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How the insect pathogen bacteria *Bacillus thuringiensis* and *Xenorhabdus/Photorhabdus* occupy their hosts

Christina Nielsen-LeRoux1, Sophie Gaudriault2,3, Nalini Ramarao1, Didier Lereclus1 and Alain Givaudan2,3

Insects are the largest group of animals on earth. Like mammals, virus, fungi, bacteria and parasites infect them. Several tissue barriers and defense mechanisms are common for vertebrates and invertebrates. Therefore some insects, notably the fly *Drosophila* and the caterpillar *Galleria mellonella*, have been used as models to study host–pathogen interactions for several insect and mammal pathogens. They are excellent tools to identify pathogen determinants and host tissue cell responses. We focus here on the comparison of effectors used by two different groups of bacterial insect pathogens to accomplish the infection process in their lepidopteran larval host: *Bacillus thuringiensis* and the nematode-associated bacteria, *Photorhabdus* and *Xenorhabdus*. The comparison reveals similarities in function and expression profiles for some genes, which suggest that such factors are conserved during evolution in order to attack the tissue encountered during the infection process.

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**Introduction**

Several entomopathogenic bacteria are known [1*,2*] but the most studied are *Bacillus thuringiensis*, *Photorhabdus* and *Xenorhabdus*. *B. thuringiensis* is a Gram-positive spore-forming bacterium that has been used for more than 50 years as bio-insecticide against insect pests in crop and forestry [3,4]. *B. thuringiensis* is closely related to *Bacillus anthracis*, the etiological agent of anthrax, and to *Bacillus cereus*, an opportunistic human pathogen causing food-borne gastroenteritis [5]. *Photorhabdus* and *Xenorhabdus* species include Gram-negative symbiotic bacteria associated with entomopathogenic nematodes also used as alternatives to pesticides to control numerous insect pests [6].

Most studies have focused on the specific insecticidal toxins produced by these bacteria (*Cry* and *Cyt* toxins for *B. thuringiensis* [3,7] and *Tc* toxins for *Photorhabdus* and *Xenorhabdus* [8]). The role of other virulence and adaptation factors has been less investigated. Here, we highlight what is actually known about the factors necessary to cope with host tissue and host defence mechanisms. We aim to point out common infection strategies used by these bacteria. The issue is also to show that insect models, larger than *Drosophila* [9], mainly lepidopteran larva can also be useful to improve the understanding of bacterial infection processes [10*],11*,12*].

**Infection process**

A bacterial pathogen must be able to colonize its host by coping with different mechanical and physical barriers as well as cellular and humoral defence mechanisms (see Box 1). In nature, *B. thuringiensis* spores or vegetative cells, are taken up by the larva by ingestion or more accidentally by wounding through the cuticle (see Figure 1 for a description of *B. thuringiensis*-insect interaction). By contrast, natural infection involving *Photorhabdus/Xenorhabdus* mainly starts from the body cavity, since they are released at that site from the nematode hosts (see Figure 1 for a description of *Photorhabdus/Xenorhabdus* infection process). Then, the two groups of bacteria discussed here will deal with the various larval compartments/tissues at different moments post infection. Therefore, we describe, in the following sections, the expression and effects of various bacterial factors, at different stages of the infectious process.

**Gut adaptation and intestinal barrier degradation**

*Photorhabdus* and *Xenorhabdus* are vectored by the nematode through the gut barrier and get direct access to the hemocoel. However, the two bacteria have orally active insecticidal toxins (see Box 2). For *B. thuringiensis*, the oral infection is the norm and success to breach the gut barrier is mainly depending on the action of *Cry* toxins, which both permit *B. thuringiensis* spores to germinate and also to weaken the midgut cells. However the relative importance of *Cry* toxins and the bacteria itself depends on the insect species [13] (see Figure 1 and Box 2 for details). In
2 Ecology

Box 1 Larval anatomy and basic defense.

