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RESEARCH ARTICLE

Transport of antibiotic resistance plasmids in porous media and the influence of surfactants

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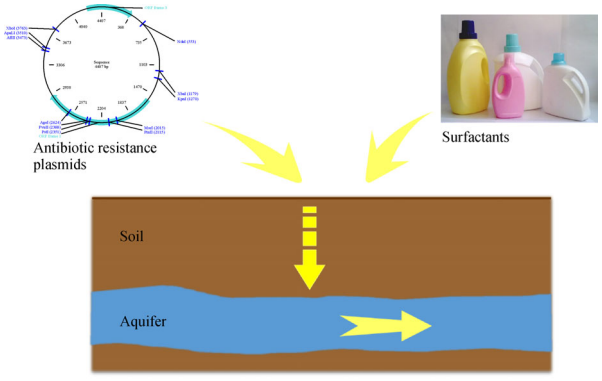
HIGHLIGHTS

- Indigenous and engineered plasmids have similar transport behavior in porous media.
- Indigenous plasmid pK5 transports similarly in quartz sand and soil.
- Anionic surfactant SDS has negligible effect on plasmid transport in porous media.
- Cationic surfactant CTAB affects plasmid transport at high concentrations.
- Indigenous plasmids may transport over significant distances in environment.

GRAPHIC ABSTRACT



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ABSTRACT

Transport of engineered antibiotic resistance plasmids in porous media has been reported to potentially cause significant spreading of antibiotic resistance in the environment. In this work, transport of an indigenous resistance plasmid pK5 in porous media was investigated through packed column experiments. At identical ionic strengths in $CaCl_2$ solutions, the breakthroughs of pK5 from soil columns were very close to those from quartz sand columns, indicating that transport of pK5 in quartz sand and soil was similar. A similarity in transport behavior was also found between pK5 and an engineered plasmid pBR322 that has approximately the same number of base pairs as pK5. The influence of surfactants, a major group of constituents in soil solutions, was examined using an engineered plasmid pcDNA3.1(+)/myc-His A. The impact of an anionic surfactant, sodium dodecyl sulfate (SDS), was negligible at concentrations up to 200 $mg \cdot L^{-1}$. Cetyltrimethyl ammonium bromide (CTAB), a cationic surfactant, was found to significantly enhance plasmid adsorption at high concentrations. However, at environmentally relevant concentrations (< 1 $mg \cdot L^{-1}$), the effect of this surfactant was also minimal. The negligible impact of surfactants and the similarity between the transport of engineered and indigenous plasmids indicate that under environmentally relevant conditions, indigenous plasmids in soil also have the potential to transport over long distances and lead to the spreading of antibiotic resistance.

1 Introduction

Antibiotics have been used in large quantities to prevent bacterial infections and to reduce diseases and promote growth for livestock and poultry in farms. Soil is one of the primary receivers of residue antibiotics. Antibiotics used as feed additives for livestock and poultry are released mainly into farmlands through manure application [1]. Antibiotics for medical purposes may be released into soil through wastewater irrigation and land application of sewage sludge [2]. The released antibiotics have caused extensive antibiotic resistance buildup in soil [3,4]. Antibiotic resistance buildup may pose risks to human health [5], as susceptible pathogenic bacteria can become resistant by

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acquiring resistance genes in soil and other environmental media. Acquisition of resistance by pathogens will compromise the effectiveness of antibiotics.

Enhanced antibiotic resistance is commonly attributed to the exchange of antibiotic resistance genes in the environment, as sensitive bacteria are more likely to acquire resistance by horizontal gene transfer than by mutations under stress of antibiotics [6,7]. Horizontal gene transfer mechanisms include transformation, transduction and conjugation. Resistance plasmids play important roles in these mechanisms. For example, transformation involves direct intake and reorganization of free plasmids by a bacterial cell [8].

Plasmids may be released either by active secretion of bacteria or due to lysis of cells in their final stage of life [9,10]. Released plasmid may spread in the environment. In particular, free resistance plasmids in soil may be dispersed downward by soil water during precipitation and wastewater irrigation, potentially reaching an aquifer. Groundwater flow in an aquifer may further carry the plasmids over a much longer distance, causing spread of resistance and posing risks to public health. Thus, understanding the transport behavior of resistance plasmids in soil is of great importance.

Plasmid adsorption to soil is one of the primary governing factors of its transport in soil. Many studies in the literature have investigated the effects of the humic substance content of soil, soil solution chemistry, surface properties of porous media, and morphology of plasmids on plasmid adsorption to soil [11–13]. In contrast, reports on the transport of free plasmids in porous media are very limited in the literature. In addition, early studies in this regard conducted experiments under very few conditions [14–16]. For example, Poté et al. performed experiments to study the transport of supercoiled and linear pLEP01 under a single flow and solution chemistry condition (600 $mg \cdot L^{-1}$ KCl) [14]. Recently, Chen et al. systematically examined the transport of eight resistance plasmid vectors (pUC18, pBlueScript II SK(+), pBR322, pFastBac HT A, pBABE-Puro, pcDNA3.1(+)/myc-His A, pcDNA3.1(+)/Flag-His A + ATM, and pAdEasy-1) in quartz sand through packed column experiments [17]. It was found that transport behavior of free plasmids is very similar to transport of colloids commonly examined in the literature (e.g., latex microspheres and bacteria). More importantly, it was demonstrated that under environmentally relevant flow and solution chemistry conditions, free plasmids may transport over significant distances in porous media. However, the authors only used engineered plasmids and quartz sand for their experiments. The transport behavior of indigenous resistance plasmids in soil is still not known.

