

Measuring growth of a phenanthrene-degrading bacterial inoculum in soil with a quantitative competitive polymerase chain reaction method

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Abstract

We measured growth of a phenanthrene-degrading bacterium, *Arthrobacter*, strain RP17, in Forbes soil, amended with 500 $\mu\text{g g}^{-1}$ phenanthrene using a quantitative competitive polymerase chain reaction method. The inoculum, which was not indigenous to Forbes soil, grew from 5.55×10^5 colony forming units (cfu) g^{-1} to 1.97×10^7 cfu g^{-1} within 100 h after the cells were added to the soil. Maximum population density was reached before the highest degradation rate was observed 150 h after the cells were added to soil. Population density remained stable even after 56% of the phenanthrene had mineralized. This study is one of the few documented examples of growth by a non-indigenous bacterium in a non-sterile soil amended with a pollutant. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Quantitative polymerase chain reaction; Phenanthrene degradation; Bioaugmentation; Bioremediation

1. Introduction

Quantification of bacterial populations in soil is difficult due to the challenges of isolating and identifying a specific population within a diverse microbial community. Specific bacterial populations are not easily separated from the rest of the microbial community through traditional culturing techniques. Many bacterial species have not yet been cultured and even if the population of interest can be cultured it is difficult to identify its members among all the other bacterial strains cultured from soil.

One approach to measuring the abundance of a specific bacterial population is to determine the concentration of 16S ribosomal genes unique to that population [1–3]. Using the polymerase chain reaction (PCR), it is possible to detect very low concentrations of 16S ribosomal genes in soil and therefore to measure low population densities in soil. Quantification of bacterial populations with a standard PCR procedure, however, is inappropriate because the amplification efficiencies vary between different envi-

ronmental samples or template concentrations [4–6]. Biases such as variations in amplification efficiencies or product-generated plateaus due to consumption of necessary reagents can be avoided by using a quantitative competitive PCR (QC-PCR) protocol [7,8]. A competitive template, with the same amplification efficiency as the target template, is included in the PCR reaction. To achieve similarity in amplification efficiency, the competitive template must contain identical primer sites and be of similar size to the target template. A linear relationship between the initial target template concentration and the QC-PCR measurements, with a slope of one, indicates that the amplification efficiencies of the competitive and target templates are equivalent [9,10]. The QC-PCR approach has been used successfully to quantify microbial populations in soil [2,3,11,12].

Though bioaugmentation, the addition of pollutant-degrading microorganisms, has proven successful for remediation of polycyclic aromatic hydrocarbons (PAHs) in soil [13–18], there are numerous cases where this strategy fails [19–21]. Analysis of such failures is often hindered because densities of pollutant-degrading organisms were not measured. Obtaining this measurement through traditional culturing techniques requires a culturing strategy which isolates only the inoculum. Such a strategy is not

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often available because PAH degradation is not limited to a narrow taxonomic group of microorganisms with specific culturing requirements. To distinguish the reason for bioaugmentation failure among the many possible mechanisms, including predation, salinity, extreme pH, lack of nutrients or low substrate availability, population densities must be quantified after inoculation. If tools to quantify the death or growth of a pollutant-degrading inoculum in a non-sterile soil were easily accessible to environmental engineers, design of bioaugmentation strategies may be improved.

The purpose of this study was to use QC-PCR to quantify the population of a phenanthrene-degrading bacterium, *Arthrobacter polychromogenes* strain RP17, in soil amended with 500 $\mu\text{g g}^{-1}$ phenanthrene. The growth of the RP17 population was subsequently related to the kinetics of phenanthrene mineralization.

2. Materials and methods

2.1. Identification of a primer specific for strain RP17

A. polychromogenes strain RP17 was isolated from Rindge soil through enrichment on mineral media containing phenanthrene as a sole carbon source. The Rindge soil series consists of poorly drained, highly organic and acidic soils that are formed in the Sacramento-San Joaquin Delta marshes. For enrichment, a sample was taken, with a shovel, from the surface layer. RP17 can use phenanthrene as its sole carbon and energy source and mineralizes the compound as demonstrated in pure culture studies with ^{14}C -labeled phenanthrene [22]. A 1000-bp segment of the 16S ribosomal gene of RP17 was sequenced using the primers 357F and 1390R and submitted to GenBank (accession number AY005127)(Table 1). Three different primers were designed for the fifth hypervariable region of the small subunit gene, of which one, RP17-988R, was selected because it did not produce a PCR product from DNA of Forbes soil that was not inoculated with RP17, while DNA from inoculated soils did produce the expected PCR fragment (Fig. 2).

