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Use of Stable Isotope Probing in Selectively Isolating Target Microbial Community Genomes from Environmental Samples for Enhancing Resolution in Ecotoxicological Assessment

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Abstract

In this study we attempted to develop a novel genomic method to selectively isolate target functional microbial genomes from environmental samples. For this purpose, stable isotope probing (SIP) was applied in selectively isolating organic pollutant-assimilating populations. When soil microbes were fed with ^{13}C-labeled biphenyl, biphenyl-utilizing cells were incorporated with the heavy carbon isotope. The heavy DNA portion was successfully separated by CsCl equilibrium density gradient. And the diversity in the heavy DNA was sufficiently reduced, being suitable for the current DNA microarray techniques to detect biphenyl-utilizing populations in the soil. In addition, we proposed a new way to get more genetic information by combining this SIP method with selective metagenomic approach. The increased selective power of these new DNA isolation methods will be expected to provide a good quality of new genetic information, which, in turn, will result in development of a variety of biomarkers that may be used in assessing ecotoxicology issues including the impacts of organic hazards, and antibiotic-resistant pathogens on human and ecological systems.

Keywords: Stable isotope probing (SIP), Ecotoxicology, Environmental samples, Functional genomics, Microarray detection limitation

The detection limitation of traditional measurement tools is one of the factors hindering our eco-toxicological efforts on microbial ecology, in particular soil microbial communities. Because the knowledge on the complex system of soil microbial ecology requires huge amounts of data and information, high throughput techniques such as DNA microarray have been considered as a promising tool for environmental and ecological studies[1,2]. However, our previous studies revealed that the diversity of soil microbial communities is too high to be analyzed by DNA microarrays, which eventually hinders the resolution of DNA microarray analysis[3]. In order for DNA microarrays to detect target microbial genes, the size of its corresponding population should be greater than 1% among the total microbial communities. Unfortunately, eco-toxicologically important microbes such as biodegrading and antibiotic-resistant ones are typically rare (less than 1%) in environment[4]. One way for solving this problem is to selectively isolate target functional microbial genomes from environmental samples so that the degree of complexity in soil microbial community can be reduced up to the level for suitable detection by ecological toxicogenomic tools such as DNA microarray chips.

In this study, we attempted to develop a method to selectively isolate target functional microbial genomes from environmental samples. For this purpose, we applied stable isotope probing (SIP) technique in selectively obtaining biphenyl-utilizing bacterial genomes.

Selective Isolation of Genomic DNA of Biphenyl-Using Microbes from a Soil Sample

To enrich target genetic information from overall microbial communities, biphenyl degrading populations were selectively labeled with isotopic stable carbon (^{13}C), and the ^{13}C-lablelled heavy DNA and RNA samples could be isolated from natural-carbon-incorporated light ^{12}C-DNA using CsCl density-gradient ultracentrifugation after (Fig. 1).

When the soil was incubated in the presence of ^{13}C-biphenyl for 3 days, very little biphenyl metabolism was observed ^{13}C-CO_2 GC-MS analysis. In this case, no heavy DNA fraction was obtained. In contrast, when incubated for 28 days, there was significant ^{13}C-CO_2 production, indicating high degree of metabolism of the added biphenyl by the soil microbes. As expected, a significant amount of heavy DNA isolated in the equilibrium density gradient (Fig. 1).

