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Natural Transformation of *Acinetobacter calcoaceticus* by Plasmid DNA Adsorbed on Sand and Groundwater Aquifer Material

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It is known that plasmid DNA and linear duplex DNA molecules adsorb to chemically purified mineral grains of sand and to particles of several clay fractions. It seemed desirable to examine whether plasmid DNA would also adsorb to nonpurified mineral materials taken from the environment and, particularly, whether adsorbed plasmid DNA would be available for natural transformation of bacteria. Therefore, microcosms consisting of chemically pure sea sand plus buffered $CaCl_2$ solution were compared with microcosms consisting of material sampled directly from a groundwater aquifer (GWA) plus groundwater (GW) with respect to the natural transformation of *Acinetobacter calcoaceticus* by mineral-associated DNA. The GWA minerals were mostly sand with inorganic precipitates and organic material plus minor quantities of silt and clay (illite and kaolinite). The amount of plasmid DNA which adsorbed to GWA (in GW) was about 80% of the amount which adsorbed to purified sand (in buffered $CaCl_2$ solution). Plasmid DNA adsorbed on sand transformed *A. calcoaceticus* significantly less efficiently than did plasmid DNA in solution. In contrast, the transformation by sand-adsorbed chromosomal DNA was as high as that by DNA in solution. In GWA/GW microcosms, the efficiency of transformation by chromosomal DNA was similar to that in sand microcosms, whereas plasmid transformation was not detectable. However, plasmid transformants were found at a low frequency when GWA was loaded with both chromosomal and plasmid DNA. Reasons for the low transformation efficiency of plasmid DNA adsorbed to mineral surfaces are discussed. Control experiments showed that the amounts of plasmid and chromosomal DNA desorbing from sand during incubation with a cell-free filtrate of a competent cell suspension did not greatly contribute to transformation in sand microcosms, suggesting that transformation occurred by direct uptake of DNA from the mineral surfaces. Taken together, the observations suggest that plasmid DNA and chromosomal DNA fragments which are adsorbed on mineral surfaces in a sedimentary or soil habitat may be available (although with different efficiencies for the two DNA species) for transformation of a naturally competent gram-negative soil bacterium.

The binding of plasmid and chromosomal DNA to mineral surfaces was recently studied with chemically purified sand and clean preparations of clay minerals, including bentonite, kaolinite, and montmorillonite (11, 17, 24). It was found that the association of polyanion DNA to these surfaces with a net negative charge was mediated by monovalent cations and even more efficiently by divalent cations (17, 24). Upon adsorption to these minerals, chromosomal DNA and plasmid DNA became highly resistant to nucleolytic degradation (11, 15, 17, 24). In an experimental approach to determine the stability of extracellular DNA in terrestrial bacterial habitats, the persistence of plasmid DNA introduced into various natural nonsterile soils was examined (21). It was found that intact plasmid DNA could be extracted from the soils up to 60 days after introduction. This DNA was active in transformation (21, 23). It was assumed that the association of DNA with soil minerals and other particulates contributed to the persistence of plasmid DNA. However, experimentally, it has not yet been examined whether plasmid DNA molecules bound on mineral surfaces can still be taken up directly by cells in the course of natural transformation in the solid-liquid interphase. Experimental studies so far have focused only on the transformation by chromosomal (linear) DNA fragments associated with chemically purified sand material or clay fractions. In particular, with respect to a possible gene transfer by free DNA in bacterial habitats, it seemed desirable to examine the adsorption of plasmid and also of chromosomal DNA to "dirty" mineral material sampled from the environment and to determine whether or not the DNA bound on this material still has transforming activity. For this purpose, we used *Acinetobacter calcoaceticus*, which has been characterized in some detail with respect to plasmid transformation (2, 14). The microcosms used were flowthrough column systems consisting of chemically pure sand in Tris-buffered $CaCl_2$ solution or of sterilized material from a natural groundwater aquifer (GWA) in groundwater (GW).

