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REVIEW

Extracellular DNA in soil and sediment: fate and ecological relevance

G. Pietramellara · J. Ascher · F. Borgogni · M. T. Ceccherini · G. Guerri · P. Nannipieri

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Abstract The review discusses origin, state and function of extracellular DNA in soils and sediments. Extracellular DNA can be released from prokaryotic and eukaryotic cells and can be protected against nuclease degradation by its adsorption on soil colloids and sand particles. Laboratory experiments have shown that DNA adsorbed by colloids and sand particles can be taken up by prokaryotic competent cells and be involved in natural transformation. Most of these experiments have been carried out under artificial conditions with pure DNA molecules and pure adsorbing matrices, but in soils and sediments, pure surface-reactive colloids are not present and DNA is present with other cellular components (wall debris, proteins, lipids, RNA, etc.) especially if released after cell lysis. The presence of inorganic compounds and organic molecules on both soil particles and DNA molecules can influence the DNA adsorption, degradation and transformation of competent cells. Extracellular DNA can be used as C, N and P sources by heterotrophic microorganisms and plays a significant role in bacterial biofilm formation. The nucleotides and nucleosides originated from the degradation of extracellular DNA can be re-assimilated by soil microorganisms. Extracellular DNA in soil can be leached and moved by water through the soil profile by capillarity. In this way, the extracellular DNA secreted by a cell can reach a competent bacterial cell far from the donor cell. Finally, the characterisation of extracellular DNA can integrate information on the composition of the microbial community of soil and sediments obtained by analysing intracellular DNA.

Keywords Extracellular DNA · Intracellular DNA · Soil and sediments · Transformation

G. Pietramellara (✉) · J. Ascher · F. Borgogni · M. T. Ceccherini · G. Guerri · P. Nannipieri
Department of Soil Science and Plant Nutrition,
University of Florence,
Piazzale delle Cascine 28,
50144 Florence, Italy
e-mail: giacomo.pietramellara@unifi.it

Introduction

The total soil DNA (tDNA) includes both intracellular (iDNA) and extracellular DNA (eDNA), with the latter originated from the former by active or passive extrusion mechanisms or by cell lysis. The eDNA can represent a relevant fraction of tDNA and thus a significant portion of the entire soil metagenome (Nielsen et al. 2006). Studies on the ecological relevance of eDNA in soils have concerned the gene transfer through transformation and interaction between eDNA molecules with the reactive surface of clay minerals, humic substances and sand particles (Nielsen et al. 2006). Only recently has the attention been directed at the role of eDNA in the formation of bacterial biofilm in soil (Whitchurch et al. 2002; Steinberger and Holden 2005; Boeckelmann et al. 2006) and at the possibility that eDNA can be transported by water through the soil profile (Agnelli et al. 2004; Ceccherini et al. 2007).

The aim of this review was to discuss the fate of eDNA, its ecological relevance as a source of genetic information and nutrients for microorganisms, its role in bacterial biofilm formation and the contribution of its genetic information for the better evaluation of the composition of microbial communities in soils. We shall also discuss the origin of eDNA, that is, how it is released from cells of roots, plant remains, fauna and microorganisms, the organisms commonly involved in soil functionality. Since

most of the available studies on eDNA concern its interaction in a pure state with pure surface-reactive colloids, the need for studying fate and functions of eDNA in situ is emphasised. We shall also discuss eDNA in sediments because: (1) their solid phase is similar to that of soil and the mechanisms protecting eDNA against nuclease can also be similar in both environments and (2) flooded agricultural soils, which are important due to the global relevance of rice as a crop, have similar properties to sediments. The bibliography on eDNA is not extensive; first studies on eDNA in aquatic environments, including sediments, were published in the eighties (DeFlaun et al. 1986, 1987; Paul et al. 1986), whereas the first reports on eDNA in soil were published by Torsvik and Goksøyr (1978) and Reanney et al. (1982). However, in order to limit the number of references, relevant reviews are cited more than the original literature.

The release of DNA molecules in soil

The release of eDNA in soil is poorly known, and for this reason, we shall mainly discuss what is known on the release of DNA from bacteria, fungal and plant cells in pure culture or in hydroponic solution.

Conditions causing cell death are important for the release of DNA molecules in soil (Ceccherini et al. 2003). Cell death of prokaryotic and eukaryotic cells can occur by physical damage of the cell, spontaneous or pathogen-induced cell lysis and necrosis, whereas cell death due to apoptosis and terminal differentiation can only occur in eukaryotic cells (Pietramellara et al. 2002).

Probably, the lysis of dead cells is the main source of eDNA in soil (Levy-Booth et al. 2007). The presence of active endonucleases can affect the postmortem integrity of DNA molecules before its release by cell lysis. However, Nielsen et al. (2007) reported that endogenous DNA degradation rapidly decreases after cell death due to cessation of nucleases synthesis and their proteolytic degradation. Indeed, DNA molecules escaped intracellular degradation and were released from decomposing tobacco and tomato leaf cells (Ceccherini et al. 2003; Potè et al. 2005, 2007).

In bacteria, the mechanism of DNA release varied with species (Lorenz et al. 1991; Dillard and Seifert 2001; Steinmoen et al. 2002). Concerning cell lysis, there are more chances for the released DNA to escape endonuclease degradation when it is released after bacterial cell lysis or when it is not caused by bacteriophages because some lytic or lysogenic bacteriophages, such as lambda and T7, encode exonucleases degrading intracellular DNA (Ahrenholtz et al. 1994). In addition to lysis, bacteria can release DNA by extrusion. This process is used as a survival strategy in *Deinococcus radiodurans* where damaged portions of genomic DNA are actively secreted and replaced via de novo synthesis (Battista 1997). The extruded DNA is generally purer than DNA released from germinating spores and lysed cells because the latter is usually associated with proteins, RNA, cell membrane residues, polysaccharides and other cell constituents (Fang and Hoh 1998; Tamayo et al. 1999). Many genera of environmental bacteria, such as *Acinetobacter*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Flavobacterium*, *Micrococcus* and *Pseudomonas*, release eDNA during growth in liquid media (Lorenz and Wackernagel 1994; Paget and Simonet 1994), and this release can be stimulated by the presence of other organisms (Matsui et al. 2003).

During biofilm formation by *Pseudomonas aeruginosa*, active eDNA extrusion involved acylated homoserine lactone signalling molecules and was inhibited by halogenated furanone (HF) (Givskov et al. 1996; Hentzer et al. 2002, 2003; Allesen-Holm et al. 2006). Another signalling system based on 2-heptyl-3-hydroxy-4-quinolone and denominated Pseudomonas quinolone signal was also involved in the extrusion of eDNA by *P. aeruginosa* (Pesci et al. 1999; McKnight et al. 2000). The DNA extrusion by *P. aeruginosa* can also occur by membrane vesicle lysis, another process regulated by quorum sensing (Allesen-Holm et al. 2006). Membrane vesicles are complex and chemically heterogeneous bilayered structures derived from the outer membrane of a wide variety of Gram-negative bacteria and retain the intrinsic lipid asymmetry of the outer membrane of the parent cell with most of the lipopolysaccharide positioned within the outer part of the membrane (Schooling and Beveridge 2006). The breaking of bacterial cell walls and membranes can also occur by autolysis (Palmen and Hellingwerf 1995) with release of both double-stranded DNAs and single-stranded RNAs (Nishimura et al. 2003).

