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The effect of bacterial growth strategies on plasmid transfer and naphthalene degradation for bioremediation☆

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Abstract

Mobilizable plasmids are extra-chromosomal, circular DNA that have contributed to the rapid evolution of bacterial genomes and have been used in environmental, biotechnological, and medicinal applications. Degradative plasmids with genetic capabilities to degrade organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), have the potential to be useful for more environmentally friendly and cost-effective remediation technologies compared to existing physical remediation methods. Genetic bioaugmentation, the addition of catabolic genes into well-adapted communities via plasmid transfer (conjugation), is being explored as a remediation approach that is sustainable and long-lasting. Here, we explored the effect of the ecological growth strategies of plasmid donors and recipients on conjugation and naphthalene degradation of two PAH-degrading plasmids, pNL1 and NAH7. Overall, both pNL1 and NAH7 showed conjugation preferences towards a slow-growing ecological growth strategy, except when NAH7 was in a mixed synthetic community. These conjugation preferences were partially described by a combination of growth strategy, GC content, and phylogenetic relatedness. Further, removal of naphthalene via plasmid-mediated degradation was consistently higher in a community consisting of recipients with a slow-growing ecological growth strategy compared to a mixed

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Paige M. Varner: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Editing. **Marco N. Allemann:** Conceptualization, Methodology, Resources, Validation, Review & editing. **Joshua K. Michener:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Review & editing. **Claudia K. Gunsch:** Conceptualization, Funding acquisition, Resources, Supervision, Review & editing.

Ethics approval

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Appendix A. Supplementary data

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community or a community consisting of fast-growing ecological growth strategy. Understanding plasmid conjugation and degradative preferences has the capacity to influence future remediation technology design and has broad implications in biomedical, environmental, and health fields.

Keywords

Bioremediation; Horizontal gene transfer; Plasmids; Ecological growth strategies

1. Introduction

Plasmids have played a key role in the rapid evolution of microorganisms because bacteria are able to transfer genes of interest to a wide range of organisms which, in turn, have greater metabolic capabilities (Frost et al., 2005; Gogarten and Townsend, 2005). Due to their transferability and provision of selective advantages, plasmids have been widely used in various fields of science and technology. For example, utilizing the transferability of plasmids to widely disseminate pollutant degradation genes in the environment has the potential to sustainably break down organic contaminants that are harmful to human and ecological health long-term (Venkata Mohan et al., 2009; Mrozik and Piotrowska-Seget, 2010). The addition of degradation genes into an well-adapted bacteria via plasmids, termed genetic bioaugmentation, can result in sustained, environmentally-friendly removal of pollutants of interest (Top et al., 2002; Top and Springael, 2003; Ikuma et al., 2012). In genetic bioaugmentation for pollutant removal a donor bacterium harboring a catabolic plasmid will transfer the plasmid to a recipient cell (transconjugant) and both donor and transconjugant can express degradation genes for removal of the contaminant. Many of these catabolic plasmids exist in the IncP incompatibility group which contains self-conjugatable plasmids that form a short, rigid pilus (Fernandez-Lopez et al., 2017). Incompatibility groups describe plasmids that are unable to coexist in a single cell due to sharing elements of the same replication system (Novick, 1987). Conjugative plasmids in the IncP group contain a Type IV secretion system (T4SS) containing a relaxosome and mating-pair formation that executes conjugative transfer of the plasmid into recipients (Lawley et al., 2003; Christie, 2016).

Though promising, genetic bioaugmentation relies on many processes that are yet to be fully understood. In particular, successful genetic bioaugmentation requires the survival of the donor organisms harboring the degradative plasmids, recipients accepting the plasmid at a significant rate, and long-term expression of degradative genes. Abiotic properties such as temperature, moisture, nutrient levels, and substrate availability have been shown to affect genetic bioaugmentation schemes. For example, conjugation has been found to be optimal at a moisture content between 40%–60% in soils and sediments (Johnsen and Kroer, 2007; Zhang et al., 2012). Temperature between 30–37 °C has also been found to be optimal for conjugation of a wide range of plasmids (Wang et al., 2014; Gao et al., 2015).

Biotic properties of both donor and recipient, however, and their effects on conjugation and plasmid-mediated degradation, have been less studied. Many of these knowledge gaps exist due to the lack of methods accessible for quantifying transfer frequencies of degradative

plasmids and identification of transconjugants (Varner and Gunsch, 2021). Some studies have indicated that similarity in GC content and phylogenetic relatedness of donor and recipient might increase the possibility of conjugation and gene expression (Thomas and Nielsen, 2005; Ikuma and Gunsch, 2012, 2013). However, studies on these factors and other biological properties are limited and are necessary to understand plasmid transfer for genetic bioaugmentation as well as other public health and biotechnological purposes.

One biological factor that is under-explored is the effect of various bacterial growth strategies on conjugation and post-transfer degradation. Historically, bacterial evolution has favored two general ecological growth strategies: high reproduction rate (r-strategists or copiotrophs) or optimal resource utilization (K-strategists or oligotrophs)(Atlas and Bartha, 1997). Depending on multiple heterogeneous abiotic and biotic factors, the ecology of an ecosystem may change over time to favor a particular life strategy which can change the functional dynamics of the microbial community and the gene pool. Additionally, growth strategies are hard to define on a broad scale because bacterial strains in the same phylum or genus can adapt various ecological growth strategies. Because of the dependence of bacterial growth on these various factors, researchers have relied on genetic relationships to determine inherent growth strategy that have been adapted by individual bacteria. For example, it has been found that a higher copy number of 16S rRNA genes in the genome is associated with a higher specific growth rate and lower carbon use efficiency (Klappenbach et al., 2000; Roller et al., 2016; Wu et al., 2017; Ortiz-Álvarez et al., 2018). The imprinting of ecological growth strategy on the genomes of bacteria via 16S rRNA copy number allows researchers to further understand the impact growth strategy has on biological functions.

Research in this area, however, is vastly under-studied and largely focuses on smaller, antibiotic resistance plasmids. In general, transfer of antibiotic resistance plasmids has mainly been studied in the context of public health, wastewater treatment systems, and the environment (Fahrenfeld et al., 2014; Partridge et al., 2018; Gothwal and Thatikonda, 2020; Nguyen et al., 2021). In one of few studies on the relationship between growth and conjugation involving a degradative plasmid, conjugation of the TOL plasmid was found to increase with the specific growth rate of both the donor and recipient (Smets et al., 1993; Seoane et al., 2010). This suggests a relationship of faster-growing bacteria (r-strategists) being able to transfer and receive degradative plasmids better than the slower-growing K-strategists. Further, researchers discovered that antibiotic resistance plasmids exhibit one of three types of transfer regimens depending on the identity of the plasmid and donor: (1) the growth stage of an organism had no effect on conjugation; (2) plasmid transfer occurred more often with donors in exponential phase of growth; and (3) plasmid transfer occurred more often with donors in stationary phase of growth. Antibiotic resistance plasmids existing within the 2nd and 3rd transfer regimen seem to have conjugation dependences or favorability for specific growth stages or bacterial growth strategies. Additionally, these transfer regimens were evident for not only the donor transferring the plasmid, but also the recipient receiving it (Sysoeva et al., 2019). Further work is needed to elucidate these relationships in larger, degradative plasmids.

To determine the relationships between growth strategy and the potential for genetic bioaugmentation success, in the present study we assessed conjugation of polycyclic

aromatic hydrocarbon (PAH)-degrading plasmids as a model. Further, we measured conjugation frequency and naphthalene degradation in simple, synthetic communities using fluorescence-based assays.

2. Methods

2.1. Strains and media

The two donor strains harboring PAH-degrading plasmids used in this study were chosen for multiple donor and plasmid properties. First, *Pseudomonas putida* G7 (G7) has a high 16S rRNA copy number compared to *Novosphingomonas aromaticivorans* F199 (F199) (Stoddard et al., 2015). Second, both plasmids are well-characterized, IncP-9 mobilizable plasmids that degrade naphthalene (Romine et al., 1999; Sota et al., 2006). Inclusion in the same incompatibility group (IncP-9) indicates the transfer system expressed in both plasmids is the same. Plasmid NAH7 harbored in G7 is significantly smaller at 83 kbp compared to plasmid pNL1 harbored in F199 which is 184 kbp. Naphthalene is the simplest PAH with two fused benzene rings and is found in abundance in creosote and is a known carcinogen (Abdo et al., 2001).

The selected recipients were *Sphingopyxis lindanitolerans* WS5A3p, *Caulobacter vibrioides* CB15, *Cupriavidus metallidurans* CH34, *Acinetobacter venetianus*, *Serratia marcescens*, and *Enterobacter cloacae*. The recipients were chosen based on multiple factors: (1) They are all Gram-negative Proteobacteria which are most able to accept IncP plasmids via conjugation; (2) They have a range of ecological growth strategies; (3) Many have been documented to have received plasmids previously (Ikuma, 2011; Redfern et al., 2019).

