APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1988, p. 1731–1737
0099-2240/88/071731-07$02.00/0

Vol. 54, No. 7

# Transduction of *Escherichia coli* by Bacteriophage P1 in Soil

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Received 25 January 1988/Accepted 11 April 1988

**Transduction of *Escherichia coli* W3110(R702) and J53(RP4) ($10^4$ to $10^5$ CFU/g of soil) by lysates of temperature-sensitive specialized transducing derivatives of bacteriophage P1 ($10^4$ to $10^5$ PFU/g of soil) (P1 Cm *cts*, containing the resistance gene for chloramphenicol, or P1 Cm *cts*::Tn*501*, containing the resistance genes for chloramphenicol and mercury [Hg]) occurred in soil amended with montmorillonite or kaolinite and adjusted to a −33-kPa water tension. In nonsterile soil, survival of introduced *E. coli* and the numbers of *E. coli* transductants resistant to chloramphenicol or Hg were independent of the clay amendment. The numbers of added *E. coli* increased more when bacteria were added in Luria broth amended with Ca and Mg (LCB) than when they were added in saline, and *E. coli* transductants were approximately 1 order of magnitude higher in LCB; however, the same proportion of *E. coli* was transduced with both types of inoculum. In sterile soil, total and transduced *E. coli* and P1 increased by 3 to 4 logs, which was followed by a plateau when they were inoculated in LCB and a gradual decrease when they were inoculated in saline. Transduction appeared to occur primarily in the first few days after addition of P1 to soil. The transfer of Hg or chloramphenicol resistance from lysogenic to nonlysogenic *E. coli* by phage P1 occurred in both sterile and nonsterile soils. On the basis of heat-induced lysis and phenotype, as well as hybridization with a DNA probe in some studies, the transductants appeared to be the *E. coli* that was added. Transduction of indigenous soil bacteria was not unequivocally demonstrated. The survival of P1, *E. coli* hosts, and transductants for at least 28 days in nonsterile soil indicated the potential for genetic transfer via transduction in soil.**

Effort is being directed worldwide towards the genetic manipulation of microorganisms (30). This biotechnology holds great promise for basic research (30), for economic exploitation in the pharmaceutical industry (24) and agriculture (18), and for the reclamation and preservation of ecological systems (5, 21). There is, however, concern about the potential risks associated with the release of genetically engineered microbes into natural environments (6, 22, 27), including the possible transfer of the novel genetic information to indigenous soil microbes. We studied gene transfer via transduction in *Escherichia coli*, a bacterium widely used in recombinant DNA studies and an inhabitant of numerous environments (30), by a specialized transducing derivative of bacteriophage P1, which is capable of lysogenizing *E. coli* and P1-sensitive mutants of several other gram-negative bacteria, including members of the genera *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, and *Citrobacter* (10, 11). By using this broad-host-range phage, the possibility of transduction of indigenous bacteria in soil could be studied. Clay amendments, nutrient levels, and microbial competition were among the environmental factors examined for their effects on transduction by P1 in soil.

Transduction may be a more important mechanism of gene transfer in soil and other natural habitats than conjugation or transformation, because the packaging of nucleic acid in a phage particle and the persistence conferred by the adsorption of phage particles on clay minerals and other particulates may represent evolutionary survival strategies (4, 17, 26, 28–32). Transduction of antibiotic resistance genes has been observed among lysogenic and nonlysogenic strains of *Staphylococcus aureus* (16) and *Streptococcus pyogenes* (13), in *S. aureus* present in the kidneys of mice (20), and in *E. coli* in the intestines of gnotobiotic mice (9). Reports of in situ transduction in aquatic environments include those of generalized transduction of *Pseudomonas aeruginosa* to streptomycin resistance (19) and plasmid transduction of the same species (25) by the generalized transducing phage F116L, and of *Vibrio parahaemolyticus* in oysters to the ability to degrade agar (3). Transduction has not been unequivocally demonstrated in soil (30).

## MATERIALS AND METHODS

**Bacteria, plasmids, and bacteriophages (Table 1).** For transduction studies, *E. coli* W3110, W3110(R702), and J53(RP4) were grown overnight at 30°C with shaking in Luria broth containing 1.0% tryptone, 0.5% yeast extract, and 0.5% NaCl. *E. coli* J53(RP4), used for the bacterial lawns on phage plaque assay plates, was prepared in the same manner.