MATERIALS AND METHODS

Bacteria and plasmids. *A. calcoaceticus* BD4 (prototrophic) was used for the isolation of chromosomal DNA and BD413 (*trpE27*) was used for transformation (10). Both strains were obtained from the Deutsche Sammlung für Mikroorganismen, Göttingen, Germany. Plasmid pKT210 is a derivative of RSF1010 carrying a chloramphenicol resistance (Cm^r) determinant (1). *Escherichia coli* DH5α (7) was used for isolation of pKT210 DNA.

Media. *A. calcoaceticus* was grown in LB broth (4) or LB broth with 20 μg of chloramphenicol ml^{-1}. For the selection of Cm^r plasmid transformants, LB agar (1.5%, wt/vol) plus 20 μg of chloramphenicol ml^{-1} was used, and for selection of chromosomal trp^+ transformants, minimal agar was used

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(3). Viable counts were determined after serial dilution and plating on LB agar. All plates were incubated overnight at 30°C. Transformations were done with competent cells suspended in transformation buffer (TB; 25 mM Tris-HCl [pH 7.5], 5 mM $CaCl_2$) or in GW.

Isolation and quantitation of DNA. Chromosomal DNA was isolated from BD4 by the method of Marmur (19). The mean fragment size was approximately 35 kb, as determined by agarose gel electrophoresis.

For the large-scale preparation of plasmid DNA with minimum contamination by plasmid multimers, the following procedure was applied. Plasmid pKT210 DNA was subjected to agarose gel electrophoresis in a 0.7% agarose gel. The monomeric supercoil plasmid DNA band was cut out and the DNA was isolated (28). A 50-μl suspension of *E. coli* DH5α (10^{11} cells ml^{-1}) in sterile water was transformed by electroporation (25 μF, 12.5 kV cm^{-1}, 200 Ω; Gene Pulser; Bio-Rad, Munich, Germany) by the method of Dower et al. (5) with 1 μl of isolated monomeric plasmid DNA. A clone containing pKT210 was grown in 100 ml of LB plus chloramphenicol for the preparation of plasmid DNA by a combined procedure that included the rapid boiling method (8) and the Qiagen purification system (Diagen, Düsseldorf, Germany). [^{3}H]thymidine-labeled pKT210 DNA was prepared as follows. *E. coli* DH5α(pKT210) was aerated at 30°C in LB broth plus chloramphenicol. At a titer of 2.1×10^8 ml^{-1}, deoxyadenosine (250 μg ml^{-1}) (to improve incorporation of thymidine) and [*methyl*-^{3}H]thymidine (74 kBq ml^{-1}) were added. At a titer of 2×10^9 ml^{-1}, cells were harvested and the plasmid DNA was isolated. The specific radioactivity of the preparation was 1.4×10^4 dpm μg of DNA^{-1}. The radioactivity was determined in a Betamatic scintillation counter (Kontron, Eding, Germany) with Optifluor scintillation fluid (Canberra-Packard, Frankfurt am Main, Germany). Nonradioactive DNA was quantitated by its fluorescence with the bisbenzimidazole dye Hoechst H33258 in an RF540 spectrofluorimeter (Shimadzu, Kyoto, Japan) as described previously (12).

Competent cells. *A. calcoaceticus* BD413 was grown to competence and stored at −80°C as described previously (14). One-milliliter aliquots were thawed at 23°C in a water bath, centrifuged (2 min at 13,000 × *g*), and washed once in TB or GW. Cells were finally suspended in TB or GW (approximately 10^9 CFU ml^{-1}). Naturally competent cells of *A. calcoaceticus* require divalent cations for DNA uptake, and this requirement is best met by Ca^{2+} (14).

Transformation in liquid. Suspensions of competent cells (0.2 ml) were incubated with pKT210 DNA (usually 5 μg ml^{-1}) or chromosomal DNA (usually 10 μg ml^{-1}) for 30 min at 23°C. DNA uptake was terminated by the addition of DNase I (100 μg ml^{-1}, final concentration). After 10 min at 30°C, cells were plated on minimal agar for the selection of prototrophic transformants and on LB agar for the determination of viable counts. In transformation experiments with plasmid DNA, cells, after DNase I treatment, were allowed to express the plasmid marker by the addition of 0.2 ml of twofold-concentrated LB broth to the transformation mixture and incubation at 30°C for 90 min before cells were plated on LB agar plus chloramphenicol. Transformation frequencies are expressed as *trp*$^+$ or Cm^r transformants per viable count. The reversion frequency of competent *A. calcoaceticus* BD413 cells to prototrophy (*trp*$^+$) was $< 8 \times 10^{-9}$, and the mutation frequency to chloramphenicol resistance was $< 6 \times 10^{-9}$. Transformation experiments were repeated at least once. The values differed by no more than 10%.

