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MiniReview

Gene transfer in soil systems using microcosms

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Abstract

Soil microcosms are a valuable tool for the study of genetic interactions between microorganisms in natural soil environments. However, many factors, some of which are better studied than others, can affect gene transfer events occurring in soil systems, and hence should be carefully considered when performing experiments in soil microcosms. This paper is a guide to setting up and evaluating gene transfer experiments, and in particular conjugation studies in soil microcosms. Particular emphasis is given to the transfer of broad-host-range (primarily clinically derived) plasmids, and natural antibiotic resistance and catabolic soil plasmids. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

Keywords: Gene transfer; Conjugation; Soil microcosm; Broad-host-range plasmid; Catabolic plasmid; Antibiotic resistance plasmid

1. Introduction

Studies of horizontal gene exchange in soil microcosms are important from several points of view. Firstly, the increasing interest in the possible spread of GMOs (genetically modified microorganisms) and antibiotic resistance traits has prompted researchers to study genetic interactions between bacteria in a number of different habitats. Secondly, horizontal gene transfer in soil may play a role in the evolution of new bacterial traits. Also, thirdly there is the possibility of applying catabolic genes to contaminated soils as a bioaugmentation strategy to enhance the biodegradation potential of microbial communities. Microcosms can be used to study these phenomena and provide easy to handle systems, which can be reproduced and set up in a controlled laboratory environment.

Laboratory microcosms are used to assess potential ecological effects which may result from the introduction of a particular organism in a particular habitat by mimicking key field parameters in a laboratory situation. For example, they may be used in the testing of potentially beneficial strain inoculants for bioremediative or agricultural purposes. Importantly, the use of microcosms in pre-release studies is an essential requirement prior to the environmental release of GMOs.

Microcosms need to be calibrated prior to use to ensure that they adequately simulate key parameters [1]. However, field calibration of a microcosm does not imply that it should be an exact analogue of a

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field environment. Instead, calibration acts as a measuring rod to show how closely related the microcosm is to the field environment. In this way, the environmental significance of data obtained from the microcosm can be assessed [2]. Hence microcosms can be used to give vital information on the survival and dispersal of bacterial strains, as well as on their ability to compete with the indigenous microbiota and their capacity for gene exchange, and on the stability of heterologous DNA in soils. A number of these considerations are discussed below. Importantly though, the ecological effects which may result from the introduction of a particular organism must be evaluated on a 'case-by-case' basis [3].

For the purpose of this review we will focus solely on studies examining plasmid-mediated gene transfer in soil microcosms. The most efficient and best studied mechanism by which plasmids are disseminated in soil is by conjugation, so only this mechanism will be discussed in this mini-review. The plasmids described are shown in Table 1.

2. Gene transfer experiments in soil microcosms

2.1. Soil microcosms

There is a need for studies to be carried out in soil systems which mimic as much as possible those parameters which exist in a natural field system. In a study examining the survival of *Pseudomonas* sp. RC1, growth chamber microcosms which simulated mean field parameters were, in general, better predictors of field behaviour than microcosms incubated continuously at 22°C [1]. Obviously a compromise has to be reached in the number of variable parameters that can be used in any given study. Too many variables will make the study unmanageable and any results obtained unclear or indecisive. This is one advantage of a microcosm, in that the variables applied can be limited so that real effects can be measured. However, there can be a danger in over-simplifying a system as dynamic as soil. Consequently, the use of simple microcosms containing only a few grams of soil must go hand in glove with more complex, scaled-up soil microcosms, to validate any results obtained.

A number of soil microcosms used to assess gene transfer have been described in the literature. These vary in complexity from very simple closed vessels containing a few grams of soil, for example in a conical flask [4] or Falcon tube [5], to more elaborate open systems such as vertical soil columns in which either the soil has to be sliced to be sampled [6] or the percolated effluent passing through the column is sampled [7]. A number of examples are given in Fig. 1.

2.2. Small and larger sized microcosms

A microcosm should be relatively simple to ensure that reliable and reproducible sampling procedures can be used. However, size is also an important consideration. Most soil microcosms described in the literature are very simple, consisting of quite small containers such as milk dilution bottles [8,9], screw-capped flasks [10], petri dishes [11] or test tubes [12] and containing only a few grams of soil.

