Annotated Bibliography on Environmental and Ecological Impacts of Transgenic Microorganisms

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Dubey, S. K. and Roy, U. 2003. Biodegradation of tributyltins (organotins) by marine bacteria. *Appl Organomet Chem* **17**: 3-8.

Many marine bacterial strains have an inherent capability to degrade toxic organotin compounds, especially tributyltins (TBTs), that enter into the environment in the form of insecticides, fungicides and antifouling paints as a result of anthropogenic and industrial activities. Significant degradation of these compounds in the ambient environment may take several years, and it is necessary to consider methods or strategies that can accelerate the degradation process. There have been few demonstrations of biological degradation of these organotin biocides exclusively in laboratory-scale experiments. Compared with the few bench-scale degradation processes, there are no reports of field-scale processes for TBT bioremediation, in spite of its serious environmental threat to nontarget organisms in the aquatic environment. Implementation of field- scale biodegradation of TBT requires inputs from biology, hydrology, geology, chemistry and civil engineering. A framework is emerging that can be adapted to develop new processes for bioremediation of toxic environmental wastes. In the case of TBT bioremediation, this framework incorporates screening and identification of natural bacterial strains, determination of optimal conditions for growth of isolates and TBT degradation, establishment of new metabolic pathways involved in TBT degradation, identification, localization and cloning of genes involved in degradation and in TBT resistance, development of suitable microbial strains using genetic manipulation techniques for practical applications and optimization of practical engineering processes for bioremediation of organotin-contaminated sites. The present review mainly addresses the aspect of TBT biodegradation with special reference to environmental sources of TBT, chemical structure and biological activity, resistant and degrading bacterial strains, possible mechanisms of resistance and degradation and the genetic and biochemical basis of TBT degradation and resistance. It also evaluates the feasibility and potential of natural and genetically modified TBT-degrading bacterial strains in field-scale experiments to bioremediate TBT-contaminated marine sites, and makes recommendations for more intensive and focused research in the area of TBT bioremediation mediated by marine bacterial strains.

Clark, I. M., Mendum, T. A. and Hirsch, P. R. 2002. The influence of the symbiotic plasmid pRL1JI on the distribution of GM rhizobia in soil and crop rhizospheres, and implications for gene flow. *Antonie Van Leeuwenhoek* **81**: 607-616.

The distribution of two genetically modified Rhizobium leguminosarum strains was investigated in the field. One, RSM2004, released in 1987, carries a Tn5 marker on its conjugative symbiotic plasmid (pSym). The second, CT0370, released at the same site in 1994, has a gusA gene integrated into its chromosome but no pSym. Plate counts indicated that the CT0370 population became established at a higher level than RSM2004. However, when peas, alfalfa and barley were grown, RSM2004 was found to outnumber CT0370 on all roots and by 100- fold on pea. Although the transfer of pSym from RSM2004 to CT0370 could be detected on plates and in microcosm studies with high inoculum densities, no transfer was detected in the field. Subsequent transfer of pSym from RSM2004 to CT0370 demonstrated that it conferred an advantage in the rhizosphere. In addition to increasing host fitness, plasmids may transfer, or mobilise other genetic elements, to other bacteria. This is more likely in sites such as the rhizosphere, where cells are active and numbers are high. The distribution of pSym and other genetic elements associated with rhizobia, in bacterial sub- populations from the soil and roots of the different plants, was investigated using PCR. The genetic elements studied were: ISRm3, an insertion element from Sinorhizobium meliloti; pSB102, a broad host range mer plasmid; the Rhizobium nodC gene (carried on pSym) and plasmid replication origins repCI and repCII. As expected, ISRm3 was detected in rhizoflora cultured from alfalfa but not the other plants. The mer gene was ubiquitous but the transfer region of pSB102 was not detected. The nodC and both repC primers amplified products from all the plants, giving further evidence for the occurrence of plasmids originating from Rhizobium in the rhizoflora of non-host plants. Despite the abundance of elements associated with transferable plasmids in rhizobia, none was detected in either inoculant strain.

Ferguson, G. C., Heinemann, J. A. and Kennedy, M. A. 2002. Gene transfer between Salmonella enterica serovar Typhimurium inside epithelial cells. *J Bacteriol* **184**: 2235-2242.

Virulence and antibiotic resistance genes transfer between bacteria by bacterial conjugation. Conjugation also mediates gene transfer from bacteria to eukaryotic organisms, including yeast and human cells. Predicting when and where genes transfer by conjugation could enhance our understanding of the risks involved in the release of genetically modified organisms, including those being developed for use as vaccines. We report here that Salmonella enterica serovar Typhimurium conjugated inside cultured human cells. The DNA transfer from donor to recipient bacteria was proportional to the probability that the two types of bacteria occupied the same cell, which was dependent on viable and invasive bacteria and on plasmid tra genes. Based on the high frequencies of gene transfer between bacteria inside human cells, we suggest that such gene transfers occur in situ. The implications of gene transfer between bacteria inside human cells, particularly in the context of antibiotic resistance, are discussed.

Hails, R. S., Hernandez-Crespo, P., Sait, S. M., Donnelly, C. A., Green, B. M. and Cory, J. S. 2002. Transmission patterns of natural and recombinant baculoviruses. *Ecology* **83**: 906-916.

The advent of genetically modified organisms such as pathogens has raised ecological questions that need to be addressed in order to assess any risks involved in their use, The baculovirus Autographa californica nucleopolyhedrovirus (AcNPV), which infects a number of lepidopteran species, has been modified to express an insect-selective toxin, This genetic modification increases the speed with which it kills its host. However, in addition to this intended feature of the modified virus, there may be other consequences for the host- pathogen interaction. We report a field experiment in which transmission patterns of the wild-type and the genetically modified baculovirus are measured within and between a model target (susceptible) and nontarget (less susceptible) lepidopteran species. Two foliar feeders were chosen: Trichoplusia ni, the cabbage looper, is highly susceptible to this pathogen, while Mamestra brassicae, the cabbage moth, is semipermissive. These two species are used as both the source and the recipients of infection for both virus types. A series of models are fitted to determine the probabilities of infection (given survival from other sources of mortality) over a 7-d period within contained field cages. Fitting these models to data illustrates teat a substantial fraction of the population escapes infection, and it is the size of the pathogen-free refuge that varies between treatments. When infected individuals from the less susceptible species die, the yield of virus is greater than from susceptible hosts, yet this does not significantly alter the risk of transmission to other hosts. In contrast, the genetically modified baculovirus always results in a lower risk of infection in the field compared to the wild type. This is because the recombinant virus causes paralysis, and as a result, the cadaver may fall from the plant before death and virus release. Hence the number of cadavers remaining on the foliage has a greater influence on transmission than the yield of virus from those cadavers.

Noordover, J. A. C., Hofmeester, J. J. M., van der Burg, J. P., de Leeuw, A., van Dijck, P. W. M., Luiten, R. G. M. and Groot, G. S. P. 2002. Containment in industrial biotechnology within wastewater treatment plants. *J Ind Microbiol Biotechnol* **28**: 65-69.

Both physical and biological containment are considered to be essential parts in the risk analysis of industrial Good Industrial Large-Scale Practice (GILSP) processes using genetically modified organisms (GMOs). Biological containment of industrial microorganisms has become a more important issue since the introduction of recombinant DNA techniques. In the event of an accidental discharge in the production plant, a large amount of organisms could be released into the wastewater treatment (WWT) system. This WWT system should therefore be considered as a part of the containment. This study demonstrates both a hydrodynamic and a microbiological model for the containment aspects of industrial WWT plants. The models are verified by measurements using industrial hosts of GILSP GMOs at full scale. Both models describe the full-scale equipment accurately. The results are supplemented with microcosm studies on survival of GMOs in defined niches. It is shown that WWT plants can be considered as useful additional parts of the containment of microorganisms, in case of an accidental discharge. The effect of drainage of an enormous amount of microorganisms (several tons) through the WWT plant into the environment is shown to be comparable to the direct drainage of a small-scale fermenter. Microcosm experiments correlate well with the survival rates in the WWT and therefore can be of use to predict the behaviour of GMOs in this environment.

Dushoff, J. and Dwyer, G. 2001. Evaluating the risks of engineered viruses: Modeling pathogen competition. *Ecol Appl* **11**: 1602-1609.

Recently there has been a great deal of interest in the potential use of genetically engineered baculoviruses as environmentally benign insecticides. Because baculoviruses often have a significant impact on the population dynamics of their hosts, any effort to assess the environmental impact of releasing engineered viruses must confront the question: Will genetically engineered baculoviruses outcompete wild-type strains, thereby altering the natural population dynamics of the host? To begin to answer this question, we develop a mathematical model of competitive interactions between genetically engineered and wild-type baculoviruses. We find that the interactions between these viruses are characterized mostly by dominance of one strain or the other, and that the chance that an engineered strain will outcompete a wild-type strain depends on its particular combination of speed of kill and infectiousness. That is, baculoviruses must kill their host to become infectious, so the faster speed of kill of most recombinant viruses confers a competitive advantage. Most such strains, however, also produce fewer infectious particles and so are less infectious. Our model shows that the extent of this decrease in infectiousness must be rather small for an engineered strain to become dominant. Nevertheless, even engineered strains that are at a substantial competitive disadvantage relative to the wild type may take decades to go extinct. An additional complicating factor is that the outcome of competition depends on the overwinter survival of these viruses, about which little is

known even for wild-type viruses. Caution is therefore necessary in predicting the outcome of competitive interactions involving introduced baculoviruses, and further work is needed in understanding pathogen overwinter survival rates.

England, L. S., Trevors, J. T. and Holmes, S. B. 2001. Extraction and detection of baculoviral DNA from lake water, detritus and forest litter. *J Appl Microbiol* **90**: 630-636.

Aims: This paper describes a quick, reproducible, sensitive method for baculoviral DNA extraction, purification and detection from freshwater and forest litter environments. Methods and Results: The extraction protocol utilizes enzymatic and chemical lysis and physical disruption. To assess the efficiency of the extraction and purification protocol, PCR was used to detect a 530 bp DNA fragment from the genome of a genetically-modified baculovirus, Choristoneura fumiferana NPVegt(-)/lacZ(+). The detection limit of PCR amplification nias routinely about 4.1 x 10(2) occlusion bodies (OBs) 450 mul(-1) lake water. Template DNA from the detritus and forest litter samples required 100-fold dilutions before use in PCR reactions. The detection limits for detritus and forest litter samples were routinely about 7.41 x 10(3) and 2.08 x 10(4) OBs 0.5 g(-1) dry weight, respectively. Conclusions: The DNA extraction and purification methodology is reproducible, sensitive and can be used in lieu of, or in conjunction with, insect bioassays. Significance and Impact of the Study: The DNA extraction and purification protocol described in this paper will facilitate risk assessment and ecological studies of both wild-type and genetically-modified baculoviruses.

Gagliardi, J. V., Buyer, J. S., Angle, J. S. and Russek-Cohen, E. 2001. Structural and functional analysis of whole-soil microbial communities for risk and efficacy testing following microbial inoculation of wheat roots in diverse soils. *Soil Biol Biochem* **33**: 25-40.

The increasing use of genetically engineered or modified microorganisms (GEMs) has led to regulations regarding the safety of their use. Intended (target) effects and unintended (non-target) effects of GEMs must currently be evaluated prior to field testing or commercial use. We present soil and rhizosphere microbial community effects testing of two GEMs, Pseudomonas chlororaphis 3732RN-L11 and Pseudomonas fluorescens 2-79RN-L3, parental strains of these organisms and an uninoculated treatment using five diverse soils planted to wheat. An assay using BIOLOG(R) GN plates measured microbial community functional responses on wheat roots with adhering soil. Overall differences using multivariate statistical methods were highest at inoculation, and these effects persisted while the inoculated organisms were detectible on selective media. Differentiation based on lacZY genes engineered to the chromosome of both GEMs was significant for the 3732 GEM in all five soils tested, but not for the 2-79 GEM in a single soil. Lactose utilization in uninoculated microbial communities varied around a low baseline value. Direct fatty acid extraction and analysis of soil from around wheat roots was also performed using a novel method. Fatty acid analysis differentiated the 3732 GEM from all other treatments, but did not distinguish the 3732 parent inoculated from uninoculated treatments. As with the BIOLOG assay, multivariate statistical differences from fatty acid analysis decreased between GEM inoculated and uninoculated populations as viable counts of the GEM declined. Neither assay showed measurable community-level effects when inoculated organisms declined below detection, though three of six soils with surviving GEM populations still had significant effects after 105 days. Published by Elsevier Science Ltd.

Girlanda, M., Perotto, S., Moenne-Loccoz, Y., Bergero, R., Lazzari, A., Defago, G., Bonfante, P. and Luppi, A. M. 2001. Impact of biocontrol Pseudomonas fluorescens CHA0 and a genetically modified derivative on the diversity of culturable fungi in the cucumber rhizosphere. *Appl Environ Microbiol* **67**: 1851-1864.