Bacteriophage P1 Cm *cts* is a temperature-sensitive specialized transducing derivative of phage P1 that is not capable of maintaining lysogeny at 42°C and above (23) and carries a gene for resistance to chloramphenicol. A variant of this phage, designated P1 Cm *cts*::Tn*501*, carries mercury (Hg) resistance genes on the Tn*501* transposon and, thus, can confer resistance to both antimicrobial agents. Lysates of the phages were prepared from *E. coli* AB1157 lysogenic for either phage P1 by heat induction (23). The cultures were grown at 30°C in LCB (Luria broth containing 0.1% glucose, 10 mM $MgSO_4 \cdot 7H_2O$, and 2 mM anhydrous $CaCl_2$) to an optical density at 600 nm of 0.15 to 0.20, shaken for 2 h at 42°C, and treated with chloroform (ca. 0.5 ml/100 ml of culture) for 1 min to release additional phages; remaining intact cells were removed by filtration through a 0.45-μm-pore-size nitrocellulose filter membrane (Millipore Corp., Bedford, Mass.) before storage of the phages in LCB at 4°C. The titers of the phages were determined on *E. coli* J53(RP4) grown on LCA (LCB plus 1.5% Bacto-Agar for the bottom agar and 0.75% agar for the top agar) and incubated at 42°C.

**Soils.** Soil, obtained from the Kitchawan Research Laboratory of the Brooklyn Botanic Garden, Ossining, N.Y., was

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TABLE 1. Some characteristics of the bacterial strains, bacteriophages, and plasmids used

| Genetic entity | Relevant genotype or phenotype | Size (MDa)[a] | Source or reference |
|---|---|---|---|
| *E. coli* | | | |
| W3110 | Prototrophic | | 2 |
| J53 | *pro met* | | 2 |
| AB1157 | *lac* | | A. Summers |
| Bacteriophage | | | |
| P1 Cm *cts* | $Cm^r$ ts[b] | 60.0 | 23 |
| P1 Cm *cts*::Tn*501* | $Cm^r$ $Hg^r$ ts | 60.3 | A. Summers |
| Plasmid | | | |
| R702 | *tra*$^+$ $Tc^r$ $Hg^r$ $Km^r$ $Sm^r$ $Su^r$ | 46.0 | 12 |
| RP4 | *tra*$^+$ $Tc^r$ $Ap^r$ $Nm^r$ $Km^r$ | 40.4 | 7 |

[a] MDa, Megadaltons.
[b] ts, Temperature sensitive.

amended with montmorillonite (Volclay, Panther Creek-Aberdeen; American Colloid Co., Skokie, Ill.) to 3, 6, or 12% (vol/vol) (3M, 6M, or 12M, respectively) or with kaolinite (Continental; R. T. Vanderbilt Co., Norwalk, Conn.) to 12% (12K). The preparation, physicochemical characteristics, and restoration of the microbial populations of these soil-clay mixtures have been described previously (1, 8).

**Enumeration of bacteria and phages.** Total soil bacteria were enumerated on soil extract agar (5 g of $KH_2PO_4$, 1 g of glucose [filter sterilized], 100 ml of soil extract, 15 g of Bacto-Agar, 1.1 ml of 1 N KOH, and 900 ml of distilled water [$dH_2O$]), gram-negative bacteria were enumerated on MacConkey agar (MAC; Difco Laboratories, Detroit, Mich.), and transduced *E. coli* and other gram-negative bacteria resistant to chloramphenicol or Hg were enumerated on MAC amended with chloramphenicol (90 μg/ml) or $HgCl_2$ (20 or 30 μM). In experiments with nonsterile soil, the titers of P1 were determined without filtration of the soil dilutions on LCA amended with 100 μg of tetracycline per ml, with tetracycline-resistant *E. coli* J53(RP4) used for the plaque assay lawns. MAC amended with tetracycline (18 μg/ml) and $HgCl_2$ (30 μM) was used in some experiments to enumerate *E. coli* J53(RP4) transduced by P1 conferring resistance to Hg. To inhibit fungi, cycloheximide (200 μg/ml) was added to all plating media, including the plaque assay medium, in studies with nonsterile soil. The antibiotics and $HgCl_2$ were sterilized by filtration (0.45-μm-pore-size filter; Millipore).

In studies with sterile soil using *E. coli* lysogenic for P1 [*E. coli* J53(RP4)(P1 Cm *cts*::Tn*501*)], Hg-resistant transductants of *E. coli* W3110 were enumerated on M9 minimal agar (6 g of $Na_2HPO_4$, 3 g of $KH_2PO_4$, 0.5 g of NaCl, 1 g of $NH_4Cl$, 15 g of Bacto-Agar, and 1 liter of $dH_2O$). After being autoclaved, filter-sterilized solutions of the following were added to yield the following final concentrations: 2 mM $MgSO_4 \cdot 7H_2O$, 0.2% glucose, 0.1 mM anhydrous $CaCl_2$, and 30 μM $HgCl_2$ (M9M). This minimal medium did not support the growth of the auxotrophic *E. coli* J53(RP4)(P1) lysogen, and it was enumerated on MAC containing tetracycline (18 μg/ml). MAC was used to enumerate total *E. coli* [*E. coli* J53(RP4)(P1), W3110, and W3110(P1)], thus allowing the numbers of *E. coli* W3110 to be calculated from the difference between the total *E. coli* and the *E. coli* J53(RP4)(P1) plus W3110(P1) counts. The titers of P1 were determined without filtration on plaque assay lawns not containing tetracycline; chloroform was added at 2% (vol/vol) to the initial soil dilutions to kill all *E. coli* before the titers were determined. In studies with nonsterile soil, lysogenic *E. coli* J53(P1 Cm *cts*) was enumerated on MAC amended with chloramphenicol (90 μg/ml), *E. coli* W3110(R702) was enumerated on MAC amended with tetracycline (25 μg/ml), *E. coli* W3110(R702)(P1) transductants were enumerated on MAC containing both chloramphenicol and tetracycline, and the phage was enumerated on tetracycline-amended LCA with *E. coli* J53(RP4) used for the lawns.