The release of DNA from fungal cells has been less investigated than that of DNA from bacterial cells despite 70% of the DNA molecules in soil being of fungal origin (Smit et al. 1999; Borneman and Hartin 2000; van Elsas et al. 2000). Herdina et al. (2004) reported that the rate of DNA breakdown in dead fungal cells is so high that the contribution of fungal DNA to eDNA pool in soil should be negligible. However, Agnelli et al. (2004) found fungal eDNA in different horizons of a forest soil (Vallombrosa, Tuscany).

Several studies have investigated the persistence of plant DNA in the terrestrial environments (Widmer et al. 1996, 1997; Paget et al. 1998; Gebhard and Smalla 1999; Ceccherini et al. 2003; Lynch et al. 2004), but the release of DNA from plant roots and plant debris is still poorly understood. This release can occur (1) after autolysis and decomposition of wilting tissues, (2) by mechanical disruption of tissues or (3) by enzymatic degradation of cell structures by plant pathogens. It is well known that

chlorophyll, proteins and RNA molecules are enzymatically degraded during leaf senescence (Thomas and Stoddart 1980; Green 1994), and this probably also occurs for the DNA molecules. In addition, the persistence of released DNA depends on the activity of extracellular nucleases released from saprophytic bacteria and fungi growing on the plant residues. Only few reports have studied the release of DNA from plastids or mitochondria (Vincent et al. 1988; Thorsness et al. 1993; Nielsen et al. 2001, 2007). Probably, mitochondrial and plastid DNA are more protected than chromosomal DNA against degradation by cytoplasmic nuclease activity due to the presence of the organellar membranes (Nielsen et al. 2006). However, Ceccherini et al. (2003) reported a 98% loss of a chloroplast *aadA* gene in ground tobacco leaf material after 72 h as compared to a 56% loss of total DNA.

Mechanical disruption of plant cell walls and membranes resulting from herbivores, farming practices or attack by pathogenic bacteria may also lead to the release of intact or damaged DNA. Ceccherini et al. (2003) reported that DNA molecules of transplastomic tobacco leaves were not degraded for 5 days when leaves were infected by *Ralstonia solanacearum*, but there was a strong decrease in the transformation frequency of the recipient bacteria (*Acinetobacter* BD413), probably because transgenic plant DNA was diluted by the presence of the pathogen DNA.

Finally, during the decomposition of dead cells and tissues, particular environmental conditions, such as rapid dissecation, low temperatures, high salt concentrations or low pH values, can affect the activity and integrity of the endoenzymes, increasing the persistence of both cells or tissues and their DNA (iDNA; Chiter et al. 2000; Hofreiter et al. 2001).

In addition to endogenous degradation, other processes such as strands breaks, baseless sites, miscoding lesions and cross-links can affect the DNA molecule, and the chances for the occurrence of these processes increase by increasing the time between cell death and cell lysis (Willersleve and Cooper 2005).

In conclusion, the release of DNA after lysis of both microbial cells and plant tissues depend on a series of processes such as DNA degradation by nucleases, nuclease degradation by proteases and protection of DNA by impurities associated to the released DNA molecule. Extracellular DNA can also be released by bacterial extrusion (Fig. 1). The study of these processes in soil and the monitoring of the size of the released eDNA are needed to better understand the origin of eDNA in soil.

Extracellular DNA adsorbed by soil components

Soils are chemically complex and spatially heterogeneous with surface-reactive particles (clay, sand, silt and humic substance) which can adsorb nucleic acids (Ranjard and Richaume 2001). By using labelled DNA, it was observed that DNA adsorption reached a maximum 1 h after DNA addition to loamy sand or to a sandy loam soil (Blum et

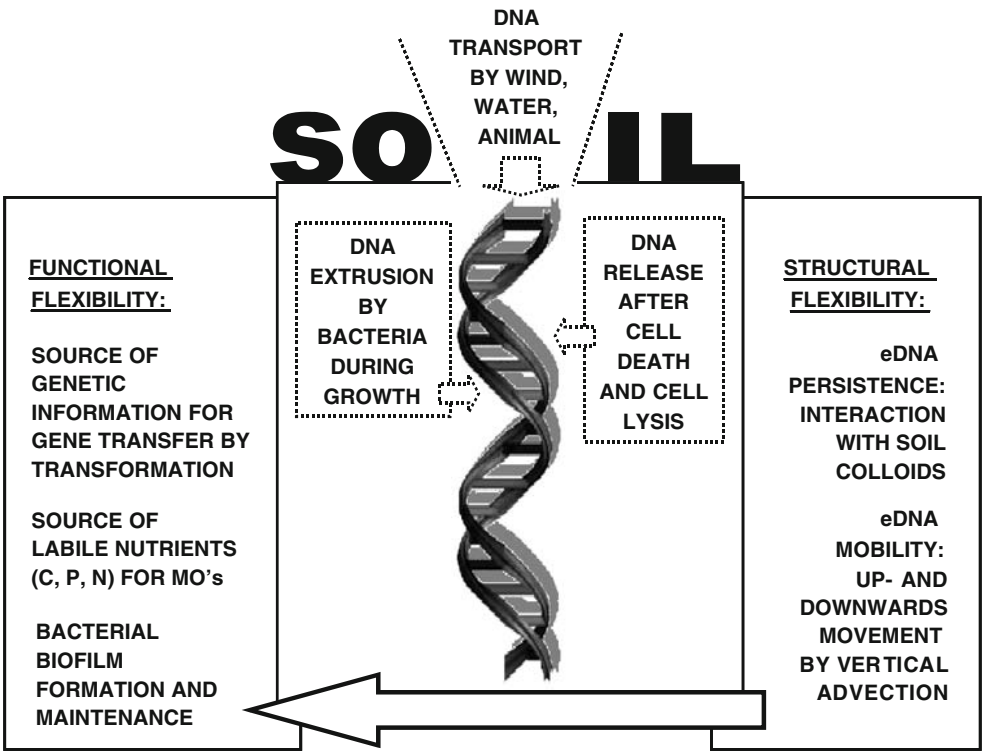

Fig. 1 Ecological relevance of extracellular DNA (*eDNA*) in soil

al. 1997). The adsorption of DNA by these surface-reactive soil particles depended not only on the length and conformation of DNA molecules but also on soil characteristics such as mineralogy, hydrophobicity or hydrophilicity of soil components, ionic composition and pH of soil (Ogram et al. 1988, 1994). Hydrophobic interaction can also be involved in the adsorption of DNA by soil particles. Indeed, water repellency is much more widespread than formerly thought (Shirtcliffe et al. 2005) because small amounts of soil organic matter (SOM) can drastically alter the surface properties, converting wettable mineral surfaces to water-repellent surfaces (Shirtcliffe et al. 2005). Here, we discuss the effects of the main hydrophilic (clays, sand minerals) and hydrophobic (humic acids) soil components on the adsorption and persistence of DNA as shown by in vitro experiments involving DNA and soil particles (Table 1). As proposed by Khanna and Stotzky (1992) and Khanna et al. (1998), we consider adsorbed DNA molecules to be retained by soil components at equilibrium conditions, whereas the residual molecules remaining attached to soil components after exhaustive water washing of those adsorbed are considered bound.

