Survival of a Genetically Engineered *Pseudomonas putida* Strain in Soil and its Potential to Conjugatively Transfer Genes to Indigenous Soil Bacteria

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Abstract

There is an interest in releasing genetically engineered bacteria into the environment as biofertilizers, biological control agents or to remediate contaminated soil. However, there is little information available on the factors affecting the persistence of genetically engineered bacteria or their potential to transfer genes to indigenous soil microorganisms. This study assessed the survival of a genetically engineered *Pseudomonas putida* strain CR30RNS (pADPTel) in bulk and rhizosphere soil and its ability to transfer genes for atrazine degradation and tellurite resistance to indigenous soil bacteria. The *P. putida* strain survived for ten weeks in bulk and rhizosphere soil at approximately 10^6 cfu per g soil. After the plants were harvested, the population of the *P. putida* strain declined to undetectable levels. However, upon the addition of water and a nutrient solution containing atrazine, the *P. putida* CR30RNS (pADPTel) population in the rhizosphere soil rebounded to a density of ca. 10^5 cfu per g soil while the population in bulk soil remained undetectable. The frequency of conjugative gene transfer to indigenous soil bacteria was assessed under laboratory conditions by the use of filter matings. Under optimal conditions a range of $1\text{-}10^{-2}$ transconjugants per recipient was observed. Recipient bacteria were identified by FAME analysis as *Rahnella aquatilis* strains. The results indicate that the genetically engineered bacteria survived better in the rhizosphere of canola than in bulk soil, and had the potential to transfer genes to indigenous soil bacteria.

Introduction

There is an interest in releasing genetically engineered bacteria into the environment as biofertilizers, biological control agents or to remediate contaminated soils [4, 5, 6]. For example, *Bradyrhizobium japonicum* was engineered to contain copies of the *nif* gene promoter from *Rhizobium meliloti*, and the *dct* genes from *Rhizobium leguminosarum* resulting in increased nitrogen uptake by soybean plants and thus increased crop yields [4]. However, few genetically engineered microorganisms intended for environmental release are commercially available due to the potential risks associated with their release. Prolonged survival of the genetically engineered microorganism and the transfer of genetic material are potential risks associated with the environmental release of a genetically engineered microorganism. Microcosms have been successfully used to study ecological mechanisms [11]. Microcosms simulate environmental conditions under contained laboratory systems and thus are feasible for assessing the risks

associated with the release of a genetically engineered microorganism. Key issues in the pre-release risk assessment of a genetically engineered microorganism are its post release fate, its affect on indigenous microbial populations, and its potential to transfer genes to indigenous soil microorganisms. Factors affecting the fate of an introduced genetically engineered microorganism are its phenotypic and genotypic properties [2, 3, 4, 8], the indigenous microbial populations [8], and the characteristics of the receiving environment [10]. Horizontal gene transfer can occur by three mechanisms: transformation, transduction, and conjugation. A considerable proportion of gene transfer in soil is accounted for by conjugation events [9]. This study assessed the survival of a genetically engineered *Pseudomonas putida* strain in soil and the rhizosphere of canola, and its potential to transfer genes to indigenous soil bacteria.

Materials and Methods

Bacterial Strains, Culture Medium, and Growth Conditions

Pseudomonas putida CR30RNS (pADPTel) was isolated from the rhizosphere of *Brassica napus* (canola) and genetically engineered by Cam Wyndham (Carleton University) to contain a 108-kb plasmid that encodes the genes for tellurite resistance and atrazine degradation. The natural resistance of strain CR30 against rifampicin, nalidixic acid, and streptomycin was increased to 100 ìg/ml by selective pressure. *Pseudomonas putida* CR30RNS (pADPTel) was cultured at 28C on glucose -atrazine (Gatz) overlay media containing the appropriate selective agents.

Indigenous soil bacteria were isolated from Watrous , SK test soil in November 1999 using a non-selective rich medium. Cultures were stored at 4C.

Survival Experiments

Survival of the genetically engineered microorganism (GEM) was assessed in soil and the rhizosphere of canola by the use of soil microcosms [1, 4]. The soil utilized for this study was obtained from a field site in Watrous, SK. Each microcosm was inoculated with *P. putida* CR30RNS (pADPTel) to a density of ca. 10^6 cfu g dry soil^{-1}. The GEM was extracted, diluted, and plated onto gatz overlay plates that were counted following five days incubation.

