The Influence of Soil Organic Matter on DNA Adsorptions on Andosols

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The influence of soil organic matter on DNA adsorption in andosols was investigated using various andosol samples including hydrogen peroxide (H₂O₂)-treated, heated (400°C), and slurry-added soils. Remarkably less DNA was adsorbed in the slurry-added soil than the original soil. The increase in soil organic matter with the addition of slurry had an obvious negative influence on the adsorption. The decrease in organic matter with H₂O₂ treatment slightly raised DNA adsorption per unit weight. Adsorption maxima estimated from a simple Langmuir equation were higher in the samples removed of organic matter by the H₂O₂ treatment and heating at 400°C than in the untreated soil, although surface area was greatly decreased by both treatments. There was no correlation between the total carbon (T-C) content and the estimated DNA adsorption maxima of any of the soil samples. These results suggest little contribution of soil organic matter to DNA adsorption in andosols.

Key words: DNA adsorption, soil organic matter, andosols, organic matter removing, manure slurry application

Most bacteria in soil are not cultivable with artificial media (21). Therefore, in several decades, these nonculturable bacteria have been detected using methods based on the hybridization or PCR amplification of DNA sequences extracted directly from soils. It is more difficult to extract DNA from volcanic ash soils (andosol), wide-spread in Japan, than from other soil types (6, 8). This has been a major problem in analyses of the composition and diversity of soil microbial communities with culture-independent methods. Thus, understanding how DNA is adsorbed by soil particles is important for developing efficient methods of extraction. Many researches have used 2:1 layer phyllosilicates such as montmorillonite as adsorptive particle to study DNA adsorption in soils (4, 5, 9, 10, 16). However, the results obtained with phyllosilicates alone are not enough to understand the behavior of DNA molecules in variable-charged soils such as andosols. Moreover, there have been few studies on DNA adsorption in natural soils (13, 14).

In a previous study (19), DNA adsorption in soils was investigated using andosol, fluvisol, and acrisol samples taken in Japan. The andosol was found to adsorb much more DNA than the fluvisol or acrisol. Next, the contribution of soil organic matter to the adsorption process was evaluated by treating soil samples with hydrogen peroxide (H₂O₂). The decrease in organic matter little affected DNA adsorption per unit weight, implying that soil organic matter may not be related to DNA adsorption in soil.

To better understand the contribution of soil organic matter to the adsorption of DNA by andosol particles, the effects of hydrogen peroxide treatment were examined. However, a small portion of organic matter is resistant even to H₂O₂. All organic matter needs to be removed from soil to study DNA adsorption in organic matter-free soil particles. In a previous

study, an andosol heated at 400°C was used to investigate inorganic anion adsorption (17). We compared DNA adsorption between the heated andosol and untreated soil to elucidate the contribution of soil organic matter to DNA adsorption. Furthermore, to investigate influences of an increase in soil organic matter content, we compared DNA adsorption between a manure slurry-amended andosol and untreated soil from neighboring fields.

Materials and Methods

Soil samples

Samples of four surface humic andosols collected in Japan were used: Chiran soil (CR soil) and Tanashi soil (TA soil) to investigate the effects of lowering soil organic content on DNA adsorption, and Miyakonojyo soil (MY soil) and MY soil amended with a large amount of manure slurry (MY+slurry soil) to examine the effects of increasing soil organic content on DNA adsorption. CR soil was a thick humic andosol taken from the top 10 cm of a field in Minamikyushu, Kagoshima. TA soil was a humic andosol from the 0–20 cm layer of a forest in Nishi-tokyo, Tokyo. The properties of the soil were described previously (18). MY soil was a humic andosol taken from the 0–20 cm layer of a field in Miyakonojyo, Miyazaki. MY+slurry soil was collected from the 0–20 cm layer of a field neighboring the MY field. This field has been amended with slurry at 60 t per 10 a. Relevant physicochemical data for the soils is given in Table 1. These soils were classified as Typic Meranudands.

Pretreatments of the soil samples

Untreated soils. The raw soil samples were sieved (pore size, 2 mm), then added with dilute NaOH solution to pH 6, and washed several times with ultra-pure water (UPW). Finally, they were freeze-dried and stored until the adsorption experiments.

Hydrogen peroxide-treated (H₂O₂-treated) soils. The freeze-dried soil samples were added with 7% H₂O₂ solution, then heated at 95°C for more than 2 hours (17). The suspensions were centrifuged at 16,000×g for 10 min, and the residue adjusted with a dilute NaOH solution to pH 6 were washed several times with UPW, then freeze-dried.

