Efficient direct purification method for high-quality DNA from soil

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ABSTRACT

Successful detection and characterization of microbacterial DNA in the environment require efficient extraction of the DNA from environmental samples and adequate purification from the coextracted contaminants that inhibit PCR. Previously reported methods for direct isolation and purification of DNA from microorganisms in soil have multiple steps, laboratory equipment's and labour-intensive, and result in loss of extracted material. This has promoted the initiative to develop an efficient, nonselective method for direct DNA extraction from the environment that is required for rapid and thorough analysis of introduced microorganisms in environmental samples and for diversity analysis of microbial community in soil. The newly developed method is based on the combination of hot-detergent treatment and commercial spin columns. Fidelity and effectiveness of the procedure were investigated by identifying several phylogenetic groups representing different components of the soil microbial community. In addition, the use of HotStar Taq DNA polymerase has provided the opportunity for simultaneous detection of a variety of microorganisms in a single tube, using multiplex PCR. The ability to simultaneously extract and amplify DNA from many types of microorganisms using a simple extraction and purification step is desirable for many applications in molecular microbial ecology.

Key words: Direct purification, soil DNA.

INTRODUCTION

It is one of the fundamental dilemmas in microbial ecology that only a fraction (0.01 to 10%) of bacteria known to occur in natural habitats can be cultivated on laboratory media (Wilson, 1997). In other words, studies on genetic composition and ecology of native bacterial populations have been considerably constrained by their dependence on culture-based methods. Environmental microbiologists have, therefore, initiated their efforts to explore the use of molecular methods to circumvent the bias of culture-dependent technique (Wilson,

1997). The direct extraction of total DNA from environmental samples has made it possible to investigate the diversity of microbial communities without prior cultivation (Miller, *et al.*, 1999; Ogram *et al.*, 1987).

PCR analysis provides a sensitive and specific means to detect and monitor microorganisms in complex environmental samples (Miller, 2001). Successful detection and characterization of microbacterial DNA in the environment require efficient extraction of the DNA from environmental samples and adequate purification from the coextracted contaminants that inhibit PCR. Soils and sediments vary greatly in chemical and organic composition. In addition, they contain abundant humic and fulvic acids that are inhibitory to Taq DNA polymerase and other enzymes (Miller, 2001). Soils are, therefore, one of the most challenging environmental matrices to obtain microbial DNA that will support PCR analysis (Miller, 2001).

Previously reported methods for direct isolation and purification of DNA from microorganisms in soil (Kuske *et al.*, 1998; Erb and Wagner-Dobler, 1993; Porteus *et al.*, 1993), have multiple steps that need laboratory equipment's and are labour-intensive, which result in loss of extracted material (Miller *et al.*, 1999). In addition, they use chemicals such as phenol, chloroform, guanidinium hydrochloride, cesium chloride and butanol that require special storage, handling, and disposal (Miller, 2001).

The current report describes a newly developed method for efficient direct purification of high-quality DNA from soil. The combination of hot-detergent treatment and the spin columns (Qiagen, Germany) conferred successful elimination of Taq polymerase inhibitors. Furthermore, it reduces the time required for sample purification to less than 30 min, compared with the standard multiple steps, labour-intensive, direct DNA extraction methods in soil. In addition, commercial spin columns use no toxic chemicals and avoids sample mix-up due to tube transfer. Finally, the use of HotStar Taq DNA polymerase has provided the opportunity for simultaneous detection of a variety of microorganisms in a single tube, using multiplex PCR.

MATERIALS AND METHODS

Site and Sample Description

Soil samples were recovered between May 1999 and June 2001 from an industrial drain in the Nile Delta named the "Defsho drain". Defsho is located at the city of Kafr El-Dawwar, 20 Km south of Alexandria, and it flows northwards until it meets the Mediterranean Sea through Abu-Kier bay. The first company dumping in Defsho is an artificial silk company, followed immediately by a factory for textile dyes and pesticides. As Defsho heads north, it accumulates wastes from various industries that represent most of the pollutant repertoire. Industrial wastes from the outlet of the dye and pesticides companies have contaminated nearby soil, surface water and water with heavy metals and Polycyclic Aromatic Hydrocarbons (PAHs) compounds and have threatened the Defsho drain as well as local ground water supplies. Each soil sample was collected and individually sealed in a sterile plastic sample bags, placed on ice, and immediately taken to the Lab. at Research Department, GEBRI, Alexandria. Then, direct DNA extraction was immediately initiated. Additional soil samples were stored at 4°C.