Clay minerals

Ogram et al. (1988) observed that clay-rich soils adsorbed more nucleic acids than clay-poor soils. Different types of clay fractions obtained from soil can adsorb and bind different amounts of DNA and have different thermodynamic properties. Coarse and organic clays obtained from an Alfisol and an Ultisol bound higher amounts of DNA than did fine and inorganic (after oxidation of organic matter by H_2O_2) clays, whereas the opposite was observed for the amount of the adsorbed DNA (Cai et al. 2006a). The DNA adsorption was endothermic for organic clays and exothermic for inorganic clays (Cai et al. 2006b). The DNA affinity was higher for pH-dependent charged particles than for permanent charged particles, and the desorption enthalpies were higher for the latter than for the former particles (Cai et al. 2006c).

Studies on the adsorption of pure DNA molecules by pure clay minerals made homoionic to cations (Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}) have shown that DNA adsorption depends on the type of clays and type of DNA (Greaves and Wilson 1969; Khanna and Stotzky 1992; Pietramellara et al. 2001), concentration and valency of the saturating cation (Greaves and Wilson 1969; Khanna and Stotzky 1992; Paget et al. 1992; Pietramellara et al. 2001) and pH value (Greaves and Wilson 1969; Khanna and Stotzky 1992; Paget et al. 1992; Pietramellara et al. 2001). Below pH 5, the phosphate moieties of DNA became protonated and can interact directly with permanent negative charges of the clays, whereas over pH 5 adsorption of DNA by negatively charged sites on clays depends on polyvalent cations (Ca^{2+}, Mg^{2+}, Al^{3+}), forming bridges between negatively charged phosphates moieties of DNA and the negative charges of clay minerals. Below pH 5, purified Ca–montmorillonite can adsorb 16 mg DNA per milligram of clay (Greaves and Wilson 1969).

Poly et al. (2000) studied the adsorption of supercoiled plasmid DNA and linear chromosomal DNA on clay minerals (montmorillonite and kaolinite) by low-temperature scanning electron microscopy. Supercoiled plasmid DNA formed bridges between clay domains by its molecular ends, whereas chromosomal linear DNA adsorbed on the clay

Table 1 Soil and sediments surface-reactive particles used to adsorb or bind DNA

Adsorbing material	pH	Cation	DNA			Reference
			Type	Molecular conformation	Purity	
Humic acid	3–4		Genomic	Linear	High	Crecchio and Stotzky 1998
Humic acid + Fe/AlOH	3–9	Fe/Al	Genomic	Linear	High	Crecchio et al. 2005
Sapropels	Acid		Genomic	Linear	High	Coolen and Overmann 2007
Native organic matter	5.8		Plasmid	Linear–supercoiled	High	Nguyen and Elimelech 2007a, b
Sand	6–8		Genomic		High	Ogram et al. 1988
Sand	7	Mix	Genomic–plasmid		High	Chamier et al. 1993
Clay (M)	3–5–9	Na/K/Ca/Mg	Genomic	Linear	High	Greaves and Wilson 1969
Clay (M)	7	Na/Ca/Mg	Genomic–plasmid	Linear–supercoiled	High	Paget et al. 1992
Clay (M)	7	Ca	Genomic–plasmid	Linear–coiled–supercoiled	High	Gallori et al. 1994
Clay (M,I,K)		Ca	Genomic	Linear–Supercoiled	High	Poly et al. 2000
Clay (M,K)	5–7	Ca	Genomic–plasmid	Linear–supercoiled	High	Pietramellara et al. 2001
Clay (M,K)	5–7	Ca	Genomic	Linear	High–low	Pietramellara et al. 2007a
Clay (M,K,G)	3–5–7	Mg	Genomic	Linear	High	Cai et al. 2006c
Clay (organic–inorganic)[a]	5–7	Ca	Genomic	Linear	High	Cai et al. 2006b

M montmorillonite, *I* illite, *K* kaolinite, *G* ghoetite

[a] Clays are extracted from soil by water sedimentation and successive oxidation by H_2O_2 to obtain the inorganic form

edges of kaolinite and on planar surface of montmorillonite. The ends of the DNA molecule are richer in negative charges than the middle part of the DNA molecule, as determined by nuclear magnetic resonance, and this different distribution, defined as a Coulombic end effect, should be considered in interpreting the adsorption of DNA on any solid surface (Stein et al. 1995). This can explain the fact that the adsorption of DNA by kaolinite or montmorillonite depends on the phosphate groups of DNA, particularly the location of acidic groups on the molecule, but not on the base composition and the type of molecular end (blunt or cohesive; Poly et al. 2000; Pietramellara et al. 2001).

Low-molecular-weight DNA was more adsorbed than high-molecular-weight DNA onto montmorillonite and kaolinite (Pietramellara et al. 2001), and chromosomal DNA was more strongly bound than plasmid DNA and RNA to clay surfaces (Franchi et al. 1999). Probably, the higher molecular mass of chromosomal DNA offers a higher number of binding sites to the surface of the clay minerals than the smaller molecular mass plasmid DNA (Pietramellara et al. 2001). Analysis of DNA–clay complexes by X-ray diffractometry and electron microscopy have shown that DNA binds primarily to the edges of clay surfaces, and the remaining DNA "tail" freely extends outwards (Paget and Simonet 1994; Khanna et al. 1998; Franchi et al. 1999). For these reasons, DNA interacted with a higher number of bonds on kaolinite than on montmorillonite (Pietramellara et al. 2001). Indeed, kaolinite presents higher ratios of edge surface/planar surface than montmorillonite, but also higher ratios of anionic exchange capacity/surface charge density, and anionic exchange capacity/cationic exchange capacity (Stotzky 1986; Paget and Simonet 1994; Alvarez et al. 1998; Franchi et al. 1999). Scatchard plot analysis of DNA adsorption on montmorillonite and kaolinite showed the existence of two different types of binding sites on clay minerals characterised by different affinities for the DNA molecule (Pietramellara et al. 2001). The number of binding sites depends not only on the type of clay but also on the type of DNA. The Scatchard plot analysis showed that adsorption of plant (*Nicotidiana* sp.), fungal (*Penicillum expansium*) and Gram-positive bacterial DNA occurred at two different sites on montmorillonite, whereas Gram-negative bacterial DNA was adsorbed at only one site. In addition, the plant DNA interacted with a higher number of sites on montmorillonite than the other types of DNA. Both Gram-positive and Gram-negative bacterial DNA, but not plant and fungal DNA, were desorbed by water washing of the clay–DNA complexes (Pietramellara, unpublished data).

Both the type and the concentration of DNA can mutually affect the adsorption of nucleic acids on montmorillonite (Crook Wyoming) made homoionic to calcium. Indeed, tobacco DNA was more adsorbed than two bacterial DNAs (one from a Gram-positive bacteria, *Bacillus subtilis* BD 1512 and one from a Gram-negative bacteria, *Acinetobacter calcoaceticus*) at low concentration, and the opposite was observed at higher DNA concentrations (Pietramellara, unpublished data).

