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Interactions between dissolved natural organic matter and adsorbed DNA and their effect on natural transformation of *Azotobacter vinelandii*

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ABSTRACT

To better understand gene transfer in the soil environment, the interactions between dissolved natural organic matter (NOM) and chromosomal or plasmid DNA adsorbed to silica surfaces were investigated. The rates of NOM adsorption onto silica surfaces coated with DNA were measured by quartz crystal microbalance (QCM) and showed a positive correlation with carboxylate group density for both soil and aquatic NOM in solutions containing either 1 mM Ca^{2+} or Mg^{2+}. Increasing total dissolved organic carbon (DOC) concentrations of the NOM solution also resulted in an increase in the adsorption rates, likely due to divalent cation complexation with NOM carboxylate groups and the phosphate backbones of the DNA. The results from Fourier transform infrared spectroscopy (FTIR) for dissolved DNA and DNA adsorbed on silica beads also suggest that adsorption may result from divalent cation complexation with the DNA's phosphate backbone. The interactions, between DNA and NOM, however, did not influence natural transformation of *Azotobacter vinelandii* by DNA. These results suggest that DNA adsorbed to NOM-coated silica or otherwise complexed with NOM remains available for natural transformation in the environment.

1. Introduction

Horizontal gene transfer can decrease or increase environmental contamination through its effects on microbial communities and their rates of adaptation to changes in their environments (Gogarten et al., 2009; Ochman et al., 2000). For example, both the ability to degrade chemical pollutants and resistance to antimicrobials have been known to develop and disseminate through horizontal gene transfer (Barlow, 2009; Springael and Top, 2004). Recent studies have suggested to include ecological exposure to human and animal antimicrobial drugs in risk assessment framework (Crane et al., 2006; Rooklidge, 2004). A more complete understanding of the physical, chemical, and biological factors that control horizontal gene transfer in the environment is likely to suggest new techniques for predicting and controlling contamination.

One mechanism of horizontal gene transfer, natural transformation, requires the uptake of extracellular DNA (Chen et al., 2005; Ochman et al., 2000). This mechanism was not originally considered important within soil environments due to the presence of DNA-degrading enzymes (Levy-Booth et al., 2007). However, there is now compelling evidence for the recalcitrance of DNA resulting from the adsorption to common soil components (Levy-Booth et al., 2007). Furthermore, from the pioneering work of Lorenz, Aardema, and Wackernagel (Lorenz et al., 1988; Lorenz and Wackernagel, 1990) and subsequent studies (e.g. Cai et al., 2006; Crecchio et al., 2005; Crecchio and Stotzky, 1998; Tebbe and Vahjen, 1993), it is apparent that DNA adsorbed to sand, clay minerals, humic acids, and soils remains available for transformation. To understand the role natural transformation plays in the evolution and adaptation of soil microbial communities, the factors that control DNA adsorption and the efficiency of natural transformation by adsorbed DNA need to be characterized. In the current work we focus on the impact of a common soil component, natural organic matter (NOM) (Levy-Booth et al., 2007) on adsorbed DNA and its effect on natural transformation.

The adsorption of DNA to soil particles, including clay minerals and silica substances, may be controlled by van der Waals interactions, electrostatic interactions, and/or cation bridging (Nguyen and Chen, 2007; Nguyen and Elimelech, 2007a; Poly et al., 1999; Sushko et al., 2006). Atomic force microscope imaging and both Monte Carlo and molecular dynamics simulations revealed the roles of van der Waals and electrostatic interactions on deposition of linear plasmid DNA fragments on charged mica surfaces (Sushko et al., 2006). Low temperature scanning electron microscopy measurements showed a compact conformation of linear chromosomal DNA in 5 mM Ca^{2+} solution. Resulting from the suppression of the double layer interactions, there was increased contact area between DNA and clay minerals (Poly et al., 1999). A model that included long-range van der Waals forces

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and double-layer interactions accurately described the adsorption of linear plasmid DNA fragments onto mica (Sushko et al., 2006).