All chemicals were purchased for Sigma Aldrich (St. Louis, MO, USA). Strains were grown at 30 °C on Caulobacter medium (CBM) consisting of 2 g/L peptone, 1 g/L yeast extract, and 0.2 g/L $MgSO_4 7{\cdot}H_2O$ unless otherwise noted. For gene insertions and selections, the following concentrations of antibiotics and selective media were used: 50 μg/L kanamycin, 25 μg/L chloramphenicol, 100 μg/L ampicillin, 100 μg/L streptomycin, and 25% w/v sucrose. For gene insertions into F199 and pNL1, conjugation and selection were achieved on R2 A medium. For gene insertions into G7 and NAH7, conjugation and selection were achieved on Luria-Bertani (LB) medium. *Escherichia coli* WM6026, the conjugation donor for gene insertions, was grown at 30 °C on LB supplemented with 300 μM diaminopimelate decarboxylase. A list of all strains used in this study and their characteristics are presented in Table 1.

To verify the relationship between growth rate and 16S rRNA copy number for the donors and recipients, growth curves at high and low nutrient conditions were conducted. Each strain was grown overnight as previously described before adding 105 cells to 5 mL of respective media (either full CBM or 1/3 diluted CBM). The growth of organisms was tracked by measuring OD600 every hour. The slopes of the growth of each strain in their exponential growth phase are reported for each media condition (Supplementary Fig. 1). The results verify that the 16S rRNA copy number of donors and recipients used in this study are indicative of their exponential growth rate in both medias used in this study.

2.2. Fluorescent protein gene insertions

To quantify conjugation via fluorescence-activated cell sorting (FACS), genes encoding constitutive fluorescence of mScarlet-I were inserted onto the NAH7 and pNL1 plasmids. A vector with the mScarlet-I protein construct under the nptII promoter from Schlechter et al. (2018) was synthesized by Genscript (Piscataway, NJ, USA) and cloned into the multiple cloning site (MCS) of the pAK405 vector for insertion into pNL1 replacing the Saro_RS17445 gene (Kaczmarczyk et al., 2012). Delivery of the vector was achieved through conjugation between *E. coli* WM6026 harboring the vector and *N. aromaticivorans* F199. Selection of *N. aromaticivorans* F199 colonies exhibiting successful gene insertion and constitutive fluorescence was achieved by following previously established methods and PCR/sequencing with primers targeting the insert region listed in Table 2 (Kaczmarczyk et al., 2012). A vector with the same mScarlet-I protein construct under the nptII promoter inserted into the MCS of the pKmobsacB vector was synthesized by Genscript for insertion into NAH7 replacing the HXC10_RS00045 gene (Schäfer et al., 1994). Delivery of the vector was again achieved through conjugation with *E. coli* WM6026 as the donor. Selection of *P. putida* G7 colonies exhibiting successful gene insertion and constitutive fluorescence was achieved following previous methods and PCR/sequencing with primers targeting the insert region listed in Table 2 (Schäfer et al., 1994).

To further assist with conjugation quantification, the chromosomes of the donors, *P. putida* G7 and *N. aromaticivorans* F199, were inserted with genes encoding constitutive fluorescence of GFP. For insertion of the construct into *N. aromaticivorans* F199, a vector based on the pAK405 vector with a GFPmut2 construct under the psyn2 promotor was synthesized for insertion into the chromosome replacing the Saro_1879 gene (Kaczmarczyk et al., 2013; Taton et al., 2014). Delivery of the vector was also achieved through conjugation with *E. coli* WM6026 as the donor. Selection of *N. aromaticivorans* F199 colonies exhibiting successful gene insertion and constitutive fluorescence was performed by following previously established methods and PCR/sequencing with primers targeting the insert region listed in Table 2 (Kaczmarczyk et al., 2012). The F199 strain fluorescing both mScarlet-I and GFPmut2 was designated JMN120 but will be referred to as F199 henceforth for. For insertion of GFP into *P. putida* G7, the pMRE-Tn7-152 vector plasmid from Schlechter et al. (2018) was used to insert the mVenus construct at the attTn7 site via transposon mutagenesis (DeBoy and Craig, 2000). Delivery of the vector was again achieved through conjugation with *E. coli* WM6026 as the donor. Selection of *P. putida* G7 colonies exhibiting successful gene insertion and constitutive fluorescence was achieved by following previously established methods and PCR/sequencing with primers targeting the insert region listed in Table 2 (Schlechter et al., 2018). Fluorescence of mScarlet-I and GFP in both donors was also validated by detecting fluorescence on the Tecan Sunrise microplate reader (Männedorf, Switzerland). The G7 strain fluorescing both mScarlet-I and mVenus was designated PVP102 but will be referred to as G7 henceforth for simplicity.

2.3. 1:1 Conjugation assays

Conjugation of pNL1 and NAH7 into recipients was first examined by adding one donor and one recipient in culture. G7, F199, and recipients were grown overnight in Caulobacter Medium (CBM) consisting of 2 g/L peptone, 1 g/L yeast extract, and 0.2 g/L $MgSO_4$ ×

$7H_2O$ at 30 °C. In the morning, donors and recipients were added in triplicate to 5 mL of CBM either with or without naphthalene. For NAH7 conjugations, G7 and recipients were added in a 1:1 ratio. For pNL1 conjugations, F199 and recipients were added in a 10:1 ratio due to the slow growth of F199 compared to most recipients. For each culture, a total of 10^6 cells were added. Naphthalene was added to the NAH7 conjugation cultures by adding naphthalene crystals to the CBM medium which ensured the medium was continually saturated with naphthalene. For the pNL1 conjugation cultures, 100 mg/L of naphthalene was added by first dissolving in acetone, adding to the culture tube, and letting the acetone evaporate — leaving naphthalene crystals in the tubes. Conjugation frequency, determined by the number of transconjugants divided by the geometric mean of the number of donors and recipients, was measured at 24 and 48 h after inoculation.

2.4. 1:2 Conjugation assays

To further assess conjugation preference and naphthalene degradation for pNL1 and NAH7, synthetic communities consisting of one donor and two recipients were created in triplicate at high or low nutrient availability. All strains were grown overnight in CBM at 30 °C before inoculation. Naphthalene dissolved in acetone was added to the glass reactors and the acetone was evaporated, leaving naphthalene crystals behind. The beginning naphthalene concentrations for pNL1 and NAH7 conjugations were 100 mg/L and 500 mg/L respectively. Reactors were then filled to 100 mL with either high nutrient medium (CBM) or low nutrient medium (CBM diluted 1:3). The resulting nitrogen and phosphorus concentrations are relevant to those seen in the environment, with the high end being representative of a biostimulation regime (Shabir et al., 2008). For NAH7 conjugations, G7 and recipients were added in a 1:1:1 ratio while for pNL1 conjugations, F199 and recipients were added in a 10:1:1 ratio. For each culture, a total of 10^5 cells were added.

Four different communities were assessed at high and low nutrient conditions: (1) Controls with no bacteria present; (2) K community containing the strains most representative of K-strategists (*S. lindanitolerans* and *C. vibrioides*); (3) Mixed (M) community containing one K- and one r-strategist (*S. lindanitolerans* and *E. cloacae*); (4) r community containing the strains most representative of r-strategists (*E. cloacae* and *S. marcescens*). Conjugation frequency, determined by the number of transconjugants divided by the geometric mean of the number of donors and recipients, was measured on days 1, 2, 4, and 8. Naphthalene saturation was monitored on days 1, 2, and 4 and a full extraction on day 8.

2.5. FACS analysis

On sample days, 500 μL of reactor media was added to 5 mL polypropylene tubes prior to sorting. Cells were sorted into 200 μL of PBS which was directly used for down-stream DNA extraction. Flow cytometric detection and sorting of cells was performed using a BD FACSAria II (San Jose, CA, USA) through the Duke Human Vaccine Institute Flow Cytometry Center at Duke University (Durham, NC, USA). The following technical settings were employed: A 70 μm nozzle and sheath fluid pressure of 70 psi with chiller; GFP was excited by a 488 nm laser and detected on the FITC-A channel with a bandpass filter of 530/30 nm; mScarlet-I was excited with a 561 nm laser and detected on the PE-Texas Red-A channel with a bandpass filter of 610/20 nm. FlowJo 10.8.1 was used for analyzing results.

Bivariate contour plots of particle FSC vs. SSC areas were employed to build a gate around the bacterial population excluding background noise. Green and red fluorescing bacterial cells were gated on bivariate contour plots using the area of PE-Texas Red vs. the area of FITC. The detection gates and example gates used in this study are depicted in Supplementary Fig. 2. Donors were gated as green and red, transconjugants were gated as red-non-green, and recipients as non-green and non-red. A total of 100,000 events were recorded and a total of 20,000 transconjugant cells were sorted when applicable under purity settings. Sorted cells were then used for DNA extraction and qPCR analysis.

