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＝Original＝

Evaluation of *Caenorhabditis elegans* as the Host in an Infection Model for Food-borne Pathogens

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The bacteriophagous nematode *Caenorhabditis elegans* has been recognized as a surrogate host for human pathogens. The aim of this study was to examine whether food-borne pathogens are pathogenic in nematodes. Young adult worms were allocated onto peptone-free medium covered with a bacterial suspension of each pathogen. The plates were incubated and as the number of live and dead worms scored at least every 24 h. Twelve of the 14 pathogenic strains, namely *Aeromonas sobria*, the diarrheagenic *Escherichia coli* strains (enteroaggregative *E. coli*, enterohemorrhagic *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli* heat-stable enterotoxin 1 gene-possessing *E. coli*, and diffusely adherent *E. coli*), *Listeria monocytogenes*, *Salmonella Enteritidis*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Yersinia enterocolitica* reduced the survival rate of worms to varying degrees. The remaining 2 strains, *Bacillus cereus* and enteroinvasive *E. coli*, did not. Thus, food-borne pathogens can infect and proliferate within bacteriophagous nematodes on peptone-free medium to the same extent as reported with conventional peptone-containing medium. However, the non-enteropathogenic *E. coli* strain HS and deletion mutants of *L. monocytogenes*, which have lost their virulence in the murine model, were also still nematocidal. Although this nematode could be an alternative host for these pathogens, the nematocidal activity of these pathogens may not necessarily reflect enteropathogenicity in humans. Pathogens and the virulence genes to be analyzed must be carefully selected before using this alternative host.

Key words: *C. elegans*, Enteric pathogen, Virulence, Screening, Infection model

Introduction

A variety of experimental models have been developed to study microbial virulence or the pathogenicity of protein toxins. However, due to increasing ethical considerations as well as economic reasons, the use of mammalian hosts is decreasing in popularity, and the establishment of effective alternative non-mammalian systems is urgently required.

There has recently been increasing interest in the soil nematode *Caenorhabditis elegans* as a possible host of human pathogenic bacteria, since it was reported by Ausubel *et al*. in 1999 that *Pseudomonas aeruginosa* caused fatal infection of *C. elegans*[31]. *C. elegans* has also been reported as a successful model for the investigation of virulence-associated factors of human pathogens such as *Burkholderia pseudomallei*[10], *Cryptococcus neoformans*[19], *Enterococcus faecalis*[27], enteropathogenic *Escherichia coli*[2, 16], *Listeria monocytogenes*[34], *P. aeruginosa*[32], *Serratia marcescens*[14], *Shigella flexneri*[6], *Staphylococcus aureus*[11, 28], and *Vibrio vulnificus*[9].

We recognized nematodes as a potential new surrogate host, and the degree of similarity between this nematode, *C. elegans,* and humans is greater than expected[17]. In the above-cited reports, the human pathogens and associated

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deletion mutants often successfully showed virulence and avirulence in the nematode as well as in the murine model. However, the worms were infected with inocula on conventional nematode growth medium, which contains peptone, raising the possibility that the inoculated pathogen would have proliferated regardless of whether it could successfully infect the nematodes and derive nutrition from the hosts; the metabolites produced by the bacteria on the medium may also have affect the nematodes. In the present study, we examined whether various food-borne pathogens are pathogenic for *C. elegans* even on medium containing no nutrients to support bacterial growth. Furthermore, the nematocidal activities of the enteric pathogens were compared with those of *E. coli* HS, an internationally well-recognized non-enteropathogenic strain[15].

Materials and Methods

Nematodes

The *C. elegans* Bristol strain N2 was kindly provided by the Caenorhabditis Genetics Center, University of Minnesota. The nematodes were maintained and propagated on nematode growth medium (NGM) using standard techniques[30].

Bacterial strains

A variety of bacterial pathogens of food-borne infectious diseases were used: *Aeromonas sobria* strain AS62[21], *Bacillus cereus* strain 91-97-13[22], enteroaggregative *E. coli* (EAggEC) strain V546[24], EAggEC heat-stable enterotoxin 1 (EAST1) gene-possessing *E. coli* (EAST1EC) O166 : H15 strain[36], enterohaemorrhagic *E. coli* (EHEC) O157: H7 strain 96-98-83[23], enteroinvasive *E. coli* (EIEC) O143 : H-strain E35990, enteropathogenic *E. coli* (EPEC) O127 : H6 strain E2348/69, enterotoxigenic *E. coli* (ETEC) O6 : H16 strain ETEC1, diffusely adherent *E. coli* (DAEC) O1 : H4 strain V64[3], *Listeria monocytogenes* strain EGD (serotype 1/2a), *Staphylococcus aureus* strain 96-55-17A, *Salmonella enterica* serovar Enteritidis phagetype 1 strain SE1, *Vibrio parahaemolyticus* serotype O3 : K6 strain VP1, and *Yersinia enterocolitica* serotype O8 strain ATCC9610. The attenuated isogenic mutants (*L. monocytogenes* Δ*hly* and *L. monocytogenes* Δ*actA*) were kindly provided by Prof. Masao Mitsuyama (Kyoto University Graduate School of Medicine). The *E. coli* strain HS was kindly provided by Prof. Alison D. O'Brien at Uniformed Services University of the Health Sciences and was used as a control since this strain had been proven to be avirulent by oral inoculation in human volunteers[15]. *E. coli* OP50 was used as a food source for the nematodes. Tryptone soya agar (Oxoid, Basingstoke, Hampshire, UK) was used to culture all the bacterial strainsused, with 0.6% yeast extract (Oxoid) added for listeria.

Nematocidal assays

Worms for the nematocidal assays were generated from eggs released after exposing adult worms to a sodium hypochlorite/sodium hydroxide solution as previously described[30]. The egg suspensions were incubated overnight at 25℃ to allow hatching, and suspensions of the L1 stage worms were centrifuged at 1,000 rpm for 1 min. The supernatant was removed and the remaining larvae were transferred onto fresh NGM plates and incubated at 25℃. Two days later each batch of 25 young adult worms was placed onto peptone-free modified nematode growth medium (mNGM) in 6.0-cm diameter plates covered with a lawn of each pathogen. In all the previous reports mentioned, the nematocidal assays were performed by introducing the worms onto NGM agar plates containing peptone, which would allow the pathogens to proliferate. The composition of the NGM was reported to influence the virulence of the pathogens[11] and metabolites produced by the bacteria from tryptophan in the medium were found to be fatal to the nematodes[2]. To exclude the effects of nutrients in the medium, the present study was performed on mNGM plates, which contained no bacterial nutrients. The bacterial lawns used for the nematocidal assays were prepared by spreading 100 μl of bacterial suspension (10 mg wet weight/100 μl of M9 buffer) on the mNGM plate. Following addition of the worms, the plates were incubated at 25℃ and live and dead worms scored at least every 24 h. A worm was considered dead when it failed to respond to a gentle touch with a worm picker. Since these worms are hermaphrodites and continue to reproduce during the assay, newly hatched progeny could have interfered with the count of the surviving worms. To avoid this, surviving adult worms

were transferred to fresh plates every 2 days. Worms that died as a result of getting stuck to the wall of the plate were eliminated from the analysis. Each assay was performed in duplicate. Nematode survival was calculated by the Kaplan–Meier method, and survival differences were tested for significance by the log rank test.

