Study of immobilization of heavy elements on biological reaction environment
-Perspective of bio-remediation of radioactive waste-

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There are some microorganisms that change the chemical state of the mobile uranyl ions to be an immobile U(IV) by the respiration reaction. Since this biotransformation reaction proceeds on the cell surface, the cell surface functions as the reaction environment. We have been studying the mechanism of the biotransformation of heavy elements by analyzing the chemical states of the immobilized heavy elements using the advanced analytical methods including XAFS, SEM and TEM. These studies can be applied to develop new techniques of separation of actinides and bio-remediation of contaminated lands. Here we introduce the recent results obtained by the experiments carried out on the nano particle formation of Ce ions on the cell surface of microorganisms, accumulation of Cs ion on the cells of soil bacteria and in basidiomycete.

We have started the exposure experiment of yeast Saccharomyce cerevsiae to the aqueous solution containing Ce ions. We found that the concentration of Ce ions decreased with increasing exposure time, while the concentration of P ions increased. We observed the cell surface by SEM and TEM, and found the nano particles containing Ce and P (Fig. 1). Since we added no P ions in the solution, the Ce adsorbed on the cell surface would react with the P ions released from the inside of cell. The crystal structure of the nano particles is determined as monazite ($CePO_4$) by selected area electron diffraction. The nano particles (~100 nm) developed on the cell surface are smaller than and differ in shape from the abiotic monazite formed by the precipitation in the solution containing Ce ions, P ions, and no yeast cell. These facts suggest that yeast cells function as bio-factory to produce the nano particles of Ce.

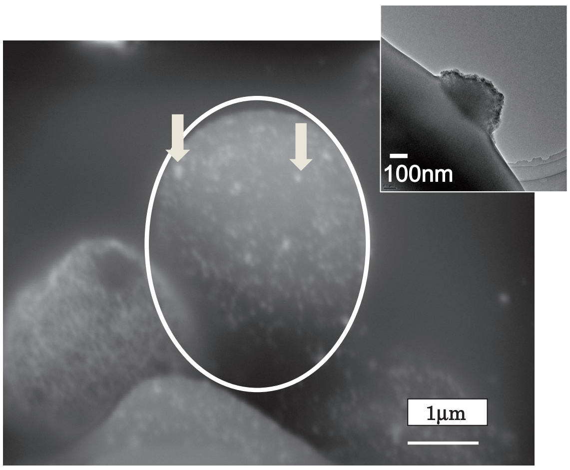

Fig.1. SEM photgraphs of Ce accumulated yeast on the cell surface for 4 days. White ellipse shows a cell, nano particles containing Ce and P by arrows. Inset shows the enlarged nano particle developed on the cell surface.

We have investigated Cs accumulation by soil bacteria of *Pseudomonas fluorescens*. Cs accumulation by *P. fluorescens* was examined both under growth and resting conditions to elucidate the interaction between Cs and bacteria. In the growth condition, *P. fluorescens* did not accumulate Cs irrespective of the presence of K. In the resting condition, the cells quickly adsorbed approximately 5μmol Cs /$g_{\text{cell dry-weight}}$ and subsequently released approximately 90% of the adsorbed Cs with 1 M CH_3COONH_4. The amount of Cs adsorption by cells of *P. fluorescens* varied with changing pH and ionic strength of the solution. These results indicate that Cs accumulation by *P. fluorescens* occurs mainly by reversible adsorption on cell walls, but not by intracellular transport under nutrient condition.

We have examined the effect of Cs on the growth of basidiomycete using *Pleurotaceae* species. The fruiting body was formed in the medium containing 500 ppm and 1000 ppm Cs (Figs. 2A and 2B). The size of the fruiting body is smaller in the medium containing higher amounts of Cs. The X-ray fluorescence spectrometry analysis of the fruiting body indicates that the fruiting body contains Cs (Fig. 2C). These results indicate that the *Pleurotaceae* species accumulates Cs in the cells. The quantitative analysis is planned in the future. The accumulation ability of microorganisms can be applied to the remediation of the contaminated land. The biological technique is eco-friendly one, so that we have a plan to develop a biological technique for remediating the contaminated land.

We also analyze the spatial distribution of radioactive Cs in soil and plant by autoradiography technique. The autoradiography image and optical one of the tree collected in Fukushima prefecture are shown in Fig. 3. Many black spots are recognized on the surface of leafs and branch of the tree. Interestingly, the black spots in the autoradiography image are detected on the leaf of dark green in optical photograph, and less spot on the light ones, suggesting that radioactive Cs accumulated only on the leaf and the branch grown before the accident and that only small amounts of radioactive Cs accumulated on the tree are transported to new grown branch and leafs. We are analyzing the distribution and the chemical states of radioactive Cs in the soil at present.

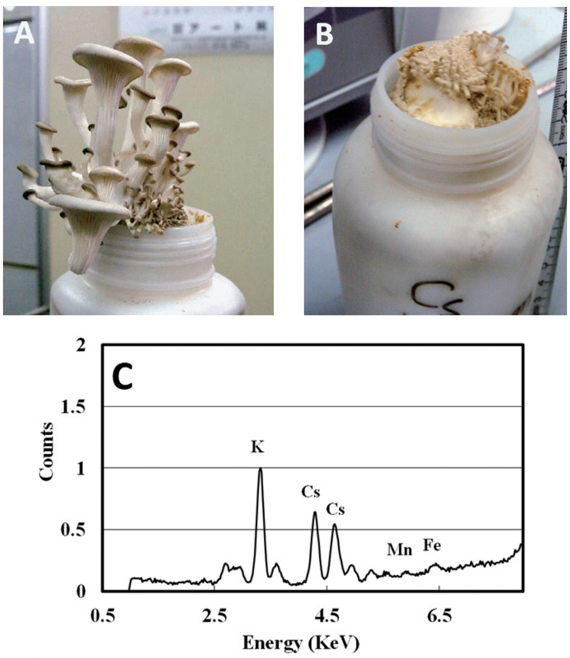

Fig.2. The photographs of *Pleurotaceae* species grown in the medium containing 500 ppm Cs (A), 1000 ppm Cs (B), and X-ray fluorescence spectrum (C).

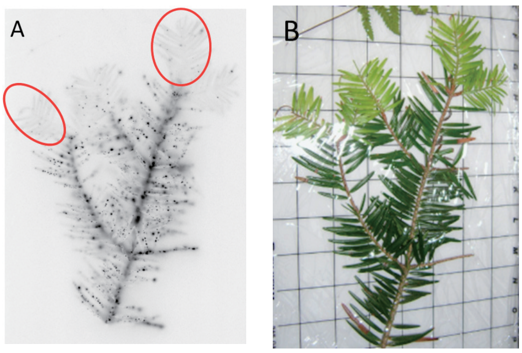

Fig.3. Autoradiograph image (right) and photograph (left) of the Torreya nucifera tree collected in Fukushima prefecture. Red circles show the leafs grown after the accident.

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