, New2 and S1. Strain identification will be done recently.

1.3 Isolation of antagonistic microbe

Seven antagonistic Bacteria against soil-born diseases were isolated from soils in Northeast China. They are Bacillus sp K4, Paenibacillus xylanexedens K2, Bacillus weihenstephanensis K5, Brevundimonas sp. K6, Brevundimonas sp. H7 Bacillus sp. K10 and Tetrathiobacter sp. J5.

1.4 Isolation of herbicides-degrading strains

Two herbicides-degrading fungi were obtained from herbicides polluted soils in Jilin. The strains are Trichoderma sp. C05 which degrade Chlorimuron-ethyl and Penicillium sp. Cy01 which degrade acetochlor.

Three herbicides-degrading Bacteria were isolated from polluted soils in Southern China. They are Mycobacterium sp. H2, Mycobacterium sp F1 and Mycobacterium sp. F2.

2. Results of N-fixing and P-solubilizing microbe

2.1 Effect of P-solubilizing microbe on insoluble phosphate solubilization

There are 11 treatments in the pot experiment containing 10 strains Px1, P47, P41, P36, K91, P62, P439, PS6, Rong1, CC2d and a control treatment (CK). In broth assay, Strain Px1 demonstrated higher efficiency to dissolve rock phosphate (RP). Strains of Px1, P47, P41, P36, K91, P439, PS6, Rong1, CC2d have got a more than 10% of RP sulubilization rate. Only P62 strain have about 10% of RP sulubilization rate in liquid medium (Table 1).

Table 1. Effect of 10 P-solubilizing strains on rock phosphate in liquid medium in 5 days

Strain	Rock Phosphate (P_2O_5%)	Available P (μg/ml)	Available P (mg/30ml)	Solubilized P %
Px1	33.4	612.2 a	18.36 a	12.10 a
P47	33.4	590.4 a	17.71 a	11.67 a
P41	33.4	565.3 ab	16.96 ab	11.17 a
P36	33.4	600.2 a	17.99 a	11.8 a
K91	33.4	558.3 ab	16.75 ab	11.03 a
P62	33.4	503.8 b	15.11 c	9.96 b
P439	22.5	353.1 c	10.59 c	10.36 b
PS6	22.5	388.2 b	11.65 b	11.39 ab
Rong1	22.5	449.1 a	13.7 a	13.40 a
CC2d	22.5	418.6 b	12.56 b	12.28 a

2.2 Effect of six P-solubilizing strains on corn biomass in pot experiment

There are 10 treatments in pot experiment, which are CK (RP), Ba5, ATCC20851, Hi6, JP3-12, K91, P403, P21-3a, Pqu and 2GW-5. The result of showed that application of 9 strains improved crop growth and increased crop biomass in vermiculite carrier combined with $Ca_3(PO_4)_2$ and rock phosphate, separately. Strain P403 have a highest biomass of 14.70 g/pot (fresh weight) and 1.56 g/pot(dry weight) for .

Strain Hi6, JP3-12, K91 and P403 got a high corn biomass in $Ca_3(PO_4)_2$ source that indicated these 4 strain have a higher ability to solubilize $Ca_3(PO_4)_2$. Strain K91, P403, Pqu and 2GW-5 increased corn biomass significantly in rock phosphate, that indicated these 4 strain have a higher capacity to solubilize rock phosphate and provide more available phosphorous than other strains(table 2).

2.3 Effect of high effective N-fixing Bactria on crop biomass

Seven N-fixing Bactria were used to compare the effects of N fixation and yield-increasing in pot experiments. The 7 associated N-fixation bacteria are H29, H8, Jing5, G-N, GDN, K7 and YM3, and sterile ATCC10084 fermentation btoth was used as control (CK).

Strain H29, H8, Jing5, G-N, GDN, K7 and YM3 increased white corn biomass significantly than control (CK). Strain H8, Jing5, GDN, K7 and YM3 increased red corn biomass significantly than control (CK). Strain H29, H8. Jing5, G-N and GDN increased wheat biomass significantly than control (CK).

3. Effects of carrier irradiation on survival rate of beneficial microbe

Five kinds of carriers including peat, corn cobs, perlite, CMF and 1:1 corn cobs/perlite mixture were used in the present study. ^{60}Co gamma irradiation was done at doses of 50 kGy and at a dose rate of 15 Gy/min. Autoclaving was done for 40 min at 121^{o}C.

The number of strain 1107 in the carriers was enumerated by plate count. Fig. 1 shows the survival of A. niger strain 1107 in various carriers sterilized using autoclaving or gamma-irradiation storage at 4^{o}C. Most of the carriers (except CMF) sterilized by gamma-irradiation gave better survival rate of inoculum during the initial 5 - 6 months shelf-life than those sterilized by autoclaving, while this benefit was not observed after 6 months.