Several studies on plasmid transfer have been carried out in 1-g soil microcosms in a test tube. However, since soil is very heterogeneous, it is questionable whether such small microcosms, which are strongly influenced by wall effects, can be representative of real field conditions. The larger a microcosm, the smaller the wall effects produced, and the better problems of heterogeneity can be avoided. We have found a 100-g soil microcosm to yield reproducible results and to be a reasonable compromise between reality and practicality [13]. Moreover, whether disturbed or undisturbed soil and what type of vessel (i.e. jars or packed columns) is used will also have an effect. Additionally, microcosm design is important. For example, a number of different strategies to maintain soil moisture can be applied such as the placing of the microcosm onto a water saturated sand bed (Fig. 1a) or the use of cotton or paper wicks connecting the microcosm soil to a water supply (Fig. 1b,c).

The microcosms described so far have been enclosed batch systems. However, a number of unenclosed soil microcosms have also been employed, such as continuous flow column reactors through which water or nutrients are percolated. Percolation of varying concentrations of 2,2-dichloropropionic acid [6] or nutrients [7] enhanced both survival and transfer of a DCPA degradative plasmid, pLF40 and

Table 1
Summary of characteristics of plasmids described in this review

Plasmid	Incompatibility group (if known)	Phenotype	References
RP4	IncP	Km^R, Tc^R, Ap^R, BHR	[12,13,34,45,47,48]
RP4p (RP4::*pat*)	IncP	Km^R, Tc^R, Ap^R, BHR	[15]
pBLK1-2 (pRK2073::Tn*5*)	IncP	Km^R, Sm^R, Sp^R, BHR	[8]
RP4::Tn*4371*	IncP	Km^R, Tc^R, Ap^R, 4-chlorobiphenyl$^+$, biphenyl$^+$, BHR	[39]
pJP4	IncP	Hg^R, 2,4-D$^+$, BHR	[30,32,41,42]
pWW0-EB62	IncP	*p*-ethyltoluene$^+$, *p*-methylbenzoate$^+$, *m*-methylbenzoate$^+$, *m*-xylene$^+$	[40]
pDN705	IncP	Tc^R, Cd^R, Zn^R, Co^R, BHR	[13]
pEMT3k (pEMT3::miniTn*5*Km1)	IncP	2,4-D$^+$, Km^R, BHR	[43,44]
pEMT1k (pEMT1::miniTn*5*Km1)	not IncP, IncN or IncW	2,4-D$^+$, Km^R, BHR	[43,44]
R57.b	IncC	Cm^R, BHR	[12]
R388::Tn*1721*	IncW	Tc^R, Tp$^+$, BHR	[7,33]
pLF40		2,2-dichloropropionate$^+$	[6]
pFT30		Tc^R	[10]
pJB5JI::Tn*5*		nif$^+$, Km^R	[30]
pIJ673		Tsr^R, Vph^R, Nm^R	[18]

Abbreviations used in review text and table: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Nm, neomycin; Sm, streptomycin; Sp, spectinomycin; Tc, tetracycline; Tp, trimethoprim; Tsr, thiostrepton; Vph, viomycin; nif, nitrogen-fixing; 2,4-D, 2,4-dichlorophenoxyacetic acid; Co, cobalt; Zn, zinc; Cd, cadmium; Hg, mercuric chloride; BHR, broad host range.

Enterobacter cloacae carrying plasmid R388::Tn*1721* respectively (Fig. 1b).

A few studies have used larger soil volumes of 50–200 g for microcosm studies [14–16], but few have used very large soil volumes of 600 g or more [17]. Cresswell et al. [18] looked at transfer of the multiple-resistance plasmid pIJ673 from *Streptomyces violaceolatus* to *Streptomyces lividans*. In this study, 200 g soil in the nutrient-amended microcosm was periodically refreshed by removal of 'spent' soil and replenishment with fresh, un-inoculated soil to maintain nutrient levels. This fed-batch system was not designed to act as a true model of field conditions, but was more accurately able to produce a dynamic system, and therefore perhaps a more realistic model to actual events observed in situ.