2.6. DNA extraction and qPCR

Transconjugants in the 1:2 conjugation assays underwent DNA extraction following sorting. DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany) following manufacturer's instructions. Lysis was carried out for 30 min at 56 °C and elution was performed twice at 25 μL. As transconjugants were sorted straight into PBS, no pelleting of cells was necessary before lysis.

The relative abundance of transconjugants in the 1:2 conjugation assays was determined by qPCR. Relative abundances were calculated by comparing the copy number-corrected starting quantity of each recipient calculated via 6-point standard curves. Taqman™ probes for each strain were developed targeting the variable V3 region of their 16S rRNA genes and primers were developed targeting the conserved area of the V3 region (Table 3). All qPCR assays were performed on a BioRad CFX96 Touch Real-Time PCR Detection System (Hercules, CA, USA) in a total reaction volume of 20 μL. Primers and probes were added to a final concentration of 250 nM with 1 μL of extracted transconjugant DNA and iTaq Universal Probes Supermix. The reaction conditions consisted of 95 °C for 3 min, followed by 39 cycles of denaturation at 95 °C for 20 s and annealing/extension at 60 °C for 30 s. Reactions were performed in triplicate and included negative controls and standards consisting of gBlocks™ of the targeted V3 region. Controls for DNA extraction and qPCR were performed containing water instead of bacteria or DNA. No contamination was found.

2.7. Naphthalene quantification

Liquid samples (3 mL) were collected at days 1, 2, and 4 from each reactor aseptically using a 25G1 needle attached to a glass syringe to extract naphthalene from the 1:2 conjugation assays. On day 8 of the experiment, naphthalene was extracted from the whole reactor by adding 100 mL of solvent to each reactor. Naphthalene was extracted using ethyl acetate and mixed for 10 seconds followed by 20 seconds and complete separation. After filtering through a 0.2 μm nylon membrane filter (Merck Millipore, Ltd.; Burlington, MA) and diluting in 50:50 acetonitrile and water, extracts were quantified using an Agilent/Varian ProStar Modular High-Performance Liquid Chromatography (HPLC) System equipped with three ProStar Solvent Delivery Modules, a 410 autosampler, a 335 UV-detector, a 363 fluorescence detector, and a Supelcosil™ LC-PAH HPLC column (100 mm × 4.6 mm, 2.6 μm particle size; Supelco, Bellefonte, PA, USA). Naphthalene concentrations were determined using fluorescence at a 7 min analysis window with 224 nm of excitation and 330 nm of emission and five-point standard curves.

2.8. Data analysis

All data were analyzed using RStudio, version 1.4.1103. Experimental replicates for all data were averaged and reported with standard error. The effect of growth strategy, GC content, and phylogenetic relatedness on conjugation frequency in 1:1 conjugation assays were analyzed using a simple linear regression to test if any of these variables had an effect on conjugation frequency. ANCOVA measurements were used to compare between naphthalene treatments for pNL1/F199 groups but not for NAH7/G7 groups due to heterogeneity of variance. All other results were analyzed using multi-way ANOVAs with post hoc Tukey's HSD and pairwise t-tests. Statistical significance was determined with a 95% confidence interval.

3. Results

3.1. Conjugation in 1:1 communities

The conjugation frequencies of pNL1 and NAH7 were assessed in 1:1 conjugation assays with each donor and recipient either in the presence or absence of naphthalene at 24 and 48 h of growth to determine: (1) conjugation preferences of the degradative plasmids; (2) whether or not naphthalene is a selective pressure for conjugation (Fig. 1). For pNL1, conjugation frequencies ranged from below detection to 0.025 (Fig. 1A). Overall, there was significantly higher conjugation of pNL1 into *S. lindanitolerans*, the recipient with the lowest 16S copy number, than *S. marcescens* ($p = 0.04$), and marginally significantly higher than *E. cloacae* ($p = 0.07$) – both of which have the highest 16S copy numbers. *C. vibrioides* harbored significantly higher amounts of pNL1 when naphthalene was present after 48 h than any other recipients throughout the study ($p < 0.001$).

For NAH7, the conjugation frequencies ranged from 0–0.7 (Fig. 1B). There were no overall significant differences in conjugation frequencies between recipient organisms. However, there was a significant decrease in conjugation frequency at 48 h compared to 24 h when naphthalene was not present ($p < 0.05$). Furthermore, the addition of naphthalene led to a significant increase in conjugation frequency across all recipients regardless of time ($p < 0.05$), suggesting that naphthalene was a selective pressure for conjugation of NAH7 but not for pNL1.

The impact of the 16S rRNA copy number of each recipient, a proxy for growth strategy, was assessed via a regression of conjugation frequency vs. 16S copy number (Fig. 2). For pNL1, there was a significant negative correlation between recipient copy number and conjugation frequency when there was naphthalene present or no naphthalene ($R^2 = 0.140$ and 0.286, $p = 0.025$ and 0.0001, respectively). ANCOVA revealed that there was no significant difference between the naphthalene and no naphthalene treatments. By contrast, for NAH7, there was a significantly negative correlation between recipient copy number and conjugation frequency when no naphthalene was present ($R^2 = 0.164$, $p = 0.014$), although this could not be tested using ANCOVA due to heterogeneity of variances. A comparison of the R^2 values between the two treatments reveal that adding naphthalene dampens any existing significant correlation ($R^2 = 0.003$, $p = 0.77$).

3.2. GC content and phylogenetic relatedness

Both GC content and phylogenetic relatedness of donor and recipient strains have been shown to have an impact on the conjugation and survival of plasmids (Thomas and Nielsen, 2005; Ikuma and Gunsch, 2012, 2013). To assess the impact of these factors on conjugation, regressions of conjugation frequency vs. recipient GC content or phylogenetic distance from the donor were assessed (Supplementary Figs. 3 and 4). All regressions for each plasmid and whether naphthalene was present were nearly identical between GC content and phylogenetic relatedness for respective groupings. These analyses reveal a significant correlation between both GC content of the recipient and phylogenetic relatedness, and conjugation frequency with one exception (R^2 ranging from 0.092 to 0.235; $p < 0.05$). When naphthalene was added, there was a non-significant positive correlation between GC content and conjugation frequency for both pNL1 and NAH7 ($R^2 = 0.092$ and 0.100, $p = 0.073$ and 0.06, respectively. Additionally, there is a range of conjugation frequencies at similar GC contents and phylogenetic relatedness with various recipient strains which suggests conjugation preferences cannot be described entirely by these two factors for these bacteria under the given experimental conditions. Furthermore, unlike previous reports suggesting preferential carbon substrate may lead to increased conjugation, naphthalene presence did not influence conjugation frequency with respect to GC content and phylogenetic relatedness.

3.3. Conjugation in 1:2 communities

Conjugation frequency of pNL1 and NAH7 was also assessed over eight days in mixed synthetic communities consisting of one donor and two recipients with varying levels of ecological growth strategies to determine transconjugant preference with either high or low levels of nutrients and conjugation preferences when there are multiple recipients present in a community (Fig. 3). For pNL1, the K community consisting of 2 recipients exhibiting K-strategy had significantly higher levels of conjugation overall compared to the mixed (M) community and the r community. Additionally, there were higher levels of conjugation frequency in the low nutrient conditions in the mixed community consisting of one r-strategist and one K-strategist, particularly on day 8 ($p < 0.05$). There were no significant differences in conjugation frequency between high and low nutrient conditions for the K or r communities. For the NAH7 plasmid, there was significantly higher levels of conjugation in the r community compared to the K community ($p < 0.01$) and marginally significant compared to the M community ($p = 0.076$). Additionally, all communities had higher levels of conjugation in the low nutrient conditions, particularly on days 4 and 8.

3.4. Relative abundances of transconjugants

The relative abundance of transconjugants was assessed via qPCR after FACS for recipients that accepted the pNL1 and NAH7 plasmids (Fig. 4). Transconjugants followed similar patterns for both pNL1 and NAH7 assays as well as when both high and low nutrients were available. First, in the K community which consisted of two K-strategists, nearly 100% of transconjugants were *S. lindanitolerans* which is the more K-strategist of the two recipients. In the mixed community consisting of one K- and one r-strategist, ~75%–100% of transconjugants were *E. cloacae* which is the r-strategist. *S. lindanitolerans* was present as

a transconjugant of pNL1 in the mixed community but was not present as a transconjugant of NAH7 in the mixed community. For the r community consisting of two r-strategists, both recipients were present as transconjugants. However, *S. marcescens*, with a lower 16S copy number, was more abundant as a pNL1 transconjugant but was less abundant than *E. cloacae* as a NAH7 transconjugant.