Mineral material and GW. The material for the sand microcosms was chemically pure sea sand purchased from Merck, Darmstadt, Germany. This material consists of 86% (wt/wt) quartz, 12% feldspar, and 2% heavy minerals (15). The sand was heated to 1,600°C and acid washed. The grain size is 0.1 to 0.3 mm. GWA material was recovered from a depth of 150 m near Hamburg, Germany. Sieved material of a grain size distribution similar to that of the sea sand was used (0.063 to 0.63 mm). Besides sand, the GWA material contained about 0.9% silt and 0.5% clay (kaolinite and illite). The particles had a brownish covering of varying intensity. The total carbon content of GWA material was 0.06% (wt/wt). GWA material was sterilized by dry heat (180°C, 5 h) before use.

GW was collected from a drinking-water well near Langen, Germany. It contained Ca^{2+} (998 μM), Mg^{2+} (187 μM), Na^+ (200 μM), K^+ (56 μM), Fe^{2+} and Fe^{3+} (19.5 μM), and Mn^{2+} (0.7 μM) (24a). The pH was 7.0, and the conductivity was 258 μS cm^{-1} (at 23°C). GW was sterilized by autoclaving for 20 min at 121°C.

Microcosms. The previously described flowthrough glass column system was used as the microcosm (13, 15). The autoclaved columns were aseptically filled with 0.7 g of mineral material (interstitial volume of the bed, 0.2 ml) and equilibrated for 15 min with TB or GW. Then columns were loaded with DNA (usually 1 to 2 μg in 0.2 ml) as described before (13, 15) and incubated for 1 h (plasmid DNA) or 2 h (chromosomal DNA). Nonadsorbed plasmid DNA was quantitatively eluted with TB or GW from the microcosm within 10 min; chromosomal DNA was eluted within 25 min (elution rate, 12 ml h^{-1}). When two types of DNA were loaded on GWA (see Table 3), the plasmid DNA was applied first.

Transformation in microcosms. A competent cell suspension in TB or GW (0.2 ml) was applied to DNA-loaded microcosms. After 30 min at 23°C, the contents of the microcosm were transferred to a tube containing 0.6 ml of LB and 0.2 ml of twofold-concentrated LB and DNase I (100 μg ml^{-1}, final concentration) for transformation with plasmid DNA. The tube was vigorously vortexed for 15 s. After 90 min at 30°C, platings for the determination of transformants and viable cells were done. In experiments with chromosomal DNA, the contents of the microcosm were vortexed in minimal medium plus DNase I and platings were done after 10 min at 30°C.

In some experiments the amount of DNA desorbing from mineral material of sand during transformation in microcosms was determined. For this purpose, a DNA-loaded microcosm was incubated with the cell-free filtrate of a competent cell suspension. This filtrate was obtained after incubation of cells of a competent culture in TB for 30 min at 23°C and subsequent centrifugation and passage of the supernatant through a 0.2-μm cellulose acetate filter (Schleicher & Schuell, Göttingen, Germany). After incubation with the DNA-loaded sand, the filtrate was eluted from the microcosm. This was done by pipetting 0.2 ml of TB on top of the sand bed and removing the filtrate by gravitational flow. The transforming activity of plasmid or chromosomal DNA in the effluent was determined after incubation of 0.1 ml of the eluted filtrate with 0.1 ml of competent cells in TB (2×10^9 ml^{-1}) for 30 min and further treatment by standard procedures for liquid transformation. In another approach, plasmid DNA-loaded sand was continuously eluted (8 ml h^{-1}) for 30 min with a cell-free filtrate of a competent cell suspension in TB. The transforming activity of released DNA was determined in the effluent fractions (0.67 ml). The