Little is known about the effects of Pseudomonas biocontrol inoculants on nontarget rhizosphere fungi. This issue was addressed using the biocontrol agent Pseudomonas fluorescens CHA0-Rif, which produces the antimicrobial polyketides 2,4- diacetylphloroglucinol (Phl) and pyoluteorin (Plt) and protects cucumber from several fungal pathogens, including Pythium spp., as well as the genetically modified derivative CHA0- Rif(pME3424). Strain CHA0-Rif(pME3424) overproduces Phl and Pit and displays improved biocontrol efficacy compared with CHA0- Rif. Cucumber was grown repeatedly in the same soil, which was left uninoculated, was inoculated with CHA0-Rif or CHA0- Rif(pME3424), or was treated with the fungicide metalaxyl (Ridomil). Treatments were applied to soil at the start of each 32-day-long cucumber growth cycle, and their effects on the diversity of the rhizosphere populations of culturable fungi were assessed at the end of the first and fifth cycles. Over 11,000 colonies were studied and assigned to 105 fungal species (plus several sterile morphotypes). The most frequently isolated fungal species (mainly belonging to the genera Paecilomyces, Phialocephala, Fusarium, Gliocladium, Perticillium, Mortierella, Verticillium, Trichoderma, Staphylotrichum, Coniothyrium, Cylindrocarpon, Myrothecium, and Monocillium) were common in the four treatments, and no fungal species was totally suppressed or found exclusively following one particular treatment. However, in each of the two growth cycles studied, significant differences

were found between treatments (e.g., between the control and the other treatments and/or between the two inoculation treatments) using discriminant analysis. Despite these differences in the composition and/or relative abundance of species in the fungal community, treatments had no effect on species diversity indices, and species abundance distributions fit the truncated lognormal function in most cases. In addition, the impact of treatments at the 32-day mark of either growth cycle was smaller than the effect of growing cucumber repeatedly in the same soil.

Glandorf, D. C. M., Verheggen, P., Jansen, T., Jorritsma, J. W., Smit, E., Leeflang, P., Wernars, K., Thomashow, L. S., Laureijs, E., Thomas-Oates, J. E., Bakker, P. and Van Loon, L. C. 2001. Effect of genetically modified Pseudomonas putida WCS358r on the fungal rhizosphere microflora of field-grown wheat. *Appl Environ Microbiol* **67**: 3371-3378.

We released genetically modified Pseudomonas putida WCS358r into the rhizospheres of wheat plants. The two genetically modified derivatives, genetically modified microorganism (GMM) 2 and GMM 8, carried the phz biosynthetic gene locus of strain P. fluorescens 2-79 and constitutively produced the antifungal compound phenazine-l-carboxylic acid (PCA). In the springs of 1997 and 1998 we sowed wheat seeds treated with either GMM 2, GMM 8, or WCS358r (approximately 10(7) CFU per seed), and measured the numbers, composition, and activities of the rhizosphere microbial populations. During both growing seasons, all three bacterial strains decreased from 10(7) CFU per g of rhizosphere sample to below the limit of detection (10(7) CFU per g) I month after harvest of the wheat plants. The phz genes were stably maintained, and PCA was detected in rhizosphere extracts of GMM-treated plants. In 1997, but not in 1998, fungal numbers in the rhizosphere, quantified on 2% malt extract agar (total filamentous fungi) and on Komada's medium (mainly Fusarium spp.), were transiently suppressed in GMM 8- treated plants. We also analyzed the effects of the GMMs on the rhizosphere fungi by using amplified ribosomal DNA restriction analysis. Introduction of any of the three bacterial strains transiently changed the composition of the rhizosphere fungal microflora. However, in both 1997 and 1998, GMM-induced effects were distinct from those of WCS358r and lasted for 40 days in 1997 and for 89 days after sowing in 1998, whereas effects induced by WCS358r were detectable for 12 (1997) or 40 (1998) days. None of the strains affected the metabolic activity of the soil microbial population (substrate- induced respiration), soil nitrification potential, cellulose decomposition, plant height, or plant yield. The results indicate that application of GMMs engineered to have improved antifungal activity can exert nontarget effects on the natural fungal microflora.

Gressel, J. 2001. Potential failsafe mechanisms against the spread and introgression of transgenic hypervirulent biocontrol fungi. *Trends Biotechnol* **19**: 149-154.

Microbial biocontrol agents are typically inefficient owing to the evolutionary necessity to be in balance with their hosts to survive. If transgenetically rendered hypervirulent, however, they could be competitive alternatives to pesticides. Potential means are delineated to prevent, contain or mitigate uncontrollable spread of hypervirulent biocontrol organisms, mutations that increase their host range, and the sexual or asexual introgression of hypervirulence genes into pathogens of other organisms. The use of asporogenic deletion mutants as a platform for generating transgenic hypervirulent biopesticides would prevent such spread. Hypervirulence genes flanked with available 'transgenetic mitigator' (TM) genes (genes that are neutral or positive to the biocontrol agent but deleterious to recombinants) would decrease virulence to non-target species.

Hellwald, K. H., Glenewinkel, D., Hauber, S. and Wittlinger, S. 2001. Complementation of CMV subgroup IA strains in replicase- mediated resistant tobacco plants after co-inoculation with different cucumoviruses. *Eur J Plant Pathol* **107**: 713-721.

Replicase-mediated tobacco plants are highly resistant to the Fny strain of Cucumber mosaic virus (CMV) and closely related subgroup IA strains. Two of these subgroup IA strains, Fny- and M-CMV, were co-inoculated with different resistance breaking cucumoviruses to nontransformed and transformed tobacco plants. RT-PCR analyses of single CMV RNAs were performed to study potential complementation of the subgroup IA strains by the resistance breaking cucumoviruses. After co-inoculation of M- CMV with PII-CMV, RNAs 1, 2 and 3 from M-CMV were detected in systemically infected leaves of control plants, whereas in noninoculated parts of replicase-mediated resistant plants only M-CMV RNAs 1 and 3 were found. Western blot studies confirmed the expression of M-CMV coat protein after co-inoculation with PII-CMV in leaves of transgenic plants. These plants also exhibited M-CMV typical yellow spots. M-CMV/TAV co-inoculated transgenic plants contained only M-CMV RNA 3, but no M-CMV RNAs 1 and 2. No M-CMV typical yellow spots were observed in these plants. Our data suggest different types of complementation of M-CMV in replicase-mediated resistant plants by PII-CMV and TAV in trans potentially leading to new RNA combinations in transformed plants compared to nontransformed plants.

Hernandez-Crespo, P., Sait, S. M., Hails, R. S. and Cory, J. S. 2001. Behavior of a recombinant baculovirus in lepidopteran hosts with different susceptibilities. *Appl Environ Microbiol* **67**: 1140-1146.

Insect pathogens, such as baculoviruses, that are used as microbial insecticides have been genetically modified to increase their speed of action. Nontarget species will often be exposed to these pathogens, and it is important to know the consequences of infection in hosts across the whole spectrum of susceptibility. Two key parameters, speed of kill and pathogen yield, are compared here for two baculoviruses, a wild-type Autographa californica nucleopolyhedrovirus (AcNPV), AcNPV clone C6, and a genetically modified AcNPV which expresses an insect-selective toxin, AcNPV-ST3, for two lepidopteran hosts which differ in susceptibility, The pathogenicity of the two viruses was equal in the less-susceptible host, Mamestra brassicae, but the recombinant was more pathogenic than the wild-type virus in the susceptible species, Trichoplusia ni. Both viruses took longer to kill the larvae of M. brassicae than to kill those of T. ni. However, whereas the larvae of T. ni mere killed more quickly by the recombinant virus, the reverse mas found to be true for the larvae of M. brassicae. Both viruses produced a greater yield in M. brassicae, and the yield of the recombinant was significantly lower than that of the wild type in both species. The virus yield increased linearly with the time taken for the insects to die. However despite the more rapid speed of kill of the wild-type AcNPV in M. brassicae, the yield was significantly lower for the recombinant virus at any given time to death. A lower yield for the recombinant virus could be the result of a reduction in replication rate. This was investigated by comparing determinations of the virus yield per unit of weight of insect cadaver. The response of the two species (to both viruses) was very different: the yield per unit of weight decreased over time for M. brassicae but increased for T. ni, The implications of these data for risk assessment of wild-type and genetically modified baculoviruses are discussed.

Milks, M. L., Leptich, M. K. and Theilmann, D. A. 2001. Recombinant and wild-type nucleopolyhedroviruses are equally fit in mixed infections. *Environ Entomol* **30**: 972-981.

The recombinant nucleopolyhedrovirus of Autographa californica Speyer (AcMNPV-AaIT) expressing the paralyzing toxin of scorpions (Androtoctonus australis Hector) kills caterpillars 30% taster than wild-type (wt) AcMNPV, and shows a great deal of promise as a bioinsecticide. Although it is generally believed that genetically modified organisms are less fit than their derived wild type and will not persist in the environment, a thorough assessment of the ecological risks associated with the release of A cMNPV-AaIT must be conducted before its commercialization. In this study, we focus on one aspect and compare the fitness of AcMNPV-AaIT and wt-AcMNPV during intra-host competition. Cabbage loopers (Trichoplusia ni Hubner) were synchronously or asynchronously infected with wt- AcMNPV and AcMNPV-AaIT, and the polymerase chain reaction was used to monitor the outcome of intra-host competition. There was no indication that AcMNPV-AaIT was. less fit than wt-AcMNPV when T. ni were synchronously fed equal doses of each NPV. Serially passing the occlusion bodies had little effect on the persistence of AcMNPV-AaIT. After seven passages, the recombinant virus was found alone or with wt-AcMNPV in 71% (12/17) of replicates whereas wt-AcMNPV occurred alone or mixed in 88% (15/17) of replicates. Dose and synchrony of infection both affected the outcome of intra-host competition. The virus with the highest dose and the first one given to T ni! had a significant competitive advantage. Finally, the AaIT insert appeared to be stable because there was no evidence that it got deleted from the recombinant genome even after AcMNPV-AaIT was serially passed seven times in T. ni. As a complementary, study, we also examined intra-host competition between two wild type NPVs, wt-AcMNPV and the single nucleocapsid (S) nucleopolyhedrovirus of T ni (TnSNPV). Our results suggest that the two viruses are equally fit during intra-host competition. Serially passing the occlusion bodies did not influence the outcome but NPV dose and synchrony of infection were again strong determinants of intra-host competition.

Sengelov, G., Kristensen, K. J., Sorensen, A. H., Kroer, N. and Sorensen, S. J. 2001. Effect of genomic location on horizontal transfer of a recombinant gene cassette between Pseudomonas strains in the rhizosphere and spermosphere of barley seedlings. *Curr Microbiol* **42**: 160-167.

The use of genetically engineered bacteria in natural environments constitutes a risk of transfer of recombinant DNA to the indigenous bacteria. However, chromosomal genes are believed to be less likely to transfer than genes on mobilizable and conjugative plasmids. To study this assumption, horizontal transfer of a recombinant gene cassette inserted into the chromosome of a Pseudomonas strutzeri strain, into a mobilizable plasmid (pAGM42), and into a conjugative plasmid (pKJK5) isolated from barley rhizosphere was investigated. Horizontal transfer efficiencies of the gene cassette inserted into a conjugative plasmid was 8.20 x 10(-3) transconjugants/(donors x recipients)(1/2) in the rhizosphere and 4.57 x 10(-2) transconjugants/(donors x recipients)(1/2) in the spermosphere. Mobilization of the plasmid pAGM42 by the plasmids RP4 and pKJK5 was also detected at high levels in the microcosms, transfer efficiencies were up to 4.36 x 10(-3) transconjugants/(donors x recipients)(1/2). Transfer of chromosomal encoded genes could not be detected in the microcosms by conjugation or transformation. However, transformation did occur by

using the same bacterial strains under laboratory conditions. The rhizosphere and especially the spermosphere thus proved to be hot spot environments providing favorable conditions for gene transfer by mobilization and conjugation, but these environments did not support transformation at a detectable level.

Bertolla, F., Kay, E. and Simonet, P. 2000. Potential dissemination of antibiotic resistance genes from transgenic plants to microorganisms. *Infect Control Hosp Epidemiol* **21**: 390-393.

Evidence that genes were transferred during evolution from plants to bacteria was obtained from nucleotide and protein sequence analyses. However, the extent of such transfers among phylogenetically distant organisms is limited by various factors, including those related to complexity of the environment and those endogenous to the bacteria, designed to prevent a drift of the genome integrity. The goal of this article is to give an overview of the potentials and limits of natural interkingdom gene transfers, with a particular focus on prokaryote. originating sequences fitting the nuclear genome of transgenic plants (Infect Control Hosp Epidemiol 2000;21:390- 393).

Milks, M. L. and Theilmann, D. A. 2000. Serial selection for resistance to a wild-type and to a genetically modified nucleopolyhedrovirus in Trichoplusia ni. *Biol Control* **19**: 283-289.

In previous work, cabbage loopers (Trichoplusia ni) evolved 22- fold resistance to the single nucleocapsid nucleopolyhedrovirus of T. ni (TnSNPV) after 26 generations of selection with the virus. The goal of the present study was to determine if T. ni could evolve resistance to the recombinant Autographa californica multiple nucleocapsid nucleopolyhedrovirus (AcMNPV- AaIT) that; expresses an insect specific neurotoxin and to determine if it was influenced by prior development of resistance to TnSNPV. To answer these questions, the T. ni line that had been exposed to TnSNPV was divided into two sublines at generation 27. One of them was serially selected for resistance to AcRMNPV-AaIT (subline TnSNPV/AcMNPV-AaIT), while the other one was mock infected with distilled water (subline TnSNPV/H2O). The same was done with the line that was used as a control from generations 1 to 26 (subline H2O/AcMNPV-AaIT and subline H2O/H2O). After 17 generations of selection with AcMNPV-AaIT, T. ni that had not been previously exposed to TnSNPV evolved only twofold resistance to AcMNPV-AaIT, However, those that had been selected with TnSNPV evolved fourfold resistance to AcMNPV-AaIT. Exposure to AcMNPV-AaIT conferred cross-resistance to TnSNPV in only one subline, subline H2O/AcMNPV-AaIT. Resistance to AcMNPV-AaIT did not affect the developmental time, pupal weight, egg production, or percentage of egg hatch of T. ni. (C) 2000 Academic Press.

