

Studies on analytical methods for amino acids to evaluate biological activities in extreme environments

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Biological activities have been detected in such terrestrial extreme environments as hot springs, submarine hydrothermal systems, deserts and polar environments. Since amino acids are essential constituents for terrestrial organisms, they could be good indicator to evaluate biological activities in extreme environments. In order to analyze amino acids in soil and rock samples, they should be extracted from mineral matrices. Hot water extraction has been widely used to extract amino acids from geological samples, but we previously found that only a small part of amino acids could be released by the method. In the present work, we examined several extraction methods for amino acid analysis, including HF digestion and HCl extraction.

Samples used were (1) soil samples in Yokohama National University (YNU) main campus (ordinary environment), (2) soil samples near the Showa Station, Antarctica, sampled by JARE 49 in 2007-8, and (3) soil samples in Atacama Desert, Chile, sampled in 2002. Procedural blank was prepared by performing the experiment procedure without samples.

Amino acid concentration in Atacama Desert soil and in Antarctica soil (except in Penguin rookeries) was much less than that in YNU campus soils.

In the HF digestion, 0.1 g of sample was heated with 3 mL of 5 M HF-0.1 M HCl in a Teflon vessel at 110 °C for 24 hours, then was acid hydrolyzed in 6 M HCl at 110 °C for 24 hours. In the HCl extraction, 0.1 g of sample was heated with 3 mL of 6 M HCl at 110 °C for 12-48 hours. In the conventional hot water extraction, 0.1 g of sample was first heated in 1.5 mL of water at 110 °C for 24 hours, and then the extract was filtered through a membrane filter: The filtrate was subjected to the HCl hydrolysis for 24 hours. In the modified hot water extraction, 0.1 g of sample was heated with 1.5 mL of water at 110 °C for 24 hours, followed by the acid-hydrolysis without filtration. All the hydrolysates were subjected to amino acid analysis by cation exchange HPLC with post-column derivatization with o-phthalaldehyde and N-acetyl-L-cysteine for fluorometric detection.

The HF digestion, the HCl extraction and the modified hot water extraction gave much higher amount of amino acids than the conventional hot water extraction. The HF digestion, however, showed higher procedural blank than the other methods. In the HCl extractions, heating for longer time yielded less amino acids. Thus the HCl extraction with shorter heating time was recommended for amino acid analysis of geological samples.

Further works, including development of high resolution, high sensitivity method for determination of amino acid enantiomers, will be done.

Keywords: Extreme environment, Amino acid, Biological activity, Extraction method, Antarctica, Atacama Desert