Journal of Industrial Microbiology (1996) 16, 62–67

Effect of alginate encapsulation and selected disinfectants on survival of and phenanthrene mineralization by *Pseudomonas* sp UG14Lr in creosote-contaminated soil

SC Weir[1,2], MA Providenti[1,3], H Lee[1] and JT Trevors[1]

[1]*Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1*

The survival and phenanthrene-mineralizing ability of free and alginate-encapsulated *Pseudomonas* sp UG14Lr cells were examined in a creosote-contaminated soil. Alginate encapsulation adversely affected both survival and phenanthrene mineralization. This was postulated to be due to concentration of water-soluble toxic compounds in the alginate beads. Toxicity studies showed that the concentrated water-soluble fraction of the creosote-contaminated soil may be toxic to *Pseudomonas* sp UG14Lr in soil with a low moisture content. Survival of alginate-encapsulated cells improved with increasing soil moisture content. Free cells survived well at a steady population of 10^8 CFU g^{-1} dry soil for 28 days in the creosote-contaminated soil. However, phenanthrene mineralization was not improved compared to the uninoculated control. This was attributed to the existence of indigenous phenanthrene-mineralizing microorganisms already present in this contaminated soil. The effect of calcium hypochlorite and Germiphene on survival of and phenanthrene mineralization by free and alginate-encapsulated *Pseudomonas* sp UG14Lr cells in creosote-contaminated soil was also studied. Addition of 0.1% (w/w dry soil) calcium hypochlorite reduced the introduced free cells to below detection limits (10 CFU g^{-1} dry soil) within 14 days, while Germiphene had no effect on cell numbers. Phenanthrene mineralization by free cells was not adversely affected by treatment with calcium hypochlorite or Germiphene. Survival of alginate-encapsulated cells after treatment with disinfectants was as poor as that without disinfection. The results show that alginate encapsulation may not be a suitable formulation for introduction of *Pseudomonas* sp UG14Lr into creosote-contaminated soils.

Keywords: alginate-encapsulated cells; *Pseudomonas*; soil; survival; biodegradation

Introduction

There has been considerable interest in genetically engineered microorganisms (GEMs) and their potential applications in agriculture and bioremediation [7]. Potential risks of releasing GEMs in the environment have prompted research on containment of microorganisms. Containment methods may include chemical disinfection with agents such as hypochlorite, benzalkonium chloride and a variety of commercially available products, physical destruction by burning and biological control using bacteriophage or protozoans. Additionally, GEMs may be controlled by use of suicide plasmids which contain genes encoding cell destruction, and which are induced by non-toxic chemicals such as lactose [2,9]. Few studies have focused on methods for microbial containment and destruction in the environment. Weir *et al* [15] studied the effects of several chemical disinfectants against *Pseudomonas fluorescens* C5t in soil. Only calcium hypochlorite, Germiphene (a mixture of quaternary ammonium compounds and ethanol) and low concentrations of benzalkonium chloride were effective at killing the introduced GEM. However, Germiphene resulted in increased respiratory activity and total aerobic heterotrophic bacterial numbers in soil [15]. *Pseudomonas aeruginosa* UG2Lr is resistant to both calcium hypochlorite and Spectrum Clear Bath in soil [16]. Donegan *et al* [4] compared treating soil with cupric oxide and streptomycin with burning to destroy *Erwinia herbicola* on bean plants. Although the chemical treatments were effective in the laboratory, they did not significantly decrease bacterial numbers in the field.

An additional concern with respect to using GEMs in the environment is the method of inoculation. Encapsulation in biopolymers such as alginate or κ-carrageenan is a likely method for introduction of GEMs to soil as they provide ease of transport, handling and storage [1,3]. Encapsulation of microbial cells in alginate or κ-carrageenan also increases survival of introduced bacteria in some agricultural soils [1,12,13]. During encapsulation, it is possible to incorporate compounds such as nutrients or clays as protective agents into the matrix to enhance inoculum survival [6]. For example, *Pseudomonas fluorescens* R2f survived for 84 days in soil microcosms when encapsulated in alginate amended with skim milk and bentonite clay [13]. *Pseudomonas aeruginosa* UG2Lr survived significantly better when encapsulated in κ-carrageenan than as free cells in a nonsterile forest soil [10]. Encapsulation also protected the introduced cells from freeze/thaw stress. Alginate encapsulation provided protection to *P. aeruginosa* UG2Lr cells inoculated into an agricultural soil [16]. Most of these studies have examined survival of encapsulated microorganisms in agricultural soils with no previous history of chemical contamination. The question arises as to the sur-

Correspondence: H Lee and JT Trevors, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2]Current address: Microbiology Service, National Institutes of Health, Bethesda, MD, USA
[3]Current address: Department of Biology, University of Ottawa, Ottawa ON, Canada
Received 18 July 1995; accepted 16 October 1995

vival and degradative activity of encapsulated microorganisms in chemically contaminated soils.

