

# ***Caenorhabditis elegans* is a model host for *Salmonella typhimurium***

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**The idea of using simple, genetically tractable host organisms to study the virulence mechanisms of pathogens dates back at least to the work of Darmon and Depraitère [1]. They proposed using the predatory amoeba *Dictyostelium discoideum* as a model host, an approach that has proved to be valid in the case of the intracellular pathogen *Legionella pneumophila* [2]. Research from the Ausubel laboratory has clearly established the nematode *Caenorhabditis elegans* as an attractive model host for the study of *Pseudomonas aeruginosa* pathogenesis [3]. *P. aeruginosa* is a bacterium that is capable of infecting plants, insects and mammals. Other pathogens with a similarly broad host range have also been shown to infect *C. elegans* [3,4]. Nevertheless, the need to determine the universality of *C. elegans* as a model host, especially with regards pathogens that have a naturally restricted host specificity, has rightly been expressed [5]. We report here that the enterobacterium *Salmonella typhimurium*, generally considered to be a highly adapted pathogen with a narrow range of target hosts [6], is capable of infecting and killing *C. elegans*. Furthermore, mutant strains that exhibit a reduced virulence in mammals were also attenuated for their virulence in *C. elegans*, showing that the nematode may constitute a useful model system for the study of this important human pathogen.**

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## **Results and discussion**

Under normal laboratory conditions, *C. elegans* feeds on *Escherichia coli* (strain OP50 [7]). When we replaced this bacterium with the strains 12023 (American Type Culture Collection catalogue number 14028), SF530 (UK1 [8]) or SL1344 [9] of *S. typhimurium*, we observed that the worms had a significantly shorter life span (Figures 1,3, and data not

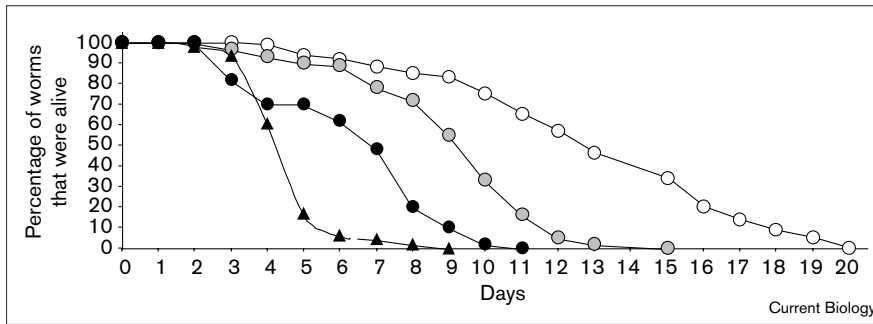
shown). Before their untimely death, the worms appeared visibly sick, but they did not show any sign of starvation.

To follow the fate of the bacteria upon ingestion, we used a strain of *S. typhimurium* that expresses the green fluorescent protein (12023 *ssaV*-GFP [10]) and is as virulent as the wild type as regards the killing of *C. elegans*. In *C. elegans*, the grinder, located in the terminal bulb of the pharynx, normally serves to break down particulate material, such as bacteria [11]. During the first day of contact with the bacteria, very few passed the grinder. After this time, increasing numbers of bacteria were seen to accumulate in the lumen of the intestine, such that after 5 days it appeared distended and full of fluorescent bacteria (Figure 2a,b). This increase in volume of the lumen was accompanied by a marked decrease in the volume of the intestinal cells. At the same time, the cells of the terminal bulb of the pharynx were progressively destroyed and their place taken up by bacteria (Figure 2c,d). The further spread of bacteria was most likely prevented by the basement membrane that surrounds the entire pharynx. Interestingly, when worms were fed on *E. coli* expressing GFP (OP50-GFP), bacteria were also found in the intestine after just 2 days, but the intestinal cells retained their normal morphology. This suggests that the shrinkage seen following colonisation by *S. typhimurium* is a specific effect and may be linked to the decreased life span of infected worms. In the presence of OP50-GFP, the pharynx was intact up until the eighth day (data not shown) and was then slowly destroyed by the bacteria, reflecting presumably the normal senescence of the worms.

The pathogenic effect of *S. typhimurium* required live bacteria: worms fed on heat-killed bacteria showed no symptoms of sickness and had a life span indistinguishable from that of worms fed on *E. coli* strain OP50 (data not shown). It appears that *S. typhimurium* is able to establish a stable colonisation of *C. elegans*. Worms were exposed to the bacteria for limited periods of time, then washed, transferred to OP50 and their survival monitored. As little as 8 hours contact with *S. typhimurium* was sufficient to reduce the survival of *C. elegans*; 24 hours contact give a time-course of survival that was very close to that of worms in permanent contact with *S. typhimurium* (Figure 1 and data not shown).

As the grinder may serve to protect *C. elegans* from potential pathogens, we hypothesised that worms with a defective grinder would be less resistant to the pathogenic effects of *S. typhimurium*. Indeed, the strain *phm-2*, which

Figure 1



Survival of *C. elegans* fed on *E. coli* and *S. typhimurium*. Wild-type worms (circles) or *phm-2* mutants (triangles) were fed on *E. coli* strain OP50 until the larval L4 stage and then kept on OP50 (open circles) or transferred to *S. typhimurium* strain 12023. These latter worms were either kept on 12023 (black symbols), or after 8 h surface sterilised with Thimerosal and returned to OP50 (grey circles). Worms were judged to be dead when they no longer responded to a light touch. In each experiment, 50 or 100 worms were used. The worms were cultivated at 25°C throughout.

