Comparison of Microbial Populations Isolated from a Variety of Soils using Different Homogenization Methods During DNA Extraction

Victoria E. Valencia (1), Mira R. Elnan (1), Nik von Atzigen (2), Suzanne J. Kennedy (2), Mark N. Brolaski (2), Michael W. Black (1), Christopher L. Kitts (1)
(1) Cal Poly-San Luis Obispo, (2) MO BIO Laboratories

ABSTRACT

Efficient DNA extraction is the key step in molecular analysis of soil for microbial ecology investigations. In fact, the choice of extraction method may have a significant effect on the data collected and mechanical lysis is generally considered best. Soils can vary greatly in their microbial diversity and load, which is influenced by factors including pH, organic content, moisture content, and texture of the soil. Consequently, careful study of the choices in mechanical lysis methods is important for obtaining reproducible data that accurately reflects the soil microbial assemblage. To assess bacterial and fungal diversity from different soils of varying characteristics, we used terminal restriction fragment length polymorphism (TRFLP) analysis, a reliable and well-established method for estimating microbial assemblage structure in environmental samples. DNA extraction of six different soil samples was evaluated using four different methods of mechanical disruption, varying the process (vortex vs. PowerLyzer™) and the matrix (glass beads vs. garnet). The use of the PowerLyzer™ generally resulted in higher DNA yields than the vortexer, as did the glass beads compared to garnet. As expected, soils containing higher sand content yielded much less DNA compared to soils rich in organic content. The effect of using the different methods on the microbial assemblage structure as assessed by TRFLP was strongly dependant on the soil type and the microbial assemblage (fungal or bacterial).

METHODS

Six soil samples were chosen to cover a range of characteristics including pH, organic carbon and nitrogen content and particle size (Table 1). DNA from each of the six soils was extracted in triplicate using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA). Mechanical lysis was performed with either a vortexer or the MO BIO PowerLyzer™, using either 0.1 mm glass beads or irregular garnet as a disruption matrix. Isolated DNA was inspected for degradation via gel electrophoresis and quantified via UV spectrophotometry. For each DNA extraction, TRFLP analysis was performed on triplicate PCR amplifications of the 16S rRNA region for bacteria and 18S-28S ITS region for fungi. Forward primers were fluorescently-labeled with Cy5 in each case. PCR triplicates from each extraction were cleaned using the Zymo DNA Clean and Concentrator-25 Kit (Zymo Research, Orange, CA) and product DNA quantified using a Bio-Tek fluorometer to measure the Cy5 incorporated label. Cleaned bacterial PCR products were digested with DpnII and the fungal product was digested with HaeIII. Excess salt was removed by ethanol precipitation and the fluorescent fragments were examined using the CEQ8000 capillary electrophoresis DNA analyzer (Beckman-Coulter, Brea, CA). The resulting TRFLP patterns were evaluated using Bray-Curtis similarity on square root scaled, normalized data with the Primer5 statistics package (Primer-E, Ivybridge, UK).

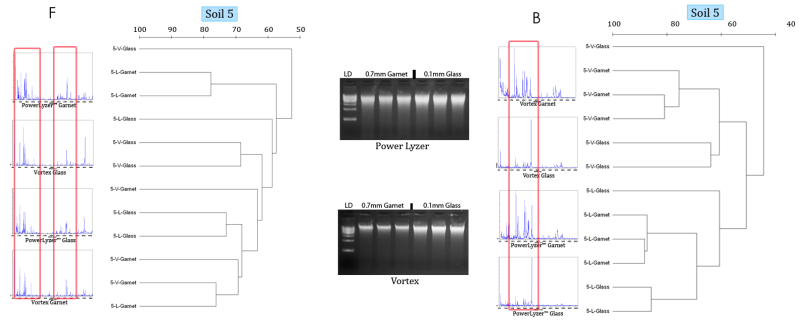

Figure 2. Comparison of 0.7mm garnet and 0.1mm glass homogenization methods and Vortex (V) versus PowerLyzer™ (L) methods for soil 5 for fungal (F) and bacterial assemblages (B) with corresponding TRFLP patterns and gel electrophoresis result for soil DNA extraction. Red boxes indicate areas in TRFLP patterns where peak heights differ between extraction methods.

