Natural Transformation of Acinetobacter sp. Strain BD413 with Cell Lysates of Acinetobacter sp., Pseudomonas fluorescens, and Burkholderia cepacia in Soil Microcosms

KAARE M. NIELSEN,1* KORNELIA SMALLA,2 AND JAN D. VAN ELSAS3

Unigen and Department of Botany, Norwegian University of Science and Technology, 7491 Trondheim, Norway1; Institut für Biochemie und Pflanzenvirologie, BBA, 38104 Braunschweig, Germany2; and Research Institute for Plant Protection, BBA, IPO-DLO, 6700 GW Wageningen, The Netherlands3

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Horizontal gene transfer can be an important mechanism for bacterial adaptation to changing environments (14, 23). Molecular studies have shown that many chromosomal genes from bacteria have a mosaic pattern (2, 5, 15, 19) presumably as a result of recombination with DNA from heterologous sources (10, 30, 31). The mechanisms causing these patterns are, however, seldom known. Natural transformation provides a mechanism of gene transfer that enables competent bacteria to generate genetic variability by “sampling” of DNA present in their surroundings. However, only a few cases of interspecies transfer of chromosomal genes between environmental isolates have been shown to occur by natural transformation. In fact, Majewski and Cohan (28) investigated barriers to transfer of chromosomal genes between environmental isolates of their relatedness based on natural transformation of an Acinetobacter species in vitro. Moreover, interactions of DNA with proteins or other cellular substances and variable methylation patterns may limit its accessibility as a source of transforming DNA for Acinetobacter cells. It is, therefore, unclear to what extent soil bacteria like Acinetobacter spp. actively access and take up DNA from divergent species in their natural habitats such as soil (27).

Studies on the transfer of chromosomal DNA in soil by natural transformation have focused mainly on transfer events with purified DNA in nutrient- or mineral-amended sterile soil (1, 11, 24). Only recently has natural transformation of chromosomal genes been shown to occur in unamended and nonsterile soil (34, 35). Here, we extend these studies by exposing a kanamycin-sensitive recipient bacterium, Acinetobacter sp. strain BD413 (pFG4) (12), to lysed cells of the nptII-containing (kanamycin-resistant [Kmr]) donor strains, Acinetobacter spp., Pseudomonas fluorescens R2f, and Burkholderia cepacia P2, in order to demonstrate that there is no efficient barrier in nonsterile soil that inhibits intra- or interspecies natural transformation of the Acinetobacter sp. with homologous DNA released from lysed bacterial cells. Detection of gene transfer was based on the restoration of a partially deleted kanamycin resistance gene (nptII) in the recipient bacterium and established models for monitoring of gene transfer in soil microcosms (12, 34, 35).

MATERIALS AND METHODS

Bacterial strains. All bacteria used in this study were originally isolated from soil and were spontaneously rifampin-resistant mutants. The strains were stored in 20% glycerol at −70°C and cultured in Luria-Bertani (LB) broth (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, 1 liter of H2O [pH 7.2]). Liquid
cultures were grown overnight at 27°C with shaking (225 rpm). Before use, 1-nl aliquots of the cultures of the recipient strain, prepared as described by Nielsen et al. (34) and stored at –70°C in 0.85% NaCl-20% glycerol, were thawed, centrifuged, and resuspended in water. Portions of 100 μl of the various transformation studies (12). Final concentration (mean ± standard deviation [SD]) of the inoculum was (6.4 ± 0.9) × 10^6 CFU/ml of water. For plating and enumeration of CFU, 1.5% agar (Oxoid, Basingstoke, England) was included in the preparation of the LBA plates with an agarose support in the central part of the plate. The antibiotics (Sigma, St. Louis, Mo.), rifampin, ampicillin, and kanamycin were added to the growth media at 50 μg ml−1. As the recipient bacterium in all transformations, Acinetobacter sp. strain BD413 (chr::KTG) [34] and P. fluorescens R21 (chr::KTG) [46], and the beta proteobacterium B. cepacia P2 (chr::KTG) [J. D. van Elsas unpublished data], with or without chromosomally inserted (chr::KTG) gene cassette, were used. Bacterial strains without the inserted KTG gene were used as controls. The absence of spontaneous mutants was confirmed by sampling microcosms after the filter transformation or, serially diluted before plating. The LBA plates used for sampling nonsterile soil were supplemented with chloramphenicol at 100 μg ml−1 to inhibit fungal growth. CFU were enumerated after a 72-h incubation period at 30°C. CFU counts refer to the soil portion, i.e., samples of 0.3 g (dry weight of soil) 

