Asian International Symposium on Agriculture

Collaboration of Agricultural Education and Research Programs for the Safety of Food and Conservation of Natural Environment

Venue: Takikawa International Memorial Hall, Kobe University
Sponsors: Faculty of Agriculture, Kobe University, RIKUSO-Kai (Alumni Association of Faculty of Agriculture, Kobe University, and KANBARA Tosao Foundation for Promotion of Science
Date: Nov. 30 (Sun) to Dec. 2 (Tue)

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Symposium Programs:

Nov. 30 (Sun) Symposium Registration at Shin-Kobe Oriental Hotel
Welcome Reception at Shin-Kobe Oriental Hotel
Dec. 1 (Mon) Symposium at Takikawa International Memorial Hall, Kobe University

[**Opening Ceremony and Morning Session**] 9:00 to 12:00

9:00-9:10 **Opening announcement**
Organizing committee
Welcome Speech
Dr. Hitoshi Ishikawa, Vice-president of Kobe University

9:10-9:40 **Introduction to Education and Research Programs of the Faculty of Agriculture, Kobe University, and Scope of the Symposium**
Dr. Shigeyuki Mayama, Dean of Faculty of Agriculture, Kobe University

9:40-10:10 **Education and Research Program under a Revitalized College of Agriculture at UP Los Banos, Philippines**
Dr. Candida B. Adalla, Dean of College of Agriculture, The University of the Philippines, Los Banos, Philippines

10:10-10:40 **Teaching and Research at the College of Agriculture and Biotechnology in China Agricultural University**
Dr. Li Jian Qiang, Vice-Dean of China Agricultural University, Beijing, P. R China

10:40-11:00 **Coffee Break**

11:00-11:30 **The Nong Lam University, Ho Chi Minh City, Vietnam: Its Education, Research and Public Service Duties and the Future Development Plan**
Dr. Bui Cach Tuyen, President of Nong Lam University, Ho Chi Minh, Vietnam

11:30-12:00 **Education and Research Programs of College of Agriculture and Life Sciences at Seoul National University**
Dr. Mooha Lee, Dean of College of Agriculture and Life Science, Seoul National University, Seoul, Korea

[**Lunch**] 12:00-13:10, Lunch Room in Takikawa International Memorial Hall

[**Afternoon Session**] 13:10-18:00

Presentations of Research Topics from Participant Universities

13:10-13:30 **Genetic Diversity of Domestic Goat**
Dr. Hideyuki Mannen, Dept. of Animal Science, Faculty of Agriculture, Kobe University

13:30-13:50 **Biotechnology in Industrial Crops**
Dr. Tomoko Hatanaka, Dept. of Plant Resource Science, Faculty of Agriculture, Kobe University

13:50-14:10 **Structure and Molecular Function of Cytochrome P450 Species in Higher Plants**
Dr. Hiromasa Imaishi, Dept. of Biological and Environmental Science, Faculty of Agriculture, Kobe University

14:10-14:30 **Germination Strategy of *Striga hermonthica*, a Root Parasitic Weed**
Dr. Yukihiro Sugimoto, Dept. of Biofunctional Chemistry, Faculty of Agriculture, Kobe University

14:30-14:50 **Coffee Break**

14:50-15:10 **Involvement of Regulatory Systems in Biocontrol of Plant Diseases by *Pseudomonas fluorescens* 2P24**
Dr. Zhnag Liqun, China Agricultural University, Beijing, P. R. China

15:10-15:30 **Current Status of Citrus Tristeza and Greening Diseases in Ho Chi Minh and Dong Nai Areas**
Dr. Le Dinh Don, Nong Lam University, Ho Chi Minh, Vietnam

15:30-15:50 **New Construction Techniques for Shallowly Buried Pipeline with Geosynthetics**
Dr. Toshinori Kawabata, Dept. of Agricultural and Environmental Engineering, Faculty of Agriculture, Kobe University

15:50-16:10 **People and their Life in South Laos - Farming System Research for Rural Development**
Dr. Nobuya Kobayashi, Food Resources Education and Research Center, Faculty of Agriculture, Kobe University

16:10-18:00 **Poster Presentations (Faculty members and graduate students from Faculty of Agriculture, Kobe University)**

[**Banquet**] 18:00-20:00, Takikawa International Memorial Hall

Dec. 2 (Tue)

[**Morning Session, Discussion and Closing Ceremony**] 9: 30-11:30

9:30-10:00	**RDE program on the Safety and Quality of Food and Food Products in the Philippines** Dr. Erlinda I. Dizon, The University of the Philippines, Los Banos, Philippines
10:00-10:30	**Pathogenomics and Asian Consortium on Systems Biology for Rice Diseases** Dr. Yong Hwan Lee, Seoul National University, Seoul, Korea
10:30-11:20	**Discussion on Future Collaboration**
11:20	**Concluding Remarks** Dr. Shigeyuki Mayama, Dean of the Faculty of Agriculture, Kobe University
11:30-13:00	**Lunch Discussion (Dean's Room, Faculty of Agriculture, Kobe University)**
13:00-	**[Visit to the Food Resources Education and Research Center, Faculty of Agriculture, Kobe University]** Dr. K. Kawano: Introduction to the Center

