
広島大学大学院生物圏科学研究科 主催

広島大学日本型（発）畜産・酪農技術開発センター / 広島大学日本食の機能開発センター 共催

Hosted by the Graduate School of Biosphere Science, Hiroshima University

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

第9回 食料・環境問題国際シンポジウム

「東南アジア・アフリカ諸国を取り巻く課題と持続可能な食料生産」

9th International Symposium on Food and Environment

“Issues facing Sustainable Food Production in Southeast Asia and Africa”

日 時：平成28年11月5日（土） 13:00 - 16:40

場 所：広島大学大学院生物圏科学研究科（生物生産学部） C206 講義室

Date: 5 November, 2016 (Sat.) 13:00 – 16:40

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

研究科長からのご挨拶

近年、地球温暖化や異常気象などの気候変動、環境汚染など様々な環境要因により農作物、畜産物および水産物の生産性の低下が危惧されています。東南アジアおよびアフリカ諸国では、これらの環境要因に加えて急激な人口の増加により安全な食料の安定的な生産、さらには健康な生活を営む上で欠かせない栄養性ならびに機能性の高い食品の確保などの食料安全保障が重要な課題となっています。このシンポジウムでは、東南アジアおよびアフリカ諸国で生じている食料生産の課題とその課題に向けた取り組み、安全・安心で機能性の高い食品の開発研究を通じて、これからの持続的な食料生産のありかたについて、活発な議論ができることを期待します。

研究科長 吉村 幸則

Greetings from the Dean

Sustainable food production and environment are of great importance especially in Southeast Asian and African countries where food resources including agricultural, animal and marine products are marketed among different countries. Stable production of safe and good-quality food is one of the greatest objectives for our research to support all the people in the world at a time when the human population is rapidly growing. This symposium aims to present overviews of current and future systems of food production, their management, and natural environment surrounding the food resources especially during this time of rapid environmental change. Some of the current research topics pursued at the Graduate School of Biosphere Science will be also presented. I hope that an active exchange of ideas and opinions can be pursued in this symposium.

Dr. Yukinori Yoshimura, Dean

Program プログラム

General Chairperson 総合司会: Hirofumi Saneoka, Vice Dean 副研究科長 実岡 寛文

13:00 広島大学大学院生物圏科学研究科とタイ・コンケン大学農学部との部局間国際交流協定締結
Formal announcement of the MOU between the Graduate School of Biosphere Science, Hiroshima University and the Faculty of Agriculture, Khon Kaen University, Thailand

13:15 **Opening Message** 開会のご挨拶 Yukinori Yoshimura, Dean 研究科長 吉村 幸則

13:20 **“The Use of Chemicals in Aquaculture in Southeast Asia: Should We Be Concerned?”**
(東南アジアの養殖漁業における化学薬品の使用：私たちは懸念すべきか?)
Dr. Erlinda Cruz Lacierda (University of the Philippines Visayas)

Chair 司会: Koichiro Kawai 河合 幸一郎 --- Page 1

14:00 **“Research Strategies on Animal Production for Climate Change”**

(気候変動に対応する家畜生産の研究戦略)

Dr. Monchai Duangjinda (Khon Kaen University)

Chair 司会: Taketo Obitsu 小櫃 剛人 --- Page 3

14:40 **Break time** 休憩

Poster presentation: Staffs supported by the 2015 Grant-in-Aid for Research, and students attending international conferences supported by the Graduate School of Biosphere Science

2015年度研究科長裁量経費による研究助成報告及び国際学会発表支援による学生の成果報告

15:00 **“Soil Salinization and Management of Salt-affected Soils in the Nile Delta of Egypt”**

(エジプトナイル・デルタ地帯における土壌の塩類化の現状とその管理)

Dr. Ahamad Mekawy (Minia University)

Chair 司会: Akihiro Ueda 上田 晃弘 --- Page 5

15:40 **“Paradigm Shift in Japanese Fermented Foods and Prebiotics:
Discovery of the Bifidogenic Function of *Aspergillus* Protease”**

(日本の発酵食品とプレバイオティクスにおけるパラダイムシフト:
麹菌プロテアーゼのビフィズス菌増殖促進作用の発見)

Dr. Norihisa Kato (Hiroshima University, Japan)