**Inoculum.** *E. coli* cells were grown in Luria broth to $10^8$ to $10^9$ cells per ml and diluted with either sterile 0.85% saline or LCB to yield approximately $10^5$ CFU/g of oven-dried soil. The phages ($10^8$ to $10^9$ PFU/ml in LCB) were similarly diluted.

**In vitro transduction.** *E. coli* J53(RP4) in 2 ml of LCB (ca. $2.4 \times 10^7$ CFU/ml) was mixed with appropriate concentrations of P1 Cm *cts*::Tn*501* to obtain the desired multiplicity of infection (MOI; i.e., the ratio of the number of PFU to the number of CFU). The phage was allowed to adsorb on the host cells for 30 min, 0.1 ml of sterile 0.2 M sodium citrate was added to chelate $Ca^{2+}$ and $Mg^{2+}$ and stop adsorption, and total and transduced *E. coli* were enumerated on MAC and on MAC containing 30 μg of chloramphenicol per ml, respectively. Transduced *E. coli* J53(RP4) was isolated for use in soil experiments involving lysogenic *E. coli* J53(RP4)(P1 Cm *cts*::Tn*501*).

**Verification of transductants from soil.** Isolates of *E. coli* from soil were evaluated for P1 transductants by testing for lysogeny of the phage. Samples (0.05 ml) of filtered lysates of each isolate, prepared by heat induction in LCB, were spotted on lawns of *E. coli* J53(RP4) on LCA. The plates were incubated at 42°C and observed for clear areas (i.e., lysis of the bacteria) where the filtrate was spotted. As a control, 0.05 ml of $dH_2O$ or lysates from verified *E. coli* J53(RP4)(P1) were spotted on each lawn. Twenty-five isolates that exhibited a lactose-positive phenotype on MAC containing $HgCl_2$ (presumptive *E. coli* transductants) from nonsterile soil and 13 isolates from sterile soil that had received P1 Cm *cts*::Tn*501* plus *E. coli* J53(RP4) were evaluated.

Fifteen presumptive *E. coli* W3110 transductants from sterile soil and eight from nonsterile soil that had received *E. coli* J53(RP4)(P1 Cm *cts*::Tn*501*) and *E. coli* W3110 were also evaluated by heat lysis. Colonies that developed from soil dilutions plated on M9M were transferred with sterile toothpicks to MAC, and those exhibiting a lactose-positive phenotype were evaluated. Fifty colonies from M9M, whose corresponding colonies on MAC containing Hg showed a lactose-negative phenotype (presumptive transductants of indigenous soil bacteria), were similarly evaluated. These individual soil isolates were also grown as a lawn on LCA and spotted with their own lysates. Presumptive transductants were also verified in some studies with a biotinylated DNA probe specific for the *repA* region of the phage P1 genome (L. R. Zeph, M. A. Onaga, and G. Stotzky, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, Q126, p. 303).

**Procedures.** Each soil tube containing 2.1 g of wet soil (approximately the equivalent of 1.7 g of oven-dried soil). *E. coli* or phage P1 or both were added in 0.2 ml of either LCB or saline, and sufficient sterile $dH_2O$ was added to bring the soil-clay mixtures to their −33-kPa water tension, which ranged from 21.1 to 24.4% water (wt/wt) (1). When sterile soils were used, they were autoclaved for 30 min at 121°C before addition of the bacteria, phage, and $dH_2O$. The soil samples were maintained at their −33-kPa water tension

TABLE 2. Frequency of transduction of *E. coli* W3110(R702) in vitro and in sterile and nonsterile soils with a lysate of phage P1 Cm *cts*

| Condition | MOI | Incubation time (min) | Transduction frequency (%)[a] |
|---|---|---|---|
| In vitro[b] | 9.5 | 30 | 66.7 |
| | 6.6 | 30 | 5.1 |
| | 2.7 | 30 | 4.8 |
| | 0.3 | 30 | 0.4 |
| Sterile soil[c] | 3.0 | 10 | 0.0 |
| | 3.0 | 180 | 0.3 |
| Nonsterile soil[d] | 3.0 | 10 | 0.0 |
| | 3.0 | 180 | 0.1 |

