Annex 3

Summary of Progress Reports on Biofertilizer and Polymer Modification
FNCA Workshop on Radiation Processing and Polymer Modification for Agricultural, Environmental and Medical Applications

November 28th - December 2nd 2022

Takasaki, Japan & Zoom Web-Meeting

Session 1: Progress Report on Biofertilizer

1. Degraded Chitosan for Animal Feed, 2. Hydrogel for Medical Application, 3. Environmental Remediation, 4. Synergistic Effect among Plant Growth Promoter (PGP), Super Water Absorbent (SWA) and Biofertilizer, 5. PGP and SWA, inclusive Process development, 6. Mutation Breeding of Microbe Using Radiation, 7. Sterilization and Sanitization Using Radiation, 8. Recycle Plastic

1) China (Prof. Zhang Ruifu, Chinese Academy of Agricultural Sciences)

Subject: 6

Short summary

Irradiation breeding of a biofertilizer strain of *Trichoderma guizhouenase NJAU4742* by X-ray and obtained a mutant with an increased ability to improve plant root development and plant growth, Identification of *Trichoderma guizhouenase* NJAU4742 secreted cedrene to promote root development, solid fermentation production of *Trichoderma guizhouenase* NJAU4742 conidia as biofertilizer, field application of *Trichoderma guizhouenase* NJAU4742-based biofertilizer in several crops.

Results

To get enhanced *Trichoderma guizhouenase* 4742 strains for different characters, breeding of this strain with X-ray radiation was carried out, Irradiation doses were 84Gy, 84Gy and 82Gy for three times, and the total was 250Gy. After irradiation, the spores of *Trichoderma guizhouenase* NJAU4742 were coated on different screening media. About 3500 different mutants were isolated and screened, a mutant with a strong ability to promote plant root development and plant growth was obtained, a volatile chemical cedrene was identified from this mutant, cedrene can promote lateral root development through the auxin signal pathway dependent manner. Different straws were compared for the solid fermentation of *Trichoderma guizhouenase* NJAU4742, and the *Trichoderma guizhouenase* NJAU4742-based biofertilizer was developed cooperated with the company. These biofertilizers were applied in the field for several crops, demonstrated the efficiency of the product.

Future plan

1) Continued application of *Trichoderma guizhouenase* NJAU4742-based biofertilizer.

2) Synergistic Effect of Super Water Absorbents and Biofertilizer

2) Indonesia (Dr. Ahmad Fathoni, Research Center for Applied Microbiology)

Subject: 4, 6

Short summary

The roles of plant growth promoting rhizobacteria (PGPR) and fungi (PGPF) for the promotion of plant

health and productivity are widely acknowledged. Indonesia, the fourth most populous country with a population of approximately 275 million, put agriculture as one of the key sectors to maintain food security. This includes emphasis on the development of biofertilizer to increase crop yield and reduce environmental damage due to chemical fertilizer and climate change. A short summary of current PGPR/PGPF research, specifically done at BRIN Indonesia will be given in this report.

Results

A wide variety of biofertilizer products were developed by researchers of BRIN. Many consisted of bacterial and fungal strains with beneficial activities such as nitrogen fixation, phosphate solubilization, herbicide and heavy metal degradation and production of valuable compounds including phytohormones, organic acid, siderophore, antimicrobial, catalase, lignocellulose, antioxidant. The application of biofertilizers could increase the yield of crops from 17% up to 33% and reduce the use of chemical fertilizer up to 50%. The commodities tested include rice, corn, melon, chili, woody plant species, etc. Improvement of soil health and plant development in marginal soil are also demonstrated for several products. Utilization of local ingredients is encouraged, for example, the use of bean sprouts as IAA precursor to improve the production of the phytohormone. However, the utilization of purified compounds to boost PGPR/PGPF growth is still unexplored and it will be a program for future research. Moreover, climate change has a significant impact on the environment and agriculture production. Therefore, improving the quality of current biofertilizers and developing new biofertilizers specific for locations and commodities are very important to be conducted in the near future. The ultimate goals are to maintain, or even increase quality and production in agricultural sectors, in order to maintain food security in national- and later global scale and to reduce land degradation due to climate change and/or chemical use.

Future plan

The development of a new biofertilizer is a priority. The use of radiation mutation in the construction of microbial mutants with beneficial properties (e.g., plant growth promoting traits, high tolerance to pests and diseases, unfavorable/extreme environmental conditions such as dryland, saline, and peatland) is aimed for future research on PGPR/PGPF. In addition, molecular/metabolic engineering (e.g., cloning) of target microbes will also be performed at BRIN. Screening would be done to select mutants with extraordinary activities or desirable traits, specifically the ones applicable to industrial scale. A product utilizing the selected mutants would be developed and further distributed, especially to impoverished community members or local farmers that will give positive feedback to food security.

3) Japan (Prof. OKAZAKI Shin, Tokyo University of Agriculture and Technology)

Subject: 4

Short summary

We isolated plant growth-promoting *Bacillus pumilus* TUAT1 and commercialized it as a biofertilizer. We found that TUAT1 could inhibit the growth of some plant fungal pathogens including *Calonectria ilicicola* which causes soybean root rot disease. When we applied the TUAT1 together with oligochitosan to soybean plants, we found that the TUAT1 could alleviate the disease occurrence of

soybean root rot disease and promote the growth of soybean.

Results

We screened plant growth-promoting strains from field soils at the Tokyo University of Agriculture and Technology (Tokyo, Japan) and isolated a strain, TUAT1, which was identified as *Bacillus pumilus*. TUAT1 promotes the growth of several plants, including that of rice and Brassica species. An inoculant for rice was developed using TUAT1 that has been commercialized as "Yume-bio" in Japan.

We found that TUAT1 could inhibit the growth of some plant fungal pathogens when cultivated together in an agar plate. To understand the biocontrol activity and mechanisms of *Bacillus pumilus* TUAT1 on different plant fungal pathogens, we evaluated biocontrol spectrum and identify the biocontrol agents of TUAT1. We found that TUAT1 could inhibit the growth of several fungal pathogens. Among them, we found that TUAT1 could inhibit strongly the growth of *Calonectria ilicicola* which causes soybean root rot disease, one of the severest soybean diseases in the world. On the pot experiment, we found that the TUAT1 could alleviate the disease occurrence of soybean root rot disease and promote the growth of soybean. Furthermore, additional treatments of soybean plants with oligochitosan together with TUAT1 could enhance the disease control as well as the seed production.

