

Caenorhabditis elegans as an Alternative Model Host for *Legionella pneumophila*, and Protective Effects of *Bifidobacterium infantis*[∇]

Tomomi Komura,¹ Chikako Yasui,¹ Hiroshi Miyamoto,² and Yoshikazu Nishikawa^{1*}

Department of Interdisciplinary Studies for Advanced Aged Society, Osaka City University Graduate School of Human Life Science, Osaka 558-8585, Japan,¹ and Department of Pathology and Microbiology, Faculty of Medicine, Saga University, Saga 849-8501, Japan²

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The survival times of *Caenorhabditis elegans* worms infected with *Legionella pneumophila* from day 7.5 or later after hatching were shorter than those of uninfected worms. However, nematodes fed bifidobacteria prior to *Legionella* infection were resistant to *Legionella*. These nematodes may act as a unique alternative host for *Legionella* research.

Legionella pneumophila, an environmental bacterium naturally found in fresh water, is the major causative agent of Legionnaires' disease (7). Fresh water amoebas, a natural host of *Legionella*, have been used as an infection model to study invasion of *Legionella* into human macrophages and subsequent intracellular growth (15). However, analyses using these protozoa have inevitably concentrated on the intracellular lifestyle of *L. pneumophila*. The fate of *Legionella* organisms in nonmammalian metazoans had not been described (10) until a very recent report by Brassinga et al. (6).

Numerous authors have reported *Caenorhabditis elegans* to be a suitable model to investigate virulence-associated factors of human pathogens (2, 8, 11, 14, 16, 20, 23, 24, 30, 31, 33). In the present study, we examined whether *C. elegans* can serve as an alternative host for *L. pneumophila*. Although the nematocidal activity of *Legionella* has been described recently, the nematodes in the previous study were infected with the pathogen on buffered charcoal yeast extract (BCYE) agar plates, which can support *Legionella* growth (6). In contrast, our experiments were independently performed on simple agar plates to exclude the possibility that the inoculated pathogen would have proliferated regardless of whether it had successfully infected the nematodes and derived nutrition from the hosts. Garsin et al. showed that nutrition available in agar plates does influence the virulence of pathogens on the medium (9). Furthermore, some pathogens produce toxic metabolites on nutrient medium *in situ* (3), and thus, we also avoided this possibility. Moreover, we focused on the effects of worm age, since *Legionella* is prone to infect elderly people.

Age at infection is likely one of the most important determinants of disease morbidity and mortality (18). Since *Legionella* organisms are prone to infect elderly people opportunistically, infections in young and older nematodes were compared. Furthermore, survival curves were compared be-

tween worms fed *Escherichia coli* OP50 (OP), an international standard food for these organisms, and those fed bifidobacteria prior to infection with *Legionella* organisms, since lactic acid bacteria exert beneficial effects on human and animal health (21).

Nematocidal assays. As a standard strain of *C. elegans*, Bristol N2 was maintained and propagated on nematode growth medium (NGM) according to standard techniques using OP (25). Tryptone soya agar (Oxoid, Basingstoke, United Kingdom) was used to culture OP at 37°C. The virulent and attenuated *L. pneumophila* strains used in this study are listed in Table 1. All attenuated strains were produced by transposon insertion into the Icm/Dot (intracellular multiplication/defect in organelle trafficking) type IV secretion system genes, which have been shown previously to be essential for virulence (5, 22). *Legionella* strains were cultured using BCYE agar plates (Oxoid) at 37°C for 2 days. *Bifidobacterium infantis* ATCC 15697 was also used to feed the worms, being one of five lactic acid bacteria that we previously found to have a longevity effect on nematodes (13). Transoligosaccharide (TOS) propionate agar (Eiken Chemical Co., Tochigi, Japan) was used to grow *B. infantis* anaerobically at 37°C (32). Bacteria were recovered from the agar plates, and each 10 mg (wet weight) of the bacteria suspended in 50 µl of M9 buffer (25) was spread onto peptone-free modified NGM (mNGM) in 5.0-cm-diameter petri dishes for feeding or infecting of *C. elegans*.

