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Current Opinion in Microbiology

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

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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 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 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

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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 forgut, 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 hemocoel (larval body cavity), which is mainly composed of hemolymph (blood). In the hemocoel 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, clothing 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 septicaemia, 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 hemocoel by degrading of the peritrophic matrix and midgut cells. Survival and development in the hemocoel 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 hemocoel [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 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 interferes 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.sussex.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 hemocoel 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' (Pir) toxins) triggering massive damage to the gut epithelium of insect [8]. 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 on 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].

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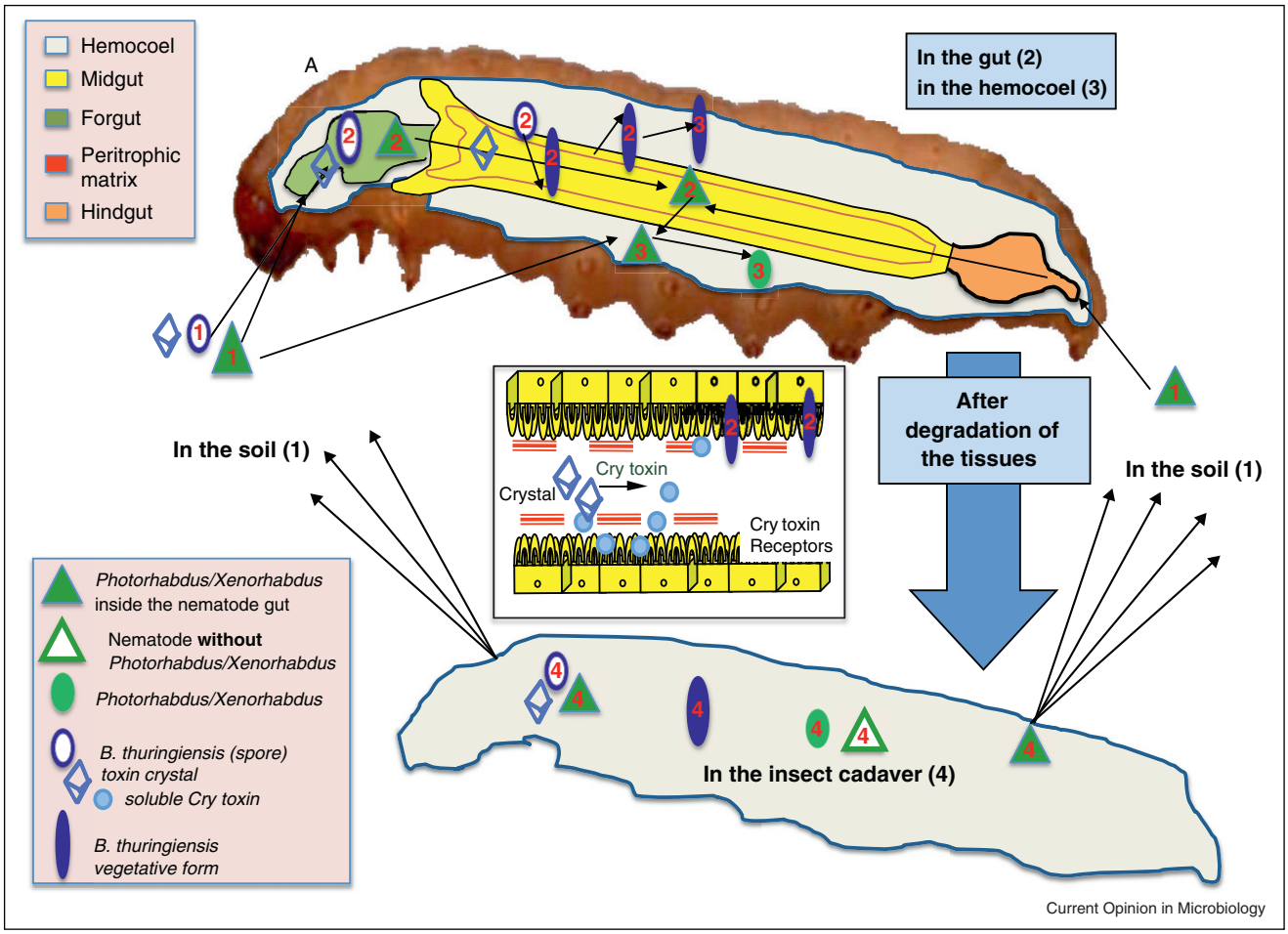
Figure 1