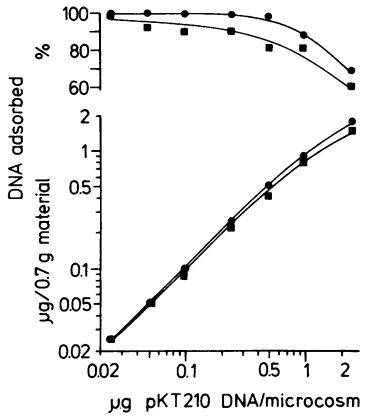

FIG. 1. Isotherms of plasmid pKT210 DNA adsorption to sand (●; bulk phase: TB) and to GWA material (■; bulk phase: GW) at 23°C.

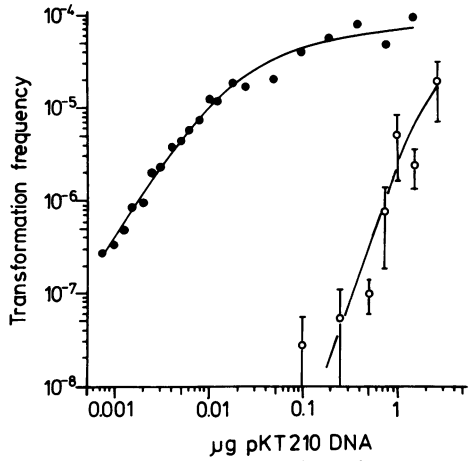

FIG. 2. Dependence of transformation of competent *A. calcoaceticus* in liquid (●; in 0.2 ml of TB; this volume corresponds to the interstitial volume of the sand bed in the microcosm) and in sand microcosms (○; 0.7 g of sand in TB) on the concentration of pKT210 DNA. For details, see Materials and Methods.

transformation experiments with microcosms were done in duplicate. The values differed by 11 to 25%.

RESULTS

Adsorption of plasmid DNA to minerals. The adsorption of plasmid DNA to sand minerals is facilitated by divalent cations (24). For quantitation of plasmid DNA binding to the minerals used in this study, a preparation of [^{3}H]thymidine-labeled plasmid pKT210 was employed. Figure 1 shows that 69% of 2.5 µg of plasmid DNA applied to a microcosm containing 0.7 g of sand in buffered $CaCl_2$ solution (TB) adsorbed within 60 min at 23°C. The nonadsorbed DNA was completely eluted from the microcosm with 1 ml of TB (corresponding to five times the interstitial volume of the sand bed). In a microcosm with GWA material as the substratum and GW as the bulk phase, plasmid DNA adsorbed only slightly less efficiently (Fig. 1); adsorption ranged from 90% (0.1 µg of DNA applied) to 59% (2.5 µg applied). This somewhat minor adsorption is probably because GW contained divalent cations only at a total concentration of 1.2 mM (see Materials and Methods), which is suboptimal for plasmid DNA adsorption to sand minerals (24). Addition of $MgCl_2$ (5 mM) to GW improved DNA adsorption to the level obtained in the sand-TB system (data not shown). The presence on GWA particles of inorganic precipitates of Fe, Ca, Mn, K, Al, and Cu (identified by chemical analysis and by scanning electron microscopy and energy-dispersive X-ray scanning of GWA material; data not shown) and perhaps organic material did not impede the formation of an association with plasmid DNA. Plasmid DNA adsorbed on GWA resisted desorption during extended elution over a period of 1 week with a salt solution prepared on the basis of the chemical analysis of GW (data not shown).

Transformation on sand. Naturally competent cells of *A. calcoaceticus* have been shown to be transformable by plasmid DNA. The plasmids were naturally occurring plasmids and recombinant derivatives thereof, including RSF1010 (2, 25), R300B (25), and a pACYC184 derivative (2). In previous experiments the highest transformation frequencies of *A. calcoaceticus* were obtained in complete medium and in Tris-HCl (pH 7.5) plus $CaCl_2$ (0.5 to 3 mM [14]). The dependence of *A. calcoaceticus* transformation in buffered $CaCl_2$ solution (TB) on the pKT210 DNA concentration was between a one-hit and a two-hit process (Fig. 2; the tangent of the inclination angle is 1.5). The DNA saturation of plasmid transformation (at 1.3×10^9 CFU ml^{-1}) was obtained at concentrations of >0.02 µg of DNA 0.2 ml^{-1} (this volume corresponds to the interstitial volume of the sand bed in the microcosms). The DNA was prepared in a way which minimizes the presence of plasmid DNA dimers (see Materials and Methods). The following experiments were performed with this plasmid DNA preparation.

