Ultrasound-mediated DNA transfer for bacteria

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ABSTRACT

In environmental microbiology, the most commonly used methods of bacterial DNA transfer are conjugation and electroporation. However, conjugation requires physical contact and cell–pilus–cell interactions; electroporation requires low-ionic strength medium and high voltage. These limitations have hampered broad applications of bacterial DNA delivery. We have employed a standard low frequency 40 kHz ultrasound bath to successfully transfer plasmid pBBR1MCS2 into \textit{Pseudomonas putida} UWC1, \textit{Escherichia coli} DH5\textalpha{} and \textit{Pseudomonas fluorescens} SBW25 with high efficiency. Under optimal conditions: ultrasound exposure time of 10 s, 50 mM CaCl\textsubscript{2}, temperature of 22°C, plasmid concentration of 0.8 ng/\mu{}l, \textit{P. putida} UWC1 cell concentration of 2.5 \times{} 10\textsuperscript{8} CFU (colony forming unit)/ml and reaction volume of 500 \mu{}l, the efficiency of ultrasound DNA delivery (UDD) was 9.8 \pm{} 2.3 \times{} 10\textsuperscript{-6} transformants per cell, which was nine times more efficient than conjugation, and even four times greater than electroporation. We have also transferred pBBR1MCS2 into \textit{E. coli} DH5\textalpha{} and \textit{P. fluorescens} SBW25 with efficiencies of 1.16 \pm{} 0.13 \times{} 10\textsuperscript{-6} and 4.33 \pm{} 0.78 \times{} 10\textsuperscript{-6} transformants per cell, respectively. Low frequency UDD can be readily scaled up, allowing for the application of UDD not only in laboratory conditions but also on an industrial scale.

INTRODUCTION

Transferring DNA to cells is a fundamental technique of molecular cloning, which has revolutionized molecular biology. In clinical practice, gene therapy and RNAi rescue is dependent on the success of DNA/RNA delivery (1). However, in environmental microbiology, the vast majority of prokaryotes (>99%) in natural environments are unculturable (2), and therefore not amenable to DNA delivery using traditional culture-dependent DNA delivery methods. Even when prokaryotes can be cultured in vitro, genetic manipulation is frequently impeded because of the lack of efficient, non-invasive and simple methods for DNA delivery. Efficient transfer of DNA to natural microbial communities, such as those found in groundwater and soils, could be used to rapidly introduce novel gene functions, and therefore change the structure and functionality of microbial communities. Delivery of regulatory genes or small regulatory RNA (sRNA) could be used to silence bacterial genes and alter bacterial behaviour, and sRNA and regulatory genes have the potential to be used as novel antibiotics against multidrug-resistant bacteria, or to inhibit bacterial biofilm formation (3). Therefore, a simple and efficient DNA/RNA delivery system is essential for the successful application of all of these technologies.

Biological methods for bacterial DNA transfer include conjugation, gene transformation and transduction (4–8). Biological methods such as conjugation and transduction usually require a specific DNA donor or host strain to achieve bacterial DNA transfer (6), while gene transformation is limited to a few naturally competent groups (8). Physical methods for gene transfer include microinjection, particle bombardment, electroporation, laser irradiation and sonoporation (9). Microinjection and particle bombardment are interference techniques: they directly penetrate the cell membrane to introduce nucleic acids into cells (9). Laser irradiation employs a laser to change cell permeability and allows DNA to be transferred (9,10), but requires physical contact of laser and cells. Most of these physical techniques are only applied to small-scale samples of eukaryotic cells. In practice, the most commonly used methods for bacterial gene transfer are...
conjugation and electroporation, along with heat shock transfer, which is mostly used with *Escherichia coli* cells. Conjugation requires physical contact of recipient and a donor with conjugative plasmid (11,12), or the participation of the third bacterium with a helper plasmid (13). Electroporation is highly efficient but requires a low-ionic strength medium and a high voltage for operation (14,15). Neither method can be adapted for a broad range of DNA delivery applications.

