

Inducible Cell Lysis System for the Study of Natural Transformation and Environmental Fate of DNA Released by Cell Death

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Two novel conditional broad-host-range cell lysis systems have been developed for the study of natural transformation in bacteria and the environmental fate of DNA released by cell death. Plasmid pDKL02 consists of lysis genes *S*, *R*, and *Rz* from bacteriophage λ under the control of the *Ptac* promoter. The addition of inducer to *Escherichia coli*, *Acinetobacter calcoaceticus*, or *Pseudomonas stutzeri* containing plasmid pDKL02 resulted in cell lysis coincident with the release of high amounts of nucleic acids into the surrounding medium. The utility of this lysis system for the study of natural transformation with DNA released from lysed cells was assessed with differentially marked but otherwise isogenic donor-recipient pairs of *P. stutzeri* JM300 and *A. calcoaceticus* BD4. Transformation frequencies obtained with lysis-released DNA and DNA purified by conventional methods and assessed by the use of antibiotic resistance (*P. stutzeri*) or amino acid prototrophy (*A. calcoaceticus*) for markers were comparable. A second cell lysis plasmid, pDKL01, contains the lysis gene *E* from bacteriophage ϕ X174 and causes lysis of *E. coli* and *P. stutzeri* bacteria by activating cellular autolysins. Whereas DNA released from pDKL02-containing bacteria persists in the culture broth for days, that from induced pDKL01-containing bacteria is degraded immediately after release. The lysis system involving pDKL02 is thus useful for the study of both the fate of DNA released naturally into the environment by dead cells and gene transfer by natural transformation in the environment in that biochemically unmanipulated DNA containing defined sequences and coding for selective phenotypes can be released into a selected environment at a specific time point. This will allow kinetic measurements that will answer some of the current ecological questions about the fate and biological potential of environmental DNA to be made.

The microbial world is characterized by the collective ability of its members to rapidly colonize and thrive in a vast range of habitats. Some of these environments are characterized by such extreme physical and/or chemical conditions that all other forms of life are excluded. The metabolic opportunism of microbes results, on the one hand, from the exceptional physiological versatility and biochemical diversity of the microbial world as a whole and, on the other, from the ability of individual populations, when under appropriate selection pressure, to rapidly evolve new metabolic potential as a result of the acquisition of new genetic information through mutation and efficient gene transfer mechanisms. Although gene transfer mechanisms such as conjugation, transduction, and transformation have been extensively studied in the laboratory and participating cellular components have been identified and characterized to a considerable extent (for reviews, see, e.g., references 4 and 14), only limited information is available on natural gene transfer in the environment (17). Since genetic flux in microbial communities is a critical component of their metabolic potential, it is important to acquire a fundamental understanding of the parameters which influence it in nature.

Transformation is the process of gene transfer in which recipient organisms take up free DNA (2) liberated by excretion or by lysis of dying cells. Since it does not involve specific

cellular interactions among donors and recipients, transformation may be the most important mechanism of microbial acquisition of DNA from distantly related organisms, perhaps even from eukaryotic organisms (19).

Several studies have reported significant amounts of dissolved DNA in environmental samples (3, 5, 11), but its heterogeneity (e.g., size and structural state) precludes definitive characterization of its chemical structure and biological activity and hence its functional significance. Though it is possible to circumvent this problem of heterogeneity by adding purified defined DNA to environmental samples, this approach suffers from its own problems. For example, the added DNA undoubtedly has a structure and composition that differ significantly from those of the DNA released directly from living, dying, or dead cells, and the microenvironments in the samples exhibit differing levels of accessibility for the added macromolecules. To address these problems, we have developed systems which can be used to study natural transformation involving DNA actually released by lysis of dying donor cells established in natural microniches.

MATERIALS AND METHODS

Bacterial strains, plasmids, culture media, and growth conditions. Bacterial strains and plasmids used in this study are given in Table 1. Luria-Bertani (LB) medium, defined medium M9, and solid media were prepared as described by Sambrook et al. (28). Minimal medium and minimal agar for transformation assays of *Acinetobacter calcoaceticus* were prepared according to the method of Cruze et al. (10). For transformation

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TABLE 1. Plasmids and bacterial strains used in this study

Plasmid or bacterial strain	Relevant genotype and phenotype ^a	Reference or source
Plasmids		
pDKL01	Km ^r Sm ^r ; lysis gene <i>E</i> of bacteriophage ϕ X174 under control of <i>Pm</i> promoter	This study
pDKL02	Km ^r ; lysis genes <i>S</i> , <i>R</i> , and <i>Rz</i> of bacteriophage λ under control of <i>Ptac</i> promoter	This study
pNM185	Km ^r Sm ^r ; RSF1010-derived broad-host-range expression vector	20
pRG1	Ap ^r ; lysis genes <i>S</i> , <i>R</i> , and <i>Rz</i> of bacteriophage λ	23
pUH12	Ap ^r ; lysis gene <i>E</i> of bacteriophage ϕ X174	15
pVLT33	Km ^r ; RSF1010- <i>lacI</i> ^q / <i>Ptac</i> hybrid broad-host-range expression vector, multiple cloning site of pUC18	13
Bacterial strains		
<i>Acinetobacter calcoaceticus</i> DSM587	Strain BD413 ivl-10 (16); Ile ⁻ Val ⁻ Leu ⁻ ; DNA donor strain for transformation of competent auxotrophic strain DSM588	DSM ^b
<i>Acinetobacter calcoaceticus</i> DSM588	Strain BD413 trpE27 (16); Trp ⁻ ; DNA donor strain for transformation of competent auxotrophic strain DSM 587	DSM
<i>Acinetobacter calcoaceticus</i> DK2	Spontaneous <i>pobA</i> mutant of <i>A. calcoaceticus</i> BD413 (16)	This study
<i>Escherichia coli</i> JM109	<i>recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 Δ(lac-proAB) λ⁻ [F' <i>traD36 proAB lacI</i>^q ZΔM15]</i>	33
<i>Escherichia coli</i> S17-1	Tp ^r Sm ^r <i>recA thi pro ΩRP4 2-Tc::Mu Km::Tn7</i>	29
<i>Pseudomonas putida</i> KT2442	Rf ^r	20
<i>Pseudomonas stutzeri</i> JM300	Naturally competent strain for transformation	8
<i>Pseudomonas stutzeri</i> DK301	Spontaneous Rf ^r mutant of <i>P. stutzeri</i> JM300	This study
<i>Pseudomonas stutzeri</i> DK304	Spontaneous Sp ^r mutant of <i>P. stutzeri</i> JM300	This study
<i>Pseudomonas testosteroni</i> DSM50244		DSM

