Executive Summary

Release Application for two genetically modified microorganisms

Two genetically modified soil bacteria are to be released in a field trial in May of 2000 as part of a collaborative transnational environmental bioremediation project funded by the EU:

1. *Pseudomonas fluorescens* strain F113::lacZYrif
2. *Pseudomonas fluorescens* strain F113rifpcb

While *Pseudomonas fluorescens* strain F113::lacZY has been released in the EU before (however only in agricultural, non-contaminated soil), the release of *Pseudomonas fluorescens* strain F113rifpcb will be a novelty. The family tree of derivatives of *Pseudomonas fluorescens* strain F113, and an overview of the strains‘ phenotypic characteristics, is depicted in Fig. 1. The wildtype bacterium, *Pseudomonas fluorescens* strain F113, is a well-known root-colonising microorganism with strong biocontrol capabilities.

The genetic modifications are

1. an insertion of the marker genes *lacZY,*derived from *Escherichia coli* K12, into the wildtype *Pseudomonas fluorescens* strain F113, resulting in *Pseudomonas fluorescens* strain F113::lacZY. This strain was then made rifampicin-resistant by spontaneous mutation, in order to further improve its unequivocal detection in soil, resulting in ***Pseudomonas fluorescens* strain F113::lacZYrif**. This strain forms distinct blue colonies on a laboratory medium, containing the artificial substrate „X-Gal“, and does not degrade xenobiotics.

2. an insertion of the biphenyl-degradation genes *bph*, derived from *Burholderia cepacia* strain LB400, into a spontaneous rifampicin-resistant mutant of the wildtype *Pseudomonas fluorescens* strain F113, resulting in ***Pseudomonas fluorescens*** **strain F113rifpcb**. This strain forms fluorescent colonies on agar plates with biphenyl as the sole source of organic carbon. In addition, colonies turn yellow when sprayed with the test substrate 2,3-dihydroxy-biphenyl. In liquid culture and in soil this strain metabolises biphenyl to CO_2, and polychlorinated biphenyls (PCBs) to the corresponding chlorobenzoates and CO_2.

The two genetically modified bacteria are not resistant against an antibiotic as a result of the genetic modification. The mentioned rifampicin resistances are spontaneous mutations achieved by selection on rifampicin-containing medium. However, *Pseudomonas fluorescens* strain F113rifpcb harbours a chromosomal herbicide (Bialaphos/phosphinothricin) resistance, which was employed as a selective marker during the genetic modification.

The release is to take place at the PCB- and petroleum hydrocarbon-contaminated property of Uniscrap A/S in Hasselager, Århus Amt.

The general purpose of the release is to document the safety of GMO bioremediation strains based on *Pseudomonas fluorescens* strain F113. The technological perspective is the development of genetically modified microbial technology addressing the problem of contaminated soil. The two strains being targetted for release are prototypes of novel genetically modified bacteria under development, consisting of a root-colonising wildtype (bacterial delivery system) with degradation genes inserted to perform bioremediation of contaminated soils after inoculation into the rhizosphere of suitable non-genetically modified plants (concept of „rhizodegradation“). For the release trial, strain F113lacZYrif serves as a non-biodegradation control strain (with the *lacZY*-genes as a marker), to quantify the strain's survival in contaminated soil. Strain F113rifpcb is to be field-tested in terms of the ecological effects of the genetic insert (survival of the construct, degradation of contaminants) in contaminated soil.

Strain F113rifpcb is the first in a series of new genetically modified rhizodegradation-bacteria: Strain F113rifpcb contains the *bph*-genes in its chromosome under the constitutive (and weakly inducible) expression control by the *bph* promoters p_{1-3}. Gene expression of *bph* is relatively weak in this strain. Boosted *bph* gene expression is not realised in this strain. Applying the step-by-step procedure, this release is to test strain F113 modified with degradation genes only, while the genetically modified boosted expression is reserved for future releases.

The specific purpose of this release trial is to answer the following research questions:

- Will the bacterial delivery system function physiologically (compatibility of plant plus bacterium in contaminated soil), i.e., will the genetically modified bacterial inoculum colonise the plant root and survive in contaminated soil under field conditions?
- Does the genetically modified system perform better than existing non-genetically modified inocula under field conditions (comparison of *Burkholderia cepacia* strain LB400, the genetic donor, and F113rifpcb for survival and bioremediation)?