Ultrasound DNA delivery (UDD) is an ideal approach for plasmid or DNA fragments delivery, which has been intensively studied in the context of eukaryotic transformation and gene therapy recent years (16–21). The mechanism of UDD is primarily based upon a cavitational effect, which physically generates reversible porosity in the cell membrane (22–24). UDD has several advantages that make it an attractive technique for DNA delivery. First, UDD can, in theory, deliver DNA or RNA to any type of cell including bacteria (25), fungi, plants (26) and mammalian cells (16,18,19,21,27,28). Second, UDD does not require ion-free media, and therefore can be applied to cells growing in natural media or human body fluids. Third, UDD is a non-invasive method, which does not require direct physical contact. However, in all previous studies, the ultrasound frequency used for transferring DNA was in the ranges of 1–3 MHz (16–21). Such frequencies have worked well with eukaryotic cells but the efficiency of bacterial transformation at these frequencies is low compared to conventional methods for the efficiency of bacterial transformation at these frequencies (25).

We report here that a standard low frequency (40 kHz) ultrasound clean bath can be used to successfully and efficiently deliver plasmid pBBR1MCS2 into *Pseudomonas putida* UWC1. Optimized conditions gave a delivery efficiency of 9.8 ± 2.3 × 10^-6 transformants per cell, which was significantly higher than the results of conjugation and even electroporation. We have also successfully transferred pBBR1MCS2 to *Escherichia coli* DH5α and *Pseudomonas fluorescens* SBW25 with 1.16 ± 0.13 × 10^-6 and 4.33 ± 0.78 × 10^-6 transformants per cell. Low frequency UDD has several advantages over high frequency UDD and other transformation methods for industrial and environmental applications. Low frequency UDD can be used to transform bacteria at room temperature and in a wide range of media. Furthermore, ultrasonic apparatus operating in the frequency range of 20–40 kHz can be readily scaled up, allowing for the application of UDD on large scales, for example, introducing DNA to natural microbial communities in order to promote enhanced biodegradation of pollutants in groundwater or wastewater treatment plants.

**MATERIALS AND METHODS**

**Chemicals and media**

All chemicals were obtained from Sigma-Aldrich Co., UK and were analytical-grade reagents unless otherwise stated. Luria–Bertani (LB) medium was used for the cultivation of bacteria. SOC medium (29) was used to recover antibiotic-resistant cells after electroporation and UDD. Ampicillin and rifampicin were used at a final concentration of 100 μg/ml. Fifty microgram per millilitre of kanamycin was used with *E. coli* DH5α and *P. putida* UWC1 and 100 μg/ml with *P. fluorescens* SBW25. LB agar containing 100 μg/ml rifampicin (LBR) was used to obtain total cell counts of *P. putida* UWC1. LB agar with 50 and 100 μg/ml kanamycin (LGBK) was used to select for transformants of *E. coli* DH5α and *P. fluorescens* SBW25. LGBK supplemented with 100 μg/ml rifampicin (LBRK) was used to identify and count *P. putida* UWC1 transformants carrying pBBR1MCS2.

**Bacteria and plasmids**

*E. coli* DH5α, *P. putida* UWC1 and *P. fluorescens* SBW25 were used as recipients of plasmid. *P. putida* UWC1 is a spontaneous rifampicin-resistant mutant of *P. putida* KT2440 (30). The broad-host-range cloning vector pBBR1MCS2 (5144 bp) (31,32), hosted in *E. coli* JM109 (pBBR1MCS2) was employed as delivery DNA. Plasmid DNA was extracted and purified from overnight cultures of *E. coli* JM109, DH5α, *P. putida* UWC1 or *P. fluorescens* SBW25 using a QIAprep Spin Miniprep kit (QIAGEN, UK). DNA concentration was determined using a GeneQuant Pro RNA/DNA calculator (Amersham Pharmacia Biotech, USA).

**Ultrasound apparatus**

Two different ultrasound systems were used with a frequency of either 40 or 850 kHz. The 40 kHz ultrasound apparatus was a standard ultrasonic cleaning bath 375 H (Langford Electronics Ltd., Coventry, UK) with a maximal electrical energy output of 75 W. The ultrasound water bath dimension was 240 × 135 × 100 (mm, L×W×H). The higher frequency ultrasound apparatus was an 850 kHz ultrasonic bath (Meinhart Ultraschalltechnik, Germany, K80-5) connected to a transducer. The K80-5 generator could generate ultrasound at four intensities, with a maximal electrical energy output of 140 W.