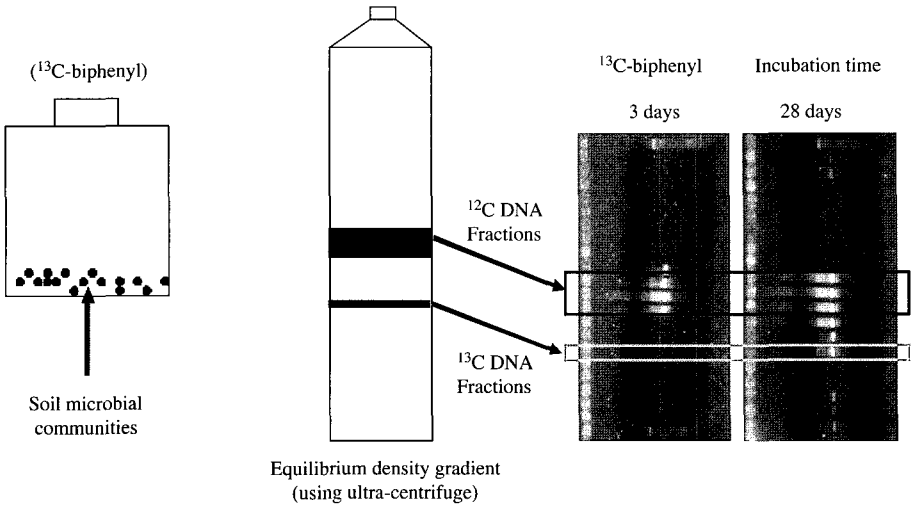

Fig. 1. Scheme for ^{13}C-biphenyl SIP method. After 28 days of incubation, a significant portion of heavy DNA was obtained from equilibrium density gradient. This was confirmed by the amplification of 16S rDNA genes from the heavy portion (in the lower box).

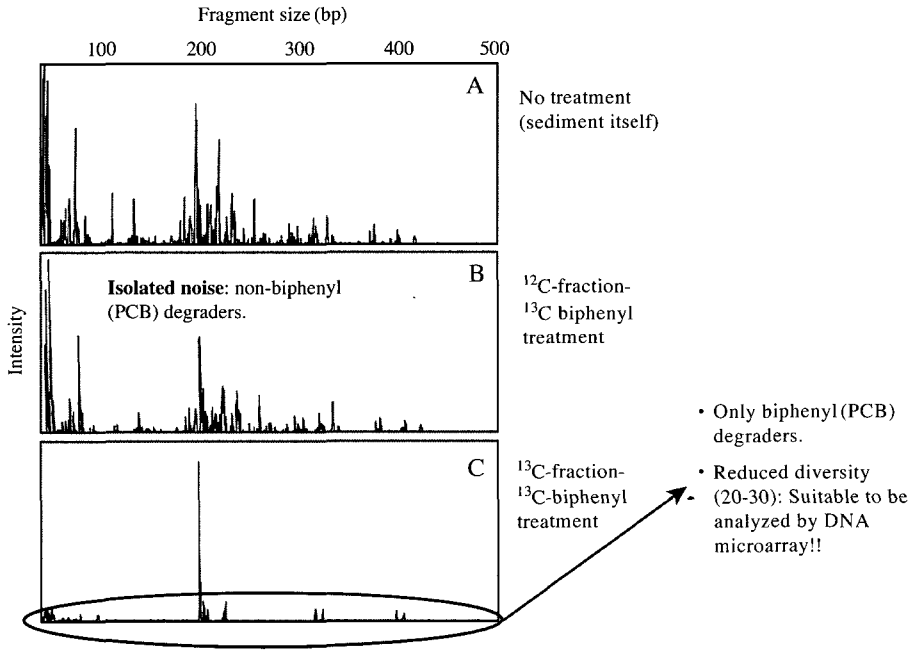

Fig. 2. ^{13}C-biphenyl SIP (stable isotope probing) reduced the diversity in biphenyl using populations.

This illustrated that the SIP method successfully isolated biphenyl-using microbial genomes from the soil sample.

Sufficient Reduction of Diversity in Biphenyl-Using Populations

To examine whether the SIP method could reduce the ecological complexity in the biphenyl using populations, we measured diversity using a standard t-RFLP (terminal-restriction fragment length polymorphisms) method (Fig. 2). Compared to the non-SIP treatment, the number of peaks in the SIP-separated DNA was reduced up to 10-15. By assuming uncertainty factor as 2, the diversity of biphenyl degrading populations was expected to be 20-30 populations. This is a significant progress since the diversity in the SIP separated DNA samples is suitable for the current DNA microarray techniques to be able to detect target genes ($>1\%$).

Discussion

The information on environmentally important microbial genes are limited. In particular, information on biodegradation genes and antibiotic-resistance

