Green Revolution

Hosted by the Graduate School of Biosphere Science, Hiroshima University

Co-hosted by the Research Center for Animal Science, the Research Center for Japanese Foods and the Research Core for Plant Science Innovation, Hiroshima University

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10th International Symposium on Food and Environment

"Status of Biotic Resources and Efforts for Sustainable Production in Asian Countries"

13 00 - 16 30 : C206

Date: 4 November, 2017 (Sat.) 13:00 – 16:30

Venue: Room C206, Graduate School of Biosphere Science, Hiroshima University

Greetings from the Dean

It is our great pleasure to commemorate the 10th anniversary of our International Symposium on Food and Environment.

Human existence largely depends upon sustainable food production in many countries. Various factors related to climate change, human population increase and change of socio-industrial structure caused by rapid development are affecting the production environment of biotic resources which consist of genetic and environmental resources. It is of our great concern how to manage and produce sustainably such valuable biotic resources for clothing and medicine as well as foods that are essential for human life. This symposium is convened to present overviews and discuss current situations, issues and challenges affecting biotic resources and the environment towards their sustainable production in Asian countries.

Dr. Yukinori Yoshimura, Dean

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	Dr. Wen-Dee Chiang	(Tunghai University	, Taiwan Chair	: Makoto) Hirayama
13:50	"Food Production and	Environment Manage	ement Sys	tems and Prol	
	Dr. Chaiyapoom Bun	chasak (Kasetsart Un	iversity, Tl Chair	hailand : Taketo	Page 4) Obitsu
14:30	Biosphere Science, H	e 2016 Grant-in-Aid for Hiroshima University 2 a University and Tungha	016		te School of
15:20	Break time "Mechanisms of Coast Case Studies at the M	al Biological Producti Iyanmar and Indones		3''	
	Dr. Kazuhiko Koike	(Hiroshima Universit	y, Japan Chair)	Yoshida
16:00	General Discussion			Chair	: Lawrence M. Liao
16:20	Closing Remarks Toshinori Nagaoka, Chair of the International Exchange Com				l Exchange Committee
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Overview of the Studies on Bioactive Peptides in Taiwan

Chiang Wen-Dee

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Bioactive peptides, which are produced by several methods including chemical synthesis and biotransformation (enzymatic hydrolysis, cell recombination and fermentation), are defined as specific protein fragments that have a positive impact on body functions and may ultimately influence health. According to their studied functional properties, bioactive peptides may be classified as antimicrobial, anti-hypertensive, immunomodulatory, hypocholesterolemic, mineral binding, anti-oxidative, blood glucose regulatory and anti-obesity. These peptides play an important role for human health. In this review, we describe the properties of bioactive peptides stated above especially those studied in Taiwan.

Based on the database of National Digital Library of Theses and Dissertations in Taiwan, there are about 700 cases of study of chemical synthetic peptides related to novel drugs and 50 cases of study of bio-transformed peptides related to nutraceuticals since 2003. According to the functionality of bio-transformed peptides, hypertensive peptides and anti-oxidative peptides are two of the most studied, with 14 and 11 cases respectively. Both mineral binding peptides and anti-obesity peptides each have seven cases. Hypocholesterolemic peptides, blood glucose regulative peptides, antimicrobial peptides and others have 2 cases each. Based on the production methods, 46 out of 50 cases have employed enzymatic hydrolysis. Three cases used recombinant cell and one case applied fermentation to produce the bioactive peptides. From the view of starting material, 18 studies are related to plant proteins while 32 are related to animal proteins. Soy and potato proteins are the major plant sources for these studies. Most of the animal proteins come from processing residue or by-product of fish and egg.

