Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Alginate beads as a storage, delivery and containment system for genetically modified PCB degrader and PCB biosensor derivatives of *Pseudomonas fluorescens* F113

B. Power, X. Liu, K.J. Germaine, D. Ryan, D. Brazil and D.N. Dowling

Department of Science and Health, Institute of Technology Carlow, Carlow, Ireland

Keywords
alginate beads, polychlorinated biphenyl biosensors, *Pseudomonas fluorescens* F113Rifpcb, rhizoremediation.

Correspondence
David N. Dowling, Department of Science and Health, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland.
E-mail: dowlingd@itcarlow.ie

2010/1019: received 24 June 2010, revised 9 December 2010 and accepted 25 February 2011

doi:10.1111/j.1365-2672.2011.04993.x

Abstract

Aims: *Pseudomonas fluorescens* F113Rifpcb is a genetically engineered rhizosphere bacterium with the potential to degrade polychlorinated biphenyls (PCBs). F113Rifpcb*gfp* and F113L::1180*gfp* are biosensor strains capable of detecting PCB bioavailability and biodegradation. The aim of this paper is to evaluate the use of alginate beads as a storage, delivery and containment system for use of these strains in PCB contaminated soils.

Methods and Results: The survival and release of *Ps. fluorescens* F113Rifpcb from alginate beads were evaluated. Two *Ps. fluorescens* F113-based biosensor strains were encapsulated, and their ability to detect 3-chlorobenzoate (3-CBA) and 3-chlorobiphenyl (3-CBP) degradation in soil was assessed. After 250 days of storage, 100% recovery of viable F113Rifpcb cells was possible. Amendments to the alginate formulation allowed for the timed release of the inoculant. Encapsulation of the F113Rifpcb cells provided a more targeted approach for the inoculation of plants and resulted in lower inoculum populations in the bulk soil, which may reduce the risk of unintentional spread of these genetically modified micro-organisms in the environment. Encapsulation of the biosensor strains in alginate beads did not interfere with their ability to detect either 3-CBA or 3-CBP degradation. In fact, detection of 3-CBP degradation was enhanced in encapsulated biosensors.

Conclusions: Alginate beads are an effective storage and delivery system for PCB degrading inocula and biosensors.

Significance and Impact of the Study: *Pseudomonas fluorescens* F113Rifpcb and the F113 derivative PCB biosensor strains have excellent potential for detecting and bioremediation of PCB contaminated soils. The alginate bead delivery system could facilitate the application of these strains as biosensors.

Introduction

Polychlorinated biphenyls (PCBs) have been identified as priority pollutants especially in soil and aquatic environments (Robinson and Lenn (1994)). Micro-organisms can degrade PCBs, but the prevailing environmental conditions govern their ability to achieve degradation (Liu and Suflita 1993). Rhizoremediation is one possible treatment method for the removal of PCBs from contaminated soils and sediments. The rhizosphere is an important interphase between plant and bacteria, where environmental conditions are somewhat more stable than in bulk soil. Here increased metabolic activity can occur including organic pollutant degradation (McGuinness and Dowling 2009).

Pseudomonas fluorescens F113 was isolated from the rhizosphere of sugar beet (Shanahan *et al.* 1992) and found to be an excellent root colonizer of other plants including tomato, pea, alfalfa and willow (Simons *et al.* 1996; Naseby and Lynch 1999; Villacieros *et al.* 2003; Rein *et al.*

2007). The genes responsible for PCB degradation from *Burkholderia xenovorans* LB400, termed the *bph* operon (Goris *et al.* 2004), were chromosomally inserted into a spontaneous rifampicin-resistant mutant of F113 (F113Rif) creating *Ps. fluorescens* F113Rifpcb (Brazil *et al.* 1995). The *bph* operon was modified by cloning a strongly inducible (nod box) promoter from *Sinorhizobium meliloti* upstream of the *bph* operon, and the cassette was subsequently chromosomally integrated into *Ps. fluorescens* F113RiflacZY, creating *Ps. fluorescens* F113L::1180 (Villacieros *et al.* 2005). Two chlorobenzoate (CBA)/PCB biosensors, *Ps. fluorescens* F113Rif*gfp and Ps. fluorescens* F113L::1180*gfp* were constructed as described in Liu *et al.* (2007). These strains were constructed by the chromosomal insertion of a promoter-reporter construct, *xyl*Sp_m::*gfp*mut3 (Moller *et al.* 1998) into *Ps. fluorescens* F113Rif and *Ps. fluorescens* F113L::1180 to create *Ps. fluorescens* F113Rif*gfp* and *Ps. fluorescens* F113L::1180*gfp*, respectively. The p_m promoter in this construct is derived from the TOL plasmid and regulates the meta-pathway of aromatic hydrocarbon degradation. The meta-pathway is induced by chlorobenzoic acid derivatives (which are also end products of the PCB degradation pathway). Induction of the p_m promoter in the *xyl*Sp_m::*gfp*mut3 construct drives the expression of the green fluorescent protein (*gfp*), which is detectable using fluorimetry or epi-fluorescent microscopy. The level of *gfp* expressed was shown to have a linear relationship with CBA concentration. As F113L::1180*gfp* can degrade PCBs, it produces chlorinated benzoate end products which in turn induce the expression of *gfp*. Therefore, this strain can actively report on its own degradation of PCBs (Liu *et al.* 2007). Biosensor *Ps. fluorescens* F113Rif*gfp* is a non-PCB degrader and so can only detect external sources of CBAs (PCB and CBA metabolites by other strains).