Soil solution includes multiple inorganic and organic constituents. Surfactants, compounds that lower interfacial tension, are a group of major organic pollutants in soil solution. They have been widely used in households and industries, e.g., as detergents, emulsifiers, foaming agents, and dispersants. Massive application of surfactants inevitably leads to its release into the environment. For example, in the past few decades, anionic surfactants have become a group of major pollutants in bodies of water, with concentrations ranging from 0.01 to over 1 $mg \cdot L^{-1}$ [18]. Surfactants are also released into soil in large quantities via wastewater irrigation and sludge application [19–21]. Surfactants by their nature can alter the surface properties of soil particles to a large extent and may dramatically affect transport of plasmids and other colloids. Indeed, distinctive adsorption kinetics for surfactant–DNA complexes and DNA molecules in surfactant solutions have been observed [22,23]. Some cationic surfactants were even found to alter DNA conformation and cause phase separation [24,25]. However, to the best of our knowledge, no study examining the effects of surfactants on plasmid transport has been reported.

The objective of this study was to investigate the transport behavior of an indigenous resistance plasmid and the influence of surfactants on plasmid transport in porous media. Both soil and quartz sand were used in packed column experiments. Two commonly used surfactants, anionic sodium dodecyl sulfate (SDS) and cationic cetyltrimethyl ammonium bromide (CTAB), were selected for examination of their possible influences.

2 Materials and methods

2.1 Plasmid preparation and characterization

Two resistance plasmids, one engineered and one indigenous, were used in this study. The engineered resistance plasmid was pcDNA3.1(+)/myc-His A (Tiandz Inc., Beijing, China), which consists of 5493 base pairs and carries both ampicillin and kanamycin resistance genes. The plasmid was extracted following the procedure described in Chen et al. [17]. Briefly, plasmids were propagated in an *Escherichia coli* host Trans-5α strain. Competent cells (10 μL) were gently mixed with 1 μg plasmid on ice and incubated for 30 min. The mixture was heat shocked for 45 s at 37°C and then cultivated in 2 mL Luria-Bertani (LB) broth for 1 h at 37°C and 200 $r \cdot min^{-1}$ to allow the bacteria to recover. Then, 100 μL of the mixture was spread onto an LB agar plate containing 100 $\mu g \cdot mL^{-1}$ ampicillin and was cultivated overnight at 37°C. A single colony was picked and added to LB broth containing 100 $\mu g \cdot mL^{-1}$ ampicillin. After cultivation overnight at 37°C with shaking at 200 $r \cdot min^{-1}$, the plasmid was extracted using EZ Spin Column Plasmid Medi-Preps Kit (UNlQ-200, Sangon Biotech Co., Ltd., Shanghai, China). The extracted plasmids were purified using the spin column of the same kit. The column was washed via centrifuging with solution provided by the manufacturer.

Ultra-pure water (Milli-Q Advantage A10, Millipore Corp., MA, USA) was used for elution after washing and air drying of the column. The plasmid solution was stored at −20°C before further experiments.

The indigenous resistance plasmid was extracted from surface soil (0–5 cm) collected from farmland at Yangge Village (39.6986 N, 116.4706 E) in the suburb of Beijing. Selective cultivation on LB agar plates using kanamycin, tetracycline, and erythromycin indicated that the soil contained bacteria resistant to these antibiotics. To extract the plasmid, 10 g of soil was mixed with 50 mL 0.85% saline and vortexed for 1 min. The supernatant was centrifuged at room temperature for 5 min at 6000 g. These two steps were repeated 3 times. The supernatants were combined and added with isopropanol to precipitate plasmids. Column Humid Acid Erasol (Tiandz Inc., Beijing, China) was used to remove humid acid from precipitated plasmids. The plasmids were then propagated in *Escherichia coli* host Trans-5α strains. Following heat shocking and cultivation in LB broth, the mixture of competent cells and plasmids was spread on LB agar plates containing kanamycin, tetracycline, or erythromycin. Single colonies from the plates were picked and added to LB broth containing the corresponding antibiotics. Following incubation, plasmids with resistance to the three antibiotics were extracted according to the procedure described above for extraction of pcDNA3.1(+)/myc-His A. The purified plasmids were again propagated in *Escherichia coli* and cultivated on plates containing the corresponding antibiotics to confirm their resistance. The plasmids were then sent to Tiandz Inc. for shotgun sequencing. Due to low copy numbers during amplification, only one kanamycin-resistant plasmid pK5 was sequenced successfully. This plasmid was found to consist of 4407 base pairs and carry kanamycin and tetracycline resistance genes (Fig. 1). The complete sequence of pK5 has been included in GenBank (number KJ792090).

The absorbance of the extracted plasmids at 260 nm and 280 nm was measured using a micro-volume UV spectrophotometer (SMA1000, Merinton Ltd., MI, USA). The A260/A280 ratios of both plasmids were between 1.8 and 2.0, indicating negligible protein contamination (plasmids contaminated with proteins would have A260/A280 ratios less than 1.8). The concentrations of plasmid solutions were derived from the absorbance at 260 nm according to the Beer–Lambert equation with an extinction coefficient of 0.02 $mL \cdot \mu g^{-1} \cdot cm^{-1}$. The concentration of indigenous plasmid pK5 extracted with the identical procedure was typically less than one tenth the concentration of pcDNA3.1(+)/myc-His A, because of an extremely low copy number of former plasmid during amplification. Zeta potentials of pcDNA3.1(+)/myc-His A in water, $CaCl_2$ and surfactant solutions were measured using a Zetasizer Nano (ZS90, Malvern Instruments Ltd., Worcestershire, UK). Zeta potentials of pK5 could not be measured as an insufficient amount of the plasmid was obtained.