Future plan

We further analyze the biocontrol agent produced by TUAT1 for understanding the biocontrol mechanisms and further application for various crops including soybean and rice in the field.

4) Malaysia (Dr. Phua Choo Kwai Hoe, Malaysian Nuclear Agency)

Subject: 3, 6, 7

Short summary

Malaysia focuses on carrier sterilization, commercialization, radiation mutagenesis, microbe beads and bioremediation. Completed activities are gamma sterilization, determination of LD_{50} of Gram positive and negative bacteria, and isolation of four *pqq* genes from mutant M100. A new multifunctional biofertilizer, M100 is commercialised. Studies on shelf life of biofertilizer treated seeds, microbe beads, and bioremediation isolates mutagenesis are in progress.

Results for 2018-2021

Gamma irradiation is central in the development of a new sterile biofertilizer carrier in Malaysia. A digestate was produced by fermenting domestic organic waste (e.g. food waste) anaerobically in a biodigester. The study showed that gamma irradiation at a dose of 50 kGy resulted in microbe free medium. The government of Malaysia, through the Ministry of Agriculture and Food Industry, encourages the use of biofertilizers, targeting to reduce by 50% of the commonly used synthetic or chemical fertilizers. To date, Nuclear Malaysia has commercialized four biofertilizer products, namely, Bioliquifert, GoGrow BioNPK Biofertilizer, M99 Biofertilizer, and Bioliquifert M100. By using these biofertilizers, farmers are able to reduce the use of chemical fertilizers, increase 8-12% of yields and 20-35% of incomes. The exhibition, public talks and promotion through social media and other media have been instrumental in sensitizing, educating, and thus encouraging farmers to include biofertilizers as their critical agroinput. Four *pqq* genes (A, B, C, D) have been isolated and determination of LD_{50} was

conducted on microbial mutant M100 resulting from mutagenesis using gamma radiation. The LD_{50} of M100 was 445.5 Gy. Mutagenesis and determination of LD_{50} of Gram-negative and Gram-positive bacteria were also conducted. Optimization of mutagenesis doses is in progress. Biofertilizer treated seeds were stored at 4°C and room temperature for the shelf-life test. Biofertilizer treated seeds stored at 4°C showed a delay in seed germination compared to those stored at room temperature. Development of slow release biofertilizer as microbe beads was carried out. The growth curve and shelf-life tests were studied. Microbe beads at 4°C and room temperature showed survival rates up to 12 months. Mutagenesis of bioremediation isolates and determination of their LD_{50} is in progress. A copyright of M99, two publications and two awards have been obtained. In conclusion, the biofertilizer project has successfully delivered R&D products into the market. New R&D activities such as mutagenesis, bioremediation and carrier developments are in progress.

Future plan

The development new carrier biofertilizer by using 50 kGy gamma irradiated digestate is planned. Application of biofertilizer and nutrient uptake analysis using drones will enhance the efficiency and effectiveness of biofertilizer application in modern agriculture. Isolation of *pqq* genes in mutants will be conducted. A guideline and protocol on mutagenesis of Gram-positive and Gram-negative bacteria are in progress. Further survival tests of biofertilizer treated seeds, as well as greenhouse and field trials of microbe beads will be conducted. Mutagenesis of bioremediation isolates and determination of their LD_{50} are in progress and will be completed.

5) Mongolia (Ms. Sunjidmaa Otgonbayar, Institute of Plant and Agricultural Sciences)

Subject: 4

Short summary

Current R&D status in 2022: Wheat seed treatment with the rhizo liquid fertilizer with tebutin (pre-sowing seed treatment with systemic fungicide) increased the yield of wheat by 6.9-9.0 center/ha and 46.3-60.4%, seed potato yield by 42.3-45.6% and yield of vegetable crops /carrot, onion, tomato, sweet pepper etc/ by 22-50 %.

Results

1. Tebutin as seed fertilizer has no significant impact on bacterial activity.
2. In 2021, plots with field germination bacterial fertilizers were 4.7-10.9% more than control, of which plot including bacterial fertilizer with 100% tebutin was higher than other plots and in 2022, plots with bacterial fertilizer were 6.1-12.2% more than control plots, of which alternative including bacterial fertilizer with 100% tebutin was higher than other plots.
3. In the average yield of 2021-2022, bacterial fertilizing plots were 6.9-9.0 centner/ha higher than the control.

Future plan

a. To dry the bacterial liquid fertilizer – freeze drying technology
b. To supply biofertilizer with additive compost materials
c. To isolate and determine the taxonomy of novel bacterial culture from rhizosphere soil

d. To study the impact of bacterial fertilizer on soil fertility- detection of nitrification, ammonification, denitrification and some enzymes

6) The Philippines (Ms. Julieta A. Anarna, University of the Philippines Los Banos)

Subject: 4

Short summary

In the Philippines BIOTECH-UPLB pioneered the development of several effective biofertilizers and contributed greatly in the research and development of microbial-based products (Azospirillum, Rhizobium, Mycorhiza and PGP) for agriculture. This year we conducted three field experiments 1. Interaction effects of nitrogen fixing inoculant with Mycorhiza, using tomato as the test crop 2. Evaluation of enhanced Bio-organic fertilizer inoculated with Bio N and Mykovam using tomato as the test crop and 3. Evaluate the yield of corn, sorghum and soybean as affected by multi-inoculation of biofertilizers

Results

The effect of the integration of nitrogen fixing bacteria and mycorrhiza was conducted under field condition using tomato as the test crop. Data obtained that plants treated with chemical fertilizer had the heaviest weight with 31. 65 tons per hectare while plants with 50% chemical fertilizer in combination with biofertilizers obtained 23.92 tons per hectare.

Formulation of enhanced bio-organic fertilizer through the addition of microbial inoculant (nitrogen fixing bacteria and mycorrhiza) was formulated to support the advocacy in organic agriculture. The formulated bio-organic fertilizer was tested in the field and compared with the conventional BOF. The data revealed that the formulated BOF obtained the highest number of fruits (922 per plot) and heaviest fruits respectively (47.75 kg/plot).

