



Quinone-based stable isotope probing for assessment of ^{13}C substrate-utilizing bacteria

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In this study, we attempted to establish quinone-stable-isotope probing (SIP) technique to link substrate-utilizing bacterial group to chemotaxonomic group in bacterial community.

To identify metabolically active bacterial group in various environments, SIP techniques combined with biomarkers have been widely utilized as an attractive method for environmental study. Quantitative approaches of the SIP technique have unique advantage to assess substrate-incorporation into bacteria. As a most major quantitative approach, SIP technique based on phospholipid-derived fatty acids (PLFA) have been applied to simultaneously assess substrate-incorporation rate into bacteria and microbial community structure. This approach is powerful to estimate the incorporation rate because of the high sensitivity due to the detection by a gas chromatograph-combustion interface-isotope ratio mass spectrometer (GC-c-IRMS). However, its phylogenetic resolution is limited by specificity of a compound-specific marker.

We focused on respiratory quinone as a biomarker. Our previous study found a good correlation between concentrations of bacteria-specific PLFAs and quinones over several orders of magnitude in various marine sediments, and the quinone method has a higher resolution (bacterial phylum level) for resolving differences in bacterial community composition more than that of bacterial PLFA. Therefore, respiratory quinones are potentially good biomarkers for quantitative approaches of the SIP technique.

The LC-APCI-MS method as molecular-mass based detection method for quinone was developed and provides useful structural information for identifying quinone molecular species in environmental samples. LC-MS/MS on hybrid triple quadrupole/linear ion trap, which enables to simultaneously identify and quantify compounds in a single analysis, can detect high molecular compounds with their isotope ions. Use of LC-MS/MS allows us to develop quinone-SIP based on molecular mass differences due to ^{13}C abundance in the quinone.

In this study, we verified carbon stable isotope of quinone compared with bulk carbon stable isotope of bacterial culture. Results indicated a good correlation between carbon stable isotope of quinone compared with bulk carbon stable isotope. However, our measurement conditions for detection of quinone isotope-ions incurred underestimation of ^{13}C abundance in the quinone. The quinone-SIP technique needs further optimization for measurement conditions of LC-MS/MS.