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Amplification of plasmid DNA bound on soil colloidal particles and clay minerals by the polymerase chain reaction

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Abstract

Polymerase chain reaction (PCR) was used to amplify a 600-base pair (bp) sequence of plasmid pGEX-2T DNA bound on soil colloidal particles from Brown soil (Alfisol) and Red soil (Ultisol), and three different minerals (goethite, kaolinite, montmorillonite). DNA bound on soil colloids, kaolinite, and montmorillonite was not amplified when the complexes were used directly but amplification occurred when the soil colloid or kaolinite-DNA complex was diluted, 10- and 20-fold. The montmorillonite-DNA complex required at least 100-fold dilution before amplification could be detected. DNA bound on goethite was amplified irrespective of whether the complex was used directly, or diluted 10- and 20-fold. The amplification of mineral-bound plasmid DNA by PCR is, therefore, markedly influenced by the type and concentration of minerals used. This information is of fundamental importance to soil molecular microbial ecology with particular reference to monitoring the fate of genetically engineered microorganisms and their recombinant DNA in soil environments.

Key words: adsorption; amplification; mineral; PCR; plasmid DNA; soil colloid

Introduction

The persistence of recombinant DNA and its potential transfer to indigenous microorganisms have raised concerns about the deliberate or accidental release of genetically engineered microorganisms (GEMs) into the environment (Lorenz and Wackernagel, 1994; Paget and Simonet, 1994). DNA molecules, released into the soil by microorganisms, are closely associated with soil constituents such as clay minerals, sand, and humic substances. Being partially protected against degradation by nucleases, such DNA molecules retain the capacity to transform competent bacterial cells (Khanna and Stotzky, 1992; Recorbet *et al*., 1993; Gallori *et al*., 1994; Blum *et al*., 1997; Crecchio and Stotzky, 1998; Stotzky, 2000; Cai *et al*., 2006). Gallori *et al*. (1994), for example, have observed that the transforming ability of chromosomal and plasmid DNA bound on Ca-montmorillonite could persist for 15 d in non-sterile soils under moist conditions. The extraction of DNA from the soil, and its amplification, have thus become an essential part of the methodology used in modern soil microbial ecology.

The increased introduction of GEMs into the environment has stimulated the focus of research into the development of sensitive methods for detecting specific genetically defined microorganisms within the complex microbial communities of natural ecosystems (Ford and Olson, 1988; Cullen and Hirsch, 1998). The most widely used technique is the amplification of specific sequences of DNA by the polymerase chain reaction (PCR). This method has been successfully applied for detecting microorganisms that are difficult to culture *in vitro* as well as tracking the fate of GEMs and particular genes that disseminate by transfer to indigenous microbes (Jain *et al*., 1988; Picard *et al*., 1992; Trevors, 1992). Using PCR and electroporation of *Escherichia coli*, Romanowski *et al*. (1993) were able to monitor extracellular plasmid DNA for up to 60 d after introduction into three different soils.

The PCR method has also been used to amplify chromosomal DNA bound on clay minerals. Alvarez *et al*. (1998), for example, have successfully amplified chromosomal DNA from *Bacillus subtilis* and calf thymus bound on montmorillonite but failed to do so when the DNA was attached to kaolinite. On the other hand, Vettori *et al*. (1996) found that montmorillonite was more efficient than kaolinite in inhibiting the amplification of bound DNA. However, the amplification of plasmid DNA bound on soil particles and oxides, and the factors controlling the process, have not been previously reported. Here we used the PCR method to amplify plasmid DNA that is bound on the fine clay fractions (colloid) of an Alfisol and an Ultisol, and on three common soil minerals including montmorillonite, kaolinite, and goethite.

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1 Materials and methods

1.1 Bacteria, media, and plasmid DNA

Plasmid pGEX-2T (GST gene fusion vector; ColE1 origin; Ap^r; *lacI*^{q}; *tac* promoter; 4.9 kb) (Smith and Johnson, 1988) was provided by D. B. Wilson (Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York). Cultures of *E. coli* TG1 containing plasmid pGEX-2T were grown overnight at 37°C in a Luria-Bertani (LB) medium (containing 10 g bacto-tryptone, 5 g bacto-yeast extract, and 10 g NaCl per liter). Plasmid DNA was prepared from *E. coli* TG1 according to the method described by Birnboim and Doly (1979).