Multi-inoculation of different BIOTECH products was used in this experiment to determine the effect on yield and nutrient content for animal feeds. In this initial experiment yield of sorghum and corn was first obtained. The heaviest weight was obtained from plants applied with full chemical fertilizers with an average weight of 13.45 kg per plot while plots applied with 50% of the recommended rate of chemical fertilizer + Biofertilizers has an average yield of 13.43 kg per plot. On the other hand, corn applied with 50%chemical fertilizers and biofertilizers response greatly with an average yield of 18.77 kg/plot.

In these three experiments conducted, biofertilizers contributed to the nutrient requirement of the test crops and can be recommended for use by farmers. Data also revealed that biofertilizers can complement the use of chemical fertilizer.

Future plan

1. Capacity building
2. Conduct more field trials.
3. Promotion and extension
4. Continue production of biofertilizers
5. Liquid formulation of Bio N (on going)

7) Thailand (Dr. Kunlayakorn Prongjunthuek, Department of Agriculture)

Subject: 4

Short summary

A study in farmer areas for further found that PGPR-I from TS13 and the LB1-3 had no difference in the growth and yield of sweet corn and waxy corn. Also, in cannabis cultivation, PGPR-I can help cannabis grow faster and has a wax coating on a leaf. Therefore, DOA's method in maize production gave higher yield and profit. Meanwhile, new methods for the preparation of microbial inoculum for the production of *Azotobacter* and *Burkholderia* were studied.

Results

Further study in farmer areas found that between PGPR-I from TS13 isolate (original) and the LB1-3 isolated from sweet corn variety Hi-brix 3 had no difference in the growth and yield of sweet corn (Hi-brix3) and waxy corn (Sweet white 25). But Chai Nat 2 (New DOA variety) had a significant difference in growth and yield.

The use of DOA's methods for producing maize and fresh cut maize produces higher yields and profits than farmers' methods. In the Pacific 789 variety, the DOA's method for fertilizer recommendation rate as soil analysis with PGPR-I biofertilizer application, maize was 225 cm height, root span 29 cm with numerous fibrous roots, 527 seeds/pod and seed weight 167 grams/pod, equivalent to a total yield of 11.25 tons/ha, with a profit of $1,721 comparison with the farmer's method which produces only 9.38 tons and has a profit of $1,170. In the case of fresh cut maize, DOA's method gave 39.87 tons/ha with $1,527 profit which over than the farmer's method (25.75 tons/ha and $787 respectively).

For cannabis cultivation, PGPR-I was found to help cannabis grow and flower faster and have a wax coating on the leaf surface. However, this study is still ongoing for the sake of clarity and can be issued as a utilization guide.

Meanwhile, new methods for the preparation of concentrated microbial inoculum for the production of *Azotobacter vinelandii* and *Burkholderia vietnamiensis* were studied. Simultaneously, a study was conducted on keeping the microbial inoculum at the proper temperature to allow the microbial to survive.

Future plan

Continue research and development of PGPR biofertilizer from the previous together with conducting product testing in farmer plots to improve, issue recommendations on the use of biofertilizers in the production of crops. Meanwhile, testing of product manufacturing and performance testing in laboratories and greenhouses. In addition, continuing the transfer of all 3 types of PGPR biofertilizer production technology to private companies. Also, develop methods for the preparation of concentrated microbial inoculum and preservation for biofertilizer production. For cooperation between the organization, the development of news PGPR biofertilizer for rice together with a study of biology and ecology of soil microorganisms in rice production systems and implementation were conducted with Rice Department.

8) Vietnam (Dr. Tran Minh Quynh, Vietnam Atomic Energy Institute)

Subject: 6, 7

Short summary

Continue to screen the potential *Trichoderma* mutants with high production cellulase with improved Endocellulase (CMCase) activity and Filter paper activity (FPase) for preparation of the probiotic for decomposing the rice straw in the field (complete).

Results

Two *Trichoderma* strains (*T. koningiopsis* VTCC 31435 and *T. reesei* VTCC 31572) with high and stable production of cellulase have been cultured and irradiated with gamma ray at the dose range from 700 to 2500 Gy to obtain the radiation induced mutants. The viability of both *Trichoderma* quickly decreased with radiation dose, and the survival spores reduced by 6.5-7.5 log at the dose of 2500 Gy, with the decimal dose (D_{10}) value was about 400 Gy.

After screening, 5 potential mutants that have outstanding CMCase and FPase activities (about 2.48 and 1.87 times compared to those of wild strains) were selected from irradiated fungal colonies. The insertion of arginine at position 413 and substitution mutation that changes amino acid from GCT (alanine) to ACT (threonine) at position 325 was found in the endoglucanase coding gene of VTCC(r) I-1 by sequencing. This mutation promoted the biosynthesis of cellulase, and its enzymatic production was stable within 5 generations without any differences in growth rate and morphology. Therefore, the culture conditions of VTCC(r) I-1 were optimized to maximize its production of cellulase for the preparation of the rice straw decomposition product (IRTr). Field tests revealed IRTr can further accelerate the degradation of rice straw.

Future plan

1. Wide area trials to evaluate the long-term effects of Rapol V biofertilizer on green tea at Thai Nguyen province (on-going).
2. Develop the BF composed of different microbes (N_2-fixation, phosphate-solubilizing, and soil conditioning bacteria) for vegetables.
3. Scale up the semi-solid fermentation to produce radiation induced mutant VTCC(r) I-1 *Trichoderma* for preparation of rice straw decomposition products
4. Evaluation of the efficiency and applicability of rice straw decomposition products in practice.
5. Dose mapping studies for radiation sterilization of health care products (masks, surgical gloves, garments…)

Session 2: Progress Report on Polymer Modification

1) Bangladesh (Dr. Salma Sultana, Bangladesh Atomic Energy Commission)

Subject: 1, 3, 5

1. Degraded Chitosan for Animal Feed

Short summary

One of the most popular meats consumed worldwide is chicken, and poultry farms are the main supplier. In recent years, chromium-rich tannery waste known as tanned skin-cut wastes (SCW) has been utilized to produce chicken feed in a number of developing nations, including Bangladesh. Chromium contamination in the food chain is primarily caused by this. LSD, or leather shaving dust, is occasionally fed straight to chickens. Chromium is quite likely to be transferred from SCW and LSD to chickens and the human body. Given the above mentioned, we looked for a substitute for conventional poultry feed. We went with degraded chitosan derived from chitin as an alternative. Chitosan with a range of molecular weights exhibits not only antibacterial activity, but also growth regulator activity. As a result, it has immense potential for usage in a variety of industries, including agriculture, medicine, and healthcare. Several nations have recently done the research and used chitosan as an additive in poultry and animal feed.

