B A
S E
Biotechnol. Agron. Soc. Environ. 1997 **1** (1), 9–16

Plasmid Transfer between Bacteria in Soil Microcosms and the Field

Jan Dirk van Elsas [1], Eric Smit [2]

[1] Research Institute for Plant Protection (IPO-DLO). P.O. Box 9060. 6700 GW Wageningen (The Netherlands).
[2] National Institute of Public Health and Environment (RIVM). P.O. Box 1. 3720 BA Bilthoven (The Netherlands).

Received 29 August 1996, accepted 13 September 1996.

In this review factors influencing conjugal plasmid transfer between bacteria and the possible role of naturally occurring selftransmissible plasmids for the dissemination of recombinant DNA in soil will be discussed. In microcosm studies, plasmid transfer between various species of introduced bacteria has been detected. Moreover, plamid transfer to indigenous soil micoorganisms was observed. Soil is an oligotrophic environment and plasmid transfer occurred mainly under conditions which were nutritionally favourable for bacteria, such as in the plant rhizosphere and in the presence of clay minerals or added nutrients. Mobilizable plasmids, lacking the ability to transfer themselves, have been reported to be transferred in the presence of selftransmissible plasmids. A study comparing conjugal transfer in microcosms with those in the field revealed that the transfer rates found in microcosms and in the field were similar. Transfer of chromosomal DNA by plasmid RP4 could only be shown on filters and was not observed in soil. Transfer of plasmids carrying biodegradative genes appeared to be favoured in the presence of the compound that can be degraded. Evidence was found for the presence of naturally-occurring selftransmissible plasmids in bacteria in the rhizosphere which could mobilize recombinant plasmids.
Keywords. Plasmid transfer, bacteria, soil, rhizosphere, biosafety, microcosm, selftransmission, mobilization, broad host range plasmid, microbial ecology, bacterial conjugation, recombinant DNA.

Transferts plasmidiques entre bactéries en microcosme de sol et au champ. Dans cet article de synthèse seront discutés d'une part, les facteurs influençant le transfert de plasmides entre bactéries par conjugaison et d'autre part, le rôle probable des plasmides autotransmissibles naturels en matière de dissémination d'ADN recombinant dans le sol. Par des études en microcosme, des transferts plasmidiques interspécifiques ont pu être mis en évidence chez des bactéries ajoutées au sol. En outre, le transfert de plasmides à des micro-organismes indigènes au sol a également pu être observé. Le sol est un milieu oligotrophe et les transferts de plasmides s'y déroulent principalement lorsque les conditions sont nutritionnellement favorables aux bactéries. C'est, par exemple, le cas à proximité des racines (rhizosphère), en présence d'argiles, ou encore lorsqu'il y a ajout de composés nutritifs. Il a été rapporté que des plasmides mobilisables mais incapables de se transférer eux-mêmes, y arrivent néanmoins en présence de plasmides autotransmissibles. Une étude comparant les taux de transfert par conjugaison, en microcosme de sol et au champ, a abouti à la conclusion que ces taux sont comparables. Quant au transfert d'ADN chromosomique par le plasmide RP4, il a pu être démontré sur filtre mais pas encore dans le sol. Lorsque les plasmides sont porteurs de gènes conférant des capacités de biodégradation, leur transfert paraît favorisé en présence du composé que ces plasmides sont à même de dégrader. Enfin, il a pu être démontré qu'il existe, parmi les bactéries de la rhizosphère, des plasmides naturels autotransmissibles capables de mobiliser des plasmides recombinants.
Mots-clé. Transfert plasmidique, bactérie, sol, rhizosphère, biosécurité, microcosme, mobilisation, autotransmission, plasmide à large gamme d'hôtes, écologie microbienne, micro-organisme génétiquement modifié, conjugaison bactérienne, ADN reconbinant.

INTRODUCTION

Bacteria with beneficial properties have been and will be applied to soils to substitute chemical pesticides in biological control of plant pathogens or to remediate organic contaminations in soil. There is currently a vast experience with unmodified bacteria released into soil to promote plant growth (Gaskins *et al.*, 1985; De Freitas, Germida, 1992; Lugtenberg, de Weger, 1992), to control plant disease (Schroth *et al.*, 1984; Keel *et al.*, 1990; Lugtenberg *et al.*, 1991) and for the degradation of a variety of polluting compounds (Alexander, 1981; Brunner *et al.*, 1985; Pipke *et al.*, 1991). One major obstacle to consistency in these applications has been the sometimes poor survival of the inoculants. A potentially significant advance has become possible with the advent of genetic modification. For instance, soil isolates well adapted to soil and endowed with heterologous beneficial genes offer great potential in the different fields of application (Doyle *et al.*, 1991; Ramos *et al.*, 1991; van Elsas *et al.*, 1991a; Waalwijk *et al.*, 1991; Fenton *et al.*, 1992). However, large-scale field trials with these organisms are still restricted because of a lack of knowledge on their fate and possible adverse effects on the ecosystem (Levin, Strauss, 1990; Wellington,

van Elsas, 1992). One particular concern is the potential for transfer of heterologous genes present in inoculant strains to members of the indigenous microbial community (Wellington, van Elsas, 1992). Several lines of evidence have suggested that such transfer might occur via transformation, transduction or conjugation, since these processes can all take place in soil under favourable conditions (Levy, Miller, 1989; Fry, Day, 1990; Wellington, van Elsas, 1992). In this paper, the focus will be on plasmid transfer by conjugation, since this has been shown to be an important gene transfer mechanism in soil. Recent reviews (Levy, Miller, 1989; Fry, Day, 1990; Wellington, van Elsas, 1992; van Elsas, Smit, 1994; Lorenz, Wackernagel, 1994) address aspects of transformational and transductional gene transfer in soil.