Worms were generated from eggs released after exposure of adult hermaphrodites to a sodium hypochlorite-sodium hydroxide solution as described previously (28). The fertilized egg suspension was incubated overnight at 25°C to allow hatching, and the suspension of larval stage 1 (L1 stage) worms was centrifuged at 156 × *g* for 1 min. The supernatant was removed, and the remaining larvae were transferred onto fresh mNGM plates covered with OP and incubated at 25°C. Because the reproductive system regulates aging in *C. elegans* (12), in order not to disturb natural pubescence, worms were fed on OP until the start of *Legionella* infection.

Nematocidal assays were begun with adult worms, which were allocated at 30 each onto mNGM plates covered with suspensions of each *Legionella* strain. Once the feeding bacte-

* Corresponding author. Mailing address: Graduate School of Human Life Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan. Phone and fax: 81-6-6605-2910. E-mail: nishikawa@life.osaka-cu.ac.jp.

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TABLE 1. *L. pneumophila* strains used in this study

Strain	Description	Reference or source
Philadelphia-1 (ATCC 33152)	Virulent, serogroup 1	ATCC
AM511	Philadelphia-1 Sm ^r r ⁻ m ⁺	17
JR32	Salt-sensitive derivative of AM511	22
LELA2883	JR32 <i>dotB2883::Tn903dIIlacZ</i> (insertion in <i>icm/dot</i> region I)	22
LELA3118	JR32 <i>dotA3118::Tn903dIIlacZ</i> (insertion in <i>icm/dot</i> region I)	22
LELA3473	JR32 <i>icmR3473::Tn903dIIlacZ</i> (insertion in <i>icm/dot</i> region II)	22
LELA4432	JR32 <i>icmE4432::Tn903dIIlacZ</i> (insertion in <i>icm/dot</i> region II)	22
LELA3244	JR32 <i>icmD3244::Tn903dIIlacZ</i> (insertion in <i>icm/dot</i> region II)	22
LELA3393	JR32 <i>icmB3393::Tn903dIIlacZ</i> (insertion in <i>icm/dot</i> region II)	22
LELA1718	JR32 <i>icmF1718::Tn903dIIlacZ</i> (insertion in <i>icm/dot</i> region II)	22

ria were switched from OP to *Legionella*, worms were given the pathogen during the entire assay time until death. The plates were incubated at 25°C, and live and dead worms were counted at least every 24 h. Worms produce progeny that develop into adults in 3 days, rendering it difficult to identify the original worms. Therefore, the original worms were transferred onto

fresh plates at approximately 2-day intervals. A worm was considered dead when it failed to respond to a gentle touch with a worm picker. Worms that died as a result of getting stuck to the wall of the plate were excluded from the analysis. All nematode survival studies were conducted by a researcher with no prior knowledge of the bacterial strains fed to the worms. Nematode survival was calculated by the Kaplan-Meier method, and survival differences were tested for significance using the log rank test.

Influence of age of *C. elegans* on *L. pneumophila* infections.

No significant differences in survival between 3-day-old worms fed OP and 3-day-old worms infected with virulent *Legionella* strains were seen (Fig. 1A). However, when the worms were infected from 7.5 days after hatching, the virulent *Legionella* strains were obviously nematocidal (Fig. 1B).

The present study is the first to show that *L. pneumophila* is virulent to elderly worms. Our previous study showed that *Salmonella enterica* is clearly virulent to both older and younger worms, although more so to elderly worms (13). These findings appear to be consistent with the epidemiological characteristics of both pathogens in humans: *Legionella* tends to infect older people in an opportunistic manner, while *Salmonella* can cause enteritis irrespective of host age.