The lepidopteran larvae commonly used for infection analysis are Galleria mellonella, Spodoptera liturata and Manduca sexta. The first insect barrier to overcome from the environment is the cuticle (skin), next the digestive tract, which is composed of a mouth, a proventriculus, a forutig and a midgut and a hindgut. A sort of protective chitin rich web called peritrophic matrix, which separates the insect midgut from direct contact to food and pathogens [64], covers a large part of this ‘tube’ (see also Figure 1). The digestive tube is surrounded by the hemocoe (larval body cavity), which is mainly composed of hemolymph (blood). In the hemocoe are located fat body cells (liver function), circulating hemocytes and humoral immune factors. Hemocytes are key elements in cellular immune responses, which include phagocytosis, nodulation and encapsulation. Humoral factors include, among others, antimicrobial peptides, lysozyme, clotting mechanisms and proteophenoxidase, which are involved in the melanization process [65]. Antimicrobial peptides and lysozyme can also be found in the gut. Thus, if an infection by B. thuringiensis results in generalized septicemia, it indicates that the bacteria have been capable to survive and develop inside the digestive tube, notably by competing with natural gut flora, and had won access to the insect hemocoe by degrading of the peritrophic matrix and midgut cells. Survival and development in the hemocoe of B. thuringiensis, Photorhabdus and Xenorhabdus indicate that these bacteria produce factors, which can cope with the above mentioned cellular and humoral immune effectors.

Beside the plasmid-encoded Cry toxins (see Box 2), the B. thuringiensis genome contains many genes, which might take part in gut pathogenesis. The role of the PlcR regulon in virulence of B. thuringiensis and B. cereus was demonstrated by using G. mellonella larvae [16]. Indeed, deletion of the global transcriptional activator, plcR, drastically reduces bacterial virulence in orally infected insects, but not by injecting the bacteria into the larval hemocoe [16,17], indicating that the PlcR regulon plays a key role in pathogenicity in the early stage of infection by oral route. PlcR controls the expression of 45 genes encoding secreted virulence factors including the pore forming enterotoxins complexes (Hbl and Nhe), hemolysins known for cytotoxic and hemolytic activities, antimicrobial peptides, and various degradative enzymes, which might allow the bacteria to develop in the intestinal environment and to damage the intestinal barrier [18]. Histological analyses indicate that B. thuringiensis colonize and damage the gut epithelium resulting in rupture of the gut integrity and the crossing of the gut barrier. Interestingly, a plcR mutant is blocked at the gut epithelial and transcriptional gfp promoter fusions showed that PlcR-regulated factors are expressed in the insect gut (Nielsen-LeRoux, unpublished data). However, deletion of enterotoxin genes (Nhe, Hbl complexes and CytK) did not significantly alter the insecticidal properties of B. thuringiensis towards three lepidopteran insects [19]. Then, further studies are

Box 2 Orally active insecticidal toxins and degradation of gut barriers, what else?