Measurement of the number of pathogens in nematodes

To recover internal bacteria from the nematodes, the method of Garsin *et al.* (2001) was used with some modifications[11]. Five worms were picked and the surface bacteria were removed by washing 4 times in 4-μl drops of M9 buffer on agar plates. Each nematode was placed in a 0.5-m*l* microtube containing 20 μl of M9 buffer and was mechanically disrupted using a microtube pestle (Scientific Specialties Inc., Lodi, CA, USA). The volume was adjusted to 500 m*l* with M9 buffer and the number of bacteria was determined using Tryptone soya agar and MacConkey agar (Nissui, Tokyo, Japan).

Results

Compared to the control worms fed with *E. coli* OP50, the international standard food for *C. elegans*, the nematodes were killed quickly after their transfer to the lawns of DAEC, EAggEC, EAST1EC, *L. monocytogenes* wild-type, *S.* Enteritidis, *S. aureus*, or *Y. enterocolitica* O8 (Figs. 1a–g). To our knowledge this is the first report to demonstrate the nematocidal activity of DAEC, EAggEC, EAST1EC, and *Y. enterocolitica*, although additional strains of each pathogen should be tested to confirm these findings. Heat-killed nematocidal bacteria strains did not kill the worms and were as safe as OP50 (data not shown). The number of bacteria recovered from worms reached 10^4 to 10^5 CFU/worm after ingestion of the nematocidal strains, except *Y. enterocolitica* (Figs. 2a–g).

EHEC, EPEC, and ETEC also killed the worms, but to a lesser extent compared to the other *E. coli* strains (Figs. 1h–j), and the number of bacteria recovered from these worms was comparatively low (Figs. 2h–j). There has been no previous report concerning the infection of worms with *A. sobria* and *V. parahaemolyticus*. Under our experimental conditions these bacteria were moderately nematocidal (Figs. 1k and l) and the number of bacteria recovered from the nematodes was also low (Figs. 2k and l).

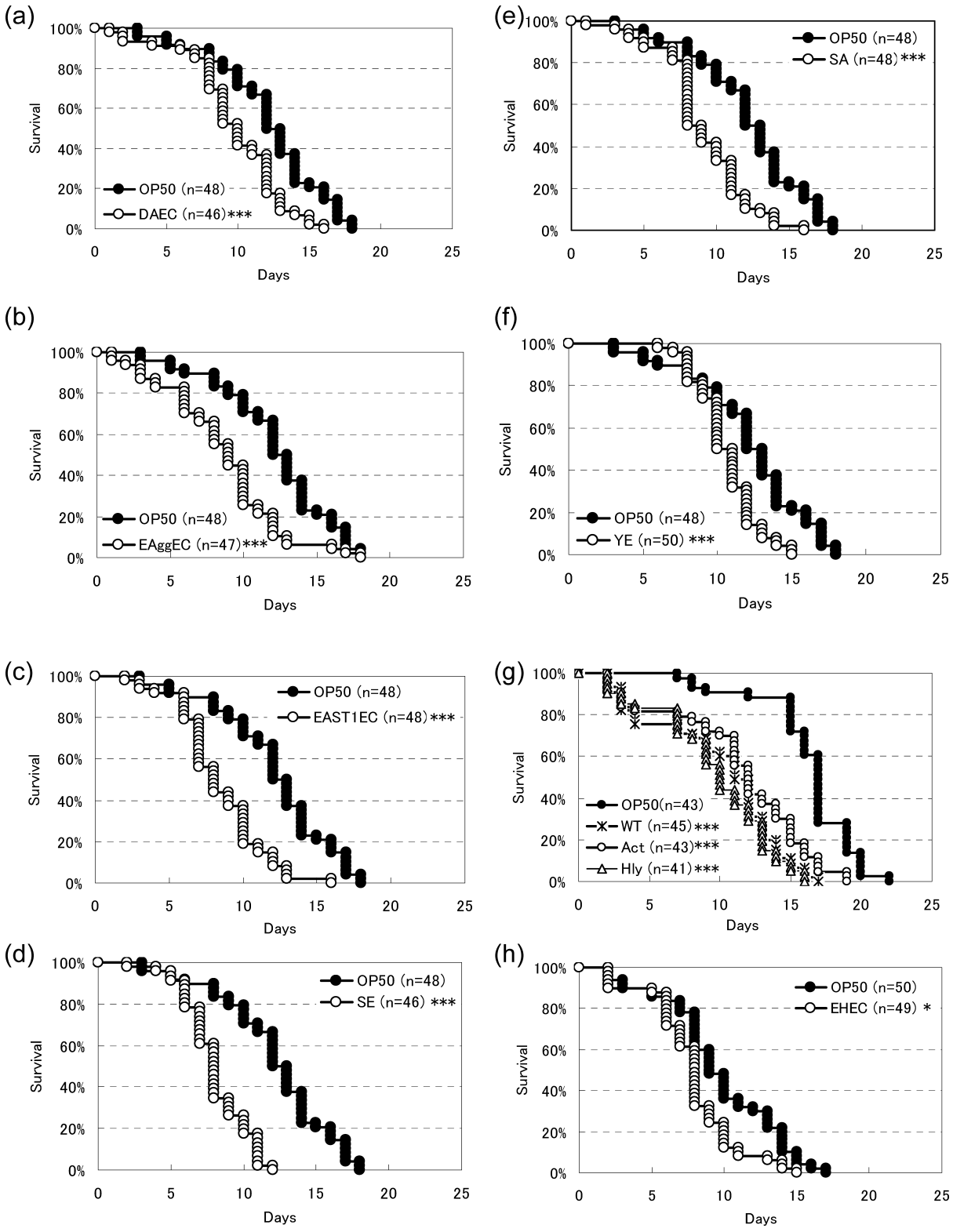
In contrast, nematodes fed *B. cereus* survived longer than the control (Fig. 1m, Fig. 2m). EIEC was avirulent in nematodes, and the lifespan of the worms was not significantly different from those grown on *E. coli* OP50 (Fig. 1n, Fig. 2n).

