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Impact of Phages on Two-Species Bacterial Communities

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A long history of experimental work has shown that addition of bacteriophages to a monoculture of bacteria leads to only a temporary depression of bacterial levels. Resistant bacteria usually become abundant, despite reduced growth rates relative to those of phage-sensitive bacteria. This restoration of high bacterial density occurs even if the phages evolve to overcome bacterial resistance. We believe that the generality of this result may be limited to monocultures, in which the resistant bacteria do not face competition from bacterial species unaffected by the phage. As a simple case, we investigated the impact of phages attacking one species in a two-species culture of bacteria. In the absence of phages, *Escherichia coli* B and *Salmonella enterica* serovar Typhimurium were stably maintained during daily serial passage in glucose minimal medium (M9). When either of two *E. coli*-specific phages (T7 or T5) was added to the mixed culture, *E. coli* became extinct or was maintained at densities that were orders of magnitude lower than those before phage introduction, even though the *E. coli* densities with phage reached high levels when *Salmonella* was absent. In contrast, the addition of a phage that attacked only *Salmonella* (SP6) led to transient decreases in the bacterial number whether *E. coli* was absent or present. These results suggest that phages can sometimes, although not always, provide long-term suppression of target bacteria.

Bacteriophages are predators of bacteria. Soon after the discovery of phages in the 1910s scientists attempted to use them as agents to cure bacterial infection (phage therapy) (5, 6). By the 1940s, phage therapy was considered a failure in the West and was abandoned in favor of antibiotics, although some Eastern European countries nurtured the technology and kept it to the present (18, 26).

Western interest in phage therapy is undergoing a revival (15, 18, 26). The greatest interest lies in using phage to treat infections, a technique that may face considerable economic hurdles because of the enormous cost of clinical trials coupled with the typically narrow host ranges of most phages. It has also been proposed that phage might be applied environmentally to depress the abundance of bacteria before they cause infection (11, 15, 21). For example, phage could be used to kill infectious bacteria in livestock feedlots (11) or on surfaces in hospitals. The advantage of this approach is that there should be fewer regulatory concerns, which should translate into a greatly reduced cost of implementation (8).

A critical obstacle for environmental phage treatment is the evolution of bacterial resistance to the phage. When large continuous cultures of bacteria have been treated with phages, resistance invariably evolves, and the concentrations of bacteria return to nearly their former levels (4, 12). This outcome has been obtained (i) despite the demonstrable fitness cost of phage resistance in many bacteria (12, 13) and (ii) even when multiple rounds of phage evolution allow the phage to enter an evolutionary arms race with bacteria (2, 20).

These in vitro laboratory experiments throw doubt on the idea that the release of phages can suppress the levels of target bacteria. Yet the experiments share one major limitation: they have all been conducted with single species of bacteria (usually *Escherichia coli*) growing essentially in monoculture (2, 12, 13, 20). Outside the laboratory, environmental populations of bacteria rarely exist in monocultures; rather, they typically exist in complex microbial communities. The ascent of phage-resistant bacteria could be profoundly affected by competition from other microbial species in ways that cannot be anticipated from monocultures. There is some support for this idea from studies of cyanobacteria in natural environments. Several studies found that cyanobacterial densities can be significantly reduced by phages (10, 22, 28). However, this result has been challenged by findings showing that cyanobacteria are largely resistant and therefore unaffected by phages (29).

Here we address the effect of interspecies competition on bacteriophage control of bacterial populations. A pair of bacterial species was maintained during serial transfers in minimal media. Phages specific to one host were introduced to observe the impact on the target species given interspecific bacterial competition.

MATERIALS AND METHODS

Strains. The bacteria used were *E. coli* B and *Salmonella enterica* serovar *Typhimurium* ATCC 14028S. The lytic phages T7 and T5 were used as *E. coli*-specific phages; SP6 was used as the *Salmonella*-specific phage. Both T phages are highly virulent and form large clear plaques on our strain of *E. coli*. T7 binds to the heptose residues in the lipopolysaccharide (7), while T5 binds to the outer membrane transporter protein FhuA (9). Competition assays have suggested that resistance to T7 comes at a high cost to the bacterial growth rate and/or competitive ability, while resistance to T5 has little or no cost in minimal media with glucose (12). SP6, a member of the same family as phage T7, forms moderate-size, clear plaques on the *Salmonella* strain used. The primary binding site of SP6 is the O antigen (23), but little is known about the cost of resistance to SP6. None of the three phage used here replicates on the heterologous host in the conditions employed (data not shown). The bacteria and phages were obtained from the collection of I. J. Molineux.

