# Stable isotope probing and its applications in soil microbial ecology

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**Abstract** Stable isotope probing (SIP), a combination of isotope labeling with the molecular biological approach, is a technique that is used to identify microorganisms in environmental samples, and at the same time, to examine microbial functions during the biogeochemical processes operating in various environmental systems. The method has the potential for wide applications in the future, since it can provide abundant information about microbial interactions and metabolic functions in complex communities. Environmental samples *in situ* or in microcosm are exposed to substrates labeled with stable isotopes and some microorganisms in these samples can metabolize the stable isotope-enriched substrates as their carbon or nitrogen resource for growth. The stable isotope assimilated by these microorganisms is then used to synthesize cellular components such as nucleic acids (DNA or RNA) and phospholipid fatty acids (PLFA). As a result, microbial identity can be linked to their functions by extracting and analyzing these stable isotope-labeled biomarkers in the microbial communities. This review introduces the range of stable isotope enriched substrates, the labeling techniques of such substrates to microorganisms, the selection criteria of appropriate biomarkers and the methods for extracting and analyzing the biomarkers, and the applications of SIP in the functional analyses of methylotrophs, bacteria of organic pollutants degradation, rhizosphere-microorganism ecology, syntrophic microorganisms and metagenomics.

## Introduction

It is a great challenge on how to link the microbial identities to their functions. Stable isotope probing (SIP), a combination of isotope labeling with molecular biological approach, provides the possibility to link the two sections. The schematic diagram of SIP technique is shown in Fig. 1.

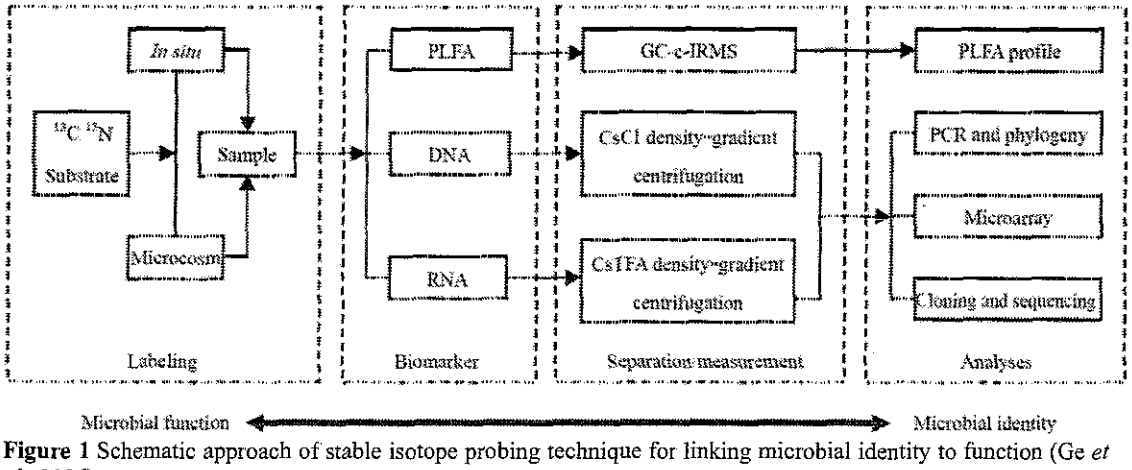


**Figure 1** Schematic approach of stable isotope probing technique for linking microbial identity to function (Ge *et al.*, 2006).

## Stable isotope and substrates

$^{13}C$ was generally used as labeling element in current SIP experiments (Boschker *et al.*, 1998; Radajewski *et al.*, 2000; Lu and Conrad, 2005). Other heavy stable isotopes, such as $^{2}H$, $^{15}N$, $^{17}O$ and $^{18}O$, have the potential as labeling element (Radajewski *et al.*, 2003). Particularly, $^{15}N$ can be used as labeling element to explore the functional groups of microorganisms involved in nitrogen biogeochemical cycle. Three groups of $^{13}C$-substrates and their applications are shown in Table 1.