DNA adsorbed on clay minerals such as montmorillonite, illite and kaolinite is protected against degradation by nucleases in soil (Greaves and Wilson 1969). Cai et al. (2006e) showed that the resistance of DNA adsorbed on kaolinite, montmorillonite, soil organic and inorganic clay to DNase I was not related to the DNA binding affinity to the solid surface but to the binding affinity of DNase I to the solid surface. Thus, kaolinite and soil inorganic clay particles showed a higher binding affinity for DNA molecule than montmorillonite and soil organic clay, but DNA adsorbed by the former was less resistant to DNase I than DNA adsorbed by the latter.

As already mentioned, the presence of pure DNA and clay minerals is rare in soil. For this reason, Pietramellara et al. (2007a) have compared the adsorption of not purified DNA and purified DNA on Ca^{2+} saturated montmorillonite and kaolinite. The presence of organic components (proteins, lipids and carbohydrates) and cellular wall debris strongly favoured the adsorption of chromosomal DNA on clay minerals, whereas the presence of cellular wall debris stimulated the binding of DNA on clays. The extracellular DNA fraction was protected against DNase by cellular wall debris, thus confirming what was already reported by Tavares and Sellsted (2001). Probably, DNA molecules have higher chances to survive in soil and to be involved in horizontal gene transfer when associated to cellular wall debris. However, the role of bacterial cell membrane debris in protecting released DNA is controversial since it was excluded by Nielsen et al. (2000), but suggested by Dupray et al. (1997) and Pietramellara et al. (2007a).

Sand particles

Sand can adsorb DNA and the adsorbed molecules are more resistant to degradation by nucleases than free DNA in solution (Lorenz and Wackernagel 1987). The adsorption of DNA on sand particles was positively correlated with its molecular weight (Ogram et al. 1988). The adsorption of calf thymus DNA or DNA from *B. subtilis* on quartz sand was maximum after 2 h at 23°C and was positively affected by NaCl or $MgCl_2$ (Aardema et al. 1983; Lorenz and Wackernagel 1987). Adsorption of supercoiled plasmid DNA on quartz surface was higher with divalent than monovalent cations (Lorenz and Wackernagel 1987; Romanowsky et al. 1991), and the use of a quartz crystal microbalance with dissipation and dynamic light scattering showed that the adsorbed DNA layer was more compact and rigid with divalent than monovalent cations even if the

former were present at lower concentrations (Nguyen and Elimelech 2007a). This occurred because the divalent cation bridges between quartz and DNA molecule caused a lower hydration of the adsorbed DNA molecule than the monovalent cations (Na^+; Nguyen and Elimelech 2007a).

Humic substances

Both chromosomal and plasmid DNA from *B. subtilis* were adsorbed to humic acids extracted from a forest soil in a pH interval from 3 to 4 (Crecchio and Stotzky 1998). DNA bound to humic acids was more resistant to DNase I degradation than free DNA, and it retained the ability to transform competent recipient cells of *B. subtilis*.

The DNA from *B. subtilis* was rapidly adsorbed (maximal adsorption after 2 h) by complexes of montmorillonite–humic acids with Al (Al–M–HA) or Fe (Fe–M–HA) hydropolymers in the pH range from 3 to 10 (Crecchio et al. 2005). The adsorption was higher on Al–M–HA than on Fe–M–HA, and no desorption occurred by washing the DNA complexes with ddH_2O, 0.1 M NaCl or 0.1 M $Na_4P_2O_7$ solution. The DNA of these complexes transformed competent cells of *B. subtilis* at a lower frequency than free DNA, but it was more protected against degradation by DNase I than free DNA.

Cai et al. (2007) observed a higher protection of DNA against degradation by nucleases when it was adsorbed by clay organic matter rather than by inorganic clays. Probably, DNA molecule penetrated through macropores of the network of the organic matter covering the clay surface, and in this way, it was less accessible to nucleases.

Both linear and supercoiled plasmid DNA adsorbed onto silica surface-coated with SOM could not be desorbed by washing with water (Nguyen and Elimelech 2007b). In addition, linear DNA formed a thicker and less compact adsorbed layer than the supercoiled DNA.

As already reported, soil water repellency mostly depends on the presence of SOM and is high when the soil particles are coated with a layer of organic material (Shirtcliffe et al. 2005). Extracellular DNA could be adsorbed on hydrophobic surface at low pH value due to denaturation of the molecule. By increasing the pH value over 5, the inner part of the DNA molecule took again the original double-strand conformation, whereas the molecular extremities remained denaturated, forming strong bonds with the hydrophobic surface, and for this reason, DNA could not be desorbed (Allemand et al. 1997).

Conformational changes of the DNA molecule

The conformation of DNA molecules can change by changing environmental conditions and during the adsorption on clay minerals. This can affect the behaviour of the DNA molecule such as its availability to bacterial transformation. Generally, changes in the conformation of the DNA molecule have been neglected in the adsorption and binding of DNA by surface-reactive soil particles. Divalent metal cations can stimulate DNA condensation (Ma and Bloomfield 1994), DNA aggregation (Duguid and Bloomfield 1995) and modification of the secondary and tertiary structure of the DNA molecule (Andrushchenko et al. 1997; Arakawa et al. 2000). Melzak et al. (1996) and Nguyen and Elimelech (2007a, b) showed that the low electrolytic strength of the aqueous solution and the presence of cations with low valency can favour the linear conformation of the DNA molecule, whereas high electrolytic strength and the presence of cations with high valency favoured the presence of the coiled form. Gueron et al. (2000) and Sun et al. (2002) showed that the condensation morphologies of the DNA molecule depend on the cations, whereas Sitko et al. (2003) reported that molecular base composition was important in the condensation process, with GC-DNA being more easily condensed than AT-DNA. The DNA molecule can collapse from 3D to 2D after its adsorption and binding on solid surface (Fang and Hoh 1998) and can change its conformation from β to Z form when bound to kaolinite, whereas the original conformation is preserved when bound to montmorillonite (Cai et al. 2006e). Molecular conformation can also change after desorption; indeed, DNA desorption from kaolinite changed the molecular conformation from β to C form as a consequence of the dehydration of the DNA molecule (Melzak et al. 1996).

Future research on the DNA adsorption

The bibliography on this topic is more extensive on clays than on humic and sand materials and, due to relevance of humic molecules in soil functionality, future research is needed to better understand the underlying mechanisms of eDNA adsorption and binding by humic molecules and the resistance of the adsorbed/bound DNA to nucleases. Changes in DNA conformation due to the used experimental conditions should be considered in adsorption /binding of eDNA by surface-reactive soil particles. Further research should also concern the role of cell impurities associated to DNA molecules after cell lysis on the adsorption /binding of eDNA and should also involve clays, of which the surfaces are covered by inorganic (e.g. iron oxides) and/or organic compounds (e.g. humic molecules), since in soil, surface-reactive particles are not present as pure particles. It is obvious that what is suggested for soil is also valid for sediments due to the similarity of surface-reactive particles of both environments. The distinction between DNA adsorption and DNA binding on surface reactive particles

of sediments should be carried out as suggested by Khanna and Stotzky (1992) for soil particles.

