X. FATE OF GENETIC MATERIAL IN SOIL

Required Readings:


Suggested Readings:


Prospects for the intentional or accidental release of genetically engineered plants and microorganisms into the environment highlight the need to understand the fate of these released organisms. The fate of a released microorganism is a matter of microbial ecology and DNA probes may be useful to identify the presence or absence of a released microorganism within the soil environment. In addition, however, the fate of the DNA itself must be known as it is possible that this DNA may be transferred to other indigenous organisms. This would result in the permanent introduction of a genetic trait, which may ultimately prove undesirable, into the soil-plant environment.

The sources of genetic material in soil are the plant, animal and microbial cells growing in or added to the soil. The total amount of DNA in soil is approximately 50-150 ug/g soil dry weight. This averages to about 8.4 fg (10\(^{-15}\) g) of DNA per bacterial cell. However, there is evidence that significant amounts of DNA are not associated with viable cells but exist in soil as naked DNA. Naked DNA may be released from dead or moribund cells or from viable cells in specific growth phases. The importance of naked DNA in soil is unclear at present but the stabilization of this DNA in the organic fraction provides the potential for a genetic trait to be maintained in the soil for long periods of time.

Microorganisms contain DNA both in chromosomal form and in plasmids. Plasmids are defined as extrachromosomal replicating molecules of DNA. They are double-stranded covalently closed circular (ccc) in shape. Plasmid DNA usually encodes for gene products that are nonessential but which can impart a specific selective advantage or provide a unique function to the cell. For example plasmids generally contain genes that code for proteins relating to resistances, catabolism, antagonism, gene transfer, and interactions between organisms. Plasmids are duplicated independently of the chromosomal DNA and may exist in microbial cells in single or in multiple copies. Multiple copies of a specific plasmid in a single cell open up the possibility that a large amount of their protein product is synthesized. Plasmids are thus ideal carriers for the
many genes needed for high-level antibiotic resistance or for the metabolism of unique food sources.

It is much easier for genetic information contained on plasmids to be transferred than if this same information were contained in the chromosome. This is because plasmids are smaller and, therefore, more easily transferred between cells. Plasmid DNA is also generally at higher concentrations in cells and thus more likely to be able to participate in various transfer processes. DNA may be transferred among microorganisms by several different mechanisms. Conjugation is the process whereby DNA is transferred from one cell to another by a mechanism that requires direct cell-to-cell contact. Transduction is the transfer of DNA from one cell (donor) to another (recipient) by a bacteriophage, i.e. a bacterial virus. Transformation is a process by which naked DNA from one cell (donor) is taken up directly by another (recipient) from the surrounding medium.

Another transfer mechanism that is exhibited by microorganisms is the insertion of DNA segments by transposons. Transposons or jumping genes are, as their name implies, highly mobile. By being contained within a transposon, a specific gene, such as one that confers resistance to an antibiotic, acquires a greatly heightened capacity to move with the transposon from one DNA molecule to another, if not from one bacterium to another. The rapid spread of antibiotic resistance among a microbial population often involves resistance genes inserted within transposons.

New techniques involving DNA isolation and characterization are currently being developed for use in soil systems. One application of these techniques is to use DNA probes to identify a specific component of the total microbial community in the soil. This identification is highly desirable to aid in the study of the fate of genetically released microorganisms. The assumption is made that the introduced microorganism contains a DNA sequence that is unique. A probe for
this unique DNA sequence can be used to detect the presence of the introduced microorganism and trace its fate or ability to survive in the soil system. The second application involves the use of the same DNA probe methodology to evaluate the fate in soil of a specific segment of DNA itself. This DNA may be an introduced gene or a gene that occurs naturally within the soil microbial population.

Two different methodological approaches have been developed to study the fate of DNA in soil and transfer of genetic information among soil microorganisms. The first method involves extracting microorganisms from soil and then breaking the cells to release the DNA. The DNA is purified and used in further studies. The problem with this approach is that microorganisms live in microenvironments that will result in different extraction efficiencies of the cells. In the second method, the cells are broken in the soil and the released DNA is then extracted from the soil. This approach is thought to provide DNA that is more representative of the total microbial community. The limitations are that often harsh methods are needed to break cells and this can degrade the quality of the DNA. Some cells are protected and may not be broken or some of the DNA is adsorbed onto soil particles or associated with humic substances. This will decrease the efficiency of extraction. The presence of humic substances and Fe in soils and sediments is also known to inhibit subsequent reactions involving restriction enzymes or the polymerase chain reaction (PCR).

To probe the total DNA in soil for a particular gene, the methodology used must meet several criteria. One, it should be rapid enough to permit the processing of the number of treatments and replicates required for ecological studies. Two, the isolated DNA must be of sufficient purity and size for precise characterization, generally by the use of restriction enzymes. Three, the probe developed must be both sensitive and specific enough to detect the presence of a particular sequence of DNA of low frequency in the complex mixture of total soil DNA.
The application of molecular biological techniques to study problems relating to the fate of DNA in soil is just beginning and there will undoubtedly be many changes and advances in the years to come.