Ultrasound may have a heating effect, so temperature was monitored throughout the experiments using a Digital Test Thermometer (Brannan Thermometers, UK).

To determine the ultrasound power in the plasmid transfer system, the temperature (T) was recorded against time (t), at 15 s intervals, over a period of 4 min using a thermocouple placed in the reaction itself. From the T versus t data, the temperature rise at zero time (dT/dt), can be estimated by either constructing a tangent to the curve at time = 0, or by curve fitting the data to a polynomial in t. The ultrasonic power actually entering the system can be obtained from this by substituting the value of (dT/dt), at time zero (obtained from either method) into the following equation (33):

\[
\text{Power} = \frac{dT_c}{dt} \frac{M}{c_p}
\]

where \(c_p\) is heat capacity of the solvent (J/kg/K) and \(M\) the mass of solvent used (kg).

Power measurements were achieved using the 1.75-ml flat-bottom glass vial (Richardsons of Leicester Ltd., UK).
Ultrasound plasmid transfer

All experiments were conducted with 5–24 replicates and means ± SE are presented. Statistical tests were performed using Excel (Microsoft, Inc.). Prior to transformation, single colonies of *P. putida* UWC1, *E. coli* DH5α or *P. fluorescens* SBW25 were inoculated and incubated in 10 ml LB broth for 13–15 h at 28°C. Solutions of 0.85% NaCl and then resuspended in 10-ml transformation medium. One, three or five hundred microlitre 0.8% NaCl was then added to 100 µl reaction mixture. Unless otherwise stated, cell concentrations were fully immersed in the ultrasound water bath, which was placed in the centre of the 375H ultrasound bath. The vials were transferred to a 1.5-ml Eppendorf tube and added to 100 µl reaction mixture. Cells were incubated in a 28°C incubator with 150 r.p.m. shaking for 2 h. Cells were diluted and plated onto appropriate media to obtain cell counts.

To attempt plasmid transfer to *P. putida* UWC1 at 850 kHz K80-5, vials containing 100 µl reaction mixture (10⁵ CFU/ml and 0.8 ng/µl of plasmid pBBR1MCS2) were placed at the centre of the ultrasound bath. The exposure time of UDD was 5–30 s for each intensity of constant ultrasound, and 30 s for ultrasound pulse. Temperature was controlled and maintained at 20°C by cycling cooling water.

The effect of UDD on plasmid integrity. To examine the effect of ultrasound on plasmids, vials containing 80 ng of pBBR1MCS2 DNA in 100 µl of 100 mM CaCl₂ solution were separately exposed to 40 kHz ultrasound for 0, 5, 10, 30, 60 s. After ultrasound treatment, each sample was transferred to a 1.5-ml Eppendorf tube and added to 10 µl 3 M sodium acetate, 200 µl absolute ethanol and 1 µl glycerol. Samples were incubated on ice for 30 min and then centrifuged at 163 000g for 10 min. The supernatant was removed and the DNA pellet was dried in an Eppendorf Concentrator 5301 (Hamburg, Germany) for 10 min. The DNA pellet was then resuspended in 10 µl deionized water. The recovered plasmid DNA was examined by agarose-ethidium bromide gel electrophoresis.

Medium effect on UDD efficiencies. To assess medium effect on UDD, three times saline washed *P. putida* UWC1 cells were mixed with plasmid DNA and resuspended in four different types of media: LB medium, phosphate buffer solution (PBS), 100 mM MgSO₄ and 100 mM CaCl₂.

Since CaCl₂ was found to enhance ultrasound plasmid transfer, it was then further investigated by performing UDD in CaCl₂ with final concentrations of 0, 1, 10, 50, 100, 200, 300 or 500 mM.

Temperature effect on UDD efficiencies. To determine the temperature effect, UDD was performed at 0, 22, 28 and 42°C.

Plasmid concentration and UDD efficiencies. UDD was carried out with plasmid DNA at final concentrations of 0, 0.08, 0.2, 0.5, 0.8, 1, 2, 5 or 8 ng/µl cells.

Cells concentration and UDD efficiencies. UDD was carried out at cell concentrations of 10⁷, 10⁸ and 10⁹ CFU/ml.

