Biology and Fertility of Soils (2024) 60:901–910
https://doi.org/10.1007/s00374-024-01841-w

ORIGINAL PAPER

Pore confinement enhances but surface adhesion reduces bacterial cell-to-cell conjugation

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Received: 13 February 2024 / Revised: 15 June 2024 / Accepted: 18 June 2024 / Published online: 28 June 2024

Abstract

As the habitats of bacteria, soil pore network and surface properties control the distribution, adhesion, and motility of bacteria in soils. These physical processes in turn influence bacterial accesses to nutrients and bacterial interactions. Our understanding on the pore- and surface-mediated bacterial interactions is currently limited. In this research, we evaluated the effects of soil pore confinement and surface adhesion on conjugation-based bacterial interactions. The interaction was measured by plasmid transfer between donor and recipient cells within the population of soil bacterium *Pseudomonas putida*. We found that the presence of porous sand media led to a net increase in conjugation frequency compared to sand-free liquid control. The increase is attributed to the facilitated effect of pore confinement on the collision of bacteria within pores. In contrast, bacterial adhesion to sand surfaces under elevated ionic strength conditions decreased the conjugation frequency as a result of mobility reduction on the surface. Such collision and adhesion mechanisms jointly drive the conjugation as a function of pore and surface properties of porous media. These results provide valuable insights into the roles of soil pores and surfaces in regulating horizontal gene transfer, an essential cell-to-cell interaction sustaining key processes of soil ecology and health.

Keywords Cell-to-cell conjugation · Soil pores · Surface adhesion · *Pseudomonas putida*

Introduction

Bacterial interactions are vital for evolutionary and ecological processes in soil (Tecon et al. 2018; Zhang et al. 2020). The interactions include horizontal gene transfer mediated by conjugative pili (Soucy et al. 2015), competition and cooperation mediated by diffusible metabolites and chemical signals (Little et al. 2008; Velicer and Vos 2009), and predatory interactions (Tecon and Or 2017). Most of the interactions occur at the scale of individual bacterial cells through physical contact in spatial domains limited to the environment surrounding bacteria (Jansson and Hofmockel 2018; Nadell et al. 2016; Zhang et al. 2020). The spatial domain limited interactions consequently affect soil bacterial community composition and many ecological processes such as microbial evolution (Jansson and Hofmockel 2018; Tecon et al. 2018).

Soil consists of size-varying pores and surface-reactive solid grains. As bacterial habitats, soil pore networks and grain surfaces control bacterial motility, adhesion, and distribution (Bailey et al. 2013; Watt et al. 2006; Chen et al. 2024). Potentially interacting bacterial microcolonies can be confined to hydrologically disconnected pores or narrow throat-connected pores (Chenu and Stotzky 2002; Foster 1988; Kuzyakov and Mason-Jones 2018). Evidence shows that 15–54% of soil porosity is inaccessible to bacterial cells (Chenu and Stotzky 2002; Kuzyakov and Mason-Jones 2018). The entrapped bacterial microcolonies may have limited dispersion for long periods in disconnected pores where conditions are unfavorable to the interactions of bacteria with their communities (Tecon and Or 2017).

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Soil may remain unsaturated for most of the time, and the aqueous phase of sandy soil is fragmented by non-capillary pores (Dechesne et al. 2010; Wang and Or 2013). Tecon et al. (2018) demonstrated that drier conditions, where the aqueous phase has poor connectivity, promoted bacterial cell-to-cell interaction. Motile bacteria were slowed under drier conditions, leading to increases in cell-to-cell contact time and thereby the conjugation rate (Berthold et al. 2016; Tecon et al. 2018). These studies suggest that soil pore networks might play a role in regulating bacterial cell-to-cell interactions in soil. However, no experimental evidence has been available to demonstrate the pore control on bacterial interactions.

Bacteria can adhere to soil surfaces when they disperse through soil pores. The adhesion restricts the motility of bacteria and affect their interactions (Massoudieh et al. 2007). The process of bacterial adhesion is governed by electrical double layers and surface roughness (Carniello et al. 2018; Song et al. 2015; Zheng et al. 2021). Many studies have shown that van der Waals and electrostatic forces determine bacterial adhesion onto soil surfaces (Chen et al. 2022; Renner and Weibel 2011). Bacteria possess net negative charges at common soil pH values (Pajerski et al. 2019; Rijnaarts et al. 1999). For gram-positive bacteria, the negative charges result from the phosphoryl groups of the teichoic acid and lipoteichoic acid tails of cell wall surfaces. For gram-negative bacteria, the ionization of the phosphoryl and carboxylate groups present in the lipopolysaccharide chains of cell surfaces play an important role in the generation of the net negative charge of the cells (Pajerski et al. 2019; Wilson et al. 2001). Consequently, more bacterial adhesion occurs on positively charged than negatively charged soil surfaces and in the solution of higher ionic strength which favors ionic shielding (Gottenbos et al. 1999; Guo et al. 2018; Oh et al. 2018). However, it is unclear how soil surface electrical properties, which vary with solution chemistry (e.g., ionic strength and pH), influence the cell-to-cell interactions and further soil microbial evolution and community diversity through conjugation events.

