The fate of introduced streptomycetes, plasmid and phage populations in a dynamic soil system

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Populations of *Streptomyces lividans* and *S. violaceolatus* were monitored in natural soil amended with nutrients. The fates of a multicopy plasmid pIJ673, and an actinophage, KC301, were determined and the extent of gene transfer was estimated. The soil was incubated for 60 d during which time 'spent' soil was periodically removed followed by addition of fresh, uninoculated soil. Maximum numbers of bacteria and phage inoculants occurred at 15 d; this correlated with a peak in the amount of plasmid DNA detected and total numbers of transconjugants recovered. A KC301 lysogen of *S. lividans* was also recovered at this time. Plasmid DNA was monitored by two methods, bead-beating and SDS/heat lysis; the latter was specific for mycelium while the former released DNA from spores and mycelium. Southern blots of soil DNA only showed the presence of plasmid DNA in SDS/heat lysis extracts from 15 d and 17 d samples, whereas positive signals were obtained throughout the experiment from bead-beaten extracts. The results confirmed that a well-developed mycelium was necessary for conjugation, phage infection, multiplication and lysogeny in soil.

Introduction

Bacterial gene transfer in soil has been investigated for a range of genera (reviewed by Stotzky & Babich, 1986; Stotzky *et al.*, 1990) and *Streptomyces* species have been studied with respect to conjugation in soil (Rafii & Crawford, 1988, 1989; Bleakley & Crawford, 1989; Wellington *et al.*, 1988, 1990a, b; Clewlow *et al.*, 1990). Genetic exchange by transformation or transduction has not yet been reported, although lysogeny has been demonstrated in nutrient-amended soil only (Herron & Wellington, 1990). Most studies have used simple batch microcosms to investigate the fate of marked plasmids and phages in soil. However, in soil the supply of nutrients is discontinuous in space and time (Nedwell & Gray, 1987), whereas the study of micro-organisms in enclosed microcosms does not allow for input of nutrients and 'turnover' of fresh soil. Since gene transfer requires an active growth phase, fluctuations in growth in response to nutrient availability may result in changes in the extent of transfer of introduced plasmids and phages within streptomycete populations.

The aim of this study was to investigate the correlation between microbial activity and genetic exchange within

a dynamic model soil system. A fed-batch microcosm was designed to allow successive addition and withdrawal of soil. Previous work with closed microcosms has shown that streptomycete growth in soil follows a cyclical pattern of germination and sporulation after inoculation into sterile nutrient-amended soil and, to a lesser extent, in nonsterile soil (Wellington *et al.*, 1990a; Herron & Wellington, 1990). If nutrient and microsite availability in the batch microcosms were limiting growth then the addition of fresh amended soil should allow further cycles of germination accompanied by a period of phage activity or plasmid transfer. In sterile nutrient-amended soil, germination reached a peak 2 d after inoculation; phage numbers also peaked at this time and transconjugants could easily be detected (Wellington *et al.*, 1990b; Herron & Wellington, 1990). Monitoring and detection of micro-organisms in environmental samples poses a number of problems (reviewed by Pickup, 1991), particularly if the organism is filamentous. A viable plate count can only be used as an indication of changes in activity. The use of spore-specific extraction techniques coupled with molecular methods for direct detection of plasmid DNA were employed in this study to establish population trends and investigate the relationship between activity and gene

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Table 1. *Inoculum used for soil pots*

Treatment (9 replicates of each):	<i>S. lividans</i> TK24 (c.f.u. g ⁻¹)	<i>S. violaceolatus</i> ISP 5438 + pIJ673 (c.f.u. g ⁻¹)	KC301 (p.f.u. g ⁻¹)	Data presented
A	1.05 × 10 ⁵	—	—	Figs 2, 4
B	—	1.04 × 10 ⁵	—	Fig. 4
C	1.05 × 10 ⁵	1.04 × 10 ⁵	—	Figs 1, 2, 6, 7
D	—	—	1.04 × 10 ⁵	Fig. 5
E	1.05 × 10 ⁵	—	1.04 × 10 ⁵	Figs 3, 5
F	—	—	—	Figs 3, 5

transfer in the filamentous, spore-forming bacteria *Streptomyces lividans* and *Streptomyces violaceolatus*.

