Can Bacteria Hunt Their Prey?
A Study of *Bdellovibrio* Chemotaxis
Towards Host Cell Supernatants

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INTRODUCTION

Members of the Bdellovibrio are predacious bacteria that attack and lyse a variety of gram negative organisms (for recent review, see Ruby, 1991). *Bdellovibrio* exhibit a bi-phasic life style in which the bacteria alternate between a free-living, motile, and non-multiplying form and an aflagellate form which multiplies within the periplasmic space of the host cell (Varon, 1980). Figure 1 is an overview of the Bdellovibrio life cycle.

After leaving the bdelloplast the rapidly motile cells are highly sensitive to starvation, they have no known storage source for carbon or energy yet they have an unusually high rate of respiration This suggests that there is a very limited time span during which bdellovibrio must find and attach to its prey cell. (Hespell, 1974). This would limit bdellovibrio distribution in nature to environments near dense prey populations unless bdellovibrios could employ a 'search' mechanism, such a positive chemotaxis towards prey.

Two things are very striking when reading the bdellovibrio literature: 1) it has been shown high concentrations of prey cells are required (10^8 cells per ml) in order to support bdellovibrio growth (Keya, 1975) and 2) all of the *Bdellovibrio* prey are gram-negative and many of them (*V. harveyi, V. fischeri, E. carotovora, S. marsescens, R. melioti, P. leiognathi, E. coli*) have been shown to produce acylated homoserine lactone signal molecules (autoinducers) as a way of regulating cell-density dependent genes (for recent review of autoinducer regulation see Fuqua, 1994).

Previous studies from the 1970's literature have shown that *Bdellovibrio* is chemotactically attracted to a few amino acids (LaMarre, 1977) and to a variety of pure compounds (Straley, 1979). There is a single report in the literature (Straley, 1977) addressing the issue of chemotaxi towards prey, indicates that *Bdellovibrio* is not attracted to prey cells. However in these experiments the prey cells and their lysates were prepared in such a way (growing to stationary phase and then washed several times) that some stationary phase products may not have been present in the 1977 chemotaxis studies, such as autoinducer and products regulated by autoinduction. We wonder if *Bdellovibrio* is chemotactically attracted to an autoinducer signal or a bacterial product regulated by an AI-type system, since chemotaxis up an AI gradient would most likely lead to a high density of prey cells.

MATERIALS AND METHODS

Bacteria. Strains of *Bdellovibrio bacteriovorus* used were the obligately predacious strain 109J and the facultatively predacious strains 6-5-S and 109J-HI (obtained from J. P. Armitage, Oxford University and M. O. Martin, Occidental College). *Escherichia coli* RP437 (provided by J. P. Armitage) is a motile and was used as a positive control in chemotaxis experiments. *Escherichia coli* ML35 and *Aquaspirillum serpens* VHL (both provided by J. P. Armitage, Oxford University) were used as prey for *B. bacteriovorus* 109J and 6-5-S respectively. To test chemotaxis towards autoinducer molecules, *E. coli* XL-1 Blue containing the plasmids pRHLI, pBLH105, and pLASI which encode for production of butyryl homoserine lactone, 3-oxo-hexanoyl homoserine lactone, and 3-oxo-tetradecanoyl homoserine lactone (provided by M. A. Parsek and B. L. Hanzelka, University of Iowa) were used. Other bacteria tested included wild-type*V. fischeri* MJ-1 (which produces 3-oxohexanoyl HSL, hexanoyl HSL, and octanoyl HSL), an autoinducer-deficient strain*V. fischeri* MJ-215 which is *luxI*$^-$, *ainS*$^-$, and *V. harveyi* (which produces 3-hydroxy-butyryl HSL) (provided by B. Hanzelka, U of Iowa and M. O. Martin, Occidental College).