For the past 15 years, several functional properties have been studied in our lab except anti-microbial peptides. One of the major functional properties studied in our lab are the anti-obesity peptides with anti-adipogenic or lipolysis-stimulating activity, which will be introduced as an example for the specific peptide production, purification and identification. Obesity has increased dramatically in the past 25 years and is considered to be a serious problem that is associated with the development of major human metabolic diseases, including non-insulin-dependent diabetes, hypertension, cancer, gallbladder disease and atherosclerosis. Adipose tissue is basically an energy reservoir in mammals that takes in glucose and free fatty acid (FFA) postprandial and stores them as triglyceride by lipogenesis. During fasting, the adipose tissue releases glycerol and FFA by lipolysis of the stored triglycerides into the circulating blood. Adipose triglyceride lipase (ATGL) predominantly performs the initial step in triglyceride hydrolysis resulting in diglyceride and FFA. Hormone-sensitive lipase

(HSL) hydrolyses triglyceride, diglyceride and monoglyceride at a ratio of 1:10:1.

In the study of anti-obesity peptides, enzymatic hydrolysis of soy protein isolated (SPI) to produce hydrolysates with anti-obesity activity (AOA) was investigated in our lab. The results indicated that enhancement of AOA for SPI by limited hydrolysis with Flavourzyme and fractionated with ultrafiltration membrane can been done. Limited enzymatic hydrolysis has been widely applied to increase the functionality of dietary protein in the food industry. If hydrolysis is not limited, and goes too far, it will usually result in a hydrolysate with uninteresting functional properties. During hydrolysis with proteases, controlling degree of hydrolysis (DH) is essential for specific properties of protein hydrolysates. The result of our study suggests that SPI should be hydrolyzed with Flavourzyme for 2 h at pH 7.0 and 50 in order to obtain hydrolysate (FH2h) with DH as close as possible to 7.42%. Extensive hydrolysis of SPI with a DH of more than 9.49% did not guarantee any further enhancement of AOA, but it did imply that limited hydrolysis was required to maintain the structure or sequence of the active peptides and to ensure functionality. To explore the AOA of SPI hydrolysate, 3T3-L1 adipocytes were applied. Intracellular triglyceride residue (TR) was employed as a marker for lipolysis in cells. The lower TR represents the better AOA. Sequential fractionation of FH2h with different molecular weight cut-off (MWCO) membrane revealed the possibility to enhance its AOA in terms of TR. The sequential fractionation of FH2h with 30-0.3 kDa MWCO membranes in order to obtain a 1 kDa retentate resulted in further enhancement of its AOA in the cells. The TR decreased significantly from 2.73 to 2.30 mole/mg protein at 400 ppm level (p < 0.05). The enhancement of bioactive activities by fractionating with different MWCO membranes has been described in many studies.

Based on the western immunoblot and immunostaining analysis, the 1 kDa retentate promotes lipolysis by increasing phosphorylation and translocation of the hormone-sensitive lipase (HSL) in 3T3-L1 adipocytes. The effect of 1 kDa retentate on the protein expression of HSL in 3T3-L1 adipocytes was determined. The cells were incubated with 50 ppm of 1 kDa retentate for 12-72 h. There was a tendency to decrease the protein expression of HSL after 24 h incubation, and there was an especially significant reduction of protein expression of HSL at 72 h incubation (p < 0.05). This might be due to the fact that some of the HSL was phosphorylated. A significant increase of phospho-HSL was observed in the cells after 48 h incubation. In addition, according to the subcellular HSL analysis after the addition of 1 kDa retentate, immunostaining of adipocytes with anti-HSL antibody was conducted. The result indicated that stimulation with 1 kDa retentate increased the immunofluorescent signal (bright ring-loop) of HSL surrounding the lipid droplet peripheries in the cells after 48 h incubation.

Further studies on purification and identification of bioactive peptides from the soy protein hydrolysate with lipolysis-stimulating activity were conducted. Gel filtration-high performance liquid chromatography (GF-HPLC) of the 1 kDa retentate derived from FH2h resulted in four fractions of GF1, GF2, GF3 and GF4. The result indicated that GF3 had highest glycerol release and significant increase of glycerol release from 395 to 488 nmol/mg protein after gel fitration of 1 kDa retentate.