1.2 Soil colloids and minerals

Samples of Brown soil (Alfisol) were taken from the 0–17 cm layer of a forested land in Tianwai Village, Taishan, Shandong Province, China, whereas those of red soil (Ultisol) were collected from the 11–40 cm layer of a cultivated land in Xianning, Hubei Province, China. Some pertinent properties of the Brown and Red soils, respectively, are pH (H_2O) 6.3 and 5.2, organic matter 42.4 and 7.2 g/kg, and cation exchange capacity 15.3 and 7.4 cmol/kg. After removing undecayed organic residues, the samples were rinsed with deionized distilled water (ddH_2O), and dispersed by adding 0.01 mol/L NaOH dropwise into pH 7–8 together with sonication. Soil colloidal components (< 2 μm) were separated by sedimentation. Part of the clays was oxidized by H_2O_2 to remove the organic matter. The clay fraction that was not treated with H_2O_2 is referred to as "organic clays", whereas the peroxide-treated fraction is denoted as "inorganic clays". After flocculation with $CaCl_2$ solution, the colloidal suspension was washed with ddH_2O and ethanol until free of chloride, and then air dried. The samples of kaolinite and montmorillonite were prepared according to the method described by Cai *et al*. (2006). Goethite was synthesized as described by Huang *et al*. (2003). The soil clay fractions and minerals were ground to pass a 100-mesh sieve (150 μm). Some of their properties are listed in Table 1.

1.3 Preparation of soil colloid- or mineral-DNA complex for amplification

Plasmid DNA (2 μg) was mixed with a 100-μl suspension (20 mg/ml) of Brown soil clay or mineral, making up a total volume of 1 ml in 10 mmol/L Tris-HCl buffer (pH 7.0). After shaking for 120 min at 25°C, the mixture was centrifuged at 20000×*g* for 20 min. The concentration of DNA in the supernatant was determined by absorbance at 260 nm. The amount adsorbed was derived from the difference between the amount of DNA initially added and that found in the supernatant. The amount of bound DNA was determined by washing the soil colloid- or mineral-DNA complex (formed after equilibrium adsorption) with 1 ml of 10 mmol/L Tris buffer (pH 7.0), centrifuging at 20000×*g* for 20 min, and measuring the absorbance of the supernatant at 260 nm. This procedure was repeated until no more DNA was detected in the supernatant. The amount of DNA bound on Brown soil colloids and minerals was calculated by subtracting the total amount of DNA in the washings from the initial amount adsorbed (before washing). The DNA complexes with Red soil colloids and minerals were prepared in 10 mmol/L NaAc-HAc buffer (pH 5.5) as described earlier.

The washed soil colloid- or mineral-DNA complexes were resuspended in 1 ml of 10 mmol/L Tris-HCl (pH 7.0) or NaAc-HAc (pH 5.5) buffer, and the bound DNA was subjected to amplification by PCR using 1 μl suspensions. Amplification was carried out on complexes before or after dilution. Soil colloid-, kaolinite-, and goethite-DNA complexes were diluted 10- and 20-fold, whereas the complex with montmorillonite was diluted 10- to 100-fold. Control experiments were carried out using free plasmid DNA and soil colloids after 10-fold dilution. No amplification was observed with soil colloids (data not shown). All amplification experiments were performed in duplicate, and each experiment was repeated thrice.

1.4 PCR amplification conditions

Amplification by PCR was performed in a total reaction volume of 25 μl in 0.2 ml polypropylene tubes using a Peltier Thermal Cycler (PTC-100, Bio-RAD Company, USA) under the following conditions: 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.001% (w/v) gelatin, 200 μmol/L deoxynucleoside triphosphates (dNTPs), 0.8 μmol/L primer, 1 μl containing different amounts of template DNA (either free or bound on soil colloid or mineral), and 1 U AmpliTaq DNA polymerase (Sangni Biological Limited Company, Shanghai, China). The primer oligonucleotides were 5′-GGCGACCATCCTCCAAAA-3′ and 5′-CGGGAGCTGCATGTGTCA-3′. After incubation at

Table 1 Selected properties of soil colloids and minerals[a]