Mating Experiments

Mating experiments were conducted using *P. putida* CR30RNS (pADPTel) as the donor organism and indigenous soil bacteria as recipient organisms. Recipient microorganisms were isolated from the test soil by a non-selective rich medium. The frequency of conjugative gene transfer to indigenous soil bacteria was assessed under laboratory conditions by the use of filter matings. The cells were recovered from the filters, diluted, and plated onto non-selective rich medium to recover possible transconjugants. The number of transconjugants was determined using selective and/or differential media that allowed the colonies to be distinguished phenotypically (Figure 1). A portion of the resulting transconjugants were isolated and identified by the MIDI system according to their FAME profiles [7].

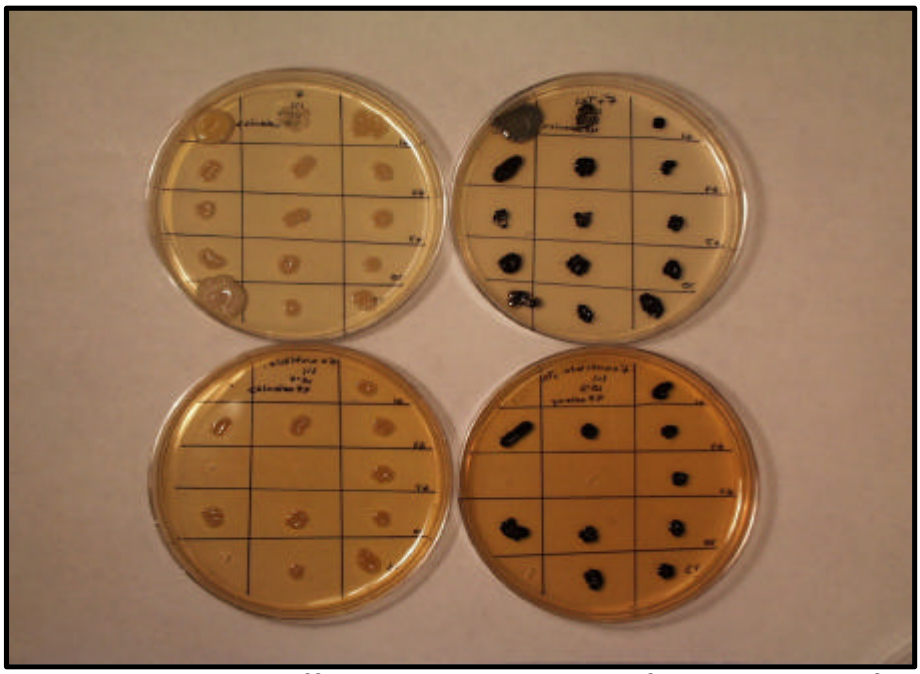


Figure 1. Selective/Differential media technique for the isolation of putative transconjugants and the determination of conjugation frequency.

Results

Survival of a Genetically Engineered *Pseudomonas putida* Strain CR30RNS (pADPTel) in Bulk and Rhizosphere Soil

P. putida CR30RNS (pADPTel) survived for ten weeks in the bulk and rhizosphere soil at ca. 10^6 cfu g soil $^{-1}$. Initially the same, however one month after the plants were harvested the population of the *P. putida* strain was ca. three $\log_{10}$ higher in the rhizosphere as compared to bulk soil. By the third month following harvest, the population of *P. putida* CR30RNS (pADPTel) had declined to undetectable levels in both the bulk soil and canola rhizosphere. However, upon the addition of water and a nutrient solution containing atrazine, the *P. putida* CR30RNS (pADPTel) population in the rhizosphere rebounded to a density of ca. 10^5 cfu g soil^{-1} while the population in the bulk soil remained undetectable (Figure 2).

