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Detection of Subterranean Biosphere by Using Phosphatase Activity

Yuki Itoh¹; Arimichi Moroi¹; Yae Edasawa¹; Yoshinori Takano²; Takeo Kaneko¹; Katsumi Marumo³,
Teturo Urabe⁴ and Kensei Kobayashi¹

¹Department of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku,
Yokohama 240-8501, Japan

²Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

³National Institute of Advanced Industrial Science and Technology, 1-1-1 Higashi, Tsukuba 305-8567,
Japan

⁴Department of Earth and Planetary Science, University of Tokyo, Hongo-Bunkyo-ku, Tokyo 113-0033,
Japan e-mail kkensei@ynu.ac.jp

A wide variety of methods have been proposed to detect microbial activities, but most of them can be applied to limited categories of terrestrial organisms. We propose here to use phosphatase activity, which seems to be an essential catalytic activity for all the terrestrial organisms, and possibly for extraterrestrial organisms. We determined phosphatase activity in core samples and chimney samples obtained in submarine hydrothermal systems located at South Mariana and at Suiyo Seamount in the Izu-Bonin Arc, Pacific Ocean, as a part of the Archaean Park Project in 2001-2003.

A 0.5-g of powdered sample was mixed with 2-mL of substrate solution (0.5 mM p-nitrophenyl phosphate in modified universal buffer; pH 6.5). After incubation at 37 °C, CaCl₂ - NaOH solution was added to stop the enzymatic reaction. Acid phosphatase activity was the formation rate of p-nitrophenol, which was calculated from the increase of absorbance at 410 nm.

Some of the chimney and core samples showed much higher activities than background activities by inorganic catalysts such as montmorillonite. Suiyo core sample showed the maximum phosphatase activity at the middle of the core, while Mariana core samples showed significant level of phosphatase activities only at the top of the core. In the case of Suiyo Seamount, hydrothermal fluid in subsurface reservoir can go through core samples. On the other hand, it hardly go through Mariana core samples, which seems to made the difference. The outer part of the chimney samples showed higher phosphatase activities than the inner part. It can explained by the facts that the inner part faces to hot fluid, while the outer part faces to ambient sea water.

The present results of phosphatase activity assay agreed with the results of organic and microbial analyses. It was shown that phosphatase activity is one of possible biomarkers for extant life.