Chair 司会: Tadashi Shimamoto 島本 整 --- Page 6

16:20 **General Discussion** 総合討論

Chair 司会: Lawrence M. Liao

16:35 **Closing Remarks** 閉会の辞

Toshinori Nagaoka, Chair of the International Exchange Committee
研究科国際交流委員長 長岡 俊徳

■ Reports of studies supported by the 2015 Grant-in-Aid for Research from the Graduate School

of Biosphere Science, Hiroshima University 2015年度研究科長裁量経費による研究助成報告

■ List of students attending international conferences supported by the Graduate School of Biosphere Science

研究科の支援による国際学会発表参加学生

The Use of Chemicals in Aquaculture in Southeast Asia: Should We Be Concerned?

Erlinda R. Cruz-Lacierda

Institute of Aquaculture, College of Fisheries and Ocean Sciences,
University of the Philippines Visayas
Miagao, Iloilo, Philippines 5023
eclacierda@yahoo.com

In the past decade 2005-2014, world fish aquaculture production had an average annual growth rate of 5.8%. In 2014, the total world aquaculture fish production was 73.8 million metric tons (MT), valued at US\$ 160.2 billion and accounts for 44.1% of the total world fisheries production. Of the 73.8 million MT produced from aquaculture, Asia remains the biggest contributor at 65.6 million MT, accounting for 89% of total world fish aquaculture production. The ASEAN (Association of Southeast Asian Nations) region contributed about 15% with Indonesia, Viet Nam, the Philippines, Myanmar, Thailand and Malaysia as the major contributors. In terms of environment, freshwater finfish culture in earthen ponds was the biggest contributor to food security and nutrition in the region, although marine finfish cage culture had been increasingly catching up.

The intensification of aquaculture in Southeast Asia resulted to frequent occurrence and emergence of several infectious diseases caused by viruses, bacteria, fungi and parasites. As a consequence, the routine use of veterinary medicines became inevitable to prevent and control disease outbreaks. Aside from disease treatment and control, various chemical and biological products were applied to soil and water during pond preparation and culture period to enhance natural aquatic productivity and maintain optimum physico-chemical parameters required for growth of the cultured animal. Chemicals were also used in pest control and transport of live animals, for breeding, health management and as feed supplement. These chemicals were classified according to its nature, function and intended purpose. The most commonly used chemicals were antimicrobials, disinfectants, chemotherapeutants, piscicides, hormones, anesthetics and for culture system preparation.

The responsible use of chemicals, particularly antibiotics, is an effective management response associated with disease epizootics. However, their widespread and indiscriminate use has resulted in many concerns including development of antibiotic-resistant pathogenic bacteria that can infect both humans and animals, potential transfer of drug resistance genes in bacteria from the aquatic environment to other bacteria, antibiotic residues in aquaculture products, potential effects on adjacent aquatic ecosystems and occupational exposure.

Government policies regulating or prohibiting the use of certain chemicals for aquaculture can help curtail the destructive consequences of chemotherapy. Guidelines on the use of chemicals in aquaculture and measures to eliminate the use of harmful chemicals have been developed by the ASEAN Member States (ASM). This guideline serves as a reference to assist aquaculturists in the use of regulated chemicals legally and properly. It also provides direction on the use of chemicals that will ensure the safety of the treated animals, end-users, consumers and the environment. The ASM has also

identified competent authorities that will regulate and monitor the use of chemicals in aquaculture. Likewise, information dissemination on the negative effects of chemicals can contribute to the decreased usage without affecting the level of aquaculture production.

Health problems and disease outbreaks are recognized as the biggest constraint to production, development and sustainability of aquaculture. Farm and health management practices focusing on disease prevention rather than treatment, maintenance of hygiene and biosecurity measures, and the responsible and effective use of chemicals could be the key to sustainability of aquaculture.

Research Strategies on Animal Production for Climate Change

Monchai Duangjinda,

Suporn Katawatin, Virote Pattarajinda, Wuttigrai Boonkum, Vibuntita Chankitisakul

Faculty of Agriculture, Khon Kaen University, Thailand

The sustainable development of animal production suitable for climate changes is a challenging concept nowadays. Environment management and housing is a common strategy to solve the heat stress in animal production. However, tradeoff with high-cost and never-ending technologies are major concern. The study on impact of heat stress in animal production has been studied in tropical countries, including Thailand; and has been studied in KKU for decades through the excellence research group and center to indicate the crucial concern of Faculty of Agriculture of KKU. Area of studies covered genetics, physiology and nutrition in varieties of livestock species.