The objective of this study was to study the effects of alginate encapsulation of *Pseudomonas* sp UG14Lr, a phenanthrene-degrading strain marked with the *luxAB* genes, on cell survival in creosote-contaminated soil [11,14]. Alginate encapsulation was previously shown to improve survival of and phenanthrene mineralization by UG14Lr in soil slurries [14]. Additionally, the effect of two chemical disinfectants, calcium hypochlorite and Germiphene, on survival and phenanthrene mineralization by UG14Lr were studied.

Materials and methods

Bacterial strain and culture conditions

Pseudomonas sp UG14Lr was derived from strain UG14 which was initially isolated from contaminated soil from a former wood treatment site [11]. A spontaneous rifampicin-resistant variant of strain UG14 was marked with the *luxAB* genes from *Vibrio harveyii* using the mini-Tn5 cassette [14] to produce strain UG14Lr. For long term maintenance, strain UG14Lr was stored at −80°C in 15% (v/v) sterile glycerol.

Preparation of free cell suspension of Pseudomonas sp UG14Lr

Cells from a frozen stock culture were grown for 48 h at 30°C on Tryptic Soy agar (TSA) (Difco Laboratories, Detroit, MI, USA) amended with 50 μg ml^{-1} each of ampicillin and rifampicin. A single colony was transferred to 50 ml GY medium (mineral salts [8] amended with 1% w/v glycerol and 0.2% w/v yeast extract) in a 250-ml Erlenmeyer flask and shaken at 200 rpm at 30°C. After 24 h, 4 ml of this culture were used to inoculate 100 ml GY medium amended with 10 μg ml^{-1} phenanthrene and incubated at 30°C and shaken at 200 rpm for 24 h. Cells were harvested by centrifugation (4000 × *g* for 10 min at 4°C), washed twice and resuspended in sterile distilled water at a concentration of 10^{10} cells ml^{-1}. Cell density was adjusted after direct counting using Petroff Hauser chamber and 1000× phase contrast magnification. Aliquots (0.5 ml) of free cell suspension were used directly for soil inoculation as described later.

Alginate encapsulation

Pseudomonas sp UG14Lr cells were encapsulated in 1.5% (w/v) alginate beads containing 1% (w/v) skim milk and 5% (w/v) montmorillonite clay as described previously [14,16]. Cells were grown in the same manner as for free cell suspensions. After harvesting, they were washed and resuspended in 5.0 ml 0.05 M PIPES buffer and added to the alginate mixture. Alginate beads were washed twice with 0.05 M PIPES buffer and dried for 24 h on sterile aluminum foil in a laminar flow cabinet. Viability of encapsulated cells was determined by first shaking five beads at 200 rpm in 5.0 ml sterile 1.0% (w/v) sodium pyrophosphate (pH 7) at 28°C for 1 h, followed by physical disruption of the beads using a pyrex brand tissue grinder (Fisher Scientific Co, Toronto, Ont, Canada). The suspension was serially diluted and 100 μl plated onto TSA. Dried beads were stored in glass bottles at 4°C in the dark.

Disinfectants

Two previously-described disinfectants were tested: calcium hypochlorite (Fluka Chemie, Switzerland) and Germiphene (Germiphene Co, Brantford, Canada), a mixture of quarternary ammonium chloride compounds [15]. Calcium hypochlorite killed *P. fluorescens* C5t in soil and inhibited respiratory activity of indigenous microorganisms. Germiphene was chosen because it killed *P. fluorescens*, but did not adversely affect the respiratory activity of indigenous microorganisms [15].

Minimal bactericidal concentration (MBC)

MBC refers to the lowest concentration needed to produce a 5 $\log_{10}$ reduction of viable bacterial numbers. The MBC values for both free and encapsulated cells were determined as described previously [15,16].

Soil characteristics and microcosm design

Creosote-contaminated soil (61% sand, 34% silt, 5% clay, 4.5% organic matter, pH 7.4) was collected from the top 0.5 m of soil at a former wood treatment facility in Ontario. This soil contained (per kg dry soil), 1400 mg polycyclic aromatic hydrocarbons, 680 mg pentachlorophenol, and 6325 mg total petroleum hydrocarbons (AG Seech, Grace Dearborn, Inc, Mississauga, Ont, Canada, personal communication). Soil was passed through a 2-mm sieve and stored at 4°C in the dark in an air-tight container until used.

