Arthrobacter globiformis and Its Bacteriophage in Soil

L. E. CASIDA, JR. AND KANG-CHIEN LIU

Department of Microbiology, The Pennsylvania State University, University Park, Pennsylvania 16802

Received for publication 15 August 1974

Bacteriophages in soil for Arthrobacter globiformis were rarely detected unless the soil was nutritionally amended and incubated. In amended soil, phage were continuously produced for at least 48 h, and this did not require the addition of host cells. Rod and spheroid stage host cells added to the amended soil encountered indigenous bacteriophage, but added phage did not encounter sensitive indigenous host cells for some time, if at all. The indigenous phage in nonincubated soil seemed to be present in a masked state which was not merely a loose physical adsorption to soil materials but required growth conditions other than lyogeny for them to increase their titers. The possibility is discussed that the indigenous host cells in nonamended soil are present in a nonsensitive spheroid state, with the cells becoming sensitive to the phage in a rate-limiting fashion as nonsynchronous outgrowth occurs for a portion of the spheroid cells.

Soil serves as a ready source for the isolation of virulent bacteriophage for many different bacteria (1). Little is known, however, about how these bacteriophage survive in soil and of the manner in which they locate and interact with their hosts in this habitat. Nor is it known how this interaction in soil is affected by pleomorphic growth cycles and dormancy states of the host bacteria. Answers to these obviously would be of interest from an ecological viewpoint, but, in addition, these answers might allow studies to be accomplished on the activities of specific bacteria in nature without isolating the bacteria. Thus, the specific bacteriophages for the bacterium are assumed to be present and operating in a defined manner in relation to their hosts, and these bacteriophages can easily be separated from the microbes and soil debris of the habitat and enumerated and evaluated by plaquing on host lawn plates of a specific bacterial species in the laboratory. The bacteriophage would thus serve as a natural internal indicator for the activities of a given bacterium in nature.

Arthrobacter globiformis was chosen as the model bacterium for this study. It occurs naturally in soil (4) and, at least in the laboratory, it demonstrates a defined but not overly complex growth cycle (3, 6, 10, 12, 13). In addition, soil is known to harbor bacteriophages for this species (5, 7, 8, 10). This bacterium and its bacteriophages were used, therefore, in the present study in an attempt to find answers to some of the more basic questions outlined above.

MATERIALS AND METHODS

Soil sample. A local Hagerstown silty clay loam (pH 6.0; plate count, 4.0 \times 10^6 at the time of use; moisture content, 25%; organic content, 3.5%; ref. 11) was used in this study. This sample consisted of the top 15 cm of soil collected directly beneath the surface vegetation, and it was stored in bulk for approximately 1 year in a sealed polyethylene bag.

Media. Most experiments used BEG broth, which contained 0.3% beef extract and 0.5% glucose. The compositions of additional media are listed in Table 2. All media components other than sugars and inorganic salts were Difco products (Difco Laboratories, Detroit, Mich.). The synthetic medium included in Table 2 was based on Lochhead and Thexton (9) and contained glucose, 1.0 g; KH\(_2\)PO\(_4\), 1.0 g; KNO\(_3\), 0.3 g; MgSO\(_4\) \cdot 7H\(_2\)O, 0.2 g; CaCl\(_2\), 0.1 g; NaCl, 0.1 g; FeCl\(_3\), 6H\(_2\)O, 0.01 g; yeast extract, 1.0 g; and distilled water, 1,000 ml. The basal layer of phage-plaquing plates was BEG medium containing 1.5% agar; the overlay for these plates was the same medium but with 1.0% agar.

A. globiformis cultures. The A. globiformis strains used in this study were American Type Culture Collection (ATCC) strains 8010 and 4336. Stock cultures were maintained on BEG medium containing 1.5% agar. Cultures of rod- and spheroid-stage cells of this bacterium were grown by inoculating a loop of cells from a slant into 50 ml of BEG broth in a 300-ml baffled-bottom Erlenmeyer flask (Bellco Glass Inc., Vineland, N.J.) and incubating by shaking at 28 C. Cells harvested at 1 day were designated rod-stage cells, and those harvested at 3 days were designated spheroid-stage cells. These cells were washed by centrifugation and resuspended to their original volume.

\(^{1}\)This research was authorized for publication as paper no. 4746 in the journal series of the Pennsylvania Agricultural Experiment Station.