3.5. Naphthalene degradation

To assess the differences in plasmid-mediated degradation of naphthalene in synthetic communities with various growth strategies, total naphthalene removal was measured at the end of the eight-day conjugation experiments (Fig. 5). For the pNL1 experiments, all communities had significantly more naphthalene removal than the control consisting of naphthalene with no donors or recipients added, suggesting increased biological removal of naphthalene over 8 days. The K community had significantly higher removal of naphthalene than the mixed and r communities ($p < 0.01$). However, there were no significant differences in removal between the mixed and r communities. In addition, we observed marginally significantly higher degradation in the low medium conditions compared to the high medium conditions ($p = 0.0875$).

For the NAH7 experiments, only the K and mixed communities had marginally significantly more naphthalene removal than the controls ($p < 0.09$). The r community had no significant biological removal of naphthalene. The K and mixed communities had marginally significantly higher removal of naphthalene compared to the r community ($p < 0.08$). However, there was no significant difference in naphthalene removal between the K and mixed communities. We also observed marginally significantly higher degradation in the low medium conditions compared to the high medium conditions ($p = 0.0538$).

4. Discussion

The conjugation of two PAH-degrading plasmids into eight recipients spanning ecological growth strategies, as determined by 16S rRNA copy numbers, was evaluated in synthetic communities. Here, conjugation was quantified using fluorescently labeled plasmids and donor chromosomes and detection/sorting via fluorescent activated cell sorting (FACS). This method sets the groundwork for additional experiments with natural communities because it allows quantification of conjugation and identification of transconjugants *in-situ* and without the need for culturing. Because only about 1% of bacteria can be cultured, this method allows for plasmid transfer tracking more accurately and with a broader range of bacteria than previously accessible (Staley and Konopka, 1985; Amann et al., 1995; Hugenholtz et al., 1998). Additionally, although using fluorescently labeled donors and plasmids paired with FACS has been used for investigating conjugation of antibiotic resistance plasmids (Klümper et al., 2015; Li et al., 2018a,b, 2020), this is the first use of this method for tracking conjugation of degradative plasmids. This method is more advantageous than previous methods necessitating qPCR because it reduces bias by not requiring DNA extraction and it is able to distinguish between live and dead cells (Redfern, 2017). Although there is bias from the Nycodenz separation of cells, studies have indicated

that 80%–90% of bacteria are recovered from soil via Nycodenz separation (Holmsgaard et al., 2011; Khalili et al., 2019).

In simple conjugation assays, one donor and one recipient were added to measure conjugation frequency both with and without the presence of the model PAH naphthalene. In general, the conjugation frequencies of the pNL1 plasmid harbored in *N. aromaticivorans* F199 were 1–2 orders of magnitude lower than the conjugation frequencies of the NAH7 plasmid harbored in *P. putida* G7. Although this phenomenon could be due to multiple different factors, the smaller size of the NAH7 plasmid may be a key contributing factor to the differences in frequency of conjugation (83 kb NAH7 plasmid as compared to the 184 kb pNL1 plasmid). Smaller plasmids are known to transfer at quicker rates and are easier to maintain in transconjugants than larger plasmids (Garbisu et al., 2017).

In the 1:1 conjugation experiments, the K-strategists were found to accept the pNL1 plasmid more frequently than the r-strategists. Particularly, *S. lindanitolerans* consistently had higher conjugation frequencies than the two recipients with highest 16S copy number: *S. marcescens* and *E. cloacae*. In addition, *C. vibrioides* had significantly higher levels of transconjugants than any other recipients, especially when naphthalene was present. This result suggests that K-strategists may be favored over the r-strategists for conjugation with the pNL1 plasmid. This result is further confirmed by the negative correlation we observed between conjugation frequency and16S rRNA copy number. This is contrary to previous studies that suggest fast-growers, such as r-strategists, can transfer plasmids more frequently (Smets et al., 1993; Seoane et al., 2010). However, those previous studies were strictly for smaller antibiotic resistance plasmids with different transfer and replication dynamics. Our results present the first indication that donors harboring degradative plasmids may in fact favor transfer into K-strategists when grown in 1:1 communities.

To assess conjugation frequencies, preferences, plasmid-mediated naphthalene degradation when there were multiple recipients present, F199 harboring pNL1 was added to three different communities consisting of two recipients with varying growth strategies. Overall, the K community consisting of two K-strategists had both higher frequencies of conjugation and higher percent removal of naphthalene. These data further support the findings from the 1:1 conjugation experiments in which pNL1 favored conjugation into K-strategists compared to r-strategists, and findings from previous studies investigating non plasmid-mediated hydrocarbon degradation (Brzeszcz et al., 2016). Interestingly, however, there was a higher relative abundance of the r-strategist, *E. cloacae*, as a transconjugant in the mixed community compared to the K-strategist, *S. lindanitolerans*. This finding suggests that fast growth may be a more critical factor in transconjugant prevalence as compared to conjugation preferences themselves. Furthermore, higher numbers of transconjugants were observed in the low nutrient conditions which might be explained by these conditions promoting more growth of the donor, F199, than the high nutrient conditions. These phenomena could describe why previous studies have found higher rates of transfer in r-strategists (Smets et al., 1993; Seoane et al., 2010). Although the competition of recipients that did not receive the plasmid were not explored, these data give insight into conjugation preferences and transconjugant competition once the recipients have taken up the plasmid of interest. Understanding relationships between conjugation and growth strategy in synthetic

communities presents strategies for implementing genetic bioaugmentation and precisely engineering environmental microbiomes in the field.

The NAH7 plasmid harbored in *P. putida* G7 was also used to explore conjugation frequencies in 1:1 assays with the donor and one recipient. In this case, there were overall no significant differences between conjugation frequencies across different species of recipients. However, the addition of naphthalene was associated with a significant increase in conjugation frequency. This trend was also observed when comparing the correlation between conjugation frequency and 16S rRNA copy number. Again, a significantly negative correlation was observed suggesting that K-strategists may also accept the NAH7 plasmid more frequently. However, this correlation was not observed in the presence of naphthalene. Therefore, the presence of naphthalene exhibited a selective pressure strong enough to diminish any effect that growth strategy might have on conjugation preferences. The increase in conjugation with the selective pressure of naphthalene supports other studies on transfer and expression of degradative plasmids (DiGiovanni et al., 1996; Ikuma and Gunsch, 2012; Zhang et al., 2012; Wang et al., 2014). However, because this phenomenon was not observed for pNL1, these data suggest some dependence on the plasmid itself and highlights the difficult for identifying generalizable framework for *in situ* microbiome engineering.

NAH7 conjugation and plasmid-mediated naphthalene degradation was also explored in more complex synthetic communities consisting of two recipients. Contrary to both the 1:1 conjugation experiments with NAH7 and the 1:2 conjugation experiments with pNL1, the r community, consisting of two r-strategists, had higher frequencies of conjugation than the K and mixed communities. Additionally, there were higher relative abundances of the r-strategists in the r and mixed communities. This change in preference compared to previous data provides some insight into differences in conjugation preference when there are mixed communities competing for resources and plasmid acceptance. In the present study, similarly to previously published studies, we observed that r-strategists might outcompete K-strategists as transconjugants, particularly when the plasmid donor is an r-strategist itself (Smets et al., 1993; Seoane et al., 2010). Interestingly, the mixed and r communities that had higher abundances of r-strategist transconjugants had less naphthalene removal than the K community consisting almost entirely of *S. lindanitolerans*, the recipient with the lowest 16S copy number. This observation supports previous findings that suggest K-strategists are able to degrade contaminants more efficiently than r-strategists (Brzeszcz et al., 2016). Thus, although conditions might favor survival of transconjugants exhibiting r-strategy, K-strategists may express plasmid genes more efficiently, a finding important to explore further for the implementation of genetic bioaugmentation for bioremediation. Finally, similar to the pNL1 experiments, the low nutrient conditions promoted significantly more conjugation in all communities compared to the high nutrient conditions. Although high nutrients would likely promote growth of the donor, G7, low nutrients may have promoted growth of transconjugants which may explain their observed increased prevalence in the community over time.

Overall, both pNL1 and NAH7 showed conjugation preferences towards K-strategists over r-strategists, except when NAH7 was in a mixed synthetic community with two

recipients. These preferences were partially described by a combination of growth strategy, GC content, and phylogenetic relatedness, supporting previous studies investigating these biological properties and their effect on conjugation (Ikuma and Gunsch, 2012, 2013; Sysoeva et al., 2019). Further, plasmid-mediated removal of naphthalene was consistently higher in communities containing K-strategists compared to either a mixed community or a community consisting of r-strategists. Although in the present experiments, some of the naphthalene degradation may be explained by favorable growth of the donor, the discrepancy between conjugation preference for r-strategists but higher degradation for K-strategists suggests that NAH7 transconjugants are likely responsible for this incremental degradation. For both plasmids in this study, lower availability of nutrients consistently promoted the highest level of conjugation and naphthalene degradation.