Transformation in soil microcosms. A sterile silt loam soil (FSO) obtained from microplots in Wageningen, The Netherlands, was used in all microcosms. The FSO soil has previously been characterized (45, 51). After sampling, the nonsterile soil was air dried and used directly or after autoclaving at 121°C. Sterile soil was obtained after gamma irradiation (4 Mrad) with a 90Co source (Gammaster BV, Ede, The Netherlands). Microcosms consisted of autoclaved polypropylene cylinders of 1-cm² surface area to which 1.2 g of soil was added, as described before (34, 35). The 7-mm-tall cylinders, made of 15-mm polypropylene centrifuge tubes (34, 35), containing the inoculated soil portions were placed on sterile agarose (1.5% [wt/vol] in water) in petri dishes. All transformations in soil were done at 20°C.

In soil microcosms, the NTSO1 strain was added to the soil in a soil microcosm by suspension of 100 μl of soil from a plate containing colonies after transformation of the sterile nonsterile soil experiment except that lysates were incubated for 0, 1, 2, 3, 4, and 5 days at 20°C before the bacterial inoculum (100 μl suspended in 5M9L25P) was added. After 24 h of incubation, the microcosms were sampled and plated, and the CFU count was determined as described above. Sterility of the lysates and nonsterile soil was confirmed by serial dilution plating of soil microcosms that received water instead of the bacterial inoculum. The absence of spontaneous mutants was confirmed by sampling microcosms receiving only the recipient inoculum and nutrients.

Stability studies using nonsterile soil were performed as described for the sterile soil experiments except that lysates were incubated for 0, 1, 2, 3, 4, 6, 8, and 24 h after addition of the recipient inoculum. In addition, lysates of Acinetobacter sp. strain BD413 (chr::KTG) were added to nonsterile soil 10-fold diluted (adjusted to 100 μl with water) and incubated for up to 8 h after addition of the bacterial recipient.

As a control to determine the possible occurrence of transformants on the selective agar plates, the various cell lysates were added to 24-h nutrient-stimulated (with 5M9L25P) soil microcosms with Acinetobacter sp. and immediately plated as described for the soil transformations. No transformants were found in these assays, indicating that any DNA released from the soil during the suspension and plating procedure did not generate transformants. Furthermore, our experimental procedures were unlikely to yield plate transformants, since sampling of bacterial cells was done at least 24 h after DNA addition (and inoculum addition) to the soil microcosms; previous studies have shown that chromosomal DNA incubated in sterile soil for more than 6 h is not available to Acinetobacter sp. for forming DNA (3).

Identification of putative transformants. Putative restoration of the nptII gene in transformants of Acinetobacter sp. (FPGA4) was assessed by restreaking colonies and amplifying genomic soil culture with primer set P1-P2 (12), using the method described by Theis et al. (39). The PCR protocol used for the PCRs included a touchdown PCR setup of 3 min denaturation at 94°C followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min, and extension at 72°C for 1 min. The PCR products were separated on 0.8% agarose gels and visualized under UV light. The presence of a band of the expected size was used as evidence of putative transformants. The nptII gene was amplified in 10-cm² plates, with antibiotics as described above for the filter transformation, or, serially diluted before plating. The LBA plates used for sampling nonsterile soil were supplemented with chloramphenicol at 100 μg ml−1 to inhibit fungal growth. CFU were enumerated after a 72-h incubation period at 30°C. CFU counts refer to the soil portion, i.e., samples of 0.3 g (dry weight of soil). Colonies obtained from nonsterile soil were identified as Acinetobacter sp. based on colony morphology (34, 35), growth rate, Biolog pattern (see below), and PCR amplification of the restored pFG4 plasmid or the original one carrying the 311-bp deletion (see below).