2.2 Column experiments

Unimin quartz sand (Unimin Corporation Co., CT, USA) with a diameter range of 417 to 600 μm and agricultural topsoil collected from Yangge Village were used as porous

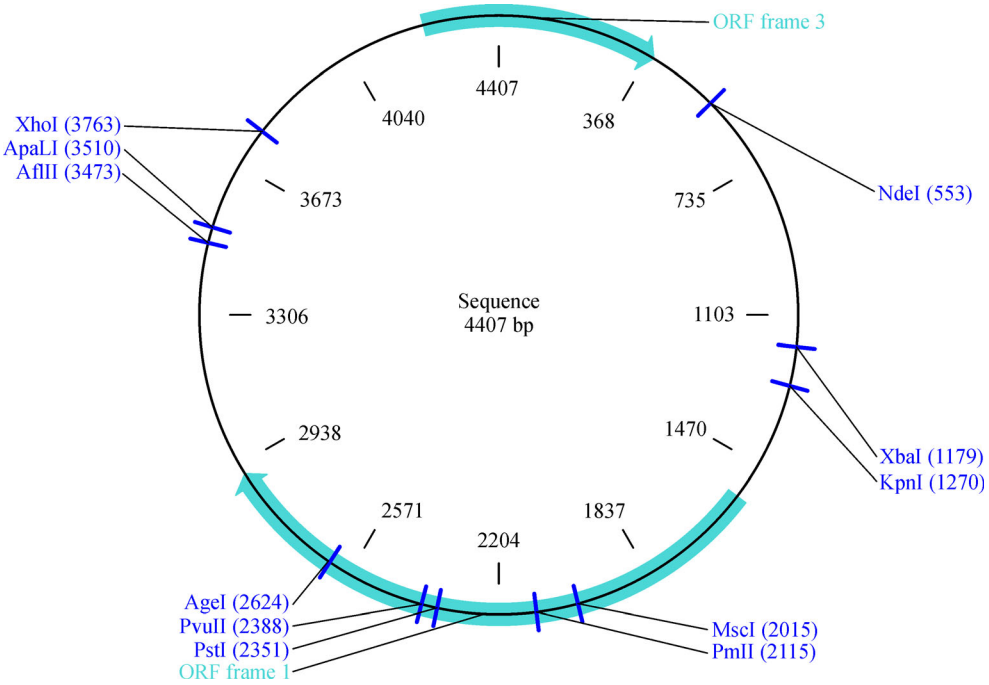

Fig. 1 Profile of the indigenous antibiotic resistance plasmid pK5

media in column experiments. The soil was air-dried and sieved to collect particles with a diameter range of 400 to 600 μm for further use. These soil particles had a pH of 7.7, a moisture content of 176 $g \cdot kg^{-1}$, a total organic content of 10 $g \cdot kg^{-1}$, and a cationic exchange capacity of 11.9 cmol $(+) \cdot kg^{-1}$. The pH value was measured at a soil-to-water ratio of 1:2.5. The total organic content was derived from the difference between total and inorganic contents. Both the total and inorganic carbon contents were determined using a TOC-VCPH SSM-5000A elemental analyzer (Shimadzu, Japan). The cationic exchange capacity was determined following the procedure described in the Chinese standard F-HZ-DZ-TR-0029.

Before use, the sand was soaked by concentrated HCl solution overnight, rinsed repeatedly with ultra-pure water, oven-dried at 105°C, and finally baked at 600°C for 4 h. Following pretreatment, the quartz sand and soil particles were wet packed into columns with an inner diameter of 1 cm and a length of 12.7 cm. To better represent actual environmental conditions, soil particles were packed without pretreatment. The porosities of the packed columns were approximately 0.45.

Transport behaviors of pK5 were examined in both quartz sand and soil at ionic strengths (IS) of 0.001, 0.006, 0.02 and 0.05 $mol \cdot L^{-1}$ in $CaCl_2$ solutions. Monovalent electrolyte (NaCl) was shown to have minimal influence on plasmid transport [17] and thus was not considered in this study. The influence of surfactants on plasmid transport was examined using SDS (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and CTAB (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) solutions of 1, 10, 50 and 200 $mg \cdot L^{-1}$. Transport behavior of plasmid pcDNA3.1(+)/myc-His A in quartz sand was examined under the effect of surfactants. In each experiment, the plasmid solutions were injected at a concentration of 5 $mg \cdot L^{-1}$ pcDNA3.1(+)/myc-His A. The effect of surfactants on pK5 transport was not examined because an insufficient amount of the plasmid was obtained.