Fifty-gram (dry weight) portions of creosote-contaminated soil were added to 250-ml glass Mason Jars. A 7-ml polypropylene scintillation vial (Fisher Scientific, Toronto, Ont, Canada) containing 5 ml 2 N NaOH was placed in the centre of each microcosm to serve as a CO_2 trap. Microcosms were amended with 3.33×10^5 dpm radiolabelled phenanthrene (phenanthrene-9-^{14}C; specific activity 13.1 mCi mM^{-1}, Sigma Chemicals, Mississauga, Ont, Canada) on day −1 (one day before inoculation) and sealed.

Cell suspensions were added dropwise to the soil surface and allowed to wet the soil for 1 h at 10°C before adding disinfectants. Dry beads were mixed in soil using a stainless steel spatula. Microcosms were treated with 1 ml of one of: sterile distilled water, 5.0% (w/v) calcium hypochlorite or 5.0% (v/v) Germiphene. The final concentration of disinfectants in the soil was 0.1% (w/v) calcium hypochlorite and 0.1% (v/v) Germiphene. The soil moisture content was 40% of the water-holding capacity (WHC) in each microcosm and soil was packed to a bulk density of 1.2 g cm^{-3}.

Periodically, samples (1.13 g soil corresponding to 1.0 g dry soil) were removed, and placed into 9.5 ml 1.0% (w/v) sodium pyrophosphate solution and were shaken for 1 h at 180 rpm. The suspension was serially diluted and plated on 1/10 TY (1.0 g tryptone, 0.5 g yeast extract, 1.0 g NaCl, 15 g agar in 1 L distilled water) amended with 5.0% (w/v) glycerol for total aerobic heterotrophic bacterial counts and on TSA amended with 50 μl ml^{-1} each of ampicillin, rifampicin and cycloheximide for counts of *Pseudomonas* sp UG14Lr. Plates were incubated at 30°C for 2 days and counted. Colonies of strain UG14Lr *luxAB*+ were confirmed using a Biomedical Image Quantifier (BIQ Bioview,

Cambridge, UK) [5]. Colonies of strain UG14Lr emit light in the presence of *n*-decanal vapors. There were no indigenous *luxAB*– positive colonies in the soil used.

Phenanthrene mineralization

Phenanthrene mineralization was measured by periodically removing and replacing the CO_2 trap as described by Providenti *et al* [11]. This also served to aerate the soil microcosms. One-millilitre samples were removed from each CO_2 trap into a 20-ml polypropylene scintillation vial (Fisher Scientific, Toronto, Ont, Canada) and neutralized by adding 1.0 ml 2 N acetic acid. Ten millilitres of Scintiverse II scintillation cocktail (Fisher Scientific) were then added. After incubating the cocktail overnight at 22°C in the dark, the amount of $^{14}CO_2$ was measured by liquid scintillation counting using a Beckman LS6000 Scintillation System (Beckman, Fullerton, CA, USA).

The amount of ^{14}C-labelled compounds remaining in each microcosm was measured to obtain a mass balance as described previously [14]. Soil was dried at 80°C for 24 h. Dried soil was mixed with a metal spatula. Samples (0.5 g) were combusted for 3 min in a model OX300 Biological Material Oxidizer (RJ Harvey Instrument Corp, Hillsdale, NJ, USA) at a temperature of 900°C. $^{14}CO_2$ produced was trapped in 20 ml Carbon 14 Cocktail (RJ Harvey Instrument Corp) in 20-ml borosilicate glass scintillation vials. The amount of radioactivity was determined by liquid scintillation counting as described above.

Soil toxicity

Experiments were conducted to determine possible reasons for poor survival of alginate-encapsulated cells in creosote-contaminated soils. Alginate beads (0.5 g containing 1×10^6 CFU) were added to 5 g creosote-contaminated soil packed to a bulk density of 1.2 g cc^{-1} in 25-ml French Square Bottles (Fisher Scientific Co, Toronto, Ont, Canada) at moisture contents varying from 20 to 120% WHC. After 5 days, 7.5 ml sterile 50 mM sodium pyrophosphate (pH 7) were added. The bottle microcosms were agitated at 200 strokes min^{-1} at 22°C for 1 h on a linear action shaker (Eberbach Corp, Ann Arbor, MI, USA). The resulting slurries were serially diluted in sterile 0.1% (w/v) sodium pyrophosphate and plated on TSA amended with 50 $\mu g\ ml^{-1}$ each of ampicillin, cycloheximide and rifampicin. Plates were incubated at 30°C for 2 days in the dark before counting CFU as described above.