5. Conclusion

The data presented herein are the first to consider the impact of ecological growth strategies on conjugative plasmid transfer and plasmid-mediated contaminant degradation. This information fills a critical research gap for the translation of genetic bioaugmentation and field application of microbiome engineering. The results from this study provide a framework for identifying the favorable conditions needed to promote the successful implementation of genetic bioaugmentation targeted to the existing recipient community present at a site. These findings are also broadly applicable to other fields beyond bioremediation design and implementation. An understanding of conditions favorable for plasmid transfer and expression can be utilized for a variety of applications including prevention of antibiotic resistance, agriculture, and other biomedical or environmental purposes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

All data analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

Abbreviations:

PAHs	polycyclic aromatic hydrocarbons
MGEs	mobile genetic elements
G7	Pseudomonas putida G7
F199	Novosphingobium aromaticivorans F199
CBM	Caulobacter medium
FACS	fluorescent-activated cell sorting
MCS	multiple cloning site

References

Abdo KM, Grumbein S, Chou BJ, Herbert R, 2001. Toxicity and carcinogenicity study in F344 rats following 2 years of whole-body exposure to naphthalene vapors. Inhal. Toxicol. 13, 931–950. 10.1080/089583701752378179. [PubMed: 11696867]

Amann RI, Ludwig W, Schleifer KH, 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59, 143–169. 10.1128/mr.59.1.143-169.1995. [PubMed: 7535888]

Atlas RM, Bartha R, 1997. Microbial Ecology: Fundamentals and Applications, fourth ed. Benjamin Cummings.

Brzeszcz J, Steliga T, Kapusta P, et al. , 2016. R-strategist versus K-strategist for the application in bioremediation of hydrocarbon-contaminated soils. Int. Biodeterioration Biodegrad. 106, 41–52. 10.1016/j.ibiod.2015.10.001.

Christie PJ, 2016. Mosiac type IV secretion systems. EcoSal Plus 7, 1–34. 10.1128/ecosalplus.ESP-0020-2015.The.

DeBoy RT, Craig NL, 2000. Target site selection by Tn7: attTn7 transcription and target activity. J. Bacteriol. 182, 3310–3313. 10.1128/JB.182.11.3310-3313.2000. [PubMed: 10809719]

DiGiovanni GD, Neilson JW, Pepper IL, Sinclair NA, 1996. Gene transfer of alcaligenes eutrophus JMP134 plasmid pJP4 to indigenous soil recipients. Appl. Environ. Microbiol. 62, 2521–2526. 10.1128/aem.62.7.2521-2526.1996. [PubMed: 8779592]

Fahrenfeld N, Knowlton K, Krometis LA, et al. , 2014. Effect of manure application on abundance of antibiotic resistance genes and their attenuation rates in soil: Field-scale mass balance approach. Environ. Sci. Technol. 48, 2643–2650. 10.1021/ES404988K/SUPPL_FILE/ES404988K_SI_001.PDF. [PubMed: 24483241]

Fernandez-Lopez R, Redondo S, Garcillan-Barcia MP, de la Cruz F, 2017. Towards a taxonomy of conjugative plasmids. Curr. Opin. Microbiol. 38, 106–113. [PubMed: 28586714]

Frost LS, Leplae R, Summers AO, Toussaint A, 2005. Mobile genetic elements: The agents of open source evolution. Nature Rev. Microbiol. 3, 722–732. [PubMed: 16138100]

Gao C, Jin X, Ren J, et al. , 2015. Bioaugmentation of DDT-contaminated soil by dissemination of the catabolic plasmid pDOD. J. Environ. Sci. 27, 42–50. 10.1016/j.jes.2014.05.045.

Garbisu C, Garaiyurrebaso O, Epelde L, et al. , 2017. Plasmid-mediated bioaugmentation for the bioremediation of contaminated soils. Front. Microbiol. 8 (1966), 10.3389/fmicb.2017.01966.

Gogarten JP, Townsend JP, 2005. Horizontal gene transfer, genome innovation and evolution. Nature Rev. Microbiol. 3, 679–687. [PubMed: 16138096]

Gothwal R, Thatikonda S, 2020. Modeling transport of antibiotic resistant bacteria in aquatic environment using stochastic differential equations. Sci. Rep. 10 (1), 1–11. 10.1038/s41598-020-72106-3. [PubMed: 31913322]

Holmsgaard PN, Norman A, Hede SC, et al. , 2011. Bias in bacterial diversity as a result of nycodenz extraction from bulk soil. Soil Biol. Biochem. 43, 2152–2159. 10.1016/j.soilbio.2011.06.019.

Hugenholtz P, Goebel BM, Pace NR, 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol. 180, 4765–4774. [PubMed: 9733676]

Ikuma K, 2011. The effect of select biological and environmental factors on the horizontal gene transfer and functionality of the TOL plasmid: A case study for genetic bioaugmentation the effect of select biological and environmental factors on the horizontal gene tran.

Ikuma K, Gunsch CK, 2012. Genetic bioaugmentation as an effective method for in situ bioremediation: Functionality of catabolic plasmids following conjugal transfers. Bioengineered 3, 236–241. 10.4161/bbug.20551. [PubMed: 22705839]

Ikuma K, Gunsch CK, 2013. Functionality of the TOL plasmid under varying environmental conditions following conjugal transfer. Appl. Microbiol. Biotechnol. 97, 395–408. 10.1007/s00253-012-3949-8. [PubMed: 22367613]

Ikuma K, Holzem RM, Gunsch CK, 2012. Impacts of organic carbon availability and recipient bacteria characteristics on the potential for TOL plasmid genetic bioaugmentation in soil slurries. Chemosphere 89, 158–163. 10.1016/j.chemosphere.2012.05.086. [PubMed: 22743182]

Johnsen AR, Kroer N, 2007. Effects of stress and other environmental factors on horizontal plasmid transfer assessed by direct quantification of discrete transfer events. FEMS Microbiol. Ecol. 59, 718–728. 10.1111/j.1574-6941.2006.00230.x. [PubMed: 17100984]

Kaczmarczyk A, Vorholt JA, Francez-Charlot A, 2012. Markerless gene deletion system for sphingomonads. Appl. Environ. Microbiol. 78, 3774–3777. 10.1128/AEM.07347-11. [PubMed: 22427496]

Kaczmarczyk A, Vorholt JA, Francez-Charlot A, 2013. Cumate-inducible gene expression system for sphingomonads and other Alphaproteobacteria. Appl. Environ. Microbiol. 79, 6795–6802. 10.1128/AEM.02296-13/SUPPL_FILE/ZAM999104843SO1.PDF. [PubMed: 23995928]

Khalili B, Weihe C, Kimball S, et al. , 2019. Optimization of a method to quantify soil bacterial abundance by flow cytometry. MSphere 4, 10.1128/msphere.00435-19.

Klappenbach JA, Dunbar JM, Schmidt TM, 2000. rRNA operon copy number reflects ecological strategies of bacteria. Appl. Environ. Microbiol. 66, 1328–1333. 10.1128/AEM.66.4.1328-1333.2000. [PubMed: 10742207]

Klümper U, Riber L, Dechesne A, et al. , 2015. Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community. ISME J. 9, 934–945. 10.1038/ismej.2014.191. [PubMed: 25333461]

Lawley TD, Klimke WA, Gubbins MJ, Frost LS, 2003. F factor conjugation is a true type IV secretion system. FEMS Microbiol. Lett. 224, 1–15. [PubMed: 12855161]

Li L, Dechesne A, He Z, et al. , 2018a. Estimating the transfer range of plasmids encoding antimicrobial resistance in a wastewater treatment plant microbial community. Environ. Sci. Technol. Lett. 5, 260–265. 10.1021/acs.estlett.8b00105.

Li L, Dechesne A, Madsen JS, et al. , 2020. Plasmids persist in a microbial community by providing fitness benefit to multiple phylotypes. ISME J. 14, 1170–1181. 10.1038/s41396-020-0596-4. [PubMed: 32020051]

Li B, Qiu Y, Zhang J, et al. , 2018b. Real-time study of rapid spread of antibiotic resistance plasmid in biofilm using microfluidics. Environ. Sci. Technol. 52, 11132–11141. 10.1021/acs.est.8b03281. [PubMed: 30179497]

Mrozik A, Piotrowska-Seget Z, 2010. Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. Res. Microbiol. 165, 363–375. 10.1016/j.micres.2009.08.001.

Nguyen AQ, Vu HP, Nguyen LN, et al. , 2021. Monitoring antibiotic resistance genes in wastewater treatment: Current strategies and future challenges. Sci. Total Environ. 783, 146964. 10.1016/J.SCITOTENV.2021.146964. [PubMed: 33866168]

Novick RP, 1987. Plasmid incompatibility. Microbiol. Rev. 51, 381–395. [PubMed: 3325793]

Ortiz-Álvarez R, Noah Fierer, De Los Ríos Asunción, et al. , 2018. Consistent changes in the taxonomic structure and functional attributes of bacterial communities during primary succession. ISME J. 10.1038/s41396-018-0076-2.