[a] Calculated as [(number of transduced *E. coli*/number of added *E. coli*) × 100].
[b] Initial *E. coli* numbers were $2.4 \times 10^7$ CFU/ml of LCB.
[c] Initial *E. coli* numbers were $3.5 \times 10^5$ CFU/g of oven-dried soil.
[d] Initial *E. coli* numbers were $1.5 \times 10^5$ CFU/g of oven-dried soil.

with sterile $dH_2O$ on the basis of periodic weight measurements. The soil tubes were incubated at 25 ± 1°C. At each sampling time, 18 ml of sterile $dH_2O$ was added to each duplicate soil tube, and serial decade dilutions were spread plated in triplicate on each medium. The plates were incubated at 30°C except the soil extract agar plates, which were incubated at 25°C, and the plaque assay plates, which were incubated at 42°C. The means ± the standard error of the means ($\bar{x}$ ± SEM) were calculated for each sampling time and expressed as $\log_{10}$ CFU or PFU/g of oven-dried soil. Most experiments were repeated at least twice, and differences in results between experiments were generally not significant.

## RESULTS

**Effects of MOI on transduction in vitro and in soil.** Transduction was determined by the acquisition of resistance to chloramphenicol or Hg by the bacteria as the result of being lysogenized by phage P1. The frequency of in vitro transduction of *E. coli* W3110(R702) by phage P1 Cm *cts* increased as the multiplicity of infection (MOI) increased (Table 2). At an MOI of 3, the transduction frequency was higher in sterile (0.3%) than nonsterile (0.1%) soil after 3 h.

**Transduction by lysates in sterile and nonsterile soils.** When nonsterile soil was inoculated with approximately $10^5$ PFU or CFU of P1 Cm *cts* and *E. coli* W3110(R702) per g of oven-dried soil in LCB, there was an increase after 1 day of approximately 1 order of magnitude in the numbers of total and gram-negative bacteria and of about 2.5 orders of magnitude in the number of *E. coli* (Fig. 1B). During the next 20 days, the numbers of total and gram-negative bacteria decreased gradually to their original levels, but the decrease in numbers of *E. coli* was slightly greater. Transductants of *E. coli* showed patterns of growth and survival similar to those of total *E. coli*, albeit at levels that were several orders of magnitude lower. The growth and survival patterns of *E. coli*, total bacteria, and gram-negative bacteria in soil without added phages were similar to those when phages were added in this and all subsequent experiments, but no chloramphenicol- or Hg-resistant *E. coli* were detected.

Three hours after inoculation, titers of P1 were below the detection level of $10^2$ PFU/g of oven-dried soil, both when the phage was added alone and when it was added with *E. coli*. This apparent decrease in phage was the result of its adsorption to soil particulates on the membrane filters during filtration of the soil dilutions (data not shown). Similar

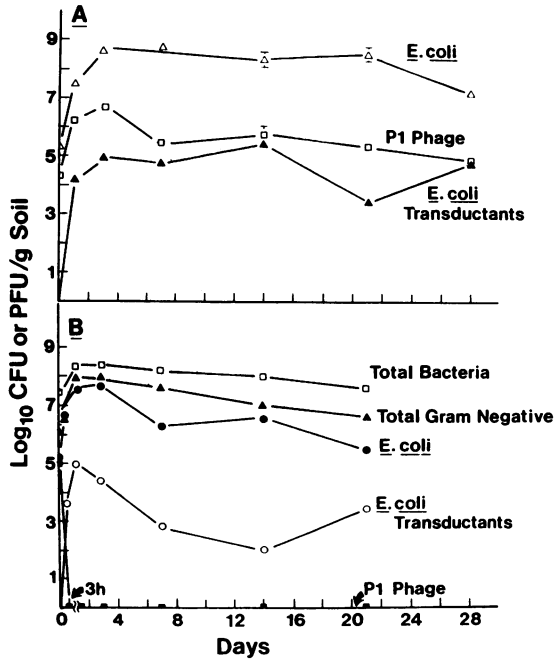


FIG. 1. Transduction of *E. coli* W3110(R702) by phage P1 Cm *cts* in sterile (A) and nonsterile (B) soils amended with 6M. Bacteria and phage inocula were added to soil in LCB on day 0. Values are means ± SEMs, which are shown if they are larger than the dimensions of the symbols.

decreases in phage P1 titers occurred after membrane filtration of both kaolinite- and montmorillonite-amended sterile soils. No decrease in titer was observed in the absence of soil, e.g., when P1 suspended in LCB was filtered or when titers of dilutions of sterile soil were determined without filtration. In subsequent experiments, titers of P1 in dilutions of nonsterile soil were determined without filtration, with LCA amended with tetracycline as the medium for plaque assay of the phage, as described in Materials and Methods.

