



## **Discrimination of Anaerobic Metabolic Pathways Using Carbon Isotopic Patterns of Amino Acids**

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The abundance and stable isotopic composition of amino acids have been broadly used to examine biogeochemical processes and ecological issues. Previous studies have demonstrated that various carbon assimilative pathways, such as autotrophy, heterotrophy and acetotrophy, could be distinguished by the carbon isotopic pattern of amino acids. However, the application of carbon isotopic pattern of amino acids in natural samples is limited, owing to the complexity of sources and processes in natural environments. Besides, the taxonomic and metabolic coverage are not fully explored, especially for anaerobic metabolisms.

Our study examined the carbon isotopic patterns of amino acids for several microorganisms bearing biogeochemical and ecological significance in anoxic environments and evaluated the possibility to discriminate the proportion of various carbon assimilative pathways in mixing cultures and environmental samples. The anaerobic strains were isolated from riverine wetlands and submarine hot springs in Taiwan and can perform sulfate reduction, elemental sulfur reduction and methanogenesis. The carbon isotopic pattern of amino acids for these anaerobic microbes was apparently similar to each other, reflecting the biosynthetic pathways of amino acids, except for that derived from the sulfate reducer utilizing acetate as carbon source. The isotopic odds observed for the acetoclastic sulfate reducer could be explained by the possibility that the synthesis of specific amino acids acquires the carboxyl group from acetate and may result in  $^{13}\text{C}$  enrichment of these amino acids. The carbon isotopic pattern of microbial amino acids of all examined strains could be well grouped into different carbon assimilative pathways through discriminant function analysis. The proportion of various metabolic activities in mixed cultures will be estimated by a Bayesian mixing model using proper anabolic end members for the carbon isotopic composition of microbial amino acids. It would be also possible to discriminate the relative importance of various carbon sources derived from different assimilation pathways by examining carbon isotopic pattern of amino acids with more characterized end members in natural systems.