The higher glycerol release also represents the better AOA. GF3 was subjected to further fractionation using a Develosil ODS-HG-5 RP-HPLC column. Four fractions, including HF1, HF2, HF3 and HF4, were also collected. HF4 was identified as the most active fraction to release glycerol at up to 582 nmol/mg protein in the adipocytes, and further fractionated by the second RP-HPLC to obtain three fractions. Both RHF4-2 and THF4-3 with glycerol release of 580 and 615 nmol/mg protein, respectively, were identified as the most potent fractions to stimulate lipolysis in the adipocytes. Peptide sequences of RHF4-2 and RHF4-3 were analyzed by LC/MS/MS. The molecular weights of RHF4-2 and RHF4-3 were 358 and 453 Da, respectively. The amino acid sequence of tripeptide RHF4-2, was composed of Leu (L) and IIe (I), potentially LLL, LLI, LIL, LIL, LII, ILI, III and III. By comparing the known amino acid sequences of two major types of soy protein, glycinin and -conglycinin, only III, ILL and LLL naturally exist in the protein. Therefore, RHF4-2 was identified as one of these three tripeptides, whereas RHF4-3 was confirmed as VHVV. In order to ensure the AOA of each purified peptide, the three tripeptides and VHVV were synthesized and their activities were further determined. The effect of RHF4-2, RHF4-3 and synthetic peptides (III, ILL, LLL and VHVV) on glycerol release in 3T3-L1 adipocytes was investigated. The synthetic peptides ILL, LLL and VHVV exhibited similar or even better glycerol release of 548, 579 and 687 nmol/mg protein, respectively, with VHVV having the highest AOA.

The in vitro effect of gastrointestinal proteases on AOA of synthetic ILL, LLL and VHVV, was also investigated. The result suggested that the gastrointestinal protease did not affect lipolysis-stimulating activity of the three novel peptides, which reveal the potential to act as anti-obesity ingredients. In order to expand the application of these peptides with AOA, an animal feeding study or clinical trial will be required in the future.

Keywords: Anti-obesity peptides; Bioactive peptide; Enzymatic hydrolysis; Glycerol release; Identification; Purification; 3T3-L1 adipocytes

Food Production and Environment Management Systems and Problems in Thailand

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Generally, the quality and quantity of food productions (animal and plant products) are related to the climate (temperature, humidity and rainfall) of each area. Thailand is located in the tropical area between latitudes 5 ° 37 'N to 20 ° 27' N and longitudes 97 ° 22 'E to 105 ° 37 'E. Most of Thailand has a "tropical wet and dry or savanna climate" type. The total area is 513,115 square kilometers or around 200,000 square miles. Both high humidity and temperature in this area have negatively affected productive performance of animals. Since the country receives a mean annual rainfall of 1,200 to 1,600 mm (47 to 63 in), however, it positively promotes high efficiency of plant and aquatic animal productions. Consequently, Thailand is the leader of shrimp and plants production in the world. Many plant productions are produced and ranked within the top ten around the world, such as rice (5), cassava (2), ginger (5), mango (3), sugar cane (4), pineapple (4), guava (4), jackfruit (3) and rubber (1). Due to the overproduction of some products (rice, cassava, sugar cane and rubber), many subsidy policies have to be implemented by the government to help the farmers.

Conversely, low productive performances of animal production are commonly found in the tropical areas. The climate of this area induces heat stress, low feed intake of animals, suppression of immunity and widespread pathogens and carriers (insects). So, the animal products (dairy cattle and pig) are not among the major agricultural products of this country. Nevertheless, raising and management of broiler chicken in Thailand have been dramatically developed since evaporative cooling system had been adapted and used to resolve the problem of heat stress. Thus, the exportation of chicken meat from Thailand is ranked around fourth or fifth in the world. Thailand exports chicken meat (both frozen and cooked meat) to Japan and EU amounting to more than 90% of the total exported products. There are some reasons that promote Thai broiler chicken industry. First, exportation of frozen meat to Japan and EU has sharply increased during the years 1970-1997. Second, the economic crisis in Thailand (started from 1997) and the consequent devaluation of the Thai currency caused high exportation of agricultural products. Third, Thailand was attacked by avian influenza (AI) in the year 2004 leading to the establishment of highly effective compartment system, high bio-security system and high quality of ready to eat products. Currently, more than 90% of total revenue of the exports come from processed products.