Conjugation is an important cell-to-cell interaction. The conjugative plasmids are transferred from donor to recipient cells through direct cell-to-cell contact through a pilus (Couturier et al. 2023; Massoudieh et al. 2007; Von Wintersdorff et al. 2016). In this study, we hypothesize that soil pores and surface electrical properties influence the conjugation process through collision and adhesion mechanisms. We employed a model system of conjugation between bacterial donor and recipient cells to observe the expression of fluorescent marker genes, which caused the donor, recipient, and transconjugant cells to fluoresce different colors. Quartz sands with uniform grain sizes and surface chemistry were added to microcosms to simulate ideal uniform porous systems. Fluorescence imaging and cell counting were adopted to enumerate various cells and calculate the conjugation frequency at different solution ionic strengths. The experiments showed that the bacterial cell interactions were promoted by bacterial collision in pore spaces but reduced by bacterial adhesion to sand surfaces. The study provides scientific insights into the roles of soil pore and surface systems in soil health and biologically mediated processes (e.g., horizontal gene transfer).

Materials and methods

Chemicals

Tryptone, yeast extract, agar powder of molecular genetic grade, sodium chloride (NaCl), and tetracycline were obtained from Fisher Scientific (Waltham, MA, USA). Potassium chloride (KCl), di-sodium hydrogen phosphate (Na_2HPO_4), and potassium phosphate (KH_2PO_4) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of reagent grade or higher purity.

Porous media

The porous media selected for the study were Ottawa sand with size ranging from 0.65 to 1.18 mm (D_{50} = 0.72 mm). The Ottawa sand was thoroughly rinsed with deionized water to remove any suspended impurities and then oven-dried at 60°C to obtain clean sand (Zhuang et al. 2009).

Bacterial strains

The soil bacterium, *Pseudomonas putida* strain KT2440 (2 μm in length and 1 μm in width), and its genetically modified strain were used as a model system to examine the effects of cell adhesion and pore confinement on cell-cell contact and conjugation (Davis et al. 2011). The wild-type, plasmid-less strain served as the recipient strain. *P. putida* KT2440::*lacI*^{q}-*pLpp-mCherry-Km*^{R} served as the donor strain with the engineered cryptic broad-host range plasmid pKJK5::P*lac*::*gfp* (Klümper et al. 2015). The plasmid-containing donor constitutively expresses the mCherry fluorescent protein as well as the *lacI*^{q} repressor of the P*lac* promoter, which prevents expression of green fluorescent protein (GFP) from the pKJK5::P*lac*::*gfp* plasmid in the donor cell (Tecon et al. 2018). Upon receipt of the plasmid, the recipient cell then becomes a transconjugant when the donor cell makes physical cell-cell contact. GFP can then be expressed in the transconjugant cells because the recipient strain lacks the *lacI*^{q} repressor (Normander et al. 1998; Tecon et al. 2018). In addition, the plasmid encodes genes

conferring resistance to tetracycline so that donor and transconjugant cells with the plasmid are resistant and can be selected on tetracycline-containing media.

To prepare bacteria for experiments, both donor and recipient strains were cultivated overnight in the Luria-Bertani (LB) broth containing tryptone at 10 g L^{-1}, yeast extract at 5 g L^{-1}, and NaCl at 10 g L^{-1} adjusted to pH 7.2 with or without tetracycline (15 μg mL^{-1}). Tetracycline (15 μg mL^{-1}) was added to the donor culture to ensure plasmid maintenance (Tecon et al. 2018). Twenty-five μL of an overnight recipient culture was transferred into a 50 mL bottle containing 20 mL of fresh LB broth. Because the donor strains grew more slowly under the conditions, 100 μL of an overnight donor culture was added into a 50 mL bottle containing 20 mL of fresh LB broth with tetracycline. Both strains were cultivated at 30 °C with shaking at 280 rpm for approximately 6 h to get cultures in early stationary phase. The cells of donor and recipient were then harvested by centrifugation at 6,043 g at 4 °C for 10 min. The cell pellets were washed three times using sterile phosphate buffered saline (PBS) after carefully removing the supernatant using a pipette and finally resuspended in 5 mL of PBS. The PBS consisted of NaCl (98 g L^{-1}), KCl (0.2 g L^{-1}), Na_2HPO_4 (1.15 g L^{-1}), and KH_2PO_4 (0.2 g L^{-1}). Cell suspensions were diluted in PBS to obtain an optical density of 1 at 600 nm (OD_{600}).