Inversely, *E. coli* strain HS reduced the worm's survival rate under our experimental conditions to the same extent as other food-borne pathogens (Fig. 1o, Fig. 2o). This strain is well recognized as a non-enteropathogenic strain representing indigenous bacterial flora and is often used as a negative control[15]. *L. monocytogenes* mutants for *hly* and *actA*, which were attenuated in their major virulence-associated genes identified in the murine model, were still pathogenic in the nematodes, to a similar extent to their parental wild-type strain (Fig. 1g, Fig. 2f).

Discussion

Twelve of the 14 food-borne pathogens examined significantly reduced the survival rate of the bacteriophagous nematode *C. elegans*, even on medium containing no peptones. Since the worms died over the course of several days, the pathogens exhibited a "slow killing" effect, with accumulation of bacteria in the intestine of the nematodes. These findings strongly support the usefulness of *C. elegans* to study food-borne pathogens, in agreement with the several reviews that have suggested the feasibility of *C. elegans* as a model to investigate virulence-associated factors of human pathogenic bacteria[1, 18, 20, 25, 26].

Nevertheless, there were some apparent anomalies in the data. The well-established enteric pathogens such as EHEC, EPEC, ETEC, and *V. parahaemolyticus* were rather modest in their nematocidal activity, and *B. cereus* and EIEC were avirulent in the worms. *A. sobria* was showed modest nematocidal activity, although the other vibrionaceae, *A. hydrophila* strains[7] and *Vibrio cholerae* El Tor Inaba[35], have previously been reported to be clearly nematocidal on NGM plates including peptones. Anyanful *et al.*[2] reported that EHEC, EIEC, EPEC, and ETEC could paralyze and kill nematodes quickly without contact by producing



toxic substances on tryptophan-containing medium, but the organisms did not produce the toxic substances on the NGM plates[2]; the organisms seemed not to infect the worms in their experiments. The reason for these discrepancies between the present data and previous reports is unclear. The pathogens might require peptones to infect worms or to produce toxic metabolites on the medium in situ, or else the temperature of the worms might be too low for these diarrheagenic *E. coli* to express the full virulence. Given the response of these pathogens, it is still uncertain whether *C. elegans* is a suitable alternative host for infection.

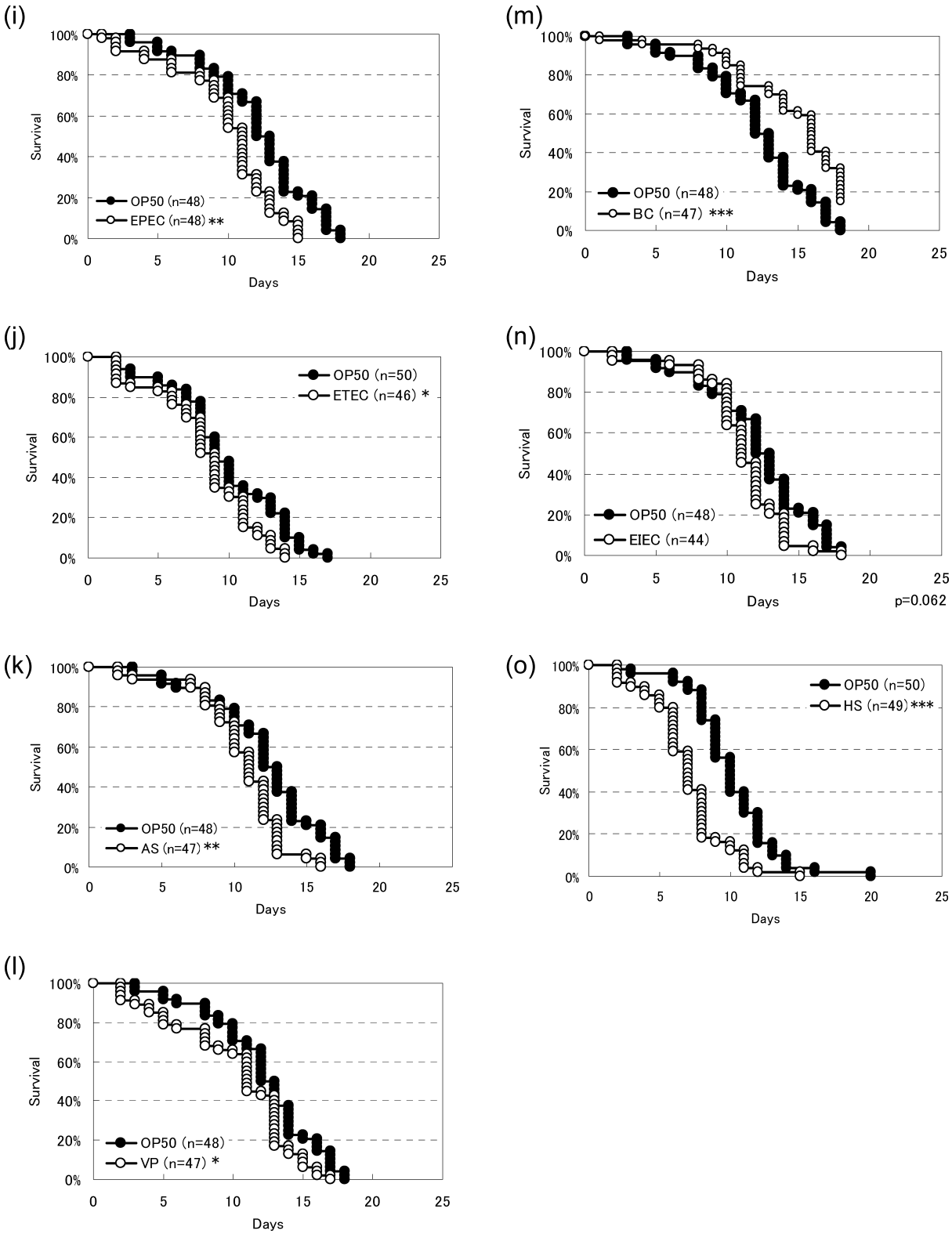
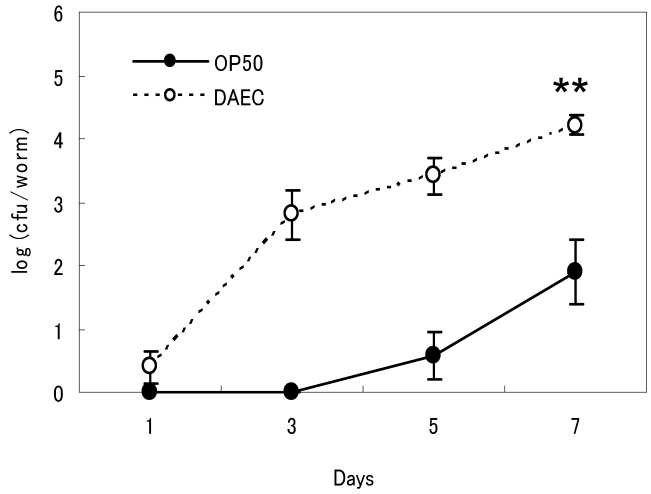
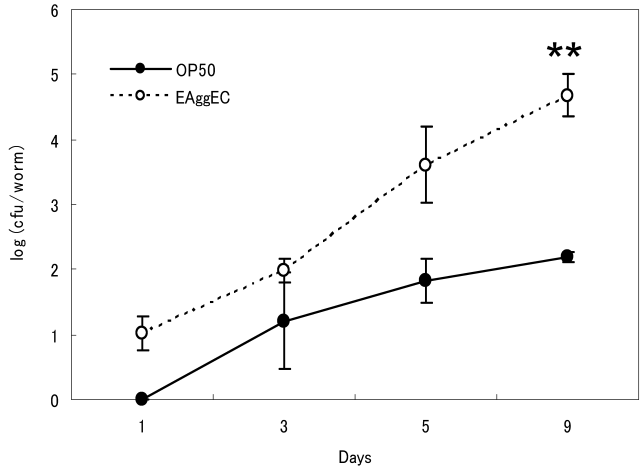