The response of heat has been investigated in animals with different fractions of Indicine and Taurine genetics from morphological, physiological, cellular and molecular responses to find the thermo-regulation mechanisms and appropriate indicator for heat-stress tolerance in animals. The sweat gland number, type, and skin distribution were considered as morphological traits. Rectal temperature (cloacal temperature), respiratory rate, PCV, H:L were considered as physiological traits. HSP, response protein were considered as cellular traits. Gene expression, SNP, nucleotide sequence, genetic markers were considered as molecular traits.

The impact of heat stress on milk production and genetic parameters in crossbred Thai-Holstein dairy cattle, Thai native chicken, Thai native beef cattle has been investigated. In terms of Taurus genetic fractions, the rate of decline of milk production was found when Holstein fraction level was more than 87.5%, and highest when Holstein fraction level was more than 98%. Temperature-humidity index, season, geographic, feed resources and quality are factors affecting animal production and genetic improvements. Heritability and genetic correlations, marker associations with production traits under heat stress were investigated for developing heat tolerance index for animal selection to cope with on-going global warming.

In dairy cattle, all variance component estimates increased with parity, with the strongest increases for effects associated with heat stress. The effect of heat stress on Thai crossbred cattle and chicken is small. Seasonality of heat stress on days open were investigated for Thai crossbred Holsteins. Calving month and season greatly affected both phenotypic and genotypic variation in days open, with a greater effect for second parity than first and for cows with $\geq 94\%$ Holstein genetics. Fewer days open in the tropics can be achieved through selective breeding of cows with $< 87.5\%$ Holsteins genetics. Days open were greatest in March (summer) and fewest in October (late rainy season) for all breed groups and parities.

In chicken, native chickens originating in a tropical environment had lower H:L and less mortality rate compared with commercial broilers. The heat stress response traits – respiratory rate (RR), cloacal temperature (CT), packed cell volume (PCV), and average daily gain (ADG), were measured for three weeks. The significant difference in PCV indicated that C2C2 chickens were less tolerant to heat stress compared with other genotypes. The RR, CT, and ADG were not significantly different among all genotypes. Since C2C2 was revealed to be heat stress sensitive, C1C1 and C1C2 could possibly be used as markers for heat-tolerant genetic lines in Thai indigenous chickens and hybrid commercial lines with no effect upon growth performance.

The effects of genetics, global changing environment, management and tropical feed resources on heat production, greenhouse gas, microbial ecology, negative energy balance, milk and meat quality of ruminants are also on-going research in KKU.

Soil Salinization and Management of Salt-affected Soils in the Nile Delta of Egypt

Ahmad Mekawy

Faculty of Botany and Microbiology, Minia University, Egypt

In Egypt, the climate is categorized as arid, thus plants are often subjected to extreme climatic factors such as high temperatures and drought. Under these conditions, dissolved salts may accumulate in soils because of the insufficient leaching of ions. An accumulation of salts in upper soil layers may be also due to unsuitable irrigation management. Furthermore, due to the intrusion of sea water in the northern Nile Delta, where rice cultivation takes place, most of agricultural lands are affected by different degrees of salinity.

For the management of salt-affected soils, the government adopted some efforts includes improving the field-drainage conditions through installation of subsurface drainage systems and recovering the desirable soil properties by applying some activities, such as sub-soiling, land leveling and gypsum amendments. Moreover, breeding for salinity tolerance in crop plants seems to be one of the most promising solutions for utilizing such soils.

Soil salinity is one of the most severe problems in agriculture. Absorption of excessive salts inhibits both root and shoot growth, reduces reproductive activity and affects viability of plants. To counter salinity stress, plant cells have several adaptive mechanisms. However, the molecular mechanisms regulating biochemical and physiological changes in response to salinity stress are not well understood.