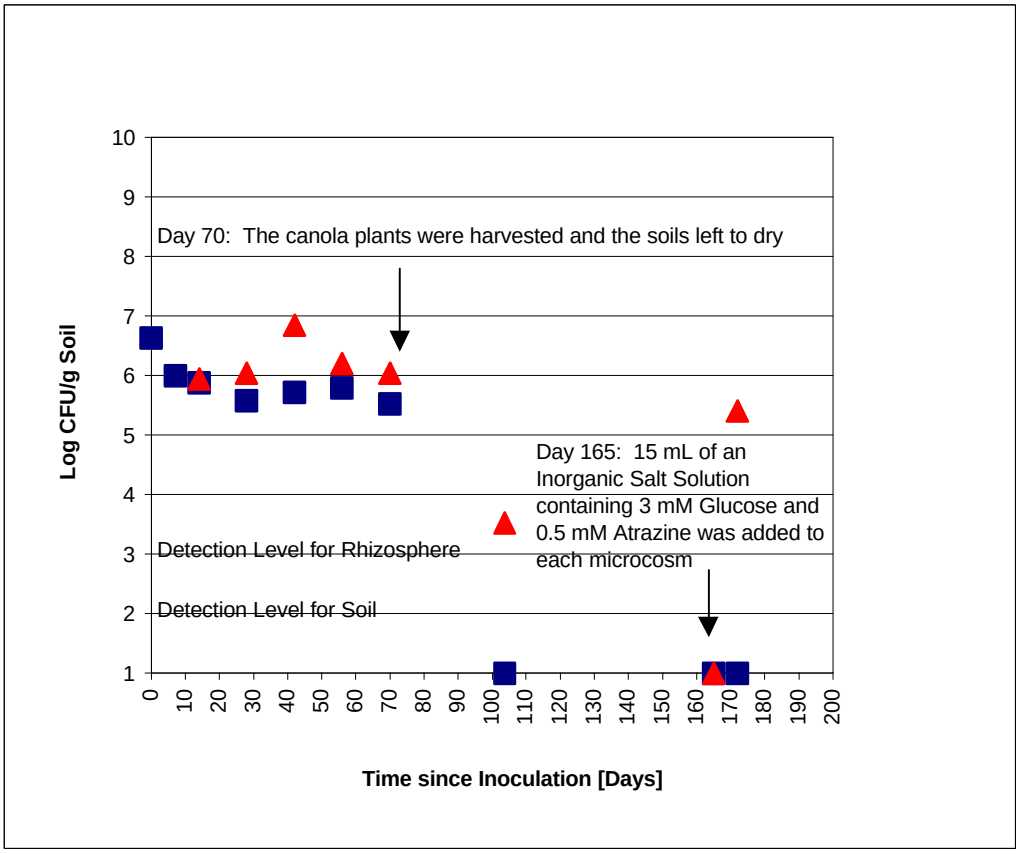
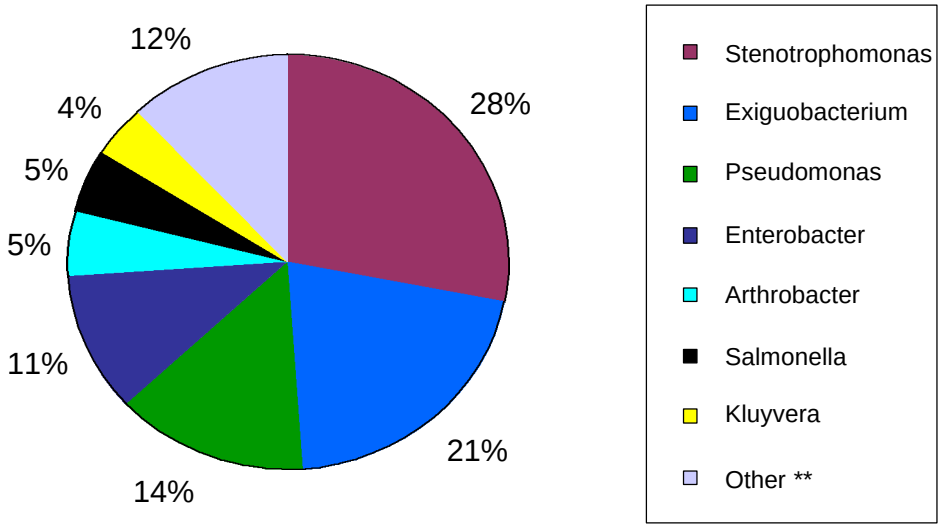


Figure 2. Survival of *Pseudomonas putida* CR30RNS(pADPTel) in the soil and the rhizosphere of the test soil. Triangles denote the rhizosphere samples and the squares denote the soil samples.