Moonan, F., Molina, J. and Mirkov, T. E. 2000. Sugarcane yellow leaf virus: An emerging virus that has evolved by recombination between luteoviral and poleroviral ancestors. *Virology* **269**: 156-171.

We have derived the genomic nucleotide sequence of an emerging virus, the Sugarcane yellow leaf virus (ScYLV), and shown that it produces one to two subgenomic RNAs. The family Luteoviridae currently includes the Luteovirus, Polerovirus, and Enamovirus genera. With the new ScYLV nucleotide sequence and existing Luteoviridae sequence information, we have utilized new phylogenetic and evolutionary methodologies to identify homologous regions of Luteoviridae genomes, which have statistically significant altered nucleotide substitution ratios and have produced a reconstructed phylogeny of the Luteoviridae. The data indicate that Pea enation mosaic virus-1 (PEMV-1), Soybean dwarf virus (SbDV), and ScYLV exhibit spatial phylogenetic variation (SPV) consistent with recombination events that have occurred between poleroviral and luteoviral ancestors, after the divergence of these two progenitor groups. The reconstructed phylogeny confirms a contention that a continuum in the derived sequence evolution of the Luteoviridae has been established by intrafamilial as well as extrafamilial RNA recombination and expands the database of recombinant Luteoviridae genomes that are currently needed to resolve better defined means for generic discrimination in the Luteoviridae (D'Arcy, C. J. and Mayo, M. 1997. Arch. Virol. 142, 1285-1287). The analyses of the nucleotide substitution ratios from a nucleotide alignment of Luteoviridae genomes substantiates the hypothesis that hot spats for RNA recombination in this virus family are associated with the known sites for the transcription of subgenomic RNAs (Miller et al. 1995. Crit Rev. Plant Sci. 14, 179-211), and provides new information that might be utilized to better design more effective means to generate transgene-mediated host resistance. (C) 2000 Academic Press.

Smith, C. R., Heinz, K. M., Sansone, C. G. and Flexner, J. L. 2000. Impact of recombinant Baculovirus applications on target heliothines and nontarget predators in cotton. *Biol Control* **19**: 201-214.

Recombinant baculoviruses have been genetically engineered to reduce the time to kill infected pests, thus reducing crop damage. In this study, wild-type viruses and recombinant viruses expressing a scorpion toxin were applied to cotton in response to larval infestations of Helicoverpa tea and Heliothis

virescens in 1997 and 1998. A chemical standard and an untreated control acted as comparison treatments. The goals of this field study were to (1) assess the efficacy of recombinant baculoviruses in protecting cotton from larval feeding damage; (2) assess the impact of recombinant virus introductions on predator densities and diversity; and (3) determine if cotton predators acquire baculovirus by consuming infected heliothines. When applications were timed at larval emergence, certain recombinant virus treatments protected cotton from damage better than wild-type virus treatments and as well as the chemical standard, Differences in efficacy between recombinant and wild-type baculoviruses were not apparent if treatments were applied 3 to 4 days after peak larval emergence. Predator densities and diversity were similar among recombinant and wild-type baculovirus treatments, whereas plots treated with the chemical standard had consistently smaller predator populations. From polymerase chain reaction analyses of predators in 1997 and 1998, 1.7 and 0.2%, respectively, of predators had consumed a virus-infected heliothine. Nine of the 26 predators carrying viral DNA were positive for recombinant virus. Additionally, 13 of the 26 predators were found to disperse 13.5 to 105 m 2 to 5 days after initial virus applications. Five of these dispersing predators (0.2% of all predators evaluated) carried recombinant viral DNA, These results suggest that the potential for the inadvertent spread of recombinant viral DNA via dispersing predators is low. (C) 2000 Academic Press.

Smith, J. M., Feil, E. J. and Smith, N. H. 2000. Population structure and evolutionary dynamics of pathogenic bacteria. *Bioessays* **22**: 1115-1122.

Evidence concerning the significance of recombination within natural bacterial populations has historically come from two main sources: multilocus enzyme electrophoresis (MLEE) and nucleotide sequence data. Here we discuss evidence from a third method, multilocus sequence typing (MLST), which is a development of MLEE based on nucleotide sequencing that combines the advantages of both approaches. MLST has confirmed both the existence of clones and the high rates of recombination for several bacterial pathogens. The data are consistent with "epidemic" population structures, where clones are superimposed upon a backdrop of frequent recombination, thus, in the short term, resisting the homogenising effect of recombination. The nature of the selective advantage of clones, however, and how this advantage relates to virulence are unclear. The current evidence also has broader implications concerning bacterial species definition, the management of antibiotic-resistant bacteria and the assessment of the dangers of releasing genetically modified organisms into the environment. (C) 2000 John Wiley & Sons, Inc.

Borja, M., Rubio, T., Scholthof, H. B. and Jackson, A. O. 1999. Restoration of wild-type virus by double recombination of tombusvirus mutants with a host transgene. *Mol Plant-Microbe Interact* **12**: 153-162.

Nicotiana benthamiana plants transformed with the coat protein gene of tomato bushy stunt virus (TBSV) failed to elicit effective virus resistance when inoculated with wildtype virus. Subsequently, R-1 and R-2 progeny from 13 transgenic lines were inoculated with a TBSV mutant containing a defective coat protein gene. Mild symptoms typical of those elicited in nontransformed plants infected with the TBSV mutant initially appeared. However, within 2 to 4 weeks, up to 20% of the transgenic plants sporadically began to develop the lethal syndrome characteristic of wild-type virus infections. RNA hybridization and immunoblot analyses of these plants and nontransformed N, benthamiana inoculated with virus from the transgenic lines indicated that wild-type virus had been regenerated by a double recombination event between the defective virus and the coat protein transgene, Similar results were obtained with a TBSV deletion mutant containing a nucleotide sequence marker, and with a chimeric cucumber necrosis virus (CNV) containing the defective TBSV coat protein gene. In both cases, purified virions contained wild-type TBSV RNA or CNV chimeric RNA derived by recombination with the transgenic coat protein mRNA, These results thus demonstrate that recombinant tombus-viruses can arise frequently from viral genes expressed in transgenic plants.

Fuchs, M., Gal-On, A., Raccah, B. and Gonsalves, D. 1999. Epidemiology of an aphid nontransmissible potyvirus in fields of nontransgenic and coat protein transgenic squash. *Transgenic Res* **8**: 429-439.

Spread of the aphid nontransmissible Zucchini yellow mosaicvirus virus (ZYMV) strain MV was monitored over two consecutive years in field plots of nontransgenic and transgenic squash expressing the coat protein (CP) gene of the aphid transmissible strain FL of Watermelon mosaic virus (WMV). The experimental approach was to mechanically inoculate plants with ZYMV strain MV and to assess subsequent transmissions, assumed to be vectored by aphids, of this strain to nonmechanically inoculated plants. Strain MV was distinguished from other ZYMV isolates by a threonine at position 10 of the CP or by a distinct electrophoretic pattern of a Nla IV- digested genomic cDNA fragment generated by RT-PCR. ZYMV strain MV was not detected in fields of nontransgenic plants, but was apparently aphid transmitted to 77 of

3,700 plants (2%) in transgenic fields. Despite the availability of numerous test plants and conditions of high disease pressure but low selection pressure, an epidemic of ZYMV strain MV did not develop in fields of transgenic plants. In contrast, the aphid transmissible ZYMV strain NY was aphid-transmitted to 99% (446/450) of transgenic plants under similar conditions. The relevance of these results in assessing environmental risks of transgenic plants expressing CP transgenes is discussed.

Hernandez-Crespo, P., Hails, R. S., Sait, S. M., Green, B. M., Carty, T. M. and Cory, J. S. 1999. Response of hosts of varying susceptibility to a recombinant baculovirus insecticide in the field. *Biol Control* **16**: 119-127.

In an attempt to improve the effectiveness of nucleopolyhedroviruses (NPV) as bioinsecticides of lepidopteran pests, the NPV of Autographa californica (AcMNPV) has been genetically modified to include a gene that expresses an insect-selective scorpion toxin, AaHIT. In a field trial, we compared the response of a highly susceptible 'target' host (Trichoplusia ni) and a representative less susceptible species (Mamestra brassicae), following spraying with wild-type AcMNPV and the genetically modified virus. Mortality and therefore risk of infection were significantly lower in the less susceptible species but did not differ between the wild-type and the recombinant viruses. Speed of kill was consistently faster in T. ni infected with the recombinant virus for samples collected 1, 3, and 5 days following spray application. The results for M. brassicae were more variable; time to death induced by both viruses was longer in M. brassicae than in T. ni but the genetically modified virus acted faster only in larvae taken 3 and 5 days following spraying. This contrasts with laboratory assays in which the recombinant virus took longer than the wild-type virus to kill M. brassicae. The results demonstrate that there are differences in virus productivity, distribution, and timing of virus release when two hosts with different susceptibilities are treated with wild-type and recombinant baculoviruses, which will impact on further rounds of virus replication. Thus, the effects of recombinant baculoviruses on less susceptible, potentially nontarget, hosts are not likely to be easy to predict, and they highlight the need for both more information on the behavior of baculoviruses in hosts of varying susceptibility and further studies on the impact of these factors on secondary transmission, (C) 1999 Academic Press.

Li, J. B., Heinz, K. M., Flexner, J. L. and McCutchen, B. F. 1999. Effects of recombinant baculoviruses on three nontarget heliothine predators. *Biol Control* **15**: 293-302.

Genetically engineered baculoviruses, relative to their wild- type progenitors, have successfully improved the time-to-kill of these arthropod-specific biopesticides. Beneficial arthropods that prey on targeted pest insects are likely the first nontarget organisms to be adversely affected by the applications of such biopesticides. The goals of this project were to assess potential risks of the recombinant baculoviruses on Solenopsis invicta, Geocoris punctipes, and Hippodamia convergens, all of which are common predators of heliothines in Texas cotton. Four recombinant Autographa californica nuclear polyhedrosis viruses (AcNPV), one Helicoverpa tea nuclear polyhedrosis virus (HzNPV), and two corresponding wild-type NPVs were used in this risk assessment study. Risks associated with these baculoviruses were determined by possible shifts in predator life history traits (rate of food consumption, travel speed, fecundity, and survival) when fed prey infected with recombinant viruses compared to prey infected with wild-type viruses or to healthy prey. We also tested for possible transmission of these viruses by predators using the polymerase chain reaction (PCR). No significant shifts in life history characteristics were detected in predators fed Heliothis virescens larvae infected with any of the seven viruses. Viral DNA was discovered using PCR in 2.3% of fire ant workers, but not from any of the queens or eggs. In G. punctipes, 13.4% of adults and 0.5% of eggs scored positive for viruses. Twelve percent of H. convergens adults were found PCR positive. Residency in all three predators tested provides a pathway which could increase the persistence of recombinant viral particles in the environment and thus may produce an indeterminable amount of risk associated with their inadvertent movement. (C) 1999 Academic Press.

Maskell, L. C., Raybould, A. F., Cooper, J. I., Edwards, M. L. and Gray, A. J. 1999. Effects of turnip mosaic virus and turnip yellow mosaic virus on the survival, growth and reproduction of wild cabbage (Brassica oleracea). *Ann Appl Biol* **135**: 401-407.

Wild plants of Brassica oleracea (wild cabbage) are commonly infected with turnip mosaic potyvirus (TuMV), turnip yellow mosaic tymovirus (TYMV) and several other viruses. A field experiment in which plants were inoculated either with TuMV or TYMV showed that virus infection significantly reduced survival, growth and reproduction. Relative to water inoculated-controls, plants infected with TYMV had greater mortality, were shorter, had a smaller leaf area and number, showed a greater amount of damage from herbivory and chlorosis, were less likely to flower and produced fewer pods and lower total seed output. Plants infected with TuMV did not appear to be adversely affected at first; however, mortality after 18 months was higher than control plants. Although TuMV infection had no effect on the number of plants

flowering, the infected plants did produce fewer pods and a lower total seed output. We conclude that both viruses can significantly affect vegetative and reproductive performance of wild cabbage and hence that introgression of virus resistance (particularly when conferred by a major gene or a transgene) from a crop might increase plant fitness in natural populations of this species. Ecological risk assessments of virus resistance transgenes must do more than survey adult plants in natural populations for the presence of the target virus. Failure to detect the virus could be due to high mortality on infection with the virus.

Snyder, W. E., Tonkyn, D. W. and Kluepfel, D. A. 1999. Transmission of a genetically engineered rhizobacterium by grasshoppers in the laboratory and field. *Ecol Appl* **9**: 245-253.

Root-colonizing bacteria have little inherent dispersal ability. However, we have shown previously that they routinely move into plant stems, sometimes at high densities, and might therefore be acquired and transmitted by foliage-feeding insects. Here we describe laboratory and field experiments on the transmission of a genetically engineered strain of the root-colonizing bacterium Pseudomonas chlororaphis (formerly aureofaciens) by a common herbivorous insect, the red-legged grasshopper Melanoplus femurrubrum, In laboratory and field microcosms, the engineered P. chlororaphis were applied to corn seeds at planting and allowed to colonize the developing roots and to invade the aerial tissues naturally. Adult M. femurrubrum, that later fed on the foliage of these plants became infested with the bacteria. The bacteria were harbored throughout the digestive tracts and elsewhere in the exposed grasshoppers and could be recovered from their frass. Grasshoppers carried the bacteria for greater than or equal to 1 wk after removal from the source of inoculum and could transmit the bacteria to new plants. In the laboratory, the likelihood of transmission declined as the proportion of grasshoppers remaining infested decreased through time. Transmission was less predictable in the field. Transport by insects may make naturally occurring soil bacteria more mobile than previously thought and could make the containment of engineered strains unlikely. However, at least in this system, microbial movement through plants and insects in the field was accurately predicted by laboratory studies, which therefore remain useful in basic research in microbial ecology, and in risk assessment.