Design. Flasks were set up with one of the following combinations: (i) a monoculture of one bacterium, (ii) a two-species culture of *E. coli* and *Salmonella*, (iii) a monoculture of one bacterium plus a phage specific to it, or (iv) a two-species culture of *E. coli* and *Salmonella* plus one phage.

Bacteria were grown in 125-ml flasks containing 10 ml of M9 minimal medium

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with 0.2% glucose and no amino acids (19). At the beginning of a serial passage line, the day 1 flask was inoculated with approximately 10^6 *E. coli* and/or *Salmonella* cells from stocks grown overnight in minimal medium. The cultures were grown with aeration in a shaking incubator at 37°C and 175 rpm for 24 h. After 24 h, 1 μl was transferred to a new flask that contained 10 ml of fresh medium. For trials in which *E. coli* was challenged with phage, 10^3 PFU of T7 or T5 was added to the day 1 flask after 11 h of bacterial growth. The delay in phage addition was necessary to keep *E. coli* from becoming extinct. For trials in which *Salmonella* was challenged, 10^6 PFU of SP6 was added to the first flask at the same time as the bacteria (no delay was necessary). All trials lasted 5 days (four transfers) or until extinction occurred.

When T7 was used to challenge *E. coli* in the presence of *Salmonella*, the *E. coli* density dropped profoundly. This drop made it difficult to maintain T7 at densities sufficient for transfer into the next flask. In one trial, 100 μl of the previous culture (instead of the usual 1 μl) was transferred between flasks to overcome this obstacle. This increase in transfer volume did not cause any noticeable change in the population dynamics compared to the other trials. In a second trial, a supplement consisting of 10^6 PFU of T7 was added on the second day and every day thereafter to the flasks otherwise transferred as described above (1-μl passages). Again this caused no noticeable change in the population dynamics compared to other trials.

Bacterial density was measured at the end of each 24-h period by plating on LB agar plates containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (40 μl of a 20-mg/ml X-Gal solution/plate; LB agar consisted of 1% NaCl, 1% Bacto tryptone, 0.5% Bacto yeast extract, and 1.5% Bacto agar). *E. coli* colonies turn blue in the presence of X-Gal, as the *lacZ*-encoded beta-galactosidase of *E. coli* hydrolyzes X-Gal. *Salmonella* colonies remain white, as they lack the enzyme. Additionally, 10^6 PFU of a phage specific to one bacterium was spread on plates to kill one of the bacterial species. Phage densities in the experimental flasks were determined by plating medium from a flask on a lawn of sensitive cells. For both bacteria and phages, the lower limit of detection was 10^3/ml; below this level an organism was unlikely to be transferred to the next flask. The densities reported below are the final densities reached by the bacteria or phages each day after 24 h of growth.

Bacterial resistance assays. Streak tests were used to test for phage resistance. Approximately 10^7 phage were spread in a line across the center of an LB agar plate and dried. A bacterial colony was then touched with a sterile loop and streaked orthogonally across the phage deposit. A control consisting of sensitive bacteria was also streaked on each plate. Colonies were scored as sensitive if the line of cell growth stopped at the line of phage and were scored as resistant if the line of cells showed no change as it crossed the line of phage.

Adsorption tests were used to assay partial phage resistance in some bacteria that were sensitive in the streak tests. Cells from a single colony were grown in a 10-ml culture under the same conditions that were used in the experimental trials. The density of cells was monitored with a Klett-Summerson photoelectric colorimeter. Once a flask reached a turbidity of 60 ($\sim 10^7$ cells/ml), a sample was plated to determine the cell density (C), and 10^6 phage were added to the flask. Five minutes after the phage were added, a sample was mixed in top agar with sensitive cells and plated to obtain a measure of the total number of phage (N_0). A 1-ml sample was also centrifuged for 1 min at 10,000 rpm to pellet the bacteria and adsorbed phage; the supernatant was plated on a lawn of bacteria to determine the number of unadsorbed phage (N_U). The adsorption rate (k) was estimated from the equation $k = -\ln(N_U/N_0)/Ct$, where t is 5 min. Adsorption assays were performed for one isolate of *E. coli* from each of the three trials in which *E. coli* was maintained in a two-species culture with T7, as well as for two isolates of the original *E. coli* stock.