The B. thuringiensis-based insecticidal products are a mixture of spores and insecticidal toxins forming a crystal inclusion. These toxins, Cry (also called delta endotoxins) and Cyt proteins, are often encoded by plasmid carried genes. They are active against larval stages of many insect orders and other invertebrates like nematodes [66]. The mode of action of Cry toxin has been extensively studied for many insect species. The general procedure is shown in Figure 1 and several families of specific midgut receptors have been identified [67]. Meanwhile other indirect roles of Cry toxins: like the binding to the peritrophic matrix [68] or the possible interaction with the commensal gut microbiota in some insects [69] need more attention. Cry toxins are present in a lower number of strains than Cry toxins and they are less specific as they interfere with several classes of cells including mammals. In many insects, the Cry toxin alone can kill the insect larvae (used in transgenic crops expression the Cry toxins) while for others the presence of the bacteria is needed [70]. Genes encoding Vip toxins (vegetative insecticidal proteins) have been found in various B. thuringiensis strains, but the expression and mode of action have been poorly studied (see [http://www.lifesci.ussex.ac.uk/Home/Neil_Crickmore/Bt] website for updated number of toxins). Although the main role of these toxins are related to gut cell interactions in the living insect, an eventual role during expression in the insect cadaver cannot be excluded. Although Xenorhabdus and Photorhabdus directly enter within hemocoe following the release by nematode hosts, these bacteria, like B. thuringiensis, have oral insecticidal toxins (such as the ‘toxin complex(es)’ (Tc) and the ‘Photorhabdus insect-related’ (Pr) toxins) triggering massive damage to the gut epithelium of insect [8]. Despite intensive research on insecticidal toxin family, their possible involvement in a precise step of the life cycle of the bacterial symbionts remains enigmatic. Indeed, P. luminescens Tc toxins inhibit phagocytosis of bacteria when added in insect hemocyte monolayers and recently, the biologically active components of the Tc toxins have been characterized as ADP-ribosyltransferases, which modify actin and Rho GTPases [71]. Because Tc toxins have also insecticidal activities after injection into insects, it is likely that the Tc-dependent inhibition of phagocytosis takes place in the hemolymph during bacterial infection. More surprisingly is the discovery of oral insecticidal activity of the universally occurring chaperon molecule GroEL in X. nematophila. GroEL protein has no effect when injected into the hemocoe or when added to cultures of insect hemocytes [72]. It is noteworthy that the XnGroEL protein and the insecticidal Tc-like complexes of the insect pathogen, Yersinia entomophaga, have chitin binding and chitinase activities, respectively. Busby et al. (2011) [73] propose that chitina protein form an integral part of the Tc complexes in Photorhabdus and Xenorhabdus as well. It is tempting to speculate that chitina activity acting on the peritrophic membrane may help toxins or bacteria to reach gut epithelial cells. According to the biology of nematode symbionts, we can also propose that chitina associated toxins may be also required in late infection in the cadaver compartment by contributing to the degradation of the insect cadaver exoskeleton. Moreover, Tc-like encoding genes have been recently found in some B. thuringiensis strains indicating a conserved and probably important function of these toxins, which would merit further attention in the B. thuringiensis life cycle [74].
General natural larval infection and life cycles of *B. thuringiensis, Photorhabdus* and *Xenorhabdus*. *B. thuringiensis* is primary an oral pathogen and the spores along with the crystal toxin are acting together to kill the insect. When ingested by a susceptible insect, the crystal inclusions are solubilized in the gut and protoxins are activated by digestive enzymes (steps 1, 2, and insert). The activated toxins (small blue circles) are then able to cross the peritrophic barrier and to bind different classes of midgut receptors (Box 2, insert and [7]). This results in the formation of pores in the epithelial midgut cells and impairment of intestinal function. This creates favorable conditions for spore-germination and development of vegetative growth (steps 2 and 3). Bacteria multiply and produce various virulence factors allowing access to the hemocoel [58]. They adapt to this new compartment and produce new virulence factors to combat the host defenses (step 3). This may allow the bacteria to cause septicemia, resulting in insect death (step 4). Within the host cadaver the *B. thuringiensis* cells have to cope with novel growth conditions to complete their development, ending with the production of resistant spores and Cry toxin crystals able to disseminate in the environment to start a new cycle of infection in another host. *Xenorhabdus* and *Photorhabdus* spp. are pathogens of numerous insects and are mutualists of nematodes from the family *Heterorhabditidae* and *Steinernematidae*, respectively. The life cycle of *Photorhabdus* and *Xenorhabdus* begins and ends with the colonization of the intestinal tract of non-feeding stage of the nematode known as the infective juvenile (IJ) [51]. Actually, the IJ stage nematodes generally use natural openings of insect larvae (mouth and anus) as main routes of entry (step 1), and end up in digestive tract (gut: step 2) before entering in the hemocoel (step 3). However, the nematode vector of *Photorhabdus*, *Heterorhabditis*, can also enter into host’s body by puncturing the larval cuticle (for review see [62]). Within the hemocoel, nematodes release their bacterial symbionts that undergo insect infection (step 3). In the insect cadaver, occur bacterial multiplication and nematode reproduction (step 4). During this life cycle, *Photorhabdus* and *Xenorhabdus* must successfully accomplish three distinct roles within the insect host: (i) overcome insect immune response, and kill insects, (ii) produce nutrients from the insect cadaver to facilitate development of the nematode, and (iii) colonize the IJ stage of the nematode [63]. After rounds of nematode reproduction, progeny nematodes receive uncharacterized environmental cues that stimulate the development of a new generation of IJs colonized by the bacteria before emerging of several hundred thousand IJs from an insect cadaver.

needed to show the precise role of the Pler-regulated factors in insect gut infection, like searched by a RT-PCR analysis [20]. In addition, to understand exactly how the bacteria cross the gut, including the peritrophic matrix (see Box 1), it is important to fully elucidate the relative impact of Cry toxins and the other *B. thuringiensis* bacterial factors, (like the metalloprotease Enhancin [21]), on these barriers.
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Inside the hemocoel
Entomopathogenic bacteria entering in the hemolymph compartment have to face the insect immune system consisting of several defensive mechanisms that parallel many aspects of the vertebrate innate immune system (see Box 1).