2.2. Construction of competitive template

A competitive template containing the 357F and RP17-

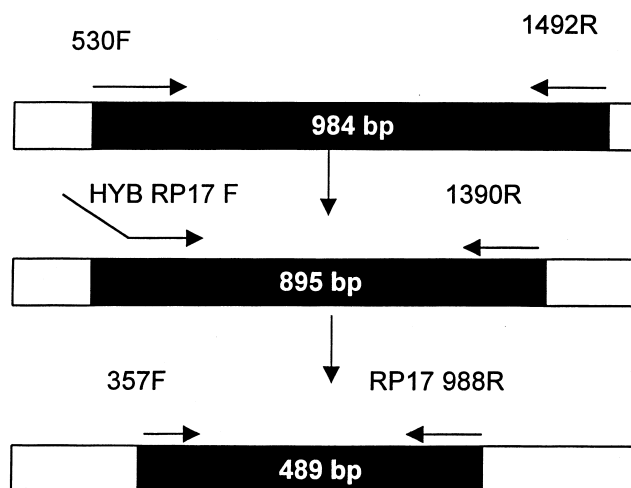


Fig. 1. Diagram of the strategy used to construct the competitive template. A 984-bp fragment was first amplified from strain RP17's genomic DNA. Subsequently, this PCR product was used to amplify a 895-bp fragment which contained the 357F primer site but not the sequence between base pairs 377 and 540 of RP17's 16S rDNA gene. The second PCR product was used as a competitive template and yielded a 489-bp product when used with primers 357F and RP17-988R.

988R primer sites, but 163 nucleotides shorter than the RP17 PCR target product, was constructed from the RP17 16S ribosomal gene using the PCR reaction (Fig. 1) [23]. Briefly, the sequence between base pairs 530 and 1492, which was obtained from a previous PCR reaction with total RP17 genomic DNA, was used in a PCR reaction with the primers 1390R and HYB-RP17, a 45-bp hybrid primer. HYB-RP17 contains the sequences corresponding to base pairs 350–377 and 540–558 of the 16S ribosomal gene of RP17. The resulting PCR product consisted of the sequence from base pairs 540 through 1390 with the sequence from base pair 350 through 377 attached at the 5' end. This PCR product was used as the competitive template.

2.3. Standard curves

To test if the competitive template could be used to quantify 16S ribosomal sequences, two different standard curves were constructed. First, a standard curve using RP17 genomic DNA ranging in concentration from 876 to 8.76 μg and 525.5 μg of competitor DNA was con-

Table 1
Primer sequences used in this study

Primer name	Primer sequence	Specificity
530F	5'-GTG CCA GCA GCC GCG GTA A-3'	Universal
1492R	5'-TAC GGY TAC CTT GTT ACG ACT T-3'	Eubacterial
HYB RP17F	5'-GGC CCA GAC TCC TAC GGG AGG CAG CAG GGC GCA AGC GTT ATC CGG-3'	Hybrid primer for RP17
1390R	5'-GAC GGG CGG TGT GTA CAA-3'	Universal
357F	5'-CTC CTA CGG GAG GCA GCA G-3'	Universal
RP17-988R	5'-GGC ACC TGT TTC CAG GTA TTA-3'	Selected <i>Arthrobacter</i> sp.

structured. PCR reactions contained 1×PCR buffer (Promega, Madison, WI, USA), 1.75 mM MgCl₂, 80 μM dNTPs, 2.5 U of Taq polymerase (Promega, Madison, WI, USA) and 50 nmol of the universal primer 357F and RP17-988R in a total volume of 50 μl (Table 1). The cycling program used was: 3 min at 94°C, followed by 27 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, and finally an extension of 7 min at 72°C. The resulting PCR reaction was separated on a 1% agarose, 1×Tris acetate EDTA, gel and stained with ethidium bromide. Intensities of the target and competitor product bands were measured using a digital camera (Biophotonics, Ann Arbor, MI, USA) and GPTools v 3.0 quantification software (Biophotonics, Ann Arbor, MI, USA). The ratio of the intensity of the target band to the intensity of the competitor band was plotted against the concentration of RP17 genomic DNA. The second standard curve tested the relationship between the competitive PCR results and the number of RP17 cells added to soil. DNA was extracted from Forbes soil immediately after the addition of an inoculum, consisting of RP17 cells ranging in concentration from 3.6×10^7 to 3.6×10^9 colony forming units (cfu) g⁻¹ soil. The protocol to extract nucleic acids from soil was identical to that described previously [11] except that the DNA was cleaned after the extraction with a silica-based mini column (Promega, Madison, WI, USA) and a 100 000-MW microcon filter (Millipore, Bedford, MA, USA). The DNA was quantified and checked for purity using a spectrophotometer (Perkin Elmer, Norwalk, CT, USA). RP17 16S rDNA was quantified in these extracts using competitive PCR reactions as described above.