Fig. 1. Survival of *C. elegans* fed with either *E. coli* OP50 or food-borne pathogens throughout their adult life from 3 days old. The pathogens illustrated in each graph are as follows: (a) DAEC, (b) EAggEC, (c) EAST1EC O166 : H15, (d) S. Enteritidis, (e) *S. aureus*, (f) *Y. enterocolitica* O8, (g) *L. monocytogenes* 1/2a and its mutants (ActA and Hly), (h) EHEC, (i) EPEC, (j)ETEC, (k) *A. sobria*, (l) *V. parahaemolyticus*, (m) *B. cereus*, (n) EIEC, and (o) non-enteropathogenic *E. coli* strain HS. Experiments (a–f, i, and k–m) were performed simultaneously, and the survival curve of the control group was comparable across these graphs. Experiment (h) was performed along with experiment (j). *, **, and *** indicate $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

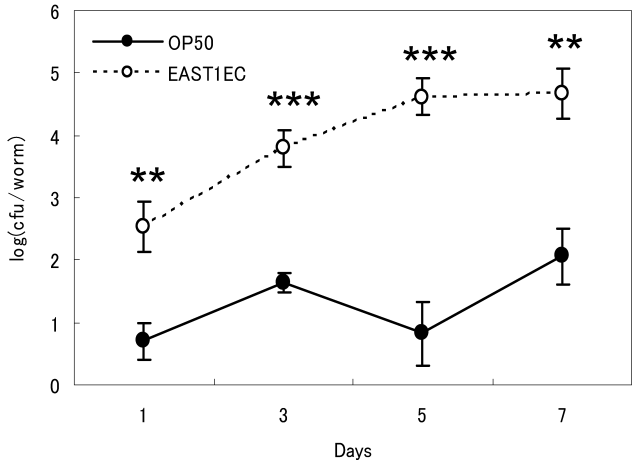
(a)


OP50
DAEC
**
log (cfu/worm)
Days

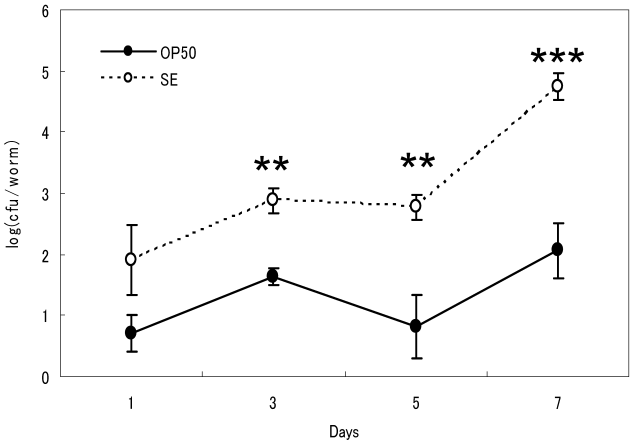
(b)


OP50
EAggEC
**
log (cfu/worm)
Days

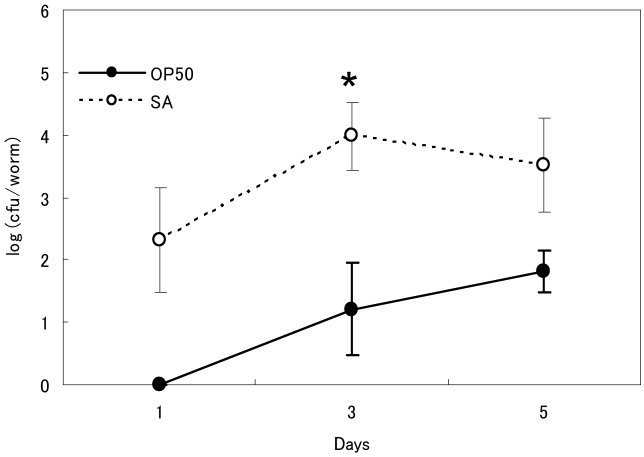
(c)


OP50
EAST1EC
**
***
***
**
log(cfu/worm)
Days

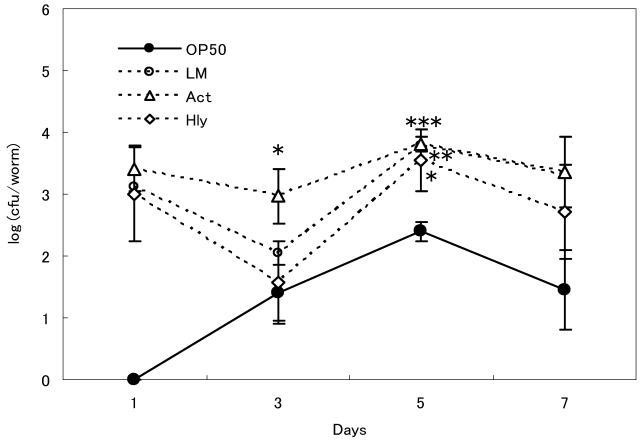
(d)


OP50
SE
**
**
***
log(cfu/worm)
Days

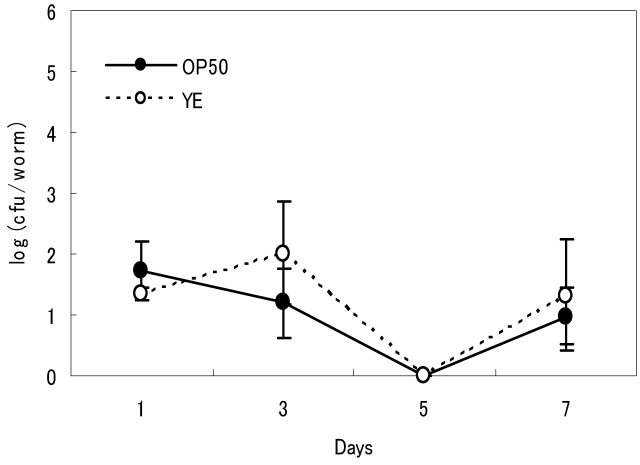
(e)