Cultivation of strains. *E. coli* and *A. serpens* were grown in LB media (10% tryptone, 5% yeast extract, 5% NaCl) and YPSC medium (0.1% yeast extract, 0.1% peptone, 0.05% anhydrous sodium acetate, 0.025% $MgSO_4$-$7H_2O$, pH 7.6 after autoclaving add 10 ml of 2.5% $CaCl_2$-$2H_2O$ per 1 L) respectively. Where indicated *E. coli* strains were also grown in M9 minimal medium (Maniatis). *B. bacteriovorus* 109J-HI was grown in PYE media (1% bacto-peptone, 0.3% yeast extract). *V. fischeri* and *V. harveyi* were grown in Sea Water Complete media. For *E coli* cells overexpressing various autoinducer synthases cells were grown to an O.D.$_{600}$ of 0.5 and then induced with 1 mM IPTG for 3 hours. All other cultures were grown overnight at room temperature with shaking.

Preparation of *Bdellovibrio* lysates. One ml of an overnight culture of prey cells was centrifuged and the pellet was resuspended in 0.5 ml of buffer (25 mM HEPES, 2 mM $CaCl_2$, pH 7.8). The washed prey cells were added to a test tube containing 0.5 ml of buffer plus 0.5 ml fresh *Bdellovibrio* lysate (24-48 hours old) and the incubated overnight at room temperature with shaking. All cultures were examined by phase contrast microscopy to insure productive infection had occurred.

Chemotaxis assays. A 'chamber' was constructed using a microscope slide and 3 coverslips (see Figure 2). The component to be tested (lysate, washed prey cells, yeast extract, etc.) was mixed with hot 4% agarose in a 1:1 ratio and then dispensed in a straight line through a syringe. The agarose line was allowed to cool and then a small piece was cut and placed in the center of the chamber. The chamber was then filled with freshly fed and washed *Bdellovibrio* lysates and the chamber was incubated for 25 minutes at room temperature. Chemotaxis chambers were visualized by eye for the formation of bacterial 'bands' around the agarose piece, indicating accumulation (positive chemotaxis) of the bacteria.

RESULTS AND DISCUSSION

To determine whether or not the chemotaxis chamber could be used as a measure of chemotactic ability, *E. coli* RP437 was tested using 0.5% yeast extract (known to attract *E. coli)* as

the chemoattractant (Table 1) and found to form a 'ring' of cells around the yeast extract/agarose block. No ring formation was observed with agarose alone. Since the chemotactic chamber seemed to be 'working' studies were initiated using *Bdellovibrio*.

Yeast extract was used again as a positive control for *Bdellovibrio* chemotaxis (Table 2) and indeed a 'ring' was formed around agarose, although not to the extent that *E. coli* RP437 formed a ring. No chemotaxis was observed towards agarose alone or with buffer/agarose. Chemotaxis was also observed towards *E. coli* ML35, *E. coli* XL1-Blue, *E. coli* XL1-Blue (pRhlI), *E. coli* XL1-Blue (pBLH105), and*E. coli* XL1-Blue (pLasI) supernatants from cultures that had been grown in LB media. However, *Bdellovibrio* also chemotaxed towards the LB media control (which is not surprising since LB contains 0.5% yeast extract). To try and get away from media containing yeast extract, the same experiment was performed using supernatants from cells grown in M9 minimal media with glucose, but the same results were obtained (Table 1).

Because autoinducer molecules are extractable in ethyl acetate, the supernatants from LB and M9 grown cells were extracted with ethyl acetate. The ethyl acetate was blown off under a stream of nitrogen gas, and the residue (containing the autoinducer molecules) was resuspended in water. This suspension was then tested for chemoattract activities. While the extraction alleviated the problem of chemtaxis towards media, no *Bdellovibrio* chemotactic activity was detected to any of the *E. coli* supernatants.

Similar studies were performed using the supernatants and extracts of *A. serpens, V. fischeri* MJ-1, *V. fischeri* MJ-215, and *V. harveyi*. Again there was chemotaxis to background media, but no chemotaxis was observed to the ethyl acetate extractable portion of the supernatants (which would contain the autoinducer signal molecules).