In each experiment, the packed sand column was first pre-equilibrated with 10 pore volumes of $CaCl_2$ or surfactant solutions of desired concentration using a syringe pump (LSP10–1B, Longer Pump Inc., Baoding, China). The absorbance at λ = 260 nm of effluent surfactant solutions following pre-equilibration was measured using a UV-vis spectrophotometer (Ultrospec 7300 pro, GE Healthcare Bio-Sciences Corp., NJ, USA). The packed soil columns were pre-equilibrated until the absorbance of the effluent at 260 nm no longer decreased (typically approximately 20 pore volumes). After pretreatment, 2 pore volumes (9 mL) of plasmid suspensions were injected into columns, followed by 2 pore volumes of elution with electrolyte or surfactant solutions of the same concentration and free of plasmids. Both injection and elution were performed in up flow mode at a flow rate of 0.15 $mL \cdot min^{-1}$, which corresponded to a pore water velocity of 6.1 $m \cdot d^{-1}$. The effluent samples were collected using a fraction collector (Retriever 500, Teledyne Isco Inc., NE, USA) at sampling intervals of five minutes. The absorbance of the effluent samples at λ = 260 nm was measured using a UV-vis spectrophotometer (Ultrospec 7300 pro, GE Healthcare Bio-Sciences Corp., NJ, USA). Preliminary measurements showed no significant difference between the absorbance of plasmid–surfactant mixed solution and the sum of the absorbance of plasmid solution and surfactant solution. No significant difference was found, either, between the absorbance of plasmid-effluents mixed solution and the sum of the absorbance of soil column effluents following pre-equilibration and plasmid solution. Therefore, the absorbance of the plasmids was derived by subtracting the measured absorbance of effluents with $CaCl_2$ or surfactant solutions alone following pre-equilibration from the absorbance of the effluent samples with plasmids, and then converting this result into plasmid concentrations using the Beer–Lambert equation. More details of the column experiments were available in Chen et al. [17].

The influent and effluent plasmid concentrations were qualitatively verified using agarose gel electrophoresis. The influent and effluent samples were mixed with Gel Loading Buffer (Sigma-Aldrich Co. LLC., MO, USA) and then loaded into a 1% agarose gel. Electrophoresis was performed at 100 V for 40 min. The gel was then visualized by a UV Gel Imaging and Analysis System (Tanon 3500, Tanon Science & Technology Co., Ltd., Shanghai, China) to confirm the integrity of plasmids and the variations of plasmid concentrations.

From effluent concentrations, adsorption rate coefficients (k) of the plasmids in transport experiments were calculated using the following equation that neglects dispersion and assumes an exponential distribution of retained plasmids with transport distance:

$$k = -\frac{v}{L} \cdot \ln \frac{C}{C_0}, \tag{1}$$

where v is the pore water velocity, L is the length of the columns, C is the steady-state or average breakthrough concentration, and C_0 is the influent plasmid concentration.

3 Results and discussion

3.1 Transport of pK5 in quartz sand

In quartz sand, breakthrough concentrations of pK5 reached a steady-state at 0.001, 0.006, and 0.05 $mol \cdot L^{-1}$ $CaCl_2$. However, at 0.02 $mol \cdot L^{-1}$ $CaCl_2$, the concentration increased continually during breakthrough (from pore volume 1 to 3), indicating a temporal decrease in the adsorption rate coefficient during injection. The temporal decrease in adsorption may be due to the so-called blocking process (the adsorbed plasmids occupy the

adsorption sites and thus block the adsorption of incoming plasmids) [26–28]. At low ionic strength (0.001 mol·L^{-1} $CaCl_2$), near complete breakthrough of injected plasmids was observed (Table 1, Fig. 2). The breakthrough concentration decreased significantly as ionic strength increased. At 0.02 and 0.05 mol·L^{-1} $CaCl_2$, only 61.1% and 20.3% of injected plasmids exited the column, respectively. The adsorption rate coefficient increased from 0.05 h^{-1} at 0.001 mol·L^{-1} $CaCl_2$ to 3.1 h^{-1} at 0.05 mol·L^{-1} $CaCl_2$ (Table 1). Increased adsorption with increasing ionic strength (commonly attributed to compression of the double layers of plasmid and sand surfaces) has also been observed in transport experiments of colloids commonly examined in the literature (e.g., latex microspheres and bacteria) [29,30].

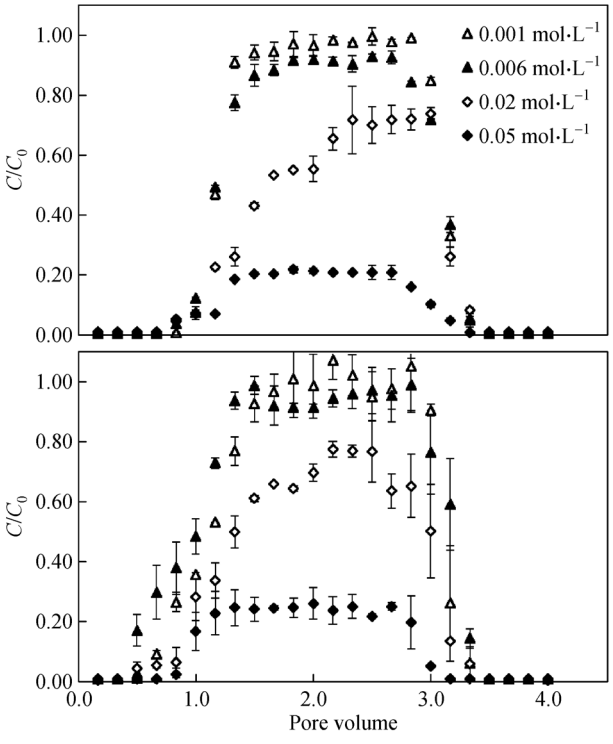

Fig. 2 Breakthrough and elution curves of pK5 in $CaCl_2$ in quartz sand (top) and soil columns (bottom)