A further experiment conducted in similar soil microcosms tested the toxicity of water-soluble compounds in creosote-contaminated soil on alginate-encapsulated cells of strain UG14Lr. Sterile distilled water (17.5 ml) was added to 20 g dry creosote-contaminated soil samples in sterile 50-ml centrifuge tubes which were shaken at 200 rpm for 1 h. The mixture was centrifuged at $5000 \times g$ for 10 min and the supernatant fluid was collected and filtered through a 4-μm pore size filter. This extract was added to 20 g of dry sandy loam soil, an agricultural soil previously described [15] which was similar in texture to the creosote-contaminated soil. The soil pellet was resuspended in 17.5 ml sterile distilled water and washed two more times. Microcosms were prepared using 5 g dry wt of the following soils at 40% WHC: (a) creosote-contaminated soil; (b) water-extracted creosote-contaminated soil; (c) sandy loam soil; (d) sandy loam soil with creosote-contaminated soil extract. Alginate beads (0.5 g dry wt containing 1×10^6 CFU) were added to each microcosm and mixed throughout the soil. Microcosms were incubated at 22°C in the dark for 5 days. Survival was measured as viable CFU as described above.

Statistical analysis

All experiments were carried out in triplicate. Results were analyzed using an IBM-compatible PC and one-way completely randomized ANOVA followed by the Student-Newman-Keuls multiple range test (Cohort, Berkeley, CA, USA) at both 5 and 1% levels of significance to determine statistically significant differences in cell counts and percentage of phenanthrene mineralized.

Results

The MBC for calcium hypochlorite against both free and alginate-encapsulated *Pseudomonas* sp UG14Lr was 0.02% (w/v). For Germiphene, the MBCs were 0.08% (v/v) and 0.1% (v/v) against free and alginate-encapsulated cells, respectively.

Survival and activity of free and alginate-encapsulated UG14Lr cells

Free *Pseudomonas* sp UG14Lr maintained a steady population near the inoculum size (10^8 CFU g^{-1} dry soil) for 28 days (Figure 1a) in creosote-contaminated soil microcosms. Alginate encapsulation adversely affected survival. Additionally, total aerobic heterotrophic populations were lower in soil amended with alginate beads. Furthermore, phenanthrene mineralization was completely suppressed in soil containing alginate beads or cells immobilized in alginate (Figure 1b). There was no statistically significant difference in mineralization between uninoculated soils and soils inoculated with free cells.

Effects of disinfectants

Calcium hypochlorite had a marked effect on survival of free *Pseudomonas* sp UG14Lr cells in creosote-contaminated soil (Figure 2a). The introduced strain was not detected after 14 days. There was no statistically significant difference in survival of free UG14Lr cells in creosote-contaminated soil with or without addition of Germiphene.

Effects of chemical treatments on phenanthrene mineralization by free cells in creosote-contaminated soil are shown in Figure 2b. Addition of Germiphene enhanced mineralization while calcium hypochlorite was inhibitory compared to no chemical treatment. However, by the end of 7 weeks, there were no significant differences between any of the treatments.

Alginate-encapsulated cells did not survive well in untreated creosote-contaminated soil. There was no significant change in cell numbers due to the addition of calcium hypochlorite. Germiphene treatment extended survival of encapsulated cells to 28 days versus 14 days in untreated creosote-contaminated soil (Figure 3a). Furthermore, mineralization was virtually absent in all bead-inoculated microcosms, although calcium hypochlorite treatment

