Short Communication

The Contribution of Soil Constituents to Adsorption of Extracellular DNA by Soils

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(Received July 24, 2008—Accepted September 9, 2008—Published online October 1, 2008)

The adsorption of DNA by an andosol was much greater than that by a fluvisol or acrisol. The decrease in soil organic matter on treatment with hydrogen peroxide (H2O2) little affected DNA adsorption per weight of the particles, implying that the organic matter is not involved in the adsorption. The acid oxalate treatment of the H2O2-treated soils decreased DNA adsorption. Among soil constituents, acid oxalate-extractable materials such as allophane and non-crystalline Al and Fe oxides are likely to make a major contribution to the adsorption of DNA.

Key words: DNA adsorption, soil DNA, hydrogen peroxide treatment, acid oxalate treatment, oxide minerals

Nucleic acids released from microorganisms and plant tissues have been shown to be adsorbed on soil particles, which alters their reactivity and susceptibility to nucleases and make them resistant to degradation[10]. Moreover, DNA molecules could retain the ability to transform competent bacteria by being bound on clay minerals and other particles[3,8,9]. With the rapid developments in the commercial production of genetically modified microorganisms and plants, great emphasis has been put on the security of genetically modified organisms (GMOs). Some genes of genetically modified crops could be excreted into the soil via the roots or released from decaying plant tissue, resulting in changes to microorganism populations and properties of the soil[11]. Therefore, a knowledge of the binding of extracellular DNA to soil constituents is essential to reveal the horizontal translocation and transformation of extracellular DNA in soil environments[12].

Understanding the adsorption of extracellular DNA on soil particles is also important for the study of biological activity and diversity in soil. Most soil bacteria can not be cultivated in artificial media[19]. These nonculturable bacteria have been detected by methods based on the hybridization or PCR amplification of DNA sequences extracted directly from soil. However, it is more difficult to extract DNA from volcanic ash soils (andosol), wide-spread in Japan, than from other types of soils[5,7]. This has been a major problem in the analysis of bacterial communities using culture-independent methods. Thus, an analysis of how DNA is adsorbed by the particles of andosol is required to improve the efficiency with which DNA is extracted from soils.

Many researches have used a 2:1 layer phyllosilicate such as montmorillonite as adsorptive particles to understand DNA adsorption in soils[8,9,13]. However, such results are inapplicable to the behavior of DNA in variable-charge soils such as andosol. Moreover, there have been few studies on DNA adsorption in natural soils[2,13]. In order to understand the contributions of various soil components to DNA adsorption, our study examined the effects of hydrogen peroxide treatment and oxalate treatment to the soil samples on the adsorption.

Three types of soil representative of Kyushu, Japan: Eutric fluvisol (Soil HT) from a paddy field in Saga city, Saga, Orthic acrisol (Soil SK) from a field in Isahaya, Nagasaki, and Humic andosol (Soil KB) from a field in Koshi, Kuma-moto, were examined. Relevant physicochemical data for the soils are given in Table 1. The raw samples were sieved (pore size, 2 mm). The samples were added to a 0.1 M NaOH or HCl solution to pH 6, then washed several times with ultra-pure water (UPW) with an electrical conductivity of <6 μS m⁻¹. Finally, the samples were freeze-dried and stored until the adsorption experiments (Untreated soils). In order to reduce the organic matter content of the soils, the freeze-dried samples were added to a 7% H2O2 solution, then heated at 95°C for more than 2 hours[16]. The suspensions were centrifuged at 16,000×g for 10 min, and the residue adjusted with a 0.1 M NaOH solution to pH 6 was washed several times with UPW, then freeze-dried (H2O2-treated soils). In order to reduce the noncrystalline Al and Fe oxide contents of the soils, the H2O2-treated samples were added to a 0.2 M acid ammonium oxalate (pH 3.0), then shaken for 4 hours in a dark room[20]. The suspensions were centrifuged at 16,000×g for 10 min, and the collected residue was added to a 5% Na2CO3 solution, standing for 16 hours. The suspensions were added to a 0.1 M HCl solution to pH 6, then washed several times with UPW. The centrifuged solid samples were freeze-dried (H2O2+oxalate-treated soils).

This study used salmon sperm DNA (10 mg mL⁻¹) supplied by Invitrogen (Carlsbad, CA, USA). The DNA solution was prepared from highly pure phenol/chloroform-extracted DNA and nuclease-free UPW and was sheared to an average size of 1,000 bp by sonication. The DNA solution was diluted to a suitable concentration with UPW for the adsorption experiments.

The diluted DNA solution was added to a 0.1-g aliquot of autoclaved soil samples. The suspension was shaken for 2 hours in an air-conditioned room at 25°C and centrifuged at

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Table 1. Chemical and physical properties of the soil samples

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample HT</th>
<th>Sample SK</th>
<th>Sample KB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification</td>
<td>Eutric fluvisol</td>
<td>Nagasaki, Japan Orthic acrisol</td>
<td>Kumamoto, Japan Humic andosol</td>
</tr>
<tr>
<td>Texture</td>
<td>Sandy loam</td>
<td>Sandy clay loam</td>
<td>Sandy clay allophane, imogolite</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>10.0</td>
<td>17.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>16.5</td>
<td>19.0</td>
<td>20.3</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>73.5</td>
<td>64.0</td>
<td>66.7</td>
</tr>
</tbody>
</table>

Maximum adsorption can be calculated by entering the empirical adsorptive data into Eq. [2].