Facing the humoral immunity
As part of the humoral response, insects produce a range of AMPs that target bacterial envelope components [22]. P. luminescens and B. thuringiensis counteract the humoral AMP response through constitutive modifications of their surface properties. In Salmonella, the addition of 4-aminoarabinose moieties on the negatively charged lipopolysaccharide LPS, requires the products of the two component system PhoPQ-regulated pmrHFIJKLM operon [23,24]. In P. luminescens, mutation of either phoP or the pmrK homologue, phoE1, resulted in both an increased sensitivity to cationic AMPs and decreased virulence towards insects [25,26]. The dlt operon encodes proteins that alinate the teichoic acids on the surface of Gram-positive bacteria, which neutralizes their negative charge and thus prevent attack by cationic AMPs. In B. cereus, and B. thuringiensis, a dlt null mutant is sensitive to molecules from the insect humoral immune system such as lysozyme, and the cationic AMP, cecropin, from the Fall Armyworm, Spodoptera frugiperda and shows reduced virulence towards lepidopteran insects [27*]. The strategy used by X. nematophila to counteract cationic AMPs is to prevent their production: cecropin transcript levels are induced after injection of non-pathogenic Salmonella enterica, but not after injection of X. nematophila [28,29]. This suppression effect is under the control of the global regulators Lrp and GpxR [30**], but the effectors responsible for the interference have yet to be identified. Many entomopathogenic bacteria secrete proteases that degrade antimicrobial peptides. The role of the B. thuringiensis metalloprotease InhA1 in inhibition of AMP activity and full virulence following injection into the hemocoel have been reported [31]. Likewise, the Photorhabdus metalloprotease PrtA destroys commercial cecropins A and B and inhibits insect antibacterial activity when inoculated within lepidoptera [32]. Therefore, both P. luminescens and B. thuringiensis/B. cereus are recognized by the insect immune system but overcome it by counteracting the action of antimicrobial peptides by multiple ways.

Facing the cellular immunity
Photorhabdus, Xenorhabdus and B. thuringiensis also interfere with insect cellular immunity. The cellular response is mediated by circulating hemocytes and includes both phagocytosis and nodulation (see Box 1). The latter response involves the aggregation of hemocytes around the invading microorganisms. Photorhabdus, Xenorhabdus and B. thuringiensis produce an array of cytolysins/hemolysins and toxins, some of which have been shown to induce necrosis or apoptosis when added to cultures of insect-immune cells (see Table 1). P. luminescens has a type III secretion system (TTSS) required for resistance to phagocytosis and encapsulation in nodules. This locus encodes an effector molecule (LopT), which is a GTPase-modifying cytoxin. Interestingly, the expression of the lopT gene coincides with the TTSS-dependent inhibition of phagocytosis in the course of insect infection, indicating a possible role for LopT to prevent uptake of P. luminescens by hemocytes [33]. By contrast, the genomes of Xenorhabdus nematophila and Xenorhabdus bovienii do not encode homologues of a dedicated TTSS [34]. Nevertheless, the X. nematophila flagellar secretion system (flagellar TTSS) is necessary for secretion of at least one non-flagellar protein, a lipase [35–37]. In addition, the flagellar regulator, FlhZ, controls the expression of the XxAXB and XhLAB hemolysins encoding-genes [36]. XxAXB is the prototype of a new family of hemolysins with apopotic and pore-forming activities in mammalian and invertebrate cells [38]. The second FlhZ-dependent hemolysin, XhLA, is a cell surface-associated hemolysin belonging to the two-partner secretion system family [39]. While XhLA and XxAXB recombinant proteins mediated lysis of insect immune cells [38,39], only XhLA has been shown to be required for the full virulence of X. nematophila in lepidoptera. B. thuringiensis also produces an hemolysin, HlyII, which is a pore forming toxin able to induce lysis of mouse macrophages and insect hemocytes by apoptosis [40]. A protein (CwpFM) with homology to cell wall peptidases in several bacteria, notably B. subtilis, was shown to be important for full virulence in Galleria, and to induce macrophage vacuolization. However its precise role during insect infection still remains to be elucidated [41]. InhA1, is a metalloprotease localized in the Bacillus exosporium, and allows B. thuringiensis spores to escape from murine macrophages [42]. A similar role in the insect cells still needs investigations. Thus, these factors allow B. thuringiensis to counteract the bactericidal properties of host phagocytic cells. As for Photorhabdus and Xenorhabdus, a mutation in the flagellar apparatus of B. thuringiensis has a pleiotropic effect. Indeed, the deletion of FlhA flagellar body protein resulted in decrease in virulence following both oral and hemocoel infection, probably owing to the combined effect of reduced cell adherence and less secreted virulence factors [43].