Methods

Bacterial strains, phage and plasmids. The strains used were as previously described for plasmid transfer studies and phage-host interactions (Wellington *et al.*, 1990a; Herron & Wellington, 1990). *Streptomyces violaceolatus* ISP 5438, transformed with pIJ673, was used as donor. pIJ673 (kindly supplied by T. Kieser, John Innes Institute, Norwich, UK) is a derivative of pIJ101, a conjugative, multicopy plasmid (Kieser *et al.*, 1982) with cloned resistance determinants against viomycin (*vph*), thiostrepton (*tsr*) and neomycin (Tn5) inserted into nonessential regions (T. Kieser, unpublished data). In soil experiments *Streptomyces lividans* TK24 (no known plasmids, streptomycin-resistant mutant) was used as a recipient. The phage KC301 (kindly supplied by K. Chater, John Innes Institute, Norwich, UK), a derivative of ϕ C31 (Lomovskaya *et al.*, 1980; Chater, 1986) contained *tsr*. TK24 was used as host both for phage propagation *in vitro* and in soil crosses. Strains were grown at 30 °C on R5 agar (Hopwood *et al.*, 1985), supplemented with appropriate antibiotics (Streptomycin, 50 µg ml⁻¹; thiostrepton, 50 µg ml⁻¹; and neomycin 10 µg ml⁻¹; all from Sigma). Filtered spore suspensions were prepared by the method of Hopwood *et al.* (1985) and stored in 20% (w/v) glycerol at -20 °C. Phage were grown overnight at 30 °C in soft nutrient agar overlays (supplemented with 8 mM-Ca(NO₃)₂ and 10 mM-MgSO₄) seeded with *S. lividans* TK24 (Hopwood *et al.*, 1985). High-titre lysates of KC301 were prepared by the method of Stuttard (1979) and stored at 4 °C.

Soil treatments. The soil used in this study was taken from a local wheatfield site at Cryfield, University of Warwick (Wellington *et al.*, 1990a). Soil was nutrient-amended with 1% (w/w) starch (soluble, BDH) and 1% (w/w) chitin (crabshell, Sigma). Six treatments were devised, each consisting of nine 200 g replicate soil pots (Table 1). Inoculants were added in distilled H₂O to give a final moisture content of 15% (w/w) (-0.67 bar). Microcosms were incubated at 22 °C, and at days 0, 2, 15, 17, 30, 32, 45, 47 and 60 one microcosm from each of the six treatments was destructively sampled. After 15 d (and every subsequent fifteenth day) the remaining microcosms were mixed and 50% of their weight replaced with fresh, uninoculated, nonsterile nutrient-amended soil. This newly constituted soil was then rewetted to 15% (w/w) moisture, thoroughly mixed and incubated further at 22 °C. Samples taken at 15, 30 and 45 d were removed before dilution of the inoculant populations with fresh soil. Soil sampled was treated as follows: three 1 g aliquots were removed and extracted with $\frac{1}{4}$ -strength Ringer's solution (Wellington *et al.*, 1990a); 100 g of soil was extracted for spore counts (Herron & Wellington, 1990); and 20 g was extracted for phage enumeration using method C of Lanning & Williams (1982). Three aliquots of the centrifugation supernatant for phage extraction

were filtered, serially diluted and assayed for KC301 in triplicate as described by Hopwood *et al.* (1985). The remaining soil was then used to calculate the soil moisture (% w/w) at each sample day. Inoculants were enumerated on RASS (Herron & Wellington, 1990), containing rifampicin (10 µg ml⁻¹, Sigma), cycloheximide (50 µg ml⁻¹, Sigma) and nystatin (50 µg ml⁻¹, Sigma). Further antibiotics were included for the enumeration of specific strains: streptomycin (10 µg ml⁻¹) for *S. lividans* TK24; neomycin (10 µg ml⁻¹) and thiostrepton (50 µg ml⁻¹) for *S. violaceolatus* ISP 5438 harbouring pIJ673; streptomycin (10 µg ml⁻¹), neomycin (10 µg ml⁻¹) and thiostrepton (50 µg ml⁻¹) for *S. lividans* TK24 transconjugants. *S. lividans* TK24 lysogens were detected using streptomycin (10 µg ml⁻¹) and thiostrepton (50 µg ml⁻¹). Indigenous streptomycetes were isolated and enumerated on RASS containing only antifungal agents.

