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Original article

Transport and survival of GFP-tagged root-colonizing microbes: Implications for rhizodegradation

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Abstract

Many recalcitrant organic contaminants pose unique and vast environmental challenges, potentially addressed through phytoremediation. Using molecular engineering to enhance enzymatic capabilities of root-colonizing microbes, additional contaminants can be targeted and contaminant fate can be altered to promote rhizosphere degradation of contaminants, which is desired among phytoremediation mechanisms. In this paper, rhizosphere bacteria were tagged with the green fluorescent protein genes (*gfp*) in order to monitor colonization, survival, and transport within the root zone and to evaluate the effectiveness of visual identification using GFP. Transport of the *gfp*-tagged root colonizers was observed to a one meter depth against a hydraulic gradient in less than 180 days revealing that plant's roots clearly enhanced movement of the recombinant strains through the rhizosphere although proliferation of the recombinant bacteria was not substantial. Over a 49-day plant growth period survival and colonization by the recombinant bacteria was monitored in soil and on roots, revealing a decreasing trend. Overall, this study showed that enhanced rhizosphere degradation is mechanistically promising, but that the specific plant-microbe pairing studied herein was not ideal. Using GFP for visual identification is not 100% efficient but provides a quick and simple marker to detect tagged microorganisms. Selection of root colonizing organisms to be engineered in enhanced rhizoremediation is a critical step in advancing the technology.

Keywords: Rhizoremediation; Phytoremediation; Rhizosphere; Recombinant bacteria; GFP; GMM

1. Introduction

Phytoremediation is well suited towards compounds that require long remediation timeframes such as compounds tightly bound to soil, as there is minimal energy input required. Phytoremediation is also suited for contaminants that exist over vast plumes or spills, being easily applied over large areas. In phytoremediation applications rhizoremediation, which is degradation of pollutants in the rhizosphere, is desired due to the in-situ contaminant destruction. In this process, plants provide the rhizosphere environment, i.e. immediate root-zone, which is a microenvironment providing selective niches for specific microbes. However, the native root-colonizing flora is not able to degrade all contaminants of interest in many cases. Therefore engineering approaches to

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introduce enhanced degradation capabilities hold great potential to degrade contaminants thought to be recalcitrant.

The rhizosphere provides environmental conditions that are advantageous for bioremediation. Anderson et al. [1] and Liste and Alexander [13] provide fundamental information on the processes involved and summaries of associated underlying studies. Plant roots can provide specific carbon sources that are readily available and degradable, altering and maintaining redox conditions and pH relative to the bulk soil, mobilizing nutrients and greatly altering moisture levels. The survival and proliferation of rhizosphere microorganisms has been shown in many studies targeting biodegradation. Plant roots also provide rhizosphere microorganisms with a large surface area on which to colonize, and survival directly on roots [10,19] and root tips [24] has been observed.

The niche provided to microorganisms by the root has been shown to increase the degradation of organic compounds such as polyaromatic hydrocarbons (PAH), benzoic acids, and benzene, toluene, and xylenes (BTEX) [12,16,21–23]. Degradation of all contaminants is not, however, enhanced. In a comparative study of contaminant fate, no significant effect due to vegetation was observed in some cases, as plants did not effectively increase degradation [2]. In other instances, the mineralization of contaminants was actually decreased [7].

Molecular engineering has the potential to generate recombinant microorganisms capable of degrading recalcitrant organic contaminants and still able to proliferate in the rhizosphere. Previous work has shown that the presence of recombinant microorganisms can significantly increase contaminant removal rates. Crowley et al. [8] demonstrated that a recombinant strain of *Pseudomonas fluorescens* 2–79 increased degradation of 2,5-dichlorobenzoate within planted, inoculated reactors. In other studies, the degradation of TCE using a wheat-colonizing bacterial strain demonstrated that engineered microbes could enhance the degradation of contaminants in the rhizosphere as compared to non-engineered strains [29], and similar findings were shown for TCE in poplar rhizosphere. Recalcitrant hydrophobic polychlorinated biphenyls (PCBs) have also been targeted in efforts showing that PCB-degrading bacteria can be generated [5], and that enzymatic activity of the engineered organism can be altered by using different promoters to improve degradation rates [28]. These studies demonstrate the benefits of incorporating engineered microorganisms and plants in a contaminant degradation system.