Other studies specifically considered the mechanisms of adsorption of DNA to soil or NOM (Cai et al., 2006; Franchi et al., 1999; Levy-Booth et al., 2007; Lu et al., 2010; Nguyen and Elimelech, 2007b). Electrostatic interactions were found to control plasmid DNA adsorption to humic substances (Levy-Booth et al., 2007) and NOM-coated silica surfaces (Nguyen and Chen, 2007; Nguyen and Elimelech, 2007a). Spectral data obtained using Fourier transform infrared spectroscopy (FTIR) suggested that the DNA phosphate backbone involved in DNA adsorption onto clay minerals or humic acids (Cai et al., 2006; Franchi et al., 1999). Specific binding of Ca^{2+} between DNA phosphate groups and surface charge groups of silica minerals was proposed to explain the strong and irreversible adsorption of plasmid DNA on silica or NOM-coated surfaces as measured by quartz crystal microbalance (QCM) (Nguyen and Chen, 2007; Nguyen and Elimelech, 2007b). In other work, the adsorption of chromosomal or plasmid DNA was controlled by ionic strength and the presence of divalent cations, due to charge screening and cation bridging respectively (Lu et al., 2010; Nguyen and Chen, 2007).

Although the effects of interactions between adsorbed DNA and dissolved unfractionated NOM on natural transformation have not, to our knowledge, been studied, one component of NOM, humic acids, has been studied. DNA bound to humic substances or to mixtures of montmorillonite–humic acid enriched with either Al or Fe transformed competent *Bacillus subtilis*, although the frequency of transformation was 10 times lower than that for free DNA (Crecchio et al., 2005; Crecchio and Stotzky, 1998). The addition of 5% humic acid to chromosomal DNA led to an average of 32% reduction in transformation frequency for *Acinetobacter* sp. strain BD413, but again did not eliminate transformation (Nielsen et al., 2000). The frequency of heat shock transformation for *Escherichia coli* DH5α also decreased from 10^{-2} to 10^{-6} as the concentration of humic acid increased from 0.01 mg/L to 100 mg/L (Tebbe and Vahjen, 1993). This last study may not be directly comparable to natural transformation experiments, because the mechanism of DNA uptake in artificial heat shock transformation is not known and is likely to be different than that used in natural transformation (Bukau et al., 1985; Sun et al., 2009). However, all of these studies indicate that NOM has the potential to attenuate the rate of natural transformation.

The objectives of this work were to study the interactions of dissolved NOM with adsorbed DNA and to probe how these interactions affect the ability of adsorbed DNA to transform bacteria. We investigated DNA adsorption to NOM using QCM for real-time determination of adsorption kinetics of the adsorbed layers and FTIR spectroscopy for identifying specific functional groups of DNA involved in adsorption. Pahokee Peat NOM and Suwannee River NOM were selected as model soil and aquatic NOM. *Azotobacter vinelandii* was chosen as a model soil bacterium because this bacterium is naturally transformable with both plasmid (Doran et al., 1987) and chromosomal DNA (Lu et al., 2010). Transformations were performed with both chromosomal and plasmid DNA in the presence of different concentrations and types of NOM.

2. Materials and methods

2.1. Solution chemistries

Dissolved NOM stocks were prepared Pahokee Peat humic acid reference material (soil NOM, IHSS) and aquatic Suwannee River NOM (SRNOM, IHSS) as previously described (Lu et al., 2010). Briefly, NOM was dissolved in DI water, stirred for 24 h, and filtered through 0.22 μm-pore-size cellulose acetate filters. The solution for coating silica beads and QCM sensors with soil NOM was prepared by diluting the filtrate to 4.6 mg C/L with 10 mM NaCl and filtering through a cellulose acetate filter again. For most other experiments dissolved NOM solutions with different DOC concentrations were diluted from NOM stocks using 20 mM MOPS buffer containing 1 mM Ca^{2+} or 1 mM Mg^{2+}. For QCM experiments, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer and Poly-L-lysine hydrobromide (PLL, Sigma) were also filtered through 0.22 μm-pore-size cellulose acetate filters and stored at 4 °C until use.

