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Comparative bacterial genomics and its use in undergraduate education

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Abstract

Photorhabdus spp. and *Xenorhabdus* spp. both form mutualistic associations with entomopathogenic nematodes and function as potent pathogenic agents towards a variety of insects. The availability of genetic approaches and the ability to grow the bacteria in standard media make *Photorhabdus* and *Xenorhabdus* promising models for the study of host–microbe interactions. A particularly attractive aspect of this system is that both harmful and beneficial interactions can be studied in a single microbe. The genome sequence of *Photorhabdus luminescens* subspecies *laumondii* strain TT01 has been completed and a genome project for *Photorhabdus asymbiotica* has been initiated. Likewise, a collaborative project to compare the genomes of two species of *Xenorhabdus* is in progress. The availability of genomic sequences for *Photorhabdus* and *Xenorhabdus* will open new avenues of investigation and enhance our understanding of the molecular mechanism by which a bacterium can function as both symbionts and pathogens. In this paper, the genomic information from the *Photorhabdus* species are compared with the genome of the related species, *Yersinia pestis*, and the usefulness of genomics in undergraduate education will provide excellent training for future scientists and assist in a better understanding of the nematode–bacterium complex.

Keywords: *Photorhabdus*; *Xenorhabdus*; Entomopathogenic nematode–bacterium complex; Mutualism; Genome; Genetics; Host–microbe interactions; College education

1. Introduction

Photorhabdus spp. and *Xenorhabdus* spp. share a symbiotic–pathogenic life cycle that is unique among the gamma subdivision of the Proteobacteria (Akhurst and Boemare, 1990; Forst and Clarke, 2002; Forst et al., 1997; Silva et al., 2002). Both bacteria are carried in the intestine of their respective symbiotic nematode host. To reproduce, the nematode invades the hemocoel of a variety of insects where the bacteria are released into the hemolymph. Here, they transit to the pathogenes stage producing a large variety of virulence factors, which rapidly kill the insect host. The bacteria grow to high cell density and secrete a plethora of degradative enzymes creating a nutrient base for nematode growth and reproduction. During nematode reproduction, the bacteria produce diverse antimicrobial and nematicidal compounds that protect the insect cadaver from invasion by other soil organisms. When the nutritional supply for nematode multiplication is exhausted, the dauer (=infective) juvenile stage of the nematode is formed which is colonized by the bacterium, reestablishing the symbiotic association.

An interesting feature of both *Xenorhabdus* and *Photorhabdus* is the formation of phenotypic variant cells that arise at low frequency during prolonged incubation. The variant cells, referred to as phase II or secondary form cells, are altered in numerous properties normally found in the so-called phase I or primary form isolated from the nematode (Boemare and Akhurst, 1988). While *Photorhabdus* and *Xenorhabdus* are closely related phylogenetically, they exhibit several major differences. For example, *Photorhabdus* is bioluminescent and produces catalase and colored pigments whereas *Xenorhabdus* spp. lack these properties (Forst and Nealson, 1996). Another salient difference

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between the genera is that the secondary variant form of *Xenorhabdus* can support growth and development of nematodes, but the secondary form of *Photorhabdus* does not support nematode growth (Bintrim and Ensign, 1998; Volgyi et al., 2000). *Photorhabdus* form mutualistic associations with *Heterorhabditis* nematodes in which the first generation adults are hermaphroditic. In contrast, *Xenorhabdus* colonize *Steinernema* nematodes in which the first and subsequent generation adults are males and females. *Xenorhabdus* spp. colonize a specialized pouch located in the anterior portion of the nematode intestine, whereas *Photorhabdus* spp. are not localized in a pouch structure but rather colonizes the upper two-third of the nematode intestine (Bird and Akhurst, 1983; Ciche and Ensign, 2003). These observations support the idea that the congruence of the symbiotic–pathogenic lifestyles of *Photorhabdus* and *Xenorhabdus* may have been a result of convergent evolution. Finally, both bacteria are highly adapted to their respective symbiotic and pathogenic hosts and have limited ability in nature to survive outside of the host environment.

Recent genomic analysis of two different subspecies of *Photorhabdus luminescens* (Thomas & Poinar) has provided insight into the diversity of genes encoding virulence factors, secreted proteins, antibiotic production, and cell surface structures. Random genomic sample sequence of *P. luminescens* subspecies *akhurstii* W14 consisted of >2000 random sequence reads (ffrench-Constant et al., 2000). The W14 strain is of particular interest since it produces a high level of cell-free oral toxicity towards the tobacco hornworm, *Manduca sexta* (L.) (Bowen et al., 1998). The first completed sequence of a *Photorhabdus* genome was recently accomplished for *P. luminescens* subspecies *laumondii* TT01, a symbiont of *H. bacteriophora* Poinar HP88 (Duchaud et al., 2003). Interestingly, *P. luminescens* TT01 does not produce cell-free oral toxicity but rather displays cell-associated toxicity (Marokhazi et al., 2003).