Selection of the native organism to receive the recombinant genes can provide a specificity regarding the survival of the recombinant organisms as well. Previous research demonstrated that native bacterial strains from poplar trees modified to degrade TCE were stable and competitive against non-engineered bacteria in the rhizosphere of poplar trees in laboratory testing [20]. However, the same strains were not competitive in the rhizosphere of wheat. Competition with other native root colonizers has been shown to decrease the density of colonization [27].

Recombinant microorganism/plant systems have potential benefits and field applications, but evaluating and then optimizing survival and transport throughout the rhizosphere are difficult and require the ability to monitor recombinant microorganisms following release with relation to effectiveness and potential proliferation. To complicate the process, use of antibiotic-resistance markers in recombinant microorganism studies has been met with opposition, and such markers are essentially banned in some countries. Therefore, simple and robust monitoring methods are needed. The use of fluorescence markers, like fluorescence genes, offers an appealing tracking method for recombinant microorganisms. The green fluorescent protein (GFP) is a visual monitoring marker and can be tested and observed with minimal disturbance to the site and the cell samples, as shown in studies using the *gfp* gene to monitor pseudomonads on barley seeds [17,26] and tomato seedling roots [4]. Use of GFP has been used to identify specific location of colonization in a root system [27]. The presence of a substrate to initiate fluorescence is not needed, and the protein is stable and continues to fluoresce even if the cells are not viable [14].

The use of GFP and other fluorescence markers may be a valuable tool, allowing colonization by recombinant microorganisms to be visualized. With this tracking method, microorganisms that have been genetically modified to degrade contaminants can be monitored with little disturbance to the cells and plant-microorganism system. In this study, rhizosphere bacteria were tagged with the green fluorescent protein genes (*gfp*) in order to monitor colonization, survival and transport within the root zone and to evaluate the effectiveness of visual identification using GFP.

2. Material and methods

2.1. Bacterial strains and growth conditions

The target native host strains included Pb1, Pb2, Pb3, and Pb5 originally isolated from the roots of poplar trees [20] and *Rhizobium* sp. 35645 obtained from the American Type Culture Collection (ATCC). *E. coli*

CC118(λpir) containing the plasmid pUT*gfp*2 was originally obtained from J.K. Jansson (Stockholm University); the plasmid carries the GFP mutant P11, derived from *gfp* gene originally found in the jellyfish *Aequorea Victoria*. *E. coli* S17-1(λpir) was used in the electroporation of the *gfp* gene-containing plasmid. The strains were grown on Luria-Bertani (LB) medium at 30 °C [20]. Kanamycin (50 μg/mL) was added to the medium when growing recombinant *gfp* strains.

2.2. Conjugation

The recipient strains (Pb1, Pb2, Pb3, Pb5, and *Rhizobium* sp. 35645) were conjugated with *E. coli* S17-1 (λpir)/pUT*gfp*2 containing *gfp*2 as described previously [29]. The three brightest fluorescing colonies were isolated based on visual observation under a UV lamp and labeled Pb3*gfp*2−1, Pb3*gfp*2−2, etc. for each different recipient culture. Restriction digests (*Not*I, 1 h, 37 °C) were performed using plasmid mini-preps (Promega) of the engineered strains to determine if chromosomal integration occurred. Strain and plasmid details are presented in Table 1.

