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Effect of amending soil with organic acids on perylene uptake into *Cucurbita pepo*

Ryouhei YOSHIHARA,^{1,3} Shinryo MATSUO,²
Toru IWATA² and Hideyuki INUI^{1,2,*}

¹ Research Center for Environmental Genomics, Kobe University,
1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan

² Graduate School of Agricultural Science, Kobe University,
1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan

³ Present address: Graduate School of Science and Engineering,
Saitama University, 255 Shimo-Okubo, Sakura-ku,
Saitama, Saitama 338-8570, Japan

(Received May 31, 2014; Accepted June 29, 2014)

The solubilization of hydrophobic organic pollutants from soil is important for the phytoremediation of contaminated soil. In this study, we used perylene as a model pollutant to evaluate the abilities of 11 organic acids to solubilize hydrophobic compounds from soil. Oxalic acid and citric acid showed high solubilization abilities, and when added to soil, they enhanced perylene uptake into *Cucurbita pepo*, likely owing to the chelating ability of these acids. © Pesticide Science Society of Japan

Keywords: hydrophobic organic pollutant, organic acid, phytoremediation.

Introduction

Hydrophobic organic pollutants (HOPs) such as dioxins and dioxin-like compounds accumulate in the environment and in the adipose tissues of animals *via* the food chain because these compounds are metabolically stable. They are adsorbed by soil and remain adsorbed for a long time.^{1–4)} Recently, phytoremediation has attracted attention as a potential low-cost method for removing HOPs from soil.⁵⁾ In particular, cucurbitaceous plants, such as *Cucurbita pepo* ssp. *pepo*, including pumpkin and zucchini, have been shown to accumulate large amounts of HOPs.^{6–11)} In previous work with *Cucurbita* species, we found that as compared to the low-accumulation cultivar *C. pepo* ssp. *ovifera* cv. Patty Green, the high-accumulation cultivar ssp. *pepo* cv. Gold Rush accumulates 165 times the amount of dioxins and dioxin-like compounds in soil culture and 13 times the amount in hydroponic culture.^{11,12)} The fact that the difference between the two cultivars is lower in hydroponic culture than in soil

culture suggests that Gold Rush solubilizes hydrophobic compounds from the soil more efficiently than Patty Green does and therefore that solubilization of HOPs from soil is an important determinant of the accumulation ability of a plant species.

Root exudates of plants have been shown to solubilize hydrophobic compounds from soil.¹³⁾ For example, organic acids present in *C. pepo* root exudates enhance the desorption and uptake of *p,p'*-dichlorodiphenyldichloroethylene by the plants.^{14–16)} On the basis of these reports, we speculated that organic acids are involved in the solubilization of HOPs from soil and therefore might improve the efficiency of the phytoremediation techniques. In this study, we investigated the solubilization of perylene, a hydrophobic polycyclic aromatic hydrocarbon (PAH), from soil in the presence of 11 organic acids to identify acids with a high ability to solubilize hydrophobic compounds. Perylene is used as a model compound for hydrophobic compounds because it shows high hydrophobicity and fluorescence, making it easy to detect. We also examined the effect of amending soil with the organic acids on perylene uptake into the plant.

Materials and Methods

1. Measurement of organic acids' ability to solubilize perylene from soil

Soil contaminated with perylene was prepared by adding 100 µL of a 200 µM solution of perylene in acetone to 100 mg of commercial soil (HYPONeX Japan Co., Osaka, Japan) and then drying the soil overnight. Aliquots (50 mg) of contaminated soil were placed in glass vials, and 1 mL of a 0.1 M solution of organic acid was added to the soil in the vials, which were then incubated at 25°C for 24 hr. After incubation, the clear supernatant was collected by centrifugation at 22,000×*g* for 10 min and mixed with an equivalent volume of 100% DMSO to measure the fluorescence of the solubilized perylene. The fluorescence of the perylene was measured at a 410-nm excitation wavelength and a 445-nm emission wavelength with a microplate reader (SH-9000, Corona Electric Co., Katsuta, Japan). We tested 11 organic acids: formic, acetic, butyric, oxalic, citric, succinic, maleic, citramalic, salicylic, ferulic, and jasmonic acids. Formic, acetic, butyric, oxalic, citric, succinic, maleic, and citramalic acids were prepared in water at a concentration of 0.1 M, and the solution pH was adjusted to 7.0 with 1 N NaOH. Salicylic and ferulic acid solutions (0.1 M) were prepared by dissolving the acids in 1 N NaOH and adjusting the pH to 7.0 with 1 N HCl. However, because a pH 7 solution of jasmonic acid produced a precipitation, a pH 9 jasmonic acid solution was used instead. The concentration of perylene was calculated from a standard curve that was created by the addition of a known concentration of perylene to the supernatant of a soil solution without perylene.

