Biol Fertil Soils (1999) 29:221–245 © Springer-Verlag 1999

REVIEW ARTICLE

M. Dröge · A. Pühler · W. Selbitschka

Horizontal gene transfer among bacteria in terrestrial and aquatic habitats as assessed by microcosm and field studies

Received: 8 July 1998

Abstract Genetic interactions among bacteria are mediated by one of the three distinct gene-exchange mechanisms: conjugation, transformation or transduction. Conjugative gene exchange relies on mobile elements, such as plasmids, which transfer between donor and recipient cells. In natural transformation, competent cells take up DNA and incorporate it into their genome. Gene transfer via transduction is mediated by bacteriophages which accidentally package donor DNA in their phage head and transfer it to recipient cells. Driven mainly by biosafety research and research into the rapid dissemination of antibiotic resistance, the evaluation of gene flux among bacteria in their natural habitats has become a focus of scientific interest in recent years. Accordingly, gene transfer has been assessed in laboratory-based studies employing model ecosystems, as well as in field experiments. Conjugative gene exchange has been shown to occur under a wide range of environmental conditions. Factors identified as conducive for conjugation include the presence of nutrients provided by the rhizosphere of plants. Studies addressing gene transfer via transformation have demonstrated that naturally transformable bacteria develop competence and take up DNA under in situ conditions. Moreover, DNA has been shown to persist to some extent in the environment, and thus be available for uptake by naturally competent cells. Gene exchange via transduction has been demonstrated under conditions of nutrient depletion and low densities of host cells. Whereas gene transfer is readily observed in the laboratory, more importantly, field studies have provided direct evidence that all three gene transfer mechanisms also occur in nature. DNA transfer frequencies observed in the environment in some cases differed considerably from those obtained under laboratory conditions. Transfers of low frequency observed in laboratory-based experiments have been readily detected in the environment in the presence of selective forces.

Key words Conjugation · Transformation · Transduction · Biosafety · Rhizobium

M. Dröge · A. Pühler (✉) · W. Selbitschka
Department of Genetics, University of Bielefeld,
P.O. Box 100131, D-33501 Bielefeld, Germany
e-mail: Puehler@genetik.uni-bielefeld.de,
Tel.: +49-521/106-5607,
Fax: +49-521/106-5626

Introduction

About 50 years have passed since the discovery that bacteria are able to exchange genetic information. Three fundamental mechanisms have been identified which mediate horizontal gene transfer (HGT) between bacteria. These are designated conjugation (Lederberg and Tatum 1946), transformation (Avery et al. 1944) and transduction (Zinder and Lederberg 1952).

Bacterial conjugation is a cell-contact-dependent parasexual process, whereby specific plasmids or transposons transfer from donor to recipient cells. Conjugative plasmids have been identified in a wide range of Gram-negative and Gram-positive bacteria. More than 25 different incompatibility groups of Gram-negative bacteria have been defined (Couturier et al. 1988) and some of these plasmids, e.g. those belonging to the incompatibility groups IncP, W, N, or C, exhibit an extraordinarily broad host range (bhv) with respect to transfer and autonomous replication (Thomas 1989). These plasmids usually transfer between most Gram-negative bacterial species. An important feature of some of these conjugative plasmids is their potential to mobilise even chromosomally located genes to recipient cells (e.g. R68.45; Haas and Holloway 1976). For detailed information about conjugation the reader is referred to Clewell (1993).

Transformation is defined as the uptake, integration and stable inheritance of cell-free DNA by bacterial and archeal cells. Natural transformation is a process whereby cells develop competence for the uptake of free DNA under certain conditions. This ability is un-

der the control of specific genes, and is a tightly regulated process. Until now, more than 40 bacterial species belonging to either the Gram-positive or the Gram-negative bacterial groups, as well as some archea such as *Methanococcus voltae*, have been shown to possess natural transformation systems (Lorenz and Wackernagel 1994). Since some of these species are promiscuous in their uptake of DNA, gene transfer via transformation between even distantly related bacteria is possible. For detailed information about natural transformation, the reader is referred to the review of Lorenz and Wackernagel (1994).

Transduction is the transfer of genetic information between donor and recipient cells that is mediated by bacterial viruses called bacteriophages [for reviews see Margolin (1987), and Weisberg (1987)]. There are two kinds of transduction mediated by two different classes of bacteriophages. In specialised transduction, only certain genes adjacent to the phage genome, which is integrated into specific sites of the bacterial chromosome, can be transferred upon excision and subsequent phage propagation. In contrast, in generalised transduction virtually any gene of a bacterial host can be transduced after accidental packaging of host DNA into a phage head. Since phages often display a narrow host range of infection, transduction is not considered to contribute essentially to gene exchange among distantly related bacteria.

While today there exists a large body of knowledge about the molecular basis of gene transfer among bacteria, less is known about how bacteria interact genetically in their natural habitats. Circumstantial evidence from population genetic studies (Smith et al. 1993) and the rapid spread of conjugative plasmids or transposons encoding antibiotic-resistance genes (van Elsas 1992; Baquero and Blazquez 1997) indicate that bacteria exchange genes in nature.

With the advent of recombinant DNA (rDNA) technology, it became possible to modify organisms such as bacteria in vitro. Applied aspects of the use of such genetically engineered microorganisms (GEMs) include the construction of bacterial strains for use as biofertilisers, for biocontrol of plant-pathogenic fungi, or for bioremediation of xenobiotic compounds. The pending release of GEMs has raised biosafety issues, and scientific as well as public concern has been expressed about the fate of recombinant bacteria and their rDNA in the environment. The potential transfer of rDNA from deliberately or accidentally released bacteria to indigenous microbes has been regarded as particularly important, since the persistence of rDNA then becomes independent of the survival of its original host in the environment, and thus potentially leads to unpredictable, long-term ecological effects. As a first step to estimate the potential for HGT between microorganisms in the environment, laboratory based studies were performed using terrestrial and aquatic model ecosystems [for reviews see Hill and Top (1998) and Ashelford et al. (1997)]. In recent years, several field studies have been conducted assessing HGT among bacteria under the complexity of fluctuating and, in some cases, synergistically interacting environmental parameters.

The aim of the present review is to summarise recent literature on HGT among bacteria in both terrestrial and aquatic habitats. In order to give a general overview, we have collected data on all three gene-exchange mechanisms: conjugation, transformation and transduction. Laboratory-based studies are presented which identified major environmentally relevant parameters influencing rates of gene transfer. Special emphasis is placed on recent reports which have addressed HGT among bacteria in the field. Finally, biosafety aspects concerning the potential for HGT from a deliberately released *Sinorhizobium meliloti* GEM are presented.

Gene transfer among bacteria in terrestrial and aquatic environments

During the last decade the issue of gene transfer among bacteria in their natural habitats has been addressed on a broader basis. Studies assessing gene exchange via conjugation, transformation or transduction, respectively, have been conducted under controlled conditions in the laboratory. For this purpose, microcosm systems have been employed containing environmental samples such as sterile and non-sterile soil or water samples. These systems have allowed researchers to analyse the influence of single environmental parameters on gene transfer among bacteria. However, it is obvious that laboratory based microcosm studies fail to mimic the complexity of fluctuating and, in some cases, synergistically interacting environmental parameters. Consequently, field studies have been performed to assess whether, and if so to what extent, HGT occurs in nature (Table 1).

These studies have identified several natural habitats as "hot spots" of genetic interactions among bacteria such as the rhizosphere (van Elsas 1992; Dröge et al. 1998) and the gut of some soil organisms [e.g. Hoffmann et al. (1998)] where nutrient availability supports locally high bacterial cell densities and positively influences metabolic activities.

Conjugation

The potential of conjugation for the exchange of genetic information among bacteria in nature is indicated by the widespread occurrence of conjugative elements, such as self-transmissible plasmids, in organisms living in various aquatic, as well as terrestrial, environments. Such mobile elements have been identified in bacterial populations inhabiting, for instance, the phytosphere of sugar beet, the rhizosphere of wheat, soils, river epilithon, marine sediments, marine air-water interfaces, marine water, marine biofilm communities, sewage, and activated sludge (see Table 2).

Table 1 Field studies assessing horizontal gene transfer (HGT) among bacteria in terrestrial and aquatic habitats

Transfer mechanism	Habitat	Donor	Recipient	Transferred DNA	Transfer detected?	Reference
Conjugation	Unplanted soil	*Escherichia coli* (IncP helper plasmid pGP527)	*Pseudomonas putida*	Mobilisable IncQ plasmid pIE723	Yes	Götz and Smalla (1997)
	Soil planted with *Lotus corniculatus*	*Mesorhizobium loti* inoculant strain	Four species related to *M. loti*	Conjugative transposon? ("symbiosis island")	Yes	Sullivan et al. (1995), Sullivan and Ronson (1998)
	Soil planted with *Pisum sativum*	Indigenous *Rhizobium leguminosarum* bv. *viciae* population	*R. leguminosarum* bv. *viciae*	Self-transmissible Sym plasmids	No	Hirsch (1996)
	Rhizosphere of sugar beet	*Pseudomonas marginalis*	*Pseudomonas aureofaciens*	Self-transmissible mercury resistance plasmid pQBR11	Yes	Lilley et al. (1994)
	Rhizosphere of wheat	*Pseudomonas fluorescens* (*bhr* helper plasmid pIO2)	Indigenous bacteria	Mobilisable IncQ plasmid pIE723	Yes	van Elsas et al. (1998)
	Crown galls of cherry seedlings	*Agrobacterium radiobacter* K84	*Agrobacterium tumefaciens*	Self-transmissible plasmid pAgK84 encoding agrocin biosynthesis and immunity	Yes	Stockwell et al. (1996)
	Phytosphere of sugar beet	Indigenous bacteria	*P. fluorescens*	Self-transmissible mercury resistance plasmids	Yes	Lilley and Bailey (1997a)
	Sewage	*E. coli*	*E. coli*	Self-transmissible R plasmid	Yes	Altherr and Kasweck (1982)
	Activated sludge	Various enteric bacteria	*E. coli* or *Shigella sonnei*	Self-transmissible R plasmids	Yes	Mach and Grimes (1982)
		Enterococcus faecalis	*E. faecalis*	Pheromone-responding plasmid pIP1017; bhr plasmid pIP501; conjugative transposon Tn*916*	Yes	Marcinek et al. (1998)
	Lake water	*Pseudomonas aeruginosa*	*P. aeruginosa*	Self-transmissible bhr plasmids R68.45 and FP5	Yes	O'Morchoe et al. (1988)
	River epilithon	*P. aeruginosa*	*P. aeruginosa*	Self-transmissible narrow host range plasmid pQM1	Yes	Bale et al. (1987, 1988a, b)
		Indigenous bacteria	*P. aeruginosa*	Self-transmissible mercury resistance plasmids	Yes	Bale et al. (1988a)
Transformation	River epilithon	Crude lysates or living cells of *Acinetobacter calcoaceticus*	Auxotrophic *A. calcoaceticus* strain	Chromosomal DNA	Yes	Williams et al. (1996)
		Crude lysates or living cells of *A. calcoaceticus*	*A. calcoaceticus*	Non-conjugative mercury resistance plasmid pQM17	Yes	Williams et al. (1996)
Transduction	Lake water	Sm-resistant *P. aeroginosa* strain lysogenic for phage F116 or lysate of F116 obtained from the strain	Non-lysogenic Sm-sensitive *P. aeruginosa* strain	Chromosomal DNA	Yes	Morrison et al. (1978)
		Non-lysogenic *P. aeruginosa* strains	Auxotrophic *P. aeruginosa* strains, lysogenic for phage DS1	Chromosomal DNA	Yes	Saye et al. (1990)
		Non-lysogenic *P. aeruginosa* strain	*P. aeruginosa* strain lysogenic for phage DS1	Non-conjugative R plasmid Rms149	Yes	Saye et al. (1987)
		Non-lysogenic *P. aeruginosa* strain	*P. aeruginosa* strain lysogenic for phage F116	Non-conjugative R plasmid Rms149	Yes	Ripp and Miller (1995)

Table 2 Incidence of conjugative plasmids in bacterial communities of terrestrial and aquatic habitats. *DP* Diparental exogenous plasmid isolation method, *iv* in vitro, *E* endogenous plasmid isolation method, *TP* triparental exogenous plasmid isolation method, *is* in situ

Habitat	Plasmid isolation method	Relevant observation	Reference
Phytosphere (sugar beet)	DP[a] (is)	Transfer of mercury resistance plasmids from indigenous bacteria to *P. fluorescens* under field conditions occurred at frequencies in the range of 10^{-2} to 1 per recipient cell, but only at a specific period of plant development. Restriction digest patterns allocated the plasmids to three different groups	Lilley and Bailey (1997a)
	DP (iv)	Seventy-nine mercury resistance plasmids of sugar beet rhizoplane and phyllosphere bacteria were exogenously isolated by using *P. putida* as the recipient. Restriction digest patterns grouped the plasmids into five distinct groups	Lilley et al. (1996)
	DP (iv)	In three independent mating experiments with *P. putida* as recipient, 23 different mercury resistance plasmids were readily isolated. Twelve of these plasmids transferred in filter matings at frequencies in the range of 10^{-3} to 1 to a *P. marginalis* recipient	Lilley et al. (1994)
	E[b]	Of a total of 435 bacterial isolates tested, 79 contained indigenous plasmids. Three of the isolates harboured self-transmissible heavy metal resistance plasmids. Another three isolates harboured plasmids which were able to mobilise IncQ plasmid RB300 into a *P. aureofaciens* recipient in triparental matings	Powell et al. (1993)
Rhizosphere (wheat)	TP[c] (iv)	Using an IncQ plasmid harbouring *E. coli* as host and a *Ralstonia eutropha* recipient, several self-transmissible plasmids with IncQ mobilising capacity were isolated from indigenous rhizosphere bacteria of young wheat plants. Transfer frequencies were about 0.5×10^{-10} per donor cell. No transconjugants were detected when donor populations derived from mature plants were used. None of three plasmids which were randomly selected for further studies could be assigned to any known incompatibility group	van Elsas et al. (1998)
Activated sludge	TP (iv)	The conjugative potential of indigenous bacterial populations of activated sludge was analysed by mobilisation of the IncQ plasmid pMOL155 from an *E. coli* donor to an *Alcaligenes eutrophus* recipient strain, mediated by the bacterial communities originating from six different wastewater treatment facilities. Three conjugative plasmids which belonged to the IncP incompatibility group were identified	Top et al. (1994)
	E	Thirty-one out of 78 coliform bacterial isolates which were obtained from various locations of an activated sludge plant were able to transfer antibiotic resistance determinants to an *E. coli* recipient	Altherr and Kasweck (1982)
Sewage and hospitals	E	Conjugative plasmids conferring multiple antibiotic resistances were identified in 7 of 9 antibiotic-resistant enteric bacteria isolated from primary sewage effluent and clinical bacterial isolates	Mach and Grimes (1982)
Marine air-water interface, marine bulk water, marine biofilm communities	DP (iv)	When using *P. putida* as the recipient, 95 mercury resistance plasmids belonging to 12 different restriction-pattern plasmid groups were isolated at frequencies ranging from 10^{-10} to 10^{-8} transconjugants per donor cell. None of these plasmids could be assigned to any known incompatibility groups. All of the newly identified plasmids were smaller than 100 kb, the majority of the plasmids were around 60 kb in size	Dahlberg et al. (1997)
Marine sediment	E	Among 34 antibiotic-resistant bacteria isolated from sediment samples which were collected downstream from two fish farms, 7 isolates were able to transfer oxytetracycline resistance to *E. coli*	Sandaa et al. (1992)
Rhizosphere (wheat)	DP (iv)	By using *P. putida, P. fluorescens* and *Enterobacter cloacae* as recipients, numerous mercury resistance plasmids were isolated from rhizosphere bacteria of young wheat plants. No transconjugants were detected when donor populations originating from mature plants were used. All of the transconjugants analysed contained plasmids with sizes between 40 kb and 50 kb. None of these plasmids could be assigned to any known incompatibility group	Smit et al. (1998)
Soil	TP (iv)	The conjugative potential of indigenous soil bacterial populations was analysed by the mobilisation of the IncQ plasmid pMOL155 from an *E. coli* donor to an *A. eutrophus* recipient strain. Of four plasmids isolated from 12 randomly selected transconjugants, one plasmid was allocated to the IncP incompatibility group, whereas the other plasmids could not be assigned to any known incompatibility group	Top et al. (1994)