Alginate, a copolymer of mannuronic and guluronic acid, is the most common material for encapsulation of micro-organisms for commercial use and has widespread environmental applications (Bettmann and Rehm 1984; Sahasrabudhe *et al.* 1988; Ivanova *et al.* 2005; Jezequel and Lebeau 2008; Tao *et al.* 2009). The alginate beads formed by the encapsulation process are dry, easy to handle, uniform, biodegradable, nontoxic and can contain a large uniform bacterial population, providing for the slow release of bacteria over long periods (Bashan 1986). Furthermore, alginate is an abundant, biodegradable and renewable marine biopolymer, making it an attractive and relatively cheap alternative to other inoculants carriers (Bashan and Gonzalez 1999). Encapsulating whole-cell biosensors in natural or synthetic polymers has been shown to be useful for the detection of other environmental pollutants (Belkin 2003; Trogl *et al.* 2005).

The objectives of this research were to determine whether alginate was a suitable carrier for F113Rifpcb and F113-based biosensors strains, for long-term storage, for release in specific environments and also as an efficient inoculant delivery system.

Materials and methods

Bacterial strains and soils used

Table 1 shows the strains used in this work. The soil used for inoculation studies was a local soil obtained from the grounds of IT Carlow and was classified as a sandy loam soil (60% sand, 33% clay and 7% silt). The soil was sieved through a 3·35-mm mesh, and rifampicin-resistant bacteria were undetectable on nutrient agar (NA) plates supplemented with 50 μg ml^{-1} rifampicin (NA Rif).

Medium and growth conditions

F113Rifpcb was maintained on NA amended with 50 μg ml^{-1} of rifampicin (NA Rif), and cultured in

Table 1 List of bacterial strains used in this study

Bacteria	Characteristics	References
Pseudomonas fluorescens F113Rifpcb	*Ps. fluorescens* Spontaneous rifampicin mutant of Ps. Fluorescens F113, *bph* operon chromosomally inserted	Brazil *et al.* (1995)
Ps. fluorescens F113Rif*gfp*	Spontaneous rifampicin-resistant mutant of *Ps. fluorescens* F113; Km^R Pm::*gfp*mut3 inserted chromosomally	Liu *et al.* (2007)
Ps. fluorescens F113L::1180*gfp*	Bp^+, Nodbox *bph* fusion, Rif^R, Km^R, Pm::*gfp*mut3 inserted chromosomally	Liu *et al.* (2007)
Pseudomonas sp. B13	Wild type; benzoate$^+$ 3-CBA$^+$	Dorn *et al.* (1974)
Rhodococcus sp. ITCBP	Ars^R, polychlorinated biphenyl degrader	Sherlock (2003) Coffey *et al.* (2009)

Bp^+, Biphenyl degrader; Rif^R, Rifampicin resistant, Km^R, Kanamycin resistant; Benzoate$^+$, benzoate degrader; 3-CBA$^+$, 3-chlorobenzoate degrader; Ars^R, Arsenate resistant.

150 ml of nutrient broth (NB) at 30°C, 100 rev min^{-1} for 24 h. *Pseudomonas fluorescens* F113Rif*gfp* or *Ps. fluorescens* F113L::1180*gfp* were maintained on NA amended with 50 μg ml^{-1} of kanamycin (NA Km) and cultured in 150 ml of NB at 30°C, 100 rev min^{-1} for 24 h.

Total viable counts (TVC) in soils were calculated by sampling 1 g of soil and preparing a standard serial dilution followed by plating on NA plates. F113Rifpcb cells were enumerated by plating on NA Rif plates. Plates were incubated at 30°C for 48 h. To ensure that only the F113Rifpcb colonies were counted, all the colonies on the NA Rif plates were sprayed with 2,3-dihydroxybiphenyl (2,3-OHBP). Colonies which developed a bright yellow coloration (because of the formation of the yellow meta-cleavage product 2-hydroxy-6-oxo-6-phenylhexa-2, 4-dienoic acid, indicating the presence of the *bph* operon) were counted and presumed to represent the inoculum level within the sample (Liu *et al.* 2007).