UDD of *E. coli* DH5α and *P. fluorescens* SBW25. The UDD conditions for *E. coli* DH5α and *P. fluorescens* SBW25 were: 10 s exposure time, 50 mM CaCl₂, 22°C, 1 ng/µl plasmid DNA of pBBR1MCS2 and cell concentrations of *E. coli* DH5α 5.8 × 10⁶ CFU/ml and *P. fluorescens* SBW25 1.1 × 10⁹ CFU/ml. The total reaction volume was 500 µl.

Conjugation and electroporation

Electroporation and conjugation were used as positive controls for plasmid transformation of *P. putida* UWC1. For electroporation, an overnight culture of *P. putida* UWC1 was washed three times and resuspended in 10% glycerol. Electroporation used similar conditions to the optimized conditions reported by Cho et al. (34). Fifty microlitres of cell suspension and 1 µl plasmid DNA (27 ng/µl) were mixed in 0.2-cm electroporation cuvettes and electroporated under conditions of 12.5 kV/cm and 25 µFD using a Bio-rad gene pulser (Bio-Rad Laboratories, UK). Cells were mixed with 950 µl of SOC immediately after electroporation and cells were incubated for 2 h at 28°C. For conjugation, 50 µl of an overnight culture of *P. putida* UWC1 (10⁹ cells/ml) and 50 µl of an overnight culture of *E. coli* JM109 (10⁶ cells/ml), harbouring pBBR1MCS2, were mixed by vortexing for 10 s and loaded onto an 0.2-µm GTBP membrane filter (Millipore, UK) and then placed on a LB agar plate. Cells were incubated for 16 h incubation at 28°C, and then washed from the filters and recovered. After electroporation or conjugation, cells were plated on LBR and LBKR plates to obtain total cell and transformant counts.

DNA manipulation and sequencing

Ultrasound transformants of *P. putida* UWC1, *E. coli* DH5α and *P. fluorescens* SBW25 (pBBR1MCS2) were verified by plasmid digestion, plasmid sequencing and 16S DNA sequencing of recipient cells. Ten transformants of each species were randomly selected from different batches of LBK plates and streaked on fresh LBK plates. Single colonies were picked from each fresh LBK plate and inoculated into LBK liquid medium. Plasmid DNA extracted from *P. putida* UWC1, *E. coli* DH5α and *P. fluorescens* SBW25 (pBBR1MCS2) and from original host *E. coli* JM109 was digested with NotI endonuclease (New England Biolabs, UK), and analysed using a 1%
agarose-ethidium bromide gel. For further confirmation, plasmids were sequenced using primer BBR1_for (5'-CCG AAG CCC AAC CTT TCA TAG AA-3').

16S DNA of *P. putida* UWC1 transformants was amplified by polymerase chain reaction (PCR) using cells from a single colony as a DNA template. The universal 16S primers for PCR were forward 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse 530r (5'-GTA TTA CCG CGG CTG CTG-3'). PCR amplifications were performed with initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 1 min and a final additional 72°C for 10 min extension. PCR products were isolated from a 1% agarose gel and purified according to the manufacturer’s instructions using a QIAquick gel extraction kit (Qiagen Co., UK). 16S PCR products were sequenced using primer 63f.

**Nucleotide sequencing and sequence analysis**

All DNA samples (PCR products or plasmids) were sequenced using dye terminator sequencing on an Applied Biosystems 3730 DNA analyzer, according to the manufacturer’s instructions. DNA sequence analysis was carried out using Blastn for confirmation of sequence homology and sequence data were aligned and edited using BioEdit to confirm correct insertions (Tom Hall, Department of Microbiology, North Carolina State University).

**RESULTS AND DISCUSSION**

**Low frequency ultrasound treatment promotes DNA delivery into bacterial cells**

Although high frequency (>-200 kHz) ultrasound has been reported to promote DNA transfer into mammalian cells (9,16–21), we found that high frequency 850 kHz ultrasound failed to deliver plasmid pBBR1MCS2 into *P. putida* UWC1 (data not shown). However, low frequency 40 kHz ultrasound did promote the transfer of plasmid pBBR1MCS2 into *P. putida* UWC1 (Figure 1A). We observed no transfer of plasmid DNA into *P. putida* UWC1 in the absence of exposure to ultrasound (Figure 1A), which excludes the possibility that transformation was due to natural competence. In all UDD experiments, we used flat-bottom glass vials. Plastic vessels can absorb ultrasound energy and round and smooth bottom vials reflect ultrasound, making the efficiency of UDD low (data not shown). As the ultrasound generator was set at the bottom of the 375H ultrasonic cleaning bath, the alignment of glass vials and generator is critical. We tried nine different positions and found that the efficiency of UDD at the centre point of the 375H water bath was higher than other points. Therefore, all UDD experiments were carried out by positioning a single vial at the centre point of the 375H bath. The ultrasound power at the centre point of the 375H bath was calculated using Equation (1), as described in materials and methods, to be 240 mW/cm² in each vial.

