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* (Coulillault 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.

**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 anesthetics (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 TD50s 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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APPENDIX

FIGURES

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.

- **a.** *S. typhi* Ty2 pSU2007.
- **b.** *S. typhi* Ty2 pSU2007, bright field.
- **c.** *S. typhimurium* SL1344 pSU2007.
- **d.** *S. typhimurium* SL1344 pSU2007, bright field.
- **e.** *E. coli* MT102 pSU2007.

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*.