Table 2 Continued

Habitat	Plasmid isolation method	Relevant observation	Reference
River epilithon	TP (iv)	Based on their capability to mobilise the IncQ deriative pD10 from a *P. putida* donor to a *P. putida* recipient strain, 54 plasmids were identified. Sizes ranged from 40 kb to >200 kb. Mobilisation frequencies were in the range of 10^{-8} to 10^{-3} per recipient. Nineteen of these plasmids conferred mercury resistance to their *P. putida* host. Four mercury resistance plasmids were identified as bhr plasmids since they transferred to representative members of β- and γ-proteobacteria	Hill et al. (1992)
	E/DP (iv)	Five out of 65 isolates of epilithic bacteria transferred mercury resistance plasmids to a *P. aeruginosa* recipient strain; plasmid transfer from mixed natural suspensions of epilithic bacteria to *P. aeruginosa* occurred readily under a wide range of environmentally relevant conditions at maximal frequencies of 2×10^{-5} per recipient. The majority of the plasmids identified (80%) were larger than 300 kb and all of them encoded mercury resistance as well as UV resistance	Rochelle et al. (1989)
	DP (is)	Numerous mercury resistance plasmids transferred in situ at frequencies of up to 3.75×10^{-6} per recipient from epilithic bacteria to *P. putida.* Nineteen of the plasmids isolated were grouped into seven groups according to their restriction digest patterns	Bale et al. (1988a)

[a] DP: isolation of conjugative plasmids employing iv or is matings between indigenous bacterial communities of a given habitat and a recipient strain. Selection is achieved by selecting for the marker of the recipient strain and randomly chosen plasmid-encoded markers such as antibiotic or heavy metal resistances. The method identifies plasmids carrying specific selectable traits and includes those plasmids which reside in the non-cultured fraction of bacteria (Bale et al. 1987; Fry and Day 1990b)
[b] E: indigenous plasmids of bacterial isolates obtained after plating of mixed bacterial suspensions from a given habitat on defined or undefined media are identified by physical extraction methods. Conjugative properties are assessed in mating experiments
[c] TP: isolation of conjugative plasmids employing IV or IS matings between indigenous bacterial communities of a given habitat, a donor strain harbouring a mobilisable plasmid which carries a selectable trait, and a recipient strain. Conjugative plasmids of indigenous bacteria may mediate transfer of the mobilisable plasmid to recipient cells. These helper plasmids may co-transfer with the mobilisable plasmid, and thus can be identified in the recipient strain. Plasmids carrying no selectable traits are identified, including those plasmids residing in the non-cultured fraction of bacteria (Bale et al. 1987; Fry and Day 1990b)

The efficiency of bacterial conjugation depends on the conjugative element as well as on both donor and recipient cells, and thus may be influenced by numerous biotic and abiotic environmental parameters. Consequently, laboratory-based studies involving in vitro as well as in situ experiments have analysed the influence of these parameters on conjugative gene exchange in terrestrial and aquatic environments. These studies have established that factors which influence transfer frequencies mainly act at the level of cell-to-cell contacts and the host cells' physiology.

Identification of environmentally relevant biotic parameters influencing conjugation

Physiological status of donor and recipient cells. The influence of the physiological status of donor and recipient cells on conjugative gene exchange has been addressed in numerous studies (e.g. Goodman et al. 1993; Smets et al. 1993; Muela et al. 1994; Sudarshana and Knudsen 1995; Arana et al. 1997). These studies have revealed that, in general, plasmid-transfer frequencies depend on the physiological status of donor rather than of recipient cells.

The influence of nutrient availability on conjugal transfer has been studied in nutrient-amended sterile soils (van Elsas et al. 1987; Top et al. 1990; Clerc and Simonet 1996; Pukall et al. 1996), on filters attached to sterile stones in either complex medium or sterile river water (Bale et al. 1987), or in nutrient-rich medium versus sterile drinking water (Sandt and Herson 1991). All of these studies have demonstrated that nutrient amendment is conducive for conjugation, and thus indicate the relevance of the physiological state of mating partners for conjugative gene exchange.

The question of the relationship between growth rates and transfer frequencies of specific plasmids was addressed by Smets et al. (1993) and Sudarshana and Knudsen (1995). Analyses of the kinetics of TOL plasmid transfer in liquid cultures between strains of *Pseudomonas putida* and *Pseudomonas aeruginosa* as donor and recipient, respectively, revealed a strong positive influence of the specific growth rate of the donor on intrinsic plasmid transfer rates (Smets et al. 1993). This dependence was confirmed by Sudarshana and Knudsen (1995), who analysed the transfer of an IncW plasmid from *Pseudomonas cepacia* to *Pseudomonas fluorescens* in sterile and non-sterile soil. Interestingly, transfer rates were found to be independent of the growth rate of recipient cells (Sudarshana and Knudsen 1995).

The dependence of plasmid transfer on the physiological status of donor cells, rather than that of recipient cells, was also obvious in several other studies (Goodman et al. 1993; Muela et al. 1994; Arana et al. 1997). The transfer of plasmids which were previously isolated from indigenous river bacteria between *Escherichia coli* strains was undetectable when the donor strain was

starved prior to mating for more than 24 h in sterile-filtered river water. In contrast, starvation of the recipient cells for even 96 h did not affect plasmid transfer (Muela et al. 1994). The transfer of various conjugative plasmids between *E. coli* donor and recipient cells under conditions of nutrient starvation revealed that starvation of the recipient strain in river water prior to mating did not influence its ability to receive and to express plasmid-encoded genes, provided that its culturability was unaffected (Arana et al. 1997). In contrast, prestarved donor cells lost their ability for plasmid transfer under the same experimental conditions. Similarly, the transfer of IncPα plasmid RP1 between marine *Vibrio* donor and recipient cells was below the limit of detection when both the donor and the recipient strain were starved prior to mating for periods longer than 14 days. When donor cells were starved for only 9 days, conjugative plasmid transfer into recipient cells starved for even 100 days was still detectable (Goodman et al. 1993).

Whereas in some systems an increase in the metabolic activity increased plasmid-transfer frequencies, no such correlation was found in other systems. Kroer et al. (1998) and Normander et al. (1998) quantified metabolic activities by the uptake of [^{3}H]leucine into donor and recipient cells and correlated the data with plasmid-transfer frequencies. Simple sand microcosms, amended with solutions containing various concentrations of isolated root exudates, were employed to study the transfer of a derivative of IncPα plasmid RP4 from a *P. fluorescens* donor strain to a species of the genus *Serratia* (Kroer et al. 1998). Interestingly, transfer rates in amended sand with cells displaying maximal metabolic activity and unamended sand which contained starved cells displaying low metabolic activity did not differ significantly. Thus, the authors hypothesised that if the metabolic activity is above a certain level which seems to be very low, bacterial conjugation takes place, and an increase in metabolic activity will not further stimulate plasmid transfer (Kroer et al. 1998). Similarly, transfer of the conjugative TOL plasmid between *P. putida* strains on leaves of bush bean yielded no correlations between transfer rates and metabolic activities (Normander et al. 1998).

Plasmid-specific properties. Expression of tra genes of conjugative elements is in most cases tightly regulated [for a review see Zatyka and Thomas (1998)]. However, transfer of some of these elements may be induced under certain environmental conditions. Expression of *tra* genes of Ti plasmids of *Agrobacterium tumefaciens*, for instance, is under quorum-sensing control. It is induced in the presence of a host plant and at a high density of *A. tumefaciens* cells carrying the Ti plasmid [for a review see Zatyka and Thomas 1998)].

Another example of a plasmid-specific property is related to the transfer of thermosensitive plasmids, which occurs at the highest frequencies at temperatures below the optimal growth temperature of their hosts (Stotzky and Babich 1986). For instance, in matings between two *Pseudomonas* strains, plasmid pQM85 displayed maximum transfer rates at 10 °C. Notably, this plasmid was isolated in matings between the indigenous community of river epilithon and a *Pseudomonas* recipient strain during winter at the same temperature (Fry and Day 1990a).

Conjugative plasmids such as IncN, IncW or IncP plasmids encode short and rigid pili. Cell contacts mediated by these kind of pili are susceptible to shearing forces compared to those mediated by the long flexible pili of, for instance, IncFII plasmids. Thus, transfer frequencies of IncP plasmids are generally higher on solid surfaces than in liquids (Thomas 1989). In liquids the transfer of plasmids encoding flexible pili is much more efficient compared to plasmids encoding short and rigid pili (Lebaron et al. 1993). However, it seems that flexible pili are disadvantageous for transfer in environmental systems containing a high solid/liquid ratio since, in contrast to IncP, IncN and IncW plasmids, no transfer of an IncFII or an IncI1 plasmid could be detected between *E. coli* strains in soil microcosms (Pukall et al. 1996).

Presence of indigenous microbes. Numerous studies have reported on a general negative influence of the presence of indigenous microbial communities on conjugative gene exchange in terrestrial (Top et al. 1990; Neilson et al. 1994) and aquatic environments (Bale et al. 1987; O'Morchoe et al. 1988). Bacteria introduced into natural habitats participate in the complex interactions between microorganisms in the microbial communities present. Those interactions may be of a competitive, antagonistic, parasitic or predatory nature (Stotzky 1989). Metabolic activity or survival of bacterial inoculants is negatively influenced, resulting in reduced transfer frequencies. Plasmid transfer was below the limit of detection in bulk soil in the presence of the indigenous community, but was observed to occur after sterilisation of the soils by autoclaving (Top et al. 1990; Neilson et al. 1994). Sterilisation of environmental samples allows inoculants to grow without competition and interactions with the indigenous microbial community (Wellington et al. 1993). It should, however, be noted that sterilisation may be associated with an increase in nutrient levels, a parameter which was shown to be conducive for conjugative gene exchange (Top et al. 1990).

In non-sterile lake water, transfer of plasmid R68.45 between *P. aeruginosa* strains was reduced in the presence of the natural microbial community compared to in autoclaved lake water (O'Morchoe et al. 1988). Conjugative transfer of the mercury resistance plasmid pQM1 between *P. aeruginosa* strains immobilised on filters which were placed face down on river stones was assessed on stones which were either scrubbed to remove the epilithic biofilm, and subsequently autoclaved, or remained untreated. Transfer frequencies on stones submerged in river water were significantly low-

er on stones covered with the natural microbial community, compared to transfer frequencies on sterile, scrubbed stones lacking the indigenous microbial community (Bale et al. 1987).

Presence of a rhizosphere. Conjugative gene exchange is stimulated in the rhizosphere compared to non-rhizosphere soil (van Elsas et al. 1988; van Elsas et al. 1989; Smit et al. 1991; Kroer et al. 1998). This finding may be attributed to the improved nutrient status of bacterial cells. Roots exude nutrients such as sugars or amino acids (Bolton et al. 1993). Nutrients may also become available when root cells which are sloughed off as roots grow into the soil are autolysed (Wellington et al. 1993). In addition, the root surface may provide microhabitats which are conducive for genetic interactions by facilitating the intimate contact of bacteria which grow as microcolonies.

The positive effect of the rhizosphere on conjugative gene transfer was obvious from the finding that transfer of the IncPα plasmid RP4 occurred in the rhizosphere of wheat but not in the corresponding non-rhizosphere soil (van Elsas et al. 1989). Transfer rates of plasmid RP4, as well as survival of both a *Pseudomonas* sp. donor and a *Pseudomonas* sp. recipient strain, decreased significantly with increasing distances from the wheat root surface (van Elsas et al. 1988). Recently, even the transfer of chromosomal markers between *P. aeruginosa* strains mediated by the IncPα plasmid R68.45, which possesses chromosome mobilising ability (cma) was observed in the rhizosphere of wheat. In unplanted soil, transfers were below the limit of detection (Troxler et al. 1997).

The conjugative transfer of IncPα plasmid RP4p from *P. fluorescens* to indigenous members of microbial soil populations in planted or unplanted soil microcosms was analysed by Smit et al. (1991). Total numbers of the colony forming units (cfu), of the donor strain as well as of the indigenous transconjugants were shown to be significantly higher in rhizosphere than in corresponding non-rhizosphere soil, 7 days after inoculation of the soil with donor cells (Smit et al. 1991). In unplanted soil microcosms, no transfer to indigenous bacteria was observed.

Similarly, transfer frequencies of plasmid RP4 from a *P. fluorescens* donor to a *Serratia* sp. recipient strain in the rhizosphere of the marsh plant *Echinochloa crusgalli* were up to 2×10^4 times higher than in unplanted sand (Kroer et al. 1998). Surprisingly, when transfer was assessed in unplanted sand versus sand amended with root exudates, no differences in transfer frequencies were observed. The authors concluded that the presence of the root, rather than the nutrients released by the roots, stimulated plasmid transfer. It was hypothesised that the presence of roots resulted in clustering of bacteria in microhabitats, leading to locally high cell densities. The intimate interaction of donor and recipient cells in these microhabitats may have enhanced transfer rates (Kroer et al. 1998).

Identification of environmentally relevant abiotic parameters influencing conjugation

Temperature. The influence of temperature on conjugative plasmid transfer has been analysed in terrestrial habitats such as soil (Richaume et al. 1989; Kinkle and Schmidt 1991; Lafuente et al. 1996), or in aquatic habitats such as river water (Bale et al. 1987), drinking water (Sandt and Herson 1991) or artificial seawater (Goodman et al. 1993). In most cases, within a certain temperature range, a positive correlation was noted between temperature and transfer frequencies. Plasmid transfer was shown to occur at low (4–15 °C) as well as high temperatures (30–40 °C), albeit at lower frequencies compared to that at an optimal temperature which is defined by both the characteristics of the plasmid and its host. For instance, plasmid pQM85 displayed maximum transfer rates at 10 °C, which is clearly not the optimal growth temperature of its *Pseudomonas* host (Fry and Day 1990a). Conversely, transfer rates of plasmid pQM1 in matings between mesophile *P. putida* strains or between psychrophilic *P. fluorescens* strains depended on the respective host strain employed (Day et al. 1992). At 5 °C for instance, plasmid transfer between psychrophilic strains was more efficient than between the mesophilic strains.

Transfer frequencies of the IncPα plasmid RP4 between *S. meliloti* strains in sterile soil microcosms increased dramatically at 30 °C, the optimal growth temperature for *S. meliloti*. Transfer frequencies were considerably lower at both lower and higher temperatures (Lafuente et al. 1996). In matings between *E. coli* carrying the self-transmissible plasmid pBLK1-2 and *Sinorhizobium fredii* recipient cells in sterile soil, maximum transfer frequencies were observed at 28 °C. Lowest frequencies were observed at the temperature extremes of 5 °C and 40 °C (Richaume et al. 1989).

In aquatic habitats, transfer of the mercury resistance plasmid pQM1 between *P. aeruginosa* strains on stones submerged in river water occurred at 6 °C, at frequencies at least 3 orders of magnitude lower than those at 20 °C (Bale et al. 1987). A pBR322 derivative was mobilised from *E. coli* to *Enterobacter cloacae* by the aid of an IncFII helper plasmid (R100–1) in sterile drinking water (Sandt and Herson 1991). Numbers of transconjugants were significantly higher at 35 °C than at 15 °C.

Interestingly, under conditions of nutrient depletion, Fernandez-Astorga et al. (1992) and Goodman et al. (1993) found that changes in temperature did not affect plasmid transfer rates. Transfer of IncPα plasmid RP1 between starved marine *Vibrio* donor and recipient cells at 4 °C and 26 °C, for instance, resulted in comparable numbers of transconjugants (Goodman et al. 1993).

In summary, the optimum temperature for plasmid transfer is determined by the host/plasmid combination. In cases where optimal transfer frequencies coincidenced with the optimal growth temperature, the re-

sults may be explained by a positive effect on the physiological states of mating partners.

Moisture. Moisture content is a key factor which influences the availability and distribution of nutrients, oxygen, etc. in soil, and thus the physiological status of bacterial cells. In addition, the moisture content of soil affects the active and passive movement of cells, and thus influences the probability of mating partners meeting or the stability of mating-pair formations. Thus, the moisture content which is optimal for plasmid transfer also depends on the corresponding donor/plasmid/recipient combination.

In matings between an *S. meliloti* strain harbouring the IncPα plasmid RP4 as donor and an *S. meliloti* recipient strain, transfer frequencies increased when sterile dry soil was adjusted to a water content of 20% (v/w) and decreased at higher moisture contents (Lafuente et al. 1996). Whereas a moisture content below 20% negatively affected the viability of bacterial cells, a moisture content above 20% negatively affected mating pair formations mediated by the IncP-encoded rigid pilus.