2.4. Measuring phenanthrene mineralization in soil

Experiments were conducted in pint-sized mason jars containing 20 g of dry weight Forbes soil (described in [24]) at a moisture content of 26.3% or -0.0222 MPa. In this study, the soil in each microcosm was spiked with 10 mg of unlabeled phenanthrene and 45 μCi of ¹⁴C-labeled phenanthrene (Sigma, St. Louis, MO, USA, >98% purity, specific activity of 59 mCi mmol⁻¹). The added solution consisted of 1.35% [¹⁴C] over [¹²C]-phenanthrene. The phenanthrene was delivered in 100 μl methylene chloride and mixed into the soil by hand with a spatula for 2 min. A small vial containing 1 ml of 0.5 N sodium hydroxide was enclosed inside the mason jar to trap CO₂ released from the soil due to respiration. The base containing ¹⁴C-labeled sodium carbonate was retrieved from the trap at regular intervals. The jar was opened during sampling to ensure that phenanthrene mineralization was not impeded by a lack of oxygen. The base trap was rinsed once with 0.5 ml of 0.5 N sodium hydroxide, the rinse was added to the first sample in a 7-ml vial, and the combined sample was counted in a liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA, USA).

2.5. Inoculating soil with *A. polychromogenes* strain RP17

Soil was inoculated with 5.55×10^5 cfu g⁻¹ soil of strain RP17 24 h after the phenanthrene was added to the soil. The inoculum was cultured in inorganic salts medium (3.47 g l⁻¹ KH₂PO₄, 4.27 g l⁻¹ K₂HPO₄, 1.23 g l⁻¹ (NH₄)₂SO₄, 0.46 g l⁻¹ MgSO₄, 17.6 mg l⁻¹ CaCl₂, 1 mg l⁻¹ FeSO₄, 3 mg l⁻¹ H₃BO₃, 2 mg l⁻¹ CoCl₂, 1 mg l⁻¹ ZnSO₄, 0.3 mg l⁻¹ MnCl₂, 0.3 mg l⁻¹ Na₂MoO₄, 0.2 mg l⁻¹ NiCl₂, 0.1 mg l⁻¹ CuCl₂) supplemented with trace organic sources (250 ng l⁻¹ starch, 250 ng l⁻¹ peptone and 250 ng l⁻¹ yeast extract) and amended with 10 mg of phenanthrene as the main carbon source. After the phenanthrene was removed from the culture through three successive washes, the culture was resuspended in phospho-saline buffer (50 mM phosphates, 0.85% NaCl, pH 6.9). One hundred μl of this suspension, containing 1.11×10^7 cfu bacteria, as determined by serial dilution plate counts, were added to the microcosm and the inoculum was stirred into the soil by hand with a spatula for 2 min.

2.6. Quantification of RP17 population in soil

The population density of RP17 in soil amended with 500 μg g⁻¹ soil was measured using the QC-PCR reaction. Twenty grams of dry weight Forbes soil was added to 16 mason jars and spiked with 500 μg g⁻¹ phenanthrene. Twenty four hours later, the soil was inoculated with 5.55×10^5 cells RP17 per gram soil in 100 μl of phospho-saline buffer and stirred into the soil by hand with a spatula for 2 min. DNA was extracted from the soils in two of the jars at each of the eight following times: 1, 39.5, 89, 136, 189, 233, 285.5 and 329.5 h after the cells were added to the soil, using the DNA extraction protocol described above. QC-PCR reactions were performed using 52.5 fg of competitor fragment.

