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DNA-based stable isotope probing: a link between community structure and function

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ABSTRACT

DNA-based molecular techniques permit the comprehensive determination of microbial diversity but generally do not reveal the relationship between the identity and the function of microorganisms. The first direct molecular technique to enable the linkage of phylogeny with function is DNA-based stable isotope probing (DNA-SIP). Applying this method first helped describe the utilization of simple compounds, such as methane, methanol or glucose and has since been used to detect microbial communities active in the utilization of a wide variety of compounds, including various xenobiotics. The principle of the method lies in providing ^{13}C-labeled substrate to a microbial community and subsequent analyses of the ^{13}C-DNA isolated from the community. Isopycnic centrifugation permits separating ^{13}C-labeled DNA of organisms that utilized the substrate from ^{12}C-DNA of the inactive majority. As the whole metagenome of active populations is isolated, its follow-up analysis provides successful taxonomic identification as well as the potential for functional gene analyses. Because of its power, DNA-SIP has become one of the leading techniques of microbial ecology research. But from other point of view, it is a labor-intensive method that requires careful attention to detail during each experimental step in order to avoid misinterpretation of results.

1 Introduction

The accurate assessment of microbial community structure and function is a central goal of much of microbial ecology research. Since less than 1% of microbes are cultivable (Torsvik and Øvreås, 2002), molecular biological methods based on molecular sequence data were the first effective tools for comprehensively determining community composition. These approaches enabled detection of uncultivable microbes. Likewise cultivable organisms are now classified with the assistance of DNA-based methods since bacterial morphology and physiology alone are not informative enough to distinguish different taxa. The most widely used phylogenetic marker has been 16S rRNA or its corresponding gene (Ludwig and Schleifer, 1994).

Although 16S rRNA analysis is an appropriate tool to describe relationships among microorganisms, phylogeny provides little information about the function of the microbe in the environment (Schloss and Handelsman, 2004). Until recently, the identification of bacteria involved in a particular process had been restricted only to cultivable bacteria, by determining which catabolic or physiological processes the organism could perform *in vitro*. Metagenomics, an approach based on analyses of environmental gene libraries, has

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broadened the scope by enabling researchers to study uncultivable bacteria as well. This method permits to link potential functions to specific microorganisms in a habitat (Rondon et al., 2000; Schmeisser et al., 2007). However, a problem can arise in some habitats, such as soil, that can be composed of many thousands of species. The metagenomic library thus may not adequately represent the diversity and may fail to notice low-abundance species. In addition, the function that has been linked to specific microorganisms is only predicted and this link needs to be demonstrated, for example, by expression studies (Wellington et al., 2003). A radical change came with the method called stable isotope probing (SIP) such as described by Boschker et al. (1998) and Radajewski et al. (2000). SIP is a cultivation independent technique whose main goal lies in linking the identity of bacteria with their function in the environment. The basis of SIP is in providing microbial community with stable isotope labeled substrate whose utilization is of interest. Microorganisms utilizing the stable isotope labeled substrate incorporate the stable isotopes into the biomass. Analyzing the components of the cell that provide phylogenetic information (biomarkers) reveals the organisms that utilize the substrate. The first biomarkers used were polar lipid derived fatty acids (PLFAs) in the study by Boschker et al. (1998). They studied sulphate reduction coupled to acetate oxidation and methane oxidation in aquatic sediments and found out that ^{13}C-labeled acetone was not utilized by widely studied *Desulfobacter* sp. but mainly by sulphate-reducing bacteria related to *Desulfotomaculum acetoxidans*. As for the methane oxidation, the work suggests that the dominant organisms involved in this process are members of genera *Methylobacter* and *Methylomicrobium*. Although Boschker et al. (1998) were the first who applied stable isotope labeling, the term SIP comes from Radajewski et al. (2000). In their study, they introduce another biomarker, DNA (therefore, DNA-based SIP or DNA-SIP). They show that after the growth of microorganisms on a ^{13}C-labeled substrate, ^{13}C-labeled DNA can be separated from ^{12}C-DNA by isopycnic (equilibrium) density gradient centrifugation in CsCl gradients. 'Heavy' (stable isotope labeled) DNA can be used to construct 16S rRNA gene clone libraries. Sequencing 16S rRNA genes enables identification or classification of the microorganisms that consumed the particular substrate. Applying this technique to explore bacteria in soil that consume methanol, Radajewski et al. (2000) found two groups of bacteria, α-Proteobacteria and *Acidobacterium*, to be involved. The third type of biomarker employed for SIP was rRNA. This approach was first published by Manefield et al. (2002). In their study, a phenol-degrading community was fed by ^{13}C-labeled phenol in an aerobic industrial bioreactor. ^{13}C-labeled RNA was separated by isopycnic density-gradient centrifugation in caesium trifluoroacetate (CsTFA) gradient and analyzed by reverse transcription-PCR (RT-PCR) and denaturing gradient gel electrophoresis (DGGE). Their results suggest that the organism dominating the acquisition of carbon from phenol is a member of the *Thauera* genus.

