Effects of ultraviolet irradiation of host and parasite on attachment of Bdellovibrio bacteriovorus to Escherichia coli
by Kathleen Forsgren Castric

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Microbiology
Montana State University

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Ultraviolet irradiation sensitivity studies of Bdellovibrio bacteriovorus 109 and its host, Escherichia coli, showed the E. coli to be more sensitive to UV irradiation than Bdellovibrio. There was no difference in the number of Bdellovibrio attached to irradiated E. coli although the fraction of survivors varied from 1 to 10^-8 host cells.

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Date Sept. 27, 1969

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ABSTRACT

Ultraviolet irradiation sensitivity studies of Bdellovibrio bacteriovorus 109 and its host, Escherichia coli, showed the E. coli to be more sensitive to UV irradiation than Bdellovibrio. There was no difference in the number of Bdellovibrio attached to irradiated E. coli although the fraction of survivors varied from 1 to 10^{-8} host cells.

INTRODUCTION

Bdellovibrio bacteriovorus is the first bacterium to be isolated that parasitizes another bacterium and replicates within the host. Its discovery by Stolp and Petzold (1962) came about because it causes a lytic reaction that is superficially similar to that caused by bacteriophage. However, Bdellovibrio plaques do not appear until two to four days after plating, whereas phage plaques appear within one day. Bdellovibrio was first shown to enter and replicate within its host by Scherff et al (1966).

The organism itself is a small, comma-shaped, gram negative bacterium, with a thick polar flagellum, sheathed its entire length. The dimensions of the organism are approximately 1.0 x 0.3 microns, varying with age and culture conditions.

Some species of Bdellovibrio have an extensive host range while others have a limited host range. For example, Bdellovibrio bacteriovorus strain W was found by Burger et al (1968) to attack Streptococcus faecalis and Lactobacillus plantarum, as well as several Enterobacteriaceae. Stolp and Starr (1963) listed 12 different bacterial strains, including Escherichia, Pseudomonas, Rhodospirillum, Erwinia, and Aerobacter species, which would serve as host for Bdellovibrio bacteriovorus used in these experiments. A limited host range was found for Bdellovibrio bacteriovorus 128 lytic on Aerobacter cloacae only (Stolp and Starr, 1963).

Bdellovibrio bacteriovorus seems to be widespread in nature, having been isolated from localities in North, Central, and South America, and in Africa, India, Israel, and Japan. It has been isolated from soil, sewage, and sea water.

Seidler and Starr (1969) propose five terms to describe the

Bdellovibrio life cycle: Attachment, penetration, elongation, fragmentation, and burst.

The initial step in attachment is a violent collision of the parasite with its host. Stolp and Petzold (1962) and Stolp and Starr (1963) suggest that the collision damaged the host cell wall, and that this is important in attachment. In support of this suggestion, Varon and Shilo (1968) found that agents which inhibit motility also inhibit attachment. After collision, the parasite begins to rotate rapidly. This movement could act as a drill (Stolp, 1968) or an arm-in-socket type action. The latter type motion would weaken the host cell wall, amking it susceptible to osmotic forces (Starr and Baigent, 1966). Burnham et al (1968) showed the first change in the host cell is a bulging of the cell wall and membrane at the point of attachment. Next, a pore is formed in the cell wall and the parasite enters the host (Scherff et al, 1966; Starr and Baigent, 1966; Burger et al, 1968).

Varon and Shilo (1968) showed that penetration of the parasite was inhibited by streptomycin, puromycin, and chloramphenicol. None of these antibiotics had any effect on attachment. Penicillin affected neither attachment nor penetration. These results indicate that mechanical forces alone are not sufficient to permit parasite penetration of the host.

Elongation begins when the parasite has entered the host and starts to grow into a long spiral or C-shaped structure. This growth continues until the Bdellovibrio completely fills the host cell (Scherff et al, 1966; Starr and Baigent, 1966; Seidler and Starr, 1968).