Plasmids are preferentially transferred by conjugation. Even non-conjugative plasmids or chromosomal genes can be transferred, via mobilization by a conjugative plasmid. Plasmid-mediated gene transfer between inoculant and indigenous bacteria in soil could result in genes being spread and established in indigenous, ecologically well-adapted new hosts. This might lead to persistence of heterologous DNA as well as expression of the gene(s) in microorganisms in soil. Whereas such a scenario might currently be looked upon as being undesirable, it could for certain applications be advantageous to establish a functional gene in a stable part of the soil or rhizosphere microflora (DiGiovanni *et al.*, 1996).

The frequency of plasmid transfer in soil as affected by soil factors has mainly been studied in soil microcosms. Soil microcosms may have fairly good predictive value for microbial events happening in the field with respect to trends, however they may also underestimate or overestimate the rate of natural processes such as bacterial inoculant survival (van Elsas *et al.*, 1991b) or activity. Hence, in addition to microcosm studies, experiments in the field can be important to acquire knowledge about the fate of bacteria and their DNA, when data obtained in microcosm investigations suggest putative biosafety problems are negligible.

This review will examine the factors in soil that affect conjugal plasmid transfer between soil bacteria, after which the potential of soil bacterial populations to act in conjugal gene transfer will be addressed. Due to space limitation, it is unavoidable that some aspects will be treated only briefly, however relevant literature for detailed information is cited.

SOIL AS A HABITAT FOR BACTERIA

Soil is composed of solid, liquid and gaseous phases. The soil solid phase represents a matrix which contains liquid and gaseous phases, where conditions commonly fluctuate. Soil is heterogeneous as to the distribution of gaseous, liquid and solid components (Smiles, 1988; van Elsas, van Overbeek, 1993). The solid phase is composed of inorganic (clay, silt and sand) and organic (humic) substances, which are complexed in aggregates. In particular, clay-organic matter complexes can have effects on bacterial survival and conjugation in soil due to their overall negatively charged surfaces and increased nutrient availability (Hattori, Hattori, 1976; Smiles, 1988). The soil matrix contains numerous crevices (pores), collectively called the soil pore network. Bacterial cells in soil pores commonly occur in water films in close association with soil surfaces. Single cells or microcolonies may be observed. In the absence of transporting agents such as water flow, growing plant roots or burrowing soil animals, bacterial movement in soil over larger than micrometer distances is limited (Trevors *et al.*, 1990). Bacterial cells are therefore confined to the very sites where they were located. Hence, conditions at the level of each individual site determine their fate and activity (Foster, 1988). Unfortunately, most studies describe bacterial fate and activity in soil at a larger scale, not providing information on individual bacterial activities.

Soil is generally poor in readily-available organic carbon (Williams, 1985). For instance, a loamy sand soil contained 0.6 mg organic C per g, but this carbon was suggested to be largely unavailable to microorganisms because it was recalcitrant or localized in sites inaccessible to soil bacteria (van Elsas, van Overbeek, 1993). The low amount of available carbon in soil precludes abundant bacterial growth and activity; only a few cellular divisions per year may be possible (Gray, Williams, 1971). Nutrients may, however, become transiently available in "hot spots" with enhanced microbial growth and activity, e.g. in decaying dead plant or animal material or in the rhizosphere.

Plant roots are sites of major carbon input into soil (Curl, Truelove, 1986; Lynch, Whipps, 1990). Water-soluble compounds like root exudates and insoluble ones like remnants of root cortex cells, may be released. For instance, for gramineous plants the soluble organic C content can be 3- to 30-fold higher in the rhizosphere than in the corresponding bulk soil. Most soluble root-released carbon is liberated near the root tip during initial root development (van Elsas, van Overbeek, 1993), whereas other sites on the root exude less carbon. Bacteria in the rhizosphere overall often show enhanced growth and activity due to the availability of organic carbon (Foster, 1988). In addition, the water flow induced by plant roots may enhance bacterial movement in the rhizosphere. Both factors are thought to stimulate cell-to-cell contacts in, for instance, microcolonies developing in the rhizosphere. Hence, the rhizosphere is a dynamic area in soil which is potentially conducive to conjugal gene transfer between soil bacteria.

