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Kinetics of Deoxyribonucleic Acid Destruction and Synthesis During Growth of *Bdellovibrio bacteriovorus* Strain 109D on *Pseudomonas putida* and *Escherichia coli*

ABDUL MATIN[1] AND SYDNEY C. RITTENBERG
University of California, Los Angeles, California 90024

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During the growth of *Bdellovibrio bacteriovorus* on *Pseudomonas putida* or *Escherichia coli* in either 10^{-3} M tris(hydroxymethyl)aminomethane or in dilute nutrient broth, the host deoxyribonucleic acid (DNA) was rapidly degraded, and by 30 to 60 min after the initiation of the bdellovibrio development cycle essentially all host DNA became nonbandable in CsCl gradients. At this stage the host DNA degradation products were nondiffusable, and there was no appreciable pool of low-molecular-weight (cold acid soluble) DNA fragments in the cells or in the suspending medium. Bdellovibrio DNA synthesis occurred only after degradation of host DNA to a nonbandable form was complete. The synthesis occurred in a continuous fashion with *P. putida* as the host and in two separate periods with *E. coli* as host. By using *E. coli* containing a ^{3}H-thymidine label, it was shown that 73%, on the average, of the thymine residues of host DNA were incorporated into bdellovibrio DNA when *E. coli* was the only source of nutrient. In the presence of dilute nutrient broth, the host cells still served as the major source of precursors for bdellovibrio DNA synthesis, with only 20% of the precursors arising from the exogenous nutrients. The data indicate an efficient and controlled utilization of host DNA by the bdellovibrio. The host DNA is apparently degraded early in the developmental cycle to oligonucleotides of intermediate molecular weight from which the biosynthetic monomers are generated only as they become needed for bdellovibrio DNA synthesis.

Although the developmental cycle of bdellovibrio growing on a host bacterium has been well characterized in a descriptive manner (20, 23), the biochemical events that characterize and permit bdellovibrio development remain largely unknown. It is clear, however, that the host suffers extensive and irreversible damage early in the bdellovibrio developmental cycle. Loss of host viability (26), inhibition of host protein and ribonucleic acid (RNA) synthesis (26), and host-membrane damage (16) manifested by an increase in permeability to small molecules are all initiated within a few minutes after attachment of the bdellovibrio to its host. The respiratory potential of the host is completely eliminated within 60 min after bdellovibrio attachment under conditions that require from 3 to 4 hr for the entire developmental cycle (16). The pattern of change in respiration rate of a population of bdellovibrio growing on *Escherichia coli* as its only source of nutrients suggested to us that all the changes mentioned above are completed before growth per se (i.e., an increase in mass) of the bdellovibrio occurs (16). To test this interpretation of the respiration data, we have examined the fate of host and bdellovibrio deoxyribonucleic acid (DNA) during the course of a developmental cycle. The results of this investigation, presented here, support our interpretation of the respiration data and provide quantitative information on the changes in the two classes of DNA.

[1] Present address: Laboratory of Microbiology, Biological Center, University, Groningen, The Netherlands.

MATERIALS AND METHODS

Organisms and growth procedures. *Pseudomonas putida*, strain N-15 (22), and *E. coli* strain ML35 (*lacI*, *lacY*) (16) were used as hosts. Both orga-

nisms were maintained on nutrient agar. Cultures of these hosts were grown in a medium of the following composition (percent, w/v): NH_4Cl, 0.1; $MgSO_4$, 0.05; Na_2HPO_4, 0.34; NaH_2PO_4, 0.11; $FeCl_3 \cdot 6H_2O$, 0.002; Casamino Acids (Difco; non-vitamin-free), 0.25; and dextrose, 0.5; *p*H 7. Cultures were started by a 1% inoculum (v/v) and were incubated with agitation for 12 to 16 hr at 30 C for *P. putida* or 37 C for *E. coli*. Such cultures reached a population of about 4×10^9 cells/ml. Cells were harvested by centrifugation at 4 C and were washed once in tris(hydroxymethyl)aminomethane (Tris) buffer, 10^{-3} M, *p*H 7.5. The washed cells were resuspended in the same buffer to the desired concentration by reference to standard curves relating viable cell count to turbidity measured with a Klett-Summerson photoelectric colorimeter.

E. coli cells containing a ^{3}H-thymidine label in their DNA were obtained by a modification of the method of Boyce and Setlow (2). A 12- to 16-hr culture was diluted with fresh medium to a population of about 8×10^8 cells/ml. The diluted culture was incubated on a rotary shaker at 37 C for 45 to 60 min. The following compounds were then added (final concentrations per milliliter): deoxyadenosine, 250 μg; thymidine, 6 μg; and thymidine-*methyl*-^3H (specific activity, 6 Ci/mmole), 3 to 4 μCi. Incubation was continued for another 40 to 50 min, which permitted one to two cell divisions and gave a final population of about 3×10^9 cells/ml. The cells were then harvested, washed, and resuspended in Tris buffer as described above. More than 90% of the radioactivity in *E. coli* cells labeled by this procedure was extractable by 0.5 N hot perchloric acid.

Attempts to label the DNA of *P. putida* by the same general procedure proved unsuccessful. Only a small amount of radioactivity was incorporated by the cells, and of this less than 10% was extractable by 0.5 N hot perchloric acid.