Conjugation experiments in microcosms

To determine if bacterial growth conditions were required for cell-to-cell conjugation, we incubated the bacteria with a 10:1 ratio of recipient-to-donor (R:D) at different initial total cell concentrations in a cell mix suspension in PBS without nutrients (no growth conditions) or in 0.1× LB media (sufficient growth conditions) (Tecon et al. 2018). The total number of conjugation events increased with the total concentrations of recipient and donor cells within 7×10^7 CFU mL^{-1} at R:D of 1:1, 5×10^8 CFU mL^{-1} at R:D of 10:1, and 5×10^9 CFU mL^{-1} at R:D of 100:1. Beyond these concentrations, the number of conjugation events reached a plateau. In this study, the total concentrations of bacterial cells could reach to 2×10^8 CFU mL^{-1} after incubation. Therefore, we set the R:D ratio as 10:1. The initial concentrations of the cell mix suspension were set as 5.5×10^4, 5.5×10^5, 5.5×10^6, 5.5×10^7, and 5.5×10^8 colony forming unit per mL (CFU mL^{-1}). After 24 h incubation, recipient, donor, and transconjugant cells were extracted and enumerated by an adapted drop plate assay (Chen et al. 2003). The efficiency of a conjugation system was described by its transfer frequency, which is commonly quantified by the ratio of the number of transconjugants at the end of the experiment to the sum of transconjugant and recipient cells.

The effect of sand on the bacterial conjugation frequency was investigated. Specifically, 3.5 g of the clean sand was added into 15-mL centrifuge tubes. The total volume of the sand was 2 cm^3 with a pore volume of 700 μL as calculated from the bulk density and sand particle density (2.7 g cm^{-3}). Fifteen μL of a suspension of recipient and donor cells with a 10:1 ratio was inoculated to 700 μL of sterile 0.1× LB broth, where the microcosms were under saturated conditions (i.e., all pores were liquid-filled without free-standing solution). The bacteria suspension and sand were then mixed evenly in microcosms and incubated at 30 °C for 24 h. The experimental conditions of the control microcosms (i.e., free liquid without sand) were set up the same as the sand microcosms. After the incubation, 6.3 mL of PBS was added to each tube. The tubes were then placed in a water-bath sonicator (FS20 Ultrasonic Cleaner, Fisher Scientific) to disperse cells for 2 min. Recipient, donor, and transconjugant cells were enumerated by an adapted drop plate assay (Chen et al. 2003). In the second set of experiments, different intensities of sonication (0-, 2-, and 10-minute sonication) were applied to the sand microcosms to detach the bacteria from the sand surfaces. In the third set of experiments, background 0.1× LB solution with different ionic strengths (0 mM, 17 mM, and 50 mM), which were obtained by adding NaCl solution or not, were used for bacterial incubation in free liquid and sand microcosms (3.5 g sand per 15-mL centrifuge tube) to examine the effect of solution ionic strength on bacterial cell-to-cell conjugation. All experiments were performed in triplicates.

Enumeration of cells with fluorescence imaging

Enumeration of donor, recipient, and transconjugant cells was conducted according to an adapted drop plate assay. Briefly, 50 μL-droplet per dilution was pipetted onto agar plates, and the plates were incubated at 30 °C overnight. The dilutions resulted in 10–300 CFU per plate for counting. The total population size, including recipient, donor, and transconjugant cells, was estimated by counting CFU on LB agar plates without tetracycline. Counts on plates with tetracycline were used to enumerate resistant donor and transconjugant cells. The difference between total and resistant population sizes gave the number of recipient cells. To discriminate donor and transconjugant cells on agar plates with tetracycline, the plates were placed in the fridge for three days to increase the mCherry signal for donors and the GFP signal for transconjugants. IVIS Lumina K w/XGI-8 Anesthesia System (PerkinElmer, Waltham, MA, USA) equipped with the spectral filters for mCherry fluorescence and GFP was used to analyze red and green colonies, respectively.

Statistical analyses

Data were compared with One-way Analysis of Variance (ANOVA). Duncan tests were applied to assess the statistical differences between mean values. SPSS 28.0 (IBM SPSS Statistics) software was used to perform spearman correlation analyses with different letters (e.g., a, b, c) annotated on graphs to indicate significant statistical differences among treatments at $p < 0.05$.