Velicer, G. J. 1999. Pleiotropic effects of adaptation to a single carbon source for growth on alternative substrates. *Appl Environ Microbiol* **65**: 264-269.

It is frequently assumed that populations of genetically modified microorganisms will perform their intended function and then disappear from the environment due to inherent fitness disadvantages resulting from their genetic alteration. However, modified organisms used in bioremediation can be expected to adapt evolutionarily to growth on the anthropogenic substrate that they are intended to degrade. If such adaptation results in improved competitiveness for alternative, naturally occurring substrates, then this will increase the likelihood that the modified organisms will persist in the environment. In this study, bacteria capable of degrading the herbicide 2,4- dichlorophenoxyacetic acid (2,4-D) were used to test the effects of evolutionary adaptation to one substrate on fitness during growth on an alternative substrate. Twenty lineages of bacteria were allowed to evolve under abundant resource conditions on either 2,4-D or succinate as their sole carbon source. The competitiveness of each evolved line was then measured relative to that of its ancestor for growth on both substrates. Only three derived lines showed a clear drop in fitness on the alternative substrate after demonstrable adaptation to their selective substrate, while five derived lines showed significant simultaneous increases in fitness on both their selective and alternative substrates. These data demonstrate that adaptation to an anthropogenic substrate ran pleiotropically increase competitiveness for an alternative natural substrate and therefore increase the likelihood that a genetically modified organism will persist in the environment.

Clerc, S. and Simonet, P. 1998. A review of available systems to investigate transfer of DNA to indigenous soil bacteria. *Antonie Van Leeuwenhoek* **73**: 15-23.

The deliberate or accidental release of genetically engineered microorganisms (GEMs) in the environment has led to some questions concerning microbial survival, transfer of DNA to the indigenous microflora and environmental consequences. Amongst horizontal gene transfer mechanisms, conjugation is probably the most frequent in the environment. With the aim of evaluating risks associated with environmental release of GEMs and their engineered DNA, studies of conjugative gene transfer between a donor strain and indigenous microflora have been conducted. Such studies required the development of a donor counterselection system to prevent growth of donor cells on transconjugant selective plates. This review summarizes the known and potential donor counterselection systems.

Cullen, D. W. and Hirsch, P. R. 1998. Simple and rapid method for direct extraction of microbial DNA from soil for PCR. *Soil Biol Biochem* **30**: 983-993.

A simple and rapid procedure for direct extraction of DNA from soils was developed to yield DNA of

a high purity and quality suitable for amplification using the polymerase chain reaction (PCR). Go-extracted humic material from soil was a major contaminant of DNA and methods were devised to overcome this problem. Oligonucleotide PCR primers were designed to detect and monitor a genetically-modified (GM) Rhizobium leguminosarum by, vicine strain RSM2004 (marked with Tn5) which had become established in Rothamsted field soils. The key steps of the procedure were alkaline-SDS buffer assisted lysis of indigenous soil bacteria in a bead-beater and the purification of extracted DNA by separate PVPP and Sephadex G-75 spin-column chromatography. The mean yield from Rothamsted soil was 25 +/- 1.7 mu g crude DNA g(-1) wet soil (i.e. 20 mu g g(-1) dry soil), sheared to fragment sizes of about 22-25 kb. The recovered DNA was easier to purify and of a higher quality, as verified by PCR amplification of a 442 bp target sequence of Tn5, than DNA extracted by a hot-SDS lysis method. The detection limit was demonstrated to be one culturable cell of RSM2004 (i.e. a single copy of Tn5) 10 mg(-1) soil against a background of 10(7) diverse non-target bacteria.

Fuchs, M., Klas, F. E., McFerson, J. R. and Gonsalves, D. 1998. Transgenic melon and squash expressing coat protein genes of aphid-borne viruses do not assist the spread of an aphid non-transmissible strain of cucumber mosaic virus in the field. *Transgenic Res* **7**: 449-462.

Transgenic melon and squash containing the coat protein (CP) gene of the aphid transmissible strain WL of cucumber mosaic cucumovirus (CMV) were grown under field conditions to determine if they would assist the spread of the aphid non- transmissible strain C of CMV, possibly through heterologous encapsidation and recombination. Transgenic melon were susceptible to CMV strain C whereas transgenic squash were resistant although the latter occasionally developed chlorotic blotches on lower leaves. Transgenic squash line ZW-20, one of the parents of commercialized cultivar Freedom II, which expresses the CP genes of the aphid transmissible strains FL of zucchini yellow mosaic (ZYMV) and watermelon mosaic virus 2 (WMV 2) potyviruses was also tested. Line ZW-20 is resistant to ZYMV and WMV 2 but is susceptible to CMV. Field experiments conducted over two consecutive years showed that aphid-vectored spread of CMV strain C did not occur from any of the CMV strain C-challenge inoculated transgenic plants to any of the uninoculated CMV-susceptible non-transgenic plants. Although CMV was detected in 3% (22/764) of the uninoculated plants, several assays including ELISA, RT-PCR-RFLP, identification of CP amino acid at position 168, and aphid transmission tests demonstrated that these CMV isolates were distinct from strain C. Instead, they were non-targeted CMV isolates that came from outside the field plots. This is the first report on field experiments designed to determine the potential of transgenic plants expressing CP genes for triggering changes in virus- vector specificity. Our results indicate that transgenic plants expressing CP genes of aphid transmissible strains of CMV, ZYMV, and WMV 2 are unlikely to mediate the spread of aphid non-transmissible strains of CMV. This finding is of practical relevance because transgenic crops expressing the three CP genes are targeted for commercial release, and because CMV is economically important, has a wide host range, and is widespread worldwide.

Giddings, G. 1998. Tansley Review No. 99 - The release of genetically engineered micro-organisms and viruses into the environment. *New Phytol* **140**: 173-184.

This review considers the reasons for, and research governing, the regulation and monitoring of genetically engineered micro- organisms and viruses (GEMs) released into the environment. The hazards associated with releasing GEMs into the environment are the creation and evolution of new pests and diseases, and damage to the ecosystem and non target species. The similarities and differences between GEMs and conventional microorganisms are discussed in relation to risk assessment. Other issues covered-include the persistence of microorganisms in the environment, transgene dispersal to non-engineered microbes and other organisms, the effects of transgenes and transformation on fitness, and the evolution of pests and pathogens that are given or acquire transgenes. Areas requiring further research are identified and recommendations for risk assessment made.

Jechlinger, W., Szostak, M. P. and Lubitz, W. 1998. Cold-sensitive E-lysis systems. *Gene* **218**: 1-7.

The release of recombinant bacteria into the environment is undesirable because of possible risks associated with the genetically modified organisms, The aim of this study was to establish a cold-sensitive killing system with a lethal gene, activated when bacteria encounter lower environmental temperatures. To obtain cold-sensitive lysis vectors, the lambda cI857 repressor/p(R) promoter expression system was combined with either the lacI/lacZpo or the phage 434 cI/p(R) system that control the expression of the lysis gene E of bacteriophage phi X174. Escherichia coli strains harbouring such suicide vectors are able to grow at 37 degrees C, but cell lysis takes place at temperatures below 30 degrees C. By replacing gene E with a beta-galactosidase reporter gene we also showed that the onset of beta-galactosidase activity corresponds with the onset of lysis at 28 degrees C, Results indicate that these newly combined promoter/repressor

systems can also be used to confer cold-sensitive expression to any gene of interest. (C) 1998 Elsevier Science B.V. All rights reserved.

Lewin, A., Jacob, D., Freytag, B. and Appel, B. 1998. Gene expression in bacteria directed by plant-specific regulatory sequences. *Transgenic Res* **7**: 403-411.

The regulation of gene expression represents a specific process which has different structural and functional requirements in different groups of organisms. It is thus assumed that regulatory sequences of eucaryotes cannot be recognized in procaryotes. This assumption is of interest for risk assessments of the environmental impact of deliberate release experiments with genetically modified organisms. In order to analyse the extent of heterologous gene expression caused by the transfer of plant-specific regulatory sequences into bacteria, we constructed fusions between plant-specific regulatory sequences and the coding regions of the luxAB genes for the luciferase of the bioluminescent bacterium Vibrio harveyi, transferred the fusions into different bacterial species and measured the luminescence to quantify the expression of the luciferase genes. The regulatory sequences investigated included (a) the 35S promoter of the Cauliflower mosaic virus, (b) the B33 promoter of a class I patatin gene of potatoes, (c) the promoter of the ST-LS1 gene of potatoes and (d) the promoter of the rolC gene of Agrobacterium rhizogenes. We could show that in addition to the 35S promoter, which has already been described as being recognized in Escherichia coli, the sequences containing the B33 and the ST-LSI promoters are recognized in bacteria. Luciferase gene expression promoted by the sequence with the ST-LSI promoter could be observed in E. coli, Yersinia enterocolitica and Agrobacterium tumefaciens. Comparison of the luminescence caused by fusions between luxAB and different promoters on the chromosome and on an endogenous plasmid of Y. enterocolitica demonstrated that the level of the heterologous gene expression caused by the fragment with the ST-LSI promoter was within the range of gene expression levels caused by endogenous promoters of Y. enterocolitica.

Richards, A., Matthews, M. and Christian, P. 1998. Ecological considerations for the environmental impact evaluation of recombinant baculovirus insecticides. *Annu Rev Entomol* **43**: 493-517.

The history of baculoviruses in insect control and the current status of recombinant baculovirus (recBV) insecticides in the laboratory and the field are briefly outlined. A conceptual model for impact evaluation is described that distinguishes between scientific impact evaluation and regulatory risk assessment. Its components are identified and reviewed in the light of existing ecological theory and experimental study under the categories of impact identification, exposure identification, and impact evaluation. Impact identification aims to identify species and populations sensitive to direct or indirect impacts by a recBV. Exposure identification examines how susceptible populations may be exposed to a recBV. Impact evaluation combines these data to predict the potential for recBV impacts in the environment.

Huang, X. P., Davis, T. R., Hughes, P. and Wood, A. 1997. Potential replication of recombinant baculoviruses in nontarget insect species: Reporter gene products as indicators of infection. *J Invertebr Pathol* **69**: 234-245.

The assessment of environmental risks associated with genetically engineered baculovirus pesticides depends on an accurate knowledge of the host range of each virus. However, studies of baculovirus host ranges based solely on symptomology may misidentify as nonhosts any species with symptomless infections. This project used recombinant viruses that allowed detection of symptomless as well as pathogenic virus infections. Seven recombinant isolates of Autographa californica nuclear polyhedrosis virus (AcMNPV), Bombyx mori nuclear polyhedrosis virus, Lymantria dispar nuclear polyhedrosis virus (LdMNPV), and Orgyia pseudotsugata nuclear polyhedrosis virus were tested by either hemocoelic injection or per os inoculation for their potential replication in 23 insect species from eight orders and 17 families. The recombinant viruses contained genes coding for beta- galactosidase, secreted alkaline phosphatase (SEAP), or luciferase under the transcriptional control of either the polyhedrin or ETL promoter. Replication was initially assessed based on detection of the reporter gene products. Results obtained with beta-galactosidase or SEAP as indicators were more consistent but less sensitive than those with luciferase. With all insects tested, much higher reporter enzyme activities were found with the beta-galactosidase reporter gene placed under the polyhedrin promoter than under the ETL promoter, As indicated by reporter enzyme activity after injection with the budded virus particles, the AcMNPV replicated in more species than did the other viruses, and the LdMNPV was the most host specific. Most of the insect species tested did not support detectable replication of any of the viruses. While an observation of symptoms of viral infection was usually concurrent with detection of reporter gene activities, with certain insect/virus combinations, little or no reporter gene activity was detected even though the feeding activity and growth rates were significantly reduced relative to those of the sham-injected controls. The results of this project provide a database for the

establishment of future environmental risk assessment protocols and guidelines with baculovirus pesticides. (C) 1997 Academic Press.

Kozdroj, J. 1997. Survival, plasmid transfer and impact of Pseudomonas fluorescens introduced into soil. *J Environ Sci Health Part A-Environ Sci Eng Toxic Hazard Subst Control* **32**: 1139-1157.

The survival of donor, recipient and transconjugant strains of Pseudomonas fluorescens, transfer of plasmid RP4 from donor to recipient, and impact of the introduced strains on indigenous microflora was determined in a sandy-loam soil. The introduced donor and recipient strains survived significantly better in sterile than in non-sterile soil where a progressive decline in their numbers with time was observed. In both soils, conjugation and plasmid RP4 transfer from donor to recipient cells was observed during the first 3 days. The transconjugant survived significantly better when it was the only strain inoculated into the soil. When introduced into soil pre- colonized by the recipient strain, the transconjugant was undetectable. The introduced strains caused stimulation of total number of indigenous heterotrophic bacteria and selected physiological groups such as proteolytic, amylolytic and ammonifying bacteria, but had no effect on the number of indigenous fungi. These results confirm the fact of limited survival of genetically modified pseudomonads introduced into soil. However, their potential for conjugation and impact on soil microflora are noteworthy from the aspect of ecological risk assessment.

Mahaffee, W. F. and Kloepper, J. W. 1997. Bacterial communities of the rhizosphere and endorhiza associated with field-grown cucumber plants inoculated with a plant growth-promoting rhizobacterium or its genetically modified derivative. *Can J Microbiol* **43**: 344-353.