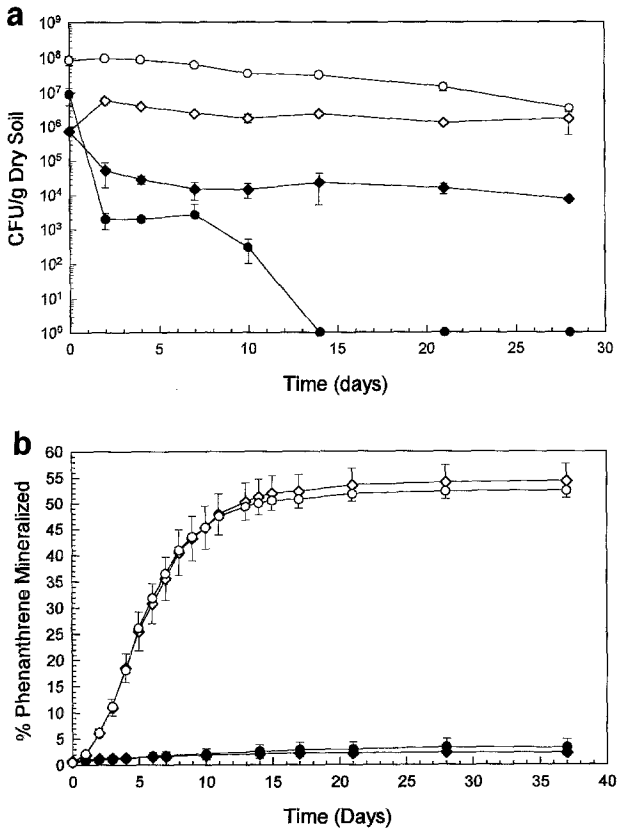

Figure 1 Survival of (a) and phenanthrene mineralization by (b) free and alginate-encapsulated *Pseudomonas* sp UG14Lr cells in creosote-contaminated soil microcosms. (○) free cells; (●) alginate-encapsulated cells; (◇) total aerobic heterotrophic bacteria (a) or phenanthrene mineralization (b) in unamended soil microcosm; (◆) total aerobic heterotrophic bacteria (a) or phenanthrene mineralization (b) in soil microcosms receiving the sterile bead amendment. Error bars are ±s.d. ($n = 3$).

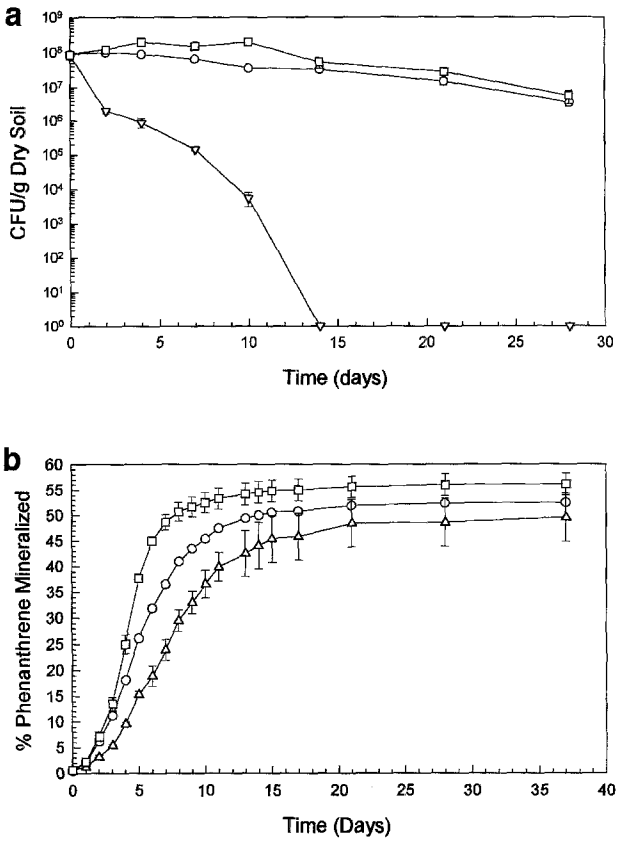

Figure 2 Survival of (a) and phenanthrene mineralization by (b) free *Pseudomonas* sp UG14Lr cells in creosote-contaminated soil microcosms treated with disinfectants. (▽) 0.1% (w/w dry soil) calcium hypochlorite; (□) 0.1% (v/w dry soil) Germiphene; (○) control, no chemical treatment. Error bars are ±s.d. ($n = 3$).

caused a slight reduction in the remaining phenanthrene mineralization (Figure 3b). In mass balance determinations, an average of 80% (range 74–87%) of the radiolabel was recovered from creosote-contaminated soil microcosms.

Survival of alginate-encapsulated cells in creosote-contaminated soil of varying moisture contents is shown in Figure 4. Survival was poor in soil with low moisture content, but improved in water-saturated soil. The highest recovery of bioluminescent cells was from soil containing 120% WHC (5.1×10^5 CFU g^{-1} dry soil) while no *Pseudomonas* sp UG14 Lr cells were recovered from soil incubated at 20% of soil WHC. The effect of water-soluble compounds from creosote-contaminated soil on survival is shown in Figure 5. The highest population was recovered from agricultural sandy loam (3.23×10^5 CFU g^{-1} dry soil), followed by water-extracted creosote-contaminated soil (1.76×10^5 CFU g^{-1} dry soil). *Pseudomonas* sp UG14Lr populations recovered from these soils were significantly higher than from creosote-contaminated soil (1.73×10^3 CFU g^{-1} dry soil) and agricultural sandy loam containing the extract (1.79×10^4 CFU g^{-1} dry soil).