2.3. Stability, expression and growth rates

The stability of the *gfp*-recombinant bacterial strains was determined using a shake flask method. The cultures were introduced into LB broth with no antibiotic. The cultures were subsequently inoculated into fresh LB broth every 24 h (approximately 16 generations/day) for seven days. The maximum specific growth rates of the recombinant *gfp*-tagged strains were determined in LB medium without antibiotics. A 150-mL flask with 50 mL of LB medium was inoculated using sterile pipette tips with 20 μL of a culture grown to an OD of 1 for each of the recombinant strains. The OD_{600} was measured in two replicate flasks every 30 min until the OD_{600} reached 0.05 and every ten minutes until the OD_{600} reached 0.5. The data collected the growth rates of each strain were calculated and compared with their corresponding host strain [20].

2.4. Colonization and survival experiments

The colonization of selected recombinant *gfp*-tagged microorganisms was tested in a 50/50 sterile mixture of potting soil (Garden Basics General Purpose Potting Soil, Walmart, USA) and local topsoil. The recombinant organisms Pb5*gfp*2−2 and *Rhizobium* sp. 35645*gfp*2−1 were selected based on similarity of growth rates to the native bacteria (Table 2). Sterile soil was used to assess the survival on the roots and in soil without competition of other soil microorganisms. Recombinant *gfp*-tagged bacterial strains were inoculated onto rooting poplar trees for a seven-week growth period. Poplar trees were prepared and inoculated with the recombinant microorganisms with slight modification to the previously described procedure [20]. In brief, the poplar cuttings were grown in washed silica sand. Once 1 to 3 inch roots had sprouted, sand was washed from cuttings, and roots were coated for 30 min with overnight bacterial cultures that was resuspended to a turbidity of 5 at 600 nm 0.1 M sodium phosphate

Table 1
Bacterial strains and plasmids utilized

Organism or plasmid	Phenotype, genotype or source	Reference
Strains		
E. coli cc118*(λpir)*	*λpir* lysogen Δ(*ara-leu*) *araD* Δ(*lacX74*) *phoA20 thi-1 rpoB argE* (am) *recA1*	Herrero [11]
E. coli S17-1*(λpir)*	RP4:2-Tc:Km^r Tn*7* *λpir* Sm^r Tp^r	V. de Lorenzo [9]
Pb1	Unknown poplar root isolate from Michigan	Shim [20]
Pb2	Unknown poplar root isolate from Michigan	Shim [20]
Pb3	Unknown poplar root isolate from Michigan	Shim [20]
Pb5	Unknown poplar root isolate from Michigan	Shim [20]
Rhizobium sp. 35645	ATCC 35645	Shim [20]
Pb1*gfp*2−1	GFP recombinant of Pb1	This study
Pb2*gfp*2−2	GFP recombinant of Pb2	This study
Pb3*gfp*2−1	GFP recombinant of Pb3	This study
Pb3*gfp*2−3	GFP recombinant of Pb3	This study
Pb5*gfp*2−2	GFP recombinant of Pb5	This study
Rhiz. sp. 35645*gfp*2−1	GFP recombinant of *Rhiz.* sp. 35645	This study
Rhiz. sp. 35645*gfp*2−2	GFP recombinant of *Rhiz.* sp. 35645	This study
Rhiz. sp. 35645*gfp*2−3	GFP recombinant of *Rhiz.* sp. 35645	This study
Plasmid		
pUT*gfp*2	Ap^r Km^r; *gfp2*	Tombolini [25]

Table 2
Host microorganisms, their growth rates and corresponding stable GFP-tagged recombinant microorganisms with comparable growth rates

Host microorganism	Host microorganism growth rate (h^{-1}) [20]	Recombinant microorganism	Recombinant microorganism growth rate (h^{-1})
Pb1	0.853	Pb1gfp2−1	1.087
Pb2	1.024	Pb2gfp2−2	0.979
Pb3	1.179	Pb3gfp2−1	0.747
		Pb3gfp2−3	1.053
Pb5	1.006	Pb5gfp2−2	0.640
Rhizobium sp. 35645	0.593	35645gfp2−1	0.732
		35645gfp2−2	0.993
		35645gfp2−3	0.757

buffer (pH 7). The trees were then replanted. Two trees were inoculated with Pb5*gfp*2−2 and two trees were inoculated with *Rhiz*. sp. 35645*gfp*2−1. The trees were planted in 250-mL containers 20 cm diameter × 25 cm tall. Following planting, the trees were watered daily with sterilized distilled water until the top layer of soil was wet. On a weekly basis the trees were given a 10-ml supplement of ¼ -strength modified Hoagland's nutrient solution. Roots from the plants were sampled after a seven-week growth period to visualize and quantify the colonization of the recombinant *gfp*-tagged cultures.