Table 2. Table 2 Effect of P-solubilizing strains on corn biomass applied with insoluble phosphates in pot experiment (g/pot).zhou city (kg/666.7m^2)

P source / Strain	$Ca_3(PO_4)_2$		Rock phospate	
	Fresh Wt	Shoot Wt	Fresh Wt	Shoot Wt
Ck(RP)	2.70 d	0.26 d	3.10 d	0.53 bc
Ba5	6.07 c	0.71 c	2.98 d	0.35 d
ATCC20851	7.28 bc	0.56 c	7.27 b	0.69 b
Hi6	8.84 bc	1.05 b	4.73 d	0.44 d
JP3-12	9.40 b	0.95 b	5.16 c	0.58 bc
K91	9.70 b	1.01 b	9.96 a	0.80 a
P403	14.70 a	1.56 a	8.8 a	0.81 a
P21-3a	6.82 c	0.78 c	4.93 d	0.39 d
Pqu	7.33 bc	0.93 b	8.27 a	0.82 a
2GW-5	6.08 c	0.39 d	6.25 c	0.82 a

RP: means rock phosphate

Table 3. Crop biomass inoculated with different strains in pot (g/pot)

Crop / Strain	White Cron		Red corn		wheat	
	Fresh Wt	Shoot Wt	Fresh Wt	Shoot Wt	Fresh Wt	Shoot Wt
CK (sterile ATCC10084)	13.6b	1.50b	10.4 b	0.98 b	5.06 b	0.89 b
H29	18.0a	1.82a	11.2 ab	0.96 b	5.99 a	0.99 a
H8	17.6a	1.79a	13.8 a	1.25 a	6.20 a	0.95 a
Jing5	16.2a	1.78a	12.8 a	1.16 ab	6.63 a	1.01 a
G-N	17.6a	1.86a	11.2 ab	0.97 b	5.75 ab	0.99 a
GDN	18.4a	1.86a	13.2 a	1.08 ab	5.90 a	1.00 a
K7	19.2a	1.96a	14.4 a	1.13 ab	5.86 a	0.79 b
YM3	17.8a	1.81a	14.6 a	1.23 a	5.41 ab	0.94 a