Maximum transfer frequencies of plasmid pBLK1-2 in matings between *E. coli* donor and *S. fredii* recipient cells in sterile soil were observed by Richaume et al. (1989) at a relatively low moisture content of 8% (v/w). Similarly, transfer of the conjugative plasmid pIJ303 between *Streptomyces* species in sterile silt loam soil was observed with highest frequencies in nutrient-amended, comparably dry soils adjusted to approximately 9% (v/w) water content (Bleakley and Crawford 1989). The authors speculated that one possible explanation was the reduced diffusion of nutrients in the dry soil leading to growth of mycelia in concentrated, nutritious microsites where hyphal fusions were promoted.

pH. In terrestrial and aquatic environments, changes in pH may affect the physiological status of parental cells and may thus influence transfer frequencies. Furthermore, binding of cells on surfaces (which often facilitates conjugative plasmid transfer) as well as the establishment of pilus-mediated cell-to-cell contact, may be influenced by changes in pH or by concomitant alterations in the concentration of cations. Studies which have addressed the influence of pH on plasmid transfer in soil (Weinberg and Stotzky 1972; Krasovsky and Stotzky 1987; Richaume et al. 1989; Lafuente et al. 1996) as well as aquatic systems (Rochelle et al. 1989) suggest that values near neutral pH are conducive for conjugal plasmid transfer.

Transfer frequencies of IncPα plasmid RP4 between *S. meliloti* strains in sterile soil were highest at pHs in the range of 7.0–8.0, and were significantly reduced at both higher and lower pHs (Lafuente et al. 1996). Analogously, Krasovsky and Stotzky (1987) who analysed transfer of chromosomal DNA between *E. coli* strains in soil by using an *E. coli* Hfr (high frequency of recombination) donor reported that both the survival of donor and recipient cells, as well as the frequencies of recombination, increased with increasing pH values from pH 4.7 towards neutrality.

The influence of pH changes on the transfer of mercury resistance plasmids previously isolated from the indigenous population of river epilithon was analysed by Rochelle et al. (1989). In matings between pseudomonads on agar plates one of these plasmids displayed a transfer optimum at pH 7.0 at optimal as well as non-optimal growth temperatures. In contrast, transfer of another plasmid was not affected by pH over the range 5.0–8.0 under an optimal growth temperature but, interestingly, displayed a clear transfer optimum at pH 6.5 at non-optimal temperatures. This result confirmed an earlier study which showed that pH and temperature may act synergistically on plasmid transfer (Singleton and Anson 1983).

Clay minerals. The presence of clay minerals such as montmorillonite (Weinberg and Stotzky 1972; Krasovsky and Stotzky 1987; Richaume et al. 1989) or bentonite (van Elsas et al. 1987) was shown to influence conjugative plasmid transfer in soil. Due to their cation-exchange capacities, clay minerals buffer soils against changes in pH (Stotzky 1986) and bind soluble organic compounds (Stotzky 1986). This is favourable for both bacterial survival and growth, and thus for conjugative gene transfer. Moreover, clay minerals provide large charged surfaces on which cells are immobilised, thus facilitating cell contacts (Stotzky 1986).

Frequencies of transfer of plasmid pBLK2-1 between *E. coli* and *S. fredii* increased significantly when montmorillonite was added to sterile sandy soil up to a concentration of 15% (Richaume at al. 1989). Higher amounts were less conducive to plasmid transfer, probably due to the excessive immobilisation of cells (Richaume et al. 1989). Sterile soil amended with bentonite and nutrients was significantly more conducive to the transfer of plasmid pFT30 in crosses between *Bacillus cereus* donor and *B. subtilis* recipient cells than soil amended with nutrients alone (van Elsas et al. 1987). No influence on the growth dynamics of donor or recipient cells was detected. Furthermore, a positive effect on plasmid transfer mediated by an alteration in pH after addition of clay was excluded. It was concluded that the physicochemical modification of the soil may have resulted in the modification of the cellular physiology or in the promotion of cell contacts and thus, may have favoured plasmid transfer (van Elsas et al. 1987). Transfer of chromosomal markers between *E. coli* strains introduced into soil was enhanced after amendment with montmorillonite, probably due to an effect on soil pH (Weinberg and Stotzky 1972). In contrast to montmorillonite amendment, addition of kaolinite, which displays a lower specific surface area as well as a lower cation-exchange capacity than montmorillonite, had no effect on conjugative transfer between *E. coli* strains in soil (Krasovsky and Stotzky 1987).

Indigenous bacteria as recipients of conjugative plasmids

Transfer to members of autochthonous bacterial communities after the inoculation of plasmid-bearing donor strains into non-sterile soil microcosms was demonstrated in several studies (Henschke and Schmidt 1990; Smit et al. 1991; Glover Glew et al. 1993; Pukall et al. 1996). For instance, Smit et al. (1991) reported on the transfer of IncPα plasmid RP4 from *P. fluorescens* to indigenous recipient cells of the genera *Pseudomonas*, *Enterobacter*, *Comamonas* as well as *Alcaligenes*, demonstrating that intergeneric gene transfer between introduced donors and genetically dissimilar recipients occurred in non-sterile soil.

Transfer of IncPα plasmid RP1 from *Pseudomonas syringae* to indigenous bacteria was observed after inoculation of leaves of bush bean (*Phaseolus vulgaris*) with the donor strain (Björklöf et al. 1995). The conjugative plasmid transferred to the genera *Pseudomonas*, *Enterobacter*, *Alcaligenes*, *Xanthomonas* as well as *Sphingobacterium*. Analysis of the transfer of IncPα plasmid RP4 from a *P. fluorescens* strain to indigenous epiphytic bacteria of bush bean revealed that the plasmid transferred to six different *Pseudomonas* spp., to *Stenotrophomonas maltophila* and to four Gram-negative isolates whose taxonomic classifications remain to be determined (Normander et al. 1998).

Plasmid transfer from *E. coli* to indigenous bacteria in the gut of the soil microarthropod *Folsomia candida* was addressed by Hoffmann et al. (1998). After feeding the microarthropods with the *E. coli* mobiliser strain S17–1, transfer of one self-transmissible and two mobilisable plasmids took place to indigenous gut bacteria. The plasmids, which were genetically tagged with a luciferase marker gene conferring a bioluminescence phenotype, were the self-transmissible IncPα plasmid derivative RP4-*luc* and the mobilisable plasmids pSUP104-*luc* or pSUP202-*luc* displaying a broad and a narrow host range of replication, respectively. A remarkable diversity of Gram-negative transconjugants carrying the tagged plasmids belonged to the α, β and γ subclasses of the proteobacteria, as determined by sequencing of 16 S rRNA genes and the BIOLOG system. This study indicated that the microarthropod gut may represent an environmental hot spot for conjugative gene transfer in soil microbial communities (Hoffmann et al. 1998).

Transfer of a 105-kb self-transmissible element carrying genes necessary for 3-chlorobenzoate (3CB) degradation, from *Pseudomonas* sp. B13 to indigenous bacteria of activated sludge was reported by Ravatn et al. (1998). Although the genetic basis for the transfer of this element is unknown, the element seems to be capable of integrating into the recipient chromosome. In laboratory-scale activated-sludge microcosms amended with 3CB, transfer into *Ralstonia eutropha* was observed (Ravatn et al. 1998).

In most of the studies cited above, the real extent of transfer probably has been underestimated due to the so-called great plate count anomaly. This phenomenon describes the well-known discrepancy between microbial numbers of environmental samples determined by microscopy employing cell-staining methods and the corresponding plate counts. Estimates of the proportion of cells culturable on standard media relative to total cell counts in environmental samples are in the range of 0.001% (for seawater) to approximately 15% (for activated sludge) [for a review see Amann et al. (1995)].

To obtain a better estimate of the transfer of conjugative plasmids to indigenous bacteria, Dahlberg et al. (1998b) used a system by which plasmid transfer to the non-cultured fraction of bacterial communities is detectable. A bhr mercury resistance plasmid was tagged with the *Aequorea victoria gfp* gene encoding the green fluorescent protein (GFP). Its fluorescence is detectable at the single-cell level by employing epifluorescence microscopy. The expression of the *gfp* gene was driven from the *l*ac promotor which was down-regulated in the donor cell by a chromosomal insertion of the *lacI* gene encoding the Lac repressor. Hence, the transfer of the plasmid, derepressed for GFP expression after transfer to indigenous bacteria, including the non-cultured fraction, could be detected by epifluorescence microscopy. After mixing *P. putida* donor cells with bulk seawater, transfer of the tagged plasmid to various cellular morphotypes at frequencies in the range of 10^{-6} to 10^{-5} per recipient cell was observed (Dahlberg et al. 1998b).

Employing a flow chamber as a model system, Christensen et al. (1998) analysed the establishment of a *stp*-tagged TOL plasmid pWWO derivative in a reconstituted biofilm community consisting of: *P. putida* PI, *Acinetobacter* sp. C6 and an unidentified isolate, D8, related to the β-subgroup of the proteobacteria. All strains were able to utilise the substrate benzyl alcohol as a sole carbon and energy source via degradation pathways similar to that of the TOL-plasmid-encoded pathway. *P. putida* PI or *P. putida* KT2442 harbouring the TOL plasmid derivative were introduced into the biofilm as plasmid donor strains. When *P. putida* PI was used as the donor, the strain established readily in the community due to an advantage in terms of growth conferred by the TOL plasmid. Transfer of the plasmid to indigenous *P. putida* recipients occurred at comparatively low frequencies compared to those obtained in plate matings. In contrast, the *P. putida* KT2442 donor strain harbouring the TOL plasmid only poorly established in the biofilm community at a 1000-fold lower rate than *P. putida* PI. Low-frequency transfer of the TOL plasmid into the indigenous *P. putida* RI strain, however, resulted in the rapid colonisation of the biofilm by transconjugants. Thus, the TOL plasmid established in the indigenous population mainly by the proliferation of transconjugants representing an optimal host-plasmid combination, rather than by high transfer frequency.

Field studies conducted in terrestrial habitats

Transfer of IncQ plasmid pIE723 from an *E. coli* donor additionally harbouring an IncP helper plasmid to a *P. putida* recipient strain under field conditions was studied by Götz and Smalla (1997). Addition of pig manure increased the mobilisation frequency at least tenfold and thus confirmed the relevance of nutrient amendment for plasmid transfer. Due to the addition of manure, mobilisation of the IncQ plasmid by indigenous plasmids of either the soil or the manure occurred (Götz and Smalla 1997).

The conducive effect of the rhizosphere on plasmid transfer already evident from laboratory based experiments was confirmed in field studies. Transfer of a mercury resistance plasmid from a *Pseudomonas marginalis* donor to a *P. aureofaciens* recipient strain was shown to occur in the rhizosphere, and at even higher frequencies on the peel, of sugar beet roots. In contrast, no transfer was detected when these bacteria were placed in soil at distances of more than 5 cm from sugar beet plants (Lilley et al. 1994).

Stockwell et al. (1996) analysed the transfer of the conjugative plasmid pAgK84 from *Agrobacterium radiobacter* to *A. tumefaciens* in crown gall tissue of cherry seedlings under field conditions. Plasmid pAgK84 encodes agrocin 84 biosynthesis and immunity, an antibiotic which blocks DNA replication of sensitive strains. *A. radiobacter* K84 is commercially used to control crown gall disease induced by the plant pathogen *A. tumefaciens*. In order to analyse the transfer rate of pAgK84 under non-selective conditions, transfer experiments were performed by using an agrocin-insensitive *A. tumefaciens* recipient strain. Surprisingly, despite the absence of selective conditions, transfer of the plasmid was detected in 4 of 13 galls investigated, at moderately high frequencies of approximately 10^{-4} transconjugants per recipient cell (Stockwell et al. 1996).

Acquisition of mercury resistance plasmids by a deliberately released genetically modified *P. fluorescens* strain from the indigenous microflora was demonstrated in the phytosphere of sugar beet (Lilley and Bailey 1997a). Transconjugants harbouring genetically distinctive mercury resistance plasmids were detected at frequencies in the range of 1.3×10^{-2} to 1 in the first year in root and leaf samples, but were shown to occur only at a specific period of plant development. It was proposed that yet unidentified signals or conditions in the rhizosphere may promote conjugal transfer (Lilley and Bailey 1997a).

Recently, mobilisation of IncQ plasmid pIE723 from *P. fluorescens* to indigenous bacteria in the rhizosphere of wheat was demonstrated under field conditions (van Elsas et al. 1998). Mobilisation was accomplished by the aid of the self-transmissible plasmid pIPO2, previously isolated from the autochthonous microflora of the wheat rhizosphere. Mobilisation of pIE723 occurred irrespective of whether helper plasmid pIPO2 was present in the same (diparental mating) or in another *P. fluorescens* host (triparental mating). Transconjugants of the indigenous community in both cases reached numbers in the range of 10^2–10^3 cfu g^{-1} soil. Ten clones were tentatively identified by the BIOLOG system as relatives of the Gram-negative bacterial species *Enterobacter aerogenes*, *Klebsiella terrigena*, *Agrobacterium radiobacter*, *Buttiaxella agrestis*, and another *Enterobacter* sp. (van Elsas et al. 1998).

Another field experiment provided evidence for horizontal transfer of an even chromosomally located symbiotic DNA region from *Mesorhizobium loti* to indigenous non-symbiotic rhizobia (Sullivan et al. 1995). The inoculant strain *M. loti*, as well as seeds of its host plant *Lotus corniculatus*, were simultaneously released into soil of a field site in New Zealand. This area was devoid of naturalised *M. loti* or other rhizobia capable of undergoing symbiosis with the plants seeded. Seven years after the release of the strain, genetically diverse *Rhizobium* strains were isolated from *L. corniculatus* root nodules. All isolates contained a symbiotic region identical to that of the inoculated strain. The authors argued that non-symbiotic rhizobia persisted in the soil and acquired symbiotic genes from the inoculant strain. The existence of such non-symbiotic rhizobia species at the release site was confirmed in following studies. Seven strains of non-symbiotic isolates belonging to four different species related to *M. loti* were identified. The newly identified symbiotic isolates belonged to one of these species (Sullivan et al. 1996). The transferred genomic region was located on a mobile element which was termed "symbiosis island" on the basis of its similarity to pathogenicity islands of Gram-negative bacterial pathogens (Sullivan and Ronson 1998). It is 500 kb in size and transferred at low frequency (5×10^{-7} per recipient) to non-symbiotic mesorhizobia in plate matings (Sullivan and Ronson 1998).

By using a direct approach for assessing the transfer of symbiotic plasmids among members of a *Rhizobium leguminosarum* population under field conditions, Selbitschka et al. (1995) constructed a *R. leguminosarum* bv. *viciae* strain specifically designed to act as the recipient for the acquisition of a symbiotic plasmid. Strain CT0370 is devoid of its symbiotic plasmid pSYM and therefore not able to induce the formation of root nodules on pea plants. The strain was genetically tagged by the stable integration of the *gus*A gene conferring β-glucuronidase activity into the chromosome. The putative donor strain *R. leguminosarum* bv. *viciae* RSM2004 (Hirsch and Spokes 1994), harbouring the conjugative, Tn*5*-tagged symbiotic plasmid pSYM::Tn*5*, which was released in a field site in the United Kingdom in 1987, had established in the natural microbial population at a density of 10^2–10^3 cfu/g^{-1} soil (Hirsch 1996). Thus, the transfer of plasmid pSYM::Tn*5* or any other *sym* plasmid from the indigenous *R. leguminosarum* bv. *viciae* field population into recipient strain CT0370 could easily be monitored by using pea as the trap plant, since root nodules harbouring transconju-

gants stained blue after inoculation with the specific substrate X-Gluc.

The recipient strain was released in 1994 and established at a density similar to that of the indigenous *R. leguminosarum* bv. *viciae* population, i.e. approximately 10^4–10^5 cfu g^{-1} soil (Hirsch 1996). More than 20 000 pea root nodules were tested for the presence of GUS-positive bacteria. No transconjugants were detected indicating that the incidence of pea-nodulating transconjugants was below the limit of detection of 2×10^{-4} (Hirsch 1997).

Field studies conducted in aquatic habitats

By employing submerged test chambers, plasmid transfer was found in the degritter tank (Altherr and Kasweck 1982), the secondary clarifier tank (Mach and Grimes 1982) as well as activated-sludge basins of wastewater treatment plants (Mach and Grimes 1982; Marcinek et al. 1998). By means of a similar experiment, O'Morchoe et al. (1988) demonstrated plasmid transfer in a freshwater environment.