Bdellovibrio bacteriovorus strain 109D (15) obtained from M. P. Starr, University of California, Davis, was used. A few key experiments were repeated using *B. bacteriovorus* strain 109J (15) with essentially identical results. The procedures used in maintaining, growing, and harvesting the bdellovibrio were identical to those described by Rittenberg and Shilo (16).

General design of experiments. Unless otherwise stated, experiments were performed in an environment in which the host cells served as the exclusive source of nutrients for the bdellovibrios, i.e., in 10^{-3} M Tris buffer, *p*H 7.5, containing 10^{-3} M $Ca(NO_3)_2$, 3.6×10^{-5} M $FeSO_4$, 3×10^{-3} M $MgSO_4$, and 6.6×10^{-5} M $MnSO_4$. A host population of 5×10^9 cells per ml was used in all experiments. Experiments were initiated by mixing equal volumes of host (1×10^{10} cells/ml) and bdellovibrio (approximately 2×10^{10} cells/ml) suspensions (zero time), and incubating at 30 C with shaking in a water bath. This ratio of bdellovibrio to host insured a rapid attack on essentially all host cells in the system. Samples were removed from the mixture at desired intervals for various measurements.

Respiration measurements. O_2 utilization was measured with a Clark-type oxygen electrode (Rank Bros., Bottisham, Cambridge, England) connected to a Sargent (model SR) recorder. The instrument was adjusted so that full-scale deflection on the recorder was equivalent to the uptake of 0.5 μmole of O_2 from a reaction mixture having a total volume of 2.0 ml. All measurements were made in a water-jacketed cell at 30 C.

Samples of experimental mixtures (1–1.5 ml) and 10^{-3} M Tris buffer to a final volume of 2 ml were added to the electrode chamber. The change in O_2 concentration of the mixture was recorded over a 2-min period. Glucose (10 μmoles) was then added and the new rate was recorded. The uptake of O_2 in the unsupplemented and glucose-supplemented systems was calculated from the slopes of the recorder traces, which were essentially linear over the period of measurement.

Estimation of host and bdellovibrio DNA. Samples (1 ml) of experimental mixtures were removed, immediately frozen at −40 C, and kept at this temperature until assayed (4–6 days). The samples were thawed, mixed immediately with 1 ml of 0.3 M saline–0.2 M ethylenediaminetetraacetate solution (*p*H 8), containing 0.5 (when host was *P. putida*) or 1.0 (when host was *E. coli*) mg of egg white lysozyme, and incubated at 37 C for 30 min. Control experiments using treatment with 3.5% Sarkosyl NL-97 for 15 min at 45 C for cell lysis showed that there was no DNA degradation during the incubation with lysozyme. A 1-ml amount of Sarkosyl solution (2% Sarkosyl NL-97 in 0.1 M Tris-0.1 M NaCl, *p*H 7.0), and 2 ml of Pronase solution (2 mg/ml; prepared by the method of Stern [24]) were then added. The suspensions, which became completely clear at this point, were then incubated at 37 C for 12 to 16 hr to allow a thorough digestion of the lysates by Pronase.

The digested lysates (4.5 ml) were dialyzed against 100× their volume of saline-citrate buffer (SC; 0.015 M saline plus 0.0015 M trisodium citrate, *p*H 7.0). Dialysis was at 5 C for 48 hr, during which time the buffer was changed twice. The lysates were recovered from the dialysis tubings, and the latter were washed twice with 1-ml volumes of SC. The final volume was made up to 8.0 ml with the same buffer; ribonuclease I (100 μg) and 125 units of ribonuclease T_1 were added, and the lysates were incubated at 37 C for 30 min.

For density gradient centrifugation, a sample of the above material containing 10 to 40 μg of DNA was mixed with 9.5 g of CsCl and enough SC to bring the final volume of the solution to 9.4 ml (initial CsCl density, about 1.73 g/cm^3). An 8.5-ml portion of this solution was centrifuged at 36,000 rev/min ($81,000 \times g$) and 27 C for 36 hr in a 50Ti rotor of a Spinco model L centrifuge.

The absorption profile of the gradients at 260 nm was recorded automatically by pumping solution at a flow rate of 0.6 ml/min from the bottom of the gradient through an LKB UVCORD connected to a Sargent model SR recorder. The resulting absorption profiles (see Fig. 2) were used to determine the quantity of DNA in the lysates. The areas under the DNA peaks of a profile were measured with a compensating planimeter (Keuffel and Esser, no. 62 0022),

and converted to amounts of DNA by reference to a standard curve obtained with known amounts of DNA banded as described above. The relation between area and DNA was linear for quantities in the gradient ranging between 14 and 150 μg.

When needed, fractions from the gradients were collected by connecting the UVCORD cell to an LKB fraction collector adjusted to give equal-time fractions. For counting, 0.05 to 0.1 ml of the fractions was pipetted onto 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.) placed at the bottom of scintillation vials. The filters were dried at 60 C for 20 min, 10 ml of a counting solution [4.0 g of 2,5-diphenyloxazole (PPO), and 0.3 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (POPOP) per liter of toluene] were added to each vial, and the samples were then counted in a Beckman spectrometer.