**Ultrasound exposure time affects cell viability, plasmid integrity and UDD**

The damage to cells which is generally associated with ultrasonic energy was first recorded in 1929 in a study of *Bacillus fisheri* in sea water (35). High power ultrasound is

![Figure 1](image-url)
P. putida.

In our experiments, controlling the power of sonication can lead to beneficial, non-lethal, effects on biological activity e.g. the promotion of bioreactor efficiency (37,38). In our experiments, prolonged exposure of P. putida UW1 to 40 kHz for more than 60s efficiently killed bacterial cells (Figure 1B), which is to be expected as low frequency ultrasound has been used for cell lysis and disruption (39-41). Exposure times of less than 30s did not significantly reduce cell viability (Figure 1B). Interestingly, exposure time of 10s resulted in higher colony counts in viability assays, which may be due to the short ultrasound treatment separating and dispersing clumped cells (Figure 1B).

Exposure of plasmids to ultrasound for 30 or 60s significantly damaged plasmid integrity and sheared plasmids into fragments (Figure 1C), which is in good agreement with observations carried out by Grokhovsky (42). However, plasmid exposure times less than 10s did not affect plasmid integrity, as assessed by gel electrophoresis (Figure 1C).

Occasionally, plasmids with 30 and 60s ultrasound exposures (Figure 1C, lanes 11 and 13) were not completely sheared; later examination indicated that in those cases the bottoms of vials and 375H were not parallel, and some the ultrasound energies might be reflected. Therefore, in order to efficiently transport ultrasound energy to the cell–plasmid mixtures, the flat bottom of vials should be parallel with the bottom of 375H ultrasonic cleaning bath.

The results of cell viability and plasmid integrity assays are in good agreement with UDD result (Figure 1), which showed that plasmid transfer occurred at a high frequency following ultrasound treatments of 5–30s. Since 30s ultrasound exposure time damaged plasmid DNA (Figure 1C) and 5s exposure time gave relatively variable results (Figure 1A), all subsequent experiments were performed with a 10s ultrasound treatment.

**Confirmation of plasmid delivery to P. putida UW1, E. coli DH5α and P. fluorescens SBW25**

We randomly picked 10 ultrasound-transformants of each species: P. putida UW1, E. coli DH5α or P. fluorescens SBW25 to confirm that kanamycin resistance arose as a result of UDD. Ten transformants of each species were confirmed by 16S rDNA sequencing of each colony, which confirmed that all 10 transformants had identical 16S rDNA to P. putida UW1, E. coli DH5α or P. fluorescens SBW25. Plasmid DNA was extracted from each of the 10 transformants. The plasmids isolated from ultrasound transformants P. putida UW1 (Figure 2), E. coli DH5α and P. fluorescens SBW25 (data not shown) were digested with NotI and compared with pBBR1MCS2 and confirmed they had identical structures. Finally, the plasmids were partially sequenced and the results confirmed that they were pBBR1MCS2.

**Addition of calcium chloride to DNA transfer media significantly enhances UDD**

We tested the effect of four different media on UDD efficiency, with P. putida UW1 as the recipient strain: LB, phosphate buffered saline (PBS), MgSO4 and CaCl2, respectively. Only CaCl2 had a significant effect on UDD (data not shown). CaCl2 concentrations lower than 10mM or greater than 500mM had no significant effect on UDD (Figure 3). The optimal CaCl2 concentration for UDD was 50mM CaCl2 (Figure 3).