Transfer of the broad host range plasmid pIP501 and the two pheromone-responsive plasmids pAD1 and pIP1017, displaying restricted host ranges, between *Enterococcus faecalis* strains in activated sludge basins of two wastewater treatment plants was analysed by Marcinek et al. (1998). The conjugative transposon Tn*916* was included in these studies. In both plants, transfer frequencies of pAD1 were below the limit of detection, whereas transfer of pIP1017 was detected. Transfer frequencies of both pIP501 and Tn*916* were higher in one plant than those obtained under laboratory conditions on agar plates, but were below the limit of detection in the other plant. These results indicated that under the specific environmental stress situations which prevailed during the experiment (low temperature, unusually diluted sewage, relatively high heavy metal concentrations) unexpectedly efficient gene transfer could occur. Transfer of pIP1017 as well as Tn*916* was more efficient at higher temperatures during summer than at lower temperatures in winter (Marcinek et al. 1998).

Conjugative transfer of IncPα plasmid R68.45 between *P. aeruginosa* strains in test chambers submerged in a lake was conducted in the absence, as well as in the presence, of the natural microbial community (O'Morchoe et al. 1988). Plasmid transfer frequencies were reduced when the indigenous microbial community was present. Much lower transfer frequencies were detected compared to those in laboratory based studies performed in parallel (O'Morchoe et al. 1988).

Bale et al. (1987, 1988a) demonstrated transfer of the narrow host range *Pseudomonas* plasmid pQM1 between *P. aeruginosa* donor and recipient strains on filters placed face down on stones submerged in a river. Under these conditions, transfer frequencies were again lower than those obtained under laboratory conditions in non-sterile river water (Bale et al. 1987). Changes in temperature had a significant effect on plasmid transfer on sterile, scrubbed stones. A linear relationship was observed between increasing river temperatures from March to November and enhanced plasmid transfer frequencies (Bale et al. 1988a). In contrast, no correlation between transfer rates and water temperature was observed when the *Pseudomonas* strains were incorporated into the epilithon bacterial community prior to mating (Bale et al. 1988b). Acquisition of mercury resistance plasmids from the indigenous bacterial community of river epilithon by a *P. putida* recipient strain immobilised on a filter placed on an epilithon-covered stone was also shown to occur (Bale et al. 1988a). Four of the plasmids isolated displayed sizes between 52 and 83 kb; three plasmids were even larger than 250 kb.

Summary

Conjugative gene transfer between introduced donor and recipient cells in terrestrial as well as aquatic, environmental samples has been readily observed under laboratory conditions. The studies discussed revealed that conjugative gene exchange occurs under a wide range of conditions, even at low temperature or under conditions of nutrient depletion. Laboratory based studies also revealed transfer of conjugative plasmids, introduced with donor strains, to indigenous bacteria.

Several field studies conducted in terrestrial as well as aquatic habitats have reported on conjugative plasmid transfer under environmental conditions. Transfer between introduced donor and recipient cells, from introduced donor cells to indigenous bacteria, as well as from indigenous bacteria to introduced recipient cells was demonstrated. The positive effects of nutrient amendment or the presence of the plant rhizosphere on gene transfer, which were identified as relevant parameters in laboratory-based studies, were confirmed in the field studies. In most cases temperature affected conjugative plasmid transfer in a similar way to that observed under laboratory conditions. Moreover, a negative impact of indigenous microbial populations on conjugative plasmid transfer between donor and recipient cells released into the environment was demonstrated. However, field studies also revealed that plasmid transfer frequencies under certain environmental conditions may differ considerably from those obtained under laboratory conditions, thus emphasising the importance of field studies.

Transformation

Basically, there are two factors which influence gene transfer via transformation in natural habitats. First, apart from bacterial species exhibiting constitutive competence, natural transformable bacteria have to de-

Table 3 Incidence of extracellular DNA in freshwater and marine environments

Habitat	Concentration of extracellular DNA (μg l^{-1})	Reference
Freshwater		
Oligotrophic[a]	1.43 ± 1.1	Paul et al. (1989)
	1.74	DeFlaun et al. (1986)
Eutrophic[b]	12.1 ± 1.2	Paul et al. (1991b)
	11.9 ± 8.9	Paul et al. (1989)
	6.97	DeFlaun et al. (1986)
Swamp[c]	7.8	DeFlaun et al. (1986)
Sediment[d]	1.0 μg g^{-1}	Ogram et al. (1987)
Marine water		
Estuarine[e]	9.4–11.6	Paul et al. (1989)
	6–44	DeFlaun et al. (1986)
Coastal	2.0–7.0[f]	Turk et al. (1992)
	5.0–15.0[g]	DeFlaun et al. (1986)
Offshore	17.1 ± 12.7[h]	Turk et al. (1992)
	4.6[g]	Paul et al. (1989)
	0.5–5.0[g]	DeFlaun et al. (1986)
Deep sea (500–1500 m)	0.2–0.5[g]	DeFlaun et al. (1986)

[a] Crystal River Springs, Kings Bay, Crystal river, Fla., samples from 0.1–0.3 m depth
[b] Medard reservoir, an eutrophic body of water collecting runoff from agricultural and phosphate mining regions, Edward Medard State Park, Valrico, Fla.
[c] Boyd Hill Nature Park, a thickly vegetated swamp adjoining Lake Maggiore, St. Petersburg, Fla.
[d] Fort Loudon Reservoir, Knoxville, Tenn.
[e] Bayboro Harbor, an eutrophic embayment of Tampa Bay, Fla.
[f] Gulf of Triest
[g] Gulf of Mexico
[h] Baltic Sea

velop competence and secondly, free DNA must be available for uptake by competent cells.

More than 40 naturally transformable bacterial species have been identified so far (Lorenz and Wackernagel 1994). Moreover, numerous studies have revealed the presence of extracellular DNA in aquatic environments (see Table 3). In soil or aquatic sediments, concentrations of extracellular DNA are difficult to determine, since procedures necessary for the desorption of DNA bound to minerals may also disrupt cells and, thus, release DNA. Using a careful DNA extraction procedure, Ogram et al. (1987) obtained approximately 1.0 μg g^{-1} high molecular weight DNA from freshwater sediment. Thus, there is a potential for gene exchange via transformation in nature.

Environmentally relevant parameters influencing the development of competence in terrestrial and aquatic environments

Most bacteria which are naturally transformable develop competence during a specific growth phase, e.g. during exponential growth or in the stationary phase. Nutrient amendment enables bacteria to attain competence due to the metabolic upshift to the logarithmic phase (e.g. *Acinetobacter calcoaceticus*; Palmen et al. 1993) or during transition from the logarithmic phase to the stationary phase (e.g. *Pseudomonas stutzeri*; Lorenz and Wackernagel 1990).

Results from in vitro experiments indicated that the development of competence by several bacterial species seems feasible under environmental conditions. *Azotobacter vinelandii*, which is an inhabitant of soils, sediments and water, developed competence in a medium which was limited in iron (Page and von Tigerstrom 1978; Page and Grant 1987). Thus, it was concluded that *A. vinelandii* may become naturally competent in soil where iron-limited growth conditions prevail (Page and Grant 1987). Moreover, Ca^{2+} concentrations required for optimal competence development of *A. vinelandii* are in the range of that of exchangeable and free Ca^{2+} concentrations frequently found in natural soils, water and sediments (Lorenz and Wackernagel 1994).

Analysis of a marine *Pseudomonas* strain (formerly *Vibrio* WJT-1C) revealed that competence started in the early exponential phase and reached an optimum at the onset of the stationary phase (Frischer et al. 1993). Temperatures ranging from 4 °C to 33 C, salinity in the range 12–50% as well as a nutrient concentration from 1% to 200% of standard media had minimal effect on competence development. It was concluded that natural plasmid transformation could occur under conditions found in tropical and subtropical estuaries (Frischer et al. 1993).

Competence development in natural habitats is poorly understood. As a first step towards examining competence under more "natural" conditions, competence development was analysed on agar plates prepared from an aqueous soil extract (Lorenz and Wackernagel 1991, 1992), in microcosms containing nutrient-amended non-sterile soil (Nielsen et al. 1997) as well as in marine water samples (Paul et al. 1991a). The results of these studies indicated that competence development is feasible in nature.

Lorenz and Wackernagel (1991, 1992) analysed the development of competence of a *P. stutzeri* strain (JM301) in the chemical environment of soil. Agar medium prepared from aqueous extracts of four different soils were employed for plate transformation assays. The extracts were limited in carbon as well as nitrogen sources. The influence of pH and temperature on transformation frequencies were also investigated. Transformation frequencies were comparable to those obtained in defined minimal medium after supplementation with carbon, nitrogen and phosphorus sources. Interestingly, an up to 290-fold increase in transformation frequencies was shown to occur under both phosphorus and nitrogen limitation. Under conditions of combined nitrogen and phosphorus limitation, or when carbon sources were limiting, no transformation was detected. Frequencies were not significantly affected by temperatures in the range of 20–37 °C. Below 20 °C, transformation frequencies decreased by a factor of approximately

2 per degree Celsius, but transformation was still detectable at 12° C. Furthermore, transformation frequencies peaked at neutral pH. The results suggested that there is a high potential for competence development of *P. stutzeri* in situ as long as nutrients are available and the pH is in the neutral to moderately alkaline range (Lorenz and Wackernagel 1992).

The natural transformation of *A. calcoaceticus* introduced with a non-competent status into non-sterile, silt loam soil microcosms occurred after amendment of soil with different nutrient solutions and chromosomal DNA (Nielsen et al. 1997). Since *A. calcoaceticus* develops competence during exponential growth (Palmen et al. 1993), the authors concluded that non-competent cells were induced by the nutrient upshift which promoted bacterial growth. The addition of DNA between 11 h and 24 h after nutrient amendment resulted in considerably lower transformation frequencies than observed after simultaneous inoculation with DNA and nutrients, probably due to the fact that growth- and activity-limiting conditions perturbed transformation after 24 h (Nielsen et al. 1997).

In an aquatic habitat, plasmid transformation of a marine *Pseudomonas* strain (formerly *Vibrio* WJT-1C) in sterile and non-sterile marine water samples was enhanced by amendment with low levels of nutrients (Paul et al. 1991a). Addition of higher levels of nutrients negatively influenced transformation compared to unamended samples. Since the strain displays highest competence during the late stationary phase, the authors suggested that the addition of low levels of nutrients enabled dormant cells to grow, and thus reach their competence state after entry into the stationary phase. In samples amended with high levels of nutrients, probably a smaller proportion of cells were in the late stationary phase compared to cells in unamended samples, thus resulting in reduced transformation frequencies (Paul et al. 1991a).

Environmentally relevant parameters influencing the persistence and availability of DNA

The persistence of free DNA in natural habitats is influenced by nucleolytic degradation and protection from degradation by adsorption to charged surfaces of, for example, minerals. Adsorbed DNA, however, may become unavailable for uptake by naturally competent bacteria and thus lose its transforming ability.

Degradation of DNA. It has been well established that free DNA is hydrolysed at substantial rates in soil (Romanowski et al. 1992; Romanowski et al. 1993a; Blum et al. 1997), in seawater (Paul et al. 1987; DeFlaun and Paul 1989; Turk et al. 1992), in freshwater environments (Paul et al. 1989), in wastewater (Phillips et al. 1989; Fibi et al. 1991) as well as in marine sediments (Maeda and Taga 1974). The hydrolysis of free DNA is due to the ubiquitous occurrence of cell-bound or free DNases produced by microorganisms so that they can utilise free DNA as a growth substrate (Greaves and Wilson 1970; Paul et al. 1988). DNase-producing microorganisms, in some cases, may account for >90% of the heterotrophic bacterial cfu of soil and aquatic environments (Maeda and Taga 1973; Maeda and Taga 1974). A positive correlation between increasing viable counts of microorganisms and increasing levels of DNase activity after addition of water to different soils was reported by Blum et al. (1997). Soil rehydration resulted in an increased availability of nutrients, and thus in growth of microorganisms excreting nucleases. Inhibition of prokaryotic and eukaryotic growth in soil by antibiotics and cycloheximide, respectively, revealed the prokaryotic origin of DNases in soil. Whereas addition of cycloheximide and water did not block the production of DNases, addition of antibiotics and water nearly completely inhibited DNase production (Blum et al. 1997).

Studies which analysed the persistence of DNA in soil showed that seeded DNA was rapidly hydrolysed but was still detectable after more than 2 months post inoculation (Romanowski et al. 1992; Romanowski et al. 1993a). After [^{3} H]thymidine-labelled plasmid DNA was seeded into non-sterile loamy sand soil, clay soil, or silty clay soil, the production of trichloracetic-acid-soluble radioactive material increased to 45%, 27% and 77% (calculated as the ratio of acid-soluble material to total radioactivity) respectively, after 60 days of incubation (Romanowski et al. 1992). In a subsequent study, Romanowski et al. (1993a) employed quantitative polymerase chain reaction (PCR) for the detection of plasmid-specific DNA sequences after long-term incubation in the same soils. Simultaneously, DNA isolated from the soils was tested for the presence of intact plasmid DNA by electroporation of *E. coli*. After 60 days of incubation, less than 0.2% of the initially introduced plasmid DNA was detectable by PCR. A more than 10 000-fold loss of transforming activity was observed by the use of the transformation assay.

Studies employing different aquatic habitats indicated that seeded DNA usually is more rapidly degraded in aquatic than in terrestrial environments. However, degradation rates amongst various habitats differ significantly. Phosphorus-32-labelled plasmid pBR322 DNA added to phosphorus-limited seawater was rapidly hydrolysed with a degradation rate of 0.4 μg DNA per liter per hour, whereas in non P-limited seawater DNA was degraded at a rate of approximately 0.002 μg DNA per l^{-1} h^{-1} (Turk et al. 1992). [^{3}H]thymidine-labelled chromosomal *E. coli* DNA seeded into oligotrophic marine surface water was nearly completely degraded after less than 12 h of incubation (Paul et al. 1987), whereas in marine sediments half-lives of DNA molecules of approximately 140 h were calculated (Maeda and Taga 1974). For [^{3}H]-labelled phage λ DNA added to oligotrophic as well as eutrophic freshwater, half-lives of approximately 4.2 h and 5.5 h, respectively, were observed (Paul et al.

1989). pBR322 DNA added to a wastewater treatment plant sample was completely converted to open circular and linear forms within 20 min (Phillips et al. 1989).

Protection of DNA. DNA is protected to some extent from degradation after its adsorption to minerals, which is considered as the most important factor determining DNA persistence in the environment.

Under most environmental conditions DNA molecules are negatively charged. Consequently, DNA adsorbs to net positively charged surfaces, such as edges of clay minerals. DNA molecules are able to bind to net negatively charged surfaces, such as the surfaces of clays, too; efficient binding is accomplished by electrostatic bridges which are built up by divalent cations such as calcium or magnesium (Lorenz and Wackernagel 1987; Lorenz and Wackernagel 1994; Paget and Simonet 1994). Repulsion between negative charges of DNA molecules and surfaces is also reduced by both an increase in the concentration of counteracting monovalent cations as well as decreasing pH values [for a review see Lorenz and Wackernagel (1994)]. If repulsion is lowered, adsorption due to non-ionic van der Waals forces seems probable (Lorenz and Wackernagel 1987). Below pH 5, adsorption to negatively charged surfaces is facilitated by protonation of DNA bases which leads to positively charged DNA molecules (Greaves and Wilson 1969).

Clay minerals (Khanna and Stotzky 1992; Paget et al. 1992) or sand (Aardema et al. 1983; Lorenz et al. 1988) display high capacities for DNA adsorption. The amount of DNA bound to the clay mineral montmorillonite increased with increasing amounts of DNA added (Khanna and Stotzky 1992), and adsorption of 30 mg of DNA to an equivalent of 1 g of montmorillonite has been reported (Paget et al. 1992). Sand displays much lower, but still considerable DNA-binding capacities. Adsorption of DNA to analytical-grade sea sand was in the range of 1.5–4.0 $\mu g\ g^{-1}1$ (Aardema et al. 1983; Lorenz et al. 1988). Binding of plasmid DNA to non-purified material taken from a groundwater aquifer was analysed by Chamier et al. (1993). The amount of bound DNA was 80% of the amount which adsorbed to chemically pure sea sand. Different types of autoclaved soils were shown to adsorb linear plasmid molecules, and binding rates of more than 1.4 $mg\ g^{-1}$ soil were reported (Ogram et al. 1994). Autoclaved marine sediments were shown to bind chromosomal DNA at a concentration of about 3.6 $\mu g\ cm^{-3}$ (Stewart et al. 1991).