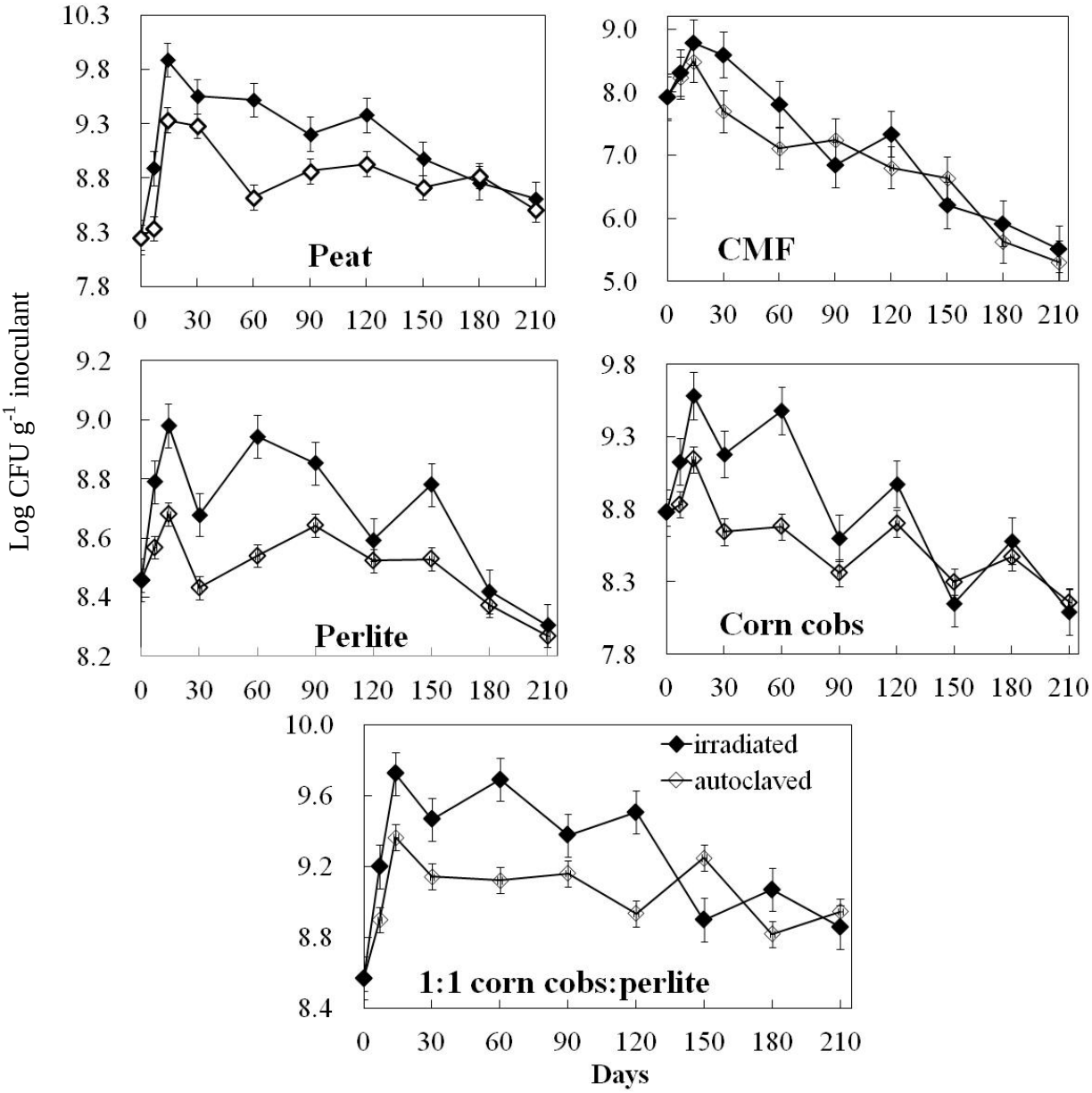


Fig.1. Survival of *A. niger* strain 1107 in various carriers sterilized using autoclaving or gamma-irradiation prior storage at 4°C. Each point represents the mean of three replicates. Vertical bars represent ±1S.D.

Effect of Oligochitosan, Vitazyme, Biofertilizer on Growth and Yield of Rice

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National Nuclear Energy Agency and Bogor Agricultural University, Indonesia

The aim of the experiment was to evaluate the effect of oligochitosan, Vitazyme, biofertilizer Azozo and their combination on growth and yield of rice cultivar Ciherang. Two kinds of oligochitosan were tested in this experiment i.e. prepared by FNCA Japan and prepared by Batan (Indonesian National Atomic Agency). Vitazyme is plant growth promoting substances and Azozo biofertilizer prepared by Laboratory of Soil Biotechnology IPB. The treatment tested were:

(1) Inorganic fertilizer NPK 100%, (2) Inorganic fertilizer NPK 50%, (3) Inorganic fertilizer NPK 50% + *Oligochitosan* (Japan), (4) Inorganic fertilizer NPK 50% + *Biofertilizer* Azozo, (5) Inorganic fertilizer NPK 50% + *Oligochitosan* (japan) + *Biofertilizer Azozo*, (6) Inorganic fertilizer NPK 50% + *Oligochitosan* BATAN, and (7) Inorganic fertilizer NPK 50% + *Vitazyme*. These treatments had four replicates. Rice seedlings were grown in pots containging 10 kg of soil.

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The dosage of inorganic fertilizer NPK was 200ppm N, 100ppm P and 100 ppmK. Oligochitosan was applied at 4 week (20 ppm) and 8 weeks (30ppm) after transplanting and 1% Vitazyme was applied at 4 and 8 weeks after transplanting. Root application of biofertilizer was applied at transplanting.

Results show that the number of tillers at the treatment NPK 50% was significantly lower than the number of tillers at the treatment 100% NPK. This means that application of inorganic fertilizer 50% NPK did not fulfill the NPK requirement by rice plant (Table 1). However, addition 50% inorganic NPK fertilizer in combination with application of oligochitosan, Vitazyme and biofertilizer increased the number of tillers (Table 1) and yield of rice significantly (Table 2). These means that oligochitosan, Vitazyme and biofertilizer inreased NPK uptake by rice plants with similar effect of application of 100% inorganic NPK fertilizer.

Conclusion

Oligochitosan, Vitazyme, and biofertilizer Azozo increased inorganic NPK fertilizer efficiecy.

Table 1. Effect of *Oligochitosan*, *Biofertilizer* and *Vitazyme* on number of tillers per pot

Treatments	Number of tillers			
	2 WAT	4 WAT	6 WAT	8 WAT
	. . . . tillers/hill....			
NPK 100%	**4.25a**	**18.00a**	**32.25a**	**32.25a**
NPK 50%	1.75b	14.00a	16.75a	14.50c
NPK 50% + *Oligochitosan* (J)	2.25b	14.75a	26.00a	22.50bc
NPK 50% + *Biofertilizer* (A)	2.50b	13.50a	23.75a	20.50bc
NPK 50% + *Oligochitosan* (J) + *Biofertilizer* (A)	2.00b	11.33a	22.33a	22.67bc
NPK 50% + *Oligochitosan* (B)	2.00b	13.00a	21.75a	21.00bc
NPK 50% + *Vitazyme*	3.00ab	17.25a	30.75a	28.00ab