Conjugal Transfer of Plasmid pADPTel from Pseudomonas putida CR30RNS (pADPTel) to Indigenous Soil Bacteria

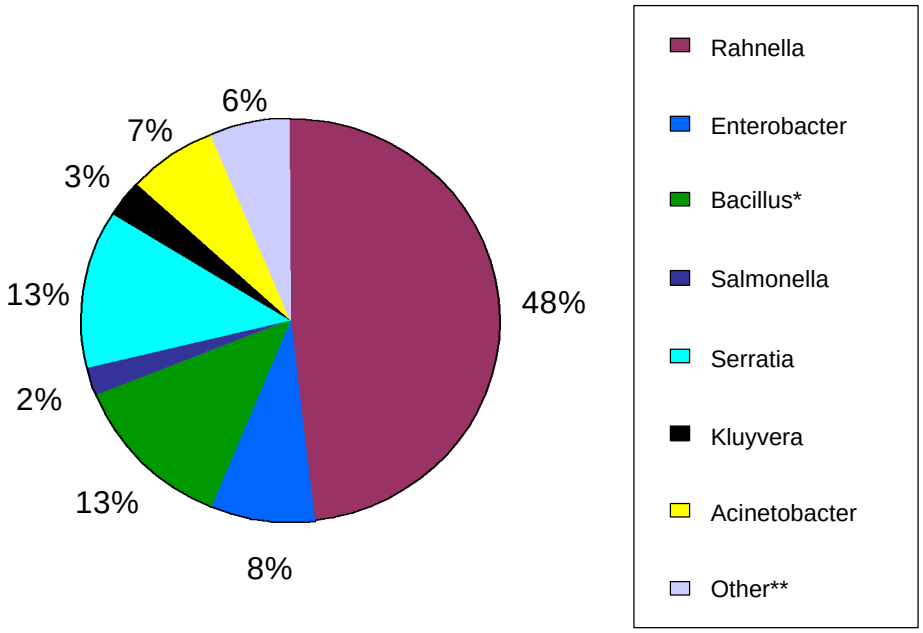
The frequency of conjugal gene transfer, of the pADPTel plasmid, to bacteria indigenous to the test soil was determined under optimal laboratory conditions. In the lab, a range of $1\text{-}10^{-2}$ transconjugants per recipient was observed.

Bacteria indigenous to the test soil were isolated and identified by the MIDI system according to their FAME profile. The vast majority of bacteria isolated from the test soil were *Stenotrophomonas* sp. (n = 16) and *Exiguobacterium* sp. (n = 12) (Figure 3). Despite the large proportions of *Stenotrophomonas* and *Exiguobacterium* species, the majority of transconjugants were identified as *Rahnella aquatilis* (n = 67) (Figure 4). No *Rahnella* species were isolated from the test soil by the non-selective rich medium.



** 4% *Bacillus*, 2% *Sphingobacterium*, 2% *Xanthomonas*, 2% *Curtobacterium*, 2% *Cellulomonas* (some have SIM < 0.5 and thus are not considered reliable)

Figure 3. Bacterial genera indigenous to the test soil.



* Includes *Bacillus* ssp. and *Paenibacillus* sp.
** 1% *Nesterenkonia*, 1% *Cellulomonas*, 1% *Microbacterium*, 1% *Pseudomonas*, 1% *Listeria*, 1% *Yersinia* (some have SIM < 0.5 and thus are not considered reliable)

Figure 4. Relative taxonomic groupings of the putative transconjugants.

Conclusions

Pseudomonas putida CR30RNS (pADPTel) survived better in the rhizosphere of canola than in bulk soil, and had the potential to transfer genes for atrazine degradation and tellurite resistance to indigenous soil bacteria at high frequencies under optimal laboratory conditions. A large portion of the putative transconjugants were identified as *Rahnella aquatilis* strains (48%) indicating that the transconjugant population was not representative of the indigenous soil bacterial population.

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