Resistant cell competition assay. To determine the cost of resistance to T7 and T5, resistant *E. coli* cells were competed against *Salmonella* in the absence of phage. The resistant *E. coli* cells were obtained as widely separated colony isolates from monocultures grown in the presence of phage. Colonies were screened to ensure that they remained resistant and were free of phage. Competition trials were initiated with 10^4 cells of each bacterium, and then the passaging protocol described above was used.

Analysis. Standard t tests were used to compare cell densities in different treatments. Analyses that treated days as independent data points were compared to analyses that treated days as nonindependent data points. Under the assumption of independence daily values were used for analysis, while under the assumption of nonindependence 5-day means were used. The two methods agreed qualitatively (although not quantitatively) concerning significance in all but one case. Both P values are reported below; the day-independent P values are given first.

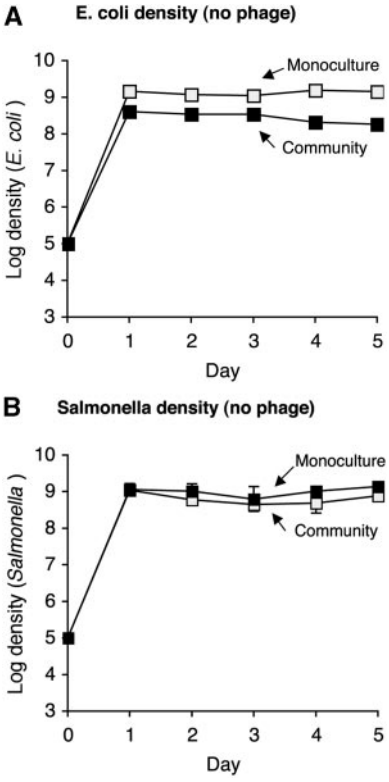

FIG. 1. Mean bacterial density by day in the absence of phage. The data are the log densities reached after 24 h of growth. (A) *E. coli* densities in monocultures ($n = 6$) (□) and in two-species cultures ($n = 5$) (■). (B) *Salmonella* densities in monocultures ($n = 3$) (□) and two-species cultures ($n = 4$) (■). The error bars indicate standard errors.

RESULTS

Without phage. In monocultures the bacteria attained densities of 10^8 to 10^9 bacteria/ml within the first day and then returned to these densities again each day for the duration of the trial (Fig. 1). The grand mean $\log_{10}$ density of *E. coli* reached 9.12 ± 0.04 bacteria/ml over six trials (Table 1). The

TABLE 1. Grand mean log densities of *E. coli* and *Salmonella*

Culture	Grand mean ± SE log density (cells/ml)	
	Monoculture	Two-species culture[a]
E. coli		
No phage	9.12 ± 0.04 (6)[b]	8.45 ± 0.05 (5)
With T7	8.19 ± 0.20 (5)[c]	Extinct (3)
		4.37 ± 0.26 (2)[c]
With T5	8.36 ± 0.24 (3)[c]	Extinct (3)
		6.34 ± 1.56 (3)[c]
Salmonella		
No phage	8.81 ± 0.07 (3)	9.00 ± 0.08 (4)
With SP6	8.58 ± 0.13 (3)	8.14 ± 0.19 (3)[c]

[a] Each value is the density of the focal bacterium (the bacterium attacked by the phage).
[b] The numbers in parentheses are numbers of cultures.
[c] The value is significantly less than the value for the corresponding no-phage control in the same column.

grand mean $\log_{10}$ density of *Salmonella* reached 8.81 ± 0.07 bacteria/ml over three trials (Table 1).