A survival experiment was completed to assess the temporal survival in both the rhizosphere and bulk soil. Inoculated trees were planted as noted in the colonization experiment. All trees were inoculated with Pb5*gfp*2−2 and planted in autoclaved soil that was the same mixture as the colonization experiment. Each week of the four-week growth period two trees (replicate reactors) were destructively sampled. From each reactor three 1-inch root and three 1-gram soil samples were taken. The samples were diluted and plated via spread plating. For each sample, duplicate plate counts were performed on selective plating techniques and using GFP to visually quantify survival. The soil and root analysis methods are detailed below.

2.5. Transport experiment

Enhanced transport of recombinant organisms by roots of poplar trees was also tested. Ten column reactors were constructed using PVC pipe, 1 m tall × 15 cm diameter. Columns were capped at the bottom and a hole drilled in the cap with a hose-barb connector used to attach latex tubing to the bottom port. Columns were affixed to a support rack; the bottom of the column was lined with geotextile (DuPont® Commercial Landscape Fabric, Lowe's, USA) at the base and then a layer of gravel to prevent clogging of the port. A 6-cm layer of sand was placed atop the gravel, and then the columns were filled to the top with potting soil (Garden Basics General Purpose Potting Soil, Walmart, USA). The soil was not sterile to assess transport in a competitive environment. The latex tubing attached to the bottom of the reactors was approximately 1.3 m long and was attached at the other end to an aspirator bottle used to water the reactors. Prior to planting the reactors, a simulated water table was established in the reactors. The aspirator bottles were placed on a higher shelf of the rack to establish hydrostatic water level in the reactors to be 15 cm below the surface of the soil. Throughout the duration of the experiment the water table was lowered to promote elongated root growth down the 1 m depth by lowering the aspirator bottles.

Poplar trees were inoculated as described above. Three trees were inoculated with Pb5*gfp*2−2, three were inoculated with *Rhiz*. sp. 35645*gfp*2−1, and four were un-inoculated controls. Each soil column received a single tree. The reactors were watered daily with approximately 500 mL of tap water to replace transpiration losses, and the water table was lowered 0.33 m every 60 days. The experiment was run for 180 days. At the end of the experiment, soil cores were removed from the reactors and soil and root samples were collected. The soil and root samples were collected from three heights of the core (0 m, 0.5 m, and 1 m depths) and microbial detection was performed as described below.

2.6. Visual GFP analysis

Root sampling was performed in all experiments described above. To extract and sample the roots the soil was loosened around the roots of the plant and one-inch sections of root were removed from the plants. The root sections were placed in sterile 15-ml screw cap test tubes and suspended in 0.1 M sterile sodium phosphate buffer. Three methods were used to detect the recombinants in the previously described experiments: epifluorescent microscopy, plate counting and visualization using a UV lamp. For epifluorescent microscopy, the microorganisms were dislodged from the one-inch sections of root suspended in buffer solution by vortexing. A total of 10 μl samples of the cells in the resulting solution were placed on slides and observed using a specific GFP filter set with an excitation wavelength of 470 nm and an emission wavelength of

525 nm (Chroma Technology Corp., Vermont, USA). Unvortexed roots were also removed from the buffer, placed on a slide and observed under the microscope. Plate counting was performed using one-inch root sections vortexed to suspend the microorganisms with serial dilutions of the solution plated onto LB media amended with kanamycin (50 μg/mL) and cyclohexamide (100 μg/mL) to control fungi. UV lamp visualization was performed using tryptic soy broth (TSB) amended with kanamycin (50 μg/mL) and cyclohexamide (100 μg/mL). Root sections were embedded into the TSB agar and incubated overnight at 30 °C. The plates were then examined for growth of *gfp*-tagged microorganisms using a hand held ultraviolet (UV) light in a dark room.