The future use of genetically modified microorganisms in the environment will be dependent on the ability to assess potential or theoretical risks associated with their introduction into natural ecosystems. To assess potential risks, several ecological parameters must be examined, including the impact of the introduced genetically modified organism on the microbial communities associated with the environment into which the introduction will occur. A 2-year field study was established to examine whether the indigenous bacterial communities of the rhizosphere and endorhiza (internal root tissues) were affected differently by the introduction of an unaltered wild type and its genetically modified derivative. Treatments consisted of the wild-type strain Pseudomonas fluorescens 89B-27 and a bioluminescent derivative GEM-8 (89B-27::Tn4431), Cucumber root or seed samples were taken 0, 7, 14, 21, 35, and 70 days after planting (DAP) in 1994 and 0, 7, 14, 28, 42, and 70 DAP in 1995. Samples were processed to examine the bacterial communities of both the rhizosphere and endorhiza. Over 7200 bacterial colonies were isolated from the rhizosphere and endorhiza and identified using the Sherlock System (Microbial ID, Inc.) for fatty acid methyl ester analysis. Community structure at the genus level was assessed using genera richness and Hill's diversity numbers, N1 and N2. The aerobic-heterotrophic bacterial community structure at the genus level did not significantly vary between treatments but did differ temporally. The data indicate that the introduction of the genetically modified derivative of 89B-27 did not pose a greater environmental risk than its unaltered wild type with respect to aerobic - heterotrophic bacterial community structure.

Natsch, A., Keel, C., Hebecker, N., Laasik, E. and Defago, G. 1997. Influence of biocontrol strain Pseudomonas fluorescens CHA0 and its antibiotic overproducing derivative on the diversity of resident root colonizing pseudomonads. *FEMS Microbiol Ecol* **23**: 341-352.

Non-target effects of biocontrol strains of Pseudomonas on the population of resident pseudomonads should he assessed prior to their large scale application in the environment. The rifampicin resistant bacterium P. fluorescens CHA0-Rif and its antibiotic overproducing derivative CHA0-Rif/pME3424 were introduced into soil microcosms and the population of resident pseudomonads colonizing cucumber roots was investigated after 10 and 52 days. Both CHA0-Rif and CHA0-Rif/pME3424 displaced a part of the resident pseudomonad population after 10 days. To investigate the population structure, utilization of 10 carbon sources and production of two exoenzymes was assessed for 5600 individual pseudomonad isolates and 1700 isolates were subjected to amplified ribosomal DNA restriction analysis of the spacer region (spacer-ARDRA). After 10 days, only the proportion of pseudomonads able to degrade L-tryptophan was reduced in treatments inoculated with either biocontrol strain. In pal allel the phenotypic diversity was reduced. These effects were only observed 10 days after inoculation, and they were similar for inoculation with CHA0-Rif and CHA0- Rif/pME3424. Changes in the population structure of resident pseudomonads on cucumber roots during plant growth were more pronounced than changes due to the inoculants. The inoculants did not affect the genotypic diversity detected with spacer- ARDRA, but the genotypic fingerprints corresponded only partially to the phenotypic profiles. Overall CHA0-Rif had a small and transient impact on the population of resident pseudomonads and the effect was essentially the same for the genetically engineered derivative CHA0-Rif/pME3424.

Palmer, S., Scanferlato, V. S., Orvos, D. R., Lacy, G. H. and Cairns, J. 1997. Survival and ecological effects of genetically engineered Erwinia carotovora in soil and aquatic microcosms. *Environ Toxicol Chem* **16**: 650-657.

The release of genetically engineered organisms into the environment has raised concerns about their potential ecological impact. In this study, genetically engineered Erwinia carotovora strains expressing varying levels of reduced phytopathogenicity and wildtype E. carotovora strains were used in aquatic and soil microcosms to assess the survival, intraspecific competition, and effects upon specific groups of indigenous bacteria. In aquatic microcosms, the densities of Erwinia genetically engineered organisms (GEMs) and wildtype strains declined and fell below the detectable limit of plate counts 15 d after the microcosms were Inoculated. In aquatic microcosms, engineered E. carotovora did not exhibit a competitive advantage over the wildtype. The effect of engineered and wildtype E. carotovora on densities of total and selected bacteria was not significantly different. Treatment with engineered E, carotovora did not change biomass values of the receiving community but did cause a transitory increase in metabolic activity. In aquatic microcosms, the inability of genetically engineered E. carotovora to persist. displace resident species, or affect the metabolic activity of aquatic communities indicates the low risk of adverse ecological consequences in aquatic ecosystems. Unlike previous investigations involving soil microcosms, densities of both the genetically engineered and wildtype E, carotovora remained at detectable levels over 60 d in both agricultural clay and forest loam soils. The type of soil significantly affected the survival of the GEM and the wildtype. The sorptive properties of clap particles. as well as low concentrations of soil nutrients and organic matter. may have contributed to the unexpected patterns of GEM and wildtype survival.

Schlimme, W., Marchiani, M., Hanselmann, K. and Jenni, B. 1997. Gene transfer between bacteria within digestive vacuoles of protozoa. *FEMS Microbiol Ecol* **23**: 239-247.

The occurrence of horizontal gene transfer between bacteria within digestive vacuoles and faecal pellets of the protozoan Tetrahymena pyriformis was investigated. More than 90% of the egested faecal pellets of T. pyriformis, added as predator to a suspension of Escherichia coli, contained viable bacteria. In a mixed population, containing donor (plasmid RP4) and recipient E. coli cells, the presence of T. pyriformis increased conjugational gene transfer by three orders of magnitude. Since the protozoa formed an average of 12-13 digestive vacuoles per cell, each protozoan had statistically egested one or more transconjugants. Thus, we show for the first time that digestive vacuoles of free-living protozoa appear to be an important ecological micro-niche, where gene transfer by conjugation (or retromobilisation) will be favoured. So far, digestive vacuoles have been ignored in genetic and ecological studies. This micro-biotope provides a selective pressure which might enhance the acquisition of virulence genes in cases of mutual interactions between genetically modified micro- organisms and wild-type pathogens. This finding is important for biosafety considerations.

Schwieger, F., Willke, B., Munch, J. C. and Tebbe, C. C. 1997. Ecological pre-release risk assessment of two genetically engineered, bioluminescent Rhizobium meliloti strains in soil column model systems. *Biol Fertil Soils* **25**: 340-348.

In order to identify potential ecological risks associated with the environmental release of two Rhizobium meliloti strains, genetically engineered with the firefly-derived luciferase gene (luc), a pre-release greenhouse investigation was conducted. The upper 4 cm of soil columns (30 cm diameter; 65 cm depth), which were filled according to the horizons of an agricultural field (loamy sand), were inoculated with seeds of Medicago sativa (alfalfa) and R. meliloti cells at approximately 5x10(6) cells.g(-1) soil. Four treatments were tested: inoculation with a non-engineered wild type strain (2011), strain L33 (luc(+)), strain LI(luc(+), recA(-)) and non-inoculated controls. The fate of the engineered strains was followed by two methods: (I) selective cultivation and subsequent detection of bioluminescent colonies and (2) PCR detection of the luc gene in DNA, directly extracted from soil. Strain R. meliloti L33 declined to 9.0x10(4) cfu.g(-1) soil within 24 weeks and to 2.8x10(3) cfu.g(-1) soil within 85 weeks in the upper 25 cm of the soil columns. Decline rates for R. meliloti L1 were not significantly different. Vertical distribution analysis of the recombinant cells after 37 weeks revealed that in three of four columns tested, the majority of cells (>98%) remained above 10 cm soil depth and no recombinant cells occurred below 20 cm depth. However, in one column all horizons below 20 cm were colonized with 2.2x10(4) to 6.8x10(4) cfu g(-1) soil. Ecological monitoring parameters included organic substance, total nitrogen, ammonium and nitrate, microbial biomass, culturable bacteria on four different growth media and the immediate utilization of 95 carbon sources (BiologGN) by soil- extracted microbial consortia. None of the parameters was specifically affected by the genetically engineered cells.

Vahjen, W., Munch, J. C. and Tebbe, C. C. 1997. Fate of three genetically engineered, biotechnologically important microorganism species in soil: impact of soil properties and intraspecies competition with nonengineered strains. *Can J Microbiol* **43**: 827-834.

The fate of a bacterium and two yeast species genetically engineered by insertion of a nucleotide sequence encoding for aprotinin was studied in three different soils. Corynebacterium glutamicum carried the recombinant gene on plasmid pUN1, Saccharomyces cerevisiae carried the gene on plasmid p707, and in Pichia angusta (formerly Hansenula polymoropha) LR9-Apr8, the gene was chromosomally inserted with eight tandem repeats. Corynebacterium glutamicum persisted longer than both yeast strains. In a sandy loam of pH 5.9, recovery rates of cultured cells were lower than in a clay silt or a silty sand, with pH values of 7.1 and 6.7, respectively. Generally, persistence at 10 degrees C was higher than at 20 degrees C. An adaptation of the genetically engineered strains resulting in higher soil persistence was not observed for any of the three species tested. Competition experiments between nonengineered and genetically engineered strains in presterilized soils revealed a reduced fitness of the engineered strains. However, a more competitive C. glutamicum pUN1 evolved after reinoculation of cells, preselected by a preceding competition experiment.

Cook, J., Bruckart, W. L., Coulson, J. R., Goettel, M. S., Humber, R. A., Lumsden, R. D., Maddox, J. V., McManus, M. L., Moore, L., Meyer, S. F., Quimby, P. C., Stack, J. P. and Vaughn, J. L. 1996. Safety of microorganisms intended for pest and plant disease control: A framework for scientific evaluation. *Biol Control* **7**: 333-351.

Microorganisms are enormous but largely untapped natural resources for biological control of pests and diseases. There are two primary reasons for their underployment for pest or disease control: (1) the technical difficulties of using microorganisms for biological control, owing to a lack of fundamental information on them and their ecology, and (2) the costs of product development and regulatory approvals required for each strain, formulation, and use. Agriculture and forestry benefit greatly from the resident communities of microorganisms responsible for naturally occurring biological control of pest species, but additional benefits are achieved by introducing/applying them when or where needed. This can be done as (1) an inoculative release, (2) an augmentative application, or (3) an inundative application. Because of their specificity, different microbial biocontrol agents typically are needed to control different pests or the same pest in different environments. Four potential adverse effects are identified as safety issues (hazards) associated with the use of microorganisms for the biological control of plant pests and diseases. These are: (1) displacement of nontarget microorganisms, (2) allergenicity to humans and other animals, (3) toxigenicity to nontarget organisms, and (4) pathogenicity to nontarget organisms. Except for allergenicity, these are the same attributes that contribute to the efficacy of microbial biocontrol agents toward the target pest species. The probability of occurrence of a particular adverse nontarget effect of a microbial biocontrol agent may be a function of geographic origin or a specific trait genetically added or modified, but the safety issues are the still the same, including whether the microorganism intended for pest or disease control is indigenous, nonindigenous (imported and released), or genetically modified by traditional or recombinant DNA (rDNA) technology. Likewise, the probability of occurrence of a particular adverse nontarget effect may vary with method of application, e.g., whether as an aerosol, soil treatment, baits, or seed treatment, and may increase with increased scale of use, but the safety issues are still the same, including whether the microorganism is used for an inoculative release or augmentative or inundative application. Existing practices for managing microorganisms in the environment (e.g., plant pathogens, Rhizobium, plant inoculants) provide experience and options for managing the risks of microorganisms applied for pest and disease control. Moreover, experience to date indicates that any adverse nontarget effects, should they occur, are likely to be short- term or transitory effects that can, if significant, be eliminated by terminating use of the microbial biocontrol agent. In contrast, production agriculture as currently practiced, such as the use of tillage and crop rotations, has significant and long-term effects on nontarget organisms, including the intentional and unintentional displacement of microorganisms. Even the decision to leave plant pests and diseases unmanaged could have significant long-term environmental effects on nontarget organisms. Potential safety issues associated with the use of microbial biocontrol must therefore be properly identified and compared with the impact of other options for managing the pest or leaving the pest unmanaged. This paper provides a scientific framework for this process. (C) 1996 Academic Press, Inc.

Heinemann, J. A., Scott, H. E. and Williams, M. 1996. Doing the conjugative two-step: Evidence of recipient autonomy in retrotransfer. *Genetics* **143**: 1425-1435.

Bidirectional exchange of genetic information, called retrotransfer, during bouts of bacterial conjugation has drawn the interest of those concerned with the risk of releasing genetically engineered microbes, the fluidity of genes among species, and the mechanism of DNA transport between cells. The phenomenon has generated two models in explanation, both of which yield highly testable predictions. The first model, called the one-step, predicts that the flow of genes from recipient bacteria to donor bacteria is mechanistically distinct from, but dependent on, conjugation between donors and recipients. The second model, called the two-step, predicts that the same genetic requirements and mechanistic constraints apply to the process of gene flow from recipients to donors as for gene flow from donors to recipients. The

requirement for expression of at least 10 plasmid-encoded genes in recipients, sensitivity of the reverse flow (recipient to donor) to restriction of DNA transferring from the donor, and the requirement of an additional 30-90 min for DNA to flow from recipients back to donors are predictions of the two-step model and directly refute the one-step model. Retrotransfer of genes to donors during conjugation remains genetically and physically indistinguishable from two successive rounds of conjugation between neighbors.

