APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1990, p. 3139–3145
0099-2240/90/103139-07$02.00/0

Vol. 56, No. 10

Mathematical Model of Plasmid Transfer between Strains of Streptomycetes in Soil Microcosms

L. J. CLEWLOW,[1] N. CRESSWELL,[2] AND E. M. H. WELLINGTON[2]*

Financial Options Research Centre, Institute for Management Research and Development,[1] and Department of Biological Sciences,[2] University of Warwick, Coventry, England CV4 7AL

Received 13 November 1989/Accepted 6 August 1990

A mathematical model was developed and used to simulate the long-term dynamics of growth and plasmid transfer in nutrient-limited soil microcosms of *Streptomyces lividans* TK24 carrying chromosomal resistance to streptomycin, *S. lividans* 1326; and *S. violaceolatus* ISP5438. Donor, recipient, and transconjugant survival was modelled by an extension to the Verhulst logistic equation which takes account of nutrient limitation, and plasmid transfer was modelled by a mass action model. Rate parameters were derived from experimental data on the early stages of the development of sterile systems. The model predicted donor, recipient, and transconjugant populations in 2.4-h (0.1-day) steps and was tested against the long-term behavior of the experimental sterile systems and independent experimental data on nonsterile systems. Bacteria were periodically enumerated onto selective media over a 20-day period. The effects of long-term nutrient-moisture depletion were correctly predicted.

Simulation and prediction of the behavior of plasmids in natural environments is important in the assessment of the advantages and disadvantages of releasing genetically engineered organisms into natural environments. Probably the greatest potential for gene transfer between microorganisms lies with conjugative plasmids. Streptomycetes are indigenous soilborne microorganisms (7) with the ability to produce useful enzymes and antibiotics (1). They may therefore become important for control of soilborne pests and maintenance of soil quality. As Knudsen et al. (5) have pointed out, because research on genetically engineered microorganisms is currently permitted initially only in contained systems, results from experiments with relatively simple microcosms must be extrapolated to highly complex natural environments. However, it is important to gain as complete an understanding as possible of simple microcosms before extrapolations can be made with confidence. In particular, mathematical models need to account for the major factors affecting the evolution of the system. Furthermore, any mathematical model will prove more practical if the parameters can be calculated in the early stages of development of the system such that long-term predictions of its evolution can be made. Application of this to a natural environment would allow, for instance, immediately after release of an organism (whether deliberate or accidental), collection of data on the basis of which predictions of the effects of the release could be made.

Our approach was to develop a model which takes account of nutrient limitation, an important factor influencing the development of streptomycetes in soil. We computed the parameters of the model by using data on the early stages of experimental sterile microcosms and investigated the ability of the model to predict the long-term behavior of the systems. As an independent test, we also investigated the applicability of the model to nonsterile systems by using parameters based on sterile systems. Details of the experimental microcosms can be found in a companion paper (9).

* Corresponding author.

MATERIALS AND METHODS

Intra- and interspecific crosses were performed by inoculation of marked strains into soil microcosms. For the intraspecific cross, *Streptomyces lividans* 1326 carrying plasmid pIJ673 acted as the donor and *S. lividans* TK24 was the recipient. For the interspecific cross, *S. violaceolatus* ISP5438 carrying plasmid pIJ673 acted as the donor with *S. lividans* TK24 as the recipient. *S. lividans* TK24 carries chromosomal resistance to streptomycin. All strains were assigned to cluster group 21 as designated by Williams et al. (11, 12). Plasmid pIJ673 carries genes that encode resistance to neomycin, thiostrepton, and viomycin. All strains were sensitive to thiostrepton, neomycin, and viomycin, and *S. lividans* 1326 and *S. violaceolatus* ISP5438 were sensitive to streptomycin. Enumeration was by the viable-count technique on arginine glycerol salts with neomycin and thiostrepton to select for the plasmid and streptomycin to counterselect for the donor genotype. The computer implementation of the model was written in C, and the program listing is available from E. M. H. Wellington.

THEORY

The mathematical model. Recent models of microbial survival and plasmid transfer have typically used simple exponential growth and decay mass action systems (2, 5). Knudsen and Hudler (4) have used logistic growth to model population size limitation, but their formulation requires knowledge of the long-term limiting population levels. We extended these models to take account of the reduction in the exponential growth rate due to loss of nutrients and/or moisture from the soil microcosms as the system evolves. This is important for streptomycetes, because it has been observed by Mayfield et al. (7) and others (10) that streptomycetes grow sporadically in soil, surviving as spores when nutrients are depleted. Our mathematical model is based on the Verhulst logistic equation (8):