Discussion

Survival and phenanthrene mineralization

Phenanthrene was mineralized in soil microcosms by both the introduced strain and the indigenous microorganisms. Greater than 50% of the radiolabelled phenanthrene added was mineralized to CO_2 after 20 days. This extent of mineralization is much higher than was found in previous studies using the same strain in creosote-contaminated soil slurries [11,14]. Free *Pseudomonas* sp UG14Lr also sustained a high population density (10^7 CFU g^{-1} dry wt soil) over 4 weeks. In soil slurries, free *Pseudomonas* sp UG14Lr populations were not detected after 19 days. This indicates the potential for *in situ* bioremediation by introduced bacteria. However, in this experiment, the indigenous population was very efficient in phenanthrene degradation.

Encapsulation adversely affected both survival of *Pseudomonas* sp UG14Lr and phenanthrene mineralization in creosote-contaminated soil microcosms. This differed from previous reports which showed that encapsulation improved survival of other bacterial strains [12,13,16]. These results also differ from those of a previous experiment which found improved survival of and phenanthrene mineralization by alginate-encapsulated *Pseudomonas* sp UG14Lr in creosote-contaminated soil slurries [14]. The improved phenanthrene mineralization seen in the previous study suggests that phenanthrene was bioavailable to cells inside the beads.

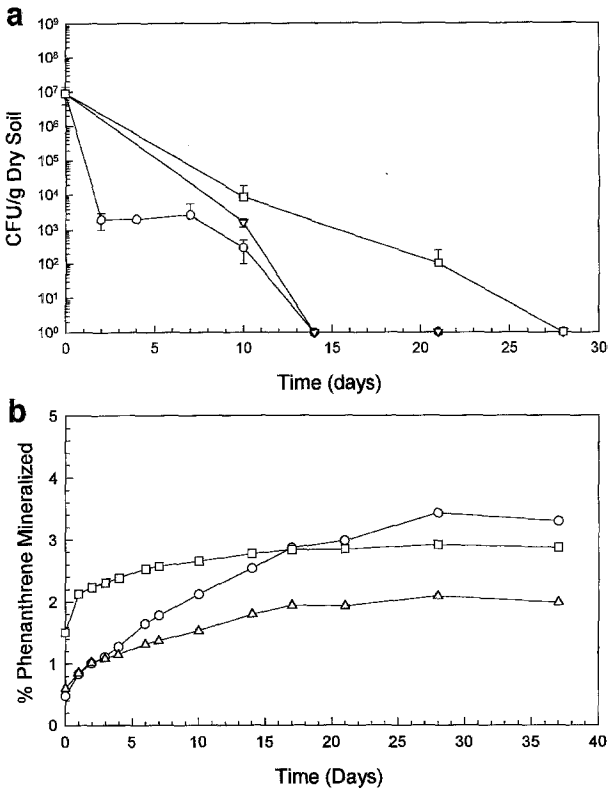

Figure 3 Survival of (a) and phenanthrene mineralization by (b) alginate-encapsulated *Pseudomonas* sp Ug14Lr in creosote-contaminated soil microcosms treated with disinfectants. (▽) 0.1% (w/w dry soil) calcium hypochlorite; (□) 0.1% (v/w dry soil) Germiphene; (○) control, no chemical treatment. Error bars are ±s.d. ($n = 3$).

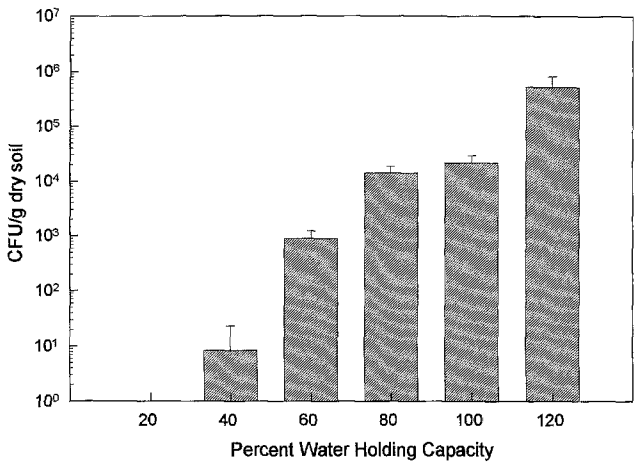

Figure 4 Survival of alginate-encapsulated *Pseudomonas* sp UG14Lr cells in creosote-contaminated soil at various soil moisture contents after 5 days incubation at 22°C. Error bars are ±s.d. ($n = 3$).

