ELSEVIER

Soil Biology & Biochemistry 39 (2007) 2977–2991

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Review Article

Cycling of extracellular DNA in the soil environment

David J. Levy-Booth[a], Rachel G. Campbell[b], Robert H. Gulden[b], Miranda M. Hart[a], Jeff R. Powell[c], John N. Klironomos[c], K. Peter Pauls[b], Clarence J. Swanton[b], Jack T. Trevors[a,*], Kari E. Dunfield[d,**]

[a]*Department of Environmental Biology, University of Guelph, Guelph, ON, Canada N1G 2W1*
[b]*Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada N1G 2W1*
[c]*Department of Integrative Biology, University of Guelph, Guelph, ON, Canada N1G 2W1*
[d]*Department of Land Resource Science, University of Guelph, Guelph, ON, Canada N1G 2W1*

Received 13 September 2006; received in revised form 14 May 2007; accepted 18 June 2007
Available online 26 July 2007

Abstract

Upon entering the soil environment, extracellular DNA is subjected to dynamic biological, physical, and chemical factors that determine its fate. This review concerns the fate of both recombinant and non-recombinant sources of DNA. A schematic of DNA cycling coupled with genetic transformation is presented to understand its behavior in soil. Extracellular DNA may persist through cation bridging onto soil minerals and humic substances, be enzymatically degraded and restricted by DNases of microbial origin, and/or enter the microbial DNA cycle through natural transformation of competent bacteria. Lateral gene transfer may disseminate DNA through the microbial community. An understanding of DNA cycling is fundamental to elucidating the fate of extracellular DNA in the soil environment.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Agriculture; Bacteria; Cycle; Degradation; DNA; Environment; Extracellular; Gene transfer; Microorganisms; Natural transformation; Persistence; Recombinant; Soil; Transgenic plants

1. Introduction

Deoxyribonucleic acid (DNA) is ubiquitous in the environment (Trevors, 1996), and can account for over 10% of extractable P in soil (Baker, 1977; Reanney et al., 1982; Turner et al., 2004; Turner and Newman, 2005). The fate of DNA in the soil environment is determined by the biological and physical properties of both DNA and soil. Although researchers have studied the individual components of DNA cycling in soil including the entry, persistence, degradation and metabolism, and genetic transformation (Table 1), there has been little attempt to develop a multidisciplinary, systematic synthesis of such studies. This review will provide an integrated analysis of these processes, and demonstrate that the fate of DNA in soil is a cyclical process that is interconnected with other nutrient cycles (Fig. 1).

DNA is found in the cells of all living organisms, and in some viruses. Extracellular, or naked DNA exists outside of the cell and is not protected by a cytoplasmic membrane. Once released into the environment, extracellular DNA may: (i) persist by binding to soil minerals and humic substances (Greaves and Wilson, 1969; Lorenz and Wackernagel, 1987; Crecchio and Stotzky, 1998), (ii) be degraded by microbial DNases and used as a nutrient for plant and microbial growth (Bowman and Cole, 1978; Romanowski et al., 1991; Paget et al., 1992; Redfield, 1993; Finkel and Kolter, 2001; Macfadyen et al., 2001; Ceccherini et al., 2003), and/or (iii) be incorporated into a bacterial genome as a possible source of genetic

*Corresponding author. Tel.: +1 519 824 4120x53367; fax: +1 519 837 0442.
**Also for correspondence. Tel.: +1 519 824 4120x58088; fax: +1 519 824 5730.
E-mail addresses: jtrevors@uoguelph.ca (J.T. Trevors), dunfield@uoguelph.ca (K.E. Dunfield).

0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2007.06.020

Table 1
Articles published after 1995 describing detection, persistence, decomposition, and transfer of recombinant DNA in soil

	References
Detection	
Sensitive detection of transgenic plant marker genes in a soil microcosm	Widmer et al. (1996)
Quantification of transgenic plant marker genes in the field	Widmer et al. (1997)
Quantification bias in DNA extraction directly from soil	Frostegård et al. (1999)
Extraction of DNA from soil	Robe et al. (2003)
Quantification of RR corn and soybean transgenes in soil	Lerat et al. (2005)
Persistance	
Persistence of *Pseudomonas aureofaciens* recombinant DNA in soil	England et al. (1997)
The fate of recombinant plant DNA in soil	Paget et al. (1998)
Long-term persistence of transgenic sugar beet DNA in the field	Gebhard and Smalla. (1999)
Protection against nuclease degradation of clay-bound DNA	Demanèche et al. (2001b)
Quantification of RR corn and soybean transgenes in leachate water	Gulden et al. (2005)
Decomposition	
Mechanism of retarded DNA degradation in nonsterile soil	Blum et al. (1997)
Degradation of DNA from transgenic tobacco leaves in soil	Ceccherini et al. (2003)
Kinetics of transgenic tomato DNA decomposition in soil microcosm	Poté et al. (2005)
Transfer	
Transformation of *Acinetobacter* sp. by transgenic sugar beet DNA	Gebhard and Smalla. (1998
HGT from transgenic sugar beet to soil bacteria under field conditions	Gebhard and Smalla. (1999
Transformation of soil bacteria by transgenic plant DNA	de Vries et al. (2001)
Transformability of DNA from transgenic tobacco leaves in soil	Ceccherini et al. (2003)
Spread of recombinant DNA from roots and pollen of transgenic potato plants.	de Vries et al. (2003)

Articles published prior to 1995 have been reviewed in Trevors (1996).

instructions (Khanna and Stotzky, 1992; Lorenz and Wackernagel, 1994; Gebhard and Smalla, 1998, 1999; Dubnau, 1999; de Vries et al., 2001; de Vries and Wackernagel, 2004). DNA makes up a large portion of the soil P pool. While DNA components may persist in soil, the genetic information provided by DNA is lost after DNA sequence restriction. Upon complete DNA degradation in soil, elemental components (e.g., C, N, O, and P) may be utilized for *de novo* DNA synthesis, or may leave the DNA cycle and enter their respective nutrient cycles. Nucleic acids (DNA and RNA) can account for 10% of humic-bound organic phosphates in mineral soils (Baker, 1977); extracellular and cell-bound DNA can account for up to 9–13% and 53% of extracted P in tundra (Turner et al., 2004) and natural wetland soil (Turner and Newman, 2005), respectively; and total DNA extracted from various soils amounts to 50–207 $\mu g\, g^{-1}$ (Reanney et al., 1982). Once released into soil DNA has several interconnected fates.

The use of transgenic plants (James, 2006) releases recombinant DNA into the environment, resulting in a recombinant DNA pool within the total extracellular DNA pool in soil (Gebhard and Smalla, 1999; Ceccherini et al., 2003; de Vries and Wackernagel, 2004; Gulden et al., 2005). The use of transgenic cropping systems has provided the impetus for the development of the molecular tools necessary to study the DNA cycle in soil (Paget and Simonet, 1994). As a result, novel molecular tools such as the *nptII* marker recovery system (de Vries and Wackernagel, 1998; de Vries et al., 2001, 2003; Meier and Wackernagel, 2003) and high-throughput soil DNA extraction and quantitative real-time PCR protocols (Lerat et al., 2005; Gulden et al., 2005) have been developed to increase the understanding of the fate of recombinant DNA in soil. The motivation for this review came from a consideration of the fate of extracellular recombinant DNA released from transgenic plants. However, the knowledge can be applied to the persistence and cycling of all DNA in soil and is not limited to recombinant DNA. The DNA cycle begins with the entry and potential persistence of DNA in soil (Table 1).

2. The entry of DNA into soil

Entry of DNA into soil is common to both DNA persistence and degradation and genetic transformation. Because of the use of transgenic crops (James, 2006; Snow et al., 2005), the unique nucleotide sequences of recombinant DNA, the knowledge of the time of introduction of these plants, and the ability to track recombinant DNA in soil (Lerat et al., 2005), plant recombinant DNA behavior can provide a framework for describing DNA cycling in soil. Plant DNA enters the soil continuously, predominantly from the sloughing off of root cap cells (Hawes, 1990; de Vries et al., 2003), as a result of pathogen colonization of below-ground biomass (Polverari et al., 2000; Kay et al., 2002), through pollen dispersal (Uribelarrea et al., 2002; de Vries et al., 2003), and during crop residue decomposition (Widmer et al., 1997; Ceccherini et al., 2003). The importance of the different mechanisms of DNA entry will differ among plant species, such as those with different life cycles and architecture. This review primarily considers annual crop plants with a single pollination event.

Plant DNA enters the rhizosphere following programmed cell death (PCD) of root cap cells that have been sloughed off during root growth (Wang et al., 1996; Greenberg, 1996; del Pozo and Lam, 1998). During early corn (*Zea maize* L.) root elongation, up to 10^3 cells per primary root are sloughed per day, depending on the density of the soil (Iijima et al., 2000). Corn root growth during this period may release up to 10^4 copies of template DNA ml^{-1} of leachate water per plant (Gulden et al. 2005). The majority of DNA in sloughed root cells is cleaved into

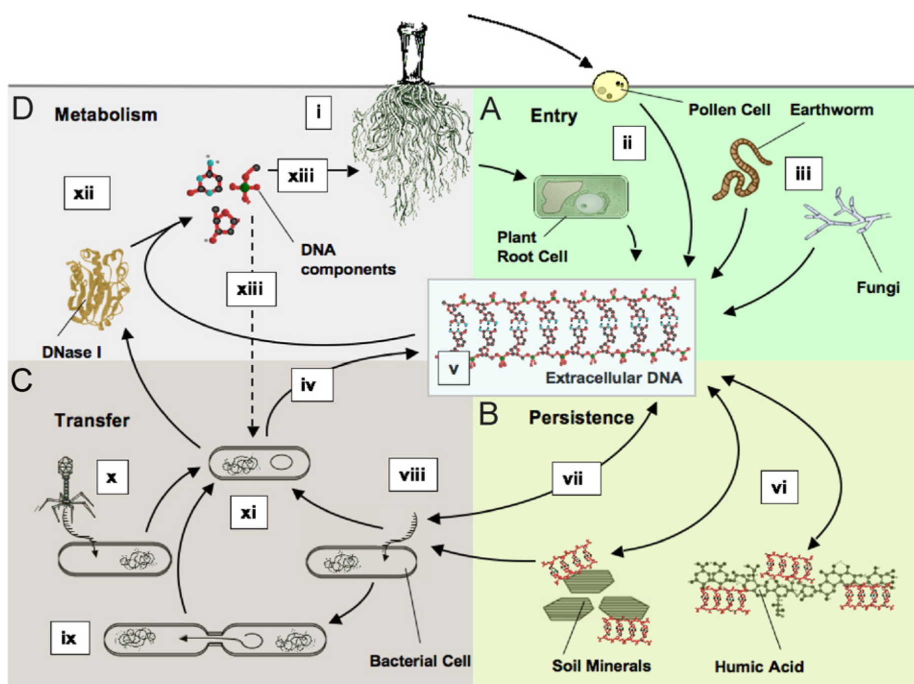

Fig. 1. The DNA cycle in soil. (A) Entry of DNA into Soil: Plant DNA is replicated during growth and cell division. During the later stages of plant development DNA degradation occurs by plant DNases *in planta* (i). Plant DNA enters soil through pollen release, cell lysis and the addition of plant materials to soils (ii). Soil fauna and fungi also release DNA into the soil environment (iii), as does bacterial lysis (iv). (B) Persistence of DNA in Soil: Following cell lysis, extracellular DNA persists in soil (v) where it can be protected by binding to soil humic acids (vi), clay minerals and sand particles in soil (vi), processes that are facilitated by cations (e.g., Ca^{2+} and Mg^{2+}) and/or low pH. *Note*: bacterial transformation by the loosely held portions of bound DNA may still occur. (C) Transfer of DNA in Soil: Competent bacteria can integrate extracellular DNA into their genome (transformation) (viii). Bacterial DNA can be transferred to recipient bacteria through a conjugation bridge (conjugation) (ix). Bacteriophages insert DNA from a donor cell directly into a recipient bacterial cell (transduction) (x). Bacterial growth and cell division replicates (amplifies) integrated DNA (xi). (D) Degradation of DNA in Soil: Unbound DNA is restricted and digested by extracellular DNases of microbial origin (xii), which are ubiquitous in the soil environment and provide oligonucleotides and nutrients that are used in metabolism by microorganisms and plants (xiii).

small fragments by endonucleases before it reaches the rhizosphere (Wang et al., 1996).