3. Results

Submission of a 1000-bp fragment (GenBank accession number AY005127) to the ribosomal database [25] revealed that strain RP17 is most closely related to *A. polychromogenes*. According to the ribosomal database, the primer designed to be specific for strain RP17, RP17-988R, recognized selected members of the *Arthrobacter agilis* subgroup, including *A. psychrolactophilus*, *A. ureafaciens*, *A. nicotinovorans*, *A. histidinovorans*, *A. ilicis* and *A. aurescens*. A PCR reaction with the RP17-988R primer in combination with the primer 357F (Table 1) resulted only in a product if RP17 cells were added to Forbes soil (Fig. 2). At least 10⁴ cells had to be added to the soil before a product could be detected. The PCR protocol, which used a limited number of amplification cycles, was not optimized for sensitivity because this study con-

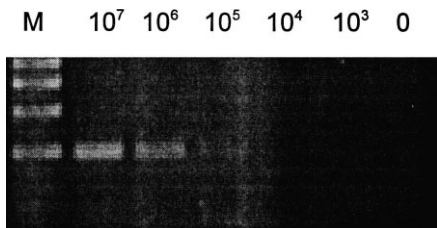


Fig. 2. Products from PCR reactions using DNA that was extracted from soil immediately after inoculation with varying densities of *Arthrobacter*, strain RP17. Primers used were 357F and RP17-988R. M=DNA marker, the numbers refer to cfu RP17 cells g^{-1} dry soil.

cerned population densities greater than 10^4 cells per gram soil.

We successfully used the end deletion protocol to construct a competitive template for use in quantification of the 16S ribosomal gene of RP17 in soil [23]. The competitive template contained the universal 357F and the RP17-988R primer sites yet missed the sequence between base pairs 377 and 540 (Fig. 1). The 489-bp PCR product resulting from the competitive template was 163 bp smaller than the 652-bp RP17 16S rDNA PCR product. We did not observe the formation of any other products, such as heteroduplexes, besides the target and competitor products (Fig. 3) [11].

The first standard curve resulted in a linear relationship between QC-PCR measurements and RP17 genomic DNA concentrations with a slope of 1.06 (Fig. 3). A slope of one indicates that the competitor and target fragments are

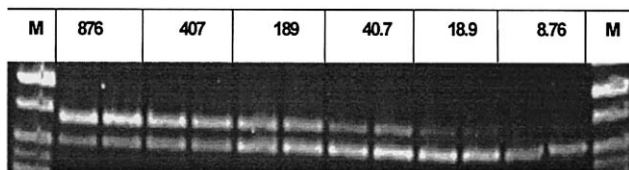
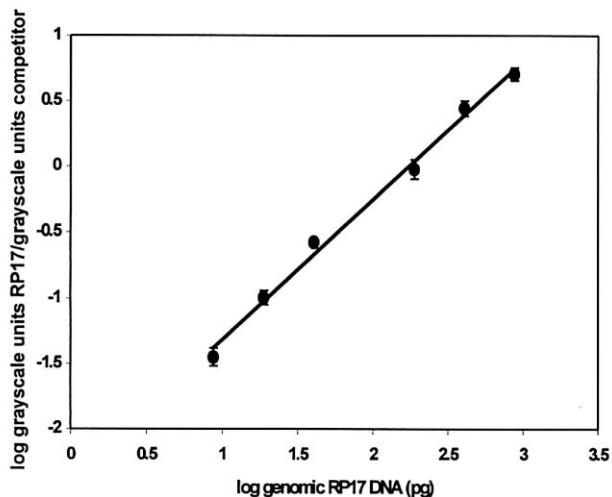


Fig. 3. Standard curve showing the relationship between QC-PCR results and the amounts of RP17 genomic DNA that were added to the reactions. The quantity of genomic DNA used to construct this curve ranged from 8.76 pg to 876 pg and 525.5 fg of competitive template was used in all reactions.