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 track (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 PlcR-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 lipopolysaccharides 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, *pbgE1*, resulted in both an increased sensitivity to cationic AMPs and decreased virulence towards insects [25,26]. The *dlt* operon encodes proteins that alanylate 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 *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 cytotoxin. Interestingly, the expression of the *lopT* gene coincides with the T3SS-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, FliZ, controls the expression of the XaxAB and XhlAB hemolysins encoding-genes [36]. XaxAB is the prototype of a new family of hemolysins with apoptotic and pore-forming activities in mammalian and invertebrate cells [38]. The second FliZ-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 *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*

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Table 1

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

Bacteria	Class of effectors		Mutant virulence[a]	Potential site(s) of effect and/or target(s) in the insect[b]			References
	Name	Short function/role		Gut	Hemocoel	Cadaver	
Regulators							
B. thuringiensis	PlcR	Quorum-sensing pleiotropic regulator, early phase of infection	I: VIR O: NON-VIR	Activates the expression of 45 genes, intestinal cell cytotoxicity			[16,18]
	NprR	Quorum-sensing pleiotropic regulator	I: VIR O: VIR			Nutriment acquisition, saprophytic development	[48,49••]
	Fur	Ferric uptake regulator	O: ATT		Regulates the expression of genes involved in iron uptake and virulence		[46]
Photorhabdus	PhoPQ	Two component system	I: NON-VIR		AMP resistance		[25]
	LuxS	Quorum-sensing auto-inducer 2 synthesis protein	I: ATT		ROS resistance		[75]
	HcaR	LysR-type transcriptional regulator	I: ATT		ROS resistance		[76]
	HexA	LysR homolog A regulator	I: ATT				[77]
Xenorhabdus	FlhD	Master regulon of the flagellar cascade	I: ATT		Production of hemolysins, motility	Production of lipases	[78]
	LrhA	LysR homolog A regulator, positive activator of *flhD*	I: ATT			Production of lipases, Tc-like toxins	[37]
	Lrp	Leucine-responsive regulatory protein, positive activator of *lrhA* and *flhD*	I: ATT		Production of hemolysins, motility	Production of lipases and antimicrobial activities	[79]
	CpxRA	Signal transduction system, positive activator of *lrhA*	I: ATT		Inhibition of hemolysin production; AMP resistance and nodules formation	Production of lipases, inhibition of protease and antimicrobial activity production	[80]
Insecticidal toxins (effect of purified toxins and/or mutants)							
B. thuringiensis	Cry	Cristal insecticidal toxins, pore forming toxins	I: Vir O: ATT/ NON-VIR	Degradation of insect intestinal epithelial cells			[7]
	Vip	Vegetative Insecticidal Proteins, pore forming toxins	O: ATT	Degradation of insect intestinal epithelial cells			[81]
Photorhabdus/ Xenorhabdus	Tc/Xpt (whole complex)	Insecticidal protein complex (proteins A, B, C)		Oral toxicity of the PP	Phagocytosis inhibition effect of the PP on insect hemocytes		[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			[85,86]
	Pit	Insecticidal toxin (Cry34 domain)			Toxicity after hemocoel injection		[87]

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Table 1 (*Continued*)