$$\dot{x} = (\alpha - \beta x)x \quad (1)$$

The effective exponential growth rate $(\alpha - \beta x)$ decreases as

the population size (x) increases. Growth or decay of the donor, recipient, and transconjugant populations is modelled by an exponential growth factor which decreases as the total population (donors, recipients, and transconjugants) size increases. Plasmid transfer is described by the mass action model of Levin et al. (6) and is proportional to the sizes of the effective donor and recipient populations. The effective donor population is the sum of the donor and transconjugant populations if the transconjugants act as plasmid donors. We therefore obtained the following set of model equations:

$$\dot{D} = [\alpha_D - \beta_D(D + R + T - D_0 - R_0 - T_0)]D \quad (2)$$

$$\dot{R} = [\alpha_R - \beta_R(D + R + T - D_0 - R_0 - T_0)]R - \gamma(D + T)R \quad (3)$$

$$\dot{T} = [\alpha_T - \beta_T(D + R + T - D_0 - R_0 - T_0)]T + \gamma(D + T)R \quad (4)$$

where D, R, and T represent the sizes of the donor, recipient, and transconjugant populations, respectively, and the subscript 0 indicates the populations at time zero. α_D, α_R, and α_T are the exponential growth rates, and β_D, β_R and β_T are growth rate reduction factors due to loss of nutrients and/or moisture. γ is the plasmid transfer rate. Note that the model allows for each of the three populations to have its own individual exponential growth rate. Furthermore, each can suffer differently from nutrient and/or moisture depletion. This extension of the Verhulst logistic equation to model nutrient consumption can be derived as follows. The rate of change of nutrients will be proportional to the size of the populations:

$$\dot{N} = -\theta_D D - \theta_R R - \theta_T T \quad (5)$$

where N is the amount of nutrient present (in appropriate units) and θ is the rate of consumption of nutrients per organism (in the same units). The effective or instantaneous growth rate (α') will be a function of the rate of consumption of nutrients, and as a first approximation, we can assume that they are directly proportional:

$$\theta = \phi\,\alpha' \quad (6)$$

where the constant of proportionality (ϕ), which we will call the growth factor, measures the efficiency with which the organism converts nutrients into growth. Substituting for θ_D, θ_R, and θ_T in equation 5 and integrating, we obtain:

$$N_0 - N = -\phi_D(D_0 - D) - \phi_R(R_0 - R) - \phi_T(T_0 - T) \quad (7)$$

If we assume that the growth factors for all three populations are equal to $\bar{\phi}$, which will apply if the organisms do not store nutrients and their abilities to utilize nutrients for growth are the same, then we obtain:

$$N_0 - N = \bar{\phi}[(D - D_0) + (R - R_0) + (T - T_0)] \quad (8)$$

If we know the amount of nutrients present, then our version of the Verhulst logistic equation, which takes account of nutrient consumption, becomes:

$$\dot{x} = [\alpha - \beta(N_0 - N)]x \quad (9)$$

Substituting for $(N_0 - N)$ in equation 8 and replacing $\beta\bar{\phi}$ with the appropriate $\beta_{\text{population}}$, we arrive at our extension to the Verhulst logistic equation.

The model in its present form does have two limitations. Firstly, if the growth reduction term exceeds the exponential growth rate (α), we obtain exponential decay of the population. However, physically, when the two terms are equal, this corresponds to nutrient nonavailability and, therefore, no growth. Consequently, the former case is not physically meaningful; we therefore reset the growth rate to zero if it becomes negative. The second limitation occurs if the total population size falls below the initial population size; if this occurs, the growth reduction term changes sign so as to increase the effective exponential growth rate. This corresponds to increasing levels of nutrients, but in the context of our model, it is not physically meaningful. However, this did not occur in any of our simulations. Neither of these limitations is serious, as they correspond to evolutionary phases outside the bounds of our model. The model makes no provision for mortality, except that the exponential growth rate (α) can be negative, giving an exponentially declining population. This is because the model is intended to simulate the behavior of viable populations in a nutrient-limited phase of growth with negligible predation on the population. This developmental phase is characterized by populations whose rates of growth decrease monotonically to zero as the nutrients are consumed. The final state of the system will consist of constant populations of dormant spores. Finally, it should be noted that the recipients and transconjugants are modelled as separate populations. It is therefore possible for the recipient population to decline if conversion to transconjugants exceeds the rate of growth.