Results

Two experiments were conducted to investigate the effects of dietary o-chitosan on growth performance in broilers. We carried out an experiment here with eight (8) pairs of broiler chicken on which we supplied normal chicken feed and radiation degraded chitosan contained chicken feed. **250** mL 200ppm degraded chitosan (o-chitosan) was sprayed with 1Kg chicken food and were mixed them homogeneously and fed to chickens. Control, chicken food was also (without o-chitosan) fed to chickens. After 12 days, 4 broiler chickens showed a higher growth rate (%) among the 8 ones which were fed normal chicken feed. On the other side, 4 chickens among the 8 others which were fed chicken feed containing degraded chitosan, also showed a higher growth rate. Lastly, according to our research, the 4 broiler chickens, fed with chicken feed containing degraded chitosan, had a comparatively higher growth rate than the 4 broilers fed with normal chicken feed.

The average growth rate (%) for chicken feed containing degraded chitosan was found higher which was 288. 79 than normal chicken feed that was 271.71. Compared to the control, body weight gain increased in the dietary o-chitosan group by 6.29%. This is the two weeks result. We could not continue our research work due to some problems. In the case of 1st experiment, after fifteen days most chickens became sick without any reason and then died. After one month we started the second experiment but we can't continue because the same problem arose and farm owner didn't agree to continue this work again. More work is required to elucidate the effect of o-chitosan on growth performance in broilers. More work is required to elucidate the effect of o-chitosan on growth performance in broilers.

Future plan

We will continue the work to investigate the o-chitosan on growth performance in broilers. We are also looking forward to set up a poultry shed/farm in the office campus in order to conduct our experiments

smoothly.

3. Prospect of metal adsorption by gamma irradiated hydrogels [Environmental Remediation]

Short summary

Heavy metals are non-biodegradable, so unlike many organic pollutants, toxic metals discharged in aquatic streams are not decomposed by microbiological activity; rather these tend to accumulate in lower plants and animals and thereby enter the food chain. More than 200 tannery industries are located in the capital city of Bangladesh and of them about 90% of tanneries are engaged in chrome tanning process. During the chrome tanning process, 40% unused chromium salts are usually discharged in the final effluents, causing a serious threat to the environment. Exposure to chromium, pentachlorophenol and other toxic pollutants increase the risk of ulcer and lung cancer. Chromium ion in liquid tanning wastes occurs in two forms; trivalent Cr^{3+} and hexavalent Cr^{6+}. Foods containing chromium (III) in little amounts can be beneficial, while excessive amounts might be hazardous. A daily intake of 50–200 g of chromium (III) is advised. The hexavalent form is 500 times more toxic than the trivalent. Copper is an element that the body needs in trace amount to function correctly. However, excessive exposure can lead to a range of health issues. For instance, simply breathing in copper can irritate our throat and nose. Oral consumption of copper may result in the following symptoms: diarrhoea, liver damage, Wilson's disease, kidney damage, and death. Considering above those facts many researchers use techniques like precipitation, ion exchange, activated carbon adsorption, and electrolytic method to remove chromium and copper from waste water. But all these methods have some limitations like high cost, low removal rate, difficulty for regeneration, and reuse. Therefore, many researchers focused on the alternative low cost effective hydrogel as an adsorbent. Here we developed gamma radiation induced hydrogels as adsorbents to adsorb chromium and copper from the aqueous solution of chromium and copper.

Results

Three different types of blend hydrogels were prepared using gamma radiation (Co-60). Starch/PEO/PSSA blend hydrogel adsorbed 64 mg Cr^{+3} from 200 ppm $Cr_2O_{3(aq)}$ solution with a removal efficiency of 60%. In the case of XG/AAc/PSSA blend hydrogel, 103 mg Cu^{+2} adsorbed from 1000ppm Cu $(NO_3)_{2(aq)}$solutions with a removal efficiency of 22% and chitosan/DMA/HEA blend hydrogel, 20 mg Cr^{+6} adsorbed from 100ppm $K_2Cr_2O_{7\ (aq)}$solutions with a removal efficiency of 40%. The prepared blend hydrogels followed the 2nd order adsorption kinetics and the experimental data well delineated the Langmuir isotherm model except for starch /PEO/PSSA hydrogel.

Future plan

- Will try to increase the metal adsorbing properties of prepared hydrogels.
- We will try to prepare some other metal adsorbing hydrogels.
- We will also try to prepare some dyes adsorbing hydrogels.

5. PGP and SWA, inclusive Process Development

Short summary

Field experiments were conducted in the fields of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh and the Atomic Energy Research Establishment (AERE), Savar,

Dhaka, Bangladesh from 2010 to till date on different types of corn, vegetables, and fruits etc. to investigate the effect of foliar application of oligo-chitosan (o-chitosan) on morphological values, growth and yields. Experiments comprised different levels of o-chitosan concentrations and were foliar applied on plants.

Results

For crops (rice, and mung bean) 50 ppm of o-chitosan and for vegetables and fruits 75 ppm o-chitosan are suitable for the highest yields. It has been noted that 50ppm o-chitosan can be treated as the best treatment for achieving the highest yield of rice and mung-bean whereas 75 ppm o- chitosan as the best treatment for achieving the highest yield of egg-plant, tomato, maize, capsicum and strawberry. Foliar application of o-chitosan increased the yield of crops (rice 7-10%, mung-bean 30%, maize 52%), vegetables (egg-plant 48%, tomato 40%), fruits (capsicum 68%, strawberry 54%) over control. Foliar application of o-chitosan shows the ability to shorten the harvest time of fruits and vegetables in contrast to control and it can also display antibiotic activity against microorganisms including bacteria and fungi on tomato and egg-plants.

Publications:

1. Rayhanur Jannat, Milon Shaha, M. Tanbir Rubayet & **Salma Sultana**, Role of Chitosan in Induction of Defense Response against Phomopsis vexans and Augmentation of Growth and Yield of Eggplant, Global Journal of Science Frontier Research: C Biological Science, Volume 18 Issue 3 Version 1.0, p. 6-16, 2018.