Results

Effects of bacterial concentration and growth on conjugation

Bacterial cells in non-growth supporting media did not exhibit conjugation as no transconjugant cells were observed at different initial cell concentrations of donor and recipient (Fig. 1A). This result is consistent with previous conjugation studies (Barr et al. 1986; Jutkina et al. 2018; Møller et al. 2017; Schuurmans et al. 2014), suggesting that growth-sufficient conditions were necessary for the observation of cell-to-cell conjugation (Fig. 1B). Therefore, all following conjugation experiments were conducted in 0.1 × LB media to support growth of the model bacterial strains rather than in non-growth supporting media. In the growth media, transconjugant cells could be detected, and the initial inoculum of recipient and donor influenced the absolute number of transconjugant cells but not the conjugation frequency. The absolute number of transconjugant cells reached 8.6×10^6 CFU mL^{-1} under the lowest initial inoculum (5.5×10^4 CFU mL^{-1}) (Fig. 1B), and increased by 0.1-, 6, 41-, and 123-fold when the initial inoculum increased to 5.5×10^5, 5.5×10^6, 5.5×10^7, and 5.5×10^8 CFU mL^{-1}, respectively (Fig. 1B). These results indicated that high initial recipient and donor cell concentrations boosted the physical contact (e.g., collision) between the bacterial cells, leading to the increase in the absolute number of transconjugant cells in the limited liquid volume. However, it is worth noting that the conjugation frequency was similar (~0.12) at each initial bacterial cell concentration except for the highest level (5.5×10^8 CFU mL^{-1}), at which the conjugation frequency was as low as 0.02 (Fig. 1C). A simplified probabilistic model was used by Tecon et al. (2018) to estimate the number of conjugation events occurring in the sand microcosms that had different total cell concentrations of recipients and donors. The number of conjugation events increased as the

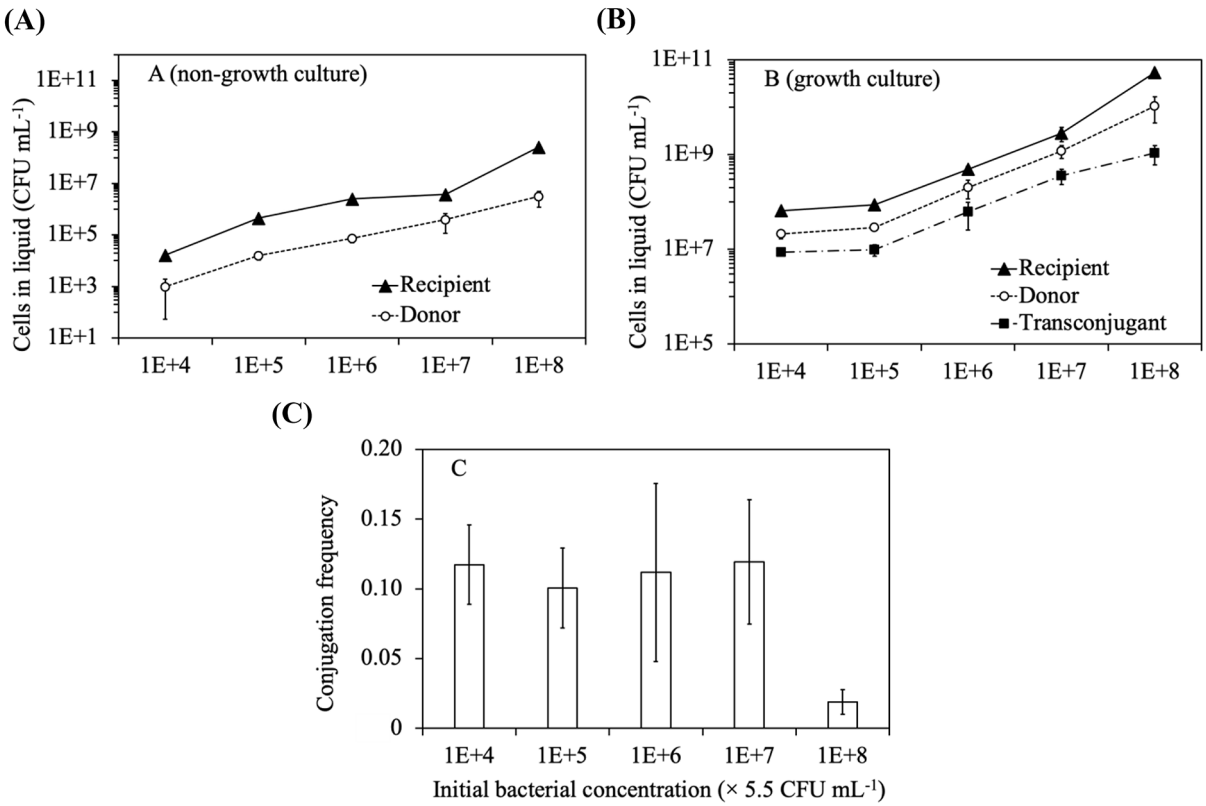

Fig. 1 Recipient, donor, and transconjugant cell concentrations in non-growth phosphate-buffered saline solution (**A**) and growth bacterial Luria-Bertani broth (**B**) as well as conjugation frequency (**C**). Conjugation frequency in Luria-Bertani broth was calculated by the quotient of transconjugants and the sum of transconjugants and recipients. Error bars represent the standard deviations of triplicate microcosms