Extracellular DNA in soil and sediments

Extracellular DNA in soil

Concentrations of eDNA in soil can range from 0.03 to 200 μg g^{-1} of soil (Table 2; Torsvik and Goksøyr 1978; Reanney et al. 1982; Selenska and Klingmueller 1992; Frostegård et al. 1999; Agnelli et al. 2004), and they decrease along the soil profile (Fritze et al. 2000; Agnelli et al. 2004, 2007).

Extracellular DNA can be leached by water, transported by vertical advection towards soil surface (Potè et al. 2003; Agnelli et al. 2004; Gulden et al. 2005; Ceccherini et al. 2007) or adsorbed more or less strongly by soil particles as shown by in vitro studies (Khanna and Stotzky 1992; Paget and Simonet 1994; Demanèche et al. 2001a; Pietramellara et al. 2001, 2007a, b). Eukaryotic DNA has a higher structural complexity and molecular size than prokaryotic DNA, and these molecular differences can affect DNA adsorption and degradation rates in soils (Pietramellara et al. 2006). Adsorption of eDNA in soil is always affected by the presence of other compounds as those released by roots. Low-molecular-weight organic acids (LMWOA), similar to those secreted from plant roots, can compete with DNA molecules for adsorption and binding on colloids if both are added together to soil (Cai et al. 2006d). However, when LMWOA were added before, DNA adsorption on soil colloids was stimulated because probably, Al^{3+} cations, solubilised by LMWOA, acted as bridges between soil colloids and the DNA molecule.

The location of the DNA release in the extracellular soil environment can be important for avoiding degradation and inactivation of the DNA molecule. For example, DNA released in microaggregates or micropores can be accessible to nucleases released from the same or other near lysed or active cells, but it may not be accessible to nucleases released by microorganisms located out of the specific micropore (Blum et al. 1997; Benedik and Strych 1998; Nielsen 2003; Nielsen et al. 2004) because during the diffusion of these enzymes to the eDNA, they can be adsorbed by soil particles or degraded by extracellular proteases (Nielsen et al. 2007). Most of the extracellular nucleases, responsible for the eDNA degradation in non-sterile soil (Blum et al. 1997; Anderson and Magdoff 2005), were associated with a restricted number of bacterial species (Benedik and Strych 1998), and these enzymes were considered to be responsible for the eDNA degradation in non-sterile soil (Blum et al. 1997). Unbound DNA in the soil solution was completely degraded by DNase I at lower concentrations (1 μg ml^{-1}) than those needed for the degradation of clay-bound DNA (Demanèche et al. 2001a). In soil microcosms, the residual amount of the target eDNA was never higher than 6% of the added amount when extractions were carried out immediately after the DNA addition to soil (Frostegård et al. 1999; Demanèche et al. 2001a).

As already mentioned, a better understanding of the behaviour of eDNA in soil requires to monitor not only its concentration and localisation but also its adsorption to surface-reactive particles, its movement by the aqueous soil phase and the complex equilibrium between active nucleases and active proteases. In addition, the type of eDNA release by cells should be also investigated. The size of the released DNA is important for detecting the target genes and the relative behaviour in the extracellular soil environment. We have not discussed here the role of eDNA in bacterial biofilms because they are mainly formed when soil is submerged by water (Pietramellara et al. 2006).

Table 2 Reports and yields of extracellular DNA (eDNA) extraction from soil, water and sediments

Environment	eDNA yields	References
Sea water particulates	568–3,163 ng/ml water	Bailiff and Karl 1991
Sea sediments	1,460–1,690 ng/g wet sediment	Dell'Anno et al. 2002
Sea sediments	670–24,300 ng/g dry sediment	Corinaldesi et al. 2005
Lakes water	5,000–70,000 ng/l water	Siuda et al. 1998; Siuda and Chrost 2000
Forest lake water	9–11 ng/ml water	England et al. 2005
Forest lake sediment	69–520 ng/g dry sediment	
Different soils	80–1,949 ng/g dry soil	Niemeyer and Gessler 2002
Forest soils	460–1,590 ng/g dry soil	Blagodatskaya et al. 2003
Forest litter	90–555 ng/0.5 g moist weight litter	England et al. 2004
Forest soil	1,700–1,900 ng/g dry soil	Agnelli et al. 2007
Forest soil	6,000 ng/g dry soil	Pietramellara et al. 2007b
Forest soil		Bladogaskaya et al. 2003; Panikov 2007

Extracellular DNA in submerged soils, sediments and bacterial biofilms

The extracellular DNA in the aquatic environment and soil sediments can be: (1) a source of genetic information to be used in gene transfer (vertical and horizontal gene transfer), (2) a source of nutrients for bacteria and (3) a constitutive component with other macromolecules, such as exopolysaccharides and proteins, in bacterial biofilms (Sutherland 2001). In the initial phase of biofilm formation, eDNA is acting as a cell-to-surface and cell-to-cell adhesion agent (Li et al. 2002; Steinberger et al. 2002); it is generally released by active extrusion and is probably protected by methylation against DNaseI digestion in the biofilm matrix of younger culture, but not in the older bacterial cultures (Bockelmann et al. 2006). Whitchurch et al. (2002) suggested that the resistance of eDNA to DNase I digestion in bacterial biofilm matrix was due to the proteases extrusion by bacteria that locally inactivated the DNase I. According to Steinberger and Holden (2005) and Bockelmann et al. (2006), further research is required to study the molecular conformation (circular or linear) of eDNA in the biofilm matrix and to determine the type of organic molecules associated with this eDNA.

Steinberger and Holden (2005) compared the fingerprints of eDNA to those of iDNA by randomly amplified polymorphic DNA analysis (RAPD) in multiple-species biofilms (*Pseudomonas aeruginosa*, *P. putida*, *Rhodococcus erythropolis* and *Variovorax paradoxus*). The eDNA production in biofilms was species-dependent and the phylogenetic information of this DNA was distinct from that of either tDNA and/or iDNA. It was suggested that the eDNA network in bacterial biofilms might be an adaptation of bacteria to the aquatic environment; the network might allow the reciprocal cooperation among different bacterial cells and the access of bacterial cells to suspended particulate organic matters (Bockelmann et al. 2006).

Dell'Anno and Danovaro (2005) reported that the concentration of eDNA into deep-sea sediments can be high (0.31±0.18 g of DNA per square metre in the top centimeters) and represents more than 90% of the tDNA (Table 2). However, this amount depends on the type of aquatic environment, being of 0.6 and 88 μg l^{-1} in oligotrophic oceanic samples and a hypereutrophic freshwater pond, respectively (Karl and Bailiff 1989). The extracellular DNA concentration of aquatic environments decreases with water depth and distance from the shore (DeFlaun et al. 1986, 1987; Paul et al. 1986, 1987); the adenine–thymine (A–T) content decreases by increasing sediment depth (Dell'Anno et al. 2002). Most of the eDNA in sediments is bound or adsorbed to particles (Dell'Anno et al. 2002; Dell'Anno and Corinaldesi 2004; Dell'Anno and Danovaro 2005). It has been calculated that about 50% of eDNA in sediments is protected against nucleases degradation (Dell'Anno et al. 2002). England et al. (2004) have shown that the persistence of chromosomic DNA from *Choristoneura fumiferaia* in sediments was shorter than that in the above water column. On the contrary, the persistence of a 530-bp DNA fragment from genome of CfMNPV*egt*$^{-}$/*lacZ*$^{+}$ added to an aquatic artificial microcosm was longer in sediments (21 days) than in the water column (24 h), probably because eDNA in water was rapidly degraded by nucleases produced by bacteria and/or bound to suspended particles (Ruiz et al. 2000; England et al. 2005). Finally, Dell'Anno and Corinaldesi (2004) reported that degradation rates of eDNA in sediments were seven to 100 times higher than those in water columns, but eDNA of sediments seemed to persist longer (days) than in water (hours) due to the higher eDNA content of the sediments.