OP50
SA
*
log (cfu/worm)
Days

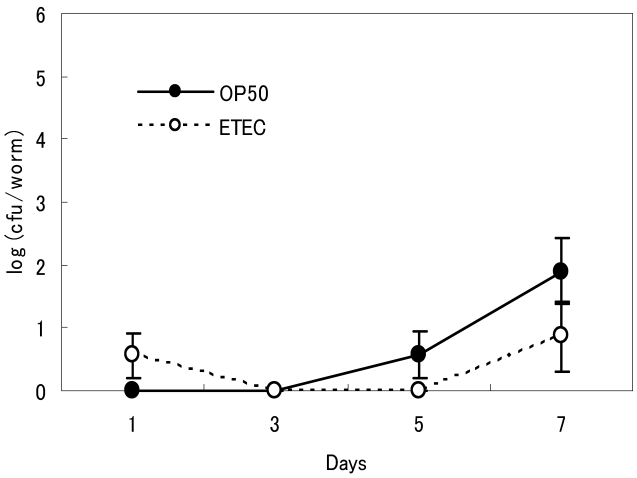
(f)


OP50
LM
Act
Hly
*
***
**
*
log (cfu/worm)
Days

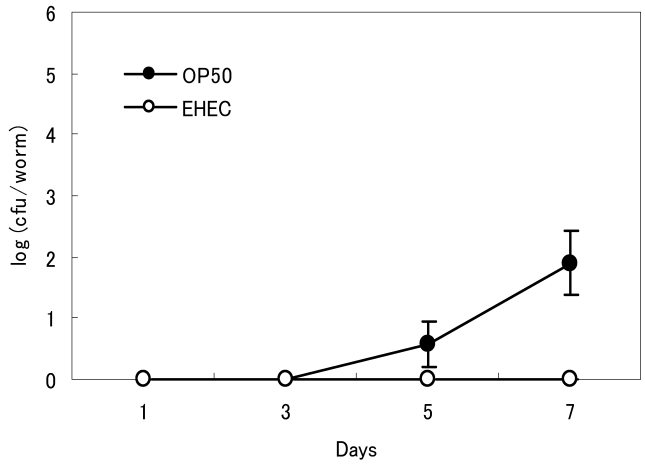
(g)


OP50
YE
log (cfu/worm)
Days
0
1
2
3
4
5
6
1
3
5
7

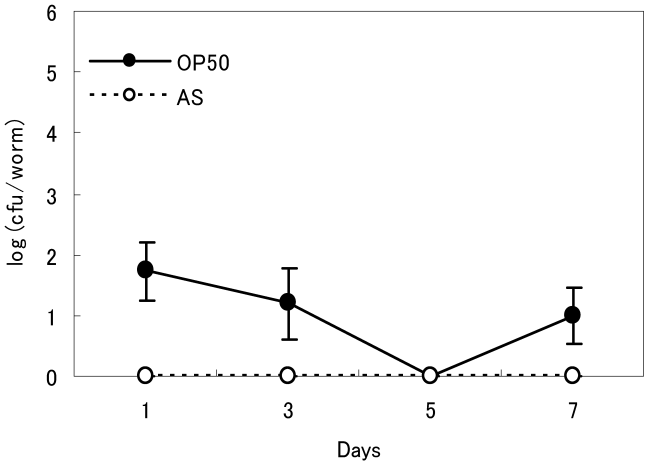
(j)


OP50
ETEC
log (cfu/worm)
Days
0
1
2
3
4
5
6
1
3
5
7

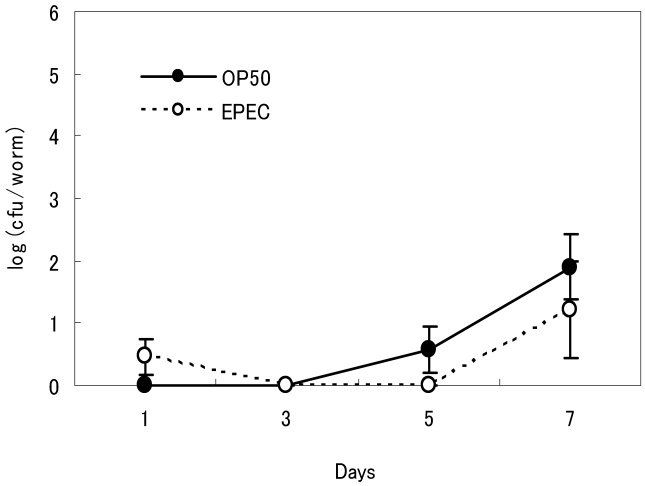
(h)


OP50
EHEC
log (cfu/worm)
Days
0
1
2
3
4
5
6
1
3
5
7

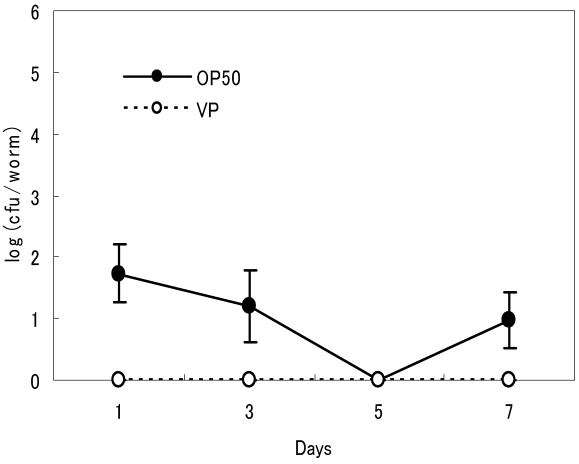
(k)