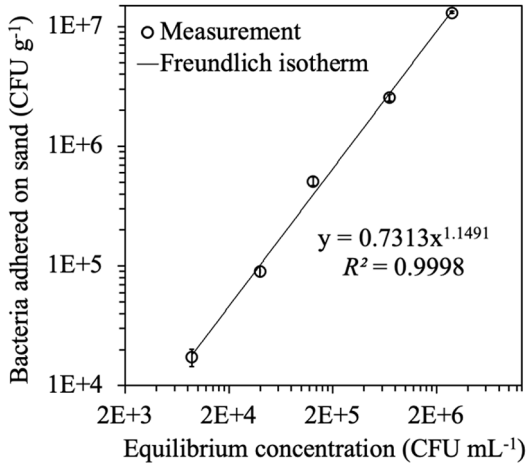

Fig. 2 Equilibrium adhesion isotherms of donor cells (*P. putida* KT2440::*lacI*^{q}-*pLpp-mCherry-Km*^{R}) to the sand at pH 7.2 in Luria-Bertani solution diluted by ten times. The Luria-Bertani broth was amended with NaCl to reach an ionic strength of 17 mM excluding the contribution of the Luria-Bertani broth. The line represents fitted curves by the Freundlich equations for sand-filled microcosms. Error bars represent the standard deviations of triplicate measurements

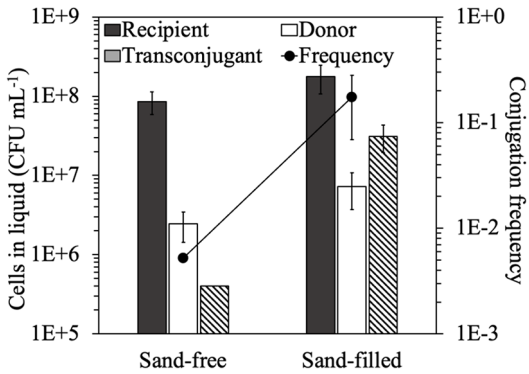

Fig. 3 The final bacterial cell concentrations of recipient, donor, and transconjugant and conjugation frequency after 24 h of incubation at 30 °C in sand-free liquid and sand-filled microcosms. Conjugation frequency was calculated as the ratio of transconjugants to the sum of transconjugants and recipients. Error bars represent the standard deviations of triplicate measurements

total cell concentrations increased from 1×10^{5} CFU mL^{-1} to 5.5×10^{8} CFU mL^{-1}, then the transconjugants reached a plateau. Although the conjugation event in our results did not reach a plateau, the increment of transconjugant concentrations at the initial bacterial concentrations of 5.5×10^{8} CFU mL^{-1} was lower than at other treatments. Consequently, the frequency of conjugation at the highest bacterial concentrations was 6-fold lower compared to other treatments (Tecon et al. 2018). In this study, LB broth was used as a growth medium for bacteria because the conjugation failed or was below detection limit in the minimal salt solutions that did not support the growth of the experimental strains.

Bacterial adhesion isotherms

The number of bacteria adhered on sand surface increased with the initial bacterial concentration (Fig. 2). The adhesion followed the Freundlich adsorption isotherm with a R^2 value of ~1.0 ($n=5$). The model fitting indicated that the K_f value of the donor cell adhesion to the clean sand was 0.73 mL g^{-1}. We assume that the donor and receipt cells have similar K_f values.

Effects of sand on bacterial conjugation

The average number of cell doublings for all three types of cells was greater in the presence of the clean sand in sand-filled microcosms compared to sand-free liquid media (Fig. 3). Bacterial cell concentration increased ~40 fold in the liquid media (corresponding to five to six cell doublings during 24 h incubation time), in comparison with the ~128-fold increase in the sand-filled microcosm (Fig. 3). Based on the results described in the previous section, initial bacterial concentration influenced the absolute number of transconjugant cells but not the conjugation frequency (Fig. 1). The absolute number of transconjugant cells and conjugation frequency were determined to evaluate the conjugation events under different environmental conditions. The conjugation frequency is a widely used indicator to determine the efficiency of plasmid transfer. The absolute number of transconjugants enumerated after 24 h of incubation increased by two orders of magnitude in the sand-filled microcosms (3.12×10^{7} CFU mL^{-1}) than in the sand-free liquid media (4.00×10^{5} CFU per mL^{-1}) (Fig. 3, gray column). The conjugation frequency in the sand-filled microcosms was about 30-fold higher than the conjugation frequency in the sand-free liquid media (~0.005) (Fig. 3, black dot). These results suggest that cell confinement in the pores between the sand grains facilitated the plasmid transfer from cell to cell.