PLASMID TRANSFER BETWEEN BACTERIA IN SOIL

Experiments in soil microcosms

A wealth of data on conjugal gene transfer between different bacteria in soil microcosms is currently available (van Elsas *et al.*, 1987, 1988ab, 1990; Richaume *et al.*, 1989, 1992; Stotzky, 1989; Henschke, Schmidt, 1990; Top *et al.*, 1990; Wellington *et al.*, 1990; Smit *et al.*, 1991, 1992). Plasmid transfer has been observed between introduced Gram-positive donor and recipient cells, e.g. different *Bacillus* spp. (van Elsas *et al.*, 1987) or actinomycetes (Wellington *et al.*, 1990). Also, the conjugative transposon Tn916 which is transferable via cell-to-cell contact (Bettram *et al.*, 1991), was transferred from introduced *Bacillus subtilis* to indigenous *Streptomyces* spp. in nutrient-amended soil (Natarajan, Oriel, 1992). Conjugal plasmid transfer has also been found with introduced Gram-negative strains, e.g. between differentially-marked *Pseudomonas fluorescens* strains (van Elsas *et al.*, 1987, 1988ab, 1990) and interspecies, e.g. from *Escherichia coli* to *Rhizobium fredii* (Richaume *et al.*, 1989). Transfer of a derivative of the selftransmissible broad-host-range plasmid RP4, RP4p, from *P. fluorescens* to indigenous bacteria was also demonstrated in several soils (Smit *et al.*, 1991; Richaume *et al.*, 1992). Several other studies reported transfer of catabolic plasmids to indigenous bacteria (Pertsova *et al.*, 1984; Fulthorpe, Wyndham, 1991; de Rore *et al.*, 1994). Recently, DiGiovanni *et al.* (1996) observed transfer of the plasmid pJP4, encoding mercury resistance and 2,4-D degradation, to indigenous bacteria in soil amended with 2,4-D. These findings highlight the potential of indigenous soil bacteria to effectively act as *in situ* plasmid recipients. In addition, mobilization of a derivative of the IncQ plasmid RSF1010, pSKTG, by plasmid RP4p present in *P. fluorescens*, to Gram-negative indigenous bacteria was demonstrated in soil (Smit *et al.*, 1992). However, transfer of a chromosomally inserted marker gene cassette, KTG, via mobilization by plasmid RP4p could not be detected in soil (Smit *et al.*, 1995).

Conjugal plasmid transfer in soil is affected by many factors (van Elsas *et al.*, 1988ab; Stotzky 1989; Bettram *et al.*, 1991). Together, these factors result in greatly reduced transfer frequencies in soil as compared to the high values obtained in e.g. filter matings (**Table 1**). Whereas it was often difficult to detect transfer in an unamended soil, transconjugants could be detected after the addition of bacterial nutrients (van Elsas *et al.*, 1988b; Top *et al.*, 1990; Wellington *et al.*, 1990). Also, soil organic matter stimulated conjugal transfer in a sterile soil (van Elsas *et al.*, 1988b), however, it lowered conjugation rates in the wheat rhizosphere under nonsterile conditions which was attributed to the stimulation of an active inhibiting fungal population (van Elsas *et al.*, 1988a). Conjugation also occurred in soil after sterilization, which removed predators and potentially inhibitory competitors (van Elsas *et al.*, 1988b; Richaume *et al.*, 1989). Moreover, the presence in soil of montmorillonitic clay minerals (van Elsas *et al.*, 1988b; Stotzky, 1989) enhanced conjugal transfer between introduced bacteria. This stimulation may have been caused by an enhanced presence of sites for bacterial adsorption (van Elsas *et al.*, 1988b). Alternatively, it might be due to a more neutral soil pH established by the clay (Stotzky, 1989). Conjugation rates in soil were often highest at moderate pH, whereas very acid conditions did not permit transfer (Richaume *et al.*, 1989; Stotzky, 1989). Soil further posed a barrier to contact between cells localized at different sites (van Elsas *et al.*, 1990), since bacteria initially introduced into different soil portions which were subsequently mixed, were less able to transfer plasmid RP4 than cells added to the same soil portion. The presence of wheat roots alleviated this barrier effect, allowing for the detection of transconjugants also in the mixed soil portions.

Importantly, conjugal transfer of plasmid RP4p was enhanced by the presence of wheat roots in a loamy sand soil, but not in some silt loam soils (Richaume *et al.*, 1992). Apparently, the already enhanced transfer rates could no longer be stimulated by the presence of plant roots. This finding indicated plant roots can indeed stimulate conjugation, but do so to a larger or smaller extent in dependency of soil factors.

Finally, selection pressure was recently found to be an important factor in the transfer of plasmids encoding catabolic genes (de Rore *et al.*, 1994, DiGiovanni *et al.*, 1996). Either transfer rates or transconjugant numbers were increased in the presence of the compound for which the plasmid contained the degradation gene. Upon introduction of *Escherichia coli* donor cells into soil, indigenous transconjugants containing the biphenyl degradative plasmid RP4::Tn4371 were only observed when the pollutant biphenyl was present (de Rore *et al.*, 1994).

