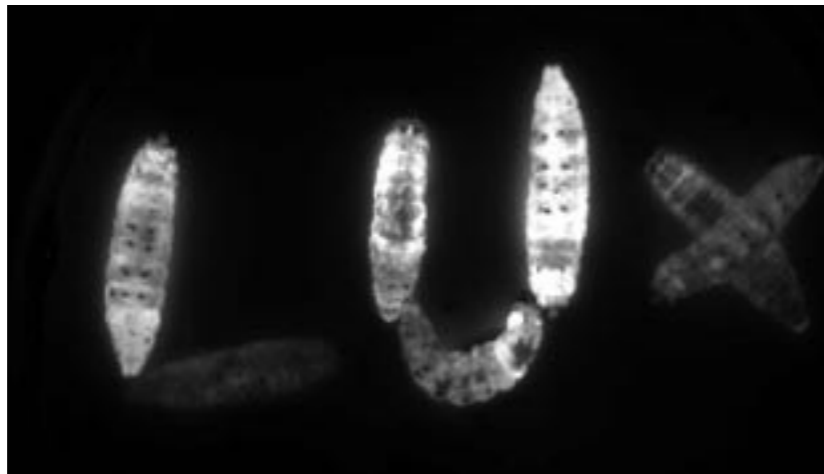


Photorhabdus: shedding light on symbioses

When living in a complex association with a nematode, the bioluminescent bacterium *Photorhabdus* can be deadly to insects, according to **Susan A. Joyce** and **David J. Clarke**.



Next time that you are on the beach, walk into the dunes and take a sample of the sandy soil within the area where the dune grass is growing. Place your soil sample in a flask, add a few insect larvae (readily available from your local bait or pet shop) and the chances are good that the insects will be dead within 2–3 days. Take these dead insects into the darkest room in your house and within 5–10 minutes you should see that some, if not all, of the insect cadavers will glow in the dark. This bioluminescence is due to the presence of the bacterium *Photorhabdus*, a highly virulent insect pathogen (entomopathogen) that you have isolated in a nematode vector from the soil. Together, *Photorhabdus* and the nematode vector (*Heterorhabditis*) form a deadly complex that is naturally lethal to insect larvae.

Photorhabdus are members of the family *Enterobacteriaceae* and are, therefore, quite closely related to familiar pathogens such as *Escherichia coli*, *Salmonella*, *Yersinia* and *Erwinia* spp. To date three species of *Photorhabdus* have been described; *P. luminescens*, *P. temperata* and *P. asymbiotica*. Although *P. luminescens* and *P. temperata* only appear to infect insects *P. asymbiotica* has been found, in a relatively small number

▲ Insect larvae infected with *Photorhabdus luminescens* photographed in a dark room using the light produced by the bacteria. The insect hosts are final instar larvae of the greater waxmoth (*Galleria mellonella*). Susan Joyce (Sand dunes, Photos.com/Jupiter)

of cases, to be associated with infections in humans. These infections, although not fatal, do result in rather serious wounds that are difficult to treat. Fortunately, these cases appear to be restricted to the southern states of the USA (in particular Texas) and the Gold Coast of Australia. However, it is rather intriguing that, under certain conditions *Photorhabdus* does appear capable of ‘jumping’ from insect to human. Interestingly, in the laboratory, most strains of *P. luminescens* and *P. temperata* do not grow at temperatures >32°C, whilst *P. asymbiotica* can grow at 37°C. Therefore, perhaps the high ambient temperature of Texas and the Gold Coast has allowed *P. asymbiotica* to adapt to growth at the relatively high temperature of humans.

Comparing the genomes of insect and human isolates of *Photorhabdus* may uncover some interesting insights into the evolution of mammalian pathogenicity in environmental micro-organisms. To this end, the genome sequence of *P. luminescens* TT01 has been available since 2003 (<http://genolist.pasteur.fr/PhotoList>) and the genome sequence of a strain of