^a Antibiotic resistances are indicated as follows: Ap^r, ampicillin resistant; Km^r, kanamycin resistant; Sm^r, streptomycin resistant; Sp^r, spectinomycin resistant; Rf^r, rifampicin resistant; Tp^r, trimethoprim resistant.

^b DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

assays of *Pseudomonas stutzeri*, LB medium was modified (9) by the addition of 1 mM MgSO₄, 0.2% (wt/vol) glucose, and 10 mM Tris hydrochloride (pH 7.5). Media were supplemented, when required, with ampicillin (100 μ g ml⁻¹), isoleucine (Ile; 250 μ g ml⁻¹), isopropyl- β -D-thiogalactopyranoside (IPTG; 20 to 80 μ g ml⁻¹), leucine (Leu; 250 μ g ml⁻¹), kanamycin (50 μ g ml⁻¹), 3-methylbenzoate (3MB; 2 mM), rifampicin (100 μ g ml⁻¹), spectinomycin (100 μ g ml⁻¹), streptomycin (100 μ g ml⁻¹), tryptophan (Trp; 50 μ g ml⁻¹), and valine (Val; 250 μ g ml⁻¹). Growth was studied by the monitoring of optical density and CFU.

DNA isolation, manipulation, and analysis. The large-scale preparation of plasmids was performed by the alkaline method of Birnboim and Doly (7). Chromosomal DNA was isolated by the method of Stanley et al. (30). Restriction endonuclease digestion, ligation, transformation, agarose gel electrophoresis, and other standard recombinant DNA techniques were carried out according to published protocols (28). Quantification of chromosomal DNA from lysates or after isolation was performed by the ethidium bromide fluorescent quantification method described by Sambrook et al. (28), and it was done with a charge-coupled device camera and E.A.S.Y. analysis software (Herolab, Wiesloch, Germany).

Lysis plasmid constructions and lysis experiments. The lysis plasmids (Fig. 1) contain the lysis genes of the *Escherichia coli* bacteriophages λ and ϕ X174. The gene *E* of bacteriophage ϕ X174 on the 1-kb *EcoRI* fragment of plasmid pUH12 was cloned into the single *EcoRI* restriction site of the broad-host-range plasmid pNM185 downstream of the *Pm* promoter, producing lysis plasmid pDKL01. To construct the lysis plasmid pDKL02, the lysis genes *R*, *Rz*, and *S* of bacteriophage λ present on the *EcoRI*-*BamHI* fragment of plasmid pRG1 were cloned downstream of the *Ptac* promoter of the broad-host-range plasmid pVLT33. To determine the efficiency of these lysis systems in a variety of bacteria, both plasmids were transferred by electroporation into the mobilizing strain *E. coli*

S17-1. The plasmids were then transferred by the filter mating procedure described by Bertram et al. (6) to several gram-negative bacterial hosts. Bacterial lysis in liquid culture was carried out routinely in LB broth at 37°C by the addition of inducers (IPTG for plasmid pDKL02 and 3MB for plasmid pDKL01). Lysis was monitored at 30 to 60 min intervals by measurement of the optical density of the culture or by plating for viable cells (CFU) on LB plates. The release of total cellular nucleic acids from cells incubated in the presence or absence of the inducer was monitored by centrifuging 100- μ l quantities of cultures at 15,000 \times *g* for 2 min at room temperature to remove cellular material and debris and by one extraction of supernatant fluids with 1 volume of phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol) to inhibit degradative reactions. An aliquot of the aqueous phase was analyzed by electrophoresis on a 0.8% (wt/vol) agarose gel.

Transformation procedure with *P. stutzeri*. Log-phase cultures of *P. stutzeri* DK304 recipient cells in modified LB broth were resuspended in fresh modified LB broth, and 1-ml aliquots were filtered on Nucleopore filters (pore size, 0.2 μ m) which were then placed on modified LB plates. Sterile chromosomal DNA extracted from *P. stutzeri* DK301(pDKL02) bacteria was applied to the cells on the filters, which were incubated cell side up for 4 h at 37°C. Transformation was terminated by the addition of 750 U of DNase I. Cells on the filters were resuspended in 1 ml of 10 mM MgSO₄, and serial dilutions were plated on selective and nonselective plates to enumerate the recipients, transformants, and spontaneous mutants. For filter transformation assays with lysis-released DNA, 20 μ l of log-phase *P. stutzeri* DK301(pDKL02) donor cells was mixed with 1 ml of log-phase *P. stutzeri* DK304 recipient cells and treated as described above, with the modification that the filters were placed on modified LB plates supplemented with 2 mM 3MB to induce lysis of donor cells.

