Behavior of Eu during culture of *Paramecium sp*. with yeast cells sorbing Eu

N. KOZAI[1]* T. OHNUKI[1], T. SATOH[2] AND F. ESAKA[3]

[1]Advanced Science Research Center, Japan Atomic Energy Agency (JAEA), Tokai, Ibaraki, 319-1195 Japan (*correspondence: kozai.naofumi@jaea.go.jp, ohnuki.toshihiko@jaea.go.jp)

[2]Dept. of Advanced Radiation Technology, JAEA, Takasaki, Gunma, 370-1292 Japan (satoh.takahiro37@jaea.go.jp)

[3]Nuclear Science and Engineering Directorate, JAEA, Tokai, Ibaraki, 319-1195 Japan (esaka.fumitaka@jaea.go.jp)

It is known that activity of microorganism has a great impact on migration of actinides released in the environments. Retardation by adsorption or precipitation on the cells is the most desirable function of bacteria. It is also known that protozoa, who prey smaller microorganism such as bacteria, are found in not only surface soil but also deep subsurface. However, no knowledge on the role of protozoa in the migration of actinides is available. The present paper investigated behavior of *Paramecium sp. (P. bursaria)* in media containing yeast sorbing Eu. Paramecium is a well-known unicellular protozoa living in freshwater environments. Yeast, *Saccharomyces cerevisiae*, was used as a food source and Eu (III) was used as simulant of trivalent actinides.

After the contact of yeast cells (dry weight *ca*. 0.1g) with a 0.5mM Eu (III) solution (25ml, pH *ca*. 6), many nano-particles of Eu with phosphate formed on yeast cells. *P. bursaria* cells were cultured with those yeast cells in an inorganic salt solution containing no phosphate (400 ml). The Eu concentration in the aqueous phase increased up to about 1 μM soon after the introducing of yeast cells but quickly decreased to an almost constant level, less than 0.1 μM, after the second day of the culture. The amount of Eu leached into the aqueous phase was less than 0.1 % of the Eu on the yeast cells. As culture time advances, membranous precipitates formed. These menbranous precipitates contained undigested and digested yeast cells and dense membranous organic substance filling gaps between those cells. Many nano-particles of Eu phosphate were observed on digested residue of yeast cells. These resutls suggest that *Paramecium sp*. do not impair actinide-mineralization action of microorganisms.

Mechanisms of iron oxidation in the thermoacidophilic crenarchaeon *Metallosphaera yellowstonii*: Field and laboratory studies suggest possible role of novel proteins

M.A. KOZUBAL, Z.J. JAY, R.E. MACUR AND W.P. INSKEEP*

Thermal Biology Institute and Department of Land Resources and Environmental Science, Monana State University, Bozeman, MT 59717 (*correspondence: binskeep@montana.edu)

The oxidation of ferrous iron is catalyzed by microorganisms as an important source of electrons for energy conservation. High-temperature geothermal systems provide natural laboratories to study mechanisms controlling the rate and formation of Fe (III)-oxides across different temperatures and geochemical gradients. *Metallosphaera yellowstonii* str. MK1 was isolated from an acidic Fe (III)-oxide mat in YNP and is an aerobic, thermoacidophilic Sulfolobales capable of growth on Fe (II), pyrite, or elemental sulfur. Importantly, 16S rRNA gene sequences related to strain MK1 (>99%) are found in many different low-pH, Fe (III)-oxide microbial mats of YNP. Analysis of draft genome sequence from *M. yellowstonii* reveals 7 copies of heme copper oxygen reductases (subunit I) in a total of 5 different terminal oxidase complexes including the *fox*A-J gene cluster (thought to be specific for Fe oxidation), as well as a novel gene coding for a putative blue multi-copper oxidase (*mco*). Gene expression screens and reverse transcriptase (RT)- quantitative (q) PCR of field samples and cultures grown on either Fe (II), pyrite or elemental sulfur show that *fox* and *mco* genes are highly up-expressed when Fe (II) serves as the electron donor. Protein sequence analysis of FoxC indicates a novel lysine-lysine or lysine-arginine heme b binding domain and is likely the cytochrome component of a heterodimer complex with FoxG serving as a ferredoxin subunit. Bioinformatic analysis of *mco* indicates that this gene codes for a novel blue multi-copper oxidase with two plastocyanin type I copper domains; only three similar sequences were found in Genbank, and appear unique to members of the Order Sulfolobales. Our results show that these thermophilic archaea have evolved a unique electron transport mechanism involved in the oxidation of Fe (II) that is not apparently reproduced in other known prokaryotes. Moreover, other thermophilic iron oxidizing archaea in these acidic habitats appear to posses different mechanisms of Fe (II)-oxidation.