During fragmentation, the spiral-shaped structure breaks into daughter

cells and with the burst they are liberated from the host.

Scherff et al (1966), using the host Pseudomonas fluorescens, reported that the life cycle from attachment to burst could be completed in one hour, with an average of 8-12 B. bacteriovorus produced in one host. Starr and Baigent (1966), working with different strains of Bdellovibrio, reported that the time for the life cycle can vary from five to six hours or longer, depending on the length of time the parasite has been without a host. Seidler and Starr (1969) reported an average of 5.7 Bd. 109 cells produced from a singly infected host.

The purpose of the following work was: To compare the ultraviolet sensitivities of Bdellovibrio bacteriovorus 109 and its host, E. coli B, and to determine whether irradiation of the host cells affected their ability to support the attachment of Bdellovibrio.

MATERIALS AND METHODS

A. Bacterial Strains

The Bdellovibrio bacteriovorus culture, Bd. 109, was obtained from M. P. Starr, University of California at Davis. The host, E. coli B, strain WM3SR, was from the stock cultures of P. D. Skaar, Montana State University.

B. Media

Yeast Peptone Broth (YPB), composed of 0.3 percent yeast extract and .06 percent peptone, was used to grow E. coli. It was also used in the preparation of overlay (0.6 percent agar) and the agar base (1.5 percent) for plaque assay. Dilute Nutrient Broth (DNB) was used to grow Bdellovibrio. It was composed of one volume Nutrient Broth plus nine volumes of distilled water containing 0.2 g/liter of $Ca(NO_3)$ and 0.01 g/liter of $MnSO_4$. The buffer used in the irradiating procedures was composed of 6 g. NA_2HPO_4, 3 g. KH_2PO_4 in one liter of distilled water.

C. Cultivation

The E. coli broth cultures were incubated on a shaker at room temperature (approximately 22 C) for 18-20 hours. The E. coli was stored on nutrient agar slants in screw cap tubes or in YPB at 4 C. The liquid cultures of Bdellovibrio were inoculated by adding 5 ml (5×10^8 cells) of a 20 hour culture of E. coli to 2 ml (2×10^6 cells) of Bdellovibrio in 20 ml of DNB. The Bd. 109 cells were taken from the top layer of stored plates which had been eluted by adding 10 ml of YPB and letting the plates stand for one hour. The broth cultures were incubated at room temperature (approximately 22 C) on the shaker for 18-20 hours. Plaque assays were

carried out by the method used for phage (Stolp and Starr, 1963). To the melted top agar was added 1 ml YPB containing 10^8 E. coli per ml and 0.1 ml of a Bdellovibrio suspension. The plates were incubated at 35 C for three days before counting the plaques. The Bdellovibrio were stored by placing a plate showing semi-confluent or confluent lysis at 4 C; transfers were made once a month.

D. Irradiation Procedures

For the irradiation of the host, an 18-20 hour culture of E. coli was centrifuged for ten minutes at low speed. The bacteria were then resuspended in 10 ml of buffer. The suspension was mixed and placed in a standard petri dish. A General Electric germicidal ultraviolet lamp was used to irradiate the sample. The distance from the center of the irradiation source to the dish was 44.5 cm. A rotary shaker was used to agitate the sample throughout the period of irradiation. All work was done under red light, and the plates were incubated in the dark at 37 C. The irradiation of Bd. 109 followed the procedure given for its host with the following modifications: For the irradiation of Bd. 109, an 18-20 hour culture was centrifuged at low speed for ten minutes. Five ml of the supernatant containing Bdellovibrio were then added to 5 ml of buffer, the suspension mixed, and placed in a petri dish. Irradiation was then carried out as described above. The plaque assay plates were incubated for three days before counting.

In performing the experiments using non-irradiated Bd. 109 and irradiated E. coli, the E. coli were treated in the manner described previously.