Stability of cell lysates in vitro and in soil. The stability of cell lysates of Acinetobacter sp. strain BD413 (chr::KTG), P. fluorescens R21 (chr::KTG), and B. cepacia P2 (chr::KTG), incubated in vitro (in water or 5% lactic acid solution) and in sterile and nonsterile soil for up to 8 days, was measured by the ability to transform freshly added Acinetobacter sp. strain BD413(pFG4). For the in vitro studies, 100 μl of each cell lysate was incubated in separate Eppendorf tubes at 20°C with either 100 μl of MilliQ water or 100 μl of a 10% (wt/vol) in water lactic acid [35] or 100 μl of sterile soil (Gammaster BV, Ede, The Netherlands). Microcosms consisted of autoclaved polypropylene cylinders of 1-cm³ volume to which 1.2 g of soil was added, as described before (34, 35). The 7-mm-tall cylinders, made of 15-mm polypropylene centrifuge tubes (34, 35), containing the inoculated soil portions were placed on sterile agarose (1.5% [wt/vol] in water) in petri dishes. All transformations in soil were done at 20°C.
primer, 0.05 µl of T4 gene 32 protein (Pharmacia), and 0.25 µl (10 U/µl) of Stoffel fragment Taq DNA polymerase (Perkin-Elmer). The sequences of primers P1 and P2 were (1286) 5’ TGC TAA AGG AAG CGG AAC 3’ and (2929) 5’ AGG TCA ACA GGC GGT AAC 3’, respectively. The primers were designed to amplify the 5’5 region from position 1236 to 2929, which includes the nptII promoter, the complete nptII gene, and the bleomycin resistance gene (12). The amplification conditions were 7 min at 95°C, then 1 min at 94°C, 1 min at 62°C, and 2 min at 72°C for 35 cycles, and finally 10 min at 72°C. DNA from a KmR Acinetobacter sp. strain BD413(pFG4) strain was used as a positive control (the expected band size of the restored gene is 1,693 bp). Colonies obtained from recipient-selective plates (the expected band size of a gene with deletion is 1,376 bp) and wild-type Acinetobacter sp. strain BD413 were used as negative controls.

To confirm the identity of KmR bacterial cells obtained from transformation studies done in nonsterile soil, colonies were routinely restreaked on transformant-selective plates. Randomly selected colonies were confirmed to have metabolic patterns in Biolog GN plates identical to that of the recipient strain BD413(pFG4). The Biolog plates were used as specified by the manufacturer and quantified on a Biolog MicroLog station (Biolog Microlog, Biolog, Inc., Hayward, Calif.).