Confirmation of phenotypes. Putative transconjugants were confirmed by extraction of plasmid DNA using the alkaline lysis procedure of Hopwood *et al.* (1985). Digestion with *Pst*I yielded a characteristic five-band restriction pattern following electrophoresis in a 1% (w/v) agarose gel (Wellington *et al.*, 1990a). Putative lysogens were probed with ³²P-labelled KC301 DNA by the colony hybridization method of Hopwood *et al.* (1985). These isolates were also checked for spontaneous phage release (Herron & Wellington, 1990).

Detection of pIJ673 using soil DNA extraction. Two methods for the recovery of plasmid DNA from soil were used; both involved modifications of the direct lysis procedures of Ogram *et al.* (1987), and the development of these methods is described in Cresswell *et al.* (1991). The first method lysed micro-organisms in the soil by SDS/heat treatment, and allowed efficient lysis of streptomycete mycelia. The second method involved bead-beating, which lysed both spores and mycelia.

SDS/heat lysis. Ten grams of soil, resuspended in 20 ml sodium phosphate buffer (0.12 M, pH 8; SPB) with 1.5% (w/v) SDS, was heated at 70 °C for 1 h then shaken on a flask shaker (maximum setting, 10 min). Samples were then centrifuged (1660 g, 10 min), the supernatant removed and the pellet re-extracted with SPB. Supernatants were pooled and centrifuged (13800 g, 20 min) and DNA in the supernatant was precipitated by the addition of 0.5 vol. polyethylene glycol 6000 (50%, w/v) and incubation at 4 °C overnight. The sample was then centrifuged (2260 g, 10 min). The resultant pellet was resuspended in 5 ml TE buffer (10 mM-Tris, 1 mM-EDTA) and extracted twice with equal volumes of TE-saturated phenol. Phases were separated by centrifugation and the phenol phase was re-extracted with TE buffer. The aqueous phases were pooled and extracted with chloroform/isoamyl alcohol (24:1, v/v). DNA was finally precipitated with ethanol at -20 °C, using standard procedures. The final DNA pellet was resuspended in 100 µl TE buffer.

Bead-beating lysis. Ten grams of soil was suspended in 25 ml SPB. The suspension was decanted into a 40 ml bead-beating bottle and glass beads (0.10-0.11 mm diameter) added to fill the bottle. Samples were subjected to five 1 min bursts of bead-beating using a Braun bead-

beater. The suspension of beads and soil was recovered and the bottle washed with 5 ml SPB, which was added to the extracted soil. Samples were then centrifuged at 1660 *g* for 10 min. The supernatant was retained and the pellet re-extracted twice with SPB by vortexing and centrifugation. Supernatants were pooled and subsequent processing was the same as for the SDS/heat lysis protocol except that phenol/chloroform/isoamyl alcohol (25:24:1, by vol.) was used for extraction.

DNA was extracted by both methods from treatments C and F from day 2 onwards at each sample time. Following extraction from soil, DNA samples (40 µl) were subjected to electrophoresis in 1% (w/v) agarose gels, following standard procedures, before being Southern blotted and probed with ³²P-labelled pIJ673 DNA. Labelling reactions were carried out using a random-primed DNA-labelling kit (Boehringer-Mannheim). Quantification of plasmid DNA was achieved by densitometric analysis of autoradiographs. A Molecular Dynamics 300A Computing Scanning Densitometer was used to obtain standard curves for known amounts of pure plasmid present on each blot, and from these the amount of plasmid DNA present in the DNA extracts could be calculated. Autoradiographs were developed after various lengths of exposure; this was necessary to optimize quantification.

Statistical analysis. Where possible, all points on graphs are means of three replicate samples counted in triplicate. Statistical analysis was done with the MINITAB statistical software package (Minitab Statistical Software, State College, PA, USA). Minimum significant differences (MSD) were calculated from analysis of variance using the Tukey-Kramer method (Petersen, 1985; Fry, 1989). Regression analysis of the survival data for *S. lividans* TK24 (treatment A); *S. violaceolatus* (treatment B) and KC301 (treatment D) was done to determine differences in the rates of decline in population numbers. In addition a theoretical rate of decline was determined by halving the population number at each dilution time starting from the recoverable counts at 0 d and assuming no growth or death occurred. Significant differences were calculated by Student's *t*-test, comparing the slopes of the lines of best fit using the equation:

$$t = \frac{b - (\text{hypothesized value})}{\text{estimated SD of } b} \quad (\text{Ryan } et al., 1985)$$