Soil colloid or mineral	OM (g/kg)	SESA (m^2/g)	CEC (cmol/kg)	Clay mineral composition
Brown organic clay	71.0	64.4	57.6	Hydromica (55%), vermiculite (30%), kaolinite (15%)
Brown inorganic clay	9.6	77.1	47.9	Hydromica (55%), vermiculite (30%), kaolinite (15%)
Red organic clay	30.3	97.4	28.4	Hydromica (35%), 1.4 nm mineral (30%), kaolinite (35%)
Red inorganic clay	8.4	110.5	23.0	Hydromica (35%), 1.4 nm mineral (30%), kaolinite (35%)
Montmorillonite	-	73.6	90.2	Montmorillonite
Kaolinite	-	22.9	7.1	Kaolinite
Goethite	-	83.0	0	Goethite

[a] Clay mineral was determined by X-ray diffraction analysis. Organic matter (OM) was determined by digestion with potassium dichromate, and cation exchange capacity (CEC) by leaching with ammonium acetate (pH 7.0) (Xiong, 1985). The specific external surface area (SESA) was measured by N_2 adsorption at 77 K and applying the BET equation (Beijing Analytical Instrument Company).

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94°C for 90 s, the reaction mixtures were cycled 30 times through the following temperature profile: 94°C for 15 s (denaturation), 58.5°C for 30 s (annealing), and 72°C for 20 s (extension), followed by one extension step at 72°C for 4 min.

The amplification products (5 μl per lane) were analyzed by electrophoresis on 2% (w/v) agarose gels at 120 V for 30 min, after which the gels were photographed with an UVP gel scanner.

2 Results and discussion

2.1 Affinity of plasmid DNA for soil colloids and minerals

Figure 1 shows the relative capacity of the Brown soil colloids and minerals to retain plasmid DNA against washing with 10 mmol/L Tris buffer (pH 7.0). Fig.2 presents the respective curves for the Red soil colloids and minerals after washing with NaAc buffer (pH 5.5). In both cases, DNA was not released further after three washes. The percentage of DNA desorbed by Tris buffer from the organic clay of Brown soil was five times higher than that from the corresponding inorganic clay. The respective figure for montmorillonite was 62.3% which was about 15 times higher than that for kaolinite (4.9%) and goethite (4.4%) (Fig.1). As shown in Fig.2, the percentage of DNA desorbed from the Red soil colloids and minerals by NaAc buffer decreased in the following order: montmorillonite (31.5%) > organic clay (23.2%) > inorganic clay (12.2%) > kaolinite (4.9%) > goethite (2.5%). The affinity of kaolinite and goethite for plasmid DNA is clearly higher than that of soil colloids and montmorillonite. Further, the DNA molecules appear to be more tightly held by inorganic clays than by organic clays.

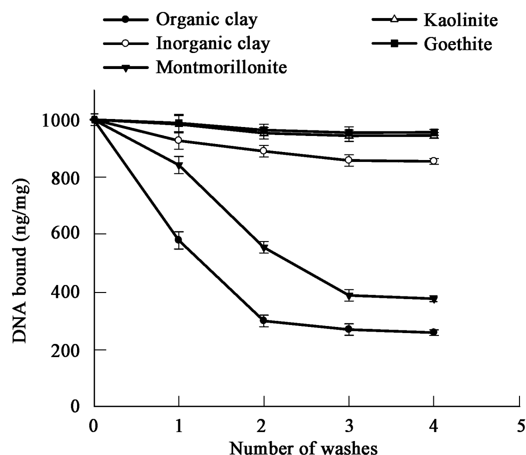

Fig. 1 Elution of plasmid DNA adsorbed on soil colloids from Brown soil and minerals with Tris buffer.

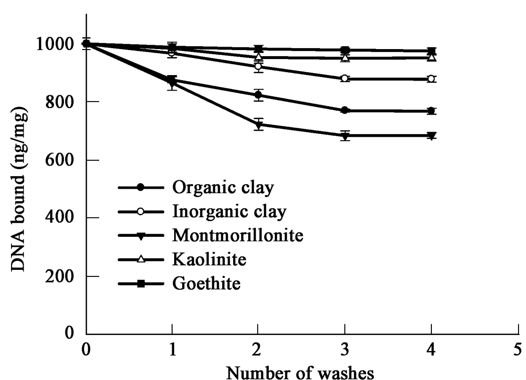

Fig. 2 Elution of plasmid DNA adsorbed on soil colloids from Red soil and minerals with NaAc buffer.