Soil used in the present study was contaminated with creosote from a wood treatment facility. The soil contained many water-soluble compounds such as pentachlorophenol which may be toxic to the introduced strain. Possibly, toxic compounds in the soil, such as pentachlorophenol, may have concentrated within the hydrophillic bead matrix due to the low moisture content (40% WHC). This would have inactivated the introduced cells before they could metabolize phenanthrene. In slurries, the toxins would be diluted below the concentration initially present in the creosote-contaminated soil. The water-soluble fraction of creosote-contaminated soil was toxic to alginate-encapsulated cells of strain UG14Lr (Figure 5). Results of experiments with different moisture contents suggest that increasing water content may dilute these toxic compounds (Figure 4). The number of *Pseudomonas* sp UG14Lr cells recovered from creosote-contaminated soil microcosms after 5 days was directly proportional to soil moisture content. Saturated soil may allow for dilution of components of creosote below toxic levels to the introduced strain. This may explain the poor survival of alginate-encapsulated *Pseudomonas* sp UG14Lr cells in soil microcosms compared to soil slurries.

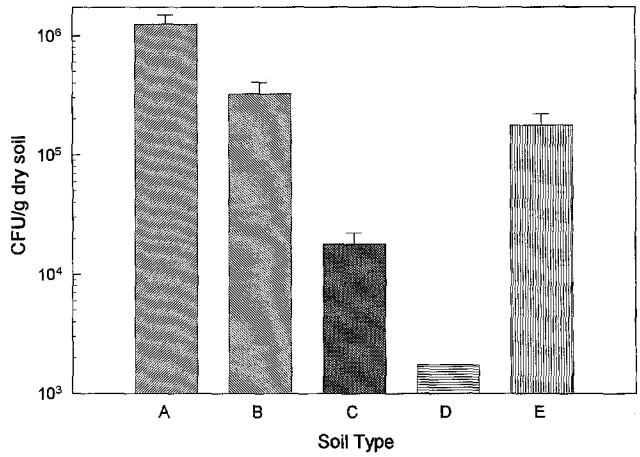

Figure 5 Survival of alginate-encapsulated *Pseudomonas* sp UG14Lr in various soils after 5 days incubation at 22°C. (A) inoculum density; (B) agricultural sandy loam soil; (C) agricultural sandy loam soil containing water extract from creosote-contaminated soil; (D) creosote-contaminated soil; (E) creosote-contaminated soil after extraction with water. Error bars are ±s.d. ($n = 3$).

A further question as to why phenanthrene mineralization was inhibited by the addition of alginate beads remains. Total culturable indigenous aerobic heterotrophic bacterial numbers were reduced 100-fold in the presence of alginate beads (Figure 1a). This contradicts previous experiments that reported an increase in total culturable aerobic heterotrophic bacteria in soil amended with alginate beads [16]. One possible reason for this may be that bacteria present in creosote-contaminated soil have been selected for their tolerance and degradative capacity for compounds that make up creosote. It is likely the microbial diversity in this soil was lower than in non-contaminated agricultural soil. Therefore, there may not be many microorganisms present capable of degrading alginate. Since alginate beads can absorb many of the compounds in creosote-contaminated soil, these compounds may be unavailable as nutrient sources for indigenous bacteria, resulting in their lower numbers.

Disinfection of free and alginate-encapsulated Pseudomonas sp UG14Lr

Free cells were killed by addition of calcium hypochlorite. Previous studies demonstrated the killing efficacy of calcium hypochlorite on free *P. fluorescens* C5t and *P. aeruginosa* UG2Lr cells in an agricultural sandy loam

soil [14,16]. However, *P. aeruginosa* UG2Lr populations recovered from an initial decline after addition of calcium hypochlorite. *Pseudomonas* sp UG14Lr populations did not recover and were not detected after 14 days. Furthermore, phenanthrene mineralization in calcium hypochlorite-treated microcosms was slightly, though not significantly, lower than in non-treated microcosms. These results indicated that calcium hypochlorite was effective at inhibiting the introduced strain without adversely affecting selected indigenous microbial activities.

Germiphene slightly increased both phenanthrene mineralization and survival of strain UG14Lr. Germiphene consists of quaternary ammonium compounds containing long chain hydrocarbons. These hydrocarbons may have been utilized by *Pseudomonas* sp UG14Lr and indigenous microorganisms, thereby allowing increased populations and metabolic activity in the soils. *Pseudomonas* sp UG14Lr numbers were slightly higher in Germiphene-treated microcosms than in non-treated microcosms for the first 15 days. It was during this time when most of the phenanthrene mineralization occurred. Previous experiments demonstrated increased respiratory activity and numbers of *P. fluorescens* C5t cells in soil treated with Germiphene [15].

