BDELLOVIBRIO BACTERIOVORUS PROTECTS *CAENORHABDITIS ELEGANS* FROM BACTERIAL PATHOGENS

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ABSTRACT

Bdellovibrio bacteriovorus is a naturally predatory bacterium that multiplies inside Gram negative prey bacteria. There is much interest in using *Bdellovibrio* as a living antibiotic to control infections by Gram negative pathogens. In recent years *Caenorhabditis elegans* has proven to be an attractive animal model of bacterial pathogenesis for a range of pathogens. We have used the *C. elegans* animal pathogenesis model to examine the ability of *B. bacteriovorus* to protect nematodes from four bacterial pathogens. In all cases, nematodes treated with *B. bacteriovorus* and the pathogen survived at a significantly higher level than nematodes treated with the pathogen alone. Treatment with *B. bacteriovorus* alone was nontoxic to the worms. We monitored the persistence of *E. coli* K-12 and *E. coli* OP50 in both *B. bacteriovorus* treated nematodes and control nematodes. *E. coli* K-12 levels were significantly lower in *B. bacteriovorus* treated nematodes than in control nematodes one day after *Bdellovibrio* exposure and *E. coli* K-12 was eliminated from the worm gut two days faster in *B. bacteriovorus* treated nematodes. *E. coli* OP50 also demonstrated significantly lower levels in *B. bacteriovorus* treated nematodes and faster elimination from the worm gut. The successful use of *B. bacteriovorus* as a therapeutic agent in *C. elegans* indicates that it may be useful as a living antibiotic in other animal systems.

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KEYWORDS

- *Bdellovibrio bacteriovorus*
- *Caenorhabditis elegans*
- pathogenesis, biocontrol
- infection model

INTRODUCTION

Bdellovibrio bacteria are intriguing because they naturally reproduce inside other Gram negative bacteria. The *Bdellovibrio* life cycle involves attachment to and penetration of prey cells, elongation inside the prey periplasm using prey components for growth, fragmentation into multiple cells, and finally, lysis of the prey cell (1). Because *Bdellovibrio* lyses prey as it multiplies, and because it cannot infect eukaryotic cells, there is growing interest in using *Bdellovibrio* as a "living antibiotic" (2).

Numerous researchers have demonstrated in vitro killing of pathogens by *Bdellovibrio,* (3, 4, 5, 6) supporting the idea of using *Bdellovibrio* to control infections. Additionally, *Bdellovibrio* has been shown to attack prey within bacterial biofilms and reduce biofilm biomass (7, 8, 9). Two studies have put the living antibiotic concept into practice, demonstrating protection against *Aeromonas hydrophila* infection in fish and protection against *Proteus penneri* infection in shrimp through the use of *Bdellovibrio*

(10, 11). Fish and shrimp mortality was significantly lower when the animals swam in water containing both the pathogen and *Bdellovibrio* as compared to animals in water containing only the pathogen. However, it was not determined whether the mechanism of *Bdellovibrio* protection was simply a reduction of the pathogen level in the water, the killing of the pathogen within the animal, or a combination of the two. Until recently, the use of *Bdellovibrio* as an *in vivo* treatment for infection has been an intriguing, but theoretical option. In 2011 Atterbury *et al.* demonstrated *Bdellovibrio* could be used therapeutically to control *Salmonella* infection in chickens without negative effects on the birds (12). This was the first study to demonstrate in vivo efficacy of *Bdellovibrio* as a treatment for bacterial infection. Here we continue the use of *Bdellovibrio* as an *in vivo* therapeutic agent, but in the *C. elegans* bacterial pathogenesis model.

In 1999 Tan *et al.* first reported the use of the nematode *C. elegans* as an animal model for bacterial pathogenesis (13). Since then numerous researchers have demonstrated that this system can be used for multiple bacterial pathogens including *Pseudomonas aeruginosa, Salmonella enterica, Serratia marcescens,* and *Staphylococcus aureus* (14, 15). Genes identified in *C. elegans* as important in pathogenesis have been confirmed in mouse models of pathogenesis, validating the use of *C. elegans* as a pathogenesis model (16). Using *C. elegans* as an animal model for pathogenesis is attractive for numerous reasons such as low cost, short generation time, complete genome sequence and ease of genetic manipulation (17). When *C. elegans* are maintained in the laboratory they are grown on Petri plates containing lawns of nonpathogenic *E. coli* OP50 as their food source and the worms typically live two weeks (18). When grown on a pathogen instead of OP50, worm survival is greatly reduced (16).