Work from this project indicate that *Bdellovibrio* is not chemoattracted to acylated homoserine lactone molecules. It would appear that the role that chemotaxis plays in the life of *Bdellovibrio* is in finding areas containing amino acids and various other compounds. This could be for two possible reasons: 1) it is a way in which *Bdellovibrio* could chemotax towards an environment that its prey might also chemotax towards or 2) perhaps these amino acids could be utilized by cellular

maintenance (but not growth) thus alleviating starvation for a time until an appropriate host cell can be found.

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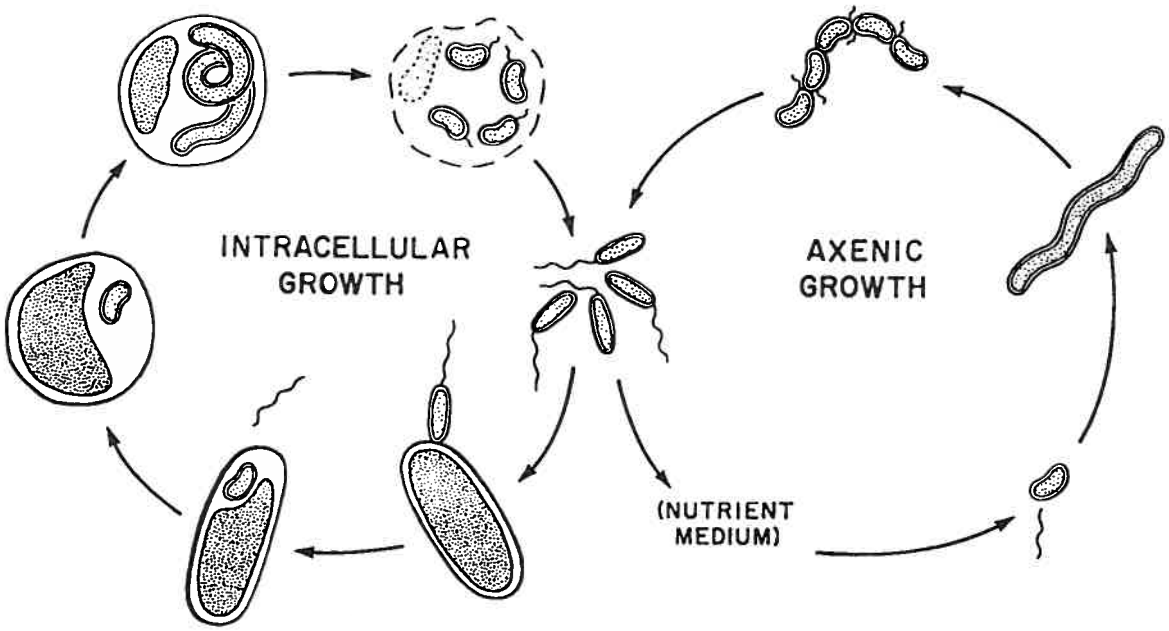

Fig. 1. The two growth patterns of *Bdellovibrio bacteriovorus* 109J. *Left*: wild-type bdellovibrios can grow only within another bacterium, alternating between an extracellular, attack-phase developmental form and an intracellular, growth phase developmental form. *Right*: variant strains can be isolated that can grow axenically when inoculated into a complex nutrient medium. Many of these variants are facultative, i.e., able to grow either intracellularly or axenically. (Ruby, 1991).