**RESULTS**

Filter transformations. To clarify the effects of increasing concentrations of cell lysates on natural transformation of the recipient bacterium Acinetobacter sp. strain BD413(pFG4) under optimized conditions in vitro, we exposed this recipient to 1, 10, and 100 µl of lysate (per filter) obtained from the KmR donor bacteria, Acinetobacter sp., P. fluorescens, and B. cepacia. The cell lysates proved to be highly efficient sources of DNA, since lysates from all three strains gave rise to high numbers of transformants (Fig. 1b). The maximum transformation frequencies obtained under optimized in vitro conditions were 3.0 × 10⁻⁵ for Acinetobacter sp., 3.5 × 10⁻⁶ for P. fluorescens R2F, and 6.3 × 10⁻⁷ for B. cepacia P2. An increase of the lysate concentration from 10⁵ to 10⁸ cells per filter increased the numbers of transformants 12- to 25-fold in all cases (Fig. 1b). To determine if the 100 µl of cell lysate used would be saturating for the recipient cells, transformations were also done with a 10-fold-concentrated cell lysate of Acinetobacter sp. (chr::KGT). This concentrate produced a significant higher number (mean ± SD) of transformants ([3.6 ± 0.3] × 10⁵ transformants; frequency, 5.8 × 10⁻⁴) compared to the normally used lysate ([1.4 ± 0.3] × 10⁴ transformants; frequency, 3.0 × 10⁻⁵). However, since the concentrated cell lysate would represent a further enrichment of an already dense cell suspension, we found the continued use of this concentrate of little value when estimating lysate availability in natural soils. Cell lysates obtained after longer periods of heat treatment, e.g., autoclaving at 120°C for 15 min, did not give rise to any transformants in our studies, presumably due to fragmentation of the DNA, generation of inactive single-stranded DNA, and complexation of DNA with the denatured cell debris. Shorter periods of heating gave occasional growth of survivors.

Filter transformations performed with purified DNA instead of lysate (at 0, 0.1, 1, 10, and 50 µg of purified DNA per filter) isolated from the same bacterial strains gave rise to numbers of transformants higher than those obtained with the lysates (Fig. 1b). However, if the numbers of transformants detected are adjusted by the estimated DNA content in the lysates (see Materials and Methods), the lysates were at least as efficient as transforming DNA; differences in transformation frequency of less than fourfold for Acinetobacter sp. DNA and less than threefold for P. fluorescens DNA were found. For B. cepacia, a higher difference in transformation frequency (8- to 15-fold) was seen; the lysate in this case also gave higher numbers of transformants compared to purified DNA.

Sterile-filtered suspensions of non-heat-treated bacterial cultures were also assayed as a source of DNA in the filter

**TABLE 1.** Natural transformation of Acinetobacter sp. strain BD413 in filter assays with sterile-filtered cell suspensions (chr::KGT) of Acinetobacter sp., P. fluorescens, and B. cepacia

<table>
<thead>
<tr>
<th>Strain (chr::KGT)</th>
<th>Mean no. of transformants (CFU) ± SD</th>
<th>No. of transformants/recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter sp. strain BD413</td>
<td>(2.4 ± 0.4) × 10⁵</td>
<td>1.2 × 10⁻⁵</td>
</tr>
<tr>
<td>P. fluorescens R2F</td>
<td>(3.1 ± 0.7) × 10⁴</td>
<td>1.3 × 10⁻⁷</td>
</tr>
<tr>
<td>B. cepacia P2</td>
<td>(2.0 ± 0.3) × 10³</td>
<td>6.9 × 10⁻²</td>
</tr>
</tbody>
</table>

FIG. 1. (a) Natural transformation of Acinetobacter sp. strain BD413(pFG4) on filter with increasing concentrations of chromosomal DNA (chr::nptII) from Acinetobacter sp. (circles), P. fluorescens R2F (squares), and B. cepacia P2 (diamonds). Filled symbols, transformants; open symbols, recipients. Triangles show recipients and transformants of Acinetobacter sp. strain BD413 (wild type) with homologous cell lysates (chr::nptII), T bars, SD. (b) Natural transformation of Acinetobacter sp. strain BD413(pFG4) on filter with increasing concentrations of cell lysates (chr::nptII) from Acinetobacter sp. (circles), P. fluorescens R2F (squares), and B. cepacia P2 (diamonds). Filled symbols, transformants; open symbols, recipients. Triangles show recipients and transformants (symbols are within symbols for Burkholderia transformants) of Acinetobacter sp. strain BD413 (wild type) with homologous cell lysates (chr::nptII). T bars, SD.
transformations to indicate a potential variability of the amount of free DNA available in the cell supernatant. However, Table 1 shows that lower frequencies were obtained with filtrates than with cell lysates (compare Table 1 to Fig. 1b for 100 μl of lysate), presumably indicating the amount of free DNA available in the supernatant compared to total DNA present in the cell debris. There were no clear differences in the recipient counts obtained after the filter transformations done with the above-described DNA sources, indicating that inhibition of recipient growth was not the cause of the variability in the number of transformants observed.