Our lab has taken advantage of the well-studied *C. elegans* bacterial pathogenesis model system to examine the use of *Bdellovibrio* to protect *C. elegans* from bacterial infection. In this study, we first established an infection in the nematode and then examined the curative effect of a brief exposure to *Bdellovibrio*. We show that worms treated with both *Bdellovibrio* and a pathogen live significantly longer than worms treated with the pathogen alone. We also demonstrate that bacterial levels are lower and cleared faster in *Bdellovibrio* treated worms than control worms. This work demonstrates that *Bdellovibrio* can be used as a therapeutic treatment for bacterial infections in a well-defined animal model.

MATERIALS AND METHODS

NEMATODE AND BACTERIAL STRAINS

Wild type *C. elegans* N2 worms were used in all nematode assays. Worms and nonpathogenic *E. coli* OP50 were supplied by the Caenorhabditis Genetics Center (Minneapolis, MN). Worms were grown on nematode growth medium (NGM) with *E. coli* OP50 as the food source (18). Pathogens tested were *E. coli* K-12, *Enterobacter aerogenes* ATCC 13048, *Pantoea agglomerans* LS005, and *Salmonella enterica serovar Typhimurium* LT2 (19). *B. bacteriovorus* HD100 was used for all biocontrol assays (20). *E. coli* HB101 was used as the non-pathogenic control in the biocontrol assays since our early work in this system used *B. bacteriovorus* 109J, which does not infect *E. coli* OP50, but does infect *E. coli* HB101.

However, all the experiments described here used *B. bacteriovorus* HD100, which does infect both *E. coli* OP50 and *E. coli* HB101. *B. bacteriovorus* HD100 was cultured using *E. coli* K-12 as prey according to standard protocols (21). *B. bacteriovorus* prey lysates were checked microscopically for active, motile *B. bacteriovorus* cells and an absence of prey cells. Prey lysates contained approximately 6 x 10^8 *B. bacteriovorus* cells per ml. The persistence assays utilized kanamycin-resistant *E. coli* K-12 derivative strain JW1863-1 (22), supplied by the *E. coli* Genetic Stock Center (New Haven, CT) and ampicillin-resistant *E. coli* OP50-GFP strain DB15, kindly supplied by J. Ewbank (Centre d'Immunologie de Marseille-Luminy, Marseille, France).

PATHOGENICITY ASSAY

Bacteria were grown overnight in LB broth and 50 µl culture was spread on 60 mm diameter NGM plates. Plates were incubated for two days at 25°C to establish bacterial lawns. *C. elegans* were reared on NGM with lawns of *E. coli* OP50 as the food source. One-day old adult worms were placed on NGM plates containing lawns of bacteria. Worm survival was monitored daily for the next nine days. Worms were considered dead when they did not respond to gentle prodding with a platinum wire. Surviving adult worms were transferred daily to fresh bacterial lawn plates to separate them from newly hatched juvenile worms. Each trial measured the survival of 30 worms per treatment.

BIOCONTROL ASSAY

Bacteria were grown overnight in LB broth and 50 µl culture was spread on NGM plates. Plates were incubated for two days at 25°C to establish bacterial lawns. *C. elegans* were reared on NGM with lawns of *E. coli* OP50 as the food source. One day old adult worms were placed on NGM plates containing lawns of a pathogen or nonpathogenic *E. coli* HB101. After exposing the worms to the pathogen or HB101 for 48 hours (32 hours for *E. coli* K-12), worms were washed three times in Ca/HEPES buffer (21) to remove external bacteria. *E. coli* K-12 treated worms were exposed to *E. coli* for 32 hours instead of 48 hours because a 48 hour exposure to *E. coli* K-12 was too toxic and killed the majority of the worms. Washed worms were suspended in 1 ml of an active *B. bacteriovorus* prey lysate or 1ml of Ca/HEPES buffer for 15 minutes. A 15 minute exposure to *B. bacteriovorus* was chosen because this is the time required for *B. bacteriovorus* to attach to prey cells (2). Then the worms were pelleted and placed on NGM plates containing lawns of the nonpathogenic *E. coli* HB101. Worms were transferred to new *E. coli* HB101 plates daily and worm survival was monitored daily for the next seven days. Each trial measured the survival of 40-50 worms per treatment.

