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An improved method for single step purification of metagenomic DNA

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Abstract An improved method for purification of intact metagenomic DNA from soil has been developed using Q-Sepharose, which purified the DNA from phenolic and humic acid contaminants in a single step. The entire procedure for purification took only 45 min. A total of 81% of DNA was recovered after purification and there was 84% reduction in humic acid contents. The purified DNA was readily digested with restriction enzymes and can be further used for molecular applications.

Keywords Metagenomic DNA · Humic acid · Phenolic compounds · DNA purification

Introduction

The microbial diversity presents an enormous and largely untapped genetic and biological pool that can be exploited for the recovery of novel genes, entire metabolic pathways and their products. In the field of biotechnology, new and improved technologies are needed to increase the speed and efficiency of discovering new molecules especially from microorganisms and to develop microbial natural products. The environmental samples from sediment soil and particularly of unusual ecosystems are important sole sources of enzymes or other related biotechnological products. Previously, these were isolated from microorganisms through conventional cultivational methods and screening of mutants, but these standard media cultivation methods can cultivate only 0.001–0.1% of the total population of bacteria in natural habitats [1–2]. Therefore, methods to extract and purify DNA from soil can play an important role. Many methods for isolation and purification of DNA from soils as well as sediments have been reported [3–6]. Extraction of total metagenomic DNA is necessarily a compromise between the vigorous extraction required for the representation of all microbial genomes and the minimization of DNA shearing. The co-extraction of inhibiting contents like polyphenols and humic acids are also a major problem because these compounds hinder the molecular biological manipulation like DNA digestion, ligation and polymerase activity [7–9].

The present study was undertaken to develop simple quick and efficient protocol for the purification of high quality unsheared chromosomal DNA from soil sample.

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Materials and methods

Extraction of metagenomic DNA

Soil samples were collected from hot springs in Himachal Pradesh, India. DNA from soil was extracted by the method of Zhou et al. (1996). Sieved fine soil (0.5 g) was extracted with 1.3 ml of extraction buffer (100 mM Tris Cl, pH 8.0, 100 mM EDTA, pH 8.0, 1.5 M NaCl, 100 mM sodium phosphate, pH 8.0, 1% CTAB). After proper mixing, 13 μl of proteinase K (10 mg/ml) was added. All eppendorf tubes were incubated horizontally at 37°C with shaking for 45 min, after that 160 μl of 20% SDS was added and vortexed for 30 sec with further incubation at 60°C for 2 hours. The sample in each eppendorf was mixed thoroughly after every 15 min interval. The samples were centrifuged at 5000 × *g* for 10 min. The supernatant was transferred into new eppendorf tubes. The remaining soil

pellets were treated three times with 400 µl of extraction buffer, 60 µl of SDS (20%) and kept at 60°C for 15 min with intermittent shaking after every 5 min. The supernatants collected from all four extractions was mixed with equal quantity of chloroform and isoamyl alcohol (24:1) and extracted three to four times. Aqueous layer was separated and precipitated with 0.6 volume of isopropanol. After centrifugation at 12,000 × *g* for 15 min, the brown pellet was washed with 70% ethanol, dried at room temperature and was dissolved in TE (10 mM Tris Cl, 1 mM EDTA, pH 8.0). DNA concentration was estimated spectrophotometrically. DNA was analyzed for restriction enzyme digestion analysis by Sau3A1 for 15 min.

Purification of DNA

Q-Sepharose (Sigma) was washed and equilibrated with 10 mM potassium phosphate buffer (pH 7.2). Finally, approximately 500 µl of solid washed beads were suspended in 1.5 ml of 10 mM potassium phosphate buffer. After mixing thoroughly the aliquots of 300 µl of Q-Sepharose in buffer were transferred in 5 eppendorf tubes and centrifuged for 1 min to separate the overlaying buffer. Different concentrations of chromosomal DNA preparation (100–500 µg) in 1 ml TE buffer were added in the eppendorf tubes containing Q-Sepharose beads and mixed properly by inverting the tubes up and down slowly for 15 min and were kept at room temperature for 5 min. The brown color in the chromosomal preparation bound instantly to the Q-Sepharose. The preparations were centrifuged at 1000 × *g* for 1 min. Supernatant containing DNA was saved for spectrophotometric analysis as well as for restriction digestion analysis of metagenomic DNA with Sau3A1as above.