951
in distilled water for addition to soil, which was to be incubated at 60% of moisture-holding capacity (MHC), but were not washed or concentrated when used in broth studies.

The lysogenic strain of 8010 occurred originally as a turbid plaque when a filtrate from a soil broth enrichment without added cells was plaque with strain 8010 on nutrient agar. A culture recovered from this plaque was purified and then handled in a manner similar to that for the nonlysogenic strain.

**Soil incubation at 60% MHC.** Portions (5 g) of soil in screw-cap tubes (18 [inner diameter] by 150 mm) were adjusted to 60% of the soil’s MHC by adding distilled water plus a washed strain 8010 cell suspension (1.7 × 10⁹ colony-forming units [CFU]/5 g of soil) and/or a carbon or nitrogen solution nutritional amendment. The tubes were incubated with caps loose at 28 C for periods of from 0 to 4 days, and then the soil from each tube was added to 100 ml of BEG broth in a 300-ml baffled-bottom flask. This was shaken for 10 min and then centrifuged at low speed. The supernatant fluid, containing the phage, was passed through a membrane filter (0.3-μm pore size; Millipore Corp., Bedford, Mass.) and then further diluted in sterile BEG broth for phage plaquing.

**Broth-soil phage enrichments.** Soil (4 g) was added to 100 ml of broth medium in a 300-ml baffled-bottom flask. In some experiments the flasks also received unwashed _A. globiformis_ cells, either rod or spheroid stage, at a final concentration in the flask of 1.7 × 10⁶ or 1.4 × 10⁶ CFU/ml, respectively; the final flask titer of added FX-1 phage was 40 plaque-forming units (PFU)/ml and of added soil-mixed phage preparation was 100 PFU/ml. These flasks were shaken at 28 C, and, at various time intervals, 5-ml samples were removed and subjected to low-speed centrifugation. The supernatant fluid was then passed through a 0.3-μm membrane filter, and the filtrate was diluted in BEG broth for phage plaque assay. Two or more flasks per treatment were included so that an overall large withdrawal of fluid volume from any one flask during sampling would not occur to affect the results.

**Cell-phage interaction in absence of soil.** To 80 ml of broth medium in a 500-ml Klett side-arm Erlenmeyer flask (Bellco Glass Inc., Vineland, N.J.) was added rod- or spheroid-stage unwashed cells at a final concentration in the flask of 10⁶ CFU/ml; the soil-mixed phage preparation, when added, was at a final titer of 10⁴ PFU/ml. These flasks were shaken, and, at various time intervals, turbidity was measured as Klett units and 5 ml-samples were withdrawn and treated as above for phage plaque assay.

For sonic treatment trials, phage suspension (1 ml), strain 8010 cell suspension (1 ml), soil (4 g), or mixtures of these were added to 100 ml of sterile BEG broth, and this was sonically treated for either 1 or 12 min in a Biosonic II oscillator (Bromwell Scientific, Inc., Rochester, N.Y.) operating at 112 W acoustic energy at the probe tip. The probe had been sterilized with alcohol.

**Phage sources and assay.** Most phage samples for plaque assay were diluted in BEG broth. One milliliter of a dilution to be plaque and 0.15 ml of strain 8010 broth culture (rod stage) were added to 2.5 ml of BEG 1% agar medium, and this was applied as an overlay on a BEG 1.5% agar basal layer. The experiments reported in Table 2, however, used nutrient broth and agar, respectively, for dilution and plating. The plates were incubated 48 h at 28 C, and then the plaques were counted. The plates were again observed at 4 days of incubation so as to note the occurrence of possible plaques not present at 2 days and whether any plaques had markedly increased in size. Periodic electron microscopy checks of phosphotungstic acid-negative stains were made of plaques resulting from soil enrichments to be sure that bacteriophage actually were causing the plaques.

Virulent phage strain FX-1 was recovered from a soil enrichment (without added host cells) that had been plaque on strain 8010 on nutrient agar. It was purified on this medium, and phage preparations were prepared by suspending the surface agar from confluent lysis plates in nutrient broth. The residual cells and agar were removed by low-speed centrifugation, and the phage suspension was passed through a sterile 0.3-μm membrane filter and refrigerated.