Samples of 1.0 ml containing 10^8 host cells were taken at different time intervals and mixed with 0.1 ml containing 10^6 Bdellovibrio. The samples were then incubated 30 minutes in the dark to allow for attachment. They were then filtered through a 0.65 micron pore size Millipore filter. The filtrate was diluted, and plated upon non-irradiated E. coli host. The filters then were placed in 5 ml of distilled water and left for 30 or 60 minutes. The distilled water with the filters was agitated, samples of the eluate were taken, and also plated upon non-irradiated E. coli. The purpose of this last step was to recover the Bd. 109 which had attached to E. coli, and could no longer pass through the filter.

For the experiments using both irradiated E. coli and Bdellovibrio, the host and parasite were irradiated separately, samples were taken at given time intervals, and the E. coli and Bdellovibrio mixed for each time interval. They were then incubated for 30 minutes to allow for attachment. The samples were filtered, the filtrate diluted, and plated upon a non-irradiated host. The filters then were placed in 5 ml of distilled water and left for 30 or 60 minutes. The distilled water with the filters was agitated, samples were taken, and also plated upon non-irradiated E. coli.

RESULTS

A. The Ultraviolet Sensitivities of *E. Coli* and *B. bacteriovorus*

Five experiments were performed to test the ultraviolet sensitivity of the *E. coli* host. The results are given in Table I and Figure 1. The change in the slope of the curve which seems to occur at about 10^{-5} survival may be due to the existence of a subpopulation of more resistant mutants.

Five experiments also were performed to test the ultraviolet sensitivity of *Bdellovibrio bacteriovorus*. The results are given in Table II and Figure 2. The survival curve appears to be linear to 10^{-6} survivors.

As can be seen by a comparison of the *B. bacteriovorus* survival curve with that of *E. coli* (superimposed on Figure 2), the ultraviolet sensitivity of *E. coli* is greater than that of *Bdellovibrio*. This is probably due, in part at least, to the larger target size.

B. The Attachment of *B. baeteriovorus* to Irradiated *E. coli*

Three experiments were performed in an attempt to assess the ability of irradiated *E. coli* to support the attachment of *Bdellovibrio*. *E. coli* was irradiated for periods of 0 to 60 seconds, and its capacity as host checked at intervals.

The *Bdellovibrio* cells were allowed to attach for thirty minutes, and then the host-parasite suspension was filtered in order to separate the unattached *Bd.* 109 which would pass through the filter. The host cells with the attached *Bdellovibrio* were then eluted and plated upon non-irradiated *E. coli*. When the filtrate was assayed, it was found that the number of *Bdellovibrio* in the filtrate remained constant with time.

TABLE I

Survival of E. coli following UV irradiation

Fraction of cells surviving UV irradiation

Time of irradiation in seconds	Experiment Number 1	2	3	4	5	Average
0	6×10^{8}(1)	7×10^{8}	1.3×10^{9}	5×10^{8}	2×10^{8}	6.6×10^{8}
10	7.16×10^{-2}					7.19×10^{-2}
20	4.34×10^{-3}	9.28×10^{-4}	8.45×10^{-3}			4.54×10^{-3}
30	2.32×10^{-4}					2.32×10^{-4}
40		3.12×10^{-6}	3.82×10^{-5}	1.0×10^{-5}	6.5×10^{-6}	1.44×10^{-5}
60	6.68×10^{-7}	4.31×10^{-9}				3.36×10^{-7}
80			1.15×10^{-8}	2.0×10^{-9}	1.0×10^{-8}	7.80×10^{-9}

(1) Viable cell count at time 0

Figure 1 Survival of *E. coli* following UV irradiation. The curve through the x's represents the average of the fraction surviving for each time interval. The dots represent the results of each individual experiment.