2.2. Carboxyl group density determination for SRNOM and soil NOM

Potentiometric titrations with NaOH were employed to determine the carboxyl group density for soil NOM and SRNOM (Collins et al., 1986). A 400-mL solution of each NOM sample was diluted from the stock to a known final concentration of ca. 6 mg C/L with 0.1 M NaCl as the background electrolyte. A 100-mL aliquot of each sample was transferred to a 250 mL three-neck vessel for titration. The sample was adjusted to pH 3.0, purged with nitrogen gas for 20 min to remove CO_2, and then continually stirred under nitrogen atmosphere during the titration. A 25.0-mL microburette filled with carbonate-free 0.005 M NaOH was used to titrate the sample to an endpoint of pH 8. Each titration was completed in 10 to 15 min. At least three replicate titrations for each NOM preparation were conducted. The pH probe was calibrated before each use. Calculation of the carboxyl group concentration from the titration results was based on the electroneutrality equation as described previously (Ritchie and Perdue, 2003). Carboxyl group densities are reported in units of milli-equivalent/g C (mequiv/g).

2.3. Preparation and characterization of DNA

Chromosomal DNA was extracted from *A. vinelandii* wildtype (strain DJ, obtained from Dr. Dennis Dean) as described previously (Lu et al., 2010). Plasmids pBR325 (purchased from DSMZ, Germany) and pDB17 ((Jacobson et al., 1989), obtained from Dr. Dennis Dean) were propagated in *E. coli* strain DH5α and purified using the QIAGEN Plasmid Maxi Kit (QIAGEN, Cat. no, 12163). DNA concentration and size were determined by Nanodrop® ND-1000 (Thermo Scientific) and gel electrophoresis, respectively. The DNA stock was divided into aliquots and stored at −20 °C; it was warmed to room temperature immediately before use and diluted in MOPS buffer with 1 mM Ca^{2+} (chromosomal DNA) or 1 mM Mg^{2+} (plasmid DNA) to a final concentration of 20 mg/L. For FTIR, extracted DNA was further concentrated to 6000 mg/L by ethanol precipitation.

2.4. Transmission electron microscopy (TEM)

Micrographs of silica bead-adsorbed DNA complexes were obtained on a TEM (JEM-2100, JEOL, Tokyo, Japan) operating at 80 kV and energy-dispersive X-ray spectroscopy was processed by Philips CM12 TEM. After DNA adsorption, silica beads were collected by centrifugation at 16,900 *g* for 1 min and resuspended in a few drops of Karnovsky's fixative for 20 min. The suspensions were applied to holey-carbon-coated copper grids of 300 mesh and stained with 2% osmium tetroxide for 15 min.

2.5. Dissolved NOM interaction with adsorbed DNA layers

Real-time monitoring of dissolved NOM deposition onto adsorbed DNA layers was performed with a QCM (D300, Q-sense AB, Gothenburg, Sweden). As described in previous publications (Lu et al., 2010; Nguyen and Elimelech, 2007b; Voinova et al., 1999), this technique allows monitoring of adsorbed wet mass of the adsorbed layers. Before each experiment, the sensor was soaked in 2% Hellmanex II (Hellma GmbH & Co. KG, Müllheim, Germany) detergent for at least 2 h, rinsed thoroughly with deionized water, dried with ultrahigh-purity N_2, and oxidized in a UV chamber (Bioforce Nanosciences, Inc., Ames, IA) for 30 min. The clean sensor was then mounted in a chamber configured as

a radial stagnant point flow cell with the temperature controlled at 25 °C.

A syringe pump in suction mode was employed to inject solutions into the sensor chamber at a speed of 0.1 mL/min. For each experiment, DI water was first introduced into the chamber until a stable baseline was obtained (1 to 2 Hz change in frequency/h). The sensor was equilibrated with electrolyte solution and then DNA was adsorbed to the sensor surface as follows: first a PLL layer was deposited, followed by a soil NOM layer, and then a DNA layer as described before (Lu et al., 2010). To investigate the interaction of dissolved NOM with the adsorbed DNA, we monitored the frequency change when solutions of either soil NOM or SRNOM were introduced into the flow cell. Dissolved NOM solution in the same buffer was injected for 20–30 min until the frequency change leveled off indicating a saturated surface. Dissolved SRNOM solutions had concentrations of 0, 10, or 100 mg C/L, while the soil NOM solution was 6 mg C/L. After each experiment, the system was washed by injecting 2% Hellmanex II for 30 min and then DI water for at least 2 h. When studying the attachment of NOM to DNA layers, the attachment rate of NOM was calculated as the initial slope for the linear portion of the frequency vs. time curve as described (Nguyen and Elimelech, 2007b).