The soil-mixed phage preparation was made as follows. Soil (12 g) was added to 300 ml of BEG broth in a 1,000-ml Erlenmeyer flask. This was shaken for 24 h at 28 C and then clarified by low-speed centrifugation. The supernatant fluid was sequentially passed through sterile 0.8- and 0.3-μm membrane filters to yield a mixed phage suspension containing 10⁴ PFU/ml as plaque on strain 8010. Phosphotungstic acid-negative stains of this preparation viewed by electron microscopy showed phage of several different morphologies, but no bacterial or other cells.

**RESULTS**

**Soil present.** We have not been successful in extracting bacteriophage for _A. globiformis_ ATCC 8010 from our soil without first incubating the soil. Adjustment of the soil to 60% of its MHC with distilled water, with or without rod-stage host cell additions, and incubating at 28 C gave a few phage in one instance but none in the second instance (Table 1). Usually, however, no phage could be detected under these conditions. In contrast, incubation of soil amended with glucose or sucrose provided extensive phage production for this bacterium, and the response was greater when washed rod-stage host cells had been initially added to the soil.

Several broth media were evaluated with the object of finding one that would more easily allow study of the phage enrichment process when host cells had not been added than was possible by incubation of the soil at 60% MHC. In addition, it was desired that the soil with its indigenous host cells and phage be dispersed and agitated in a liquid medium, so that the effects of spatial discontinuities separating the phage from their hosts would be lessened. A
medium containing 0.3% beef extract and 0.5% glucose (i.e., BEG medium), inoculated and shaken with 4 g of soil (host cells not added), yielded $2.7 \times 10^4$ PFU/ml at 3 days of enrichment (Table 2). This medium, therefore, was used in most of the succeeding trials.

BEG broths were inoculated with 4 g of soil, with and without additions of host cells or phage, and shaken 1 to 2 days at 28°C. The soil not receiving added phage or host cells continuously produced phage over the time periods tested (Fig. 1 and 2). The added soil-mixed phage preparation, however, apparently encountered few if any sensitive indigenous host cells in the soil, and a delay in addition of the phage until 6 h of incubation had occurred did not change this picture. The pure laboratory strain of virulent phage (FX-1) did encounter some sensitive indigenous host cells in the soil (Fig. 2), but not until sometime between 14 and 24 h of incubation; this was shown to be a repeatable phenomenon. This in situ phage production by FX-1 was sensitive to sonic treatment. A 1-min sonic treatment at zero time of the broth containing the soil and FX-1 virtually eliminated this response. In contrast, the indigenous phage production occurring in the soil enrichment without added phage or host cells withstood a 12-min sonic treatment.

Rod-stage cells of A. globiformis ATCC strains 8010 and 4336 responded alike in phage production when they were added to soil enrichments (Fig. 2). Note that the soil incubated with strain 4336 was plaqued on strain 8010. Both bacterial strains vigorously produced phage starting at about 8 h and extending to about 14 h of incubation. In contrast, the added

| Nutrient amendment | Host cells not added (days of incubation) | Host cells added (days of incubation) |
|--------------------|------------------------------------------|----------------------------------------|
|                    | 0*                                        |                                       |
| None               | 40                                        | 0                                      |
| None*              | 0                                        | 0                                      |
| Starch*            | 0                                        | 40                                     |
| Glucose            | $3.6 \times 10^3$                         | $7.2 \times 10^4$                      |
| Sucrose            | $6.6 \times 10^4$                         | $3.8 \times 10^4$                      |
| (NH₄)₂SO₄*         | 120                                       | 180                                    |
| NaNO₃             | 60                                        | 20                                     |
| Urea               | 20                                        | 20                                     |

*This 0-h value represents a consistent result obtained in several trials.
*ND, Not determined.
*Separate but similar experiment using 25 g of soil in polyethylene-covered glass tumbler.
*Starch, glucose, and sucrose added at 50 mg/5 g of soil.
*(NH₄)₂SO₄, NaNO₃, and urea added at 5 mg/5 g of soil.

| Medium*              | PFU/ml*                             |
|----------------------|-------------------------------------|
| Distilled water      | 0                                   |
| Nutrient broth       | 93                                  |
| Nutrient broth, 0.6% CaCO₃ | 105                              |
| 1% glucose           |                                     |
| Nutrient broth, 0.5% NaCl | 145                              |
| 0.5% Peptone         | 178                                 |
| 1.0% Peptone         | 185                                 |
| 1.0% Peptone, 1.0% NaCl | 110                             |
| 0.3% Beef extract    | $3.0 \times 10^3$                     |
| 0.3% Beef extract, 0.5% glucose | $2.7 \times 10^4$                   |
| Synthetic medium*    | $6.4 \times 10^4$                     |
| Heart infusion broth*| 16                                  |

