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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 foodborne 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 larvae 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 woundong 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
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**Box 1 Larval anatomy and basic defense.**

The lepidopteran larvae commonly used for infection analysis are *Galleria mellonella*, *Spodoptera littoralis* 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 proventricule, a foragut, 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 hemocoeel (larval body cavity), which is mainly composed of hemolymph (blood). In the hemocoeel 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 prophenoloxidase, 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 sepsicaemia, 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 hemocoeel by degrading of the peritrophic matrix and midgut cells. Survival and development in the hemocoeel of *B. thuringiensis*, *Photorhabdus* and *Xenorhabdus* indicate that these bacteria produce factors, which can cope with the above mentioned cellular and humoral immune effectors.

The gut, the newly germinated vegetative bacteria need to compete with the commensal gut microbiota and to resist to host factors like antimicrobial peptides in order to persist in the larval gut lumen. The factors involved in these resistance mechanisms are surface properties like the lysozyme, resistant peptidoglycan structure of *B. thuringiensis* and the production of bacteriocins [14], which can also explain the reduction of the gut microbiota predominantly composed of *Enterococcus* sp. in lepidopteran larvae [15].

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 hemocoeel [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.

**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 insert 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. Cyt 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 hemocoeel following the release by nematode hosts, these bacteria, like *B. thuringiensis*, have oral insecticidal toxins (such as the ‘toxin complexes’ (*Tc*) and the *Photorhabdus* insect-related (*Ph*) toxins) triggering massive damage to the gut epithelium of insect [9]. Despite intensive research on insecticidal toxin family, their reliable involvement in a precise step of the life cycle of the bacterial symbionts remained 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 hemocoel 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 chitinase proteins form an integral part of the *Tc* complexes in *Photorhabdus* and *Xenorhabdus* as well. It is tempting to speculate that chitinase 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 chitinase 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].

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
needed to show the precise role of the PtcR-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-aminoarabino moieties on the negatively charged lipopolysaccharides LPS, requires the products of the two component system PhoPQ-regulated pmrHIJKLM operon [23,24]. In P. luminescens, mutation of either phoP or the pmrK homologue, phgE1, 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 CpxR [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 Photobacterium 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
Photobacterium, 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. Photobacterium, 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 encapulation in nodules. This locus encodes an effector molecule (Lop’T), which is a GTPase-modifying cytotoxin. Interestingly, the expression of the lop’T gene coincides with the TTSS-dependent inhibition of phagocytosis in the course of insect infection, indicating a possible role for Lop’T 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 XaxAB and XhlAB hemolysins encoding-genes [36]. XaxAB 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 XaxAB 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 Photobacterium 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 Photobacterium (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</th>
<th>Potential site(s) of effect and/or target(s) in the insect</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 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>
<td>[16,18]</td>
</tr>
<tr>
<td></td>
<td>NprR Quorum-sensing pleiotropic regulator</td>
<td>I: VIR O: VIR</td>
<td>Regulates the expression of genes involved in iron uptake and virulence</td>
<td>[48,49**]</td>
</tr>
<tr>
<td></td>
<td>Fur Ferric uptake regulator</td>
<td>O: ATT</td>
<td>Nutriment acquisition, saprophytic development</td>
<td>[46]</td>
</tr>
<tr>
<td><strong>Photorhabdus</strong></td>
<td>PhoPQ Two component system</td>
<td>I: NON-VIR</td>
<td>AMP resistance</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>LuxS Quorum-sensing auto-inducer 2 synthesis protein</td>
<td>I: ATT</td>
<td>ROS resistance</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>HcaR LysR-type transcriptional regulator</td>
<td>I: ATT</td>
<td>ROS resistance</td>
<td>[76]</td>
</tr>
<tr>
<td></td>
<td>HexA LysR homolog A regulator</td>
<td>I: ATT</td>
<td>Production of lipases</td>
<td>[77]</td>
</tr>
<tr>
<td><strong>Xenorhabdus</strong></td>
<td>FhD Master regulon of the flagellar cascade</td>
<td>I: ATT</td>
<td>Production of hemolysins, motility</td>
<td>[78]</td>
</tr>
<tr>
<td></td>
<td>LrhA LysR homolog A regulator, positive activator of fhd</td>
<td>I: ATT</td>
<td>Production of hemolysins, motility</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td>Lrp Leucine-responsive regulatory protein, positive activator of lrhA and fhd</td>
<td>I: ATT</td>
<td>Production of lipases and antimicrobial activities</td>
<td>[79]</td>
</tr>
<tr>
<td></td>
<td>CpxRA Signal transduction system, positive activator of lrhA</td>
<td>I: ATT</td>
<td>Inhibition of hemolysin production; AMP resistance and nodules formation</td>
<td>[80]</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Class of effectors</th>
<th>Mutant virulence</th>
<th>Potential site(s) of effect and/or target(s) in the insect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vip Vegetative Insecticidal Proteins, pore forming toxins</td>
<td>O: ATT</td>
<td>Degradation of insect intestinal epithelial cells</td>
<td>[81]</td>
</tr>
<tr>
<td><strong>Photorhabdus/ Xenorhabdus</strong></td>
<td>Tc/Xpt (whole complex) Insecticidal protein complex (proteins A, B, C)</td>
<td>Oral toxicity of the PP</td>
<td>Phagocytosis inhibition effect of the PP on insect hemocytes</td>
<td>[71,82–84]</td>
</tr>
<tr>
<td><strong>Photorhabdus</strong></td>
<td>TcA Tetrameric homoprotein forming a pore and probably involved in TcC translocation</td>
<td>After oral ingestion of the PP, degradation of insect intestinal epithelial cells</td>
<td>Degradation of intestinal epithelial cells; expressed in the gut</td>
<td>[11*,$83]</td>
</tr>
<tr>
<td></td>
<td>PirAB Insecticidal toxin (Cry and juvenile hormone esterase domains)</td>
<td>Oral toxicity of the PP</td>
<td></td>
<td>[85,86]</td>
</tr>
<tr>
<td></td>
<td>Pit Insecticidal toxin (Cry34 domain)</td>
<td></td>
<td>Toxicity after hemocoel injection</td>
<td>[87]</td>
</tr>
</tbody>
</table>
### Table 1 (Continued)