Competing with the host for iron
Another part of innate host response to infection is the sequestering of iron sources to limit bacterial growth [44]. Several factors involved in iron acquisition contribute to the virulence of B. thuringiensis and Photorhabdus (see Table 1). The recently identified surface protein of B. thuringiensis, IlsA [12**], is involved in iron uptake from ferritin and heme. An IVET system and gfp transcriptional fusions showed that the ilsA gene is specifically transcribed when bacteria are within the hemocoel and the ilsA mutant is affected in its virulence towards Galleria
Table 1  

**B. thuringiensis, Photorhabdus and Xenorhabdus effectors influencing the infection process in lepidopteran larvae**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Class of effectors</th>
<th>Mutant virulence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Potential site(s) of effect and/or target(s) in the insect&lt;sup&gt;b&lt;/sup&gt;</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulators</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. thuringiensis</em></td>
<td>PlcR</td>
<td>Quorum-sensing pleiotropic regulator, early phase of infection</td>
<td>I: VIR O: NON-VIR</td>
<td>Activates the expression of 45 genes, intestinal cell cytotoxicity</td>
</tr>
<tr>
<td></td>
<td>NprR</td>
<td>Quorum-sensing pleiotropic regulator</td>
<td>I: VIR O: VIR</td>
<td>Nutriment acquisition, saprophytic development</td>
</tr>
<tr>
<td></td>
<td>Fur</td>
<td>Ferric uptake regulator</td>
<td>O: ATT</td>
<td>Regulates the expression of genes involved in iron uptake and virulence</td>
</tr>
<tr>
<td><em>Photorhabdus</em></td>
<td>PhoPQ</td>
<td>Two component system</td>
<td>I: NON-VIR</td>
<td>AMP resistance</td>
</tr>
<tr>
<td></td>
<td>LuxS</td>
<td>Quorum-sensing auto-inducer 2 synthesis protein</td>
<td>I: ATT</td>
<td>ROS resistance</td>
</tr>
<tr>
<td></td>
<td>HcaR</td>
<td>LysR-type transcriptional regulator</td>
<td>I: ATT</td>
<td>ROS resistance</td>
</tr>
<tr>
<td></td>
<td>HexA</td>
<td>LysR homolog A regulator</td>
<td>I: ATT</td>
<td>Production of lipases and antimicrobial activities</td>
</tr>
<tr>
<td><em>Xenorhabdus</em></td>
<td>FhD</td>
<td>Master regulon of the flagellar cascade</td>
<td>I: ATT</td>
<td>Inhibition of protease and antimicrobial activity production</td>
</tr>
<tr>
<td></td>
<td>LrhA</td>
<td>LysR homolog A regulator, positive activator of fhdI</td>
<td>I: ATT</td>
<td>Production of hemolysins, motility</td>
</tr>
<tr>
<td></td>
<td>Lrp</td>
<td>Leucine-responsive regulatory protein, positive activator of lrhA and fhdI</td>
<td>I: ATT</td>
<td>Production of hemolysins, motility</td>
</tr>
<tr>
<td></td>
<td>CpxRA</td>
<td>Signal transduction system, positive activator of lrhA</td>
<td>I: ATT</td>
<td>Production of lipases</td>
</tr>
</tbody>
</table>