Langer and colleagues suggested that the mechanism of sonophoresis with low frequency ultrasound, such as 20kHz, is primarily due to cavitational effects (43-45). We propose a mechanism of ultrasound-mediated plasmid transfer of bacteria in which plasmid transfer is enhanced in the presence of CaCl2 (Figure 4): Plasmid DNA and bacteria are initially well-mixed in an aqueous solution (Figure 4A). The addition of CaCl2 causes changes in the
conformation of plasmid DNA or cellular membrane structures that promote transformation (Figure 4B). As low frequency 40 kHz ultrasound is applied to the solution the transmitted energy causes temporary porosity in the cell membrane, which enables the plasmids to enter through the pores (Figure 4C). When the ultrasound is switched off the cell membrane repairs itself and the transformed cell retains the plasmid DNA (Figure 4D). Bacteria acquire new functions, such as growing on a selective medium, after taking up plasmid DNA (Figure 4E).

Temperature affects UDD efficiency

The efficiency of UDD to _P. putida_ UWC1 at 22°C was higher than UDD efficiency at 0, 28 or 42°C (Figure 5). However, the reason for this remains unclear. We monitored the temperature of DNA transfer media during 10 s ultrasound, and found that short-term ultrasound treatment did not increase the temperature of cell–plasmid reaction solutions (data not shown), which rules out the possibility that thermal effects associated with ultrasound treatment affected UDD.

The effects of plasmid and cell concentration on UDD

UDD efficiency was highest at plasmid concentrations of 0.8 ng/µl with decreasing efficiency at lower and higher values (Figure 6). Cell concentrations of 6.0 ± 0.9 × 10⁷ CFU/ml or less did not give a significant number of transformants at a plasmid concentration of 0.8 ng/µl (Table 1). However, increasing concentration of plasmids up to 8 ng/µl did generate a few transformants. Since UDD requires the interaction of cells and DNA, as cells concentration was low, the chance that plasmids meet cells significantly decreased. This may suggest that the number of collisions between cells and plasmids is important for UDD. Cell concentrations of 5.3 ± 1.3 × 10⁸ and 3.2 ± 0.3 × 10⁹ CFU/ml gave UDD efficiencies of 1.1 ± 0.4 × 10⁻⁶ and 4.2 ± 2.4 × 10⁻⁶ transformants per
These findings show that the efficiency of UDD in bacterial cells is affected by the cells population density and the concentration of plasmid DNA used during the sonoporation process.

To ensure the 40 kHz ultrasonic cleaning bath can deliver DNA to *P. putida* UWC1, we have also attempted to transfer plasmids to *E. coli* DH5α and *P. fluorescens* SBW25. Using the optimal conditions based on UDD experiments of *P. putida* UWC1, we have successfully delivered pBBR1MCS2 to *E. coli* and *P. fluorescens* SBW25 with efficiencies of $1.16 \pm 0.13 \times 10^6$ and $4.33 \pm 0.78 \times 10^6$ transformants per cell, respectively (Figure 7A and B). To improve UDD efficiency of *E. coli* DH5α and *P. fluorescens* SBW25, more work in the future is needed, since the optimal conditions for *P. putida* UWC1 may not necessarily be the best for *E. coli* DH5α and *P. fluorescens* SBW25E.

UDD delivered plasmids to *E. coli* DH5α or *P. fluorescens* SBW25

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Comparison of conjugation, electroporation and UDD of *P. putida* UWC1

Under optimal conditions for *P. putida* UWC1: ultrasound exposure time of 10 s, 50 mM CaCl$_2$, temperature of 22°C, plasmid concentration of 0.8 ng/µl, *P. putida* UWC1 cell concentration of $2.5 \times 10^9$ CFU/ml and reaction volume of 500 µl, the efficiency of UDD was $9.8 \pm 2.3 \times 10^{-6}$ transformants per cell. UDD efficiency was compared with two traditional plasmid transfer methods: conjugation and electroporation, and was found to be nine times higher than conjugation and up to four times higher than electroporation (Figure 8).

The ultrasound apparatus used in this study was a standard ultrasound bath. Further investigation of the effects of ultrasound frequency, intensity, alignment and distribution on UDD may well lead to further
improvements in transformation efficiency, and allow this process to be scaled to an industrial level. Our results have shown that UDD is a viable technology for bacterial transformation, which is far less dependent on the ionic strength of the medium than electroporation, allowing it to be used with bacteria growing in complex environments and under more natural conditions. We believe UDD will open up numerous new opportunities for DNA and RNA delivery into a wide range of microorganisms.

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