Plant DNA may enter the rhizosphere during root colonization by pathogens (Polverari et al., 2000; Kay et al., 2002). For example, plant DNA was released following *Xanthomonas campestris* pv. *vesicatoria* colonization of pepper (*Capsicum annuum* L.) plants (Polverari et al., 2000). Unlike cells undergoing PCD, nuclear DNA was not fragmented during pathogen colonization, even when pathogen colonization induced advanced stages of hypersensitive cell death (Polverari et al., 2000). The release of intact DNA into the rhizosphere during pathogen colonization may be important because of the potential of colonizing bacteria to be transformed (Bertolla et al., 1999; Kay et al., 2002).

The role of pollen in the entry of DNA into soil depends on the species and life cycle stage of the plant (McPartlan and Dale, 1994; Saeglitz et al., 2000; Uribelarrea et al., 2002; Meier and Wackernagel, 2003). However, pollen dispersal can account for a noteworthy fraction of DNA addition to soil. A (2*n*) sugar beet (*Beta vulgaris* L.) pollen grain contains 13.4 pg DNA (Turbin et al., 1965), which can enter the soil following microbial degradation of the pollen wall. For example, within a sugar beet crop the mean volume of air-borne pollen was 4208 pollen grains m^{-3} (Scott, 1970). Therefore, sugar beet pollen could potentially add about 5.6×10^4 pg DNA m^{-3}, or about 3.6×10^4 complete (2*n*) sugar beet genomes m^{-3} (Arumuganathan and Earle, 1991), into the surrounding soil. The method of pollination for each plant will determine the amount of DNA that enters the soil during pollen dispersal and the distance the DNA travels. The movement of DNA from self-pollinating crops such as soybean is likely negligible, but wind-transported pollen from species such as potato (*Solanum tuberosum* L.), sugar beet, and canola (*Brassica napus* L.), have been found at distances of about 10 m (McPartlan and Dale, 1994), 200 m (Saeglitz et al., 2000), and 1.5 km (Timmons et al., 1995), respectively. The spread of DNA to soil via pollen is temporally limited, i.e., it occurs solely during anthesis.

Crop residue and leaf litter decomposition are temporally limited pathways of DNA entry in soil. DNA is largely degraded *in planta* within 5 days of fresh plant tissue addition to soil at 21.5 °C (Poté et al., 2005). The decomposition of plant tissues by microbial enzymes facilitates the release of undegraded DNA into the rhizosphere (Widmer et al., 1997; Paget et al., 1998; Gebhard and Smalla, 1999; Dilly et al., 2001; Ceccherini et al., 2003), which is accessible to decomposing microorganisms (Ceccherini et al., 2003). In general, DNA release during

plant residue decomposition is poorly characterized. In temperate climates and agricultural systems, the entry of crop residue DNA into soil is believed to follow seasonal oscillations following patterns of plant growth and senescence, while in tropical systems the entry of DNA in soil may be continuous.

Microorganisms also contribute to the soil DNA pool. Soil bacteria such as *Acinetobacter* spp. and *B. subtilis* release DNA into their environment through secretion of both plasmid and chromosomal DNA (Lorenz et al., 1991; Hamilton et al., 2005; Backert and Meyer, 2006), infection by bacteriophages (Redfield, 1988), and cell lysis (Lorenz et al., 1991; Steinmoen et al., 2002). Release of DNA from living bacterial cells occurs under periods of nutritional stress in pure cultures (Lorenz et al., 1991). For example, *Bacillus subtilis* cultured on minimal growth medium, $0.1\,\mu g\,DNA\,ml^{-1}$, releases DNA during the lag phase of growth (Lorenz et al., 1991). Active secretion of DNA from bacteria has not been demonstrated in soil, but may occur due to nutrient limitations in the soil environment. Bacterial cell lysis is primarily responsible for the presence of the microbial DNA pool in soil and is recognized to aid in the survival of closely related strains under nutrient limited conditions (Recorbet et al., 1993; England et al., 1995, 1997). In the decline phase of bacterial growth, lysing *B. subtilis* cells released $2.8\,\mu g\,DNA\,ml^{-1}$ in minimal medium (Lorenz et al., 1991). The end result of DNA entry into the soil is a pool of extracellular DNA in the interstitial soil solution. Regardless of the source of extracellular DNA in soil, its fate is either persistence, degradation for use in DNA cycling, or entry into the lateral gene transfer network via natural transformation.

3. Persistence of extracellular DNA in soil

The persistence of extracellular DNA is influenced by the soil environment, i.e. chemical, physical and biological properties of the soil. DNA persists in soil by adsorbing to soil minerals (sand and clay), humic substances, and organomineral complexes. The adsorption of DNA to mineral and humic substances protects it from extracellular, microbial DNases and nucleases, which degrade unbound DNA in the soil solution (Greaves and Wilson, 1969; Lorenz and Wackernagel, 1987; Khanna and Stotzky, 1992; Paget et al., 1992; Blum et al., 1997; Paget et al., 1998; Crecchio and Stotzky, 1998; Demanèche et al., 2001a, b). In a loamy sand and a sandy loam soil, DNA adsorption reached a maximum 1 h after DNA entry (Blum et al., 1997). Up to 80% of the 300 ng of ^{3}H-labelled DNA introduced these soils was adsorbed, depending on binding site availability (Blum et al., 1997). Binding sites of the soil matrix can promote DNA persistence in two ways, (i) by protecting bound DNA from nuclease mediated enzymatic hydrolysation (Crecchio and Stotzky, 1998) and (ii) by adsorbing DNases and nucleases, thereby reducing the potential for enzymatic DNA restriction (Tebbe and Vahjen, 1993; Blum et al., 1997). Binding inhibits the ability of extracellular DNases to hydrolyze extracellular DNA through partial deactivation and physical separation of the enzymes from their substrate (Khanna and Stotzky, 1992; Demanèche et al., 2001b). However, if DNases saturate binding sites of clay particles adjacent to adsorbed DNA, then portions of the DNA may also be degraded (Demanèche et al., 2001b). Therefore, DNA adsorption to the soil matrix is primarily influenced by soil characteristics such as: concentration of humic substances, soil mineralogy, cation concentration, and soil pH.

Humic substances, including humins, humic acids (HA) and fulvic acids, are the products of enzymatic degradation of numerous molecules including lipids, amino acids, peptides and polysaccharides (Schnitzer, 1982; Stevenson, 1994). The aromatic portions of humic substances are thought to arise primarily from degradation of celluolignic substances (Stevenson, 1994). They are resistant to microbial degradation and are characterized by a high degree of spatial heterogeneity (Stevenson, 1994), and the ability to bind DNA through cation bridging (Crecchio and Stotzky, 1998). Crecchio and Stotzky (1998) showed that 2 mg HA, which are characterized operationally by their insolubility in acids and organic solvents, bound about $30\,\mu g$ of *B. subtilis* DNA, whereas 4 mg HA bound about $40\,\mu g$. Binding sites of humic substances are formed when phenolic hydroxyl groups within these compounds are ionized. Acidic functional groups of humic substances arise mainly from carboxylic acid, and are binding sites (Stevenson, 1994).

The number of binding sites and the binding mechanism play a role in determining a soil particle's DNA binding capacity. For example, sand binds DNA in lesser amounts than clay minerals primarily due to differences in surface area. Montmorillonite and kaolinite clays have specific surface areas of $812\,m^{2}\,g^{-1}$ (Petersen et al., 1996) and $10.97\,m^{2}\,g^{-1}$ (Slater et al., 2006), respectively, whereas Ottawa grade sand has a surface area of $0.0485\,m^{2}\,g^{-1}$ (Slater et al., 2006). As a result, the capacity of sand to bind DNA is at least three orders of magnitude less than that of clay (Lorenz and Wackernagel, 1987, 1992; Romanowski et al., 1991; Khanna and Stotzky, 1992; Paget et al., 1992, Blum et al., 1997). Silt, an intermediate class of soil particle, theoretically has a DNA binding capacity between that of sand and clay, but has not been characterized. Due to the high number of binding sites per unit volume, the primary mineralogical characteristic that determines the DNA binding capacity of soil is clay content. For example, following the application of $20\,\mu g$ of DNA into a sandy loam soil (6.2% clay) adsorbed 40% more DNA than a sandy soil (0.1% clay) (Blum et al., 1997).

Not all clay minerals have equal DNA binding capacity. Montmorillonite (2:1 (Si:Al) expanding clay mineral) has about 67 times the specific surface area and about 23 times greater cation exchange capacity than kaolinite (1:1 (Si:Al) non-expanding clay mineral) (Poly et al., 2000), although the surface charge density of kaolinite is about 2.5 times

greater than montmorillonite (Pietramellara et al., 2001). Purified montmorillonite may adsorb more than its own weight in DNA (Greaves and Wilson, 1969). Researchers have found that montmorillonite adsorbed a higher amount of DNA than kaolinite (Khanna and Stotzky, 1992; Pietramellara et al., 2001). A maximum of 59.4% of 50 μg chromosomal *B. subtilis* DNA added to montmorillonite was adsorbed, versus 31.4% in kaolinite (Pietramellara et al., 2001). This trend was reversed at low divalent cation concentrations (Poly et al., 2000), potentially due to the greater surface charge density of kaolinite (Pietramellara et al., 2001). Complexes of montmorillonite clay, HA and Al or Fe are believed to be the primary forms of organization of the soil matrix, and organomineral-DNA binding was proposed to be the most important mechanism of DNA persistence in soil (Crecchio et al., 2005).

The adsorption of DNA to sand is enhanced by cation concentrations (Lorenz and Wackernagel, 1987; Romanowski et al., 1991; Lorenz and Wackernagel, 1992; Paget et al., 1992). For example, the Mg^{2+} concentration needed for half-maximum adsorption of DNA in 0.7 g of sand was 0.5 mM (Romanowski et al., 1991), where saturation of sand occurred at Mg^{2+} concentrations above 50 mM (Lorenz and Wackernagel, 1987). It is notable that divalent cation concentrations of 0.4–0.5 mM are present in most natural soil environments (Ogram et al., 1988; Romanowski et al., 1991). Divalent cations (e.g., Mg^{2+} and Ca^{2+}) are more effective at forming bridges than monovalent cations (e.g., K^+ and Na^+) (Lorenz and Wackernagel, 1987; Romanowski et al., 1991). At least 70–100 times greater concentrations of monovalent cations than divalent cations were required to bind the same amount of plasmid DNA to sand (Romanowski et al., 1991).

