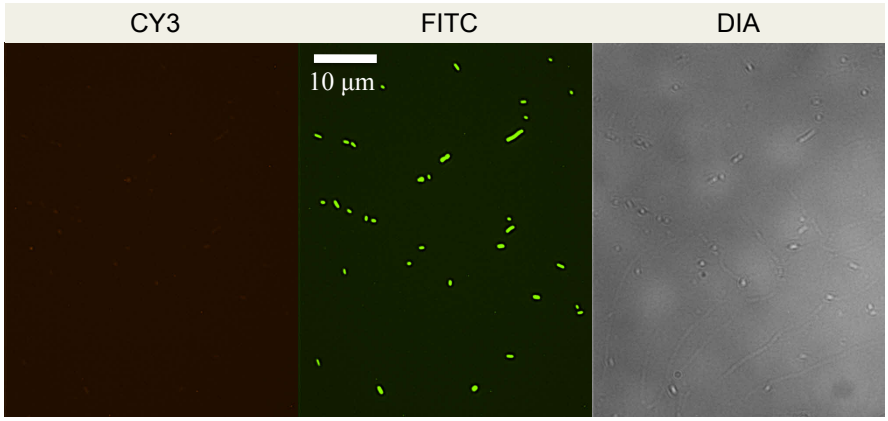
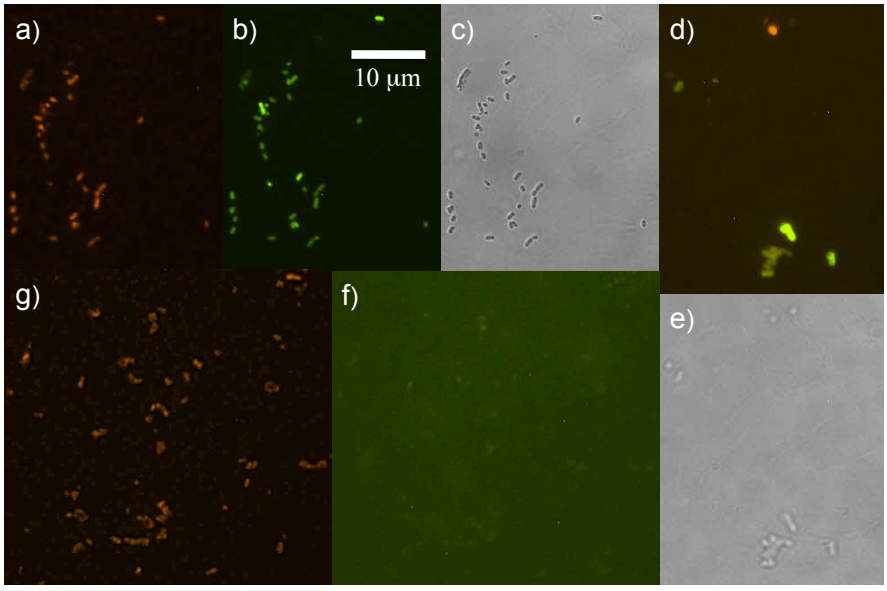
***Supplemental Information* for " Spatially organized films from *Bdellovibrio bacteriovorus* prey lysates" by Ferguson *et al.***

**Part I** Control Images for Fluorescence *In Situ* Hybridization Experiments

**Supplemental Figure 1. *E. coli* fluorescence *in situ* hybridization (FISH) control images.** These control FISH images show that *B. bacteriovorus* specific and gamma-proteobacteria specific probes do not cross hybridize in *E. coli*. The panel shows FISH images for planktonic *E. coli* ML 35 with BDE525 probe (left; orange/red), GAM42a probe (center; green), and in brightfield (right). The scale bar (center panel) applies all images.




**Supplemental Figure 2. *B. bacteriovorus* prey lysate fluorescence *in situ* hybridization control images.** These control FISH images show that the *B. bacteriovorus* specific and gammaproteobacteria specific probes do not cross hybridize for bdellovibrios in a cleared co-culture with *E. coli* ML 35. The panels show FISH on a planktonic HD *B. bacteriovorus* prey lysate. Prey lysate cells hybridized to BDE525 and EUB388 probes in Cy3 (a), FITC (b), and brightfield (c); a bdelloplast and prey cells hybridized with BDE525 and GAM42a probes in overlap of Cy3 and FITC (d) and brightfield (e); HD bdellovibrios hybridized to BDE525 and GAM42a probes in FITC (f) and Cy3 (g), respectively. The 10 μm scale bar in (b) applies to each image.