In addition, breakthrough concentrations of pK5 at a particular ionic strength were similar to those of pBR322, which has 4361 base pairs (whereas pK5 has 4407 base pairs) [17]. At 0.001, 0.006, 0.02, and 0.05 mol·L^{-1} $CaCl_2$, 95.9%, 89.3%, 61.1%, and 20.3% of injected pK5 exited the column, respectively, whereas under the same column conditions 97.7%, 90.1%, 67.4%, and 21.5% of injected pBR322 exited the column at these ionic strengths, respectively. This observation is consistent with the finding in Chen et al. that plasmid size is the primary governor of plasmid transport in quartz sand under the same column conditions [17]. Under the same experimental conditions, 99.2%, 93.2%, 75.4%, and 21.9% of injected pcDNA3.1 (+)/myc-His A exited the column at 0.001, 0.006, 0.02, and 0.05 mol·L^{-1} $CaCl_2$ [17], respectively. At a particular ionic strength, slightly more injected pcDNA3.1(+)/myc-His A exited the column than pK5. This is consistent with the fact that pcDNA3.1(+)/myc-His A has more base pairs and likely a large particle size. Less adsorption of large plasmids is expected by colloid filtration theory, as the effects of surface properties of plasmids are minimal [17]. Furthermore, pK5, pBR322, and pcDNA3.1(+)/myc-His A all demonstrated a steady-state breakthrough at 0.001, 0.006, and 0.05 mol·L^{-1} $CaCl_2$ and increasing breakthrough at 0.02 mol·L^{-1} $CaCl_2$ during injection. These results indicate that the transport behavior of the indigenous plasmid pK5 is highly similar to that of engineered plasmids. Thus, engineered plasmids can be used as a surrogate to examine factors that affect the transport of indigenous plasmids (such as pK5).

3.2 Transport of pK5 in soil

Breakthrough of pK5 from soil columns was nearly complete at 0.001 and 0.006 mol·L^{-1} $CaCl_2$ (Fig. 2). At 0.02 mol·L^{-1} $CaCl_2$, a continual increase in breakthrough concentration was also observed. At 0.02 and 0.05 mol·L^{-1} $CaCl_2$, 68.7% and 24.5% of injected pK5 exited the column, respectively. The exited fractions were very close to those of pK5 in quartz sand (Table 1). These results indicate that transport of pK5 in quartz sand and the soil was similar and that the effects of the differences in surface properties of quartz sand and the soil were minimal.

At 0.006 mol·L^{-1} in $CaCl_2$, which is higher than the ionic strength of soil solutions and groundwater in many places [31], the breakthrough concentration of the indigenous plasmid was approximately 90% of the influent concentrations at a transport distance of 12.7 cm in quartz sand. This breakthrough concentration translates into an adsorption rate coefficient of 0.18 h^{-1} (Table 1). The

Table 1 Fractions exiting columns and adsorption rate coefficients (k) of indigenous resistance plasmid pK5 at different ionic strength (in $CaCl_2$) in porous media

Porous media	0.001 mol·L^{-1}		0.006 mol·L^{-1}		0.02 mol·L^{-1}		0.05 mol·L^{-1}	
	Exited fractions (%)	k (h^{-1})	Exited fractions (%)	k (h^{-1})	Exited fractions (%)	k (h^{-1})	Exited fractions (%)	k (h^{-1})
Quartz sand	95.9±1.3	0.05±0.02	89.3±0.1	0.18±0.02	61.1±2.7	1.1±0.1	20.3±0.2	3.1±0.0
Soil	102.3±2.5	N/A	109.2±6.0	N/A	68.7±2.5	0.79±0.04	24.5±1.5	2.8±0.1

breakthrough concentration in soil was over 100% at 0.006 mol·L^{-1} in $CaCl_2$, which made the translation into the adsorption rate coefficient not applicable (Table 1). Assuming a constant adsorption rate coefficient of 0.18 h^{-1} over a transport distance in a subsurface environment, plasmid concentrations would still be over 10% of the source concentration at a distance of 3 m. This means that the indigenous resistance plasmid may transport over a considerable distance in soil and may have the potential to reach underlying aquifers. In addition, as observed by Chen et al. [17], significant fractions of adsorbed plasmids could be desorbed at reduced ionic strengths, which means that on occasions of irrigation and rainfall, adsorbed plasmids may be desorbed and transported to even longer distances. Hence, it is highly likely that indigenous plasmids may transport over a noticeable distance and have the potential to spread antibiotic resistance to much larger regions in the environment.

3.3 Influence of surfactants on plasmid transport

Because pK5 demonstrated transport behavior similar to that of engineered plasmids and this indigenous plasmid had very low copy numbers during amplification, the effects of surfactants on plasmid transport were examined in quartz sand using the engineered plasmid pcDNA3.1 (+)/myc-His A. In the absence of $CaCl_2$, breakthrough of pcDNA3.1(+)/myc-His A was nearly complete as SDS concentrations increased from 1 mg·L^{-1} to 200 mg·L^{-1} (Fig. 3). In fact, breakthrough curves at different SDS concentrations were nearly identical in shape. The adsorption rate coefficient showed no clear trend and ranged from 0.03 to 0.13 h^{-1} (Table 2). Clearly, the influence of SDS on plasmid transport was negligible at most.