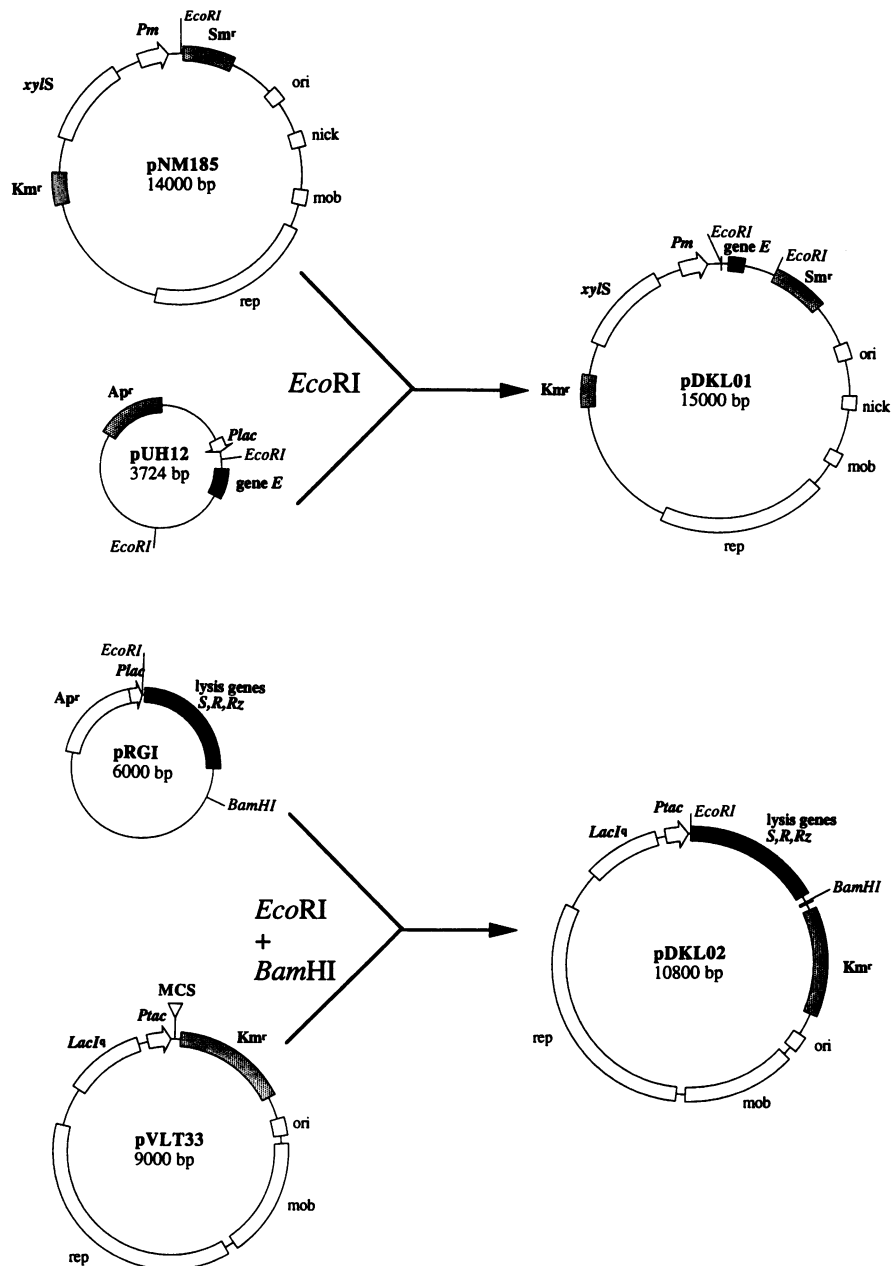


FIG. 1. Construction of inducible cell lysis plasmids pDKL01 and pDKL02. Construction details are described in the text. Abbreviations: *Pm*, promoter of the TOL plasmid *meta* pathway operon; *xylS*, gene of cognate regulator of *Pm*; *gene E*, lysis gene of bacteriophage ϕ X174; *Plac*, promoter of the *lac* operon; *Ptac*, *Plac* and *Ptac* hybrid promoter; *lacI^q*, high-affinity mutant repressor protein of the *lac* operon; MCS, multiple cloning site; genes *S*, *R*, and *Rz*, lysis genes of bacteriophage λ ; *Km^r*, kanamycin resistance; *Sm^r*, streptomycin resistance; *ori*, origin of replication; *nic*, relaxation nick site; *rep*, genes of vegetative replication functions; *mob*, genes of mobilization functions.

Transformation procedure with *A. calcoaceticus*. *A. calcoaceticus* DSM587 was grown in LB broth at 30°C to the beginning of stationary growth phase, and 300- μ l aliquots in 20% (vol/vol) glycerol were shock-frozen in liquid N₂ and stored at -70°C. For transformation, an aliquot of competent culture was thawed in a water bath at 30°C, and isolated DNA from *A. calcoaceticus* DSM588(pDKL02) dissolved in LB broth was added in volumes up to 50 μ l. For transformation with lysis-released DNA, *A. calcoaceticus* DSM588(pDKL02) donor bacteria were grown in LB broth to early log phase and cell

lysis was induced by addition of IPTG (final concentration, 80 μ g ml⁻¹). After 3 h of incubation at 37°C, an aliquot (up to 50 μ l) of the raw lysate was directly added to the competent cells. After 60 min of incubation at 30°C, transformation was terminated by the addition of DNase I (final concentration, 10 μ g ml⁻¹). After an additional 10-min incubation at 30°C, the culture was diluted into minimal medium. Prototrophic transformants were enumerated by plating them onto mineral medium, plasmid transformants were enumerated by plating them onto mineral medium containing Leu, Ile, Val, and Km,

TABLE 2. Induction of cell lysis in gram-negative bacteria

Bacterial strains	Reduction in cell viability in bacteria carrying ^a :	
	pDKL01	pDKL02
<i>A. calcoaceticus</i> DSM587	ND	++
<i>A. calcoaceticus</i> DK2 ^b	-	ND
<i>E. coli</i> S17-1	+++	+++
<i>P. putida</i> KT2442	+	++
<i>P. stutzeri</i> JM300	++	++
<i>P. testosteroni</i> DSM50244	++	ND

^a Viability reduced by ≥ 1 (+), ≥ 2 (++), or ≥ 3 (+++) orders of magnitude following induction of expression of the plasmid-carried lysis genes by 3MB or IPTG. -, no reduction in viability; ND, not determined. No reductions in cell viability were observed when 3MB or IPTG was added to bacteria without the lysis plasmid.