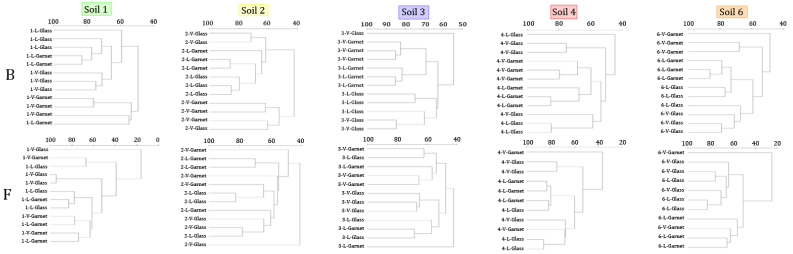

Figure 3. Comparison of 0.7mm garnet and 0.1mm glass homogenization methods and Vortex (V) versus PowerLyzer™ (L) bashing methods for soils 1-4 and soil 6. TRFLP analysis of extractions using different methods indicated small differences in peak distribution for bacterial assemblages in most soils, and differences in some soils with fungal assemblages.

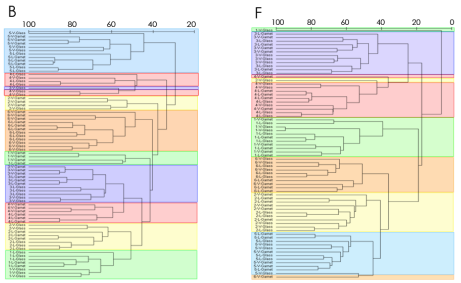

Figure 4. Comparison of 0.7mm garnet and 0.1mm glass homogenization methods and Vortex (V) versus PowerLyzer™ (L) bashing methods across all 6 soils for fungal (F) and bacterial (B) assemblages. Different colored boxes indicate each soil type. Differences observed between the methods were overshadowed by those found between soil types. The majority of the extractions grouped primarily by soil type. Soil types 1, 2, and 4 for bacterial assemblages were split, either by the vortex with garnet (soils1 and 2) or by the bead homogenization method (soil 4).

Table 1. Soil characteristics for each of the six soils used for DNA extraction and subsequent TRFLP analysis. Total N (%)=Total Nitrogen content, Total C (%)=Total Carbon content.

Soil ID	pH	Total N (%)	Total C (%)	USDA texture by Feel	%Clay
1	8.74	0.011	10.35	Sandy Loam	8
2	8.27	0.116	1.244	Sandy Loam	18
3	8.08	0.157	2.502	Clay	45
4	7.97	0.334	4.948	Silty Clay	40
5	8.75	0.142	1.437	Sandy Loam	14
6	8.41	0.213	2.285	Sandy Loam	16

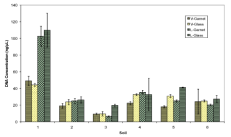

Figure 1. Extracted DNA concentrations from soils 1-6 compared by Vortex (V) and PowerLyzer™ (L) kits and by bead beating method (0.7mm garnet beads and 0.1mm glass beads). Soil 1 showed significantly more DNA per extraction with the PowerLyzer™ method.

Table 2. Summary comparison between pairs of DNA extraction methods for each soil as detailed in figures 2 and 3. Separate tables for Bacterial (B) and Fungal (F) data are presented. Vortex (V) and PowerLyzer™ (L) methods are compared with each other and by matrix used. Clear differences between methods are denoted "Y", unless there were fewer than 3 TRFLP replicates (Ym). When replicate TRFLP data between DNA extraction methods was not clearly different this was marked

B

Soil	1	2	3	4	5	6
V-Garnet vs. V-Glass	Y	N	Y	Y	Y	Y
V-Garnet vs. L-Garnet	N	Y	Y	Y	Y	Y
L-Garnet vs. L-Glass	N	Y	Y	Y	Y	Y
L-Glass vs. V-Glass	Y	N	N	N	Y	Y

F

Soil	1	2	3	4	5	6
V-Garnet vs. V-Glass	N	Y	Y	N	Y	Ym
V-Garnet vs. L-Garnet	N	N	N	Ym	Y	N
L-Garnet vs. L-Glass	N	Y	N	N	Y	Ym
L-Glass vs. V-Glass	N	N	Y	Ym	Y	N

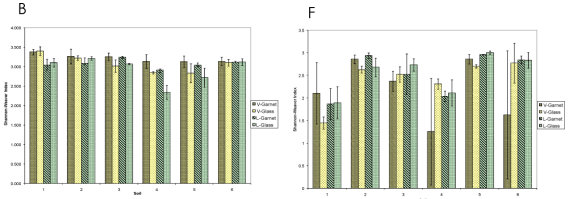

Figure 5. Shannon-Weaver Index of diversity for Bacterial (B) and Fungal (F) assemblages calculated from TRFLP patterns. Extractions of soils 1-6 were compared by Vortex (V) and PowerLyzer™ (L) extractions kits and also by bead beating methods (0.7mm garnet beads and 0.1 mm glass beads.) The Fungal plot shows large error bars on soils 4 and 6 for V-Garnet samples, because these samples were only completed in duplicate. Bacterial assemblages exhibit a decreasing diversity index corresponding to and increase in DNA isolated (e.g. compare soil 1 Shannon-Weaver with the corresponding soil 1 DNA concentrations in Figure 1). There were few differences between the methods when comparing the total number of peaks per TRFLP pattern (data not shown).