E. COLI PERSISTENCE IN *C. ELEGANS*

Nematodes were exposed to an antibiotic-resistant strain of *E. coli* (32 hour exposure for kanamycin-resistant *E. coli* K-12 derivative JW1863-1 or 48 hour exposure for ampicillin-resistant *E. coli* OP50-GFP strain DB15) followed by three washes in Ca/HEPES buffer. The washed worms were suspended for 15 minutes in either 1 ml of an active *B. bacteriovorus* prey lysate or 1 ml of Ca/HEPES buffer, then pelleted and placed on NGM plates with *E. coli* HB101 lawns. Worms were transferred daily on to fresh *E. coli* HB101 plates as described above for the biocontrol assays. Numbers of internal bacteria persisting in the nematodes after *B. bacteriovorus* or buffer exposure were determined daily using the protocol of Garsin *et al.* (23) with the following modifications. Briefly, 5 worms were placed on a LB agar plate containing the appropriate antibiotic (50 µg/ml) and washed twice with 4 µl

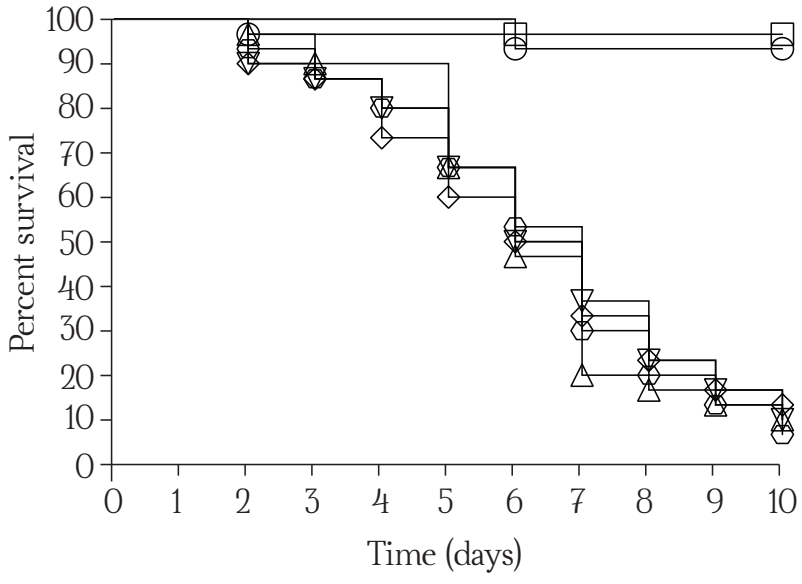

Figure 1. Survival curves for *C. elegans* exposed to *E. coli* OP50 (□), *E. coli* HB101 (○), *E. coli* K-12 (▽), *E. aerogenes* (△), *P. agglomerans* (◇), and S. *enterica* (⬡). Data are from one trial representative of two independent trials.

M9 medium to remove surface bacteria. Washed worms were suspended in 20 µl M9 medium and ground with a pestle. 30 µl of M9 medium was added to the worm solution to bring the total volume up to 50 µl; the solution was diluted in Ca/HEPES buffer and plated on LB agar containing the appropriate antibiotic (50 µg/ml) for bacterial enumeration.

STATISTICS

Kaplan-Meir survival analysis followed by pairwise logrank tests (24, 25, 26) was used to analyze *C. elegans* survival over time. The Mann Whitney test was used to analyze *E. coli* persistence data. Data analyses were performed using GraphPad Prism® 4 (27). The significance level for all statistical analyses was set at $a = 0.05$.

RESULTS

PATHOGENICITY ASSAY

We tested the pathogenicity of four species of bacteria, comparing them to the standard, nonpathogenic *E. coli* OP50 routinely used to maintain *C. elegans*. All four species tested were pathogenic when compared to *E. coli* OP50, greatly reducing worm survival (Fig. 1). The pairwise comparisons examining worm survival between the four pathogens indicated that all four pathogens were similar in pathogenicity (p=0.9926). We also tested *E. coli* HB101 and found it to be nonpathogenic. Worm survival on *E. coli* HB101 was not significantly different from worm survival on *E. coli* OP50 (p=0.5482). Worms grown on all four pathogens survived significantly less than worms grown on *E. coli* OP50 (p<0.001) and worms grown on all four pathogens survived significantly less than worms grown on *E. coli* HB101 (p<0.001). We proceeded to use *E. coli* HB101 as the *C. elegans* food source when monitoring worm survival in our biocontrol assays rather than *E. coli* OP50 since our early work in this system used *B. bacteriovorus* strain

Table 1 P values for pairwise comparisons in the biocontrol assay survival curves.

