Competitive Fitness of Isolates Enriched on Phenanthrene Sorbed to Model Phases

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We evaluated the competitive fitness of three phenanthrene-mineralizing isolates that were provided with nonsorbed or sorbed phenanthrene in competition experiments. One isolate had a clear advantage when presented with Amberlite IRC-50-sorbed phenanthrene, yet none were favored when presented with nonsorbed or Amberlite XAD-7-sorbed phenanthrene. Our results indicate that contaminant availability alone does not determine the isolates’ competitive fitness.

For some time, it has been recognized that the sorption of hydrophobic organic contaminants strongly influences their biodegradation. Several studies have revealed that contaminant biodegradation rates can be restricted by the rate of desorption from solid phases (4, 15, 19), yet there is an increasing number of examples in which contaminant mineralization rates exceed desorption (3, 6, 11, 16–18). Mechanistically, this observation has been attributed to the differential abilities of microorganisms to access bound contaminants (10, 16, 17). One example contrasts two naphthalene-degrading organisms that differ in their ability to access sorbed naphthalene (10). Naphthalene mineralization kinetics indicated that Alcaligenes sp. strain NP-Alk is limited by contaminant desorption whereas Pseudomonas putida ATCC 17484 promotes naphthalene desorption. More recently, Tang et al. (17) set out to specifically isolate microorganisms capable of growth on sorbed or nonsorbed phenanthrene. Their strategy yielded two isolates that mineralized nonsorbed phenanthrene; however, only the isolate enriched on sorbed phenanthrene could mineralize sorbed phenanthrene. Clearly, contaminant-degrading microorganisms can differ in their ability to access bound substrates.

In a prior study, enrichments were carried out to isolate bacteria capable of growth on phenanthrene under various bioavailability conditions (9). It was found that a single soil inoculum yielded isolates differing in their ability to degrade sorbed phenanthrene. Molecular analysis of the enrichments revealed that distinct microbial populations were selectively enriched under conditions of different phenanthrene bioavailability (8). Specifically, several Burkholderia spp. were enriched when phenanthrene was provided nonsorbed and availability was not limiting (8, 9). In enrichments where phenanthrene was provided sorbed to a carboxylic acid-based cation-exchange resin that reduced phenanthrene bioavailability (Amberlite IRC-50), different Burkholderia spp. were selected (8). Presorbing phenanthrene to a polycrylate resin that reduced phenanthrene bioavailability further (Biobeads SM7) yielded Mycobacterium-dominated enrichments. These findings suggest that uniquely adapted microbial populations can be selectively enriched from the same inoculum simply by varying phenanthrene bioavailability. We conducted competition experiments to test the hypothesis that these populations were selected under specific conditions because of their specialization, resulting in increased fitness.

The model organic phases Amberlite IRC-50 and Amberlite XAD-7 (Rohm and Haas, Philadelphia, PA) are porous resins with large surface areas available for phenanthrene sorption. Although Grosser et al. (9) used Biobeads SM7 (Rohm and Haas, Philadelphia, PA), we used the chemically identical XAD-7 resin (Rohm and Haas) due to the discontinued production of SM7. Using a protocol detailed by Grosser et al. (9), solid phases were preloaded with [9-14C]phenanthrene (purity, >98%; specific activity, 59.5 mCi mmol⁻¹) and unlabeled phenanthrene to yield 10,000 dpm g⁻¹ phenanthrene (Sigma Chemical, St. Louis, MO). To measure phenanthrene loading, 0.5 g of each resin, four replicates each, was combusted in a biological oxidizer (model OX-300; R.J. Harvey Instrument, Hillsdale, NJ). Combustion and scintillation counting (Packard Tricarb CA2200; Packard Instruments, Meriden, CT) of the solid phases revealed phenanthrene loading of 3.1 mg g⁻¹ IRC-50 and 7.0 mg g⁻¹ XAD-7.

The microorganisms used for the competition experiments were isolated based on their ability to grow on sorbed or nonsorbed phenanthrene as a sole source of carbon and energy (8, 9). Burkholderia sp. strain S4.11 (GenBank accession no. AF247495) was enriched in treatments containing phenanthrene alone and in treatments with a nonsorptive solid phase that did not influence bioavailability (sand). Burkholderia sp. strain A6.33GD (GenBank accession no. AF247492) was isolated from treatments containing IRC-50-sorbed phenanthrene. Mycobacterium sp. strain SM7.6.1 (GenBank accession no. AF247497) was isolated on phenanthrene sorbed to Biobeads SM7. To initiate competition experiments, colonies from phenanthrene-supplemented yeast extract-peptone-glucose plates (5, 12) were resuspended in a modified soil solution equivalent medium (1, 5) to an optical density at 500 nm of 0.01. Based on direct cell counts, this was equivalent to 1 × 10⁸ to 2 × 10⁸ cells ml⁻¹. Each isolate was added to 125-ml Erlemeyer flasks containing 50 ml soil solution equivalent me-

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dium, yielding an initial population density of \(1 \times 10^5\) to \(2 \times 10^6\) cells ml\(^{-1}\). This inoculum level did not yield PCR products when the molecular methods described below were used, thus ensuring that any PCR products obtained during competition experiments were due to microbial growth.