**Part II**: PCR and Sanger Sequencing of DNA from Prey Lysates, Young Films, and Mature Films

***Methods***

DNA Extraction

DNA extraction of the *E. coli* or *B. bacteriovorus* was performed using the QIAGEN or similar DNeasy Kit as described therein.

Polymerization Chain Reaction (PCR)

Amplification of the *B. bacteriovorus hit* gene (Bd0108) and portion of the 16S rRNA gene were performed using primers listed below in Table S.1. The *E. coli* primers were utilized in our earlier publication.(1) Thermocycler conditions were as follows for *hit* gene amplification: initial denaturation of the DNA; 2 min. at 94°C; 32 cycles of denaturation at 94°C for 30 sec.; annealing at 61.5°C for 30 sec.; extension at 68° C for 1 min.; and hold at 4°C. For the 16S rRNA gene amplification, thermocycler conditions were as follows: initial denaturation of the DNA; 2 min. at 94°C; 32 cycles of denaturation at 94°C for 30 sec.; annealing at 52°C for 30 sec.; extension at 68° C for 1 min., and hold at 4°C. *E. coli* PCR followed conditions reported previously.(1)

Gel Electrophoresis and Extraction

After the PCR was completed, the samples were analyzed by electrophoresis through a 1% agarose gel containing 0.5 ng/mL ethidium bromide. Gels were imaged using a Bio-Rad gel Doc XR imaging system. To extract PCR-amplified DNA products separated by gel electrophoresis for sequencing, we used the PureLink Quick Gel Extraction and PCR Purification Combo Kit (Life Technologies).

Sanger Sequencing

Gel separated and extracted DNA product samples were sent to Alpha Biolabs, Inc. in Burlingame, California for Sanger sequencing. Samples were initially sent at a concentration of 20-60 ng/µL of DNA with high 260/280 nm wavelength absorption (based on Nanodrop measurements) along with 60-120 ng/µL primers.

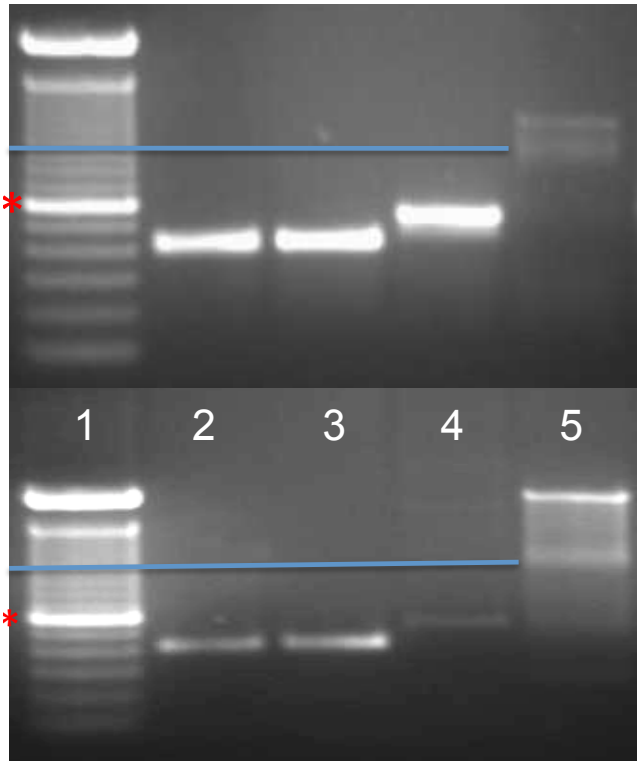
**Supplemental Table 1**. Primers for PCR and sequencing.