In sterile soil, the increase in numbers of *E. coli* W3110(R702), W3110(R702)(P1), and phage P1 was greater than in nonsterile soil (Fig. 1A). However, the numbers of transduced *E. coli* W3110(R702) did not exceed $10^5$ CFU/g of oven-dried soil, which was significantly lower than the maximum numbers observed with transduced *E. coli* J53(RP4) in sterile soil (Fig. 2).

The growth and survival of *E. coli* J53(RP4) introduced in LCB and of the resultant Hg-resistant *E. coli* J53(RP4) transductants were also different in sterile and nonsterile soils (Fig. 2). In sterile soil, growth of total and transduced *E. coli* J53(RP4) was rapid, attaining a maximum in excess of $10^8$ CFU/g of oven-dried soil by day 4, and this maximum was maintained for 28 days. In nonsterile soil, maximum *E. coli* J53(RP4) counts were 1 to 2 orders of magnitude lower, and a gradual decline occurred after day 4.

**Effects of nutrient additions on transduction by lysates in soil.** In sterile soil inoculated with *E. coli* J53(RP4) and P1 Cm *cts*::Tn*501* in LCB, the numbers of *E. coli* J53(RP4) and the titers of phage P1 rapidly increased by 3 to 3.5 orders of magnitude and reached a plateau by day 4 (Fig. 3). When bacteria were inoculated in saline, their numbers increased more gradually until day 14 and then declined by 2 orders of magnitude during the next 12 days, and the titers of P1 decreased initially but then gradually increased to approxi-

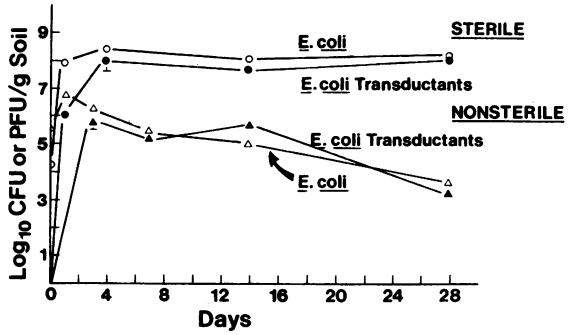


FIG. 2. Comparison of transduction of *E. coli* J53(RP4) by phage P1 Cm cts::Tn*501* in sterile and nonsterile soils amended with 6M. Bacteria and phage inocula were added in LCB on day 0. Values are means ± SEMs, which are shown if they are larger than the dimensions of the symbols.

mately the level of the original inoculum of $7.8 \times 10^4$ PFU/g of oven-dried soil. The numbers of transduced *E. coli* increased to about $10^8$ CFU/g of oven-dried soil when the bacteria were inoculated in LCB and to between $10^4$ and $10^5$ CFU/g when they were inoculated in saline.

In nonsterile soil, the numbers of transduced *E. coli* were the same 1 day after inoculation ($3.5 \times 10^3$ CFU/g of soil) in saline or LCB, but after 4 days, numbers of *E. coli* J53(RP4) transductants were nearly 1 order of magnitude higher with the LCB inoculum (Fig. 4). Thereafter, the rate of decrease in the number of transductants was similar with both types of inocula. Inoculation in LCB resulted in an initial growth of both total gram-negative bacteria and introduced *E. coli*, but the subsequent decline in the numbers of *E. coli* was similar with both inocula and paralleled that of the transductants. The gradual decrease in the titer of P1 was similar with both inocula during the 28-day incubation.

**Effects of clay minerals on transduction by lysates in soil.** The multiplication, survival, and maximum numbers of *E. coli* J53(RP4) and *E. coli* J53(RP4) transductants were similar in nonsterile 3M (Fig. 4B), 6M (Fig. 2), and 12K (data not shown) soils. Transduction in 12M soil was also evaluated, but growth of indigenous Hg-resistant soil bacteria that produced slime and spreading colonies on the selective medium prevented enumeration of *E. coli* transductants. The survival of P1 Cm cts::Tn*501* was determined only for the 3M and 12K soils, because techniques for monitoring this phage in nonsterile soil had not been developed when the

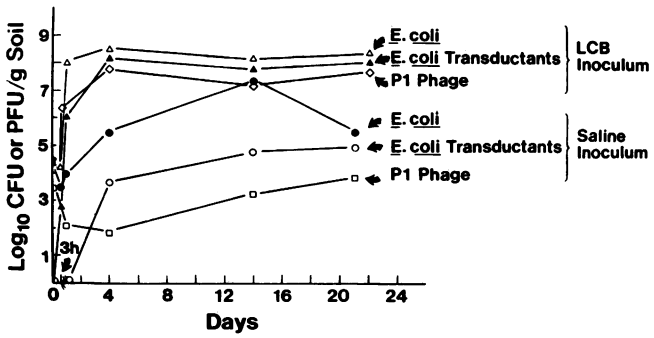


FIG. 3. Comparison of the effects on transduction of inoculating *E. coli* J53(RP4) and phage P1 Cm cts::Tn*501* in saline or LCB into sterile soil amended with 6M. Values are means ± SEMs, which are within the dimensions of the symbols.