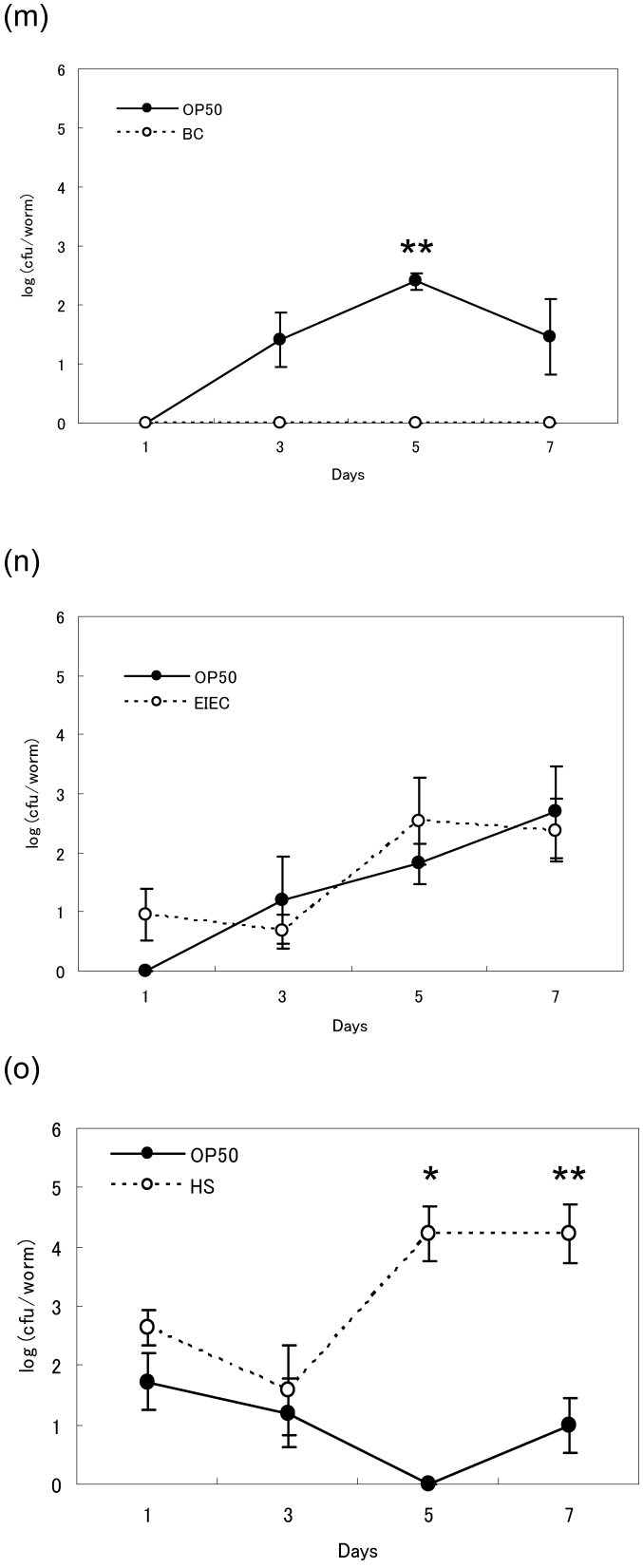

OP50
AS
log (cfu/worm)
Days
0
1
2
3
4
5
6
1
3
5
7

(i)


OP50
EPEC
log (cfu/worm)
Days
0
1
2
3
4
5
6
1
3
5
7

(l)


OP50
VP
log (cfu/worm)
Days
0
1
2
3
4
5
6
1
3
5
7



The *B. cereus* used in this study is a causative agent of emetic-syndrome food poisoning by intradietetic intoxication[22]. Therefore, direct feeding would most likely not result in nematocidal activity. Alternatively, *B. cereus* is a natural inhabitant in soil and is possibly a natural food for the soil nematode *C. elegans* rather than *E. coli* OP50 and this perhaps accounts for the longevity of worms fed *B. cereus*[12].

Borgonie *et al*. reported that the intestinal lumen of the bacteriophagous nematode was surrounded by a peritrophic-like membrane which is likely to be chitinous and protects the epithelial surface[5]. To date no reports have demonstrated invasion by human pathogenic bacteria into the cytoplasm of nematode gut cells or penetration into the body cavity, except in the terminal phases of infection[20]. Normally EIEC is able to enter into a cell and escape the extracellular host-defense system. We therefore believe that EIEC could not pass through the peritrophic-like membrane or enter the nematode gut cells, and thus became exposed to antibacterial factors in the worm's gut, indicating that the EIEC used in this study is not fatal to nematodes.

The *E. coli* HS and *L. monocytogenes* attenuated mutants were pathogenic in our experiments. Several genes of *P. aeruginosa*, *S. aureus*, *S. marcescens*, and *Yersinia pestis* have been newly identified and found to be involved in the pathogenicity of *C. elegans*, and some have also been found to be essential for virulence in the

Fig. 2. Number of bacteria recovered from *C. elegans* fed with either each pathogenor *E. coli* OP50. Nematodes were transferred to plates on which pathogens were spread. After the days indicated, the nematodes were washed and homogenized. The resulting suspension was diluted and plated onto Tryptone soya agar and MacConkey agar plates. The number of colony-forming units per worm was calculated. The pathogens illustrated in each graph are as follows: (a) DAEC, (b) EAggEC, (c) EAST1EC O166 : H15, (d) *S.* Enteritidis, (e) *S. aureus*, (f) *L. monocytogenes* 1/2a wild-type and the deletion mutants (ActA and Hly), (g) *Y. enterocolitica* O8 (h) EHEC, (i) EPEC, (j) ETEC, (k) *A. sobria*, (l) *V. parahaemolyticus*, (m) *B. cereus*, (n) EIEC, and (o) non-enteropathogenic *E. coli* strain HS. Experiments (a, h, i, and j), (b and e), (c and d), (f and m), and (g, k, l, and o) were performed simultaneously, and the control in each group is comparable across these graphs. All results represent the mean±standard error of the mean. *, **, and *** indicate $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

mammalian system[4, 14, 29, 33]. However, the bacterial pathogenicity observed in *C. elegans* models may reflect other virulence-related aspects rather than enteropathogenicity; although the current observations do not necessarily exclude the possibility that the characteristics associated with nematocidal activity may be prerequisites for some human enteropathogens. The genes *hly* or *actA* are critical for the intracellular parasitism of *L. monocytogenes* in mice. The *hly* gene encodes a pore-forming toxin, listeriolysin O, which is the major virulence factor implicated in the escape of this bacterium fromphagocytic vacuoles, and the product of the *actA* gene mediates actin-based motility in the cytoplasm. However, Thomsen *et al*. (2006) reported that intracellular life was not important for listeria in *C. elegans*. *L. monocytogenes* seems to be resistant to humoral antibacterial factors in the worm[34]. At present it remains unclear whether the properties associated with nematocidal activity play an important role in the virulence of listeria in mammals, although virulence factors PrfA and DegU have been found to be essential for the expression of virulence in *C. elegans* as well as in mice[34].

The numbers of bacteria recovered from the worms were comparatively high after ingestion of the nematocidal strains, except *Y. enterocolitica*. The other Yersinia, *Y. pestis* and *Y. pseudotuberculosis*, were previously reported to kill nematodes by forming a biofilm around the worm's mouth to inhibit food intake[8]. *Y. enterocolitica* might also behave similarly and the number of organisms recovered from the worms was low.