Guest Participants from the Collaborative Universities

From Philippines
Dr. Candida B. Adalla, Dean of College of Agriculture, The University of the Philippines, Los Banos, Laguna Philippines 4031, Philippines
Dr. Erlinda I. Dizon, Institute of Food Science and Technology, The University of the Philippines, Los Banos, Laguna Philippines 4031, Philippines

From P. R. China
Dr. Li Jian Qiang, Vice-Dean of China Agricultural University, Beijing, P. R. China
Dr. Zhang Liqun, China Agricultural University, Beijing, P. R. China

From Vietnam
Dr. Bui Cach Tuyen, Rector of Nong Lam University, Ho Chi Minh City, Vietnam
Dr. Le Dinh Don, Vice Chairman, Department of Biotechnology, Nong Lam University, Ho Chi Minh City, Vietnam
Dr. Tran Tan Viet, Deputy Dean, Faculty of Agriculture, Nong Lam University, Ho Chi Minh City, Vietnam

From Korea
Dr. Mooha Lee, Dean of College of Agriculture and Life Science, Seoul National University, Seoul, Korea
Dr. Yong Hwan Lee, College of Agriculture and Life Science, Seoul National University, Seoul, Korea

Local Organizing Committee

Dr. Shigeyuki Mayama, Dr. Chiharu Nakamura, Dr. Hisashi Horio,
Dr. Kazunori Uchida, Dr. Masashi Mizuno, Dr. Fumio Mukai,
Dr. Takashige Ishii, Dr. Makio Takeda and Dr. Hiroshi Yamagata

Plenary Lectures

Introduction to Education and Research Programs in Faculty of Agriculture, Kobe University, and Scope of the Symposium

Dr. Shigeyuki Mayama
Dean of Faculty of Agriculture, Kobe University,
Kobe 657-8501, Japan

We all now face serious problems such as over-population, global warming, environmental destruction, food shortage and crisis of food safety that all threaten not only our daily livings but also our lives in the 21st century. Under this unfavorable circumstances, importance of agricultural education and research is ever-increasing because it is expected to serve for human welfare by providing knowledge and technology needed to ensure sustainable food production, utilization and environmental conservation. Agricultural research has recently made significant advances to invent new bio-industries through the development of innovative biotechnology and its efficient utilization.

Agricultural science is a multidisciplinary science covering wide aspects of food science, environmental science and life science, and plays pivotal roles for sustainable human life today and tomorrow. Our Faculty of Agriculture, Kobe University, aiming at creating constructive measures for collective solution and reward to social requirements, makes every endeavor in providing new and high-level education and research programs.

Our campus lies on the hill-side of Rokko mountains in the city of Kobe, famous for an international trade port, and is enriched with a vast agricultural area extending from Seto-inland sea in the south to the Japan Sea in the north. At present nearly 1,000 foreign students are attending in Kobe University, and Our Faculty is accepting more than 70 of them. We have already developed educational and research collaborations with several Asian countries including China, Korea, Vietnam and Philippines and also offer a variety of training courses in biotechnology, environmental science and plant protection to technologists and scientists from developing countries all over the world through intimate collaboration with Japan International Cooperation Agency (JICA). Our aim is to offer ample educational and research opportunities to students and provide our society with talented and dedicated young people having profound knowledge and wide field of vision through the promotion of international collaboration and information-exchange network.

The Asian International Symposium on Agriculture (AISA) is organized and dedicated to provide a platform and portfolio for the promotion of collaboration of agricultural education and research programs for the safety of food and conservation of natural environment in Asian countries. Scientific knowledge must be disseminated and accompanied with responsibility. As we face a global food and environmental problems, agricultural education and research community has more responsibility to advance our scientific knowledge and technology and engage in ongoing and active collaboration, especially in this region of the world. I warmly and proudly welcome you to this Symposium, which is a continuation of our academic cooperation as sister universities. All of you are indispensable members of the Symposium. I hope the collective participation of all delegates will uphold and augment the success of the Symposium.

Education and Research Program under a Revitalized College of Agriculture at University of Philippines Los Banos, Philippines

Dr. Candida B. Adalla
Dean of College of Agriculture, The University of Philippines,
Los Banos, Laguna 4031, Philippines

The UPLB College of Agriculture (UPLBCA) was organized in 1909 from its humble beginning where classes were held in borrowed tents. It evolved into its present status as the Center of Excellence in Agricultural Education in the Philippines. In the early years, it has contributed to the rapid development of agricultural science and education not only in the Philippines but also in the South East Asia (SEA).

Recent developments, particularly the enactment of the Agriculture and Fisheries Modernization Act (AFMA) of 1997 (RA 8435) and the global economic crisis led to the redirection of academic and research programs of the UPLBCA. The reorganization of the College necessitated the merger of related units into functional clusters to attain the desired synergy of strength, sharing of resources to insure the required depth and breadth of CA curriculum that must be made relevant and equally competitive with emerging degree programs at the UPLB campus.

Research-wise, the College must respond to the national agenda of AFMA particularly those related to attaining food self-sufficiency, food security, income generation and preservation of the environment. These are met by conducting researches in close coordination with national research coordinating agencies like the Philippine Council for Agriculture and Resources, Research and Development (PCCARD) and the Department of Agriculture's Bureau of Agricultural Research (DA-BAR). Its research and development initiatives range from traditional varietal improvement methods to high-end researches using modern biotechnology tools. From the traditional discipline-based investigation, UPLBCA is now shifting to the team or inter-disciplinary and participatory methodologies.

