- Short communication

Real-Time Quantification and Length Polymorphism (LPM) Analysis of Bacterial and Fungal DNA from Environmental Soil Samples Extracted by Different Methods

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Quantitative estimation as well as diversity analysis of soil microbial population especially bacteria and fungi are important since these are the major groups of microbial communities.[1,2] Thus the quantitative estimation and diversity analysis of both bacteria and fungi in soil will allow us for efficient monitoring of the environmental as well as microbial processes. Culture independent method such as the extraction of DNA from natural environment has now become a useful and an attractive alternative for the study of different microbial communities in soil.[3-5] Comparative study of these extraction methods had been carried out on the basis of PCR,[6] spectroscopy, gel electrophoresis[7] and competitive PCR. However, all of these methods have certain limitations which resist the accurate estimations of biomass. PCR based quantification however has the disadvantage of relying on end-point measurement of the amount of DNA produced, which makes it difficult to deduce the initial concentration of template DNA.[8] On the other hand, without extensive purification, spectroscopic A_{260} cannot be an accurate measure for the DNA concentration. This is due to the overlap in the 260 nm absorbance range by co-extracted humic contaminants.[9] Gel electrophoresis is mainly qualitative or at best semi-quantitative approach. Competitive PCR has also the disadvantage due to the requirement of time and resource consuming post PCR analysis.[10] Thus although various suitable extraction protocols have already been established, they still suffer lack of precision as well as reliability for the quantification of the extracted DNA. In this study, real-time quantitative PCR (RTQ-PCR) was used for the comparative study of different DNA extraction methods for quantifying soil bacterial and fungal biomass in terms of target gene copy number. Length polymorphism (LPM) pattern was also observed for diversity analysis as a tool to evaluate the extraction efficiency of these methods.

Two different environmental samples woodland (WL) and grassland (GL), collected from the campus of the Yokohama National University, Japan, were chosen to extract DNA. After removing all visible roots and other materials from the soil samples, they were sieved (2-mm mesh) and then stored at -20ºC. DNA extraction was carried out by three different protocols: bead mill homogenization (BB),[9] grinding in the presence of liquid nitrogen (LN)[11] and hot

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detergent sodium dodecyl sulfate (SDS) based enzymatic lysis combined with bead beating (ENZ-BB).[7] However for all the methods tested, purification was carried out using spin column packed with Polyvinyl polipyrrolidone (PVPP) and Sepharose-4B to remove humic acids[9] and low molecular weight RNA[12] from the extracted DNA. 16S [27f (5'AGAGTTTGATCMTGGCTCAG-3') and 519r (5'-GWATTACCGCGGCKGCTG-3')] and 18S [NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS2(5'-GCTGCTGGCACCAGACTTGC-3')] rDNA specific universal bacterial (B) and fungal primers (F) were used respectively to quantify the target bacterial and fungal DNA by LightCycler™ PCR and detection system (Roche Diagnostic, Mannheim, Germany). For LPM analysis, to obtain a high resolution (1~2 bp) in chain length, pre-cast Spreadex® EL 1200 Mini Gel (Elchrom Scientific, Switzerland) was used.

Bead beating method resulted in highest target DNA copy number both for bacteria (5.38×10^9 and 4.01×10^8 copies/µl for woodland and grassland samples, respectively) and fungi (6.16×10^8 and 2.7×10^8 copies/µl for woodland and grassland samples, respectively) as shown in Figure 1 and 2. Liquid nitrogen method showed more or less comparable values with bead beating method, however, enzymatic lysis combined with bead beating resulted,

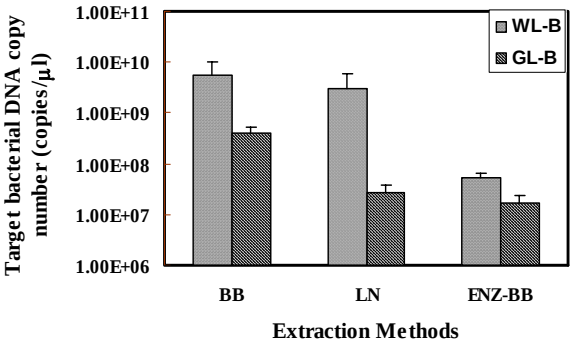

Figure 1. Quantitative estimation of bacterial target DNA copy number in woodland and grassland soil extracted by different extraction methods.

in lowest bacterial and fungal target DNA copy number in both woodland and grassland samples. In enzymatic lysis combined with bead beating method simultaneous involvement of mechanical force and enzymatic activity might release i) nuclease during the earlier steps or ii) might reinforce the interaction between DNA and soil particles probably by modifying soil structure which might thereby enhance adsorption of DNA to soil particles.