Sonication effect and conjugation location in pores

Sonication was used to detach cells from the sand surfaces after the incubation experiments. Before the detachment test, each strain was exposed to sonication in sand-free liquid media to assess the impact of sonication on the viability of cells. No significant difference in cell number was observed among the recipient, donor, and transconjugant cells before and after the sonication ($p>0.05$, Fig. 4A). The total cell concentrations of all three bacterial strains detached from the sand after 24 h incubation increased by 2.5–4.7 times and 3.0-4.6 times after the sonic treatments for 2 min and 10 min, respectively (Fig. 4B). There was no significant difference in transconjugants under the sonication of different time lengths in the sand-filled microcosms

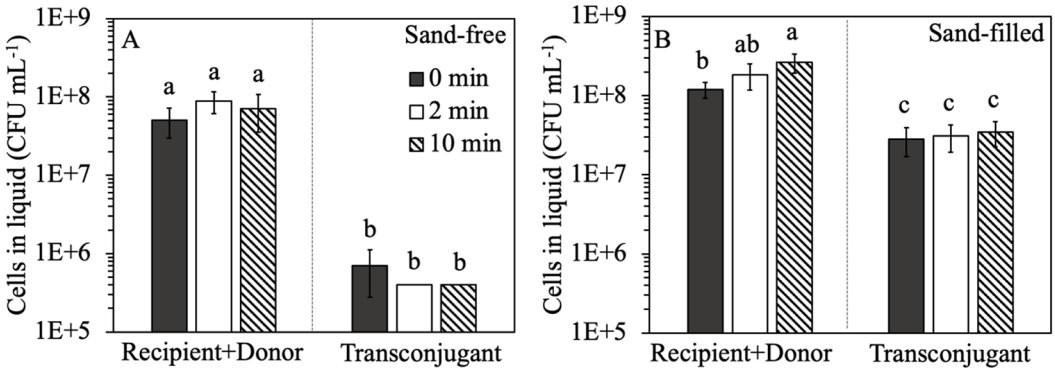

Fig. 4 Recipient, donor, and transconjugant cell concentrations after 24 h incubation in (**A**) sand-free liquid media and (**B**) sand-filled microcosms under different time lengths of exposures to sonication (0 min, 2 min, and 10 min). Error bars represent the standard deviations of triplicate microcosms. Different letters (a, b, c) were annotated on graphs to indicate the statistical significance of differences among treatments at $p < 0.05$

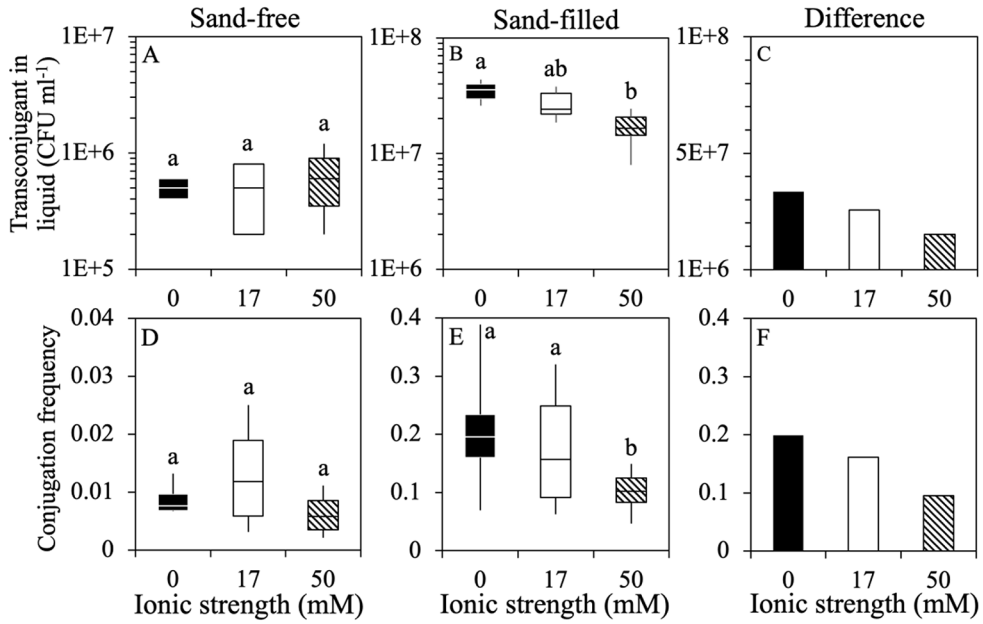

Fig. 5 Transconjugant cell concentrations (**A** and **B**), conjugation frequency (**D** and **E**) after 24 h incubation in sand-free liquid media and in sand-filled microcosms with different ionic strengths (0 mM, 17 mM, and 50 mM, which only reflect the ionic strength of added NaCl), and their difference between sand-free liquid media and sand-filled microcosms (**C** and **F**). Error bars represent the standard deviations of triplicate measurements. Different letters (a, b, c) are annotated on graphs to indicate statistical significance among treatments at $p < 0.05$

(Fig. 4B). These results suggest that sonication could detach bacteria from the sand surfaces but had minimal effect on bacterial viability. In addition, most of the detached cells from the sand surfaces were donor and recipient cells, and there was no transconjugant cells. This result indicate that the conjugation occurred in the aqueous phase and not on the sand surface.