2.3. *Microcosms for studying bacterial interactions in the vicinity of plant roots*

Two examples of soil microcosms incorporating plants are given in Fig. 1. For both examples, complete destruction of the microcosm was needed for sampling and hence this could only be done once per experiment. Hence, such systems may be unsuitable for longer term experiments where continuous sampling is required. In this case, many identical microcosms (one for each sampling in duplicate or triplicate) are used and set up at the start of an experiment.

The microcosm in Fig. 1a contained up to 40 g soil. In this system, surface sterilised plant seeds were placed on top of the nylon gauze and covered with gravel to protect them from drying. The entire system was then incubated until a root mat had developed and the shoots sprouted to 10 cm in height. The soil block was frozen in liquid nitrogen and then sampled using a thin slicing technique on a microtome. The bacterial distribution could then be determined in individual slices at increasing distance from the root mat. Fig. 1c shows a slightly different soil column containing only 10–12 g soil. By contrast, whole plant seedlings were planted into this microcosm at the beginning of the experiment and subsequently, the complete core was withdrawn and sampled, including the whole plants.

Larger, more complex rhizosphere microcosms also require destruction of the plants for sampling.

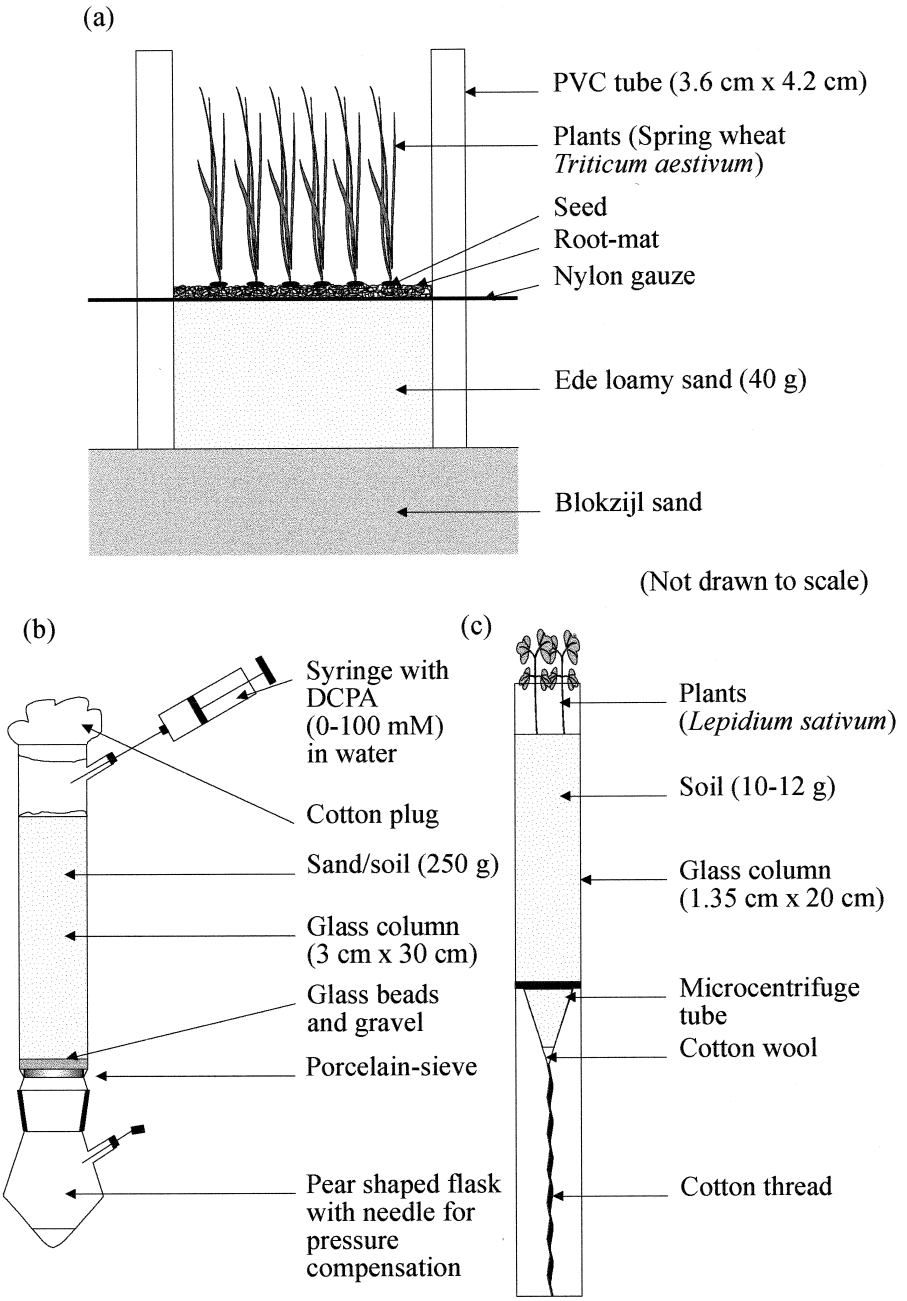

Fig. 1. Examples of three different soil microcosms with/without rhizosphere, used for studying survival, distribution of and gene transfer between bacteria in soil. (Figures (a), (b) and (c) were redrawn from [53], [6], and [4] respectively.) a: A soil chamber for studying bacterial populations in close vicinity to plant roots, sitting on a layer of Blokzijl sand to regulate the moisture tension. The root-mat developed was analyzed using a thin-slicing technique. b: A vertical soil microcosm in a glass column percolated with varying concentrations of 2,2-dichloropropionic acid. Effluent samples percolated through the microcosm were collected and sampled from the receptor flask. c: A column microcosm which can be used with or without plants from which the whole soil core was removed and sampled at the end of the experiment.

However, if sufficient plants can be added to a system, only a small number need to be destroyed at each sampling and the microcosm can be used over a much longer period of time [17]. Also, if larger plants are used, soil in close proximity to the root surface can be shaken off and sampled instead of using the whole plant [15].

2.4. Bulk soil