2. Nusrat Jahan Nitu, Md. Mahidul Islam Masum, Rayhanur Jannat, **Salma Sultana,** Md. Khurshed Alam Bhuiyan, Application of chitosan and *Trichoderma* against soil-borne pathogens and their effect on yield of tomato, International Journal of Biosciences (IJB), Vol.9 No. 1, p. 10-24, 2016.

3. Salma Sultana, Mahfuza Islam, Mst Afifa Khatun, Md Afzal Hassain and Roksana Huque, Effect of Foliar Application of Oligo-chitosan on Growth, Yield and Quality of Tomato and Eggplant, *Asian Journal of Agricultural Research* ISSN 1819-1894, Vol. 11(2), p.36-42, 2017.

Future plan

The plan which we were supposed to accomplish in 2020 and 2021, the work was postponed due to the Covid-19 pandemic situation. For Covid-19, the last week of March to July 2020 and February to July 2021 was a totally lockdown in our country. During the lockdown period, we couldn't attend our office and our all research activities were stopped. We have already discussed with our higher authority and collaborator (BARI) regarding Field-level trials. We have been able to extend our collaboration with BINA to commercialize our PGP. From BINA it would be very much easy for us to reach the farmers. We have been trying on building up awareness about using the environment friendly agricultural practice.

2) China (Dr. Hongjuan MA, Shanghai University)

Subject: 2

Short summary

Polymer modification with irradiation technology and their applications in uranium extraction, solar-driven desalination, ^{177}Lu-labeled microspheres for internal radiation therapy and ion sieve composite

adsorption materials were ongoing investigated. Irradiation technology with EB and ^{60}Co holds a huge market and demand in China and will maintain continuous growth. New materials based on radiation technology have broad application space in the future.

Results

1. Porous structure in raspberry-like nanofibers with rearranged AO groups cyclic imide dioxime nanoparticles was constructed to improve the AO utilization ratio during U adsorption. The adsorption capacity of U was determined to be 11.39 mg-U/g-ads with AO utilization ratio of 1/74.
2. Covalent cross-linking induced by EB irradiation resulted new photothermal PMF with superstability, durability, fabricability and portability. The evaporation rate can achieve 1.65 kg m^{-2} h^{-1} in pure water, and 1.54 kg m^{-2} h^{-1} for textile wastewater under one sun illumination (1 kW m-2), which breakthrough the theoretical limit of planar evaporators. The PMF was tested with 12 cycles, the evaporation rate remained stable and the average evaporation rate could reach 1.48 kg m^{-2} h^{-1}.
3. A hybrid-integrated radioactive microsphere strategy using lutetium-177 coordinated polymeric microspheres was fabricated via radiation-induced graft polymerization for imaging-guided locoregional intravascular brachytherapy. ^{177}Lu-PCMs can be visualized via SPECT to validate the in vivo biodistribution and retention in real time, achieving precise delivery, effective anti-cancer treatment, and a distinguished safety profile without degradation, ectopic embolization, and adverse reactions.
4. Ion sieve composite adsorption material prepared with radiation cross-linking exhibited good adsorption capacity and long term serves and can be used for lithium extraction from salt lake.

Future plan

1. Research and development of new uranium extraction materials with higher capacity, pilot scale of materials (100 kg), uranium extraction (1 kg).
2. Continuous fabrication of photothermal fabrics with EB irradiation (10 m^2), marine test at least 1 week.
3. Pilot scale of ^{177}Lu-labeled microspheres for internal radiation therapy and clinical validation.
4. Development of Ti-Ion Sieve Composite. Lithium extraction from seawater.

3) Indonesia (Dr. Tita Puspitasari, Research Organization for Nuclear Energy)

Subject: 1, 2, 8

Short summary

R&D for Polymer Modification in terms of application for animal feed, hydrogel for medical applications, and recycle plastics is still ongoing, even though struggling in the process of establishing a new organization namely BRIN. The Research Organization for Nuclear Energy, which was previously named BATAN has got the mandate to conduct research on Nuclear Technology as a core technology. Furthermore, for the application matter, we need to collaborate with the appropriate institute. In this regard, some aspects should be adjusted regarding a human resource, facilities, financial support, research topics, administration procedure etc.

Results

1. Application of oligochitosan on dairy cattle was conducted. The results show that oligochitosan could

maintain the health of dairy cattle through preventing damage on cardiovascular cells as a result of temperature differences in the low and medium plains. Furthermore, the oligochitosan also has the ability to regulate lipid homeostasis through good energy management.

2. Gamma-irradiated PEGDA-chitosan hydrogel for control release of ciprofloxacin was developed. The result shows that PEGDA-chitosan hydrogels released more ciprofloxacin than that PEGDA hydrogels and have similar inhibition zones with positive control. The PEGDA-chitosan also showa promising
3. The development of new products based on recycle plastics was elaborated. There are two kinds of products such as compatibilizers for wood plastics composite as mechanical recycling and fuels as chemical recycling mechanisms. All are assisted by radiation processing.

Future plan

1. Continuing to complete the data sheet of the effect of oligochitosan onto various parameters such as immunity, histology, and reproduction of animal and keep effort to get funding support
2. Continuing to study the toxicity test of PDGA-chitosan, and conducting the development of Drug Delivery System based on alginate.
3. Continuing to reach the proof of concept of recycling polymer waste.

4) Japan (Dr. TAGUCHI Mitsumasa, National Institutes for Quantum Science and Technology)

Subject: 2

Short summary

Our research aim is to create highly original and functional biodevices by ionizing radiations. The microtopography and physical and chemical properties of the biomaterials can be freely and three-dimensionally controlled from nm to μm by utilizing the uniqueness and superiority of radiations. We are conducting research and development of cutting-edge medical devices such as three-dimensional cell culture substrates, nanosensors, and microfluidic chips based on biocompatible polymers.

Results

1) Functional cell culture substrates

We have shown that radiation crosslinking is mainly due to hydroxyl radicals from the decomposition of water molecules from the analysis of acid hydrolysis products of gelatin hydrogels. It was found that cell-sensing sites (arginine-glycine-aspartic acid sequence) for cell adhesion remained even after the irradiation. Based on the radiation crosslinking mechanism, proteins were crosslinked by radiations to produce hydrogels covering a wide elastic modulus range of biological tissues. Furthermore, culture experiments were carried out using various types of lineage cells such as fibroblasts, epithelial cells and myoblasts, primary cells, and cancer cells to demonstrate the effectiveness of the radiation crosslinked hydrogel.