C. elegans is useful for studying the relationship between innate immunity and pathogens because the nematode lacks an adaptive immune system. Using the worms on agar plates containing no bacterial nutrients, we recently found that lactic acid bacteria can enhance the host's defense against salmonella in *C. elegans* and prolong lifespan[13]. Although *C. elegans* does not have phagocytes specialized for innate host defense, it produces a variety of humoral antibiotic substances such as lysozymes, caenopores, lipase, lectins and C3-like thioester-containing proteins, and defensin-like antibiotic peptides[1, 18, 20, 25, 26]. These substances in the nematodes might be recognized as a category of the digestive enzymes of mammals. Bacteria resistant to these antibacterial substances are likely to be nematocidal irrespective of their enteropathogenicity in humans. Consequently, *C. elegans* may be more suitable to study systemic infection-causing pathogens which have to contend with humoral factors of the host.

In conclusion, the use of nematodes as an alternative host for screening bacterial strains for attenuated mutants is likely to result in the reducing the need for experimental mammalian hosts. However, in the case of enteric pathogens, careful selection of bacterial species and strains is required, as is analysis of the etiological significance of nematocidal virulence of human enteric pathogens in humans.

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References

1) Alegado, R. A., Campbell, M. C., Chen, W. C., Slutz, S. S. and Tan, M. W.: Characterization of mediators of microbial virulence and innate immunity using the *Caenorhabditis elegans* host-pathogen model. Cell Microbiol., **5**, 435–444 (2003).
2) Anyanful, A., Dolan-Livengood, J. M., Lewis, T., Sheth, S., Dezalia, M. N., Sherman, M. A., Kalman, L. V., Benian, G. M. and Kalman, D.: Paralysis and killing of *Caenorhabditis elegans* by enteropathogenic *Escherichia coli* requires the bacterial tryptophanase gene. Mol. Microbiol., **57**, 988–1007 (2005).
3) Arikawa, K., Meraz, I. M., Nishikawa, Y., Ogasawara, J. and Hase, A.: Interleukin-8 secretion by epithelial cells infected with diffusely adherent *Escherichia coli*

possessing Afa adhesin-coding genes. Microbiol. Immunol., **49**, 493–503 (2005).

4) Begun, J., Sifri, C. D., Goldman, S., Calderwood, S. B. and Ausubel, F. M.: *Staphylococcus aureus* virulence factors identified by using a high-throughput *Caenorhabditis elegans*-killing model. Infect. Immun., **73**, 872–877 (2005).
5) Borgonie, G., Claeys, M., Vanfletteren, J., DeWaele, D. and Coomans, A.: Presence of peritrophic-like membranes in the intestine of three bacteriophagous nematodes (Nematoda: Rhabditida). Fundam. Appl. Nematol., **18**, 227–233 (1995).
6) Burton, E. A., Pendergast, A. M. and Aballay, A.: The Caenorhabditis elegans ABL-1 tyrosine kinase is required for *Shigella flexneri* pathogenesis. Appl. Environ. Microbiol., **72**, 5043–5051 (2006).
7) Couillault, C. and Ewbank, J. J.: Diverse bacteria are pathogens of *Caenorhabditis elegans*. Infect. Immun., **70**, 4705–4707 (2002).
8) Darby, C., Hsu, J. W., Ghori, N. and Falkow, S.: *Caenorhabditis elegans*: plague bacteria biofilm blocks food intake. Nature, **417**, 243–244 (2002).
9) Dhakal, B. K., Lee, W., Kim, Y. R., Choy, H. E., Ahnn, J. and Rhee, J. H.: *Caenorhabditis elegans* as a simple model host for *Vibrio vulnificus* infection. Biochem. Biophys. Res. Commun., **346**, 751–757 (2006).
10) Gan, Y. H., Chua, K. L., Chua, H. H., Liu, B., Hii, C. S., Chong, H. L. and Tan, P.: Characterization of *Burkholderia pseudomallei* infection and identification of novel virulence factors using a *Caenorhabditis elegans* host system. Mol. Microbiol., **44**, 1185–1197 (2002).
11) Garsin, D. A., Sifri, C. D., Mylonakis, E., Qin, X., Singh, K. V., Murray, B. E., Calderwood, S. B. and Ausubel, F. M.: A simple model host for identifying Gram-positive virulence factors. Proc. Natl. Acad. Sci. U.S.A., **98**, 10892–10897 (2001).
12) Garsin, D. A., Villanueva, J. M., Begun, J., Kim, D. H., Sifri, C. D., Calderwood, S. B., Ruvkun, G. and Ausubel, F. M.: Long-lived *C. elegans daf-2* mutants are resistant to bacterial pathogens. Science, **300**, 1921 (2003).
13) Ikeda, T., Yasui, C., Hoshino, K., Arikawa, K. and Nishikawa, Y.: Influence of lactic acid bacteria on longevity of *Caenorhabditis elegans* and host defense against *Salmonella entetica* serovar Enteritidis. Appl. Environ. Microbiol., **73**, 6404–6409 (2007).
14) Kurz, C. L., Chauvet, S., Andres, E., Aurouze, M., Vallet, I., Michel, G. P., Uh, M., Celli, J., Filloux, A., De Bentzmann, S., Steinmetz, I., Hoffmann, J. A., Finlay, B. B., Gorvel, J. P., Ferrandon, D. and Ewbank, J. J.: Virulence factors of the human opportunistic pathogen *Serratia marcescens* identified by *in vivo* screening. EMBO J., **22**, 1451–1460 (2003).
15) Levine, M. M., Bergquist, E. J., Nalin, D. R., Waterman, D. H., Hornick, R. B., Young, C. R. and Sotman, S.: *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. Lancet, **1**, 1119–1122 (1978).
16) Mellies, J. L., Barron, A. M., Haack, K. R., Korson, A. S. and Oldridge, D. A.: The global regulator Ler is necessary for enteropathogenic *Escherichia coli* colonization of *Caenorhabditis elegans*. Infect. Immun., **74**, 64–72 (2006).
17) Mushegian, A. R., Garey, J. R., Martin, J. and Liu, L. X.: Large-scale taxonomic profiling of eukaryotic model organisms: a comparison of orthologous proteins encoded by the human, fly, nematode, and yeast genomes. Genome Res., **8**, 590–598 (1998).
18) Mylonakis, E., Ausubel, F. M., Tang, R. J. and Calderwood, S. B.: The art of serendipity: killing of *Caenorhabditis elegans* by human pathogens as a model of bacterial and fungal pathogenesis. Expert. Rev. Anti-infect. Ther., **1**, 167–173 (2003).
19) Mylonakis, E., Idnurm, A., Moreno, R., El Khoury, J., Rottman, J. B., Ausubel, F. M., Heitman, J. and Calderwood, S. B.: *Cryptococcus neoformans* Kin1 protein kinase homologue, identified through a *Caenorhabditis elegans* screen, promotes virulence in mammals. Mol. Microbiol., **54**, 407–419 (2004).
20) Nicholas, H. R. and Hodgkin, J.: Responses to infection and possible recognition strategies in the innate immune system of *Caenorhabditis elegans*. Mol. Immunol., **41**, 479–493 (2004).
21) Nishikawa, Y., Hase, A., Ogawasara, J., Scotland, S. M., Smith, H. R. and Kimura, T.: Adhesion to and invasion of human colon carcinoma Caco-2 cells by *Aeromonas* strains. J. Med. Microbiol., **40**, 55–61 (1994).
22) Nishikawa, Y., Kramer, J. M., Hanaoka, M. and Yasukawa, A.: Evaluation of serotyping, biotyping, plasmid banding pattern analysis, and HEp-2 vacuolation factor assay in the epidemiological investigation of *Bacillus cereus* emetic-syndrome food poisoning. Int J. Food Microbiol, **31**, 149–159 (1996).
23) Nishikawa, Y., Hase, A., Ogasawara, J., Cheasty, T., Willshaw, G. A., Smith, H. R., Tatsumi, Y. and Yasukawa, A.: Phage typing and DNA-based comparison of strains of enterohemorrhagic *Escherichia coli* O157 from apparently sporadic infections in Osaka City, Japan, 1996. Jpn. J. Infect. Dis., **54**, 140–143 (2001).
24) Nishikawa, Y., Zhou, Z., Hase, A., Ogasawara, J., Kitase, T., Abe, N., Nakamura, H., Wada, T., Ishii, E., Haruki, K. and Team, S.: Diarrheagenic *Escherichia coli* isolated from stools of sporadic cases of diarrheal illness in Osaka City, Japan between 1997 and 2000: prevalence of enteroaggregative *E. coli* heat-stable enterotoxin 1 gene-possessing *E. coli*. Jpn. J. Infect. Dis., **55**, 183–190 (2002).
25) Nishikawa, Y., Hoshino, K. and Ikeda, T.: Innate immunity in *Caenorhabditis elegans* and the application to immunonutrition research. J. Human Life Sci., **4**, 51–74 (2005).
26) Schulenburg, H., Kurz, C. L. and Ewbank, J. J.: Evolution of the innate immune system: the worm perspective. Immunol. Rev., **198**, 36–58 (2004).
27) Sifri, C. D., Mylonakis, E., Singh, K. V., Qin, X., Garsin, D. A., Murray, B. E., Ausubel, F. M. and Calderwood, S. B.: Virulence effect of *Enterococcus faecalis* protease