Only a handful of conjugative gene transfer experiments in field situations have been carried out in bulk soil using defined donor and/or recipient strain inoculants [19,20]. Hence, laboratory-based soil systems have almost exclusively been used to demonstrate that the potential for gene transfer in the natural soil environment exists. One of the predominant reasons for this must be the difficulty in identifying suitable phenotypic/genotypic markers encoded by natural soil plasmids. This has meant that in many cases GMOs are the only option for use in mating experiments to ensure adequate selection against the indigenous soil microflora. However, the use of GMOs in a field situation is costly in time and also financially speaking where rigorous application procedures to obtain permission to do field release experiments are required. Another problem is that transfer frequencies observed in the field situation are often below the levels of detection, perhaps because of spatial separation of donor and recipient cells in soil. For example, in a 3-year study looking at plasmid transfer from a genetically modified *Rhizobium* inoculant to indigenous soil *Rhizobium* species in the field, no in situ gene transfer could be observed, although transfer could successfully be demonstrated to occur in laboratory matings [21]. Hence the dearth of field data has led to the use of soil microcosms without calibration to field systems to verify the authenticity of the data produced. Therefore in many respects, it is impossible to evaluate how well data from laboratory systems reflects those from natural soil systems. A number of field experiments are reportedly under way however [22].

Retrospective field evidence for in situ gene transfer in the soil environment has been shown [23,24]. Such studies infer that horizontal gene transfer has taken place where a highly conserved gene/plasmid is shown to be shared by a group of taxonomically diverse hosts. However, no conclusive relationship between transfer and time can be made, and any quantification of transfer frequency is rather limited. This is perhaps not the case for a study by Lilley and Bailey though [20] where a narrow window for permissive transfer was observed in the sugarbeet rhizosphere environment. Interestingly, this study suggested that plasmid transfer to an introduced recombinant *Pseudomonas fluorescens* strain from the indigenous bacterial population occurred as a result of plant signals at a very precise time in the plants growth and development. The scarcity of available data on the impact of such environmental signals on conjugation contrasts strongly with the important consequences they may have on the dissemination of genetic information in field situations. Therefore results from microcosms have to be evaluated from a very critical viewpoint, with the realisation that there may be many unknown factors which cannot be taken into account and could not be mimicked in the microcosm set-up.

