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

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Preventive effects of mung bean seed coat extract on metabolic syndrome in high fat-fed obese mice

Noriyuki Yanaka, Thanutchaporn Kumrungsee, Sudathip Sae-tan

Graduate School of Biosphere Science, Hiroshima University,
Higashi-Hiroshima 739-8528, Japan

mung bean

, Thanutchaporn Kumrungsee, Sudathip Sae-tan

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mungbean seed coat water extract, MSWE

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Taxonomy of freshwater red algae (Rhodophyta) of Vietnam

LIAO Lawrence Manzano

Graduate School of Biosphere Science, Hiroshima University,
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Field sampling in selected sites in northern Vietnam and examination and critical revision of old collections have revealed at least seven species of freshwater red algae, increasing the number of species considerably and adding to the knowledge of red algal biodiversity in the country. On-going laboratory study will result to one major publication next year as well as presentations at the Asia-Pacific Phycological Forum in Sapporo in 2020.

The collaborating scientist from Vietnam visited Hiroshima University to receive training in identification methods in 2018 using collected samples from Vietnam and to procure important taxonomic references. To help fulfill the goals for international cooperative research, the research leader visited Vietnam National University in 2019 to join field sampling and to conduct a short workshop on algal identification and specimen curation for 30 students and researchers at the host university. Recruitment of potential graduate students was also possible after an introductory information presentation on Hiroshima University was given.

Development of simple measuring technique for methane production from native goats in Philippines

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Methane produced from digestive tract of ruminant animals is one of the greenhouse gasses, and mitigating the methane emission from ruminants is globally important issues. To build basic data base, the measurement of the exact emission rate from ruminants has been conducted over the world. I tried to develop simple measurement techniques as an international collaboration study with researchers in Visayas State University (VSU), Philippines. First, respiration gas around the mouth of native sheep, instead of goats, in VSU farm was collected into a small plastic bag for 10 seconds. Then, the gas was transferred into an evacuated grass tube. The methane and carbon dioxide concentration in the tube was measured with gas-chromatography at Hiroshima University. The methane concentration in the collected gas was measurable but lower than that expected, because collection time of respiration gas was not enough to obtain eructation gas which contains high methane. However, simple estimation of the methane emission seems to be possible with improving collection methods of respiration gas.

Ocean bottom agitation to increase primary productivity in Hiroshima Bay, and trial production of the agitator

Kazuhiko Koike[1], Shizuka O-hara[1], Ken-ichiro Furuya[2], Takafumi Seta[3]

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2017
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Lack of oyster seed stocks has been serious problem in Hiroshima Bay since 2017. This is due to insufficient phytoplankton growth during the oyster spawning season at the bay. To overcome this, in June 2018, an alliance of Hiroshima University, The City of Hiroshima and Hiroshima Oyster farmers' Union tried a series of "ocean bottom agitation" (total 6 days, done by 9 boats) in June 2018. The strategy was; to increase phytoplankton population in the water columns by suspending and geminating their resting cells submerging in the ocean sediment, and to increase inorganic nutrients accumulating in the bottom layer, and thus to result in fertilizing culture grounds. Following to the preliminary field survey and laboratory experiment to investigate quality and quantity of the resting cells on the target ground and to know which phytoplankton species could be occurring after agitation, actual field operations were done during June 4th-6th (off Kusatsu, Hiroshima) and June 11th-14th (off Eba, Hiroshima). Before and after the agitations,

photosynthetic activity and population biomass of phytoplankton species, as well as inorganic nutrient distributions and ocean bottom environments, were monitored. In the result, notable increases of the phytoplankton activity and population were recognized after the operation. Since such bottom agitation was revealed to be effective to induce phytoplankton increase, an autonomous solar-powered agitator was custom built and planned to install on the oyster farm of Hiroshima Bay (by July 2019). These our trials were introduced and broadcasted in a RCC TV program, and under preparation for a journal submission.

Determination of trace inorganic ions in seawater samples by ion chromatography using reversed-phase C18 column and application for the sea surface microlayer

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C18

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C18

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Conservative Mixing

2019 7

We report the determination of trace inorganic anion

The analysis of high ascorbic acid biosynthesis mechanism in acerola towards breeding ascorbic acid rich plants.

Marina SUEKAWA

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Higashi-Hiroshima 739-8528, Japan

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(Ascorbic acid; AsA)

AsA

AsA AsA

AsA AsA

AsA 2 AsA 6

3 AsA

3 1 2

3 AsA AsA

AsA 3

3

AsA

AsA

High ascorbic acid (AsA) plants have high nutrient value and environmental stress tolerance. However, it is not easy to enhance AsA contents in plants due to the existence of multiple biosynthesis pathways and unclear of rate-limiting enzyme for AsA biosynthesis. The aim of this study is elucidating the high AsA accumE1 (0 (c)1(iA(v)(a)2(y))5(hen)6(i)5ah(a)2nism34Tw -27202 Tw T(bi)Tj