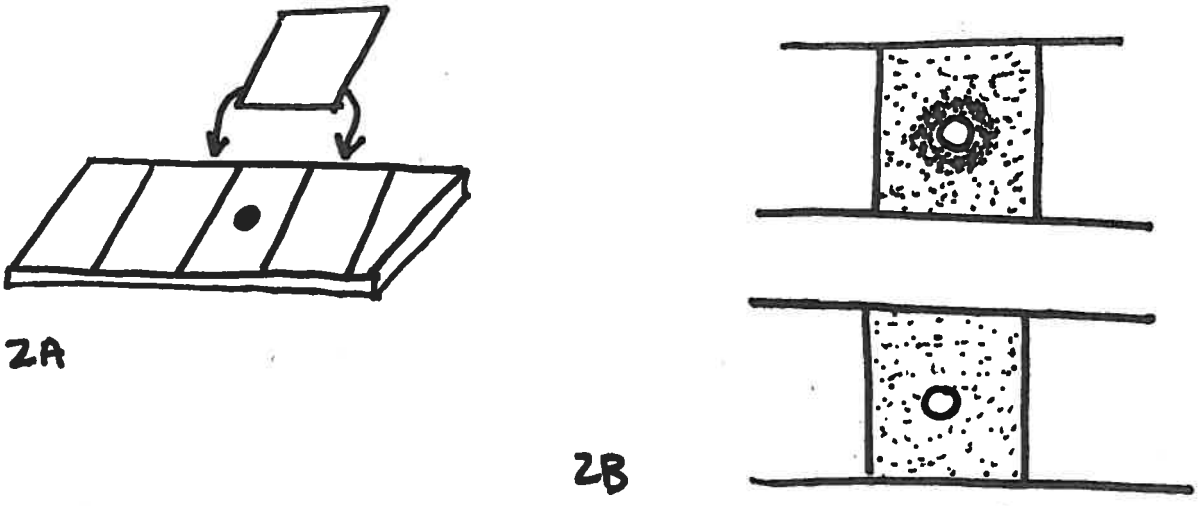

Fig. 2. Chemotaxis chamber assay. 2A. A piece of agarose containing the test substance is placed in between two coverslips on a glass slide (held in place by a strip of high vacuum grease). Another slide is overlaid, forming a chamber. 50 µl of bacteria are added to the chamber and incubated for 25 minutes at room temperature, after which the slide chamber was held up to the light and read for chemotactic activity. 2B. If the substance is a chemoattractant, the motile cells will begin to accumulate around the agarose block as the test substance diffuses out of the block (top). If the test substance is not chemoattractive, the cells will remain uniformly distributed along the chamber (bottom). The chamber assay was developed by J. P. Armitage.

Table 1. Chemotaxis by *Bdellovibrio* to *E. coli* supernatants and extracts.

	E. coli RP437	*Bdellovibrio*[a] sub. in LB[b]	*Bdellovibrio* sub. in M9	*Bdellovibrio* sub. LB extract	*Bdellovibrio* sub. M9 extract
Yeast Extract (+)	+++[c]	++	++	++	++
agarose (-)	-0-	-0-	-0-	-0-	-0-
media or media extract	+++	++	++	-0-	-0-
E. coli ML35	ND	++	++	-0-	-0-
E. coli XL-1 Blue	ND	++	++	-0-	-0-
" " pRhlI	ND	++	++	-0-	-0-
" " pBLH105	ND	++	++	-0-	-0-
" " pLasI	ND	++	++	-0-	-0-

[a] *Bdellovibrio bacteriovorous* strains 109J, 6-5-S, and 109J-HI all exhibited similar behavior

[b] Refers to what media the substrate bacteria were grown in, extract indicates the ethyl acetate soluble portion of the supernatant

[c] Results were scored as follows: +++, strong positive chemotaxis observed; ++, somewhat strong chemotaxis observed; +, weak chemotaxis observed; -0-, no chemotaxis observed; ND, not determined

Table 2. Chemotaxis by *Bdellovibrio* to non-*E. coli* supernatants and extracts.

	E. coli RP437	*Bdellovibrio*[a] supernatant	*Bdellovibrio* supernatant extract
Yeast extract (+)	+++[c]	++	++
agarose (-)	-0-	-0-	-0-
YPSC	ND	++	-0-
Aquasprillum in YPSC	ND	++	-0-
SWC	ND	++	-0-
V. fischeri MJ-1 in SWC	ND	++	-0-
V. fischeri 215 in SWC	ND	++	-0-
V. harveyi in SWC	ND	++	-0-

[a c] As in Table 1

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