*Host cells not added.
* Diluted in nutrient broth and plaqued on nutrient agar.
* See Materials and Methods.
* The values for PFU per milliliter at 1, 2, and 4 days, respectively, were 10, 5, and 6.

spheroid cells of strain 8010 delayed phage production during the first 9 h (rate compared to the soil-only control; Fig. 1), but then continuously produced phage at least through 24 h of incubation. When the soil-mixed phage preparation and rod-stage host cells of 8010 were added simultaneously to the soil and the soil was incubated, the phage production curve closely resembled that obtained when only the bacteria were added without adding phage (see Fig. 1 and 2). Thus, in this case, the actual source of the phage inoculum (whether added or indigenous) producing the additional phage was not clear.
being liberated in the soil as a result of growth-related processes or simply were being physically desorbed from a masked state on the soil debris, flasks containing distilled water or broth medium were incubated at 4°C, and filtrates prepared at intervals during incubation were enriched for 24 h at 28°C, as above, with a pure culture of strain 8010. The cold-temperature soil incubation comprised, sequentially, shaking for 13 h, stationary for 11 h, and final shaking for 6 h. No phage were detected in these samples, nor were phage present in the final pure bacterial culture enrichment broths.

**Soil absent.** Some of the above results could be evaluated by studying the phage-host interactions in the absence of soil. Strain 8010 and its lysogenic counterpart were added to broth to provide $10^4$ CFU/ml; the soil-mixed phage preparation was added to yield 1 PFU/2.0 $\times 10^4$ CFU. These were shaken at 28°C, and samples withdrawn at intervals were plated on the nonlysogenic strain. During most of the duration of the phage liberation stages, the lysogenic and nonlysogenic strains produced phage in a similar manner (Fig. 3). However, the rod-stage inoculum cells started phage release at about 8 to 9 h, whereas the spheroid-stage inoculum cells delayed until some time between 13 and 15

![Graph](https://example.com/graph.png)

**FIG. 1. Phage production in shaken BEG broth soil enrichments in the presence and absence of added soil-mixed phage preparation or rod- or spheroid-stage cells of A. globiformis ATCC 8010. Plaqued on strain 8010. Symbols: Soil only, •; soil-mixed phage preparation plus soil, O; rod-stage cells plus soil, ■; spheroid stage cells plus soil, □.**

The shaken soil enrichments to which neither phage nor host cells had been added were found to have less than 1 free PFU/ml present at 3 h, but at least 1 PFU/ml at 4 h (sometimes delayed to 5 h). This was shown by adding 1-ml portions of the soil filtrates, recovered at these times, to broth flasks freshly inoculated with rod-stage cells of strain 8010 (soil not present). Shaking of these flasks for 24 h revealed no phage PFU for the enrichment of the 3-h filtrates, but a wide range of PFU titers (up to $5 \times 10^8$) for the enrichments of the 4- and 5-h filtrates. To determine whether the phage were

![Graph](https://example.com/graph2.png)

**FIG. 2. Phage production in shaken BEG broth soil enrichments in the presence and absence of added phage FX-1 or rod-stage cells of A. globiformis ATCC strains 8010 and 4336. Plaqued on strain 8010. Symbols: Soil only, •; strain FX-1 phage plus soil, O; A. globiformis 4336 cells plus soil, ■; A. globiformis 8010 cells plus soil, □.**
These results for the rod-stage cells approximately correspond to those for rod-stage cells in the presence of soil (Fig. 1 and 2), but the initiation of phage release for the spheroid-stage cells in Fig. 3 (absence of soil) seems to be delayed approximately 3 to 5 h as compared with the results in the presence of soil (Fig. 1). In the absence of added phage, both rod- and spheroid-stage cells of the lysogenic strain produced almost no phage in this broth medium; 12 PFU/ml were detected at 18 h for the rod-stage cells, and 30 PFU/ml at 12 h for the spheroid-stage cells. At other incubation times only 1 or 2 PFU/ml were detected. Both the lysogenic and nonlysogenic strains grew at a similar rate in this medium; Fig. 4 shows growth for rod- and spheroid-stage cell inocula of the nonlysogenic strain. As compared to BEG broth, considerably more growth of both the lysogenic and nonlyso-
genic strains occurred in heart infusion broth, and the growth of both strains was equivalent in this medium. The delay for growth initiation by the spheroid-stage cells was still present, however, in this medium. Figure 5 presents growth results and phage production resulting from lysogeny when lysogenic strain, spheroid-stage cells were used as inoculum; rod-stage cells were not tested for phage production. Addition of the
mixed-soil phage preparation did not materially change this picture for either growth or phage production. In contrast to these results, the spheroid-stage cells of the nonlysogenic strain (rod cells not tested) were virtually immune to added phage in the heart infusion broth. Growth as measured by turbidity was not altered, and the added phage were barely detectable or not detectable in samples taken during incubation.