At a CTAB concentration of 1 mg·L^{-1}, 96.9% of injected plasmid exited the column (Fig. 3). As CTAB concentration increased to 10 and 50 mg·L^{-1}, the fractions of plasmids exiting the column were only 38.0% and 13.5%, respectively. As CTAB concentration increased further to 200 mg·L^{-1}, the fraction of plasmids exiting the column increased to 57.2%. These results indicate that CTAB has a profound impact on transport of pcDNA3.1 (+)/myc-His A and the magnitude of the impact is dependent on the concentration of CTAB.

The difference in the influence of SDS and CTAB on plasmid transport may be explained by the difference in the interaction of the two surfactants with plasmid. The pcDNA3.1(+)/myc-His A plasmid had negative zeta potentials of −49.0±9.0 mV in aqueous solution, indicating that the plasmid is negatively charged. The negative charges emanate from the dissociation of the phosphate groups on the plasmid. As an anionic surfactant, SDS also carries negative charges. Thus, electrostatic repulsion exists between SDS and plasmid DNA molecules and does not significantly alter the overall surface charges of the DNA molecules. In fact, zeta potentials of the pcDNA3.1(+)/myc-His A plasmid ranged from −43.9 to −53.8 mV in SDS solutions.

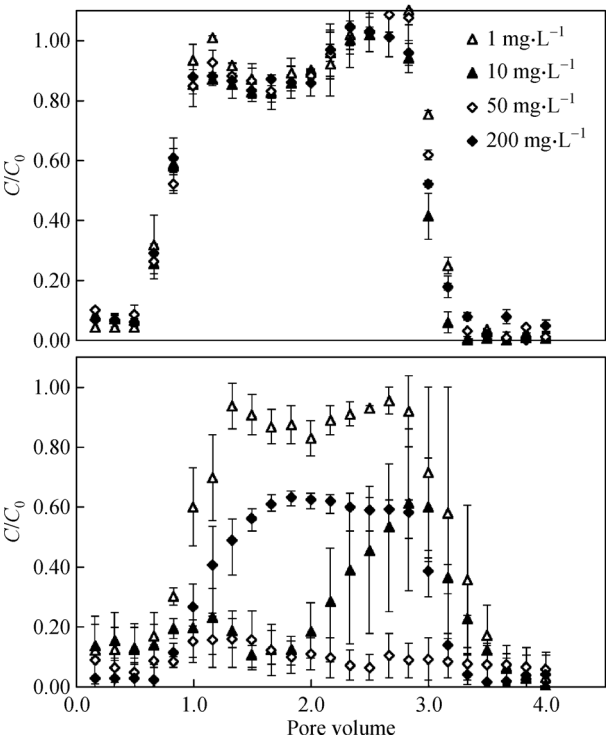

Fig. 3 Breakthrough and elution curves of pcDNA3.1(+)/myc-His A in quartz sand columns in the presence of SDS (top) and CTAB (bottom)

In contrast, CTAB molecules carry cationic surface active groups (CTA^+) and bind tightly with plasmid DNA molecules due to electrostatic attraction. Typically, several CTA^+ and one DNA molecule form complexes [25], altering the surface charges of the DNA molecules. The zeta potential of the pcDNA3.1(+)/myc-His A plasmid in absence of $CaCl_2$ increased from −41.6 mV to 78.1 mV as CTAB concentration increased from 1 to 200 mg·L^{-1}. The increasing zeta potentials at CTAB concentrations from 1 to 50 mg·L^{-1} could explain the increasing adsorption rate coefficients, as the surface of Unimin quartz sand is negatively charged (Table 2). A decrease in the adsorption rate coefficient from 4.3 to 1.0 h^{-1} as CTAB concentration increased further from 50 to 200 mg·L^{-1} may be due to the accumulation of positive charges at the surface of quartz sand by CTAB pre-absorption, which again leads to electrostatic repulsion between plasmid–surfactant complexes and sand surfaces.

Agarose gel electrophoresis results of effluent samples of pcDNA3.1(+)/myc-His A from transport experiments under the influence of SDS and CTAB are shown in Figs. 4 and 5, respectively. The fluorescence intensity of the bands was well consistent with the breakthrough curves. Bands of plasmids in effluents at 50 mg·L^{-1} CTAB were nearly invisible, corresponding to the low breakthroughs in the

Table 2 Zeta potentials, fractions exiting column, and adsorption rate coefficients (k) of pcDNA3.1(+)/myc-His A in the presence of surfactants

Surfactant	Concentration ($mg \cdot L^{-1}$)	Zeta potential (mV)	Exited fractions (%)	k (h^{-1})
SDS	1	−43.9±11.2	99.8±0.2	0.03±0.01
	10	−44.4±2.3	97.9±0.0	0.13±0.01
	50	−46.5±3.9	99.1±0.2	0.07±0.01
	200	−53.8±1.0	98.9±0.1	0.11±0.02
CTAB	1	−41.6±0.4	96.9±0.6	0.21±0.05
	10	−27.1±6.7	38.0±0.3	2.3±0.1
	50	22.6±9.1	13.5±0.4	4.3±0.4
	200	78.1±13.5	57.2±0.2	1.0±0.0

