SESSION 4:
LESSONS LEARNED AND REMAINING CHALLENGES

RISK ANALYSIS OF SOIL-PLANT HORIZONTAL GENE TRANSFER

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1. Introduction

The introduction to modern agricultural practice of genetically modified plants (GMPs) has raised a series of questions and concerns that have been debated within the political and scientific communities in recent years (20).[1] Although biological products could offer great potential benefits to agriculture, such as the reduced use of pesticides and fertilizers, there are many uncertainties about the risks of the introduction of transgenic plants into the open environment and the ecological impact of engineered genes. This is based on the hypothesis that if the genetic alteration is transferred to other organisms, in particular microbial recipients, it could be disseminated into the natural habitat, with unpredictable consequences (10).

Studies of the ecological impact of engineered genes have failed to provide a clear consensus about whether GMPs represent an actual risk to the environment, since the data are too few and contrasting. These uncertainties reflect our limited knowledge of the microbial ecology of natural habitats, in particular of agricultural soil which contains and nourishes more than a billion cells per mg of earth (19). Although there has been extensive research on the effects of adding or removing species in communities of higher plants and animals, little information is available for microbial communities. Therefore, an accurate assessment of the risks of GMPs requires an increase of studies on the fate of transgenes after their release into the wild and their influence on natural bacterial communities.

The purpose of this paper is to discuss the possibility of horizontal gene transfer (HGT) from plants to soil microorganisms and the mediating effects of soil mineral components, mainly clay particles, on the transfer processes.

2. Mechanisms of Horizontal Gene Transfer between Bacteria

Horizontal transfer of genetic information between unrelated bacteria has been widely demonstrated in laboratory conditions and in natural systems (2, 21). Recent analyses of the composition of bacterial genomes show that considerable portions of bacterial chromosomes consist of exogenous DNA (about 20% of the genome in *Synechocystis* and 15% in *Escherichia coli*), indicating that the transfer of chromosomal DNA fragments between bacterial species is an important mechanism in their evolution (4). Other studies have shown that genes have been transferred from plants to bacteria during evolution (3).

As plants do not have an identified mechanism of host-range gene transfer, except for pollen hybridization with related species, the possibilities of HGT from plants to bacteria can be explored by considering what is known about the mechanisms of HGT between bacteria.

[1] The number(s) between parenthesis indicate the specific literature reference as listed at the end of this paper.

There are three major mechanisms of bacterial genetic exchange**: Conjugation**, **Transformation** and **Transduction** (Fig.1).

Conjugation (Cg) is a process of genetic exchange between bacteria that requires contact between the two cells. Cg was discovered in *Escherichia. coli* by Lederberg and Tatum in 1946, and has been demonstrated in many other gram-positive and gram-negative bacteria. It was the first mechanism of HGT to be studied for genetically modified micro-organisms. It requires the cytoplasmic presence of a circular DNA molecule, a plasmid, which codes for the transfer functions, although both plasmid and chromosomal genes can be transferred. In addition to specific functions for DNA transfer, plasmids codify for many other activities, such as resistance to antibiotics and heavy metals, which can be transferred between bacteria that are very distantly related. Moreover, these broad-host range (BHR) plasmids can mediate the exchange of genetic information even between prokaryotic and eukaryotic cells.

The best known example is the transfer of DNA from *Agrobacterium tumefaciens* to certain plants. *A. tumefaciens* causes a well-known plant disease which consists in the formation of tumors (22). Tumoral transformation is due to incorporation into the host plant's genome of a portion of DNA of plasmid Ti ("Tumor inducing") in the bacterium. The incorporated part (T-DNA) contains genes which control the synthesis of hormones necessary for the formation of tumors; hence the bacterium is called a "natural genetic engineer".

Therefore, conjugation could have played, and could continue to play (plasmids have been detected in plant mitochondria), a significant role in the transfer of genetic information between distant bacterial species, as well as between bacteria and eukaryotic cells. This could explain, at least in part, the numerous discrepancies found in phylogenetic trees.