The planned release trial is conducted by the consortium of the EU project „Rhizodegradation“, as a part of the project‘s work programme. The partners of the „Rhizodegradation“ project are:

Ulrich Karlson, project coordinator and responsible for the release trial

Dept. of Microbial Ecology and Biotechnology, National Environmental Research Institute, POBox 358, DK–4000 Roskilde

David N. Dowling

Department of Appl. Biol. & Chem., Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland

Fergal O'Gara

BIOMERIT Research Centre, Microbiology Department, National University of Ireland, Cork, Ireland

Rafael Rivilla

Dept. Biología, Universidad Autónoma, UAM, Cantoblanco, E–28049 Madrid

Martin Bittens

WCI Umwelttechnik, Hauptstraße 45A, D–30974 Wennigsen

Stephen Francesconi

Nova Research Inc., c/o. Naval Research Laboratory, Code 6115, 4555 Overlook Ave, Washington, D.C. 20375-5321, U.S.A.

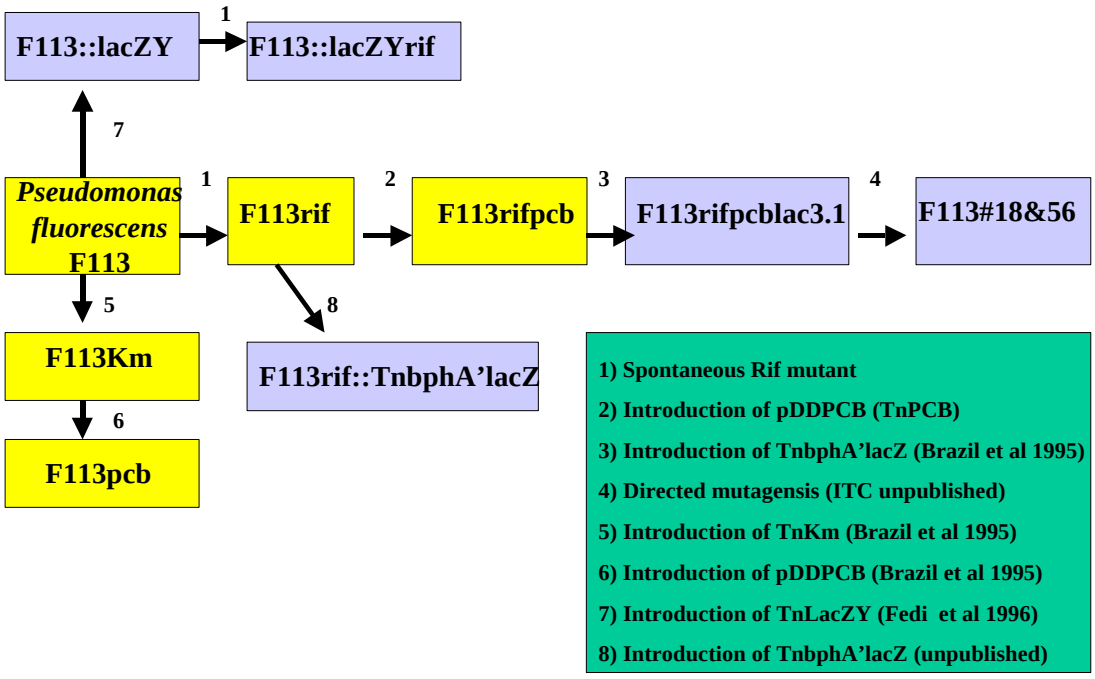
Hap Pritchard

U.S. Naval Research Laboratory, Environ. Quality Sciences Sect., 4555 Overlook Ave, Washington, D.C. 20375, U.S.A.

H.C. Pedersen

Dept. of Seed Technology, Danisco Seed, Højbygaardvej 14, DK-4960 Holeby

Fig. 1: Family tree of derivatives of *Pseudomonas fluorescens* strain F113 and their phenotypic characteristics. Abbreviations: DAPG, diacetylphloroglucinol production; HCN, cyanide production; Rf, rifampicin resistance; Km, kanamycin resistance; Spc, spectomycin resistance; LacZ, ß-galactosidase activity; BP, growth on biphenyl; 2,3-OHBP, colour reaction with 2,3-dihydroxy-biphenyl.



Strain	Phenotypes								
F113	DAPG	HCN	Biocontrol						
F113rif	DAPG	HCN	Biocontrol	Rif					
F113rifpcb	DAPG	HCN	Biocontrol	Rif	2,3-OHBP	BP			
F113rifpcblac3.1	DAPG	HCN	Biocontrol	Rif	2,3-OHBP	BP	LacZ	Spc	
F113#18 &56	DAPG	HCN	Biocontrol	Rif	2,3-OHBP*	BP*	LacZ	Spc	
F113Km	DAPG	HCN	Biocontrol	Km					
F113pcb	DAPG	HCN	Biocontrol	Km	2,3-OHBP	BP			
F113::lacZY	DAPG	HCN	Biocontrol	LacZ	Growth on lactose				
F113::lacZYrif	DAPG	HCN	Biocontrol	LacZ	Growth on lactose		Rif		