To check the transformation efficiency of the plasmid-har-}

bonding *Acinetobacter* sp. strain BD413(pFG4) as a recipient for chromosomal DNA and lysates, we compared the transformation frequencies obtained with this strain to frequencies for the wild-type *Acinetobacter* sp. strain BD413. In transformation studies with purified homologous DNA, the wild-type strain was significantly more transformable than the plasmid-bearing strain when high concentrations of DNA were used (Fig. 1a). This observation could be due to deleterious crossover events in the pFG4 recipient since a recombination of the restored kanamycin resistance-conferring plasmid into a newly generated chromosomal nptII-bearing recipient would cause inactivation of the selected marker gene. However, such a relationship was not found when lysates were used, and the plasmid-bearing strain proved to be a more efficient recipient for lysates than the wild-type strain (Fig. 1b). The presence, or generation during uptake, of more fragmented DNA in the cell lysates, possibly impeding transformation of the wild-type recipient, which requires larger fragments for integration into the chromosome (40), could account for this observation.

**Transformation in soil microcosms.** Given the high numbers of bacterial cells in soil (10^9 to 10^{10} bacteria per g of soil), dead bacteria could potentially contribute significantly to the gene pool of chromosomal DNA present in this environment. To elucidate the potential of cell lysates obtained from common soil and rhizosphere bacteria to function as DNA sources for natural transformation, we exposed the recipient bacterium *Acinetobacter* sp. strain BD413(pFG4) to cell lysates obtained from the Km^r (chr::KTG) donor bacteria, *Acinetobacter* sp. strain BD413, *P. fluorescens* R2f, and *B. cepacia* P2. For transformation in sterile and nonsterile soil, the recipient was added to the soil and incubated for 24 h before addition of nutrient solution and lysates. As seen from Table 2, freshly added cell lysates obtained from all three different species were able to transform the recipient *Acinetobacter* sp. strain BD413(pFG4) residing in sterile soil. The homologous cell lysates were, however, 4- to 16-fold more efficient for transformation than lysates produced from the heterologous strains. Recipient *Acinetobacter* cells residing in nonsterile soil were recalcitrant to transformation with heterologous cell lysates. The homologous lyse, however, transformed the recipient at a frequency of 1.1 × 10^{-6}, corresponding to 1.9 × 10^{-7} transformants per lysed cell (Table 2).

Microcosms with added inoculum, nutrients, and the wild-type strain lysates, or only lysates and nutrients, were used as controls for spontaneous mutations and contamination. None of these treatments produced any colonies that were identified as *Acinetobacter* spp. on transformant-selective plates. In addition, selected colonies obtained from transformant-selective plates were restreaked and confirmed to be *Acinetobacter* sp. strain BD413(pFG4) by the metabolic pattern obtained on

### TABLE 2. Natural transformation and restoration of a 317-bp deleted nptII gene in *Acinetobacter* sp. strain BD413(pFG4) residing in sterile and nonsterile soil microcosms for 24 h, with added cell lysates of *Acinetobacter* sp., *P. fluorescens*, and *B. cepacia*^a^

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th><em>Acinetobacter</em> sp. strain BD413</th>
<th><em>P. fluorescens</em> R2f</th>
<th><em>B. cepacia</em> P2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of transformants (CFU)^a^</td>
<td>No. of transformants/recipient</td>
<td>No. of transformants/lysed cell</td>
</tr>
<tr>
<td></td>
<td>Mean no. of transformants (CFU) ± SD</td>
<td>Mean no. of transformants (CFU) ± SD</td>
<td>Mean no. of transformants (CFU) ± SD</td>
</tr>
<tr>
<td>0</td>
<td>1,073</td>
<td>9.9 × 10^{-6}</td>
<td>4.8 × 10^{-6}</td>
</tr>
<tr>
<td>1</td>
<td>73</td>
<td>5.6 × 10^{-7}</td>
<td>3.2 × 10^{-7}</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>1.2 × 10^{-7}</td>
<td>9.3 × 10^{-8}</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>8.8 × 10^{-8}</td>
<td>8.0 × 10^{-8}</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>4.5 × 10^{-8}</td>
<td>4.4 × 10^{-8}</td>
</tr>
</tbody>
</table>