Based on the amount of phenanthrene sorbed, flasks containing sorbed phenanthrene received 0.81 g IRC-50 or 0.36 g XAD-7. For treatments with no solid phase, labeled and unlabeled phenanthrene (2.5 mg) dissolved in acetone were added directly to sterile flasks. Flasks were covered with rubber stoppers with a suspended cup containing 0.25 ml of 0.5 N NaOH to trap \(^{14}\)CO\(_2\). Base traps were exchanged regularly and replaced with fresh NaOH. The removed NaOH was added to 1 ml ScintiSafe Plus 50% (Fisher Scientific, Fair Lawn, NJ) for scintillation counting. The isolates’ ability to grow alone on sorbed and nonsorbed phenanthrene was tested in parallel with competition experiments in identical manners.

At regular intervals, well-mixed 1-ml subsamples, including solid phases, were removed and immediately frozen at \(-20^\circ\)C. DNA was extracted from 0.5-ml subsamples using a FastDNA SPIN kit for soil (BIO 101, Vista, CA) with shaking in a Savant FastPrep instrument (Savant, Farmingdale, NY). The extraction procedure was optimized to compensate for differences in cell lysis efficiency among \(\textit{Burkholderia}\) spp. and the \(\textit{Mycobacterium}\) sp. (2). A 1:1:1 mixture of the three isolates (\(1 \times 10^8\) to \(2 \times 10^8\) cells each) was prepared and subjected to multiple bead beating cycles. Tubes were shaken at maximum speed, 6.5 m s\(^{-1}\), for 45 s for one to four cycles. DNA yield and shearing were evaluated by UV spectrophotometry and electrophoresis in a 1% SeaKem GTG agarose gel (FMC BioProducts, Rockland, ME) stained with ethidium bromide. Two bead beating cycles resulted in an optimal balance between DNA yield and minimal shearing (data not shown).

A portion of the 16S rRNA gene was amplified from DNA extracts by using primers 1070f and 1392r-GC (7). PCR mixtures (50 \(\mu\)l) contained 5 \(\mu\)l of template DNA, 50 mM KCl, 10

**FIG. 1.** Denaturing gradient gel with PCR products from \(\textit{Burkholderia}\) sp. strain A6.33GD, \(\textit{Burkholderia}\) sp. strain S4.11, \(\textit{Mycobacterium}\) sp. strain SM7.6.1, and a 1:1:1 mixture of all three isolates. The pattern generated from the mixture in lane 4 includes two heteroduplexes (HD) formed from the \(\textit{Burkholderia}\) spp.

**FIG. 2.** Phenanthrene mineralization kinetics (A) and molecular analysis (B) of competition experiments without solid phases (nonsorbed phenanthrene). The PCR-DGGE results for biomass samples taken at times B\(_1\) (lanes 1 and 3) and B\(_2\) (lanes 2 and 4) from replicate competitions (Mix A and Mix B) are shown in panel B. Error bars represent standard errors for duplicate treatments. HD, heteroduplex.
mM Tris HCl (pH 8.3), 1.5 mM MgCl$_2$, 200 𝜇M deoxynucleoside triphosphates, 0.5 𝜇M of each primer, and 1.25 U Taq polymerase (Fisher Biotech, Fair Lawn, NJ). The PCR program included a 2-min denaturation step at 94°C, followed by 25 cycles of 94°C, 55°C, and 72°C for 45 s each, and a 7-min final extension. The PCR products and a mass ladder (Gibco BRL, Grand Island, NY) were visualized with a 3% NuSieve 3:1 agarose gel (FMC BioProducts, Rockland, ME) following ethidium bromide staining.

Denaturing gradient gel electrophoresis (DGGE) (13) was used to track microbial populations during competition experiments. The specific methods used were as described previously (5, 7). Gradient gels contained 8% acrylamide with a urea formamide gradient of 40 to 60%. The PCR products (120 ng) were added to wells and electrophoresed at a constant voltage of 80 mV for 12 h. Gels were stained with SYBR green II (Molecular Probes, Eugene, OR) and photographed. When DNA extracts were subjected to PCR-DGGE, bands from each isolate could be clearly distinguished, providing a rapid means of differentiating the phenotypically indistinguishable *Burkholderia* spp. (Fig. 1). The primary DGGE band from the *Mycobacterium* isolate was not as intense as those of the *Burkholderia* strains, likely reflecting the difficulty in lysing mycobacterial cells (2). Two heteroduplexes in the mixture lane (Fig. 1, lane 4) were always observed when both *Burkholderia* strains were present in detectable numbers.

