

## Biological systems in closed-ecosystem-creature and its environment

TOMITA-YOKOTANI, Kaori<sup>1\*</sup>

<sup>1</sup>University of Tsukuba

Living creatures on the earth have been evolved since its origin a long time ago. They equip several important functions affecting each other. Knowledge on those functions and interaction of the ecology is essential for secure design of a closed-ecosystem with limited number of living species under the harsh environments, such as space and deep sea or desert. Organic substances can produce the ground on the surface of our earth. The mechanisms of soil production are very important matter in all the cases of bio-ecosystems. After the production of many species of creatures, the interactive functions among their organisms have important mechanisms during the evolution. It thinks that the establishment of the ecosystem to have been equipped with the higher feature in providing the place of the advanced specialized field research information about each biological system and its functions detailed feature when the human being tried artificial ecosystem becomes possible. In this time, I will discuss about several research field, microorganisms, plants, mammal, human food, and several creatures interactions and technology.

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## Space Experiment of Space Inflatable Terrarium

KISHIMOTO, Naoko<sup>1\*</sup>

<sup>1</sup>Setsunan University

We propose space inflatable structures which internal pressure is kept 1atm at space environment. Space experiment on the ISS is scheduled in FY2012.

Keywords: Inflatable Structure, Terarium, Space Experiment

## Evaluation of foods in Nostoc sp. HK-01

KIMURA, Yasuko<sup>1\*</sup>, TOMITA-YOKOTANI, Kaori<sup>2</sup>, KATO Hiroshi<sup>3</sup>

<sup>1</sup>Jumonji University, <sup>2</sup>University of Tsukuba, <sup>3</sup>Mie University

We have been studying future space agriculture in Mars. A cyanobacteria, Nostoc sp. HK-01, has several outer space environmental tolerance. Space environment is very a severe closed ecosystem for us. It has a possibility that this species of cyanobacteria is able to use as some foods under the emergency or severe environment. We will study the proposal of utilization of cyanobacteria, Nostoc sp HK-01, for the variation of meal under the serious environment.

Keywords: Cyanobacteria,, Nostoc sp. HK-01, food

## Importance of the microbe management in Space foods

KATAYAMA, Naomi<sup>1\*</sup>, Megumi Sofue<sup>1</sup>

<sup>1</sup>Nagoya Women's University

### [Background]

The long-term space stay era is coming. A space station and future moon base, the life-support system in Mars station become very important. It is important that we fix the living environment. The accumulation of data will be carried out by more and more researchers from now on. We build the mini-earth in the space, and the human needs fundamental researches to become able to live on other planets. Furthermore, I will advance to the applied study to perform a real experiment in space. The meal is very important so that an astronaut accomplishes a duty lively well. It is clear from the impression of the astronaut the meal "is pleasure in space duty", and "to be time for rest". However, like this important meal may be going to take the life of the astronaut in space. In other words it is the food poisoning outbreak due to the microbe. It is required in the long-term space stay era to think about food safety, relief.

### [Purpose]

Therefore this study think about the microbe crisis control in the cooking process. For example, in a spaceship, on the moon base, a plant factory in Mars station, we thought about the microbe crisis control performed in the future.

### [Method]

We examine the past food poisoning example that happened on the earth and clarify what kind of situation food poisoning was caused in. We will do check about crisis control effect. We think about the measurement of crisis control important point of the plant factory and/or cooking process in the space.

### [Result and Discussion]

As for the food poisoning caused on the earth, for example in Tokyo, there was the really most outbreak number in July, in the past 10 years. The number of the food poisoning patients had most in May, the cases were 12.6 cases and 332 people on the average. Noro-virus was most high number (995) of the species of food-poisoning bacteria in the H21, in Tokyo. We need to check about compound food which was many food processing. Many people's hand and finger touch the food. The pollution of the bacteria from a human finger is becoming the problem because of the food poisoning. Because of the sterilization about the indigenous bacteria in the fingers of the astronaut is need of space food manuals.

### [Example]

#### Staphylococcus aureus

Man's respiratory pages, skin and superficial wounds are common sources of *S. aureus*. *S. aureus* is allowed to grow in foods, it can produce a toxin which is heat stable and may not be destroyed. Good personal hygiene while handing foods will help keep *S. aureus* out of foods.