Growth form in soil. Studies of soil samples were performed with a scanning electron microscope (SEM) to obtain a qualitative picture of the evolutionary profile of the soil microcosms. The studies were performed on sterile soil with a monoinoculum of 1.0×10^6 donor *S. lividans* ISP5438 spores carrying plasmid pIJ673. Sterile soil was used so that other spores and mycelial growth would not interfere with the observations, as it was not possible to differentiate among spores from different species. Eight SEM stubs, four at the day 3 stage and four at the day 4 stage, each holding approximately 0.1 g of soil, were sputter coated with a gold-palladium alloy and examined with a JEOL 330A SEM. Preliminary observations indicated that finding ungerminated spores was difficult. Once germination had begun, it was possible, although still difficult, to find one or two spores; this could be done only at the day 2 stage. However, it was much easier to find mycelial growth at the day 3 stage and beyond. It was therefore decided to conduct more detailed observations of the day 3 and 4 stages. The observations proceeded as follows. A stub was placed in the SEM, the approximate position of the center of the stub was located, and the SEM micrometer positions were noted. The position of the field of view was then adjusted to lie at one corner of a 1-cm^2 region centered on the stub (the stubs were approximately 2.5 cm in diameter). The magnification of the SEM was set so that the field of view was 100 μm^2. The field of view was then altered in 1-mm steps both horizontally and vertically so that 100 views of the 1-cm^2 square region of the stub were observed. Each field of view was studied carefully for signs of mycelial growth and differentiation, and the field of view was classified as X (no soil), S (soil only), L (low growth), M (medium growth), or H (high growth). The results are shown in Table 1. On day 3, an average of 13% differentiation was observed, whereas on day four, 69% was seen.

Further evidence of the evolutionary profile of the systems comes from work by Herron and Wellington (3). They have developed a method of biomass extraction specific for spores. Preliminary results indicate that spore numbers decline from day 0 until day 3, when they begin to increase again, and by day 5, they are increasing rapidly. We can therefore say that at around day 2 spores had just begun to

TABLE 1. SEM observations of soil samples[a]

Day	Stub	% of views with[b]:			
		S	L	M	H
3	1	78.69	6.56	3.28	11.48
	2	67.78	18.64	8.47	5.08
	3	84.06	10.14	4.35	1.45
	4	83.33	2.38	4.76	9.52
	Mean	78.47	9.43	5.22	6.88
	SD	6.50	5.98	1.96	3.90
4	5	70.89	11.39	10.13	7.59
	6	70.15	16.42	5.97	7.46
	7	78.87	5.63	8.45	7.04
	8	73.56	10.34	6.90	9.19
	Mean	73.37	11.0	7.85	7.83
	SD	3.42	3.83	1.58	0.81

[a] S, soil only; L, low growth; M, medium growth; H, high growth.
[b] Percentage of the number of observations of views containing soil in each category.

germinate and the germ tubes were, on average, a few micrometers long. Transfer frequencies for the experimental soil systems at that point were typically on the order of 1%. We can estimate the expected transfer frequency by calculating the probability of two spores being within a few micrometers of each other, assuming that they are homogeneously distributed throughout the soil. The probability function which is applicable is related to a probability system termed urn models (13) and is called the hypergeometric function. If we consider 1 g of soil, we may estimate its volume on the basis of its density, which is typically 3.9×10^5 g/m^3 (7) and indicates a volume for 1 g of soil of 2.56 cm^3. The volume of a single spore is approximately 1 μm^3 (7), and there were 1.0×10^6 spores per g of soil. The hypergeometric function will give the probability of obtaining a certain number of spores in a given volume with a known concentration of spores. We may calculate the concentration of spores in terms of spore volumes, that is, the number of spores in 1 g of soil over the total possible number of spore volumes in the volume occupied by 1 g of soil. If we assume the average length of hyphae observed at day 2 is 1 μm, the probability that another spore is with a sphere of this radius from a spore is effectively zero (approximately 1.0×10^{-159079}). We must consider mycelial growth of over 60 μm before the probability becomes larger than 1%. Mycelial growth of this extent has certainly not occurred extensively at day 2; therefore, to explain the much higher transfer frequencies obtained, we must postulate that the spores tend to be clustered much closer together. This is supported by SEM observations which showed that spores tended to cluster in crevices in soil particles. We may explain this qualitatively by noting that when spores are inoculated into dry soil, the water carrying them tends to collect and be absorbed into the soil particle crevices, thus drawing the spores into these crevices. Further support comes from an analysis of soil particle sizes indicating a composition (dry weight) of 63.6% sand, 18.4% silt, 11.7% clay, and 6.2% loss on ignition. This shows that typical soil particles are either of the same order of size as spores or much larger. The largest proportion of particles will consequently have crevices suitable for spore clustering. Another explanation sometimes proposed for nonuniform distribution of spores in suspension is clumping. However, our probability calculation shows that for the concentration levels used, the probability that spores or small spore chains will meet to clump together is effectively zero (the suspensions were filtered to remove large mycelial fragments and spore chains). This assumes that the spores remain uniformly distributed in suspension after mixing until inoculation. If the spores are allowed to settle on either the surface of the water or the bottom of the container, then the probability of clumping will be greatly enhanced. This was not allowed to occur in our experiments. Spore clustering has important consequences for plasmid transfer. After the initial transfer due to meeting of early mycelia, further growth up to a few hundred micrometers will not lead to any further meeting of effective donors and recipients, assuming that the plasmid will be distributed throughout the whole mycelial mass following a single transfer event, which will occur if the plasmid contains a spread function, as pIJ673 does. We may therefore assume that transfer ceases after approximately day 2 until the second generation of spores germinates and begins to grow.