<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>Xenorhabdus</strong></td>
<td>GroEL</td>
<td>Molecular chaperone</td>
<td>Oral toxicity of the PP Chitin binding activity</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>MxA</td>
<td>Soluble monomeric pilin</td>
<td>Oral toxicity of the PP</td>
<td>Pore forming-toxin activity of PP</td>
</tr>
<tr>
<td><strong>Hemolysins</strong></td>
<td>B. thuringiensis</td>
<td>Hbl</td>
<td>Tripartite toxin composed of 3 proteins B, L1 and L2. Pore forming toxin.</td>
<td>O: VIR Hemolytic and cytotoxic to epithelial (mammals)</td>
</tr>
<tr>
<td></td>
<td>HlyII</td>
<td>Pore forming toxin, virulence factor</td>
<td>I: ATT</td>
<td>Induces apoptosis of insect hemocytes, Hemolytic activity in low-iron conditions (Photorhabdus) or in high-iron conditions (Xenorhabdus)</td>
</tr>
<tr>
<td>Photorhabdus/</td>
<td>XylA/XhlA</td>
<td>Hemolysin belonging to the two-partner secretion (TPS) family</td>
<td>I: VIR (Photo) I: ATT (Xeno)</td>
<td></td>
</tr>
<tr>
<td><strong>Xenorhabdus</strong></td>
<td>XaxAB</td>
<td>Binary cytotoxin</td>
<td>I: VIR</td>
<td>Necrotic and apoptotic activities on insect hemocytes Late expression in cadaver</td>
</tr>
<tr>
<td><strong>Effectors interfering with eukaryotic signals</strong></td>
<td>B. thuringiensis</td>
<td>SPS</td>
<td>Involved with Glucose-6-phosphate uptake</td>
<td>I: VIR Specifically expressed in the gut O: VIR</td>
</tr>
<tr>
<td></td>
<td>IlbA</td>
<td>Involved in iron acquisition from heme and ferritin</td>
<td>I: ATT O: ATT</td>
<td>Specifically expressed in the hemocoel, iron regulated</td>
</tr>
<tr>
<td>Photorhabdus</td>
<td>YrC</td>
<td>Iron dicitrate uptake</td>
<td>O: ATT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cif</td>
<td>Cyclomodulin</td>
<td>I: VIR</td>
<td>PP triggers inhibition of proliferation and apoptosis in insect cell</td>
</tr>
<tr>
<td></td>
<td>ScTC</td>
<td>Secretin of a type 3 secretion system (TTSS)</td>
<td>I: VIR</td>
<td>Nodule formation and phagocytosis by macrophage cells</td>
</tr>
<tr>
<td></td>
<td>LopT</td>
<td>Effector of the type 3 secretion system (TTSS)</td>
<td>I: VIR</td>
<td>PP triggers the release of RhoA and Rac from insect cell membrane</td>
</tr>
<tr>
<td></td>
<td>Mcf</td>
<td>Protein with BH3 domain, which is sufficient to allow Escherichia coli both to persist within and kill an insect (makes the caterpillars floppy)</td>
<td>I: VIR</td>
<td>PP triggers apoptosis in the midgut epithelium PP triggers apoptosis in hemocytes and inhibition of phagocytosis</td>
</tr>
<tr>
<td></td>
<td>ExbD</td>
<td>Component of the TonB complex involved in uptake of siderophores</td>
<td>I: ATT</td>
<td>Virulence of the mutant rescued by FeCl&lt;sub&gt;3&lt;/sub&gt; injection</td>
</tr>
<tr>
<td></td>
<td>YfeABCD</td>
<td>Divalent cation transporter</td>
<td>I: ATT</td>
<td>Virulence of the mutant rescued by FeCl&lt;sub&gt;3&lt;/sub&gt; injection</td>
</tr>
<tr>
<td><strong>Antimicrobial molecules</strong></td>
<td>Photorhabdus</td>
<td>Hydroxy-stilbene</td>
<td>Antimicrobial molecule whose production is phenylalanine ammonia-lyase (SliA) and polyketide synthetase-dependant</td>
<td>I: ATT (sliA mutant) Inhibition of phenoloxidase activation Antibiotic activity against Gram negative and positive bacteria</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Mutant virulence