Comparison of the rates of plasmid transfer between bacteria in microcosms and in the field

The rates of transfer of IncP and IncQ plasmids between introduced fluorescent pseudomonads were compared in the rhizosphere of wheat plants growing in Ede loamy sand (van Elsas *et al.*, 1988a; Smit *et al.*, 1992), in soil microcosms and in the same soil in a field microplot. *Pseudomonas fluorescens* R2f donor bacteria with plasmid RP4 or RP4p, with or without an IncQ plasmid (pSKTG or RSF1010), were used. The marked plasmid versions (RP4p and pSKTG) were used in the microcosms, whereas the unmodified plasmids (RP4 and RSF1010) were released. Donor counterselection was performed by using the donor-specific lytic bacteriophage ΦR2f as described in Smit *et*

Table 1. Frequencies of transfer of IncP and IncQ plasmids between *Pseudomonas fluorescens* strains in filter matings, and from a *P. fluorescens* donor to indigenous bacteria in the wheat rhizosphere, in soil microcosms and in the field — *Fréquences de transfert de plasmides IncP et IncQ déterminées soit entre souches de Pseudomonas fluorescens lors de croisements sur filtres, soit entre le donneur P. fluorescens et les bactéries indigènes à la rhizosphère du froment lors d'expériences en microcosme de sol et au champ.*

Experiment	**Estimated transfer frequency**	
	IncP	**IncQ**
Filter mating		
Donor with RP4 or RP4p **(1)**	10^{-1}	ND **(4)**
Donor with RP4 and RSF1010 **(2)**	10^{-1}–10^{-2}	10^{-1}–10^{-2}
Donor with RP4p and pSKTG **(2)**	10^{-1}–10^{-2}	10^{-1}–10^{-2}
Soil microcosms (wheat rhizoshere) **(3)**		
Donor with RP4p	10^{-3}	ND
Donor with RP4p and pSKTG	10^{-3}	10^{-3}
Donor with pSKTG	ND	BD **(5)**
Field (wheat rhizosphere)		
Donor with RP4	10^{-3}	ND
Donor with RP4 and RSF1010	10^{-3}	3×10^{-3}
Donor with RSF1010	ND	BD

(1) RP4 and RP4p are self-transmissible broad host range IncP plasmids. RP4p was constructed by insertion of a hybridization marker into RP4 (Smit *et al.*, 1991, 1992). **(2)** RSF1010 and pSKTG are broad host range mobilizable IncQ plasmids. The RSF1010 derived pSKTG contains extra selectable and hybridization markers (Smit *et al.*, 1992). **(3)** Ede loamy sand soil planted with wheat was used in microcosms and the field, over 7–10 days; donor cells were added at 10^7 cfu per g soil. Details in Smit *et al.* (1992, 1995). **(4)** ND = not detected because the measures are not possible in these situations. **(5)** BD = below detection limit (about 10^2 cfu per g soil).

al. (1991, 1992). The results (**Table 1**) indicated that both the IncP and the IncQ plasmids (from donors with an IncP plasmid) were spread into the indigenous bacterial community, at frequencies (per donor) 100-fold lower than those observed in filter matings. The IncQ plasmids were not transferred in the absence of the mobilizing IncP plasmid in the donor. The transfer rates appeared very similar between microcosms and field. Hence, we concluded that these short-term experiments in soil microcosms were clearly predictive of events taking place over the same time span in the field. However, it is still possible that small microcosms with plants are not suitable for long-term experiments due to differences in plant development between microcosms and the field.

SCREENING FOR NATURAL ELEMENTS CONFERRING PLASMID TRANSFER CAPABILITY IN SOIL BACTERIA

Plasmid mobilization mediated by selftransmissible plasmids naturally present in soil bacteria is a potentially unwanted process. Experiments in soil microcosms in which both selftransmissible and mobilizable plasmids were present, indicate that mobilization can take place in soil (Smit *et al.*, 1992) and although transfer of a mobilizable plasmid to the indigenous microflora without adding the Tra genes simultaneously was not detected, this could merely be a limitation of the experimental detection limit. Selftransmissible plasmids have been isolated from the environment (Fry, Day, 1990; Top *et al.*, 1990; Top, 1992; Kobayashi, Bailey, 1994; Lilley *et al.*, 1994; Hill *et al.*, 1995) of which some have been shown to be able to mobilize IncQ plasmids (Hill *et al.*, 1992; Top, 1992). Such plasmids might enhance the dissemination of recombinant genes from introduced strains to the natural microflora in the environment.

To better understand the gene mobilizing capacity of soil bacterial populations, we have used exogenous triparental mobilization (Fry, Day, 1990; Top, 1992) to obtain mobilizing genetic elements from soil bacteria (Smit *et al.*, 1994). The criterion used was the capacity of the indigenous bacterial community to mobilize an IncQ plasmid from an *Escherichia coli* donor to an *Alcaligenes eutrophus* recipient strain (see **figure 1** for an explanation of the method used). A number of mobilizer plasmids was obtained at extremely low frequencies, from wheat rhizosphere soil (**Table 2**) but not from bulk soil. The characteristics of three of these plasmids are currently being unraveled by various methods. Molecular analysis revealed these plasmids were medium-sized (45–60 kb), and not classifiable as members of known broad host range incompatability groups (IncQ, IncP, IncN, IncW). A screening for antibiotic and heavy metal resistances revealed that none of the plasmids contained a selectable marker. The plasmids were therefore marked with a selectable transposon marker in order to facilitate plasmid