^b *pobA* spontaneous mutant defective in degradation of 3MB.

and total cells were counted by plating them onto mineral medium containing Leu, Ile, and Val.

RESULTS

Characterization of the inducible cell lysis systems. Two types of the lysis system were assessed for their utility. The first is based on gene *E* of bacteriophage ϕ X174, whose product induces cellular autolysins which lead to pore formation in the bacterial membrane, thereby liberating cytoplasmic material (31). The other system consists of lysis genes *S*, *R*, and *Rz* from bacteriophage λ under the control of the *P_{tac}* promoter. The *S* gene product causes lesions in the cytoplasmic membrane through which the murein-degrading activity coded for by the *R* gene escapes to the periplasm, while the *Rz* gene product may be an endopeptidase that cleaves the oligopeptide cross-

links in the peptidoglycan and/or between peptidoglycan and the outer membrane (34). The construction of plasmid pDKL01 containing lysis gene *E* of bacteriophage ϕ X174 under the control of the positively regulated *P_m* promoter of the *meta* pathway operon of the TOL plasmid (32) is shown in Fig. 1. Transcription from the *P_m* promoter is induced by the *xylS* gene product after activation by effector molecules such as benzoate and its derivatives (24). Plasmid pDKL02 (Fig. 1) is a similar broad-host-range plasmid containing lysis genes *S*, *R*, and *Rz* of bacteriophage λ under the control of the *P_{tac}* promoter which is activated by addition of IPTG. No reduction in the viability of *A. calcoaceticus* was observed after induction of lysis plasmid pDKL01, whereas for all other bacteria tested, viability was reduced by more than two orders of magnitude after the addition of the appropriate inducer (Table 2).

Substantial amounts of nucleic acids from *A. calcoaceticus* DSM587 (Fig. 2) and *E. coli* S17-1 (Fig. 3A), both carrying pDKL02, and from *P. stutzeri* JM300 (Fig. 4A), also carrying this plasmid, began to be liberated 90 min and 180 min, respectively, after the addition of the inducer. The released DNA persisted for more than 5 days in culture broth without any reduction in its transforming activity (data not shown). In contrast, induction of *E. coli* S17-1 (Fig. 3B) and *P. stutzeri* JM300 (Fig. 4B), both containing pDKL01, resulted in the release of nucleic acids that were rapidly degraded in the culture broth. This latter system is therefore unsuitable for studying the biological fate of released DNA over extended periods of time.

In order to assess the utility for transformation experiments of DNA that has undergone lysis gene-mediated release, we determined whether or not cell lysates obtained by this procedure negatively affected recipient cells, e.g., through the action of the released lysis proteins. Increasing amounts of cell lysate from donor bacteria induced in the early log phase were added to 300 μ l of log-phase recipient cells, and after 1 h of

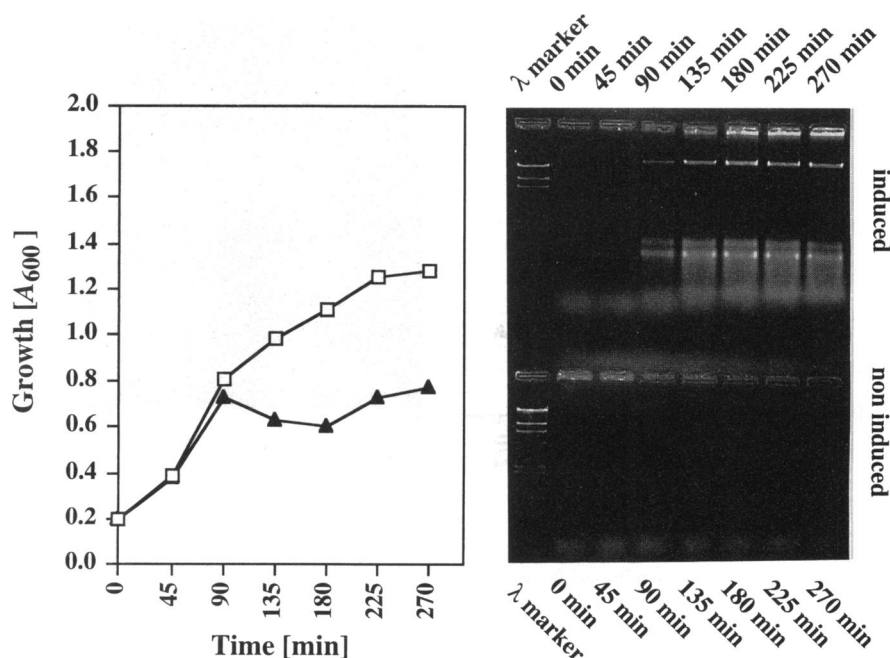


FIG. 2. Nucleic acid release from *A. calcoaceticus* DSM587(pDKL02) bacteria. Bacterial growth was monitored by following the A_{600} of the bacterial culture. Monitoring of released nucleic acids by agarose gel electrophoresis was performed as described in Materials and Methods. Open squares indicate noninduced cultures; closed triangles indicate induced cultures. The DNA marker was *Hind*III-digested λ DNA.