2.2 Amplification of free and bound plasmid DNA

Figures.3a and 3b show the agarose-gel electrophoresis patterns of the PCR-amplified products of free plasmid DNA and the same DNA bound on three minerals and the organic and inorganic clays from the Brown and Red soils. DNA amplification was not observed for the original (undiluted) complexes with soil colloids, montmorillonite, and kaolinite. Appreciable amplification, however, occurred after 10- or 20-fold dilution of the soil colloid- and kaolinite-DNA complexes. This suggests that the amplification process is markedly influenced by the concentration of suspended solids (here clay particles). Similarly, Tsai and Olson (1992) observed that PCR amplification of soil-extracted DNA was inhibited by co-extracted humic acids in the suspension. The lack of amplification of DNA bound on soil colloid- or mineral-DNA complexes may be ascribed to the adsorption of Taq DNA polymerase by clay particles, and the resultant reduction of its catalytic activity. As for montmorillonite-DNA complex at pH 7.0, there was no amplification even after the 100-fold dilution (Fig.3a, lane 16), although the same complex at pH 5.5 was amplified after diluting 100-fold (Fig.3b, lane 16). Similarly, Vettori *et al.* (1996) observed that a greater dilution (20-fold) was required to amplify the complex of

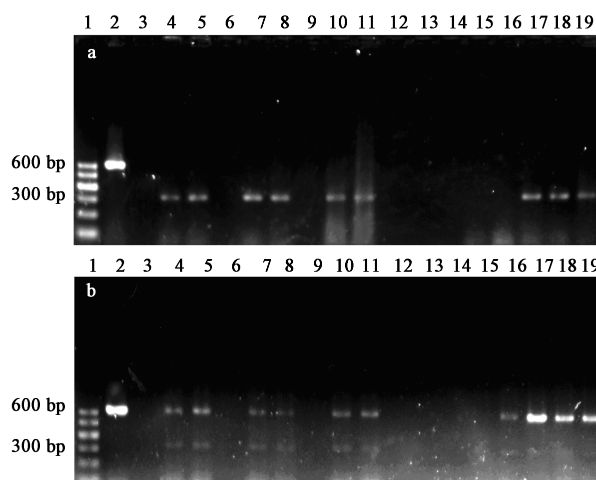

Fig. 3 PCR amplification of plasmid DNA, free and bound on soil colloids from Brown soil and minerals (a) and Red soil and minerals (b). lane 1: molecular mass marker (100 base pair (bp) DNA ladder); lane 2: plasmid DNA alone; lanes 3–5: undiluted, 10-fold, and 20-fold diluted organic clay-DNA complex, respectively; lanes 6–8: undiluted, 10-fold, and 20-fold diluted inorganic clay-DNA complex, respectively; lanes 9–11: undiluted, 10-fold, and 20-fold diluted kaolinite-DNA complex, respectively; lanes 12–16: undiluted, 10-fold, 20-fold, 50-fold, and 100-fold diluted montmorillonite-DNA complex, respectively; lanes 17–19: undiluted, 10-fold, and 20-fold diluted goethite-DNA complex, respectively.

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chromosomal DNA with Wyoming montmorillonite than its counterpart with kaolinite (10-fold). Montmorillonite was highly effective in inhibiting amplification presumably because of its large propensity for binding and deactivating AmpliTaq DNA polymerase (Vettori *et al*., 1999). On the other hand, DNA bound by goethite at both pH 5.5 and pH 7.0 was amplified whether the complex was used directly, or diluted 10- and 20-fold. Thus, the extent to which minerals inhibit amplification of surface-bound DNA varies with mineral type, especially the surface charge characteristics.

The fragments of PCR amplification products of free plasmid DNA prepared at pH 5.5 and pH 7.0 were both expected 600 base pair (bp) (Figs.3a and 3b, lane 2). However, the fragments of the amplified DNA products bound by kaolinite and Red soil colloids at pH 5.5 occurred at 600 and 300 bp (Fig.3b), whereas only the 300 bp fragment was observed for the amplified DNA complexes with minerals and Brown soil colloids at pH 7.0 (Fig.3a). The presence of the 300 bp fragment (actual fragment size, 600 bp) may be ascribed to the large negative charge of soil colloidal particles at pH 7.0, enabling their DNA complexes to move relatively fast in the electric field.

3 Conclusions

Our investigation confirmed that PCR technique can be used to amplify plasmid DNA bound on soil colloids and minerals. The extent to which amplification was inhibited varies with the type of soil clay and mineral on which the plasmid DNA was bound. The greatest extent of inhibition was found for DNA bound on montmorillonite, whereas goethite did not inhibit amplification of bound DNA. Therefore, the type of mineral must be considered in using the PCR method to amplify DNA directly extracted from natural habitats.

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