Another species of *Photorhabdus*, *P. asymbiotica* Fischer–LeSaux et al., has been isolated from human wounds and does not appear to be associated with a nematode host (Akhurst et al., 1996). It is not clear whether *P. asymbiotica* is carried to the human host via an invertebrate organism such as in the case of a spider bite or exists as a free-living environmental bacterium. The genome of *P. asymbiotica* ATCC43949 is presently being sequenced at the Sanger Institute (www.sanger.ac.uk/Projects/P_asymbiotica/). Comparison of the completed genomes of *P. luminescens* and *P. asymbiotica* will provide an excellent opportunity to elucidate genetic similarities and differences in *Photorhabdus* species that appear to have distinctly disparate life cycles. Comparative genomics will further allow us to address the question of whether new genes were acquired by *P. asymbiotica* that allowed it to infect mammalian hosts. In addition, the comparison between a mutualistic and a putative free-living species of *Photorhabdus* should provide insights into the molecular nature of symbiotic host–microbe interactions. Finally, a collaborative genome sequencing project of two different species of *Xenorhabdus*, *X. nematophila* (Poinar & Thomas) and *X. bovienii* Akhurst, has been recently initiated (http://xenorhabdus.danforthcenter.org/). *X. nematophila* is the symbiont of the nematode *Steinernema carpocapsae* (Weiser), whereas the *X. bovienii* species was recently isolated from a potentially new steinernematid species related to *S. feltiae* (Filipjev) and *S. kushidai* Mamiya (Spiridonov et al., 2004).

Yersinia pestis (Lehmann & Neumann) belongs to the same phylogenetic clade as *Photorhabdus* and *Xenorhabdus*, and its life cycle is similar in several aspects to the latter bacteria. *Y. pestis* is carried into the digestive tract of fleas, that serve as vectors for the bacterium between mammalian hosts. When the infected flea bites a mammalian host, the bacterium is inoculated intradermally into the blood that flows from the wound. At this stage, the bacterium transits from the digestive tract environment of the flea to its pathogenic phase in the host blood stream. *Y. pestis* is believed to have recently evolved from the mammalian enteropathogen *Y. pseudotuberculosis* (Lehmann & Neumann), and like *Xenorhabdus* and *Photorhabdus*, has limited ability to survive outside of the host environment. The genome sequence of two different strains of *Y. pestis* has recently been published (Deng et al., 2002; Parkhill et al., 2001), and the genome sequence of a third strain of *Y. pestis* (*Y. pestis* biovar *mediaevails* strain 91001) has been deposited under the Accession No. NC_005810.

We will focus on *Photorhabdus* species and compare available genomic information with that of *Y. pestis*. In addition, we will discuss how bacterial genomes can be used in undergraduate education.

2. General aspects of the genomes of *Photorhabdus* spp.

Photorhabdus luminescens TT01, sequenced by the Pasteur group headed by Frank Kunst, possesses the largest genome (5.6 Mb) so far sequenced within the Enterobacteriacae family (Table 1). The genome of *P. asymbiotica* is estimated to be 5.5 Mb, suggesting that the larger genomic size is characteristic of this genus. The average genome size of most members of this family is approximately 4.6 Mb (Table 1). The genomes of *X. nematophila* and *X. bovienii*

Table 1
Representative genomes of the gamma subdivision of the Proteobacteria

Organism	Strain	Lifestyle[a]	Genome size (Mb)	G + C content
P. luminescens	TT01	P/S	5.6	42.8%
P. asymbiotica[b]	ATCC 43494	P	5.5	42%
E. coli	O157:H7	P	5.5	50.8%
S. typhimurium	LT2	P	4.8	53%
E. coli	K12	F	4.6	50.8%
Y. pestis	CO92	P	4.6	47.6%
X. nematophila[b]	ATCC 19061	P/S	4.5	~45%
X. bovienii[b]	85831	P/S	4.2	~45%
P. mirabilis[b]	NA	P	3.9	NA
B. floridanus	NA	ES	0.71	27.4%

[a] P/S = pathogenic and symbiotic; P = pathogenic; F = free-living; ES = endosymbiotic.
[b] Genomic sequence analysis is in progress.