The dominant mechanism of DNA adsorption to the soil matrix differs depending on soil pH. Both soil constituents and DNA have a net negative charge above pH 5 [isoelectric point of DNA (Theng, 1979)], and thus require cations to mediate adsorption (Fig. 2a) (Greaves and Wilson, 1969, 1970). Below pH 5, the phosphate moieties of DNA become protonated, resulting in a net positive charge and DNA adsorption may occur without the need for cation bridging (Fig. 2b) (Greaves and Wilson, 1969). At soil pH greater than 5, DNA has a net negative charge in the soil solution and thus, the concentration and valence charge of cations effect DNA persistence. Cations are thought to bridge the negatively charged phosphate moieties of DNA molecules and the negatively charged moieties of soil particles, including clay minerals (Fig. 2a) and HA. Differences in the location of DNA adsorption to montmorillonite clay have also been found to be pH dependent. Below pH 5 adsorption of DNA was greater on internal clay surfaces; above pH 5 DNA was confined to the external surfaces where exchangeable cations were located (Greaves and Wilson, 1969). This is believed to occur by protonation of hydroxyl groups on clay external surfaces at low pH values (Theng, 1979).

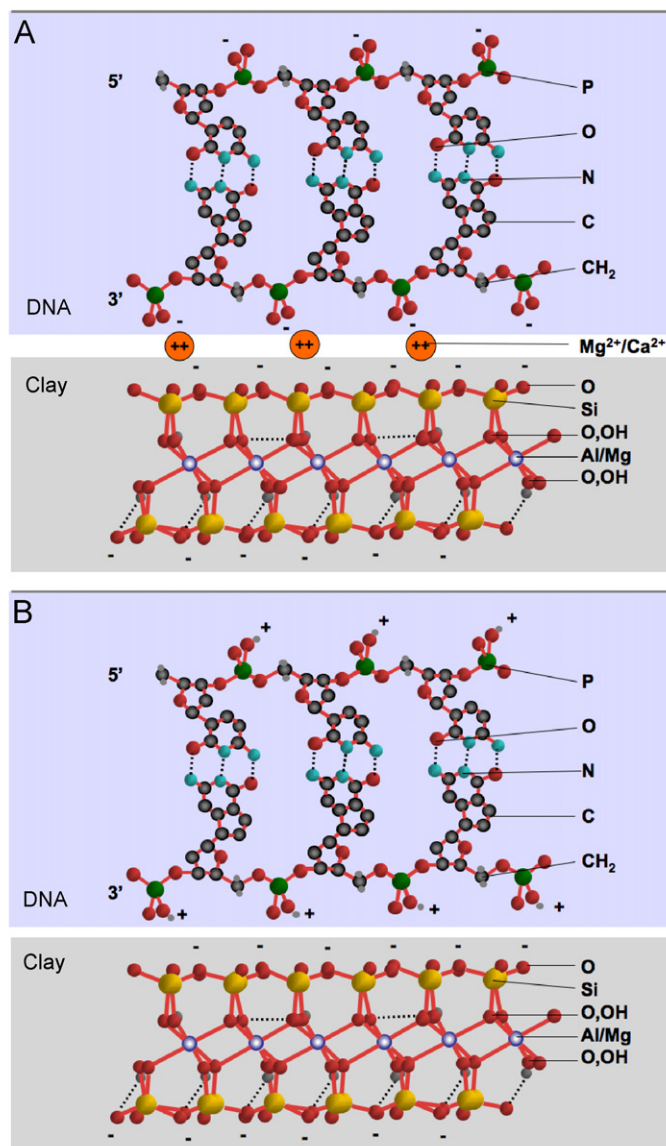

Fig. 2. The mechanism of adsorption of extracellular DNA to external clay mineral (montmorillonite) surface moieties depends on pH. (A) At pH >5, adsorption is believed to be facilitated by cation bridging. (B) At pH<5, adsorption is believed to occur without mediation by cations, as DNA is positively charge below its isolelectric point (pH 5).

Regardless of the net charge of DNA in the soil solution, pH influences the DNA binding capacity of soil (Lorenz and Wackernagel, 1987; Romanowski et al., 1991; Crecchio and Stotzky, 1998). About 2 μg more DNA bound per mg HA at pH 3 than at pH 4 (Crecchio and Stotzy, 1998). In 0.5 mM of Mg^{2+}, the maximum percent of DNA adsorbed to sand particles (about 79%) occurred at a pH 5, and declined linearly until pH 9, where about 5% of DNA was adsorbed (Romanowski et al., 1991). These data suggest the greater negative charge of DNA and the soil matrix with increasing pH reduces efficiency of cations to mediate adsorption, due to increased electrostatic repulsion between DNA and the soil matrix. Lorenz and Wackernagel (1987) observed that DNA bound to sand was

greatest at pH 9 in 0.2 M of Mg^{2+}. Taken together, the DNA binding capacity of soil is influenced by an interaction between soil texture, cation concentration and soil pH. The understanding of DNA adsorption processes would benefit from additional studies of these interactions.

DNA persistence and the rate of DNA degradation in soil have been elucidated primarily in microcosm studies in controlled environments. But natural soils are complex and heterogeneous. Confirmation that the factors and their interactions that affect DNA persistence in microcosm studies apply to natural soils is vital to the understanding of DNA persistence in soil. Variability in soil constituents and microenvironments in field soils causes the persistence of DNA to be difficult to predict (Romanowski et al., 1991, 1992; Paget et al., 1992; Recorbet et al., 1993). Therefore it is understandable that studies monitoring recombinant DNA persistence in field soil have ranged from 77 days (Widmer et al., 1997) to at least 2 years (Gebhard and Smalla, 1999) (Table 2). The environmental fate of recombinant DNA is influenced by both persistence and the rate of DNA degradation in soil (Lorenz and Wackernagel, 1994; Wackernagel, 1996; Meier and Wackernagel, 2003).

4. The DNA cycle in soil

DNA persistence and degradation are the major processes that completes the DNA cycle in soil. Degradation of DNA can be both intra- or extracellular. After uptake, living cells and can synthesize DNA via two main pathways: DNA salvage and *de novo* synthesis (Fig. 3). Due to the energy requirements of *de novo* nucleotide synthesis (Henderson and Paterson, 1973; Stouthamer, 1979), we suggest that the DNA salvage pathway, the synthesis of DNA from partial DNA breakdown products, plays a fundamental role in the soil environment (Kornberg, 1974; Redfield, 1993).

4.1. DNA degradation in soil

DNA degradation begins with the enzymatic restriction of high molecular weight, double-stranded DNA (Greaves and Wilson, 1970; Blum et al., 1997). Briefly, DNA is cleaved into smaller duplex DNA fragments by microbial restriction endonuclease I in soil (Blum et al., 1997; Demanèche, et al., 2001b) to about 400 bp oligonucleotides. Studies in pure culture have shown complete restriction to about 7 bp sequences (Mishra, 2002), and hydrolysis to single nucleotides by DNases (Kornberg, 1974), however no studies have shown this process occurs in soil. An important result of DNA restriction is the loss of genetic information.

The primary mechanism for extracellular DNA degradation in soil is believed to be bacterial DNases (Blum et al., 1997). Soil bacteria actively secrete nucleases into the soil to increase the rate at which the nutrients in extracellular soil DNA become accessible. The stimulation of microbial growth and production of DNase in soil occurs rapidly following DNA entry. Blum et al. (1997) found that the number of viable microorganisms increased one order of magnitude 12 h after DNA entry to loamy soil and, during this time, about 68% of the 50 μg DNA added to loamy soil was degraded. Data, based on target DNA sequence persistence also indicate that oligonucleotide restriction is rapid in soil (Blum et al., 1997; Ceccherini et al., 2003; Herdina et al., 2004; Poté et al., 2005). For example,

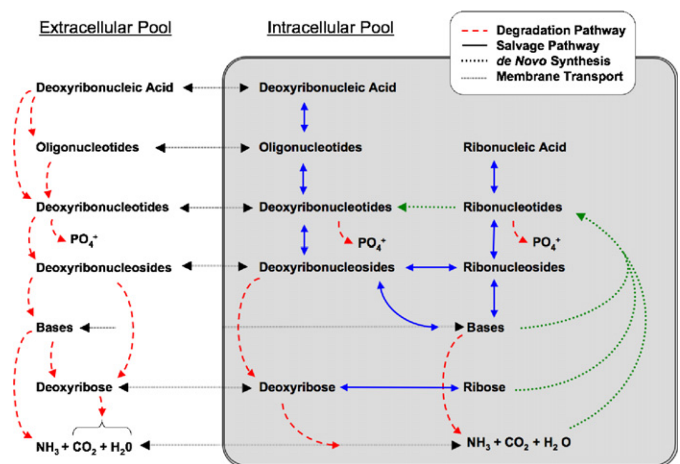

Fig. 3. In the DNA metabolism cycle in soil extracellular DNA restriction and degradation by microbial DNases is linked to the uptake of DNA and DNA components, which are metabolized in the nucleotide salvage and *de novo* synthesis pathways. Intermediates in these pathways may also be passively or actively secreted into the soil environment, completing the cycle.

Table 2
Persistence of recombinant DNA in field soils

Species	Gene	Conditions	Duration	References
Corn (*Zea maize* L.)	*cryIA(b)*	4200 kg biomass composted with manure	7 days	Guan et al. (2005)
Tobacco (*Nicotiana tabacum* L.)	*nptII*	10 g biomass buried at 10 cm in silt loam soil	77 days	Widmer et al. (1997)
Potato (*Solanum tuberosum* L.)	*nptII*	2–5 g biomass in fine sandy loam soil surface	137 days	Widmer et al. (1997)
Tobacco	*aacCl*	Whole plants in situ from sandy clay-loam soil	1 year	Paget et al. (1998)
Poplar (*Populus* sp.)	*nptII*	5 g buried at 5–10 cm in sandy loam soil	1 year	Hay et al. (2002)
Potato	*nptII*	Pollen	1 year	de Vries et al. (2003)
Sugar Beet (*Beta vulgaris L.*)	*nptII*	Whole plants shredded and mixed into silt loam soil	2 years	Gebhard and Smalla. (1999)

85.8 pg of extracellular fungal DNA from *Gaeumannomyces graminis* var. *tritici* was 92% degraded after 4 days in soil (Herdina et al., 2004).

DNA sequence degradation is influenced by DNase concentration. Oligonucleotides may be degraded to mononucleotides by DNases, which catalyze the hydrolytic cleavage of phosphodiester linkages in the DNA backbone (Kornberg, 1974; Mishra, 2002). Unbound DNA in the soil solution is completely degraded by DNase I concentrations above 1 $\mu g\,ml^{-1}$, while clay-bound DNA was recoverable at DNase I concentrations of 10 $\mu g\,ml^{-1}$ (Demanèche et al., 2001b). DNA can bind weakly to soil particles such as sand (Greaves and Wilson, 1969), which allows extracellular-ends of the compound to be degraded by adjacent DNases. Romanowski et al. (1991) found that below DNase I concentrations of 10000 $\mu g\,ml^{-1}$ almost all DNA was recovered from sand. Soil particles protect DNA from DNase I concentrations that would otherwise lead to its degradation, however above a threshold concentration DNases may saturate soil particle binding sites adjacent to bound DNA (Demanèche et al., 2001b).