Pathogen	Comparison					
	HB101 vs. HB101 +Bd[a]	HB101 vs. Pathogen	HB101 vs. Pathogen +Bd	HB101 +Bd vs. Pathogen	HB101 +Bd vs. Pathogen +Bd	Pathogen vs. Pathogen +Bd
E. coli K-12	0.4958	<0.0001	0.0047	<0.0001	0.0412	<0.0001
E. aerogenes	0.4402	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P. agglomerans	0.7376	<0.0001	0.0207	<0.0001	0.0098	<0.0001
S. enterica	0.7318	<0.0001	0.1901	<0.0001	0.3292	<0.0001

[a]Bd indicates *Bdellovibrio*

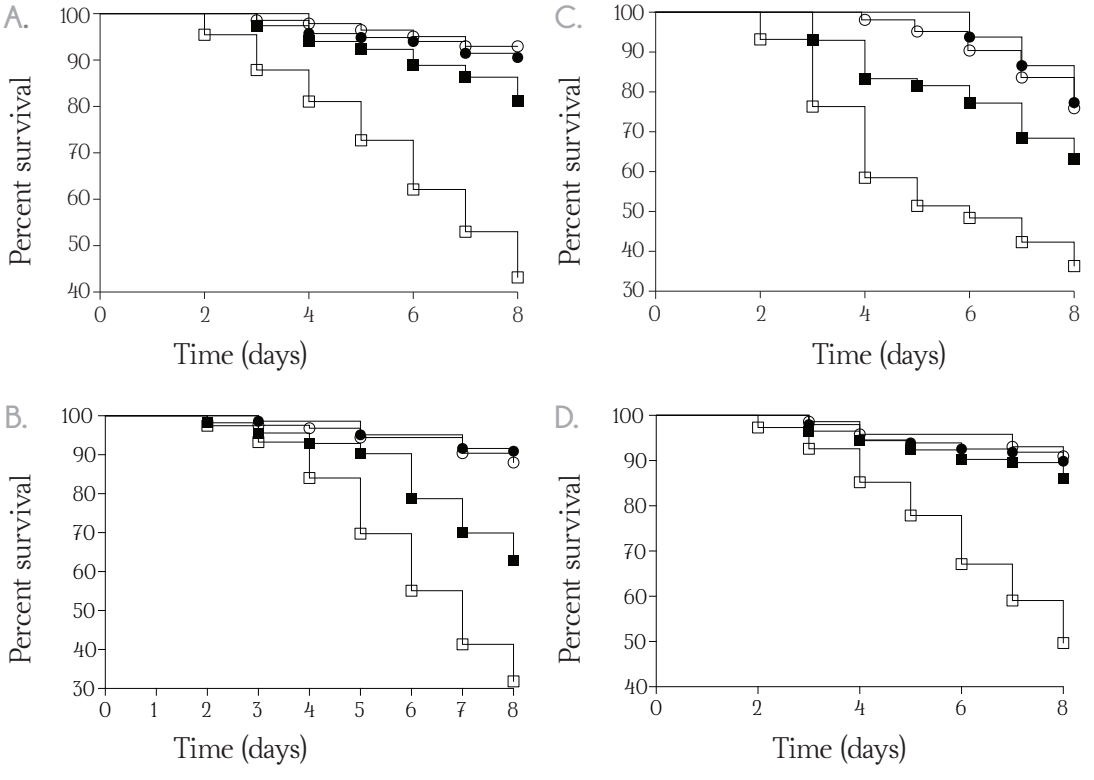

Fig. 2. Survival curves for *C. elegans* exposed to (a) *E. coli* K-12 (b) *E. aerogenes* (c) *P. agglomerans* and (d) *S. enterica*. Worms were treated with nonpathogenic *E. coli* HB101 (○), HB101 and *Bdellovibrio* (●), pathogen (□), or pathogen and *Bdellovibrio* (■). Worms were exposed to *Bdellovibrio* or control buffer on day one. Data are from three independent trials for each pathogen.