To examine whether the virulence of *Legionella* correlates with its ability to accumulate in the nematodes, the number of organisms in the nematodes was determined according to the

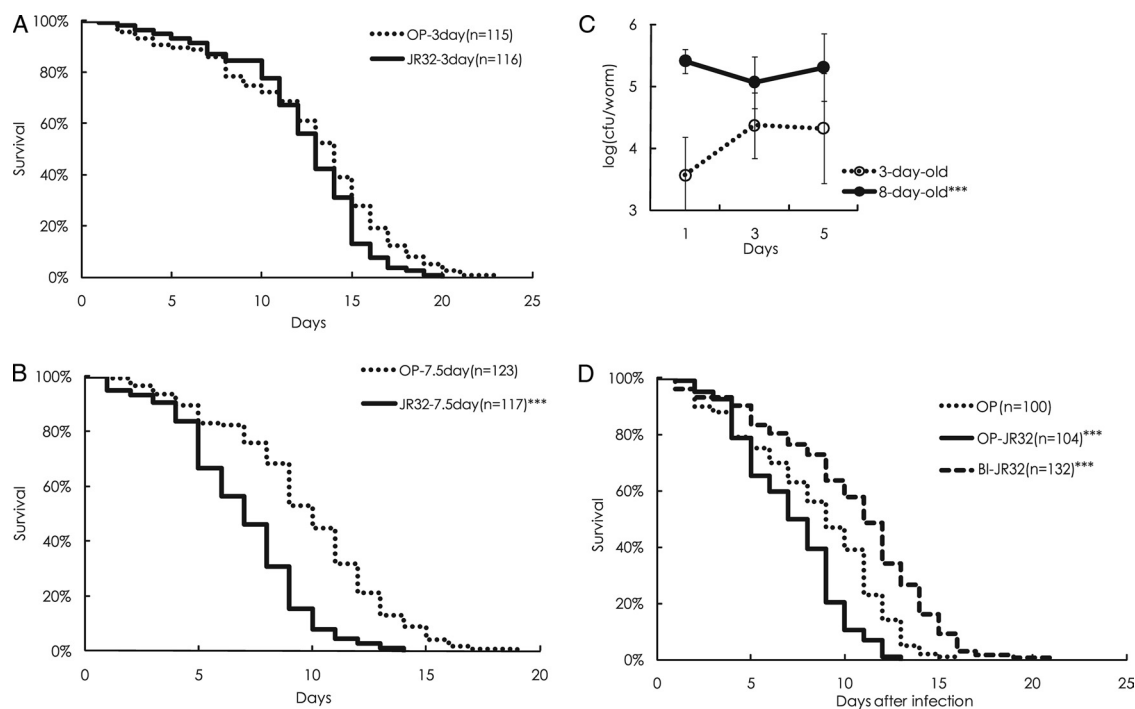


FIG. 1. Survival of nematodes infected with *L. pneumophila*. (A and B) At 3 days (A) or 7.5 days (B) of age, nematodes were transferred onto agar plates covered with the virulent *L. pneumophila* strain JR32. The survival curves were compared with those of worms fed on OP. (C) The number of *Legionella* cells recovered from the nematodes that started ingesting *Legionella* at 3 days of age was significantly lower than the number recovered from worms infected at 8 days of age. The results are presented as the means \pm the standard errors, and the data were analyzed by two-factor factorial analysis of variance (ANOVA). (D) Adult worms fed a diet of bifidobacteria from 3 days of age for 5 days were transferred onto *Legionella* plates for infection, and survival curves were drawn. Nematodes fed bifidobacteria (BI) were significantly more resistant than controls to the pathogen (JR). All experiments were performed twice in duplicate or triplicate. *** indicates statistical significance at a *P* value of <0.001.