The paper will focus on the on-going reorganization of the College and its implications to its current and future education and research programs.

Teaching and Research at the College of Agriculture and Biotechnology in China Agricultural University

Dr. Li Jian Qiang
Associate Dean of College of Agriculture and Biotechnology,
China Agricultural University,
Beijing 100094, P. R. China

China agricultural university is a leading agricultural education and research institution in China. The history of the university traces back to 1905 as the College of Agriculture in the Capital University (the present Peking University), the oldest university in China. Under the jurisdiction of the Ministry of Education in China, the university offers a wide range of subjects majoring in agriculture, biology, engineering, veterinary medicine, economics, management, humanities and social science, etc. It boasts 5 academicians of the Chinese Academy of Sciences, 5 academicians of the Chinese Academy of Engineering Sciences, 286 professors, and 504 associate professors. The number of students has kept rising over the past years. Currently, 4,067 graduate students and 14,400 undergraduate students are enrolled in 13 colleges in the university. China Agricultural University has established the friendship and cooperative relationship with more than 100 universities in the United States, Japan, Germany, Canada, England, and 20 other countries. The university is dedicated to creating colorful campus life and educationally purposeful experiences for students that promote involvement, learning, and success. There are more than 70 student clubs and organizations actively involved in politics, economics, science and technology, civilization, sports, and so on.

The College of Agriculture and Biotechnology is the largest college in China Agricultural University. Our students choose from a diverse array of undergraduate and graduate degrees and programs. Some 1,500 undergraduate students and 1,100 graduate students study in the college, in such fields as genetics and plant breeding, crop production, seed science, crop physiology, plant pathology, entomology, fruit science, vegetable science, and ornamental horticulture. Our faculty, consisting of 2 academicians of the Chinese Academy of Engineering Sciences, 50 professors, and 90 associate professors, conduct basic and problem-solving research in a wide range of fields. The College of Agriculture and Biotechnology, armed with advanced equipments, rich facilities, as well as considerable funding, has been recognized around the country for its interdisciplinary teaching, research and extension efforts. It has built on tight cooperation and international exchange programs with the United States, Japan, and Australia, etc.

China is a large agricultural country. Agricultural production is most important basis for social stability and continuous development of Chinese economy. In the 21st century, Chinese agriculture is confronted with three big tasks. The first is to develop grain production and ensure food safety. The second is to enhance the farmers' income and boost rural economy. The third is to improve ecological environment and achieve sustained development of agriculture. Crop science, plant protection and horticulture have been playing essential roles in dealing with these challenges. The College of Agriculture and

Biotechnology in China Agricultural University would like to make efforts in cultural exchange and cooperation in science and education with international peers and institutions.

The Nong Lam University, Ho Chi Minh City, Vietnam: Its Education, Research and Public Service Duties and the Future Development Plan.

Dr. Bui Cach Tuyen,
Rector of the Nong Lam University,
Ho Chi Minh City, Vietnam

Nong Lam University (NLU), Hochiminh City, Vietnam founded in 1955, starting with three disciplines: Agronomy, Animal Science &Veterinary Medicine, and Forestry. Gradually, other disciplines were added such as Fishery, Agricultural Engineering and Agricultural Economics. In 1985, the College of Forestry in Dong Nai province was merged with the Faculty of Forestry of NLU to form the bigger entity dealing with forestry. Since 2000, with the approval of the Ministry of Education, NLU is being transformed into comprehensive university to satisfy the new social demand. Until now, NLU created about 20,000 alumni working mainly in more than 30 southern provinces of Vietnam contributing to the regional successful production of world top commodities - in term of quantity- such as rice, coffee, black pepper, rubber, tea, cashew nut, etc. for domestic consumption and export.

So far, NLU is organized into 11 faculties with 50 departments, 3 university-attached departments, 13 research or training centers. NLU has relation with more than fifty international universities/colleges/institutes or organizations.

Besides the task of providing education for more than fourteen thousands students, the scientific research of NLU is among the top in southern universities. The public service of the NLU is widely recognized by the society.

With the new strategic development plan, NLU has been trying to pursuit qualified educational programs, applicable scientific researches and effective public services, especially in agricultural extension.

Education and Research Programs of College of Agriculture and Life Sciences at Seoul National University

Dr. Mooha Lee
Dean, College of Agriculture and Life Sciences, Seoul national University, Seoul 151-741, Korea

The goal of the College of Agriculture and Life Sciences at Seoul National University is to produce well-educated professionals who will work in the agricultural and related fields, thus contributing to the development of agriculture and life sciences both at the national and global levels. The College focuses on the training of researchers, educators, and managers who have a sense of mission for the agricultural field. Emphasis is placed on the study of fundamental theories in both agriculture and life sciences as well as on their practical applications to the agricultural industry.

Throughout the century-long history of education and research, the College of Agriculture and Life Sciences at Seoul National University has been leading Korean agriculture. We are well known to the world by its dedication to development and dissemination of knowledge in many academic and professional fields. Most of our 20,000 graduates are playing pivotal role in various areas. Our alumni are recognized to be the most devoted and qualified group in the Korean agricultural society.