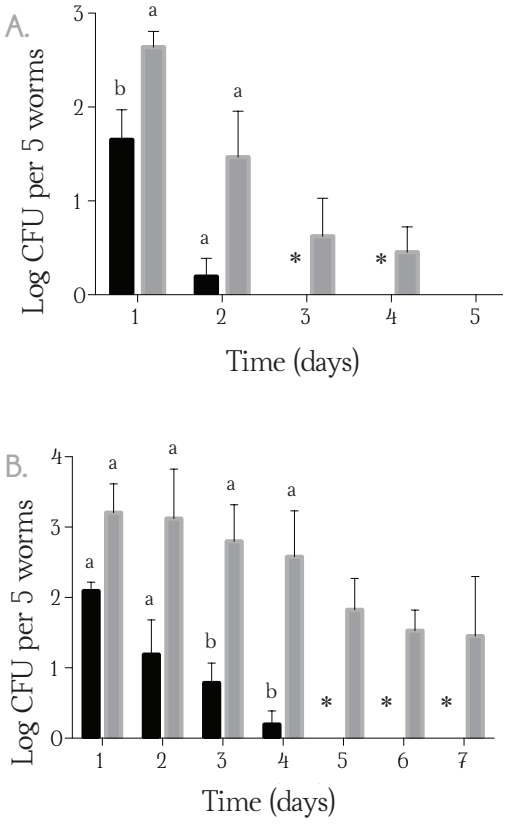

Fig. 3. Persistence of antibiotic-resistant derivatives of (a) *E. coli* K-12 and (b) *E. coli* OP50 within *C. elegans* treated with *Bdellovibrio* (black bars) or control buffer (grey bars). Worms were treated with *Bdellovibrio* or control buffer on day zero. Values with the same letter for a single time are not significantly different ($p \leq 0.05$). Asterisks indicate values with zero variance and thus these days were excluded from analysis. Log transformed data are from four independent trials and error bars indicate standard error.

109J, which did not prey on *E. coli* OP50.

BIOCONTROL ASSAY

To determine whether *B. bacteriovorus* could protect nematodes from bacterial pathogens, we established infections in the nematodes, briefly treated infected worms with *B. bacteriovorus*, placed worms on non-pathogenic *E. coli* HB101, and monitored worm survival for seven days. For all four pathogens tested, worm survival was significantly improved when worms were treated with *B. bacteriovorus* as compared to the pathogen alone (Fig. 2). For each pathogen, the pairwise comparison between worms treated with the pathogen alone and worms treated with both the pathogen and *Bdellovibrio* was highly significant (Table 1). Worm survival was unaffected by *B. bacteriovorus* treatment when worms were grown on nonpathogenic *E. coli* HB101 (Table 1), demonstrating that *B. bacteriovorus* is nontoxic to worms. *Bdellovibrio* and pathogen treated worms had significantly longer survival than worms treated with the pathogen alone. However, for three of the four pathogens, *Bdellovibrio* treatment was unable to restore the same level of worm survival as with the nonpathogenic *E. coli* HB101 control, and there were still significant survival differences between control worms and pathogen plus *Bdellovibrio* treated worms. *S. enterica* infection was the only one completely rescued by *Bdellovibrio* with no significant difference in survival curves between control worms and *S. enterica* plus *Bdellovibrio* treated worms (Table 1).

E. COLI PERSISTENCE IN *C. ELEGANS*

We also monitored the persistence of one of the four pathogens (a kanamycin-resistant derivative of *E. coli* K-12) as well as ampicillin-resistant *E. coli* OP50 in both

Bdellovibrio treated and control worms. One day after exposure to *Bdellovibrio* or a control buffer, *E. coli* K-12 levels were significantly lower in worms treated with *Bdellovibrio* compared to control worms (Fig. 3A). Levels of pathogenic *E. coli* K-12 decreased to undetectable levels in worms three days after *Bdellovibrio* treatment, while it took five days for pathogenic *E. coli* to drop below detectable levels in control worms. *E. coli* OP50 showed a similar trend in that bacterial levels were lower in *Bdellovibrio* treated worms, although a significant difference between *Bdellovibrio* treated and control worms was not detected until three days after *Bdellovibrio* treatment (Fig. 3B). *E. coli* OP50 was also cleared to undetectable levels faster in *Bdellovibrio* treated worms and *E. coli* OP50, unlike *E. coli* K-12, persisted in the control worms for the entire seven day experiment. The limit of pathogen detection was five CFU per five worms.