^a Inoculation time of lysates in sterile soil before addition of the *Acinetobacter* sp.(pFG4 LeptII), suspended in 5M9L2SP. The microcosms with added bacteria were incubated further for 24 h at 20°C before plating and enumeration of CFU.

^b The variabilty of the CFU of transformants and recipients, as determined by the coefficient of variation (SD/mean), was between 0.3 and 0.4 (mean values).

^c The number of lysed cells is given as the CFU count of the bacterial suspension used for preparation of the lysates.

^d ND, not detected.
TABLE 4. Natural transformation and restoration of a 317-bp deleted nptII gene in Acinetobacter sp. strain BD413(pFG4) with cell lysates (chr::KTG) of the Acinetobacter sp., P. fluorescens, and B. cepacia, incubated for up to 24 h in nonsterile soil before recipient inoculation

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Acinetobacter sp. BD413</th>
<th>P. fluorescens R2f</th>
<th>B. cepacia P2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of transformants/ (CFU)</td>
<td>No. of transformants/ lyzed cell</td>
<td>No. of transformants/ (CFU)</td>
</tr>
<tr>
<td>0</td>
<td>276</td>
<td>&lt;2.2 x 10^6</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>148</td>
<td>1.7 x 10^-6</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>6.2 x 10^-7</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>2.1 x 10^-7</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>2.1 x 10^-7</td>
<td>ND</td>
</tr>
<tr>
<td>24</td>
<td>ND</td>
<td>&lt;2.2 x 10^-6</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Incubation time of lysates in nonsterile soil before addition of Acinetobacter sp. (pFG4 ΔnptII) (suspended in 5M9L25P). The microcosms with added bacteria were incubated further for 24 h at 20°C before plating and enumeration of CFU.

b The variability of the CFU of transformants and recipients, as determined by the coefficient of variation (SD/mean), was between 0.3 and 0.4 (mean values).

c The number of lysed cells is given as the CFU count of the bacterial suspension used for the preparation of the lysates.

d ND, not detected.

Biolog GN plates and by the presence of the restored nptII gene sequence as verified by PCR.

Availability of cell lysates for natural transformation in soil.
To clarify the time period during which cell lysates would be available as a source of transforming DNA in soil, the lysates were incubated for increasing amounts of time in sterile (0 to 4 days) and nonsterile (0 to 24 h) soil before addition of the bacterial recipient. As can be seen in Table 3, cell lysates (chr::KTG) incubated in sterile soil were available as transforming DNA for the recipient cells for 3 days for P. fluorescens and B. cepacia lysates or 4 days with Acinetobacter sp. lysates. The highest transformation frequencies were always produced with freshly added lysate to soil. Lysates incubated for 1 day in soil had an average remaining transforming capability of 40%. In nonsterile soil (Table 4), the lysates became rapidly inactivated, and restoration of the nptII gene was seen only with lysates of P. fluorescens and B. cepacia incubated for up to 4 h in soil and with lysates from Acinetobacter sp. incubated for up to 8 h in soil. Incubation of the lysates for 1 h in nonsterile soil reduced their transforming ability by 31% on average. For the Acinetobacter sp. cell lysates, a 10-fold-diluted lysate was also assayed in nonsterile soil. Transformation with this lysate was detectable only for 2 h in soil. After 1 h in soil, the diluted lysate had a remaining transforming activity of 36%.