Now, at the doorstep of the new millenium, our aspiration to preeminence in teaching, research, and service has never been greater. We are evolving and expanding our role to meet the changing needs of national and international communities. As a college of a national university, we recognize that our mission is to apply the results of our academic endeavors in service to the community, the nation, and the world. We are committed to strive for a better research environment where we can provide adequate facilities, equipments, and resources to fulfill the mission.

Oral Presentations

Genetic Diversity of Domestic Goat

Dr. Hideyuki Mannen
Department of Animal Science, Faculty of Agriculture, Kobe University

The domestic goat (*Capra hircus*) often is dismissed as the “poor man’s cow” for its ability to thrive on meager fodder and cope with harsh environments. However, in fact, this species is one of most considerable livestock in the world. The origins and genetic diversity have been investigated by fossil remains and morphological studies, but this issues are still unclear. The understanding and information of the origins and the diversity would be important in point of genetic resource conservation. In my laboratory, we have been studied genetic diversity of Asian domestic goats using DNA variations. In this symposium, I will explain our work for the origins and genetic diversity of domestic goats using molecular techniques.

Biotechnology in Industrial Crops

Dr. Tomoko Hatanaka
Department of Plant Resource Science, Faculty of Agriculture, Kobe University

Soybean is not only a food crop but also an important industrial crop. As an oil crop, it produces the biggest amount of plant oil in the world. The fatty acid profile of soybean oil shows high linoleic and linolenic acids, which implies that it can be appropriate for industrial uses. In many cases, soybean oil is chemically epoxidized and used for plasticizers, coatings, paints, etc. Vernolic acid is one of natural epoxy fatty acids. This unusual fatty acid is enriched in the seed oils of several Asteraceae genera, including Vernonia, Stokesia, and Crepis, but not in soybean. Recently three epoxygenase genes that can convert linoleic acid to vernolic acid have been cloned from those species. Interestingly, these epoxygenase genes had high similarities to delta-12 desaturases that convert oleic acid to linoleic acid. Furthermore, it has been suggested that transgenic soybean plants expressing the introduced epoxygenase gene produce vernolic acid, thus they can be used for the direct production of vernolic acid without extra chemical processes. Here, I would like to introduce our attempts to improve soybean oil for industrial uses using molecular techniques.

Structure and Molecular Function of Cytochrome P450 Species in Higher Plants

Dr. Hiromasa Imaishi
Department of Biological and Environmental Science, Faculty of Agriculture, Kobe University

Cytochrome P450 (P450 or CYP) monooxygenases play an important role in the oxidative metabolism of a variety of lipophilic compounds including secondary metabolites as well as foreign chemicals in higher plants. We isolated CYP703A1, CYP92B1 and CYP76J1 cDNA clones from petunia. A high level of the transcript of each of cyp703A1, cyp92B1 were found at the early stage of flower development,while CYP76J1 expressed in leaves. CYP703A1, CYP92B1 and CYP76J1 cDNA clones were expressed in the yeast *Saccharomyces cerevisiae* AH22 cells under the control of alcohol dehydrogenase I promoter and terminator. The recombinant yeast microsomes containing each of CYP703A1, CYP92B1 and CYP76J1 hemoproteins were found to metabolize lauric acid to give a hydroxylated metabolite, while CYP76J1 also metabolized capric acids. We discuss about physiological functions of hydroxylated fatty acids in higher plants.

Germination Strategy of *Striga hermonthica*, a Root Parasitic Weed

Dr. Yukihiro Sugimoto
Department of Biofunctional Chemistry, Faculty of Agriculture,
Kobe University

Striga hermonthica is a root parasitic weed that reduces yields of many important cereals in semi-arid sub-Saharan Africa. Seeds of *Striga* require a period of pre-treatment 'conditioning' in a warm moist environment for several days before they have the potential to germinate. Germination occurs in response to an exogenous stimulant, which is exuded from the roots of host and some non-host plants. Involvement of ethylene biosynthesis in *Striga* germination has been well documented. To further understand the regulation of ethylene production during *Striga* seed germination, ACC synthase and ACC oxidase genes were cloned, and temporal changes of expression of these genes during conditioning and after seed treatment with a germination stimulant were investigated. Based on the results, the germination strategy of *S. hermonthica* under field conditions is discussed.

Involvement of Regulatory Systems in Biocontrol of Plant Diseases by *Pseudomonas fluorescens* 2P24

Dr. Zhang Liqun
Department of Plant Pathology, China Agricultural University,
Beijing 100094, P. R. China

Pseudomonas fluorescens 2P24 is a biocontrol agent isolated from wheat field in Shandong province, China. This strain produces several antifungal compounds, such as 2,4-diacetylphloroglucinol (2,4-DAPG), hydrogen cyanide and siderophore, and protects crops against several soil-borne diseases, such as wheat take-all caused by *Gaeumannomyces graminis* var. *tritici*, tomato bacterial wilt caused by *Ralstonia solanacearum* and cotton damping-off caused by *Rhizoctonia solani*. Molecular genetic analysis revealed that strain 2P24 contained GacS/GacA two component regulatory system and quorum-sensing regulatory system. The antifungal production was found to be under the regulation of GacS/GacA two component regulatory system and the colonization activity was partially controlled by the quorum-sensing system. Chromosome deletion of any genes involved in these regulation systems resulted the significant decline of biocontrol activity of 2P24. Our results support a preliminary conclusion that both two regulatory systems play important roles in the disease suppression by *P. fluorescens* 2P24.