Preparation of alginate encapsulated F113 derivative strains

The cell immobilization technique described by Bashan (1986) and Bettmann and Rehm (1984) was employed. Ten millilitres of overnight (O/N) cultures were added to 90 ml sterile 3% sodium alginate and mixed well. The mixture was then extruded through a 5-ml sterile syringe into a sterile 2% calcium chloride solution. The beads were allowed to harden for 30 min and were then harvested after washing with distilled water.

Survival of F113Rifpcb in alginate beads

Survival of F113Rifpcb encapsulated in alginate beads was monitored in beads stored in unsealed petri dishes at room temperature under nonsterile conditions. For cell enumeration, 10 beads were dissolved in 5 ml 2% sodium citrate (30 min shaking at 100 rev min^{-1}, followed by 1 min vortexing). Enumeration of both TVC and F113Rifpcb within the beads was carried out.

Effect of various additives on the survival of F113Rifpcb in alginate beads

The effect of incorporation of additives on the levels of F113Rifpcb in alginate beads was assessed. The additives were either 10% w/v skim milk powder (SMP) or soil extract (SE). Therefore, there were three treatments in total (i) alginate in distilled water (control), (ii) alginate + 10% SMP and (iii) alginate + SE. To prepare these, three 10-ml O/N cultures were grown. The cells were collected by centrifuging at 1700 ***g*** for 13 min. Cell pellets were resuspended in one of the following alginate mixtures (i) 3% alginate in distilled water (control), (ii) 3% alginate in soil extract (supernatant of 40 g soil with 150 ml distilled water centrifuged at 1700 ***g*** for 13 min) and (iii) 3% alginate with 10% w/v SMP. Each flask was shaken at 100 rev min^{-1} for 1 h, and the alginate beads were prepared as described before. Five of each bead type, control, SE and SMP were placed into separate petri dishes containing 40 g of sieved soil and then stored at room temperature.

Effect of inoculation method on the survival of F113Rifpcb in soil and colonization level in Alfalfa

Microcosms were prepared to evaluate various inoculation techniques with respect to rhizosphere colonization of alfalfa (*Medicago sativa*) by F113Rifpcb. The inoculation experiments were carried out in individual propagators, which were maintained at 18°C, with an 18/6-h light–dark regime, for 28 days. The experiment was carried out in pots which contained 150 g sieved soil, with a single alfalfa seed planted 1·5 cm below the surface. The inoculation techniques assessed were (i) spray inoculation – 3 ml of an O/N culture were sprayed on the soil surface at Day 0, (ii) alginate beads – a single bead containing F113Rifpcb was planted adjacent to the seed and (iii) F113rifpcb/seed co-encapsulation – the alginate encapsulated F113Rifpcb – was prepared as before, except a seed was placed at the top of the syringe, and the culture/alginate mixture was allowed to engulf the seed prior to contact with the $CaCl_2$. Control pots consisted of a F113Rifpcb encapsulated bead placed 1·5 cm below the soil surface in pots with no plants. Rhizosphere counts were carried out on a wet weight basis where the seedling was gently removed from the pot and excess soil was shaken off. The root and closely adhering soil were placed in 9 ml Ringers, vortexed for 2 min and then TVC and F113Rifpcb counts were carried out. TVC and F113Rifpcb counts in control pots were also carried out using 1 g soil samples.

Evaluation of PCB biosensor activity when encapsulated in alginate beads

Thirty alginate beads encapsulating either biosensor *Ps. fluorescens* F113Rif*gfp* or biosensor *Ps. fluorescens* F113L::1180*gfp* were introduced into 10-g samples of soil amended with 100 ppm 3-CBA or 3-chlorobiphenyl (3-CBP). For the purposes of comparison, nonencapsulated cells were introduced into similar soils at a rate of 1×10^8 cells g^{-1} soil.

To evaluate the effect of other CBA and PCB degraders on the encapsulated biosensors ability to detect these compounds in soil, similarly prepared soil samples were

set up that were first inoculated with either the 3-CBA degrader strain *Pseudomonas* sp. strain B13 or the PCB degrader strain *Rhodococcus* sp. ITCBP. Controls were set up using the biosensor or the CBA/PCB degraders alone.

Biosensor cells were visualized under a Nikon E400 epi-fluorescent microscope equipped with a 100-W mercury short arc photo-optic lamp and two filters with the excitation wavelength of 465–495 and 450–490 nm, respectively. Lucia® imaging software (ver. 4.6; Lucia, Prague, Czech Republic) was used to capture and process microscopic images. Nonfluorescent cells were visualized by staining with 0·1% acridine orange for 1 min which stained the bacterial cells red.

Statistical analysis

Results are averages of triplicate sampling, and standard deviations are expressed at the $P < 0{\cdot}05$ level of significance.