Determination of model parameters. The model parameters may be calculated from experimental data by making certain observations about known states of the system. Firstly, in the first few days after inoculation of the spores into the soil, the growth of the donor and recipient populations will be purely exponential, since significant loss of nutrients or moisture will not have occurred. Secondly, any increase in the transconjugant numbers must be due to transfer alone, since the new transconjugants will not have had time to sporulate. Therefore, assuming that donor and recipients initially grow exponentially, we may solve the exponential growth equations for donor and recipient populations and rearrange them to obtain:

$$\alpha_D = \frac{\log_e(D_t/D_0)}{t} \tag{10}$$

$$\alpha_R = \frac{log_e(R_t/R_0)}{t} \tag{11}$$

The transconjugant transfer equation can be solved if we note that the transconjugant population size will be small compared with the initial donor population size, and we can therefore approximate the equation to the following:

$$\dot{T} = \gamma DR \tag{12}$$

Substituting for D and R in equation 12, integrating, and rearranging for γ yields:

$$\gamma = \frac{(T_t - T_0)(\alpha_D + \alpha_R)}{D_0R_0\{\exp[(\alpha_D + \alpha_r)t] - 1\}} \tag{13}$$

The experimental data indicate that exponential growth continues up to around day 7. We can thus calculate the transconjugant growth rate in the same way as for the donor and recipient populations by assuming purely exponential growth from day 2:

$$\alpha_T = \frac{\log_e(T_t/T_0)}{t} \tag{14}$$

The β factors may be calculated at the first point at which a change in the growth rate becomes apparent:

$$\beta_D = \frac{\alpha_D - \log_e(D_t/D_0)}{D + R + T - D_0 - R_0 - T_0} \tag{15}$$

$$\beta_R = \frac{\alpha_R - \log_e(R_t/R_0)}{D + R + T - D_0 - R_0 - T_0} \tag{16}$$

$$\beta_T = \frac{\alpha_T - \log_e(T_t/T_0)}{D + R + T - D_0 - R_0 - T_0} \quad (17)$$

On the basis of soil observations, we would expect germination of the next generation of spores to occur around day 5 and extensive mycelial development, and thus the potential for more plasmid transfer, to occur between days 6 and 7. However, experimental data showed that at this point the populations tend to limit values because of nutrient or moisture limitations. We can postulate, therefore, that extensive mycelial growth is not likely to occur under these poor conditions and any additional plasmid transfer will be minimal.

To summarize, the donors and recipients grow exponentially up to around day 7, at which point competition for the reducing nutrient and/or moisture levels begins to slow the growth rate. The transconjugants are created by a period of transfer from day 0 up to day 2, after which they begin to grow exponentially until at day 7 grow rate reduction begins to occur as for the donor and recipient populations. We call this type of simulation phased. We also study simulations in which all of the parameters act for all of the simulation; we call this unphased simulation.

RESULTS

Experiment A was an intraspecies transfer between *S. lividans* 1326 and TK24 in sterile soil. Experiment B was an interspecies transfer between *S. violaceolatus* ISP5438 and *S. lividans* TK24 in sterile soil. Computer simulations were performed for experiment A amended-soil data and for both amended- and unamended-soil data from experiment B. The experiment A unamended-soil data were not modelled, since no significant change in the populations occurred after day 2 because of the lack of nutrients in the unamended soil. The model curves were calculated at 2.4-h (0.1-day) intervals. Figures 1 to 7 show comparisons of the various simulations with the experimental data.

Experiments A and B cannot be directly compared, because the initial inoculum levels in experiment A are approximately 10 times greater and also, as noted above, experiment A was an intraspecies cross while experiment B was an interspecies cross.

As expected, since the donor and recipient are similar (TK24 was derived from 1326) they performed similarly in experiment A, although the recipient population suffered a delay before noticeable growth occurred. We expected that the initial exponential growth rate of the recipient in experiment A amended soil would be similar for the amended soil in experiment B, since the systems were essentially the same in the early stages. However, the initial growth rate of the recipient in experiment A was approximately half that for experiment B; note, however, that the growth rate in experiment A was still very close to exponential, so that the difference was not due to nutrient limitation. It was, therefore, probably due to the higher population loading. Indeed, it may be that nutrient limitation effects occurred from day zero. This point is discussed more fully later.