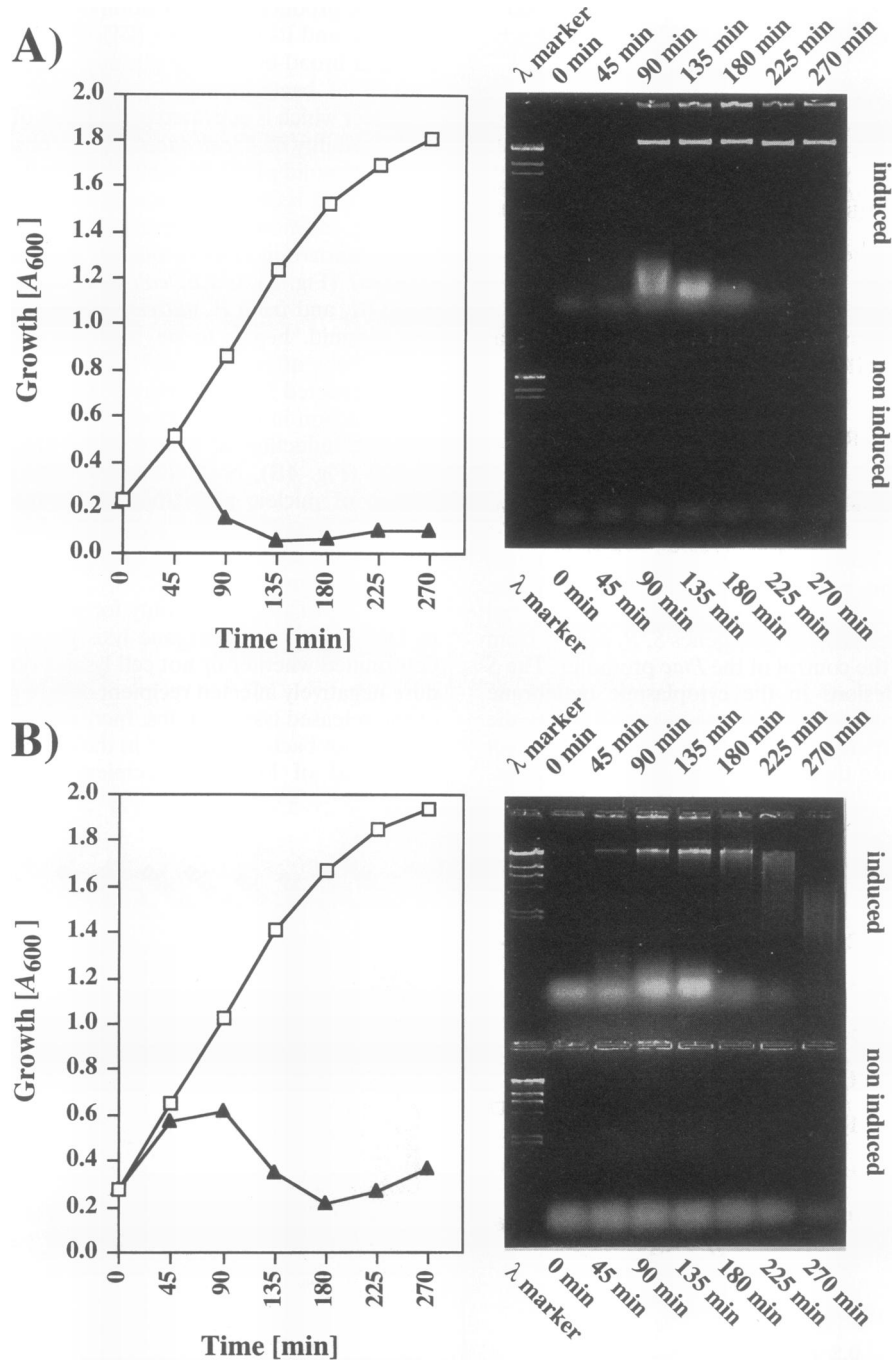


FIG. 3. Nucleic acid release from *E. coli* S17-1(pDKL02) (A) and *E. coli* S17-1(pDKL01) (B) bacteria. Details are as in the legend for Fig. 2. Note the rapid loss of high-molecular-weight DNA released from pDKL01-carrying bacteria.