In communities containing both bacteria and no phage, the bacterial densities followed roughly the same pattern that they followed in monocultures (Fig. 1). Within the first day the log density of each bacterial species reached 8 to 9 bacteria/ml, and then the density rose to this value every day thereafter. The grand mean log density of *E. coli* was 8.45 ± 0.06 bacteria/ml over five trials (Table 1). The grand mean log density of *Salmonella* was 9.00 ± 0.08 bacteria/ml over four trials (Table 1). In a two-species culture, therefore, *E. coli* attained approximately one-fifth the mean density that it attained in a monoculture, a significant reduction (for days independent, $P < 0.001$; for days dependent, $P < 0.001$). The *Salmonella* density, in contrast, was slightly higher in a two-species culture than in a monoculture; the increase is marginally significant ($P = 0.04$) if days are treated as independent and insignificant ($P = 0.18$) if days are treated as nonindependent.

In view of the finding that the density of *Salmonella* was not reduced in the presence of high *E. coli* densities, we tested whether either bacterium was feeding on a metabolite of the other species. Monocultures of each bacterium were maintained at saturation for 1 day in the original medium. The spent medium was filtered and inoculated with the same bacterium or the other bacterium (filters were rinsed in sterile water first to remove soluble chemicals that might have affected growth). No substantial difference was observed between the growth rates when the bacteria were inoculated into their own spent medium or spent medium of the other species (data not shown). The same outcome was obtained with media from bacteria maintained at saturation for 3 days before filtering. It should be noted that this assay tests for metabolites of bacteria in the stationary phase, not metabolites that are limited to bacteria during rapid growth.

Additionally, we tested the ability of each bacterium to invade a population of the other species. Two-species flasks were set up in which the initial density of one bacterium was 4 orders of magnitude lower than that of its competitor. When *Salmonella* was rare initially, the mean log density of this organism rose to 8.03 bacteria/ml for 5 days (one trial). When *E. coli* was rare initially, in one trial it attained a mean log density of 7.03 bacteria/ml for 5 days. However, in two later trials, the density of *E. coli* remained less than 10^5 cells/ml and the organism was driven to extinction on the fifth day. These data suggest that *Salmonella* is able to invade a population even when it is rare but that *E. coli* is sometimes unable to enter a population if it is at a numerical disadvantage.

***E. coli* challenged with T7.** In monoculture, phage T7 had little long-term effect, and the *E. coli* density increased to high values (Fig. 2, upper panel). The grand mean log density of *E. coli* reached 8.19 ± 0.20 bacteria/ml every day in five trials (Table 1), which was less than 1 order of magnitude less than the monoculture density in the absence of phage, although the difference is highly significant ($P < 0.001$ and $P = 0.007$). Ten colonies from day 2 in each of two trials all tested as T7 resistant. However, sensitive cells must also have been present as T7 was maintained for 5 days at a grand mean log density of 8.03 ± 0.25 particles/ml.

In contrast, when competing with *Salmonella*, *E. coli* was not able to reach a high density when it was challenged with T7. *E. coli* became extinct in three of six trials, twice on the first day and once on the second day. In one trial T7 became extinct on day 2, but even in the subsequent absence of the phage, the log density of *E. coli* did not reach a value higher than 5.0 bacteria/ml in the remaining 3 days. In one trial each, *E. coli* and phage were maintained for 5 days by passaging 100 μl daily or adding phage daily (as described in Materials and Methods). In these two trials the levels of *E. coli* remained low, with a grand mean log density of only 4.37 ±0.26 bacteria/ml (Table 1 and Fig. 2, lower panel). In the trial in which 100 μl was passaged, T7 was lost on the sixth day, but the *E. coli* levels stayed low until the trial was ended on day 7. Cells remained sensitive to T7 for the three trials in which *E. coli* was maintained (10 of 10 colonies from day 2 of each trial were sensitive as determined by the streak test). Adsorption tests with an isolate from each of these three trials failed to detect any reduction in phage cell attachment rates that could serve as an alternative to complete resistance.