Partridge SR, Kwong SM, Firth N, Jensen SO, 2018. Mobile genetic elements associated with antimicrobial resistance. Clin. Microbiol. Rev. 31, 10.1128/CMR.00088-17.

Redfern LK, 2017. Microbial communities and polyciclic aromatic hydrocarbons: Exposure related adaptations in environmental microbiomes and their potential for bioremediation.

Redfern LK, Gardner CM, Hodzic E, et al. , 2019. A new framework for approaching precision bioremediation of PAH contaminated soils. J. Hazard. Mater. 378, 10.1016/j.jhazmat.2019.120859.

Roller BRK, Stoddard SF, Schmidt TM, 2016. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. Nat. Microbiol. 1, 1–7. 10.1038/nmicrobiol.2016.160.

Romine MF, Stillwell LC, Wong KK, et al. , 1999. Complete sequence of a 184-kilobase catabolic plasmid from sphingomonas aromaticivorans F199. J. Bacteriol. 181, 1585–1602. 10.1128/jb.181.5.1585-1602.1999. [PubMed: 10049392]

Schäfer A, Tauch A, Jäger W, et al. , 1994. Small mobilizable multi-purpose cloning vectors derived from the escherichia coli plasmids pk18 and pk19: Selection of defined deletions in the chromosome of corynebacterium glutamicum. Gene 145, 69–73. 10.1016/0378-1119(94)90324-7. [PubMed: 8045426]

Schlechter RO, Jun H, Bernach M, et al. , 2018. Chromatic bacteria – A broad host-range plasmid and chromosomal insertion toolbox for fluorescent protein expression in bacteria. Front. Microbiol. 9, 1–14. 10.3389/fmicb.2018.03052. [PubMed: 29403456]

Seoane J, Yankelevich T, Dechesne A, et al. , 2010. An individual-based approach to explain plasmid invasion in bacterial populations. FEMS Microbiol. Ecol. 75, 17–27. 10.1111/j.1574-6941.2010.00994.x. [PubMed: 21091520]

Shabir G, Afzal M, Anwar F, et al. , 2008. Biodegradation of kerosene in soil by a mixed bacterial culture under different nutrient conditions. Int. Biodeterioration Biodegrad. 61, 161–166. 10.1016/j.ibiod.2007.06.003.

Smets BF, Rittmann BE, Stahl DA, 1993. The specific growth rate of pseudomonas putida PAW1 influences the conjugal transfer rate of the TOL plasmid. Appl. Environ. Microbiol. 59, 3430–3437. 10.1128/aem.59.10.3430-3437.1993. [PubMed: 8250565]

Sota M, Yano H, Ono A, et al. , 2006. Genomic and functional analysis of the IncP-9 naphthalene-catabolic plasmid NAH7 and its transposon Tn4655 suggests catabolic gene spread by a tyrosine recombinase. J. Bacteriol. 188, 4057–4067. 10.1128/JB.00185-06. [PubMed: 16707697]

Staley JT, Konopka A, 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu. Rev. Microbiol. 39, 321–346. [PubMed: 3904603]

Stoddard SF, Smith BJ, Hein R, et al. , 2015. Rrndb: Improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic Acids Res. 43, D593–D598. 10.1093/nar/gku1201. [PubMed: 25414355]

Sysoeva TA, Kim Y, Rodriguez J, et al. , 2019. Growth-stage-dependent regulation of conjugation. AIChE J. 66, 1–10. 10.1002/aic.16848.

Taton A, Unglaub F, Wright NE, et al. , 2014. Broad-host-range vector system for synthetic biology and biotechnology in cyanobacteria. Nucleic Acids Res. 42, e136. 10.1093/NAR/GKU673. [PubMed: 25074377]

Thomas CM, Nielsen KM, 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature Rev. Microbiol. 3, 711–721. 10.1038/nrmicro1234. [PubMed: 16138099]

Top EM, Springael D, 2003. The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds. Curr. Opin. Biotechnol. 14, 262–269. 10.1016/S0958-1669(03)00066-1. [PubMed: 12849778]

Top EM, Springael D, Boon N, 2002. Catabolic mobile genetic elements and their potential use in bioaugmentation of polluted soils and waters. FEMS Microbiol. Ecol. 42, 199–208. 10.1111/j.1574-6941.2002.tb01009.x. [PubMed: 19709279]

Varner PM, Gunsch CK, 2021. Properties affecting transfer and expression of degradative plasmids for the purpose of bioremediation. Biodegradation 32, 361–375. 10.1007/s10532-021-09950-1. [PubMed: 34046775]

Venkata Mohan S, Falkentoft C, Venkata Nancharaiah Y, et al. , 2009. Bioaugmentation of microbial communities in laboratory and pilot scale sequencing batch biofilm reactors using the

TOL plasmid. Bioresour. Technol. 100, 1746–1753. 10.1016/j.biortech.2008.09.048. [PubMed: 19010662]

Wang Y, Kou S, Jiang Q, et al. , 2014. Factors affecting transfer of degradative plasmids between bacteria in soils. Appl. Soil Ecol. 84, 254–261. 10.1016/j.apsoil.2014.07.009.

Wu L, Yang Y, Chen S, et al. , 2017. Microbial functional trait of rRNA operon copy numbers increases with organic levels in anaerobic digesters. ISME J. 11, 2874–2878. 10.1038/ismej.2017.135. [PubMed: 28841202]

Zhang Q, Wang B, Cao Z, Yu Y, 2012. Plasmid-mediated bioaugmentation for the degradation of chlorpyrifos in soil. J. Hazard. Mater. 221–222, 178–184. 10.1016/j.jhazmat.2012.04.024.

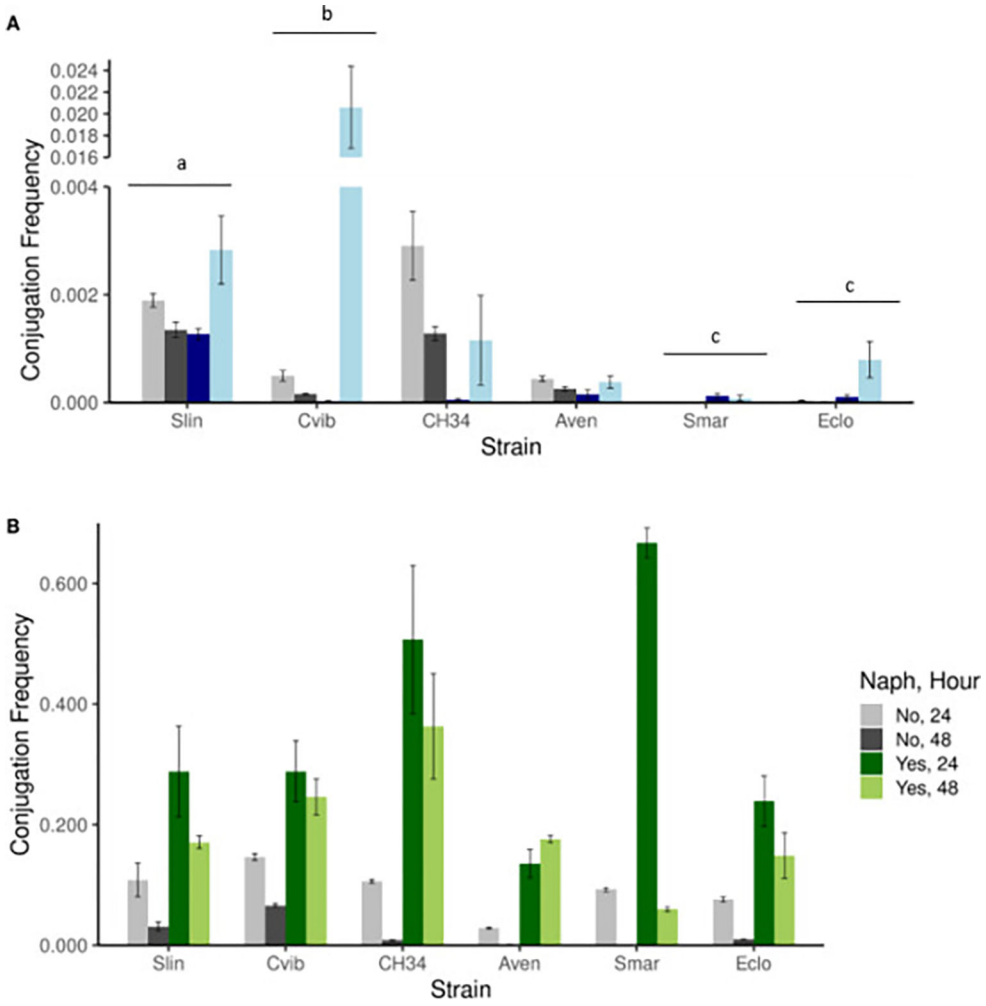

Fig. 1.