Kinkel, L. L., Wilson, M. and Lindow, S. E. 1996. Utility of microcosm studies for predicting phylloplane bacterium population sizes in the field. *Appl Environ Microbiol* **62**: 3413-3423.

Population sizes of two ice nucleation-active strains of Pseudomonas syringae a ere compared on leaves in controlled environments and in the field to determine the ability of microcosm studies to predict plant habitat preferences in the field. The P. syringae strains investigated were the parental strains of recombinant deletion mutant strains deficient in ice nucleation activity that had been field tested for their ability to control plant frost injury. The population size of the P. syringae strains was measured after inoculation at three field locations on up to 40 of the same plant species that were studied in the growth chamber. There was seldom a significant relationship between the mean population size of a given P. syringae strain incubated under either wet or dry conditions in microcosms and the mean population size which could be recovered from the same species when inoculated in the field. Specifically, on some plant species, the population size recovered from leaves in the field was substantially greater than from that species in a controlled environment, while for other plant species field populations were significantly smaller than those observed under controlled conditions. Population sizes of inoculated P. syringae strains, however, were frequently highly positively correlated with the indigenous bacterial population size on the same plant species in the held, suggesting that the ability of a particular plant species to support introduced bacterial strains is correlated with its ability to support large bacterial populations or that indigenous bacteria enhance the survival of introduced strains. Microcosm studies therefore seem most effective at assessing possible differences between parental and recombinant strains under a given environmental regime but are limited in their ability to predict the specific population sizes or plant habitat preferences of bacteria on leaves under field conditions.

Klijn, N., Weerkamp, A. H. and deVos, W. M. 1996. Biosafety assessment of the application of genetically modified Lactococcus lactis spp in the production of fermented milk products. *Syst Appl Microbiol* **18**: 486-492.

The past decade has shown enormous progress in the understanding of microbial genetics and has resulted in the ability to genetically modify lactic acid bacteria for the production of fermented foods. However, before these genetically modified micro-organisms can be applied in the actual manufacture of fermented foods the biological safety aspects related to their use in fermentations, release into the environment and consumption by animals and humans need to be assessed. Because the direct application of generically modified micro-organisms in food involves the intentional intake of usually high numbers of live microorganisms by the consumer, the biosafety assessment is especially complicated. The assessment of the risk, i.e. the product of hazard and exposure, was started by monitoring the survival and the dissemination of industrial lactococci in products, the environment and the human gastrointestinal tract. The transfer of generic information was evaluated by analysing conjugal transfer in different environments (during fermentation and cheese making). Transfer of pAM beta 1 between environmental isolates belonging to different species of Lactococcus was established, resulting in a better knowledge of the ecological fare of genetic information from lactococci. Based on the low survival in the environment and the regular gene transfer rates it was concluded that consumption of lactococci that are modified by deletion of genetic information or another selfcloning procedure in a fermented milk product, will not influence the potential hazard of the consumption.

Natsch, A., Keel, C., Troxler, J., Zala, M., VonAlbertini, N. and Defago, G. 1996. Importance of preferential flow and soil management in vertical transport of a biocontrol strain of Pseudomonas fluorescens in structured field soil. *Appl Environ Microbiol* **62**: 33-40.

The large-scale release of wild-type or genetically modified bacteria into the environment for control of plant diseases or for bioremediation entails the potential risk of groundwater contamination by these microorganisms, For a model study on patterns of vertical transport of bacteria under field conditions, the biocontrol strain Pseudomonas fluorescens CHA0, marked with a spontaneous resistance to rifampin (CHA0-Rif), was applied to a grass-clover ley plot (rotation grassland) and a wheat plot. Immediately after bacterial application, heavy precipitation was simulated by sprinkling, over a period of 8 h, 40 mm of water containing the mobile tracer potassium bromide and the dye Brilliant Blue FCF to identify channels of preferential flow, One day later, a 150-cm-deep soil trench was dug and soil profiles were prepared. Soil samples were extracted at different depths of the profiles and analyzed for the number of CHA0-Rif cells and

the concentration of bromide and Brilliant Blue FCF, Dye coverage in the soil profiles was estimated by image analysis. CHA0 was present at 10(8) CFU/g in the surface soil, and 10(6) to 10(7) CFU/g of CHA0 was detected along macropores between 10 and 150 cm deep, Similarly, the concentration of the tracer bromide along the macropores remained at the same level below 20 cm deep, Dye coverage in lower soil layers was higher in the ley than in the wheat plot, In nonstained parts of the profiles, the number of CHA0-Rif cells was substantially smaller and the bromide concentration was below the detection limit in most samples, We conclude that after heavy rainfall, released bacteria are rapidly transported in large numbers through the channels of preferential flow to deeper soil layers, Under these conditions, the transport of CHA0-Rif is similar to that of the conservative tracer bromide and is affected by cultural practice.

Prosser, J. I., Killham, K., Glover, L. A. and Rattray, E. A. S. 1996. Luminescence-based systems for detection of bacteria in the environment. *Crit Rev Biotechnol* **16**: 157-183.

The development of techniques for detection and tracking of microorganisms in natural environments has been accelerated by the requirement for assessment of the risks associated with environmental release of genetically engineered microbial inocula. Molecular marker systems are particularly appropriate for such studies and luminescence-based markers have the broadest range of applications, involving the introduction of prokaryotic (lux) or eukaryotic (luc) genes for the enzyme luciferase. Lux or luc genes can be detected on the basis of unique DNA sequences by gene probing and PCR amplification, but the major advantage of luminescence-based systems is the ability to detect light emitted by marked organisms or by luciferase activity in cell-free extracts. Luminescent colonies can be detected by eye, providing distinction from colonies of indigenous organisms, and the sensitivity of plate counting can be increased greatly by CCD imaging. Single cells or microcolonies of luminescent organisms can also be detected in environmental samples by CCD image-enhanced microscopy, facilitating study of their spatial distribution. The metabolic activity of luminescence-marked populations can be quantified by luminometry and does not require extraction of cells or laboratory growth. Metabolic activity, and potential activity, of marked organisms therefore can be measured during colonization of soil particles and plant material in real time without disturbing the colonization process. In comparison with traditional activity techniques, luminometry provides significant increases in sensitivity, accuracy, and, most importantly, selectivity, as activity can be measured in the presence of indigenous microbial communities. The sensitivity, speed, and convenience of luminescence measurements make this a powerful technique that is being applied to the study of an increasingly wide range of ecological problems. These include microbial survival and recovery, microbial predation, plant pathogenicity, phylloplane and rhizosphere colonization and reporting of gene expression in environmental samples.

Deleij, F., Sutton, E. J., Whipps, J. M., Fenlon, J. S. and Lynch, J. M. 1995. Impact of Field Release of Genetically-Modified Pseudomonas- Fluorescens on Indigenous Microbial-Populations of Wheat. *Appl Environ Microbiol* **61**: 3443-3453.

In a field release experiment, an isolate of Pseudomonas fluorescens, which was chromosomally modified with two reporter gene cassettes (lacZY and Kan(r)-xylE), was applied to spring wheat as a seed coating and subsequently as a foliar spray. The wild-type strain was isolated from the phylloplane of sugar beet but was found to be a common colonizer of both the rizosphere and phylloplane of wheat as well. The impact on the indigenous microbial populations resulting from release of this genetically modified microorganism (GMM) was compared with the impact of the unmodified, wild-type strain and a nontreated control until 1 month after harvest of the crop. The release of the P. fluorescens GMM and the unmodified, wild-type strain resulted in significant but transient perturbations of some of the culturable components of the indigenous microbial communities that inhabited the rhizosphere and phylloplane of wheat, but no significant perturbations of the indigenous culturable microbial populations in nonrhizosphere soil were found. Fast-growing organisms that did not produce resting structures (for example, fluorescent pseudomonads and yeasts) seemed to be most sensitive to perturbation. In terms of hazard and risk to the environment, the observed microbial perturbations that resulted from this GMM release may be considered minor for several reasons. First, the recombinant P. fluorescens strain caused changes that were, in general, not significantly different from those caused by the unmodified wild-type strain; second, perturbations resulting from bacterial inoculations were mainly small; and third, the release of bacteria had no obvious effects on plant growth and plant health.

Gillespie, K. M., Angle, J. S. and Hill, R. L. 1995. Runoff Losses of Pseudomonas-Aureofaciens (Laczy) from Soil. *FEMS Microbiol Ecol* **17**: 239-245.

Leaching of genetically modified microorganisms (GMMs) through soil profiles is generally not a significant concern, since GMMs typically remain near the soil surface following application. The presence of high numbers of GMMs at the soil surface, however, suggests that losses via runoff may occur. Traditional

methods of plating nonlabeled bacteria lack precision and are thus seldom used to monitor runoff losses. To examine whether lacZY, a common genetic marker, could be used to evaluate bacterial runoff from soil, a lacZY(+) strain of Pseudomonas aureofaciens 3732 RN-L11 was used at three different pH levels, with and without wheat (Triticum aestivum L.) cover in a greenhouse experiment. Twelve times over a 245 day period, soils were subjected to simulated rainfall of 84 mm h(-1) for a 15 min duration. Runoff losses and survival were quantified at each time. Pseudomonas aureofaciens survived for the longest period at a soil pH of 7; survival was reduced at lower pHs. The number of cells in runoff were usually related to the number of cells surviving in the soil. When high soil populations were present, runoff losses often exceeded 10(10) cfu event(-1). When the soil population declined to low or undetectable levels, the runoff contained fewer organisms. Runoff losses of 10(8) cfu event(-1), however, were observed during one runoff event even when the soil population was below the detection limit. This study indicates that lacZY is an effective marker, and that runoff of GMMs may be an important mechanism for movement to nontarget environments.

Heinz, K. M., McCutchen, B. F., Herrmann, R., Parrella, M. P. and Hammock, B. D. 1995. Direct Effects of Recombinant Nuclear Polyhedrosis Viruses on Selected Nontarget Organisms. *J Econ Entomol* **88**: 259-264.

A limitation to effective field use of naturally occurring nuclear polyhedrosis viruses (NPVs) is the slow rate at which they kill their host. In making NPVs a more attractive pest management tool, this problem has been addressed by modifying NPVs genetically to express insecticidal proteins resulting in substantial increases in their speed of action. One concern associated with these recombinant NPVs, however, is their effects on nontarget insects associated with pests targeted for control by applications of NPVs. Our studies evaluated the direct effects of wild-type Autographa californica NPV (AcNPV) and a recombinant AcNPV (AcAaIT) on three insects beneficial to production agriculture. The recombinant NPV expresses an insect-selective neurotoxin, AaIT, which was isolated from tire scorpion, Androctonus australis Hector. Two generalist predators, Chrysoperla carnea Stephens and Orius insidiosus (Say), were not adversely affected by feeding on larvae of Heliothis virescens (F.) infected with AcAaIT. Similarly, no adverse effects were detected in the honey bee, Apis mellifera L., when injected with wild-type or recombinant NPVs. Results from this study may provide a foundation upon which potential risks associated with genetically engineered NPVs may be evaluated on a limited scale in greenhouse or field experiments.

Kaplan, I. B., Shintaku, M. H., Zhang, L., Marsh, L. E. and Palukaitis, P. 1995. Complementation of Virus Movement in Transgenic Tobacco Expressing Cucumber Mosaic-Virus 3a Gene. *Virology* **209**: 188-199.

Tobacco plants were transformed with the cucumber mosaic cucumovirus (CMV) 3a gene and the in planta-expressed 3a protein was detected immunologically. The 3a protein was predominantly localized in a subcellular fraction corresponding to the cytosol. Two frameshift and four deletion mutants were created within the 3a open reading frame of CMV RNA 3. Five of these mutants, containing an N-terminal, large central, or C- terminal 70-amino-acid deletion could not infect nontransformed tobacco plants, but could infect the 3a transgenic tobacco plants, and generally accumulated to wild-type levels. The sixth mutant, lacking the C-terminal 43 amino acids of the 3a protein, was able to infect nontransformed tobacco plants. A delay in accumulation of viral RNA in both the inoculated and the systemically infected leaves was demonstrated for one of the mutants. Thus, the CMV 3a protein is a virus movement protein, the functions of which can be complemented in a transgenic plant The CMV 3a transgenic plants were able to complement the long-distance movement of a pseudorecombinant cucumovirus defective for this function in tobacco, as well as the cell-to-cell, but not the long-distance, movement of two other related viruses. However, these transgenic plants were unable to complement the long-distance movement of viruses from several other taxonomic groups that could move cell to cell but not long distance in tobacco. (C) 1995 Academic Press, Inc.

Scott, E. M., Rattray, E. A. S., Prosser, J. I., Killham, K., Glover, L. A., Lynch, J. M. and Bazin, M. J. 1995. A Mathematical-Model for Dispersal of Bacterial Inoculants Colonizing the Wheat Rhizosphere. *Soil Biol Biochem* **27**: 1307-1318.

A mathematical model has been constructed to describe bacterial growth and movement in the rhizosphere. In the model, bacteria are introduced into the soil on inoculated seeds and growth occurs, after seed germination, on material produced as root exudates. Movement of substrates away from the rhizosphere into the bulk soil is by diffusion and microbial movement is mediated by carriage on the root surface. The relationship between specific growth rate and substrate concentration is described by Monod kinetics and death occurs at a constant specific rate. An important component of the model is treatment of the effects of matric potential on the distribution and activity of bacteria in different microhabitats. Simulation of the model quantifies the distribution of both bacteria and substrate with depth and time in the rhizosphere

and demonstrates significant differences between substrate concentrations at high and low matric potentials. Sensitivity analysis of model predictions indicates the parameters which govern microbial growth to be more important determinants of microbial movement than plant-associated parameters. Predictions of the model compared well with experimental data on microbial movement in the rhizosphere of wheat plants grown in microcosms, and inoculated with luminescence-marked Pseudomonas fluorescens, and provide the basis for quantitative risk assessment following environmental release of genetically- engineered microorganisms.