Current status of Citrus Tristeza and Greening diseases in Ho Chi Minh and Dong Nai areas

Tuyen Bui Cach, Don Le Dinh and Thuan Tu Thi My Nong Lam University, Ho Chi Minh City, Vietnam

More than five hundreds of Citrus leaf samples were subjected to PCR and ELISA analyses for detection of *Citrus Tristeza Virus* and *Liberobacter sp.*. The samples were collected from Citrus fields in Hochiminh City and Pummelo fields in Tan Binh-Dong Nai, and also from 11 seedling nurseries. Results indicated that Tristeza virus and Greening bacterium were in all of Citrus fields sampled. There were 40% Citrus plants infected with both Tristeza and Greening. In seedling nurseries, there were more than 60% Citrus infected with CTV, but only 2% Pummelo infected. We suggest that infected-seedlings, which are selling, should be a cause of expanding CTV and Greening diseases to fields. The management of disease-free Citrus seedling program should be based on a strict sanitation plan.

New Construction Techniques for Shallowly Buried Pipeline with Geosynthetics

Dr. Toshinori Kawabata
Dept. of Agricultural and Environmental Engineering, Faculty of Agriculture, Kobe University

In recent years, many old open channels for irrigation are being replaced with pipelines. However, pipelines have to be installed in deep position, because buoyancy acts on the pipe for the ground water level keeps near the ground level in alluvial plain. Sheet piles were commonly used for pipeline construction with deep excavation. And total costs of pipeline construction become expensive. Therefore we have to reduce the cost. And we suggested the new method for shallow cover for buried pipeline using geosynthetics. In this symposium, especially, results of large scale shaking table tests and centrifuge model tests that noticed the behavior of buried pipeline during liquefaction of the ground are introduced.

People and their Life in South Laos - Farming System Research for Rural Development

Dr. Nobuya Kobayashi
Food Resources Education and Research Center, Faculty of Agriculture,
Kobe University

It is well understood from more than 30 years experience of agricultural and rural development in Asian countries that inhabitants' own consciousness and voluntary dealing are indispensable to the development of inhabitants' life and agriculture. In any rural development undertaking, it is important to observe carefully and extract the potential of life-style and farming system based on the traditional knowledge of inhabitants themselves. We have conducted field survey to learn the inhabitants' experiences and knowledge that have been accumulated for many generations. I herein report a case study in Savannakhet Province, Lao Peoples' Democratic Republic aiming at sustainable agricultural development.

RDE Program on the Safety and Quality of Food and Food Products in the Philippines

Erlinda I. Dizon
Institute of Food Science and Technology, College of Agriculture,
The University of Philippines,
Los Banos, Laguna 4031, Philippines

In view of the global trade liberalization, the Philippine government created a national network that will implement programs to ensure safety and quality food and food products. The Philippines food exports had experienced rejections and detentions due sometimes for non-compliance to the standards set by the importing countries. In this regard, the food scientists can contribute greatly for the success of the food industry to answer the global concerns on the safety and quality of food for human consumption. The Institute of Food Science and Technology (IFST) at the University of the Philippines Los Banos (UPLB) is tasked to teach, and to conduct research and extension activities in pursuit of safe and quality of foods. The proposed research program includes the role of IFST for the development of the small and medium scale enterprises and the national food industry as a whole. The paper also presents the goal, objectives and strategies to meet the envisioned targets of the proposed program. Exchange of scientists between and among countries to do cooperative research is included in the plan. Results of the RDE program will be of benefit to developing countries like the Philippines in attaining global competitiveness.

Pathogenomics and Asian Consortium on Systems Biology for Rice Diseases

Dr. Yong-Hwan Lee
School of Agricultural Biotechnology, Seoul national University,
Seoul 151-742, Korea

Rice blast, caused by *Magnaporthe grisea*, is considered as a model system for studying fungal pathogen-plant interactions. Although much has been known about pre-penetration stages, little is known about molecular mechanisms and genes responsible for infectious growth and reproduction of the fungus in host tissues to complete the disease cycle. To elucidate the mechanisms involved in the late stages of pathogenesis at genomics level, expressed sequence tag (EST) analysis was conducted to identify *in planta* expressed *M. grisea* genes as well as rice genes involved in defense responses against infection by the blast fungus. Two different cDNA libraries were constructed from rice leaves infected with *M. grisea* strain KJ201. Four thousands clones were sequenced and 2,315 non-redundant ESTs were identified. Bioinformatic analysis and transcriptional profiling of the fungal genes expressed *in planta* will be discussed. In the second approach to understand pathogenicity factors of this fungus at whole genome level, *Agrobacterium tumefaciens*-mediated transformation (ATMT) of *M. grisea* was developed as an insertional mutagenesis tool. A mutant bank of transformants generated by ATMT is being constructed and phenomics data including loss or gain of virulence, cultural characteristics, infection-related morphogenesis and pathogenicity are being recorded in the mutants database. Current progress on characterizing transformants in this project will be presented. In addition to pathogenomics research in my lab, the proposal on Asian Consortium on Systems Biology for Rice Diseases will be presented.