Direct Soil DNA Extraction

Hot-detergent method (Ogram *et al.*, 1987) was used for direct DNA extraction from soil samples. Soil 0.5 g was added to 0.5 ml of 2x TENS buffer (1x TENS is 50 mM Tris-HCl pH 8.0, 20 mM EDTA, 100 mM

NaCl, 1% (w/v) sodium dodecyl sulfate). Samples were vortexed briefly, and incubated at 70°C for 10 min. They were then centrifuged at 12,000 x*g* for 10 min at room temperature. Finally, the supernatant, containing DNA, was subjected to ethanol precipitation.

Modified Method for Soil DNA Purification

The following modified method is based on the combination of the hot detergent method (Ogram *et al.,* 1987) and the use QIAamp spin columns (Qiagen, Germany). It is noteworthy that all columns and buffers, described herein, are provided with the aforementioned kit. The hot-detergent method was carried out and the supernatant, which contains soil DNA, was collected and loaded onto QIAamp spin columns rather than performing ethanol precipitation as described by the method (Ogram *et al.,* 1987). Five hundred micro liters of buffer AW1, provided with the kit, were added to the QIAamp spin column, closed, centrifuged for 1 min, and placed in a clean 2ml collection tube. The tube containing the filtrate was discarded. This step was repeated with AW2 buffer. DNA was finally eluted with the addition of 50 µl of nuclease-free H_2O.

PCR Amplification of Soil DNA

Purified soil DNA was tested by PCR amplification of rDNA gene fragments of several phylogenetic groups representing different components of the soil microbial community. These groups include members with a broad range of microbial cell size, composition, and growth habitats (Table 1).

For the detection of Polycyclic Aromatic Hydrocarbons (PAHs) degrading native genes, primers specific for *nahAc* gene were employed (Table 2). The *nahAc* gene encodes the large subunit of the iron-sulfur component of naphthalene dioxygenase, the initial enzyme in the naphthalene catabolic pathway (Herrick *et al.,* 1993).

DNA amplifications were carried in an ABI GeneAmp PCR system 9700 cycler with a denaturing step at 95°C for 5 min and the step cycle program set for 45 cycles (with a cycle consisting of denaturing 94°C for 30s, annealing at 55°C for 30s and extension step at 72°C for 30s), followed by a final extension step at 72°C for 10 min.

Table (1): PCR product sizes used to detect phylogenetic groups of native microorganisms and plant material in Defsho soil.

Organism group	Primer sequences (5`-3`)	Reference	Amplified product (bp)
Bacteria	AGAGTTTGATCCTGGCTCAG TGCGGCTGGATCACCTCCTT	Wilson (1997)	1492
Bacillus	ATTAGATACCTGGTAGTCC TACCTTGTTACGACTTTA	Wilson (1997)	723
Plants	AACTGGAGGAAGTGTGGG AGGAGGTGATCCAACCGC	Wilson (1997)	315
Fungi	GACCCTATGCAGCTTCTACT TTATCCCTAGCGTAACTTTAT	Wilson (1997)	735

Table (2): PCR primers used to detect nahAc gene in Defsho soil.

Primer sequences (5`-3`)	Reference	Amplified product (bp)
GTTTGCAGCTATCACGGCTG TTCGAACAAATGGTGGTAGC	Herrick *et al.* (1993)	520

RESULTS AND DISCUSSION

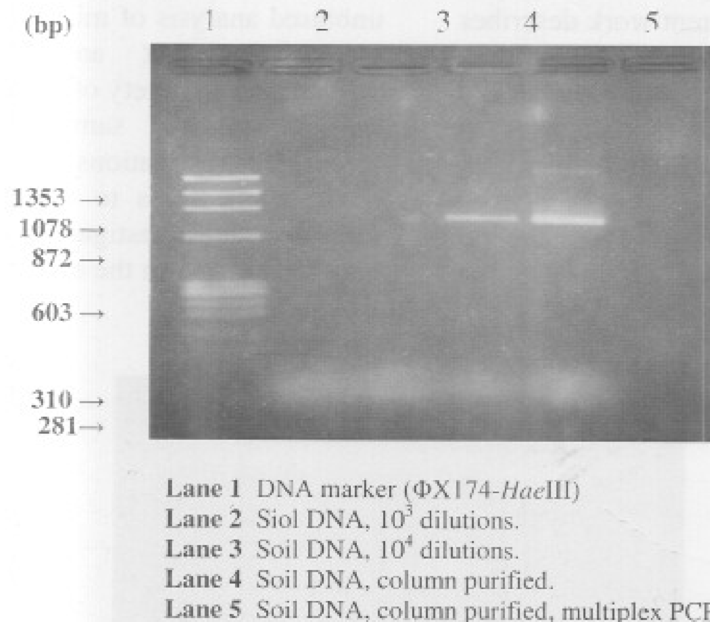
Soil DNA was prepared using hot-detergent method (see materials and methods). The purified soil DNA was then tested by PCR amplification of rDNA gene fragments of several phylogenetic groups representing different components of the soil microbial community (Fig. 1). No amplified products were detected suggesting that the soil DNA contains impurities that inhibit *Taq* polymerase. This has promoted the initiative to dilute these samples, hoping to dilute the impurities to a level of non-inhibitory to *Taq* polymerase. The fact that even with one thousand time dilutions, no amplified products were detected (Fig. 1, Lanes: 2 & 3), suggesting that these impurities are present in high concentrations. In addition, parameters of the PCR and primer concentrations were modified without success. These negative results were not surprising in the light of previous reports, that materials native to the sediments, such as humic substances and mineral constituents could be inhibitory to *Taq* polymerase of extracted DNA (Miller, 2001).