Clegg, C. D., Vanelsas, J. D., Anderson, J. M. and Lappinscott, H. M. 1994. Assessment of the Role of a Terrestrial Isopod in the Survival of a Genetically-Modified Pseudomonad and Its Detection Using the Polymerase Chain-Reaction. *FEMS Microbiol Ecol* **15**: 161-168.

The effects of a terrestrial isopod, Porcellio scaber, on the survival of a genetically modified pseudomonad were studied. Pseudomonas fluorescens KTG was inoculated onto ash leaf litter and supplied to populations of P. scaber. Plate counts were lower in fresh faeces than the ash leaf litter for P. fluorescens KTG, and higher counts were detected in the faeces for the total bacterial population. When faeces were aged by incubation for up to 7 days at 15-17 degrees C, plate counts for P. fluorescens KTG increased during the first day to a level similar to those in the corresponding ash leaf litter, and remained relatively constant thereafter. The total bacterial population in the faeces continued to increase steadily over the 7 days, whilst remaining at a constant level in the ash leaf litter during the same period. Counts of bacteria in faecal material showed that P. fluorescens KTG was present for 6 days after the isopods had fed on inoculated litter although transit times of food through the gut were as little as 5 h. The implications for GEMMO dispersal of bacterial retention in the gut is considered. The polymerase chain reaction was utilised in the detection of the inserted DNA. Positive amplification of the inserted DNA sequence of P. fluorescens KTG was achieved in ash leaf litter, fresh faeces, and faeces from animals which were supplied uninoculated litter for one day after feeding on the inoculated litter. However, plate counts were more sensitive than the polymerase chain reaction in detecting P. fluorescens KTG in the faeces. Our findings suggest that when the GEMMO is ingested by the woodlouse it can survive within the guts and faeces. This has implications for risk assessment of genetically modified bacteria in terrestrial environments.

Danneberg, G. and Mieschendahl, M. 1994. Growth of Genetically-Modified Escherichia-Coli K-12 Cells in Sewage-Sludge of 2 Different Sewage-Treatment Plants. *Acta Hydrochim Hydrobiol* **22**: 10-12.

The release of genetically engineered microorganisms may occur from research laboratories or production plants. The most important pathway of release is via the sewage system and the sewage treatment plants into surface waters. The growth of these organisms might represent a risk to the environment. Therefore the growth of a genetically engineered strain of E. coli K-12 was investigated. Aqueous supernatant from sewage sludge of two domestic sewage plants was used as medium. This medium was treated with sterile filtration and amended with nutrients. The test strain E coli K-12 W3110i(q)M15Nal(r)(pBR322) did not show permanent growth under the conditions employed. The result is interpreted by the presence of bacteriophages and the poor nutritional conditions in the medium.

Fujimura, H., Sakuma, Y. and Amann, E. 1994. Survival of Genetically-Engineered and Wild-Type Strains of the Yeast Saccharomyces-Cerevisiae under Simulated Environmental- Conditions - a Contribution on Risk Assessment. *J Appl Bacteriol* **77**: 689-693.

A genetically-engineered strain of Saccharomyces cerevisiae employed for the industrial production of the human coagulation Factor XIIIa (rhFXIIIa) was used for a survival study under simulated environmental conditions. The homologous strain devoid of the recombinant plasmid and the homologous strain bearing the 2 mu m-based vector plasmid without the rhFXIIIa- encoding DNA insert were compared. The strains were introduced into natural soil/water suspension, into soil/medium suspension and into waste water. After intervals, samples of cell suspensions were taken and viable cell numbers were determined by plating on antibiotic-containing medium. In addition, a non- radioactive technique involving enhanced chemiluminescence was employed to detect plasmid-bearing yeast cells. The rhFXIIIa expression plasmid showed a high stability during the simulated environmental condition. No differences in survival rates, however, could be detected for the plasmid-bearing and plasmid- less strains under the three conditions tested, suggesting that the presence of plasmid does not confer selective advantages on the survival of the yeast cells. It is concluded that, even after accidental release of the engineered yeast cells into the environment, elimination rates would be comparable to those for non-recombinant yeast strains.

Velkov, V. V. 1994. Introduction of Genetically-Modified Microorganisms into the Environment - Prospects and Risk. *Genetika* **30**: 581-592.

An attempt was made to assess the prospects and risks of introduction of genetically modified

microorganisms (GMM) into the environment. In particular, the prospects of GMM introduction into agriculture, food industry, health service, and biotechnology of environmental protection are discussed, as well as various types of its potential adverse consequences. From the point of view of rational assessments of the risks of GMM introduction, we analyzed the physiological, biochemical, and molecular biological mechanisms underlying the viability of microorganisms in the environment, where they exist in a state of deep starvation. Data on the dynamics of GMM introduced into soil and aquatic ecosystems, as well as on gene transfer in microbial ecosystems were also analyzed. In general, the following conclusions were made: (1) GMM introduction into the environment is actually promising in terms of expected benefits. Apparently, GMM introduction for environmental bioremediation and for the control of agricultural pests are the most promising of them; (2) the probability of the replacement of natural soil and water microorganisms by the introduced GMM is low; (3) the probability of the massive spread of foreign genes from the introduced GMM in natural microbial populations is negligible and may be reduced to zero.; (4) the probability of negative effects of GMM on ecological characteristics of natural ecosystems (inhibition or activation of certain groups of natural microorganisms, formation of toxic or genotoxic metabolic products of introduced GMM) is real and causes the greatest concern in respect to potential negative consequences of GMM introduction. Conditions providing ecological safety of GMM introduction into the environment were formulated.

Lecoq, H., Ravelonandro, M., Wipfscheibel, C., Monsion, M., Raccah, B. and Dunez, J. 1993. Aphid Transmission of a Non-Aphid-Transmissible Strain of Zucchini Yellow Mosaic Potyvirus from Transgenic Plants Expressing the Capsid Protein of Plum Pox Potyvirus. *Mol Plant-Microbe Interact* **6**: 403-406.

Transgenic Nicotiana benthamiana plants expressing the coat protein of an aphid-transmissible strain of plum pox potyvirus (PPV) were infected by a non-aphid-transmissible strain of zucchini yellow mosaic potyvirus (ZYMV-NAT) in which the coat protein has a D-T-G amino acid triplet instead of the D-A-G triplet essential for aphid transmission. The aphid vector Myzus persicae could acquire and transmit ZYMV-NAT from these plants but not from infected N. benthamiana control plants that were not transformed or that were transformed but not expressing the PPV coat protein. The aphid-transmitted ZYMV subcultures were shown still to be non-aphid-transmissible from plants not expressing PPV coat protein, which indicated that their transmission was not due to RNA recombination or to reversion to the aphid-transmissible type. In immunosorbent electron microscopy experiments using the decoration technique, virus particles in the infected control plants could be coated only with ZYMV antibodies, while virus particles in the infected transgenic plants expressing the PPV coat protein could be coated not only with ZYMV antibodies but also in part with PPV antibodies. This suggests that aphid transmission of ZYMV-NAT occurred through heterologous encapsidation. These results indicate a potential risk of releasing genetically engineered plants into the environment.

Molin, S., Boe, L., Jensen, L. B., Kristensen, C. S., Givskov, M., Ramos, J. L. and Bej, A. K. 1993. Suicidal Genetic Elements and Their Use in Biological Containment of Bacteria. *Annu Rev Microbiol* **47**: 139-166.

The potential risks of unintentional releases of genetically modified organisms, and the lack of predictable behavior of these in the environment, are the subject of considerable concern. This concern is accentuated in connection with the next phase of gene technology comprising deliberate releases. The possibilities of reducing such potential risks and increasing the predictability of the organisms are discussed for genetically engineered bacteria. Different approaches towards designing disabled strains without seriously reducing their beneficial effects are presented. Principally two types of strain design are discussed: actively contained bacteria based on the introduction of controlled suicide systems, and passively contained strains based on genetic interference with their survival under environmental-stress conditions.

Schardl, C. L. and Siegel, M. R. 1993. Molecular-Genetics of Epichloe-Typhina and Acremonium-Coenophialum. *Agric Ecosyst Environ* **44**: 169-185.

The close associations between grasses and endophytes, whereby the fungi constitute maternally inherited components of the symbiotic entities (symbiota), make genetic engineering and other genetic manipulations of the endophytes an attractive approach to improvement of forage, pasture and turf-grass cultivars. A number of protective alkaloids are produced by the endophytes. Although insecticidal, nematocidal and antimicrobial metabolites produced by the mycosymbionts are desirable agronomic characteristics, anti-mammalian activities which are problematic for livestock farmers need to be mitigated. As a necessary prerequisite for endophyte genetic engineering, the authors have developed a transformation protocol for the tall fescue symbiont Acremonium coenophialum Morgan-Jones et Gams. The approach used was to introduce the antibiotic resistance gene, hph, which was obtained from the bacterium Escherichia coli and modified in vitro to allow expression in fungi. Successful incorporation of this 'chimeric'

gene into the fungal genome was detected by resistance of the transformants to hygromycin B and by DNA hybridization analysis. The wide variation in the amounts and/or types of protective alkaloids produced by different isolates of Epichloe typhina (Fr.) Tulasne (Acremonium typhinum Morgan-Jones et Gams), and other Acremonium spp., raises the prospect of employing the sexual cycle of E. typhina as an alternative genetic system for development of new endophyte strains. However, there is a lack of phenotypically variable characters to follow in Mendelian crosses. Therefore, restriction fragment length polymorphisms should be identified and used for genetic mapping. Preliminary studies of this approach are presented. Genetic manipulations and other approaches to cultivar development based on the endophytes should be done with an understanding of their evolutionary biology and the basis for host preference. Previous studies have added much to the understanding of the alkaloid production potential of different isolates, and the degree of host specificity of both choke pathogens and non-pathogenic endophytes. This paper presents a phylogenetic analysis using DNA sequence information. The results support four conclusions: (1) the endophytes have evolved from E. typhina; (2) the endophytes do not have a strict coevolutionary relationship with their grass hosts; (3) loline production, characteristic of certain symbiota, is not a good indicator of phylogenetic affinities of the fungal symbionts; (4) there is a close relationship between isolates of A. coenophialum from tall fescue, and Acremonium lolii Latch, Samuels et Christensen from perennial ryegrass and a non-pathogenic A. typhinum from red fescue.

Thorpe, I. S., Killham, K., Prosser, J. I. and Glover, L. A. 1993. Novel Method for the Study of the Population-Dynamics of a Genetically Modified Microorganism in the Gut of the Earthworm Lumbricus-Terrestris. *Biol Fertil Soils* **15**: 55-59.

A laboratory microcosm study was used to investigate the survival and population dynamics of genetically modified microorganisms (GMM) in the gut of Lumbricus terrestris. Three methods of axenic earthworm production were investigated. An antibiotic mixture of streptomycin and cycloheximide was introduced either passively, mixed with sterile soil or cellulose, or actively, by teflon catheter. Worms treated by all methods lost weight but this was least for the catheter method which was also the only method to produce axenic earthworms. Axenic earthworms were used to determine the effect of competition with indigenous gut bacteria on ingested GMM. The GMM used was Pseudomonas fluorescens, strain 10586/FAC510, with chromosomally inserted Lux genes for bioluminescence, and chromosomal resistance to rifampicin. The bacteria were grown up to the mid-exponential phase before inoculation into earthworms. Bacteria in faecal material were enumerated by dilution plate counting using selective agar. The GMM were re- isolated from the casts of both antibiotic-treated and untreated earthworms. Lower concentrations of GMM and higher concentrations of indigenous bacteria in the casts of untreated compared to antibiotic-treated earthworms suggested that competition is a fundamental control on population dynamics of the introduced bacterial inocula ingested by earthworms. The catheter method, developed in this study, is proposed as a technique to contribute to the risk assessment of environmental release of GMM.

Tirodimos, I., Pretoriusguth, I. M., Priefer, U., Tsaftaris, A. and Tsiftsoglou, A. S. 1993. Methods of Assessment of Transfer of a Gentamicin-Resistance Gene (Aacc1) from Genetically Engineered Microorganisms into Agriculturally Important Soil Bacteria. *Appl Microbiol Biotechnol* **38**: 526-530.

In order to assess the risk associated with the deliberate release of genetically engineered microorganisms (GEMs) into the agricultural environment, the transfer of plasmids between bacterial strains was investigated under laboratory conditions. Genetically modified Rhizobium leguminosarum and Agrobacterium tumefaciens strains carrying the gentamycin acetyltransferase resistance gene (aacC1) on various plasmids were investigated for their ability to transfer the aacC1 gene to their wild-type (w. t.) counterparts, as well as to Pseudomonas syringae. Conjugation experiments between the various strains, were carried out after the relevant characteristics and conditions for selective growth of each bacterial strain had been ascertained. After conjugations on filters had been completed, the putative transconjugants were grown in media containing antibiotics and assessed for the presence of aacC1 gene by: (a) DNA plasmid profile; (b) expression of AAC(3)-I enzyme activity; (c) colony hybridization using a P-32-labelled DNA probe complementary to the aacC1 gene. The results obtained indicate that transfer of the aacC1 gene from genetically modified strains of R. leguminosarum into a plasmid-free strain of A. tumefaciens occurred via self-transmissible plasmids. Alternatively, genetically modified A. tumefaciens bearing the aacC1 gene on plasmids acquired from R. leguminosarum strains, transferred it ineffectively to a hardly detectable frequency. No transfer of the aacc1 gene from genetically modified R. leguminosarum or A. tumefaciens strains into P. syringae has been observed. These data indicate that in the absence of the RP4 element, genetically modified A. tumefaciens is not able to efficiently transfer aacC1 into w.t. R. leguminosarum and P. syringae.