RESULTS AND DISCUSSION

The soils we examined all came from around the San Luis Obispo, CA area (Table 1) and covered a range of organic content (1.2% – 10%), pH (5.4 – 8.1) and particle size (8% – 45% clay). Traditionally, evaluations of DNA extraction methods are based on DNA yield. In most soils we tested there was little obvious difference in DNA yields between extraction methods (Figure 1). The clear exception was soil 1, the highest organic content soil, where considerably more DNA was extracted with the PowerLyzer™ compared to vortex methods, regardless of matrix. Careful examination also reveals a trend to increased DNA yield with the PowerLyzer™ and/or glass beads across most soil types. All methods appeared to result in minimal amounts of DNA shearing (Figure 2, center)

TRFLP analysis was used to evaluate the apparent bacterial and fungal assemblages represented by the extracted DNA. If the extraction methods were equivalent then the TRFLP patterns should not differ more than the variation present in the method. We compared TRFLP patterns by cluster analysis of Bray-Curtis similarity for each soil (Figures 2 and 3). TRFLP data from replicate extracts generally clustered together, indicating our methods were reproducible. To clarify evaluations of extraction methods we assessed pair-wise comparisons of extraction methods to determine differences (Table 2). When all three replicate extractions clustered separately between the methods compared we marked this as representing a difference between methods (Y). If separate clusters were not observed we marked the methods as not making a difference (N). Two sets of fungal TRFLP data were incomplete so there was some question as to differences in apparent assemblage structure (Ym).

Different extraction methods commonly resulted in different apparent bacterial assemblages, indicating that most soils harbor bacteria with different DNA extraction efficiencies. Conversely, fewer extraction methods resulted in different apparent fungal assemblages. When all soil types and extraction methods were analyzed together, bacterial and fungal TRFLP data clustered primarily by soil type (Figure 4), indicating that differences in microbial assemblage structure between soils are larger than those created by the choice of a DNA extraction method. Notable exceptions were observed in bacterial assemblages, where two separate groups were observed in each of the soil types 1, 2 and 4. In soil types 1 and 2, the vortex method with garnet appeared to be responsible for this separation while soil 4 was split based upon the matrix alone (glass vs. garnet).

The apparent microbial diversity seen in each extraction was analyzed using the Shannon-Weaver index (Figure 5). Differences in Shannon-Weaver diversity between DNA extraction methods were apparent for some soils and depended on the soil type and microbial assemblage investigated. Correlating with increased DNA yield, decreases in Shannon-Weaver diversity were most common when the PowerLyzer™ and glass bead method was employed to the bacterial assemblages (Figure 5A). This may be explained by a mechanism whereby the more efficient DNA extraction method results in more DNA from dominant populations of difficult to lyse soil bacteria (e.g. spore formers), resulting in a more skewed species distribution. Conversely, DNA from more easily lysed organisms (e.g. Gram negative bacteria) may be degraded by harsher extraction procedures, causing these organisms to lose representation in the TRFLP pattern. In contrast, the PowerLyzer™ tended to increase diversity in fungal assemblages (Figure 5B) which may be a result of overall increases in DNA that could allow for detection of under represented species. To relate higher DNA yield more directly to microbial diversity, an analysis of specific TRF peak differences and a comparison of DNA sequences would be required.

CONCLUSIONS

- The PowerLyzer® with glass bead matrix method resulted in a higher DNA yield for most soils tested, especially the high organic content soil.
- The PowerLyzer® with glass bead matrix method resulted in a lower apparent diversity of bacteria and higher apparent diversity of fungi for most soils tested, especially the high organic content soil.
- Choice of DNA extraction method altered the apparent bacterial assemblage structure (assessed via TRFLP) in most soils tested.
- Choice of DNA extraction method altered the apparent fungal assemblage structure (assessed via TRFLP) in some soils but not in others.
- In most cases the choice of DNA extraction method did not affect the detection of differences in microbial assemblage structure between soils.
- The vortex based DNA extraction methods produced higher amounts of variation in apparent microbial assemblage structure compared to the PowerLyzer™ methods.

Acknowledgements

We would like to thank Alice Hamrick for her technical and logistical support, the Cal Poly EBI for materials, supplies and use of equipment, the Cal Poly UBL for supplies and financial support, and Craig Stubler for collecting and characterizing the six soils used in this study.

References

Liu, WT et al. "Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA" Appl. Environ. Microbiol. Vol 63-11: 4516-4522.