WAT =weeks after transplanting (J-Japan; B =Batan)

Table 2. Effect of *Oligochitosan*, *Biofertilizer* and *Vitazyme* on Yield of Rice

Treatments	Yield g/pot
NPK 100%	**46.25a**
NPK 50%	20.32b
NPK 50% + *Oligochitosan* (J)	40.13a
NPK 50% + *Biofertilizer* (A)	37.50a
NPK 50% + *Oligochitosan* (J) + *Biofertilizer* (A)	36.43a
NPK 50% + *Oligochitosan* (B)	35.02a
NPK 50% + *Vitazyme*	40.69a

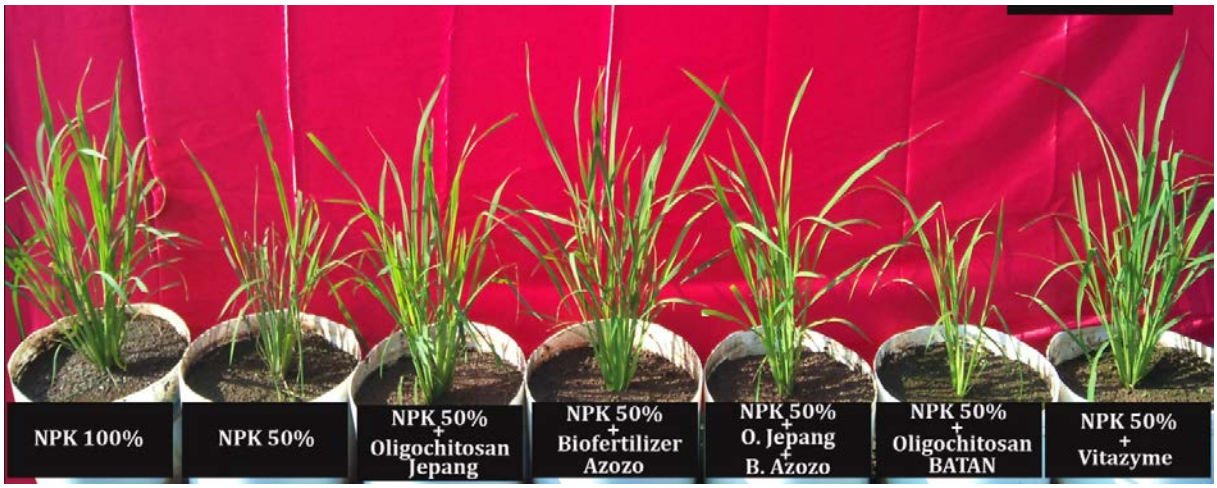

Figure 1. Effect of inorganic NPK fertilizer, *Oligochitosan*, *Biofertilizer* and *Vitazyme* on growth of rice plant

Activities Supporting the Advancement of Malaysia's Biofertilizer Industry

Phua Choo Kwai Hoe, Khairuddin Abdul Rahim, Ahmad Nazrul Abd Wahid
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There has been an increasing number of small-scale farmers and even the established plantation industry in Malaysia that have shown keen interest on biofertilizers and bioorganic fertilizers as their agricultural inputs. At the same time, there are many products claimed as "biofertilizers" in the market. However, most of these products are found to be only bioorganic fertilizers or organic fertilizers. Empty fruit bunches (EFB) – waste products from the oil palm industry are the major materials for composting and producing organic fertilizers. Many of these producers have made claims that their products are biofertilizer products. Due to the market demands, the price of EFB has increased. Apart of that, currently there are more research institutions focusing on biofertilizer development. Thus, there seems to be an increase on public acceptance of biofertilizers, organic fertilizer or bioorganic fertilizers in the country.

In Malaysia, gamma irradiation has been used by universities, research institutions and bioorganic fertilizer companies for substrates or carrier sterilization. Malaysian Agri Hi-Tech Sdn. Bhd. (MYAGRI) is one of Malaysia's biofertilizer companies actively utilizing the gamma irradiation facility of Malaysian Nuclear Agency (Nuclear Malaysia), the MINTec-SINAGAMA, to irradiate substrates in its bioproducts formulations. In 2012, one thousand kilogram of vermiculite had been irradiated using this facility. MYGRI utilizes one nitrogen fixing bacterial inoculant generated through R&D of Nuclear Malaysia in one of its products, agriCare®ORGANIC-N. The company produced about 40t of the product in 2011, with a reportedly increasing demand until the present. Carrier for production of MF-Biopellet, a bioorganic fertilizer product from Nuclear Malaysia was irradiated at 35 to 50 kGy for purpose of sterilization.