Rice, *Oryza sativa* L. is one of the most important crop species and the major food crop for much of the world's population. Since rice is a relatively salt-sensitive crop species, it is important to understand the mode of adaptation to salinity stress in order to produce new salinity tolerant rice varieties with increased productivity. In Egypt, soil salinization is becoming increasingly challenging for agriculture, and the response of many Egyptian rice cultivars to salinity stress has not been clearly studied. Thus, we conducted a study to investigate the physiological responses of two important local rice cultivars to salinity stress and to elucidate differences in the mechanisms of salinity tolerance between them by comparing the growth parameters, Na⁺ and K⁺ accumulation and the expression profiles of some genes encoding Na⁺ and K⁺ transport protein. Also, isolation and characterization of genes which could be involved in salinity tolerance were conducted to reveal other adaptation strategies of this cultivar in response to salinity stress.

“Paradigm Shift in Japanese Fermented Foods and Prebiotics: Discovery of the Bifidogenic Function of *Aspergillus* Protease”

Norihisa Kato

The Research Center for Japanese Foods, Graduate School of Biosphere Science,
Hiroshima University

Gut microbiota play several health beneficial roles for their host such as the digestion of food, immunomodulation and protection from pathogenic infection (1). They also continuously produce various bioactive metabolites including organic acids such as propionate and n-butyrate, which have been considered to modulate colonic cell proliferation, mucins, IgA and pH (1). Accordingly, intestinal microbiota dysbiosis often induces several disorders (1). Among the microflora, *Bifidobacterium* is an important probiotic thought to be involved in positive health benefits to the human host and preventive effects on several disorders such as inflammatory bowel disease, colon cancer, allergy, constipation, liver disease, insulin resistance, etc. (1). Recent studies suggest that *Bifidobacterium* exhibits antioxidant potential, and acts via a number of mechanisms to improve brain health. A reduction of *Bifidobacterium* in terms of number and diversity is one of the most notable changes in elderly populations. This may lead to a reduction in gut function and immune response, and potentially increased susceptibility to diseases. It is well known that the abundance of colon *Bifidobacterium* is elevated by consumption of prebiotics such as inulin and oligosaccharides.

Aspergillus species have been widely used for the production of a variety of Japanese fermented foods such as miso, soy sauce, sake, sake lees, etc. However, there is limited study on the application of *Aspergillus* species for the production of functional foods beneficial for health. In a recent study, we observed a marked elevation in cecum *Bifidobacterium* and organic acids in rats fed *Aspergillus*-fermented burdock compared to burdock powder (2). Similar effects were found in rats fed with water-soluble fraction from the fermented burdock and other *Aspergillus*-fermented foods such as malted rice and multi-grain malt. Since the water-soluble fraction from the fermented burdock contained substantial amount of extracellular proteases derived from *Aspergillus*, we postulated that the bifidogenic effects might be associated with *Aspergillus*-derived proteases. To test the hypothesis, rats were fed a diet containing 0.1% protease A ‘Amano’ SD (*Aspergillus oryzae*, Amano Enzyme Inc.) (Amano protease) and the cecum microflora was measured by Real-time PCR. The results showed a marked elevation in cecum *Bifidobacterium* (3). Since the Amano protease preparation is a mixture of several proteases, we set out to identify the active protease responsible for the bifidogenic effect. Our results showed an acid protease (AcP) derived from *A. oryzae* to exhibit a potent bifidogenic effect that was lost when protease was inactivated (4). Our findings provide an insight into novel applications of an acid protease derived from *Aspergillus* as a functional food supplement for colon health.

Currently, the concept of prebiotic, as defined by Roberfroid (5), is “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health”. The present study may open up a new concept of probiotic, and the *A. oryzae*-derived AcP may be considered as a new type of probiotic. Furthermore, it is of great interest that very small amount of AcP (0.0384%) is able to elevate *Bifidobacterium*, compared with 5 to 10% of dietary inulin and oligosaccharides necessary to elevate *Bifidobacterium*. Further study is in progress to elucidate the underlying mechanisms by which the presence of AcP elevates the *Bifidobacterium* levels in the colon. It is also of great interest to investigate the response of colon *Bifidobacterium* to consumption of *A. oryzae*-fermented Japanese foods such as miso, sake flake, etc. which contain several *Aspergillus* proteases. Intriguingly, recent meta-genome analysis by scientists in University of Tokyo have reported that the composition of the Japanese gut microbiome showed more abundant in the genus *Bifidobacterium* than other nations (6). Possibly, the reason might be, at least in part, ascribed to daily consumption of *Aspergillus*-fermented foods by Japanese. On the other hands, there is growing evidence suggesting the beneficial effect of probiotics in the fields of animal husbandry and fisheries. Thus, it is of great interest to investigate the potential application of *Aspergillus* for raising farm animals and fishes.