<sup>b</sup> Potential site(s) of effect and/or target(s) in the insect
<table>
<thead>
<tr>
<th>Organism</th>
<th>Monomeridene-acetone</th>
<th>Monoterpenoid compound</th>
<th>PP inactivates phenoloxidase and phospholipase A2</th>
<th>Antimicrobial molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenorhabdus</td>
<td>Phage-tail bacteriocin encoded by a P2-like tail synthesis gene cluster</td>
<td>Proteins required for the D-alylation of lipoteichoic acids (confers positive charge)</td>
<td>Resistance to AMPs</td>
<td>[27*]</td>
</tr>
<tr>
<td>Xenorhabdicin</td>
<td>Cell Wall peptidase</td>
<td>Cell Wall peptidase</td>
<td>Resistance to AMPs</td>
<td>[27*]</td>
</tr>
<tr>
<td>FliA</td>
<td>Basal component of the flagella apparatus</td>
<td>Early phase of infection, PteR regulated</td>
<td>Resistance to AMPs</td>
<td>[27*]</td>
</tr>
<tr>
<td>Photorhabdus</td>
<td>PbgP1P2P3P3E1E3E3</td>
<td>Responsible for the biosynthesis of L-aminoarabinose and its ligation onto the lipid A moiety of LPS</td>
<td>Resistance to AMPs</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>B. thuringiensis FliA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. thuringiensis CwpFM</td>
<td>Cell Wall peptidase</td>
<td>Cell Wall peptidase</td>
<td>Resistance to AMPs</td>
<td>[27*]</td>
</tr>
<tr>
<td>B. thuringiensis InhA1</td>
<td>Metalloprotease</td>
<td>Metalloprotease</td>
<td>Resistance to AMP, allows bacterial escape from macrophages</td>
<td>[31,42]</td>
</tr>
<tr>
<td>InhA2</td>
<td>Metalloprotease</td>
<td>Metalloprotease</td>
<td>Resistance to AMP</td>
<td>[97]</td>
</tr>
<tr>
<td>InhA3</td>
<td>Metalloprotease</td>
<td>Metalloprotease</td>
<td>Resistance to AMP</td>
<td>[97]</td>
</tr>
<tr>
<td>NprA</td>
<td>Metalloprotease</td>
<td>Metalloprotease</td>
<td>Resistance to AMP</td>
<td>[97]</td>
</tr>
<tr>
<td>Clp</td>
<td>Proteases ClpP1 (Intracellular ATP-protease activity)</td>
<td>Proteases ClpP1</td>
<td>Early phase of infection, PteR regulated</td>
<td>[27*]</td>
</tr>
<tr>
<td>Photorhabdus</td>
<td>PrtA Serralysin-like metalloprotease</td>
<td>Serralysin-like metalloprotease</td>
<td>Degradation of AMPs by the PP</td>
<td>[101,21]</td>
</tr>
<tr>
<td>PrtS</td>
<td>M4 metalloprotease</td>
<td>M4 metalloprotease</td>
<td>Induction of melanization reaction by the PP</td>
<td>[103]</td>
</tr>
<tr>
<td>SodA</td>
<td>Superoxide dismutase</td>
<td>Superoxide dismutase</td>
<td>Resistance to oxidative stress</td>
<td>[76]</td>
</tr>
</tbody>
</table>

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

AMP: anti-microbial peptides; ROS: reactive oxygen species; PP: purified protein.
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[45**]. 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 xshF, 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 xshBA 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 basal 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 phenoloxidase 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 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 coinfections, 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 mammalian 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


2. Jurat-Fuentes J, Jackson T: Bacterial entomopathogens. In Insect Pathology, edn Second. Edited by Vega F, Kaya H: Elsevier; 2012:286-349. 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 regulon 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.


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This study demonstrated that the dlt mutant of Bacillus cereus has a attenuated phenotype when injected into the hemocoele of Galleria mellonella and Spodoptera littoralis. It is also the first report of the presence of a dlt-like locus in Gram-negative bacteria including Photorhabdus luminescens.


This review emphasizes regulatory cascades involved in coordinating transitions between various stages of the X. nematophila life cycle: infection, reproduction and transmission.


This study shows a visual in vivo localized expression, using GFP promoter fusion, of a factor responding to iron depleted environment of the insect hemocoele. It also highlights for the first time the interaction of a bacterial surface protein and host ferritin.


This article highlights that the pathogenic and necrotrophic lifestyles of B. thuringiensis occur successively during the infectious cycle. It shows that the quorum sensor NprR triggers the production of a lipopeptide allowing B. thuringiensis to survive and eventually sporulate in the host cadaver.


The R-type bacteriocin (xenorhabdincin) is shown to be produced in the hemocoele of insects infected with Xenorhabdus nematophila. Xenorhabdincin 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.


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