TABLE 2. Mean and maximum survival days of worms infected with *L. pneumophila* strains

Bacterial strain	Nematode age (days) at start of infection	Mean survival time ^a (days) ± SE	Maximum survival time (days)	No. of nematodes (no. of experiments) ^b
<i>E. coli</i> OP (control)	3.0	13.20 ± 0.45	23	115 (2)
<i>L. pneumophila</i> JR32	3.0	12.80 ± 0.34	20	116 (2)
<i>E. coli</i> OP (control)	7.5	10.20 ± 0.33	19	123 (2)
<i>L. pneumophila</i> JR32	7.5	7.40 ± 0.26***	14	117 (2)
<i>E. coli</i> OP (control)	7.5	10.17 ± 0.44	17	70 (1)
<i>L. pneumophila</i> LELA2883	7.5	10.50 ± 0.54	20	69 (1)
<i>L. pneumophila</i> LELA3118	7.5	10.00 ± 0.53	21	72 (1)
<i>L. pneumophila</i> LELA3473	7.5	9.76 ± 0.49	19	72 (1)
<i>L. pneumophila</i> LELA4432	7.5	9.62 ± 0.49	18	74 (1)
<i>L. pneumophila</i> LELA3244	7.5	11.12 ± 0.51	18	60 (1)
<i>L. pneumophila</i> LELA3393	7.5	9.64 ± 0.44	16	65 (1)
<i>L. pneumophila</i> LELA1718 ^c	7.5	8.74 ± 0.43*	15	66 (1)
<i>E. coli</i> OP (control)	7.5	9.14 ± 0.38	17	78 (1)
<i>L. pneumophila</i> LELA1718 ^c	7.5	9.49 ± 0.38	15	70 (1)
<i>E. coli</i> OP (control)	8.0	9.13 ± 0.45	14	48 (1)
<i>L. pneumophila</i> LELA1718 ^c	8.0	7.33 ± 0.42**	13	48 (1)
<i>L. pneumophila</i> LELA4432	8.0	10.00 ± 0.52	18	46 (1)

^a *, **, and *** indicate a statistically significant difference from the control at *P* values of <0.05, <0.01, and <0.001, respectively.

^b Each experiment was performed in duplicate or triplicate.

^c When worms were infected with LELA1718 at 7.5 days after hatching, the strain showed weak virulence in one of two experiments. The 8-day-old worms seemed to be more vulnerable to the strain.

method of Garsin et al. (9) with some modifications. Each nematode was mechanically disrupted using a microtube pestle (Scientific Specialties Inc., Lodi, CA). The number of *Legionella* cells was determined using BCYE agar plates supplemented with polymyxin B at 80 IU/ml. The number of *Legionella* cells recovered from worms infected from 3 days after hatching was smaller than the number recovered from worms infected from 8 days after hatching (Fig. 1C); this logarithmic difference cannot be explained by the difference in body size (the area of the worm's projection) between elderly and young worms since the bodies of the 8-day-old worms were 20% larger than those of the 3-day-old worms.

Although there are no data to explain the vulnerability of the older worms, the ability of the older worms to adapt to microbes might have deteriorated, a possibility consistent with the finding that the level of heat shock protein 16 in 16-day-old worms responding to heat stress had declined to about 50% of that in 4-day-old young adults (34). Since nematodes have to switch distinct genes on and off to defend against and digest different species of ingested pathogens (35), it is likely that the worms that first encountered the *Legionella* cells when they were young were able to respond effectively to *Legionella* and that the 8-day-old worms were not.

Virulence of transposon insertion mutants in nematodes.

On the basis of these findings, 7.5- to 8-day-old nematodes were used as the hosts to examine the virulence of the *Legionella* strains. Although the parent strain JR32 showed significant nematocidal activity, all the genetically attenuated mutants, having been demonstrated to be less virulent in the lungs of guinea pigs (19) or in human macrophages (22), were considered nonpathogenic, similar to OP (Table 2). Interestingly, the attenuated mutant LELA1718, which is reportedly cytotoxic in contrast to the other attenuated mutants in a cytotoxicity assay with HL-60-derived human macrophages (22), showed modest virulence in the nematodes compared to other avirulent mutants.