Results and discussion

The humic acid materials in soil have similar charge characteristics as of DNA resulting in their co-purification evident by the extraction being brown in colour. Humic acid content also interferes in DNA quantification since they exhibit absorbance both at 230 nm and 260 nm, the later used to quantitate DNA. We have developed an improved method for purification of metagenomic DNA after which intact unsheared DNA was obtained that could be easily digested with restriction enzymes followed by ligation for metagenomic library construction. Previously, many methods for purification of metgenomic DNA have been reported, which are either expensive or taken longer time for purification [10–14]. Purification of metagenomic DNA using Sephadex G-200 columns was carried out [15], but the recovery of DNA was less. No DNA was recovered in the flow through and after elution with TE buffer containing high salt (10 mM Tris Cl, 1 mM EDTA, 100 mM NaCl, pH 8.0), only 65–73% of DNA was recovered which was sheared. An additional step for salt removal is required after this purification. Polyvinyl polypyrolidone (PVPP) was also used for reduction of humic acid as reported earlier [17–18]. Both these methods (PVPP and Sephadex G-200) took longer for purification and purity of DNA was also not satisfactory as evaluated by calculating the % recovery and % reduction in humic acid (Table 1). Present improved method can purify DNA in 45 min without running any column chromatographic step. With lower concentration of DNA (100 µg), the recovery of chromosomal DNA was nearly 30–34% of the initial DNA concentration used for DNA purification, while the increase in concentration of DNA to Q Sepharose ratio resulted in enhanced recovery of DNA. At higher DNA concentration (300–500 µg), most of the polyphenols, humic acid and small fragments of DNA bound to the Q-Sepharose immediately, whereas high molecular weight DNA remained unbound and was separated. A total of 81% of chromosomal DNA was recovered when 300 µg of DNA was used for purification as calculated spectrophotometrically (Table 1). The concentration of DNA before and after purification was 1.94 µg/µl and 1.37 µg/µl respectively. To evaluate the purity of extracted DNA, absorbance ratio at $A_{260/230}$ (DNA/humic acid) and $A_{260/280}$ for (DNA/protein) were determined (Table 1). Total reduction in humic acid was nearly 84% using Q–Sepharose according to absorbance measured at 230 nm before DNA purification (0.912) and after purification (0.143). Thus DNA was sufficiently purified using Q-Sepharose ($A_{260/230 =}$ 1.65) as high absorbance ratio (A260/230 > 1.2) is indicative of pure DNA. Similarly high absorbance ratio ($A_{260/280}$ > 1.7) is an indicative of pure DNA [16]. For all the estimations mean values of triplicates were calculated. Purified DNA was digested easily while non-purified DNA was not digested (Fig. 1), therefore indicating that humic acids present in non-purified metagenomic DNA hindered the restriction digestion of DNA, which was digested easily

Table 1 Efficacy of DNA purification as evaluated by % DNA recovery and % reduction in humic acid

Purification method	% DNA recovery[a]	% Reduction in humic acid[a]	OD 260/230	OD 260/280
No purification	100	0	0.32	0.82
Q-Sepharose	81	84	1.65	1.86
Sephadex G-200	73	65	0.67	1.62
PVPP	67	70	0.69	1.75

[a] All the experiments were carried out in triplicates and the values of standard deviation were in range of 2.2–9.7%

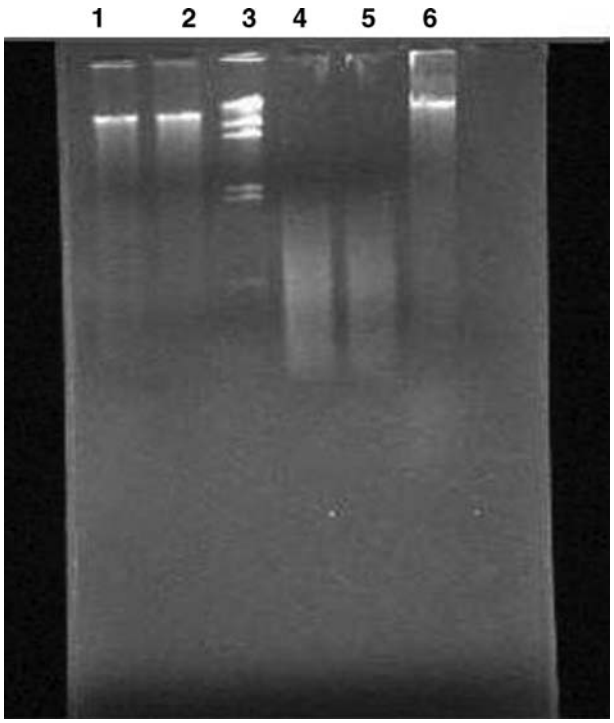

Fig. 1 Restriction fragment analysis of chromosomal DNA on 0.8% agarose gel. Lane 1: Intact undigested metagenomic DNA before purification, Lane 2: Metagenomic DNA (unpurified, containing humic acid) subjected to Sau 3A1 digestion, Lane 3: λ Hind III digest (molecular marker), Lane 4–5: Metagenomic DNA (Q-Sepharose purified) subjected to Sau 3A1 digestion, Lane 6: Intact undigested metagenomic DNA after Q-Sepharose purification

after purification by this method. Purified and restriction enzyme digested DNA could be ligated easily.