**Insecticidal toxins (effect of purified toxins and/or mutants)**

| *Photorhabdus/ Xenorhabdus* | Tc/Xpt (whole complex) | Insecticidal protein complex (proteins A, B, C) | Oral toxicity of the PP | Degradation of intestinal epithelial cells | [71,82–84] |
| *Photorhabdus* | TcA                | Tetrameric homoprotein forming a pore and probably involved in TcC translocation | After oral ingestion of the PP, degradation of insect intestinal epithelial cells | Degradation of intestinal epithelial cells; expressed in the gut | [11*,83] |
|             | PirAB              | Insecticidal toxin (Cry and juvenile hormone esterase domains) | Oral toxicity of the PP | Toxicity after hemocoel injection | [85,86] |
|             | Pit                | Insecticidal toxin (Cry34 domain) | | | [87] |
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Mutant virulencea</th>
<th>Class of effectors</th>
<th>Potential site(s) of effect and/or target(s) in the insectb</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Xenorhabdus</strong></td>
<td></td>
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<tr>
<td>GroEL</td>
<td>Oral toxicity of the PP</td>
<td>Molecular chaperone</td>
<td></td>
<td>[72]</td>
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<tr>
<td>MxA</td>
<td>Chitin binding activity</td>
<td>Soluble monomeric pilin</td>
<td></td>
<td>[88]</td>
</tr>
<tr>
<td><strong>Hemolysins</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><strong>B. thuringiensis</strong></td>
<td></td>
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</tr>
<tr>
<td>HlyII</td>
<td>Induces apoptosis of insect hemocytes, Hemolytic activity in low-iron conditions (Photobacterium) or in high-iron conditions (Xenorhabdus)</td>
<td>Pore forming toxin, virulence factor</td>
<td>I: ATT</td>
<td>[40,90]</td>
</tr>
<tr>
<td><strong>Photobacterium/ Xenorhabdus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhIA/XhIA</td>
<td>Hemolytic activity in low-iron conditions (Photobacterium) or in high-iron conditions (Xenorhabdus)</td>
<td>Hemolysin belonging to the two-partner secretion (TPS) family</td>
<td>I: VIR (Photo)</td>
<td>[10**,39,91]</td>
</tr>
<tr>
<td><strong>Xenorhabdus</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>XaxAB</td>
<td>Necrotic and apoptotic activities on insect hemocytes</td>
<td>Binary cytotoxin</td>
<td>I: VIR</td>
<td>[10**,38]</td>
</tr>
<tr>
<td><strong>Effectors interfering with eukaryotic signals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B. thuringiensis</strong></td>
<td></td>
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</tr>
<tr>
<td>SPS</td>
<td>Specifically expressed in the gut</td>
<td>Involved with Glucose-6-phosphate uptake</td>
<td>I: VIR</td>
<td>[104]</td>
</tr>
<tr>
<td>IlsA</td>
<td>Specifically expressed in the hemocoel, iron regulated</td>
<td>Involved in iron acquisition from heme and ferritin</td>
<td>I: ATT</td>
<td>[12**,45**]</td>
</tr>
<tr>
<td><strong>Photobacterium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yvrc</td>
<td>PP triggers inhibition of proliferation and apoptosis in insect cell</td>
<td>Iron dicitrate uptake</td>
<td>O: ATT</td>
<td>[92]</td>
</tr>
<tr>
<td><strong>Antimicrobial molecules</strong></td>
<td></td>
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</tr>
<tr>
<td><strong>Photobacterium</strong></td>
<td></td>
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</tr>
<tr>
<td>Hydroxy-stilbene</td>
<td>Inhibition of phenoloxidase activation</td>
<td>Antimicrobial molecule whose production is phenylalanine ammonia-lyase (SilA) and polyketide synthetase-dependant</td>
<td>I: ATT (silA mutant)</td>
<td>[55]</td>
</tr>
</tbody>
</table>
### Xenorhabdus

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzylidene-acetone</td>
<td>Monoterpenoid compound</td>
<td>Inactivates phenoloxidase and phospholipase A2</td>
</tr>
<tr>
<td>Xenorhabdicin</td>
<td>Phage-tail bacteriocin encoded by a P2-like tail synthesis gene cluster</td>
<td>Protects the nematode against Gram negative competitors</td>
</tr>
</tbody>
</table>

### Enzymes involved in envelope modification and membrane proteins

<table>
<thead>
<tr>
<th>B. thuringiensis</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dlt ABCD</td>
<td>Proteins required for the D-alanylation of lipoteichoic acids (confers positive charge)</td>
</tr>
<tr>
<td>CwpFM</td>
<td>Cell Wall peptidase</td>
</tr>
<tr>
<td>FlhA</td>
<td>Basal component of the flagella apparatus</td>
</tr>
<tr>
<td>PbgP1P2P3P3E1E3E3</td>
<td>Responsible for the biosynthesis of L-aminoarabinose and its ligation onto the lipid A moiety of LPS</td>
</tr>
</tbody>
</table>