Radioactivity escaping into the medium. Samples of experimental mixtures (2 ml) were centrifuged at 25,000 × *g* for 15 min to sediment all cells, and 0.5-ml samples of the resulting supernatant solutions were then counted.

Radioactivity of cold acid extracts. Samples (0.5 ml) of experimental mixtures were quickly mixed with 0.5 ml and 3.0 ml of chilled 1 N and 0.5 N $HClO_4$, respectively, and held on ice for 30 min. A 1-ml portion of these mixtures was filtered through a 0.45-μm membrane filter (Millipore). The filter was washed with two 1-ml portions of chilled 0.5 N $HClO_4$. The combined filtrate and washings were neutralized with 1.5 ml of 1 N NaOH and counted. Radioactivity of these samples minus radioactivity in the medium at corresponding times gives the radioactivity of the cold acid-extractable cell fraction.

Radioactivity of the nondialyzable fraction of lysed samples. Samples (1 ml) of the experimental mixture were lysed and dialyzed as described for the preparation of samples for gradient centrifugation. The dialysis bags were placed on a bed of polyethylene glycol to concentrate the lysates to about 2 ml. The lysate was removed, the bags washed with buffer, and the combined lysates and washings were made up to 5 ml. A 1-ml sample was mixed with 2 ml of 1 N $HClO_4$, heated at 80 to 85 C for 10 min, neutralized with 1 N NaOH, and counted.

Radioactivity of hot acid-extractable and precipitable fractions of whole cells. A 1-ml amount of a cell suspension (2.5 × 10^9 cells) was mixed with 1.0 ml of 1 N $HClO_4$ and heated as described above. The sample was then centrifuged at 12,000 × *g* for 10 min. The supernatant solution was drained off, neutralized with 1 N NaOH, and counted. The pellet was suspended in 2 ml of 0.5 N $HClO_4$, and the suspension was neutralized with 1 N NaOH and counted.

Radioactivity of all samples except those of the gradient fractions was measured by scintillation counting in a solution (13) of: toluene, 3 liters; Triton X-100 (Sigma), 1 liter; PPO, 16 g; and POPOP, 200 mg.

Chemicals. Sarkosyl was obtained from Geigy Chemical Corp., Ardsley, N.Y.; CsCl (optical grade) from Harshaw Chemical Co., Cleveland, Ohio; and PPO and POPOP from Packard Instrument Co., Downers Grove, Ill. All other special chemicals were purchased from Calbiochem, Los Angeles, Calif.

RESULTS

Developmental cycle: B. bacteriovorus 109D growing on P. putida. The development of *B. bacteriovorus* 109D on *P. putida* in Tris buffer proceeded normally as judged by phase-contrast microscopy. At a multiplicity of two bdellovibrio per host, there was rapid attachment and penetration; some host cells became spheroplasts by 20 min, and 60 to 70% by 30 min. Some uninfected host cells remained in the mixture, however, until as late as 130 to 140 min. Most hosts (60–70%) lysed between 3 and 3.5 hr, but complete lysis was not observed until 5 to 5.5 hr. The development pattern was very similar when dilute nutrient broth (21) was substituted for Tris. However, in the latter menstruum synchrony was somewhat better, and a greater percent of host lysed between 3 and 3.5 hours. In either environment development was less synchronous than with *E. coli* ML35 as host (16).

The unsupplemented and glucose-supplemented respiration patterns over a complete developmental cycle are shown in Fig. 1. After a small initial decrease over the first 60 min, the respiration rate of the unsupplemented mixture increased steadily, reaching a max-

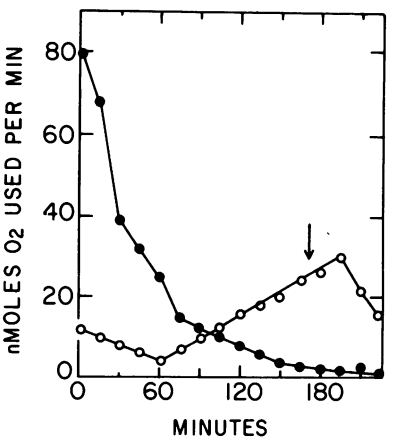

FIG. 1. *Unsupplemented and glucose-supplemented respiration patterns during development of B. bacteriovorus strain 109D growing on P. putida. Bdellovibrio and host suspensions in Tris buffer were mixed and shaken at 30 C. Samples (1 ml), containing 1 × 10^{10} bdellovibrio and 5 × 10^9 P. putida at zero time, were removed at intervals and their respiration rates* (○) *and the stimulation of these rates by 10 μmoles of glucose* (●) *were determined. Arrow indicates onset of lysis.*

imum at about 200 min concomitantly with the onset of lysis of the host. The rate then decreased, reaching a stationary value by about 250 min. The rate of glucose respiration by the mixture decreased rapidly. By 60 min, there was a 70% reduction of the system's potential to oxidize glucose. Thereafter, the decay of glucose respiration was less rapid but eventually reached completion. In some experiments, a small initial increase in the rate of glucose respiration preceded its decay. Since glucose does not appreciably stimulate respiration of *B. bacteriovorus* strain 109D, changes in the glucose respiration rate during the developmental cycle can be attributed to changes occurring in the host. The observed respiratory patterns are similar in most respects to those described for the development of *B. bacteriovorus* strain 109J on *E. coli* strain ML35 (16), and it may be presumed that early damage to the host cell membrane occurs also in the *B. bacteriovorus* 109D-*P. putida* system.