Several studies demonstrated protection against nucleolytic degradation by adsorption of DNA to clay minerals (Khanna and Stotzky 1992; Paget et al. 1992; Gallori et al. 1994), sea sand (Aardema et al. 1983; Lorenz et al. 1988; Romanowski et al. 1991), material taken from a groundwater aquifer (Romanowski et al. 1993b) as well as non-sterile soil (Blum et al. 1997).

In order to analyse the protection of DNA by adsorption to minerals, Khanna and Stotzky (1992) employed transformation assays. When montmorillonite-DNA complexes previously incubated with DNaseI were mixed with competent *B. subtilis* cells, transformation occurred but was strongly inhibited (by ca. 90%) compared to in experiments performed in the absence of DNaseI (Khanna and Stotzky 1992). However, when the same amount of free chromosomal DNA was mixed with an approximately fourfold lower amount of free DNaseI, nearly complete inhibition of transformation was reported (ca. 99.8%). Transformation of competent *B. subtilis* cells by chromosomal DNA adsorbed to sand particles of sand columns in the presence of DNaseI was unaffected at concentrations up to 1 μg DNaseI ml^{-1} (Lorenz et al. 1988). In contrast, transformation by chromosomal DNA in liquid culture was strongly inhibited at a 20-fold lower concentration of DNaseI.

DNA adsorbed to sterile groundwater aquifer material was approximately 1000-fold more resistant to DNaseI than free plasmid DNA in sterile, filtered groundwater when the production of acid-soluble radioactivity mediated by DNaseI degradation of [^{3}H]thymidine-labelled plasmid DNA was assessed (Romanowski et al. 1993b). Blum et al. (1997) employed a procedure for the reisolation of [^{3}H]thymidine-labelled phage P22 DNA adsorbed to non-sterile soil particles or dissolved in the interstitial solution of non-sterile soils. The amount of DNA in the fraction of non-adsorbed DNA declined rapidly within 24 h after DNA addition to the soils. In contrast, the amount of DNA in the fraction of adsorbed DNA declined at a much slower rate. These results suggested that adsorbed DNA was protected from degradation by endonucleases produced by indigenous bacteria (Blum et al. 1997).

Availability of adsorbed DNA for transformation. Several studies indicated that transformation of naturally transformable bacteria occurred in the solid/liquid interface on minerals, i.e. competent bacteria captured adsorbed DNA directly from mineral surfaces (Lorenz et al. 1988, Lorenz and Wackernagel 1990; Chamier et al. 1993, Romanowski et al. 1993b). Romanowski et al. (1993b) employed a column containing chromosomal DNA adsorbed to groundwater aquifer material. The column was incubated for 30 min with a cell-free filtrate of a competent *B. subtilis* cell suspension. Columns were eluted and transforming activities of effluents were assayed by transformation of *B. subtilis*. Transforming activities of effluents were only approximately 12% of the total transforming activity in microcosms, quantified after the same time of incubation. Apparently, only a minor proportion of competent cells in the microcosms were transformed by desorbed DNA. Interestingly, in the case of *B. subtilis,* transformation by chromosomal DNA was up to 50-fold enhanced in the solid/liquid interfaces in sand microcosms compared to that measured by liquid-culture assays (Lorenz et al. 1988). In contrast, transformation efficiencies of *P. stut-*

zeri (Lorenz and Wackernagel 1990) as well as *A. calcoaceticus* (Chamier et al. 1993) by sand-adsorbed chromosomal DNA were comparable to those observed when free DNA was employed. However, the transformation of *A. calcoaceticus* by sand-adsorbed plasmid DNA was significantly reduced compared to its transformation by free plasmid DNA (Chamier et al. 1993).

In contrast to the above-mentioned studies which indicated that adsorbed DNA was available for natural transformation, Stewart et al. (1991) reported that DNA added to marine sediment was largely unavailable for uptake by competent bacteria. The authors analysed the transformation of *P. stutzeri* ZoBell by chromosomal DNA adsorbed to marine sediment. Loading of sterile autoclaved marine sediment with DNA in the range of 0–1.0 μg/cm^{-3}, did not result in a significant increase in transformation frequencies. However, when sediments were pre-loaded with an excess of calf-thymus DNA and, thus, saturated, transformation frequencies increased with increasing DNA concentrations.

Transformation of indigenous bacteria

Little is known about that proportion of bacteria of a given community which are naturally transformable. Culturable heterotrophic bacteria of various marine environments were tested for their ability to take up DNA in filter transformation assays (Frischer et al. 1994). Three out of 30 and 15 out of 105 of the isolates tested were transformed by plasmid and chromosomal DNA, respectively. Isolates transformed by plasmid DNA included members of the genera *Vibrio* and *Pseudomonas*, from which representatives are known that possess natural transformation systems. Moreover, isolates of unknown identity displayed the capability of natural transformation, too. Plasmid transformation of mixed marine populations from surface and deep water, sediments, marine sponges, holothurians and the coral surface layer, on filters placed on solid artificial seawater media, was also shown to occur in 5 of 14 samples (Frischer et al. 1994).

Filter transformation of bacteria, extracted from freshly collected marine sediments, by DNA previously isolated from rifampicin-resistant bacterial inhabitants of the sediment was demonstrated by Stewart and Sinigalliano (1990). Numbers of rifampicin-resistant colonies in DNA-treated samples were reproducibly threefold higher than numbers of colonies showing spontaneous resistance to rifampicin in untreated samples.

Field studies in terrestrial and aquatic environments

Compared to the number of laboratory based studies, only few data have been published dealing with transformation of bacteria in the field. To our knowledge, so far no study has reported the transformation of bacteria in terrestrial habitats. Recently, one study demonstrated the natural transformation of bacteria in a freshwater habitat.

Transformation of *A. calcoaceticus* by chromosomal and plasmid DNA in rivers was addressed by Williams et al. (1996). A crude lysate or living donor cells of a prototrophic *A. calcoaceticus* donor strain and an auxotrophic *A. calcoaceticus* recipient strain were immobilised on separate filters. These were placed together on the surface of sterile slate disks which were submerged in a river. Transformation to prototrophy by crude lysates was observed in three different rivers, and differed in frequencies ranging from 10^{-3} to 10^{-6} per recipient. Transformation by DNA released from living donor cells was analysed in one of the rivers, and the transformation frequencies were two orders of magnitude lower than those observed when crude lysates were employed. Transformation frequencies observed when using crude lysates or living donor cells significantly increased with temperature. At the temperature optimal for in situ transformation, transformation frequencies were comparable to those obtained under laboratory conditions on agar plates. No transformation was detected during the winter at water temperatures between 2 °C and 6 °C. Transformation rates under field conditions and those observed in parallel experiments conducted under controlled laboratory conditions in river-water microcosms differed significantly. Furthermore, there was a high degree of variability in transformation frequencies in replicate experiments conducted under field conditions. Plasmid transformation of an *A. calcoaceticus* recipient strain by crude lysates and living donor cells of an *A. calcoaceticus* donor strain, harbouring the non-conjugative mercury resistance plasmid pQM17, on submerged sterile stones was also reported (Williams et al. 1996).

Moreover, evidence was provided for transformation of the auxotrophic *A. calcoaceticus* strain previously incorporated into the indigenous microbial community of river epilithon (Williams et al. 1996). Filters carrying a crude lysate of the prototrophic *A. calcoaceticus* donor strain were placed in contact with the epilithic bacterial community containing the auxotrophic *A. calcoaceticus* recipient strain on slate disks. Prototrophic transformants arose at a frequency of 1.0×10^{-4} on the disks submerged in the river.

Summary

Laboratory studies have revealed that naturally transformable bacteria develop competence under in situ conditions. Thus, the occurrence of competent bacteria in the environment can be considered as a certainty. DNA was shown to be protected from degradation by nucleases after adsorption to minerals. Although DNA seeded into soil was hydrolysed at substantial rates, it was still detectable at low levels after even several

weeks. In aquatic habitats, hydrolysis of free DNA is often more rapid than in terrestrial habitats. Nonetheless, under certain circumstances, such as in marine sediments, prolonged persistence of DNA has been reported. Thus, free DNA may persist long enough to be available for natural transformation. Whereas the in situ transformation of bacteria under laboratory conditions has been reported on several occasions, data about transformation under field conditions are still scarce. However, transformation of *A. calcoaceticus* was shown to occur in freshwater environments.

Transduction

The potential for gene transfer via transduction in nature may be inferred from studies which reported on high concentrations of bacteria and phages in terrestrial as well as aquatic habitats (e.g. Germida and Casida 1983; Wommack et al. 1992; Paul et al. 1993; Campbell et al. 1995). Early studies which addressed the question of the prevalence of phages in nature employed methods such as plating of environmental samples on plates containing different indicator bacteria. For soil, the plating technique is still the method of choice, since it is difficult to prepare soil samples suitable for analyses by, for example, microscopy. Phages have been found in the range of $0–10^7$ plaque-forming units (pfu) per gram of soil (see Table 4). In aquatic habitats, the transmission electron microscopy technique is frequently used to estimate concentrations of viruses. By employing this method, workers observed high concentrations of viruses, in the range of $10^3–10^8$ pfu ml^{-1} (or cm^{-3}), in marine waters, marine sediment, lake water, sewage or activated sludge (see Table 5). From morphological analyses it was concluded that a large proportion of these viruses are bacteriophages (e.g. Proctor and Fuhrman 1990; Wommack et al. 1992).

For estimations of bacterial numbers in terrestrial and aquatic habitats, either the direct plate count method, cell-staining methods using, e.g. acridine orange (Wommack et al. 1992), or in situ hybridisation methods (Amann et al. 1995) have been used. Different soils such as clay soil (Devanas and Stotzky 1988; Stotzky 1989), forest soil (Kauri 1978), desert soil (Skujins 1984) or fertile mollisol (Metting 1993) were shown to harbour bacterial in the range of $10^4–10^9$ cells g^{-1}. In aquatic systems such as freshwater lakes and streams (Brayton et al. 1987; O'Morchoe et al. 1988), marine waters (Iriberri et al. 1987; Wommack et al. 1992), river mud (Fjerdugstad 1975), wastewater, and activated sludge (Altherr and Kasweck 1982; Fujita and Ike 1994), bacterial counts were in the range of $10^3–10^9$ bacteria ml^{-1}.

Environmentally relevant biotic parameters influencing phage adsorption and propagation

Compared to studies assessing gene exchange in natural habitats via conjugation and transformation, only few studies have addressed gene transfer via transduction. Transduction in natural habitats is influenced by environmental parameters affecting adsorption of phages to their specific host cells as well as phage propagation. Employing in vitro as well as in situ experiments, biotic as well as abiotic parameters modulating both processes have been analysed.

Table 4 Prevalence of bacteriophages in soils. *pfu* Plaque-forming units

Indicator host(s)	pfu[a] (g^{-1} soil)	Reference
P. putida	$0–10^4$	Campbell et al. (1995)
Ensifer adhaerans	$0–10^4$	Germida and Casida (1983)
Actinomycetes	$0–10^4$	Lanning and Williams (1982)
Arthrobacter soil isolates	$0–10^3$	Germida and Casida (1981)
Bacillus stearothermophilus	4×10^7	Reanney and Marsh (1973)

[a] pfu of bacteriophages were determined employing phage propagation on indicator bacteria

Table 5 Prevalence of virus particles in aquatic ecosystems

Habitat	Phage concentration[a] (ml^{-1})	Reference
Marine waters	$1.7 \times 10^6–1.2 \times 10^7$	Paul et al. (1993)
	$2.0 \times 10^6–1.4 \times 10^8$	Wommack et al. (1992)
	$1.2 \times 10^6–3.5 \times 10^7$	Hara et al. (1991)
	$2.4 \times 10^5–3.4 \times 10^7$	Paul et al. (1991b)
	$5.0 \times 10^5–1.3 \times 10^7$	Bratbak et al. (1990)
	$10^3–10^8$	Proctor and Fuhrman (1990)
	$5.0 \times 10^6–1.5 \times 10^7$	Berg et al. (1989)
Marine sediment	$1.35 \times 10^8–5.3 \times 10^8$ cm^{-3}	Paul et al. (1993)
Lake water	2.5×10^8	Berg et al. (1989)
Sewage	2.2×10^7	Ewert and Paynter (1980)
Activated sludge	9.5×10^7	Ewert and Paynter (1980)

[a] Phage concentrations were estimated by employing transmission electron microscopy

Physiological status of the host cell Phage propagation requires the metabolic machinery of host cells and, consequently, efficient transduction is probably negatively influenced under growth-limiting conditions. Starved cells display considerable physiological alterations compared to growing cells [for a review see Kolter et al. (1993)], which may include changes in phage-receptor structures and thus, may influence adsorption of phages (Kokjohn et al. 1991). Adsorption of phages F116L and UT1 to *P. aeruginosa*, was not affected after even more than 40 days of starvation in river water (Kokjohn et al. 1991). However, phage multiplication was significantly impaired when host cells were inoculated into sterile river water and starved for at least 20 h prior to infection. The latency period was significantly extended and the burst size was significantly reduced compared to experiments conducted with non-starved cells in a rich medium.

Similar results were reported by Schrader et al. (1997) who examined the influence of starvation of *P. aeruginosa* and *E. coli* on bacteriophage infection and multiplication. The study revealed that several, but not all, phages are able to infect, and to replicate in, stationary phase cells, albeit with increased latency periods as well as reduced burst sizes. For instance, *P. aeruginosa* starved in buffer for approximately 24 h was infected with either the virulent, generalised transducing phage, UT1, or with the temperate phage, ACQ. Phage progeny was detected in both cases but latency periods increased approximately two- and threefold, respectively, and the mean burst sizes were tenfold lower than in experiments with host cells grown exponentially. Surprisingly, phage ACQ was able to infect and to multiply in stationary-phase *P. aeruginosa* cells starved for even 5 years in sterile lake water. Latency periods were extended threefold whereas the burst size was effectively unchanged, compared with host cells starved for 24 h. It remains to be determined whether this result was due to the presence of a significant subpopulation of metabolically active bacteria among the long-term starved *P. aeruginosa* cells. In contrast, phage BLB, a temperate, generalised transducing phage of *P. aeruginosa,* was unable to multiply in stationary phase host cells.

Zeph et al. (1988) addressed the question of whether the increased metabolic activity of host cells due to the addition of nutrients may result in higher transduction frequencies in soil. The authors reported on transduction of *E. coli* by a genetically marked derivative of phage P1 in soil. When phages and recipient cells were added in a nutrient-rich medium to sterile soil, significantly higher numbers of phages, recipient cells and transductants were recovered compared to experiments in which the components were added in saline. However, transduction frequencies were essentially the same. In non-sterile soil, numbers of transductants were only slightly higher when phages and recipients were added in a nutrient-rich medium. Again, transduction frequencies were not affected by nutrient amendment (Zeph et al. 1988). Hence, addition of nutrients did not affect transduction in non-sterile soils (Zeph et al. 1988; Stotzky 1989).

Host cell density. The question of a potential threshold value of host cell density below which effective viral propagation is impaired was addressed by Wiggins and Alexander (1985). The existence of such a threshold was inferred from the observation that the numbers of several virulent phages of *Staphylococcus aureus*, *B. subtilis* and *E. coli* did not increase in culture, unless a cell density greater than approximately 10^4 host cells ml^{-1} was reached (Wiggins and Alexander 1985). In contrast, no such threshold was reported by Kokjohn et al. (1991) when using *P. aeruginosa* as the host strain and a different experimental setting which allowed the authors to assess the influence of host cell densities on infection separately from their effect on phage propagation. The authors analysed phage/host interactions between the generalised transducing phages F116L and E79, as well as UT1, and their host, *P. aeruginosa* in a nutrient-rich medium. Phage propagation was analysed at host cell densities in the range of 10^1–10^5 cfu ml^{-1} at a multiplicity of intection (moi) of approximately 1. No threshold value necessary for phage propagation was detected, which was observed at even a cell density as low as 100 cfu ml (Kokjohn et al. 1991). Thus, effective interactions between *P. aeruginosa* and its specific phages occurred at cell densities of *P. aeruginosa* frequently found in natural freshwater ecosystems.

Marsh and Wellington (1992) addressed the question of a potential threshold value of host cell density below which effective viral propagation in soil is impaired. The temperate phage ϕC31 was co-inoculated at a density of 2.0×10^4 pfu g^{-1} soil with its Gram-positive host strain *Streptomyces lividans* KT24 at cell densities in the range of 2.0×10^0 to 2.0×10^5 cfu/g^{-1} soil. The moisture content of the soil was adjusted to about 40% of its water holding capacity. A minimum density of 2.0×10^3 cfu g^{-1} soil was required, below which neither phage propagation nor lysogenisation of host cells was detectable.

