Journal of Microbiological Methods 115 (2015) 64–66

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

Co-extraction of DNA and PLFA from soil samples

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ARTICLE INFO

Article history:
Received 11 May 2015
Accepted 11 May 2015
Available online 28 May 2015

Keywords:
DNA
PLFA
Co-extraction
16S rRNA sequencing
Soil

ABSTRACT

Lipid/DNA co-extraction from one sample is attractive in limiting biases associated with microbial community analysis from separate extractions. We sought to enhance established co-extraction methods and use high-throughput 16S rRNA sequencing to identify preferentially extracted taxa from co-extracted DNA. Co-extraction results in low DNA yields and distinct community structure changes.

Culture-independent techniques are routinely used to analyze microbial communities. The two most commonly used culture-independent techniques are lipid analysis (Vestal and White, 1989) and nucleic acid analysis (Ogram et al., 1987). Phospholipid ester-linked fatty acid (PLFA) analysis can provide information on microbial biomass, physiological stress, community structure, and in some cases taxonomic identification through indicator biomarkers (Kehrmeyer et al., 1996; Tunlid et al., 1989). Nucleic acid analysis is often performed through sequencing of marker genes or shotgun metagenomic approaches. Nucleic acid analyses can yield information on species diversity, community structure, and functional potential of microbial communities (Robe et al., 2003; Roose-Amsaleg et al., 2001). Nucleic acid and lipid extraction involve direct extraction of these molecules from an environmental sample. In most cases, two extraction techniques are performed to study the lipids and nucleic acids in a sample. Since every extraction technique has its own biases (Hazen et al., 2013; Miller et al., 1999; Roose-Amsaleg et al., 2001; Zhou et al., 1996), using different extractions to study lipids and DNA could increase the associated biases. It would be advantageous if lipids and nucleic acids could be extracted from the same environmental sample using a single technique. Co-extraction is attractive as it would yield more representative results and limit some of the biases associated with separate extractions. Several groups have developed techniques to recover DNA from the aqueous phase of the modified Bligh and Dyer PLFA extraction technique (Bligh and Dyer, 1959; White et al., 1979).

The modified Bligh and Dyer method is commonly used to extract lipids from environmental samples, involving a solvent extraction and phase separation (Bligh and Dyer, 1959). The organics phase contains solvent-extracted total lipids and is further processed for PLFA recovery. The aqueous phase is generally discarded; however it contains water-soluble lipids and other macromolecules, including nucleic acids. Previous studies have recovered DNA from this aqueous phase (Kehrmeyer et al., 1996; Malave-Orengo et al., 2010; Villanueva et al., 2004; White et al., 2003), but in many cases the yields were quite low. Kehrmeyer et al. (1996) successfully recovered DNA from the aqueous phase of a PLFA extraction. They used radio-labeled DNA to show that DNA partitions into the aqueous phase during lipid extraction. Kehrmeyer et al. (1996) reported DNA yields from their co-extraction technique, but only for cultured microbes, not for DNA extracted from environmental microbes. The approach of Kehrmeyer et al. (1996) was subsequently applied to extract lipids and DNA from a microbial mat samples (Villanueva et al., 2004). In this method DNA was precipitated from the aqueous phase with no further purification. However, the amount of DNA recovered by this method was often quite low.

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http://dx.doi.org/10.1016/j.mimet.2015.05.018

Table 1
DNA concentrations for extractions done for different methods. DP corresponds to PLFA/DNA co-extractions. PS corresponds to PowerSoil extractions. Kehrmeyer extractions were data from Kehrmeyer et al. (1996).

	Avg. nanodrop 260/280	Avg. nanodrop 260/230	Avg. ngs DNA/g soil (Qubit)
PD-MTT	1.323 ± 0.076	0.827 ± 0.098	11.05 ± 13.91
PD-MTC	1.688 ± 0.152	0.848 ± 0.220	0.88 ± 0.66
PD-STPJ	1.533 ± 0.017	0.783 ± 0.052	1.41 ± 0.97
PD-STDI	1.516 ± 0.061	0.832 ± 0.096	1.66 ± 0.61
PS-MTT	1.555 ± 0.032	1.250 ± 0.053	20,570.0 ± 2011.2
PS-MTC	1.363 ± 0.060	1.016 ± 0.251	5719.9 ± 647.1
PS-STPJ	1.330 ± 0.096	1.003 ± 0.054	5419.8. ± 698.3
PS-STDI	1.48 ± 0.288	0.830 ± 0.207	9874.3 ± 8038.5
Kehrmeyer, *Pseudomonas fluorescens*	Not reported	Not reported	2.28 ± 0.6
Kehrmeyer, *Pseudomonas putida*	Not reported	Not reported	1.19 ± 0.1

The goal of this was twofold. First, we sought improve the previously developed co-extraction techniques by including a DNA extraction step in an effort to achieve reliable extraction of high concentrations of DNA from soil samples. We also sought to assess the diversity of the extracted community using massively-parallel 16S rRNA sequencing.