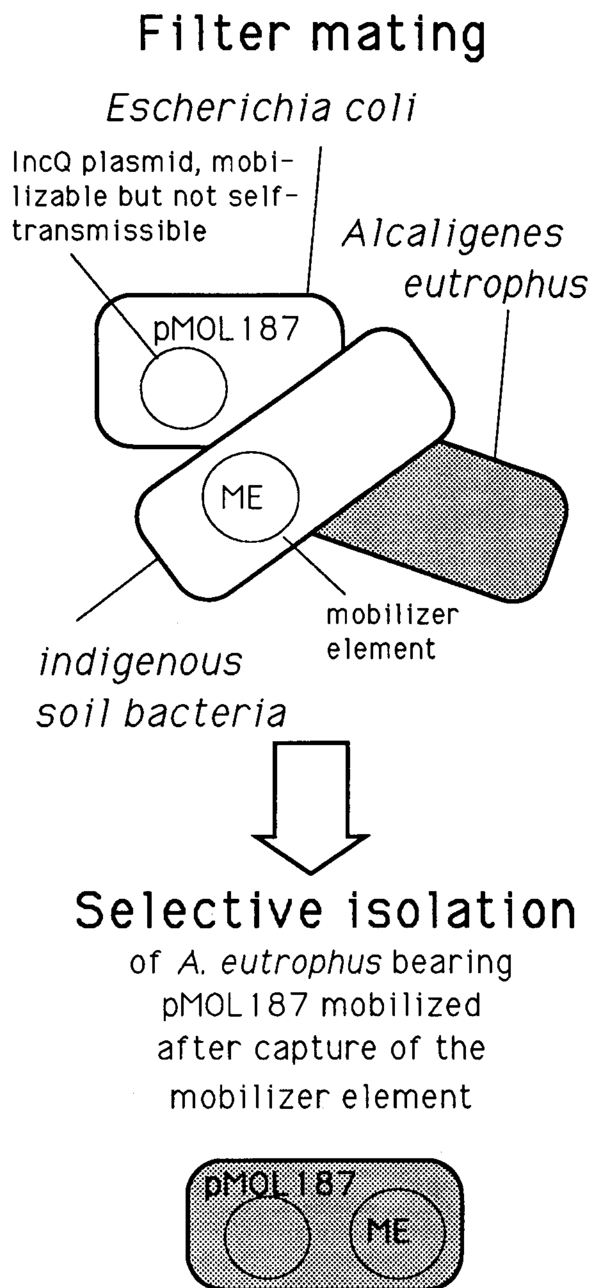

Figure 1. Schematic representation of the tri-parental mating assay for the isolation of plasmids with mobilizing capacity from the bacterial population from soil — *Représentation schématique d'une expérience de croisement triparental ayant pour but d'isoler de la population bactérienne du sol les plasmides dotés d'une capacité mobilisatrice.*

transfer studies. They were shown to transfer into a range of different Gram-negative hosts, including *Alcaligenes eutrophus*, *Pseudomonas fluorescens* and *Escherichia coli*, confirming their broad host range amongst Gram-negative bacteria. They further mobilized the IncQ plasmid pSUP104 at high frequency between members of these species. **Table 2** summarizes these findings.

We surmised these plasmids were originally present in a minor part of the total bacterial population in the rhizosphere, resulting in low isolation frequencies. When present in the total donor population in filter mating assays, they were shown to mobilize IncQ plasmids at high frequency, pinpointing their possible role in gene or plasmid mobilization in the rhizosphere. Recent research using PCR for the detection of sequences of selftransmissible plasmids in soil also presents strong evidence for the presence of selftransmissible plasmids in soil (Götz *et al.*, 1996). Further study of the prevalence of these plasmids in the rhizosphere microflora under different soil conditions will shed light on the ecological significance of gene (plasmid)-mobilizing capacity present in bacterial populations in soil.

CONCLUSIONS

Current insight suggests that conjugal plasmid transfer does occur in soil provided conditions are conducive to bacterial activities. The conclusive evidence which pinpoints the soil factors that enhance bacterial conjugation has been reviewed (Stotzky, 1989) and was briefly treated here.

Due to the large surface provided by the soil matrix and its gross oligotrophy, soil poses particular barriers to conjugal plasmid transfer between bacteria. These barriers to gene transfer are firstly physical, i.e. the presence of structured particulate matter in soil impairs free mixing and movement of bacterial cells, and secondly nutritional, i.e. the transfer of genes is hampered due to the lack of energy or carbon sources needed for expression of genes involved in transfer. Soil factors which reduce bacterial population densities, such as predation by protozoa, competition, severe drought or freezing/thawing, also affect gene transfer rates negatively by reducing the sizes and activities of donor, recipient and transconjugant populations. On the other hand, soil potentially enhances plasmid transfer rates between cells in close proximity, since certain plasmids transfer better at surfaces. Further, the barriers to gene transfer may be alleviated under conditions in which bacterial growth and activity is possible. A paradigm of such hot spots for bacterial activity is the rhizosphere. The enhanced nutrient input and water fluxes in the rhizosphere were shown to stimulate conjugal gene transfer between pseudomonads (van Elsas *et al.*, 1988a).