Results

Pseudomonas fluorescens F113Rifpcb was successfully encapsulated in calcium alginate beads that were *c.* 3 mm in diameter. The initial inoculum level used to prepare the beads contained $4{\cdot}6 \times 10^8$ CFU ml^{-1}. The beads were stored in a sealed petri dish, at room temperature under nonsterile conditions. Analysis of the beads 1 day after preparation showed that they contained $1{\cdot}0 \times 10^5$ CFU bead^{-1}. Forty days after preparation, the counts of F113Rifpcb within the beads dropped to 35% of that at day 1 (Fig. 1). The population of F113Rifpcb within the beads began to increase and by day 75, the population was nine times greater than those at day 1. Activity of the F113Rifpcb population could be detected by spraying the beads with 2,3-OHBP, which resulted in the beads turning yellow because of the enzymatic conversion of 2,3-OHBP to 2-hydroxy-6-oxo-6-phenylhexa-2, 4-dienoic acid by F113Rifpcb cells. After 75 days, there was a steady decline in the inoculum population. By day 265, the inoculum population had fallen to two times greater than that at day 1.

The effect of various additives on the survival of F113Rifpcb within the beads was assessed (Fig. 2). The additives were the addition of 10% SMP, aqueous SE and control beads with no additives. By day 30, the addition of 10% SMP had significantly increased the numbers of F113Rifpcb cells released into the soil compared to the SE and control beads. Only after 50 days did the SE amendment significantly increase the inoculum release compared to the control beads. By day 80, the inoculum level in the soil was similar for all three bead types.

An experiment was carried out to compare the effect of inoculation method on the colonization efficiency of alfalfa by F113Rifpcb. The results showed that F113Rifpcb colonized alfalfa equally well using the soil spray or F113Rifpcb/seed co-encapsulation method (Fig. 3a). However, the F113Rifpcb/seed co-encapsulation method resulted in significantly lower F113Rifpcb counts in the nonrhizosphere soil (Fig. 3b). The F113Rifpcb encapsulation method also resulted in significantly lower counts in the nonrhizosphere soil, which lead to slower colonization of the alfalfa roots. However, by 28 days there was no significant difference between the inoculum levels on the roots of alfalfa plants using the three inoculation methods. With the spray inoculation method, the inoculum became a dominant bacterial strain in the soil (2–9% of the TVC in the rhizosphere and 6–27% of the TVC in nonrhizosphere soil). Both F113Rifpcb encapsulation and F113Rifpcb/seed co-encapsulation methods lead to inoculum populations corresponding to 0·03–2% of the TVC in the rhizosphere and 0·01–0·08% of the TVC in nonrhizosphere soil.

Similar levels of Gfp expression were observed in free living and encapsulated biosensor strains F113Rif*gfp* and F113L::1180*gfp* when placed into 3-CBA and 3-CBP

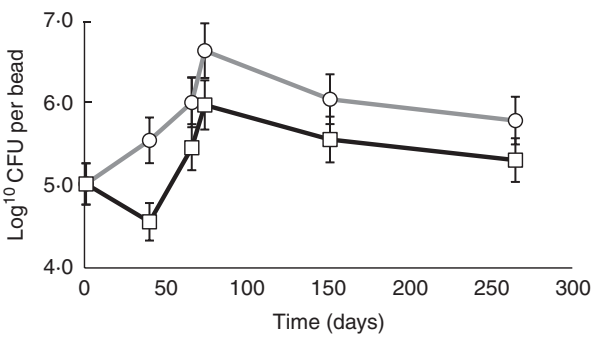

Figure 1 Survival of *Pseudomonas fluorescens* F113Rifpcb in alginate beads stored under ambient, nonsterile conditions. (○) Total viable counts and (□) F113Rifpcb.

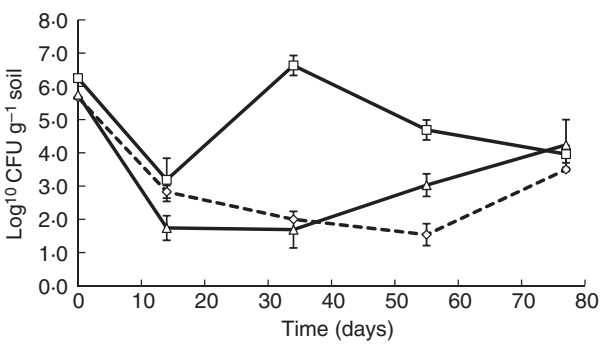

Figure 2 Release of F113Rifpcb into soil from alginate formulations. (□) Skim milk powder; (Δ) Soil extract and (◇) Control.