The kinetics of DNA sequence degradation are affected by temperature. Gulden et al. (2005) determined that the half-life of extracellular DNA target sequences in soil leachate water (soil solution that has exited the system through downward percolation) decreased with an increase in temperature and suggested that sequence degradation was an enzymatic process based on the reaction rates. Furthermore, DNA degradation slows considerably in frozen soils. Although some researchers consider the process of decomposition to cease at 0 °C, Henriksen and Breland (1999) found appreciable microbial activity at 0 °C and below, and Diaz-Raviña et al. (1994) found that the minimum temperature of microbial DNA degradation correlated with the lowest temperature that bacteria were able to extract thymidine from frozen soil (−8.4 °C). Under temperatures of 0 °C most DNA undergoes slow turnover rates, but in microniches of unfrozen water may be degraded by metabolically active microorganisms. Although enzymatic degradation of DNA is believed to decline or cease in frozen or desiccated soils, processes such as chemical hydrolysis, chemical oxidation, and DNA cross-linking will degrade DNA (Hofreiter et al., 2001). Willerslev et al. (2004) reported that interstrand crosslinks would prevent DNA amplification by PCR after about 400,000 years. The theoretical long-term persistence of DNA in soil is supported by the discovery of intact ancient DNA molecules, such as 13,775-year-old-mammoth DNA in permafrost soil (Greenwood et al., 1999). The G+C content may affect DNA degradation kinetics in frozen soil. Cytosine is particularly sensitive to hydrolytic deamination (Shapiro, 1981) and DNA from high G+C Gram-positive Actinobacteria has been shown to persist longer in frozen soil than DNA from low G+C Gram-positive Clostridiaceae (Hofreiter et al., 2001). This could have consequences for genetic studies of microbial diversity in frozen and tundra soils.

The deoxyribonucleotides resulting from enzymatic DNA restriction are further broken down to moieties and elemental constituents. This portion of the DNA metabolism cycle is a parallel cycle that can occur intracellularly or in the soil solution, and is not well understood. Nucleotides are central to DNA salvage, as intracellular deoxyribonucleotides are directly incorporated into DNA synthesis. The low solubility coefficient of nucleotides prevents passive transport through cellular membranes (Kornberg, 1974). However, bacteria may actively transport them into their cells in periods of nutritional stress (Dubnau, 1999). Due to the increased solubility of nucleosides, they are more likely to be transported into bacterial cells (Stewart and Carlson, 1986; Lorenz and Wackernagel, 1994). Nucleotides may be hydrolyzed to form nucleosides by catabolic enzymes excreted into the soil matrix by bacterial species such as *Bacillus subtilis* (Jensen, 1978). Nucleosides can be further cleaved into the base (purine or pyrimidine) moiety and deoxyribose. Bases can enter the DNA salvage pathway, or be metabolized to uric acid, which can be degraded by *Bacillus fastidious* (Bongaerts and Vogels, 1976) and other soil bacteria (Imsenecki and Popova, 1971). In potato tuber cells, 47.4% of the carbon from the pyrimidine base uracil was recovered as CO_2 indicated complete metabolism; however, the pyrimidine base cytocine was not degraded (Katahira and Ashihara, 2002). This demonstrates that pools of DNA constituents remain in the cell for *de novo* nucleotide synthesis. The final breakdown products of DNA degradation can be taken up by microorganisms (Finkel and Kolter, 2001; Macfadyen et al., 2001) and plants (Hirose and Ashihara, 1984; Katahira and Ashihara, 2002) and can be re-incorporated into nucleotides, or further degraded into PO_4^{3-}, CO_2, NH_3 (Pearson et al., 1941; Bongaerts and Vogels, 1976) and enter their respective nutrient cycles. Some of these nutrients may be re-assimilated into DNA through *de novo* synthesis (Vogels and van Der Drift, 1976; Finkel and Kolter, 2001; Turner and Newman, 2005). Because *de novo* nucleotide synthesis is inhibited by the presence of DNA precursor moieties (Henderson, 1972), nucleotide salvage, in part, dictates the rate of *de novo* synthesis component of the DNA metabolism cycle.

4.2. Salvage of nucleotides from soil

The nucleotide salvage pathway provides multiple points of closure in the DNA cycle (Fig. 3). The salvage pathway allows partial DNA breakdown products such as nucleotides, nucleosides, ribose, and bases to be re-assimilated into nucleic acids without further degradation of the moieties after entering a living cell. The benefit of these multiple points of closure is illustrated by considering the energy requirements of nucleotide salvage compared to *de novo* nucleotide synthesis. Nucleotide synthesis is metabolically expensive, consuming 70% of the total energy required for DNA synthesis (Ingraham et al., 1983). The synthesis of a single nucleotide from ribose, amino acids,

bases, CO_2, and NH_3 requires the equivalent of at least 60 ATP molecules while the conversion of a salvaged nucleoside to a nucleotide requires only a single ATP (Kornberg, 1974; Stouthamer, 1979). Unlike most enzymatic systems, nucleases do not catalyze reversible reactions, as the condensation of nucleotides into oligonucleotides is catalyzed by a different group of enzymes (i.e., DNA polymerases) (Kornberg, 1974; Mishra, 2002). Further degradation of nucleotides yields molecules that may not be used in *de novo* nucleotide synthesis. While nucleotide salvage has not been observed in soil, it is possible it occurs based on the knowledge gained from laboratory studies (Finkel and Kolter, 2001; Macfadyen et al., 2001; Katahira and Ashihara, 2002; Mishra, 2002) and because the soil environment is nutrient poor, yet contains extracellular DNA.

Ninety percent of nucleosides taken up by potato tuber cells were converted into nucleotides by nucleoside kinases and nucleoside phosphotransferases and incorporated into nucleic acids (Katahira and Ashihara, 2002). However, the majority of the salvaged nucleotides were used to synthesize RNA, possibly due to differences in RNA and DNA requirements in growing potato tubers (Katahira and Ashihara, 2002). Following RNA degradation, ribonucleotides should remain in the intracellular pool, allowing for their conversion to deoxyribonucleotides through the action of ribonucleotide reductases (Kornberg, 1974; Katahira and Ashihara, 2002). For information regarding the role of RNA in nucleotide salvage and *de novo* synthesis, refer to Kornberg (1974), Katahira and Ashihara (2002), and Geigenberger et al. (2005). Nucleotide salvage is a common cellular process observed across taxonomic groups (Stouthamer, 1979; Ingraham et al., 1983; Katahira and Ashihara, 2002; Mishra, 2002) including many bacteria and plants.

5. DNA transformation in soil bacteria

Natural transformation, or genetic transformation, is the second component for completing the extracellular DNA cycle in soil. This process is the only known mechanism by which prokaryotes are able to take up, incorporate, and express extracellular DNA. To date, natural transformation has been shown to occur in about 90 different species of prokaryotes including the soil microorganisms *Bacillus subtilis* and *Acinetobacter* spp. (de Vries and Wackernagel, 2004). Natural transformation is comprised of four main steps: (i) binding of extracellular DNA to the cell surface of competent cells, (ii) uptake through the cell wall and/or membrane, (iii) integration into the bacterial genome, and (iv) expression of the foreign DNA. There are significant barriers to each of these steps that dictate the occurrence and frequency of successful natural transformation. In soil, degradation and adsorption to soil minerals and humic substances limits the availability of extracellular DNA for natural transformation. Growth state and species diversity may also limit transformation of soil bacterial populations. Once extracellular DNA is bound to the bacterial surface, transport into the cell depends on its competence, which is a regulated physiological state that allows uptake of macromolecular DNA (Paget and Simonet, 1994; Lorenz and Wackernagel, 1994; Dubnau, 1999; Thomas and Nielsen, 2005). Integration of foreign DNA into the genome is also influenced by a number of factors including sequence homology between the genomic DNA and the foreign strand. DNA transformation and DNA metabolism are linked as failure to overcome any of these barriers typically renders the foreign DNA susceptible to digestion.

If extracellular DNA is successfully integrated into the bacterial genome through natural transformation, then transduction and conjugation may spread the DNA through the lateral gene transfer network (Fig. 1). Transduction involves the transfer of DNA between bacteria via bacteriophages, which incorporate chromosomal or plasmid bacterial DNA upon infection and transfer this DNA to recipient bacterial cells during subsequent infection. Conjugation is the direct transfer of genetic material from a donor bacterial cell to a recipient cell via a conjugation bridge or sex pilus. These two mechanisms of lateral gene transfer allow transfer of genes between living mature bacterial cells only, so that gene movement is restricted to intracellular prokaryotic DNA. Lateral gene transfer has been extensively researched and is the subject of numerous reviews, including Lorenz and Wackernagel (1994), England and Trevors (2003), Chen and Dubnau (2004), de Vries and Wackernagel (2004).

5.1. Binding and uptake of extracellular DNA

Competence varies among species, strains or isolates (Sikorski et al., 2002; de Vries and Wackernagel, 2004; Thomas and Nielsen, 2005). Developing natural competence is energy-demanding for cells, involving the synthesis of 20–50 proteins, and is induced by factors such as growth conditions, population density and nutrient status (limiting or excessive) (Lorenz and Wackernagel, 1994; Thomas and Nielsen, 2005). In a bacterial population, competence can range from near zero to almost 100% depending on the species or strain involved (Thomas and Nielsen, 2005). For example, *Pseudomonas aeruginosa* appears to be non-competent (van Schaik et al., 2005), while endosymbiotic bacteria such as *Neisseria* spp. and *Vibrio parahemolytica* have been shown to be constitutively competent (Stewart, 1989; Lorenz and Wackernagel, 1994; Paget and Simonet, 1994).

The factors that regulate the competence of bacterial cells are numerous making it difficult to predict whether a given strain or isolate is, or will become competent (Thomas and Nielsen, 2005). Growth stage can influence whether a bacterial cell will be competent, however this appears to depend on species. Some bacteria such as *Pseudomonas* spp. (Carlson et al., 1983) and *Acinetobacter* spp. (Palmen et al., 1994) become competent during the

exponential or early stationary growth phase, while others such as *B. subtilis* develop competence post-exponentially (Albano et al., 1987). In addition, some bacterial species (e.g., *Streptococcus* spp. and *Bacillus* spp.) require a critical concentration of soluble competence factors, which are small peptides that include pheromones (Magnuson et al., 1994; Solomon et al., 1995; Håvarstein et al., 1996). The induction of competence by these peptides is thought to occur primarily at high population densities. Bulk soil often does not have conditions that are advantageous for bacterial growth and competence development. Most bacteria in soil live in a state of dormancy (Nielsen et al., 1997a, b), and those that do not are limited in their ability to enter exponential growth phase (Bertolla et al., 1997). Despite these limitations, some soil bacteria such as *Pseudomonas fluorescens* can undergo natural transformation in soil at population densities of $2.4 \times 10^8 \pm 9.8 \times 10^7$ cells g dry soil^{-1} (Demanèche et al., 2001a). However, Lorenz and Wackernagel (1991) suggest that the induction of competence in soil is not dependent on cell density, but nutrient availability. In soil, competence has been shown in hotspots where exponential growth is more common due to increased nutrient availability, such as microaggregates and the plant rhizosphere (Lynch, 1982; Lorenz and Wackernagel, 1991; Richaume et al., 1992; Lilley et al., 1994; Paget and Simonet, 1994; Lilley and Bailey, 1997; Nielsen et al., 2001; Nielsen and van Elsas, 2001).

Once bacteria become competent, natural transformation begins when double-stranded DNA non-covalently binds to sites on the cell surface (Dubnau, 1999). The number of DNA binding sites varies with the microorganism. For example, the soil bacteria *B. subtilis* has been found to have about 50 sites per competent cell (Dubnau and Cirigliano, 1972; Singh, 1972; Dubnau, 1999). The number of DNA binding sites is not known for many soil bacteria, including *Acinetobacter* sp. strain BD413, the naturally-competent model strain used in many transformation studies. *Acinetobacter* sp. BD413 has been shown to bind extracellular DNA indiscriminately from various sources, including plant DNA (Widmer et al., 1996, 1997; de Vries and Wackernagel, 1998; Gebhard and Smalla, 1998, 1999; Nielsen et al., 2000; Nielsen and van Elsas, 2001; de Vries et al., 2001, 2003; Iwaki and Arakawa, 2006). Once double-stranded DNA is bound to the cell surface it must be taken up into the cell or remain susceptible to shearing and DNase digestion. Various DNA uptake mechanisms in Gram-negative and Gram-positive bacteria have been elucidated. The main difference between these two types of bacteria is the presence of an outer membrane in Gram-negatives, which must be crossed before transport across the cytoplasmic membrane can occur. In many Gram-positive and Gram-negative microorganisms, including *B. subtilis*, *S. pneumoniae* and *Acinetobacter* sp. strain BD413, only single stranded DNA is transported into the bacterial cell (Palmen et al., 1993; Dubnau, 1997; Dubnau, 1999).