* To whom correspondence should be addressed.

E-mail: hinui@kobe-u.ac.jp

Published online August 14, 2014

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2. Measurement of perylene uptake into plants

A sample of autoclaved commercial soil described above sieved to a particle size of <2 mm was spiked with an equal amount of either acetone or a 1 mM acetone solution of perylene. After evaporation of the acetone, 45 g of the spiked soil was placed in a glass beaker, and 55 mL of tap water was added. Seeds of *C. pepo* ssp. *ovifera* cultivar Patty Green (Johnny's Selected Seeds, Albion, ME), which had been soaked in tap water at 4°C overnight, were sown and then incubated for 11 days under 16-hr-light and 8-hr-dark conditions at 25°C. Then an aqueous solution (40 mL) containing either 0.1 M citric acid and 0.1 M oxalic acid and adjusted to pH 7.0 was added to the soil. After incubation for 2 days, the stems of the plant were sampled. The same amount of 50% DMSO as the weight of the stems was added to the stems, which were then ground with a mortar and pestle. Perylene fluorescence at 410-nm excitation and 445-nm emission was measured in the supernatants that were obtained by centrifugation of the ground stems at 22,000×g for 10 min, as described above.

Results

1. Solubilization of soil-adsorbed perylene by organic acid solutions

The abilities of 11 organic acid solutions to solubilize perylene from soil were evaluated by preparing soil contaminated with perylene and measuring the quantity of the compound solubilized from the soil by the acids (Fig. 1). After incubation of the soil with water alone, the perylene concentration in the supernatant was 0.9 nM, whereas the perylene concentrations after incubation with oxalic acid and citric acid were approximately 98.6 and 81.4 nM, respectively. These results suggest that oxalic acid and citric acid readily solubilized perylene from the soil. Citramalic acid solubilized a small amount of perylene (supernatant concentration 7.4 nM), but none of the other organic acid solutions showed significant solubilization ability.

Fig. 1. Solubilization of perylene from soil by organic acid solutions. Values are means $\pm$ SDs ($n=3$). Asterisks indicate significant differences as determined by Student's *t*-test (* $p<0.01$, ** $p<0.05$).

We also investigated the time course of perylene solubilization with citric acid and oxalic acid and found that under our experimental conditions, the amount of solubilized perylene continued to increase throughout the 24-hr incubation period (Fig. 2). This suggests that solubilization of perylene was relatively slow.

2. Enhancement of perylene uptake into plants by organic acids

To confirm that perylene uptake into plants was enhanced by oxalic acid and citric acid, we cultivated *C. pepo* in perylene-contaminated soil treated with these organic acids. The uptake of perylene into the plant from the soil was significantly increased by the addition of citric acid (Fig. 3). Uptake was also increased by the addition of oxalic acid, although the increase was not statistically significant. These observations suggest that organic acids with a high ability to solubilize perylene from the soil can enhance its uptake into plant.

Discussion

Our results regarding the solubilization of soil-adsorbed perylene by organic acid solutions indicate that oxalic acid and citric acid have high perylene-solubilization abilities. White *et al.* reported that oxalic acid and citric acid enhance *p,p'*-dichlorod-

Fig. 2. Time course of the solubilization of perylene from soil by citric acid and oxalic acid. Values are means $\pm$ SDs ($n=3$).

Fig. 3. Accumulation of perylene in the stems of *C. pepo* ssp. *ovifera* cv. Patty Green plants grown in soil amended with organic acids. Values are means $\pm$ SDs ($n=3-4$). Asterisks indicate significant differences as determined by Student's *t*-test ($p<0.01$).

iphenyldichloroethylene desorption from soil and uptake from soil by plants.^{14,15)} Taken together, these results suggest that these two organic acids may be effective for the phytoremediation of soil contaminated with hydrophobic pollutants. In addition, citramalic acid, which solubilized a small amount of perylene from the soil, has been shown to be present in root exudates of sugar beets and to solubilize soil phosphorus.¹⁷⁾ Again, these findings suggest that citramalic acid also contributes to the solubilization of hydrophobic compounds from the soil and may enhance the uptake of hydrophobic pollutants from the soil by plants.

In our evaluation of the perylene-solubilization ability of 11 organic acids, we did not identify any specific association between chemical structure and solubilization ability, except that the presence of multiple carboxyl groups conferred a higher solubilization ability. Oxalic acid and citric acid are well known to chelate metal ions in soil. In addition, Yang *et al.* showed that the desorption of weathered PAHs is increased by the presence of citric acid and other chelating agents.¹⁸⁾ These investigators speculated that chelating agents may disrupt soil humic macro-molecule-metal ion-mineral linkages by releasing metal ions from the soil; specifically, these investigators reported that disruption of these linkages results in the release of soil organic matter and that PAHs can also be released into the aqueous phase. Moreover, the reduction of cross-linking in the soil organic matter phase changes the soil structure and enhances the diffusion of PAHs.¹⁸⁾ In our study, we found that the supernatants obtained from soils treated with oxalic acid, citric acid, and citramalic acid were muddy (data not shown). This observation suggests that the high perylene-solubilization abilities of these three organic acids may involve disruption of the soil structure as a result of their chelation properties. We intend to clarify the relationship between chemical structure and solubilization ability in future work.