In 2011, Ministry of Science, Technology and Innovation, Malaysia (MOSTI) funded a total of RM 195,000.00 for Malaysian Nuclear Agency biofertilizer project with the title "Quantification of improvement of phosphorus and nitrogen nutrition of vegetable crops from phosphate solubilizing bacteria and rhizobacteria interaction using isotopic tracer technology(02-03-01-SF0051)." This two years project will be completed in year 2013. The project involved development phosphate solubilizing bacteria into various form of biofertilizer products such as pellet, liquid and seed treatment that could enhance P and N uptake and create healthy rhizosphere and increase crop productivity. Assessment of the products was implemented through greenhouse and field trials for nutrient uptake studies using ^{15}N isotope and on synergistic interaction between phosphate solubilizing bacteria with plant growth promoting rhizobacteria.

Two isolates, namely AP2 and AP3, isolated from compost samples were used in this project. Isolate AP2 is a plant growth promoter and phosphate solubilizing bacteria and isolate AP3 is a phosphate solubilizing bacteria. Both of these isolates have been found to have the ability to solubilize potassium, too. With that there is an added value to these

multifunctional biofertilizer isolates.

MF-BIOPELLET is a pellet form product containing isolates AP2 and AP3. "Natural Farming" composting EFB material was developed as a pellet-form product. This product was gamma sterilised at 50 kGy at MINTec-SINAGAMA, and used as a carier for the biofertilizer inoculum. This product had been tested on tomato, chinese cabbage, spinach, as well as on green and white mustard in greenhouse trials. Results showed the product enhanced the growth of the plants. Field trial was also conducted in Cameron Highlands, Pahang, know for its extensive vegetable farms in Malaysia. Shelf life study was conducted by storing these products at room temperatures (28 $\pm$ 2 ^{o}C) and low temperatures (9 $\pm$ 2 ^{o}C) for six months. At the end of the storage period, products kept at low temperatures showed more viable cells as compared to the products at room temperatures.

Development of biofertilizer for use in seed treatments for okra seeds were carried out by mixing isolate AP 2 and isolate AP 3 with adhesives. Maximum germination rates and log of viable cells were observed when treated with polyethylene glycol 4000 (PEG) mixed with AP2 and AP3. In a greenhouse experiment, okra seeds treatment with isolate AP2 and AP3 with PEG showed enhancement of the growth and N uptake via N_2 fixation.

The above isolates were also formulated as liquid inoculants, and introduced into a fertigation system in an effort to reduce usage of chemical fertilizers. A greenhouse trial was conducted to evaluate the effectiveness of liquid biofertilizers on tomato plants grown under a fertigation system. Result showed combination of liquid biofertilizers could reduce the usage of chemical fertilizers.

There are two ongoing greenhouse trials on rice plants. Greenhouse trial I is to study effectiveness of liquid biofertilizers on nutrient uptake, utilizing ^{15}N isotope as tracer. Greenhouse trial II is to study effectiveness of biofertilizers with irradiated oligochitosan (sample form Japan) on the growth of rice plants.

Field trial at MADA, Kedah started in October 2012. Biofertilizers were tested on two field plots of aerobic and non-aerobic rice, for the investigation of plant-soil-microbe interaction and assessment of N nutrient uptake efficiency using ^{15}N isotopic tracer.

In the future, biofertilizer development will focus on improvement of biofertilizer inoculant and investigating synergistic effects of biofertilizer and oligochitosan or other bioproducts in greenhouse or field experiments.

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MF-BIOPELLET

Fertigation system using liquid biofertilizer

Effects of biofertilizer and irradiated oligochitosan on rice plant and nutrient uptake study using ^{15}N isotopic tracer in a greenhouse trial.

Introduction of Rhizobacterial Fertilizer

Delgermaa Bongosuren, Plant Science Agricultural Research Institute (PSARI)


Rhizobacterial Fertilizer

Plant growth promoting rhizobacteria (PGPR) are free living bacteria (*Azospirillum, Azotobacter, Azoarcus*) commonly found in soils and association with roots at plants, including important agricultural crops such as wheat. There are some bacteria convert atmospheric nitrogen into soil and some bacteria help in solubilization of insoluble phosphates and improve P uptake from soil. The institute has successfully launched its biofertilizer product under the trade name of Rhizobacterial fertilizer since 2001, which reduces the input of fertilizers and increases crop yield. The Rhizobacterial fertilizer is low cost and environment friendly product and can be used to enhance all crops as well as soil fertility.

At present, the 15-20 ton bio-fertilizer produced annually under the laboratory conditions and distributed to farmers in major crop production provinces including Selenge, Bulgan, Uvs and Tuv in over 10,000 ha.

The wheat grain yield increases by 25,3-49,4 %, under irrigated and 30% under rain fed conditions. Application of 6 kg/ha Rhizobacterial fertilizer has highest grain yield of wheat.