2.7. Molecular analysis

PCR analysis was performed on 16 cultures isolated from experiments above. Prior to PCR, a chromosomal DNA extraction procedure was completed as previously described with some modifications [15]. One millimeter of overnight culture was centrifuged in 1.5-mL microcentrifuge tubes for five minutes at $7.2 \times 10^3 \times g$. A total of 30 μL of 25% SDS was added to the cell suspension. The amount of DNA extracted was quantified using a Cary 50 UV-Vis spectrophotometer at wavelengths of 260 nm and 280 nm, with the ratio of OD_{260}/OD_{280} indicating the purity of the DNA, the ratio of OD_{260}/OD_{280} showed the purity of the DNA with respect to protein or phenol contamination. If the OD_{260}/OD_{280} ratio was between 1.8 and 2.0 the concentration of DNA was calculated. After quantification, the DNA suspension was diluted to an appropriate concentration so that between 0.5–1 μg could be used in PCR reactions.

Using the *gfp* primers AVGFPF (forward) and AVGFPR (reverse), a 614-bp sequence of the *gfp* gene was amplified during PCR [18]. The plasmid pUT*gfp*2 containing the inserted *gfp* was used as a positive control. Pb5*gfp*2–2 and *Rhiz*. sp. 35645*gfp*2–1, the two original cultures used as inoculants in the experiments, were also used to verify the PCR products. PCR-quality water was used as a negative control. Taq DNA Polymerase (Eppendorf) was used according to the manufacturer's protocol. The total reaction volume was 100 μL. An optimized PCR protocol was used that consisted of a denaturation step at 94 °C for 5 min, a 1-min denaturation step at 94 °C, a 1-min primer annealing step at 55 °C, and a 1-min primer extension step at 72 °C (30 cycles) and ending with a final extension step at 72 °C for 10 min.

To visualize the PCR results, the products were run on 4% agarose gels for 120 min at 50 volts. The gels were stained with ethidium bromide and visualized under a UV light. The images of the gels were captured using a Kodak 290 digital zoom camera and analyzed using Kodak 1D 3.6.1 software.

3. Results

3.1. Construction of gfp-expressing strains

The plasmid, pUT*gfp*2 was successfully transformed into *E. coli* S17-1(λpir), a donor *E. coli*, using electroporation. The recipient strains (Pb1, Pb2, Pb3, Pb5, and *Rhizobium* sp. 35645) were conjugated with the donor *E. coli* (containing pUT*gfp*2), which created recombinant, root-colonizing bacteria that possessed *gfp*2. Restriction digestion analysis of plasmid mini-preps of the recombinant cultures showed that *gfp*2 genes were integrated into the chromosome rather than into an indigenous plasmid by the lack of presences of *Not*I fragments. The recombinant bacteria were named Pb1*gfp*2–1, Pb2*gfp*2–2, and so on for each of the respective host microorganisms, Table 1. Active expression of the *gfp*2 genes was determined by visual observation of green-colored colonies following conjugation and emission of fluorescence when excited at wavelengths of 396 nm.

3.2. Stability of gfp2 expression and specific growth rates

The stability of GFP expression was determined by monitoring fluorescence of recombinant cultures prepared each day by serially diluting the preceding culture (without antibiotics). GFP expression was stable for at least 7 days as determined by visual observation of green colony color and emission of green fluorescence under UV light. Assuming cultures had eight hours of exponential growth per day, the *gfp*2 genes were constitutively expressed for more than 112 generations.