Surface soil was sampled from four different sites, two from Middle Tennessee and two from South Texas. Samples from Middle Tennessee were labeled MTT and MTC. Samples from South Texas are labeled STPJ and STDI. Ten grams of each soil sample were extracted using the modified Bligh and Dyer method (Bligh and Dyer, 1959). Recovered aqueous phases were subjected to DNA extraction following the modified Miller Method (Hazen et al., 2010). Briefly, one volume of Miller SDS buffer was added to the aqueous phase and extracted with Phenol:Chlorofom:Isoamyl Alcohol. The rest of the Modified Miller DNA extraction was performed according to previously described protocols (Hazen et al., 2010). The Power Soil DNA extraction kit (MoBio) was used to compare DNA yields and community diversity as it has been shown to be a robust method for DNA extraction from soils (Mahmoudi et al., 2011). Extractions were performed in quadruplicate from each soil sample.

Quality of extracted DNA was determined by measuring 260/280 and 260/230 ratios. Concentration of DNA was determined using Qubit Fluorometric Quantitation (Life Technologies, Carlsbad CA). High-throughput 16S rRNA sequencing was performed on the Illumina MiSeq according to the protocol (Caporaso et al., 2010, 2012). The resulting DNA sequences were analyzed using the QIIME version 1.8.0 pipeline according to standard protocols (Techtmann et al., 2015).

While, measurable DNA was recovered from the aqueous phase of the PLFA co-extractions, the DNA yields were much lower compared to the PowerSoil DNA extractions (Table 1). DNA yields from the aqueous phase extractions ranged from 0.88 ng/g of soil to 11.0 ng/g of soil (Table 1). These yields are similar to the numbers reported by Kehrmeyer et al. (1996). However, the DNA yields for the PowerSoil extraction were three orders of magnitude higher (5419 ng/10 g of soil to 20,570 ng/g of soil). The quality of the extracted DNA, as determined by measuring the 260/280 ratio was similar between co-extraction and PowerSoil methods.

16S rRNA genes were amplified and sequenced from DNA extracts from both extraction techniques. To determine if the co-extraction recovered a similar number of operational taxonomic units (OTUs), student's t-test was used to compare differences in the alpha diversity between extraction techniques. There were significant differences in Observed Species, Shannon, and phylogenetic alpha diversity metrics between extraction methods, with the PowerSoil method recovering a higher diversity (Fig. 1A–C) (Observed species; p-value = 0.000026, Shannon diversity; p-value = 0.00022 and PD whole tree diversity; p-value = 0.000044).

To identify if the DNA extraction methods resulted in different community structure, non-metric multidimensional scaling of weighted UniFrac distances was used to compare the communities recovered from each extraction method (Fig. 2). PERMANOVA was used to identify if these differences were statistically significant. There were significant differences in the microbial community structure from each of the extraction methods. Analysis of taxa plots indicated that while the dominant classes were consistently present in all samples, the abundance of these classes were different between the two extraction techniques (Fig. 2B).

These findings indicate that it is difficult to co-extract high concentrations DNA from the aqueous phase of the lipid extraction. Our diversity analysis points to significant differences in the recovered diversity in comparison to standard techniques such as the PowerSoil. This finding is not surprising, as other studies have shown that biases exist between different extraction methods (Hurt et al., 2014; Vishnivetskaya et al., 2014). Many of the differences between these extraction techniques could be due to the very low DNA yields from the PLFA/DNA co-extraction. The PLFA/DNA co-extraction remains an attractive technique. However, our work further demonstrates the many hurdles that must be overcome before this approach is more widely applicable.

Acknowledgments

This research was supported by contract A13-0119-001 Deep Sea Basin Microbiology between the University of Tennessee and BP America.

