

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

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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 tempereture 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.

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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 N₂-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 N₂-fixing heterocyst and express to stabilize intra-and outer-cellular condition under desiccation in N₂-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 N₂-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 N₂-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

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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

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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

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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

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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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