References:

- (1) Liu et al. Impact of high fat diets, prebiotics and probiotics on gut microbiota and immune function, with relevance to elderly populations. *Nutr Aging* 3: 171–192 (2015)
- (2) Okazaki et al. Burdock fermented by *Aspergillus awamori* elevates cecal *Bifidobacterium*, and reduces fecal deoxycholic acid and adipose tissue weight in rats fed a high-fat diet. *Biosci Biotechnol Biochem* 77: 53-57 (2013)
- (3) Yang et al. Beneficial effects of protease preparations derived from *Aspergillus* on the colonic luminal environment in rats consuming a high-fat diet. *Biomed Rep* 3: 715-720 (2015)
- (4) Kato et al. Discovery of *Aspergillus*-derived acid protease as a novel bifidogenic factor (Invited lecture). 16th International Nutrition & Diagnostic Conference, October 5, 2016, Prague.
- (5) Roberfroid. Prebiotics the concept revisited. *J Nutr* 137: 830S-837S (2007)
- (6) Nishijima et al. The gut microbiome of healthy Japanese and its microbial and functional uniqueness. *DNA Res* 23: 125–133 (2016)

平成27年度
研究科長裁量経費による研究助成

Reports of studies supported by Grant-in-Aid for Research from the Graduate
School of Biosphere Science, Hiroshima University

成果報告書



平成28年11月5日

November 5, 2016

広島大学大学院生物圏科学研究科

Graduate School of Biosphere Science, Hiroshima University

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平成27年度研究科長裁量経費による助成研究一覧

Reports of studies supported by Grant-in-Aid for Research from the Graduate School of
Biosphere Science, Hiroshima University

助成区分	研究課題名	研究代表者
基盤研究サポート Grant-in-Aid for Fundamental Research	環境ウイルスのキャラクタリゼーションに関する基礎 研究 A study on characterization of novel environmental viruses	中井 敏博 Toshihiro Nakai
	ウシの行動反応と気質関連遺伝子多型との関係 Temperament Related Polymorphisms Associated with Behaviors in Cattle	豊後 貴嗣 Takashi Bungo
	クラスタ構造の根を形成する植物の低リン適応特性 の解明 Study on the properties of low P tolerance of plants forming root-cluster structures.	和崎 淳 Jun Wasaki

Isolation and characterization of novel environmental viruses

Toshihiro Nakai¹, Patrick Fatsi¹, Takeshi Naganuma², and Takato Saito²

¹ *Fish Pathology Laboratory, Graduate School of Biosphere Science*

² *Marine Ecosystem Dynamics Laboratory, Graduate School of Biosphere Science*

Current topics on environmental viruses, which “bacteriophage (phage)” can be a good therapeutic agent for bacterial infections in animals as well as human beings and “giant viruses” (nucleocytoplasmic large DNA viruses) infecting *Acanthamoeba* are discovered one after another recently, are very attractive to all microbiologists. In the present study, we tried to isolate and characterize novel phages and giant viruses from seawaters. The results obtained are summarized as follows.

We successfully isolated phages infecting a gliding bacterium *Tenacibaculum maritimum*, which has caused severe mortality in cultured marine fishes worldwide. All of the phage isolates (n=8) were icosahedral head measuring about 120 nm in diameter with a long contractile tail (about 150 nm), which is larger than T4 phage in Myoviridae. The genome size was about 225 kbp (GC% 29.7) with the number of predicting ORFs of 306 or 308, some sequences of which were closely related to *Sphingobacterium* (= *Sphingomonas*) *paucimobilis* phage PAU; a large T4-like myovirus. Interestingly, our phage designated as “PTm” has a very unique structure consisting of several fibers (about 70 nm long) on the head. Based on phage adherence experiments, we speculated that the fiber functions as a sensor searching for host bacterial surface.