genes and the quorum-sensing locus *fsr* in *Caenorhabditis elegans* and mice. Infect. Immun., **70**, 5647–5650 (2002).
28) Sifri, C. D., Begun, J., Ausubel, F. M. and Calderwood, S. B.: *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. Infect. Immun., **71**, 2208–2217 (2003).
29) Styer, K. L., Hopkins, G. W., Bartra, S. S., Plano, G. V., Frothingham, R. and Aballay, A.: *Yersinia pestis* kills *Caenorhabditis elegans* by a biofilm-independent process that involves novel virulence factors. EMBO Rep., **6**, 992–997 (2005).
30) Sulston, J. and Hodgkin, J.: Methods, the Nematode *Caenorhabditis elegans*. Wood, W. B. and Researchers, The community of *C. elegans* researchers (eds.), 587–606, Cold Spring Harbor Laboratory Press, New York (1988).
31) Tan, M. W., Mahajan-Miklos, S. and Ausubel, F. M.: Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. Proc. Natl. Acad. Sci. U.S.A., **96**, 715–720 (1999).
32) Tan, M. W., Rahme, L. G., Sternberg, J. A., Tompkins, R. G. and Ausubel, F. M.: *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. Proc. Natl. Acad. Sci. U.S.A., **96**, 2408–2413 (1999).
33) Tan, M. W. and Ausubel, F. M.: *Caenorhabditis elegans*: a model genetic host to study *Pseudomonas aeruginosa* pathogenesis. Curr. Opin. Microbiol., **3**, 29–34 (2000).
34) Thomsen, L. E., Slutz, S. S., Tan, M. W. and Ingmer, H.: *Caenorhabditis elegans* is a model host for *Listeria monocytogenes*. Appl. Environ. Microbiol., **72**, 1700–1701 (2006).
35) Vaitkevicius, K., Lindmark, B., Ou, G., Song, T., Toma, C., Iwanaga, M., Zhu, J., Andersson, A., Hammarstrom, M. L., Tuck, S. and Wai, S. N.: A *Vibrio cholerae* protease needed for killing of *Caenorhabditis elegans* has a role in protection from natural predator grazing. Proc. Natl. Acad. Sci. U.S.A., **103**, 9280–9285 (2006).
36) Zhou, Z., Ogasawara, J., Nishikawa, Y., Seto, Y., Helander, A., Hase, A., Iritani, N., Nakamura, H., Arikawa, K., Kai, A., Kamata, Y., Hoshi, H. and Haruki, K.: An outbreak of gastroenteritis in Osaka, Japan due to *Escherichia coli* serogroup O166: H15 that had a coding gene for enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1). Epidemiol. Infect., **128**, 363–371 (2002).

食中毒菌の感染実験宿主としての *Caenorhabditis elegans* の評価

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細菌食性の土壌線虫である *Caenorhabditis elegans*（線虫）が，ヒト病原体の代替宿主として認められつつある．本研究の目的は，食中毒細菌が線虫に病原性を示すか否か検討することにある．ペプトンを含まない寒天上に各種の病原細菌を塗りつけた後，若い成虫を移して摂取させた．24 時間ごとに線虫の生死を確認し，生残率を経時的に調べた．供試した 14 種の病原体のうちセレウスと腸管侵入性大腸菌を除く 12 菌種（各種下痢原性大腸菌，黄色ブドウ球菌，腸炎ビブリオ，サルモネラ，リステリア，エロモナス，エルシニア）が，線虫の生残率を対照群に比べて有意に低下させた．既に報告されたペプトンを含む寒天上で実施された線虫の感染実験と同様に，線虫に病原性を示す菌は，ペプトンを含まない寒天上でも線虫に感染し，その体内で増殖していることが明らかとなった．しかしながら，ヒトに病原性を示さない大腸菌 HS 株や，マウスに対する病原性を失ったリステリアの変異株が線虫に病原性を示した．線虫は食中毒菌の病原性を検討するうえで有用であるが，線虫に対する病原性がヒトへの腸管病原性を必ずしも反映するわけではない．線虫を代替宿主として利用するに当たっては，病原菌の種類や解析する病原遺伝子を注意深く選定し実験することが求められる．