2) Smart peptide nanoparticles

Peptides composed of aromatic amino acid residues were synthesized to form nanoparticles suitable for diagnostic and drug delivery system (DDS) applications. γ-irradiated peptide nanoparticles showed positive surface potential, and maintained biodegradability. The peptide nanoparticles were stable in

water and phosphate buffer. The surface potential of the peptide nanoparticles could be changed to negative by using fluorescent labeling reagents, and the fluorescently labeled peptide nanoparticles were taken up into HeLa cells.

3) Microfluidic chips

Multiple layers of MDPS chips were irradiated with electron beams to introduce a cross-linked structure between the layers. Due to the high penetrating range of the radiation, multiple microfluidic chips could be integrated in a single irradiation. This technology does not require the use of chemicals and does not allow chemicals such as solvents to enter the chip, thus ensuring accurate analysis.

Future plan

We investigate the radiation crosslinking technique to develop functional bio-devices in medical applications. In particular, we aim to develop 3D cell culture hydrogels that can be applied to drug discovery and regenerative medicine, nanosensors that can be used in MRI and PET diagnostics, and microfluidic chips that can perform multiple types of simultaneous tests on a palm-sized chip, and implement into society.

5) Kazakhstan (Mr. Nurkassimov Azat Kanatovich, JSC "Park of Nuclear Technologies)

Subject: -

Short summary

The object of the research is modified cabling and wiring products (CWP) by radiation cross-linking of polymer cable insulation in a protective atmosphere.

This material will be used in the field of mining and alternative energy sources, in particular in the production of solar panels, which requires only radiation-crosslinked polyethylene insulation and the production of oil-submersible cables.

Results

The use of polymer cable insulation modification in a protective medium, as compared to the air medium, contributed to:

- Increase in the portion of the gel fraction for practically all polymer samples, with some exceptions;
- Improvement of physical and chemical properties, such as heat resistance of the polymer, electrical resistance, etc.;
- The dominance of the crosslinking process over the destruction process in doses up to 125 kg.

The use of the ELV-4 electron acceleration unit contributes to a more productive manufacturing process of cable and wire products and improves operational characteristics.

Based on the results, it is planned to create our own production of oil-submersible and photovoltaic cables using the technology described in this paper.

Future plan

1. Output to production capacity
2. Promotion of the product in the market of cable and wire products
3. Conducting research work on the creation of self-regulating heating cables based on PTC conductive polymers

4. Creating new job

6) Malaysia (Ms. Maznah Binti Mahmud, Malaysian Nuclear Agency)

Subject: 1, 4, 5

Short summary

Gamma ray is used to induce degradation of chitosan and carrageenan through polysaccharide chain scission without changing their molecular structure backbone. Low molecular weight chitosan, LMCt (Mw 5000 Da) indicated promising results as an animal booster meanwhile low molecular weight of carrageenan, LMCarra (Mw 10000 – 20000 Da) showed good potential as a plant growth promoter. The irradiation dose, dose rate, polymer form (dry, solution or wet) and chemical presence are factors affecting the degradation rate of chitosan and carrageenan. As for super water absorbent (SWA) preparation, gamma ray inducing polymer crosslinking to form a permanent 3D network demonstrated as a moisture retainer in soil and proved reducing the irrigation water consumption in agriculture. The property of SWA can be modified according to the application requirement by varying the irradiation dose, polymer composition/molecular weight/type, combination reaction, dose rate etc. Pot test and small-scale study of LMCt (animal feed supplement), LMCarra and SWA were carried out. Next, the study on up-scale production and collaboration with farmers and industry are initiatives will be focused on.

Results

Irradiation of chitosan solution by gamma at a dose of 25 - 50kGy produces low molecular weight chitosan (LMCt) with an average molecular weight (Mw) of 5000 Da. The addition of 0.05% LMCt in dietary supplement of tilapia showed the best result on final weight, growth rate, feed conversion ratio, protein efficiency ratio and survival rate, respectively.

As lobsters are slow and periodic feeders, LMCt was added (20 ml of 0.05% LMCt in 100L tank size) into the tank instead of mixed with feed. By adding LMCt in drinks healed unhealthy chicken back to normal. LMCt also inhibits the growth of *E coli*. The antimicrobial property of LMCt reduced as the storage time increased.

The synergistic study of SWA and PGP indicated great result on enhancing Chinese kale growth by up to 22% compared to Control (without SWA and without PGP). But there is an insignificant difference in fresh biomass and leaf area between treated and untreated Chinese kale. The biobased PGP showed better performance than commercial PGP after combined with SWA. Application of SWA in pot can reduce the irrigation frequency.

The LMCarra exhibits PGP activity on mustard and kale. 80 ppm of LMCarra treatment attained the highest yield and fresh biomass on mustard and kale, respectively compared to other concentrations and Control (without PGP). All LMCarra treatments demonstrated insignificant difference in root length of both mustard and kale. The growth rate (plant height after 32 days) of kale after being treated with 80, 20 and 60 ppm are 21 – 40.6% compared to Control. Meanwhile for mustard, treatment of 100 and 80 ppm indicated plant growth rate (plant height after 33 days) of 13% and 15.5%, respectively compared to Control. The 80 ppm LMCarra indicates a promising PGP effect on mustard and kale among other

concentrations when compared to Control.

Future plan

In 2023, we will carry out studies on LMCt (animal feed additive), synergistic study and LMCarra (PGP), respectively:

i. To gain more specific data on the growth and performance of poultry
ii. To approach farmers and industry to observe the potential of LMCt in the real field.
iii. To diversify the application of LMCarra on angiosperms plants.
iv. To complete the storage study of LMCarra in various storage environments with vary chemical preservative types and concentrations.
v. To establish the procedure of an up-scale process of LMCt and LMCarra
vi. To establish and produce procedures on application of PGP and synergy SWAPGP.