Effects of solution ionic strength on conjugation

Background 0.1× LB broth solutions with different ionic strengths (0 mM, 17 mM, and 50 mM) adjusted with NaCl had no significant influence on bacterial growth in sand-free liquid media or sand-filled microcosms. Likewise, the absolute number of transconjugant cells had no significant variation with ionic strength in sand-free liquid media (Fig. 5A). In comparison, the absolute number of transconjugant cells increased with decreasing ionic strength in the sand-filled microcosms (Fig. 5B). Low ionic strength (0–17 mM) promoted the conjugation, and the transconjugant cell number reached the highest value at 0 mM (3.55×10^7 CFU mL^{-1}). To examine if high ionic strength hindered conjugation, we calculated the difference in the transconjugant cell number between the sand-free liquid media and the sand-filled

microcosms at each ionic strength (Fig. 5C). The first column represents the difference in transconjugant concentration at 0 mM ionic strength between sand-free liquid media and sand-filled microcosms. The difference reduced as solution ionic strength increased from 0 mM to 17 mM by 6.7% and from 0 mM to 50 mM by 40%.

The conjugation frequency was not impacted by ionic strength in sand-free liquid media (Fig. 5D), but decreased with increasing ionic strength in the sand-filled microcosms (Fig. 5E). The highest conjugation frequency of ~0.20 in the sand-filled microcosms was reached at ionic strength of 0 mM (Fig. 5E). The difference in conjugation frequency between the sand-free liquid media and the sand-filled microcosms decreased with increasing ionic strength (Fig. 5F).

Discussion

Pore-enhanced conjugation

The presence of sand grains in the aqueous phase increased the number of transconjugant cells compared with that in the free liquid media, corresponding to the greater conjugation frequency observed in the sand-filled microcosms (Fig. 3). Pores between sand grains increased bacterial conjugation events compared to sand-free liquid without pore confinements, regardless of whether electrostatic repulsion or attraction existed on the sand surfaces. This result is attributed to the compartmentalization of the aqueous phase in pores between the sand grains (Fig. 6). Bacteria have evolved a large array of motility mechanisms to promote colonization in the environment (Jarrell and McBride 2008; Miyata et al. 2020; Raina et al. 2019). In aqueous environments, bacterial swimming motility using a single or multiple flagella is a well characterized mechanism (Berg and Anderson 1973; Nakamura and Minamino 2019; Silverman and Simon 1974). Cells swim freely by rotating their flagellar filament(s) (Nakamura and Minamino 2019). When all the flagella in a cell spin counterclockwise, the filaments form a bundle behind the cell that pushes it forward roughly in a straight line (Turner et al. 2000; Wadhwa and Berg 2022). Once cells encounter obstacles or sense a change in the concentration gradient of a chemical attractant/repellant, flagella switch their direction of rotation to a new direction that could create opportunity for donor-recipient collision (Berg and Brown 1972; Larsen et al. 1974). *P. putida*, used in this study, is a multi-flagellated species that has five to seven flagella at one pole (Harwood et al. 1989). The motility of the cells generally follows a straight line in the aqueous environment, but cells reorient their direction once encountering the sand surfaces (Turner et al. 2000; Wadhwa and Berg 2022). The aqueous phase within the porous sand media was typically disconnected, forming isolated water-filled compartments (Fig. 6). Each 'compartment' has a smaller volume and a shorter cell-to-cell distance than that in the sand-free aqueous cell suspensions. According to relevant theory proposed by Subramanian and Davis (1979), the confinement of pore walls might increase collision and decrease cell diffusivity, leading to increase in cell conjugation frequency. This is because the pore confinement can result in inhomogeneity of cell particles across pores, leading to pressure anisotropy that favors cell collision frequency compared to the bulk liquid phase at the same cell concentrations. Less cell diffusivity along the pore axis relative to bulk liquid phase might also contribute to the pore-enhanced collision and in turn conjugation. *P. putida* requires direct contact (< 1 μm of donor-recipient distance) with rigid pilus to transfer plasmids (Seoane et al. 2011). As a result, conjugation events occurred more frequently in pore spaces, where bacterial cell concentration and of cell collision probability were higher compared to pore-free aqueous media (Fig. 6). Electrostatic repulsion between the like-charged bacteria and sand surface might be another factor influencing bacterial movement direction and thereby leading to conjugation events. In this study, the uniform sand medium used to quantify the influences of pores and surface properties on cell-to-cell conjugation does not completely represent the ranges of natural soil properties. Therefore, future investigations should quantify the impacts of additional soil components and properties, such as clays, which can change soil pore size distribution, soil surface reactivity, and water retention and conductivity.