Amount and composition of DNA species. The DNA contents of the bacteria used in this study (Table 1) were determined as described on lysates of standardized suspensions of the individual organisms. Buoyant densities were determined from banding patterns in cesium chloride with *E. coli* strain ML35 DNA (1.710 g/cm^3 [17]) used as the marker; the guanine plus cytosine (G + C) contents were calculated from the buoyant densities (17). The data reported in Table 1 are in good agreement with values in the literature (9, 11, 19).

Kinetics of DNA degradation and synthesis during growth of B. bacteriovorus 109D on P. putida. As expected from the buoyant densities, the DNA of *B. bacteriovorus* 109D and the DNA of *P. putida* were clearly separated by gradient centrifugation. Figure 2 shows the absorption profile at 260 nm after centrifugation in cesium chloride of a lysed sample of a *B. bacteriovorus* strain 109D-*P. putida* mixture. The sample was removed immediately after mixing the two populations and was processed and centrifuged as described, except that the lysate was not treated with ribonucleases. The diffuse band of 260 nm-absorbing material at the bottom of the gradient is due mainly to RNA since most of it disappears on treatment of the lysate with ribonucleases. The sharply defined bands near the middle of the gradient represent the two DNA species, the heavier being *P. putida* DNA, and the lighter bdellovibrio DNA. These bands disappear if the lysate is treated with deoxyribonuclease. The diffuse band at the top of the gradient is presumably due primarily to protein.

TABLE 1. *Composition and content of DNA of experimental organisms*

Organism	Buoyant density (g/cm^3)	G + C (mole %)	Content (μg/10^{10} cells)
Bdellovibrio bacteriovorus 109D	1.710	50	31.5
B. bacteriovorus 109J	1.710	50	32.0
Escherichia coli ML35 (*lacI, lacY*)	1.710	50	80
Pseudomonas putida N-15	1.722	63	64

Figures 3 and 4 show the time course of the changes in the two species of DNA during a developmental cycle in Tris buffer. The host DNA became nonbandable early in the cycle. A decrease could be detected as early as 15 min; by 30 min only 30% of the host DNA remained bandable; and by 150 min, none was detectable.

The amount of bandable bdellovibrio DNA remained essentially unchanged during the first 60 min of the cycle (Fig. 4). Some increase was observed between 60 and 150 min, but 80% of the total increase occurred after 150 min.

When dilute nutrient broth was used in place of Tris buffer as the suspending menstruum, the patterns of host DNA breakdown and bdellovibrio DNA synthesis were very similar (Fig. 4). The more clear-cut temporal separation of degradation and synthesis in this medium is probably a reflection of improved synchrony of development (*see above*).

The data (Fig. 4) also show the total amount of DNA synthesized. It is noteworthy that the amount of DNA synthesized in dilute nutrient broth, 58 μg/ml, was identical, within the limits of accuracy of the measurements, to

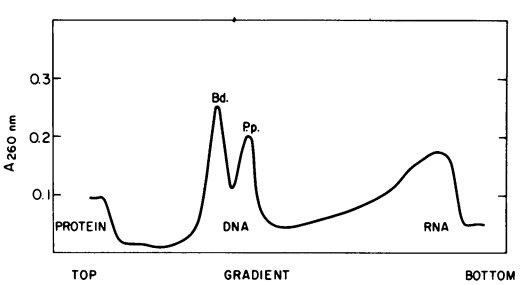

FIG. 2. *Absorption profile at 260 nm of CsCl gradient prepared from a zero-hour sample of a B. bacteriovorus (Bd.) strain 109D-P. putida (P.p.) mixture. The sample (1 ml) containing 1.5 × 10^{10} bdellovibrio and 5 × 10^9 P. putida was removed immediately after the suspensions were mixed and was processed as described in text.*

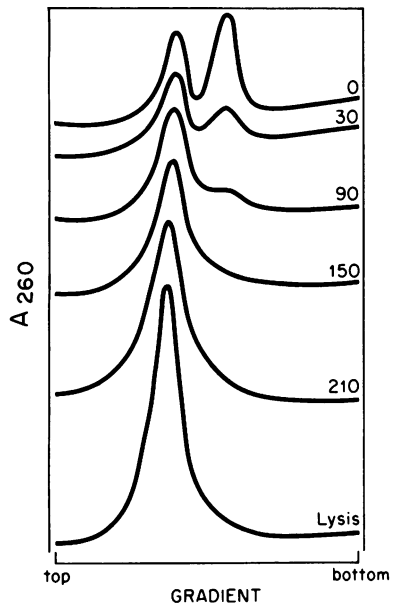

FIG. 3. *Absorption profiles at 260 nm of CsCl gradients prepared from a B. bacteriovorus strain 109D-P. putida experimental mixture. Suspensions were mixed and shaken at 30 C; 1-ml samples containing 1 × 10^{10} bdellovibrio and 5 × 10^{9} P. putida at zero time were removed at the times shown and processed as described. Only the DNA region of the gradients is shown.*