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Table 1. Chemical and physical properties of the andosol samples

	Sample MY	Sample MY+slurry	Sample TA	Sample CR	Sample KB*
Location	Miyazaki		Tokyo	Kagoshima	Kumamoto
Clay content (%)	10.6		23.9	23.5	13.0
Texture	Sandy loam		Clay loam	Clay loam	Sandy loam
pH (H ₂ O)	5.3	6.4	5.3	6.1	6.9
pH (KCl)	4.9	5.2	4.5	5.3	6.0
Total carbon content (%)	2.4	8.3	8.3	12.0	9.6

* The data for Sample KB was quoted from our previous study (19).

400°C heated soils. TA soil samples were heated at 400°C for 4 hrs in an electric oven (17). The solid added with UPW was adjusted to pH 6, then washed several times with UPW. The centrifuged solid samples were finally freeze-dried.

DNA sample

This study used salmon sperm DNA (average size, 1,000 bp) supplied by Invitrogen (Carlsbad, CA, USA). The DNA solution was diluted to a suitable concentration with UPW for the adsorption experiments.

DNA Adsorption Experiments

The diluted DNA solution (volume: 1.0 mL) was added to 0.1-g aliquots of autoclaved soil samples. The initial DNA concentration ranged from 0 to 400 mg L⁻¹. The suspension was shaken for 2 hours in an air-conditioned room at 25±1°C and centrifuged at 16,000×g for 20 min. The DNA in the supernatant was quantified fluorometrically using the PicoGreen quantification method (7). The amount of DNA adsorbed was calculated from the difference between the amount added and the amount remaining in the solution.

DNA adsorption by a variety of solid particles is affected by factors such as the solution's pH and ionic strength (1, 11). However, because each soil sample was adjusted to pH 6 and repeatedly washed with UPW, we did not consider these factors to be relevant in the adsorption experiments.

The affinity of the soil samples for DNA was determined with maximum adsorption ($A_{\max}$) from the Langmuir equation, shown below;

$$A = \frac{k \cdot A_{\max} \cdot C}{1 + k \cdot C} \quad [1]$$

where A is each DNA's adsorption, k is an adsorptive equilibrium constant, and C is each DNA's equilibrium concentration. From Eq. [1], we then obtain,

$$\frac{C}{A} = \frac{1}{k \cdot A_{\max}} + \frac{1}{A_{\max}} \cdot C \quad [2]$$

Maximum adsorption can be calculated approximately by applying the empirical adsorptive data to Eq. [2]. The significance of correlation coefficients in regression analyses was evaluated with a t -test.

Results

Comparison of DNA adsorption among a variety of andosols

Fig. 1 shows DNA adsorption in the untreated samples of MY, MY+slurry, TA, and CR soils. DNA adsorption in the andosol is also indicated in Table 3 with each $A_{\max}$ value estimated from the Langmuir equation. For comparison, data (19) on another humic andosol (KB soil), a fluvisol and an acrisol are also listed in Table 3. Almost all of the DNA (<200 mg L⁻¹) was adsorbed by untreated MY soil particles. The adsorption increased in proportion with the DNA concentration in untreated TA soil. The adsorption maxima in

the untreated andosols estimated with the Langmuir equation decreased in the order: KB soil>MY soil>TA soil>CR soil (Table 3). This order can not be explained simply by the total carbon (T-C) content, clay fraction content and pH of the soils, listed in Table 1. Ogram *et al.* (13, 14), using three soils taken in the USA, also found that DNA adsorption had no correlation with the chemical and physical properties of the soils.

The effects of an increase in soil organic content on DNA adsorption were evaluated by comparing MY soil with MY+slurry soil (Fig. 1). The total carbon content of MY+slurry soil was 8.3%, whereas that of MY soil was 2.4% (Table 1). The DNA adsorption by MY+slurry soil was remarkably less than that by MY soil. The increase in soil organic matter due to the slurry had an obvious negative influence on DNA adsorption in the soil. This result was consistent with the finding that almost all DNA was extracted from the same MY+slurry soil in the study of microbial communities while much DNA could not be extracted from the untreated MY soil (Sakai, *personal communication*).

Fig. 1. DNA adsorption in each treated andosol.