5.2. DNA integration

Successful natural transformation requires the integration and expression of the foreign DNA fragment. Only one strand of the DNA molecule enters the cytoplasm in bacteria (Barany et al., 1983; Dubnau, 1999; Chen and Dubnau, 2004). If the foreign molecule is plasmid DNA, it may be reconstituted and maintained. DNA may also be integrated into the host's chromosome. Foreign DNA in the cytoplasm that is not integrated is degraded quickly by nucleases and enters the internal DNA metabolism cycle.

Successful integration and expression of the foreign DNA molecule depends mainly on the presence of homologous regions between the foreign strand and the chromosomal DNA (Lopez et al., 1982; Carlson et al., 1983, 1984; de Vries and Wackernagel, 2002). Homologous recombination, or DNA crossover, is a DNA exchange mechanism between two similar nucleotide sequences. Typically, regions between 25 and 200 base pairs in length of high sequence similarity are sufficient for successful integration of foreign DNA (Thomas and Nielsen, 2005). Under optimal environmental or experimental conditions with sufficient homology, successful integration of internalized DNA fragments occurs with a frequency between 0.1% in *Acinetobacter baylyi* to 25–50% in *B. subtilis* and *S. pneumoniae* (Palmen and Hellingwerf, 1997; Thomas and Nielsen, 2005). Illegitimate recombination events, integration of two non-homologous DNA sequences (de Vries and Wackernagel, 2002), occur at lower frequencies even in the most indiscriminate bacterial strains (de Vries et al., 2001; Nielsen, 2003; Thomas and Nielsen, 2005). For example, de Vries and Wackernagel (2002) found that integration of non-homologous DNA into the genome of *Acinetobacter* sp. strain BD413 was at least 10^9-fold lower than that of homologous DNA. Yet, when this foreign DNA was linked to a segment of DNA homologous to the recipient, integration through ''homology-facilitated illegitimate recombination'' increased by at least 5 orders of magnitude (de Vries and Wackernagel, 2002).

Most transgenic plants have been engineered with genetic constructs that include bacterial genes, such as *neomycin phosphotransferase II* (*nptII*), which confers tolerance to the antibiotic kanamycin to the plant. Detection of recombinant DNA in soil (Romanowski et al., 1992; Trevors, 1996; Widmer et al., 1996; Paget et al., 1998) has encouraged research into the transfer of transgenes to non-target organisms. An intact *nptII* gene has been found to transform the competent bacterial strain of *Acinetobacter* sp. BD413 engineered to contain a *nptII* gene with an internal deletion in soil via homologous recombination (Widmer et al., 1996, 1997; de Vries and Wackernagel, 1998; Gebhard and Smalla, 1998, 1999; Nielsen et al., 2000; Nielsen and van Elsas, 2001; de Vries et al., 2001, 2003; Iwaki and Arakawa, 2006). The observation that homologous recombination could repair a deletion in the *nptII* gene and restore kanamycin resistance led to the development of marker-rescue

transformation, which is a sensitive assay for the detection of *nptII* genes (de Vries and Wackernagel, 1998). It is widely considered that the impact of *nptII* transfer from transgenic plants to bacteria is negligible since resistance to kanamycin is already widespread in soil microorganisms (Cole and Elkan, 1979; van Elsas and Pereira, 1986; Henschke and Schmidt, 1990; Smalla et al., 1993; Dröge et al., 1998) and such transfer would not result in a selective advantage (Nap et al, 1992). The transfer of recombinant DNA into the soil microbial community remains a public concern due to the chance it might result in new antibiotic resistant bacteria, which could include human pathogens (Daniell, 1999; Daniell et al., 2001; Puchta, 2000; Snow et al., 2005). However, this is not scientifically justified, since kanamycin is not used in medical or animal husbandry applications (Bennett et al., 2004). None-the-less kanomycin-resistance genes as selection markers for transformation of plants are being phased out in favor of other genes such as the regeneration-enhancing gene *ipt* that are more acceptable (Ebinuma et al., 1997; Kunkel et al., 1999; Endo et al., 2002).

Other bacterial genes such as the *cp4 epsps* gene, which confers resistance to glyphosate and was originally isolated from *Agrobacterium* sp. strain CP4 (Padgette et al., 1995), have been incorporated into many crops. Transfer of the *cp4 epsps* transgene is of interest due to high potential for homology between the transgene and the bacterial *epsps* (or *aroA*) genes. Many soil bacteria are also naturally susceptible to glyphosate. For example, Moorman et al. (1992) showed that growth of *Bradyrhizobium japonicum* strains USDA 110, 123 and 138 was partially inhibited by 1.0 mM and completely inhibited by 5.0 mM glyphosate. A BLAST search of the *cp4 epsps* gene revealed fragment homology to several bacterial genes involved in the shikimate pathway. For example, the *3-phosphoshikimate-1-carboxyvinyltransferase* gene of *Mesorhizobium loti* showed a homology of 79–84% to the *cp4 epsps*, while the *aroA* gene of *B. japonicum* showed 84–96% homology (Table 3). *B. japonicum* and *M. loti* are the symbionts responsible for nitrogen fixation in soybean (*Glycine max* (L.) Merr.) and lotus, respectively (Somasegaran and Hoben, 1994). It is not clear whether *B. japonicum* and *M. loti* can develop competence, although their close relative *Sinorhizobium meliloti* is competent to DNA uptake (de Vries and Wackernagel, 2004). However, due to the role of Rhizobiaceae in nodulation and potential susceptibility to glyphosate, as well as their close proximity to root exudates that contain plant recombinant DNA (Gulden et al., 2005), they should be targets for investigating transgenic DNA uptake via natural transformation.

Transfer of recombinant DNA from transgenic plants to competent soil bacteria has yet to be detected in the field (Nielsen et al., 1998; Paget et al., 1998; de Vries and Wackernagel, 2004). However, investigations of the transfer of recombinant DNA in field settings continues because controlled studies in soil microcosms have shown natural transformation to occur. For example, Paget and Simonet (1994) described multiple investigations of transformation efficiency (binding, uptake and integration) of DNA bound to clay (montmorillonite) (Khanna and Stotzky, 1992; Paget et al., 1992), sand (Lorenz et al., 1988), and mixtures of these particle size classes in microcosms (Chamier et al., 1993). This research has shown that desorption of DNA bound to soil particles does not always need to occur for successful natural transformation, but adsorption of DNA to soil particles reduces the frequency of transformation (Khanna and Stotzky, 1992). For example, the transformation frequency of *B. subtilis* in non-sterile soil was observed to be about 1 order of magnitude lower than in liquid cultures (Gallori et al., 1994). Nielsen et al. (1997b) also found an inhibitory effect of soil on transformation and DNA availability, depending on the soil type. In their study, *Acinetobacter* sp. strain BD413 cells were transformed at a rate that was about 3 orders of magnitude higher (and for a longer period of time) in a silt loam (frequency about 10^{-4}) than in a loamy sand (frequency about 10^{-7}) without added nutrients (Nielsen et al., 1997b). Sikorski et al. (1998) observed that *P. stutzeri* cells were transformed in soil by both bound DNA and DNA in the liquid fraction of the soil, but that the dissolved DNA from the liquid fraction accounted for only 10% of the overall transformation events. It was also shown that the

Table 3
Percent homology of non-overlapping bacterial gene fragments to the *cp4 epsps* gene

Microorganism	No. of non-overlapping regions	Fragment size (bp)	Homology (%)
Sinorhizobium meliloti 1021	1	1278	84
Mesorhizobium loti MAFF303099	4	684	79
		156	82
		144	81
		117	84
Pseudomonas putida KT2440	1	63	85
Bradyrhizobium japonicum USDA 110	4	54	85
		53	84
		28	96
		26	96

transformation frequency and the quantity of a DNA sequence in a soil microcosm followed linear relationship (Sikorski et al., 1998).

Successful natural transformation alone may not be sufficient to maintain the newly incorporated gene in a functional state. For long-term introgression, successfully incorporated foreign DNA must confer a selective advantage in conjunction with limited consequences to fitness (Ochman et al., 2000) as bacterial genomes often delete non-essential DNA (Andersson and Kurland, 1998; Andersson and Andersson, 1999; Ochman et al., 2000). If a selective advantage arises and fitness is not affected, then complete introgression and amplification of the foreign DNA within the transformed species is likely. Once this occurs, the other mechanisms of lateral gene transfer (conjugation and transduction) may spread the gene among bacterial populations and species rapidly leading to further amplification of the newly acquired DNA sequence. Conjugation especially tends to occur more frequently (Dröge et al., 1998) and could thus be more difficult to contain than natural transformation or transduction. Dröge et al. (1998) lists the examples showing that the environment in the plant rhizosphere (van Elsas et al., 1988, 1989; Lilley et al., 1994; Troxler et al., 1997; Lilley and Bailey, 1997) and the root nodule (Pretorius-Güth et al., 1990; Dröge et al., 1998) is conducive to conjugation. Yet without selective pressure, it is possible the incorporated DNA will be lost within a few bacterial generations (Yenofsky et al., 1990; Nielsen et al., 1997, 2000).

Although natural transformation of recombinant DNA from transgenic plants to endogenous soil bacteria has not been detected in vivo, current methods have not been sufficiently sensitive to monitor such low frequency events (Heinemann and Traavik, 2004). Marker-rescue transformation developed by de Vries and Wackernagel (1998) provides a sensitive assay for detecting the *nptII* gene in soil, albeit under highly artificial conditions, such as the use of constituently competent bacterial strains to detect transformation. Nielsen et al. (2000) used this assay to detect the transformation of *Acinetobacter* sp. strain BD413 by the *nptII* gene from transgenic plants. The possibility exists to extend the use of this transformation assay to detect the transformation of soil bacteria by other recombinant genes in transgenic plants, such as the *cp4 epsps* gene conferring tolerance to glyphosate. Despite the current lack of evidence of gene transfer from plants to microbes in the field, continued monitoring for these events using the best methods available should be conducted as the global use of transgenic crops shows no sign of decline. Moreover to mitigate the risk of natural transformation, the origin, function and sequence of transgenes should be considered on a case-by-case basis.

6. Conclusions

In the proposed extracellular DNA cycle, a DNA molecule has three potential fates upon entry into the soil, (i) persistence through binding to the soil matrix, (ii) metabolism through DNA sequence restriction, or (iii) natural transformation through uptake and integration into competent soil microorganisms. This review has focussed on DNA behavior in agricultural systems, however, the concept that the activity of extracellular DNA in soil as a cyclical process can be applied to understanding the fate of DNA in other environments, or used in other disciplines (e.g., environmental forensics).