2.6. FTIR spectroscopic measurements of adsorbed DNA

IR absorption spectra were acquired using a Perkin–Elmer Spotlight 400 imaging spectrometer in point mode using a single element HgCdTe detector cooled by liquid nitrogen. Dissolved chromosomal DNA (6000 mg/L), plain silica beads, and DNA-adsorbed silica beads were measured. Background spectra were first acquired using two CaF_2 substrate slides in the absence of sample. A 10 μL sample was then dropped onto one CaF_2 substrate slide and covered with another slide. The background and sample single beam spectra were collected using a 100 μm × 100 μm aperture at 2 cm^{-1} resolution with 100 scans at the same sampling location. A mirror scan speed of 1 cm/s was employed to record data over the free scanning spectral range of the instrument (7200–0 cm^{-1}) and Norton–Beer medium apodization was used during the Fourier transformation. Spectra were analyzed using SpecManager software from Advanced Chemistry Development (ACD Labs, Toronto Ontario). FTIR experiments with NOM-coated silica beads were attempted. However, the peaks from NOM overwhelmed the DNA signals.

2.7. Natural transformation assays

For natural transformations the recipient strain was *A. vinelandii* DJ77, which contains a deletion in the *nifH* gene that prevents nitrogen fixation ((Jacobson et al., 1989), obtained from Dr. Dennis Dean). Competent (transformable) cells of DJ77 were prepared by growth on modified (no molybdenum) Burke's medium plates with addition of 0.013 mol/L ammonium acetate at 30 °C for 2 days and then in liquid media of the same composition, shaking at 170 rpm for 18 to 20 h (Jacobson et al., 1989). The cells were centrifuged and washed twice with MOPS buffer before being resuspended in MOPS buffer with 1 mM Ca^{2+} or Mg^{2+} for transformation with chromosomal or plasmid DNA, respectively. Transformations were conducted by mixing 200 μL of competent cells, 200 μL of MOPS buffer with the specified salt, dissolved SRNOM or soil NOM, and dissolved or adsorbed chromosomal DNA or dissolved plasmid DNA (target mass of 2 μg DNA) sequentially. After incubating at room temperature for 20–30 min, the mixture was diluted and plated in duplicate on selective and non-selective media for transformed and viable cell counts respectively. Selective media were Burke's medium for chromosomal DNA transformations and Burke's medium with 20 μg/mL carbenicillin and 0.013 mol/L ammonium for plasmid DNA, while the non-selective medium was Burke's medium with 0.013 mol/L ammonium. Plates were grown at 30 °C for 3–5 days. Transformation frequency was determined by dividing the number of transformed cells by that of viable cells. There were at least three replicates (parallel transformations) for each condition within a batch of competent cells. Independent biological replicates are reported separately. For transformation with plasmid DNA only dissolved plasmids pDB17 and pBR325 were conducted due to the difficulty in extracting and purifying enough plasmid DNA for adsorption experiments.

3. Results and discussion

3.1. Adsorbed DNA characterization

After the DNA-coating procedure, we were able to observe a layer around silica beads in TEM micrographs (Fig. 1). Energy-dispersive X-ray spectroscopy for multiple locations in this layer confirmed that it contained DNA, based on the presence of phosphorus, for which DNA was the only source in these samples.

3.2. Dissolved NOM interaction with adsorbed DNA layer

Fig. 2a and b shows how the adsorption rates (expressed in Hz/min) of dissolved NOM onto an adsorbed DNA layer were affected by NOM carboxyl group density and NOM concentration at pH 7.2. In the absence of NOM, changes due to interaction of buffer solution with the DNA layer were also plotted (i.e. zero carboxylate group density or zero DOC in Fig. 2a and b, respectively).