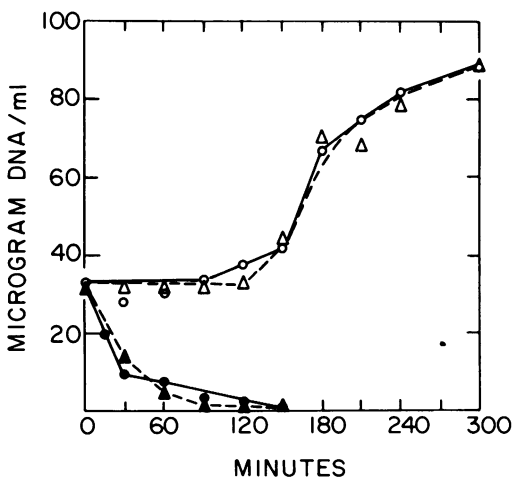

FIG. 4. *Kinetics of host DNA breakdown (●) and bdellovibrio DNA synthesis (O) during a developmental cycle of B. bacteriovorus strain 109D growing on P. putida in Tris buffer (——) or in dilute nutrient broth (-----). P. putida (1 × 10^{10}/ml) and B. bacteriovorus (2 × 10^{10} ml) suspensions were mixed and shaken at 30 C; samples were removed at intervals and their DNA contents were determined by CsCl density gradient centrifugation. Each point represents an average of at least two separate determinations, the results of which deviated by less than 5%.*

that synthesized in Tris buffer: 57 μg/ml. From the data in Table 1 and in Fig. 4, it can be calculated that the net synthesis of DNA in these experiments corresponds to an increase of about 1.8 × 10^{10} bdellovibrios/ml, and represents a yield of 3.6 bdellovibrios/host cell. Assuming that only one bdellovibrio grew per host, this is equivalent to a "burst size" of 4.6, a number similar to burst sizes reported by Varon and Shilo (28) and Seidler and Starr (18) for *E. coli*, an organism of approximately the same size as *P. putida*.

Kinetics of DNA degradation and synthesis during growth of B. bacteriovorus 109D on E. coli ML35. The DNA species of *E. coli* ML35 and *B. bacteriovorus* 109D, having equal buoyant densities, cannot be separated by density gradient centrifugation. It proved possible, however, to follow the fate of the two DNA species by growing *B. bacteriovorus* on *E. coli* which had ^{3}H-thymidine label in its DNA. In the experiment shown (Fig. 5), the quantities of bdellovibrio DNA and *E. coli* DNA were equal at the time of mixing the two suspensions, about 40 μg/ml each. By 45 min after mixing, the total bandable DNA in the system had decreased to 40 μg/ml, i.e., a quantity equal to the starting quantity of either species. Over the same period of time, the radioactivity of the bandable DNA had decreased by 96%. The only reasonable interpretation of the data is that almost all of the *E.*

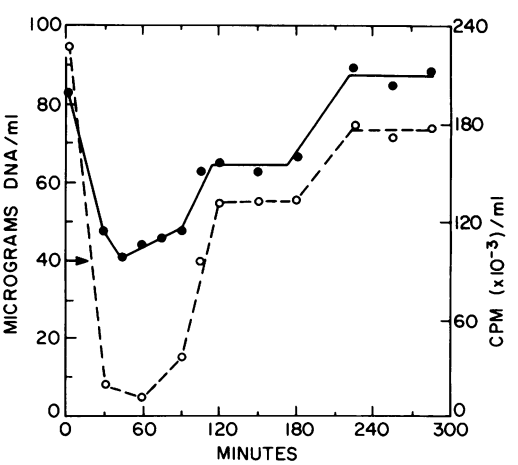

FIG. 5. *Kinetics of DNA degradation and synthesis during growth of B. bacteriovorus strain 109D on ^{3}H-thymidine-labeled E. coli. Labeled host (1 × 10^{10} cells/ml, 46,000 counts/min) and unlabeled bdellovibrio (2.6 × 10^{10} cells/ml) suspensions were mixed and shaken at 30 C. Samples were removed at intervals and their DNA (●) contents and radioactivity (O) were determined. Arrow indicates the amount of bdellovibrio DNA present in the mixture at zero time.*

coli DNA had been degraded. Furthermore, the data indicate that the bdellovibrio DNA does not increase in quantity over the first 45 min of the infection cycle. Qualitatively, the early pattern is the same as that observed with *P. putida;* quantitatively, degradation of *E. coli* DNA is somewhat more rapid. Essentially identical results were obtained when the experiment was repeated with *B. bacteriovorus* strain 109J instead of 109D.

The thymidine label lost from the DNA band was not detected as radioactive components in any other region of the CsCl gradients. A possible explanation for this disappearance is that the products of host DNA breakdown are diffusible and were lost during the dialysis step (see Materials and Methods) before CsCl centrifugation. However, determination of the total radioactivity of the lysates from the 30-, 45-, and 60-min samples after the dialysis step but before CsCl centrifugation showed that over 90% of the added radioactivity was nondialyzable. The results eliminated the above possibility. The only other explanation is that the products of host DNA breakdown are distributed throughout the gradient and escape detection against the background count because of dilution.