#### <Control measures of Staphylococcus aureus>

- 1) Keep hot food hot and cold food cold ( below 5 centigrade and above 60 centigrade)
- 2) reheat food to steaming hot before serving ( at least 75 centigrade)
- 3) cook food heat to at least 75centigrade
- 4) wash and dry the hands properly
- 5) avoid handling food without gloves

#### Clostridium botulinum

It has caused death in approximately 30 percent of the case; and it occurs mostly in home-canned foods. Cl. Botulinum can exist as a heat-resistant spore, and can grow and produce a neurotoxin in under processed, home-canned foods. The botulinum toxin is destroyed by boiling the food for 10 minutes.

#### <Control measures of Clostridium botulinum>

- 1) keep hot food hot and cold food cold ( below 5 centigrade and above 60 centigrade)
- 2) bottle only high acid fruits at home
- 3) add citric acid
- 4) When making vegetable in oil or flavoured oils to use only acidified or dried vegetables

Keywords: Space foods, microbe, food poisoning, HACCP, Crisis control manual

## Development in Large Scale Image Program about Carbon Circulation

ARAI, Mayumi<sup>1\*</sup>, CHIKAKIYO Takeshi<sup>1</sup>, IKEBE Yasushi<sup>1</sup>, MATSUYAMA Momoyo<sup>1</sup>

<sup>1</sup>National Museum of Emerging Science and Innovation(Miraikan)

A family animation program featuring our relationship with life and our planet.

Strange things start to happen around our main character Naoko, as aliens come to Earth in pursuit of the C-atom created in outer space. Naoko is shown some peculiar images a number of times, and she begins to realize the intrinsic link between her and other living beings. Through immersive dome imagery and music, "Encounter with Earth" offers the experience of looking at ourselves and our planet from a different perspective.

Experiencing this program, you may notice that humans are a part of the "cycling" system that links life forms and the Earth. And yet the program not only presents science to understand the Earth system but also gives you an opportunity to encounter new perspectives on yourself by looking the Earth from the space.

One of the most noteworthy features of this program is that it provides a special experience of watching the Earth from an atomic-microscopic perspective to a macroscopic perspective (as seen by the naked eye), and a global perspective of the Earth overall from outer space. These three perspectives provide the opportunity to cultivate a scientific perspective to understand the relationship between humans and the Earth. Moreover, the dome theater environment and the animation imagery popular to family audiences provides unique discoveries and surprises for children as well as adults.

Keywords: Carbon Circulation, Space, Earth, Science Communication, Animation, Large Scale Image Program

## Serious environment tolerance in cyanobacteria, Nostoc sp. HK-01

IGARASHI, yuichi<sup>1\*</sup>, Mayumi Arai<sup>2</sup>, Haruka Fujishiro<sup>1</sup>, TOMITA-YOKOTANI, Kaori<sup>1</sup>, Seigo Sato<sup>1</sup>, Hiroshi Kato<sup>3</sup>, Masayuki Ohmori<sup>4</sup>

<sup>1</sup>University of Tsukuba, <sup>2</sup>National Museum of Emerging Science and Innovation, <sup>3</sup>Mie University, <sup>4</sup>University of Chuo

We have already reported the growth of terrestrial cyanobacterium, Nostoc sp., on the Martian Regolith Simulant (MRS) and its vacuum tolerance as one of our challenges in this century to inhabit Mars. Here, we examined high-temperature tolerance, UV tolerance, gamma-ray tolerance, heavy particle beam tolerance of Nostoc sp.HK-01 to indicate its survivability in space-environment. All the cyanobacterial cells could live under the environment, high temperature, UV, gamma-ray, heavy particle beam. After the exposed cell, they are difficult to live under the environment, high temperature in 24h. The several severe environment tolerances in the dry material, cyanobacteria, Nostoc sp. HK-01, were investigated for future space utilization and an environment of some emergency situation.

## Study of effective utilization of the Nitrogen-fixing terrestrial cyanobacterium based on the desiccation-related genes.

KATOHI, Hiroshi<sup>1\*</sup>

<sup>1</sup>Div. Plant Func. Gen., Life Sci. Res. Center, Mie Univ.