An increase in the carboxylate group density for SRNOM and soil NOM from 180 ± 4 meq/g to 210 ± 1 meq/g more than doubled the rate of attachment of NOM on the DNA layer (Fig. 2a). Divalent Ca^{2+} cations were present in our experiments at 1 mM and could form inner-sphere complexes with the NOM carboxylate groups and the phosphate backbone of the chromosomal DNA, as previously suggested (Gavryushov, 2008; Iskrenova-Tchoukova et al., 2010; Kankia, 2000). These strong inner complexes facilitate the adsorption of NOM to the DNA layer. Dissolved NOM interactions with adsorbed plasmid DNA (both pBR325 and pDB17) were assessed in the presence of 1 mM Mg^{2+} because Mg^{2+} instead of Ca^{2+} was required for the detection of natural transformation by plasmid DNA. For Mg^{2+} we suspect that an outer-sphere complexation mechanism would dominate resulting from the strongly bound water molecules within the inner-sphere of the hydration shell. The formation of weak outer-sphere complexes by

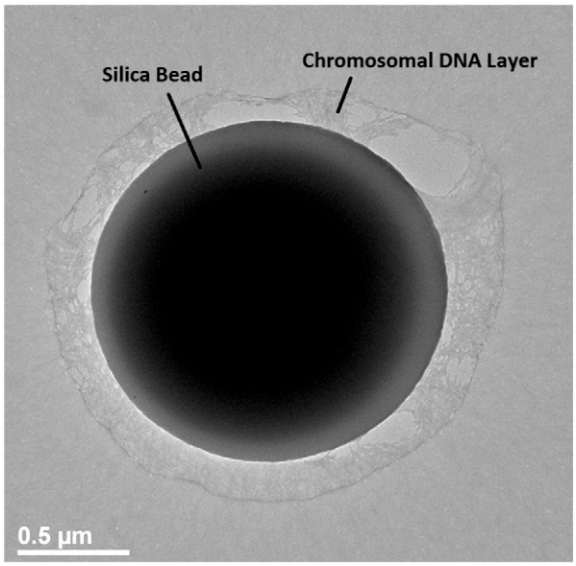

Fig. 1. Characterization of DNA adsorbed on silica beads. Micrograph of a silica bead with adsorbed DNA, as observed by osmium staining and TEM. The diameter of the silica beads based on TEM was ca. 1.6 μm.

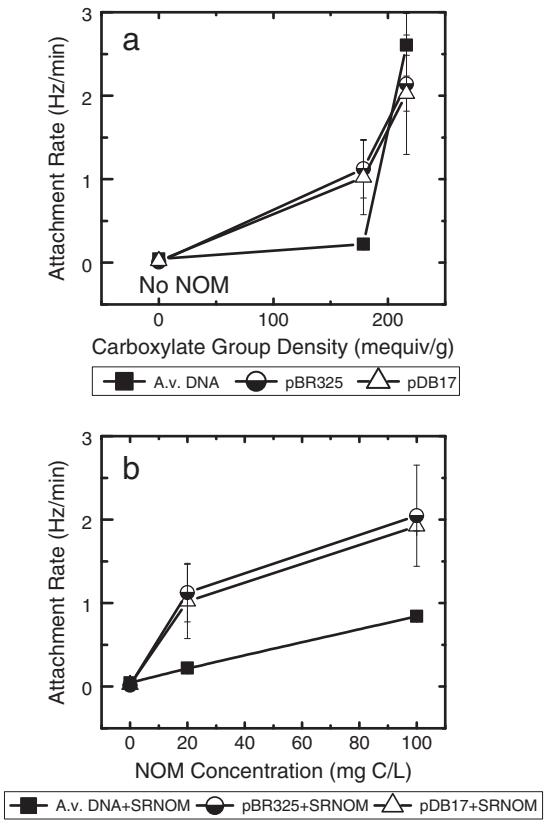

Fig. 2. Adsorption of dissolved NOM to DNA-coated surfaces. a) Attachment rate vs. NOM carboxyl group density at pH 7.2. b) Adsorption rate vs. the concentration of dissolved NOM (mg C/L). The attachment rate was calculated as the initial slope for the linear portion of the frequency vs. time curve at around 2.89 min. The standard deviations were from the replicates of same conditions. 20 mM MOPS buffer containing 1 mM Ca^{2+} was used in chromosomal DNA experiments (A.v. DNA), while plasmid DNA experiments used 20 mM MOPS buffer containing 1 mM Mg^{2+}.

