

## Gene transfer in microbial biofilms

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### ABSTRACT

Quantification and prediction of gene transfer in natural environments is required for reliable assessment of risks associated with the transfer of recombinant DNA in genetically engineered microbial inocula, and for a greater understanding of the mechanisms controlling transfer of genetic material. Published models of gene transfer describe plasmid transfer in homogeneous, liquid culture systems and do not consider the effects of surface growth or biofilm formation, which will be important in the majority of natural environments. To address this issue, a mathematical model has been constructed which describes bacterial plasmid transfer during colonisation of solid substrata. The model considers growth, colony expansion and substrate utilisation and generates predictions regarding changes in the numbers of donor, recipient and transconjugant cells. The model demonstrates the interactions between colony expansion, donor and recipient inoculum sizes and initial nutrient concentration in determining the final number of transconjugants, and explains a number of features observed in experimental studies of plasmid transfer during growth on particulate material.

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### Introduction

Microbial growth on surfaces and the formation of biofilms provide, in many ways, ideal conditions for gene transfer. Concentration of cells at the surface increase the chances of cell-cell contact while the dynamic nature of biofilms, with attachment of freely suspended cells, surface growth and desorption of cells, increase the probability of encounters between donors and recipients. The widespread occurrence of surface-attached populations in natural environments necessitates study of gene transfer on surfaces for both prediction of gene transfer frequencies and for increased understanding of the mechanisms controlling gene transfer in the environments. These, in turn, are essential for the assessment of risks associated with, for example, the spread of antibiotic resistance genes in hospitals and the transfer of recombinant genes to indigenous microbial populations following the introduction of genetically engineered microorganisms to terrestrial or aquatic environments.

Gene transfer may occur through transformation by naked DNA, transduction or conjugation. The last of these has been most commonly studied and will be the focus of this article, although many of the concepts discussed below are applicable to all gene transfer processes. Quantification and mathematical modelling of plasmid transfer by conjugation must consider the probability with which donor and recipient cells meet and the subsequent ability of donors to transfer, and recipient cells to receive plasmid DNA.

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## Gene Transfer in Liquid Culture

The earliest theoretical models of gene transfer considered plasmid transfer in liquid culture during exponential growth. Thus, Levin *et al.* [5] proposed a model based on the assumption that plasmid transfer is dependent on random encounters between donor cells (including transconjugants) and recipients such that production of new transconjugants during a particular time interval is related directly to the numbers of donors and recipients. If cells are not growing, this may be represented by the following equations:

$$D_t = D_{t-1}$$

$$T_t = T_{t-1} + \tau[(D_{t-1} + T_{t-1})R_{t-1}]$$

$$R_t = R_{t-1} - \tau[(D_{t-1} + T_{t-1})R_{t-1}]$$

where  $D$ ,  $R$  and  $T$  represent numbers of donor, recipient and transconjugant cells at time intervals  $t$  and  $t-1$ . Thus, the increase in the number of transformed cells during the chosen time interval is dependent on the number of encounters between donors (including transformants) and recipients and, hence, on the respective cell numbers. The parameter  $T$ , termed the gene transfer frequency constant, quantifies the probability of plasmid transfer following contact between donor and recipient. It is, therefore, presumably related to the physiology of donor and recipient cells and was termed 'fertility' by Levin *et al.* [5]. The model can be modified to take into account exponential growth in batch culture, leading to the following equations:

$$D_t = D_{t-1}e^{\mu dt}$$

$$T_t = T_{t-1}e^{\mu dt} + \tau[(D_{t-1} + T_{t-1})R_{t-1}]$$

$$R_t = R_{t-1}e^{\mu dt} - \tau[(D_{t-1} + T_{t-1})R_{t-1}]$$

where  $\mu$  is specific growth rate during exponential growth and  $dt$  is the time interval over which transformation events are being measured.

## Gene Transfer in the Rhizosphere and Phyllosphere

Although the above model is useful in describing plasmid transfer in homogeneous liquid culture, Levin *et al.* [5] pointed out its inability to predict events where the spatial distribution of cells is not homogeneous, including situations involving surface growth. Their model has, however, been modified to describe gene transfer in heterogeneous natural environments. For example, Knudsen *et al.* [3] modelled plasmid transfer in the rhizosphere and phyllosphere. They considered variation in the physiology of natural populations by replacing specific growth rate constants with 'growth-death' rate constants, which were positive or negative depending on whether the population was growing or dying exponentially. The predictions of the model were tested for plasmid transfer between two pseudomonads in rhizosphere and phyllosphere microcosms under a range of environmental

conditions. Survival rates for donors and recipients and gene transfer rates were determined experimentally and then used to generate predictions from the model, giving good agreement between experimental and predicted results.