One of the major difficulties in studying gene transfer events in soil is the rarity of the events studied. Transfers still detectable *in vitro* due to the lack of background, often escape detection in soil, due to the lower frequencies of transfer and the higher background found when applying selection. An additional complication is the lack of culturability of a major part of the bacterial population in soil, which impairs the detection of potential non-culturable excipients by conventional means. However, low-frequency events may be detectable if strong selection for the plasmid is applied, such as found by de Rore *et al.* (1994).

Table 2. Frequencies of mobilization of IncQ plasmids in filter matings — *Fréquences de mobilisation de plasmides IncQ lors de croisements sur filtres* :

1. Tri-parental mating with a mixed bacterial population obtained from the wheat rhizosphere in order to isolate naturally-occurring plasmids with mobilizing capacity (see also **figure 1**) — *Croisement triparental au moyen d'une population bactérienne mixte issue de la rhizosphère du froment dans le but d'en isoler des plasmides naturels dotés d'une capacité mobilisatrice (voir également* **figure 1**).
2. Tri-parental mating between different bacterial strains using one of the isolated new mobilizer elements obtained by the tri-parental mating assay in 1 — *Croisement triparental entre diverses espèces bactériennes en ayant recours à l'un des éléments mobilisateurs nouvellement isolés lors de l'essai de croisement décrit en* 1.
3. Di-parental mating between *P. fluorescens* pSUP104 and the mobilizer with *E. coli* as recipient. (Smit *et al.*, 1994) — *Croisement diparental entre le donneur P. fluorescens porteur de pSUP104 et l'accepteur E. coli porteur de l'élément mobilisateur.*

Experiment	Frequency of mobilization (per donor)
1. Mobilization of pMOL187 from *Escheria coli* to *Alcaligenes eutrophus* by indigenous mobilizers	5×10^{-11}
2. Triparental mobilization of pSUP104 from *E. coli* to *Pseudomonas fluorescens* by new mobilizer element delivered by *A. eutrophus*	3×10^{-1}
3. Direct mobilization of pSUP104 from *P. fluorescens* to *E. coli*	5×10^{-3}

pMOL187, pSUP104: mobilizable broad host range IncQ plasmids (Smit *et al.*, 1992; Top, 1992).

The close match between data obtained in the rhizosphere of wheat grown in microcosms and in the field suggested that at least, for the short time span used, microcosms could predict conjugal plasmid transfer rates in the field. Finally, natural mobilizer elements were found in the wheat rhizosphere, which might be involved in *in situ* gene mobilization. The role of selftransmissible plasmids in the soil bacterial community is up to now only speculative; these plasmids might provide their hosts with a means to genetically adapt to changing environmental conditions. The ecological relevance of these plasmids is still under investigation.

Acknowledgements

This work was supported by the EU-BIOTECH programme (Grant no. BIO2CT920491). We thank Anneke Wolters for excellent technical assistance and Max Mergeay for providing the triparental plasmid isolation system.

Bibliography

Alexander M (1981). Biodegration of chemicals of environmental concern. *Science* **211**, 132–138.

Bettram J, Strätz H, Dürre P (1991). Natural transfer of conjugative transposon Tn916 between Gram+ and Gram- bacteria. *J. Bacteriol.* **173**, 443–448.

Brunner W, Sutherland FH, Focht DD (1985). Enhanced biodegradation of polychlorinated biphenyls in soil by analog enrichment and bacterial inoculation. *J. Environ. Qual.* **14**, 324–328.

Curl AE, Truelove B (1986). "The Rhizosphere". Springer Verlag, Berlin.

De Freitas JR, Germida J (1992). Growth promotion of winter wheat by fluorescent *Pseudomonads* under field conditions. *Soil Biol. Biochem.* **24**, 1137–1146.

de Rore H, Demolder K, De Wilde K, Top E, Houwen F, Verstraete W (1994). Transfer of the catabolic plasmid RP4::Tn4371 to indigenous soil bacteria and its effect on respiration and biphenyl breakdown. *FEMS Microbiol. Ecol.* **15**, 71–78.

DiGiovanni GD, Neilson JW, Pepper IL, Sinclair NA (1996). Gene transfer of *Alcaligenes euthrophus* JMP134 plasmid pJP4 to indigenous soil recipients. *Appl. Environ. Microbiol.* **62**, 2521–2526.

Doyle JD, Short KA, Stotzky G, King RJ, Seidler RJ, Olsen RH (1991). Ecologically significant effects of *Pseudomonas putida* PPO301 (pRO103) genetically engineered to degrade 2,4-dichlorophenoxyacetate, on microbial populations and processes in soil. *Can. J. Microbiol.* **37**, 682–691.

Fenton AM, Stephens PM, Crowley J, O'Callaghan M, O'Gara F (1992). Exploitation of gene(s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a *Pseudomonas* strain. *Appl. Environ. Microbiol.* **58**, 3873–3878.

Foster RC (1988). Microenvironments of soil microorganisms. *Biol. Fertil. Soils* **6**, 189–203.