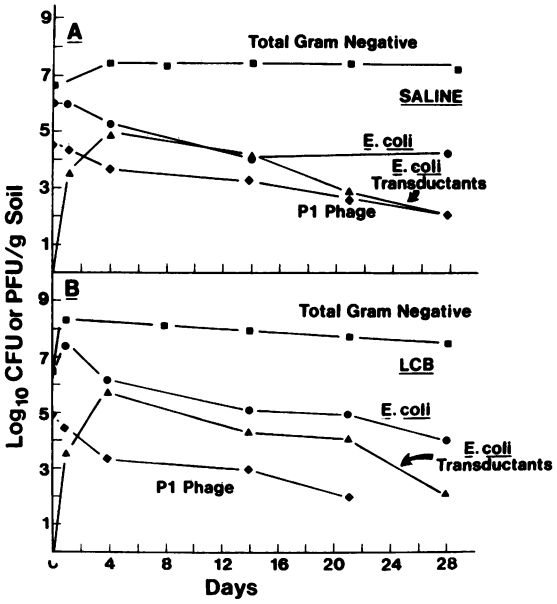


FIG. 4. Comparison of the effects on transduction of inoculating *E. coli* J53(RP4) and phage P1 Cm cts::Tn*501* in saline (A) or LCB (B) into nonsterile soil amended with 3M. Values are means ± SEMs, which are within the dimensions of the symbols.

studies with the 6M soil were conducted. The titer of P1 decreased in the 3M soil until it was below detectable levels (less than $10^2$ PFU/g of oven-dried soil; 0.1 ml of the 1:10 soil-water dilution was added to 2.5 ml of top agar) after day 13. In the 12K soil, P1 was no longer detectable after day 8.

**Transduction by lysogenic *E. coli* in sterile and nonsterile soils.** When *E. coli* J53(RP4) lysogenic for P1 Cm cts::Tn*501* and nonlysogenic *E. coli* W3110 were inoculated separately in LCB into sterile soil, free phage P1 and transductants of *E. coli* W3110 were detected (Fig. 5A). The number of transductants reached between $10^5$ and $10^6$ CFU/g of oven-dried soil, and the release of P1, probably from both the added lysogen and transduced *E. coli* W3110, resulted in $10^3$ to $10^4$ PFU/g of soil in the first few days after inoculation; these levels were maintained throughout the 22 days of incubation. The survival patterns of the introduced lysogen and *E. coli* W3110 were similar during the first 8 days after inoculation, but then the numbers of *E. coli* W3110 declined.

When *E. coli* J53(P1 Cm cts) and nonlysogenic *E. coli* W3110(R702) were added in LCB to nonsterile soil, *E. coli* W3110(R702) transductants and free phage were detected only on day 1 at, respectively, $10^2$ CFU and $10^4$ PFU/g of oven-dried soil (Fig. 5B). Both *E. coli* strains decreased in number after multiplying for the first 1 to 2 days.

**Verification of *E. coli* and indigenous soil isolates as P1 lysogens.** All 15 isolates of Hg-resistant *E. coli* W3110 from sterile soil to which both lysogenic *E. coli* J53(RP4)(P1) and nonlysogenic *E. coli* W3110 had been added were demonstrated by heat induction of lysis to be P1 transductants. Fourteen of the *E. coli* W3110(P1) isolates also acquired resistance to tetracycline, apparently through transfer of the RP4 plasmid, because these isolates also acquired resistance to ampicillin. Similarly, all presumed transductants of *E. coli* W3110(R702) from nonsterile soil produced heat-induced phage P1 lysates. In addition, phage P1 was detected in all heat-induced cultures of the presumptive transductants of *E. coli* J53(RP4) isolated from sterile and nonsterile soils that