Most of eDNA in sediments derived from bacterial active extrusion, rather than from cellular lysis, has a high molecular size and often contains gene sequences different from those of the intracellular 16S rRNA (Whitchurch et al. 2002; Corinaldesi et al. 2005).

The eDNA of aquatic environments can be derived from far sources. The *cry1Ab* gene (codifying for protein δ-endotoxin and derived from *Bacillus thuringensis krustaky*) inserted and expressed in corn cropped near a river was found in the river surface water and sediments 82 km far away from the crop site (Douville et al. 2007).

Ecological relevance of extracellular DNA in soil and sediments

The eDNA in soil can have an ecological relevance similar to that already mentioned for sediment. Indeed, also in soil, it can be an extracellular gene pool available for bacterial transformation and can be used as a source of nutrients and/or nucleotides for synthesis of DNA by soil microflora (Finkel and Kolter 2001; Wackernagel 2006; Levy-Booth et al. 2007). In addition, the phylogenetic information of eDNA can integrate those of iDNA for a better evaluation of the composition of microbial communities of soils (Pietramellara et al. 2007b).

The persistence of eDNA is important for its ecological role, and in the surface layer (0–15 cm) of soil, it can range from a few days to several years (Nielsen et al. 2004). The persistence of chromosomal DNA from baculovirus *Choristoneura fumiferana* (C*f*MNPVegt^{-}/*lacZ*$^{+}$) added to a forest litter microcosm, sediment and water column was monitored by polymerase chain reaction (PCR) analysis and found to be the longest (3 months) in the forest litter microcosm (England et al. 2005). Levy-Booth et al. (2007) suggested that in frozen or desiccated soil samples, enzymatic degradation of eDNA markedly decreased the concentration of eDNA without excluding the occurrence of intracellular processes such as

degradation by chemical hydrolysis, chemical oxidation or DNA cross-linking (Willereslev and Cooper 2005). The base composition, methylation and the conformation of the DNA molecule can affect its degradation. Indeed, positive correlation between the G+C content of the DNA molecule and its resistance to degradation was shown by Hofreiter et al. (2001). The presence of single-strand configuration can also protect the DNA molecule from enzymatic degradation (Nielsen et al. 2007). The only study monitoring the persistence of fungal DNA in soil was carried out by Herdina et al (2004) who observed a rapid degradation of eDNA from *Gaeumannomyces graminis* (var. *tritici*).

The use of transgenic plants in agriculture has promoted studies on the fate of transgenic plant DNA in soil by monitoring the inserted marker genes (Lynch et al. 2004). The persistence of this DNA in both microcosm and field conditions depends on environmental conditions stimulating microbial activity such as soil moisture and temperature (Widmer et al. 1996, 1997; Paget and Simonet 1997; Gebhard and Smalla 1999; Bruinsma et al. 2003; DeVries et al. 2003). The degradation rate of transgenic plant DNA is expected to be identical to that of DNA of the conventional variety, unless the transgenes have caused metabolic changes in the plant with production of plant tissues more or less susceptible to decomposition (Lynch et al. 2004). The persistence of an entire transgenic cassette of rhizomania-resistant sugar beet plants was monitored by extracting tDNA from soil treated with transgenic litter or from soil taken from plots planted with transgenic plants (Gebhard and Smalla 1999). The extracted DNA was amplified with three different primers. Six months after the introduction of the litter into soil, the construct-specific DNA was detected with all three primer sets; however, after 18 and 24 months, PCR amplification only occurred with one primer, suggesting that the construct was partially degraded. DeVries et al. (2003) observed the persistence of DNA from transgenic potato in the field site for 8 months; they further observed its transforming potential up to 4 years of soil storage at 4°C, moisture and darkness. However, both Gebhard and Smalla (1999) and DeVries et al. (2003) did not evaluate if the transgenic DNA persisted as eDNA bound to soil particles, as iDNA of plant cells or as iDNA of transformed bacterial cells. Transgenic plant DNA can be degraded not only by soil microorganisms degrading the debris but also during the senescence preceding leaves fall (Ceccherini et al. 2003). However, despite both degradation processes, measurable amounts of transgenic plant DNA were released and were available for bacterial transformation (Ceccherini et al. 2003).

The presence of stable C and N isotopes has been monitored to determine the age of tDNA and eDNA in soil. The age and the mean residence time of eDNA from the top to the deep horizon of a forest soil profile ranged from 33,000 to 38,000 years before present (YBP) and from 100,000 to 900,000 years, respectively (Agnelli et al. 2007). It was suggested to use these values with a certain caution since eDNA isotopic signatures might have been affected by fossil C associated to reagents used in the purification of eDNA even if these reagents were not revealed by gas chromatography–mass spectrometry analysis. The natural abundance of δ13C, δ15N of tDNA of nine different soils was influenced by isotopic composition of soil organic matter (Schwartz et al. 2007).

Due to the large discrepancy in the number of studies on plant and bacterial eDNA versus fungal eDNA, future research should reduce this gap due to the importance of fungal communities in soil. In addition, future research should also concern ancient DNA fragments extracted from animal, plant or microbial fossils of soil so as to use them as a natural DNA clock and get insights in the biological history of soil (Willerslev et al. 2004a,b; Willerslev and Cooper 2005). In this contest, the use of paleo-soil or paleo-horizon might be helpful.

Extracellular DNA as a source of nutrients

Extracellular DNA, whose concentration can range from 0.2 to 44 $\mu g\ l^{-1}$ in various marine and aquatic environments (Finkel and Kolter 2001) (Table 2) and from 10% to 60% of tDNA pool in a forest soil profile (Agnelli et al. 2004), is a P-rich molecule (10%, *w*/*w*; Turner et al. 2004; Turner and Newman 2005) that can also release C and N upon its complete degradation by microorganisms. Dell'Anno and Danovaro (2004, 2005) calculated that the use of eDNA alone can supply 4.7 and 47% of the prokaryotic C, N and P daily needs in sediments, respectively.

Both heterologous and homologous eDNA can be taken up by *Haemophilis influenzae* and *Nesseria gonorrhoeae* through membrane proteins and used for natural transformation, for repairing genomic DNA damaged portions or as nutrient sources (Sthouthamer 1979; Finkel and Kolter 2001; Mishra 2002; Levy-Booth et al. 2007). Despite the different possibilities for use, probably, eDNA is taken up by bacterial cells by only one mechanism. If this hypothesis is true, we do not know if the system for de novo synthesis or nutrient acquisition arose first and then evolved into the system for genetic transformation or vice versa. The ability to shift from the use of eDNA as a source of genetic informations to that as a source of nutrients can be an environmental advantage in relation to bacterial needs.