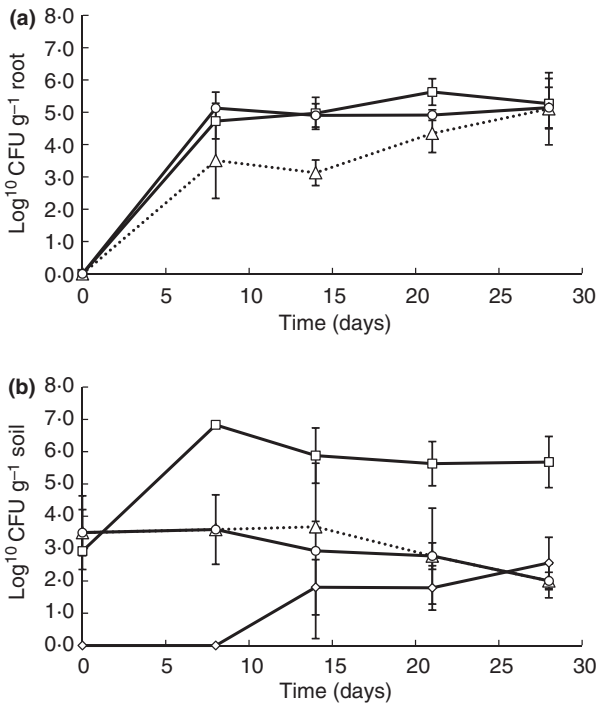

Figure 3 (a) Colonization of alfalfa roots by F113Rifpcb and (b) survival of F113Rifpcb in soil, using the various inoculation methods. (□) Soil spray; (Δ) F113Rifpcb encapsulated bead; (○) F113Rifpcb/seed co-encapsulation and (◇) Control F113Rifpcb encapsulated bead with no seeds.

Table 2 Percentage of cells expressing Gfp in 3-CBA or 3-CBP amended bulk soil 10 days following inoculation

	Nonencapsulated		Alginate encapsulated	
	3-CBA	3-CBP	3-CBA	3-CBP
F113L::1180*gfp*	100 (328) SD:0	30 (410) SD:11·9	100 (174) SD:0	30 (585) SD:11·6
F113Rif*gfp*	100 (416) SD:0	0·01 (444) SD:0	100 (307) SD:0	0·01 (427) SD:0
F113Rif*gfp* & *Pseudomonas* B13	0 (263) SD:0	Nd	0 (281) SD:0	Nd
F113L::1180*gfp* & *Pseudomonas* B13	0 (475) SD:0	0 (519) SD:0	0 (415) SD:0	10 (268) SD:4·6
F113Rif*gfp* & *Rhodococcus* ITCBP	Nd	30 (279) SD:14·9	Nd	50 (567) SD:14·2

Numbers in brackets correspond to total number of cells visualized i.e. 100%, Nd, not determined; SD, standard deviation; 3-CBA, 3-chlorobenzoate; 3-CBP, 3-chlorobiphenyl.

amended soil (Table 2). Almost all of the biosensors cells reported the presence of 3-CBA in 3-CBA amended soil, regardless of whether they were encapsulated or non-encapsulated. In contrast, in 3-CBP amended soil less than a third of the F113L::1180*gfp* biosensor cells were fluorescent in both free living and encapsulated samples. In 3-CBP amended soil, Gfp expression in F113Rif*gfp* (non-PCB degrader) fell to background levels of 0·01%. This background level of fluorescence was also noted in nonamended soils.

To test the effect of other CBA degraders on the sensing ability of the biosensors, they were each co-inoculated with the CBA degrader *Pseudomonas* sp. B13 and placed into 3-CBA amended soil. At the start of the experiment, Gfp expression could be observed in both biosensor strains (*c.* 80%), proving that they detected the initial presence of 3-CBA (Table 2) (Fig. 4a). However, after 10 days, no fluorescent cells could be visualized in the nonencapsulated biosensor samples, while 10% of the encapsulated biosensor cells were still expressing Gfp. To test the effect of other PCB degraders on the sensing ability of the F113Rif*gfp* biosensor, it was co-inoculated with the PCB degrader *Rhodococcus* sp. ITCBP and placed into 3-CBP amended soil. After 10 days, *c.* 30% of the non-encapsulated biosensor cells were expressing Gfp. However, a greater percentage of biosensors cells were expressing Gfp [*c.* 50% (with 100% = 567 cells visualized)] within the alginate beads (Table 2) (Fig. 4b). No fluorescent cells were visualized in the control samples using a pure culture of either F113Rif*gfp* or *Rhodococcus* sp. ITCBP.

Discussion

Under suitable storage conditions, *Ps. fluorescens* has been shown to survive in alginate beads for up to 14 years (Bashan and Gonzalez 1999) and for effective colonization of plants, inocula within alginate beads should be above 10^5 CFU bead^{-1} (Bashan 1986). The genetically modified micro-organisms *Ps. fluorescens* F113Rifpcb survived above this threshold in alginate beads for more than 250 days under ambient storage conditions. A related strain, F113LacZY, also displayed similar survival patterns in alginate but over a much shorter time period (Russo *et al.* 1996).