Transformation with various amounts of plasmid DNA adsorbed on sand in TB was obtained in the microcosm (Fig. 2). The efficiency was lower and the dependence on DNA concentration was steeper than in liquid transformations (Fig. 2). Also, the DNA saturation of transformation in the sand microcosm was shifted toward a DNA concentration that was about 100 times higher than in liquid transformations.

In control experiments, microcosms containing plasmid DNA-loaded sand were incubated for 30 min with the cell-free filtrate of a competent cell suspension instead of the cell suspension itself. The DNA desorbing from sand during this incubation period and present in the microcosm effluent was quantitated by a separate transformation assay. With this effluent, the number of transformants obtained was only about 1/10 of the number directly found on sand in the microcosm (Table 1, experiment A). In another experiment, the plasmid DNA-loaded sand was eluted (8 ml h^{-1}) for 30 min with a cell-free filtrate of a competent cell suspension in TB (six effluent fractions of 0.67 ml each). The transforming activity in the fractions ranged from 0.9×10^{-7} to 2.3×10^{-7}. After 30 min of elution, the transforming activity obtained in the microcosm was still 7.1×10^{-7}. These data indicate that transformation in the sand microcosm occurred mainly by direct uptake of plasmid molecules from the mineral surfaces and that a minor portion of cells was transformed by desorbed DNA in the bulk phase.

In contrast to the observations made with plasmid DNA, the transformation efficiency of chromosomal DNA on sand was as high as in liquid (Table 1, experiment B). Transformation by DNA desorbing from sand during 30 min of incubation with a cell-free filtrate of a competent cell sus-

TABLE 1. Transformation with plasmid pKT210 DNA (Cm^r) and chromosomal DNA (trp^+) in sand microcosms and in liquid

Expt (DNA)	μg of DNA		Selected marker	Transformation frequency (transformants/viable count)[a]
	Applied	Adsorbed		
A				
Sand microcosm (plasmid)	1.0	0.83	Cm^r	4.4×10^{-7}
Effluent[b]			Cm^r	3.7×10^{-8}
Sand microcosm (plasmid)	0.5	0.49	Cm^r	1.4×10^{-7}
Effluent[b]			Cm^r	$<2.4 \times 10^{-8}$
B				
Sand microcosm (chromosomal)	2.0	1.57	trp^+	5.7×10^{-4}
Liquid	2.0		trp^+	5.2×10^{-4}
C				
Sand microcosm (chromosomal)	2.0	1.55	trp^+	5.7×10^{-4}
Effluent 1[b]			trp^+	8.4×10^{-5}
Effluent 2[c]			trp^+	3.8×10^{-5}

[a] Transformation was performed at 23°C for 30 min in TB; for details, refer to Materials and Methods.
[b] The microcosm was incubated with 0.2 ml of the cell-free filtrate of a competent cell suspension for 30 min at 23°C. The liquid was eluted and used to transform competent *A. calcoaceticus* cells (see Materials and Methods).
[c] After elution, the microcosm was again incubated with 0.2 ml of a cell-free filtrate of a competent suspension for 30 min at 23°C. The liquid was once again eluted and used to transform competent *A. calcoaceticus* cells.

pension was again low in comparison with the transformation frequency obtained directly in the microcosm (Table 1, experiment C), supporting the interpretation of a direct uptake of DNA from mineral grains.