Results

Inoculant population levels in treatment C (Table 1), inoculated with both *S. lividans* TK24 and *S. violaceolatus* ISP 5438, were maintained during the first 15 d (Fig. 1*a, b*). Subsequently the titre of both organisms decreased as the inoculum was diluted by the addition of fresh soil. Counts obtained from the Ringer's dilution plate method were uniformly higher than those obtained by the spore extraction method. At 60 d inoculants were undetectable by dilution plating but both strains were readily enumerated by spore extraction (Fig. 1*a, b*). *S. lividans* TK24 reached a peak at 15 d, before declining in a step-wise manner for the remainder of the experiment (Fig. 2*a, b*) and this trend was reproduced in other treatments (data not shown). Indigenous streptomycetes showed fluctuations in counts between 10⁷ and 10⁸ c.f.u. (g soil)⁻¹, reaching a peak at 17 d (Fig. 3*a, b*), whereas inoculant populations peaked at 15 d, as did transconjugant numbers and plasmid DNA (see Fig. 6*a, b*).

Fig. 1. Growth and survival of *S. lividans* TK24 (■) and *S. violaceolatus* ISP5438 harbouring plasmid pIJ673 (▨) in treatment C: (a) spore-specific extraction method; (b) dilution plate method. The asterisks indicate dilution time.

Significant growth of either *S. lividans* or *S. violaceolatus* was not detected after 15 d. The rate of decline in *S. lividans* TK24, *S. violaceolatus* ISP 5438 and the phage KC301 was significantly greater ($P < 0.01$) than the predicted decline, as shown by regression analysis (Fig. 4*a, b, c*). This indicates that the dilution effect was not the sole factor contributing to the decline in inoculant population numbers. The survival of KC301 was similar to that of its host; phage numbers declined throughout the 60 d experiment and after 15 d counts from soils containing KC301 (treatments D and E) were indistinguishable from those of indigenous phage in treatment F (Fig. 5*a*). Indigenous actinophage were undetectable at 0 d but were detected at 2 d; this population declined significantly between 2 d and 15 d ($P < 0.05$), probably due to a reduction in soil moisture content over the initial 15 d incubation. Indigenous phage numbers were maximal at 17 d after the system was 'fed' and rewetted at 15 d; this corresponded with the maximum numbers of recoverable indigenous streptomycetes (Fig. 5*b*). After 17 d, indigenous phage numbers declined during the

Fig. 2. Proportional change in the populations of *S. lividans* TK24 in treatments A (□---□) and C (△—△): (a) spore-specific extraction method; (b) dilution plate method. The arrows indicate dilution time. X_0 is the count at time 0, X_t that at time t . X is c.f.u. (g dry wt soil) $^{-1}$.

remainder of the experiment, corresponding with a significant decline in indigenous streptomycete population (Fig. 3a). Each time the soil was 'fed' phage counts increased; the increases at these points were not significant ($P < 0.05$) for KC301 but were markedly so for indigenous actinophage between 15 d, 17 d and 30 d, 32 d (Fig. 5a). One thiostrepton-resistant *S. lividans* TK24 colony was detected at 15 d from treatment E, using the spore extraction method; it was confirmed as a lysogen by screening for spontaneous phage release and by probing with ^{32}P -labelled KC301 DNA. Problems occurred with fungal contamination at low dilutions of the final suspension of the spore extraction method, thus hindering the screening of these plates for lysogens. No streptomycetes were isolated from control soils that were resistant to the antibiotics used to select for inoculant genotypes, with the exception of streptomycin and rifampicin used to select for *S. lividans* TK24; such indigenous colonies were differentiated from *S. lividans* TK24 by colony pigmentation.

Transconjugants were readily detected using both extraction methods from treatment C (Fig. 6a). Counts peaked at around 15 d, before declining to undetectable

Fig. 3. (a) Comparison of population levels of *S. lividans* TK24 and indigenous streptomycetes: *S. lividans* enumerated by the spore-specific extraction method (▨) and the dilution plate method (■) in treatment E; indigenous streptomycetes enumerated by the dilution plate method in treatment F (□). Asterisks indicate dilution time. (b) Proportional change in populations of *S. lividans* TK24 and indigenous streptomycetes: *S. lividans* TK24 enumerated by the spore-specific (△—△) and dilution plate (□---□) methods; indigenous streptomycetes enumerated by the dilution plate method (○---○). Arrows indicate dilution time. X_0 is the count at time 0, X_t that at time t . X is c.f.u. (g dry wt soil) $^{-1}$.

levels, although one colony was detected at 60 d using the spore extraction method.