Kroer, N. and Coffin, R. B. 1992. Microbial Trophic Interactions in Aquatic Microcosms Designed for Testing Genetically Engineered Microorganisms - a Field Comparison. *Microb Ecol* **23**: 143-157.

Microcosms may potentially be used as tools for evaluating the fate and effects of genetically engineered microorganisms released into the environment. Extrapolation of data to the field, however, requires that the correspondence between microcosm and field is known. Microbial trophic interactions within the microbial loop were compared quantitatively and qualitatively between field and microcosms containing estuarine water with and without intact sediment cores. The comparison showed that whereas proportions between trophic levels in microcosms were qualitatively similar to those in the field, rates of microbial processes were from 25 to 40% lower in microcosms. Nitrogen cycling was disrupted in microcosms incubated in the dark to eliminate primary production. Examination of the microbial parameters further suggests that sediment in microcosms may be an important factor regulating the bacterial trophic level. These results demonstrate that analysis of microbial trophic interactions is a sensitive method for the field comparison of aquatic microcosms and a potentially useful tool in the risk assessment of genetically engineered microorganisms.

Nybroe, O., Christoffersen, K. and Riemann, B. 1992. Survival of Bacillus-Licheniformis in Seawater Model-Ecosystems. *Appl Environ Microbiol* **58**: 252-259.

The fate of Bacillus licheniformis DSM 13 was monitored after introduction into laboratory microcosms and mesocosms established in the Knebel Vig estuary, Denmark. The model organism was detected by a combination of immunofluorescence microscopy and nonselective plating followed by colony blotting. This allowed simultaneous quantification of intact cells and culturable cells. B. licheniformis DSM 13 adapted poorly to the conditions in filtered (0.2-mu-m-pore-size filter) seawater. Results from additional microcosm studies using natural seawater demonstrated that protozoan grazing also was important in regulating the population of the introduced model organism. In experiments using mesocosms, B. licheniformis DSM 13 also showed a rapid die-off. The introduction of the organism led to increased nutrient levels and to increased growth of both autotrophic and heterotrophic components of the plankton community compared with those of control enclosures. Thereby, a more intensive predation impact on the bacterioplankton community was induced. The combination of microcosm and mesocosm experiments provides a scenario in which the influence of single biotic and abiotic factors on survival of introduced organisms can be tested and in which the effect of the introduction on ecosystem structure and function can be evaluated. This test concept might prove useful in risk assessment of genetically modified microorganisms.

Seidler, R. J. 1992. Evaluation of Methods for Detecting Ecological Effects from Genetically Engineered Microorganisms and Microbial Pest- Control Agents in Terrestrial Systems. *Biotechnol Adv* **10**: 149-178.

This report summarizes and evaluates research from several laboratories that deals with the detection of ecological effects induced through exposure of microbes or plants to genetically engineered microorganisms (GEMs) and microbial pest control agents (MPCAs). Some 27 potential endpoints for measuring effects have been studied. Perturbations induced by GEMs have been detected in about one-half of these endpoints. Detectable effects have been recorded for over half of the 16 species of bacteria and fungi studied. The effects caused by GEMs and MPCAs include inhibition of beneficial mycorrhizal fungi growing on Douglas fir seedling roots, depression in plant root and shoot growth, inhibition of predatory soil protozoa, accumulation of a toxic metabolite during biodegradation that inhibits soil fungi, increased microbial community respiration due to rapid lignin breakdown in soil, and the displacement of a broad group of gram-negative bacteria that inhabit the root surface of cereal crops. These effects were usually, but not always, of short duration. However, some of the changes were irreversible during the observation time of days, weeks, or in one case, months.

Sobecky, P. A., Schell, M. A., Moran, M. A. and Hodson, R. E. 1992. Adaptation of Model Genetically Engineered Microorganisms to Lake Water - Growth-Rate Enhancements and Plasmid Loss. *Appl Environ Microbiol* **58**: 3630-3637.

When a genetically engineered microorganism (GEM) is released into a natural ecosystem, its survival, and hence its potential environmental impact, depends on its genetic stability and potential for growth under highly oligotrophic conditions. In this study, we compared plasmid stability and potential for growth on low concentrations of organic nutrients of strains of Pseudomonas putida serving as model GEMs. Plasmid-free and plasmid-bearing (NAH7) prototrophic isogenic strains and two amino-acid auxotrophs, all containing antibiotic resistance markers, were held physically separate from but in chemical contact with lake water containing the natural bacterium-sized microbial populations. Cells were reisolated at

intervals over a 2-month period to determine the percent retaining the plasmid and the specific growth rate on various media. Plasmid stability in lake water was strongly strain specific; the NAH7 plasmid was stably maintained by the prototrophic strain for the duration of the test but was lost within 24 h by both of the auxotrophs. Specific growth rates of reisolates, compared with those of the corresponding non-lake water-exposed strains (i.e., parental strains), were not different when measured in rich medium (Luria-Bertani broth). However, specific growth rates were 42, 55, and 63% higher in reisolates of auxotrophs and the plasmid-free prototroph, respectively, when measured in 10-fold-diluted medium after exposure of 15 days or longer to lake water. Moreover, lake water-exposed strains grew actively when reintroduced into sterile lake water (28- to 33-fold increase in numbers over 7 days), while the corresponding unadapted parental strains exhibited no growth over the same period. Postrelease adaptations to oligotrophic environments tend to increase the fitness of GEMs for survival and establishment of populations in aquatic ecosystems. Such genetic changes might not be detectable by using standard laboratory characterizations of parental strains yet may be critical to assessments of a particular GEM's efficacy or the risks associated with its release into the environment.

Bolton, H., Fredrickson, J. K., Bentjen, S. A., Workman, D. J., Li, S. W. and Thomas, J. M. 1991. Field Calibration of Soil-Core Microcosms - Fate of a Genetically Altered Rhizobacterium. *Microb Ecol* **21**: 163-173.

Microcosms containing intact soil-cores are a potential tool for assessing the risks of the release of genetically engineered microorganisms (GEMs) to the environment. Before microcosms become a standard assessment tool, however, they must first be calibrated to ensure that they adequately simulate key parameters in the field. Four systems were compared: intact soil-core microcosms located in the laboratory at ambient temperature and in a growth chamber with temperature fluctuations that simulated average conditions in the field, field lysimeters, and field plots. These four systems were inoculated with rifampicin-resistant Pseudomonas sp. and planted to winter wheat. Populations of the Pseudomonas sp. in soil decreased more rapidly at ambient temperature, but population size at the three-leaf stage of wheat growth was the same in all four systems. Populations of the Pseudomonas sp. on the rhizoplane of wheat were the same at the three-leaf stage in all four systems, and colonization with depth at the final boot stage-sampling was also similar. In general, microcosms incubated at ambient temperature in the laboratory or in the growth chamber were similar to those in the field with respect to survival of and colonization of the rhizoplane by the introduced Pseudomonas sp.

Bolton, H., Fredrickson, J. K., Thomas, J. M., Li, S. W., Workman, D. J., Bentjen, S. A. and Smith, J. L. 1991. Field Calibration of Soil-Core Microcosms - Ecosystem Structural and Functional Comparisons. *Microb Ecol* **21**: 175-189.

Microcosms containing intact soil-cores are a potential biotechnology risk assessment tool for assessing the ecological effects of genetically engineered microorganisms before they are released to the field; however, microcosms must first be calibrated to ensure that they adequately simulate key field parameters. Soil-core microcosms were compared with the field in terms of ecological response to the introduction of a large inoculum of a rifampicin-resistant rhizobacterium, Pseudomonas sp. RC1. RC1 was inoculated into intact soil-core microcosms incubated in the laboratory at ambient temperature (22-degrees-C) and in a growth chamber with temperature fluctuations that mimicked average field values, as well as into field lysimeters and plots. The effect of the introduced bacterium on ecosystem structure, including wheat rhizoplane populations of total and fluorescent pseudomonads, total heterotrophic bacteria, and the diversity of total heterotrophic bacteria, was determined. Fluorescent pseudomonads were present on the rhizoplane in significantly lower numbers in soil inoculated with RC1, in both microcosms and the field. Conditions for microbial growth appeared to be most favorable in the growth chamber microcosm, as evidenced by higher populations of heterotrophs and a greater species diversity on the rhizoplane at the three-leaf stage of wheat growth. Ecosystem functional parameters, as determined by soil dehydrogenase activity, plant biomass production, and N-15-fertilizer uptake by wheat, were different in the four systems. The stimulation of soil dehydrogenase activity by the addition of alfalfa was greater in the microcosms than in the field. In general, growth chamber microcosms, which simulated average field temperatures, were better predictors of field behavior than microcosms incubated continuously at 22-degrees-C.

Donegan, K., Seidler, R. and Matyac, C. 1991. Physical and Chemical Control of Released Microorganisms at Field Sites. *Can J Microbiol* **37**: 708-712.

An important consideration in the environmental release of a genetically engineered microorganism is the capability for reduction or elimination of microorganism populations once their function is completed or if adverse environmental effects are observed. In this study the decontamination treatments of burning and biocide application, alone and in combination with tilling, were evaluated for their ability to reduce

populations of bacteria released on the phylloplane. Field plots of bush beans (Phaseolus vulgaris), sprayed with the bacterium Erwinia herbicola, received the following treatments: control; control + till; burn; burn + till; Kocide (cupric hydroxide); Kocide + till; Agri-Strep (streptomycin sulfate); and Agri-Strep + till. Leaves and soil from the plots were sampled -1, 1, 5, 8, 12, 15, 19, and 27 days after application of the decontamination treatments. Burning produced a significant reduction in the number of E. herbicola, whereas tilling, alone or in combination with the biocide treatments, stimulated a significant increase in E. herbicola populations, which persisted for several weeks. The individual treatments of the biocides, Kocide and Agri-Strep, produced a rate of decline in E. herbicola populations that did not significantly differ from that of the control treatment.

Sandt, C. H. and Herson, D. S. 1991. Mobilization of the Genetically Engineered Plasmid Phsv106 from Escherichia-Coli Hb101(Phsv106) to Enterobacter-Cloacae in Drinking-Water. *Appl Environ Microbiol* **57**: 194-200.

We have used triparental matings to demonstrate transfer (mobilization) of the nonconjugative genetically engineered plasmid pHSV106, which contains the thymidine kinase gene of herpes simplex virus cloned into pBR322, from Escherichia coli HB101 to an environmental isolate of Enterobacter cloacae in sterile drinking water. This is the first demonstration of a two-step mobilization of a genetically engineered plasmid in any type of fresh water, including drinking water. Transfer was mediated by R plasmid R100-1 of E. coli ED2149(R100-1). Matings in drinking water at 15, 25, and 35-degrees-C yielded recombinants, the number of which increased with increasing temperature. Numbers of recombinants obtained were 2 orders of magnitude lower than those obtained from matings in Trypticase soy broth. High concentrations of parental organisms (2.6 x 10(8) to 2.0 x 10(9) CFU/ml) were required. During 1 week of incubation in drinking water, numbers of parental organisms and recombinants resulting from mobilization remained constant in the absence of indigenous organisms and declined in their presence. Using oligonucleotide probes for the cloned foreign DNA (thymidine kinase gene) and plasmid vector DNA (ampicillin resistance gene), we demonstrated that both genes were transferred to E. cloacae in the mobilization process. In one recombinant selected for detailed study, the plasmids containing these genes differed in size from all forms of pHSV106 present in E. coli HB101(pHSV106), indicating that DNA rearrangement had occurred. This recombinant maintained its plasmids in unchanged form for 15 days in drinking water. A second rearrangement occurred during serial passage of this recombinant on selective media. The possibility of rearrangements complicates risk assessment because antibiotic resistance gene DNA and cloned DNA can become dissociated, making the foreign DNA more difficult to detect.

Broker, M. 1990. A Study on the Survival of Wild-Type, Laboratory and Recombinant Strains of the Baker Yeast Saccharomyces Cerevisiae under Sterile and Nonsterile Conditions. *Zentbl Hyg Umweltmed* **190**: 547-557.

Laboratory strains of Saccharomyces cerevisiae transformed with an expression vector coding for the human coagulation protein FXIIIa (transglutaminase) under the control of a GAL promoter element have been constructed. Experiments were carried out to assess the potential biological risk of this genetically engineered microorganism. We analyzed the survival of this transgenic strain in comparison to the plasmid-free homologous strain and a wild-type isolate under various conditions. No differences could be detected in the survival rate under sterile or nonsterile conditions. In soil suspensions and waste water with limiting amounts of nutrients, the number of living yeast cells declined continously over a period of several weeks. In the presence of nutrients, the autochtonic microflora overgrew the yeast and the yeast died rapidly. The results indicate, that the transgenic yeast strain behaves like wild-type strains and the plasmid-free laboratory strain and has no properties which would make it fitter under environmental conditons, which are unappropriate for baker yeast. The results presented in this paper indicate a rapid disappearance of the recombinant yeast strain under natural conditions.

Kluepfel, D. A., Kline, E. L., Mueller, J., Drahos, D. J., Barry, G. and Hemming, B. C. 1990. Evaluation of the Risks Associated with the Release of Soil- Borne Genetically Engineered Bacteria into the Environment. *Abstr Pap Am Chem Soc* **199**: 155-AGRO.

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