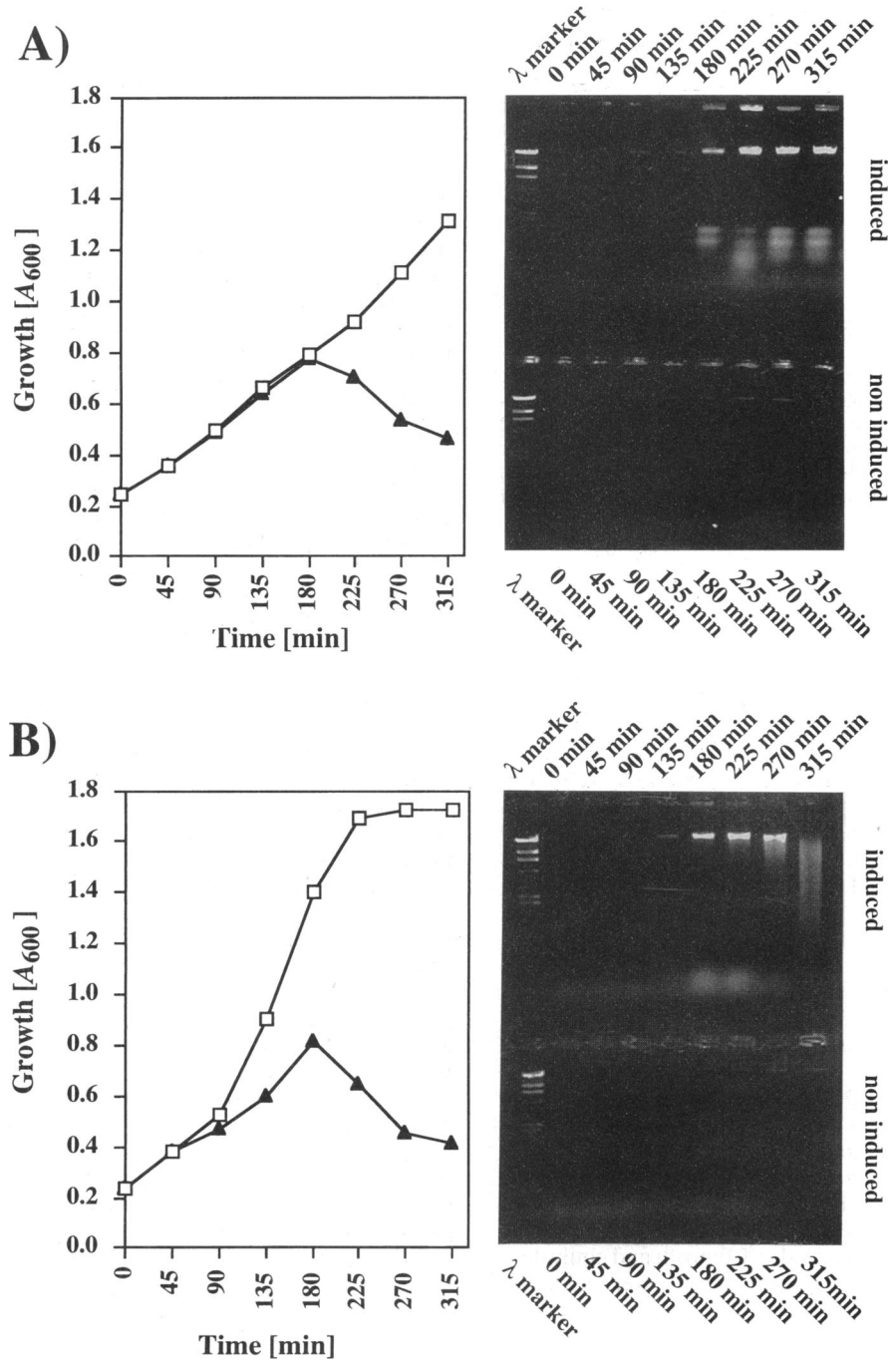


FIG. 4. Nucleic acid release from *P. stutzeri* JM300(pDKL02) (A) and *P. stutzeri* JM300(pDKL01) (B) bacteria. Details are as in the legend for Fig. 2.

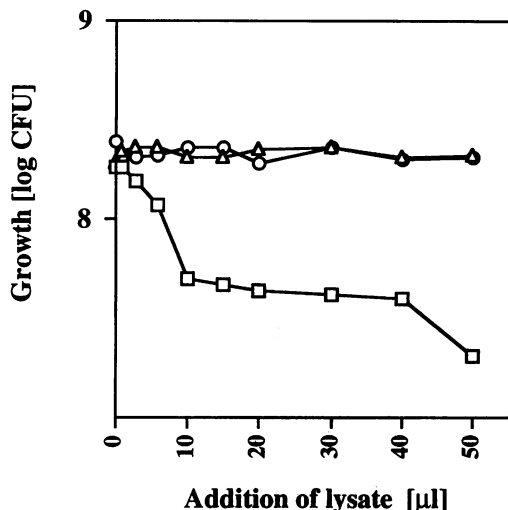


FIG. 5. Effect of donor cell lysates on *A. calcoaceticus* DSM587, *E. coli* S17-1, and *P. stutzeri* JM300 bacteria. Lysates prepared by induction of the appropriate donor strain carrying cell lysis system pDKL02 were added to exponentially growing recipient cells and incubated for 60 min at 37°C prior to determining viable counts. Symbols: triangles, *A. calcoaceticus* DSM587; circles, *E. coli* S17-1; squares, *P. stutzeri* JM300.

incubation at 37°C, the viable counts were determined. No influence on the viability of the recipients was observed after lysates of *E. coli* S17-1(pDKL02) and *A. calcoaceticus* DSM587(pDKL02) were incubated respectively with *E. coli* S17-1 and *A. calcoaceticus* DSM587 cells in lysate-to-cell ratios of up to 1:6 (vol/vol) (Fig. 5). On the other hand, *P. stutzeri* JM300 bacteria were very sensitive to cell lysates of *P. stutzeri* JM300(pDKL02) (Fig. 5). Crude extracts lacking lysis proteins had no effect on viability at a crude extract-to-cell ratio of 1:10 (vol/vol), indicating that the inhibitory effect observed is caused by lysis proteins. Transformation studies with high levels of released or purified DNA were therefore limited to *A. calcoaceticus*.

The lysis efficiency of *A. calcoaceticus* DSM587(pDKL02) and *E. coli* S17-1(pDKL02) bacteria depended on the growth phase: it was highest in the exponential phase and became progressively lower in the stationary phase (Fig. 6 and unpublished data).