Plasmid-bearing strains were also monitored using soil DNA extractions. Total soil DNA was isolated from treatments C and F using the bead-beating and SDS/heat lysis extraction methods (Fig. 7a-d). Plasmid DNA in bead-beaten extracts from treatment C was quantified by densitometric analysis (Fig. 6b). In SDS/heat lysis extracts plasmid DNA was only detected at 15 d and 17 d in soil from treatments B (data not shown) and C (Fig. 7b). The signals at 17 d obtained from the SDS/heat lysis extracts were much weaker than those from 15 d extracts (Fig. 7b). Soil at 15 d was extracted prior to the removal of 'spent' soil and addition of fresh nutrient-amended soil. In extracts obtained by bead-beating (treatment C),

Fig. 4. Theoretical and actual rates of decline in inoculant spore and total propagule counts for (a) *S. lividans* TK24 in treatment A, (b) *S. violaceolatus* harbouring pIJ673 in treatment B, and (c) phage KC301 in treatment D. Spore counts, observed (—); theoretical (---); total counts, observed (.....); theoretical (---); phage counts, observed (---); theoretical (---).

plasmid signal was detected throughout the experiment, with a peak yield of plasmid at 15 d (Fig. 6 b, 7 b, 7 d). During the experiment, maximum signal for plasmid DNA was consistently obtained at 15 d. No signals were obtained from treatment F soil extracted by either method.

Discussion

During the first 15 d of the experiment, populations of all streptomycete inoculants were maintained. The rapid subsequent decline in inoculant populations indicated that they were unable to colonize nonsterile fresh soil and substrates. Perhaps this was due to available sites in the fresh soil being occupied by the resident microbial population. Comparison with a theoretical decline, based on halving populations at each dilution point, showed that death and predation of streptomycetes may also have been affecting survival, causing a significant increase in the rate of decline. The data suggest that the

Fig. 5. Correlations between phage and host populations in soil. (a) Fate of KC301 and indigenous phage in treatments D, E and F: KC301 in treatment D (■); KC301 + TK24 in treatment E (▨); indigenous phage in treatment F (■). Asterisks indicate dilution time. (b) Comparison of changes in indigenous streptomycete and phage populations in treatment F. □—□, Streptomycetes enumerated by the dilution plate count method; △---△, phage. Arrows indicate dilution time.

inoculants were present in the mycelial state after the initial 15 d incubation period, but it is difficult to predict if plasmid transfer occurred to any significant extent after this phase. Continued recovery of transconjugants may be due to their growth and sporulation. Secondary plasmid transfer events via the indigenous streptomycete population may also have taken place. Studies using SDS/heat and bead-beating lysis methods have shown that the cellular origin of streptomycete plasmid DNA can be determined in soil (Cresswell *et al.*, 1991). Detection of plasmid DNA from soil-borne spores using SDS/heat lysis required a minimum of approximately 10^6 spores (g soil) $^{-1}$. Detection using bead-beating gave two orders of magnitude greater sensitivity for detection of plasmid DNA from spores. As the total numbers of plasmid-containing spores in the treatments did not exceed 10^6 c.f.u. g $^{-1}$ (Fig. 1 a, b) the inoculant level was below that of the minimum detection by SDS/lysis. Plasmid was only detected in extracts obtained from SDS/heat lysis at 15 d and 17 d in soil from treatments B and C; the data indicate that the inoculum had

Fig. 6. (a) Detection of *S. lividans* TK24 transconjugants in treatment C by the dilution plate (■) and spore-specific extraction (⊠) methods; (b) detection of plasmid DNA in bead-beaten extracts from treatment C (⊠). Asterisks indicate dilution time.

germinated and after 2 d produced sufficient mycelial growth to be detected by SDS/heat lysis. No plasmid DNA was detected at 2 d in soil extracted by SDS/heat lysis from treatment C, although the initial inoculum size of plasmid-containing host was 1.04×10^5 c.f.u. (g soil) $^{-1}$ (Table 1). Cresswell *et al.* (1991) detected plasmid in SDS/heat lysis extracts from sterile soil inoculated with 1×10^4 spores g $^{-1}$ and incubated for 2 d at 21 °C. It is therefore possible that much of the *S. violaceolatus* ISP 5438 inoculum was still present as spores at 2 d in the nonsterile soil used in this study. No further fluctuations in plasmid DNA were observed in the SDS/heat lysis extracts, suggesting that no significant regrowth of plasmid-containing mycelia occurred. The bead-beaten extracts also contained most plasmid DNA at 15 d (Fig. 7d) but there was evidence for further growth and sporulation before 45 d (Figs 1a and 6b).