Synthesis of bdellovibrio DNA, which started at about 45 min, took place in two almost equal steps separated by a period of little or no synthesis (Fig. 5). The first of these occurred between 45 and 105 min, and the second between 180 and 225 min. Radioactivity reappeared in the DNA concurrent with synthesis, and this reappearance also exhibited a biphasic pattern. A plot of the specific activity of newly synthesized DNA as a function of time (Fig. 6) shows that, during the first period of synthesis, the specific activity of the newly synthesized DNA was constant and almost equal to the initial specific activity of the *E. coli* DNA. During the second period, the specific activity of this DNA fell to about 65% of that of the initial host DNA.

It is clear that the breakdown products of host DNA were utilized for synthesis of bdellovibrio DNA. In the experiments shown (Fig. 5), 80% of the radioactivity initially in the methyl group of host thymine residues was incorporated into bdellovibrio DNA. In other experiments this value ranged from 60 to 80%, and averaged 73%. If the other DNA bases were as efficiently used, only 20% of newly synthesized bdellovibrio DNA in the experiment cited was derived from host components other than DNA. The non-DNA host precursors were apparently utilized mainly during the second period of synthesis, because it was at this time that the specific activity of newly synthesized bdellovibrio DNA decreased as compared to that of the initial host DNA.

A comparison was made of the quantity of bdellovibrio DNA synthesized by using ^{3}H-thymidine-labeled *E. coli* as host and Tris or dilute nutrient broth as the suspending medium. The amount of DNA formed under the two conditions was about the same (Table 2), as is also the case for *B. bacteriovorus* 109D growing on *P. putida* (Fig. 4). However, the specific activity of the DNA of bdellovibrios from dilute nutrient broth was about 20% less than that of bdellovibrios from Tris (Table 2).

Localization of degraded host DNA. The efficiency of host DNA degradation and resynthesis into bdellovibrio DNA, and the temporal separation of these processes, raises the question whether the products of DNA breakdown are released into the external environment and then reincorporated into the bdellovibrio, or whether they are confined within the boundary of the host cell wall. To decide this point, a mixture of ^{3}H-thymidine-labeled *E. coli* and bdellovibrio in Tris was sampled at intervals during the developmental cycle, the samples were centrifuged, and the radioactivity in the supernatant liquid was determined. The results show (Fig. 7) that there was a slow release of label into solution which accelerated somewhat towards the end of the developmental cycle. At all stages, however, a major portion of the label remained in the cells. Of particular significance, at 45 and 60 min, when almost all radioactivity had disap-

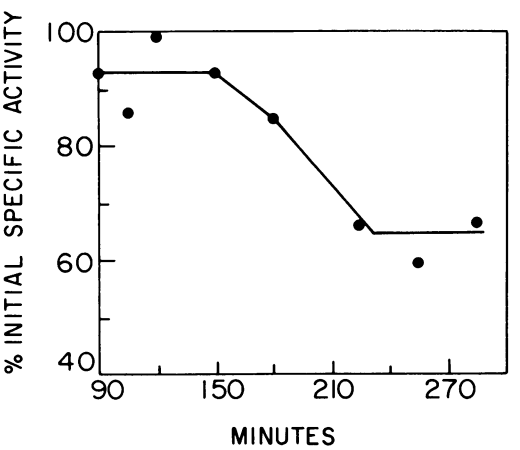

FIG. 6. *Specific activity of newly synthesized bdellovibrio DNA during its growth on ^{3}H-thymidine-labeled E. coli, as percent of initial specific activity of E. coli DNA (580 counts per min per μg). Data from experiment described in Fig. 5. Newly synthesized DNA (after 60 min) equals total DNA minus initial bdellovibrio DNA.*

TABLE 2. *DNA synthesis during growth of B. bacteriovorus strain 109D on ^{3}H-thymidine-labeled E. coli in Tris buffer and in dilute nutrient broth (NB)*

Experiment in	DNA (μg/ml of culture)			*B. bacteriovorus* DNA formed (μg/ml)	Radioactivity of DNA[a]		Specific activity of *B. bacteriovorus* DNA[b]
	Initial		Final (*B. bacteriovorus*)		Initial (*E. coli*)	Final (*B. bacteriovorus*)	
	E. coli	*B. bacteriovorus*					
Tris	40	57	110	53	169,000	110,000 (65)[c]	2,000
NB	40	52	104	52	160,000	85,000 (53)	1,600

[a] Counts per minute per milliliter.
[b] Counts per minute per microgram of DNA synthesized.
[c] Numbers in parentheses are percent of host radioactivity incorporated by the bdellovibrios.

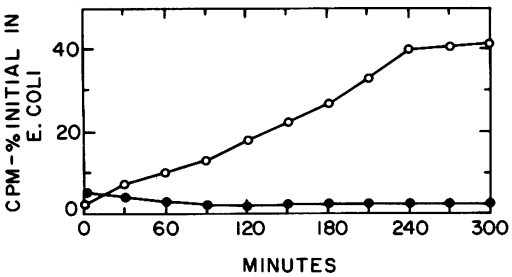

FIG. 7. *Release of radioactivity into the medium during growth of B. bacteriovorus strain 109D on ^{3}H-thymidine-labeled E. coli. Suspensions of bdellovibrio (2.6 × 10^{10} cells/ml) and E. coli (1 × 10^{10} cells/ml; 46,000 counts/min) were mixed, shaken at 30 C, and sampled at intervals for analyses (see Materials and Methods). O, Radioactivity of supernatant fluid; ●, radioactivity of cold acid-extractable material.*

peared from the bandable DNA (Fig. 5), only 10% of the radioactivity had been released from the cells. This result is consistent with the finding that host DNA degradation products at this stage are not dialyzable. The results imply that there is no early build-up of a pool of nucleosides or mononucleotides, and, in fact, no significant amount of radioactivity could be extracted by cold acid treatment of cells at any stage of the infection cycle (Fig. 7).