Commercialization of Rhizobacterial fertilizer/wheat yield/

№	Provinces	Total area /ha	Yields t/ha	Yield addition	
				t/ha	%
1	Selenge province 2002-2005	2,400	1.27	0.38-0.45	49.4
2	Bulgan province 2003-2009	4,000	1.62	0.42-0.7	35.2-58.3
3	Darkhan-Uul province 2005-2011	2,000	1.94	0.21	25.3
4	Uvs province 2003-2005	1,500	2.54	0.50	30.0


Without biofertilizer With biofertilizer


Without biofertilizer With biofertilizer

Determination of Microbial Population of *Bio N*™ Carrier and Storage Through Gamma Irradiation Sterilization

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Rationale

UPLB through National Institute of Molecular Biology and Biotechnology (BIOTECH), has provided its resources in the development of alternative fertilizer technologies that enhance and sustain crop production. BIOTECH has developed microbial-based fertilizers that are safe to use and which have suggested promise in improving the socio-economic benefits of intended clients. One of the developed microbial technologies is the *Bio N* technology.

The microorganism which was isolated from the roots of the local grass talahib (*Saccharumspontaneum* L.), and further developed into an effective inoculant for rice and corn has been packaged and registered under the brand name, *Bio N*. *Bio N* is a microbial inoculant in powder form that contains two species of nitrogen-fixing bacteria which have been recognized by farmer users to improve the yield of rice and corn as well as reduce the inorganic fertilizer application for these crops. The study aims the development of better carriers for concentrates (source of the bacteria distributed to the Mixing Plants) and regular *Bio N* (ready-to-use form of the biofertilizer) using gamma irradiation sterilization of carrier. It also aims to ensure maintenance and/or improve the average population of the inoculant strains while in storage.

Methodology

Bio N inoculants and concentrates are being mass produced at BIOTECH and being reconstituted in the different *Bio N* Mixing Plant. *Bio N* carriers are composed of soil and charcoal. Production required large volume of soil and charcoal. The product has two components namely, the solid carrier (soil + charcoal) and the bacteria. The solid component is finely pulverized, mixed and steam sterilized for three days at 1 hour each day. (Figure 1).

One hundred sixty five gram packs of *Bio N* carrier (composed of soil and charcoal) were exposed to ionizing radiation at doses 0, 10, 20, 30 and 40 kilo Gray (kGy) respectively. Irradiation process was carried out at the ^{60}Co irradiation facility of the PNRI. Irradiated samples were analyzed for the presence of viable cells/spores of aerobic bacteria (Total Plate Count).The colony forming units per gram (CFUs/g) of the samples were also determined.

Fig. 1 Preparation for sterilization of *Bio N* carrier using steam sterilizer

Determination of irradiation sterilization dose of *Bio N*^{TM} carrier

The *Bio N* carrier composed of soil and charcoal are the major materials in production. The characteristic of the carrier is very essential to keep the microorganisms for longer life span and deliver to the end users with good quality. This study was carried out to improve the quality of *Bio N* by sterilizing its substrate (soil and charcoal) using gamma irradiation Table 1 shows the results of microbial count on *Bio N* carrier subjected to different doses of irradiation. The bacterial population and mold count were high on non-irradiated carrier (0kGy). On the other hand, samples irradiated at 10kGy had a mold count of < 850. Results obtained from samples of irradiated carrier at 20, 30, 40 and kGy were found to be sterile. From this result, the irradiation sterilization dose of *Bio N* carrier was set at 20kGy.

Survival and Growth of *Bio N* organisms in irradiated carrier

The carrier material is an important factor in Bio N production, since this is where microorganisms grow and multiply. The sterile carrier improves the quality of the product and survival of the microorganisms. In the production of *Bio N* carrier were sterilized by autoclaving and this is the limiting factor for large scale production.

A research project on The Use of Nuclear Techniques on the Assessment and Improvement of Biofertilizer *Bio N* was undertaken by The Philippine Nuclear Research Institute (PNRI) under its collaborative framework with the Japanese government sponsored by FNCA and collaboration with BIOTECH-UPLB. Through this collaboration sterilization of *Bio N* carrier through gamma irradiation was realized. Study was conducted to determine the survival and growth of *Bio N* organisms (Table 2). In the conventional sterilization process of *Bio N* the lifespan of the product is only six months while in the gamma irradiation the lifespan is ten months.

Comparative analysis of irradiation and heat autoclave sterilization

Table 2 shows the efficiency of using gamma irradiation technique compared to autoclave treatment. In gamma irradiation technique sterilization of carrier is sixteen thousand packets per week while in heat autoclave sterilization of carrier is five thousand packets. The texture of carrier is dry and therefore ready to use. On the contrary the cost of irradiated carrier is higher than with the heat treatment. Gamma irradiation technique is more efficient in the large scale production of *Bio N* carrier.