Using DNA microarray from a terrestrial cyanobacterium *Anabaena* sp. PCC7120, the typical desiccation-responsible genes were selected and the gene-disruptant were characterized. All of typical gene-disruptants showed low viability under desiccation using cells grown in N<sub>2</sub>-free medium. These results may suggest that desiccation-tolerant genes contain nitrogen fixation relating genes, are expressed irrespective of nitrogen content to protect desiccation sensitive N<sub>2</sub>-fixing heterocyst and express to stabilize intra-and outer-cellular condition under desiccation in N<sub>2</sub>-rich condition. We were also shown that the no-inducible photosynthesis gene, *psb28*, was related to desiccation tolerant. *Psb28* protein associates with photosystem II but function of *Psb28* is not enough to understand.

Desiccation tolerant N<sub>2</sub>-fixing cyanobacterium, *Nostoc commune* is related to the *Anabaena*. The *Nostoc* has ability to use scientific research for desiccation tolerance system, food and soil for plantation. These abilities expect to improve devastating soil to nutrient-rich soil including space agriculture. So it was tried to isolate the *Nostoc* and succeeded to cultivate the *Nostoc* axenically. To confirm ability of the *Nostoc* soil, the *Nostoc* was used plantation as nutrient containing plate. The result of difference plant growth between N<sub>2</sub>-deficient plate and cyanobacterial mat is now in progress.

Keywords: Desiccation, cyanobacteria, gene analysis, bioremediation, agriculture, tolerance

## The interaction between tall fescue and endophyte in severe environments

YOKOSHIMA, Mika<sup>1\*</sup>, TOMITA-YOKOTANI Kaori<sup>2</sup>, CHIDA Yukari<sup>2</sup>, SATO Seigo<sup>2</sup>

<sup>1</sup>College of agrobiological resource sciences, University of Tsukuba, <sup>2</sup>Graduate school of life and environmental sciences, University of Tsukuba

Endophytes are the micro-organisms that are present in plants' intercellular spaces and each of them has symbiotic relationship with its host plant. In a narrow sense, endophytes refer to filamentous fungi which live inside rice plants. It has been known that endophyte-infected plants show enhanced resistance to multiple-stress, diseases and insects. One of endophyte-plant associations is Neotyphodium-tall fescue(Festuca arundinacea) one.

The purpose of this experiment is to understand the interaction between the endophyte and the plant by investigating the changes and phenomena of the endophyte-infected plant in depth which is exposed to severe environments.

Keywords: endophyte, tall fescue, severe environment



## Utilization of functional woody plant in closed bio-ecosystem

CHIDA, Yukari<sup>1\*</sup>, Kyohei Motohashi<sup>1</sup>, Seigo Sato<sup>1</sup>, Kaori Tomita-Yokotani<sup>1</sup>

<sup>1</sup>University of Tsukuba

Woody plant has several utilizations, the production of excess oxygen, woody materials for living cabin, provision of biomass and recycling in the atmosphere in closed ecosystems. In addition of them, there are several woody plants as herbal medicine. We have already reported the existence a tree line, *Prunus* sp., which has a high allelopathic activity and several functions. Here we will show the utilization usage of this tree line under the environment in closed ecosystem.

## Chemical analysis in a silicon uptake-deficient mutant *lsi1* of rice

SUZUKI, Toshisada<sup>1\*</sup>, Azusa Yamahata<sup>1</sup>, Takeshi Katayama<sup>1</sup>, Taketa Shin<sup>2</sup>, Masahiko Ichii<sup>1</sup>

<sup>1</sup>Faculty of Agriculture, Kagawa University, <sup>2</sup>Institute of Plant Science and Resources, Okayama University

A rice mutant *lsi1* accumulates less silicon in the shoots than a wild type rice (cv. Oochikara). The mutant *lsi1* and the wild type were planted in seedling cases, lignin contents in leaf and stem tissues were determined with Klason method. Lignin contents in leaves and stems of *lsi1* were higher than those of the wild type. Lignin contents in leaves and stems of *lsi1* planted in silicon addition soil (Si+) were lower than those in control soil (Si-). These results suggested that silicon accumulation in rice might have a negative influence on the deposition of lignin and formation of the secondary cell walls, and thus it affected mechanical strength of rice.