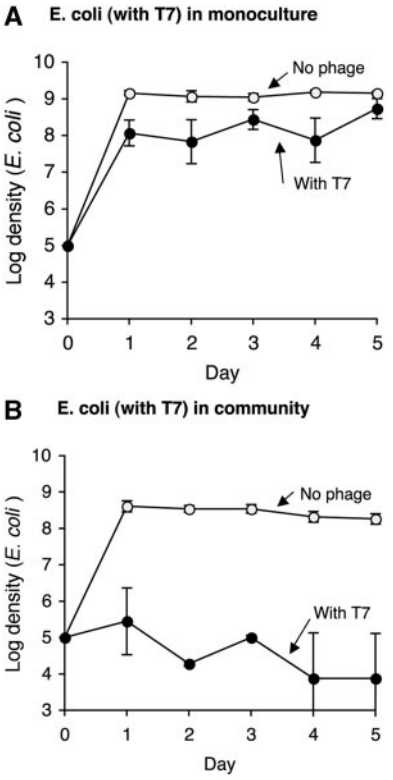

FIG. 2. *E. coli* mean densities when organisms were cultured with phage T7. The data are the log densities reached after 24 h of growth. (A) *E. coli* densities in monocultures with T7 ($n = 5$) (●). (B) *E. coli* densities in two-species cultures with T7 (●); values are the means of the two trials in which *E. coli* and T7 were maintained. Each panel also shows bacterial densities in controls in which phage were absent (from Fig. 1) (○). The error bars indicate standard errors.

T7-resistant *E. coli* showed a competitive cost initially; however, this cost was compensated with time. When resistant *E. coli* obtained after 8 h of exposure to T7 was competed against *Salmonella* in the absence of phage, *E. coli* was driven to extinction after 3 days. In a similar trial with *E. coli* first exposed to T7 for 24 h, *E. coli* survived for 5 days in mixtures with *Salmonella*, but the density never got above 10^5 cells/ml. The

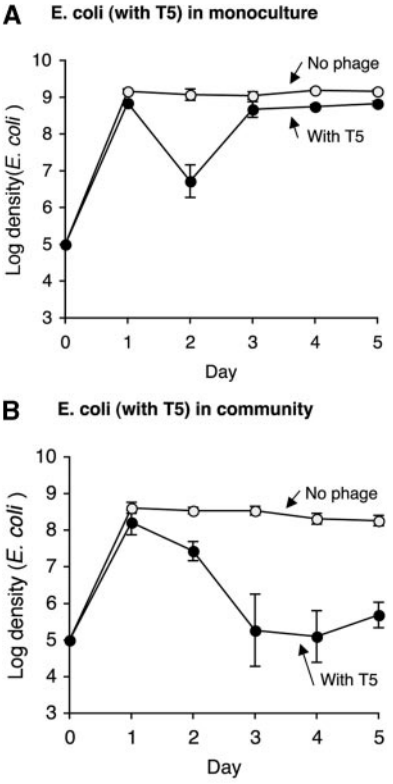

FIG. 3. *E. coli* mean densities when organisms were cultured with phage T5. The data are the log densities reached after 24 h of growth. (A) *E. coli* densities in monocultures with T5 ($n = 3$) (●). (B) *E. coli* densities in two-species cultures with T5 (●). E (T5) in a two-species culture is from the three trials in which *E. coli* was maintained for 5 days. Each panel also shows the bacterial densities in controls in which phage were absent (from Fig. 1) (○). The error bars indicate standard errors.

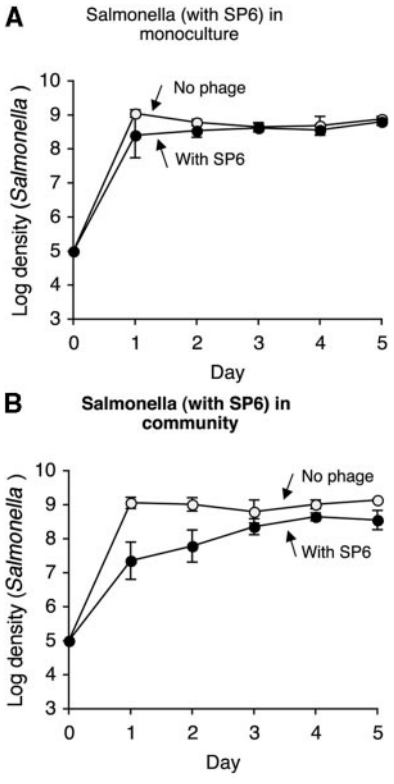

FIG. 4. *Salmonella* mean densities when organisms were cultured with phage SP6. The data are the log densities reached after 24 h of growth. (A) *Salmonella* densities in monocultures with SP6 ($n = 3$) (●). (B) *Salmonella* densities in two-species cultures with SP6 ($n = 3$) (●). Each panel also shows the bacterial densities in controls in which phage were absent (from Fig. 1) (○). The error bars indicate standard errors.

density of *E. coli* that was grown with T7 for 5 days rose to 10^7 cells/ml against *Salmonella* within the first day.