Phage titres increased when the soil was 'fed' at 15 d and 30 d, in a manner similar to that reported by Germida (1986), indicating that the virus was propagating in a host which must have been in the mycelial state (Lomovskaya *et al.*, 1972) (Fig. 5a). Previous studies (Herron & Wellington, 1990; Wellington *et al.*, 1990a) indicated that in nonsterile unamended soil systems, germination of *S. lividans* TK24 was very limited. However, a significant increase in c.f.u. was observed in nonsterile nutrient-amended soil (Wellington *et al.*,

1990a). In the present study growth occurred within the first 15 d but no significant increase in inocula population levels was observed. The soil used in this study was collected at a time when the indigenous bacterial populations were high and the soil was not dried below 7% (w/w) moisture content. This may have resulted in a lack of growth and establishment of the inoculum due to the increased competition from indigenous microflora.

It was also clearly demonstrated that *S. lividans* TK24 and *S. violaceolatus* ISP 5438 did not spread and colonize nonsterile nutrient-amended soil but appeared to compete poorly with indigenous bacteria. This may reflect the long-term maintenance of these strains under laboratory conditions. *S. lividans* is also a streptomycin-resistant mutant, which can result in reduced fitness (Garrette & Wittman, 1974). The congruence between different methods for monitoring plasmid stability and survival in soil was also clearly demonstrated. The extraction and probing of soil DNA provided a sensitive method for monitoring plasmid in soil. The use of differential extraction techniques indicated that plasmid-containing strains were present mainly as spores after 30 d, which was supported by spore counts for treatment C (Fig. 1a). All the monitoring techniques showed that inoculant populations peaked at 15 d, and this coincided with the highest number of transconjugants recovered together with the appearance of one lysogen. Also at this time the highest level of plasmid DNA in the mycelial phase was detected by the SDS/heat lysis extraction procedure.

The fed-batch system was not designed as a true model of soil field conditions but it did, by dilution and withdrawal, provide a dynamic system resulting in population fluctuations. It has been demonstrated that direct monitoring of a plasmid can be achieved and provides data on population changes where plate counts failed; this was also demonstrated by Selenska & Klingmüller (1991a, b) for plasmid-encoded *nif* and chromosomally inserted Tn5 genes in an *Enterobacter agglomerans* host. KC301 numbers did show periodic increases in titre which correlated with addition of fresh soil and nutrients. These fluctuations were analogous to the rise and fall of phage titres predicted by a mathematical model of actinophage-host interactions in soil (Williams *et al.*, 1986; Manchester, 1986). The paucity of lysogens isolated related to the decline in phage and host numbers below the threshold level predicted by previous batch systems (Herron, 1991). This suggested that most phage infections were virulent, which may be indicative of old or senescing mycelium becoming infected. Phage infection, under laboratory conditions, is highest when using exponentially growing hyphal tips (Lomovskaya *et al.*, 1972). This point highlights how little is known about the response of a

Fig. 7. Agarose gels of DNA extracted from soil and corresponding Southern blots probed with radiolabelled pIJ673 DNA. (a, b) SDS/heat lysis extracts of DNA from treatment C; (c, d) bead-beaten extracts of DNA from treatment C. λ. *Hind*III-restricted λ DNA size markers. Distinct DNA bands are not clearly visible in the agarose gels, especially for SDS/heat lysis extracts, because of the humic acid and phenolic contamination; this ran ahead of the DNA and removed much of the ethidium bromide with a tendency to smear the samples.

phage to the metabolic activity of its host. Future work will go on to study the effect of different nutrient regimes on the ability of phage KC301 to lysogenize or lyse a streptomycete host *in vitro*.

Previous studies of streptomycete survival in soil have indicated that spores survive well in nonsterile soil (Wang *et al.*, 1989). In the present study there was evidence of death and/or predation of spores and mycelium. It is possible that addition of fresh nutrients caused spores to germinate, making them more vulnerable to phage infection and predation. This may be a more realistic model of soil field conditions compared to batch systems where no fresh nutrients are added.

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