Another subject was to find phages lytic to nanobacteria, i.e., bacteria smaller than 0.22 μ m in size. We first tried to isolate nanobacteria from seawaters collected around Tanegashima and Yakushima islands by Yoyoshio-Marun (Hiroshima University Research Vessel), and obtained three nanobacteria-like isolates on agar plates (Marine Agar) after filtration with triple 0.2- μ m membrane filters. However, TEM observation revealed that the representative cells after cultured in broth were slender (about 0.35 μ m in width) but more than 1 μ m in length, suggesting that these are mero-type nanobacteria. In addition, we found a temperate phage (myovirus-like) in the culture of a nanobacteria strain, but not virulent phages.

We also tried to detect giant virus from seawaters using a new culture system of *Acanthamoeba*. The above-mentioned seawater samples (each 20 L) filtered through the 0.22- μ m membrane filter or amoeba culture inoculated with the filtrates were subjected to PCR amplification with published giant virus-specific primers and TEM observation, but regrettably no giant viruses have been found so far.

Acknowledgement: This study was supported by Grant-in-Aid for Research from the Graduate School of Biosphere Science, Hiroshima University.

Temperament Related Polymorphisms Associated with Behaviors in Cattle

Takashi BUNGO

(Laboratory of Animal Behavior & Physiology)

The behavioral trait is one of the important points when handling livestock. Some studies in mammals revealed that variations in monoamines and their related genes are involved in behavioral traits mostly related to temperament in animals. The objectives of the present study were investigated the possible role of these genes on temperament and behavioral traits or performance in (1) dairy cows and (2) Japanese Black calves. Blood samples were collected for DNA extraction and genotyping was carried out using PCR-RFLP method. Two monoamine related genes, namely monoamine oxidase A (*MAOA*) or serotonin receptor 1B (*5-HT1B*) was subjected in this work.

(1) The traits or performance were evaluated by scores of temperament questionnaire, milk component or frequency of refusing the excessive entry to an automatic milking system. By analyzing the association between the polymorphisms and temperament scores, cows with *5-HT1B* polymorphism showed high score of "Taking the lead". Also, it was detected that cows with *5-HT1B* polymorphism tended to be lower milk fat. Cows carrying *MAOA* polymorphism had much frequency of refusing the excessive entry to the automatic milking system. These results provide an alternative for breeding selection of Holstein cow towards improving manageability on the automatic milking system.

(2) At 2, 4 and 6 weeks of age, play behavior (gallop, turn, leap, buck, head-shake and butting to objects) in calves were monitored visually and recorded. In the frequencies of gallop, leap, head-shake and butting to objects, the effect of polymorphism was significant, whereas age and an interaction between polymorphism and ages was not significant. In the amount of turn, both the effect for polymorphism or ages was not significant but age \times polymorphism interaction was significant. Also, a post hoc test detected that calves with polymorphism exhibited high frequency of turn at 6 weeks of age. Amount of buck showed a similar tendency as turn. These findings suggest that *MAOA* polymorphism, but not age affects the frequency of play behavior in Japanese Black calves.

Study on the properties of low P tolerance of plants forming root-cluster structures.

Department of Environmental Dynamics and Management
Graduate School of Biosphere Science

Jun WASAKI

Phosphorus (P) is one of the most essential elements for plants. In general, fertilization of P is required for cultivated lands because of the low mobility of P in the soils. However, it is estimated that the economical available P rock resources remains only for recent several decades. The exhausting P sources have to be concerned for sustainable crop production. Plants frequently face to the P deficiency, therefore, plants have evolved many strategies to grow under low P conditions.

Part of low P tolerant plants has an ability to form root clusters. Cluster roots are consisted with highly dense rootlets within 1 cm in length gathered in narrow part of secondary roots. Dauciform roots are consisted with dense root hairs in a short lateral roots. It has been suggested that these root clustered structures not only increase the root surface but also have a specific function 'exudative burst' of P mobilizing substances, such as organic acids and acid phosphatase (APase). However, the molecular mechanisms for the low P tolerance and interaction between low P stress and other elements are still unclear. The aim of this study is to analyze the effects of cluster roots on the low P tolerance and accumulation of other minerals.