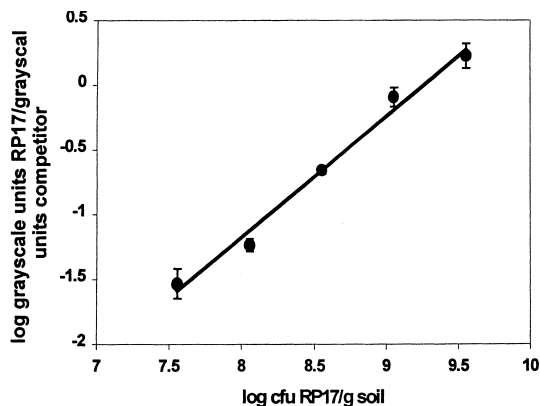


Fig. 4. Standard curve showing the relationship between QC-PCR results and the density of *Arthrobacter* strain RP17 cells added to soil. The cell density of the inocula ranged from 3.6×10^7 to 3.6×10^9 cfu g^{-1} .

amplified with equal efficiency and is a prerequisite for using a competitive template in quantitative PCR. Standard curves, such as this one, are often used as evidence to support the use of quantitative PCR to accurately assess the absolute concentration of the target sequence. Models of PCR amplification, however, suggest linear standard curves with slopes approximating one, only ensure accurate determination of the relative target sequence concentration because a shift of the curve cannot be detected in real experiments as there is no reference point [9,10]. Though quantification of ethidium bromide-stained DNA in agarose can be complicated by the fact that the intensity of the stain can saturate even at low concentrations of DNA, this appeared not to be a problem in constructing the standard curve. The signal from the stain saturated above approximately 100 ng of DNA (data not shown). It was not necessary to load dilutions of the PCR products on to the gel to ensure that the concentrations of DNA were below this limit because the errors in the DNA concentration estimates were so small that they did not affect the position or shape of the standard curve.

A second standard curve representing the relationship between a range of cell concentrations in soil and QC-PCR measurements using 52.5 fg of competitor template also was linear and had a slope of approximately one (0.93) (Fig. 4). This relationship suggested that quantitative PCR can be used to measure the concentration of RP17 cells in soil despite the variation in DNA extraction efficiency and the purity of the extracts.

The mineralization curve of $500 \mu g g^{-1}$ phenanthrene in soil inoculated with 5.55×10^5 cfu g^{-1} cells of RP17 resembled Monod degradation kinetics involving population growth (Fig. 5) [26]. An initial lag in the mineralization rate was followed by an exponential rise, which reached a maximum after approximately 200 h. The lag phase and the exponential increase in phenanthrene mineralization were likely due to growth of the population of RP17 and not induction because the inoculum was cultured on phenanthrene prior to being added to the soil. Subse-

quently, the mineralization rate declined in a bi-phasic exponential pattern, indicating substrate availability was influenced by both degradation and desorption. Only 3% of phenanthrene spiked into Forbes soil that was not inoculated with strain RP17 mineralized (Fig. 5).

The population density of RP17 increased from 5.55×10^5 cfu g⁻¹ soil to 1.97×10^7 cfu g⁻¹ soil by 89 h after the bacteria were added to soil (Fig. 6). The population reached a maximum just before the highest degradation rate was observed, indicating that degradation activity per cell was still increasing even though growth had ceased. Throughout the remainder of the experiment, the population remained stable between 2 and 4×10^7 cfu g⁻¹ soil. Deviation between these values may be entirely due to variance inherent in the method, and does not necessarily represent fluctuations in the population of RP17. Stabilization of the population density while degradation of phenanthrene was declining suggests the cells were going dormant, or were maintaining themselves on an alternative carbon and energy source.

4. Discussion

Quantitative PCR is a commonly used method for measuring the concentration of a DNA sequence. Since the first reports on quantification of cytokine mRNA and DNA in MLA-144 cells, a primate T-cell line [27], the method has been used in hundreds of studies. Debate continues on the use of QC-PCR to determine absolute quantities of DNA in a sample [9,10], because a standard curve with a slope of one, though a prerequisite, is not a guarantee that the amplification efficiencies of the competitor and target templates are equivalent. Constant change in amplification efficiencies of the target and competitor templates may result in a standard curve with a slope of one but also will induce a parallel shift in the standard curve so that absolute quantification of a DNA sequence is in-