DNA was then purified using the modified method which is based on the combination of the hot detergent-method and QIAamp spin columns. The detection of amplified product, similar to the expected size of Bacillus (Table 1 and Fig. 1, Lane 4) suggests that the use of these columns has successfully eliminated Taq polymerase inhibitors. This suggests that commercial columns are effective in purifying the extracted soil DNA from other contaminants that coextracted with DNA from soil without the need for further purification. This suggestion is further supported by the fact that QIAamp purified soil DNA does not require dilution to bring the impurities to the non-inhibitory level of *Taq* polymerase. The newly developed method reduces the time required for sample extraction and purification to less than 30 min, compared with the standard multiple steps and labour-intensive direct DNA extraction methods in soil. Furthermore, these spin columns use no toxic chemicals and avoids sample mix-up due to tube transfer.

The use of HotStarTaq DNA polymerase has provided the opportunity for simultaneous detection of a variety of microorganisms in a single tube, using multiplex PCR (Fig. 1, Lane 5). The detection of two amplified products of approximately 1492 and 723 bp, corresponding to Bacteria and Bacillus respectively, suggests that the newly developed method allows for simultaneous detection of several phylogenetic groups in a single reaction. In addition, the fact that the full-length of the 16S rDNA gene, 1492 bp, can be amplified from soil DNA demonstrates the integrity and excellent quality of purified soil DNA.

The direct extraction of total DNA from environmental samples has made it possible to investigate the diversity of microbial communities without prior cultivation. In this regard, PCR detection of several microbial groups native to soils was investigated with DNA extracted from the Defsho soil (Fig. 2).

All target microbial groups (Table 1) were easily detected in standard (Fig. 2) as well as multiplex PCR assays (Fig. 1). Amplified products of approximately 1492, 723 and 315 bp, respectively, were detected (Fig. 2, Lanes: 2, 3, 4). These amplified products are consistent with rDNA of Bacteria, Bacillus and plants (Wilson, 1997). On the other hand, the product specific for fungi was not detected (Fig. 2, Lane 5). This is in agreement with previous results (Porteus *et al.*, 1993; Herrick *et al.*, 1993). The detection of several microbial communities in soil recovered from Defsho promotes future molecular characterization of these diverse microbial groups.



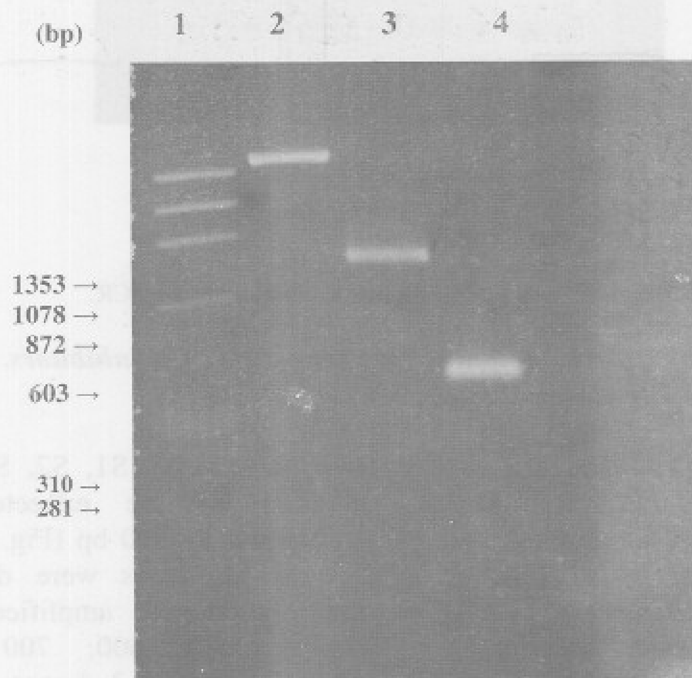
Lane 1 DNA marker (ΦX174-*Hae*III)
Lane 2 Siol DNA, 10^3 dilutions.
Lane 3 Soil DNA, 10^4 dilutions.
Lane 4 Soil DNA, column purified.
Lane 5 Soil DNA, column purified, multiplex PCR.