In conclusion, we report a quick modified method for purification of intact and good quality of metagenomic DNA from soil samples with one-step addition to the method of Zhou et al (1996).

References

1. Ward, D. M., Bateson, M. M., Weller, R., & Ruff-Roberts, A. L. (1992). Ribosomal RNA analysis of microorganism as occur in nature. *Advances in Microbial Ecology, 12*, 219–286.
2. Pace, N. R., Angert, E. R., Delong, E. F., Schmidt, T. M., & Wickham, O. S. (1993). New perspective on the natural microbial world. In: R. H. Baltz, B. D. Hegeman, & P. L. Skatrud (Eds.), *Industrial microorganisms: Basic and applied molecular genetics* (pp. 77–84). Washington: ASM.
3. More, M. I., Herrick, J. B., Silva, M. C., Ghiorse, W. C., & Madsen, E. L. (1994). Quantitative cell lysis of indigenous microorganisms and rapid extraction of DNA from sediments. *Applied and Environmental Microbiology, 60*, 1572–1580.
4. Clegg, C. D., Ritz, K., & Griffith, B. S. (1997). Direct isolation of microbial community DNA from humified upland soils. *Applied Microbiology, 25*, 30–33.
5. Luna, M. G., Anno, D. A., & Danovaro, R. (2006). DNA extraction procedure: A critical issue for bacterial diversity assessment in marine sediments. *Environmental Microbiology, 8*, 308–320.
6. Niemi, M. R., Heiskanen, I., Wallenius, K., & Lindström, K. (2001). Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. *Journal of Microbiological Methods, 45*, 155–165.
7. Holben, W. E., Jansson, J. K., Chelm, B. K., & Tidje, J. M. (1988). DNA probe method for the detection of specific microorganisms in the soil community. *Applied and Environmental Microbiology, 54*, 703–711.
8. Steffan, R. J., & Atlas, R. M. (1998). DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. *Applied and Environmental Microbiology, 54*, 2185–2191.
9. Ranjan, R., Grover, A., Kapardar, R. K., & Sharma, R. (2005). Isolation of novel lipolytic genes from uncultured bacteria of pond water. *Biochemical and Biophysical Research Communications, 335*, 57–65.
10. Bertrand, H., Poly, F., Van, T. V., Lombard, N., Nalin, R., Vogel, T. M., & Simonet, P. (2005). High molecular weight DNA recovery from soils prerequisite for biotechnological metagenomic library construction. *Journal of Microbiological Methods, 62*, 1–11.
11. Yeates, C., Gilling, M. R., Davison, A. D., Altaviilla, N., & Veal, D. A. (1998). Methods for microbial DNA extraction from soil for PCR amplification. *Biological Procedures Online, 1*, 40–47.
12. Roose-Amsaleg, C. L., Garnier-Sillam, E., & Harry, M. (2001). Extraction and purification of microbial DNA from soil and sediment samples. *Applied Soil Ecology, 18*, 47–60.
13. Santosa, D. A. (2001). Rapid extraction and purification of environmental DNA for molecular cloning application and molecular diversity studies. *Molecular Biotechnology, 17*, 59–64.
14. Lakay, F. M., Botha, A., & Prior, B. A. (2006). Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. *Journal of Applied Microbiology, 100*, 1365–1373.
15. Miller, D. N., Bryant, J. E., Madsen, E. L., & Ghiorse, W. C. (1999). Evaluation and optimization of DNA extraction and purification procedure for soil and sediments sample. *Applied and Environmental Microbiology, 65*, 4715–4724.
16. Zhou, J., Bruns, M. A., & Tiedje, J. M. (1996). DNA recovery from soils of diverse composition. *Applied and Environmental Microbiology, 62*, 316–322.
17. Wise, M. G., Mcarthur, J. V., & Shimkets L. J. (1997). Bacterial diversity of Carolina Bay as determined by 16S rRNA gene analysis: Confirmation of Novel Taxa. *Applied and Environmental Microbiology, 63*, 1505–1514.
18. Holben, W. E, Jansson, J. K., Chelm B. K., & Tiedje, J. M. (1988). DNA probe method for the detection of specific microorganisms in the soil bacterial community. *Applied and Environmental Microbiology, 54*, 703–711.