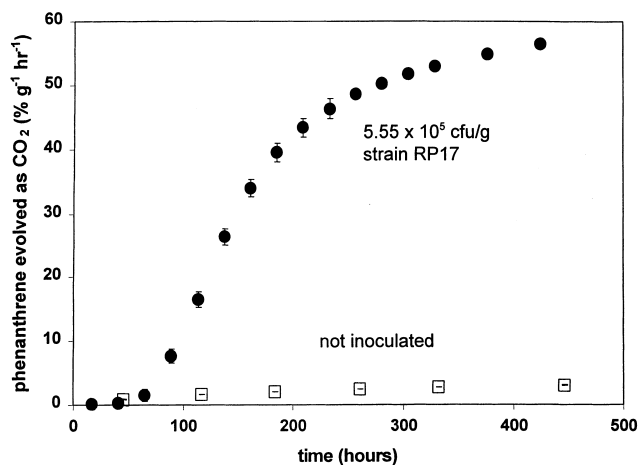


Fig. 5. Cumulative phenanthrene degradation of soil spiked with $500 \mu\text{g g}^{-1}$ phenanthrene and either inoculated with 5.55×10^5 cfu g⁻¹ cells of *Arthrobacter* strain RP17 (●) or not inoculated (□).

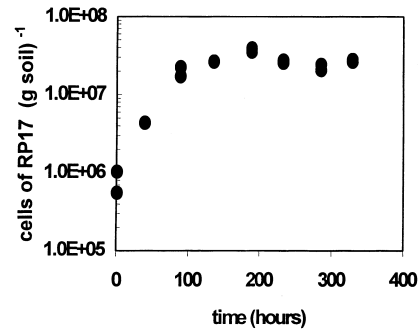


Fig. 6. Change in the population density of *Arthrobacter*, strain RP17, as measured with QC-PCR, in soil spiked with $500 \mu\text{g g}^{-1}$ phenanthrene and inoculated with 5.55×10^5 cfu g⁻¹.

accurate. Even in this case QC-PCR is suitable for assessing relative changes in the concentration of a specific DNA sequence.

QC-PCR recently has been used in several studies to measure the abundance or survival of a bacterium or fungus in soil. For instance, the method was used in measuring the cell density of *Sphingomonas chlorophenolica* in pentachlorophenol-contaminated soil [3]. The bacterium could be detected down to a density of 10^3 cfu g⁻¹. A genetically modified fungus, *Trichoderma virens* (GvT6), was enumerated, down to a level of 10^2 cfu, in soil with QC-PCR [12]. The abundance in soil of the 16S ribosomal gene of the uncultured bacterial strain EA25, a relative of members in the *Planctomyces* and *Chlamydia* genera, was estimated to be 2.17×10^8 copies per gram soil using QC-PCR [11]. Also, in samples taken from soil hot spots, the number of *Pseudomonas* bacteria cultured on Gould's S1 media corresponded well to the concentration of *Pseudomonas* DNA detected with a QC-PCR method [28]. Our results demonstrate that QC-PCR successfully documented the growth of RP17 in non-sterile Forbes soil amended with 0.5 mg kg^{-1} phenanthrene.

QC-PCR is particularly well suited for monitoring the population density of an inoculated organism. Relative differences in QC-PCR measurements can be related to absolute changes in the population density because initial inocula densities are known. Furthermore, in a negative control, DNA from uninoculated soil can be used in a PCR reaction, to ensure that the inoculum specific primer only recognizes the inoculum and not populations indigenous to the soil. This negative control is unavailable when studying indigenous microbial populations. Finally, growth measurements are not invalidated by substantial variance inherent in the QC-PCR method because the increases in the 16S ribosomal gene concentration of a growing inoculum are large. In our study, for instance, the population increased more than 35-fold.

Growth of inoculated bacteria has been demonstrated in sterile soils [19,29,30]. Sterile soils contain large amounts of available carbon, in the form of dead biomass, and bacteria inoculated into sterile soils are not subjected to predation and competition pressures. Therefore, it is diffi-

cult to attribute the growth of inocula in experiments using sterile soils to the degradation of the pollutant instead of other carbon sources. Extreme caution must be exercised to relate findings made in sterile soil microcosms to potential bioaugmentation success.