Environmentally relevant abiotic parameters influencing phage adsorption and propagation

Temperature, pH and moisture content of soils. Abiotic parameters such as temperature, pH and moisture content can have significant effects on the physiological status of host cells, thus resulting in changed latency periods and burst sizes. Beside a general influence on the metabolic state of host cells, changing temperatures can affect phage adsorption in natural habitats, since many phages display optimal temperatures for this process (Seeley and Primrose 1980). Changes in pH are also likely to influence adsorption of phages to host cells. Moreover, changes in pH may influence the attachment of phages to clay minerals (Stotzky et al. 1981; Lipson and Stotzky 1987), thus affecting the survival of these

virions in the environment (Stotzky 1989). Changes in the moisture content of soils will influence the movement of phages, and thus the probability of phage/host cell interactions.

Ionic strength. Many phages require adequate concentrations of cations such as calcium or magnesium ions for an effective adsorption to their host cell (Hayes 1964). This may be explained by the capacity of ions to reduce the electrostatic repulsion forces between phages and host cells (e.g. Primrose et al. 1982; Stotzky 1986; Marsh and Wellington 1994). Both phages and bacteria are negatively charged under field conditions (Stotzky et al. 1981; Lipson and Stotzky 1984, 1987). However, whether changes in concentrations of different ions in various habitats affect transduction frequencies needs to be determined.

Presence of surfaces. Phages may adsorb to clay minerals such as montmorillonite or kaolinite. The degree of adsorption depends on the chemical composition of clay minerals which determines their net charge as well as on the charge of the phage envelope. For instance, in contrast to phage P1 which had a higher affinity to montmorillonite than to kaolinite, phage F116 of *P. aeruginosa* did not tightly adsorb to these minerals (Yin et al. 1997). Clay minerals may enhance phage survival due to protection against inactivation by, e.g., proteases (Zeph et al. 1988; Stotzky 1989; Yin et al. 1997). Furthermore, clay minerals act as concentrating surfaces, not only for phages but also for their host strains and nutrients (Stotzky 1986). The resulting combination of bacterial growth and the intimate interactions between phages and bacteria may enhance the probability of phage adsorption and propagation and, thus, transduction. An excess of clay minerals, however, may counteract transduction due to a limitation of bacterial as well as phage movement, resulting in a lower probability of contacts between phages and hosts (Stotzky 1989). Moreover, attachment of bacteria may reduce the number of accessible phage receptors on host cell surfaces (Stotzky 1989).

In order to determine whether the presence of clay particles is conducive to phage/host interactions, various amounts of the clay minerals mackaloid and montmorillonite were added to mixtures of phage F116 and cells of its specific host *P. aeruginosa* (Ripp and Miller 1995). Subsequently, plaque formation efficiencies (PFE) were determined. At low concentrations of both types of clays, PFE were greatly enhanced, relative to control experiments in the absence of clay. Hence, phage/host interactions were promoted by the presence of clay. Increasing clay concentrations, however, resulted in a steady decline in PFE.

Zeph et al. (1988) demonstrated the lysogenic conversion of *E. coli* by a genetically tagged derivative phage, P1, in soil. Multiplication, survival and maximum numbers of recipient as well as lysogenised cells were similar in both unamended non-sterile soil or non-sterile soils amended with different amounts of montmorillonite or kaolinite. In soil amended with montmorillonite, the infectious phage particles persisted significantly longer than in soil amended with kaolinite (Zeph et al. 1988).

Transduction as a viable mechanism for gene transfer among microbes on plant lamellar surfaces was reported by Kidambi et al. (1994). A *P. aeruginosa* recipient strain lysogenic with respect to the general transducing phage F116, and a *P. aeruginosa* donor strain containing the non-conjugative antibiotic resistance plasmid Rms149, were co-inoculated on sterile bean and soybean leaves. Following inoculation, transductants harbouring plasmid Rms149 were observed on 90% of the leaves studied.

Transduction of indigenous bacteria of natural communities

Cell-free lysates of the two generalised transducing phages of *P. aeruginosa*, UT1 and F116, obtained after infection of a *P. aeruginosa* strain harbouring plasmid Rms149, were incubated with lake microorganisms concentrated from 10 l samples (Ripp et al. 1994). Previous studies had revealed that 1–20% of these microorganisms belonged to the *P. aeruginosa* group (Miller 1992). Plasmid-Rms149-harbouring transductants were obtained with the aid of both phages at similar frequencies, i.e. at ca. 10^{-3} per phage, at a moi of 0.1 and 0.05, respectively (Ripp et al. 1994).

Field studies in terrestrial and aquatic environments

Whereas numerous field studies have been performed in freshwater habitats employing the use of test chambers (Morrison et al. 1978; Saye et al. 1987, Saye and Miller 1989, 1990; Ripp and Miller 1995), so far no study has reported the transduction of bacteria under field conditions in terrestrial habitats.

Transduction of streptomycin resistance (Sm^R) by phage F116 from a spontaneous Sm^R mutant of *P. aeruginosa* to a *P. aeruginosa* recipient strain in test chambers submerged in a lake was analysed by Morrison et al. (1978). Transduction by phage lysates occurred at similar frequencies as in parallel in vitro experiments. Interestingly, transduction rates by spontaneously induced phages of the donor strain were comparable to those obtained when phage lysates were used (Morrison et al. 1978).

Transduction of chromosomal markers from non-lysogenic *P. aeruginosa* donor to *P. aeruginosa* recipient strains, lysogenic with respect to phage DS1, in test chambers submerged in a lake, as well as the reciprocal exchange of chromosomal markers between lysogenic and non-lysogenic cells, was reported (Saye et al. 1990). Hence, lysogenic strains were not only able to serve as a source for transducing phages, but were also able to

act as recipients. Laboratory based microcosm studies performed in parallel confirmed the reciprocal gene exchange between non-lysogenic and lysogenic *P. aeruginosa* strains in lake water. Interestingly, transductants of the lysogenic strains occurred ten to 100-fold more frequently than transductants of non-lysogenic strains. This result was explained by the improved survival of lysogenic strains due to their immunity against destruction by free phages. The authors concluded that lysogeny has the potential of increasing the size and the flexibility of the gene pool available to natural populations of bacteria (Saye et al. 1990). It is worth noting that many bacteria isolated from soils as well as aquatic habitats were shown to harbour temperate phages (Miller and Sayler 1992). For instance, Ogunseitan et al. (1992) reported that up to 40% of *P. aeruginosa* in natural ecosystems contained DNA sequences homologous to phage genomes.

Transduction of plasmid Rms149 between *Pseudomonas* strains was demonstrated in test chambers in lake water (Saye et al. 1987). Test chambers containing sterile lake water were inoculated with either: (1) phage DS1 lysates obtained from a lysogenic *P. aeruginosa* strain carrying the non-conjugative antibiotic resistance plasmid Rms149 and a non-lysogenic *P. aeruginosa* recipient strain, (2) the plasmid-harbouring lysogenic donor as well as the non-lysogenic recipient strain, or (3) a non-lysogenic *P. aeruginosa* donor strain harbouring the plasmid and a *P. aeruginosa* recipient strain, lysogenic for phage DS1. Transductants were only obtained employing the non-lysogenic donor and the lysogenic recipient strain. The cell density in the corresponding test chambers was similar to that of naturally occurring *Pseudomonas* concentrations. These results demonstrated once more the importance of lysogeny for gene transfer among bacteria in the environment. Experiments using the non-lysogenic plasmid donor and the lysogenic recipient strain were conducted in the presence of the indigenous microbial community of the freshwater habitat. Under these conditions, the survival of donor and recipient cells, the stability of free phages, as well as the numbers of recoverable transductants, were greatly reduced compared to in sterile lake water (Saye et al. 1987).

Ripp and Miller (1995) reported that the aggregation of bacteriophages and bacterial cells on particulate matter can increase the probability of progeny phages and transducing particles infecting new host cells. The authors analysed the transduction of plasmid Rms149 between *P. aeruginosa* strains in test chambers submerged in a lake. The chambers were filled with sterilised lake water sampled either from a depth of 6 m or from the surface of the lake. The concentration of the particulate matter in water from deeper samples was approximately 14-fold higher than in the surface water. After incubation of the chambers in the lake, 100 times more transductants were detected in chambers containing the sample from a depth of 6 m than in those containing the surface water sample. The aggregation of bacteriophages and bacterial cells on the particulate matter thus may have increased the probability of progeny phages and transducing particles finding and infecting of new host cells (Ripp and Miller 1995).

Summary

Laboratory studies have indicated that phages adsorbed and even multiplied in bacterial populations starved for even several years. However, latency periods as well as burst sizes were lower under starvation conditions compared to those observed in a nutrient-rich medium. These results suggested that transduction is feasible in nutrient-poor habitats. Studies which addressed the influence of the density of host cells on phage/host interactions suggested that these interactions occur at low cell densities. The presence of clay minerals was shown to favour both phage survival and phage/host interactions. Under laboratory conditions, transduction was demonstrated in soil as well as on the surface of leaves.

Field experiments indicated that transduction is an important mechanism for the exchange of genetic information among bacteria in aquatic systems, such as freshwater habitats. Additionally, these field studies revealed that the presence of solid surfaces facilitates phage/host interactions and enhances transfer frequencies. Lysogeny was identified as particularly advantageous for the acquisition of DNA.

Release of a genetically modified *S. meliloti* strain – biosafety considerations concerning the potential of HGT

To illustrate the potential for the horizontal transmission of rDNA of a released GEM in the light of the gene transfer studies described above, the following test case briefly considers a hypothetical example of a biofertiliser introduced into the environment to enhance crop yield. The microorganism is an *S. meliloti* strain genetically modified for enhanced symbiotic nitrogen fixation. In order to reduce the probability of lateral transfer, the rDNA is integrated in the chromosome of the GEM rather than into a self-transmissible or mobilisable plasmid. What about the potential for transfer of rDNA after environmental release of this GEM?

Laboratory based studies have demonstrated that conjugative plasmids with the ability to mobilise chromosomal markers, such as R68.45, may mediate the exchange of chromosomal genes between *S. meliloti* strains (Kondorosi et al. 1977). In addition, genetic transformation (Courtois et al. 1988) as well as general transduction (Finan et al. 1984) of *S. meliloti* have been shown to be viable gene-transfer mechanisms. Hence, the rDNA of a released *S. meliloti* GEM may be subject to transfer to indigenous *S. meliloti* bacteria via each of

the three known types of gene transfer. Considering the extraordinary broad host range of transfer systems such as that of IncP plasmids (Thomas 1989) which were shown to occur in different soils (Götz et al. 1996), and taking into account the promiscuity of uptake systems of some naturally transformable bacteria, rDNA could be transmitted to a wide range of autochthonous microbes.

The test case GEM, *S. meliloti*, is a soil bacterium which positively responds to the presence of rhizospheres, in particular that of its host plant alfalfa. Thus, *S. meliloti* lives in a habitat where conditions such as nutrient availability, locally high cell densities, solid surfaces, etc., prevail, which have been shown to favour genetic interactions (e.g. van Elsas 1992; Dröge et al. 1998). Provided that the GEM is released on a large scale, it may be introduced, for example into different soil types, which may include soils whose physicochemical characteristics are favourable for gene exchange. In the absence of the host plant, *S. meliloti* will persist for some time in the soil. During that time, the GEM is exposed to seasonal variations of numerous biotic and abiotic factors which may create conditions identified as conducive for gene exchange.

Gene transfer studies with *S. meliloti* performed under more natural conditions have already established that conjugative gene exchange occurs in soil (Lafuente et al. 1996) as well as in root nodules of the host plant alfalfa (Pretorius-Güth et al. 1990). There is no reason to assume that transduction or transformation would not work under field conditions as well. Hence, it is conceivable that transfer of rDNA from the test case GEM, *S. meliloti*, to autochthonous soil bacteria under field conditions may occur.

In order to minimise the probability of rDNA transfer, strategies have been developed to link rDNA with genes encoding cell-killing functions which are inactive in the original host but active after transfer into recipients, i.e. members of autochthonous microbial communities (Diaz et al. 1994). However, such strategies provide no guarantee for confining rDNA to the original host since such genes, like any other structural genes, are subject to inactivation by mutations (Molin et al. 1993).

Conclusions

During the last decade a substantial body of knowledge has accumulated on how bacteria interact genetically in their natural environments. Gene transfer experiments performed in model ecosystems or under field conditions demonstrated that, under certain circumstances, genes were readily transferred. This refers in particular to habitats such as the phytosphere where conditions prevail which were identified as conducive for gene exchange (e.g. van Elsas 1992; Dröge et al. 1998).

The results of gene transfer studies presented in this review may suggest that there is extensive gene flux among bacteria in nature. However, there are natural barriers that act at the level of transfer as well as at the level of maintenance of the transferred gene in the host cell [for an overview see Miller and Levy (1989)] which counteract the free and unlimited exchange of genes. Examples of systems which counteract the establishment of transferred DNA in a host are restriction/modification or mismatch repair systems. The occurrence of distinct species in nature is indicative of the occurrence of such mechanisms. Bacteria have evolved mechanisms rendering them capable of retaining their integrity as species, which would otherwise be disrupted by extensive gene exchange.

In the test case example outlined above, the potential for rDNA transfer from the GEM to autochthonous microbial populations has been considered. Of course, gene transfer could also take place from the autochthonous community into a released GEM. Such transfers would also be of biosafety relevance since the transferred DNA might alter the ecological fitness of the released strain (Lilley and Bailey 1997b). It is obvious that there is no way to prevent the transfer of DNA into a released GEM, and it seems hardly feasible that the transfer of rDNA from the GEM to the indigenous microbial community can be avoided. Thus, once a GEM has been released, it must be taken into account that the transfer of rDNA may occur. All available information indicates that if such a transfer occurs, it will probably occur at a low frequency. However, the frequency of transfer of rDNA to indigenous bacteria itself is of minor importance since even rare transfer events may be selected for if the transferred gene confers an advantageous trait. This was particularly evident from the transfer of a mobile element carrying symbiotic genes from a released *M. loti* strain to non-symbiotic *M. loti*-related soil bacteria. Although the element transferred between *M. loti* species at low frequencies under optimal conditions in the laboratory, transconjugants of indigenous non-symbiotic rhizobia were readily selected in the field (Sullivan et al. 1995). Furthermore, the study of Søren Molin's group clearly demonstrated that a TOL plasmid encoding an advantageous trait readily established in a biofilm community. Plasmid establishment occurred independently of the plasmid's original host after low frequency transfer into a member of the biofilm community, which resulted in an advantageous plasmid/host combination (Christensen et al. 1998).

Despite the fact that a considerable amount of information is now available on how bacteria exchange genes in natural environments, numerous issues remain to be addressed in future research. This applies in particular to our lack of knowledge about the selective forces which act in natural environments. Selective processes ultimately influence whether the transfer of rDNA will result in an environmental impact. Partly due to the lack of appropriate methods there is a paucity of knowledge about the organisms which interact genetically (donor cells and recipient cells), the envi-

ronmental relevance of conjugative plasmids or phages as vehicles of transferred DNA, or the conditions under which genes are transferred. For instance, the incidence of conjugative bhr plasmids displaying cma properties and their contribution to gene mobilisation within microbial communities are largely unexplored. Due to the fact that most bacterial species in microbial communities are simply unknown, there is a paucity of knowledge about the proportion of bacterial species of a given microbial community which possess natural transformation systems. Another issue which is largely unexplored refers to the question how frequently do naturally transformable bacteria become competent in the environment? Furthermore, the incidence of transducing phages displaying a broad host range of infection and propagation is unknown. Most of the transducing phages isolated so far display a narrow host range, indicating that transduction contributes little to interspecific or intergeneric gene transfer among bacteria. However, due to the limited number of studies addressing this issue, the proportion of transducing phages which mediate HGT among unrelated bacteria remains to be determined. Finally, the question has to be addressed to what extent non-cultured bacteria serve as donors or recipients of DNA. Recent developments in marker gene technology in conjunction with suitable gene mobilisation and detection systems (e.g. Dahlberg et al. 1998a; Strätz et al. 1996) make it feasible to address this question in the near future.

Acknowledgements This work was supported by a grant of the German Ministry for Education, Science, Research and Technology (ref. no. 0311223).