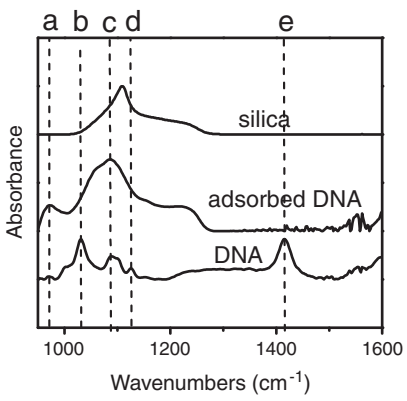

Fig. 3. FTIR spectroscopy of silica (top), DNA adsorbed on silica (middle) and dissolved chromosomal DNA (bottom), all in the presence of 1 mM Ca^{2+}. The observed vibrational modes are 971 (a: DNA backbone absorption peak), 1030 (b: DNA backbone absorption peak), 1087 (c: symmetrical PO_2^- stretching mode), 1126 (d: asymmetric PO_2^- stretching mode), and 1416 (e: base pair absorption peak).

Mg^{2+} and carboxylate groups of NOM has been showed previously (Iskrenova-Tchoukova et al., 2010; Kalinichev and Kirkpatrick, 2007). In both Ca^{2+} and Mg^{2+} cases, however, metal cation interactions with carboxylate moieties from the NOM and the DNA phosphate backbone most likely control DNA adsorption to NOM or NOM adsorption to DNA. By the same reasoning the adsorption rates of dissolved NOM onto adsorbed chromosomal or plasmid DNA layer increased with DOC concentrations (Fig. 2b).

DNA has been shown to form surface complexes at the silica–water interface in previous research (Lu et al., 2010), and our IR spectra confirmed the same based on differences between the IR spectrum for DNA and the spectrum of DNA adsorbed to silica (Fig. 3). For example, the absorption peaks at 970, 1032 and 1086 cm^{-1} correspond to vibrational modes associated with the DNA backbone. Specifically, the 970 and 1032 cm^{-1} absorption peaks are assigned to the DNA backbone and the symmetrical PO_2^- stretch is assigned to the 1086 cm^{-1} peak (Mao et al., 1994). Shifts in peak positions upon adsorption or otherwise significant changes in these peaks are indicative of interactions of the DNA backbone or phosphate groups with the silica surface. Most significantly, we observed the disappearance of the peak at 1126 cm^{-1} (assigned as the asymmetric PO_2^- stretching mode) upon adsorption of DNA to silica (Mao et al., 1994). The IR spectra for chromosomal DNA adsorption to silica spheres were similar to the results shown by Mao et al. for DNA adsorbed to crystalline silica (Mao et al., 1994). Consistent with previous findings, we note the importance of the phosphate groups in adsorption onto silica beads in the presence of Ca^{2+}. These types of interactions have been observed for chromosomal DNA extracted from *B. subtilis* BD1512 on clay surfaces, where the importance of phosphate interaction with clay was observed to be through cationic bridges with the clay planar surface or with positive charges on the clay edges (Khanna et al., 1998). Dissolved Ca^{2+} was also found via IR spectroscopy to bind to both phosphate groups and base pairs of calf thymus DNA (Hackl et al., 2005). We also observed DNA interactions with silica beyond the phosphate backbone. The absorption peak at 1416 cm^{-1}, corresponding to base pairs, diminished after adsorption. In addition to indicating interaction at the base pairs, the loss of intensity of this peak may indicate structural changes in the adsorbed DNA. At a minimum, these results indicate that chromosomal DNA probably interacts with surfaces at multiple sites including its phosphate backbone in the presence of Ca^{2+}. Though the major peaks are identified, there is also some discordance between the spectra of DNA in solution and adsorbed DNA. Recently, it has become clear that the spectra recorded from microscopic samples (here, beads) can show sharp deviations from those recorded in bulk at specific frequencies due to anomalous dispersion and focusing optics (Davis et al., 2010, 2011). While a complete optical model and solution for the spherical case are under development, it is not possible to draw further quantitative conclusions at this point from the data.