In experiment B with amended soil, the donor strain outperformed the recipient strain in the early stages, when nutrients and moisture were plentiful, but as the nutrients and moisture declined, the recipient strain began to perform slightly better than the donor strain. An interesting point is that in both the amended- and unamended-soil experiments the donor growth rate increased slightly after day 2 while the recipient growth rate decreased. The transconjugant population appeared to be still increasing after day 15. However, although we expected it to be still increasing

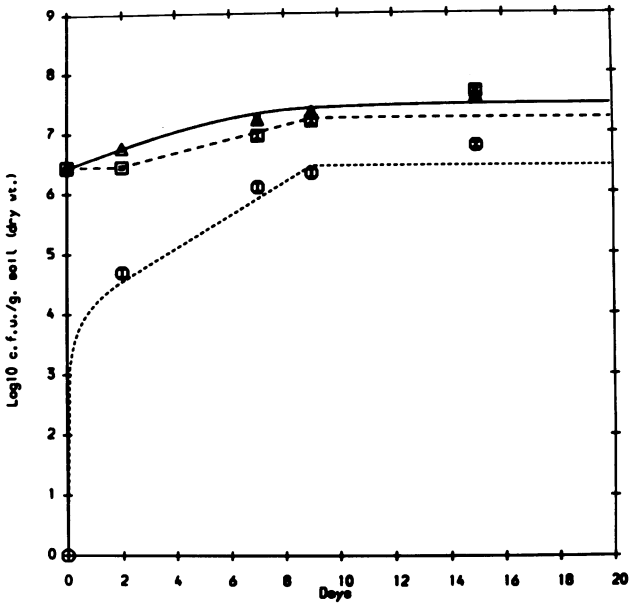

FIG. 1. Comparison of an experimental intraspecific cross (experiment A) between *S. lividans* 1326 and TK24 in sterile amended soil and a phased computer simulation. ——, Model donor; — —, model recipient; - - -, model transconjugant; △, experimental donor; □, experimental recipient; ○, experimental transconjugant.

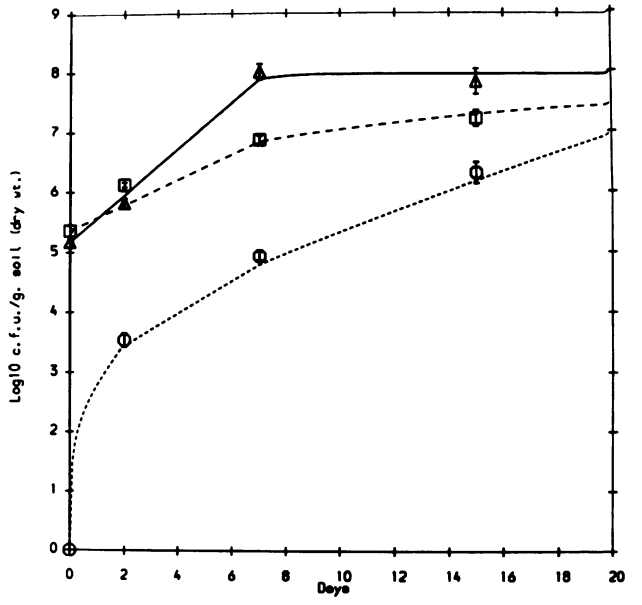

FIG. 2. Comparison of an experimental interspecific cross (experiment B) between *S. violaceolatus* ISP5438 and *S. lividans* TK24 in sterile amended soil and a phased computer simulation. ——, Model donor; — —, model recipient; - - -, model transconjugant; △, experimental donor; □, experimental recipient; ○, experimental transconjugant.

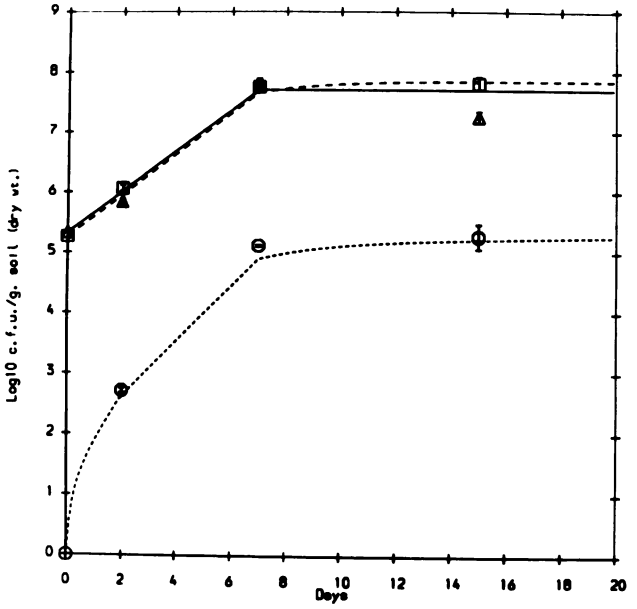

FIG. 3. Comparison of an experimental interspecific cross (experiment B) between *S. violaceolatus* ISP5438 and *S. lividans* TK24 in sterile unamended soil and a phased computer simulation. ——, Model donor; — —, model recipient; - - -, model transconjugant; △, experimental donor; □, experimental recipient; ○, experimental transconjugant.