Fig. 1 indicates the DNA adsorption isotherms for each soil sample. For all the samples, the relation between adsorption and the final concentration of DNA significantly fitted a simple linear Langmuir equation (Eq. 2) (Table 2). These isotherms were categorized as the L- or H-type13, indicating strong affinity between the DNA molecules and the surface of soil particles. Furthermore, these isotherms showed that the adsorbed DNA molecules are likely to form monomolecular layers on the surface of soil particles, ruling out the precipitation or coagulation of DNA molecules.

In the original or untreated samples, Soil KB adsorbed twice more DNA (amount per soil weight) than the other two soils (Fig. 1). The adsorption was greater in the untreated Soil SK than in Soil HT. The adsorption maxima in the untreated soils estimated with the Langmuir equation decreased in the order: Soil KB > Soil SK > Soil HT (Table 2). Because there was no marked difference among the particle-size distribution of the three soils (Table 1), the variation in adsorption among these soils should not be attributed to particle size distribution. Table 1 indicates that the carbon content and acid oxalate extractable Al, Fe, and Si of Soil KB (andosol) which had extensive capacity to adsorb DNA, were much greater than those of the other soils. Therefore, it is...
likely that the adsorption is related to the amount of organic matter or acid oxalate-extractable oxide minerals in the soil.

The effect of soil organic matter on DNA adsorption was investigated by reducing the organic matter content of the soils with H$_2$O$_2$ treatment. Total carbon content decreased with the H$_2$O$_2$ treatment, to 0.3 and 0.4% in Soil HT and SK, respectively. The total carbon content of the H$_2$O$_2$-treated Soil KB was about one third (3.6%) of that of the untreated soil. Since these soils seem to contain little carbonate, the decrease in total carbon content can be regarded as a decrease in soil organic matter.

DNA adsorption in the H$_2$O$_2$-treated Soil HT and SK was similar to that in the untreated soils (Fig. 1). The estimated DNA adsorption maxima did not differ significantly between the H$_2$O$_2$-treated soils and the untreated soils (Table 2). In Soil KB, the H$_2$O$_2$-treatment increased slightly DNA adsorption (Fig. 1) and the adsorption maxima to 5.72 mg g$^{-1}$ (Table 2). The apparent slight increase in DNA adsorption with the H$_2$O$_2$-treatment would be caused by the formation of new adsorptive sites due to H$_2$O$_2$-induced exposure of Al and Fe constituents occluded or trapped by the organic matter.

From the present results, we conclude that soil organic matter has no positive influence on DNA adsorption. That is, soil organic matter is not likely to adsorb DNA molecules. Ogram et al.\textsuperscript{13} and Cai et al.\textsuperscript{21} also stated that soil organic matter seems to make no contribution to the adsorption of DNA.

Next, by reducing the mineral content of the H$_2$O$_2$-treated soils with acid oxalate extraction, we investigated whether oxide minerals contribute to the adsorption. The reduction in acid oxalate-extractable Al, Fe, and Si caused by the H$_2$O$_2$+oxalate treatment should be similar to the amount of acid oxalate-extractable metals in the original soils shown in Table 1.

In Soil HT, no apparent difference in DNA adsorption was observed between the H$_2$O$_2$+oxalate-treated sample and the untreated sample (Fig. 1). The adsorption decreased with the H$_2$O$_2$+oxalate treatment in Soil SK (Fig. 1). The adsorption in the H$_2$O$_2$+oxalate-treated KB sample was about half that in the untreated soils (Fig. 1). The estimated adsorption maxima fell to 2.62 mg g$^{-1}$ (Table 2). These results indicated that minerals such as allophane and noncrystalline Al and Fe constituents which are soluble or extractable with acid oxalate have a major role in the adsorption of DNA in soils.

The percentage of the decrease in DNA adsorption with the H$_2$O$_2$+oxalate treatment declined in the order, Soil KB>>Soil SK>>Soil HT. This order may be related to the smaller amounts of oxalate-extractable Al, Fe, and Si in soil HT and soil SK than in soil KB. In other words, the acid oxalate treatment had extensive negative effects on DNA adsorption in the soils which contained greater amounts of oxalate-extractable Al, Fe, and Si. This implies that the oxide minerals in soil are among the most important adsorbents for DNA molecules.

However, we have no evidence of DNA adsorption by noncrystalline (oxalate-extractable) oxides such as allophone. There have been only a few studies on crystalline oxides such as goethite\textsuperscript{15} and silica\textsuperscript{16}. Studies on DNA adsorption to amorphous clay minerals such as allophane and various Al and Fe oxides rather than on phyllosilicates should help to reveal the behavior of extracellular DNA molecules in soils in humid regions such as Japan.

Acknowledgements

The authors thank Assoc. Prof. S-I. Wada, Kyushu University for advice on this study, and Dr. H. Kubodera, National Agricultural Research Center for the Kyushu Okinawa Region for helping with the analysis of soil properties. This research was supported by a Grant-in-aid for Scientific Research from the Japan Society for the Promotion of Science (1930044).

References