3.3. Natural transformation

The transformation frequency for different batches of competent cells showed substantial variation, with differences of up to 5.4×10^{-4} (see for example adsorbed chromosomal DNA transformation frequencies in the absence of NOM in Fig. 4). Similar variation was observed in our previous work (Lu et al., 2010). Because of this we have presented the transformation results by batch of competent cells (i.e., competent cells prepared on the same day from the same plate) rather than averaging all values from the same condition. Each point represents triplicate transformations prepared from the same batch. The transformation frequencies ranged from 1.4×10^{-5} to 5.2×10^{-4} with chromosomal DNA (adsorbed and dissolved) and from 9.1×10^{-7} to 1.1×10^{-4} with dissolved pDB17. These values are of the same magnitude as previous reports looking at different parameters affecting transformation of several *A. vinelandii* strains (Table 1).

Comparing transformation frequencies across NOM types (soil and aquatic, Fig. 4a) and concentrations (Fig. 4b) within a single batch, the differences are relatively small compared to the differences between batches; the largest difference observed for a given type of DNA is 3.6×10^{-4}, and the average difference is only 1.0×10^{-4}. Furthermore, there is no consistent trend in transformation frequencies with NOM type or concentration. Based on these data, although dissolved NOM interacted with adsorbed DNA, this interaction did

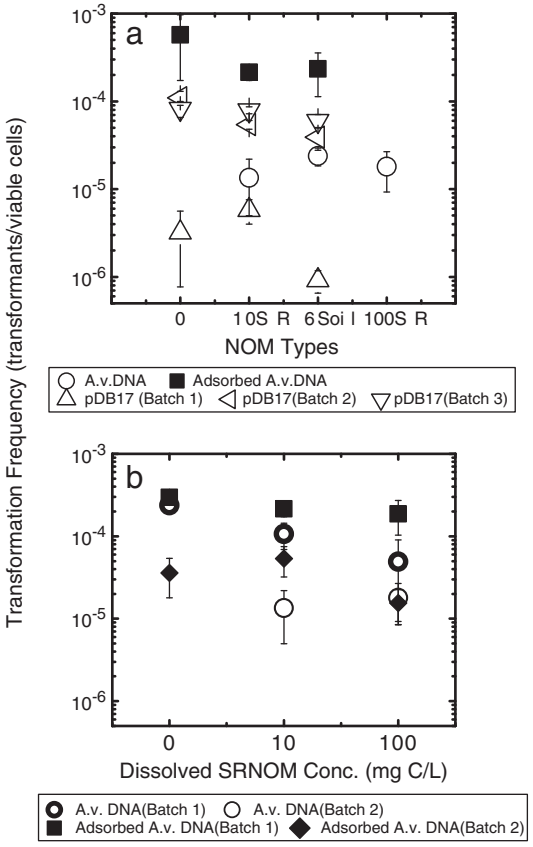

Fig. 4. Effect of NOM on natural transformation of *A. vinelandii*. a) Effect of NOM type on natural transformation; b) Effect of NOM concentration on natural transformation. Transformation experiments were conducted with dissolved or adsorbed chromosomal DNA in MOPS buffer containing 1 mM Ca^{2+} at pH 7.2 and with plasmid pDB17 DNA in MOPS with 1 mM Mg^{2+}. The transformation frequency was calculated as the ratio of transformants to viable cells. Each data point was calculated from at least three replicates from the same batch of competent cells. Independent biological replicates, from separate batches of competent cells, are shown separately due to substantial variation amongst batches. The DNA concentration was approximately 5 μg/mL and DNA was dissolved in solution unless specified.

not prevent or even strongly influence natural transformation of *A. vinelandii*.