Conjugation frequency of (A) pNL1 harbored in F199 and (B) NAH7 harbored in G7 in 1:1 conjugation assays ordered from lowest 16S copy number to highest. Bars are colored by whether naphthalene was added and at which hour the sample was taken. Letters above bars indicate populations that are significantly or nearly-significantly different from one another. For B, all naphthalene-added communities had significantly higher conjugation frequency than communities with no naphthalene. “Slin” indicates *S. lindanitolerans*; “Cvib” indicates *C. vibrioides*; “CH34” indicates *C. metallidurans* CH34; “Aven” indicates *A. venetianus*; “Smar” indicates *S. marcescens*; “Eclo” indicates *E. cloacae*. Error bars indicate standard error ($n = 3$).

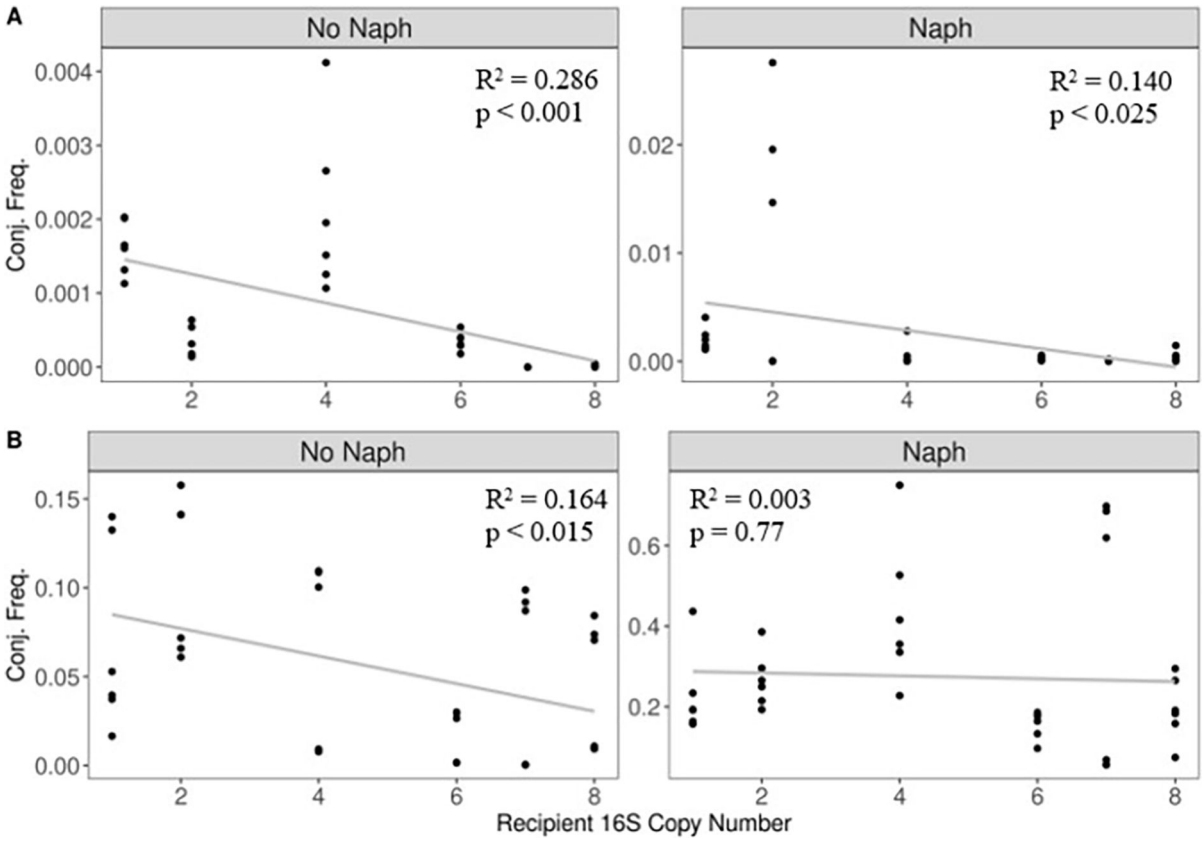

Fig. 2.

Regressions of conjugation frequency vs. 16S rRNA copy number of the recipient for (A) pNL1 harbored in F199 and (B) NAH7 harbored in G7. R^2 values and *p*-values are reported for each regression which are separated by whether naphthalene was present.

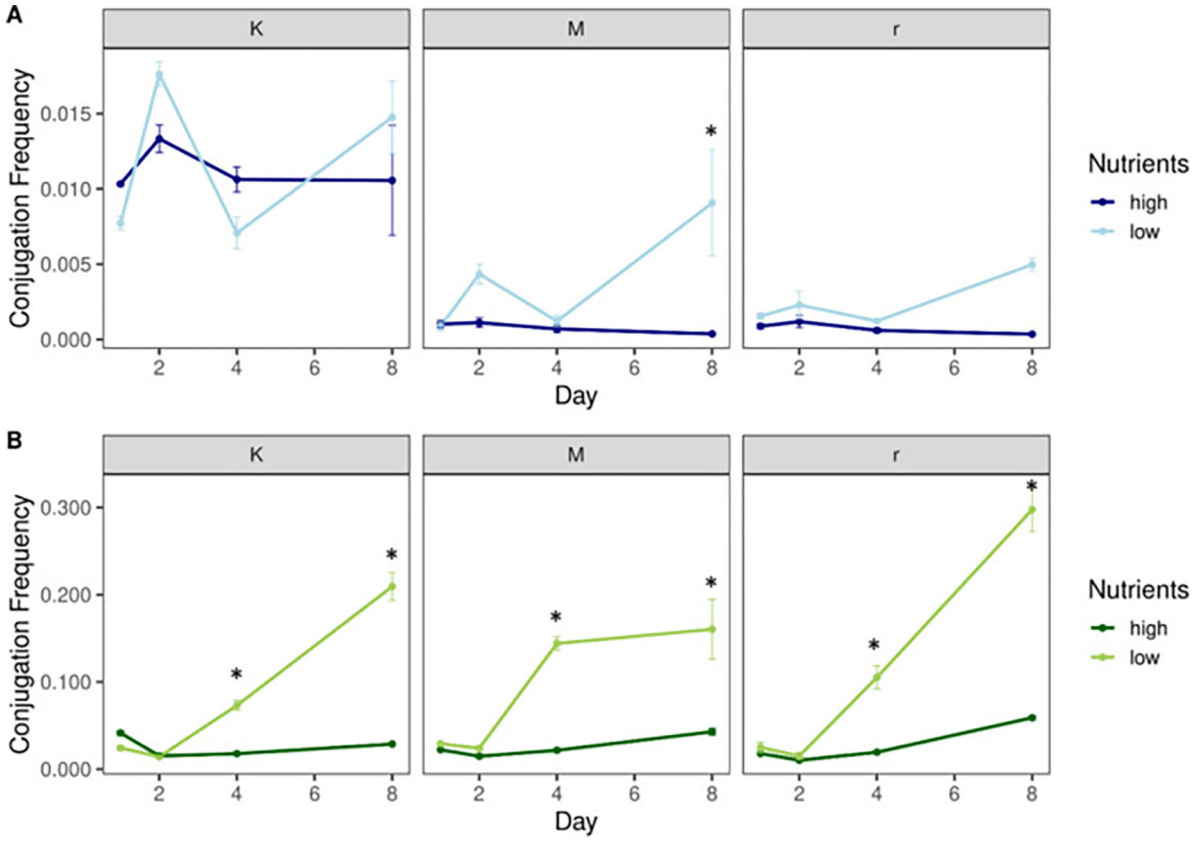

Fig. 3.

Conjugation frequency of (A) pNL1 harbored in F199 and (B) NAH7 harbored in G7 in 1:2 conjugation assays colored by high or low nutrient levels. Stars indicate significant difference in conjugation frequency between high and low nutrients per day ($p < 0.05$). "K" represents the K community consisting of *S. lindanitolerans* and *C. vibrioides*; "M" represents the mixed community consisting of *S. lindanitolerans* and *E. cloacae*; "r" represents the r community consisting of *S. marcescens* and *E. cloacae*. Error bars represent standard error ($n = 3$).