The model of Levin *et al.* [5] has also been modified by Clewlow *et al.* [2], who replaced exponential growth terms with the logistic equation to describe nutrient limited growth in soil. Again, good agreement was obtained between experimental and predicted behaviour for gene transfer between streptomycetes in soil microcosms.

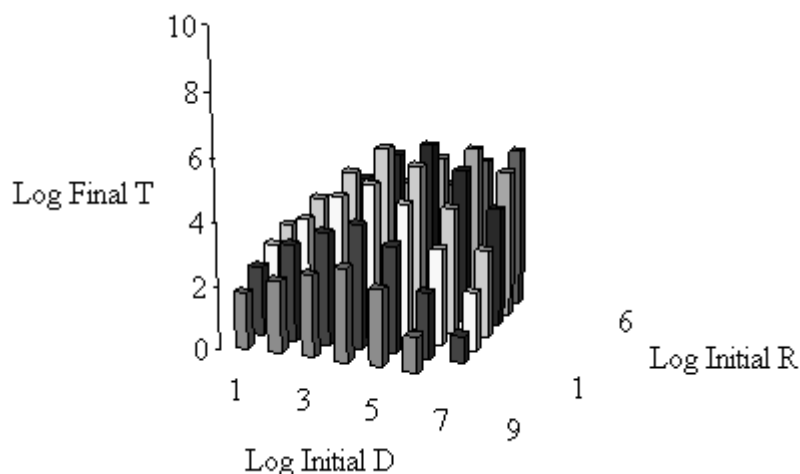
### **Gene Transfer on Surfaces**

Modifications of the model of Levin *et al.* [5] to describe gene transfer in the rhizosphere and phyllosphere do not take into account the effects of spatial heterogeneity resulting from colonisation of soil, root or leaf surfaces. In fact, the high cell inocula used in the experimental studies of Knudsen *et al.* [3] and Clewlow *et al.* [2] effectively reduce heterogeneity and increase analogy with liquid culture systems. Simonsen [7], however, demonstrated experimentally that growth of donors and recipients on an agar surface affected gene transfer rates. Plasmid transfer rates were similar to those in liquid culture with high cell inocula but were significantly reduced at low cell inocula where larger intercellular distances greatly reduced the number of encounters between donors and recipients.

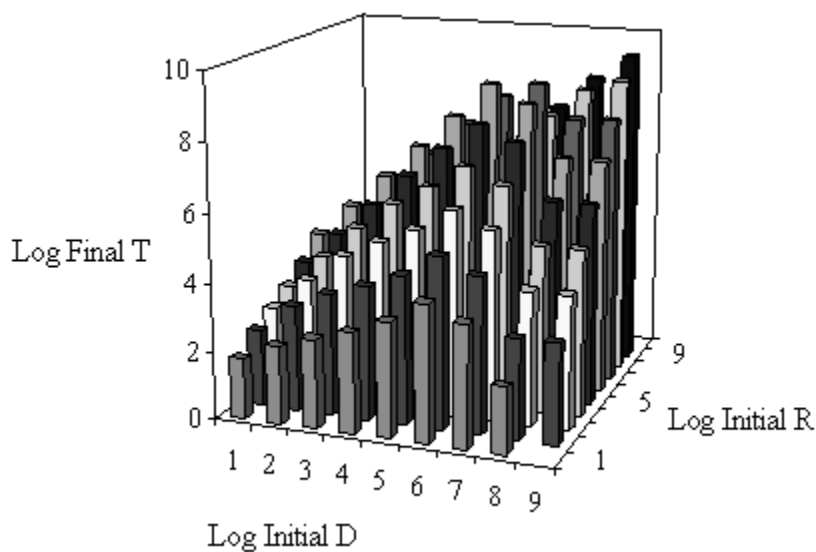
Certain aspects of the effects of spatial heterogeneity on gene transfer may be modelled by consideration of the early stages of colonisation of an agar surface. Following inoculation of donors and recipients, colonies are formed, assumed to be circular, which increase in diameter until encountering another colony. Plasmid transfer occurs when colonies of donor and recipient cells meet and the cells within transconjugant colonies thus formed can act as donors. Using relatively simple assumptions and simple kinetics for rates of colony expansion on agar [6], this approach may be used to assess the effects of initial donor and recipient cell concentrations on the numbers of transconjugants after a particular period of incubation on a solid surface.