Uptake of exogenous nucleosides. Unlabeled *E. coli* and unlabeled *B. bacteriovorus* 109D were mixed in Tris buffer to give initial cell concentrations of 5 × 10^9 and 1 × 10^{10} cells/ml, respectively. The host DNA contained about 7.6 μg of thymidine residues per ml. The mixture was incubated at 30 C until all host cells had spheroplasted (about 45 min), at which time ^{3}H-thymidine (0.073 μg/ml; specific activity 6 Ci/mmole) was added to the buffer. Incubation was continued until lysis of *E. coli* was complete. A sample of the bdellovibrio was then fractionated in CsCl, and the radioactivity of the various fractions was determined (Table 3). Only about 6%, or 0.004 μg/ml, of the exogenously added thymidine was incorporated by the bdellovibrio. It can be assumed (Fig. 5) that about 80%, or 6 μg/ml, of thymidine residues from host DNA had been incorporated into bdellovibrio DNA. Thus, little equilibration occurred between thymidine in the external medium and the breakdown products of host DNA generated within the spheroplasts. This indicated that either no appreciable pool of thymidine *per se* was created by host DNA degradation or, if such a pool existed, it was almost completely isolated from the exogenous thymidine.

This experiment was repeated, adding ^{14}C-adenosine (8 μg/ml; specific activity 53.3 mCi/mmole) instead of thymidine. Incorporation of the nucleoside was also low (Table 3).

Effect of streptomycin and mitomycin C on changes in host and parasite DNA. In the presence of streptomycin (200 μg/ml), added at time zero, *B. bacteriovorus* 109D attached to *P. putida* but failed to penetrate. The endogenous respiration rate of the infection mixture slowly decayed. The potential to oxidize glucose remained unchanged during the first hour and decreased by less than 30% in 5 hr. These results agree qualitatively with previously described effects of streptomycin on

TABLE 3. *Incorporation of added ^{3}H-thymidine and ^{14}C-adenosine into B. bacteriovorus strain 109D growing on E. coli in Tris buffer*

Determination	^{3}H-thymidine		^{14}C-adenosine	
	Amt[a]	%	Amt[a]	%
Counts added	144	100	55.6	100
Counts incorporated .	8.5	6.0	3.9	7.0
in RNA	ND[b]		2.6	4.7
in DNA	6.7	4.7	0.83	1.5
in protein	1.8	1.3	0.47	0.8

[a] Counts per minute (× 10^{-4}) per milliliter.
[b] None detectable above background level.

bdellovibrio (16, 27). In addition, no detectable destruction of host DNA occurred within 200 min; after 5 hr, 70% of the host DNA was still bandable (Table 4). There was no destruction or synthesis of bdellovibrio DNA.

In the presence of mitomycin C (5 μg/ml, added at time zero), an antibiotic known to inhibit DNA synthesis at low concentrations (6), there was rapid attachment and penetration of the bdellovibrio into *P. putida*. Spheroplast formation of the host occurred, but at a slower pace than in controls without the antibiotic. Both the endogenous respiration rate and the potential to respire glucose decreased with time, but not as rapidly as in the absence of the antibiotic. Host DNA was completely degraded at or before 165 min; no synthesis of bdellovibrio DNA occurred (Table 4).

DISCUSSION

The data presented reveal a striking feature of bdellovibrio attack on its host, namely the early and rapid destruction of host DNA. With both *E. coli* and *P. putida* as host, destruction of the host DNA reached completion within the first 45 to 60 min of the bdellovibrio developmental cycle. Furthermore, synthesis of bdellovibrio DNA did not occur until degradation of host DNA was complete. In this respect infection by bdellovibrio is similar to the lytic cycle of T5 phage development, in which destruction of host DNA precedes synthesis of phage DNA (3, 14).

The mechanism of destruction of host DNA during bdellovibrio development is not known. One possibility is that damage to the host cell membrane, which is initiated early after attachment (16), disrupts the internal organization of the host cell. A consequence of this disruption could be that host deoxyribonucleases which normally remain compartmentalized can act on host DNA. It is noteworthy that physical and biochemical evidence (1, 4, 5, 25) indicates a functional attachment of the DNA of *E. coli* and other bacteria to the cell membrane. Cell membrane has also been postulated to be the primary target for the action of colicins (12), individual types of which are known to duplicate (12) one or more of the early effects of bdellovibrio attack on its host, namely inhibition of DNA, RNA, and protein synthesis (26), inhibition of host respiration (16), and degradation of host DNA.