Transforming activity of DNA released by induced cell lysis. Natural transformation of *A. calcoaceticus* DSM587 (auxotrophic for isoleucine, valine, and leucine) was investigated with DNA purified from *A. calcoaceticus* DSM588(pDKL02) (auxotrophic for tryptophan) and DNA from the same donor strain after the release of the DNA by induced cell lysis. Increasing amounts of purified DNA dissolved in LB broth and equivalent amounts of DNA released from lysed bacteria were added to naturally competent *A. calcoaceticus* DSM587 recipient cells. Both types of DNA transformed the recipient to prototrophy with the same efficiency (Fig. 7). Both types of DNA also produced identical transformation frequencies with the pDKL02 lysis plasmid for the *A. calcoaceticus* recipient cells (selection for kanamycin resistance). These frequencies were 75% lower than those obtained with the chromosomal marker. Cotransfer of both plasmid and chromosomal markers was relatively rare (fewer than 10^{-7} transformants per viable count).

Transformation of *P. stutzeri* DK304 with DNA released from lysed donor cells of *P. stutzeri* DK301(pDKL02) and used

in the lysate at a lysate-to-recipient ratio of 1:50 to minimize the inhibitory effect of the lysate took place at a frequency of $2.1 \times 10^{-6} \pm 0.1 \times 10^{-6}$ transformants per µg of DNA. The same amount of purified DNA produced a transformation frequency of $5.7 \times 10^{-7} \pm 0.2 \times 10^{-7}$ transformants per µg of DNA. These data suggest that lysis-released DNA is as efficient as purified DNA in natural transformation.

DISCUSSION

DNA in living cells is presumed to be complexed with a variety of divalent cations, DNA-binding proteins, and RNA and to exist in physical and chemical forms that differ from those which characterize DNA purified by classical procedures involving harsh denaturing conditions. Changes in DNA structure and function associated with the loss or gain of complexing agents following release into the environment have so far not been adequately characterized. As a result, it has so far not been possible to throw light on the biological significance, if any, of environmental DNA. Moreover, it has been difficult, in an experimental sense, to approach transformation with lysis-released DNA in situ.

An experimental system that would allow such an analysis would depend on the release of precisely defined DNA molecules carrying selective markers. These molecules would be comparable to those of free DNA in natural environments (12, 22) and would be released according to signals provided by the experimenter. We developed two inducible cell lysis systems in pursuit of a system with these characteristics. The first one involved plasmid pDKL01 and the lysis gene *E* from bacteriophage φX174. This gene activates cellular autolysins (18) that cause formation of a single pore at the cell pole or at the midpoint through which cytoplasmic contents are released (34). The DNA released by this system was highly instable, presumably because of the nuclease activity in the lysates induced with the cellular autolysis system. The half-life of the released DNA was less than 1 h, so the cell lysis system with pDKL01 is not useful for transformation studies. However, the increased nuclease activity induced by autolysis may be of physiological significance and should be taken into account when the persistence of DNA in the environment is studied with externally added purified DNA (for examples, see references 25–27). In any case, the lysis gene *E* may be useful for the construction of biological containment systems, since killing is accompanied by the destruction of released genetic information.

The second cell lysis system involved plasmid pDKL02, which contains the lysis genes *S*, *R*, and *Rz* of bacteriophage λ, and provided released DNA that stably maintained its physical integrity and transforming activity. DNA released by this system was tested with two naturally competent recipient bacteria, namely *P. stutzeri* and *A. calcoaceticus*. The former gave low transformation frequencies and was killed at recipient suspension-to-lysate ratios of lower than 50:1 (vol/vol) by the λ lysis proteins or cellular products released by the action of the lysis proteins. On the other hand, *A. calcoaceticus* was not sensitive to lysates and exhibited high transformation frequencies, thereby providing high reproducibility in transformation experiments.

Under the conditions in our experiments, the transformation frequencies of prototrophic phenotypes resulting from a DNase-sensitive process involving lysis-released DNA and DNA purified by standard procedures were nearly identical. This would suggest that lysis-released DNA is as efficient as purified DNA in natural transformation. The difference in