Fig. (1): Effect of the use of QIAamp columns for removal of PCR inhibitors. Bacterial primers were used to amplify bacterial DNA from soil.

The successful attempt to design a new improved method to purify DNA from soil, has promoted the initiative to amplify and detect PAHs catabolic genes in their native populations. For the detection of PAHs degrading native genes, primers specific for *nahAc* gene were employed. The *nahAc* gene encodes the large subunit of the iron-sulfur component of naphthalene dioxygenase, the initial enzyme in the naphthalene catabolic pathway. The above new modified method for direct DNA extraction from soil was used to amplify and detect *nahAc* from different soils taken from the Defsho site (Fig. 3). These soils were designated, S1, S2, S3, S4 and S5. In addition to the expected amplicon of approximately 520 bp (Fig. 3, Lanes 2, 4, 5), several amplicons were detected. Sizes of these additional amplified products were approximately 600, 700 and 900 bp, respectively (Fig. 3, Lanes: 2, 3, 6). The fact that the primers employed for the detection of *nahAc* are both highly specific for detection of *nahAc* and at the same time allow the detection of related, but unknown *nahAc* genes suggests that the additional amplicons represent a mixture of *nahAc* sequences amplified from a number of different native bacterial strains. In

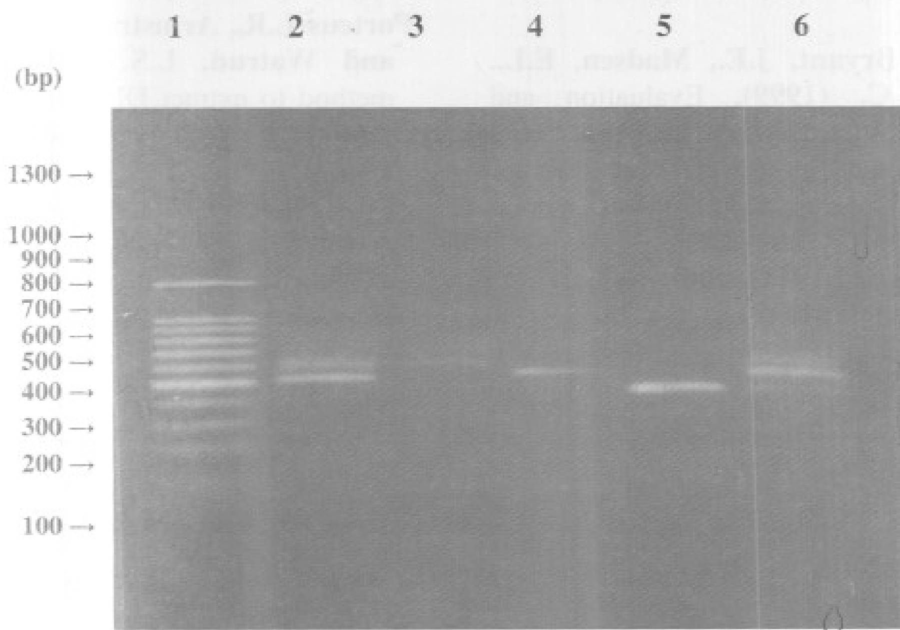
addition, PCR conditions were carried out at stringent conditions to amplify *nahAc* genes and *nahAc*-related unknown genes. The detection of *nahAc*-related genes suggests that these genes may have evolved from *nahAc* gene and/or genes that are employed in alternative pathway in naphthalene catabolism. This suggestion is supported by the previous study of Herrick *et al.* (1993).