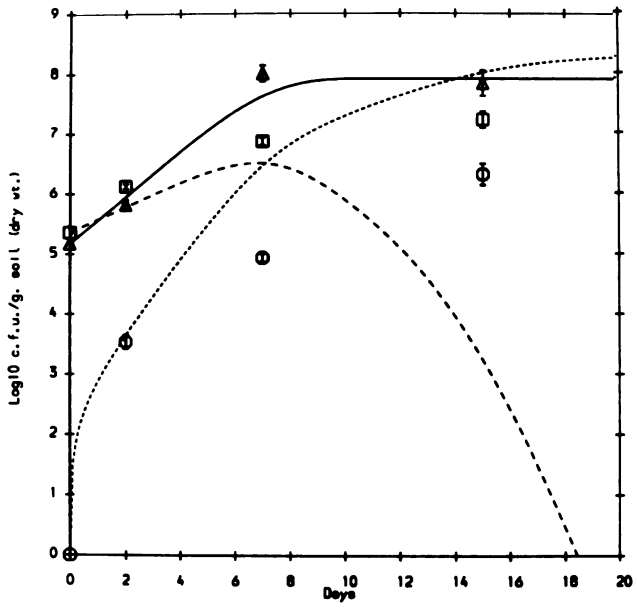

FIG. 5. Comparison of an experimental interspecific cross (experiment B) between *S. violaceolatus* ISP5438 and *S. lividans* TK24 in sterile amended soil and an unphased computer simulation. ——, Model donor; — —, model recipient; - - -, model transconjugant; △, experimental donor; □, experimental recipient; ○, experimental transconjugant.

slightly since transconjugants are essentially recipients with the plasmid, the actual rate of growth is probably not as high as the model indicates and another datum point at around day 11 would probably lead to a higher growth reduction factor and thus a lower growth rate in the later stages.

In experiment B, we expected the initial exponential growth rates of donors and recipients to be similar in the amended- and unamended-soil systems, since both had plentiful supplies of nutrients initially. We know this because all of the populations grew purely exponentially up to day 7. However, the recipient exponential growth rate in amended

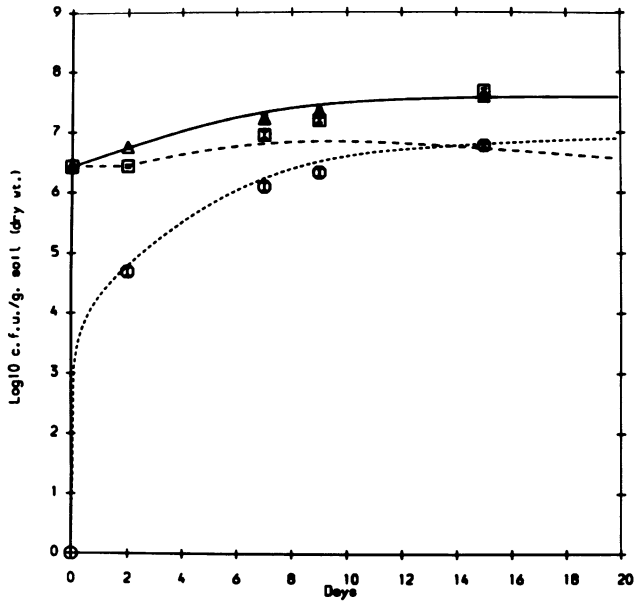

FIG. 4. Comparison of an experimental intraspecific cross (experiment A) between *S. lividans* 1326 and TK24 in sterile amended soil and an unphased computer simulation. ——, Model donor; — —, model recipient; - - -, model transconjugant; △, experimental donor; □, experimental recipient; ○, experimental transconjugant.

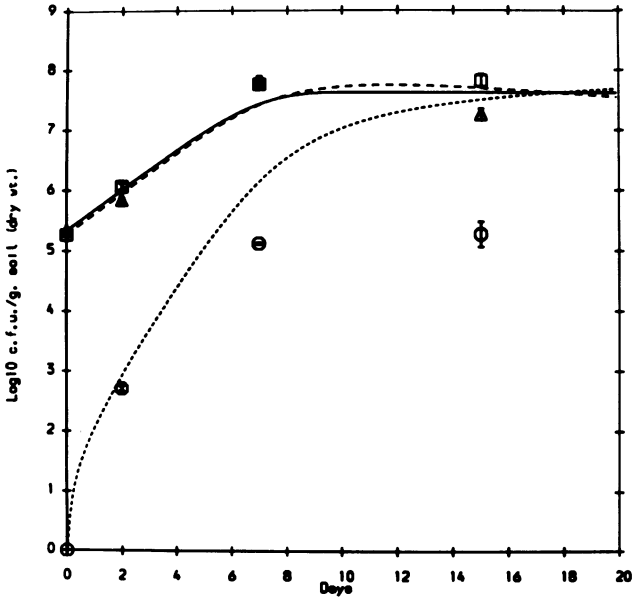

FIG. 6. Comparison of an experimental interspecific cross (experiment B) between *S. violaceolatus* ISP5438 and *S. lividans* TK24 in sterile unamended soil and an unphased computer simulation. ——, Model donor; — —, model recipient; - - -, model transconjugant; △, experimental donor; □, experimental recipient; ○, experimental transconjugant.