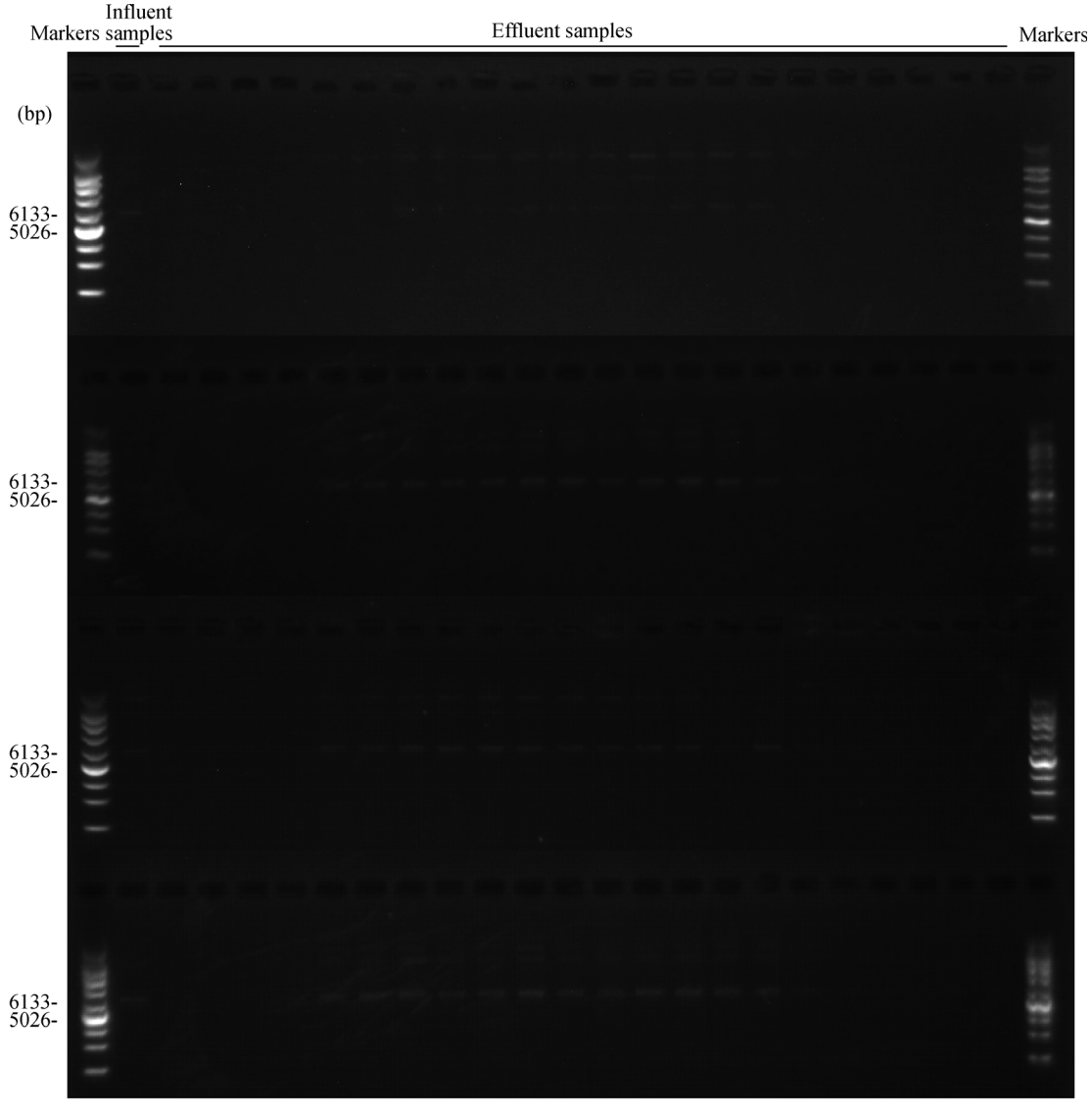

Fig. 4 Agarose gel electrophoresis results of pcDNA3.1(+)/myc-His A in 1, 10, 50 and 200 $mg \cdot L^{-1}$ (from top to bottom) SDS solutions transporting through quartz sand columns

