



# *Caenorhabditis elegans* for the study of host–pathogen interactions

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The molecular basis of the pathogenicity of infectious agents, and of the corresponding mechanisms of host defence, can be studied using model systems<sup>1</sup>. Intuitively, the more closely related a model of an infectious disease is to the natural pathology, the greater its relevance, hence the reliance on rodents and mammalian tissue culture for *in vivo* and *in vitro* studies of human disease, respectively.

Although it is clear that the exquisite host specificity of certain pathogens, particularly viruses such as HIV and intracellular bacterial pathogens such as *Listeria* and *Salmonella*, restricts the range of possible model hosts, many other pathogens exhibit considerable promiscuity. The plant pathogen *Agrobacterium tumefaciens*, for example, can also infect humans<sup>2</sup>. This suggests that there could be common strategies of microbial pathogenesis, regardless of the host. Conversely, although adaptive immunity is specific to vertebrates, it has become apparent that the underlying mechanisms of innate antimicrobial defences are evolutionarily ancient and have been highly conserved during evolution<sup>3</sup>. There is, therefore, reason to believe that useful insights into host–pathogen interactions can be gained from the use of invertebrates as model hosts, and there are many reasons to promote their use when compared, for example, with the murine model (Table 1).

One of the simplest invertebrate models is the nematode worm *Caenorhabditis elegans*. This small hermaphroditic animal, which grows to 1 mm in length and is normally found in the soil, has been the object of intense study for more than 20 years<sup>4</sup>. *C. elegans* possesses several advantages as a model, including simple growth conditions, rapid generation time with an invariant cell lineage, and the fact that the genetic and molecular tools used for its manipulation are well developed. Importantly, it was the first multicellular animal for which the genome sequence was completed<sup>5</sup>. The *C. elegans* research community is well served by the comprehensive database ACeDB ([http://www.sanger.ac.uk/Projects/C\\_elegans/](http://www.sanger.ac.uk/Projects/C_elegans/)), which integrates genetic and

The nematode worm *Caenorhabditis elegans*, for which the complete genome sequence is available, has several other advantages as an experimental system, and has already been widely used as a model for the study of vertebrate biology. Recent investigations have revealed that *C. elegans* could also be an extremely useful model system in the study of bacterial pathogenesis and have reinforced the notion that common virulence and host defence mechanisms exist.

molecular data, and a catholic Web server maintained by L. Avery (<http://elegans.swmed.edu>). It has become clear that many of the discoveries made with *C. elegans* are relevant to the study of higher organisms. This extends beyond fundamental cellular processes such as transcription, translation, DNA replication and cellular metabolism. For this reason, and because of its intrinsic practical advan-

tages, *C. elegans* has proved to be an invaluable tool for understanding vertebrate neuronal growth and pathfinding, apoptosis and intra- and intercellular signalling pathways, to give but a few examples<sup>4</sup>. Recent studies on the interaction between *C. elegans* and the enterobacterium *Pseudomonas aeruginosa* (reviewed in Ref. 6), suggest that it also represents a useful model for the study of pathogenesis and host defences.

*P. aeruginosa*, an opportunistic human pathogen, is capable of infecting a broad range of both animal and plant hosts. The strain PA14 can kill *C. elegans* in one of two ways. So-called ‘fast killing’ is caused by a diffusible phenazine toxin that provokes a lethal oxidative stress<sup>7</sup>. The bacteria produce this toxin when they are cultured on high-osmolarity medium. If the nematode is exposed to bacteria that have been grown on minimal medium, ‘slow killing’ occurs. This appears to be the consequence of a bacterial infection and takes days rather than hours. By isolating and characterizing *P. aeruginosa* mutants that exhibited reduced virulence in *C. elegans*, Ausubel and colleagues have been able to demonstrate that different genes act as virulence factors in the two pathologies. Significantly, the majority of the virulence factors important in the fast or slow killing of *C. elegans* are also implicated in the virulence of *P. aeruginosa* in a mammalian model system, underlining the existence of common virulence mechanisms<sup>8</sup>. Strikingly, *C. elegans* mutants with an altered sensitivity to oxidative stress exhibit an altered susceptibility to fast killing. Additionally, *C. elegans* mutants that lack P-glycoproteins, which function as toxin efflux pumps, were shown to be more sensitive to

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**Table 1. Comparison between the mouse and nematode as models for the study of host-pathogen interactions**