Another possibility is that, upon successful attachment, the bdellovibrio releases deoxyribonucleases into the host cell. These enzymes could pre-exist in the bdellovibrio or be induced after attachment. Induction of a DNA endonuclease and an oligonucleotide diesterase upon infection of *E. coli* with T2 phage has been demonstrated (10; S. Bose and N. Nossel, Fed. Proc., p. 272, 1964). The inhibition of host DNA destruction by streptomycin might suggest that protein synthesis is required. However, streptomycin also inhibits early damage to the host cell membrane (16), and its effect on DNA degradation could be a secondary effect.

As yet, there is little basis for choosing between these and perhaps other alternatives. The few studies on degradative enzymes of bdellovibrio have focused on proteases (21, 23) and lysozyme-like enzymes (23). Although both types of activity have been detected in bdellovibrio, there is no direct evidence that implicates these enzymes in specific aspects of host destruction.

Another noteworthy feature of the bdellovibrio growth is the remarkable degree to which host DNA serves as a precursor for synthesis of bdellovibrio DNA. If the efficiency of uptake of host thymidine is representative of the uptake of all DNA components, as much as 80% of host DNA is incorporated into bdellovibrio DNA. This is in contrast to the T-even phage infections of *E. coli* in which less than 30% of the phage progeny nitrogen and phosphorus is derived from the host DNA (7). The host cell is apparently the major source of bdellovibrio DNA precursors even in the presence of exogenous nutrients. Similar total amounts of bdellovibrio DNA are synthesized in Tris buffer and in dilute nutrient broth during its development on either *P. putida* or *E. coli*, and the uptake of a thymidine label from *E. coli* into bdellovibrio DNA is depressed only some 20% by the exogenous nutrients. Thus, a maximum of 20% of the thymidine could have been synthesized from exogenous compounds.

TABLE 4. *Effect of streptomycin and mitomycin C on DNA degradation and synthesis during growth of B. bacteriovorus on P. putida in Tris buffer*

Antibiotic	Time[a] (min)	DNA (μg/ml)	
		P. putida	*B. bacteriovorus*
Streptomycin (200 μg/ml)	0	35	45
	200	32	45
	300	26	45
Mitomycin C (5 μg/ml)	0	35	40
	165	Trace	40
	300	Trace	40

[a] Time after mixing bdellovibrio and host populations; inhibitors present from zero-time on.

The apparent efficiency of conversion of host DNA to bdellovibrio DNA does not imply an inability of bdellovibrio to synthesize DNA components de novo. Most strains of bdellovibrio, including the two used in these studies, have a G + C content of approximately 50 moles percent (19) and can grow on hosts with G + C contents ranging from very low to very high. This in itself indicates that bdellovibrio can synthesize all DNA components from other host cell components or from exogenous precursors or both. In our experiments, DNA synthesis was about equally efficient during growth on *P. putida* or *E. coli*, which have DNA species of different compositions, although the kinetics of DNA synthesis in the two hosts differ. With *P. putida*, synthesis of bdellovibrio DNA occurs in a continuous fashion. With *E. coli*, bdellovibrio DNA synthesis is discontinuous, occurring in two separate periods. The biphasic pattern of synthesis with *E. coli* as the host can be explained in at least two ways. It could represent two distinct rounds of DNA replication separated in time. Alternatively, it could be explained by the exhaustion of some DNA precursor followed by an inductive period in which biosynthetic enzymes for the formation of that precursor are produced. In either case, a biphasic pattern would not be observed if bdellovibrio development were not proceeding synchronously in the host population. This may explain the difference in the pattern of DNA synthesis observed in experiments with *E. coli* as compared to *P. putida* as host since better synchrony was achieved in the former growth system.

The clear separation in time between host DNA degradation and bdellovibrio DNA synthesis points to a precise control by the bdellovibrio over the degradative process. The data indicate a rapid breakdown of host DNA to polynucleotides of intermediate molecular weight, followed by a subsequent slower production of monomeric units that enter into biosynthesis. This pattern is similar to that described for the kinetics of *E. coli* DNA breakdown by phage T4 (8, 29); endonuclease activity which begins within 5 min after infection reduces the size of the *E. coli* genome to pieces of about 200 million molecular weight and then to pieces of about 1 million molecular weight. This is followed by rapid exonuclease activity with no detectable formation of acid-insoluble products below molecular weight of 1 million.

The early breakdown products of host DNA are not dialyzable, not bandable, but not cold acid-soluble. These DNA fragments are retained within the confines of the host cell wall; they sediment with the cells. Consequently they are reserved for later use by the bdellovibrio and are not dissipated into the environment. The initial breakdown products must be further degraded at least to the mononucleotide stage before their resynthesis into bdellovibrio DNA is possible. Yet, there is never an appreciable pool of mononucleotides or small polynucleotides associated with the bdellovibrio-host complex. The rate of breakdown of polynucleotides to mononucleotides must be in essential balance with the rate of DNA synthesis. The control is not perfect since there is a 20 to 40% loss of potential precursors; nevertheless, it is impressive. Thus it appears that the bdellovibrio has evolved a mechanism for arresting host DNA breakdown initially at the oligonucleotide stage and generating monomers from these only as they become needed for synthesis. With such a mechanism, and perhaps similar control over degradation of other classes of host macromolecules, the selective advantage derived by the bdellovibrio from its periplasmic location during growth is obvious. Having killed its prey it can feast on the remains at its own pace and without competition. The details of how this careful control is exercised remain to be elucidated.

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