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Short communication

Influence of naphthalene biodegradation on the adhesion of *Pseudomonas putida* NCIB 9816-4 to a naphthalene-contaminated soil

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ABSTRACT

In an earlier study, *Pseudomonas putida* NCIB 9816-4, which was one of the most studied naphthalene-degrading bacteria, showed the preferred adhesion to the naphthalene-contaminated soil when it was in the exponential growth phase. The adhesion was found to take place through a hydrophobic interaction. We postulated that the surface hydrophobicity of *P. putida* NCIB 9816-4 in the exponential growth phase might be increased during the uptake of naphthalene, which caused the preferred adhesion to the naphthalene-contaminated soil. To verify this postulate, a plasmid-cured strain of *P. putida* NCIB 9816-4 was obtained in this study and compared with the wild-type for adhesion to the naphthalene-contaminated soil. Only the wild-type in the exponential growth phase showed increased adhesion to naphthalene-contaminated soil. The water contact angles of the two strains were measured in the presence and in the absence of naphthalene as indices of surface hydrophobicity. The water contact angle of the wild-type increased in the presence of naphthalene, whereas that of the cured strain did not change. We conclude that the uptake of naphthalene during naphthalene biodegradation in the exponential growth phase of *P. putida* NCIB 9816-4 made the cell surface more hydrophobic, resulting in increased adhesion to naphthalene-contaminated soil.

1. Introduction

In situ bioremediation has been recognized as an economic technology for the remediation of the soil contaminated with polycyclic aromatic hydrocarbons (PAHs) [1]. However, it has limited application because contaminants in the deep soil are hard to be treated. Biological clogging, which is known to occur during in situ bioremediation, inhibits the microbial transport through the soil matrix and the biodegradation of contaminants due to the retarded transport of oxygen [2]. Therefore, the interactions between microorganisms and the surface of contaminated soil need to be thoroughly understood, especially with respect to the initial adhesion of cells to the soil surface [3].

In an earlier study, we investigated the effect of naphthalene, a model PAH compound, on the adhesion of a naphthalene-degrading bacterium to the soil surface [4]. We used *Pseudomonas putida* NCIB 9816-4 as a microbial strain capable of degrading naphthalene. It contains the NAH plasmid carrying genes for enzymes involved in naphthalene degradation, especially the conversion of naphthalene to salicylate [5]. *P. putida* DK-1, which does not degrade naphthalene, was used as a comparative control strain. *P. putida* NCIB 9816-4 in the exponential growth phase showed the preferred adhesion to the naphthalene-contaminated soil, whereas the adhesion of *P. putida* DK-1 was not affected by naphthalene. Bacterial adhesion to hydrocarbons (BATH) assay [6] was used to determine the hydrophobicity of the cell surface and the soil surface. BATH assay is to measure the distribution equilibrium of a species of interest between the aqueous phase and an organic solvent. The culture medium without a carbon source and hexadecane were used as the aqueous phase and the organic solvent, respectively. The soil contaminated with naphthalene exhibited the higher hydrophobicity than the uncontaminated soil. Microbial adhesion, especially the initial adhesion to the naphthalene-contaminated soil, was found to take place through a hydrophobic interaction. We postulated that the surface hydrophobicity of *P. putida* NCIB 9816-4 in the exponential growth phase might be increased during the uptake of naphthalene, which caused the preferred adhesion to the naphthalene-contaminated soil.

The purpose of this study was to verify this postulate. A plasmid-cured strain of *P. putida* NCIB 9816-4, which had lost the ability to degrade naphthalene because of the loss of the NAH plasmid, was compared with the wild-type for adhesion to the naphthalene-contaminated soil. To determine the change in cell surface hydrophobicity due to naphthalene uptake, the water

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Table 1
Physical and chemical properties of soil used in this study [4,8].

Properties	Values
Organic carbon	0.53%
pH in water	7.61
CEC (cation exchange capacity)	4.92 me/100 g soil
Sand	70.60%
Silt	18.32%
Clay	11.08%
Hydrophobicity[a]	20.11 ± 3.17% for uncontaminated soil 31.91 ± 2.52% for naphthalene-contaminated soil

[a] The soil was ground using a ball mill for the measurement of the hydrophobicity. BATH assay was used to determine the hydrophobicity of soil surface. When A and B are defined as the soil concentration in the aqueous phase prior to and after partitioning, respectively, the hydrophobicity data are expressed as $[(A-B)/A] \times 100$.

contact angles of both strains were measured in the absence and in the presence of naphthalene. The BATH assay reported in [4] is not appropriate for this study. Naphthalene adsorption can occur on the cell surface in the case of a microbe with the capability for naphthalene uptake. Naphthalene is highly soluble in organic solvents, and cells with naphthalene adsorbed lose the naphthalene immediately when they are put into an organic solvent in the BATH assay. Hence, the change in cell surface hydrophobicity due to naphthalene uptake cannot be determined by the BATH assay.

2. Experimental

Refer to [4] for the composition of the minimal medium and the culture condition. To obtain a plasmid-cured strain without the ability to degrade naphthalene, *P. putida* NCIB 9816-4 was cultivated in glucose minimal medium for 24 h, and then 0.5 ml of the cell culture was transferred to fresh glucose minimal medium. After repeating the transfer 28 times, a sample of the cell culture was spread onto an LB plate. After incubation for 1 day, single colonies on the LB plate were transferred onto minimal medium plates, which were then incubated in a desiccator filled with naphthalene vapor. One colony on the LB plate failed to grow on minimal medium. To investigate the genotype of the resultant mutant, its plasmid DNA, if any, was isolated. The NAH plasmid (Genbank accession number AF491307) of *P. putida* NCIB 9816-4 was isolated and digested with *Eco*RI. The absence of a plasmid DNA in the cured strain was confirmed by gel electrophoresis [7].