Transformation on GWA minerals. GWA microcosms were loaded with 1 μg of pKT210 DNA dissolved in GW (0.2 ml). Plasmid transformation of *A. calcoaceticus* cells in the microcosm was not detectable (transformation frequency, $<6 \times 10^{-9}$; limit of detection), whereas transformation with an equivalent amount of plasmid DNA was efficient in GW (2.5×10^{-6}). In contrast, chromosomal DNA adsorbed on GWA transformed cells (Table 2). The transformation frequency was somewhat lower than in liquid, either TB or GW (Table 2), or on sand (cf. Tables 1, experiments B and C, and 2). The strong difference between the transformation efficiencies of linear DNA (chromosomal) and plasmid DNA (which is highly enriched for the selected Cm^r marker compared with the trp^+ marker in chromosomal DNA) was further examined by using GWA on which both DNAs were loaded. Table 3 confirms that adsorbed chromosomal DNA transformed almost as efficiently as in liquid, whereas transformation by plasmid DNA, which was solely adsorbed on GWA material, dropped below the detection limit. However, when plasmid DNA and chromosomal DNA were both adsorbed on the same GWA material, plasmid transformants were repeatedly obtained, although at a frequency of only 3.7×10^{-8} (Table 3). The experiment presented in Table 3 suggests that the reason for the specifically low efficiency of plasmid transformation on GWA material (and probably on sand [Table 1, experiment A, and Fig. 1]) compared with the transformation by chromosomal DNA on the minerals may be related to the different physicochemical properties of plasmid DNA molecules compared with linear duplex DNA or with the specific mode of plasmid transformation (see Discussion).

TABLE 2. Transformation of competent *A. calcoaceticus* with chromosomal DNA in GWA/GW microcosms and in liquid

Expt	μg of DNA		Transformation frequency (10^{-4} trp^+ cells/viable cell)
	Applied	Adsorbed[a]	
GWA/GW microcosm	1.0	0.56	0.29
	2.0	1.36	1.30
GW (0.2 ml)	2.0		3.20
Buffer (0.2 ml in TB)	2.0		6.70

[a] The amount of adsorbed DNA was calculated following fluorimetric determination of the DNA eluting from the microcosm at the end of the period for DNA adsorption.

DISCUSSION

The GWA material and the GW used in the microcosm studies were recovered from the environment. For the purpose of the experiments, the samples were sterilized, which may have modified the surfaces of the minerals and some constituents of the GW. In the presence of GW, the adsorption capacity of this material for plasmid DNA molecules and linear duplex DNA was almost as high as the DNA binding capacity of a model sediment system consisting of chemically purified sea sand and a buffered solution of $CaCl_2$ (Fig. 1; Tables 1 and 2). This indicates that the presence of inorganic precipitates and perhaps organic components on the mineral material does not hinder DNA from adsorbing to the surfaces. Furthermore, the results demonstrate that the ionic milieu of water sampled from the environment is favorable for the association of plasmid and linear duplex DNA with the minerals. The presence of Ca^{2+} (0.998 mM) and Mg^{2+} (0.187 mM), which are about 100 times more effective than monovalent cations in promoting DNA binding to quartz, clay, and other minerals (15, 17, 24), has probably contributed mainly to DNA binding in GW. Adsorption of linear duplex DNA to sediments and soils has also been observed in other studies (6, 20).

DNA bound on GWA material or on sand was active in transformation of naturally competent cells of *A. calcoaceticus*. However, the binding to minerals decreased the transforming activity of plasmid DNA much more strongly than the transforming activity of chromosomal DNA (Fig. 2;

TABLE 3. Transformation with plasmid pKT210 DNA and chromosomal DNA in a GWA/GW microcosm and in GW[a]

Expt	μg of plasmid DNA		μg of chromosomal DNA		Selected marker	Transformation frequency
	Applied	Adsorbed	Applied	Adsorbed		
GWA/GW microcosm			2.0	1.34	*trp*$^+$	1.1×10^{-4}
	1.0	0.70			Cm^r	$<6.0 \times 10^{-9}$
	1.0	0.88	2.0	1.08	*trp*$^+$	2.6×10^{-4}
					Cm^r	3.7×10^{-8}
GW			2.0		*trp*$^+$	9.3×10^{-4}
	1.0		2.0		*trp*$^+$	3.7×10^{-4}
					Cm^r	3.1×10^{-5}

[a] For details, see footnotes to Table 1 and Materials and Methods.