Poster Presentations

Genotypic analyses of pandemic *Vibrio parahaemolyticus* and novel PCR-based identification method for the pandemic strains

Masatoshi Okura
Department of Animal Science, Graduate School of Science and Technology, Kobe University

A total of 54 *Vibrio parahaemolyticus* strains including pandemic O3:K6 strains and newly emerged O4:K68, O1:K25, O1:K26 and O1:K untypeable strains (collectively referred to as “pandemic group” hereafter) were examined for their pulsed-field gel electrophoresis (PFGE) and arbitrarily primed-PCR (AP-PCR) profiles, and for the presence or absence of genetic marker DNA sequences, *toxRS/new* or *orf8* , that had been reported elsewhere to be specific to the pandemic group. Both PFGE and AP-PCR analyses indicated that all strains of the pandemic group formed a distinct genotypic cluster, suggesting that they originated from the same clone. In addition to the pandemic group, four O3:K6 strains that did not possess the thermostable direct hemolysin gene (*tdh*) also belonged to this cluster and possessed the *toxRS/new* sequence. However, three O3:K6 strains that clearly belonged to the pandemic group by PFGE and AP-PCR did not possess *orf8* sequence. The evidence suggests that neither of *toxRS/new* nor *orf8* sequence makes reliable gene markers for definite identification of the pandemic group. We therefore developed a novel PCR assay specific to the pandemic group. The assay employs an oligonucleotide primer pair derived from the group-specific sequences of an AP-PCR fragment. With this assay, the pandemic group was distinguished from other *V.parahaemolyticus* strains successfully by yielding a 235 bp specific PCR amplicon.

Effects of Repeated Subculturing and Prolonged Storage at Room Temperature of Enterohemorrhagic *Escherichia coli* O157:H7 on Pulsed-Field Gel Electrophoresis Profiles

Atsushi Iguchi
Doctoral course in Department of Bioscience,
Graduate school of Science and Technology, Kobe University

In order to determine relatedness among bacterial isolates involved in an outbreak of infectious disease, the isolates should be typed by strain level after identification at species level. With recent progress in molecular biology, several newer molecular methods have been developed for the strain typing. Among them, pulsed-field gel electrophoresis (PFGE) is considered to be an excellent approach, and is employed in many epidemiological investigations of food or water-borne outbreaks and nosocomial infections caused by bacterial strains such as methicillin-resistant Staphylococcus aureus and enterohemorrhagic Escherichia coli (EHEC). Variations in PFGE fragment patterns in single strain that had been cultured repeatedly over time were reported for several bacterial species. For example, an extensive genotypic change occurred in a strain of Campylobacter coli after extended subculturing under standard in vitro culture conditions. However to date EHEC O157:H7 has hardly been evaluated in this regard. We therefore examined the variability of PFGE patterns of three clinical isolates of EHEC O157:H7 through repeated subculturing and prolonged storage *in vitro*. Three clinical strains of EHEC O157:H7 that were subcultured repeatedly or stored at room temperature over a 25-week period showed appreciable variations in their PFGE fragment patterns. Through repeated subculturing and prolonged storage, the various patterns different from the original pattern were observed, but the fragment pattern differences were 5 fragments at most. The variations could be explained by a couple of spontaneous genetic events at most, thus did not invalidate their genetic lineage of the strains. This in turn suggests that the apparent genotypic diversity as seen in PFGE profiling of EHEC O157:H7 isolates is not due to genetic instability of the isolates.

Designing a New Forestry for Sustainable Management of Landscape-level Biodiversity

Hiroaki Ishii[1] and Shingo Taniguchi [2]
[1] Graduate School of Science and Technology, Kobe University
[2] Tajima Center, Hyogo Prefectural Forest and Forest Products Research Institute

We propose to design a more sustainable forest management plan that also promotes biodiveristy at both the stand and landscape scales. The plan involves implementation of new silvicultural techniques that enhances stand structural complexity, especially that of the forest canopy in an effort to create a more diverse habitat structure for forest dwelling organisms. We also propose a more structured zonation of forest lands creating buffer zones around conservation areas and corridors for large mammal movement. Such an integrated forest management plan should be implemented in order to create a sustainable forest landscape that also supports high biodiversity.

Reseach and development on alternative composting of waste materials: Catalytic effect of certain inorganic minerals present in fresh volcanic ash on humification of several plant residues

Socorro Asuncion Bulosan-Atendido[1], Takeshi Suzuki[2], Hiroo Otsuka[2] and Nobuhide Fujitake[2]