Microcosms containing lysates of wild-type strains of the three donor bacteria, nutrients and the inoculant, or microcosms with lysates and nutrients were used as controls. These microcosms did not give rise to any Acinetobacter transformant colonies (see above). Colonies restreaked from transformant and recipient-selective plates were PCR amplified to confirm recombinational repair of the nptII gene. Figure 2 shows the PCR amplification of DNA from colonies growing on transformant-selective plates transformed with either of the three lysates and colonies growing on the corresponding recipient-selective LBA plates.

Stability of cell lysates in vitro. To clarify the stability of transforming DNA in the cell lysates over time, portions of the lysates of the three bacteria were stored in Eppendorf tubes with added water or 5% humic acid. Transformation assays with the water-suspended lysates sampled at day 1, 2, 4, and 8 did not reveal any clear changes in the transforming ability of the lysates (Fig. 3). The addition of 5% humic acid to the lysates affected their transforming activity, giving consistently lower transformation frequencies, and a 32% average reduction was seen. This reduction might be explained by a physical inhibition of the filter transformation by the dark brown humic acid solution. Thus, clear effects of humic acids on protection of DNA (7) or inhibition of transformation (48) were not found.

DISCUSSION
In this study, we used lysates obtained from common soil bacteria (3, 4, 22) to demonstrate that Acinetobacter sp. cells...
can efficiently access genetic information in cell debris of various bacterial genera present in soil. Based on homologous recombination-mediated restoration of a kanamycin resistance gene (nptII) in the recipient Acinetobacter sp. (pFG4), the uptake of chromosomal gene fragments present in lysates (chr::Km) of Acinetobacter sp., P. fluorescens R2f, and B. cepacia P2 was shown to occur at frequencies between $10^{-5}$ and $10^{-6}$ in vitro. A 10-fold drop in the frequencies was seen in sterile soil, and a further reduction from 5- to more than 100-fold was noted in nonsterile soil. The homologous lysate transformed the recipient at a frequency of $1.1 \times 10^{-6}$ in nonsterile soil. This frequency is similar (when judged determined per microgram of DNA used) to that obtained in studies using purified DNA in nonsterile soil (34; K. M. Nielsen, T. B. Løkken, and A. M. Bones, unpublished data). On average, a less than 10-fold difference in the capability of restoration of the antibiotic resistance gene (via homologous recombination) was seen between isogenic and heterogenic lysates. The reduced transformation frequencies obtained for the heterologous lysates can be caused by differences in the degree of homology to the recipient genome or reduced accessibility of the nptII gene due to cellular debris. Homology to the heterologous strains is found only in the pFG4 plasmid in the recipient Acinetobacter sp., whereas homology to the isogenic lysate is also displayed by the recipient chromosome. Indeed, filter transformations with plasmid-harbouring recipients gave, on average, 10-fold-higher numbers of transformants with isogenic lysates than the wild-type recipients; these numbers of transformants were comparable to those obtained with the heterogenic lysates. Up to 1 µg of free DNA (per g of dry soil) has been estimated to be present in soil (of a total of approximately 90 µg of DNA/g of dry soil [49]). Torsvik et al. (50) estimated that at least 4,000 different bacterial types composed the majority of the bacterial diversity seen in soil. Our 14-fold-higher inoculum concentration (compared to the estimated average population size) could be successfully transformed in nonsterile soil with DNA corresponding to the amount found in the lysates of fewer than five clones. Acinetobacter sp. has also been transformed in vitro with the nptII marker gene present in transgenic plants (8, 12), and its DNA uptake is regarded as nonspecific (40, 41). Due to the heterogeneity of the DNA donors used here, it was unclear if DNA escaping their cells would be exploited efficiently as a source of genetic information by Acinetobacter spp. populations in soil. The results obtained both in vitro and in situ indicate that the lack of DNA purity and the variable cellular background did not reduce their transforming ability, which for the isogenic strain was similar to that obtained with purified DNA (Fig. 1a). Kloos et al. (20) also observed equally efficient transformation of Acinetobacter spp. on filters when inducing lysis of donor cells. DNA may be associated with the bacterial slime layer and thereby become stabilized and still be a source of extracellular DNA. Catlin (6) reported transformation of Neisseria meningitidis by DNA from cells and from culture slime, and Junji (18) prepared crude extracts of cells for natural transformation of Acinetobacter spp. by heating sodium dodecyl sulfate-treated cell suspensions at 60°C for 1 h. The apparently enhanced transformation efficiency of the cell lysates compared to the purified DNA on filters might be due to underestimation of the amount of free chromosomal DNA present in the cell supernatant. The sterile-filtered cultures of the different donors all contained high amounts of free DNA, as shown in the transformation assay (Table 1), indicating that none of the bacteria secreted high amounts of DNAse during in vitro growth. Thus, the inactivation of the transforming activity of the cell lysates seen in soil seems to be due not to any introduced DNAse activity but rather to indigenous activity in soil or other abiotic factors and mechanisms of DNA inactivation present in soil.