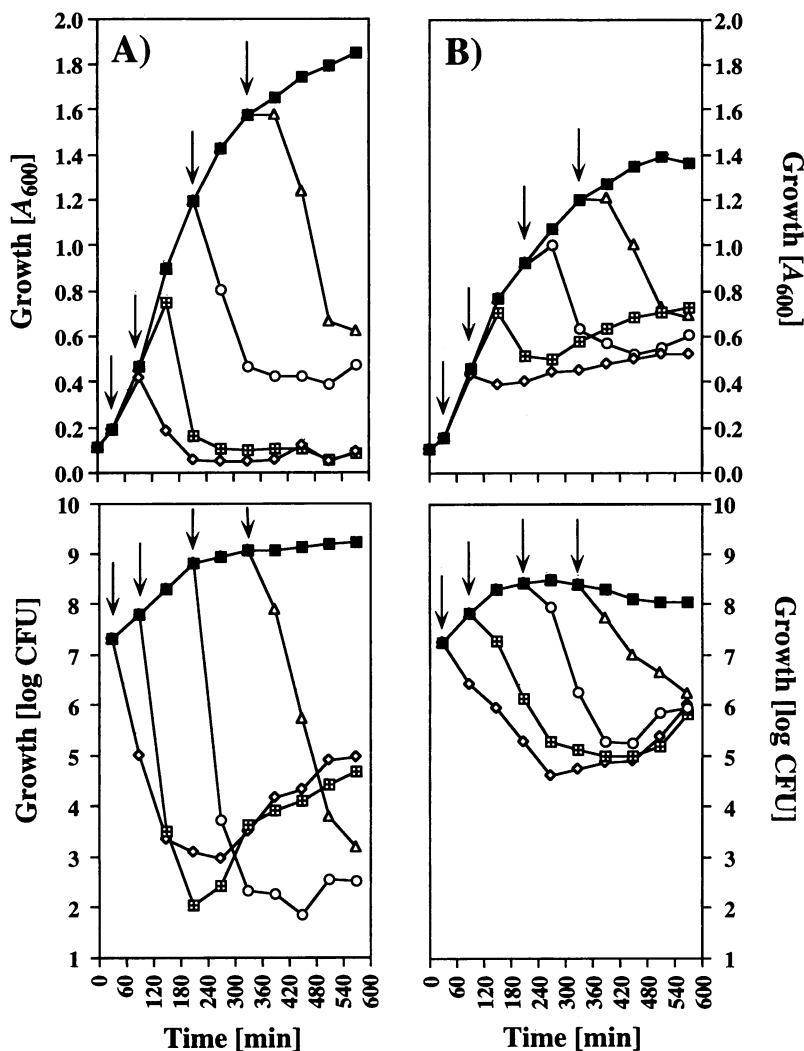


FIG. 6. Induction of cell lysis in *E. coli* S17-1(pDKL02) (A) and *A. calcoaceticus* DSM587(pDKL02) (B) bacteria during the growth phase. Bacterial growth was monitored by measuring the A_{600} of cell cultures and the number of CFU. The effect on growth became progressively lower in the stationary phase, and no effect was observed when cultures were induced in the late stationary phase (for *E. coli* S17-1, $A_{600} \geq 1.9$; for *A. calcoaceticus* DSM587, $A_{600} \geq 1.4$). Closed symbols represent the growth of noninduced cultures, whereas open symbols represent the growth of induced cultures (each type of symbol represents one culture). The arrows indicate additions of inducer at different stages of growth.

relative frequencies of plasmid DNA uptake (selection for kanamycin resistance) versus chromosomal marker acquisition (selection for prototrophic growth) could depend on the type of selection markers, as was described in a previous study (21).

There are at least three differences between lysis-released DNA and purified DNA that may influence transformation efficiencies, namely, size, chemical composition, and structure (see above). In the case of lysis-released DNA, the presence of cell debris and proteins released during lysis could also be a factor. Since purified DNA is subject to physical and chemical handling procedures known to fragment DNA molecules, it is presumed shorter and less efficient at transformation than lysis-released DNA. However, there is presumably an optimum transformation size for DNA above which frequencies fall. The high viscosities associated with high-molecular-weight DNA may also reduce transformation efficiency. The influence of cellular DNA complexing agents like DNA-binding proteins, RNA, and divalent cations on transformation and their fates over time are not known, and the potential effects of released

cellular proteins are also not known, although it has been reported that released proteins stabilize the nuclease activity of *Serratia marcescens* (1). The genetic tools developed in this study will enable the design of experiments to shed light on these aspects and permit transformation by lysis-released DNA to be studied in situ. Our current studies involve the analysis of the physical, chemical, and biological fate of marker DNA molecules (plasmid chromosomal segments carrying defined genetic markers) liberated from bacteria, labelled with appropriate precursors, and incorporated into environmental samples (water, soil, and sediment) prior to lysis. These studies will provide information on the length of time released DNA remains biologically active in different compartments under different prevailing conditions. Analysis of transformation under such conditions, following incorporation of essential isogenic donor and recipient cells of *A. calcoaceticus* into microbial communities of various complexities, will permit the characterization of the ecological parameters that influence transformation and hence gene flux in microbial communities.

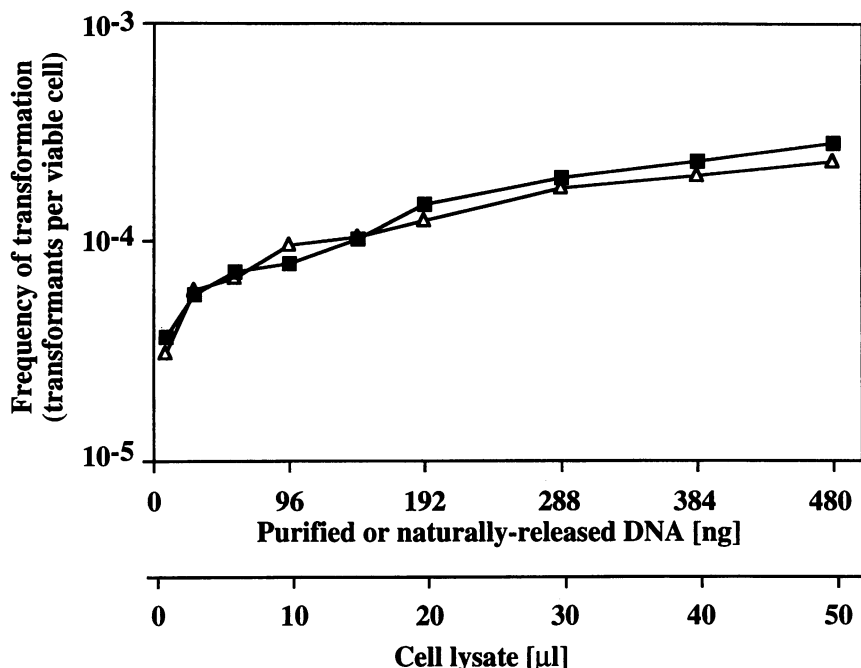


FIG. 7. Natural transformation with purified and lysis-released DNA. Competent *A. calcoaceticus* DSM587 cells were transformed with increasing amounts of purified or lysis-released DNA obtained from *A. calcoaceticus* DSM588(pDKL02). Purified DNA was dissolved in LB broth; identical volumes and concentrations of DNA (isolated or lysis-released) were added to 300- μ l aliquots of competent cells. Transformation frequencies are expressed as prototrophic transformants per recipient. Spontaneous mutation to prototrophic phenotype was fewer than 10^{-9} per recipient cell. The data are the means from two independent experiments. Open triangles indicate transformants with purified DNA; closed squares indicate transformants with lysis-released DNA.

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