Both the rod- and spheroid-stage cells of strain 8010 were quite resistant to sonic treatment; the CFU per milliliter were identical for cells not sonically treated and cells sonically treated for 12 min. The phage, however, displayed some sensitivity towards sonic treatment. Phage strain FX-1 showed 97% survival of PFU per milliliter with 1 min of sonic treatment and 43% with 12 min; the respective survival values for the soil-mixed phage preparation were 84 and 50%. Enrichment in shaken culture was not involved in these determinations, and soil was not present. The nonsonically treated, soil-mixed phage preparation was relatively resistant to the shaking involved in broth incubations; shaking in BEG broth in the absence of host cells or soil gave percent survivals of the phage at 5, 12, and 24 h, respectively, of 60, 35, and 31%.

**Rod- and spheroid-stage cells.** Rod- and spheroid-stage strain 8010 cells were used as inocula in these studies. The rod cells were from near the end of the logarithmic growth phase, and they presented a Klett value of approximately 256 units and a plate count of $1.7 \times 10^8$/ml. The respective values for the spheroid cells, which received two additional days of incubation, were similar, being 226 units and $1.4 \times 10^9$/ml. In addition to this age disparity, these cells differed in their morphology. The rod cells were short, gram-negative rods surrounded by what appears to be a slime layer. The spheroid cells were gram-positive coccoid rods slightly pointed at each end and surrounded by a thin, gram-negative casing. The delay in growth initiation observed when the latter cells were used as inoculum (see Fig. 4) seems to be due to the time required for pleomorphic outgrowth of these cells. The outgrowth at 12 h is shown in Fig. 6, which corresponds to a point on the growth curve (Fig. 4) when growth increase is not yet measurable as turbidity.

**DISCUSSION**

Bacteriophages for _A. globiformis_ ATCC strain 8010 were rarely detected in our soil unless the soil was amended nutritionally and incubated. This was true for soil incubated with water at 60% of the soil’s MHC and for soil shaken in water. Addition of washed host cells did not change this picture. In contrast, addition of glucose or sucrose to soil at 60% of MHC, with or without a simultaneous addition of host cells, stimulated a bacteriophage titer buildup to approximately $10^4$ to $10^5$ PFU/g of soil by the second day of incubation. Amendment of the soil with nitrogen-containing compounds, however, was less stimulatory to the production of phage.

The sequence of events and the activities of the phage and host cells occurring during soil incubation were more easily studied by using shaken- aerated broth enrichments with soil, and, for approximately the first 24 h, these enrichments seemed to be representative of the 60% MHC soil incubations. Evaluation of several broth media showed that a medium comprised of 0.3% beef extract and 0.5% glucose (i.e., BEG broth) provided acceptable phage titers in the shaken soils as plated with _A. globiformis_ ATCC strain 8010. When this medium was used for growing strain 8010 in the absence of soil, a reasonable growth rate and total amount of growth were obtained, although these were less than those obtained with a nutritionally richer medium such as heart infusion broth.

Soil shaken in BEG broth (host cells or phage not added) started to produce phage which plaqued on strain 8010 at about 6 h of incubation, and this phage production continued for 48 h or longer. However, a decrease in the phage production rate usually occurred by about 24 h (Fig. 2). Host cells of strains 8010 and 4336 added to this soil enrichment encountered bacteriophage, as shown by phage titers greater than with the soil alone, starting at about 8 to 9 h of incubation. In contrast, phage added to the soil (host cells not added) did not seem to encounter sensitive host cells for some time, if at all. Laboratory strain FX-1 lytic phage interacted with the indigenous host cells in the soil starting some time after 14 h of incubation (Fig. 2; rate compared with the soil-only control), but a soil-mixed phage preparation that had originally been recovered from this soil did not find sensitive host cells (Fig. 1). The reaction of the indigenous host cells in soil to the added FX-1 phage seemed to be a different phenomenon from that of the interaction of the indigenous phage and indigenous host cells already in the soil. Thus, sonic treatment of the mixture of soil and phage FX-1 before incubation destroyed the phage production response to this phage in the soil, but the phage production by the indigenous phage and
host cells was not affected by the sonic treatment. Pure culture studies showed that both the rod- and spheroid-stage cells of strain 8010 were quite resistant to the sonic treatment levels used in this study, and that both the FX-1 phage strain and the soil-mixed phage preparation were relatively resistant to sonic treatment.