Concluding Remarks

Gamma irradiation has an important role to improve the quality of *Bio N* carrier and prolong the lifespan of microorganism. The use of gamma irradiation sterilization is very efficient and practical especially in large scale production.

Table 1. Microbial count of *Bio N* carrier in different doses of irradiation

Sample	Bacterial count (CFU/g)	Yeast Count (CFU/g)	Mold Count CFU/g)
Non-irradiated	1.96×10^{6}	< 1	6.7×10^{3}
10 kGy	< 1	< 1	< 850
20 kGy	< 1	< 1	< 1
30 kGy	< 1	< 1	< 1
40 kGy	< 1	< 1	< 1
50 kGy	< 1	< 1	< 1

Table 2. Survival and Growth of *Bio N* organisms in irradiated carrier

Incubation period (month)	Cell number per gram
1	1×10^8
2	1×10^8
3	$1X\ 10^8$
4	1×10^7
5	2×10^7
6	1×10^7
7	$1.8X\ 10^6$
8	2.5×10^6
9	6.6×10^6
10	7.3×10^6

Table 3. Comparison of using gamma irradiation technique with heat autoclave method for sterilization of carrier (BIOTECH-UPLB)

Particulars	Irradiated Carrier	Heat Autoclave Carrier
Efficiency	16,000 packets per week	5,000 per week
Texture of the carrier	Dry and ready to use	Wet and laborious
Cost per packet	PhP 2.70	PhP 1.03

Introduction of Bio N Carrier Sterilization Using Gamma Irradiation at PNRI 2012

Determination of irradiation sterilization dose of *Bio N* carrier

The characteristic of the carrier is very essential to keep the microorganisms for longer life span and deliver to the end users with good quality. This study was carried out to improve the quality of *Bio N* by sterilizing its substrate (soil and charcoal) using gamma irradiation.

Preparation of *Bio N* Carrier for Gamma Irradiation

1. Coordination and scheduling with the Philippine Nuclear Research Institute (PNRI) regarding gamma sterilization of carriers since they are the sole agency of the government mandated to advance and regulate the safe and peaceful applications of nuclear science and technology in the Philippines.

2. Consultation with the PNRI staff on the proper materials and positioning of the carrier for each box. The size of the box is 25 x 36 x 26 comprising of 120 packets of *Bio N* carrier equivalent to 19.8 kg per box.

3. A total of 2 tons of *Bio N* carrier was transported to PNRI for gamma irradiation for one load.

4. The dosage used in this sterilization was at 20kGy unlike the previous recommendation which was 30kGy on lower sterilization dose was conducted since the initial process in the preparation of the carrier undergone solar and oven drying that can kill/lessen the microorganisms in the soil

Samples of *Bio N* carrier submitted to PNRI for gamma irradiation

***Bio N* boxes already subjected to gamma irradiation**

Biofertilizer Application in Thailand

Phatchayaphon Meunchang and Achara Nuntagit, Department of Agriculture (DOA)

In 2012, we succeed to developed 4 types of biofertilizer. We found the specific indigenous isolates of rhizobium for 50 species of leguminous plants. The rhizobium biofertilizer for soybean peanut and green gram were produced in commercial scale for 30 years. PGPR biofertilizer were developed for use in maize, rice, sugar cane and cassava production. PGPR biofertilizer apply reduced chemical fertilizer at approximately 25% from recommendation rate. At present, we are developing novel mutant strains of Azospirillum, Azotobacter and Beijerinckia by means of mutation breeding using E beam irradiation. We selected 3 strains of each genus. They showed increase of nitrogen fixation activity at 25% rather than parent strains by ARA. Mycorrhizal biofertilizer produced to application within vegetables, fruits, and rubber. It enhances water and nutrients absorption by developing fungi filamentous. Utilization of mycorrhizal biofertilizer decreases crop production costs at 25%. Today, it is very popular use in rubber and fruit productions. We are now developing novel biofertilizer for chili, asparagus and oil palm.

Integrated use of oligochitsan and PGPR biofertilizer were searched using several rice cultivers in pot experiment. The oligochitsan application without fertilization did not increase height of the two rice varieties, CP304 hybrid and Jusmine 105 wild type. Whereas, in same condition using of PGPR II for rice increased height of rice variety CP304 at 15% and Jusmine 105 at 20%. Use of irradiated oligochitsan without fertilization increased tiller number of the rice varieties, CP304 hybrid and Jusmine 105 wild type, at 26 and 20% respectively. Whereas, PGPR increased tiller number of the rice varieties CP304 and Jusmine105, at 170 and 75%, respectively. These results indicated that oligochitosan promotes tiller number of rice. This phenomenum is very similar to PGPR II under the lower fertilization condition. The response to oligochitosan and PGPR II in the hybrid variety was higher than that of wild type.