The pathogenicity of *L. pneumophila* in *C. elegans* seems to correlate well with that in macrophages, and the nematode could serve as a unique host of *Legionella* spp. Indeed, several genes of human pathogens have been recently discovered to be involved in virulence in *C. elegans*, and some of these are also required for virulence in mammalian systems (4, 16, 26, 29). However, the pathogenesis of infections with *Legionella* spp. in nematodes remains to be elucidated. The numbers of organisms recovered from the worms were not significantly different among the three *Legionella* strains (JR32, LELA1718, and LELA4432) that showed different levels of pathogenicity. Since the nematodes actively take in bacteria, the differences in virulence would not result in a difference in bacterial number as long as the levels of bacterial resistance to antimicrobial peptides in the worms did not differ among the bacterial strains. Functional defects in the Icm/Dot type IV secretion system could have decreased the secretion of toxic effectors from the attenuated mutants and consequently increased worm survival. Programmed cell death in *C. elegans* appears to be an important defense response against pathogen attack and stress (1). Since Icm/Dot plays a role to inhibit programmed cell death (6), the attenuated mutants appeared not to reduce worm survival.

Influence of bifidobacteria on resistance against *Legionella* infection. Three-day-old worms were assigned to either a control group that continued to be fed OP or to a group that was fed bifidobacteria for 5 days. The 8-day-old worms were then transferred onto mNGM covered with *Legionella*. Nematodes fed bifidobacteria were clearly resistant to subsequent *Legionella* infection, in contrast to nematodes fed OP before the *Legionella* infection, and nematodes fed bifidobacteria survived longer than the control worms fed OP throughout the experiment (Fig. 1D); however, the numbers of *Legionella* organisms recovered from the worms showed no significant difference between groups fed with bifidobacteria or OP. These results are similar to our previous findings that lactic acid bacteria are

effective in increasing both the longevity of *C. elegans* and the host defense against *Salmonella* (13). Our ongoing experiments using mutated nematodes suggest that bifidobacteria enhance the worms' host defense via the PMK-1/p38 mitogen-activated protein kinase signaling pathway (unpublished results), which plays an important role in the innate immunity of *C. elegans* (27).

In conclusion, we found that *L. pneumophila* significantly reduced the life span of *C. elegans* via *icm/dot*-associated pathogenicity when the pathogen infected older worms. Since the worms died over the course of several days, the pathogen is considered to have caused what is known as slow killing (30). *C. elegans* can be an additional unique model to study opportunistic infection with *Legionella* spp.