The maximum specific growth rates determined for the recombinant *gfp*-tagged microorganisms were found to be similar to the host strains (Table 2). This experiment was not a conclusive study of the growth rates and metabolic properties of the recombinants, rather this served as a metric to select individual recombinants to carry forward to the colonization and survival experiments. A comparison of the determined growth rates to those of the host strains show that there is minimal difference among the engineered strains and the

non-engineered hosts, so *gfp*2 insertions caused little metabolic impact on the recombinant microorganisms.

3.3. Colonization and survival experiments

After four weeks of growth, roots from the trees inoculated with Pb5*gfp*2–2 revealed colonization at a density of 2×10^4 to 26×10^4 colonies per inch of root length. The roots from the trees inoculated with *Rhizobium* sp. 35645*gfp*2–1 did not show measurable colonization after the four-week growth period, suggesting that survival was linked to plant-microbe specificity, for root colonizing capability of the *Rhizobium* sp. 35645 was not fostered in the poplar rhizosphere.

Over the 7-week survival experiment, the Pb5*gfp*2–2 inocula clearly survived in the presence of the poplar cuttings, measured on roots and in bulk soil (Fig. 1). A decreasing trend in the density with time was noted in both rhizosphere samples and bulk soil samples. Throughout the 49-day growth period the density of Pb5*gfp*2–2 in the rhizosphere compared to the bulk soil appeared slightly higher, however, colonization was considerably more dense on the roots in terms of area or volume, as the units for the root colonization (CFU per inch root length) represent a much smaller volume and surface area than the 1-gram soil sample. Overall the difference between the rhizosphere and bulk soil survival was not significant, but the average density in the rhizosphere (per inch) was higher than in the surrounding soil (per gram) in all reactors tested. There was no observable impact on plant health and growth among inoculated plants. There has not been extensive research on the Pb series of bacteria as to their

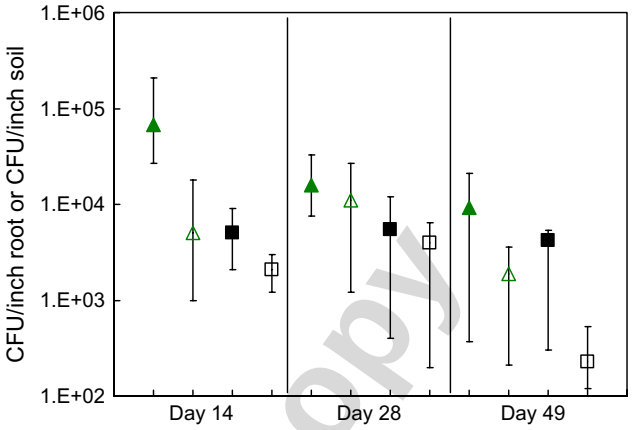

Fig. 1. Survival results throughout 49 days of growth. Only Pb5*gfp*2–2 showed survival, all *Rhizobium*-inoculated samples were negative. Two reactors were sacrificed at each time period, with three soil and root samples taken from each reactor: triangles (△, ▲) represent average concentration of colonies per inch of root, and squares (□, ■) represent average concentration of colonies per gram of soil. The open symbols (△, □) represent one reactor and filled symbols (▲, ■) represent the second reactor sampled. Error bars represent the high and low values determined via plate counts ($n = 3$ samples, analyzed in duplicate).

impacts on the plant, and the *Rhizobium* sp. strain used has not been shown nor is thought to form nodules on poplar trees. Selected colonies isolated from the rhizosphere samples had the *gfp* gene insert as shown using PCR (Fig. 2).