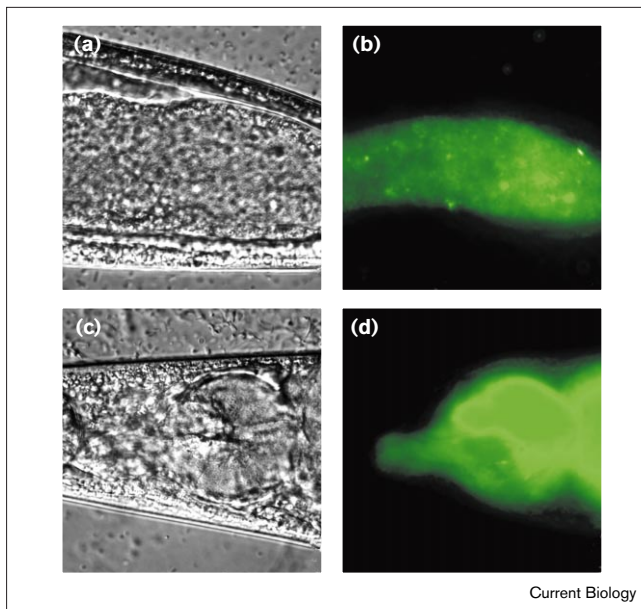
has an abnormal terminal bulb [12], was significantly more susceptible (Figure 1). During the infection of both wild-type and *phm-2* worms, the bacteria did not invade the intestinal cells but remained extracellular, in contrast to the situation seen during infection of their mammalian hosts [13]. As mentioned above, the strain 12023 *ssaV*-GFP was as virulent as its parental strain with regards the infection of *C. elegans*. The *ssaV* gene is one of the 13 genes that make up the *Salmonella* pathogenicity island 2 (SPI2; [14]), and encodes a type III secretion apparatus. SPI2 is a major determinant of *Salmonella* pathogenicity in mice, but is apparently dispensable during the infection of

*C. elegans*. It remains to be determined whether other SPI2 genes, or those involved in the SPI1 type III secretion system [15], are needed for full pathogenicity in the nematode model.

An important aspect of the physiology of *S. typhimurium* is its capacity to withstand acid pH, both during its passage through the alimentary tract and subsequently when it is within a phagolysosome [13]. We therefore tested two strains harbouring mutations in the genes *fur-1* [16] and *ompR* [17], which are known to be involved in different aspects of acid tolerance in *S. typhimurium*. There was a significant reduction in virulence of both mutants when compared with the parental strain (Figure 3). The stationary-phase sigma factor sigma S (RpoS) has been shown to be required for a sustained acid-tolerance response in virulent *S. typhimurium* [18]. The *rpoS* mutant strain JF2690 was found also to be significantly attenuated for its virulence in *C. elegans* (data not shown). Independently, the laboratory of F. Ausubel has also shown that numerous strains of *S. typhimurium* are able to infect *C. elegans* and has identified mutants that are attenuated for their virulence [19].

*S. typhimurium* is an enteropathogenic bacterium that represents a major public health problem. Many innovative strategies have been devised for understanding its mode of action and its interactions with host cells [20,21]. The finding that it is capable of infecting *C. elegans*, and that genes important for its full pathogenicity in vertebrates also play a role during infection of *C. elegans*, opens the possibility of taking a new genetic approach to study *S. typhimurium*. For example, the direct screening of *S. typhimurium* mutants for those that exhibit an attenuation of their virulence is highly feasible. This method has already been applied with great success to *P. aeruginosa* [3]. It may aid the understanding of certain aspects of *S. typhimurium* biology, and the cell biology of its interaction with host cells, such as the epithelial damage that it provokes in mammals [22] or its survival in the intestinal tract, areas of ongoing research [23].

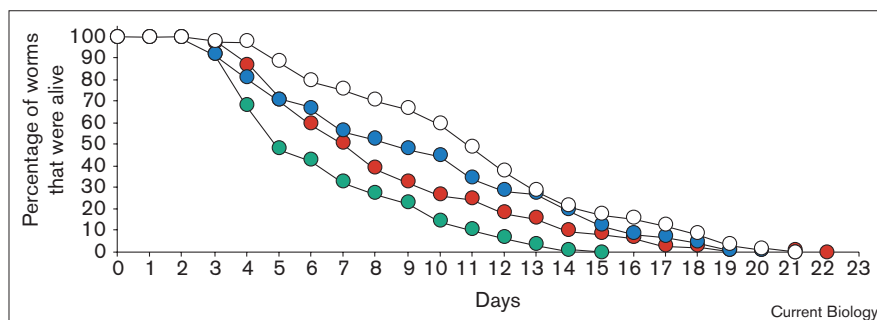
Figure 2



Accumulation of *S. typhimurium* within the intestine and pharynx of *C. elegans*. (a,c) Nomarski and (b,d) fluorescence photomicrographs of the (a,b) posterior and (c,d) anterior of a worm after contact for 5 days with GFP-expressing *S. typhimurium* [10]. The intestine and terminal bulb of the pharynx can be seen to be full of intact bacteria.

Figure 3

Survival of *C. elegans* fed on *S. typhimurium* acid-sensitive mutants. Wild-type worms were fed on *E. coli* strain OP50 until the L4 stage and then kept on OP50 (open circles) or transferred to *S. typhimurium* strain UK1 (green), or *fur-1* (red; JF588) and *ompR* (blue; JF2757) mutants, and the infection followed as in Figure 1. The results are representative of four independent experiments, with 50 worms tested under each condition.



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