Organic acids and APase secreted from cluster roots of hydroponically cultured *Hakea laurina*, a Proteaceae plant of Southwest Australia, were analyzed. Metabolomic analysis by using CE-MS revealed that citrate, malate and isocitrate were dominant and specific in cluster root exudates among known anions. Additionally, unidentified anions at m/z 137.0 and 95.0 were 1st and 4th abundant peaks found in the root exudates of cluster roots. APase activities of root exudates of immature and mature cluster roots were significantly high than normal roots. These results suggest that the formation of cluster roots is important strategy in the *Hakea* to uptake from unavailable forms of P.

P and N concentrations of mature and senescent leaves of *Helicia cochinchinensis* and neighbored trees grown at Miyajima were measured. P concentration of senescent leaves was lower than mature leaves in all tested trees. To estimate the ability of P retranslocation from old leaves to expanding leaves, P remobilization efficiency (PRE) was calculated from the differences of P concentration between mature and senescent leaves. PRE of *Helicia* was 53-63%, which was higher than neighbored trees (8-46%) excepting *Michelia compressa* (68%). It was indicated that the demand of P against N was the lowest in *Helicia* among tested plants. It was suggested that the P remobilization and low demand of P in the mature leaves contributed to the low P tolerance of *Helicia*.

Mineral concentration for many mineral elements in *Helicia* and neighbored trees were analyzed by ICP-MS. Mature leaves of *Helicia* contained higher concentration of Al and Mn than other plants. The concentration of these elements of neighbored trees were tended higher in the individuals close to *Helicia* than the same species grown without influences of *Helicia*. Principle component analysis revealed that mineral composition of leaves of neighbored trees was influenced by *Helicia*. The increased concentration in neighbored trees was frequently found in multivalent cations, suggesting that the function of organic acids secreted from cluster roots of *Helicia* plants mobilized the cations and stimulated the accumulation by neighbored plants.

**List of students attending international conferences
supported by the Graduate School of Biosphere Science**

No.	Grade Level	Name	Title of Presentation
1	M1	堀田 聡史 SATOSHI HORITA	Combination effect of sucrose fatty acid esters and polysorbates, on the stability of O/W emulsion
2	M1	荒谷 友美 TOMOMI ARATANI	Molecular phylogenetic analysis of <i>Sus scrofa</i> and the origin of SNP for quantitative trait
3	M2	高原 貴 KAN TAKAHARA	Effect of low dose of alcohol on DMH-induced rats colon cancer
4	M2	竹内 佳子 KEIKO TAKEUCHI	Analysis of genetic structure and diversity of Japanese wild boar and reference populations
5	M2	西川 萌美 MOEMI NISHIKAWA	Reaction of Cathelicidin-2 secreted from goats milk leukocytes to lipopolysaccharide
6	M2	浦上 真治 SHINJI URAGAMI	Effect of dietary vitamin B6 on gene expression in skeletal muscle of rats
7	M2	曾我部 知史 TOMOCHIKA SOGABE	Effects of water and disaccharide on the mechanical glass transition of porous amorphous starch solids
8	M2	稲生 雄大 YUDAI INABU	Plasma concentrations of glucagon-like peptide 1 and 2 in calves fed calf starters containing lactose
9	M2	黒澤 祐人 YUTO KUROSAWA	Study on domestication process of Asian junglefowls
10	D1	石橋 ちなみ CHINAMI ISHIBASHI	Freeze-thaw stability of O/W emulsions: Influence of crystallization behavior of fats
11	D2	RISKI AGUNG LESTARIADI	Coping up with the risk, increasing sustainability; strategies for small-scale shrimp farming in Indonesia
12	D2	楊 永寿 YANG YONGSHOU	Consumption of protease preparations derived from <i>Aspergillus</i> markedly elevates cecal <i>Bifidobacterium</i> and organic acids in rats fed a high-fat diet
13	D3	黄 安琪 HUANG ANQI	Effects of artificial insemination on exosome localization and its protein level in sperm storage tubules of hen oviduct
14	D3	笈野 哲史 SATOSHI TOMANO	Genetic diversity of the big-fin reef squid, <i>Sepioteuthis lessoniana</i> around Japan
15	D3	TRAN VAN HUNG	Effect of dietary fibers on intestinal barrier defects and inflammation in mice and human intestinal epithelial cells
16	D3	矢吹 友佳理 YUKARI YABUKI	Regulators of ribosome synthesis, Rrs1 and Ebp2, are involved in the secretory response at the nuclear envelope