Mechanisms of Coastal Biological Production in Southeast Asia and Some Concerns: Case Studies at the Myanmar and Indonesian Coasts

Kazuhiko Koike

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As the top growth center of the world, fisheries in Southeast Asian countries are of high importance and hence seeking better and suitable ways are on-going. The FAO has recommended a comprehensive management approach of oceans and other aquatic environments as the "Blue Growth", which aims towards implementing sustainable capture fisheries and aquaculture methods while the coastal environments are conserved.

The author has been engaging in coastal environmental researches in the top 1 and 3 countries in SE Asia, namely Indonesia and Myanmar, and clarified their coastal characters which may largely contribute biological productions there. In this presentation, these results will be given and will be taken as an opportunity to discuss possible coastal conditions that may decrease natural biological productions.

Myanmar is the third among the fish producing countries in ASEAN and more than 50% of the capture fisheries rely on coastal marine fisheries. To understand the mechanisms underlying the high production there, oceanographic and phytoplankton surveys, including primary productivity measurements were conducted near an active fishing ground near Myeik City. Three surveys, one in each of the representative seasons and covering the characteristic coastal environments, showed well-defined seasonality in primary production and phytoplankton occurrence. The end of the dry season was the most productive, with productivity of 2.59 ± 1.56 g C m⁻² day⁻¹ and high concentration of chlorophyll a. Whereas primary productivity was low at the onset of the dry season, 1.36 ± 0.77 g C m⁻² day⁻¹, this low primary production might be compensated by activation of microbial food chains originating from high dissolved organic carbon from the mangrove sediments. The rainy season exhibited the lowest production, 6.6% of the end of the dry season, due to the extensive discharge of turbid water from the rivers which lowered the euphotic layer depth and resulted in an unusually high diffuse attenuation coefficient of 2.30 ± 1.03 m⁻¹. This incident of turbid water may be related to soil erosion from inland deforestation and mangrove deterioration.

Based upon the awareness of lowered coastal production due to inland and mangrove deforestations, this phenomenon has been more intensively surveyed along the Indonesian coast because the country is notable for such events as extensive deforestations. The survey is under progress, but a new insight was revealed during the survey about "the true contribution of the mangrove forest toward coastal productivity." This concept intends to clarify the true mechanisms occurring within the mangrove forests and creeks, in which the microphytobenthos (unicellular microalgae inhabiting on the sediment surfaces) possibly contributes to coastal production, and not limited within the mangrove ecosystem.

We believe the achievement of our study will further emphasize the importance of mangrove conservation for sustainable fisheries. The latest data will be shown in the presentation.

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Graduate School of Biosphere Science, Hiroshima University

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Research on acclimazation of prepubertal Holstein heifers for hot summer season

International Comparative Study on Management of Basin-scale Water Quality and Quantity

Tamiji Yamamoto¹ Doddi Yudianto², and Peter Davey³

1: Graduate School of Biosphere Science, Hiroshima University, Director of Center for Restoration of Basin Ecosystem and Environment

The authors have conducted the project "Cooperative Education of "Peace" and "Environment" in the Framework of International University Consortium INU (International Network of Universities)" by the support of Inter-University Exchange Project of Japan Society for the Promotion of Science during 2011-2015. During the four-year project period, we conducted international summer school for master course students in the field of aquatic environmental sciences every year. More than 140 peoples including students and teachers in total came together from the 7 oversea schools which have agreement with Hiroshima University. In parallel, Parahyangan Catholic University in Indonesia and Griffith University in Australia have also held their summer schools twice in each. Students and teachers of Hiroshima University attended these summer schools.