In summary, survival of encapsulated *Pseudomonas* sp UG14Lr was poor in creosote-contaminated soil microcosms. Addition of disinfectants did not further affect the rate of decline of the introduced bacteria. Previously, alginate encapsulation was found to improve survival and activity of strain UG14Lr cells in creosote-contaminated soil slurries [16]. The results of this study indicate that alginate encapsulation may not be a feasible method of inoculation of bacteria into chemically contaminated soil under conditions similar to those used in this study. However, free cells survived well in creosote-contaminated soil. Additionally, phenanthrene mineralization in soil was greater than previously reported for slurries [11,14]. These results are encouraging for using microorganisms for biodegradation of pollutants *in situ*. Our results showed also that in historically contaminated soils which are already populated with indigenous microorganisms capable of degrading the pollutants, bioaugmentation with another microbial culture may not be necessary.

Acknowledgements

This research was supported by a grant from the Ontario Ministry of Environment and Energy to JTT and HL, and a Natural Sciences and Engineering Research Council (NSERC) of Canada Research grant to JTT. SC Weir was the recipient of a graduate scholarship from NSERC. We thank AG Seech who kindly provided the creosote-contaminated soil and Dr M Griffiths for allowing the use of the BIQ.

References

1 Bashan Y. 1986. Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. Appl Environ Microbiol 51: 1089–1098.
2 Bej AK, MH Perlin and RM Atlas. 1988. Model suicide vector for containment of genetically engineered microorganisms. Appl Environ Microbiol 54: 2472–2477.
3 Cassidy MB, H Lee and JT Trevors. 1995. Environmental applications of immobilized microbial cells. J Ind Microbiol (in press).
4 Donegan K, R Seidler and C Matyac. 1991. Physical and chemical control of released microorganisms at field sites. Can J Microbiol 37: 708–712.
5 Flemming CA, KT Leung, H Lee, JT Trevors and CW Greer. 1994. Survival of *lux-lac* marked biosurfactant-producing *Pseudomonas aeruginosa* UG2L in soil: monitored by non-selective plating and polymerase chain reaction. Appl Environ Microbiol 60: 1606–1613.
6 Heijnen CE, CH Hok-A-Hin and JA van Veen. 1992. Improvements to the use of bentonite clay as a protective agent, increasing survival levels of bacteria introduced into soil. Soil Biol Biochem 24: 533–538.
7 Jackman SC, H Lee and JT Trevors. 1992. Survival, detection and containment of bacteria. Microb Releases 1: 125–154.
8 Kiyohara H, K Nagao and K Yano. 1982. Rapid screen for bacteria degrading water-insoluble, solid hydrocarbons on agar plates. Appl Environ Microbiol 43: 454–457.
9 Knudsen SM and OH Karlstrom. 1991. Development of efficient suicide mechanisms for biological containment of bacteria. Appl Environ Microbiol 57: 85–92.
10 Leung K, MB Cassidy, SB Holmes, H Lee and JT Trevors. 1995. Survival of K-carrageenan-encapsulated and unencapsulated *Pseudomonas aeruginosa* UG2Lr cells in forest soil monitored by polymerase chain reaction and spread plating. FEMS Microb Ecol 16: 71–82.
11 Providenti MA, CW Greer, H Lee and JT Trevors. 1995. Phenanthrene mineralization by *Pseudomonas* sp UG14. World J Microbiol Biotechnol 11: 271–279.
12 Trevors JT, JD van Elsas, H Lee and AC Wolters. 1993. Survival of alginate-encapsulated *Pseudomonas fluorescens* cells in soil. Appl Microbiol Biotechnol 39: 637–643.
13 van Elsas JD, JT Trevors, D Jain, AC Wolters, CE Heijnen and LS van Overbeek. 1992. Survival of, and root colonization by, alginate-encapsulated *Pseudomonas fluorescens* cells following introduction into soil. Biol Fertil Soils 14: 14–22.
14 Weir SC, SP Dupuis, MA Providenti, H Lee and JT Trevors. 1995. Nutrient enhanced survival of and phenanthrene mineralization by alginate-encapsulated and free *Pseudomonas* sp UG14Lr cells in creosote-contaminated soil slurries. Appl Microbiol Biotechnol 43: 946–951.
15 Weir SC, H Lee and JT Trevors. 1994. Survival and respiratory activity of genetically engineered *Pseudomonas* spp exposed to antimicrobial agents in broth and soil. Microb Releases 2: 239–245.
16 Weir SC, H Lee and JT Trevors. 1995. Survival of free and encapsulated *Pseudomonas aeruginosa* UG2Lr in soil treated with disinfectants. J Appl Bacteriol (in press).