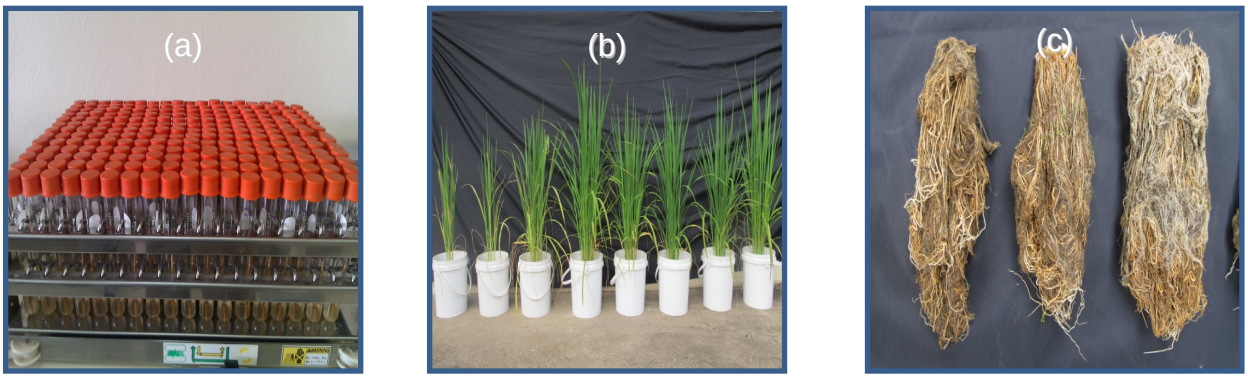


Fig 1 Nitrogenase activity tested for selected novel mutant strains by e-beam (a)

Integrate used of irradiation Oligochitosan and PGPR biofertilizer were searched in rice (b)

The root of rice used irradiation Oligochitosan and PGPR biofertilizer (c)

Summary of Vietnam Country Report in 2012

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

1. Biofertilzer development

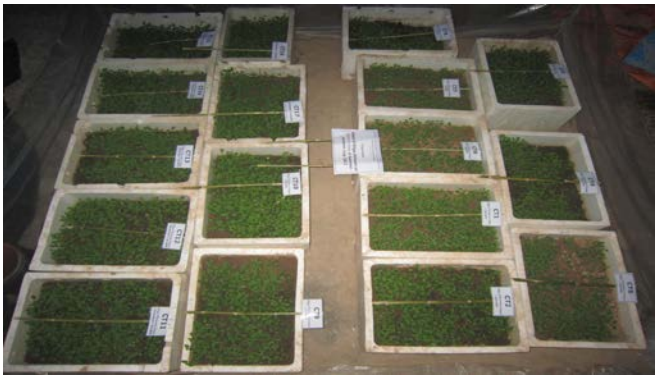
Sandy soil is poor in organic matter, nutrition elements and have low moisture. Agricultural production in sandy soil is difficult and unstable. Biofertilizer will improve the agricultural production in sandy soil. 27 microbial strains includes N-fixing, P-solubilizing, Cilicate solubilizing microorganism and polysacharide producing microbes are selected for biofertilizer production used in sandy soil. Technology for microbial inoculants applied for sandy soil are developed. It contained *Bradyrhizobium japonicum, Bacillus megaterium, Paenibacillus castaneae* and *Lipomyces starkeyi* with the density of more than 10^8 CFU/gram. Green house and field experiments are conducted to evaluate the effect of biofertilizer on soil fertility, growth and yield of peanut and cashew. The results showed that biofertilizer and cover crops improved the soil moisture, increase the density of beneficial microorganisms in the soil, increased yield component and yield of peanut and cashew from 17.02% to 24.36%. Biofertilizer bring more profit for farmers.

2. Experiments of synergy effect between biofertilizer and irradiated oligochitosan

Irradiated oligochitosan from Japan is added to broth medium for Azotobacter, Bacillus, streptomyces and yeast with concentration of 1 to 5 ppm. The effect of oligochitosan on growth of tested microbes in the broth is evaluated by plate count method. The results showed no effect of oligochitosan on growth of tested microbes in all concentration from 1 to 5 ppm.

Synergy effect of oligochitosan and biofertilizer on growth of cabbage are tested in the green house. It showed, that oligochitosan and biofertilizer have the synergy effect on growth and yield of cabbage. Application of oligochitosan and biofertilizer can save 50% recommended NPK rate and increase the cabbage yield of 27,81% to control applied 100 % recommended NPK rate.

Field experiments on the effect of biofertilizer on peanut growing in sand soil



Experiment on effect of oligochitosan on cabbage