| Organism and Gene | Figure | primers | Amplicon size (bp) |
|---|---|---|---|
| *E. coli* ¥ | S.3-S.5 | 5'-TTTCAGCATGTCGGCATC-3'§<br>5'-CTGGAGTTGAATCATCAG-3'† | 411 |
| Bd2269 serine protease | S.3 | 5'-TACGTGAGTACCGTGACCT-3'§<br>5'-GATCACTAGAACGCGCTCT-3'† | 517 |
| Bd0108 *hit* | S.3 | 5'-GAAGGCTATTGCATCGCGTGTG-3'§<br>5'-GTCGTCATCACACGCTGCAGAT-3'† | 1047 |
| *Bd0108 *hit* | S.4(b)<br>S.5(a) | 5'-GAATCGGAGGGTTTCAGGGC-3'§<br>a:5'-GGTCGCAATACGGCCGCGG-3'†<br>b:5'-GCGGCCTTCGTGGCTACTTTGG-3'† | a: 662<br>b: 765 |
| *Bd_r01 16S rRNA | S.4, S.5 | 5'-CGGGGAAAGCTTTCGGGTGAGT-3'§<br>5'-TTGACCCCTTCAGTGACG-3'† | 764 |

***** Also used for sequencing. ¥ Based on *E. coli* K-12 substr. MG 1655 complete genome
§ Complementary to the template strand † Complementary to the coding strand

***Results and Discussion***

Before turning to FISH and atomic force microscopies to probe these films, our first sets of experiments utilized DNA-based methods. To identify the species present in the young and mature films, we employed total DNA extraction and PCR/gel electrophoresis methods with species-specific primers. Supplemental Figure 3 shows DNA amplification products of 2-day old films grown from an unfiltered prey lysate of *B. bacteriovorus* 109J (upper gel) and our environmental *B. bacteriovorus* isolate (lower gel) with *E. coli* ML 35 prey.

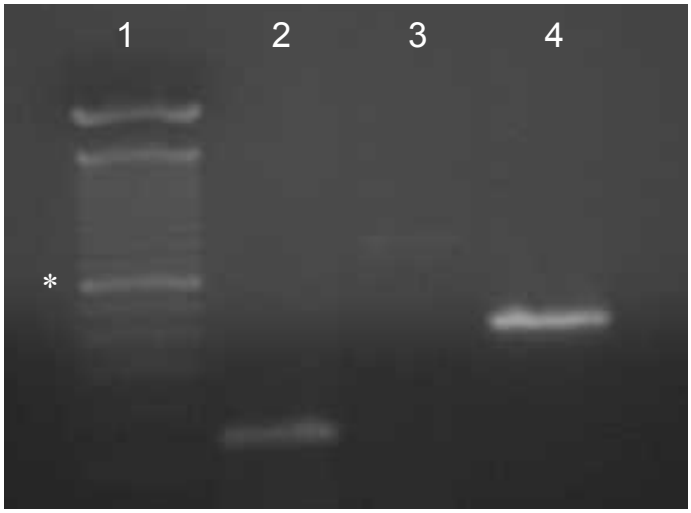



**Supplemental Figure 3. PCR Amplification of Genes from a Young Film.** Gel electrophoresis of PCR products from DNA extracted from two-day old films of *B. bacteriovorus* 109J (top) and our *B. bacteriovorus* environmental isolate (bottom) prey lysates. Lane (1) contains the 100 bp ladder (red asterisk at 600 bp, blue line at 1000 bp) while lane (2) serves as a control that shows *E. coli* cells with *E. coli* primers. The remaining lanes contain the amplification products of young film cells with (3) *E. coli* primers, (4) a serine protease housekeeping gene (*B. bacteriovorus* Bd2269), and (5) a portion of the *hit* locus (containing *B. bacteriovorus* Bd0108 in full).

In both gel images, comparison of lanes 2 and 3 shows that *E. coli* prey are still present in the bacterial film after two days. Lane 4 is a *Bdellovibrio* serine protease housekeeping gene, which indicates that *Bdellovibrio* is also present in both films. Lane 5 is the product of amplification of the *hit* locus (Bd0108), part of the *hit* locus (Bd0108-Bd0121) that is tied to the predation phenotype. Further evidence that our environmental isolate is *B. bacteriovorus* comes from PCR product sequencing (*vide infra*).