The incorporation of various additives into the sodium alginate solution has been shown to enhance inoculant survival and populations within alginate beads (Yu *et al.* 2001). Bashan *et al.* (2002) found the addition of SMP (0·75%) significantly increased the levels of the plant growth promoter (PGP) strain *Azospirillium brasilense* within alginate beads and showed that these beads degraded faster in soil than beads without SMP, resulting in a more rapid release of the inoculum. Vancov *et al.* (2005) found that the addition of 1% SMP to alginate encapsulated *Rhodoccoccus* strain NI86/21 increased their degradation rate of atrazine and enhanced the survival rate of the strain under storage conditions. In the current

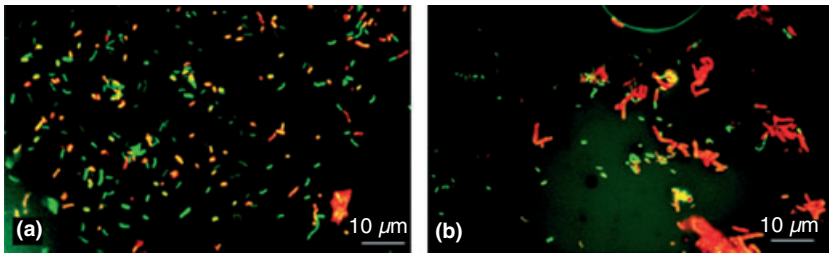

Figure 4 Alginate encapsulated biosensor F113Rif*gfp* co-inoculated with (a) the 3-chlorobenzoate (3-CBA) degrader *Pseudomonas* sp. B13 in 3-CBA amended soil (time: 0) and (b) the polychlorinated biphenyls degrader *Rhodococcus* sp. ITCBP in 3-chlorobiphenyl amended soil (time: 10 days). Green cells are F113Rif*gfp*, and red cells are non-*gfp* expressing cells.

study, the incorporation of 10% SMP significantly enhanced the release of F113rifpcb into the soil. The addition of SE increased inoculum level but only after 30 days. This may be because of the presence of complex compounds such as humic acid that provided extra nutrients to the bacterial cells. Young *et al.* (2006) found that the addition of humic acid (1%) to beads maintained inoculum levels of 10^5 CFU bead^{-1} (10^8 CFU g^{-1}) for up to 5 months of storage. Ivanova *et al.* (2005) showed that population levels of up to 10^9 CFU bead^{-1} could be maintained in alginate beads through the addition of starch coupled with drying the beads to *c.* 7% water.

The spray inoculation and F113rifpcb/seed co-encapsulation techniques were equally effective at establishing high inoculum counts on the roots of plants, resulting in *c.* 10^5 CFU g^{-1} root tissue. Bashan *et al.* (2002) found similar colonization levels when microalginate beads were used to inoculate tomato and wheat plants with the PGP strain *A. brasilense*. However, Young *et al.* (2006) found that encapsulation of their inoculum leads to enhanced plant root colonization (10^5–10^7 CFU cm^{-2} of root) compared with free cell inoculation (10^3–10^4 CFU cm^{-2} root).

Alginate encapsulation significantly increases inoculant robustness, storage shelf-life and requires minimum control of environmental storage conditions, therefore reducing the associated cost and handling labour (Stephen and Rask 2000). With the concerns over the release of genetically engineered micro-organisms, the encapsulation technique provides a more a targeted approach for plant inoculation compared to soil spraying and has potential for environmental applications (Cassidy *et al.* 1996).

A good inoculation technique must ensure that the inoculum not only establishes itself but also that it expresses the desired traits (Ramos *et al.* 1994). Encapsulation of strains in alginate does not appear to affect either plant growth promotion traits or xenobiotic degradation ability (Reed and Glick 2005). Tao *et al.* (2009) showed that beads prepared with 3% sodium alginate beads have good mass transfer performance and allowed polyaromatic hydrocarbons such as phenanthrene to enter the bead and come into contact with the degrading inoculum.

In the current study, encapsulation of the two *gfp*-based PCB biosensors (F113Rif*gfp* and F113L::1180*gfp*) in 3% calcium alginate beads did not adversely affect their ability to detect 3-CBA in amended soil nor did it affect biodegradation rate of 3-CBP by F113L::1180*gfp* in amended soil. This suggests that both CBAs and PCBs effectively transfer through the alginate matrix. CBAs are rapidly degraded by indigenous soil microbes which can prevent the biosensors from detecting active PCB biodegradation. The results from this study showed that in the presence of CBA and CBP degraders, biosensor *gfp* expression levels were higher within the beads compared to free living cells. This higher level of *gfp* expression is probably because of niche exclusion, where the existing biosensor population prevents the proliferation of indigenous CBA degraders inside the alginate bead, resulting in a build up of CBA within the bead, leading to greater induction of the biosensor.