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REFERENCES

- Aballay, A., and F. M. Ausubel. 2001. Programmed cell death mediated by *ced-3* and *ced-4* protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. *Proc. Natl. Acad. Sci. U. S. A.* **98**:2735–2739.
- Aballay, A., P. Yorgey, and F. M. Ausubel. 2000. *Salmonella typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans*. *Curr. Biol.* **10**:1539–1542.
- Anyanful, A., J. M. Dolan-Livengood, T. Lewis, S. Sheth, M. N. Dezalia, M. A. Sherman, L. V. Kalman, G. M. Benian, and D. Kalman. 2005. Paralysis and killing of *Caenorhabditis elegans* by enteropathogenic *Escherichia coli* requires the bacterial tryptophanase gene. *Mol. Microbiol.* **57**:988–1007.
- Begun, J., C. D. Sifri, S. Goldman, S. B. Calderwood, and F. M. Ausubel. 2005. *Staphylococcus aureus* virulence factors identified by using a high-throughput *Caenorhabditis elegans*-killing model. *Infect. Immun.* **73**:872–877.
- Berger, K. H., and R. R. Isberg. 1993. Two distinct defects in intracellular growth complemented by a single genetic locus in *Legionella pneumophila*. *Mol. Microbiol.* **7**:7–19.
- Brassinga, A. K., J. M. Kinchen, M. E. Cupp, S. R. Day, P. S. Hoffman, and C. D. Sifri. 2010. *Caenorhabditis* is a metazoan host for *Legionella*. *Cell. Microbiol.* **12**:343–361.
- Fields, B. S., R. F. Benson, and R. E. Besser. 2002. *Legionella* and Legionnaires' disease: 25 years of investigation. *Clin. Microbiol. Rev.* **15**:506–526.
- Gan, Y. H., K. L. Chua, H. H. Chua, B. Liu, C. S. Hii, H. L. Chong, and P. Tan. 2002. Characterization of *Burkholderia pseudomallei* infection and identification of novel virulence factors using a *Caenorhabditis elegans* host system. *Mol. Microbiol.* **44**:1185–1197.
- Garsin, D. A., C. D. Sifri, E. Mylonakis, X. Qin, K. V. Singh, B. E. Murray, S. B. Calderwood, and F. M. Ausubel. 2001. A simple model host for identifying Gram-positive virulence factors. *Proc. Natl. Acad. Sci. U. S. A.* **98**:10892–10897.
- Hilbi, H., S. S. Weber, C. Ragaz, Y. Nyfeler, and S. Urwyler. 2007. Environmental predators as models for bacterial pathogenesis. *Environ. Microbiol.* **9**:563–575.
- Hoshino, K., C. Yasui, T. Ikeda, K. Arikawa, H. Toshima, and Y. Nishikawa. 2008. Evaluation of *Caenorhabditis elegans* as the host in an infection model for food-borne pathogens. *Jpn. J. Food Microbiol.* **25**:137–147.
- Hsin, H., and C. Kenyon. 1999. Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* **399**:362–366.
- Ikeda, T., C. Yasui, K. Hoshino, K. Arikawa, and Y. Nishikawa. 2007. Influence of lactic acid bacteria on longevity of *Caenorhabditis elegans* and host defense against *Salmonella enterica* serovar Enteritidis. *Appl. Environ. Microbiol.* **73**:6404–6409.
- Joshua, G. W., A. V. Karlyshev, M. P. Smith, K. E. Isherwood, R. W. Titball, and B. W. Wren. 2003. A *Caenorhabditis elegans* model of *Yersinia* infection: biofilm formation on a biotic surface. *Microbiology* **149**:3221–3229.
- Jules, M., and C. Buchrieser. 2007. *Legionella pneumophila* adaptation to intracellular life and the host response: clues from genomics and transcriptomics. *FEBS Lett.* **581**:2829–2838.
- Kurz, C. L., S. Chauvet, E. Andres, M. Aurouze, I. Vallet, G. P. Michel, M. Uh, J. Celli, A. Filloux, S. De Bentzmann, I. Steinmetz, J. A. Hoffmann, B. B. Finlay, J. P. Gorvel, D. Ferrandon, and J. J. Ewbank. 2003. Virulence factors of the human opportunistic pathogen *Serratia marcescens* identified by in vivo screening. *EMBO J.* **22**:1451–1460.
- McKinney, R. M., H. W. Wilkinson, H. M. Sommers, B. J. Fikes, K. R. Sasseville, M. M. Yungbluth, and J. S. Wolf. 1980. *Legionella pneumophila* serogroup six: isolation from cases of legionellosis, identification by immunofluorescence staining, and immunological response to infection. *J. Clin. Microbiol.* **12**:395–401.