P. asymbiotica has just been completed at the Sanger Centre (www.sanger.ac.uk/Projects/P_asymbiotica/).

Bioluminescence

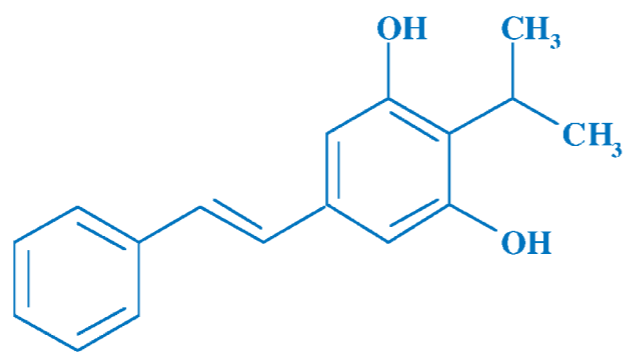
One of the most striking features of *Photorhabdus* bacteria is that they are bioluminescent and, indeed, it is this feature that gave the bacteria their name, i.e. *photo* (referring to the ability to produce light) and *rhabdus* (rod). *Photorhabdus* have a *lux* operon that contains all of the genes required for the production of light from fatty acids and molecular O₂, i.e. *luxCDABE*. The biochemistry of light production in bacteria is well described and it involves the conversion of a fatty acid into an aldehyde through the action of a fatty acid reductase (encoded by *luxC* and *luxD*). This reaction requires a supply of electrons that are delivered to the enzyme by the reduced electron

carrier flavin mononucleotide (FMN). The aldehyde is then bound to the luciferase enzyme (encoded by *luxA* and *luxB*) which, together with O₂, forms an unstable ternary complex that rapidly decomposes, releasing H₂O, fatty acid and energy in the form of photons. The requirement for electrons suggests that one function of light production may be to act as an electron shunt that allows the recycling of essential electron carriers without the production of ATP and/or biomass. Although bacterial bioluminescence is quite common in the marine environment, *Photorhabdus* is the only terrestrial bacterium that is known to produce light. In the sea, many light-producing bacteria are involved in symbioses with marine animals and the role of bacterial bioluminescence can vary from protection (counter-illumination in the *Vibrio*–squid symbiosis) to the

attraction of a mate. Unfortunately the role of bioluminescence in *Photorhabdus* is not yet understood, although, as all isolated *Photorhabdus* strains produce light, it is likely that there is a strong selection for this activity.

The life cycle

Photorhabdus are normally found colonizing the guts of the infective juvenile (IJ) stage of the nematode *Heterorhabditis*. The IJ is a soil-dwelling, motile, non-feeding stage of the nematode whose role is to actively seek out and infect susceptible insect larvae and release the *Photorhabdus* into the insect bloodstream. Here the bacteria begin to divide whilst producing a wide range of toxins and hydrolytic enzymes that serve to kill the insect within 2–3 days and facilitate the conversion of the internal organs and tissues of the insect into an environment where



▲ The molecular structure of 3'-5'-dihydroxy-4-isopropylstilbene (ST). David Clarke

◀ A hand infected with *P. asymbiotica*. John Gerrard / Nick Waterfield

the nematode can reproduce. During development, the nematodes feed on the *Photorhabdus* biomass within the insect cadaver and after 2–3 generations of reproduction, environmental conditions stimulate the development of IJs that are colonized by *Photorhabdus* before the IJ emerges from the insect cadaver into the soil. A single IJ infecting the insect will result in >100,000 IJs emerging from the insect cadaver 2–3 weeks later. The interaction between *Photorhabdus* and *Heterorhabditis* is, in effect, a highly effective symbiosis of pathogens and it is so effective that it is produced and marketed by several companies as a biocontrol agent for a variety of insect pests, in particular the weevil. Therefore, this bacterium has the ability to act as a pathogen in one host (insect) and a mutualist in another host (nematode).

Pathogenicity and toxin production

Photorhabdus is extremely virulent and, in some insect hosts, injection of a single cell is sufficient to kill the host within 2–3 days. *Photorhabdus* are capable of killing a wide range of insects from the Orders Coleoptera (beetles), Lepidoptera (moths, butterflies) and Diptera (flies). However, in the environment the host range appears to be limited by the ability of the IJ to penetrate the potential host. *Photorhabdus* grow exponentially in the insect, achieving high cell densities within the host [approx. 10^9 c.f.u. per *Galleria mellonella* (greater waxmoth) larva] and converting all of the internal organs and tissues of the insect into a biomass that is required for the growth and development of the nematode partner. There is a close correlation between the growth rate of *Photorhabdus* and the time taken to kill the insect, suggesting that pathogenicity is dependent on bacterial growth.

Insects have a very sophisticated innate immune system that has striking parallels with the innate immune system in vertebrates. This system is highly effective at killing bacteria,

and *Photorhabdus* produce toxins that target the cellular branch of innate immunity. Analysis of genome sequence data has revealed that *Photorhabdus* encodes an impressive array of toxins. Indeed studies in *Photorhabdus* have resulted in the identification of a new family of toxins that have since been shown to be found in other bacteria, including mammalian pathogens. The Tc toxins from this family are large protein complexes that can be poisonous when fed to insect larvae. When cloned into the model plant *Arabidopsis*, the Tc toxins provide protection against insect pests, suggesting a potential alternative to Bt toxin.