In a recent report by Liu *et al.* (2010), there was a positive correlation between the PCB levels within three different PCB contaminated environmental samples and the percentage of biosensor F113L::1180 cells that were fluorescent. The level of *gfp* expression within F113L::1180*gfp* in these samples correlated positively with the PCB concentration. The containment of the biosensor cell within the alginate beads allows for easy recovery and visualization of the biosensor cells from environmental matrices and may provide a practical approach for the assessment of biodegradation potential of PCB contaminated soils.

Acknowledgements

This work was funded in part by EU contracts QLK3-CT2000-00164 & QLRT-2001-00101 and the HEA funded TSR III project 'Agribiotics'.

References

Bashan, Y. (1986) Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. *Appl Environ Microbiol* **51**, 1089–1098.

Bashan, Y. and Gonzalez, L.E. (1999) Long-term survival of the plant-growth-promoting bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* in dry alginate inoculant. *Appl Microbiol Biotechnol* **51**, 262–266.

Bashan, Y., Hernandez, J.-P., Leyva, L.A. and Bacilio, M. (2002) Alginate microbeads as inoculants carriers for plant growth promoting bacteria. *Biol Fertil Soils* **35**, 359–368.

Belkin, S. (2003) Microbial whole-cell sensing systems of environmental pollutants. *Curr Opin Microbiol* **6**, 206–212.

Bettmann, H. and Rehm, H.J. (1984) Degradation of phenol by polymer entrapped microorganisms. *Appl Microbiol Biotechnol* **20**, 285–290.

Brazil, G.M., Kenefick, L., Callanan, M., Haro, A., De Lorenzo, V., Dowling, D.N. and O'Gara, F. (1995) Construction of a rhizosphere Peudomonad with potential to degrade polychlorinated biphenyls and detection of *bph* gene expression in the rhizosphere. *Appl Environ Microbiol* **61**, 1946–1952.

Cassidy, M.B., Lee, H. and Trevors, J.T. (1996) Environmental applications of immobilized microbial cells: a review. *J Ind Microbiol* **16**, 79–101.

Coffey, L., Clarke, A., Duggan, P., Tambling, K., Horgan, S., Dowling, D. and O'Reilly, C. (2009) Isolation of identical nitrilase genes from multiple bacterial strains and real-time PCR detection of the genes from soils provides evidence of horizontal gene transfer. *Arch Microbiol* **191**, 761–771.

Dorn, E., Hellwig, M., Reineke, W. and Knackmuss, H.J. (1974) Isolation and characterization of a 3-chlorobenzoate degrading Peudomonad. *Arch Microbiol* **99**, 61–70.

Goris, J., De Vos, P., Caballero-Mellado, J., Park, J., Falsen, E., Quensen, J.F. III, Tiedje, J.M. and Van Damme, P. (2004) Classification of the biphenyl-and polychlorinated biphenyl-degrading strain LB400T and relatives as *Burkholderia xenovorans* sp. nov. *Int J Syst Evol Microbiol* **54**, 1677–1681.

Ivanova, E., Teunou, E. and Poncelet, D. (2005) Alginate based macrocaPules as inoculants carriers for production of nitrogen biofertilisers. In Proceedings of the Balkan scientific conference of Biology. Bulgaria, Plovdiv May 19–21, 2005. pp. 90–108.

Jezequel, K. and Lebeau, T. (2008) Soil bioaugmentation by free and immobilised bacteria to reduce potentially phytoavailable cadmium. *Bioresour Technol* **99**, 690–698.

Liu, S. and Suflita, J.M. (1993) Ecology and evolution of microbial populations for bioremediation. *Trends Biotechnol* **11**, 344–352.

Liu, X., Germaine, K.J., Ryan, D. and Dowling, D.N. (2007) Development of an alginate encapsulated *gfp*-based biosensor for detecting the bioavailability and biodegradation of polychlorinated biphenyls and chlorobenzoates. *J Environ Eng Landsc Manage* **4**, 261–268.

Liu, X., Germaine, K.J., Ryan, D. and Dowling, D.N. (2010) Genetically modified Pseudomonas biosensing biodegraders to detect PCB and chlorobenzoate bioavailability and biodegradation in contaminated soils. *Bioeng Bugs* **1**, 1–9.

McGuinness, M. and Dowling, D. (2009) Plant-associated bacterial degradation of toxic organic compounds in soil. *Int J Environ Res Public Health* **6**, 2226–2247.

Moller, S., Sternberg, C., Andersen, J.B., Christensen, B., Ramos, J., Givskow, M. and Molin, S. (1998) In situ gene expression in mixed-culture biofilms: evidence of metabolic interactions between community members. *Appl Environ Microbiol* **64**, 721–732.