3. Factors affecting gene transfer in soil

Besides microcosm size and design, a number of other factors can strongly influence gene transfer events in the microcosm. These include inoculum treatment [25], its mode of application [26], the concentration at which it is added and the sampling method used.

3.1. Soil treatment

Prior to its use in the microcosm, soil may undergo one or more of the following treatments: drying, sieving, sterilisation, adjustment to standard moisture content and/or amendment with nutrients and/or salts. Additional parameters which can be set prior to an experiment include temperature, pH, ionic composition and microbial competition with other inoculated strains or, with the indigenous soil microflora.

Microcosms consisting of undisturbed soil samples are ideal as they best mimic real soil conditions. Angle et al. [27] compared the survival of *lacZY* marked *Pseudomonas aeruginosa* strains in two different mi-

crocosms and in a field plot. One microcosm consisted of 50 g of dried and sieved soil in a 160-ml dilution bottle (disturbed soil), while the other one consisted of an undisturbed soil column. They showed that the population size of *P. aeruginosa* declined at a significantly greater rate in the disturbed soil microcosm than in the intact soil core microcosm or in the field. The destruction of the soil aggregates by drying and sieving was thought to be the cause of the poorer survival of the strain. Therefore, since survival also influences conjugative plasmid transfer, it may be important to re-evaluate results of conjugation studies obtained with dried and sieved soil!

3.2. Sterile versus non-sterile soil

Transfer frequencies are almost always higher in sterile soil than in non-sterile soil. This seems to be due to better survival of the donor and/or recipient strains because of lack of competition with indigenous microorganisms and a generally higher soil nutrient content after sterilisation. In addition, more efficient conjugational transfer occurs thanks to the higher microbial metabolic state in the presence of more available nutrients. Hence, the use of sterile soil represents a compromise between strict laboratory and in situ conditions [28]. Obviously, sterile soil is a very artificial environment, and results obtained using it can only be used as a first indication for further research rather than to draw important ecological conclusions.

Transfer of Tn*5*-marked symbiotic plasmids (pSym) has been observed in sterile soil microcosms between *Rhizobium leguminosarum* biovar *trifolii* and specific soil bacteria [29] at frequencies of 10^{-4} per donor strain. For transfer of the pea symbiotic plasmid pJB5JI in non-sterile soil between *Rhizobium fredii* and a pSym-cured strain of *R. leguminosarum* [30], highest transfer frequencies of 10^{-4} per recipient were obtained when soil temperatures (28°C) and moisture levels (30–35%) were optimal for rhizobial growth. Plasmid transfer frequencies were also increased by the presence of pea rhizosphere, by SBM (dried soyabean meal) additions and by increasing the inoculum density but, in each case, the increase was no more than one order of magnitude.

3.3. Spatial separation between donor and recipient strains

Soil is a heterogeneous system which may act as a physical barrier to bacterial cell-to-cell contact. Hence, an important factor for conjugative gene transfer in soil is that the bacteria reach and maintain high enough cell numbers to facilitate cell-to-cell contact between donor and recipient strains. The ability to maintain high numbers and to migrate through soil depends on the characteristics of each species and is an important factor in the process of genetic transfer [31]. Hence it seems likely that genetic transfer will be limited to the area of dispersal of donor and recipient strains and has been shown to be primarily influenced by the amount of water present which aids the mobility and survival of cells [11]. For example, experiments by Van Elsas et al. [16] have shown that plasmid transfer did not occur between donor and recipient organisms introduced separately into soil samples which were subsequently mixed. However, more recent microcosm studies have shown that dispersal of inoculated bacteria by mesobiota such as earthworms can enhance gene dissemination in deeper soil layers [32].