The extracellular DNA cycle in soil is not a closed system; it is continually replenished by DNA that is released into soil by living and decomposing organisms. In addition, DNA is removed from the cycle through extracellular degradation or uptake by organisms to be reincorporated into DNA (salvage and transformation) or other molecules (degradation). The fact that this cycle is an open, interconnected system in the soil is vital for two reasons. First, its components are energy and nutrient rich. Degraded DNA or salvaged by-products play an important role for growth of microorganisms and plants in nutrient-deprived soils. Second, an intact DNA sequence carries a genetic message that may be picked up by foreign soil microorganisms through natural transformation and thereby cross the species barrier. Under specific conditions this may result in intercellular persistence and amplification of foreign DNA sequences within the microbial lateral gene transfer network and may result in unforeseen environmental impacts.

The soil environment provides numerous environmental and physiological barriers that prevent gene transfer to soil microorganisms such as rapid degradation of extracellular DNA in soil, limited DNA uptake, and lack of integration and persistence in the recipient genome. Nonetheless, natural transformation of foreign DNA into native soil microbial populations is an important component of prokaryotic evolution. The homology of recombinant DNA to microbial genes and the selective pressure of transgenic cropping systems have led to concerns regarding the release of transgenic plants into the environment. However, once the genetic message is degraded, recombinant DNA can be considered equivalent to other extracellular DNA in the soil and should not be considered an environmental concern.

Investigations into recombinant DNA release in soil have often focused on specific elements of DNA behavior. There is a need to integrate these elements to enhance our understanding of the fate of DNA in the soil environment more accurately predict potential risks associated with recombinant DNA release in soil. In this review, we have attempted to synthesize the current knowledge in such a manner suggest that future research into the activity of DNA in soil, including environmental risk assessments regarding the release of recombinant DNA in the environment, continue to employ an interdisciplinary approach that focuses on the relationships between the individual components of the extracellular DNA cycle in soil.

Acknowledgments

Financial support provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic Grant, Discovery Grant, Post-Doctoral Fellowship, and Graduate Scholarships. Infrastructure and equipment support provided by the Canadian Foundation for Innovation and the Ontario Ministry of Research and Innovation. The authors also wish to express their gratitude to Drs. S. Lerat and L. England for their assistance.

References

Albano, M., Hahn, J., Dubnau, D., 1987. Expression of competence genes in *Bacillus subtilis*. Journal of Bacteriology 169, 3110–3117.

Andersson, J.O., Andersson, S.G.E., 1999. Insights into the evolutionary process of genome degradation. Current Opinion in Genetics & Development 9, 664–671.

Andersson, S.G.E., Kurland, C.G., 1998. Reductive evolution of resident genomes. Trends in Microbiology 6, 263–268.

Arumuganathan, K., Earle, E.D., 1991. Nuclear DNA content of some important plant species. Plant Molecular Biology Reporter 9, 211–215.

Backert, S., Meyer, T.F., 2006. Type IV secretion systems and their effectors in bacterial pathogenesis. Current Opinions in Microbiology 9, 207–217.

Baker, R.T., 1977. Humic acid-associated organic phosphate. New Zealand Journal of Science 20, 439–441.

Barany, F., Kahn, M.E., Smith, H.O., 1983. Directional transport and integration of donor DNA in *Haemophilus influenzae* transformation. Proceedings of the National Academy of Sciences of the United States of America 80, 7274–7278.

Bennett, P.M., Livesey, C.T., Nathwani, D., Reeves, D.S., Saunders, J.R., Wise, R., 2004. An assessment of the risks associated with the use of antibiotic resistance genes in genetically modified plants: report of the working party of the British Society for Antimicrobial Chemotherapy. Journal of Antimicrobial Chemotherapy 53, 418–431.

Bertolla, F., van Gijsegem, F., Nesme, X., Simonet, P., 1997. Conditions for natural transformation of *Ralstonia solanacearum*. Applied and Environmental Microbiology 63, 4965–4968.

Bertolla, F., Frostegard, A., Brito, B., Nesme, X., Simonet, P., 1999. During infection of its host, the plant pathogen *Ralstonia solanacearum* naturally develops a state of competence and exchanges genetic material. Molecular Plant–Microbe Interactions 12, 467–472.

Blum, S.A.E., Lorenz, M.G., Wackernagel, W., 1997. Mechanism of retarded DNA degradation and prokaryotic origin of DNases in nonsterile soils. Systematic and Applied Microbiology 20, 513–521.

Bongaerts, G.P.A., Vogels, G.D., 1976. Uric acid degradation by *Bacillus fastidious* strains. Journal of Bacteriology 125, 689–697.

Bowman, R.A., Cole, C.V., 1978. Transformations of organic phosphorus substrates in soils as evaluated by $NaHCO_3$ extraction. Soil Science 125, 49–54.

Carlson, C., Pierson, L.S., Rosen, J.J., Ingraham, J.L., 1983. *Pseudomonas stutzeri* and related species undergo natural transformation. Journal of Bacteriology 153, 93–99.

Carlson, C.A., Steenbergen, S.A., Ingraham, J.L., 1984. Natural transformation of *Pseudomonas stutzeri* by plasmids that contain cloned fragments of chromosomal deoxyribonucleic acid. Archives of Microbiology 40, 134–138.

Ceccherini, M., Poté, J., Kay, E., Van, V.T., Marechal, J., Pietramellara, G., Nannipieri, P., Vogel, T.M., Simonet, P., 2003. Degradation and transformability of DNA from transgenic leaves. Applied and Environmental Microbiology 69, 673–678.

Chamier, B., Lorenz, M.G., Wackernagel, W., 1993. Natural transformation of *Acinetobacter calcoaceticus* by plasmid DNA adsorbed on sand and groundwater aquifer material. Applied and Environmental Microbiology 59, 1662–1667.

Chen, I., Dubnau, D., 2004. DNA uptake during bacterial transformation. Nature Reviews Microbiology 2, 241–249.

Cole, M.A., Elkan, G.H., 1979. Multiple antibiotic resistance in *Rhizobium japonicum*. Applied and Environmental Microbiology 37, 867–870.

Crecchio, C., Stotzky, G., 1998. Binding of DNA on humic acids: effect on transformation of *Bacillus subtilis* and resistance to DNase. Soil Biology & Biochemistry 30, 1060–1067.

Crecchio, C., Ruggiero, P., Curci, M., Colombo, C., Palumbo, G., Stotzky, G., 2005. Binding of DNA from *bacillus subtilis* on montmorillonite–humic acids–aluminum or iron hydroxypolymers. Soil Science Society of America Journal 69, 834–841.

Daniell, H., 1999. GM crops: public perception and scientific solutions. Trends in Plant Science 4, 467–469.

Daniell, H., Muthukumar, B., Lee, S.B., 2001. Marker free transgenic plants: engineering the chloroplast genome without the use of antibiotic selection. Current Genetics 39, 109–116.

del Pozo, O., Lam, E., 1998. Caspases and programmed cell death in the hypersensitive response of plants to pathogens. Current Biology 8, 1129–1132.

Demanèche, S., Kay, E., Gourbière, F., Simonet, P., 2001a. Natural transformation of *Pseudomonas fluorescens* and *Agrobacterium tumefaciens* in soil. Applied and Environmental Microbiology 67, 2617–2621.

Demanèche, S., Jocteur-Monrozier, L., Quiquampoix, H., Simonet, P., 2001b. Evaluation of biological and physical protection against nuclease degradation of clay-bound plasmid DNA. Applied and Environmental Microbiology 67, 293–299.

de Vries, J., Wackernagel, W., 1998. Detection of *nptII* (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue transformation. Molecular & General Genetics 257, 606–613.

de Vries, J., Wackernagel, W., 2002. Integration of foreign DNA during natural transformation of *Acinetobacter* sp. by homology-facilitated illegitimate recombination. Proceedings of the National Academy of Sciences of the United States of America 99, 2094–2099.

de Vries, J., Wackernagel, W., 2004. Microbial horizontal gene transfer and the DNA release from transgenic crop plants. Plant and Soil 266, 91–104.

de Vries, J., Meier, P., Wackernagel, W., 2001. The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. FEMS Microbiology Letters 195, 211–215.

de Vries, J., Heine, M., Harms, K., Wackernagel, W., 2003. Spread of recombinant DNA by roots and pollen of transgenic potato plants, identified by highly specific biomonitoring using natural transformation of an *Acinetobacter* sp. Applied and Environmental Microbiology 69, 4455–4462.

Diaz-Raviña, M., Frostegaard, A., Baaaath, E., 1994. Thymidine, leucine and acetate incorporation into soil bacterial assemblages at different temperatures. FEMS Microbiology Ecology 14, 221–232.

Dilly, O., Bartsch, S., Rosenbrock, P., Buscot, F., Munch, J.C., 2001. Shifts in physiological capabilities of the microbiota during the decomposition of leaf litter in a black alder (*Alnus glutinosa* (Gaertn.) L.) forest. Soil Biology & Biochemistry 33, 921–930.

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

Dubnau, D., 1997. Binding and transport of transforming DNA by *Bacillus subtilis*: the role of type-IV pilin-like proteins—a review. Gene 192, 191–198.

Dubnau, D., 1999. DNA uptake in bacteria. Annual Review of Microbiology 53, 217–244.

Dubnau, D., Cirigliano, C., 1972. Fate of transforming DNA following uptake by competent *Bacillus subtilis*. IV. The endwise attachment and uptake of transforming DNA. Journal of Molecular Biology 64, 31–46.

Ebinuma, H., Sugita, K., Matsunaga, E., Yamakado, M., 1997. Selection of marker-free transgenic plants using the isopentenyl transferase gene. Proceedings of the National Academy of Sciences of the United States of America 94, 2117–2121.

Endo, S., Sugita, K., Sakai, M., Tanaka, H., Ebinuma, H., 2002. Single-step transformation for generating marker-free transgenic rice using the *ipt*-type MAT vector system. The Plant Journal 30, 115–122.

England, L.S., Trevors, J.T., 2003. The microbial DNA cycle in soil. Biology Forum 96, 317–326.

England, L.S., Lee, H., Trevors, J.T., 1995. Recombinant and wildtype *Pseudomonas aureofaciens* strains introduced to soil: effect on cellulose and straw decomposition. Molecular Ecology 4, 221–230.

England, L.S., Lee, H., Trevors, J.T., 1997. Persistence of *Pseudomonas aureofaciens* strains and DNA in soil. Soil Biology & Biochemistry 29, 1521–1527.

Finkel, S.E., Kolter, R., 2001. DNA as a nutrient: novel roll for bacterial competence gene homologs. Journal of Bacteriology 183, 6288–6293.

Frostegård, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., Simonet, P., 1999. Quantification of bias related to the extraction of DNA directly from soils. Applied and Environmental Microbiology 65, 5409–5420.

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

Gebhard, F., Smalla, K., 1998. Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. Applied and Environmental Microbiology 64, 1550–1554.

Gebhard, F., Smalla, K., 1999. Monitoring field releases of transgenic modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbial Ecology 28, 261–272.

Geigenberger, P., Regierer, B., Nunes-Nesi, A., Leisse, A., Urbanczyk-Wochniak, E., Springer, F., van Dongen, J.T., Kossmann, J., Ferniea, A.R., 2005. Inhibition of *de novo* pyrimidine synthesis in growing potato tubers leads to a compensatory stimulation of the pyrimidine salvage pathway and a subsequent increase in biosynthetic performance. The Plant Cell 17, 2077–2088.

Greaves, M.P., Wilson, M.J., 1969. The adsorption of nucleic acids by montmorillonite. Soil Biology & Biochemistry 1, 317–323.

Greaves, M.P., Wilson, M.J., 1970. The degradation of nucleic acids and montmorillonite–nucleic-acid complexes by soil microorganisms. Soil Biology & Biochemistry 2, 257–268.