3.4. Transport of root colonizers

Following 180 days of growth in non-sterile soil, root samples at depths of 0 m, 0.5 m, and 1 m were

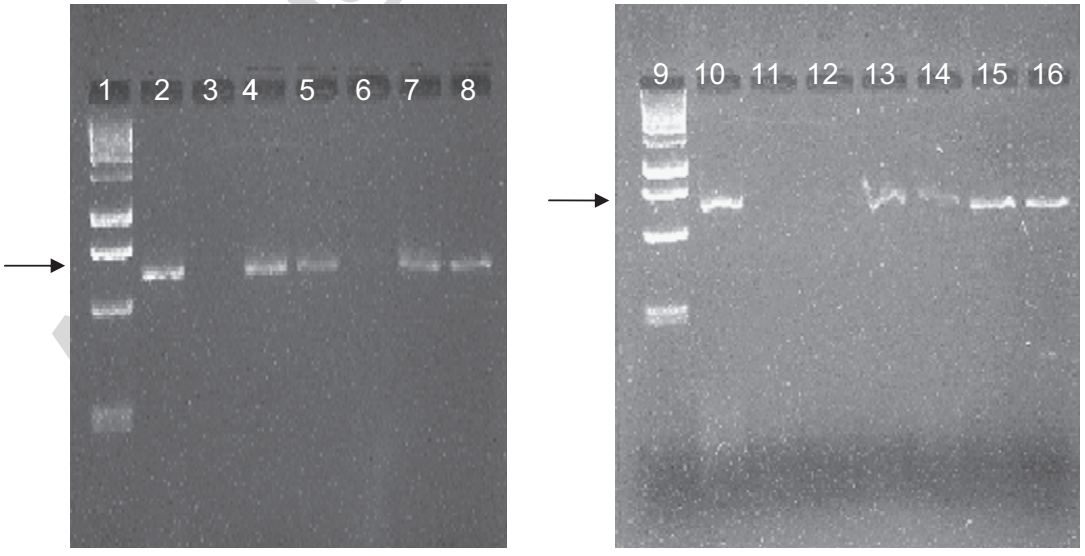

Fig. 2. PCR of representative isolated colonies from the survival experiment and transport experiments. Details of lane designations shown in Table 3.

Table 3
Description of isolates selected for PCR, as shown in Fig. 2

PCR Lanes	Details/experiment	Culture inoculated
1 & 9	1 kb molecular weight marker	NA
2 & 10	Positive PCR controls	Pb5gfp2–2
3 & 11	Negative controls	Pb5TOM no gfp genes
4	Isolate from 0.5 m depth/transport reactor Pb2	Pb5gfp2–2
5	Isolate from 1 m depth/transport reactor Pb3	Pb5gfp2–2
6	Isolate from 1 m depth/transport reactor Pb2	Pb5gfp2–2
7	Isolate from 0.5 m depth/transport reactor Rhiz1	*Rhizobium* sp. 35645gfp2–1
8	Isolate from 1 m depth/transport reactor Rhiz3	*Rhizobium* sp. 35645gfp2–1
12	Negative control *P. fluorescens* 2–79TOM	
13–16	Isolates from survival experiment with time at the various sampling intervals	Pb5gfp2–2

examined. At 0 m depth all of the plants, inoculated and non-inoculated, showed no presence of recombinant bacteria or the *gfp* gene. At 0.5 m and 1 m depths, the plants that were inoculated with Pb5*gfp*2–2 and *Rhizobium* sp. 35645*gfp*2–1 showed the presence of recombinant bacteria, and the control reactors showed none. The numbers of *gfp*-tagged microorganisms found after 180 days were minimal and thus densities of recombinant microorganisms were marginal. Visual assessment of roots was performed using epifluorescence microscopy with a specific GFP filter set (Fig. 3). Plate counts were performed on samples from the reactors and the counts also were not significant although, PCR showed microorganisms isolated from the soil did maintain the *gfp* gene insert, as shown in Fig. 2 and Table 2. By performing PCR on cultures isolated via visual GFP screening, it was evident that quickly screening soil-borne and root-associate isolates for the *gfp* gene using only fluorescence is useful but not 100% accurate. Some fluorescing isolates lacked *gfp*. Visual evaluation provided a way to quickly and easily screen for recombinant microorganisms maintaining a specific gene group. However, to provide more solid confirmation for the presence of the target genes, another method can be performed, such as PCR, antibiotic selectivity, or a second reporter such as *lux*. Transport of *gfp*-tagged rhizosphere bacteria clearly occurred, and while the majority of the isolates selected based upon florescence activity were confirmed via molecular methods, the density of recombinants after 1 meter transport against the hydraulic gradient in a competitive environment for 180 days was modest.