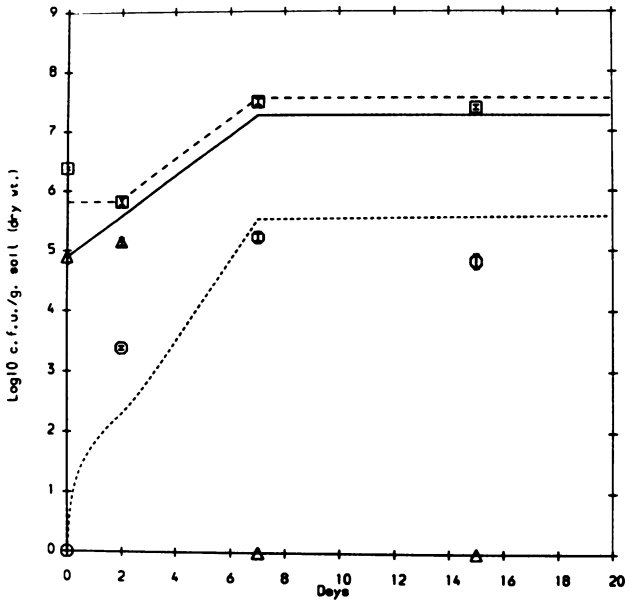

FIG. 7. Comparison of an experimental interspecific cross (experiment C) between *S. violaceolatus* ISP5438 and *S. lividans* TK24 in nonsterile amended soil and a phased computer simulation. ——, Model donor; — —, model recipient; - - -, model transconjugant; △, experimental donor; □, experimental recipient; ○, experimental transconjugant.

soil was half of that in unamended soil. In fact, when the growth rates between days 0 and 2 were calculated, they were similar in the amended and unamended soils. The lower growth rate for the recipients in amended soil was, therefore, an artifact of the assumption that because one population continued to grow exponentially up to day 7 then all populations will do so. This will only be true, however, if all of the populations behave similarly in nutrient-rich environments. We would also expect the growth rate reduction factors to be similar for both amended and unamended soils, since this just expresses the reaction of the populations to reduced nutrient levels. However, the recipient growth rate reduction factor was approximately half that of the donors. These facts indicate that the recipient population performed better at reduced nutrient levels. This behavior of the recipient population also occurred in the transconjugant population with a reduced exponential growth rate and a reduced growth rate reduction factor in amended soil relative to unamended soil.

Further analysis of the model showed that the growth rate reduction factors could be included from the start of growth of the populations without greatly affecting the fit of the model to the experimental data. This indicates that the effect of the loss of nutrients and moisture does indeed follow the behavior assumed by the model. However, study of plasmid transfer behavior in the model for experiment B showed that pure transfer based on the initial transfer rate resulted in transconjugant levels much higher than those obtained experimentally (Fig. 5 and 6). The recipient population decline in Fig. 5 was because the rate of plasmid transfer exceeded the rate of growth of the recipient population, as noted in the description of the mathematical model. The situation is similar for combined transfer and growth of transconjugants. This indicates that, in reality, the transfer rate must decrease as the system evolves for an interspecies cross. For an intraspecies cross, the transfer rate appears to increase slightly, which would be expected, since transfer between organisms of the same species tend to be better than between different species (Fig. 4). However, in both intraspecies and interspecies crosses, the proportion of effective donors which were recipients with the plasmid increased as the system evolved. This indicates that the transconjugants did not contribute to the effective donors as much as would be expected in an interspecies cross.

As a further test of the validity of the model, the parameters obtained from experiment B were applied to experimental data obtained from an identical interspecies cross performed in nonsterile soil (experiment C). The parameters for the unamended experiment B soil were applied to the amended nonsterile soil because sterilization of the soil effectively releases nutrients and we had to mimic this by amending the nonsterile soil. The recipient population showed an initial decrease which our model cannot take into account. However, since we obtained exponential growth up to day 7 for experiment B and the recipient population in experiment C did increase after day 2, we may apply exponential growth after day 2. The donor population also caused problems, since it was not clear from the experimental data whether it remained constant or fell to zero. However, the poor performance of the donor in competitive or nutrient-limited environments is confirmed by these data. For this reason, our model overestimates the long-term donor population when the experiment B parameters are used. The model does predict the recipient and, perhaps more importantly, transconjugant numbers quite well, especially the long-term limiting population levels.