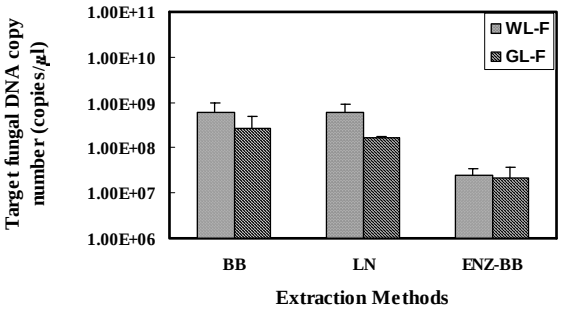

Figure 2. Quantitative estimation of fungal target DNA copy number in woodland and grassland soil extracted by different extraction methods.

Some differences were also observed among these three methods when the LPM patterns of amplified 16S rDNA genes were considered as shown in Figure 3 and 4. For woodland soil, all the methods showed bands around 540, 533, 524, 504 and 478 bp. A band just above 478 bp was observed in case of bead beating and liquid nitrogen method with a slightly higher intensity of bands in case of bead beating method (lane 2 in Fig. 3). For grassland soil, bead beating showed a somewhat strong band at 540 bp (lane 2 in Fig. 4) while this band in DNA samples extracted by the two other methods could not

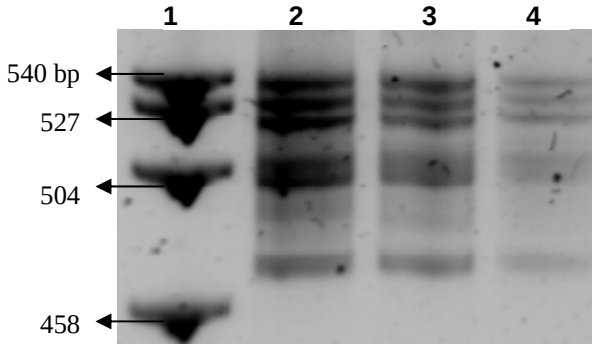

Figure 3. LPM pattern of woodland soil bacterial DNA amplified by 16S rDNA specific universal bacterial primers. Lanes: 1, M3 marker; 2, 3, 4, soil DNA extracted by BB, LN and ENZ-BB methods, respectively.

be clearly detected. Such variations in the intensity of the bands obtained for the grassland soil using different extraction methods suggests a preferential

DNA recovery or preferential DNA amplification by bead beating method. However, unlike the LPM pattern of soil bacterial DNA amplified by universal 16S rDNA primers, soil fungal DNA using 18S rDNA primer did not show any significant LPM pattern on high resolution gel electrophoresis suggesting that LPM analysis using 18S rDNA may not be suitable for diversity analysis. However, on the basis of high values obtained by RTQ-PCR both for bacteria and fungi in the present study, the bead beating method is expected to be appropriate for different type of environmental samples having a wide variety of microbial biomass.

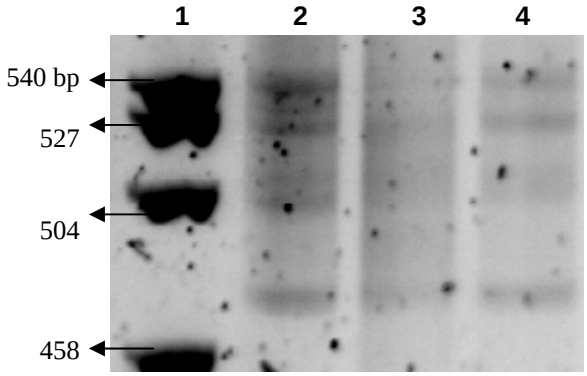

Figure 4. LPM pattern of grassland soil bacterial DNA amplified by 16S rDNA specific universal bacterial primers. Lanes: 1, M3 marker, 2, 3, 4, soil DNA extracted by BB, LN and ENZ-BB methods, respectively.

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