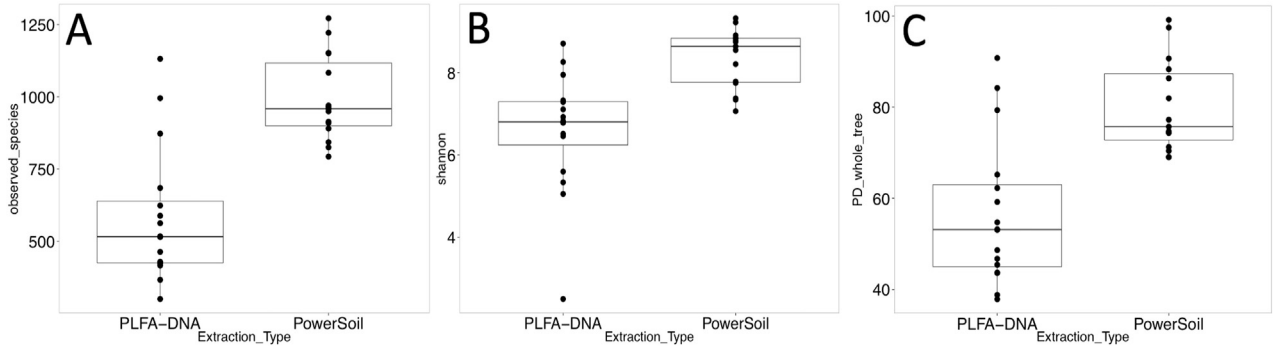

Fig. 1. (A–C) Alpha diversity comparing extraction type for all four sampling sites. t-Tests were used to identity if differences between extraction type were statistically significant. (A) Observed species (p-value = 0.000026), (B) Shannon diversity (p-value = 0.00022), (C) phylogenetic diversity (p-value = 0.000044).

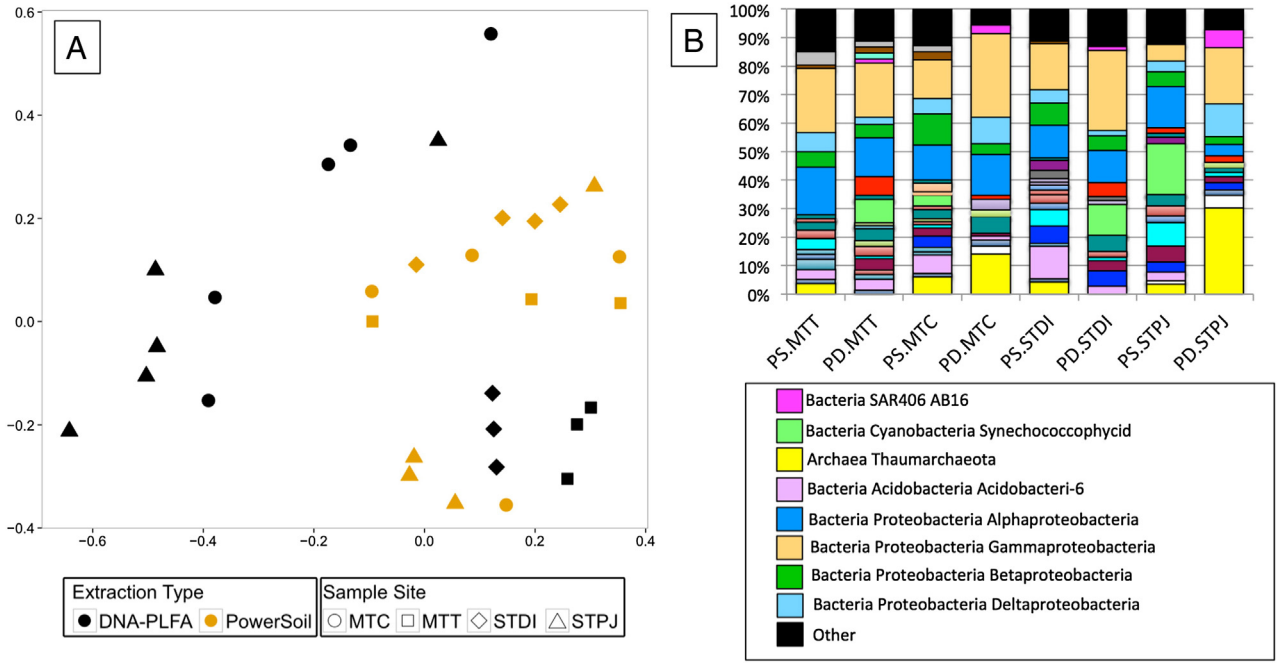

Fig. 2. Differences in diversity between extraction techniques. (A) NDMS of weighted UniFrac distances (stress: 0.23). Colors correspond to extraction type and shape corresponds to sampling location. PERMANOVA was used to identify if differences in community structure between extraction types were statistically significant (R^2 value = 0.59873; p = 0.001). (B) The relative abundance of each order was plotted for each sample. Sequences from each replicate extraction were merged and taxon plot was drawn. Taxa whose relative abundance was less than 1% were merged into the 'Other' category. The most dominant taxa are shown in the figure legend.

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