7) Mongolia (Dr. Chinzorig RADNAABAZAR, National University of Mongolia)

Subject: 2, 3, 4, 7

Short summary

In 2022 we worked to get PVA and starch-based SWA (5kGy irradiated SWA shows 368 times water retention capacity) preparation and PVA and keratin-based hydrogel were prepared with a freeze-thaw method. preparation. In more, we studied prolonged storage and total bacterial count reduction in Mongolian traditional dairy product – small curd due to effects of X-ray irradiation.

Results

- Hydrogel for Medical Application subject is now in an ongoing study. We prepared PVA-based hydrogel with keratin and tested it on the NIH3T3 cell line for wound healing but the results were not significant compared with PVA alone. In the previous year, we synthesized ZnO nanoparticles and applied them to the hydrogel.
- Environmental remediation is standardized in Mongolia, after mining certain ores the private companies remediate mined using endemic plants with or without biofertilizer. In 2021, we prepared Cryogel-Zeolite composite material and tested on a laboratory scale. Pb adsorption rate was 87.47 mg/g after 20 hrs, room temperature.
- PVA and acrylic acid-based SWA prepared using starch with 1-7 kGy x-ray irradiator and water retention potential is reached 334, 348, and 368 times each on 3kGy, 5 kGy, and 7 kGy irradiation.
- Mongolian traditional dairy product – small curd irradiated with 1 kGy the total bacterial counts were reduced from $4.1*10^6$ control group to $3*10^6$, and in the case of 3 kGy total counts were reduced to $1*10^6$, and for the 5 kGy the bacterial counts were significantly reduced to $2*10^5$ colony forming units.

Future plan

1. Post-harvest treatment of basic vegetables (potato, carrot, garlic, onion,) with irradiation to reduce losses during preservation.
2. Meat treatment to improve hygiene and sanitation.
3. Promote and educate farmers and small businesses, provide nuclear technological information to

the public as a safe.

8) The Philippines (Dr. Charito T. Aranilla, Philippine Nuclear Research Institute)

Subject: 2, 5, 8

2. Hydrogel for Medical Application

Short summary

The proposal for the Clinical Trials and Upscaling Production Trials of the hemostatic agents has been approved and granted funding of USD 350,000 by the Department of Science and Technology. Linkages with stakeholders such as Clinical Research Organization, Technology Adapter, Clinical Trial Centers, and the GMP toll manufacturing company has been established. The project will commence on December 1, 2022. The successful implementation of the said project will facilitate the commercialization of the radiation-processed hemostatic agents.

Results

The proposal titled Radiation-engineered Hemostatic Agents as Life-saving Devices (REHEAL): Clinical investigations of safety and efficacy in the management of traumatic bleeding in emergency settings" was submitted last March 2022 and got approved in June 2022 by the Department of Science and Technology with a funding grant of USD350,000.00. The project's objectives are to investigate the safety and efficacy in managing traumatic bleeding, to conduct upscale production in a GMP facility, to establish semi-commercial scale radiation processing, and to validate and substantiate a 25 kGy sterilization dose. Meetings and engagements with the stakeholders were conducted to introduce the project and discuss the implementation plans. The project will be implemented on December 1, 2022.

Future plan

For 2023, all activities related to the clinical trial and GMP production trial will be conducted. A production cost analysis will be conducted in collaboration with the Business Development Section of PNRI to assess the marketability of the products The technology transfer process will be initiated by end of 2023.

5. PGP and SWA, inclusive Process Development

Short summary

The effects of long term-storage and retrogradation on the gel properties and stability of the starch/polyacrylic SWA were studied. This phenomenon affects the functional properties and shelf-life of any starch-based materials; the SWA was no exception. Additives such as CaCl2 and low-temperature storage are recommended to control this effect.

Results

The effects of long-term storage and retrogradation on the stability of the gel properties of radiation-synthesized super water absorbents (SWA) made from cassava starch and acrylic acid were studied. Retrogradation is a property of starch that occurs when the disaggregated polysaccharides amylose and amylopectin in gelatinized starch eventually reassemble over time into a more ordered crystalline structure. This phenomenon affects the functional properties and shelf-life of any starch-based materials,

and the SWA was no exception. Differential scanning calorimetry (DSC) and transmission electron microscopy (TEM) analyses were used to explore the retrogradation phenomenon. TEM and scanning area electron diffraction (SAED) images revealed the presence of starch nanocrystallites and an increase in the crystalline state in the SWA matrix after 24 months of storage. Enthalpy changes in DSC analysis also confirmed the retrogradation of aged SWA samples. However, no sharp peak was observed in XRD diffractograms, mainly due to the nanostarches present in the SWA. The most critical property affected by starch retrogradation in SWA is the degree of swelling. The degree of swelling of SWA was significantly reduced from 148 to 30 g/g while the gel fraction remained stable after 24 mos. Additives such as $CaCl_2$ and low-temperature storage are recommended to control this effect. Suppression of starch retrogradation in the optimized SWA is crucial to prolong its marketable life.

Future plan

Since this project has officially ended in June 2022, a new proposal will be submitted to the Department of Science and Technology to obtain funding for Technology promotion activities.

8. Recycle Plastic

Short summary

The World Bank Group estimates that the Philippines produces 1.1 million tons of PE, PP, and PET plastic waste, of which only 28% is recycled and mostly PET. Due to this, there is approximately a 790-890-million-dollar loss in material value per year. The technical factors affecting the low recycling rates of PE and PP waste are due to variations in plastic quality and composition and the commingling of the different resin types in municipal waste coupled with the poor thermo-mechanical properties of used-up plastics. There needs to be more efficient and integrable technological intervention. Furthermore, addressing multiple problems like these typically compounds costs.

The Philippines, through the PNRI, looks towards the possible application of radiation processing of polymers in tackling the plastic recycling problem. Ionizing radiation can be used in the modification of polymers with very distinct effects like crosslinking, grafting, oxidation, and degradation. These are readily applicable to polymeric materials like plastics.

Results

Our current project, which has an active collaboration with the IAEA, Envirotech Waste Recycling Inc., DOST-ITDI, and QST-Japan, applies post-radiation reactive extrusion, or PREX. In this approach, radiation in forming radicals and oxidative species is augmented with extrusion to support the reactive species in enhancing miscibility and inducing crosslinks.

Mixed plastic from municipal waste containing HDPE, PP, LDPE, and multi-layer packaging was provided by our collaborator, Envirotech, and locally available abaca fibers were irradiated and compounded using a melt mixer to produce specimen composites for testing. The generation of reactive species on both the plastic and fibers generated a flexural strength increase of 25% at 20% fiber loading compared to a 4% increase using only pristine fibers.