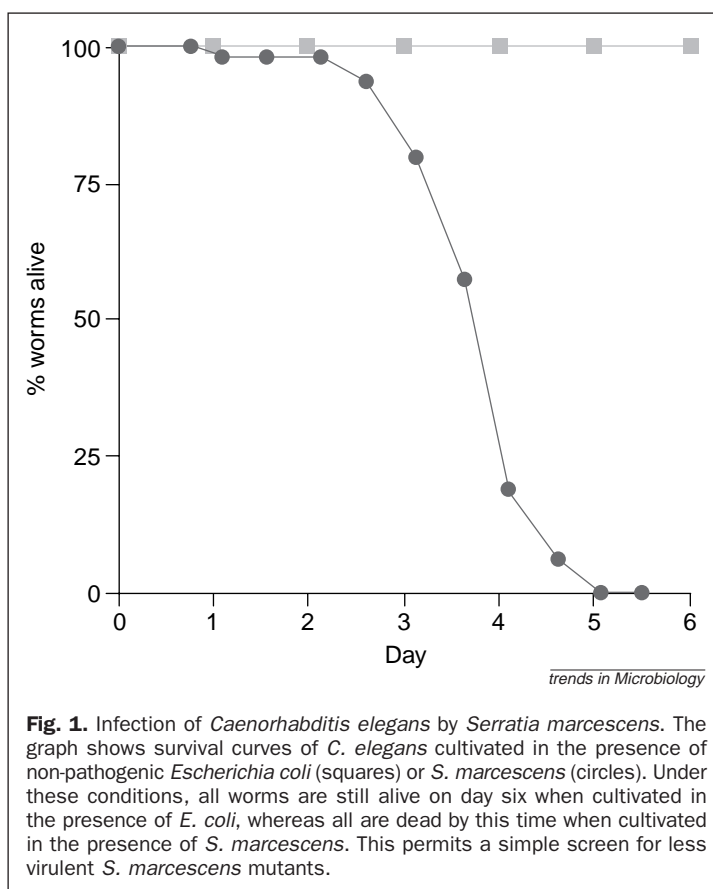
| Property                           | Mouse               | <i>Caenorhabditis elegans</i> |
|------------------------------------|---------------------|-------------------------------|
| Organismal complexity              | High                | Low                           |
| Cost                               | High                | Low                           |
| Size                               | 10 cm               | 1 mm                          |
| Life cycle                         | 8 weeks             | 3 days                        |
| Reproduction                       | Cross fertilization | Self fertilization            |
| Genetic variation <sup>a</sup>     | Moderate to high    | Negligible                    |
| Genome sequence                    | 2003 (?)            | Complete                      |
| Saturating mutagenesis             | Impossible          | Routine                       |
| Genetic mapping                    | Possible            | Routine                       |
| Generation of transgenic lines     | Several months      | Several days                  |
| Generation of functional knockouts | Several months      | Several days <sup>b</sup>     |
| Positional cloning                 | Difficult           | Routine                       |
| Known pathogens                    | Many                | Few <sup>c</sup>              |
| Adaptive immunity                  | Yes                 | No                            |
| Innate immunity                    | Yes                 | Yes                           |
| Biological relevance               | Confirmed           | Potential                     |

<sup>a</sup>Between individuals of the same strain.  
<sup>b</sup>By RNA-triggered gene silencing<sup>13</sup>.  
<sup>c</sup>Ref. 10.

fast killing. In the case of the *P. aeruginosa* strain PAO1, a different, as-yet-undefined toxin, this time under the control of the quorum-sensing regulators LasR and RhIR, provokes a lethal paralysis of *C. elegans* within minutes<sup>9</sup>. Random mutagenesis of *C. elegans* and selection of resistant mutants led to the finding that the protein EGL-9, which is of unknown function but is conserved in vertebrates, is important for susceptibility to paralysis<sup>9</sup>. Interestingly, although fast killing by PA14 is not dependent upon LasR, slow killing is. Indeed, *lasR* is one of 12 *P. aeruginosa* mutants found to be less pathogenic in plant, invertebrate and vertebrate model systems<sup>8</sup>. Taken together, these results illustrate how a genetically amenable system can contribute to the elucidation of the mechanisms of host defence<sup>10</sup>.

Clearly, the use of *C. elegans* as a model for studying pathogenicity will be limited to those pathogens that are able to infect the nematode. Luckily, in this respect, its susceptibility to *P. aeruginosa* appears not to be an isolated case. A second opportunistic human pathogen, *Serratia*, is also pathogenic to nematodes<sup>11</sup>. In humans, *Serratia marcescens* can cause meningitis, endocarditis and pyelonephritis. In the past three decades, there has been a steady increase in nosocomial *S. marcescens* infections, particularly in neonates and immunocompromised patients. Although numerous studies of the mechanisms of its pathogenicity have been undertaken, it is a matter of concern that many *S. marcescens* strains are resistant to multiple antibiotics<sup>12</sup>; current drugs have been unable to stem the increase in incidence of these infections, which can be life threatening. This underscores the need for the development of new investigative approaches.

Like *P. aeruginosa*, *S. marcescens* is capable of killing *C. elegans* either by a toxin-based mechanism (L. Carta, pers. commun.) or following the establishment



**Fig. 1.** Infection of *Caenorhabditis elegans* by *Serratia marcescens*. The graph shows survival curves of *C. elegans* cultivated in the presence of non-pathogenic *Escherichia coli* (squares) or *S. marcescens* (circles). Under these conditions, all worms are still alive on day six when cultivated in the presence of *E. coli*, whereas all are dead by this time when cultivated in the presence of *S. marcescens*. This permits a simple screen for less virulent *S. marcescens* mutants.

of an infection. In the latter case, the bacteria are able to survive within the usually hostile environment of the nematode intestine and proliferate and spread, leading to a systemic infection that kills the host (Fig. 1; C.L. Kurz and J.J. Ewbank, unpublished). Under standard assay conditions, the progression of

the infection is highly reproducible. This allows one to screen *S. marcescens* mutants for those showing reduced virulence. Worms can be cultivated in either 96-well microtitre plates or 24-well culture plates, and thousands of bacterial clones can be individually tested. Such an approach is impossible with mice, owing to constraints of space and the number of animals required. Screens of a *S. marcescens* mutant library produced by transposon insertion (J. Celli, M. Uh and B.B. Finlay, pers. commun.) suggest that, in *C. elegans*, a significant number of genes are involved in virulence. The characterization of these mutants will reveal whether genes encoding potential effectors of pathogenicity, such as chitinases or nucleases, or genes encoding regulators of such virulence factors, have been mutated. Additionally, it is possible to carry out reciprocal screens of *C. elegans* mutants for those displaying enhanced or reduced resistance. The characterization of such mutants should shed light on the host factors involved in defence mechanisms. This capacity to take a two-sided genetic approach holds great promise for the study of the interaction between pathogen and host.

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