Phosphatase activity in soils in the vicinity of Syowa Station

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In late years brisk biological activity is reported at extreme environment of the atmosphere, pole area, earth crust deep part, and knowledge of the earth biosphere is extending. Underground biosphere, deep-sea hydrothermal systems, upper air, chilly environment are listed as extreme environments. A number of methods are proposed to detect frontiers of biosphere, including culture methods, direct imaging, analysis of bioorganic compounds, and analysis of stable isotopic ratio. We examined novel biospheres in subsurface submarine hydrothermal systems by analyzing amino acids and their optical activity and enzymatic activities.

Among many enzymes, we selected phosphatase. Alkaline and acid phosphatase hydrolyze phosphate esters, which are essential bioorganic compounds as nucleic acids and cell membrane constituents. Thus phosphatases are universal for terrestrial organisms. In addition, phosphatases are staboe and and easy to assay. Therefore we measured phosphatase activityies as biomarkers of extreme environment biospheres. Here we measured phosphatase activities of surface soil samples obtained near Syowa Base, Antarctica, extremely cold and dry environments.

Eight samples near Syowa Base were used together with ordinary surface soil samples of YNU campus. E. coli alkaline phosphatase was used as a standard enzyme. Determination of phosphatase activities were measured by the method of Tabatabai (1982) as follows: Substrate solution (p-nitrophenyl phosphate in MUB buffer solution (pH 6.5 or pH 8.0)) was added to each powdered soil sample and the mixture was incubated for 1h hour in a water bath at 310 K. The reaction was terminated by the addition of 0.5M CaCl2-NaOH, and the solution was filtered through a 0.20-micrometer membrane filter. The absorbance of the reaction product (p-nitrophenol) at 410 nm was measured with a spectrometer: Increasing rate of the absorbance at pH 6.5 was referred as ACP (acid phosphatase activity), and that at pH 8.0 as ALP (alkaline phosphatase activity). To extract enzymes, Tris-HCl buffer solutions (pH 9.0) was added to soil samples and stirred for one hour. Activity of extracted enzymes are measured by the fluorometric method, where 4-methylumbelliferyl phosphate was used as substance. We mixed extract and the substance solution (pH 8.0), and incubated at each temperature between 297 K and 343 K. Activity was calculated by the fluorescence intensity of 4-methylumbelliferone at the wavelength of 451 nm.

Phosphatase activity was confirmed from some of Antarctic soils.ALP activity of Antarctic soils ranged 2.00 - 26.1 nmol/(min g), and ACP ranged 2.01 - 23.7 nmol/(min g). Activity of extracts from Antactic soils showed maximum at 338 K, while that from the campus showed maximum at 358 K, which reflects the characteristics of host organisms of the enzymes. It can be said that phosphatase activity is among good indicators of biosphere frontiers.