DISCUSSION

Overall, the fit of the model, especially the early plasmid transfer phase and the growth rate reduction phase, is good, indicating that the assumptions and approximate methods of calculating the parameters work well. The longer-term nutrient-limited population levels are also predicted well. Application of the parameters calculated from the sterile-system data to the nonsterile system indicates that the overall behaviors are similar, and therefore, prediction of the behavior of more complex nonsterile systems may be possible with this model. A problem which is apparent in Fig. 7 is that if the donor population is inhibited by one of the natural populations present such that its numbers fall rapidly after inoculation, this will not be modelled by parameters calculated from sterile-microcosm data. However, as Fig. 7 shows, this does not adversely affect the prediction of transconjugant numbers. This is probably because, as Freter et al. (2) have noted, transconjugant-to-recipient transfer is probably the more important after the transconjugants have begun to grow. We should note that higher time resolution data, for example, daily values, would allow the parameters to be estimated much more accurately. This is especially important for the beta factors which are calculated from the initial deviations from exponential growth. With more datum points, a least-squares regression could be performed, leading to a more accurate value and, consequently, better prediction of the limiting population levels.

An important experimental observation of streptomycetes is that they grow sporadically in natural soil environments, surviving as spores when nutrients are scarce (7, 10). This implies that spread of a plasmid from a released organism to the natural population will occur initially in a burst, and the new transconjugants will then survive in spore form until the

next burst of growth. This behavior is directly analogous to a batch microcosm in which nutrients and moisture are not replenished as the system evolves. Furthermore, we have shown that this can be modelled by a straightforward extension of the Verhulst logistic equation. Our model also has the advantage that predictions of the final population levels at the end of the initial burst of growth can be made by using experimental data collected immediately after inoculation or release of the donor.

ACKNOWLEDGMENTS

We gratefully acknowledge grants received from the Science and Engineering Council (L.J.C.) and the Natural Environment Research Council (N.C.)

LITERATURE CITED

1. **Berdy, J.** 1980. Recent advances in and prospects of antibiotic research. Process Biochem. **15**:28–36.
2. **Freter, R., R. R. Freter, and H. Brickner.** 1983. Experimental and mathematical models of *Escherichia coli* plasmid transfer in vitro and in vivo. Infect. Immun. **39**:60–84.
3. **Herron, P. R., and E. M. H. Wellington.** 1990. New method for extraction of streptomycete spores from soil and application to the study of lysogeny in sterile amended and nonsterile soil. Appl. Environ. Microbiol. **56**:1406–1412.
4. **Knudsen, G. R., and G. W. Hudler.** 1987. Use of a computer simulation model to evaluate a plant disease biocontrol agent. Ecol. Modell. **35**:45–62.
5. **Knudsen, G. R., M. V. Walter, L. A. Porteous, V. J. Prince, J. L. Armstrong, and R. J. Seidler.** 1988. Predictive model of conjugative plasmid transfer in the rhizosphere and phyllosphere. Appl. Environ. Microbiol. **54**:343–347.
6. **Levin, B. R., F. M. Stewart, and V. A. Rice.** 1979. The kinetics of conjugative plasmid transmission: fit of a simple mass action model. Plasmid **2**:247–260.
7. **Mayfield, C. I., S. T. Williams, S. M. Ruddick, and H. L. Hatfield.** 1972. Studies on the ecology of actinomycetes in soil. IV. Observations on the form and growth of streptomycetes in soil. Soil Biol. Biochem. **4**:79–91.
8. **Verhulst, P. F.** 1845. Nouv. Mem. R. Bruxelles **18**:1–20.
9. **Wellington, E. M. H., N. Cresswell, and V. A. Saunders.** 1990. Growth and survival of streptomycete inoculants and extent of plasmid transfer in sterile and nonsterile soil. Appl. Environ. Microbiol. **56**:1413–1419.
10. **Williams, S. T.** 1978. Streptomycetes in soil ecosystems, p. 137–144. *In* M. Mordarski, W. Kuryloicz, and J. Jeljaszewicz, (ed.), *Nocardia* and *Streptomyces*. Gustav Fischer Verlag, New York.
11. **Williams, S. T., M. Goodfellow, G. Alderson, E. M. H. Wellington, P. H. A. Sneath, and M. J. Sackin.** 1983. Numerical classification of *Streptomyces* and related genera. J. Gen. Microbiol. **129**:1743–1813.
12. **Williams, S. T., M. Goodfellow, E. M. H. Wellington, J. C. Vickers, G. Alderson, P. H. A. Sneath, M. J. Sackin, and A. M. Mortimer.** 1983. A probability matrix for identification of some *Streptomyces*. J. Gen. Microbiol. **129**:1815–1830.
13. **M. Woodroofe.** 1975. Probability with applications. McGraw-Hill Book Co., New York.