3.4. Presence of nutrients

Importantly, the survival and persistence of GMOs, as well as conjugative DNA transfer has been shown to be greatly influenced by the addition of nutrients. Top et al. [13] showed that mobilisation of the IncP plasmid pDN705 from *Escherichia coli* to *Alcaligenes eutrophus* was only detected in a sterile sandy soil at 20°C when nutrients were added (5.0×10^{-7} per recipient after 4 days). In a richer sterile sandy loam soil, transfer occurred at similar frequencies in both nutrient-amended and non-amended soil (1×10^{-5} per recipient after 1 day) but no transfer was observed in non-sterile soil unless nutrient amended (6.9×10^{-7} per recipient after 5 days).

In soil slurry, greatest transfer of the plasmid R388::Tn*1721* between *Pseudomonas cepacia* strains was observed at 35°C when enriched with a nutrient supplement [33]. Conversely, low numbers of transconjugants were observed in low nutrient or pH stress, even when initial donor and recipient numbers

were maintained at high levels. A similar positive effect of nutrients on the number of transconjugants formed was also seen for transfer of R388::Tn*1721* in a continuous-flow soil column [7], for transfer of plasmid RP4 and (retro)mobilisation of an IncQ vector in sterile and non-sterile soil microcosms [34] and for transfer of IncP, IncN, and IncW plasmids in sterile soil [35].

3.5. *Presence of a selective pressure such as heavy metals or recalcitrant organics*

Researchers have become aware that conjugative plasmid transfer may play an important role in the acquisition of heavy metal resistance and degradative traits by bacteria in natural ecosystems exposed to high concentrations of heavy metals and novel, man-made recalcitrant organics. Kinkle et al. [36] showed that the 2,4-D degradative and Hg^R plasmid pJP4 was transferred from an introduced *Bradyrhizobium japonicum* strain to several introduced *Bradyrhizobium* sp. strains in non-sterile soil. However, the addition of mercury (up to 50 mg kg^{-1} soil) had no apparent stimulatory effect on the number of transconjugants obtained.

Direct and triparental mobilisation, and retromobilisation of the heavy metal resistance gene cassette *czc* (Co^R, Zn^R, Cd^R) from *E. coli* or *Pseudomonas putida* to *A. eutrophus* has also been investigated in sterile and non-sterile soil microcosms [13,34,37,38]. These studies showed that the presence of heavy metals in the soil positively influenced the number of transconjugants found in sterile soil. However, this positive effect was not clearly demonstrated in non-sterile soil. Ratios of transconjugants to recipients were sometimes higher in sterile polluted soil than in plate matings.

In contrast to the results with heavy metal contaminated soils, the presence of certain man-made organic substrates has clearly been shown to enhance the number of transconjugants obtained which express the catabolic genes in non-sterile soil. De Rore et al. [39] studied the transfer of a non-recombinant biphenyl degradative transposon located on RP4, to indigenous bacteria in sandy soil. The plasmid was transferred to bacteria belonging to a number of different genera and in spite of the fast disappearance of the donor, the number of transconjugants was higher in biphenyl spiked soil than in non-treated soil (5.9×10^4 g^{-1} soil compared to 4.1×10^2 g^{-1} after 26 days). In contrast, transfer of the catabolic plasmid pWWO-EB62, encoding degradation of ethylbenzoate, between introduced strains in sterilised soil with and without addition of ethylbenzoate was only detected when the donor and recipient strains were of the same species [40]. A positive effect of ethylbenzoate on the number of transconjugants obtained was not observed. On the contrary, a negative effect was seen, i.e. a lag phase in the formation of transconjugants, which was probably due to toxic effects of the chemical on the recipient strain.

The effect of selective pressure exerted by 2,4-D on the dissemination of pJP4 in soil was studied by Neilsson et al. [41] and diGiovanni et al. [42]. Neilsson et al. [41] found that transfer of pJP4 from *A. eutrophus* JMP134 to *Variovorax paradoxus* in nonsterile soil amended with 100 ppm 2,4-D occurred at 10^{-6} per parent strain after 48 h. diGiovanni et al. [42] examined the frequency of plasmid transfer to indigenous bacteria, and observed that transconjugants were only detected at high concentrations of 2,4-D (1000 ppm), and not at 100 ppm or 500 ppm. In both studies [41,42], the plasmid donor strain survived well thereby compromising the ability to assess frequencies of plasmid transfer to indigenous bacteria. Indeed, the donor strain by itself could readily deplete the carbon source required for the selection of transconjugants in the 2,4-D enriched soil.