Tables 2 and 3). This observation was also made with preparations of another plasmid and another naturally competent soil bacterium (22). It is a novel finding. The observation was made because quantitative transformations with DNA in liquid and DNA adsorbed on minerals in the microcosm system were compared (Fig. 2; Tables 1 [experiment B], 2, and 3). The DNA concentration dependence of plasmid transformation, which was between a one-hit and a two-hit process in liquid, changed to about a three-hit process on sand (Fig. 2). On GWA, transformation by plasmid DNA occurred at a low frequency only when chromosomal DNA was also present on the mineral surfaces (Table 3). The reason for the strong decrease of the transforming activity of plasmid DNA upon association with mineral surfaces is not clear. Poor transformation of a *Vibrio* sp. by plasmid DNA was also observed in sterilized marine sediment microcosms (9), whereas chromosomal transformation of a *Vibrio* sp. and *Pseudomonas stutzeri* ZoBell occurred at high frequency in marine or freshwater sediments (9, 26, 27). Perhaps circular and particularly supercoiled molecules bind to sites on mineral surfaces where removal by cells in the course of DNA uptake is less easy than removal of linear duplex DNA molecules (such as the chromosomal DNA). It is also conceivable that the chance of a cell finding a DNA molecule on the mineral surface is proportional to the target size of the DNA molecule. The outer surface of a DNA molecule becomes smaller upon folding. Thus, the target size may be considered greater for a linear than for a supercoiled DNA molecule of the same mass. Another reason for the decrease of the transformation frequency by plasmid DNA adsorbed on a mineral surface may be related to the fact that *A. calcoaceticus* requires more than a single plasmid molecule for successful transformation (Fig. 2). It is likely that the diffusional movement of DNA molecules and cells in the three dimensions of a solvent provides a higher probability of producing active collisions (between, e.g., a cell and two plasmid molecules) than the probability provided when the DNA molecules are fixed by adsorption to the two-dimensional sphere of a mineral surface and when only the cell can move. Clearly, additional experimentation is required to settle this point. Such studies may also provide insight into the fact that the presence of chromosomal DNA on the GWA material improved transformation by plasmid DNA.

The observation that DNA associated with GWA material transformed the soil bacterium *A. calcoaceticus* suggests that chromosomal and even plasmid DNA on these mineral surfaces may be available for uptake by cells, although with different efficiencies. Previously, the uptake of chromosomal DNA from chemically purified sand by *Bacillus subtilis* and *P. stutzeri* was shown (13, 16). In other experiments it was found that during adsorption chromosomal DNA was distributed between purified sand and clay minerals (17). Sand-adsorbed (13, 16) as well as clay-adsorbed (11, 18) chromosomal DNA is active in transformation. Clay-adsorbed chromosomal DNA seems to be taken up by *B. subtilis* directly from the mineral surface (18). As outlined in the introduction, the binding of DNA to mineral surfaces is accompanied by an increased protection of the DNA against DNases. As shown recently, the binding of plasmid DNA to GWA material increased the resistance against DNase I about 1,000-fold compared with DNA in solution (22).

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REFERENCES

1. **Bagdasarian, M., R. Lurz, B. Rückert, F. C. H. Franklin, M. M. Bagdasarian, J. Frey, and K. N. Timmis.** 1981. Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in *Pseudomonas*. Gene **16**:237–247.
2. **Bruns, S., K. Reipschläger, M. G. Lorenz, and W. Wackernagel.** 1992. Characterization of natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter calcoaceticus* by chromosomal and plasmid DNA, p. 115–126. *In* M. J. Gauthier (ed.), Gene transfers and environment. Springer-Verlag KG, Berlin.
3. **Cruze, J. A., J. T. Singer, and W. R. Finnerty.** 1979. Conditions for quantitative transformation in *Acinetobacter calcoaceticus*. Curr. Microbiol. **3**:129–132.
4. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16**:6127–6145.
6. **Greaves, M. P., and M. J. Wilson.** 1969. The adsorption of nucleic acids by montmorillonite. Soil Biol. Biochem. **1**:317–323.
7. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
8. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**:193–197.
9. **Jeffrey, W. H., J. H. Paul, and G. J. Stewart.** 1990. Natural transformation of a marine *Vibrio* species by plasmid DNA. Microb. Ecol. **19**:259–268.
10. **Juni, E., and A. Janik.** 1969. Transformation of *Acinetobacter calco-aceticus* (*Bacterium anitratum*). J. Bacteriol. **98**:281–288.