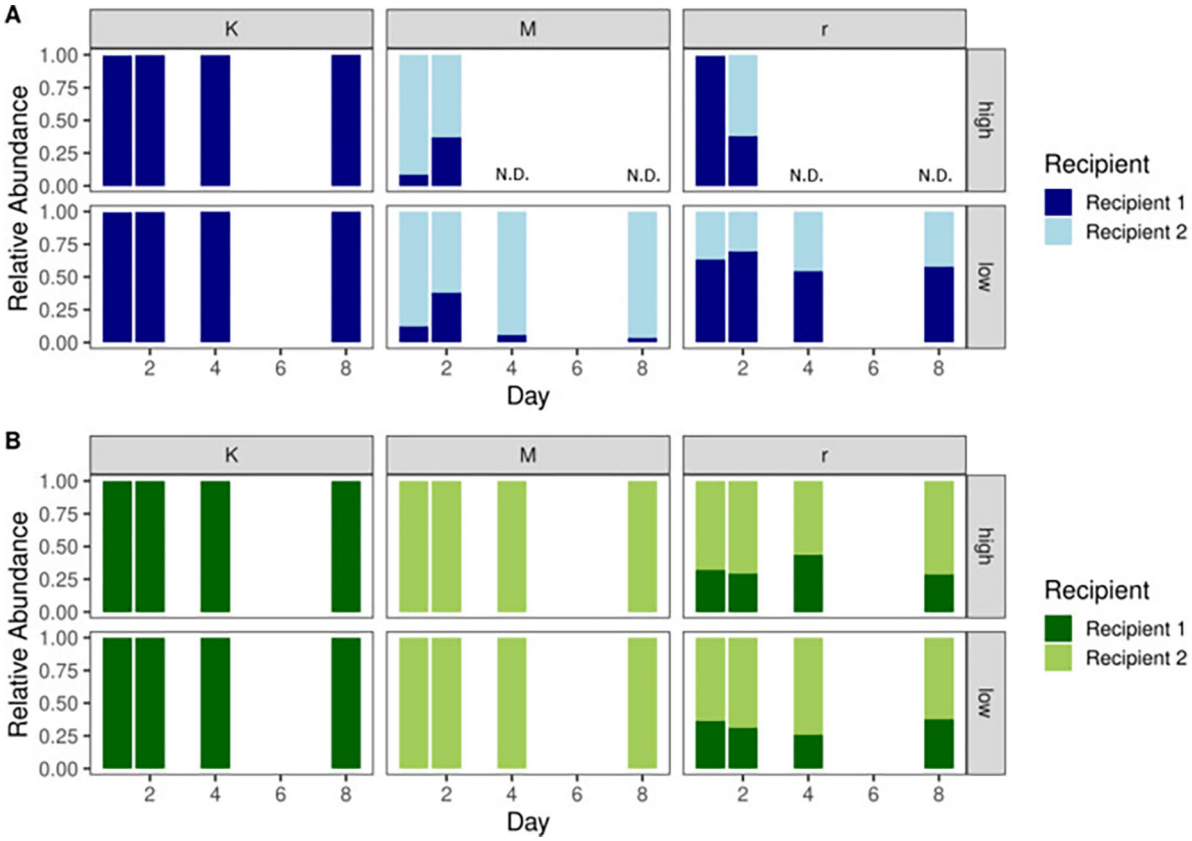

Fig. 4.

Relative abundances of transconjugants in 1:2 synthetic communities at high and low nutrients for (A) pNL1 harbored in F199 and (B) NAH7 harbored in G7. Recipient 1 has the lower 16S copy number and Recipient 2 has the higher 16S copy number. For K community: Recipient 1 is *S. lindanitolerans* and Recipient 2 is *C. vibrioides*. For M community: Recipient 1 is *S. lindanitolerans* and Recipient 2 is *E. cloacae*. For r community: Recipient 1 is *S. marcescens* and Recipient 2 is *E. cloacae*. "N.D." indicates no transconjugants detected.

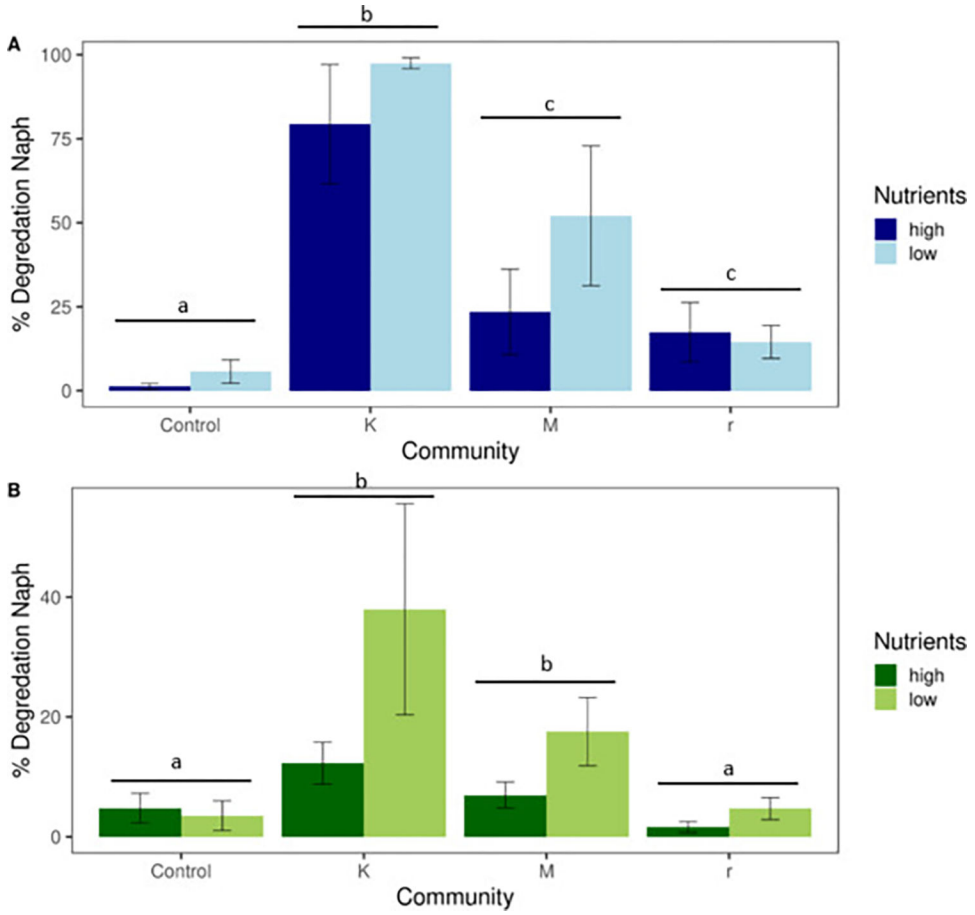

Fig. 5.

Percent change in naphthalene for the synthetic communities for (A) pNL1 harbored in F199 and (B) NAH7 harbored in G7. Letters above bars indicate populations that are significantly ($p < 0.05$) or nearly-significantly ($p < 0.09$) different from one another. "K" represents the K community consisting of *S. lindanitolerans* and *C. vibrioides*; "M" represents the mixed community consisting of *S. lindanitolerans* and *E. cloacae*; "r" represents the r community consisting of *S. marcescens* and *E. cloacae*. Error bars represent standard error ($n = 3$).

Table 1

Bacterial strains and plasmids used in this study. Relevant properties such as the 16S rRNA copy number and fluorescent protein genes are reported.

Strain or Plasmid	Relevant Properties	Reference
Pseudomonas putida G7	donor harboring NAH7; 16S copy number: 7	ATCC 17485
Novosphingobium aromaticivorans F199	donor harboring pNL1; 16S copy number: 3	DSM 12444
Sphingopyxis lindanitolerans	recipient; 16S copy number: 1	ATCC TSD-188
Caulobacter vibrioides	recipient; 16S copy number: 2	ATCC 19089
Cupriavidus metallidurans CH34	recipient; 16S copy number: 4	ATCC 43123
Acinetobacter venetianus	recipient; 16S copy number: 6	ATCC 31012
Serratia marcescens	recipient; 16S copy number: 7	ATCC 13880
Enterobacter cloacae	recipient; 16S copy number: 8	ATCC 13047
NAH7	degrades naphthalene, ohenanthrene, anthracene	Sota et al. 2006
pNL1	degrades naphthalene, fluorene	Romine et al. 1999
JMN120	*N. aromaticivorans* F199; mScarlet-I, GFPmut2, kan^R	This work
PVP102	*P. putida* G7; mScarlet-I, mVenus, kan^R, cam^R	This work

Table 2

Primers used to validate successful insertion of fluorescent protein gene constructs.

Insertion Target	Forward Primer	Reverse Primer
pNL1	CCCTTCCGCGTTCCACTCG	CCATACGGCGGCGAGTTGC
NAH7	ATGCGCCGAGCTTCTTTTTCAAT	CTTTCTCAGGGCCGTGTTGGG
F199 chromosome	TGCCGATCACCTACCTCAAG	AGAAGACTGGTGTGATCGGC
G7 chromosome	GCTGGCGCCGATCCTTTACAC	CTCACTTCGGCCGTCTTTATCACA

Table 3

Primers and probes used in qPCR assays.

Primer/Probe	Sequence
V3 Forward Primer	AKGAATTGACGGGGRCCY
V3 Reverse Primer	TCACRACACGAGCTGACG
S. lindanitolerans probe	CAGAACCTTACCAGCGTTTGACATCCTTG
C. vibrioides probe	CTTACCACCTTTTGACATGCCTGGACCGC
S. marcescens probe	CTTTACCAGAGATGGATTGGTGCCTTCGG
E. cloacae probe	CCACAGAACTTTCCAGAGATGGATTGGTG