**Table 1** $^{13}C$ substrates and their applications (Ge *et al.*, 2006)

| Group | $^{13}C$-substrates | Applications |
|---|---|---|
| $^{13}CO_2$ | $CO_2$ | Effect of plant-microorganism interactions on carbon cycling in terrestrial ecosystem and turnover rate of carbon in soil |
| $^{13}C$ methylic compounds | Methane, Methanol, Methyl chloride and Methyl bromide | Study in methylotrophs |
| $^{13}C$ multicarbon compounds | Phenol, Salicylate, Naphthalene, Phenanthrene, 2,2'- Dichlorobiphenyl, Propionate, Acetate, Glucose, Caffeine, Toluene | Study of biodegradation of organic pollutants |

**Biomarker**

Three biomarkers, PLFA, DNA and RNA, were generally used in SIP experiments. The first applied biomarker is PLFA (Boschker *et al.*, 1998). The $^{13}C$ ratio ($\delta^{13}C$) of different PLFAs was determined by gas chromatograph-combustion-isotope ratio mass spectrometry (GC-c-IRMS). Then the microorganisms represented by $^{13}C$-labeled PLFA were the specific microorganisms incorporating such $^{13}C$-substrate. However, because many PLFAs are ubiquitous to all organisms, the method was limited to qualifying gross changes in microbial communities.

DNA and RNA as biomarkers can transfer more genetic and taxonomic information. Because of the higher buoyant density, nucleic acids (DNA or RNA) enriched $^{13}C$ can be fractionated by caesium chloride (CsCl or caesium trifluoroacetate, CsTFA) density gradient centrifuge (Radajewski *et al.*, 2000; Manefield *et al.*, 2002) Then the $^{13}C$-labeled nucleic acids can be analysed through different molecular approaches, such as polymerase chain reaction (PCR) and phylogenetic analysis, microarray analysis and metagenomic analysis. The main limitation of using DNA as a biomarker is that the synthesis of $^{13}C$-labeled DNA needs long incubation periods, which results in two disadvantages that (1) the experiment cost will increase and (2) some of the $^{13}C$ will assimilate by non-targeted organisms which do not directly use this $^{13}C$-substrate. Using RNA as a biomarker can decrease incubation periods, since RNA became $^{13}C$-labeled much more rapidly than DNA. The combination use of DNA and RNA seems to be a promising method in future application. $^{13}C$ RNA can be used to identify the instant microbial functional groups and $^{13}C$ DNA to trace the flow of $^{13}C$ in the microbial food web for examining the interaction of different microorganisms (Lueders *et al.*, 2004b)

**Labeling technique**

Two labeling methods, directly labeling microorganisms and indirectly labeling microorganisms by labeling plants, were generally used. The gaseous, liquid and solid $^{13}C$-substrates can be used in directly labeling to research methylotrophs (Radajewski *et al.*, 2000) and organic waste degradation bacteria (Singleton *et al.*, 2005) $^{13}CO_2$ was generally used in indirectly labeling to examine the process occurred in plant-soil-microorganism system, such as to evaluate the effect of environmental factors on C flow and determine the key microorganisms on this process (Lu and Conrad, 2005). Both directly labeling and indirectly labeling could be operated *in situ* (Johnson *et al.*, 2005) or in microcosom (Lu and Conrad, 2005)

**Applications**

SIP has been applied in the functional analyses of methylotrophs (Radajewski *et al.*, 2000), bacteria of organic pollutants degradation (Singleton *et al.*, 2005), rhizosphere-microorganism ecology (Lu and Conrad, 2005), syntrophic microorganisms (Lueders *et al.*, 2004a) and metagenomics (Lu and Conrad, 2005). The method has the potential for wide applications in the future, since it can provide abundant information about microbial interactions and metabolic functions in complex communities.

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