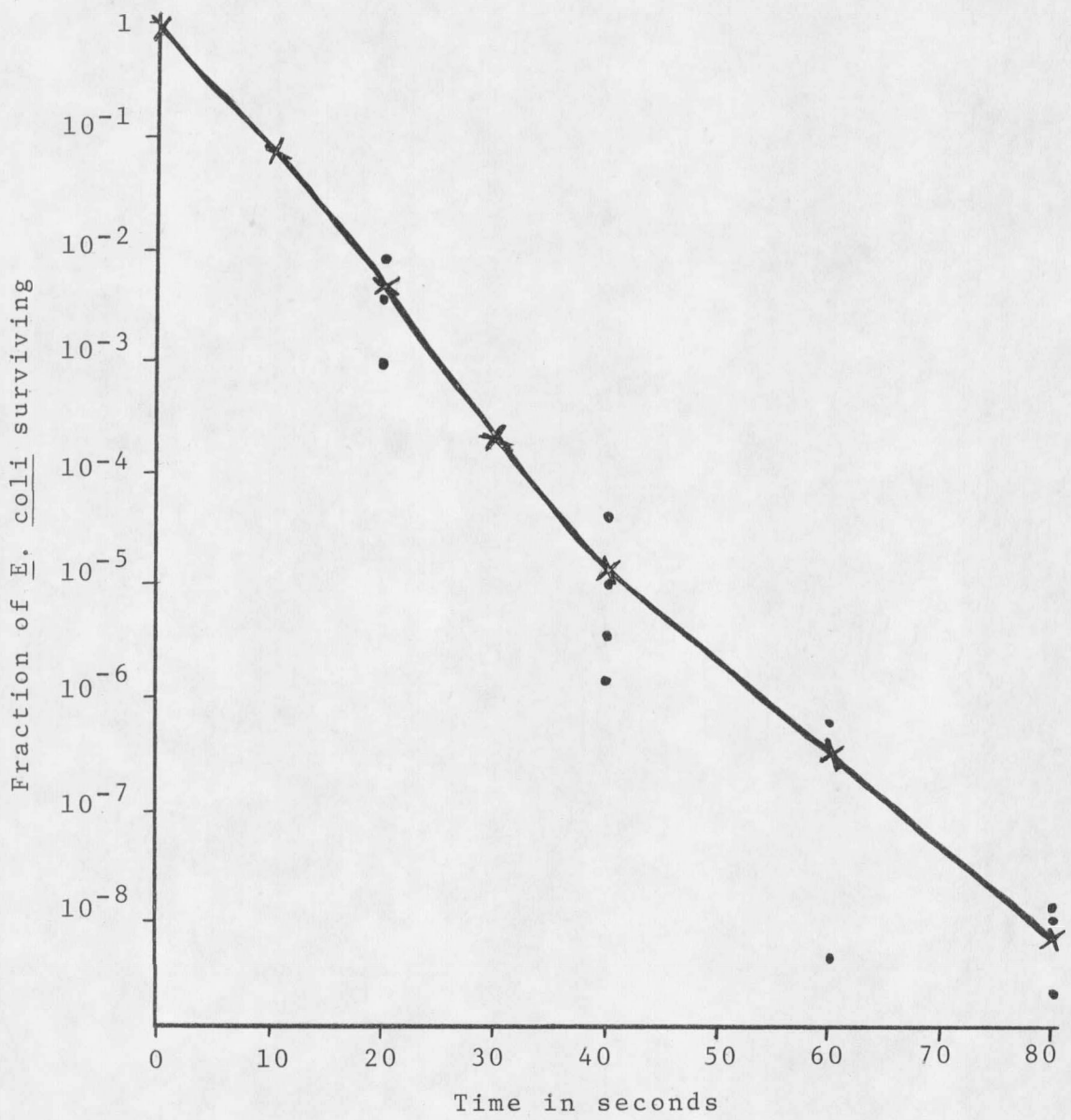

Figure 1

Survival of E. coli following UV irradiation

TABLE II

Survival of Bd. 109 following UV irradiation

Fraction of cells surviving UV irradiation

Time of irradiation in seconds	Experiment Number 1	2	3	4	5	Average
0	2.0×10^{5}(1)	3.5×10^{6}	1.5×10^{7}	1.2×10^{7}	4.8×10^{7}	1.6×10^{7}
15	1.3×10^{-2}	7.7×10^{-3}	2.92×10^{-2}	6.5×10^{-2}	1.25×10^{-3}	2.3×10^{-2}
30	3.0×10^{-3}	8.5×10^{-4}	1.33×10^{-3}	8.75×10^{-4}	1.33×10^{-4}	1.08×10^{-4}
45			2.0×10^{-6}	8.34×10^{-5}	1.46×10^{-5}	3.34×10^{-5}
60	1.0×10^{-5}		2.0×10^{-7}	8.3×10^{-8}	8.3×10^{-8}	2.56×10^{-6}

(1) Viable plaque count at time 0

Figure 2 Survival of Bd. 109 following UV irradiation. The dashed line through the circles represents the average of the fraction of Bd. 109 surviving for each time interval. The dots represent the results of each individual experiment, and the straight line represents the average number of E. coli survivors.