Overall, the rates and extents of phenanthrene mineralization were greatest without solid phase and progressively lower

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**FIG. 3.** Mineralization kinetics (A) and molecular analysis (B) of competition experiments with phenanthrene sorbed to IRC-50 solid phase. The PCR-DGGE results (B) are for biomass samples taken at time $B_1$. Lanes 1 and 2 represent duplicate treatments containing only *Burkholderia* sp. strain A6.33GD, and lanes 3 and 4 represent replicate competitions containing all three isolates. Error bars represent standard errors for duplicate treatments.

**FIG. 4.** Phenanthrene mineralization kinetics (A) and molecular analysis (B) of competition experiments with phenanthrene sorbed to XAD-7 solid phase. The PCR-DGGE results for biomass samples taken at times $B_1$ (lanes 1 and 3) and $B_2$ (lanes 2 and 4) from replicate competitions (Mix A and Mix B) are shown in panel B. Biomass sample $B_2$ was taken at hour 694. Error bars represent standard errors for duplicate treatments. HD, heteroduplex.
with IRC-50- and XAD-7-sorbed phenanthrene (Fig. 2 to 4). Competition experiments using phenanthrene with no solid phase yielded the highest rates of mineralization (Fig. 2A). Phenanthrene mineralization patterns observed with the \textit{Burkholderia} spp. individually mirror those observed in the competition experiment, whereas the incubation containing only the \textit{Mycobacterium} sp. yielded a reduced rate and extent of mineralization. Although all isolates are capable of mineralizing phenanthrene independently, only \textit{Burkholderia} sp. were evident in competition replicates (Fig. 2B). The slower-growing \textit{Mycobacterium} sp. was undetected in competition experiments with nonsorbed phenanthrene.

Competition experiments with IRC-50-sorbed phenanthrene revealed preferential selection of the \textit{Burkholderia} strain enriched on that solid phase. After 280 h of incubation, only the IRC-50-selected \textit{Burkholderia} sp., isolate A6.33GD, mineralized phenanthrene when added alone (Fig. 3A). The mineralization kinetics were similar in the competition experiment after an increased lag period. Growth by isolate A6.33GD exclusively was evident in the mixture (Fig. 3B). Further, no PCR products were detected when isolates S4.11 and SM7.6.1 were inoculated alone (data not shown).

When XAD-7-sorbed phenanthrene was used, a superior competitor did not emerge. All isolates were capable of phenanthrene mineralization, albeit at the lowest rates observed (Fig. 4A). In contrast to our hypothesis, \textit{Mycobacterium} sp. strain SM7.6.1 did not emerge as the predominant phenanthrene-mineralizing microorganism in this competition experiment (Fig. 4B). This was, however, the only situation in which a DGGE band contributed by isolate SM7.6.1 was observed. In one replicate (Mix A), a faint band was observed at the expected position below the band for isolate S4.11 (Fig. 1). Given the nonquantitative nature of PCR and the fact that we do not know how efficient our extraction procedure is, we cannot conclude that isolate SM7.6.1 is less competitive than the \textit{Burkholderia} sp. on XAD-7-sorbed phenanthrene. However, it does appear that the \textit{Mycobacterium} sp. is competitive only in the presence of XAD-7.

We set out to test the hypothesis that soil microbial populations specialized at degrading bound contaminants demonstrate increased fitness over other isolates when placed together in direct competition experiments. Two isolates were unable to mineralize phenanthrene when sorbed to IRC-50 (Fig. 3), yet all isolates could mineralize XAD-7-sorbed phenanthrene (Fig. 4) despite the fact that phenanthrene was more strongly bound to this phase (9). Therefore, if sorption strength is the main determinant of competitive fitness, then all isolates that mineralize XAD-7-sorbed phenanthrene should also be able to mineralize IRC-50-bound phenanthrene, yet this is not what we observed. Our results imply that the surface chemistry of the solid phase influences which organism(s) is capable of phenanthrene mineralization alone and in direct competition experiments. However, we are presently unable to determine whether the ability to mineralize sorbed phenanthrene is due to microbial attachment to solid phases, enhanced desorption through biosurfactant production, or another mechanism(s) (14). Adaptation to different degrees of phenanthrene sorption apparently does not, by itself, govern the competitive fitness of these phenanthrene-degrading isolates.

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REFERENCES


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