Examples of simulations are illustrated in Fig. 1a. At high cell inocula, confluent growth occurs. The situation is analogous to that in liquid culture and conjugation is maximal. As recipient inoculum is reduced, conjugation is reduced, due to greater initial intercellular distances and consequent greater requirement for colony expansion to enable encounters between donors and recipients. This reduction in gene transfer, however, depends on the donor:recipient ratio due to the contrasting effects of initial intercellular distances, which determines the extent of colony expansion required for conjugation, and the time at which substrate is completely utilised, which will decrease with increasing numbers of both donors and recipients. Thus, donor cell inoculum at which maximum transconjugant numbers are achieved increases with increasing recipient inoculum. For low donor inocula, conjugation will be low at low recipient numbers due to large initial intracellular distances and formation of a low number of large colonies, many of which may not meet within the incubation period or prior to exhaustion of nutrients. As donor:recipient ratio decreases, through greater recipient inocula, final transconjugant number increases due to formation of more recipient colonies, increasing the number of encounters with donor colonies. At the highest recipient inocula, however, transconjugant number will decrease due to exhaustion of nutrients before all recipients have encountered donor colonies.

a.



b.



**Fig. 1.** Predicted final numbers of transconjugants following inoculation of agar with varying numbers and ratios of donor and recipient cells and subsequent incubation for 9 hr. Substrate concentration in (b) is 1% of that in (a).

A factor of significance in experimental studies of gene transfer is nutrient concentration and gene transfer in microcosm studies is frequently undetectable in the absence of nutrient amendment (e.g., [2, 8]). This reduction in gene transfer is predicted by simulation of the model for similar donor and recipient inocula to those in Fig. 1a, but with an initial nutrient concentration reduced by a factor of 100. At low recipient and donor inocula, final transconjugant numbers are the same at the two nutrient concentrations (Fig. 1b). Under these conditions, gene transfer was limited by the large intracellular distances and the requirement for significant colony expansion for donor and recipient colonies to meet. At the higher cell inocula, however, final transconjugant numbers are reduced due to more rapid exhaustion of nutrients, decreasing the period of colony expansion and reducing the number of encounters between donors and recipient colonies.

A factor of significance in experimental studies of gene transfer is nutrient concentration and gene transfer in microcosm studies is frequently undetectable in the absence of nutrient amendment. This reduction in gene transfer is predicted by simulation of the model for similar donor and recipient inocula to those in Fig. 1a, but with an initial nutrient concentration reduced by a factor of 100. At the higher recipient inoculum level, final transconjugant number is significantly reduced at the higher donor inocula. This is due to earlier exhaustion of nutrients by the high donor inoculum, reducing colony expansion and, hence, the number of encounters between donors and recipients. At lower donor inocula, final transconjugant numbers are similar at the two nutrient concentrations. Under these conditions, colony expansion during the incubation period will not be limited by exhaustion of nutrients and the number of colony counters will be similar to that at higher nutrient concentration. At the lower recipient inoculum level ( $10^3$ ), no plasmid transfer was predicted.

## Conclusion

The above theoretical model introduces one feature of biofilm growth, initial microcolony expansion, and demonstrates its effect on transconjugant production at a range of cell inocula. It demonstrates the importance of the relationship between colony expansion, initial intercellular distances and substrate utilisation in determining the levels of gene transfer. It also explains one general finding of experimental studies of gene transfer in soil microcosms, the reduction in plasmid transfer at lower nutrient concentrations. The model is supported by data obtained from an experimental model designed specifically to test the underlying assumptions of the model and reported elsewhere (Lagido *et al.*, in preparation). The model is not designed or formulated to describe gene transfer in more complex, multilayer biofilms or in natural environments. In such mature biofilms, gene transfer will depend on the ability of cells and plasmids to move within the biofilm, on the physiological activity of donor and recipient cells and on the significant spatial and temporal heterogeneity existing in such environments. In addition, it does not incorporate the effect of other populations, not participating in gene transfer, which will be competing for nutrients.

The two basic processes required for accurate modelling of gene transfer, the probability of encounters between donors and recipients and their subsequent ability to conjugate, require consideration of all factors controlling cell concentration, cell movement and cell separation. In natural environments, reliable quantitative information on these factors is not available and models incorporating all of these factors would be impossible to test critically. The function of the model described here is not to mimic exactly the natural situation, but to highlight the fundamental mechanisms affecting gene transfer of attached populations. It also suggests caution in the ways in which gene transfer is quantified, which most commonly involves use of gene transfer frequency, calculated as the ratio between transformants and numbers of either donors or recipients. This value, in itself, suffers from a lack of consideration of the multiplicity of factors affecting gene transfer and, for particulate systems, ignores the two factors whose importance is illustrated by the theoretical model. Effectively, it lumps together all of the physical, biological and environmental factors which contribute to cell movement and physiology leading to plasmid transfer.

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