Naseby, D.C. and Lynch, J.M. (1999) Effects of *Pseudomonas fluorescens* on ecological functions in the pea rhizosphere are dependent on pH. *Microbiol Ecol* **37**, 248–256.

Ramos, J.L., Díaz, E., Dowling, D.N., de Lorenzo, V., Molin, S., O'Gara, F., Ramos, C. and Timmis, K.N. (1994) The behaviour of bacteria designed for biodegradation. *BioTech* **12**, 1349–1356.

Reed, M.L. and Glick, B.R. (2005) Growth of canola (*Brassica napus*) in the presence of plant growth promoting bacteria and either copper or polycyclic aromatic hydrocarbons. *Can J Microbiol* **51**, 1061–1069.

Rein, A., Fernqvist, M.M., Mayer, P., Trapp, S., Bittens, M. and Karlson, U.G. (2007) Degradation of PCB congeners by bacterial strains. *Appl Environ Microbiol* **77**, 469–481.

Robinson, G.K. and Lenn, M.J. (1994) The bioremediation of polychlorinated biphenyls (PCBs): problems and perspectives. *Biotechnol Genet Eng Rev* **12**, 139–188.

Russo, A., Moënne-Loccoz, Y., Fedi, S., Higgins, P., Fenton, A., Dowling, D.N., O'Regan, M. and O'Gara, F. (1996) Improved delivery of biocontrol *Pseudomonas* and their antifungal metabolites using alginate polymers. *Appl Microbiol Biotechnol* **44**, 740–745.

Sahasrabudhe, S.R., Modi, A.J. and Modi, V.V. (1988) Dehalogenation of 3-chlorobenzoate by immobilized *Pseudomonas* sp. B13 cells. *Biotech Bioeng* **31**, 889–893.

Shanahan, P., O'Sullivan, D.J., SimPon, P., Glennon, J.D. and O'Gara, F. (1992) Isolation of 2, 4-diacetylphloroglucinol from a fluorescent Peudomonad and investigation of physiological parameters influencing its production. *Appl Environ Microbiol* **58**, 353–358.

Sherlock, O. (2003) A molecular based detection system for a PCB degrading *Pseudomonas* and isolation of variants of the *bphC* gene. PhD thesis, Institute of Technology Carlow, Carlow, Ireland.

Simons, M., Vanderbij, A.J., Brand, I., de Weger, L.A., Wijffelman, C.A. and Lugtenberg, B.J.J. (1996) Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. *MPMI* **9**, 600–607.

Stephen, J.H. and Rask, H.M. (2000) Inoculum production and formulation. *Field Crop Res* **65**, 249–258.

Tao, X.Q., Lu, G.N., Liu, J.P., Li, T. and Yang, L.N. (2009) Rapid degradation of phenanthrene by using *Sphingomonas* sp. GY2B immobilised in calcium alginate gel beads. *Int J Environ Res Public Health* **6**, 2470–2480.

Trogl, J., Ripp, S., Kuncova, G., Sayler, G.S., Churava, A., Parik, P., Demnerova, K., Halova, J. *et al.* (2005) Selectivity of

whole cell optical biosensor with immobilized bioreporter *Pseudomonas fluorescens* HK44. *Sen Actuat* **107**, 98–103.

Vancov, T., Jury, K. and Van Zweiten, L. (2005) Atrazine degradation by encapsulated *Rhodococcus erythropolis* NI86. *J Appl Microbiol* **99**, 767–775.

Villacieros, M., Power, B., Sánchez-Contreras, M., Lloret, J., Oruezabal, R.I., Martín, M., Fernández-Piñas, F., Bonilla, I. *et al.* (2003) Colonization behaviour of *Pseudomonas fluorescens* and *Sinorhizobium meliloti* in the alfalfa (*Medicago sativa*) rhizosphere. *Plant Soil* **251**, 47–54.

Villacieros, M., Whelan, C., Mackova, M., Molgaard, J., Sánchez-Contreras, M., Lloret, J., Aguirre de Cárcer, D., Oruezábal, R.I. *et al.* (2005) Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens* F113 derivatives, using a *Sinorhizobium meliloti* nod system to drive *bph* gene expression. *Appl Environ Microbiol* **71**, 2687–2694.

Young, C.C., Rekha, P.D., Lai, W.A. and Arun, A.B. (2006) Encapsulation of plant growth promoting bacteria in alginate beads enriched with humic acid. *Biotech Bioeng* **95**, 76–83.

Yu, W.K., Yim, T.B., Lee, K.Y. and Heo, T.R. (2001) Effect of skim milk-alginate beads on survival rate of *Bifidobacteria*. *Biotechnol Bioproc Eng* **6**, 133–138.