Fry JC, Day MJ (1990). "Bacterial genetics in the natural environment". Chapman and Hall, London.

Fulthorpe RR, Whyndham RC (1991). Transfer and expression of the catabolic plasmid pBRC60 in wild bacterial recipients in a freshwater ecosystem. *Appl. Environ. Microbiol.* **57**, 1546–1553.

Gaskins MH, Albrecht SL, Hubbel DH (1985). Rhizosphere bacteria and their use to increase plant productivity. *Agric. Ecosyst. Environ.* **12**, 99–116.

Götz A, Pukall R, Smit E, Tietze E, Prager R, Tschape H, van Elsas JD, Smalla K (1996). Detection and characterisation of

broad-host-range plasmids in environmental bacteria by PCR. *Appl. Environ. Microbiol.* **62**, 2621–2628.

Gray TRG, Williams ST (1971). Microbial productivity in soil. *In* "Microbes and biological productivity. 21st Symposium Soc. Gen. Microbiol.", pp. 255–286. Cambridge University Press, UK.

Hattori T, Hattori R (1976). The physical environment in soil microbiology: an attempt to extend principles of microbiology to soil microorganisms. *CRC Crit. Rev. Microbiol.* **4**, 423–461.

Henschke RB, Schmidt FRJ (1990). Plasmid mobilization from genetically engineered bacteria to members of the indigenous microflora. *Curr. Microbiol.* **20**, 105–110.

Hill KE, Weigthman AJ, Fry JC (1992). Isolation and screening of plasmids from the epilithon which mobilize recombinant plasmid pD10. *Appl. Environ. Microbiol.* **58**, 1292–1300.

Hill KE, Fry JC, Weightman AJ, Day MJ, Bradley DJ, Cousland B (1995). Retrotransfer of IncP-like plasmids from aquatic bacteria. *Lett. Appl. Microbiol.* **20**, 317–322.

Keel C, Wirthner P, Oberhansli T, Voisard C, Burger U, Haas D, Defago G (1990). Pseudomonads as antagonists of plant pathogens in the rhizosphere: role of the antibiotic 2,4-diacetylphloroglucinol in the suppression of black root rot of tobacco. *Symbiosis* **9**, 327–341.

Kobayashi N, Bailey MJ (1994). Plasmids isolated from the sugar beet phyllosphere show little or no homology to molecular probes currently available for plasmid typing. *Microbiology* **140**, 289–296.

Levin MA, Strauss B (1990). "Risk assessment in genetic engineering". McGraw-Hill, New York.

Levy SB, Miller R (1989). "Gene transfer in the environment". McGraw-Hill, New York.

Lilley AK, Fry JC, Day M, Bailey MJ (1994). *In situ* transfer of an exogenously isolated plasmid between *Pseudomonas* spp. in sugar beet rhizosphere. *Microbiology* **140**, 27–33.

Lorenz M, Wackernagel W (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev.* **58**, 563–602.

Lugtenberg BJJ, de Weger LA, Bennet JW (1991). Microbial stimulation of plant growth and protection of disease. *Curr. Opin. Biotechnol.* **2**, 457–464.

Lugtenberg BJJ, de Weger LA (1992). Plant root colonization by *Pseudomonas* species. *In* "*Pseudomonas* molecular biology and biotechnology" (E. Galli, S. Silver, B. Witholt, eds.), pp. 13–20. American Society of Microbiology, Washington DC.

Lynch JM, Whipps JM (1990). Substrate flow in the rhizosphere. *Plant Soil* **129**, 1–10.

Natarajan MR, Oriel P (1992). Transfer of transposon Tn916 from *B. subtilus* into a natural soil population. *Appl. Environ. Microbiol.* **58**, 2701–2703.

Pertsova RN, Kunc F, Golovleva LA (1984). Degradation of 3-chlorobenzoate in soil by pseudomonads carrying biodegradative plasmids. *Folia Microbiol.* **29**, 242–247.

Pipke R, Wagner-Döbler I, Timmis KN, Dwyer DF (1992). Survival and function of a genetically engineered pseudomonad in aquatic sediment microcosms. *Appl. Environ. Microbiol.* **58**, 1259–1265.

Ramos JL, Duque E, Ramos-Gonzales MI (1991). Survival in soil of a herbicide resistant *Pseudomonas putida* strain bearing a recombinant TOL plasmid. *Appl. Environ. Microbiol.* **57**, 260–266.

Richaume A, Angle JS, Sadowski MJ (1989). Influence of soil variables on *in situ* plasmid transfer from *Escherichia coli* to *Rhizobium fredii*. *Appl. Environ. Microbiol.* **55**, 1730–1734.

Richaume A, Smit E, Faurie G, van Elsas JD (1992). Influence of soil type on the transfer of plasmid RP4 from *Pseudomonas fluorescens* to introduced recipient and to indigenous bacteria. *FEMS Microbiol. Ecol.* **101**, 281–292.

Schroth MN, Loper JE, Hildebrand DC (1984). Bacteria as biocontrol agents of plant disease. *In* "Current perspectives in microbial ecology" (M.J. Klug, C.A. Reddy, eds.), pp. 362–369. Am. Soc. Microbiol., Washington DC.