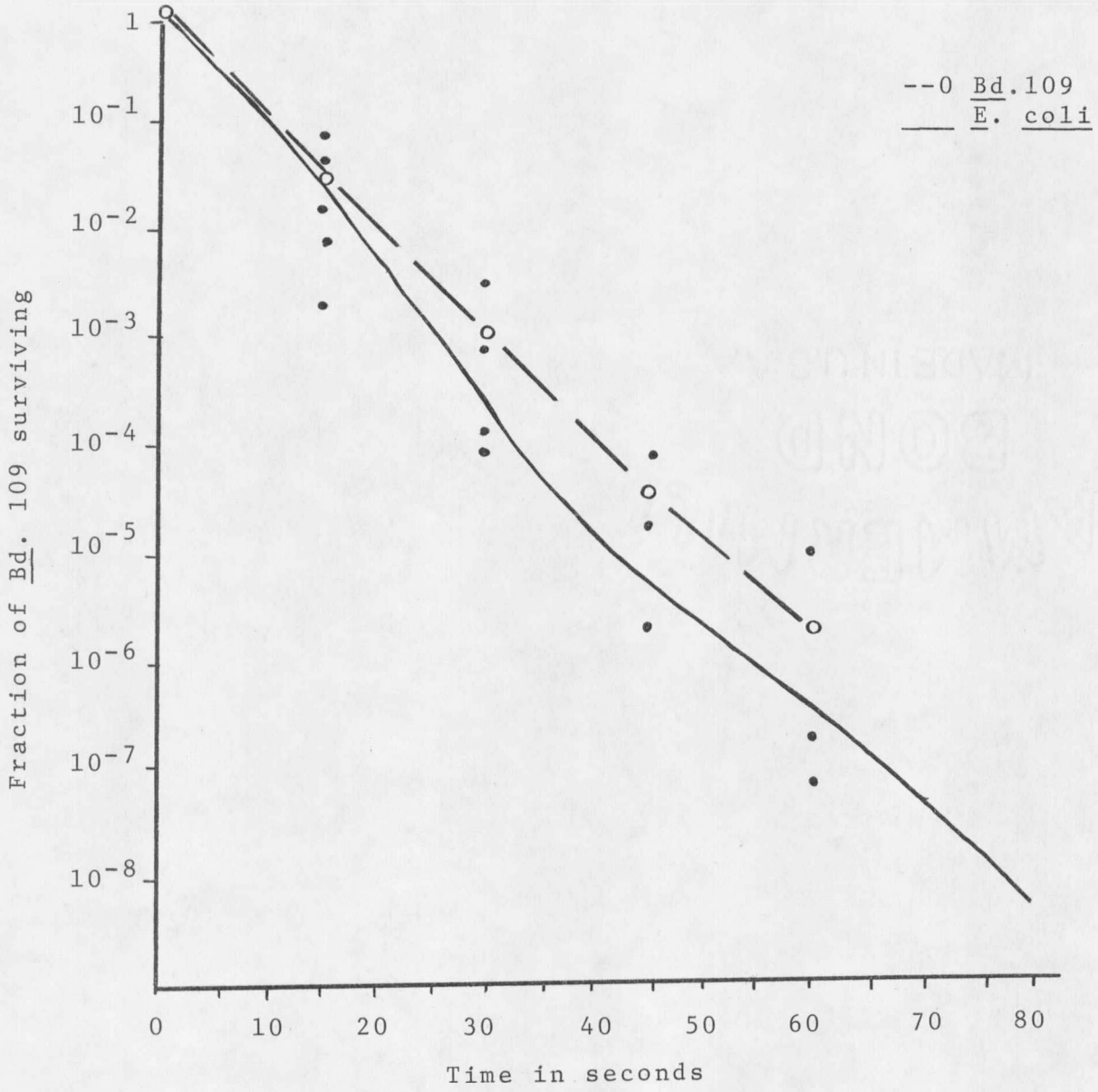

Figure 2

Survival of Bd. 109 following UV irradiation

The number of plaque forming units retained by the filter (Table III) did not decrease markedly even after exposure of the host to irradiation which should have killed all but about 2.2×10^3 host cells. Therefore, it is safe to conclude that the Bdellovibrio had attached to the irradiated host cells. Penetration and growth were not measured in these experiments but it is probable that they did occur. In support of this argument Varon and Shilo (1968) demonstrated that within thirty minutes 90 percent of the attached Bdellovibrio had penetrated their host cells. Therefore, in order to form plaques the Bdellovibrio would have to be using the irradiated cells as hosts. Otherwise they would have to leave the irradiated host and enter a non-irradiated host in order to form plaques.

Two trials were made in an effort to see what the results of plating irradiated Bd. 109 on irradiated E. coli would be. Since Bd. 109 will grow on irradiated E. coli (Stolp, 1968), it would be expected that the survival curve would drop as did that of the irradiated Bd. 109 on non-irradiated E. coli. This was found to happen. For this experiment, Bdellovibrio and E. coli were irradiated, attachment allowed to proceed, and the cells were then filtered. A reduction in the