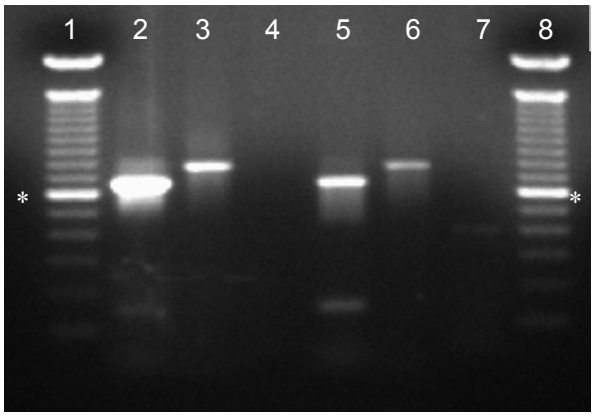
For both *B. bacteriovorus* strains the expected PCR product at 1047 bp containing the Bd0108 *hit* gene is produced in lane 5; a second larger product can also be seen, indicating that there may be genetic variability at the *hit* locus even at this early stage in film development. Importantly, the young film developed from our environmental isolate is confirmed as *B. bacteriovorus*. A control gel image to test cross hybridization of selected primers is provided in S. Figure 4.

**Supplemental Figure 4. Control gel electrophoresis of PCR products from DNA extracted from a liquid culture of *E. coli* ML 35.** This 4-lane gel of PCR products shows the capabilities of the primers that we picked to probe the film cells' genes by DNA extraction with gel electrophoresis. The lanes are (1) 100 bp ladder where the asterisks mark 600 bp, (2) planktonic *E. coli* with *Bb* Bd0108 primers, (3), planktonic *E. coli* with *Bb* 16S rRNA primers, and (4) planktonic *E. coli* with *E. coli* primers. There is some nonspecific binding of *B. bacteriovorus* 16S rRNA primers for *E. coli* cells in lane 3, but it is not a strong reaction.




Turning next to the mature film, DNA from the inner and outer regions of the mature film was isolated and amplified using *hit* gene (Bd0108) or 16S rRNA *B. bacteriovorus* or *E. coli* primers (S. Figure 5).

**Supplemental Figure 5. PCR and gel electrophoresis of DNA from inner and outer regions of a mature film.** Samples from inner (lanes 2-4) and outer regions (lanes 5-7) of mature films were analyzed for the presence of *Bdellovibrio* or *E. coli* genes. Lanes 2 and 5 show amplicons from the *hit* gene Bd0108; lanes 3 and 6 show amplicons of the *Bdellovibrio* 16S rRNA; lanes 4 and 7 show products of amplification using *E. coli* genomic sequence primers. Lanes 1 and 8 contain a 100 bp ladder, where the asterisk marks the strong band at 600 bp. *B. bacteriovorus* clearly is present in both the inner and outer regions, while *E. coli* only exists in the outer (weak band in this gel image). In other replicates of this experiment, we sometimes detected *E. coli* in the inner region as well as the outer region but these were for mature films of varying ages.




Both the inner and outer regions clearly contain both the *Bdellovibrio* 16S rRNA gene and the *hit* gene, indicating that *Bdellovibrio* cells are present in both the inner yellow region and the outer white one. We further explored the *hit* gene in both inner and outer regions of the mature film by Sanger sequencing, and these results follow.

The *E. coli* primers on the other hand amplify only the sample from the outer region, and that weakly. In other replicates of this experiment, a weak band is sometimes seen in the inner

region as well. Since the primers generally appear to amplify *E. coli* DNA successfully when large numbers of *E. coli* cells are present (i.e. S. Figure 3) we hypothesize that the number of *E. coli* cells is modest, variable between experiments, and also spatially and temporally variable across the film.

Sequencing of *hit* locus from *B. bacteriovorus* 109J grown on *E. coli* ML 35 cleared prey lysate (planktonic cells)
The *hit* gene (Bd0108) is 306 bp and a 100% match to the published sequence of *B. bacteriovorus* HD 100. This control demonstrates that the *hit* gene is wild-type in sequence in the prey lysate from which the mature film is derived.

Sequencing of *hit* locus from inner and outer regions of the mature film developed from *B. bacteriovorus* 109J / *E. coli* ML 35 prey lysate
The sequence of the *hit* gene was wild-type in cells from the inner regions of four independent films and in cells from the outer region of three independent films. Note that replicates of this experiment were initiated with *B. bacteriovorus* sampled from both PFU clearings and nonPFU or multiply-passaged prey lysates, but the origin of the *Bdellovibrio* had no effect upon the result. Nonetheless, as we did not sample each and every cell from the mature film's inner and outer regions, it is possible that other low-frequency variants for the *hit* gene were not observed.