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Nowadays, recycling and utilization of various waste materials such as crop plant residues, refuse food, lumber wastes and so on needs great attention. At the same time, reducing the concentration of carbon dioxide in the air to reduce global warming effect was also deemed necessary. One way of fixing carbon in the soil for a long term period is to return plant residue carbon, brought forth by photosynthesis, to the soil and accumulate it as undecomposable soil organic matter or humus. Soil organic matter can be fractionated methodologically into fulvic acid (FA), humic acid (HA) and humin (Kumada 1987). Humic acids are the most studied group of humic substances (Cresser et al. 1993). They are an aggregate of various humified molecules and have different properties and humification degrees. Humification of humic acid from plant residue is considered to proceed from Type Rp - Type P - Type B - Type A, the last type being regarded as the most humified form of soil HAs, having aromatic compounds and functional groups which render them as the most stable (Kumada, 1987). Type A humic acid in surface layers of Andosol has a lifetime of some thousand years (Arai et al 1986 as edited by Wada, 1986) although the mechanism of its genesis is not clear. If Type A humic acid can be created from various waste materials (e.g. composting) and then apply them in soils, plant residue carbon may stay for a much longer period in the soil. At the same time, it could serve as good quality compost for growing crops. Volcanic ash can act as catalyst on the genesis of Type A humic acid derived from some plant residues (Otsuka et al, 1994). However, it was still unknown which inorganic component of volcanic ash can be the source of this catalytic reaction. In order to clarify this, a model thermal incubation experiment of three plant residues rice straw, broad leaf and pine tree saw dusts with Sakurajima volcanic ash was performed. Addition of phosphate and lowered pH condition was also employed. At the same time, inorganic minerals Fe, Mn and Al in the forms of oxides, hydroxides and sulfates were also incubated with the three plant residues. The temperature was set at 90°C and incubation period lasted up to 180 days. Daily water addition was supervised and pH was monitored throughout the incubation period and humus composition was analyzed at the end of each incubation period. It was found that pH and phosphate affects the formation of Type A humic acid from three plant residues with Sakurajima volcanic ash. Their influence was on the transformation of Fe and Al present in the volcanic ash into a more extractable form. The relatively small amounts of Fe and Al in higher pH values and their reaction with phosphate rendered these components ineffective in hastening the humification process. Meanwhile, Type A humic acids were formed in all plant residues incubated with $Al_2(SO_4)_3$, FeO(OH) and MnO_2 after 180 days of incubation

period. It was concluded that inorganic compounds of Fe, Al and Mn contributed to the hastening of the humification process of plant residues and that each element have different effects depending on their crystallinity, solubility and valency.

MDL1 and *MDL2*, Dicer homologs in *Magnaporthe oryzae* are involved in the morphological development and pathogenicity of the fungus.

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By database search, we identified two Dicer homologs in the genome of *Magnaporthe oryzae*, and tentatively named them *Magnaporthe* Dicer like (*MDL*)*1* and *2* . *MDL1* and *2* were 5682 and 4732 bp long in length, respectively, and contained RNaseIII and helicase domains that were characteristic to Dicer-like proteins found in animals and plants. To gain insights into roles of *MDL1* and *2* in *M. oryzae*, we disrupted those genes by homologous recombination. The *MDL1* and *MDL2* disruptants showed significantly reduction, but not complete loss, of GFP silencing induced by hairpin GFP RNA, supporting the idea that the Dicer-like genes were involved in RNA silencing in *M. oryzae*. Interestingly, phenotypic changes including anomalous spore shape (*MDL1*), less sporulation (*MDL2*), slow mycelium growth (*MDL2*) and less fertility (*MDL2*), were observed in the disruptants. Moreover, the *MDL2* disruptants showed less virulence on the host plants while the *MDL1* disruptants were as virulent as wild type. These findings indicated that the two Dicer-like enzymes, MDL1 and MDL2 were involved in fungal development and virulence of *M. oryzae* through slightly different pathways.

Comparative studies on the induced resistance of *Pseudomonas fluorescens* against microorganisms and insect pests in tomato

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Pseudomonas fluorescens is one of the prominent biocontrol agent in practical application. Apart from direct antagonistic activity against plant pathogens, the Pseudomonads have been shown to induce systemic resistance in plants against some pathogens and insects. Nitric oxide (NO) has been shown to play a critical signaling role in the activation of plant defense responses. In rhizobacteria *Pseudomonas,* NO can be produced through dissmilatory denitrification. Therefore, we have examined if increasing NO production of pseudomonads improves their efficiency of biocontrol against disease and insect. The endogenous nitric oxide reductase gene of a biocontrol agent, *P. fluorescens* T5, was disrupted by homologous recombination using a suicide plasmid. Three *Nor* gene disruptants were obtained, which were confirmed by Southern hybridization. NO assay by fluorescence DAF-2 showed that the transformants increased the production of NO by 2.9, 3.3, and 3.2 times over the wild type, respectively. To test induced resistance against pathogen, tomato seeds were coated with T5 and the NO overproducing transformants and sown in soil infested with *R. solanacearum*. Infection with *R. solanacearum* was obviously reduced in tomato shoots treated with the NO overproducing transformants compared with the wild type. Fourteen days after treatment, the rate of healthy shoots was 20- 30% higher in tomato treated with the transformants than with the wild type. When 4-day-old adult of the ladybird beetle (*Epilachna vigintioctopunctata*) was released, however, no significant difference was detected in damaged leaf area between tomato seedlings treated with the NO overproducing transformants and those with the wild type. These results suggest that the overproduction of NO enhances the ability of *P. fluorescens* to suppress soil-born disease but not to protect tomato against the attack by the ladybird beetle.