number of cells was found both in the non-attached cells which passed through the filter and in the attached Bdellovibrio which remained on the filter. No viable Bdellovibrio could be recovered from the filter after 15 seconds or from the filtrate after 30 seconds. At 30 seconds there was an average of .11 percent survivors so the small number of viable cells remaining were probably lost in the filtration and elution process. Table IV gives the results of the two experiments.

TABLE III

Survival of Bd. 109 absorbed to UV killed E. Coli

Plaque Counts of Bd. 109

Period of exposure of E. coli to UV in seconds	Experiment Number 1	2	3
0	1.7×10^5	1.1×10^2	
15	5.0×10^4	1.0×10^2	1.1×10^4
30	7.2×10^5		1.0×10^5
45	4.0×10^4	7.9×10^3	1.0×10^3
60	2.0×10^5	7.0×10^3	3.0×10^3

TABLE IV

Survival of irradiated Bd. 109 attached to irradiated E. coli

Period of exposure to UV in seconds	Plaque Counts of Bd. 109			
	Unattached Experiment Number		Attached Experiment Number	
	1	2	1	2
0	8.9×10^5	7×10^4	1.4×10^4	3×10^4
15	6.7×10^3	8.1×10^3	52	1.3×10^3
30	9	60		

DISCUSSION

The ultraviolet sensitivities of Bdellovibrio bacteriovorus 109 and its host, Escherichia coli, were compared. The E. coli was more sensitive to irradiation than the Bdellovibrio. The E. coli probably has a larger target size than Bd. 109 which would account for its greater sensitivity.