4. Discussion

This study demonstrates the successful integration of *gfp* into microorganisms isolated from the rhizosphere of the poplar tree. These recombinant microbes are stable in terms of growth and expression of *gfp* in lab tests. GFP fluorescence along with kanamycin resistance is a dual marker system that allows further experimenting on the confirmation of *gfp*-tagged microorganisms in laboratory studies.

While earlier work has shown significant degradation of TCE by other recombinants of these same host organisms Pb1, Pb2, etc. [6,20], this study shows that survival was not optimized over a longer period. The optimization of the plant-bacteria pairing is a critical step in the future of enhanced rhizodegradation. The results from these experiments give more insight on the

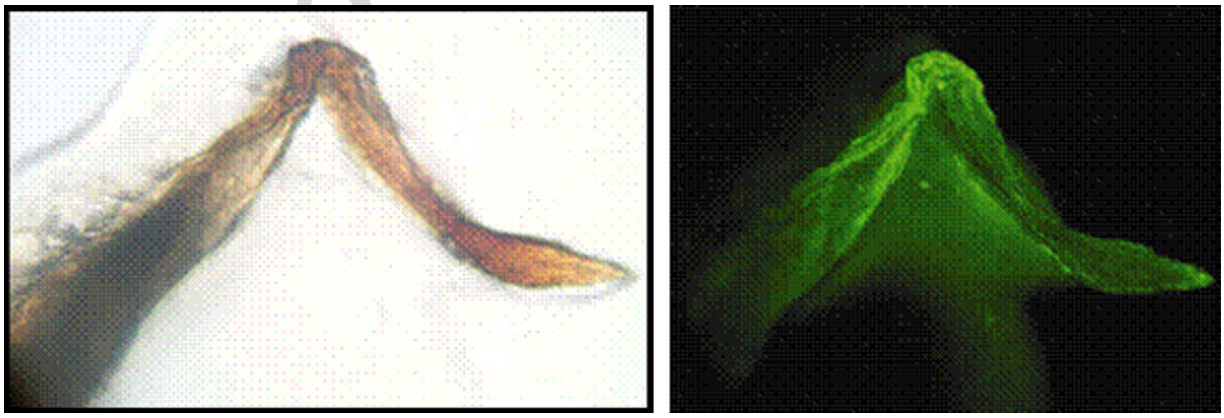

Fig. 3. Photographs of poplar roots from the transport study using Pb5*gfp*2–2. The first photograph is a poplar root using visible light. The second photograph of the same root was taken under epifluorescence microscopy using a specific GFP filter set, with excitation at 470 nm and emission at 525 nm.

interactions between plants and recombinants in a phytoremediation system and demonstrate that the use of GFP is a potential technique for monitoring recombinants in the rhizosphere.

Using the GFP marker tools developed, the engineered root colonizers were shown to be transported 1 meter with the growth of roots against the hydraulic gradient in soil. Microbial transport at this scale against the advection of water has not been previously shown. This transport is encouraging, providing evidence that inoculating roots with proficient root colonizers may provide a low-cost, low energy method to deliver recombinant bacteria throughout a soil profile.

Overall, the ability to detect and enumerate recombinant microorganisms in the rhizosphere without a selective antibiotic will greatly advance the use of recombinant microorganisms in the environment. The use of GFP and other fluorescence markers may be a valuable tool because it allows for visualization of the recombinant microorganism colonies. Recently, similar tools have been utilized for evaluating survival of endophytic bacteria, engineered to degrade contaminants while colonizing xylem tissues [3]. With this tracking method, microorganisms that have been genetically modified to degrade contaminants can be monitored with little disturbance to the cells and plant-microorganism system.

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