Future plan

Conduct research to elucidate the mechanisms of the process and optimize parameters to maximize

property improvement.

9) Thailand (Dr. Phiriyatorn SUWANMALA, Thailand Institute of Nuclear Technology)

Subject: 5, 8

5. PGP and SWA, inclusive Process Development

Short summary

Super water absorbent beads (SWA beads) were successfully prepared in a laboratory scale as well as in a semi-pilot scale. Unlike the first generation of SWA (with irregular shape and size), SWA beads are relatively uniform both in terms of size and shape. The preparation processes for SWA beads are more convenient and practical, eliminating four major processes (cutting, drying, grinding and size-sorting) which are highly time- and energy-consuming.

Results

SWA beads were successfully synthesized in a laboratory scale as well as a semi-pilot scale by radiation-induced graft polymerization of acrylic acid (AA) onto cassava starch microbeads. The swelling capacity of SWA beads was approximately 360 times of its original dry weight, which is higher than that of the SWA hydrogel (the 1st generation of SWA produced by TINT). The process made it possible to prepare SWA beads with regular size and shape. The preparation of SWA beads in an up-scaling production resulted in a more simplified and practical process, while simultaneously saving a lot of time, energy as well as manpower.

Future plan

For a future plan, an up-scaling preparation of SWA beads will be performed in order to prepare SWA beads in a large amount. The prepared SWA beads will be used for field tests in arid areas with trees with high economic values such as young rubber and chili trees. The prepared SWA beads will also be tested for its biodegradability using the soil burial technique.

8. Recycle Plastic

Short summary

Thailand is working on a project titled “Fabrication of recycled plastic composites with neutron shielding properties”. The project aims to use turn local plastic waste (caps of water bottle, mostly HDPE) into composites with neutron shielding properties. The concept of the project has been proven by both a number of published research works as well as a fact that there are similar products commercially available in the market, mostly in China and the USA.

Results

Preliminary experiments and results have also shown that composites made from recycled HDPE can offer neutron shielding properties. The project is currently in TRL 2 and is undergoing processes to finish TRL 3 at the end of 2022, at the earliest. The experts (from the IAEA) offer a recommendation for the project to use compounds (made from recycled plastic) produced by private companies to make sure that the raw material can offer more consistent properties. Hence, a collaboration with private companies has been initiated. CirPlas is an SME company in Thailand who is collecting plastic wastes

and turning them into compounds to be used to create different products. CirPlas will be the official supplier of the raw material for Thailand's project.

Future plan

For a future plant, additives such as boron (B) and bismuth (Bi) will be used to mix with plastic waste to prepare recycled plastic composite with neutron shielding properties. Currently, PE sheets loaded with boron (B) (for neutron shielding purposes) are not commercially available in Thailand. They are available only via import from foreign countries, mostly China and USA. Hence, the cost is very expensive, especially for purchase with a small volume. The major goal of this project is to reduce plastic waste by recycling them into light-weight materials with neutron shielding properties. The prepared composites can be used as a shielding for the Neutron Imaging Analysis laboratory at TINT.

10) Vietnam (Dr. Nguyen Ngoc Duy, Vietnam Atomic Energy Institute)

Subject: 1, 3

Short summary

- Se nanoparticles (SeNPs) had a stimulant effect against ESM disease caused by *Vibrio parahaemolyticus* in shrimp. The immunostimulant effect was the highest at a concentration in the range of 1-2 mg/kg. The growth rate and survival rate of shrimp fed 2 mg/kg SeNPs increased by 11% and 29.2%, respectively, compared with the control.
- Electronic beam irradiation at a dose of 1 kGy in the presence of 5 mM H2O2 combined with biological treatment has reduced the color degree, COD, BOD, TOC, and pH of wastewater after 3 days of implementation and achieved grade B QCVN 13 2015.
- The Ag nanoparticlesTiO_2 photocatalysts with a size in the range of 2–30 nm were successfully synthesized by the EB irradiation method. The photocatalytic activity of the Ag nanopartilces/TiO_2 material depended on the initial Ag concentration. The Ag nanoparticles/TiO_2 photocatalyst with 1 % Ag concentration was suitable to achieve the highest RhB degradation efficiency.

Results

- Feed for white-leg shrimp containing nano selenium with concentrations in the range of 0.5 - 2.0 mg/kg was prepared. Nano selenium has a stimulant effect against ESM disease caused by *Vibrio parahaemolyticus* in shrimp. The immuno-stimulant effect increased with increasing nano selenium content and the highest effect was achieved in the concentration range of 1-2 mg/kg. Nano selenium has a growth stimulating effect. The growth rate and survival rate of shrimp fed 2 mg/kg SeNPs increased by 11% and 29.2%, respectively, compared with the control.
- Azo-dyes have been treated by electron beam irradiation combined with H_2O_2 oxidizing agent. The dose of 1 kGy combined with 5 mM H_2O_2 was selected for further biological treatment. Studied on the treatment of azo dyes in textile dyeing wastewater by biological methods with irradiated samples and combined treatment with H_2O_2 with the following results: COD, BOD_5, and TOC of wastewater samples all decreased over time of biological treatment. Azo dye wastewater samples met discharge standards after 3 days of biological treatment.
- The Ag nano/TiO_2 photocatalysts were successfully synthesized by the EB irradiation method. The

size of Ag nanoparticles was in the range of 2-30 nm. Photocatalytic activity of the Ag nano/TiO_2 material depended on the initial Ag concentration. The Ag nano/TiO_2 photocatalyst with 1 % Ag concentration was suitable to achieve the highest RhB degradation efficiency. Under the irradiation of visible light, the photocatalytic efficiency of the Ag 1%/TiO2 material was 98% compared to 65% for the TiO2 after 2.5 h of lighting time.

Future plan

- Synthesis of alloy Ag-Cu nanoparticles/diatomite by electron beam method using chitosan as a stabilizer for use as antibacterial and antifungal substances in agriculture.
- Synthesis of Cu nanoparticles/TiO_2 and Cu-Ag nanoparticles/TiO_2 by electron beam irradiation for photodegradation of organic pollutants in water.
- Study on the fabrication of nanogels by the electron beam irradiation method from natural polymers for applications in medicine