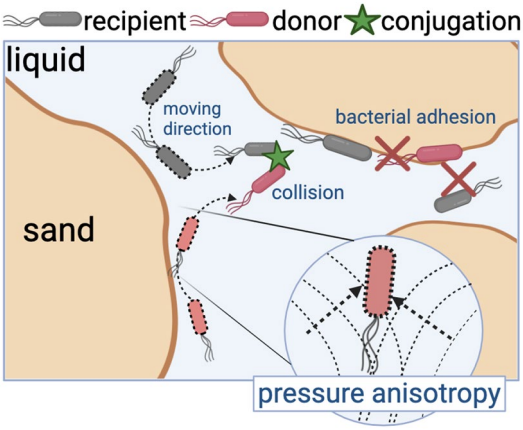

Fig. 6 Conceptual diagram of sand impact on bacterial conjugation

Adhesion-reduced conjugation

Surface adhesion inhibited the conjugation because the bacteria were spatially separated from the pore liquid and had much less mobility. Bacterial adhesion on soil surfaces was reported to form biofilm, which is considered as a hot spot for plasmid transfer (Sutherland 2001). However, our results showed that more bacterial adhesion resulted in less plasmid transfer (Fig. 5). The bacterial plasmid transfer competence on sand surface does not occur in all cells of a population. More bacteria can adhere on the sand surfaces at higher ionic strength, and the adhesion facilitates the formation of discontinuous patches of biofilms on the sand surface under the growth conditions (He et al. 2020). The discontinuous patches might lead to infrequent events of plasmid transfer between donor and recipient cells which appears at the edge of bacterial colonies on sand surfaces (Koraimann and Wagner 2014; Reisner et al. 2012). However, the plasmid transfers from some donor to recipient cells but not from all, reducing conjugation frequency (Koraimann and Wagner 2014). In addition, bacterial cells, such as *Pseudomonas* strains, align themselves in ways that would maximize contact area on the sand surface (Wadhwa and Berg 2022). During this process, motile bacteria differentiate into an aligned chain of cells before the growing chains further develop fibers and bundles on the sand surface (Honda et al. 2015; Mamou et al. 2016; Mendelson 1999). These well-aligned structures could promote sliding of a colony on the sand surface where the swimming behavior of bacteria is not efficient, leading to a stronger and more stable attachment (Van Gestel et al. 2015; Yaman et al. 2019). However, surface adhesion fixed the bacterial cells, contributing to the loss of their motility. The plasmid used in this study, the conjugative IncP-1 plasmid pKJK5, can facilitate two single cells attached together (Røder et al. 2013). The fixed cells on the sand surface at higher ionic strength conditions had lower collision rate with the cells swimming in the pore liquid due to limited pilus length (Honda et al. 2015; Mamou et al. 2016; Mendelson 1999; Van Gestel et al. 2015; Yaman et al. 2019). As a result, the conjugation frequency of bacteria decreased once they were adhered on sand.

The isoelectric point of *P. putida* KT2440::*lacI*^{q}-*pLpp-mCherry-Km*^{R} was between 4 and 6 (Hanna 2007). In the experimental liquid with pH 7.2, the net surface charge of the bacterial donor cells was negative. A similar characteristic was assumed for the receipt cells since the donor and receipt cells share the same surface properties. Increasing ionic strength in aqueous solution shields surface electronic charges of both the bacteria and the sand. The shielding decreases the electrostatic repulsion of the negatively charged bacteria to the negatively charged sand (Zhuang and Jin 2003). Thus, the bacterial adhesion on the sand increased with solution ionic strength, leading to a decrease in conjugation frequency in the sand-filled microcosms.

Environmental implications

Our results show that soil pore and surface systems are key to gene transfer between bacterial cells, which influences the evolution of microbial communities. The transfer process is mediated by the factors (e.g., pores, water content, and surface charges) that control bacterial distribution between liquid and solid phases. Our results indicated that cell-to-cell collision determines conjugation frequency. Pore spaces favored the collision of bacteria while the adhesion of bacteria to sand surfaces limited their motility and in turn reduced the collision frequency. These findings based on a simple sand system advance the understanding of soil physicochemical control on genetic interactions of microbes. This study provides implications for the importance of soil pore and surface heterogeneities on soil microbial evolution and diversity. In natural soils, the physical environment affects bacterial motility and dispersion, thereby conjugation. For example, bacteria can move much more efficiently and over larger distances through water flow than by their endogenous motility. Thus, pore water saturation significantly impacts the potential for cell-to-cell contact and conjugation. It is worth noting that the results in this study were observed using a uniform sand medium. Future investigations should be made under environmental conditions more relevant to natural soils, such as variable pore water saturation, size-varying soil pores, and surface-reactive particles (e.g., clays and metal oxides).

Acknowledgements We thank Arnaud Dechesne at Technical University of Denmark for providing the *P. putida* KT2440 and *P. putida* KT2440::*lacI*^{q}-*pLpp-mCherry-Km*^{R}. We also thank the editor and reviewers for their highly valuable comments which improved the data interpretation.

Funding This work was supported by U.S. Department of Agriculture's National Institute of Food and Agriculture funds (USDA-NIFA 2018-67019-27792).

Data availability Data will be made available on request from the authors.

Declarations

Conflict of interest The authors declare no competing interests.

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