11. **Khanna, M., and G. Stotzky.** 1992. Transformation of *Bacillus subtilis* by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA. Appl. Environ. Microbiol. **58:**1930–1939.
12. **Labarca, C. A., and K. Paigen.** 1980. A simple, rapid and sensitive DNA assay procedure. Anal. Biochem. **102:**344–352.
13. **Lorenz, M. G., B. W. Aardema, and W. Wackernagel.** 1988. Highly efficient genetic transformation of *Bacillus subtilis* attached to sand grains. J. Gen. Microbiol. **134:**107–112.
14. **Lorenz, M. G., K. Reipschläger, and W. Wackernagel.** 1992. Plasmid transformation of naturally competent *Acinetobacter calcoaceticus* in non-sterile soil extract and groundwater. Arch. Microbiol. **157:**355–360.
15. **Lorenz, M. G., and W. Wackernagel.** 1987. Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. Appl. Environ. Microbiol. **53:**2948–2952.
16. **Lorenz, M. G., and W. Wackernagel.** 1990. Natural genetic transformation of *Pseudomonas stutzeri* by sand-adsorbed DNA. Arch. Microbiol. **154:**380–385.
17. **Lorenz, M. G., and W. Wackernagel.** 1992. DNA binding to various clay minerals and retarded enzymatic degradation of DNA in a sand/clay microcosm, p. 103–113. *In* M. J. Gauthier (ed.), Gene transfers and environment. Springer-Verlag KG, Berlin.
18. **Lorenz, M. G., and W. Wackernagel.** Transformation as a mechanism for bacterial gene transfer in soil and sediment—studies with a sand/clay microcosm and the cyanobacterium *Synechocystis* OL50. *In* Proceedings of the Sixth International Symposium on Microbial Ecology (ISME-6), in press.
19. **Marmur, J.** 1961. A procedure for isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3:**208–218.
20. **Ogram, A., G. S. Sayler, D. Gustin, and R. J. Lewis.** 1988. DNA adsorption to soils and sediments. Environ. Sci. Technol. **22:**982–984.
21. **Romanowski, G., M. G. Lorenz, G. Sayler, and W. Wackernagel.** 1992. Persistence of free plasmid DNA in soil monitored by various methods, including a transformation assay. Appl. Environ. Microbiol. **58:**3012–3019.
22. **Romanowski, G., M. G. Lorenz, and W. Wackernagel.** Plasmid DNA in a groundwater aquifer microcosm: adsorption, DNase resistance and natural genetic transformation of *Bacillus subtilis*. Mol. Ecol., in press.
23. **Romanowski, G., M. G. Lorenz, and W. Wackernagel.** Unpublished data.
24. **Romanowski, G., M. G. Lorenz, and W. Wackernagel.** 1991. Adsorption of plasmid DNA to mineral surfaces and protection against DNase I. Appl. Environ. Microbiol. **57:**1057–1061.

24a. **Rötlich, H.** Personal communication.

25. **Singer, J. T., J. J. van Tuijl, and W. R. Finnerty.** 1986. Transformation and mobilization of cloning vectors in *Acinetobacter* spp. J. Bacteriol. **165:**301–303.
26. **Stewart, G. J., and C. D. Sinigalliano.** 1990. Detection of horizontal gene transfer by natural transformation in native and introduced species of bacteria in marine and synthetic sediments. Appl. Environ. Microbiol. **56:**1818–1824.
27. **Stewart, G. J., C. D. Sinigalliano, and K. A. Garko.** 1991. Binding of exogenous DNA to marine sediments and the effect of DNA/sediment binding on natural transformation of *Pseudomonas stutzeri* strain ZoBell in sediment columns. FEMS Microbiol. Ecol. **85:**1–8.
28. **Weichenhan, D.** 1991. Fast recovery of DNA from agarose gels by centrifugation through blotting paper. Trends Genet. **7:**109.