Behavior of Shallowly buried Pipe on applying Traffic Load by D.E.M. Analyses

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Recently, the constructions of shallowly buried flexible pipelines are increasing to reduce the cost of pipeline construction. However, the shallowly buried pipelines have a risk of large deformation caused by traffic loads. Our previous research pointed that the risk of the concentrated earth pressure acting on the top of the pipe due to traffic loads. In this poster, numerical analyses were carried out on the behavior of buried flexible pipe with shallow depth of cover and with conventional depth under traffic load. The pipe diameters are 2000mm and backfill materials are sand or gravel. In addition, as new techniques for distribution of traffic loads, it was suggested that the using of the sandbags in the backfill materials to reduce the loads acting on the pipe top. As a result, it was found that the concentrated traffic loads acting on the pipe top were distributed by using sandbags.

Development of porcine primordial follicles in xenografts

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In the mammalian ovaries, primordial follicles are the largest storage of ovarian follicles containing non-growing small oocytes. The mechanisms that control the initiation of development of primordial follicles have not been understood well. Small oocytes in primordial follicles of newborn rodents grow in the cultured ovary or ovarian tissue. For domestic animals, however, culture systems for primordial follicles have not been valid. Xenotransplantation of ovarian tissues to immunodeficient mice can be a substitute of an effective culture system for the development of primordial follicles. This experiment was conducted to study the development of pig primordial follicles in xenografts. Effect of the gender of host mice on the follicular development and oocyte growth in xenografts were also examined. Cortical slices containing only primordial follicles were collected from the ovaries of 6-month-old gilts (n = 8) and 10-day-old piglets (n = 6). Each slice was cut into 2 pieces; one was fixed for histological examination and the other was transplanted into SCID (severe combined immune deficiency) mice. After 2 months, the grafts were recovered and processed for histological examination. Histological examination confirmed that the cortical slices contained only primordial follicles before transplantation. After transplantation, 47% (171/364) of primordial follicles from adult pig ovaries survived in the xenografts but none of those developed to primary follicles or beyond. On the other hand, in the xenografts of newborn pig ovaries, 13.5, 9.7 and 0.3% of 1,122 follicles developed to the primary, secondary and the antral stages, respectively, in male SCID mice. In the xenografts in female SCID mice, there was no antral follicle although secondary follicles (6.1%) were observed among a total of 1,042 follicles. Oocyte growth and follicular development were significantly higher in male and ovariectomized female than that in intact female host mice ($p<0.05$). The results showed that primordial follicles of 6-month-old pig ovaries survived but did not develop in xenografts, whereas newborn pig primordial follicles developed to the antral stage. This suggests that non-growing oocytes in adult pig ovaries have been altered the requirements for initiating their growth.

Regulation of chromosome condensation by histone H3 phosphorylation during maturation in pig oocytes

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When oocytes resume meiosis, chromosomes start to condense and Cdc2 kinase becomes activated. However, recent findings show that the chromosome condensation does not always correlate with Cdc2 kinase activity in pig oocytes. The objectives of this study were to examine 1) the correlation between chromosome condensation and histone H3 phosphorylation at serine 10 (Ser10) during meiotic maturation of pig oocytes, and 2) the effects of protein phosphatase 1/2A (PP1/PP2A) inhibitors on the chromosome condensation and examine the involvement of Cdc2 kinase, MAP kinase and histone H3 kinase in this process. Phosphorylation of histone H3 (Ser10) was first detected in the clump of condensed chromosomes at the diakinesis stage and maintained until metaphase II. The kinase assay showed that histone H3 kinase activity was low in oocytes at the germinal vesicle stage, increased at the diakinesis stage, and high activity was maintained until metaphase II. Treatment with okadaic acid (OA) or calyculin-A (CL-A), the PP1/PP2A inhibitors, induced rapid chromosome condensation with histone H3 (Ser10) phosphorylation after 2 hr. Both histone H3 kinase and MAP kinase were activated in the treated oocytes, although Cdc2 kinase was not activated. In the oocytes treated with CL-A and the MEK inhibitor, U0126, neither Cdc2 kinase nor MAP kinase were activated, although histone H3 kinase was still activated and chromosomes condensed with histone H3 (Ser10) phosphorylation. These results suggest that phosphorylation of histone H3 (Ser10) occurs in condensed chromosomes during maturation in pig oocytes. Furthermore, the chromosome condensation is correlated with histone H3 kinase activity, but not with Cdc2 kinase and MAP kinase activities.

Non-destructive Visualization of Internal Structures of Model Foods for Detecting Foreign Materials by Electrical Impedance Tomography

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The application of Electrical Impedance Tomography (EIT) for visualizing the internal structure of a model food containing a void and foreign materials was investigated. As model foods, agar gel, electrolyte solution, raw hamburger meat and sliced ham were tested and a Teflon plastic cylinder and a copper pipe were adopted as foreign materials and were embedded in model foods. In the EIT system, the neighboring method was adopted and the voltage differences between the adjacent pairs of 16 electrodes placed on the surface of the model foods were measured. Finite Element Method (FEM) and Newton-Rapson optimization algorithm could computationally solve an inverse problem for image reconstruction of the electrical resistivity distribution. As a result, a clear image of a copper pipe or a void (hole) in agar gel, hamburger meat and ham could be obtained with a difference image between static image and the reference image excluding foreign materials and voids. For a plastic cylinder in the solution, a much clearer image could be obtained without a difference image. However, in the cases of hamburger meat and ham, neither the position nor the size of the foreign materials agreed accurately with the original position and the actual size of them. Because the measurement accuracy was strongly influenced by non-homogeneous distribution of the electrical current in foods due to the thickness and the composition of the foods.