Extracellular DNA as a source of genetic information

Several reviews have discussed the factors affecting genetic transformation, competence development and DNA uptake processes by bacterial cells (Saunders and Saunders 1988;

Stewart 1992; Lorenz and Wackernagel 1994; Paget and Simonet 1994; Pietramellara et al. 2006). Bacterial transformation can only occur if the bacterial cell is in a particular physiological state named "competence". The development of competence is usually monitored by detecting bacterial transformation, which involves binding of exogenous DNA on the external surface of the bacterial cell, DNA uptake usually as a single strand, insertion of the single strand in the chromosome or double strand plasmid reconstruction and gene expression. Competence is physiologically regulated and inducible in several naturally transformable bacterial species by factors such as nutrient availability, calcium concentration, pH value and temperature (Lorenz and Wackernagel 1994; Solomon and Grossman 1996), whereas constitutive competence has been found only in *N. gonorrhoeae* (Sparling 1996). In Gram-positive bacteria, competence is usually induced and controlled by competence factors, which are secreted into the medium (Saunders and Saunders 1988) and have to reach a certain concentration to be effective (Stewart 1992). On the contrary, competence of Gram-negative bacteria is usually internally regulated (Stewart 1992; Paget and Simonet 1994).

In many competent bacterial species, uptake of DNA only occurs after digestion of eDNA into smaller fragments by endonucleases of the bacterial membrane, probably because only small DNA fragments can be transported across cell membrane (Stewart and Carlson 1986; Lorenz and Wackernagel 1994; Dubnau 1999). However, the size of the DNA fragments is important for the transformation of the recipient competent bacterial cell because too small DNA fragments could carry a trunked copy of the gene of interest (Zawadzki and Cohan 1995). In addition, bacterial transformation occurs more with homologous than heterologous chromosomal DNA, since the former has more chances to be integrated into the host's chromosome by normal recombination processes. However, plasmid DNA without homology to the host can be recircularised by mismatch repair if multiple copies are taken up (Doran et al. 1987; Frischer et al. 1994), allowing transformation between taxonomically distant donor and recipient bacteria. For example, Paul et al. (1992) demonstrated transfer of a small non-conjugative plasmid from *Escherichia coli* to a marine *Vibrio* by natural transformation.

It is generally believed that bacterial transformation by eDNA in soil occurs at very low frequency, often below the detection limit (Gebhard and Smalla 1999; Thomas and Nielsen 2005). Since natural competence has been generally studied with culturable bacteria, it is reasonable to suppose that the number of bacterial species capable of being transformed in soil is higher than generally believed due to the large prevalence of unculturable over culturable bacteria in soil (Mercier et al. 2006). Lorenz and Wackernagel (1991) suggested that the induction of competence in soil is not related to cell density but to nutrient availability. If this hypothesis is true, bacterial transformation should be higher in soil hot spots such as the rhizosphere, residuesphere, bacterial biofilms and preferential flow pathways where nutrient concentration can be higher than in bulk soil; these sites are also characterised by a high richness and evenness of microbial communities (Beven and Germann 1982; Flury and Flühler 1994; Bundt et al. 2001; Pietramellara et al. 2002). However, different bacterial species (almost 90), including soil bacteria as *B. subtilis* and *Acinetobacter* sp., can be transformed (DeVries and Wackernagel 2004). Transfer of genes of eDNA can also occur in soil by electrotransformation due to thunderstorms and lightning discharges (Lurquin 1997). Indeed, Demanèche et al. (2001b) showed that electrical field and current could make bacterial cell membranes more permeable, permitting the penetration of eDNA into the non-competent bacterial cells.

Bacterial transformation can have important implications on the fate of eDNA and on bacterial evolution in soil. Indeed, once bacterial transformation has occurred, the genetic information of eDNA can be successively spread into the soil bacterial population by conjugation and/or transduction (Nielsen et al. 2007). Despite the relevance of bacterial transformation in the bacterial evolution, the occurrence of the process in soil has been poorly studied, whereas more information are available on transformation of specific competent bacteria by eDNA adsorbed/bound to surface-reactive soil particles. Khanna and Stotzky (1992) showed natural transformation of *B. subtilis BD 630* with chromosomal DNA of *B. subtilis* BD 1512 adsorbed to montmorillonite. Chromosomal and plasmid DNA bound to montmorillonite and kaolinite retained the ability to transform competent bacterial cells after three to four and one to two cycles of wetting and air drying, respectively (Pietramellara et al. 1997). Six hours of wetting were required for the clay–DNA complexes to regain transforming activity. It was hypothesised that changes in structural conformation of the DNA molecule occurred under the dry condition, making the DNA molecule unable to transform competent bacterial cells. Vettori et al. (1996) and Alvarez et al. (1998) reported that DNA from *B. subtilis* or calf thymus DNA bound to montmorillonite could be amplified by PCR and suggested that both adsorbed DNAs could transform bacterial competent cells. However, plasmid DNA bound on kaolinite, illite and montmorillonite was only partially available for transformation of *Acinetobacter* sp. BD413, and there was a positive correlation between the availability of DNA to competent bacterial cells and the availability of DNA to nuclease degradation (Demaneché et al. 2001a).

Pietramellara et al. (2007a) have compared the transforming frequency of not pure and pure DNA both adsorbed on Ca saturated montmorillonite and kaolinite.

Cellular wall debris (cwd) stimulated bacterial transformation when the not pure DNA+cwd was adsorbed on clay fractions, whereas the presence of organic molecules such as proteins, lipids and carbohydrates (released after cell lysis) negatively affected bacterial transformation. As previously reported, cwd can partially protect the DNA molecule against nuclease degradation.

Both chromosomal and plasmid DNA bound on humic acids transformed competent cells of *B. subtilis* in in vitro experiments and were more protected against degradation by DNase I than free DNA (Crecchio and Stotzky 1998). Competent cells of *B. subtilis* were transformed by chromosomal DNA bound on Al or Fe–montmorillonite–humic acids complexes (Crecchio et al. 2005).

Adsorption of bacterial DNA on sand particles increased transformation frequencies of *B. subtilis* by 20 to 50 times with respect to values of bacterial DNA in liquid culture (Lorenz et al. 1988). Transformation frequencies of *Pseudomonas stutzeri* and *Acinetobacter* sp. cells were also higher with plasmid DNA adorbed by sand particles than with free DNA, particularly at suboptimal temperatures (Lorenz and Wackernagel 1990).

In conclusion, both the development of bacterial competence and the frequency of bacterial transformation in soil are poorly known and future research is needed even if it is difficult to design suitable approaches for studying these processes in situ.

