Electronic Journal of Biotechnology ISSN: 0717-3458 Vol.6 No.2, Issue of August 15, 2003

Received January 23, 2003 / Accepted July 15, 2003

SHORT COMMUNICATION

Infection of *Caenorhabditis elegans* by *Salmonella typhi* Ty2

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Financial support: Vice-Rectoria de Investigación y Estudios Avanzados, Universidad Católica de Valparaíso. Proyecto D.I.UCV 122.770.2002.

Keywords: *Caenorhabditis elegans*, invasiveness, *Salmonella typhi* Ty2.

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* (Couillault 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 paseudomallei* (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^r). *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 37ºC.

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 (TD_{50}).

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 anesthesic (Aballay et al. 2000). After the worms ceased to move they were observed by epifluoresence 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 TD_{50} 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 TD_{50}'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).

ACKNOWLEDGMENTS

We thank Dr. Guido Mora (Pontificia Universidad Católica de Chile) who provided *S. typhi* Ty2, Dr. Alejandro Aballay (Harvard Medical School) for *C. elegans*, *S. typhimurium* SL1344 and *E. coli* OP50, Dr. Soren Mölin (Molecular Ecology Group, BioCentrum-DTU, Denmark) for *E. coli* MT102 pSU2007 and Dr. Juan Kuznar (Universidad de Valparaíso) for use of epifluorescence microscopy facilities.

REFERENCES

Aballay, A.; Yorgety, P. and Ausubel, F.M. *Salmonella typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans*. *Current Biology*, November 2000, vol. 10, no. 23, p. 1539-1542.

Aballay, A. and Ausubel, F.M. Programmed cell death mediated by ced-3 and ced-4 protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. *Proceedings of the National Academy of Sciences of the United States of America*, February 2001, vol. 98, no. 5, p. 2735-2739.

Brenner, S. The genetics of *Caenorhabditis elegans*. *Genetics*, May 1974, vol. 77, p. 71-94.

Couillault, C. and Ewbank, J.J. Diverse bacteria are pathogens of *Caenorhabditis elegans*. *Infection and Immunity*, August 2002, vol. 70, no. 8, p. 4705-4707.

Chadfield, M.; Permin, A.; Nasen, P. and Bisgaard, M. Investigation of the parasitic nematode *Ascaridia galli* (Shrank, 1788) as a potential vector for *Salmonella enterica* dissemination in poultry. *Parasitology Research*, April 2001, vol. 87, no. 4, p. 317-325.

Gallagher, L.A. and Manoil, C. *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. *Journal of Bacteriology*, November 2001, vol. 183, no. 21, p. 6207-6214.

Miller, J.H. *Experiments in Molecular Genetics*. Cold Spring Harbor Press, 1972. 466 p. ISBN 0-87969-106-9.

O'Quinn, A.L.; Wiegand, E.M. and Jeddeloh, J.A. *Burkholderia pseudomallei* kills the nematode *Caenorhabditis elegans* using an endotoxin-mediated paralysis. *Cellular Microbiology*, June 2001, vol. 3, no. 6, p. 381-393.

Tesser, B.; Aranda E. and Mora G.C. *Salmonella typhi* es capaz de sobrevivir en el interior de protozoos ambientales. In: *XXIII Congreso Chileno de Microbiología*. (28th-30th November, 2001, Tomé, Chile). Abstracts, 2001. p. 52.

Pascopella, L.; Raupach, R.; Ghori, N; Manack, D.; Falkow, S. and Small, P.L.C. Host restriction phenotypes of *Salmonella typhi* and *Salmonella gallinarum*. *Infection and Immunity*, November 1995, vol. 63, no. 11, p. 4329-4335.

Pier, G.B.; Grout, M.; Zaidi, T.; Meluleni, G.; Muesschenborn; S.S.; Banting, G.; Ratcliff, R.; Evans, M.J. and Colledge, W.H. *Salmonella typhi* uses CFTR to enter intestinal epithelial cells. *Nature*, May 1998, vol. 393, no. 7, p. 79-82.