Greenberg, J.T., 1996. Programmed cell death: a way of life for plants. Proceeding of the National Academy of Science of the United States of America 93, 12094–12097.

Greenwood, A.D., Capelli, C., Possnert, G., Pääbo, S., 1999. Nuclear DNA sequences from late Pleistocene megafauna. Molecular Biology and Evolution 16, 1466–1473.

Guan, J., Spencer, J.L., Ma, B.L., 2005. The fate of the recombinant DNA in corn during composting. Journal of Environmental Science and Health Part B 40, 463–473.

Gulden, R.H., Lerat, S., Hart, M.M., Powell, J.T., Trevors, J.T., Pauls, K.P., Klironomos, J.N., Swanton, C.J., 2005. Quantitation of transgenic plant DNA in leachate water: real-time polymerase chain reaction analysis. Journal of Agricultural and Food Chemistry 53, 5858–5865.

Hamilton, H.L., Dominguez, N.M., Schwartz, K.J., Hackett, K.T., Dillard, J.P., 2005. *Neisseria gonorrhoeae* secretes chromosomal DNA via a novel type IV secretion system. Molecular Microbiology 55, 1704–1721.

Håvarstein, L.S., Gaustad, P., Nes, I.F., Morrison, D.A., 1996. Identification of the streptococcal competence-pheromone receptor. Molecular Microbiology 21, 863–869.

Hawes, M.C., 1990. Living plant cells released from the root cap: a regulator of microbial populations in the rhizosphere? Plant and Soil 129, 19–27.

Hay, I., Morency, M.J., Séguin, A., 2002. Assessing the persistence of DNA in decomposing leaves of genetically modified poplar trees. Canadian Journal of Forest Resources 32, 977–982.

Heinemann, J.A., Traavik, T., 2004. Problems in monitoring horizontal gene transfer in field trials of transgenic plants. Nature Biotechnology 22, 1105–1109.

Henderson, J.F., 1972. Regulation of Purine Biosynthesis, vol. 170. American Chemical Society Monograph, Washington, DC.

Henderson, J.F., Paterson, A.R.P., 1973. Nucleotide Metabolism: An Introduction. Academic Press, London.

Henriksen, T.M., Breland, T.A., 1999. Decomposition of crop residues in the field: evaluation of a simulation model developed from microcosm studies. Soil Biology & Biochemistry 31, 1423–1434.

Henschke, R.B., Schmidt, F.R.J., 1990. Screening of soil bacteria for plasmids carrying antibiotic resistance. Biology and Fertility of Soils 9, 257–260.

Herdina, Neate, S., Jabaji-Hare, S., Ophel-Keller, K., 2004. Persistence of DNA of *Gaeumannomyces graminis* var. *tritici* in soil as measured by a DNA-based assay. FEMS Microbiology Ecology 47, 143–152.

Hirose, F., Ashihara, H., 1984. Interrelationship between the pentose phosphate pathway and nucleotide synthesis in cultured cells of *Catharanthus roseus*. Plant Science Letters 35, 123–126.

Hofreiter, M., Serre, D., Poinar, H.N., Kuch, M., Pääbo, S., 2001. Ancient DNA. Nature Reviews Genetics 2, 353–360.

Iijima, M., Griffiths, B., Bengou, G., 2000. Sloughing of cap cells and carbon exudation from maize seedling roots in compacted sand. New Phytologist 145, 477–482.

Imsenecki, A.A., Popova, L.S., 1971. Decomposition of uric acid by aerobic bacteria isolated from soil. Mikrobiologiia 40, 269–274.

Ingraham, J.L., Maaloe, O., Neidhardt, F.C., 1983. Growth of the Bacterial Cell. Sinauer, Sunderland, MA, 435pp.

Iwaki, M., Arakawa, Y., 2006. Transformation of Acinetobacter sp. BD413 with DNA from commercially available genetically modified potato and papaya. Letters in Applied Microbiology 43, 215–221.

James, C., 2006. Preview: Global status of Commercialized Biotech/GM Crops: 2005. ISAAA Brief 35. ISAAA, Ithaca, NY.

Jensen, K.F., 1978. Two purine-nucleoside phosphorylases in *Bacillus subtilis*: purification and some properties of the adenosine-specific phosphorylase. Biochimica et Biophysica Acta 523, 346–356.

Katahira, R., Ashihara, H., 2002. Profiles of pyrimidine biosynthesis, salvage and degradation in disks of potato (*Solanum tuberosum* L.) tubers. Planta 215, 821–828.

Kay, E., Vogel, T.M., Bertolla, F., Nalin, R., Simonet, P., 2002. In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. Applied and Environmental Microbiology 68, 3345–3351.

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. Applied Environmental Microbiology 58, 1930–1939.

Kornberg, A., 1974. DNA Synthesis. W.H. Freeman and Company, San Francisco.

Kunkel, T., Niu, Q., Chan, Y., Chua, N., 1999. Inducible isopentenyl transferase as a high-efficiency marker for plant transformation. Nature Biotechnology 17, 916–919.

Lerat, S., England, L.S., Vincent, M.L., Pauls, K.P., Swanton, C.J., Klironomos, J.N., Trevors, J.T., 2005. Real-time polymerase chain reaction (PCR) quantification of the transgenes for Roundup Ready corn and Roundup Ready soybean in soil samples. Journal of Agricultural and Food Chemistry 53, 1337–1342.

Lilley, A.K., Bailey, M.J., 1997. The acquisition of indigenous plasmids by a genetically marked pseudomonad population colonizing the sugar beet phytosphere is related to local environmental conditions. Applied and Environmental Microbiology 63, 1577–1583.

Lilley, A.K., Fry, J.C., Day, M.J., Bailey, M.J., 1994. In situ transfer of an exogenously isolated plasmid between *Pseudomonas* spp. in sugar beet rhizosphere. Microbiology 140, 27–33.

Lopez, P., Espinosa, M., Strassi, D.L., Lacks, S.A., 1982. Facilitation of plasmid transfer in *Streptococcus pneumoniae* by chromosomal homology. Journal of Bacteriology 150, 692–701.

Lorenz, M.G., Wackernagel, W., 1987. Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. Applied and Environmental Microbiology 53, 2948–2952.

Lorenz, M.G., Wackernagel, W., 1991. High frequency of natural genetic transformation of Pseudomonas stutzeri in soil extract supplemented with a carbon/energy and phosphorus source. Applied and Environmental Microbiology 57, 1246–1251.

Lorenz, M.G., Wackernagel, W., 1992. DNA binding to various clay minerals and retarded enzymatic degradation of DNA in a sand/clay microcosm. In: Gautheir, M.J. (Ed.), Gene Transfers and Environment. Springer, New York, USA, pp. 103–113.

Lorenz, M.G., Wackernagel, W., 1994. Bacterial gene transfer by natural genetic transformation in the environment. Microbiological Reviews 58, 563–602.

Lorenz, M.G., Aardema, B.W., Wackernagel, W., 1988. Highly efficient genetic transformation of *Bacillus subtilis* attached to sand grains. Journal of Genetic Microbiology 134, 107–122.

Lorenz, M.G., Gerjets, D., Wackernagel, W., 1991. Release of transforming plasmid and chromosomal DNA from two cultured soil bacteria. Archives of Microbiology 156, 319–326.

Lynch, J.M., 1982. Limits to bacterial growth in soil. Journal of General Microbiology 128, 405–410.

Macfadyen, L.P., Chen, D., Vo., H.C., Liao, D., Sinotte, R., Redfield, R.J., 2001. Competence development by *Haemophilus influenzae* in regulated by the availability of nucleic acid precursors. Molecular Microbiology 40, 700–707.

Magnuson, R., Solomon, J., Grossman, A.D., 1994. Biochemical and genetic characterization of a competence pheromone in *B. subtilis*. Cell 77, 207–216.

McPartlan, H.C., Dale, P.J., 1994. An assessment of gene transfer by pollen from field-grown transgenic potatoes to non-transgenic potatoes and related species. Transgenic Research 3, 216–225.

Meier, P., Wackernagel, W., 2003. Monitoring the spread of recombinant DNA from field plots with transgenic sugar beet plants by PCR and natural transformation of *Pseudomonas stutzeri*. Transgenic Research 12, 293–304.

Mishra, N.C., 2002. Nucleases: Molecular biology and applications. Wiley, Hoboken, NJ, pp. 45–60.

Moorman, T.B., Becerril, J.M., Lydon, J., Duke, S.O., 1992. Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains after treatment with glyphosate. Journal of Agricultural and Food Chemistry 40, 289–293.

Nap, J.P., Bijvoet, J., Stiekema, W.J., 1992. Biosafety of kanamycin-resistant transgenic plants. Transgenic Research 3, 216–225.

Nielsen, K.M., 2003. An assessment of factors affecting the likelihood of horizontal gene transfer of recombinant plant DNA to bacterial recipients in the soil and rhizosphere. Collection of Biosafety Reviews 1, 96–149.

Nielsen, K.M., van Elsas, J.D., 2001. Stimulatory effects of compounds present in the rhizosphere on natural transformation of *Acinetobacter* sp. BD413 in soil. Soil Biology & Biochemistry 33, 345–357.

Nielsen, K.M., Bones, A.M., van Elsas, J.D., 1997a. Induced natural transformation of *Acinetobacter calcoaceticus* in soil microcosms. Applied and Environmental Microbiology 63, 3972–3977.

Nielsen, K.M., van Weerelt, M.D., Berg, T.N., Bones, A.M., Hagler, A.N., van Elsas, J.D., 1997b. Natural transformation and availability of transforming DNA to *Acinetobacter calcoaceticus* in soil microcosms. Applied and Environmental Microbiology 63, 1945–1952.

Nielsen, K.M., Bones, A.M., Smalla, K., van Elsas, J.D., 1998. Horizontal gene transfer from transgenic plants to terrestrial bacteria—a rare event? FEMS Microbiology Reviews 22, 79–103.

Nielsen, K.M., van Elsas, J.D., Smalla, K., 2000. Transformation of *Acinetobacter* sp. strain BD413(pFG4Δ*nptII*) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Applied and Environmental Microbiology 66, 1237–1242.

Nielsen, K.M., van Elsas, J.D., Smalla, K., 2001. Dynamics, horizontal transfer and selection of novel DNA in bacterial populations in the phytosphere of transgenic plants. Annals of Microbiology 51, 79–94.

Ochman, H., Larwrence, J.G., Griosman, A.G., 2000. Lateral gene transfer and the nature of bacterial innovation. Nature 405, 299–304.

Ogram, A., Sayler, G.S., Gustin, D., Lewis, R.J., 1988. DNA adsorption to soils and sediments. Environmental Science and Technology 22, 982–984.

Padgette, S.R., Kolacz, K.H., Delannay, X., Re, D.B., LaVallee, B.J., Tinius, C.N., Rhodes, W.K., Otero, Y.I., Barry, G.F., Eichholtz, D.A., Peschke, V.M., Nida, D.L., Taylor, N.B., Kishore, G.M., 1995. Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Science 35, 1451–1461.

Paget, E., Simonet, P., 1994. On track of natural transformation in soil. FEMS Microbial Ecology 15, 109–118.

Paget, E., Monrozier, L.J., Simonet, P., 1992. Adsorption of DNA on clay minerals: protection against DNase I and influence on gene transfer. FEMS Microbiology Letters 97, 31–40.

Paget, E., Lebrun, M., Freyssinet, G., Simonet, P., 1998. The fate of recombinant plant DNA in soil. European Journal of Soil Biology 34, 81–88.

Palmen, R., Hellingwerf, K.J., 1997. Uptake and processing of DNA by *Acinetobacter calcoaceticus*—a review. Gene 192, 179–190.