The effect of removing organic matter on DNA adsorption in the andosols

The effects of soil organic matter on DNA adsorption in the soils were investigated by reducing the organic matter contents of the soils with H₂O₂ treatment (Fig. 1). Total carbon content decreased with the H₂O₂ treatment from 2.4 to 0.12% in MY soil, from 8.3 to 2.2% in TA soil and from 12.0 to 0.9% in CR soil (Table 1 and 2). Since these soils seem to contain little carbonate, the decrease in T-C contents can be regarded as a decrease in soil organic matter. DNA adsorption was greater in the H₂O₂-treated MY and CR soils than in the untreated soils (Fig. 1). The estimated DNA adsorption maxima slightly increased from 4.17 mg g⁻¹ in untreated MY soil to 4.42 mg g⁻¹ in the H₂O₂-treated soil (Table 3). In TA soil, the specific surface area of the H₂O₂-treated soil was 109 m² g⁻¹, remarkably less than the untreated soil (Table 2). The contribution of organic matter to soil surface area would be great. Fig. 1 shows that more DNA was adsorbed in the H₂O₂-treated TA soil than in the untreated soil. The adsorption maximum of the H₂O₂-treated TA soil, 4.71 mg g⁻¹ was higher than that of the untreated soil, 3.19 mg g⁻¹, although surface area was greatly decreased by the treatment. Also, the decrease in organic matter content of KB soil little affected DNA adsorption per unit weight, in the H₂O₂-treated soil particles (19). Similarly, H₂O₂-treatment of a clay fraction of Alfisol increased significantly DNA adsorption (2). From the present results, we conclude that soil organic matter has no positive influence on DNA adsorption.

As Table 2 shows that a small portion of organic matter is resistant even to H₂O₂ treatment, it is necessary to remove all organic matter from soil in order to assess DNA adsorption in organic matter-free soil particles. We compared DNA adsorption between TA andosol heated at 400°C and untreated soil to reveal the absolute contribution of soil organic matter to DNA adsorption. The heat treatment

Table 2. DNA adsorption maxima and chemical properties of the treated TA andosols

	Untreated	H ₂ O ₂ treated	400°C heated
Total carbon content (%)	8.25	2.17	0.03
Acid oxalate extractable			
Al (%)	4.21	4.70	4.55
Fe (%)	1.75	2.14	1.87
Specific surface area (m ² g ⁻¹)*	215	109	60.7
DNA adsorption maximum per weight (mg g ⁻¹)**	3.19	4.71	4.92
DNA adsorption maximum per surface area (mg m ⁻²)	0.0148	0.0432	0.0811

* N₂-BET method (Data from Saeki and Matsumoto (17)).

** estimated by the simple Langmuir equation.

reduced the soil T-C content to 0.03%. The specific surface area of the heated soil was one third that of the original soil, and also less than that of the H₂O₂-treated soil (Table 2). Nevertheless, DNA adsorption was significantly greater in the heated TA soil than untreated soil (Fig. 1). The DNA adsorption maximum of the particles was raised from 3.19 mg g⁻¹ to 4.92 mg g⁻¹ by the heat treatment. Moreover, DNA adsorption per specific surface area of the heated soil was 5 fold that of the original soil (Table 2).

These results suggested a negative relationship between soil organic matter content and the adsorption of DNA molecules in soils. Thus, we investigated the relationship between the T-C content and the estimated DNA adsorption maxima (Table 3) of all the soil samples used in the present study with a simple linear regression model. However, a statistical analysis of the data resulted in no correlation between the two values (Fig. 2).

Table 3. Estimated Langmuir equation parameters for each treated soil sample

Samples		DNA adsorption maxima <i>A</i> _{max} (mg g ⁻¹)	Constant <i>k</i>	Correlation coefficient <i>r</i>	Sample No. <i>n</i>
Andosol					
Miyakonojyo (MY)	untreated	4.17	0.013	0.960 (<i>p</i> <0.01)	6
	H ₂ O ₂ treated	4.42	0.021	0.983 (<i>p</i> <0.001)	6
MY+slurry	untreated	1.80	1.039	0.997 (<i>p</i> <0.001)	11
Tanashi (TA)	untreated	3.19	0.024	0.835 (<i>p</i> <0.1)	5
	H ₂ O ₂ treated	4.71	0.054	0.987 (<i>p</i> <0.001)	7
	400°C heated	4.92	0.222	0.995 (<i>p</i> <0.001)	7
Chiran (CR)	untreated	2.76	0.011	0.970 (<i>p</i> <0.001)	10
	H ₂ O ₂ treated	3.27	0.058	0.853 (<i>p</i> <0.01)	11
Kumamoto* (KB)	untreated	5.65	0.286	0.984 (<i>p</i> <0.001)	18
	H ₂ O ₂ treated	5.72	0.578	0.959 (<i>p</i> <0.001)	18
Fluvisol*					
Saga	untreated	2.76	0.091	0.995 (<i>p</i> <0.001)	10
	H ₂ O ₂ treated	2.69	0.081	0.993 (<i>p</i> <0.001)	18
Acrisol*					
Nagasaki	untreated	3.47	0.083	0.982 (<i>p</i> <0.001)	12
	H ₂ O ₂ treated	3.42	0.148	0.982 (<i>p</i> <0.001)	17

* Data from our previous study (19).