APPENDIX

FIGURES

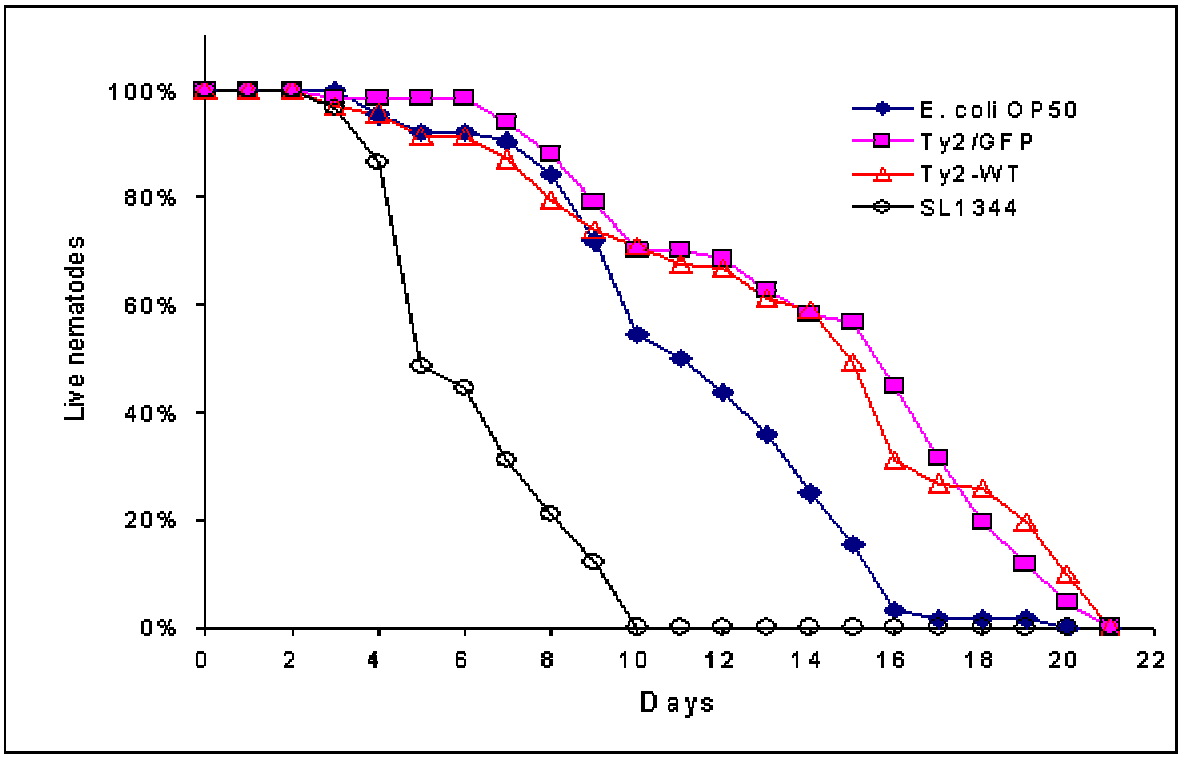

Figure 1. Mortality of *C. elegans* in the presence of *E. coli* OP50 (◆); *S. typhi* Ty2/GFP (■); *S. typhi* Ty2 (△); *S. typhimurium* SL1344 (○).

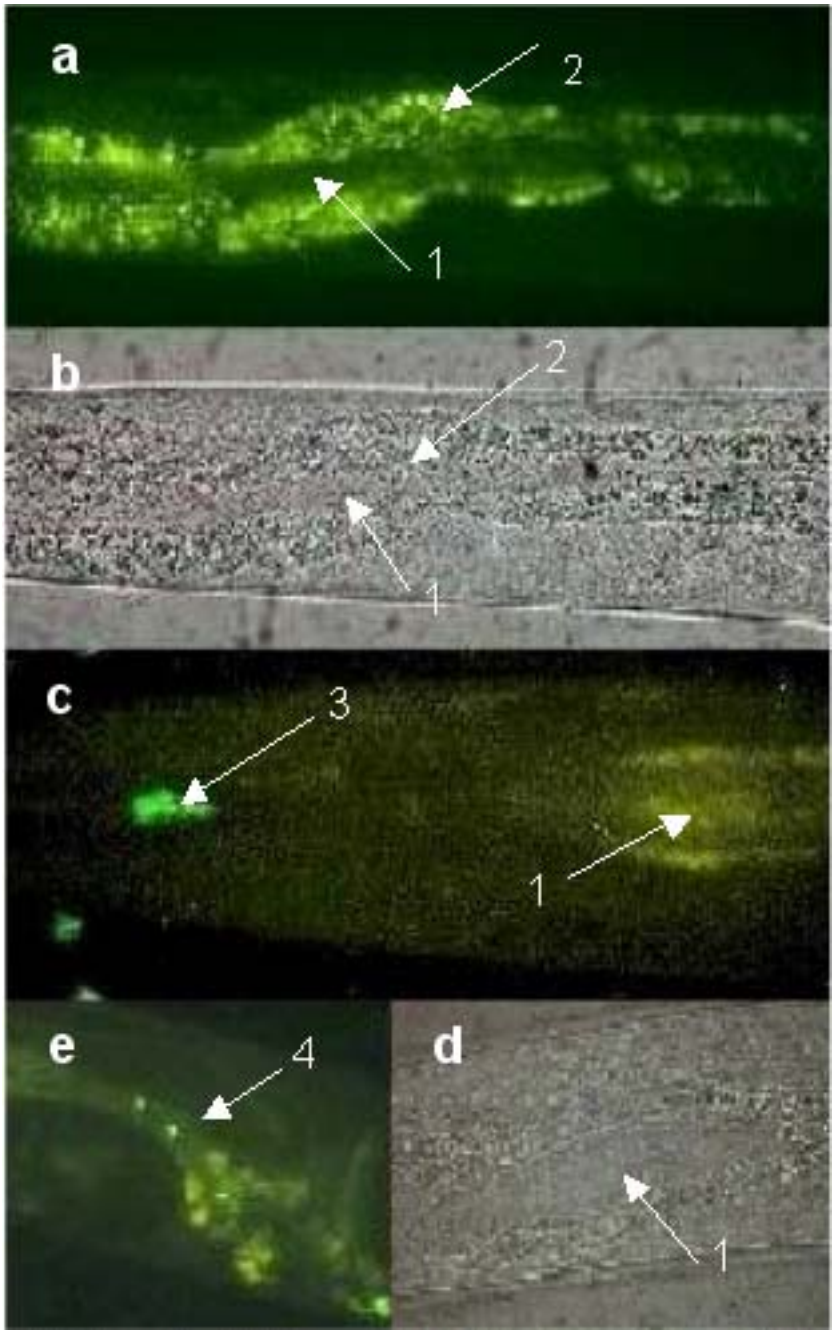

Figure 2. Location of GFP tagged bacteria in the *C. elegans* intestine.

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.
2. Intestinal lining invaded by *S. typhi* Ty2 pSU2007.
3. *S. typhimurium* SL1344 pSU2007 in the pharynx of *C. elegans*.
4. *E. coli* MT102 pSU2007 in the intestine of *C. elegans*.