Infection of Caenorhabditis elegans by Salmonella typhi Ty2

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Several serovars of Salmonella infect and kill the nematode C. elegans. However, here we report that Salmonella typhi Ty2, a representative strain of this human pathogen, readily infects the intestinal lining of C. elegans without significantly affecting its viability. Our observation suggests extending the use of the C. elegans model system for the study of host parasite relationships, to address problems concerning the biology of S. typhi.

The nematode Caenorhabditis elegans has been used as a model system to study bacterial pathogenesis due to ease of manipulation and a detailed knowledge of its biology. Several bacterial pathogens, both Gram positive and Gram negative, have been reported to infect and kill C. elegans (Couliault and Ewbank, 2002). Recently, C. elegans has been used to elucidate molecular mechanisms of virulence in Pseudomonas aeruginosa (Gallagher and Manoil, 2001) infection by Burkholderia pseudomallei (O’Quinn et al. 2001) and S. typhimurium, a bacterium that persistently infects the C. elegans intestine and finally kills the nematode (Aballay et al. 2000; Aballay and Ausubel, 2001). Furthermore S. enteritidis and S. dublin have also been shown to kill C. elegans (Aballay et al. 2000). On the other hand, S. typhi is considered to be a pathogen restricted to human hosts (Pascopella, et al. 1995) and therefore not many cell or animal systems are available to study S. typhi pathogenesis.

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Here, we report that the *S. typhi* Ty2 WHO reference strain does not kill *C. elegans* but can infect the nematode’s intestinal lining. Consequently, *C. elegans* is suitable for exploring cell invasion by *S. typhi* and possibly its persistence in this host.

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**METHODS**

**Growth of bacteria and *C. elegans***

Both Wild Type (WT) and Green Fluorescent Protein (GFP) tagged bacteria were used. The latter contained the plasmid pSU2007 that codes for GFP and Kanamycin resistance (Km’). *S. typhi* Ty2 WT, *S. typhi* Ty2 pSU2007, *S. typhimurium* SL1344, *S. typhimurium* SL1344 pSU2007, *Escherichia coli* MT102 pSU2007 and *E. coli* OP50 were grown in Luria-Bertani medium (Miller, 1972) at 37°C.

The nematode *C. elegans* WT N2 Bristol was propagated on NG agar, fed with *E. coli* OP50 (Brenner, 1974).

**Mortality assays**

Assays were performed according to Aballay et al. 2000. Dead nematodes were counted every 24 hrs. and removed from the assay plates. Thus, we determined the time it takes for 50% of the nematodes to die (TD50).

**Epifluorescence microscopy**

Nematodes infected with different GFP-tagged bacteria, were suspended in M9 salts solution (Miller, 1972) for 10 min., centrifuged and finally suspended in M9 with 30 mM sodium azide, used as anesthetic (Aballay et al. 2000). After the worms ceased to move they were observed by epifluorescence microscopy at 460-490 nm using a Olympus BX 60 microscope. Images were obtained using an Olympus C3030-Zoom digital camera. A total of 50 specimens were examined, coming from four independent *C. elegans* – *S. typhi* Ty2 plates.

**RESULTS AND DISCUSSION**

Recently, Aballay et al. 2000 have reported a TD50 of 7.6 +/- 0.7 days for a nosocomial isolate of *S. typhi* (strain 469) in a 10 day experiment designed to assay killing by *S. typhimurium* SL1344.

However, when assaying the WHO reference strain *S. typhi* Ty2 we found that it does not kill *C. elegans* in a 22 day assay (Figure 1). We found TD50’s of 14.94 days for *S. typhi* Ty2 WT, 15.56 days for *S. typhi* Ty2 pSU2007, 11 days for *E. coli* OP50 and 4.97 days for *S. typhimurium* SL1344.

No swelling of the intestine that was observed in *S. typhi* Ty2 infected *C. elegans* (Figure 2a and Figure 2b) in contrast with *S. typhimurium* SL1344 infected nematodes (Figure 2c and Figure 2d). In addition, we saw that *S. typhi* Ty2 invades the worm’s intestinal lining (Figure 2a). This is consistent with a reduced reproductive rate we observed for *C. elegans* grown in *S. typhi* Ty2 (48.2 worms/ml/day) when compared to the reproductive rate of *E. coli* OP50 grown nematodes (96.2 worms/ml/day). These results suggest that nematodes, such as *C. elegans*, might act as temporal reservoirs for this bacterium. In this respect, Tesser et al. 2001 have reported carriage of *S. typhi* inside environmental protozoa, which act as potential reservoirs.

The fact that *C. elegans* infected with *S. typhi* remains viable and active suggests that bacterivorous nematodes might play a role in the dispersal of *S. typhi*. We are currently testing this possibility in view of recent evidence (Chadfield et al. 2001) indicating that the poultry parasitic nematode *Ascaridia galli* is involved in the dispersal of *S. typhimurium*. In this case, the bacterium infects *A. galli* but does not kill it, thus promoting its own dissemination.

Finally, the *C. elegans - S. typhi* Ty2 association allows to address questions about invasiveness of *S. typhi* in a whole organism system, with the added advantage of the detailed knowledge pertaining the genetics and molecular biology of *C. elegans*. This is a complementary approach to a simpler cultured cell system expressing a surface receptor for *S. typhi* that has been described earlier (Pier et al. 1998). Furthermore, the *C. elegans* system could be useful in elucidating differences in host specific adaptations between *S. typhi* and *S. typhimurium*, considering that the latter remains in the intestinal tract during the lethal infection of *C. elegans* (Aballay et al. 2000).

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REFERENCES


Figure 1. Mortality of *C. elegans* in the presence of *E. coli* OP50 (■); *S. typhi* Ty2/GFP (●); *S. typhi* Ty2 (▲); *S. typhimurium* SL1344 (○).
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Figure 2. Location of GFP tagged bacteria in the *C. elegans* intestine.


1. Intestinal lumen.
3. *S. typhimurium* SL1344 pSU2007 in the pharynx of *C. elegans*.
4. *E. coli* MT102 pSU2007 in the intestine of *C. elegans*.