***E. coli* challenged with T5.** In monoculture, T5 had little effect on *E. coli* densities, as observed for T7 (Fig. 3, upper panel). The grand mean log density of *E. coli* with T5 reached 8.36 ± 0.24 cells/ml, which was less than 1 order of magnitude less than the density of *E. coli* in the absence of phage, although again the difference is significant ($P = 0.003$ and $P < 0.001$). In a resistance test 90% (27/30) of the *E. coli* colonies were resistant to T5 on day 3. The T5 densities peaked on day 2, and then T5 became extinct on day 4 in all trials.

T5 had a strong impact on *E. coli* densities in the *E. coli*-*Salmonella* two-species culture. In three trials, *E. coli* was driven to extinction, once on day 2, once on day 4, and once on day 5. In these trials T5 was maintained as long as *E. coli* was present. In three other trials, *E. coli* was maintained at low levels, with a grand mean log density of 6.34 ± 1.56 cells/ml. This density is significantly lower ($P = 0.008$) than the density of *E. coli* in a two-species culture without phage (Fig. 3, lower panel). T5 was lost once on day 4 and twice on day 5. In all of these cases the *E. coli* density remained low even in the absence of phage. Surprisingly, cells from day 3 of each trial all tested sensitive to T5 (10 of 10 colonies per trial).

To ensure that our two-species culture results were not an artifact of T5 resistance never appearing in the population, we ran two further trials. In these trials, *E. coli* was allowed to evolve resistance to T5 for 3 h before *Salmonella* was added. *E. coli* was maintained for 5 days at a grand mean log density of 5.88 ± 0.83 cells/ml, which was not significantly different from the values in the other two-species T5 culture trials ($P = 0.23$). One-half of the colonies on day 3 tested resistant.

E. coli showed no competitive cost to T5 resistance in the absence of phage. In a trial initiated with *E. coli* that had grown with phage for 8 h, the density of *E. coli* rose to 10^7 cells/ml within 1 day, and this level was maintained for 5 days. At the end of the 5 days the *E. coli* still tested resistant to T5. If resistance to T5 had a cost, one would expect the resistant *E. coli* cells to be replaced by sensitive cells in the absence of phage.

***Salmonella* challenged with SP6.** In monoculture *Salmonella* reached high densities whether SP6 was present or absent (Fig. 4, upper panel). In the presence of the phage, the grand mean log density of *Salmonella* was 8.58 ± 0.13 cells/ml in three trials (Table 1), which was not significantly different from the mean log density of *Salmonella* alone ($P = 0.07$ and $P = 0.18$). In resistance tests on day 3 of the three trials, all 30 colonies tested resistant. However, sensitive cells must also have been present as SP6 was maintained in all trials at a mean log density of 6.56 ± 0.45 particles/ml.

Salmonella reached a high density in the presence of SP6 even in competition with *E. coli* (Fig. 4, lower panel). In competition SP6 reduced the density of *Salmonella* for 2 days, but by the third day the density of *Salmonella* was back to densities equivalent to those observed in the absence of phage. The grand mean log density of *Salmonella* with SP6 was 8.14 ± 0.19 cells/ml in three trials, which was significantly lower than the density of *Salmonella* in the absence of phage ($P < 0.001$ and $P = 0.01$) but was a reduction of less than 1 order of magnitude. In resistance tests on day 3 of the three trials, all 30 colonies tested resistant. However, some sensitive cells must have been present as SP6 was maintained in two trials at a grand mean log density of 8.03 ± 0.27 particles/ml. In one trial, phage were lost on the third day and added back on the fourth day. On the fifth day of this trial, the log density of the phage reached 6.49 particles/ml, and there was no obvious change in the pattern of *Salmonella* density.