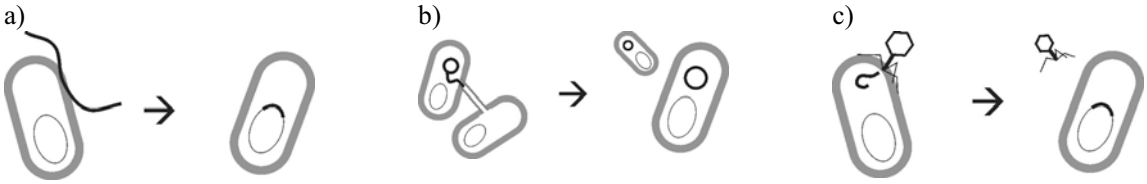

Fig. 1. Schematic representation of the mechanisms of: a) Transformation, b) Conjugation, and c) Transduction.

Transformation (Tf) is the process in which a piece of naked DNA penetrates a recipient bacterial cell, in a physiological state known as "competence", and becomes incorporated into its genome. Tf was the first mechanism of gene transfer studied (6), and its description led to the discovery of DNA as the carrier of genetic information (1). Tf has been demonstrated in more than 40 species of bacteria inhabiting a wide range of environments, such as soil, water and plants.

Although Tf was the first reported instance of bacterial gene transfer in a natural environment (the blood of mice), it was long considered unimportant as a mechanism of HGT in natural habitats. This was due to the two main factors that control the process: the development of competence and the availability of DNA for uptake. Studies carried out in the last ten years have demonstrated that both conditions can occur in soil, and that transformation could be an important mechanism of HGT. The development of competence is highly plausible for two reasons: 1) the presence in soils of protected "microniches" (21), where "competence factors" can accumulate to a concentration that allows the generation on the cells of "transformosomes" for DNA uptake (competence under "external control"); 2) the stressful

environmental conditions that usually predominate in soil (limiting concentrations of nutrients or growth factors) can promote competence under "internal control".

Regarding the presence of naked transforming DNA, several studies have shown that DNA in soil can originate from many sources, including lysis of dead cells and the active release of DNA during the "competence" phase of bacterial cells. Observations on the fate of extracellular DNA in natural environments indicate that, despite the presence of various biotic (nucleases) and abiotic (pH, dryness) degrading factors, DNA can persist for a long time as a result of its interactions with soil particles, mainly clay minerals, without losing its ability to transform competent bacterial cells (15). In other words, the interaction (adsorption/binding) of DNA on clay minerals does not prevent its biological activity but rather enhances its persistence in the environment.

Transduction (Td), discovered by Zinder and Lederberg in 1952, is a process that allows the transfer of genes from one bacterium to another through the intervention of virus particles, bacteriophages or "phages". Td has been shown to occur under laboratory conditions in more than 50 bacterial species. However, its importance as a mechanism of HGT in natural habitats, and specifically in soil, is not well understood (21). The major restrictions to Td in soil appear to be due to the necessity of sufficient concentrations of bacteria and phages and to the limited host-range infectivity of phages. Nevertheless, high concentrations of bacteria and bacteriophages have been detected in different types of soil, and phages with host ranges that cross species have been reported. Hence, it is quite possible that Td can occur in the soil environment. Moreover, studies in different laboratories in the last two decades have indicated that the survival and persistence of virus particles in soil is greatly affected by the presence of clay minerals. Adsorption of phages on clay particles protects them against inactivation, e.g. by UV radiation and biodegradation, thus enabling them to persist in terrestrial habitats. Bacteriophages adsorbed on clays are also able to transduce bacteria, indicating that adsorption does not eliminate the viral activity (16).

All these observations indicate that Td could be an important method of HGT in soil.

3. Possibilities of HGT from Transgenic Plants to Soil Bacteria

Recent studies in different laboratories throughout the world have demonstrated that exogenous genes can be transferred, albeit at very low frequencies, from various transgenic plants (sugar beet, potato, tobacco) to soil bacteria (*Acinetobacter* sp., *Pseudomonas stutzeri*) (5, 11, 17). However, the authors have clearly indicated that many physical and biological barriers or factors can hinder HGT between distantly related organisms, thus rendering the risks of spreading transgenic traits (genes or DNA fragments) from GMPs to soil bacteria negligible.