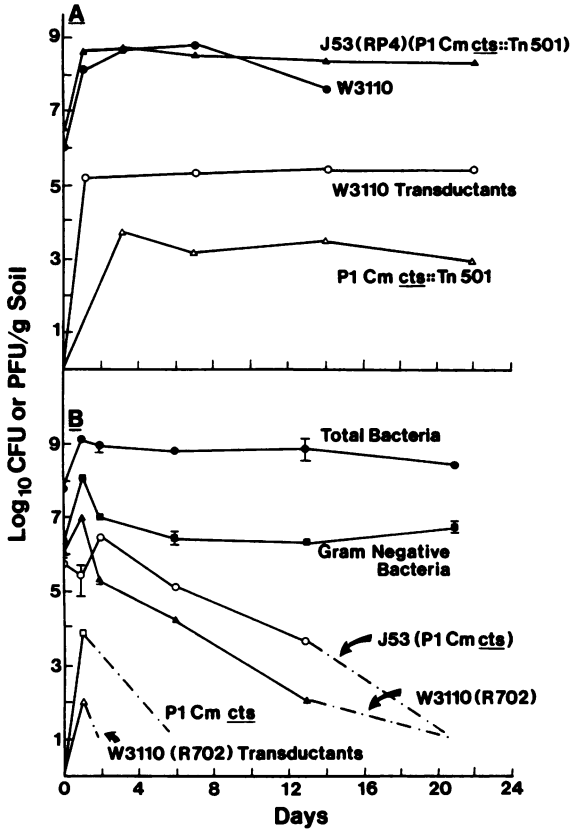


FIG. 5. Transduction in sterile (A) and nonsterile (B) soils amended with 6M that had received *E. coli* J53(RP4) lysogenic for phage P1 Cm cts::Tn*501* and nonlysogenic *E. coli* W3110 (sterile) or *E. coli* J53 lysogenic for phage P1 Cm cts and nonlysogenic *E. coli* W3110(R702) (nonsterile). Bacteria and phages were added in LCB on day 0. Values are means ± SEMs, which are shown if they are larger than the dimensions of the symbols.

had received free phage P1 Cm cts::Tn*501*. The presence of the phages was further confirmed with the DNA probe (data not shown).

None of the lactose-negative isolates from nonsterile soil produced lytic phage P1 or reacted with the DNA probe, although all isolates grew on the Hg-amended medium. However, the number of Hg-resistant indigenous bacteria in these soils was about $10^4$ CFU/g of soil. Consequently, no unequivocal data were obtained to indicate that phage P1 lysogenized indigenous soil bacteria.

## DISCUSSION

Transduction of *E. coli* W3110(R702) and J53(RP4) by lysates of phage P1 occurred in nonsterile soil, even though extensive multiplication of P1 was not observed, suggesting that infection by P1 probably occurred primarily during the first 1 to 2 days after inoculation. The appearance of transduced *E. coli* as early as 3 h after inoculation, with maximum numbers of transductants detected by day 1, further indicated that transduction by P1 after the first few days was minimal. Isolates of *E. coli* from soil were confirmed as transductants both by their abilities to grow on selective media and to produce P1 lysates after heat induction and, in some studies, with a DNA probe.

The transduction of *E. coli* J53(RP4) in nonsterile soil was not significantly affected by the type or amount of clay added. In contrast, the transfer of plasmids via conjugation in soils was enhanced at relatively high concentrations (e.g., 12%) of montmorillonite (M. A. Devanas and G. Stotzky, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, Q125, p. 302), as was the transfer of chromosomal DNA (15, 33). Montmorillonite has a general stimulatory effect on the growth of bacteria in pure culture and in soil (29, 32), primarily because this clay buffers the pH. Concentrations of montmorillonite in excess of the 6% used in this study need to be tested for their influence on transduction by P1.

The presence of organic nutrients did not affect transduction in nonsterile soil. When *E. coli* J53(RP4) and phage P1 were inoculated into nonsterile soil in saline, there was no increase in the numbers of *E. coli*, and the numbers of transductants were only slightly less than when they were inoculated in LCB and the host multiplied initially. Even though the numbers of *E. coli* J53(RP4) and *E. coli* J53(RP4) transductants were higher with the LCB inoculum, the percentage of the *E. coli* population that was resistant to Hg on day 4, apparently as the result of P1 transduction, was identical, at 67%, when added in either saline or LCB. This may have been the result of the similar gradual decrease in P1 titer over 28 days with both inocula. Inasmuch as phages are usually capable of infecting only metabolically active bacteria, a significant segment of the *E. coli* population in the saline inoculum must have been in this state, even while the majority of the host cells were probably in the stationary or death phase. The phage, therefore, was capable of infecting cells and establishing lysogeny under the relatively low nutritional conditions that presumably exist in natural soil. In contrast, in sterile soil, the absence of competitors for the available nutrients resulted in more transductants with the LCB than with the saline inoculum.

When *E. coli* J53(RP4) lysogenic for P1 was inoculated with *E. coli* W3110 into sterile soil, Hg-resistant transductants of W3110 were detected and confirmed by heat induction of phage P1 and with a DNA probe. In nonsterile soil, the presence of indigenous soil microorganisms greatly reduced the number of chloramphenicol-resistant *E. coli* W3110(R702) transductants detected. Nevertheless, these results demonstrated that phage P1 released from one lysogenic host in soil can lysogenize susceptible bacterial cells, despite the initial low titer of free phage and low MOI.