All of the three biomarkers described above have been widely used, each being most appropriate to different situations and experimental objectives. As Dumont and Murrell (2005) and Friedrich (2006) summarize, PLFA-SIP has the greatest sensitivity, however, its use is limited by the low phylogenetic resolution of PLFAs. PLFA-SIP is also advantageous for determining relative abundance of bacteria versus fungi that utilized the ^{13}C-substrate. On the other hand, DNA and rRNA are the most taxonomically informative biomarkers and offer the ability of identifying a broad spectrum of microorganisms involved in a particular process. RNA-based SIP (or RNA-SIP) is the more sensitive one of the two as the copy number of rRNA in an actively metabolizing cell is much higher than rRNA gene copies. According to Manefield et al. (2007), RNA labeling with ^{13}C is 6.5 times faster than DNA labeling and, unlike with DNA-SIP, it does not require cell division. Nonetheless, DNA-SIP is deservedly considered unique in linking the identity of microbes with their physiological role in the environment (Friedrich, 2006); not only 16S rRNA genes can be analyzed, but because stable isotope labeled DNA represents the entire genomes of active populations, also functional genes, i.e. genes encoding key enzymes of metabolic pathways, within these populations can be analyzed. The combination of DNA-SIP with metagenomic library approaches thus eliminates the weakness of these approaches, i.e. they may easily overlook low abundant parts of the community that perform the process of interest (Wellington et al., 2003). Due to entire genomes being present in the isolated stable isotope labeled DNA even complete operons can be identified combining DNA-SIP with metagenomic analysis. This has been demonstrated by Dumont et al. (2006) with the methane monooxygenase operon. In addition, works using DNA-SIP together with RNA-SIP (Lueders et al., 2004a; Bernard et al., 2007; Héry et al., 2008) or PLFA-SIP (Webster et al., 2006) have been published taking advantage of DNA-SIP's value as a tool for linking microbial identity and functional genes with metabolic activity combined with the increased sensitivity of the other methods.

Other techniques have also been developed in the last decade that combine the identification of both cultivable and uncultivable microbes with their metabolic capabilities (Dumont and Murrell, 2005). These techniques include the combination of fluorescent *in situ* hybridization (FISH) with microautoradiography (Lee et al., 1999) and the isotope array (Adamczyk et al., 2003). Unlike DNA-SIP, both these methods are based on radioactive isotope labeled substrate incorporation into biomass. Moreover, as Whitby et al. (2001) referred to the former, it is not straightforward as high background fluorescence and non-specific binding of the probes can be encountered.

In this review, we consider applications of DNA-SIP for linking community structure with its function in microbial ecology and highlight its application to bioremediation studies.

2. Methodological considerations of DNA-SIP

DNA-SIP relies on tracking stable isotopes from specific substrates into the DNA of microorganisms that grow and metabolize the substrate. Isopycnic centrifugation then permits separation of stable isotope labeled DNA from the DNA of microbes inactive in the utilization of the substrate. Once the labeled DNA is separated, community profiling (fingerprinting) can be carried out with techniques like terminal-