DISCUSSION

While many have used *C. elegans* as a model for bacterial pathogenesis, we have extended that model to investigate control of four bacterial pathogens by *Bdellovibrio*. The non-vertebrate *C. elegans* has many advantages as an animal model for *Bdellovibrio* infection control studies including short life span, ease of manipulation, low cost, consumption of bacteria as food, and absence of ethical concerns. Our work in *C. elegans* supports and extends earlier work using *Bdellovibrio* as a therapeutic agent to control bacterial infections in chickens (12). Interestingly, the one log reduction in S. enterica by *Bdellovibrio* in chickens is similar to the reduction in *E. coli* K-12 levels we demonstrated in *C. elegans* (Fig. 3A). In agreement with the chicken study, our work demonstrated improved animal health with a single, discrete dose of *Bdellovibrio*. Using *Bdellovibrio* to control infection is often compared to bacteriophage therapy with *Bdellovibrio* having the advantage of a wider prey range than phage (2). Indeed, similar to our results, one group has demonstrated the ability of phage to protect *C. elegans* from *Salmonella* infection (28) confirming the robustness of the *C. elegans* model.

Our pathogenicity assay results demonstrate a clear difference in nematode survival between the four pathogens tested and the two non-pathogenic *E. coli* strains (Fig. 1). This highly significant survival difference is also reflected in the biocontrol assay comparing the HB101 treated worms with the pathogen treated worms (Fig. 2). Although *E. coli* K-12 is typically considered to be nonpathogenic in animal models and our referring to *E. coli* K-12 as a pathogen may seem inaccurate, others have also demonstrated that *E. coli* K-12 is pathogenic in *C. elegans* (29). *E. coli* OP50 is the strain typically used as a nonpathogenic food source for *C. elegans*; however we have demonstrated that *E. coli* strain HB101 is also nonpathogenic. Similar nematode survival curves between OP50 and HB101 have also been demonstrated by researchers examining the effect of bacterial nutrition on *C. elegans* lifespan (30). Interestingly, when survival is examined beyond ten days, worms live longer on HB101 compared to survival on OP50 (30).

Although *Bdellovibrio* provided intermediate protection from most pathogens, the significant improvement in survival along with the complete protection of *Salmonella*

treated worms clearly demonstrates the protective ability of *Bdellovibrio* in this system (Fig. 2 and Table 1). The variation in *Bdellovibrio* protection of *C. elegans* from pathogens may be due to the difference in bacterial colonization of the worms. *S. enterica* serovar Typhimurium kills worms through a persistent intestinal colonization while *E. coli* kills through a non-persistent intestinal colonization (16). The ability of *S. enterica* to multiply within and distend the worm intestinal lumen, establishing a persistent infection after the worms are no longer being fed *S. enterica* cells (31), may provide a more concentrated source of pathogen cells to support increased *Bdellovibrio* growth and predation, leading to complete recovery from infection. Interestingly, these data suggest that the more numerous the pathogen cells are in the host, the more effective *Bdellovibrio* treatment may be for resolving the infection.

We followed the persistence of two *E. coli* strains in this system using antibiotic-resistant derivatives of *E. coli* K-12 and *E. coli* OP50 to examine the effect of *Bdellovibrio* on *E. coli* clearance from the worm. Pathogenic *E. coli* K-12 levels were significantly lower in *Bdellovibrio* treated worms one day after treatment and *E. coli* K-12 was cleared from the worms two days quicker in *Bdellovibrio* treated worms (Fig. 3A). This marked reduction in pathogenic *E. coli* levels by *Bdellovibrio* was enough to significantly improve worm survival, but not enough to restore worm survival back to the level seen in non-pathogen treated control worms (Table 1). Our results are based on a single, 15 minute exposure of the worms to *Bdellovibrio* and increased survival may occur with longer or repeated exposures of the worms to *Bdellovibrio.* We chose a 15 minute exposure to allow time for *Bdellovibrio* to attach to prey cells and begin invasion of the prey cell (2). Even without *Bdellovibrio* treatment, *E. coli* K-12 was cleared from the worms, in agreement with earlier research demonstrating that pathogenic *E. coli* does not establish a persistent infection in worms (16). Levels of nonpathogenic *E. coli* OP50 were also significantly lower and cleared faster in *Bdellovibrio* treated worms (Fig. 3B). However, unlike *E. coli* K-12, nonpathogenic *E. coli* OP50 was able to persist in the control worms for seven days. The levels of *E. coli* OP50 we detected in control worms on day one agree closely with those found by others investigating viable *E. coli* OP50 counts in *C. elegans* lysates (30), validating our work in this system.