Attachment of Bdellovibrio to E. coli was not changed regardless of the numbers of survivors of E. coli. Varon and Shilo (1968) demonstrated that E. coli hosts which had been irradiated would allow the attachment of Bd. 109. Stolp (1968) has said that Bdellovibrio will grow on host cells which have been irradiated. However, he did not indicate which strain of Bdellovibrio was used, nor did he publish any supporting data. In view of Stolp's work, there is the possibility that the plaques formed in these experiments were due to growth on irradiated Bdellovibrio.

The attachment sites for B. bacteriovorus are not in any way damaged by ultraviolet light, as they are by heat, chloroform, or toluene (Stolp, 1968).

Shilo and Bruff (1965) isolated a strain of Bdellovibrio that retained its parasitic capabilities when grown on host-free media. Seidler and Starr (1969) were able to grow Bdellovibrio when the host and parasite were suspended in a buffer system. This argues that one of the main contributions of the host is an essential nutrient, or nutrients. It would also explain the ability of a UV killed cell with its receptor sites intact to support the growth of Bdellovibrio.

The survival curve obtained when both irradiated E. coli and Bdellovibrio are used is predictable, since Bd. 109 can attach and presumably grow on E. coli. The curve, as expected, follows the survival curve of

irradiated Bd. 109 on non-irradiated E. coli.

SUMMARY

Ultraviolet irradiation sensitivity studies of Bdellovibrio bacteriovorus 109 and its host, Escherichia coli, showed the E. coli to be more sensitive to UV irradiation than Bdellovibrio. There was no difference in the number of Bdellovibrio attached to irradiated E. coli although the fraction of survivors varied from 1 to 10^{-8} host cells.

LITERATURE CITED

Burger, A., G. Drews and Rita Ladwig. 1968. Wirtskreis und Infections-cyclus eines neu isolierten Bdellovibrio bacteriovorus-Stammes. Arch. Mikrobiol. 61: 261-279.

Burnham, J.C., T. Hasimoto, and S.F. Conti. 1968. Electron Microscopic Observations on the Penetration of Bdellovibrio bacteriovorus into Gram-negative Bacterial Hosts. J. Bacteriol. 96: 1366-1381.

Scherff, R.H., J.E. DeVay, and T. Carroll. 1966. Ultrastructure of Host Parasite Relationships Involving Reproduction of Bdellovibrio bacteriovorus in Host Bacteria. Phytopathology. 56: 627-632.

Seidler, R.J., and M.P. Starr. 1969. Factors Affecting the Intracellular Parasitic Growth of Bdellovibrio bacteriovorus Developing Within Escherichia coli. J. Bacteriol. 97: 912-923.

Shilo, M. 1966. Predatory Bacteria. Sci. J. 2: 33-37.

Shilo, M., and Barbara Bruff. 1965. Lysis of Gram-Negative Bacteria by Host-Independent Ectoparasitic Bdellovibrio bacteriovorus Isolates. J. Gen. Microbiol. 40: 317-328.

Starr, M.P., and N. Baigent. 1966. Parasitic Interaction of Bdellovibrio bacteriovorus with Other Bacteria. J. Bacteriol. 91: 2006-2017.

Stolp, H., and H. Petzold. 1962. Untersuchungen uber einen Obligat Parasitischen Mikroorganism mit Lytischer Aktivitat fur Pseudomas-Bakterien. Phytopathol. Z. 45: 364-390.

Stolp, H., and M.P. Starr. 1963. Bdellovibrio bacteriovorus gen. et. sp. n., A Predatory Ectoparasitic, and Bacteriolytic Microorganism. Antonie van Leeuwenhoek J. Microbiol. Serol. 29: 217-248.

Stolp, H. 1968. Bdellovibrio bacteriovorus-ein rauberischer Bakterien-parasit. Naturwissenschaften. 55: 57-63.

Varon, M., and M. Shilo. 1968. Interaction of Bdellovibrio bacteriovorus and Host Bacteria. J. Bacteriol. 95: 744-753.