Study of the regulation of potassium transport mediated via *Pota1* in plants

Sho Nishida, Jun Wasaki

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Higashi-Hiroshima 739-8528, Japan

Pota1

739-8528

Pota1

Pota1

K^+ *NPF7.3*

SKOR *Pota1* Pota1 K^+

Pota1

Pota1 Pota1

K^+ Pota1

Pota1 K^+

The purpose of this study is to reveal the molecular mechanism of the regulation of potassium translocate from roots to shoots mediated by "Pota1" encoding a transcription factor (TF) in *Arabidopsis thaliana*. We conducted transcriptome analyses for *Pota1* knockout mutant and the wild type plants, and found that "*NPF7.3*" and "*SKOR*", which are responsible for root-to-shoot K^+ translocation, are down-regulated at transcript level in Pota1 mutant compared to the wild type, indicating that Pota1 is a TF regulating the expression of these K^+ transporter genes. In addition, it was found that the activity of Pota1 is regulated through the change of splicing pattern in response to low potassium. Meanwhile, the transcriptome analyses and phenotype analyses revealed that Pota1 is also involved in the root hair development in response to low potassium, indicating that Pota1 is responsible for the morphogenesis for the efficient K^+ absorption. Our study suggests that Pota1 plays multiple roles in K^+ absorption and translocation.

Establishment of Genetic DNA Makers for Camel hybrids, and their applications

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Camelus dromedarius *Camelus bactrianus* *Camelus ferus* 3

mtDNA D-loop mtDNA 2 mtDNA

In order to detect the hybrid camels between the *Camelus dromedaries* (One humped camel) and *Camelus bactrianus* (two humped camel) which were domesticated, we did DNA sequences of mitochondrial D-loop regions for two species of camels in Kazakhstan farmers and Japanese Zoological gardens. In this study, 1.2kb mitochondrial DNA (mtDNA) D-loop region from hair of camels were analyzed to reveal it.

From the results of the molecular phylogenetic tree, the genetic relationship of Japanese camels was

Grant

chicks.

Development of simple and efficient oral vaccine using bacterial antigen

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LPS

Wang
DNA *Mol Ther.*, 2015 LPS LPScap
FITC LPS FITC-LPScap FITC
LPS FITC-LPScap EDTA
FITC-LPS
FITC-LPS LPScap 0.63% LPS
RAW264.7 100μg FITC-LPScap FITC-LPS
100μg cap 630μg FITC-LPS 24
FITC 630μg FITC-LPS 100μg FITC-LPScap
FITC PCR LPS
IL -10 LPScap
IL-10 LPScap

We developed a functional material to more efficiently occur immune responses against bacterial infection by encapsulation of lipopolysaccharide (LPS) which is one of cell wall components of gram-negative bacteria from *Escherichia coli.* First, LPS nanocapsule (LPScap) was synthesized using a modification of the method of Wang et al (Wang *et al., Mol Ther.,* 2015). Using fluorescein isothiocyanate (FITC)-conjugated LPS (FITC-LPS), we determined that approximately 6.3 μg of LPS was incorporated into 1 mg of LPScap, corresponding to a LPS encapsulation rate of 0.63 %. Next, to investigate whether LPScap can exert on immune cells, we examined the effect of LPScap on the uptake and interleukin (IL)-10 mRNA expression level using RAW264.7 macrophage. The uptake of FITC-LPS and FITC-LPScap by RAW264.7 macrophage was investigated using flow cytometric analysis. The proportion of $FITC^+$ cells was significantly increased in a stimulation of 100 μg FITC-LPScap compared with a stimulation of 630 μg unencapsuled FITC-LPS. Moreover, a result of real-time qPCR analysis showed that IL-10 mRNA expression in cells stimulated by LPScap was higher as compared with cells stimulated by unencapsuled LPS. In future research, we plan to investigate LPScap efficacy for animal body by oral administration of LPScap into mice.

Structural and functional role during nitric oxide detoxification in bacterial cytochrome c'.

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Biosci. Biochem. Biotechnol. *Extremophiles*

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Bull. Chem. Soc. Jpn.

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Essex Michael A. Hough
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This study aims to reveal the NO-binding properties in extremophilic cytochromes c', which are related to the protection or detoxification against nitrosative stress. Through NO-binding assay and structural analysis, we found that the heme environment of cyochromes c' was essential for the control of NO-binding affinity and thermal stability. These insights will shed more light on understanding of the function in cytochromes *c'* (Yamane-Koshizawa et al. *Biosci. Biochem. Biotechnol.* 2018, Suka et al. *Extremophiles* 2019, Yamanaka et al. *Bull. Chem. Soc. Jpn.* 2019). In addition, a multiple research team from Essex University, Oregon University, and Hiroshima University identified that cytochromes P460 and c' from *Methylococcus capsulatus* (Bath) showed similar β-sheet structures with changing the functional role around the heme. This result will provide insights relevant to enzyme redesign for synthetic enzymology and engineering of proteins (Adams et al. *Chem. Sci.* 2019).