C. elegans appears to be an ideal model system for refining and exploring the use of *Bdellovibrio* as a therapeutic agent. Since *C. elegans* is a bacteriovore, exposure of the worms to pathogenic bacteria is simple and easy. The lower growth temperatures favored by *C. elegans* (20–25°C) compared to birds and mammals coupled with *Bdellovibrio's* optimal growth temperature of 28°C makes *C. elegans* an attractive animal system to investigate the use of *Bdellovibrio* as a biocontrol agent. We administered *Bdellovibrio* as a liquid treatment for precise, controlled dosing, but worms could also be treated with *Bdellovibrio* through placement on plaque plates (17) containing both the pathogen and *Bdellovibrio.* Our work prepares the way for future experiments with *C. elegans* and *Bdellovibrio* to examine additional pathogens, dosage and frequency of *Bdellovibrio* treatment, persistence of *Bdellovibrio* in worms, effect (if any) of *Bdellovibrio* on worm morphology, as well as other variables.

While an intriguing hypothesis, the use of *Bdellovibrio* as a feasible therapeutic agent has only been demonstrated *in vivo* in chickens against *Salmonella* (12). Here we extend

that work by demonstrating significantly increased nematode protection from four different pathogens through *Bdellovibrio* treatment. In addition to being a well-studied pathogenesis model, *C. elegans* are much more tractable than chickens and our results lay the groundwork for future *Bdellovibrio* biocontrol studies in *C. elegans*. The presence of *Bdellovibrio* as a member of a healthy gut community in children (32), along with its lack of toxicity in birds and nematodes, suggests that it holds potential for therapeutic use. Our demonstration of protection by *Bdellovibrio* against multiple bacterial pathogens in the well-studied *C. elegans* pathogenesis model strengthens the validity of *Bdellovibrio* as a promising, future therapeutic agent.

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REFERENCES

1. Sockett, R. E. 2009. Predatory lifestyle of *Bdellovibrio bacteriovorus*. *Annu. Rev. Microbiol.* 63:523–539.

2. Sockett, R. E. & Lambert, C. 2004. *Bdellovibrio* as therapeutic agents: a predatory renaissance? *Nat. Rev. Microbiol.* 2:669–675.

3. Fratamico, P. M. & Cooke, P. H. 1996. Isolation of *Bdellovibrios* that prey on *Escherichia coli* O157:H7 and *Salmonella* species and application for removal of prey from stainless steel surfaces. *J. Food Safety* 16:161–173.

4. Lu, F. & Cai, J. 2010. The protective effect of *Bdellovibrio*-and-like organisms (BALO) on tilapia fish fillets against *Salmonella enterica ssp. enterica* serovar Typhimurium. *Lett. Appl. Microbiol.* 51:625–631.

5. Dashiff, A. & Kadouri, D. E. 2011. Predation of oral pathogens by *Bdellovibrio bacteriovorus* 109J. *Mol. Oral Microbiol.* 26:19–34.

6. Van Essche, M., Quirynen, M., Sliepen, I., Loozen, G., Boon, N., Van Eldere, J., & Teughels, W. 2011. Killing of anaerobic pathogens by predatory bacteria. *Mol. Oral Microbiol.* 26:52–61.

7. Kadouri, D. & O'Toole, G. A. 2005. Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. *Appl. Environ. Microbiol.* 71:4044–4051.

8. Van Essche, M., Quirynen, M., Sliepen, I., Van Eldere, J., & Teugels, W. 2009. *Bdellovibrio bacteriovorus* attacks *Aggregatibacter actinomycetemcomitans*. *J. Dent. Res.* 88:182–186.

9. Dashiff, A., Junka, R. A., Libera, M., & Kadouri, D. E. 2010. Predation of human pathogens by the predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*. *J. Appl. Microbiol.* 110:431–444.

10. Chu, W. H. & Zhu, W. 2009. Isolation of *Bdellovibrio* as biological therapeutic agents used for the treatment of *Aeromonas hydrophila* infection in fish. *Zoonoses Public Health* 57:258–264.

11. Cao, H., He, S., Lu, L., Yang, X., & Chen, B. 2014. Identification of *Proteus penneri* isolate as the causal agent of red body disease of the cultured white shrimp *Penaeus vannamei* and its control with *Bdellovibrio bacteriovorus*. *Antonie van Leeuwenhoek* 105:423–430.

12. Atterbury, R. J., Hobley, L., Till, R., Lambert, C., Capeness, M. J., Lerner, T. R., Fenton, A. K., Barrow, P., & Sockett, R. E. 2011. Effects of orally administered *Bdellovibrio bacteriovorus* on the well-being and *Salmonella* colonization of young chicks. *Appl. Environ. Microbiol.* 77:5794–5803.