Mutualism and signal production

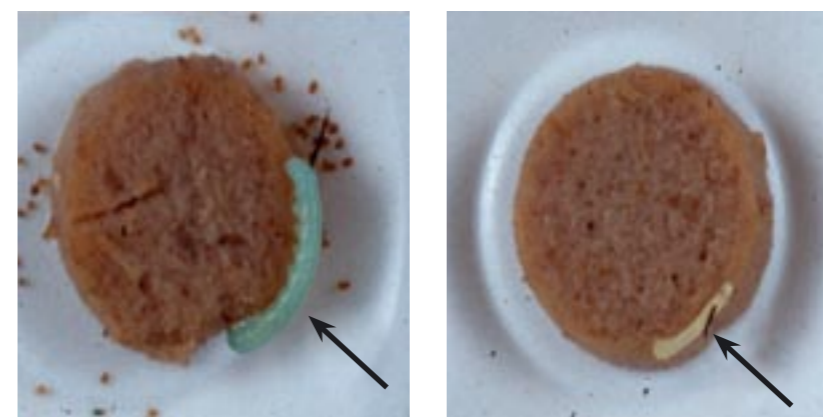
The *Photorhabdus*–*Heterorhabditis* association is highly specific, and nutritional interactions do make a contribution to this specificity. Therefore, a particular isolate of *Heterorhabditis* cannot feed on all *Photorhabdus* bacteria. Nutritional interactions are common in many mutualistic associations, e.g. amino acid exchange between plant cells and *Rhizobium bacteroides*, but the details of these interactions are not yet known in the *Photorhabdus*–*Heterorhabditis* system. In addition, successful colonization of the IJ is essential for both partners in the association, and it has been shown that IJs will only be colonized by the nematodes' cognate bacterial partner (or in some cases a very close relative). *Photorhabdus* is transmitted to the IJ via the mother in a very complex process that results in her infection and death.

During the stationary phase of growth, *Photorhabdus* produces several small bioactive molecules, including a redox-active, anthraquinone pigment and a stilbene molecule, 3'-5'-dihydroxy-4-isopropylstilbene (ST). The ST molecule is produced in large quantities when *Photorhabdus* are grown in either LB broth or insect larvae, and it was originally identified as an antibiotic. It was suggested that



◀ GFP-tagged *Photorhabdus* in the gut of a *Heterorhabditis* IJ. Bars, 50 µm (left) and 20 µm (right). Catherine Easom

▼ The Tc toxins are orally toxic to larvae of *Manduca sexta* (the tobacco hornworm moth). In a typical bioassay, 2nd instar insect larvae are allowed to feed on a wheatgerm-based diet that can be treated with preparations of the Tc toxin complex. The larvae do not feed on the food with the Tc complex added (right) and fail to develop compared with controls (left). Nick Waterfield



From a more fundamental perspective, we expect that the tripartite interaction between *Photorhabdus*, *Heterorhabditis* and insect will also help us to answer a very important question: what is the real role of such bioactive molecules in nature?

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the role of ST was to protect the insect cadaver from saprophytic organisms in the soil. However, recent studies have shown that ST is also an inter-kingdom signal that is involved in controlling the development of *Heterorhabditis* into the IJ stage. The IJ is developmentally analogous to the dauer stage in the model nematode *Caenorhabditis elegans*. In *C. elegans*, dauer formation is a response to the low availability of food and/or high nematode numbers. Therefore, it is possible that ST is perceived by *Heterorhabditis* as an indication that food (i.e. bacteria) is plentiful. The ST molecule is multipotent and has also been shown to dampen the insect immune system so that the nematode and bacteria are invisible to the insect immune system post-infection.

The future is bright (or should that be light-producing!)

Photorhabdus is a Gram-negative bacterium that is equipped for life as a

mutualist in one host and as a pathogen in another, including the occasional human. Its complex life cycle, relatedness to important mammalian pathogens and amenability to genetic manipulation have contributed to the development of this genus as an exciting model for studying bacteria–host interactions. However, *Photorhabdus* is also quickly emerging as a source of novel bioactive compounds. In total there are 22 genetic loci in the genome of *P. luminescens* TT01 that contain genes predicted to be involved in the production of small bioactives, including polyketide synthases (PKS), non-ribosomal peptide synthases (NRPS) or PKS-NRPS. These loci occupy 7% of the TT01 genome compared to 4% in *Streptomyces*, the organism responsible for the production of many important antibiotics. Therefore, it is expected that screening libraries of *Photorhabdus* DNA will identify new bioactive compounds, including toxins and antibiotics.

Further reading

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