Production of extracellular DNA in Acinetobacter spp., Burkholderia sp., and Pseudomonas spp. is known to occur (25, 26, 39, 42). From the differences seen between the transforming activity of the purified DNA, sterile-filtered cell supernatants, and cell lysates of B. cepacia P2 and P. fluorescens R2f, it can be suggested that B. cepacia liberates more free chromosomal DNA than P. fluorescens during in vitro growth since the CFU counts of their lysates were comparable. Fragments of chromosomal bacterial DNA have been shown to persist in soil for weeks (9, 43). However, this physical stability has not been reflected in similar data demonstrating the long-term biological activity (e.g., transforming activity) of chromosomal DNA in soil. Bacterial DNA introduced into nonsterile soil has been found to be active as transforming DNA for only a few hours (34). Thus, there is a clear discrepancy between the detected physical and the functional significance of DNA in soil (20). Pure DNA is initially hydrolyzed at substantial rates when introduced to soil. The half-life of purified DNA added to soil has been estimated to be 9 to 28 h, depending on the soil’s mineral composition (28). The presence of cell debris may be important for the protection of crude DNA against enzymatic hydrolysis and its interaction with the soil matrix. The half-life of DNA associated with dead bacterial cells may therefore differ substantially from estimates obtained with purified DNA. For instance, the half-life of DNA present in bacterial cells in deep-sea sediments was found to be several days (38).

Our data demonstrate that cell lysates are available as a source of transforming DNA for Acinetobacter spp. populations in sterile or nonsterile soil for considerable time periods: P. fluorescens and B. cepacia lysates were available for up to 3 days/4 h and isogenic lysates of the Acinetobacter sp. were available for up to 4 days/8 h. Lysates incubated for 1 day in soil had an average transforming capability of 30 to 40% of the initial value. The relative availability of the DNA present in the lysates was enhanced over time compared to the availability of naked chromosomal DNA for natural transformation of Acinetobacter spp. in the same sterile and nonsterile FSL soil (34; Nielsen et al., unpublished data), indicating that the presence of cell debris may protect DNA from inactivation in soil.

The decreasing transformation frequencies over time were likely to be caused by DNA fragmentation, degradation by nucleases, and possible inactivation of DNA by binding to soil substances. Shorter DNA fragments are known to be less efficient for natural transformation than high-molecular-weight DNA (40).

Thus, we conclude that cell lysates in which DNA may be adhering to polysaccharides, proteins, and/or membranes are generally not inhibitory to natural transformation of Acinetobacter spp. in soil and may, in addition, protect DNA from rapid inactivation. Furthermore, if DNA homology is present (21, 29, 32, 47, 53), gene transfer by natural transformation might provide populations of Acinetobacter cells with a mechanism for generating genetic variability (e.g., mosaic genes) by enabling them to take up chromosomal DNA released from various bacterial donors in their surroundings.

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