Smiles DE (1988). Aspects of the physical environment of soil organisms. *Biol. Fertil. Soils* **6**, 204–215.

Smit E, van Elsas JD, van Veen JA, de Vos WM (1991). Detection of plasmid transfer from *Pseudomonas fluorescens* to indigenous bacteria using a phage for donor-counter selection. *Appl. Environ. Microbiol.* **57**, 3482–3488.

Smit E, Venne D, van Elsas JD (1992). Effects of co-transfer and retro-transfer on the mobilization of a genetically engineered IncQ plasmid between bacteria on filters and in soil. *Appl. Environ. Microbiol.* **59**, 2257–2263.

Smit E, Ten Thij GJ, Wolters A, van Elsas JD (1994). Effect of plant roots and polluting compounds on gene transfer and the occurrence of selftransmissible plasmids. *In* "Horizontal gene transfer. Mechanisms and implications. Proceedings of the workshop in Bielefeld, Germany, July 25–27, 1994" (M.J. Klug, C.A. Reddy, eds.), pp. 115–118. Center for Interdisciplinary Research, University of Bielefeld.

Smit E, Wolters A, van Elsas JD (1995). Genetic stability, conjugal transfer and expression of heterologous DNA inserted into different plasmids and the genome of *Pseudomonas fluorescens*. *Rev. Microbiol.* **26**, 169–179.

Stotzky G (1989). Gene transfer among bacteria in soil. *In* "Gene transfer in the natural environment" (S.B. Levy, R.V. Miller, eds.), pp. 165–222. McGraw-Hill, New York.

Top E, Mergeay M, Springael D, Verstraete W (1990). Gene escape model: transfer of heavy metal resistance genes from *Escherichia coli* to *Alcaligenes eutrophus* on agar plates and in soil. *Appl. Environ. Microbiol.* **56**, 2471–2479.

Top E (1992). "Horizontal gene transfer in the environment and the significance of broad host range plasmids". Ph. D. Thesis, Gent Univ., Belgium.

Trevors JT, van Elsas JD, van Overbeek LS, Starodub ME (1990). Transport of a genetically engineered *Pseudomonas fluorescens* strain through a soil microcosm. *Appl. Environ. Microbiol.* **56**, 401–408.

van Elsas JD, Govaert JM, Van Veen JA (1987). Transfer of plasmid pFT30 between bacilli in soil as influenced by bacterial population dynamics and soil condition. *Soil Biol. Biochem.* **19**, 639–647.

van Elsas JD, Trevors JT, Starodub ME (1988a). Bacterial conjugation between *pseudomonads* in the rhizosphere of wheat. *FEMS Microbiol. Ecol.* **53**, 299–306.

van Elsas JD, Trevors JT, Starodub ME (1988b). Plasmid transfer in soil and rhizosphere. *In* "Risk assessment for deliberate releases" (W. Klingmuller, ed.), pp. 89–99. Springer Verlag, Heidelberg, Germany.

van Elsas JD, Trevors JT, Starodub ME, Van Overbeek LS (1990). Transfer of plasmid RP4 between pseudomonads after introduction into soil: influence of spatial and temporal aspects of inoculation. *FEMS Microbiol. Ecol.* **73**, 1–12.

van Elsas JD, van Overbeek LS, Feldmann AM, Dullemans AM, de Leeuw O (1991a). Survival of a genetically engineered *Pseudomonas fluorescens* in soil in competition with the parent strain. *FEMS Microbiol. Ecol.* **85**, 53–64.

van Elsas JD, Heijnen CE, van Veen JA (1991b). The fate of introduced genetically engineered microorganisms (GEMs) in soil and microcosms and the field; impact of soil structural aspects. *In* "Biological monitoring of genetically engineered plants and microbes" (D.R. MacKenzie, S.C. Henry, eds.), pp. 67–79. Agricult. Res. Inst., Bethesda MD.

van Elsas JD, van Overbeek LS (1993). Bacterial responses to soil stimuli. *In* "Starvation in bacteria" (S. Kjelleberg, ed.), pp. 55–79. Plenum Press, New York.

van Elsas JD, Smit E (1994). *In* "Molecular ecology of rhizosphere microorganisms" (F. O'Gara, D.N. Dowling, B. Boesten, eds.), pp. 151–164. VCH Publisher, New York.

Waalwijk C, Dullemans A, Maat C (1991). Construction of bioinsecticidal rhizosphere isolate of *Pseudomonas fluorescens*. *FEMS Microbiol. Lett.* **77**, 257–264.

Wellington EMH, Cresswell N, Saunders VA (1990). Growth and survival of *Streptomycetes* inoculants and extent of plasmid transfer in sterile and nonsterile soil. *Appl. Environ. Microbiol.* **56**, 1413–1419.

Wellington EMH, van Elsas JD (1992). "Gene transfer among microorganisms in the natural environment". Pergamon Press, London.

Williams ST (1985). Oligotrophy in soil: fact or fiction? *In* "Bacteria in the natural environment: the effect of nutrient conditions" (M. Fletcher, G. Floodgate, eds.), pp. 81–110. Academic Press, London.

(56 ref.)