This study is among the first to show a pollutant-degrading inoculum can grow in a non-sterile soil. Other studies in which inoculated pollutant-degrading populations were monitored showed declines in population densities. A pentachlorophenol-degrading *Sphingomonas* sp. UG30 population declined in a sandy loam soil, as measured by most probable number PCR from 1.8×10^8 to approximately 2×10^4 cells g^{-1} soil over 50 days [31]. Cell density of *S. chlorophenolica* strain RA2 in Skalborg soil amended with $100 \text{ mg } g^{-1}$ declined, according to QC-PCR results, from 10^8 cfu to 8×10^6 cfu in 42 days [3]. Most probable number counts from soils amended with PCP and immobilized *Rhodococcus chlorophenolicus* PCP-1 cells showed a decline from 1×10^9 to 1×10^6 bacteria g^{-1} soil [1]. Initially, however, the results suggest a slight increase in PCP-mineralizing populations. MPN does not have the ability to distinguish between indigenous PCP-mineralizing populations and strain PCP-1 and therefore it is not possible to draw conclusions about the population dynamics of strain PCP-1. Finally, introduced populations of phenanthrene-mineralizing *Pseudomonas* sp. UG14Gr, containing the green fluorescent protein as a marker, declined from 1×10^9 to approximately 1×10^4 regardless of nitrogen amendment or moisture content [32].

Because introduced bacteria are usually not capable of growth in polluted environments, high inoculum densities (10^8 cfu g^{-1} soil or higher) are required for successful bioaugmentation efforts. For instance, high concentrations of cells were used to accelerate the mineralization of pentachlorophenol in sandy loam, peaty soils [15] and sandy subsoil microcosms [18], 2,4-D in sandy loam microcosms [17] and biphenyl and polychlorinated biphenyl in Altamont soil [13]. In [3], a treatment amended with $30 \text{ mg } g^{-1}$ pentachlorophenol and inoculated with 10^6 cfu *S. chlorophenolica* strain RA2 was included. The population density of this inoculum remained stable, 1.3×10^6 cfu being present after 10^5 days [3]. Because the introduced populations do not survive inoculation only improves the degradation rate temporarily.

Competitive quantitative PCR is an appropriate method to test the ability of a bacterial inoculum to survive in soil. Degradation by the inoculum must be sustained over extended periods of time to successfully remediate aged pollutants because bioavailability is limited by slow desorption rates [33]. Large hydrophobic molecules, such as phenanthrene, diffuse slowly from small inner aggregate pores or organic matter, to become available to microorganisms [34,35]. Therefore, bacteria in soil are often exposed to very low concentrations of phenanthrene. The current practice of isolating contaminant-degrading bacteria through enrichment on high substrate concentrations is

unlikely to yield an inoculum for effective bioaugmentation. Other traits, such as production of surfactants or the ability to attach to surfaces, may be important for remediation success. Instead of microorganisms that degrade pollutants rapidly in pure culture, cultures that can survive in non-sterile soil for extended periods of time should be selected for bioaugmentation trials.

The low initial cell density used in our study may have been important in creating conditions under which RP17 cells could grow. Resources necessary for growth and survival of bacteria in soil are generally limited. These scarce resources may be depleted if the soil is overpopulated due to inoculation with high cell densities. The opportunity to grow may allow the small fraction of the inoculum that is placed in microsites suitable for long term survival to become predominant. Cells that have grown in soil, as opposed to the culturing flask, may have physiological traits, such as extracellular polysaccharides, that promote long term survival in soil. A bioaugmentation strategy should be optimized such that microorganisms inoculated into micro-habitats not suitable for long term survival are prevented from using limited resources. Consequently, in designing remediation strategies that aim to establish a population that is viable for a longer period of time, a low inoculation density might be considered.

Bioaugmentation remains a controversial approach to remediating contaminated soils and may be applicable to only a few pollution problems. Frequently, what appears to be successful bioaugmentation can be attributed to stimulation of biodegradation by nutrients added with the inoculum rather than by the inoculum itself [36]. Often, the bioavailability of the pollutant and not the indigenous, contaminant-degrading population limits degradation rates [19]. Furthermore, placing the inoculum into those microsites of soil, where the majority of the contamination is likely to occur, remains a formidable challenge [21]. Elucidation of the parameters that define the ecological niche that is made available because of soil pollution is presently among the most important research challenges [37]. Identification of the microbial habitat on a microscale is an integral part of this challenge [38]. As our knowledge of the fate of inocula in soil continues to grow, bioaugmentation efforts will increasingly become more effective.

In this study, we documented growth of strain RP17 in non-sterile, sieved soils with freshly added phenanthrene. Though our findings did not resolve the difficulties of using bioaugmentation to remediate pollution out in the field, we have used a promising approach for quantifying a bacterial inoculum after it has been added to soil. Applying QC-PCR to screen isolates for their ability to thrive in polluted soil can help in the design of improved bioaugmentation strategies.

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