Transfer of two other 2,4-D degradative plasmids, pEMT1 and pEMT3, isolated from agricultural soil [43] was recently investigated in soil microcosms [44]. Transfer of plasmid pEMT1k (a kanamycin marked derivative of pEMT1) to the indigenous microbial populations was observed in non-amended and 2,4-D-amended soil though 2,4-D amendment had an enhancing effect on transconjugant numbers (2×10^2 per donor in 2,4-D treated soil compared to 3.9×10^{-3} per donor in non-treated soil after 5 days). Transfer of plasmid pEMT3k was only observed in 2,4-D treated soil (one per donor after 5 days). These results show that a selective pressure in a soil habitat can strongly affect the extent of gene spread and hence needs to be considered as an influential factor in each case.

3.6. Soil type

Soil type has been shown to greatly influence conjugal transfer in soil. Hence bacterial strains and plasmids might then be expected to exhibit different survival and transfer characteristics in different soils [13]. For example, intergeneric transfer of plasmid pBLK1-2 occurred in soil but was strongly influenced by soil variables such as clay, organic matter, soil pH, soil moisture and soil incubation temperature [8]. Maximum transfer frequencies per recipient were obtained at 15% clay content, 5% organic matter, pH 7.25, 8% moisture, and a soil incubation temperature of 28°C. Soils containing high clay and organic matter [15], sandy soil with a high clay and silt contents [12], a sandy loam [13] and bentonite amended soil [45] were all shown to enhance transfer of IncP plasmids. This is thought to be due to an enhancing of cell-to-cell contact required for conjugal transfer in finer textured soil. The 4.4-kb *Bacillus cereus* plasmid pFT30 isolated from soil [10] transferred at a frequency of 1×10^{-6} per donor in laboratory matings between *Bacillus* sp. In sterile loamy sand, this transfer frequency declined to 0.7×10^{-7} and no transfer was observed in unamended non-sterile soil. However in the presence of bentonite clay, plasmid transfer occurred at a frequency of 0.9×10^{-7} in non-sterile soil and survival of the recipient strain was concomitantly enhanced. When a more efficient transfer/soil system was used, for example the pea symbiotic plasmid pJB5J1 and a silt loam soil [30], transfer was demonstrated to occur in non-sterile and unamended soil.

3.7. Influence of plant rhizosphere

Plant roots in rhizosphere soil have been shown to be just as influential on gene transfer as soil type. This is primarily due to the large amount of nutrient-rich plant root exudates which are released into the soil. Foster et al. [46] showed that bacterial growth was stimulated in soil as far as 1–2 mm from the root, although the effect was most pronounced at 0–50 μm from the root surface. Notably, enhancement of transfer of RP4 [47] was found to occur just around the plant root area and a marked decrease in frequency was observed with increasing distance from the root. However, although transfer was detected in soil in a model wheat rhizosphere microcosm, no transfer was detected in non-rhizosphere soil [45], unless the soil was nutrient amended [48]. In addition, there may also be some evidence for migration of bacterial populations towards the plant roots due to the flux of soil water induced by the plants themselves [16].

3.8. Temperature

Temperature has been shown to greatly affect gene transfer frequencies. For example, plasmids RP4 and R57.b transferred at high frequencies (10^{-2}) at 30°C, but at low frequencies at 20°C in a number of different sterile soils [12]. (It is unclear from this reference how these particular transfer frequencies were estimated.) This observation has probably been biased however, by the use of *E. coli* as the donor strain in this study which has a high optimal growth temperature. Hence, indigenous soil strains are likely to produce higher transfer frequencies at more environmentally relevant soil temperatures than *E. coli*. This has been shown very distinctly by plasmids indigenous to river epilithon such as pQM1, pQM3 and pQM4 which transferred optimally at 25, 20 and 15°C respectively [49].