DISCUSSION

Our study addressed the use of phages to control bacterial densities in the external environment (phage "prophylaxis") (15). Previous work did not reveal a long-term effect of phages on bacterial densities, but in that work monocultures (single strains of bacteria) were used (2, 12, 13, 20). We studied phages in experimental environments with two bacteria, only one of which was susceptible to the phage. Our rationale was that competition between bacterial species may enhance the effectiveness of phage prophylaxis because bacterial resistance to phages can decrease the competitive ability of bacteria (12, 13). Our results suggest that the cost of resistance sometimes allows phages to suppress and maintain low densities of a susceptible bacterium when a resistant competitor is present.

In the two-bacterium cultures, phages were far more effective at maintaining low densities of *E. coli* than at maintaining low densities of *Salmonella*. The basis for this difference remains unclear. One explanation is that only *E. coli* truly experienced competition; the density of *Salmonella* was actually higher in the presence of *E. coli* than in a monoculture. Thus, any cost of resistance would have had a larger impact on *E. coli* densities than on *Salmonella* densities in competition. Alternatively, the difference between *E. coli* and the *Salmonella* control may have stemmed from the particular phages used and the types of bacterial resistance that they selected.

In contrast to previous work, in which single bacterial species were used, our results suggest that phages have the potential to reduce bacterial density in the environment. However, our study provides only a modest extension of previous work toward the overwhelming complexity that comprises real bacterial communities, and elaborations of our design are desirable as well. A straightforward extension of our work is to use communities consisting of three or more bacterial species. As communities are expanded to include more species, the equilibrium densities of individual species may drop to such low values that they no longer maintain phage populations, a problem that could be surmounted by periodic addition of high phage densities. Further realism would be added by allowing biofilm growth on surfaces, which adds the complexities of spatial population structure, a semisolid matrix through which phages must diffuse, and highly variable physiological states of bacteria. Work on phages in biofilms already indicates that biofilms present special challenges to phage growth and maintenance (27). Thus, the addition of greater complexity to these experiments, while increasing the realism, will also pose greater challenges to understanding.

Our results pertain to the use of phage to prevent, rather than treat, bacterial infection. When infections are treated, bacterial resistance is of little concern if the immune system is able to eliminate bacteria at levels below a threshold density (15). Thus, phage enable the infection to be controlled by killing enough cells to allow the immune response to eliminate the other cells; results of several experimental studies suggest that phage can be effectively used to treat bacterial infections (1, 3, 14, 16, 17, 21, 24, 25). The dynamics of environmental phage prophylaxis are thus vastly different than the dynamics of phage therapy (14). However, due to the medical practice regulations, phage may have more immediate application in the prevention of infection.