Due to termination of the Japan-supported exchange program in 2015, we faced to fund shortage for the activity, and the activity center moved to Parahyangan Catholic University. Then, we shifted our activity to exchange information in research field, because it would less spent much money compared to student exchange program. During the summer schools so far, we have already recognized that the way of management of water quality and quantity is much different in each country. In Indonesia, rivers are just the place of garbage dump site because of poor garbage collection system. This is also the cause of drinking water shortage. In Australia, repetition of drought and flood may have their water management difficult. On the contrary, we have been storing several excellent technologies in the fields of civil engineering, water management and ecosystem restoration in Japan.

Therefore, in the present project, we proposed to spend fund for attendance to the largest conference "International Seminar on Water Resilience in a Changing World" in Indonesia for comparison of the diversion system of river water and management system of water quantity and quality in the water cycle of different countries. The senior author, Tamiji Yamamoto, attended the conference as a scientific committee member. There were three sub-topics, "Water Conservation and Risk and Impact of Extreme Event", "Water Security for All", and "Water Governance and Partnership", and the total number of papers submitted were 95.

By hearing excellent presentations and through discussion with presenters, we could exchange information. Other than Parahyangan Catholic University, presentations were done from other Indonesian universities, Hassanuddin University, Gadjah Mada University, Bandung Institute of Technology, Brawijaya University, Diponegoro University, Indonesia University, and North Sumatera University, which have agreements with Hiroshima University. Several presentations were from Netherland, Koria and Japan. To pursue our collaborative research regarding management of water quantity and quality in the basin scales, information exchange like this time would be very important.

Being not different from our expectation at the initial stage of our collaboration, we recognized that technologies and knowledge of Japan in the fields of water purification, garbage collection and processing, and restoration of ecosystem are at the top level in the world. These sophisticated technologies and systems we have developed in Japan could contribute restoration of water environment in other countries by transferring the technologies.

The senior author has experience of an industrial development advisor of Hiroshima City, a council member of Hiroshima Recycle Initiative, and an advisor of Chugoku New Business Conference. In these relationship, now we are planning to hold an international seminar in Hiroshima or Higashi-Hiroshima.

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^{3:} Environmental Protection, Program Director Bach of Environmental Management and Specialization Convener Masters of Environment, Griffith University, Australia

Research on acclimatization of prepubertal Holstein heifers for hot summer season

Miki Okita, Yuzo Kurokawa, Takashi Bungo (Graduate School of Biosphere Science, Hiroshima University)

Recently, we found that raised dairy cow's rectal temperature and respiration rate because of hot summer tended to lower with time during hot summer season (Okita et al. 2014). It implied that cows gradually acclimatize to thermal condition in summer. There is little information for the acclimatization in cattle. The objective of the present study was to investigate the changes in physiological parameters and dairy gain in Holstein heifers during summer. The experiments were conducted from 28 June to 3 July (period 1), 25 July to 30 July (period 2), 23 August to 28 August (period 3) and 20 September to 25 September (period 4) in 2016. We used 4 or 5 Holstein heifers (initial age =

The mean temperature-humidity index (THI) during period 1, 2, 3, and 4 were 73.9, 77.0, 75.7 and 69.6, respectively. We measured dry matter intake, body weight, heart rate, rectal temperature, respiration rate and skin temperatures. Samples of jugular vein blood were collected to determine the plasma glucose, total cholesterol (T-Cho), alanine amino transferase (ALT) and alkaline phosphatase (ALP) with a biochemistry analyzer.

8.6±0.9 months, initial body weight = 295±32 kg). All heifers were fed colored guineagrass and concentrate to meet

the heifer's growth rate of 0.9kg/day.

Dry matter intake for periods 3 and 4 were significantly lower than that for period 1 and 2, though body weight gain did not differ among the experimental periods. Rectal temperature and respiration rate for period 2 and 3 were significantly higher than those for period 4. Skin temperature for period 2 was significantly higher than that for period1, 3 and 4. Plasma ALT, T-Cho and Hematocrit for period 3 were significantly lower than those for period 1, 2 and 4. ALP for period 2 and 3 were significantly lower than that for period 4.