Palmen, R., Buijsman, P., Hellingwerf, K.J., 1994. Physiological regulation of competence induction for natural transformation in *Acinetobacter calcoaceticus*. Archives of Microbiology 162, 344–351.

Palmen, R., Vosman, B., Buijman, P., Breek, C.K., Hellingwerf, K.J., 1993. Physiological characterization of natural transformation in *Acinetobacter calcoaceticus*. Journal of General Microbiology 139, 295–305.

Pearson, R.W., Norman, A.G., Ho, C., 1941. The mineralization of the organic phosphorous of various compounds in soil. Proceedings of the Soil Science Society of America 6, 168–175.

Petersen, L.W., Moldrup, P., Jacobsen, O.H., Rolston, D.E., 1996. Relations between specific surface area and soil physical and chemical properties. Soil Science 161, 9–21.

Pietramellara, G., Franchi, M., Gallori, E., Nannipieri, P., 2001. Effect of molecular characteristics of DNA on its adsorption and binding on homoionic montmorillonite and kaolinite. Biology and Fertility of Soils 33, 402–409.

Polverari, A., Buonaurio, R., Guiderdone, S., Pezatti, M., Marte, M., 2000. Ultrastructural observations and DNA degradation analysis of pepper leaves undergoing a hypersensitive reaction to *Xanthomonas campestris* p.v. *vesicatoria*. European Journal of Plant Pathology 106, 423–431.

Poly, F., Chenu, C., Simonet, P., Rouiller, J., Monrozier, L.J., 2000. Differences between linear chromosomal and supercoiled plasmid DNA in their mechanisms and extent of adsorption on clay minerals. Langmuir 16, 1233–1238.

Poté, J., Rossé, P., Rosselli, W., Van, V.T., Wildi, W., 2005. Kinetics of mass and DNA decomposition in tomato leaves. Chemosphere 61, 677–684.

Pretorius-Güth, I.M., Pühler, A., Simon, R., 1990. Conjugal transfer of megaplasmid 2 between *Rhizobium meliloti* strains in alfalfa nodules. Applied and Environmental Microbiology 56, 2354–2359.

Puchta, H., 2000. Removing selectable marker genes: taking the shortcut. Trends in Plant Science 5, 273–274.

Reanney, D.C., Roberts, W.P., Kelly, W.J., 1982. Genetic interactions among microbial communities. In: Bull, A.T.S., Slater, J.H. (Eds.), Microbial Interactions and Communities. Academic Press, London, pp. 287–322.

Recorbet, G., Picard, C., Normand, P., Simonet, P., 1993. Kinetics of the persistence of chromosomal DNA from genetically engineered *Escherichia coli* introduced into soil. Applied and Environmental Microbiology 59, 4289–4294.

Redfield, R.J., 1988. Evolution of bacterial transformation: is sex with dead cells ever better than no sex at all? Genetics 116, 213–221.

Redfield, R.J., 1993. Genes for breakfast: the have-your-cake-and-eat-it-too of bacterial transformation. Journal of Heredity 84, 400–404.

Richaume, A., Smit, E., Faurie, G., van Elsas, J.D., 1992. Influence of soil type on the transfer of plasmid RP4p from *Pseudomonas fluorescens* to introduced recipient and to indigenous bacteria. FEMS Microbiology Ecology 101, 281–291.

Robe, P., Nalin, R., Capellano, C., Vogel, T.M., Simonet, P., 2003. Extraction of DNA from soil. European Journal of Soil Biology 39, 183–190.

Romanowski, G., Lorenz, M.G., Wackernagel, W., 1991. Adsorption of plasmid DNA to mineral surfaces and protection against DNase I. Applied and Environmental Microbiology 57, 1057–1061.

Romanowski, G., Lorenz, M.G., Sayler, G., Wackernagel, W., 1992. Persistence of free plasmid DNA in soil monitored by various methods, including a transformation assay. Applied and Environmental Microbiology 58, 3012–3019.

Saeglitz, C., Pohl, M., Bartsch, D., 2000. Monitoring gene flow from transgenic sugar beet using cytoplasmic male-sterile bait plants. Molecular Ecology 9, 2035–2040.

Schnitzer, M., 1982. Organic matter characterization. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. American Society of Agronomy, Inc. (ASA) and Soil Science Society of America, Inc. (SSSA), Madison, WI.

Scott, R.K., 1970. The effect of weather on the concentration of pollen within sugar-beet crops. Annals of Applied Biology 66, 119–127.

Shapiro, R., 1981. Damage to DNA caused by hydrolysis. In: Seeberg, E., Kleppe, K. (Eds.), Chromosome Damage and Repair. Plenum Press, New York, NY, pp. 3–18.

Sikorski, J., Graupner, S., Lorenz, M.G., Wackernagel, W., 1998. Natural genetic transformation of *Pseudomonas stutzeri* in a non-sterile soil. Microbiology 144, 569–576.

Sikorski, J., Möhle, M., Wackernagel, W., 2002. Identification of complex composition, strong strain diversity and directional selection in local *Pseudomonas stutzeri* populations from marine sediments and soils. Environmental Microbiology 4, 465–476.

Singh, R.N., 1972. Number of deoxyribonucleic acid uptake sites in competent cells of *Bacillus subtilis*. Journal of Bacteriology 110, 266–272.

Slater, L., Ntarlagiannis, D., Wishart, D., 2006. On the relationship between induced polarization and surface area in metal–sand and clay–sand mixtures. Geophysics 71, A1–A5.

Smalla, K., van Overbeek, L.S., Pukall, R., van Elsas, J.D., 1993. Prevalence of *nptII* and Tn5 in kanamycin-resistant bacteria from different environments. FEMS Microbiology Ecology 13, 47–58.

Snow, A.A., Andow, D.A., Gepts, P., Hallerman, E.M., Power, A., Tiedje, J.M., Wolfenbarger, L.L., 2005. Genetically engineered organisms and the environment: current status and recommendations. Ecological Applications 15, 377–404.

Solomon, J.M., Magnuson, R., Srivastava, A., Grossman, A.D., 1995. Convergent sensing pathways mediate response to two extracellular competence factors in *Bacillus subtilis*. Genes & Development 9, 547–558.

Somasegaran, P., Hoben, H.J., 1994. Handbook for Rhizobia: Methods in Legume–Rhizobium Technology. Springer, Germany, 450pp.

Steinmoen, H., Knutsen, E., Håvarstein, L.S., 2002. Induction of natural competence in *Streptococcus pneumoniae* triggers lysis and DNA release from a subfraction of the cell population. Proceedings of the National Academy of Science of the United States of America 99, 7681–7686.

Stevenson, F.J., 1994. Humus Chemistry. Genesis, Composition, Reactions, second ed. Wiley, New York, NY.

Stewart, G.J., 1989. The mechanism of natural transformation. In: Levy, S.B., Miller, R.V. (Eds.), Gene Transfer in the Environment. McGraw-Hill, New York, pp. 139–164.

Stewart, G.J., Carlson, C.A., 1986. The biology of natural transformation. Annual Reviews of Microbiology 40, 211–235.

Stouthamer, A.H., 1979. The search for correlation between theoretical and experimental growth yields. International Review of Biochemistry 21, 1–47.

Tebbe, C.C., Vahjen, W., 1993. Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. Applied and Environmental Microbiology 59, 2657–2665.

Theng, B.K.G., 1979. Formation and Properties of Clay–Polymer Complexes. Elsevier, Amsterdam (pp. 3–35).

Thomas, C.M., Nielsen, K.M., 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature Reviews Microbiology 3, 711–721.

Timmons, A.M., O'Brian, E.T., Charters, Y.M., Dubbels, S.J., Wilkinson, M.J., 1995. Assessing the risks of wind pollination form fields of genetically modified *Brassica napus* ssp *oleifera*. Euphytica 85, 417–423.

Trevors, J.T., 1996. DNA in soil: adsorption, genetic transformation, molecular evolution and genetic microchip. Antonie van Leeuwenhoek 70, 1–10.

Troxler, J., Azelvandre, P., Zala, M., Defago, G., Hass, D., 1997. Conjugative transfer of chromosomal genes between fluorescent pseudomonads in the rhizosphere of wheat. Applied and Environmental Microbiology 63, 213–219.

Turbin, N.B., Bormotov, V.E., Sabchenko, V.K., Matoshko, I.V., 1965. Nucleic acid content in pollen of diploid and tetraploid sugar beet. Doklady Akademii Nauk SSSR 161, 463–465.

Turner, B.L., Newman, S., 2005. Phosphorus cycling in wetland soils: the importance of phosphate diesters. Journal of Environmental Quality 34, 1921–1929.

Turner, B.L., Baxter, R., Mahieu, N., Sjögersten, S., Whitton, B.A., 2004. Phosphorus compounds in subarctic Fennoscandian soils at the mountain birch (*Betula pubescens*)—tundra ecotone. Soil Biology & Biochemistry 36, 815–823.

Uribelarrea, M., Cárcova, J., Otegui, M.E., Westgate, M.E., 2002. Pollen production, pollination dynamics, and kernel set in maize. Crop Science 42, 1910–1918.

van Elsas, J.D., Pereira, T.P.R.R., 1986. Occurrence of antibiotic resistance among bacilli in Brazilian soils and the possible involvement of resistance plasmids. Plant and Soil 94, 213–226.

van Elsas, J.D., Trevors, J.T., Starodub, M.E., 1988. Bacterial conjugation between pseudomonads in the rhizosphere of wheat. FEMS Microbiology Ecology 53, 299–306.

van Elsas, J.D., Nikkel, M., van Overbeek, L.S., 1989. Detection of plasmid RP4 transfer in soil and rhizosphere, and the occurrence of homology to RP4 in soil bacteria. Current Microbiology 19, 375–381.

van Schaik, E.J., Giltner, C.L., Audette, G.F., Keizer, D.W., Bautista, D.L., Slupsky, C.M., Sykes, B.D., Irvin, R.T., 2005. DNA binding: a novel function of *Pseudomonas aeruginosa* type IV pili. Journal of Bacteriology 187, 1455–1464.

Vogels, G.D., van Der Drift, C., 1976. Degradation of purines and pyrimidines by microorganisms. Bacteriological Reviews 40, 403–468.

Wackernagel, W., 1996. Persistence of DNA in the environment and its potential for bacterial genetic transformation. In: Schmidt, E.R., Hankeln, T. (Eds.), Transgenic Organisms and Biosafety. Horizontal Gene Transfer, Stability of DNA, and Expression of Transgenes. Springer, Heidelberg, Germany, pp. 137–146.

Wang, H., Li, J., Bostock, R.M., Gilchrist, D.G., 1996. Apoptosis: a functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. Plant Cell 8, 375–391.

Widmer, F., Seidler, R.J., Watrud, L.S., 1996. Sensitive detection of transgenic plant marker gene persistence in soil microcosms. Molecular Ecology 5, 603–613.

Widmer, F., Seidler, R.J., Donegan, K.K., Reed, G.L., 1997. Quantification of transgenic plant marker gene persistence in the field. Molecular Ecology 6, 1–7.

Willerslev, E., Hansen, A.J., Ronn, R., Brand, T.B., Barnes, I., Wiuf, C., Gilichinsky, D.A., Mitchell, D., Cooper, A., 2004. Long-term persistence of bacterial DNA. Current Biology 14, R9–R10.

Yenofsky, R.L., Fine, M., Pellow, J.W., 1990. A mutant neomycin phosphotransferase II gene reduces the resistance of transformants to antibiotic selection pressure. Proceedings of the National Academy of Science USA 87, 3435–3439.