The finding that natural transformation was not prevented by the interactions between DNA and NOM is consistent with prior studies that demonstrated that transformation can occur in the presence of humic acids (Crecchio et al., 2005; Crecchio and Stotzky, 1998). However, in those studies a decrease in transformation frequency was observed in the presence of humic acids, while our research shows no difference in the frequency upon addition of NOM. Possible explanations for this discrepancy include at least two that are methodological. First, the microorganism employed to study transformation differed between the studies. Crecchio et al. used the Gram-positive *B. subtilis*, which has a different membrane structure from the Gram-negative *A. vinelandii* used in the current work. Differences in the membrane structure require differences in DNA uptake (Chen and Dubnau, 2004). Second, Crecchio et al. used dissolved DNA as their control, whereas in the current work we compared DNA adsorbed to silica beads with and without NOM, thus removing any general influence of adsorption on transformation frequency. The literature is conflicted on whether adsorption to other surfaces will decrease natural transformation in *B. subtilis* (Gallori et al., 1994; Lorenz et al., 1988).

The amount of chromosomal DNA in our natural transformation tests was based on saturating DNA concentrations for *A. vinelandii* strain UW10 (Doran et al., 1987), raising the concern that the DNA–NOM interactions could be reducing the DNA available for transformation without affecting the observed transformation frequency. Natural transformation experiments were repeated with 0.05 μg/mL DNA (a 100-fold reduction) in the presence of dissolved NOM. At this concentration the transformation frequency would be dependent on the amount of DNA (Doran et al., 1987). We found that even at the lower concentration of DNA, *A. vinelandii* DJ77 was still transformed at similar frequencies with or without 100 mg C/L of dissolved SRNOM, on average 4.1×10^{-5} (with a standard deviation of 1.5×10^{-5}) and 4.1×10^{-5} (with a standard deviation of 4.4×10^{-5}), respectively.

The lack of impact on transformation frequency has at least two potential explanations. It may simply reflect incomplete NOM coverage of the DNA surface, despite a 20-fold excess of NOM. Alternatively, it is possible that different structural elements of the DNA interact with NOM than with the cell surface. Since the initial contact between DNA and a competent cell is not understood at a molecular level (Krüger and Stingl, 2011), it is difficult to assess this possibility. Even for heavily studied model organisms, where some of the proteins interacting with DNA and affecting natural transformation have been identified, such as *Neisseria meningitidis* (PilQ (Assalkhou et al., 2007) and PilG (Lång et al., 2009)) and *B. subtilis* (ComEA (Provvedi and Dubnau, 1999)), the specific mechanism of initial DNA binding is not yet known.

Table 1
Comparison of transformation frequencies for *A. vinelandii*.

Strain	DNA	Transformation frequency [a]	Reference
UW10	Chromosomal	5.0×10^{-5}–7.0×10^{-2}	Doran et al. (1987)
UW	Chromosomal	5.0×10^{-8}–7.0×10^{-3}	Page and Grant (1987)
DJ77	Chromosomal	1.1×10^{-5}–1.1×10^{-3}	Lu et al. (2010)
DJ77	Chromosomal	1.4×10^{-5}–5.2×10^{-4}	This study
ATCC12837	Plasmid	3.0×10^{-4}–5.7×10^{-2}/μg DNA	Glick et al. (1985)
UW10	Plasmid	1.0×10^{-6}–2.0×10^{-3}	Doran et al. (1987)
DJ77	Plasmid	9.1×10^{-7}–1.1×10^{-4}	This study

[a] Transformation frequencies were calculated as numbers of successful transformants divided by numbers of viable cells, normalized to the amount of DNA where noted.

4. Environmental implications

As a mechanism of gene transfer, natural transformation plays an important role in the evolution of soil microbial communities and thereby influences their ability to degrade contaminants and to resist antimicrobials. This study demonstrated interactions between extracellular DNA and dissolved NOM via the phosphate groups in the DNA backbone. However, these interactions did not reduce the availability of the DNA for transformation of *A. vinelandii*, supporting the idea that adsorbed DNA provides an environmental reservoir of genetic potential for soil microorganisms.

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