### Extra-cellular enzymes

<table>
<thead>
<tr>
<th>B. thuringiensis</th>
<th>Description</th>
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<tbody>
<tr>
<td>InhA1</td>
<td>Metalloprotease</td>
</tr>
<tr>
<td>InhA2</td>
<td>Metalloprotease</td>
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<tr>
<td>InhA3</td>
<td>Metalloprotease</td>
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<tr>
<td>NprA</td>
<td>Metalloprotease</td>
</tr>
<tr>
<td>Clp</td>
<td>Proteases ClpP1 (Intracellular ATP-protease activity)</td>
</tr>
<tr>
<td>MpbE</td>
<td>Enhancer</td>
</tr>
<tr>
<td>PrtA</td>
<td>Serralysin-like metalloprotease</td>
</tr>
<tr>
<td>PrtS</td>
<td>M4 metalloprotease</td>
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<tr>
<td>SodA</td>
<td>Superoxide dismutase</td>
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<th>Action</th>
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<tbody>
<tr>
<td>PP</td>
<td>Resistance to AMPs</td>
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<td></td>
<td>Resistance to AMPs</td>
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<tr>
<td>Pertinax</td>
<td>Resistance to AMPs, allows bacterial escape from macrophages</td>
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<tr>
<td></td>
<td>Resistance to AMP</td>
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<tr>
<td></td>
<td>Belongs to the NprR regulon</td>
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<td></td>
<td>NprR regulon, degrades host tissue components</td>
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<tr>
<td></td>
<td>Potentially involved in nutrient acquisition</td>
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<tr>
<td>PP</td>
<td>Implicated in stress resistance and growth at low temperature</td>
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<tr>
<td>PP</td>
<td>Degradation of AMPs by the PP</td>
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<td></td>
<td>Localization in conjunctive tissue in dead insect</td>
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<tr>
<td>PP</td>
<td>Induction of melanization reaction by the PP</td>
</tr>
<tr>
<td>PP</td>
<td>Resistance to oxidative stress</td>
</tr>
</tbody>
</table>

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*a* by injection into the hemocoel; *O:* per os; *VIR:* full virulence; *ATT:* attenuated; *NON-VIR:* non virulent at tested doses.

*b* AMP: anti-microbial peptides; ROS: reactive oxygen species; PP: purified protein.
The regulation of the hlyII hemolysin gene mentioned above might involve the ferric uptake repressor, Fur [46]. HlyII induces lysis of cells known to sequester iron during infection, and may thus participate in the release of iron. Furthermore, it was proposed that iron might constitute a signal to bacteria for the transition between the living insect infection and the cadaveric stage. Indeed, Jubelin et al. [10**] found that expression of shbF, a X. nematophila siderophore-related gene, was strongly upregulated in the early stages of infected larvae and downregulated in moribund larvae. The monitoring of virulence gene expression during insect infection process also revealed that xaxAB and xhBA hemolysin genes are expressed concomitantly with the increase in iron availability detected at the time of insect death suggesting that iron constitutes a signal governing X. nematophila adaptation to shifting host environments.

In the cadaver
Degradation of the host tissues
In addition to the PlcR-regulated proteins, B. thuringiensis strains produce several chitinases [47], which might be involved in degradation of the chitin rich peritrophic matrix and parts of cuticle. The recently described quorum sensor NprR activates the transcription of a gene encoding an extracellular protease, NprA, which is the main extracellular component during sporulation (in vitro) [48]. The massive production of this neutral protease might provide nutrients allowing the bacterial population to achieve a complete sporulation, which takes place during the late stage of the infection in the insect cadaver. Moreover, it was shown that NprR controls the expression of several genes encoding chitinases. These enzymes and a lipopeptide also regulated by NprR, contribute to the development of B. thuringiensis during the late stage of infection promoting the saprophytic lifestyle of B. thuringiensis [49**]. Likewise, the metalloprotease InhA1 is produced during stationary phase [50] and may then contribute to the degradation of host tissue. Xenorhabdus and Photorhabdus also secrete several proteins such as proteases, lipases, hemolysins and chitinases that may participate in the transformation of the insect cadaver into nutrients [30**,51]. Immuno-histochemistry revealed that on the basolateral side of the gut (facing the hemocoel), P. luminescens abundantly secretes two virulence factors, the gut-active toxin complex A (Tca) and the metalloprotease, PrtA, which may facilitate the rapid destruction of the gut tissue. Moreover, PrtA is detectable in the late step of septicemia when the insect is already dead [11] and may consequently participate in the digestion of host components. By contrast, a recent study shows that an ortholog of Photorhabdus PrtA peptidase is produced by Xenorhabdus in the early stage of infection in Galleria mellonella [52].