Bacteria	Class of effectors		Mutant virulence[a]	Potential site(s) of effect and/or target(s) in the insect[b]			References
	Name	Short function/role		Gut	Hemocoel	Cadaver	
Xenorhabdus	GroEL	Molecular chaperone		Oral toxicity of the PP Chitin binding activity			[72]
	MrxA	Soluble monomeric pilin		Oral toxicity of the PP	Pore forming-toxin activity of PP		[88]
Hemolysins							
B. thuringiensis	Hbl	Tripartite toxin composed of 3 proteins B, L1 and L2. Pore forming toxin.	O: VIR	Hemolytic and cytotoxic to epithelial (mammals)			[19,89]
	HlyII	Pore forming toxin, virulence factor	I: ATT		Induces apoptosis of insect hemocytes,		[40,90]
Photorhabdus/ *Xenorhabdus*	PhlA/XhlA	Hemolysin belonging to the two-partner secretion (TPS) family	I: VIR (*Photo*) I: ATT (*Xeno*)		Hemolytic activity in low-iron conditions (*Photorhabdus*) or in high-iron conditions (*Xenorhabdus*)		[10••,39,91]
Xenorhabdus	XaxAB	Binary cytotoxin	I: VIR		Necrotic and apoptotic activities on insect hemocytes	Late expression in cadaver	[10••,38]
Effectors interfering with eukaryotic signals							
B. thuringiensis	SPS	Involved with Glucose-6-phosphate uptake	I: VIR O: VIR	Specifically expressed in the gut			[104]
	IlsA	Involved in iron acquisition from heme and ferritin	I: ATT O: ATT		Specifically expressed in the hemocoel, iron regulated		[12••,45••]
	Yvrc	Iron dicitrate uptake	O: ATT				[92]
Photorhabdus	Cif	Cyclomodulin	I: VIR		PP triggers inhibition of proliferation and apoptosis in insect cell		[93]
	SctC	Secretin of a type 3 secretion system (TTSS)	I: VIR		Nodule formation and phagocytosis by macrophage cells		[33]
	LopT	Effector of the type 3 secretion system (TTSS)	I: VIR		PP triggers the release of RhoA and Rac from insect cell membrane		[33]
	Mcf	Protein with BH3 domain, which is sufficient to allow *Escherichia coli* both to persist within and kill an insect (makes the caterpillars floppy)		PP triggers apoptosis in the midgut epithelium	PP triggers apoptosis in hemocytes and inhibition of phagocytosis		[94,95]
	ExbD	Component of the TonB complex involved in uptake of siderophores	I: ATT		Virulence of the mutant rescued by $FeCl_3$ injection		[96]
	YfeABCD	Divalent cation transporter	I: ATT		Virulence of the mutant rescued by $FeCl_3$ injection		[96]
Antimicrobial molecules							
Photorhabdus	Hydroxy-stilbene	Antimicrobial molecule whose production is phenylalanine ammonia-lyase (StlA) and polyketide synthetase-dependant	I: ATT (*sltA* mutant)		Inhibition of phenoloxidase activation	Antibiotic activity against Gram negative and positive bacteria	[55]

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Xenorhabdus	Benzylidene-acetone	Monoterpenoid compound			PP inactivates phenoloxidase and phospholipase A2	Antimicrobial molecule	[56]
	Xenorhabdicin	Phage-tail bacteriocin encoded by a P2-like tail synthesis gene cluster				Protects the nematode against Gram negative competitors	[54•]
Enzymes involved in envelop modification and membrane proteins							
B. thuringiensis	Dlt ABCD	Proteins required for the D-alanylation of lipoteichoic acids (confers positive charge)	I: NON-VIR O: ATT		Resistance to AMPs		[27•]
	CwpFM	Cell Wall peptidase	I: NON-VIR	Adhesion to epithelial cells, biofilm formation	Macrophage vacuolization		[41]
	FlhA	Basal component of the flagella apparatus	O: ATT I: ATT	Allows motility and adhesion to intestinal cells	Inhibits production of Phospholipase/hemolysins		[43]
Photorhabdus	PbgP1P2P3P3E1E3E3	Responsible for the biosynthesis of L-aminoarabinose and its ligation onto the lipid A moiety of LPS	I: ATT		Resistance to AMPs		[26]
Extra-cellular enzymes							
B. thuringiensis	InhA1	Metalloprotease	I: ATT O: VIR		Resistance to AMP, allows bacterial escape from macrophages		[31,42]
	InhA2	Metalloprotease	I: ATT O: ATT	Early phase of infection, PlcR regulated	Resistance to AMP		[97]
	InhA3	Metalloprotease	I: VIR		Belongs to the NprR regulon		[49••,98]
	NprA	Metalloprotease	I: VIR O: VIR		NprR regulon, degrades host tissue components	Potentially involved in nutriment acquisition	[49••] [48] [99]
	Clp	Proteases ClpP1 (Intracellular ATP-protease activity)	I: ATT		Implicated in stress resistance and growth at low temperature		[100]
	MpbE	Enhancine	O: ATT	Expressed in the gut			[101,21]
Photorhabdus	PrtA	Serralysin-like metalloprotease			Degradation of AMPs by the PP	Localization in conjunctive tissue in dead insect	[11•,32,102]
	PrtS	M4 metalloprotease			Induction of melanization reaction by the PP		[103]
	SodA	Superoxide dismutase	I: ATT		Resistance to oxidative stress		[76]

[a] I: 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.

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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 *xhbF*, 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 *xhlBA* 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-dependent 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

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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.

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- • of special interest
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