### INTRODUCTION

Rice (*Oriza sativa* L.) is the most effective silicon accumulating plant. Silicon absorbed in rice tissues contributes to enhance their strength, hardness, and resistance to disease and insects. The rice mutant *lsi1* (low silicon rice 1) was isolated from sodium azide-treated seeds of a wild type rice (cv. Oochikara). This mutant accumulates less silicon in the shoots than the wild type. The gene (*Lsi 1*) was predicted to encode a membrane protein which controls silicon transport in rice. Molecular and physiological studies of the mutant *lsi1* have contributed to clarify the silicon accumulation system and the biotic resistance role such as pests and disease. Mechanical strength of rice is an important trait that affects grain yield and quality. The trait is associated with the contents of lignin, cellulose, hemicellulose, and silicon. However, it is unknown that correlation of quantity and quality between lignin and silicon in rice tissues. In this study, we compared lignin contents between the rice mutant *lsi1* and the wild type to understand the mechanism controlling the mechanical strength.

### RESULTS AND DISCUSSIONS

The wild type and *lsi1* were planted in 6.1 kg of soil with 400 g of silica gel (Si+) and without silica gel (Si-: control soil) in seedling case. The leaf and stems were ground into powder by a Wiley mill. The powder (40-80 mesh) was extracted with a mixture of ethanol-benzene (1:2, v/v) for 6 h in Soxhlet extractors. The defatted powder was treated with pepsin to remove proteins. The amounts of lignin in the leaves and stems of the wild type and *lsi1* were determined by Klason method. The ash was determined with a muffle furnace at 600°C. In control soil (Si-), the lignin contents in leaves and stems of the mutant *lsi1* were 17.6% and 19.4%, respectively, whereas those of the wild type were 14.2% and 16.6%, respectively. The lignin contents in leaves and stems of *lsi1* were higher than those of the wild type. In silicon addition soil (Si+), the lignin contents in leaves and stems of *lsi1*, and those of the wild type were 14.7%, 18.0%, 12.1% and 14.4%, respectively. The lignin contents in *lsi1* and the wild type planted in the silicon addition soil (Si+) were lower than those in the control soil (Si-). The amount of ash in the leaves and stems of both the wild type and *lsi1* were determined. In the control soil (Si-), the ash contents in leaves and stems of the wild type were 11.5% and 5.68%, respectively. The mutant *lsi1* contained little amount of silica in leaves and stems compared with the wild type (< 2.0%). In the silicon addition soil (Si+), the ash content in leaves and stems of the wild type were about 2 fold higher than those in the control soil (Si-). While the ash contents of *lsi1* were also increased than those in the control soil (Si-), the content in leaves and stems were ca 5% in silicon addition soil (Si+). Nakata et al. reported that silicon accumulation in the wild-type leaves cultivated with silicon amendment (Si+) was enhanced over three-fold compared with that the control. These results suggested that silicon accumulation in rice might have a negative influence on the deposition of lignin and formation of the secondary cell walls, and thus it affected mechanical strength of rice.

Keywords: lignin, silicon, *lsi1*

## Piezoelectric biosensor for the estimation of environments in a closed-ecosystem

ABE, Tomoko<sup>1\*</sup>, TORII Hitomi<sup>2</sup>, YAMANA Masao<sup>1</sup>

<sup>1</sup>School of Science and Engineering, Tokyo Denki University, <sup>2</sup>Graduate School of Science and Engineering, Tokyo Denki University

In recent years, piezoelectric quartz systems have been used in analytical chemistry because their oscillating frequencies are sensitive and have wide range. A quartz crystal microbalance (QCM), which is a nanogram mass sensing device, has been applied to determine gases, ions, and some biomolecule. These studies are based on the fact that the resonant frequency change of the quartz crystal corresponds to mass change on the crystal surface.

In this study, we used the QCM as a transducer for the measurement of cell growth. As a result of the current experiment, both the dry cell weight and the living cell weight were linearly proportional to the frequency change in the range of  $10^{-8}$  to  $10^{-5}$  gram when our QCM sensor was used for the measurement. Namely, the measurement of bacterial cell weight is possible within this range using the sensor. Furthermore, we have succeeded in living cell adhesion to the gold surface of the quartz crystal in our own QCM system. Although cells adhered to the surface under growth media containing serum, a stable unchanging oscillation frequency occurred. In the device, living cells serve as the sensing element, where cellular mass and viscoelasticity affect the frequency of the crystal.

We aim to construct the simple piezoelectric biosensor for the estimation of ecological environments (e.g., water, soil, or air pollution containing cytotoxic activity). Because the cell adhesion change can be monitored as frequency change of the quartz in the system, this biosensor will be also useful for the real time identification or screening of biologically active drugs or biological molecules that affect cell adhesion. This method will also be variable to analyze the behavior of cells in the closed-ecosystem.

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