No P1 lysogens of indigenous soil bacteria were confirmed by heat induction of Hg-resistant isolates or with the DNA probe, even though the host range of phage P1 includes phage-sensitive mutants of such genera as *Klebsiella* and *Enterobacter*, which contain species that are normal soil inhabitants (11). If P1 transductants of indigenous bacteria were formed in soil, their numbers were too low to detect on the $10^{-2}$ soil dilution plates that were the source of the isolates. Even isolates that were incubated at 42°C only long enough to induce lysis by the temperature-sensitive P1 and then returned to 25°C to continue growth (thereby avoiding any growth inhibition that may occur at 42°C) failed to produce free phage P1.

An exact determination of transduction frequency in soil was not possible, except early in the incubation, because it was not possible to distinguish between increases in numbers of transductants with time resulting from the multiplication of *E. coli* transduced early in the incubation and increases resulting from sequential infection by P1. In sterile soil at an MOI of 3, the lower transduction frequency of 0.3% at 3 h, compared with the approximately 5% frequency in vitro after only 30 min, was probably the result of the lower numbers of host and phage added to soil and of more

contact between the phage and host in liquid than in structured soil. In nonsterile soil, the transduction frequency at 3 h was even lower (0.1%) than in sterile soil, indicating that indigenous soil microbes interfered with the ability of the phage to transduce the added *E. coli* or with the survival of the transductants.

Several factors affected the survival of P1 in soil. The numbers of free phage P1 remained higher in sterile soil, apparently because the host cells multiplied more extensively in sterile than in nonsterile soil. There was no increase in P1 titer in nonsterile soil receiving free phage in either a saline or LCB inoculum, even though *E. coli* J53(RP4) was present in excess of $10^6$ CFU/g of soil, probably because there was little multiplication of the host. Biological inactivation of the phages may have also been responsible for their gradual decrease in nonsterile soil, in contrast to their persistence in sterile soil containing only *E. coli* hosts. The increases in phage titers in both sterile and nonsterile soils when lysogenic *E. coli* was added were probably the result of reinfection and phage multiplication after spontaneous lysis of *E. coli* lysogens, which, in pure culture, occurs at a rate of one in every $10^5$ to $10^6$ P1-containing lysogens (23).

Host cell density may also have been involved in the extent of phage multiplication, because in pure culture, approximately $10^4$ CFU of *S. aureus*, *Bacillus subtilis*, or *E. coli* per ml were required before any increase in their respective phages was detected (34). Multiplication of *E. coli* J53(RP4) in sterile soil was more gradual when the bacteria were inoculated in saline than when they were inoculated in LCB, and the pattern of increase in P1 when it was inoculated in saline suggested that a minimum of $10^5$ to $10^6$ CFU of *E. coli* J53(RP4) per g of soil was required before phage P1 multiplication was detected. An estimate of this minimum level in sterile soil that received LCB inocula was not possible with the sampling times used, because multiplication of *E. coli* J53(RP4) was too rapid.

Montmorillonite protects phages and other viruses from inactivation (26, 28, 29, 32), as was confirmed with phage P1 in these studies. The numbers of P1 remained significantly higher in nonsterile 3M than in 12K soil, although the titers of the phage decreased in both soil-clay mixtures. The numbers of P1 added without host cells also remained higher in sterile soil amended with montmorillonite than in soil amended with kaolinite.

The presence of $Mg^{2+}$ and $Ca^{2+}$ at near optimal levels in soil samples receiving inocula in LCB apparently promoted adsorption of the phage to host cells (10), thereby contributing to higher phage titers. Soil samples receiving inocula in saline contained only the $Mg^{2+}$ and $Ca^{2+}$ naturally present in the soil-clay mixtures, and these concentrations may not have been optimal for adsorption. Furthermore, lysogenic bacteria generally undergo spontaneous induction of phage lysis more readily in a nutrient-rich environment (14), which could have increased P1 titers in soil receiving LCB inocula, although this was not evident in studies with nonsterile soil.

The results of these studies demonstrated that transfer of genetic information by transduction can occur in soil and that the resultant lysogens are capable of surviving in soil for at least 28 days. These results complement those of in situ transduction studies in aquatic ecosytems (3, 19, 25) and the recent report of transduction of *E. coli* in soil (J. J. Germida and G. G. Khachatourians, Abstr. Annu. Meet. Soil Sci. Soc. Am. 1986, S3, p. 179). Gene transfer occurred after the addition of either lysates of transducing phage or lysogenic bacteria to both sterile and nonsterile soils, which was similar to the results obtained in sterile river water microcosms (19). Thus, the potential importance of transduction as a method of gene transfer in natural ecosystems is being established. However, more studies are needed to clarify the environmental factors that influence this process, to determine whether indigenous bacteria are transduced by added phage, and to develop experimental protocols for assessing the risk that transduction poses in the release of recombinant bacteria to the environment.

## ACKNOWLEDGMENTS

This study was supported in part by cooperative agreements CR812484, CR813431, and CR813650 between the U.S. Environmental Protection Agency-Corvallis Environmental Research Laboratory and New York University.

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