3.9. Earthworm activity

A major limiting factor in the dispersal of bacteria and their genes introduced into soil is their poor mobility through the soil system. Meso- and macrobiota can however contribute to the dispersal of bacteria and plasmids in soil, as recently shown by Daane et al. [32]. In a 40 cm high soil column plasmid pJP4 transferred from *P. fluorescens* to the indigenous bacteria in the higher layers, but the number of transconjugants was significantly increased in the deeper layers of the soil columns containing one earthworm. Hence, the effect of earthworm activity on the numbers of transconjugants, their vertical distribution, and their diversity should be considered in future risk assessment and other plasmid transfer studies in soil.

3.10. Plasmids used for the transfer experiments

There are a predominance of plasmid transfer

studies in soil microcosms which have utilised the broad-host-range (BHR) or so-called promiscuous plasmids [50] belonging to the IncC, IncN, IncP, IncQ and IncW groups. The IncP plasmids in particular have specific characteristics which have made them the plasmid of first choice in many cases when studying gene transfer in natural environments. IncP plasmids transfer at very high frequencies, have an extremely broad host-range and encode a range of selective markers such as resistance to kanamycin and tetracycline (e.g. RP4) or mercuric chloride (e.g. pJP4) which have made them easy to select in soil environments. Many of these plasmids (with the exception of pJP4) were not isolated from a soil environment, but were derived from clinical sources. Hence, they are perhaps less appropriate for use in microcosms which pertain to mimic natural field populations. Perhaps the most interesting plasmids in such studies are those which have been isolated from the soil of interest. Such natural plasmids include antibiotic resistance, heavy metal resistance, and catabolic plasmids. However, relatively few studies have been conducted in natural soils using indigenous soil microbes with realistic population densities and incubation conditions [16,19,51].

3.11. Estimation of transfer frequencies

Transfer frequencies are estimated as a ratio of transconjugant counts to donors or recipients. A number of studies in soil microcosms still make the assumption however that all transconjugants observed arise as a direct result of transfer occurring in the soil. In the initial stages of an experiment this will indeed be the case. However, there will be subsequent survival, growth and die-off of transconjugants in competition with donors, recipients and indigenous soil microorganisms. Increases in transconjugant numbers with time are therefore likely to result from replication of the initial transconjugants, particularly in instances where selective pressure actively promotes growth of these transconjugants. Consequently, sampling time after inoculation will play an important role in transconjugant numbers observed and in any subsequent interpretation of these counts.

Conjugal transfer of thiostrepton resistance plasmids has been reported between *Streptomycetes* colonising sterile soil microcosms [52] and in nutrient amended and non-amended non-sterile soil [51]. In this last study, plasmid transfer in soil was comparable to that obtained on laboratory media, although after day two in the soil microcosm, frequencies mainly reflected growth and sporulation of transconjugants. After day two in this case, 'transfer frequency' does not reflect the frequency of actual conjugative transfer and use of the term 'transfer frequency' is incorrect. The transconjugant to donor/recipient 'ratio' can still be calculated after day two, but this should remain defined as a 'ratio' and be distinct from the true estimate of transfer frequency.

4. In conclusion

Microcosm studies are invaluable to our understanding of how field systems and the dynamics of such systems work. Hence, the use of microcosms is fundamental to research in microbial ecology if we are to gain in our understanding of how plasmid mediated gene dispersal occurs and to what extent it affects the microbial community structure and dynamics in the natural soil environment. Numerous studies have shown that many factors affect the rate of gene transfer in soil, and can be used to draw useful insights into the design of new microcosm studies. It has also been shown that selective pressure exerted by a xenobiotic compound can greatly increase the populations of bacteria that have acquired genes that encode mineralisation of or resistance to that compound. This should not be overlooked when assessing plasmid dissemination in natural environments.

In conclusion, comparison of several studies has revealed that the standard soil microcosm does not exist. Therefore one has to be very careful when drawing conclusions based on comparisons made between different microcosm studies which have been performed under even slightly different environmental conditions. We hope that our list of factors which may directly or indirectly influence the extent of conjugative gene transfer occurring in soil can be used as a guideline for setting up future soil microcosm studies, and that this will aid the researcher in evaluating the data obtained from such studies.

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