The phage production in soil not receiving added host cells or phage did not seem to be merely a physical release of phage which had been masked by adsorption to soil materials, because phage titers did not build up in the soil when it was incubated in the cold. In addition, sonic treatment of the soil did not release additional phage over those in nonsonically treated soil.

The strain 8010 cells used in this study were either gram-negative, short rods (rod-stage cells) taken near the end of their logarithmic growth or gram-positive spheroids (spheroid-stage cells) from late in the maximal stationary phase of growth. On inoculation into fresh BEG medium, the latter cells delayed growth for approximately 6 h longer than did the rod cells. This extra time was required for pleomorphic outgrowth of the spheroids (Fig. 6) before initiating a rapid growth phase. A lysogenic strain of 8010 was also used in these studies and, as concerns spheroid pleomorphic outgrowth and growth of the rod cells, it behaved in a manner similar to that of the nonlysogenic strain. The choice of bacterial cell numbers (either rod or spheroid stage, lysogenic or nonlysogenic) to be added to the soil or used as inoculum in pure culture studies was quite empirical because, although Arthrobacter species are thought to be quite numerous in soil (2), it was not known how many A. globiformis-like cells might be in soil that could respond to phage that plaque on strain 8010. Likewise, the numbers of phage PFU to be added to soil or pure bacterial cultures were open to question. Since the phage numbers recoverable from soil were nil or very low unless incubation with nutrients was employed, it was decided to use a low ratio of PFU to CFU in the pure culture experiments. The final ratio when soil was not present was approximately 1 PFU/2 × 10^4 CFU.

A comparison of Fig. 3 and 4 for pure cultures in the absence of soil shows that, for both rod- and spheroid-stage cells used as inoculum, phage production is initiated as the rod cells start to multiply and is rapid thereafter. The above-mentioned delay for spheroid pleomorphic outgrowth thus also applies as a delay for phage production. Figure 3, in addition, shows that the lysogenic strain in BEG broth with added phage responds with growth and phage production in a manner similar to that of the nonlysogenic strain.

The delay for phage production by indigenous soil phage acting on spheroid-stage cells added to the soil, and the shorter delay for added rod-stage cells, are shown in Fig. 1. After these delays, however, phage production in both cases is rapid, with rates roughly equivalent to those resulting when 100 PFU of the soil-mixed prepa-
ration per ml are reacted with 10^4 CFU of strain 8010 per ml (Fig. 3). These rates are considerably greater, however, than those for the interaction of indigenous phage with indigenous host cells (Fig. 1 and 2). The latter rates could indicate that the sensitive host cell level naturally present in soil actually is quite low. From this viewpoint it is of interest that the 24-h indigenous phage production titers in Fig. 1 and 2 (host cells and phage not added) can be duplicated (experiment not reported) by shaking a mixture of 80 CFU of strain 8010 rod-stage cells per ml and 250 PFU of the soil-mixed phage preparation per ml for 24 h in BEG broth. A low available host cell number could also explain the inability of the added soil-mixed phage preparation to locate sensitive host cells, and of added strain FX-1 phage to find sensitive host cells until some time after 14 h of incubation. A low sensitive host cell level, however, does not necessarily mean that the total numbers of host cells are low. It is generally assumed that Arthrobacter species occur naturally in soil in the spheroid (coccoid) stage (4) and, although the present study has shown a defined time requirement for spheroid outgrowth, nothing is known about the frequency with which this outgrowth occurs in nature. In other words, the proportion of the spheroid population that does not respond with outgrowth to a given environmental stimulus is not known. A non-synchronous spheroid outgrowth in nature, however, could provide a continuous but rate-limiting source of low numbers of cells sensitive to phage and, thus, might explain the present results. The survival of this bacterium in nature thus could well depend on there being a reservoir of spheroid cells which do not undergo outgrowth even though growth conditions may have improved.