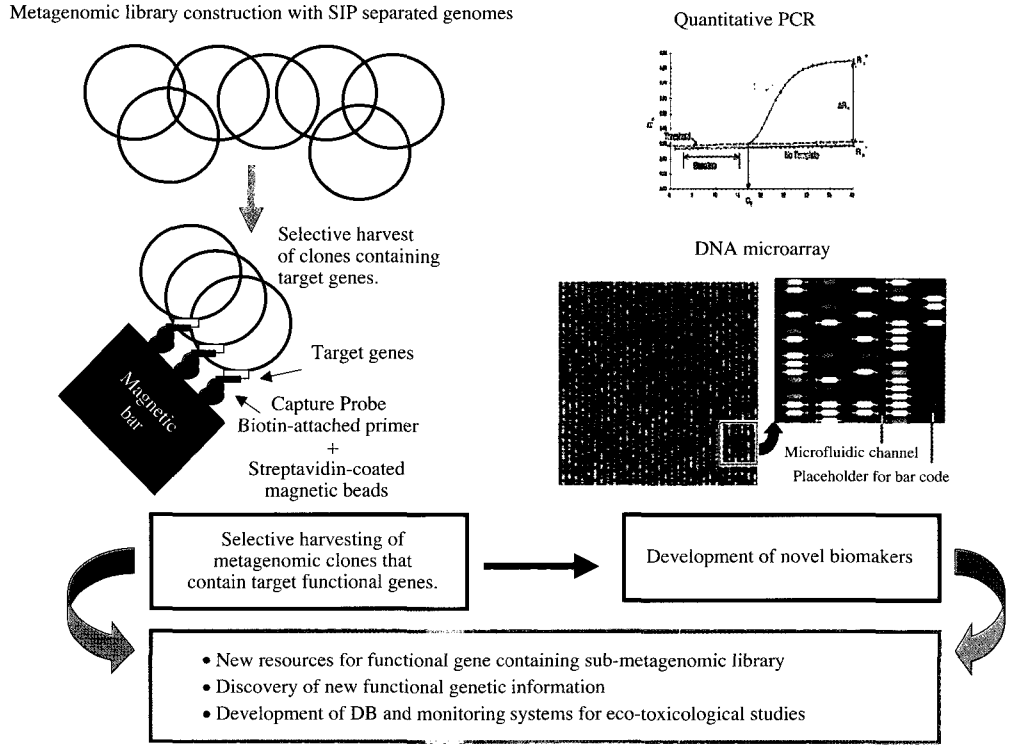

Fig. 3. Proposed idea to expand the applicability of the SIP method in environmental toxicogenomics tools.

genes are very limited. The problem was partially due to the fact that the traditional ecology method was culture-dependent. Since only a few can grow on laboratory culture media, the current culture dependent methods cannot capture enough genetic information from nature. So called environmental genomics is a novel way to capture genetic diversity in nature. Craig Venter actually used metagenomic approach to explore microbial diversity in ocean[5]. However, the price for sequencing long insertions in metagenomic clones is extremely high. Therefore, as proposed in Figure 3, we can use biotin-labeled primers and magnetic beads (so-called Catch Probe) in selectively harvest only metagenomic clones that contain target genes. Once one gets enough new genetic information, it will be much efficient and sure to develop target-specific and accurate biomarker sequences, which can be used in variety of environmental toxicogenomic tools including real-time PCR and DNA microarray chips.

Methods

Materials and Microcosm Experiment

Soil came from a U.S. Air Force base, which was contaminated with PCB (polychlorinated biphenyls). ^{13}C-biphenyl was purchased from Sigma. Five gram of soil was added in each 160 mL serum bottle. ^{13}C-biphenyl was added and the serum bottle was sealed with an air-tight butyl rubber cap. The prepared microcosm bottles were incubated in a dark room without mixing. Each day ^{13}C-CO_2 was measured using GC-MS.

SIP

From the soil, DNA was extracted using Zhou's method[6]. The protocol of Radajewski *et al.*[7] was used to separate and analyze ^{13}C-enriched RNA. Briefly, 500 ng of total DNA was loaded onto CsCl density gradients (buoyant density 1.55 g/mL) and spun in a TLA120.2 rotor in an Optima TLX ultracentrifuge (Beckman Coulter) at 64,000 rpm (150,000 × g) and 20°C for 36 h. Gradients were fractionated from below by displacement with water by using a syringe pump and DNA was isolated from gradient fractions by precipitation with isopropanol. The DNA samples were then polymerase chain reaction (PCR) amplified using primers 27F and 1429R (for bacterial 16S rRNA genes), and products separated on 0.8% agarose gels. Gels were stained with SYBR gold nucleic acid gel stain (Molecular Probes) and visualized by a

UV transillumination. The diversity of 16S rRNA gene PCR products were determined by a standard t-RFLP method (http://rdp8.cme.msu.edu/html/t-rflp_jul02.html) with one enzyme.

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