The size, the shape, and the surface appendages of *P. putida* strains were analyzed using TEM (JEM 1010, JEOL). The number density of viable cells was measured by plate counting using LB plates. Naphthalene concentrations in the supernatants of soil slurries were estimated by measuring the absorbance at 254 nm after filtration through a 0.2-μm PTFE syringe filter.

To measure the water contact angle of the cell surface, microbial cells were harvested by centrifugation and washed three times with a phosphate buffer, which contained (per liter) 3.0 g K_2HPO_4, 1.5 g KH_2PO_4, 0.01 g NaCl. The cells suspended in the phosphate buffer were vacuum-filtered through a 0.45 μm nitrocellulose membrane filter (type HA, Millipore®, USA) to create a bacterial layer. The water contact angle of the cell surface was measured using the DSA 100 system (Krűss GmbH, Germany) [6]. At least three replicate bacterial layers were used for each measurement, and their mean value is presented.

The soil used in all experiments was taken from an uncontaminated area near a coal mine in Samchok, Kangwon-Do, South Korea. The soil sample was air-dried and was passed through a series of sieves. Physical and chemical properties of the test soil were measured by The Jeil Analysis Center (Bucheon, South Korea), and are summarized in Table 1 [8]. Refer to [4] for the methods to prepare soil suspension and to perform cell adhesion experiments.

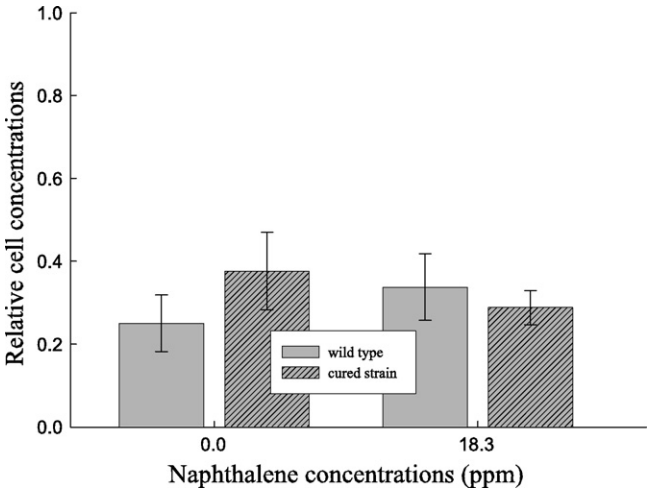

Fig. 1. Degrees of cell adhesion to the soil for *P. putida* NCIB 9816-4 and the cured strain in the exponential phase. The degree of cell adhesion is denoted as the ratio of the adhered cell concentration to the initial cell concentration.

Table 2
Water contact angles of the two strains in the exponential phase.

Strain	Presence of naphthalene in the washing medium	Water contact angle (°)
P. putida NCIB 9816-4	No	20.4 ± 1.6
	Yes	25.7 ± 0.3
Cured strain	No	22.0 ± 2.8
	Yes	20.0 ± 0.6

3. Results and discussion

The NAH plasmid with *Eco*RI restriction sites is shown in the supplementary material, Fig. S1. When the NAH plasmid was digested with *Eco*RI, numerous small DNA fragments were observed by gel electrophoresis, as shown in the supplementary material, Fig. S2 (see lane C). No plasmid DNA was found in the plasmid-cured strain (see lane D in Fig. S2). Hence, the cured strain could not degrade naphthalene and therefore did not grow taking advantage of naphthalene as the sole carbon source.

The average diameter and length of the rod-shaped *P. putida* NCIB 9816-4 and its plasmid-cured strain in the exponential phase are given in the supplementary material, Table S1. Their values were quite similar although the average surface area of the plasmid-cured strain was ~15% larger than that of the wild-type. Therefore, the change in the cell surface area cannot be a reason for the increased adhesion of the wild-type to the naphthalene-contaminated soil.

Degrees of cell adhesion to the soil are shown in Fig. 1. The wild-type strain (*P. putida* NCIB 9816-4) showed increased adhesion to naphthalene-contaminated soil, whereas the adhesion of the plasmid-cured strain was not affected by contamination with naphthalene. The water contact angles of the two strains are summarized in Table 2. The wild-type strain washed with naphthalene-containing minimal medium had a higher contact angle than the wild-type strain washed with naphthalene-free minimal medium. However, the contact angle of the cured strain was not affected by the presence of naphthalene in the washing medium. We conclude that the uptake of naphthalene during its biodegradation increased the surface hydrophobicity of *P. putida* NCIB 9816-4 through naphthalene adsorption on the cell surface, and this caused the increased cell adhesion to the naphthalene-contaminated soil.

4. Conclusions

A plasmid-cured strain of *P. putida* NCIB 9816-4 that had lost its ability to degrade naphthalene as a result of the loss of the NAH plasmid was obtained. This cured strain was compared with the wild-type for cell adhesion to the soil surface. In the exponential growth phase, the wild-type showed increased adhesion to naphthalene-contaminated soil, whereas the plasmid-cured strain did not. The water contact angle was measured as an index of surface hydrophobicity. The wild-type washed with naphthalene-containing medium had a greater contact angle than that washed with naphthalene-free medium. However, the contact angle of the cured strain was not affected by the presence of naphthalene. Therefore, it can be concluded that naphthalene uptake during naphthalene biodegradation increased the hydrophobicity of *P. putida* NCIB 9816-4 through the adsorption of naphthalene onto the cell surface, which caused increased adhesion to naphthalene-contaminated soil.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2009.07.009.

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