- Miller, E., and N. Gay. 1997. Effect of age on outcome and epidemiology of infectious diseases. *Biologicals* **25**:137–142.
- Miyamoto, H., S. Yoshida, H. Taniguchi, and H. A. Shuman. 2003. Virulence conversion of *Legionella pneumophila* by conjugal transfer of chromosomal DNA. *J. Bacteriol.* **185**:6712–6718.
- Mylonakis, E., A. Idnurm, R. Moreno, J. El Khoury, J. B. Rottman, F. M. Ausubel, J. Heitman, and S. B. Calderwood. 2004. *Cryptococcus neoformans* Kin1 protein kinase homologue, identified through a *Caenorhabditis elegans* screen, promotes virulence in mammals. *Mol. Microbiol.* **54**:407–419.
- Naidu, A. S., W. R. Bidlack, and R. A. Clemens. 1999. Probiotic spectra of lactic acid bacteria (LAB). *Crit. Rev. Food Sci. Nutr.* **39**:13–126.
- Sadosky, A. B., L. A. Wiater, and H. A. Shuman. 1993. Identification of *Legionella pneumophila* genes required for growth within and killing of human macrophages. *Infect. Immun.* **61**:5361–5373.
- Sifri, C. D., J. Begun, F. M. Ausubel, and S. B. Calderwood. 2003. *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. *Infect. Immun.* **71**:2208–2217.
- Sifri, C. D., E. Mylonakis, K. V. Singh, X. Qin, D. A. Garsin, B. E. Murray, F. M. Ausubel, and S. B. Calderwood. 2002. Virulence effect of *Enterococcus faecalis* protease genes and the quorum-sensing locus *fsr* in *Caenorhabditis elegans* and mice. *Infect. Immun.* **70**:5647–5650.
- Stiernagle, T. 1999. Maintenance of *C. elegans*, p. 51–67. In I. A. Hope (ed.), *C. elegans*: a practical approach. Oxford University Press, New York, NY.
- Styer, K. L., G. W. Hopkins, S. S. Bartra, G. V. Plano, R. Frothingham, and A. Aballay. 2005. *Yersinia pestis* kills *Caenorhabditis elegans* by a biofilm-independent process that involves novel virulence factors. *EMBO Rep.* **6**:992–997.
- Styer, K. L., V. Singh, E. Macosko, S. E. Steele, C. I. Bargmann, and A. Aballay. 2008. Innate immunity in *Caenorhabditis elegans* is regulated by neurons expressing NPR-1/GPCR. *Science* **322**:460–464.
- Stulston, J., and J. Hodgkin. 1988. Methods, p. 587–606. In W. B. Wood (ed.), *The nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Tan, M. W., and F. M. Ausubel. 2000. *Caenorhabditis elegans*: a model genetic host to study *Pseudomonas aeruginosa* pathogenesis. *Curr. Opin. Microbiol.* **3**:29–34.
- Tan, M. W., S. Mahajan-Miklos, and F. M. Ausubel. 1999. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **96**:715–720.
- Tan, M. W., L. G. Rahme, J. A. Sternberg, R. G. Tompkins, and F. M. Ausubel. 1999. *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. *Proc. Natl. Acad. Sci. U. S. A.* **96**:2408–2413.
- Thitaram, S. N., G. R. Siragusa, and A. Hinton, Jr. 2005. *Bifidobacterium*-selective isolation and enumeration from chicken caeca by a modified oligosaccharide antibiotic-selective agar medium. *Lett. Appl. Microbiol.* **41**:355–360.
- Thomsen, L. E., S. S. Slutz, M. W. Tan, and H. Ingmer. 2006. *Caenorhabditis elegans* is a model host for *Listeria monocytogenes*. *Appl. Environ. Microbiol.* **72**:1700–1701.
- Walker, G. A., T. M. White, G. McColl, N. L. Jenkins, S. Babich, E. P. Candido, T. E. Johnson, and G. J. Lithgow. 2001. Heat shock protein accumulation is upregulated in a long-lived mutant of *Caenorhabditis elegans*. *J. Gerontol. A Biol. Sci. Med. Sci.* **56**:B281–B287.
- Wong, D., D. Bazopoulou, N. Pujol, N. Tavernarakis, and J. J. Ewbank. 2007. Genome-wide investigation reveals pathogen-specific and shared signatures in the response of *Caenorhabditis elegans* to infection. *Genome Biol.* **8**:R194.