References

Aardema BW, Lorenz MG, Krumbein WE (1983) Protection of sediment-adsorbed transforming DNA against enzymatic inactivation. Appl Environ Microbiol 46:417–420

Altherr MR, Kasweck KL (1982) In situ studies with membrane diffusion chambers of antibiotic resistance transfer in *Escherichia coli*. Appl Environ Microbiol 44:838–843

Amann RI, Ludwig W, Schleifer K-H (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Arana JI, Justo A, Muela A, Pocino M, Iriberri J, Barcina I (1997) Influence of a survival process in a freshwater system upon plasmid transfer between *Escherichia coli* strains. Microb Ecol 33:41–49

Ashelford KE, Fry JC, Day MJ, Hill KE, Learner MA, Marchesi JR, Perkins CD, Weightman AJ (1997) Using microcosms to study gene transfer in aquatic habitats. FEMS Microbiol Ecol 23:81–94

Avery OT, MacLeod CM, McCarty M (1944) Studies on the chemical nature of the substance-inducing transformation of pneumococcal types. J Exp Med 79:137–158

Bale MJ, Fry JC, Day MJ (1987) Plasmid transfer between strains of *Pseudomonas aeruginosa* on membrane filters attached to river stones. J Gen Microbiol 133:3099–3107

Bale MJ, Fry JC, Day MJ (1988a) Transfer and occurrence of large mercury resistance plasmids in river epilithon. Appl Environ Microbiol 54:972–978

Bale MJ, Day MJ, Fry JC (1988b) Novel method for studying plasmid transfer in undisturbed river epilithon. Appl Environ Microbiol 54:2756–2758

Baquero F, Blázquez J (1997) Evolution of antibiotic resistance. Trends Ecol Evol 12:482–487

Berg Ø, Børsheim KY, Bratbak G, Heldal M (1989) High abundance of viruses found in aquatic environments. Nature 340:467–468

Björklöf K, Suoniemi A, Haahtela K, Romantschuk M (1995) High frequency of conjugation versus plasmid segregation of RP1 in epiphytic *Pseudomonas syringae* populations. Microbiology 141:2719–2727

Bleakley BH, Crawford DL (1989) The effect of varying moisture and nutrient levels on the transfer of a conjugative plasmid between *Streptomyces* species in soil. Can J Microbiol 35:544–549

Blum SAE, Lorenz MG, Wackernagel W (1997) Mechanism of retarded DNA degradation and prokaryotic origin of DNases in nonsterile soils. Syst Appl Microbiol 20:513–521

Bolton H Jr, Fredrickson JK, Elliott LF (1993) Microbial ecology of the rhizosphere. In: Metting FB Jr (ed) Soil microbial ecology. Dekker, New York, pp 27–63

Bratbak G, Heldal M, Norland S, Thingstad TF (1990) Viruses as partners in spring bloom microbial trophodynamics. Appl Environ Microbiol 56:1400–1405

Brayton PR, Tamplin ML, Huq A, Colwell RR (1987) Enumeration of *Vibrio cholerae* 01 in Bangladesh waters by fluorescent-antibody direct viable count. Appl Environ Microbiol 53:2862–2865

Campbell JIA, Albrechtsen M, Sørensen J (1995) Large *Pseudomonas* phages isolated from barley rhizosphere. FEMS Microbiol Ecol 18:63–74

Chamier B, Lorenz MG, Wackernagel W (1993) Natural transformation of *Acinetobacter calcoaceticus* by plasmid DNA adsorbed on sand and groundwater aquifer material. Appl Environ Microbiol 59:1662–1667

Christensen BB, Sternberg C, Andersen JB, Eberl L, Møller S, Givskov M, Molin S (1998) Establishment of new genetic traits in a microbial biofilm community. Appl Environ Microbiol 64:2247–2255

Clerc S, Simonet P (1996) Efficiency of the transfer of a pSAM-derivative plasmid between two strains of *Streptomyces lividans* in conditions ranging from agar slants to non-sterile soil microcosms. FEMS Microbiol Ecol 21:157–165

Clewell DB (ed) (1993) Bacterial conjugation. Plenum, New York

Courtois J, Courtois B, Guillaume J (1988) High-frequency transformation of *Rhizobium meliloti*. J Bacteriol 170:5925–5927

Couturier M, Bex F, Bergquist PL, Maas WK (1988) Identification and classification of bacterial plasmids. Microbiol Rev 52:375–395

Dahlberg C, Linberg C, Torsvik VL, Hermansson M (1997) Conjugative plasmids isolated from bacteria in marine environments show various degrees of homology to each other and are not closely related to well-characterized plasmids. Appl Environ Microbiol 63:4692–4697

Dahlberg C, Bergström M, Andreason M, Christensen BB, Molin S, Hermansson M (1998a) Interspecies bacterial conjugation by plasmids from marine environments visualized by *gfp* expression. Mol Biol Evol 15:385–390

Dahlberg C, Bergström M, Hermansson M (1998b) In situ detection of high levels of horizontal plasmid transfer in marine bacterial communities. Appl Environ Microbiol 64:2670–2675

Day MJ, Fry JC, Diaper J (1992) Effect of host cell physiology on plasmid transfer in river epilithon. In: Gauthier MJ (ed) Gene transfers and environment. Springer, Berlin Heidelberg New York, pp 35–50

DeFlaun MF, Paul JH (1989) Detection of exogenous gene sequences in dissolved DNA from aquatic environments. Microb Ecol 18:21–28

DeFlaun MF, Paul JH, Davis D (1986) Simplified method for dissolved-DNA determination from aquatic environments. Appl Environ Microbiol 52:654–659

Devanas MA, Stotzky G (1988) Survival of genetically engineered microbes in the environment: effect of host/vector relationship. In: Pierce G (ed) Developments in industrial microbiology. Elsevier, Amsterdam, pp 287–296

Diaz E, Munthali M, Lorenzo V de, Timmis KN (1994) Universal barrier to lateral spread of specific genes among microorganisms. Mol Microbiol 13:855–861

Dröge M, Pühler A, Selbitschka W (1998) Horizontal gene transfer as a biosafety issue: a natural phenomenon of public concern. J Biotechnol 64:75–90

Elsas JD van (1992) Antibiotic resistance gene transfer in the environment: an overview. In: Wellington EMH, van Elsas JD (eds) Genetic interactions among microorganisms in the natural environment. Pergamon, Oxford, pp 17–39

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

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

Elsas JD van, Nikkel M, Overbeek LS van (1989) Detection of plasmid RP4 transfer in soil and rhizosphere, and the occurrence of homology to RP4 in soil bacteria. Curr Microbiol 19:375–381

Elsas JD van, McSpadden Gardener BB, Wolters AC, Smit E (1998) Isolation, characterization, and transfer of cryptic gene-mobilizing plasmids in the wheat rhizosphere. Appl Environ Microbiol 64:880–889

Ewert DL, Paynter MJB (1980) Enumeration of bacteriophages and host bacteria in sewage and the activated sludge treatment process. Appl Environ Microbiol 39:576–583

Fernandez-Astorga A, Muela A, Cisterna R, Iriberri J, Barcina I (1992) Biotic and abiotic factors affecting plasmid transfer in *Escherichia coli* strains. Appl Environ Microbiol 58:392–398

Fibi MR, Bröker M, Schulz R, Johannsen R, Zettlmeissl G (1991) Inactivation of recombinant plasmid DNA from a human erythropoietin-producing cell mouse line grown on a large scale. Appl Microbiol Biotechnol 35:622–630

Finan TM, Hartwieg E, LeMieux K, Bergman K, Walker GC, Signer ER (1984) General transduction in *Rhizobium meliloti*. J Bacteriol 159:120–124

Fjerdugstad E (1975) Bacteria and fungi. In: Whitton BA (ed) River ecology. University of California Press, Berkeley, pp 129–140

Frischer ME, Thurmond JM, Paul JH (1993) Factors affecting competence in a high frequency of transformation marine *Vibrio*. J Gen Microbiol 139:753–761

Frischer ME, Stewart GJ, Paul JH (1994) Plasmid transfer to indigenous marine bacterial populations by natural transformation. FEMS Microbiol Ecol 15:127–136

Fry JC, Day MJ (1990a) Plasmid transfer in the epilithon. In: Fry JC, Day MJ (eds) Bacterial genetics in natural environments. Chapman and Hall, London, pp 172–181

Fry JC, Day MJ (1990b) Plasmid transfer and the release of genetically engineered bacteria in nature: a discussion and summary. In: Fry JC, Day MJ (eds) Bacterial genetics in natural environments. Chapman and Hall, London, pp 243–250

Fujita M, Ike M (1994) Wastewater treatment using genetically engineered microorganisms. Technomic, Lancaster

Gallori E, Bazzicalupo M, Dal Canto L, Fani R, Nannipieri P, Vettori C, Stotzky G (1994) Transformation of *Bacillus subtilis* by DNA bound on clay in non-sterile soil. FEMS Microbiol Ecol 15:119–126

Germida JJ, Casida LE (1981) Isolation of *Arthrobacter* bacteriophage from soil. Appl Environ Microbiol 41:1389–1393

Germida JJ, Casida LE (1983) *Ensifer adhaerens* predatory activity against other bacteria in soil, as monitored by indirect phage analysis. Appl Environ Microbiol 45:1380–1388

Glover Glew J, Angle JC, Sadowsky MJ (1993) In vivo transfer of pR68.45 from *Pseudomonas aeruginosa* into indigenous soil bacteria. Microb Releases 1:237–241

Goodman AE, Hild E, Marshall KC, Hermansson M (1993) Conjugative plasmid transfer between bacteria under simulated marine oligotrophic conditions. Appl Environ Microbiol 59:1035–1040

Götz A, Smalla K (1997) Manure enhances plasmid mobilization and survival of *Pseudomonas putida* introduced into field soil. Appl Environ Microbiol 63:1980–1986

Götz A, Pukall R, Smit E, Tietze E, Prager R, Tschäpe H, Elsas JD van, Smalla K (1996) Detection and characterization of broad-host-range plasmids in environmental bacteria by PCR. Appl Environ Microbiol 62:2621–2628

Greaves MP, Wilson MJ (1969) The adsorption of nucleic-acids by montmorillonite. Soil Biol Biochem 1:317–323

Greaves MP, Wilson MJ (1970) The degradation of nucleic-acids and montmorillonite-nucleic-acid complexes by soil microorganisms. Soil Biol Biochem 2:257–268

Haas D, Holloway B (1976) R factor variants with enhanced sex factor activity in *Pseudomonas aeruginosa*. Mol Gen Genet 144:243–251

Hara S, Terauchi K, Koike I (1991) Abundance of viruses in marine waters: assessment by epifluorescence and transmission electron microscopy. Appl Environ Microbiol 57:2731–2734

Hayes W (1964) The genetics of bacteria and their viruses. Wiley, New York

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

Hill KE, Top EM (1998) Gene transfer in soil systems using microcosms. FEMS Microbiol Ecol 25:319–329

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

Hirsch P (1996) Population dynamics of indigenous and genetically modified rhizobia in the field. New Phytol 133:159–171

Hirsch P (1997) Acquisition of genes from indigenous bacteria by inoculant strains at long-term release sites. In: Hoeveler A, Cresti M (eds) Biotechnology (1992–1994). Final report, vol I. Office for Official Publications of the European Communities, Brussels pp 231–234

Hirsch PR, Spokes JR (1994) Survival and dispersion of genetically modified rhizobia in the field and genetic interactions with native strains. FEMS Microbiol Ecol 15:147–160

Hoffmann A, Thimm T, Dröge M, Moore ERB, Munch JC, Tebbe CC (1998) Intergeneric transfer of conjugative and mobilizable plasmids harbored by *Escherichia coli* in the gut of the soil microarthropod *Folsomia candida* (Collembola). Appl Environ Microbiol 64:2652–2659

Iriberri J, Unanue M, Barcina I, Egea L (1987) Seasonal variation in population density and heterotrophic activity of attached and free-living bacteria in coastal waters. Appl Environ Microbiol 53:2308–2314

Kauri T (1978) Aerobic bacteria in the horizons of a beech forest soil. In: Loutit MW, Miles JAR (eds) Microbial ecology. Springer, Berlin Heidelberg New York, pp 110–112

Khanna M, Stotzky G (1992) Transformation of *Bacillus subtilis* by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA. Appl Environ Microbiol 58:1930–1939

Kidambi SP, Ripp S, Miller RV (1994) Evidence for phage-mediated gene transfer among *Pseudomonas aeruginosa* strains on the phylloplane. Appl Environ Microbiol 60:496–500

Kinkle BK, Schmidt EL (1991) Transfer of the pea symbiotic plasmid pJB5JI in nonsterile soil. Appl Environ Microbiol 57:3264–3269

Kokjohn TA, Sayler GS, Miller RV (1991) Attachment and replication of *Pseudomonas aeruginosa* bacteriophages under conditions simulating aquatic environments. J Gen Microbiol 137:661–666

Kolter R, Siegele DA, Tormo A (1993) The stationary phase of the bacterial life cycle. Annu Rev Microbiol 47:855–874

Kondorosi A, Kiss GB, Forrai T, Vincze E, Banfalvi Z (1977) Circular linkage map of *Rhizobium meliloti* chromosome. Nature 268:525–527

Krasovsky VN, Stotzky G (1987) Conjugation and genetic recombination in *Escherichia coli* in sterile and nonsterile soil. Soil Biol Biochem 19:631–638

Kroer N, Barkay T, Sørensen S, Weber D (1998) Effect of root exudates and bacterial metabolic activity on conjugal gene transfer in the rhizosphere of a marsh plant. FEMS Microbiol Ecol 25:375–384

Lafuente R, Maymo-Gatell X, Mas-Castella J, Guerrero R (1996) Influence of environmental factors on plasmid transfer in soil microcosms. Curr Microbiol 32:213–220

Lanning S, Williams ST (1982) Methods for direct isolation and enumeration of actinophages in soil. J Gen Microbiol 128:2063–2071

Lebaron P, Roux V, Lett MC, Baleux B (1993) Effects of pili rigidity and energy availability on conjugative plasmid transfer in aquatic environments. Microb Releases 2:127–133

Lederberg J, Tatum EL (1946) Gene recombination in *Escherichia coli*. Nature 158:558

Lilley AK, Bailey MJ (1997a) The acquisition of indigenous plasmids by a genetically marked pseudomonad population colonizing the sugar beet phytosphere is related to local environmental conditions. Appl Environ Microbiol 63:1577–1583

Lilley AK, Bailey MJ (1997b) Impact of plasmid pQBR103 acquisition an carriage on the phytosphere fitness of *Pseudomonas fluorescens* SWB25:burden or benefit. Appl Environ Microbiol 63:1584–1587

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

Lilley AK, Bailey MJ, Day MJ, Fry JC (1996) Diversity of mercury resistance plasmids obtained by exogenous isolation from the bacteria of sugar beet in three successive years. FEMS Microbiol Ecol 20:211–227

Lipson SM, Stotzky G (1984) Adsorption of viruses to particulates: possible effects on virus survival. In: Misra AH, Polasa H (eds) Viral ecology. South Asia, New Delhi, pp 165–178

Lipson SM, Stotzky G (1987) Interactions between viruses and clay minerals. In: Rao VC, Melnick JL (eds) Human viruses in sediments, sludges and soils. CRC, Boca Raton, Fla., pp 198–229

Lorenz MG, Wackernagel W (1987) Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. Appl Environ Microbiol 53:2948–2952

Lorenz MG, Wackernagel W (1990) Natural genetic transformation of *Pseudomonas stutzeri* by sand-adsorbed DNA. Arch Microbiol 154:380–385

Lorenz MG, Wackernagel W (1991) High frequency of natural genetic transformation of *Pseudomonas stutzeri* in soil extract supplemented with a carbon/energy and phosphorus source. Appl Environ Microbiol 57:1246–1251

Lorenz MG, Wackernagel W (1992) Stimulation of natural genetic transformation of *Pseudomonas stutzeri* in extracts of various soils by nitrogen or phosphorus limitation and influence of temperature and pH. Microb Releases 1:173–176

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

Lorenz MG, Aardema BW, Wackernagel W (1988) Highly efficient genetic transformation of *Bacillus subtilis* attached to sand grains. J Gen Microbiol 134:107–112

Mach PA, Grimes DJ (1982) R-plasmid transfer in a wastewater treatment plant. Appl Environ Microbiol 44:1395–1403

Maeda M, Taga N (1973) Deoxyribonuclease activity in seawater and sediment. Mar Biol 20:58–63

Maeda M, Taga N (1974) Occurrence and distribution of deoxyribonucleic acid-hydrolyzing bacteria in sea water. J Exp Mar Biol Ecol 14:157–169

Marcinek H, Wirth R, Muscholl-Silberhorn A, Gauer M (1998) *Enterococcus faecalis* gene transfer under natural conditions in municipal sewage water treatment plants. Appl Environ Microbiol 64:626–632