In conclusion, the current work describes a newly developed method for direct purification of high-quality soil DNA. The newly developed method has made it possible to detect the presence of different microbial groups in soil recovered from the Defsho's site and to detect and amplify PAHs catabolic genes in their native populations. Finally, the use of HotStarTaq DNA polymerase has provided the opportunity for simultaneous detection of a variety of microorganisms in a single tube, using multiplex PCR. The ability to simultaneously extract and amplify DNA from many types of microorganisms using simple extraction and purification step is desirable for many applications in molecular microbial ecology. It is especially useful for unbiased analysis of microbial communities in the environment and for simultaneous detection of a variety of target microorganisms in environmental samples collected during forensic investigations. In addition, it has broad applications to agricultural, industrial, forensic and investigative needs to monitor microorganisms in the environment.



Lane 1 DNA marker DNA marker (ΦX174-*Hae*III).
Lane 2 16S rDNA bacterial gene.
Lane 3 16S rDNA Bacillus gene.
Lane 4 18S rDNA Plants gene.

Fig. (2): PCR amplified products of DNA purified from soil to detect microorganisms and plant materials.



Lane 1 DNA marker (100 bp ladder).
Lane 2 Soil Sample S1.
Lane 3 Soil Sample S2.
Lane 4 Soil Sample S3.
Lane 5 Soil Sample S4.
Lane 6 Soil Sample S5.

Fig. (3): PCR amplification and detection of nahAc in Defsho's soil.

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REFERENCES

Erb, R.W. and Wagner-Dobler I. (1993). Detection of polychlorinated Biphenyl degradation genes in polluted sediments by direct DNA extraction and polymerase chain reaction. Applied and Environmental Microbiology, 59: 4065-4073.

Herrick J.B., Madsen EL, Batt C.A and Ghiorse W.C. (1993). Ploymerase chain reaction of Naphthalene-catabolic and 16S rRNA gene sequence from indigenous sediment. Applied and environmental microbiology, 61:687-694.

Kuske, C.R., Banton, K.L., Adorada, D.L., Stark, P.C., Hill K.K., and Jackson, P.J. (1998). Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. Applied and Environmental Microbiology, 64: 2463-2472.

Miller D.N. (2001). Evaluation of gel filtration resins for the removal of PCR-

inhibitory substances from soils and sediments. J. of Microbiol. Methods, 44: 49-58.

Miller, D.N., Bryant, J.E., Madsen, E.L., Ghiorse, W.C. (1999). Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Applied and Environmental Microbiology, 65: 4715-4724.

Ogram, A., Sayler G.S. and Barkay, T. (1987). The extraction and purification of microbial DNA from sediments. J. Microbiol. Methods. 7: 43-51.

Porteus L.R.. Armstrong, J.L., Seider, R.J., and Watrud, L.S. (1993). An effective method to extract DNA from environmental samples for polymerase chain reaction amplification and DNA fingerprint analysis. Curr. Microbiol. 29: 301-307.

Wilson, I.G. (1997). Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63: 3741-3751.

الملخص العربي

طريقة مباشرة وفعالة لعزل وتنقية المادة الوراثية في التربة

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يعتبر التحليل الجزيئي الميكروبي للتربة من أهم التحاليل في مجال دراسة التنوع الميكروبي البيئي ، ونظرا لوجود العديد من المواد الكيماوية المثبطة لعمليه تفاعل البلمرة المتسلسل (PCR) مما يحول دون وجود دراسة شاملة للتنوع الجزيئي المكيروبي للتربة ، فإن الهدف من هذه الدراسة هو التوصل إلي طريقة مباشرة فعالة لعزل وتنقية المادة الوراثية من التربة بحيث تكون مماثلة للتنوع البيئي الجزيئي .

أوضحت الدراسة أنه باستخدام الطريقة الجديدة التي تعتمد علي التخلص من المثبطات الموجودة في التربة والتي تحول دون عمل (PCR) ، كما تم اختصار المدة الزمنية المطلوبة للعزل والتنقية إلي ثلاثين دقيقة مقارنة بالطرق الأخرى التي تستغرق أياما ، بالإضافة إلي ان الطريقة الجديدة لا تستخدم أي مواد كيماوية ، التي تستدعي وجود أماكن خاصة للحفظ والعمل والتخلص الآمن منها .

وقد تم استخدام هذه الطريقة الجديدة في تحديد التنوع الميكروبي داخل تربة ملوثة بمختلف مصانع الدباغة ، حيث تم تحديد وجود المادة الوراثية الخاصة بـ Bacillus ، والنباتات ، كما قد تم استخدام الطريقة الجديدة في عزل أحد الجينات *nahac* والذي يساعد في التخلص الحيوي من مركبات PAHS الخاصة بالمخلفات الصناعية .