<u>List of Poster Presentations by Students</u>

Tunghai University 東海大学						
Name		Title of presentation				
Ying-Chu Shin 施 映竹		Evaluation of soy protein hydrolysate with calcium-chelating activity in Caco-2 cell				
Chia-Chun Chan 詹 佳純		Ultrasound-assisted ethanol extracts from guava leaves and their antioxidant and blood glucose regulation activities in C2C12 cells with insulin resistant				
Hiroshima University 広島大学						
Grade Level	Name	Title of presentation				
M1	Saki Ishimaru 石丸 彩樹	Effect of calcium salts of medium-chain fatty acids on performance and plasma hormone concentrations in lactating dairy cows				
M1	Yui Takao 髙尾 結衣	Effect of calcium salts of medium-chain fatty acid supplements of feeding behavior and milking activity in lactating dairy cows in an automatic milking system				
M1	Maho Ito 伊藤 真穂	Molecular phylogenetic analysis and morphological features of Ryukyu Wild Boar in Japan				
M1	Taiki Fuji 藤 太稀	Functional morphology of cephalothoracic sucker of Caligus				
M1	Yuya Furukawa 古川雄也	The diffrence of phosphorus uptake among natural variations of Arabidopsis thaliana				
M1	Shin-ya Morishima 森島 慎也	Observation for zooxanthellae in feces expelled from tridacnine shellfishes				
M1	Azusa Endo 遠藤 梓	Strict pair-bond and stable territories of the vagabond butterflyfish, Chaetodon vagabundus performing spawning migration				
M2	Masami Koide 小出昌美	Phylogenetic and photosynthetic characters of zooxanthellae (dinoflagellate genus Symbiodinium)				
M2	Tadashi Okamura 岡村 惟史	Helicia cochinchinensis: an "Influencer" for mineral uptake of surrounding plants under low phosphate by forming cluster root				
M2	Kazuki Yoshida 吉田 和貴	Comparison of size parameters of the invasive moon snail Laguncula pulchella between northern and southern Japan				
M2	Tomomi Ebara 頴原 智美	Glass transition and texture properties of hazelnut				
M2	Masahiro Yamaguchi 山口 将大	Consumption of fructo-oligosaccharides increases ethanol levels in intestines and tissues of rats				
M2	Kaho Honma 本間 香帆	Genetic diversity and origin of native chicken in Madagascar				
M2	Misaki Fujisawa 藤澤 美咲	The cleaner's mimic <i>Aspidontus taeniatus</i> utilizes the effect of aggressive mimicry only when it is small				
M2	Sumana Chuamnakthong	The effects of mild salinity and osmotic pretreatment on salt acclimation in rice				

Grade Level	Name	Title of presentation	
D1	Shizuka O-hara 小原 静夏	Spatiotemporal changes of primary production related with environmental factors and <i>in situ</i> photosynthetic factors of phytoplankton communities in the Bingo-Nada (the Seto Inland Sea)	
D1	Qin Dong	Study on physiological characteristics of germination of plants differing in phytate content	
D1	Fumika Ito 伊藤 文香	DNA polymorphism on a tool for molecular discrimination in animal species	
D2	Chinami Ishibashi 石橋 ちなみ	In situ observation of template effects of emulsifiers with different fatty acid moieties	
D3	Sunday Oluwatoyin Michael	Selective determination of Lipid hydroperoxides in natural waters using a fluorescent probe	
D3	Fongin Suwalee	Effect of maltodextrin on the water sorption and glass transition of freeze-dried mango pulp and its application to other dry fruits	
D3	Dwi Eva	Gender differences and dietary supplemental Vitamin B6: Impact on colon	
	Nirmagustina	luminal environment	
D3	Dissanayaka Mudiyanselage Samantha Bandara	Response to phosphorus deficiency of two rice genotypes with contrasting tolerance is determined by plasticity of root growth and leaf phosphorus remobilization	
	Dissanayaka	as determined by plasticity of root grown and real phosphorus remobilization	
D3	Yang Yongshou 楊 永寿	Bifidogenic effect of Aspergillus-derived acid protease	