Margolin P (1987) Generalized transduction. In: Ingraham JL, Low KB, Majasanik B, Schaechter M, Umbarga HE, Neidhardt FC (eds) *Escherichia coli* and *Salmonella typhimurium*. Cellular and molecular biology. American Society for Microbiology, Washington, DC, pp 1154–1168

Marsh P, Wellington EMH (1992) Interactions between acinophage and their streptomycete hosts in soil and the fate of phage-borne genes. In: Gauthier MJ (ed) Gene transfers and environment. Springer, Berlin Heidelberg New York, pp135–142

Marsh P, Wellington EMH (1994) Phage/host interactions in soil. FEMS Microbiol Ecol 15:99–108

Metting FB Jr (1993) Structure and physiological ecology of soil microbial communities. In: Metting FB Jr (ed) Soil microbial ecology. Dekker, New York, pp 3–25

Miller RV (1992) Methods for evaluating transduction: an overview with environmental considerations. In: Levin MA, Seidler RJ, Rogul M (eds) Microbial ecology: principles, methods, and applications. McGraw-Hill, New York, pp 229–251

Miller RV, Levy SB (1989) Horizontal gene transfer in relation to environmental release of genetically engineered microorganisms. In: Levy SB, Miller RV (eds) Gene transfer in the environment. McGraw-Hill, New York, pp 405–420

Miller RV, Sayler GS (1992) Bacteriophage/host interactions in aquatic systems. In: Wellington EMH, Elsas JD van (eds) Genetic interactions among microorganisms in the natural environment. Pergamon, Oxford, pp 176–193

Molin S, Boe L, Jensen LB, Kristensen CS, Givskov M, Ramos JL, Bej AK (1993) Suicidal genetic elements and their use in biological containment of bacteria. Annu Rev Microbiol 47:139–166

Morrison WD, Miller RV, Sayler GS (1978) Frequency of F116-mediated transduction of *Pseudomonas aeruginosa* in a freshwater environment. Appl Environ Microbiol 36:724–730

Muela A, Pocino M, Arana I, Justo JI, Iriberri J, Barcina I (1994) Effect of growth phase and parental cell survival in river water on plasmid transfer between *Escherichia coli* strains. Appl Environ Microbiol 60:4273–4278

Neilson JW, Josephson KL, Pepper IL, Arnold RB, Di Giovanni GD, Sinclair NA (1994) Frequency of horizontal gene transfer of a large catabolic plasmid (pJP4) in soil. Appl Environ Microbiol 60:4053–4058

Nielsen KM, Bones AM, Elsas JD van (1997) Induced natural transformation of *Acinetobacter calcoaceticus* in soil microcosms. Appl Environ Microbiol 63:3972–3977

Normander B, Christensen BB, Molin S, Kroer, N (1998) Effect of bacterial distribution and activity on conjugal gene transfer on the phylloplane of the bush bean (*Phaseolus vulgaris*). Appl Environ Microbiol 64:1902–1909

Ogram A, Sayler GS, Barkay T (1987) The extraction and purification of microbial DNA from sediments. J Microbiol Methods 7:57–66

Ogram AV, Mathot ML, Harsh JB, Boyle J, Pettigrew CA Jr (1994) Effects of DNA polymer length on its adsorption to soils. Appl Environ Microbiol 60:393–396

Ogunseitan OA, Sayler GS, Miller RV (1992) Application of DNA probes to analysis of bacteriophage distribution patterns in the environment. Appl Environ Microbiol 58:2046–2052

O'Morchoe SB, Ogunseitan O, Sayler GS, Miller RV (1988) Conjugal transfer of R68.45 and FP5 between *Pseudomonas aeruginosa* in a natural freshwater environment. Appl Environ Microbiol 54:1923–1929

Page WJ, Tigerstrom M von (1978) Induction of transformation competence in *Azotobacter vinelandii* iron-limited cultures. Can J Microbiol 24:1590–1594

Page WJ, Grant GA (1987) Effect of mineral iron on the development of transformation competence in *Azotobacter vinelandii*. FEMS Microbiol Lett 41:257–261

Paget E, Simonet P (1994) On the track of natural transformation in soil. FEMS Microbiol Ecol 15:109–118

Paget E, Simonet LJ, Monrozier P (1992) Adsorption of DNA on clay minerals: protection against DNaseI and influence on gene transfer. FEMS Microbiol Lett 97:31–40

Palmen R, Vosman B, Buijsman P, Breek CKD, Hellingwerf KJ (1993) Physiological characterization of natural transformation in *Acinetobacter calcoaceticus*. J Gen Microbiol 139:295–305

Paul JH, Jeffrey WH, DeFlaun MF (1987) Dynamics of extracellular DNA in the marine environment. Appl Environ Microbiol 53:170–179

Paul JH, DeFlaun MF, Jeffrey WH (1988) Mechanisms of DNA utilization by estuarine bacterial populations. Appl Environ Microbiol 54:1682–1688

Paul JH, Jeffrey WH, David AW, DeFlaun MF, Cazares LH (1989) Turnover of extracellular DNA in eutrophic and oligotrophic freshwater environments of southwest Florida. Appl Environ Microbiol 55:1823–1828

Paul JH, Frischer ME, Thurmond JM (1991a) Gene transfer in marine water column and sediment microcosms by natural plasmid transformation. Appl Environ Microbiol 57:1509–1515

Paul JH, Jiang SC, Rose JB (1991b) Concentration of viruses and dissolved DNA from aquatic environments by vortex flow filtration. Appl Environ Microbiol 57:2197–2204

Paul JH, Rose JB, Jiang SC, Kellogg, CA, Dickson L (1993) Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl Environ Microbiol 59:718–724

Phillips SJ, Dalgarn DS, Young SK (1989) Recombinant DNA in wastewater: pBR322 degradation kinetics. J Water Pollut Control Fed 61:1588–1595

Powell BJ, Purdy KJ, Thompson IP, Bailey MJ (1993) Demonstration of *tra*$^{+}$ plasmid activity in bacteria indigenous to the phyllosphere of sugar beet; gene transfer to a recombinant pseudomonad. FEMS Microbiol Ecol 12:195–206

Pretorius-Güth I-M, Pühler A, Simon R (1990) Conjugal transfer of megaplasmid 2 between *Rhizobium meliloti* strains in alfalfa nodules. Appl Environ Microbiol 56:2354–2359

Primrose SB, Seeley ND, Logan KB, Nicolson JW (1982) Methods for studying aquatic bacteriophage ecology. Appl Environ Microbiol 43:694–701

Proctor LM, Fuhrman JA (1990) Viral mortality of marine bacteria and cyanobacteria. Nature 343:60–62

Pukall R, Tschäpe H, Smalla K (1996) Monitoring the spread of broad host and narrow host range plasmids in soil microcosms. FEMS Microbiol Ecol 20:53–66

Ravatn R, Zehnder AJB, Meer JR van der (1998) Low-frequency horizontal transfer of an element containing the chlorocatechol degradation genes from *Pseudomonas* sp. strain B13 to *Pseudomonas pu*tida F1 and to indigenous bacteria in laboratory-scale activated-sludge microcosms. Appl Environ Microbiol 64:2126–2132

Reanney DC, Marsh SCN (1973) The ecology of viruses attacking *Bacillus stearothermophilus* in soil. Soil Biol Biochem 5:399–408

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

Ripp S, Miller RV (1995) Effects of suspended particulates on the frequency of transduction among *Pseudomonas aeruginosa* in a freshwater environment. Appl Environ Microbiol 61:1214–1219

Ripp S, Ogunseitan OA, Miller RV (1994) Transduction of a freshwater microbial community by a new *Pseudomonas aeruginosa* generalized transducing phage, UT1. Mol Ecol 3:121–126

Rochelle PA, Fry JC, Day MJ (1989) Factors affecting conjugal transfer of plasmids encoding mercury resistance from pure cultures and mixed natural suspensions of epilithic bacteria. J Gen Microbiol 135:409–424

Romanowski G, Lorenz MG, Wackernagel W (1991) Adsorption of plasmid DNA to mineral surfaces and protection against DNaseI. Appl Environ Microbiol 57:1057–1061

Romanowski G, Lorenz MG, Sayler G, Wackernagel W (1992) Persistence of free plasmid DNA in soil monitored by various methods, including a transformation assay. Appl Environ Microbiol 58:3012–3019

Romanowski G, Lorenz MG, Wackernagel W (1993a) Use of polymerase chain reaction and electroporation of *Escherichia coli* to monitor the persistence of extracellular plasmid DNA introduced into natural soils. Appl Environ Microbiol 59:3438–3446

Romanowski G, Lorenz MG, Wackernagel W (1993b) Plasmid DNA in a groundwater aquifer microcosm – adsorption, DNase resistance and natural genetic transformation of *Bacillus subtilis*. Mol Ecol 2:171–181

Sandaa RA, Torsvik VL, Goksoyr J (1992) Transferable drug resistance in bacteria from fish-farm sediments. Can J Microbiol 38:1061–1065

Sandt CH, Herson DS (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

Saye DJ, Miller RV (1989) The aquatic environment: consideration of horizontal gene transmission in a diversified habitat. In: Levy SB, Miller RV (eds) Gene transfer in the environment. McGraw-Hill, New York, pp 223–259

Saye DJ, Ogunseitan O, Sayler GS, Miller RV (1987) Potential for transduction of plasmids in a natural freshwater environment: effect of plasmid donor concentration and a natural microbial community on transduction in *Pseudomonas aeruginosa*. Appl Environ Microbiol 53:987–995

Saye DJ, Ogunseitan OA, Sayler GS, Miller RV (1990) Transduction of linked chromosomal genes between *Pseudomonas aeruginosa* strains during incubation in situ in a freshwater habitat. Appl Environ Microbiol 56:140–145

Schrader HS, Schrader JO, Walker JJ, Wolf TA, Nickerson KW, Kokjohn TA (1997) Bacteriophage infection and multiplication occur in *Pseudomonas aeruginosa* starved for 5 years. Can J Microbiol 43:1157–1163

Seeley ND, Primrose SB (1980) The effect of temperature on the ecology of aquatic bacteriophages. J Gen Virol 46:87–95

Selbitschka W, Jording W, Nieman S, Schmidt R, Pühler A, Mendum T, Hirsch P (1995) Construction and characterization of a *Rhizobium leguminosarum* biovar *viciae* strain designed to assess horizontal gene transfer in the environment. FEMS Microbiol Lett 128:255–263

Singleton P, Anson AE (1983) Effect of pH on conjugal transfer at low temperatures. Appl Environ Microbiol 46:291–292

Skujins J (1984) Microbial ecology of desert soils. In: Marshall KC (ed) Advances in microbial ecology. Plenum, New York, pp 49–91

Smets BF, Rittmann BE, Stahl DA (1993) The specific growth rate of *Pseudomonas putida* PAW1 influences the conjugal transfer rate of the TOL plasmid. Appl Environ Microbiol 59:3430–3437

Smit E, Elsas JD van, Veen JA van, deVos WM (1991) Detection of plasmid transfer from *Pseudomonas fluorescens* to indigenous bacteria in soil by using bacteriophage ϕR2f for donor counterselection. Appl Environ Microbiol 57:3482–3488

Smit E, Wolters A, Elsas JD van (1998) Self-transmissible mercury resistance plasmids with gene-mobilizing capacity in soil bacterial populations: influence of wheat roots and mercury addition. Appl Environ Microbiol 64:1210–1219

Smith JM, Smith NH, O'Rourke M, Spratt BG (1993) How clonal are bacteria? Proc Natl Acad Sci USA 90:4384–4388

Stewart GJ, Sinigalliano CD (1990) Detection of horizontal gene transfer by natural transformation in native and introduced species of bacteria in marine and synthetic sediments. Appl Environ Microbiol 56:1818–1824

Stewart GJ, Sinigalliano CD, Garko KA (1991) Binding of exogenous DNA to marine sediments and the effect of DNA/sediment binding on natural transformation of *Pseudomonas stutzeri* strain ZoBell in sediment columns. FEMS Microbiol Ecol 85:1–8

Stockwell VO, Kawalek MD, Moore LW, Loper JE (1996) Transfer of pAgK84 from the biocontrol agent *Agrobacterium radiobacter* K84 to *A. tumefaciens* under field conditions. Phytopathology 86:31–37

Stotzky G (1986) Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. In: Huang PM, Schnitzer M (eds) Interaction of soil minerals with natural organics and microbes. Soil Science Society of America, Madison, Wis., pp 305–328

Stotzky G (1989) Gene transfer among bacteria in soil. In: Levy SB, Miller RV (eds) Gene transfer in the environment. McGraw-Hill, New York, pp 165–222

Stotzky G, Babich H (1986) Survival of, and genetic transfer by, genetically engineered bacteria in natural environments. Adv Appl Microbiol 31:93–138

Stotzky G, Schiffenbauer M, Lipson SM, Yu BH (1981) Surface interactions between viruses and clay minerals and microbes: mechanisms and implications. In: Goddard M, Butler M (eds) Viruses and wastewater treatment. Pergamon, Oxford, pp 199–204

Strätz M, Mau M, Timmis KN (1996) System to study horizontal gene exchange among microorganisms without cultivation of recipients. Mol Microbiol 22:207–215

Sudarshana P, Knudsen GR (1995) Effect of parental growth on dynamics of conjugative plasmid transfer in the pea spermosphere. Appl Environ Microbiol 61:3136–3141

Sullivan JT, Patrick HN, Lowther WL, Scott DB, Ronson CW (1995) Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. Proc Natl Acad Sci USA 92:8985–8989

Sullivan JT, Eardly BD, Berkum P van, Ronson CW (1996) Four unnamed species of nonsymbiotic rhizobia isolated from the rhizosphere of *Lotus corniculatus*. Appl Environ Microbiol 62:2818–2825

Sullivan JT, Ronson CW (1998) Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a *phe*-tRNA gene. Proc Natl Acad Sci USA 95:5145–5149

Thomas CM (ed) (1989) Promiscuous plasmids of Gram-negative bacteria. Academic Press, Harcourt Brace Jovanovich, London

Top E, De Smet I, Verstraete W, Dijkmanns R, Mergeay M (1994) Exogenous isolation of mobilizing plasmids from polluted soils and sludges. Appl Environ Microbiol 60:831–839

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 samples. Appl Environ Microbiol 56:2471–2479

Troxler J, Azelvandre P, Zala M, Defago G, Haas D (1997) Conjugative transfer of chromosomal genes between fluorescent pseudomonads in the rhizosphere of wheat. Appl Environ Microbiol 63:213–219

Turk V, Rehnstam A-S, Lundberg E, Hagström A (1992) Release of bacterial DNA by marine nanoflagellates, an intermediate step in phosphorus regeneration. Appl Environ Microbiol 58:3744–3750

Weinberg SR, Stotzky G (1972) Conjugation and genetic recombination of *Escherichia coli* in soil. Soil Biol Biochem 4:171–180

Weisberg RA (1987) Specialized transduction. In: Ingraham JL, Low KB, Majasanik B, Schaechter M, Umbarga HE, Neidhardt FC (eds) *Escherichia coli* and *Salmonella typhimurium* cellular and molecular biology. American Society for Microbiology, Washington, DC, pp 1169–1176

Wellington EMH, Herron PR, Cresswell N (1993) Gene transfer in terrestrial environments and the survival of bacterial inoculants in soil. In: Edwards C (ed) Monitoring genetically manipulated microorganisms in the environment. Wiley, Chichester, pp 137–169

Wiggins BA, Alexander M (1985) Minimum bacterial density for bacteriophage replication: implications for significance of bacteriophages in natural ecosystems. Appl Environ Microbiol 49:19–23

Williams HG, Day MJ, Fry JC, Stewart GJ (1996) Natural transformation in river epilithon. Appl Environ Microbiol 62:2994–2998

Wommack KE, Hill RT, Kessel M, Russek-Cohen E, Colwell RR (1992) Distribution of viruses in the Chesapeake bay. Appl Environ Microbiol 58:2965–2970

Yin X, Zeph LR, Stotzky G (1997) A simple method for enumerating bacteriophages in soil. Can J Microbiol 43:461–466

Zatyka M, Thomas CM (1998) Control of genes for conjugative transfer of plasmids and other mobile elements. FEMS Microbiol Rev 21:291–319

Zeph LR, Onaga MA, Stotzky G (1988) Transduction of *Escherichia coli* by bacteriophage P1 in soil. Appl Environ Microbiol 54:1731–1737

Zinder ND, Lederberg J (1952) Gene exchange in *Salmonella*. J Bacteriol 64:679–699