13. Tan, M. W., Mahajan-Miklos, S., & Ausubel, F. M. 1999. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian

bacterial pathogenesis. *Proc. Natl. Acad. Sci.* USA 96:715–720.

14. Glavis-Bloom, J., Muhammed, M. and Mylonakis, E. (2012). Of model hosts and man: using *Caenorhabditis elegans, Drosophila melanogaster* and *Galleria mellonella* as model hosts for infectious disease research. *Adv Exp Med Biol*, 710, 11–17.

15. Marsh, E. K. and May, R. C. (2012). *Caenorhabditis elegans*, a model organism for investigating immunity. *Appl Environ Microbiol*, 78(7), 2075–2081.

16. Sifri, C. D., Begun, J. & Ausubel, F. M. 2005. The worm has turned – microbial virulence modeled in *Caenorhabditis elegans*. *Trends Microbiol.* 13:119–127.

17. Kurz, C. L. & Ewbank, J. J. 2000. *Caenorhabditis elegans* for the study of host–pathogen interactions. *Trends Microbiol.* 8:142–144.

18. Stiernagle, T. 2006. Maintenance of *C. elegans*. In WormBook, ed. The *C. elegans* research community. doi.10.1895.wormbook.1.101.1.

19. Rogosky, A. M., Moak, P. L. & Emmert, E. A. B. 2006. Differential predation by *Bdellovibrio bacteriovorus* 109J. *Curr. Microbiol.* 52:81–85.

20. Rendulic, S., Jagtap, P., Rosinus, A., Eppinger, M., Baar, C., Lanz, C., Keller, H., Lambert, C., Evans K. J., Goesmann, A., Meyer, F., Sockett, R. E. & Schuster, S. C. 2004. A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. *Science* 303:689–692.

21. Lambert, C. & Sockett, R. E. 2008. Laboratory maintenance of *Bdellovibrio*. *Curr. Protocol. Microbiol.* 7:Unit 7B.2.

22. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., & Mori, H. 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* 2:2006.0008.

23. Garsin, D. A., Sifri, C. D., Mylonakis, E., Qin, X., Singh, K. V., Murray, B. E., Calderwood, S. B., & Ausubel, F. M. 2001. A simple model host for identifying Gram-positive virulence factors. *Proc. Natl. Acad. Sci.* USA 98:10892–10897.

24. Altman, D. G. 1991. Practical statistics for medical research. London: Chapman and Hall.

25. Bland, J. M. & Altman, D. G. 1998. Survival probabilities (the Kaplan–Meier method). *BMJ* 317:1572.

26. Bland, J. M. & Altman, D. G. 2004. The logrank test. *BMJ* 328:1073.

27. Motulsky, H. J. 2003. Prism 4 statistics guide – statistical analyses for laboratory and clinical researchers. SanDiego CA: GraphPad Software Inc.

28. Santander, J. & Robeson, J. 2004. Bacteriophage prophylaxis against *Salmonella enteritidis* and *Salmonella pullorum* using *Caenorhabditis elegans* as an assay system. *Electron. J. Biotechnol.* 7:206–209.

29. Browning, D. F., Wells, T. J., Franca, F. L. S., Morris, F. C., Sevastsyanovich, Y. R., Bryant, J. A., Johnson, M. D., Lund, P. A., Cunningham, A. F., Hobman, J. L., May, R. C., Webber, M. A., & Henderson, I. R. 2013. Laboratory adapted *Escherichia coli* K-12 becomes a pathogen of *Caenorhabditis elegans* upon restoration of O antigen biosynthesis. *Mol. Microbiol.* 87:939–950.

30. So, S., Tokumaru, T., Miyahara, K., & Ohshima, Y. 2011. Control of lifespan by food bacteria, nutrient limitation and pathogenicity of food in *C. elegans*. *Mech. Ageing Dev.* 132:210–212.

31. Aballay, A., Yorgey, P., & Ausubel, F. M. 2000. *Salmonella typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans*. *Curr Biol.* 10:1539–1542.

32. Iebba, V., Santangelo, F., Totino, V., Nicoletti, M., Gagliardi, A., DeBaise, R. V., Cucchiara, S., Nencioni, L., Conte, M. P., & Schippa, S. 2013. Higher prevalence and abundance of *Bdellovibrio bacteriovorus* in the human gut of healthy subjects. *PLoS One* 8:e61608.