These barriers mainly involve the processes of release and persistence of plant DNA in the soil environment, the uptake of heterologous plant DNA by competent bacterial cells and the phenotypic expression of plant DNA.

With regard to the availability of DNA, studies in different laboratories and in different experimental conditions (including open field tests) have indicated that transgenic plant DNA can persist in the soil for up to 2-3 years, particularly in soils rich in organic matter and clay particles on which nucleic acid can adsorb (12, 18), without inhibiting its availability to competent bacteria. Recently, we developed a very sensitive method to detect plant DNA deriving from GMPs which contained a segment of DNA homologous to bacterial DNA carrying the genes for resistance to the antibiotic kanamycin ("transgenic

cassette"); this method was based on the direct extraction of DNA from the soil and its specific amplification by the PCR technique.

Once the plant DNA is available, it must be taken up and internalized by competent bacterial cells. These processes are genetically and environmentally controlled. Although the ability to develop competence under natural conditions has been demonstrated for only a few soil bacteria, the presence in soils of specific microniches, such as aggregates or rizospheres, could permit bacteria to develop competence (21). This suggests that transformation can occur in soils, even if at very low frequencies because of the numerous steps involved in the entire process (10). In fact, the internalization and maintenance of transgenic plant DNA in bacterial cells requires its incorporation into the bacterial chromosome, or its autonomous replication as a plasmid, and the expression of the genetic traits. The incorporation of exogenous genes into the recipient bacterial genome is strictly dependent on sequence homology between the two DNAs (plant and bacterium), and it seems that the degree of homology is the main factor determining the success of stable incorporation of transgenic DNA in bacteria (10). It is assumed that heterologous DNA that penetrates the bacterial wall is rapidly degraded by the bacterial cell's restriction enzyme system. However, in many cases, transformation is mediated by single-strand DNA and thus is not affected by restriction enzymes (9). Moreover, some bacterial species can take up DNA independently of its sequence (14), and bacterial mutants of genes involved in DNA repair processes show less stringent homology requirements (7). Last but not least, many GMPs have been engineered by the insertion of bacterial DNA, which could lead to increased stabilization of plant DNA in bacteria. This is the case of transgenic plants transformed with so-called "gene cassettes", genetic constructions containing bacterial traits like the genes for resistance to antibiotics (13).

The problem of the phenotypic expression of transgenic DNA in competent bacterial cells also represents a strong barrier to successful HGT from plants to bacteria. However, we cannot exclude *a priori* that gene construction can be expressed in bacteria. Indeed, bacterial resistance to antibiotics is developed by the acquisition of resistance genes from heterologous bacterial sources, i.e. by the transfer of plasmids. As many transgenic plants contain both the genes of specific interest and a gene conferring resistance to an antibiotic (for the detection of transformed cells), there is the possibility that these genes could be taken up by indigenous soil bacteria. Moreover, the recent techniques of introduction of genes to plant chloroplasts ("transplastomic plants") could favour the expression of these genes in bacteria because of the prokaryotic-like nature of the chloroplast compartment (8).

4. Conclusions

The results of studies of HGT from transgenic plants to soil bacteria seem to indicate that successful transfer events are extremely rare and that possible risks associated with such events are negligible. Nevertheless, it must be emphasized that this conclusion is based on a small number of observations and indications reported in the scientific literature. As previously mentioned, the main problem in this field is our limited knowledge of the ecology of microbial communities in natural habitats, specifically in soils; thus we probably cannot predict and correctly quantify the actual occurrence of interkingdom gene transfer. Only increased knowledge of the processes regulating the function of microbial communities in different habitats will allow us to predict the consequences of the introduction of transgenic crops into the environment. Until then, we must proceed on a "case-by-case" basis.

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