As compared to incubation with water, incubation with citric acid or oxalic acid for 2 days resulted in the accumulation of 2 to 3 times as much perylene in the stems of *C. pepo*. The fact that the solubilization of perylene was relatively slow indicates that long-duration incubation with organic acids will result in increased accumulation because the high concentration in the rhizosphere will increase perylene uptake by the plants. Non-cucurbitaceous plants may also be able to accumulate HOPs in their aerial parts when grown in soil treated with citric, oxalic, or citramalic acid, as suggested by the fact that komatsuna, soybean, and tomato plants accumulate dieldrin from quartz sand spiked with this pesticide.⁹⁾

In this study, we tested the solubilization ability of a hydrophobic compound from soil by 11 organic acids by measuring the fluorescence of perylene solubilized from soil. Perylene uptake from soil into *C. pepo* was enhanced by the addition of the

organic acids that showed high solubilization abilities. We expect the method used in this study to be useful for identifying novel factors for the solubilization of hydrophobic pollutants from soil. However, this study should be repeated with different contaminants to determine which acids produce the largest solubilization effect for each compound. In addition, we did not investigate the optimal acid concentration, which must be determined to permit practical use of this approach for the bioremediation of contaminated sites.

Acknowledgements

We are grateful to all the members of the Environmental Material Science Laboratory (Graduate School of Agricultural Science, Kobe University) for their support. This work was funded by the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant-in-Aid for Scientific Research A No. 23241028).

References

- 1) Y. Hashimoto: *J. Pestic. Sci.* **30**, 397–402 (2005).
- 2) R. Uegaki, N. Seike and T. Otani: *Chemosphere* **65**, 1537–1543 (2006).
- 3) I. Hilber, P. Mäder, R. Schulin and G. S. Wyss: *Chemosphere* **73**, 954–961 (2008).
- 4) R. Weber, A. Watson, M. Forter and F. Oliaei: *Waste Manag. Res.* **29**, 107–121 (2011).
- 5) A. C. Dietz and J. L. Schnoor: *Environ. Health Perspect.* **109** (Suppl. 1), 163–168 (2001).
- 6) A. Hülster, J. F. Müller and H. Marschner: *Environ. Sci. Technol.* **28**, 1110–1115 (1994).
- 7) J. C. White, X. Wang, M. P. Gent, W. Iannucci-Berger, B. D. Eitzer, N. P. Schultes, M. Arienzo and M. I. Mattina: *Environ. Sci. Technol.* **37**, 4368–4373 (2003).
- 8) M. I. Mattina, B. D. Eitzer, W. Iannucci-Berger, W. Y. Lee and J. C. White: *Environ. Toxicol. Chem.* **23**, 2756–2762 (2004).
- 9) T. Otani, N. Seike and Y. Sakata: *Soil Sci. Plant Nutr.* **53**, 86–94 (2007).
- 10) M. L. W. Aslund, A. Rutter, K. J. Reimer and B. A. Zeeb: *Sci. Total Environ.* **405**, 14–25 (2008).
- 11) H. Inui, T. Wakai, K. Gion, Y. S. Kim and H. Eun: *Chemosphere* **73**, 1602–1607 (2008).
- 12) H. Inui, T. Wakai, K. Gion, K. Yamazaki, Y. S. Kim and H. Eun: *Biosci. Biotechnol. Biochem.* **75**, 705–710 (2011).
- 13) A. Hülster and H. Marschner: *Organohalogen Compd.* **20**, 31–34 (1994).
- 14) J. C. White, M. I. Mattina, W. Y. Lee, B. D. Eitzer and W. Iannucci-Berger: *Environ. Pollut.* **124**, 71–80 (2003).
- 15) J. C. White and B. D. Kottler: *Environ. Toxicol. Chem.* **21**, 550–556 (2002).
- 16) X. Wang, J. C. White, M. P. Gent, W. Iannucci-Berger, B. D. Eitzer and M. I. Mattina: *Int. J. Phytoremediation* **6**, 363–385 (2004).
- 17) R. Khorassani, U. Hettwer, A. Ratzinger, B. Steingrobe, P. Karlovsky and N. Claassen: *BMC Plant Biol.* **11**, 121 (2011).
- 18) Y. Yang, D. Ratté, B. F. Smets, J. J. Pignatello and D. Grasso: *Chemosphere* **43**, 1013–1021 (2001).