The *hit* sequences from the outer region of two additional films (1 PFU and 1 nonPFU derived) were not clearly wild type. Interestingly, these mutated amplified PCR products were not easily deciphered as frame shifts or straightforward insertion/deletions because of the presence of a mixture of overlapping sequences, reflecting more than one related *Bdellovibrio* harboring mutations at the *hit* site in addition to wild type in the outer region.(2)

Sanger sequencing results reveal that both regions of the mature film contain *Bdellovibrio* cells that are wild-type in sequence for the *hit* gene (Table S.2). The *hit* gene is known to be critical for host interaction, mainly host invasion and penetration, but the entire *hit* locus may have many ways to impact change on the *Bdellovibrio* lifestyle especially when confined to a surface. Notably, in both cases the *hit* amplicon is 662 bp long as predicted, with no indication of a major insertion or deletion that might be responsible for the presence of the HI phenotype (Table S.2). Though the inner region and outer regions both contain significant populations of cells with the wild-type *hit* gene for *B. bacteriovorus*, because we do not utilize whole-genome sequencing in this investigation we cannot comment on possible mutations in other locations.

**Supplemental Table 2.** Summary of Sanger sequencing results for *B. bacteriovorus (Bb)* 109J *hit* gene Bd0108.

| Cells sampled | Bd0108 genotype | *Bb* history before sampling |
|---|---|---|
| planktonic | wild-type | 1 PFU |
| inner region of mature film | wild-type | 2 PFU and 2 nonPFU |
| outer region of mature film | wild-type | 3 PFU |
| | unclear | 1 PFU and 1 nonPFU |

In summary, while these methods clearly show that both regions of the mature film contain *Bdellovibrio bacteriovorus* and that many of these cells possess the wild-type *hit* gene, the PCR/gel results leave ambiguous the question of the presence of *E. coli* in the two regions. For these reasons, we turned to microscopy to probe the films for cell species-specific identification using FISH microscopy and cell morphology (and film spatial topography) using atomic force microscopy measurements.

Sequencing of 16S rRNA gene locus from *B. bacteriovorus* environmental isolate (cleared lysate of *E. coli* ML 35)

To augment the FISH results and predation phenotype assays, we sought additional evidence that our environmental isolate was indeed *B. bacteriovorus*. We collected cells from a cleared nonPFU prey lysate and extracted total DNA for PCR/gel electrophoresis with 16S rRNA primers. Isolation and sequencing of the PCR product revealed a near-perfect match to the 16S rRNA of *B. bacteriovorus* Tiberius and *B. bacteriovorus* HD100 (featuring only a 1 bp mismatch in each), yielding further support for identifying the environmental isolate as *B. bacteriovorus*. In addition, we aligned our partial sequence against a longer partial sequence of 109J, finding only a 1 bp mismatch (Chart S.1.).

**Supplemental Chart 1**. The 16S rRNA partial gene sequence of our environmental isolate that identifies it as *Bdellovibrio bacteriovorus*. A partial sequence of 16S rRNA gene for *B. bacteriovorus* 109J with length 1076 bp (Accession Number AY094125) is presented in black, lowercase letters. In yellow highlight is 379 bp of our nominally 764 bp PCR product for this gene. There is a 1 bp mismatch indicated by the red, un-highlighted letter. The best next matches of our sequence against microbial whole genome searches at the NCBI are the rRNA genes in *B. bacteriovorus* HD 100 and *B. bacteriovorus* str. Tiberius, respectively, where each match also shows a 1 bp mismatch.

agtggcgcacgggtgaggaacgcgtggataatctgccttagagtgggggataactagtcg
aaagattagctaataccgcataagaccacaggagctgcggctctaggggtcaaaggtttt
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**SUPPLEMENTAL INFORMATION REFERENCES**


1. **Ferguson MA**, **Schmitt JL**, **Sindhurakar AR**, **Volle C**, **Núñez ME**, **Spain EM**. 2008. Rapid isolation of host-independent *Bdellovibrio bacteriovorus*. J. Microbiol. Methods **73**:279–281.

2. **Wurtzel O**, **Dori-Bachash M**, **Pietrokovski S**, **Jurkevitch E**, **Sorek R**. 2010. Mutation Detection with Next-Generation Resequencing through a Mediator Genome. PLoS ONE **5**:e15628.