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# R-Plasmid Transfer in Soil

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Bacterial genetics owes much of its success to the use of pure laboratory cultures which have been isolated from soil, sediment, water, plants, animals and the human body. However, very litle information is available on bacterial gene transfer and organism stability in the soil environment. This paucity of information also makes it difficult to predict how adapted and genetically engineered bacteria will influence the soil environment if they are released into an uncontained location. On the other hand, it is difficult to predict how the dynamic physical, chemical and biological factors in the soil environment will influence the usefulness of a single or mixed population of bacteria released into soil.

A study was undertaken to assess the capability of Escherichia coli to undergo conjugation in the soil environment. This organism was chosen as a model test organism because of the large number of genetic studies conducted on this species. In addition, E. coli can enter and survive in the soil environment when it is applied in sewage sludge and in animal waste.

## MATERIALS AND METHODS

Escherichia coli K12 711 (nalidixic acid resistant, lactose-negative, $F^-$ plasmidless) was used as a recipient in conjugal gene transfer experiments in soil. The E. coli MA527 donor strain contained a 60 x $10^6$ dalton (Mdal) R-plasmid that encodes for both streptomycin and tetracycline resistance. E. coli MA527 has been previously described by Altherr & Kasweck (1982) and Trevors & Oddie (1986).

Donor and recipient cells were grown overnight at 37°C in LB broth, centrifuged, suspended in sterile distilled water and inoculated into 2 g of freshly collected sandy loam soil (pH 6.8), contained in glass vials, and incubated under a variety of environmental conditions for 24 h in the laboratory to simulate the natural soil environment. Serial dilutions of the soil sample were made by adding sterile 0.15 M, pH 7 phosphate buffer to the soil and mixing the contents in a Mickle apparatus for 3 min. Transconjugants were enumerated on MacConkeys agar

containing 200 µg/ml nalidixic acid (inhibited donor cell growth) and 50 µg/ml tetracycline (inhibited recipient cell growth). Plates were incubated at 37°C for 48 h. Only transconjugants were able to grow on this medium. In addition, all transconjugants were lactose-negative, as the recipient was a lactose-negative strain. This provided an additional means of selection. R-plasmids were detected in transconjugants using ethidium bromide staining-ultraviolet transillumination of agarose gels loaded with cleared cell lysates as described by Trevors and Oddie (1986).

## RESULTS AND DISCUSSION

R-plasmid transfer values are widely used to estimate the frequency at which a complex mating population transfers conjugative plasmids. The plasmid transfer frequencies summarized in Table 1 range from $3.2 \times 10^{-8}$ to $5.0 \times 10^{-9}$. A transfer frequency of $10^{-8}$ or $10^{-9}$ may appear low. However, it is probably adequate for the purpose of genetic recombination in soil; considering that a g of soil is a relatively small mass, and that it can contain millions of bacterial cells, the transfer frequencies are more than satisfactory to allow bacterial populations to adapt, and respond to chemical, physical and biological stresses. Also, conjugation represents only one mechanism of gene transfer in bacteria. Transformation has been described for Bacillus subtilis in soil (Graham & Istock 1978, 1979). However, transduction in soil has not been demonstrated to date.

Table 1. Frequency of R-plasmid transfer in aerobically incubated non-sterile soil (pH 6.8) after 24 h

| Incubation conditions in soil samples | Plasmid transfer frequency[a] |
|---|---|
| 22°C, donor to recipient ratio of 1:1, 80% of WHC | $3.2 \times 10^{-8}$ |
| 22°C, donor to recipient ratio of 1:10, 80% of WHC | $5.0 \times 10^{-9}$ |
| 22°C, donor to recipient ratio of 1:100, 80% of WHC | 0 |
| 22°C, donor to recipient ratio of 1:1, 20% of WHC | $5.0 \times 10^{-9}$ |

[a]Ratio of transconjugants to initial number of donor cells. Donor cells were plated on LB agar just prior to their addition to the soil sample. Colony forming units were counted after 24 h incubation at 37°C.
WHC (water holding capacity of soil)

Studies conducted in sterile soil reported that E. coli could conjugate and survive in this environment (Trevors & Oddie 1986; Weinberg and Stotzky 1972). However, sterile soil devoid of millions of microbial cells competing for physical space and nutrients does not exist in nature.

The results presented in Table 1 also revealed that E. coli can conjugate at 22°C under both moist (80% of the soils' water holding capacity) and dry (20%) conditions. Since the optimum growth temperature for E. coli is 37°C, it is noteworthy that conjugation occurred at 15°C below the optimal growth temperature. This is important because in the soil environment, temperature can be a fluctuating dynamic parameter that exerts a significant degree of control on microbial activities. A study by Wamsley (1966) reported that mating-pair formation by E. coli did not occur below 24°C. Achtman (1975) also reported that conjugation in E. coli may consist of mating aggregates of cells rather than pairs of donor and recipient cells.

The observations reported in the present study are more significant when combined with the recent observation of Smith et al. (1985). Their study revealed that E. coli cells were readily translocated through vertical soil profiles, where they have the potential of reaching and contaminating the groundwater. The number of bacterial strains known to contain conjugative and non-conjugative plasmids is increasing yearly. Bacterial plasmids which are non-conjugative can be mobilized by conjugative plasmids that physically recombine with them. The integrated plasmid is then transferred as a co-integrated structure. It is clear that little is currently known about environmental bacterial genetics, even though it plays a significant role in the adaptation and evolution of soil bacteria.

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