Fig. 2. The relationship between total carbon (T-C) content and DNA adsorption maxima estimated with the Langmuir equation.

Discussion

The contributions of soil organic matter to DNA adsorption

The DNA adsorption increased with the removal of organic matter by the treatment with H_2O_2 and heating at 400°C although surface area was greatly decreased by both treatments. Furthermore, the increase in soil organic matter due to the slurry had a clear negative influence on DNA adsorption in the soil. In summary, soil organic matter had no positive influence on DNA adsorption in the andosols. Ogram *et al.* (13) stated that the contribution of organic carbon content to the adsorption of DNA was very small. In soil in which the clay fraction was dominated by permanently charged minerals, organic matter seemed to make no contribution to DNA adsorption (1). The apparent increase in adsorption by H_2O_2 -treatment and heating at 400°C would be caused by the formation of new adsorptive sites due to the appearance of Al and Fe oxides trapped in organic matter by these treatments.

Soil organic matter is composed of a variety of organic compounds. Among them, humic acids may be the only compounds which can be isolated from soils and supply solid surfaces for DNA adsorption. Nevertheless, there have been few reports about DNA adsorption by one representative material of soil organic matters, humic acids (3, 20). In weak-acidic conditions, DNA molecules were adsorbed by humic acid (3, 20). We also observed that a humic acid supplied by Wako Chemicals (Osaka, Japan) adsorbed a remarkable amount of the same DNA molecules as used in the present study (Saeki, unpublished data). Most (70 to 80%) of the DNA adsorbed by the humic acid could not be extracted with distilled water, a 0.1 M NaCl solution, or DNA buffer (pH 4.0) (3), implying that DNA molecules bind to the humic acid surface. The surface chemical properties of organic matter *in situ* may be considerably different from those of humic acids extracted from soils.

Mechanisms and sites of DNA adsorption in andosols

There are three possible mechanisms of DNA adsorption by soil particles. These mechanisms are, in fact, complicated although their contributions would depend greatly on the conditions. Phosphate groups on the edge of DNA molecule may bind via a ligand exchange reaction directly to OH groups of Al or Fe oxide minerals in soils, as illustrated in Fig. 3a. Some researchers have also considered that DNA molecules bind to OH groups on the edge of phyllosilicates such as montmorillonite (9, 11, 15). A previous study (19) indicated that oxide minerals in soils are one of the most important adsorbents for DNA molecules, because adsorption was lowered when Fe or Al oxides including allophane were removed from the H_2O_2 -treated soil by an acid-oxalate

Fig. 3. Conceptual figures of DNA adsorption on soil particles.

extraction. Khanna and Stotzky (9) and Paget *et al.* (15) suggested the adsorption mechanism shown in Fig. 3c, where the DNA molecules associate with the surface of negatively charged materials such as phyllosilicates via a bridging of cations. This mechanism of adsorption might possibly occur in soil organic matter. As shown in Fig. 3d, DNA molecules might directly bind to soil organic matter. However, we concluded that the DNA molecules were not likely to bind directly to the surface of the organic matter, given from that DNA adsorption increased with the removal of soil organic matter and decreased with the addition of organic materials to the soil.

The treatment with H₂O₂ and heating at 400°C removed most of the soil organic matter, resulting in a decrease in the apparent surface area of the soil (Table 2) because of the elimination of fine pores into which N₂ molecules used to determine specific surface area, can enter. Nevertheless, DNA adsorption in the andosols increased with these treatments. There are two explanations for these results. First, the number of soil surface sites for DNA adsorption was not necessarily proportional to the specific surface areas of the soil because DNA molecules can not enter the small pores into which N₂ molecules diffuse. This concept is illustrated in Fig. 3b. Ogram *et al.* (13) reported that more short strands of DNA molecules were more adsorbed on the soil particles than the long strands, while this event was not observed in the sand. They explained that it would be more difficult for the long strands of DNA to enter soil pores than the short strands. Second, these treatments form new adsorptive sites due to the appearance of Al and Fe oxides trapped in organic matter. Our previous study indicated that oxide minerals in soils are one of the most important adsorbents for DNA molecules (19). Consequently, DNA adsorption would depend on the qualitative conditions of the soil particle's surfaces rather than simply on the specific surface area of the particles. However, the only studies of DNA adsorption by oxide minerals have been with goethite (2) and silica (12). Studies with other oxide minerals such as allophane will be required to compare the behavior of extracellular DNA molecules in volcanic ash soils.

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