Elimination of competitors and emergence from the cadaver
The current view is that, in the insect cadaver, the numerous antimicrobial molecules (bacteriocins, secondary metabolites) produced by Xenorhabdus and Photorhabdus eliminate bacteria that might antagonize the growth of the nematode partner and compete for nutrient resources [53]. This was actually demonstrated for the phage-tail like bacteriocin of X. nematophila that is required for protecting the nematode partner, Steinernema carpocapsae, against bacterial competitors in G. mellonella [54*]. Interestingly, among those antimicrobial molecules, the hydroxy-stilbene, produced by P. luminescens, and the benzylideneacetone, produced by X. nematophila, have a dual function both as inhibitors of the phospholipase activity and as antimicrobials against microbial competitors in the insect cadaver [55,56]. Chitinases of Xenorhabdus and Photorhabdus may also be required at the late stage of infection in the cadaver to inhibit fungi invaders [57]. Likewise, a number of antimicrobial products including the PlcR-regulated peptides Spp may play a role both in the gut environment and in the cadaver to compete against invaders and commensal organisms. This also suggests that some PlcR-regulated factors might be functional at various steps during the B. thuringiensis lifecycle. It has been demonstrated that a NprR-dependant lipopeptide is essential to ensure the survival of B. thuringiensis in the insect cadaver [49**]. However, the precise role of this molecule remains to be elucidated. The NprR-regulated chitinases could also contribute to the degradation of the insect cadaver exoskeleton and to the dispersion of B. thuringiensis spores and crystals in the environment [58].

Future challenges
Over the past, the field of insect–microbes interaction has made spectacular progress revealing the sophisticated dialogue between bacterial pathogens and the infected host cells. Insects provide powerful infection models to identify virulence and adaptation factors and toxins from pathogenic bacteria [12**,59]. Here we show that three entomopathogenic bacteria share common effectors although parts of their infection strategies are different (Table 1 and Figure 1). Some factors contribute to pathogenesis at several infection sites and stages, suggesting both a local adaptation and different regulatory mechanisms of these effectors. Notably, the role of pore-forming hemolysins and metalloproteases are crucial elements, as also recently reported for another entomopathogenic bacteria, Pseudomonas entomophila [60]. Today, the challenge is to study the properties of bacteria in the dynamic context of their interaction with host throughout the infection process. We believe that these insect models can be used to study various cutting edge aspects like co-infections, notably the interplay with commensal flora and other pathogens. Furthermore, insects provide an experimentally tractable organism and intra-vital imaging techniques enable the temporal and spatial visualization of events occurring within the full complexity of the living hosts. Meanwhile, to reach a real level of pathogen–insect dialogue, genetics tools and genome
sequencing of the lepidopteran infection models (Spodoptera littoralis, Galleria mellonella, Manduca sexta) are needed. To increase the understanding of the infectious cycle at the tissue level, it is tempting to propose that these bacteria and the caterpillar host are further tools in ‘tissue microbiology’ [61]. Indeed, by the association of several molecular, cellular, histo-pathological and imaging approaches, these small animals permit to visualize various tissue barriers on the same histological sample section. In addition, as several factors essential for tissue degradation and host cell death are similar to mammal pathogen effectors ( pore-forming toxins and immune evasion compounds), the study of the bacteria–insect interactions is possibly a powerful approach to provide new valuable insights into factors and mechanisms involved in human pathogenesis.

Acknowledgements

Thanks to INRA departments MICA and SPE for financial support; and to former and actual group members as well as to the many colleagues whose work is contributing to the exciting area of science highlighted in this review.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest
•• of outstanding interest


A nice overview of bacterial insect pathogen issues with future studies and that highlights especially results from studies involving Drosophila immune responses to a few infective bacteria of this insect.


This new review (book chapter, most parts have free Web access) gives a real good update to the best studied bacterial insect pathogens, including the three highlighted in this review. Especially the mode of action of B. thuringiensis Cry toxins are nicely illustrated.


This is the first study investigating the dynamics of virulence gene expression during insect infection and nematode association by carrying out real-time expression analysis using an unstable GFP. These findings shed further light on the role of the flagellar region in the Xenorhabdus life cycle and on the role of iron as a signal governing bacterial adaptation to shifting host environments.


For the first time, this study shows the specific location of Photorhabdus luminescens between the extracellular matrix and basal membrane of the midgut epithelium during infection of Manduca sexta. Bacterial expression of the gut-active Toxin complex A (Tca) and the RTX-like metalloprotease (Prta) in this gut niche is also documented.


Describes a successful use of a positive promoter trap system (IVET) for identification of genes expressed during the infection process in Galleria mellonella larvae.


10 Ecology


55. The R-type bacteriocin (xenorhabdcin) is shown to be produced in the hemocoel of insects infected with Xenorhabdus nemataphila. Xenorhabd- dicin was required for killing the potential competitor Photorhabdus luminescens and protecting the nematode partner.


Bacterial insect pathogens and their hosts
Nielsen-LeRoux et al.


This review is not concerned with insect pathogens but highlights the new concept of ‘tissue microbiology’ covering several approaches needed to get an overall understanding of an infection process.


Ecology