Bacterial transformation by extracellular DNA in sediments

The bibliography on bacterial transformation is more extensive in water than in sediments (Matsui et al. 2001). A plasmid containing the green fluorescent protein gene (*gfp*) was taken up by indigenous bacteria isolated from two Japanese rivers (Maruyama et al. 2006). Paul et al. (1991) suggested that transformation of *Vibrio* spp. probably occurred with higher frequencies in the water column than in the sediment and in the estuarine locations with higher nutrients input than in offshore waters. Plasmid transformation using *E. coli* donor cells and hFT vibrio recipients has been observed in aquatic environments (Paul and Williams 2004), and contrary to what was generally thought, *E. coli* can develop natural competence in freshwater (Baur et al. 1996). However, the transfer of non-conjugative plasmids between *E. coli* donor cells and hFT *Vibrio* recipients is DNase-sensitive, and thus, the contact between the two cells is essential for the occurrence of transformation process in marine and aquatic environments (Paul and Williams 2004). Molin and Tolker-Nielsen (2003) suggested that probably, bacteria did not develop competence in young biofilms because there was no accumulation to a sufficient high concentration of the competence-stimulating signal molecules. However, in old biofilms, the conditions for bacterial transformation are also unfavourable because the diffusion of competence factors is not favoured by the solid-like extracellular matrix and bacterial cells are generally non-metabolically active. Therefore, intermediate (not young and not old) biofilms probably support the best conditions for bacterial transformation when both the recipient bacteria and the donor cell are present. The lysis of bacterial cells in a bacterial biofilm can produce a local DNA concentration higher than 100 μg ml^{-1} (Hendrickx et al. 2003).

Both chromosomal and plasmid DNA adsorbed on sea sand particles transformed *A. calcoaceticus* cells (Chamier et al. 1993); transformation frequencies of plasmid DNA adsorbed on sand particles were significantly lower than those of plasmid DNA in solution, whereas the opposite occurred for chromosomal DNA.

Extracellular DNA: methods, structure and functionality of microbial communities in soil and sediments

Pietramellara et al. (2007b) have proposed to characterise eDNA of soil for a better evaluation of the composition of microbial communities by considering what was reported by Agnelli et al. (2004) and Corinaldesi et al. (2005) who showed that eDNA from soil and aquatic sediments, respectively, can contain gene sequences different from those of the respective iDNA. The characterisation of tDNA, iDNA and eDNA has been also proposed by Steinberger and Holden (2005) for a multiple-species unsaturated biofilm cultivated in laboratory.

Three different approaches can be used to estimate the amount of eDNA in soil and sediments: (1) by subtracting the amount of iDNA from tDNA, (2) by extracting eDNA and (3) by plotting the amount of microbial double strand DNA (*y*-axis) versus microbial biomass (*x*-axis) when microbial growth is stimulated by adding available nutrients (Blagodatskaya et al. 2003; Panikov 2007). In the case of a significant and positive correlation, the extrapolation of microbial biomass to zero gives a positive intercept on the *y*-axis corresponding to the amount of eDNA in soil.

Despite the existence of several methods for the extraction of iDNA from soil (Robe et al. 2003; Bakken and Frostegård 2006; Whitehous and Hannah 2007; Lakay et al. 2007), a few methods have been proposed to extract eDNA from soils and sediments; the latter ones are usually based on the absence of harsh chemicals in the extracting solution or physical disruption steps so as to avoid cell lysis. The mild DNA desorption is usually obtained by slightly alkaline solutions (pH 8) like sodium phosphate, Tris–ethylenediaminetetraacetic acid (EDTA) and Tris–HCl buffer (Ogram et al. 1987; Blum et al. 1997; Marstorp and Witter 1999; Niemeyer and Gessler 2002; Agnelli et al.

2004, 2007; Wackernagel 2006). The main scope of these extractions is to eliminate eDNA before the extraction of iDNA without considering any degradation of eDNA during extraction.

Concerning the direct extarction of eDNA from soil and sediments, an efficient extraction method should: (1) extract high quantity of eDNA so that the extracted eDNA is representative of that present in situ; (2) avoid changes in the extracted DNA so that it can be analysed by molecular techniques and (3) be done simultaneously or sequentially with iDNA of the same sample so as to permit comparative analysis (Corinaldesi et al. 2005). In addition, an efficient procedure to extract eDNA from soil should also include DNase inhibitors like aurintricarboxylic acid and EDTA to prevent this degradation (Marstorp and Witter 1999; Niemeyer and Gessler 2002). Agnelli et al. (2004, 2007) extracted eDNA from soil using the pioneer method proposed by Ogram et al. (1987). Pietramellara et al. (2007b) have scaled up this eDNA extraction and purification protocol and tested the new procedure on soil samples taken from horizons of a forest soil profile; the soil samples covered a wide range of physical, chemical and microbiological properties. England et al. (2004) have extracted eDNA from forest litter by sodium pyrophosphate (pH 8.0) with successive purifications of the extracted DNA by Sephadex G75 and Glass milk.

Extracellular DNA has been extracted from aquatic microcosms (England et al. 2004) and from sediments (Corinaldesi et al. 2005) by sodium pyrophosphate (pH 8); the extracted eDNA has been precipitated by adding an equal volume of isopropanol and one tenth volume of 5 M NaCl, previously filtered through 0.2-μm pore size membrane filter (Anotop 25; Whatman), to eliminate viruses and bacterial cells and successively precipitated with cetyltrimethylammonium bromide solution.

Only a few studies have characterised eDNA extracted from soil and future research should concern characterisation of eDNA extracted from soil differing in properties and management. Genetic information of eDNA should be compared with those of iDNA to evaluate the contribution of eDNA to the determination of the composition of soil microbial community.

Conclusions

Extracellular DNA can be adsorbed by surface-reactive particles in soils and sediments, being protected against degradation by nucleases and maintaining the capacity to transform competent bacterial cells. According to Gebhard and Smalla (1999) and Thomas and Nielsen (2005), bacterial transformation by eDNA can occur in soil at very low frequencies. However, natural competence has been studied with culturable bacteria and it is reasonable to hypothesise that transformation in soil may be more important than generally supposed due to the large prevalence of unculturable bacteria in soil. Protection against nuclease degradation can be due not only to the adsorption of DNA by surface-reactive particles but also to adsorption of nucleases by these particles with the consequent decrease in enzyme activity (Khanna and Stotzky 1992; Demanèche et al. 2001a). The purine and pyrimidine content of DNA may affect its enzyme degradation in soil (Levy-Booth et al. 2007). Most of the information on eDNA adsorption and binding by surface-reactive particles and on the role of these eDNA fractions in bacterial transformation derives from laboratory experiments under conditions different from those occurring in situ. Therefore, further research is needed to study the release and the behaviour of eDNA in situ and/or in laboratory conditions more similar to those occurring in situ (Pietramellara et al. 2007a,b).

Further research should also concern the origin, fate and persistence of fungal eDNA so as to reduce the existing gap with plant and bacterial DNA in soil and sediments.

Extracellular DNA can be also used as a source of nutrients or as a material in the formation of bacterial biofilms.

Extracellular DNA has been extracted from soil, sediments and water and characterised by molecular fingerprinting techniques. The characterisation of eDNA can give further information on the composition of microbial communities of soil and sediments. The estimation of the age of eDNA by isotopic signatures is problematic because traces of organic solvents, that is carbon fuel, can affect ^{13}C and ^{14}C abundances (Agnelli et al. 2007). Extracellular DNA can be leached or moved up by capillarity from a lower to a higher soil horizon, and thus, eDNA can reach a microbial cell far from the donor cell. This may have important implications in gene transfer between cells located in different microhabitats. However, more research is needed to evaluate the ecological role of eDNA in soil and sediments under different environmental conditions.

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