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REFERENCES

1. **Biswas, B. S. Adhya, P. Washart. B. Paul, A. Trostel, B. Powell, R. Carlton, and C. Merril.** 2002. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Infect. Immun. **70:**204–210.
2. **Buckling, A., and P. Rainey.** 2002. Antagonistic coevolution between a bacterium and a bacteriophage. Proc. R. Soc. Lond. B **269:**931–936.
3. **Bull, J., B. Levin, T. DeRouin, N. Walker, and C. Bloch.** 2002. Dynamics of success and failure in phage and antibiotic therapy in experimental infections. BMC Microbiol. **2:**35.
4. **Chao, L., B. Levin, and F. Stewart.** 1977. A complex two-species culture in a simple habitat: an experimental study with bacteria and phage. Ecology **58:**369–378.
5. **D'Herelle, F.** 1926. Bacteriophage and its behavior, p. 490–541. Williams and Wilkins, Baltimore Md.
6. **Eaton, M., and S. Bayne-Jones.** 1934. Bacteriophage therapy. JAMA **103:** 1769–1776, 1847–1853, and 1934–1939.
7. **Goldberg, E., L. Grinius, and L. Letellier.** 1994. Recognition, attachment and injection, p. 347–356. *In* C. Mathews et al. (ed.), Molecular biology of bacteriophage T4. American Society for Microbiology, Washington, DC.
8. **Goodridge, L., and T. Abedon.** 2003. Bacteriophage biocontrol and bioprocessing: Application of phage therapy to industry. SIM News **53:**254–262.
9. **Hantke, K., and V. Braun.** 1978. Functional interaction of the *tonA*/*tonB* receptor system in *Escherichia coli*. J. Bacteriol. **135:**190–197.
10. **Hennes, K., C. Suttle, and A. Chan.** 1995. Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. Appl. Environ. Microbiol. **61:**3623–3627.
11. **Huff, W., G. Huff, N., Rath, J. Balog, and A. Donoghue.** 2002. Prevention of *Escherichia coli* infection in broiler chickens with a bacteriophage aerosol spray. Poult. Sci. **81:**1486–1491.
12. **Lenski, R., and B. Levin.** 1985. Constraints on the coevolution of bacteria and virulent phage: a model, some experiments and predictions for natural communities. Am. Nat. **125:**585–602.
13. **Lenski, R.** 1988. Dynamics of interactions between bacteria and virulent bacteriophage. Adv. Microb. Ecol. **10:**1–44.
14. **Levin, B., and J. Bull.** 1996. Phage therapy revisited: the population biology of a bacterial infection and its treatment with bacteriophage and antibiotics. Am. Nat. **147:**881–898.
15. **Levin, B., and J. Bull.** 2004. Population and evolutionary dynamics of phage therapy. Nat. Rev. Microbiol. **2:**166–173.
16. **Matsuzaki, S., M. Yasuda, H. Nishikawa, M. Kuroda, T. Ujihara, T. Shuin, Y. Shen, Z. Jin, S. Fujimoto, M. Nasimuzzaman, H. Wakiguchi, S. Sughiara, T. Suguira, S. Koda, A. Muraoka, and S. Imai.** 2003. Experimental protection of mice against lethal *Staphylococcus aureus* infection by novel bacteriophage phi MR11. J. Infect. Dis. **187:**613–624.
17. **Merril, C., B. Biswas, R. Carlton, N. Jensen, G. Creed, S. Zullo, and S. Adhya.** 1996. Long-circulating bacteriophage as antibacterial agents. Proc. Natl. Acad. Sci. USA **93:**3188–3192.

18. **Merril, C., D. Scholl, and S. Adhya.** 2003. The prospect for bacteriophage therapy in Western medicine. Nat. Rev. Drug Discov. **2:**489–497.
19. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Mizoguchi, K., M. Morita, C. Fischer, M. Yoichi, Y. Tanji, and H. Unno.** 2002. Coevolution of bacteriophage PP01 and *Escherichia coli* O157:H7 in continuous culture. Appl. Environ. Microbiol. **69:**170–176.
21. **Nakai, T., and S. Park.** 2002. Bacteriophage therapy of infectious diseases in aquaculture. Res. Microbiol. **153:**13–18.
22. **Proctor, L., and J. Fuhrman.** 1990. Viral mortality of marine bacteria and cyanobacteria. Nature **343:**60–62.
23. **Scholl, D., J. Kieleczawa, P. Kemp, J. Rush, C. Richardson, C. Merril, S. Adyha, and I. Molineux.** 2004. Genomic analysis of bacteriophages SP6 and K1-5, an estranged subgroup of the T7 supergroup. J. Mol. Biol. **335:**1151–1171.
24. **Smith, H., and M. Huiggins.** 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves piglets and lambs. J. Gen. Microbiol. **129:**2659–2675.
25. **Soothill, J.** 1992. Treatment of experimental infections of mice with bacteriophage. J. Med. Microbiol. **37:**258–262.
26. **Summers, W.** 2002. Bacteriophage therapy. Annu. Rev. Microbiol. **55:**437–471.
27. **Sutherland, I., K. Hughes, L. Skillman, and K. Tait.** 2004. The interaction of phage and biofilms. FEMS Microbiol. Lett. **232:**1–6.
28. **Suttle, C., and A. Chan.** 1994. Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. Appl. Environ. Microbiol. **60:**3167–3174.
29. **Waterbury, J., and F. Valois.** 1993. Resistance to cooccurring phages enables marine *Synechococcus* communities to coexist with cyanophage abundant in seawater. Appl. Environ. Microbiol. **59:**3393–3399.