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## Japan Astrobiology Mars Project (JAMP)

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The liquid water is considered to be an critical factor for life. Gibbs free energy is another factor that should be counted to sustain life for long duration. The Gibbs free energy is obtained by reaction between reductant and oxdidant, or from any other non-equilibrium state of matter. As an example, aerobic organisms use carbohydrate and oxygen for getting Gibbs free energy. Many types of chemoautotrophic mechanisms are known for the process as well. On Mars surface, methane and oxidative compound such as ferric oxide or sulfate are found, and they can be a sourse of Gibbs free energy. Iron-dependent methan oxidizing bacteria was found in marine emvironment on Earth (1). This finding suggests possible presence of methane-oxidizing bateria on Mars surface, if local thermal environment and other resources permit proliferation and metabolism of the bacteria during limited portion of time period.

Our project aims to search for the methane-oxidizing microbes on Mars surface. Martian soil will be sampled from a depth of about 5 or 10 cm below the surface, where organisms are supposed to be protected from harsh hyper-oxidative environment of Mars surface. Small particles less than 0.1 mm are sieved from the sample, before transferred to analysis section by a microactuator. The particles are stained by cocktail of fluorescence reagents, and examined by a fluorescence microscope.

Combination of fluorescence dyes is selected to identify life forms from the soil sample. Intercalating fluorescence dye such as SyberGreen is used to detect genetic compounds such as DNA. Membrane specific dye or the combination of dyes is used to detect membrane surrounding the cell. Substrate dye that emits fluorescence upon cleavage by the catalytic reaction is used to detect the catalytic activity of the cell. A combination of staining reagents is chosen based on the definition of life. DNA or genetic material is required for replication of life form. Membrane separating cell from ambient leads to identification of individual. Catalytic reaction of enzymes drives metabolism. The combination is useful also for detecting pre-biotic organic material as well as remnant of ancient life.

Hydrolysis of the polymers in the cell followed by HPLC or soft ionization MS for amino acid analysis is effective in examining whether Martian life is identical or different from terrestrial life. The number and type of the amino acids as well as chirality will be analyzed to distinguish if the polymers are contamination made by Earth-related life form.

Reference: (1) E. J. Beal, et al (2009) Science 325, 184-187

Keywords: Life search, Mars, microbe, methane oxidizing bacteria, fluorescence microscope