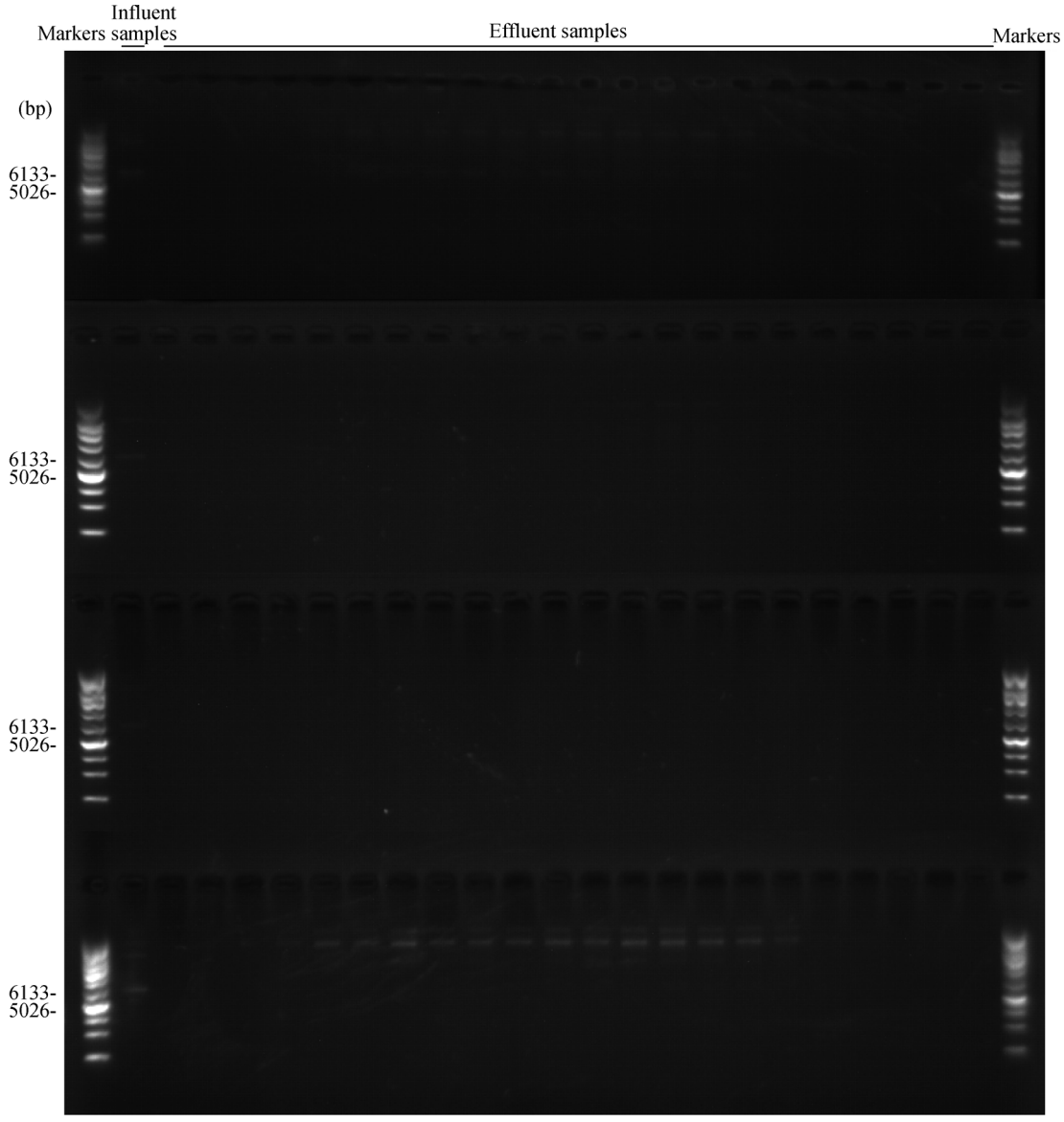

Fig. 5 Agarose gel electrophoresis results of pcDNA3.1(+)/myc-His A in 1, 10, 50 and 200 mg·L^{-1} (from top to bottom) CTAB solutions transporting through quartz sand columns

column experiment. At 200 mg·L^{-1} CTAB, bands of the highest fluorescence intensity in effluent samples were of different electrophoretic velocities from that in influent sample, which may result from changes in the conformation or surface charges of surfactant–DNA complexes after the processes at the interface of quartz sand [25].

Reports on the occurrence of surfactants in water environments are scarce in the literature. At certain sites (e.g., sewage discharge points), high surfactant concentrations (several tens mg·L^{-1}) were found [32,33]. However, at these sites, the surfactant constituents were mainly anionic. This is understandable as anionic surfactants are the primary group of surfactants used in the household (e.g., as detergents). As demonstrated above, anionic surfactants had no influence on plasmid transport even at very high concentrations. The limited reports also indicate that surfactant concentrations were typically below 1 mg·L^{-1} in most water environments [32,34,35]. At this concentration level, even cationic surfactants would have minimal influence on plasmid transport. Hence, under environmentally relevant concentrations, the impact of surfactants on plasmid transport is likely negligible.

It is worth noting that this work did not examine the sorption of surfactants by quartz sand. Preliminary experiments indicated that sorption of SDS by quartz sand was negligible, whereas sorption of CTAB was significant and concentration-dependent (data not shown). Future research is warranted to systematically examine the interaction between CTAB and quartz sand and to elucidate its impact on plasmid transport.

4 Conclusions

Transport behaviors of an indigenous plasmid pK5 in quartz sand and soil were investigated by column experiments. The indigenous plasmid pK5 had similar transport behavior as engineered plasmids in quartz sand, indicating the rationality of using engineered plasmids as a surrogate for examining the transport of indigenous plasmids. Furthermore, transport of pK5 in quartz sand and soil was also similar. Using the adsorption rate coefficient 0.18 h^{-1} of pK5 in quartz sand at 0.006 $mol \cdot L^{-1}$ $CaCl_2$, it can be estimated that indigenous plasmids could transport over a distance of 3 m in a subsurface environment. Indigenous plasmids have the potential to reach aquifers and spread antibiotic resistance farther under environmental conditions.

This study also demonstrated that anionic and cationic surfactants have a completely different influence on plasmid transport in porous media. Anionic surfactant SDS hardly influence the transport behavior of plasmid even at concentrations up to 200 $mg \cdot L^{-1}$, where cationic surfactant CTAB dramatically alter the transport behavior of plasmid at concentrations over 50 $mg \cdot L^{-1}$. Electrostatic interactions between surface active groups and DNA molecules are likely the dominant governor of plasmid transport in the presence of CTAB. As concentrations of surfactants are typically lower than 1 $mg \cdot L^{-1}$ in most aquatic environments, the influence of surfactants on plasmid transport will be negligible in most cases. The presence of surfactants in a subsurface environment will have limited effect on the high mobility of antibiotic resistance plasmids.

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