An alternate explanation for our results would be that the phage production in soil observed when neither host cells nor phage were added represents in total the results of lysogeny for the in situ cells or, alternatively, an initial production of phage through lysogeny with these phage then acting in a virulent manner on other sensitive host cells. These do not seem to apply in our study, and this conclusion is based on the opposing results obtained with the use of BEG and heart infusion broths, and on the fact that lysogeny as a means of inoculating sensitive host cells would impose too long a time delay before phage production could be initiated by the latter cells. Soil incubated in heart infusion broth (host cells and phage not added) produced only barely detectable levels of phage as contrasted with the phage yields produced in BEG broth (Table 2; Fig. 1 and 2). When soil was not present, the lysogenic strain of 8010 without addition of phage produced easily detectable levels of phage in heart infusion broth (Fig. 5), but barely detectable levels or no phage in BEG broth. Phage production in soil thus more closely resembles that associated with the nonlysogenic strain. Obviously, however, we used only one lysogenic strain in this study and, of course, do not know whether the soil harbors other lysogenic strains that would act in an entirely different manner under our experimental conditions.

Based on the foregoing discussions taken as a whole, it would seem that in non-nutritionally amended soil the numbers of naturally occurring bacteriophage capable of plaquing on A. globiformis ATCC 8010 are low but not nil. Their numbers, however, cannot be precisely quantified, because they appear to be masked in some manner, other than through lysogeny or a loose physical adsorption to soil materials, so that they cannot be washed from the soil but, still, are available with the proper time delays for reaction with added rod- or spheroid-stage host cells. The naturally occurring host cells in soil for these phage either are present in very low numbers or are present in a form insensitive to the phage. In the latter case, incubation with added nutrients would nonsynchronously change them into a sensitive state so that at any one time only a portion of the cells could interact with the phage.

ACKNOWLEDGMENTS

This work was supported by contract NGR 39-009-180 with the National Aeronautics and Space Administration.

We thank J. A. Novitsky for able technical assistance and stimulating discussions concerning portions of this study.

LITERATURE CITED

1. Adams, M. K. 1959. Bacteriophages. Interciences Publishers Inc., New York.
2. Boylen, C. W. 1973. Survival of Arthrobacter crystallopoites during prolonged periods of extreme desiccation. J. Bacteriol. 113:55-57.
3. Chaplin, C. E. 1957. Life cycles in Arthrobacter pascens and Arthrobacter terrigenus. Can. J. Microbiol. 3:103-106.
4. Conn, H. J. 1948. The most abundant groups of bacteria in soil. Bacteriol. Rev. 12:257-273.
5. Conn, H. J., E. J. Bottcher, and C. Randall. 1945. The value of bacteriophage in classifying certain soil bacteria. J. Bacteriol. 49:359-373.
6. Conn, H. J., and L. Dimmick. 1947. Soil bacteria similar in morphology to Mycobacterium and Corynebacterium. J. Bacteriol. 54:291-303.
7. Einck, K. H., P. A. Pattee, J. G. Holt, C. Hagedorn, J. A. Miller, and D. L. Berryhill. 1973. Isolation and characterization of a bacteriophage of Arthrobacter globiformis. J. Virol. 12:1031-1033.
A. GLOBIFORMIS PHAGE IN SOIL

8. Gillespie, D. C. 1960. Isolation of bacteriophage for *Arthrobacter globiformis*. Can. J. Microbiol. 6:477-478.
9. Lochhead, A. G., and R. H. Thexton. 1952. Qualitative studies of soil microorganisms. X. Bacteria requiring vitamin B₁₂ as growth factor. J. Bacteriol. 63:219-226.
10. Mulder, E. G., and J. Antheunisse. 1963. Morphology, physiology and ecology of *Arthrobacter*. Ann. Inst. Pasteur (Paris) 105:46-74.
11. Pramer, D., and E. L. Schmidt. 1964. Experimental soil microbiology. Burgess Publishing Co., Minneapolis.
12. Stevenson, I. L. 1961. Growth studies on *Arthrobacter globiformis*. Can. J. Microbiol. 7:569-575.
13. Veldkamp, H., G. van den Berg, and L. P. T. M. Zevenhuizen. 1963. Glutamic acid production by *Arthrobacter globiformis*. Antonie van Leeuwenhoek J. Microbiol. Serol. 29:35-51.