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The Diversity of Insect-bacteria Interactions and its Applications for Disease Control

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Abstract

Prokaryotic microorganisms are widespread in all environments on Earth, establishing diverse interactions with many eukaryotic taxa, including insects. These associations may be symbiotic, pathogenic and vectoring. Independently of the type of interaction, each association starts with the adhesion of the microorganism to the host, entry and "invasion" of the host, then progresses to establishment and dissemination within the host, by avoiding host immune responses, and concludes with transmission back to the environment or to a new host. Advances in genomics and genetics have allowed the dissection of these processes and provided important information on the elements driving the shaping of the members of each association. Furthermore, many mechanisms involved in the establishment of the associations have been scrutinised, along with the development of new methods for the management of insect populations.

Introduction

Insects are the most diverse group of animals with over a million different species found almost in every habitat, except the sea (Vilmos *et al.*, 1998). Due to their widespread distribution, insects are inevitably associated with an extremely large variety of microscopic life forms, including viruses, bacteria, fungi, protozoa, nematodes

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Abbreviations: AMP, antimicrobial peptides; Bt, *Bacillus thuringiensis* toxins; CI, cytoplasmic incompatibility; Cry, crystal toxin; Cyt, cytolytic toxin; EPN, entomopathogenic nematodes; IJ, infective juvenile; LPS, lipopolysaccharide; PCR, polymerase chain reaction; PVC, *Photorhabdus* virulence cassettes; T3SS, type III secretion system; Tc, toxin complex.

and multicellular parasites. Although some of these microorganisms exhibit a rather wide host range, many associations are highly specialised and involve not only certain insect species but also particular life stages of the insect host (Aronson *et al.*, 1986). This is a reflection of the properties of the ecological niche occupied by the insects and the associated microbes, the needs of the insect or the microorganism, and the genetic mechanisms used by the microorganism to establish the interaction. The purpose of this review is to present an overview of the diversity of associations between insects and bacteria and their potential and current applications. In the first part, we describe different types of interactions between these groups of organisms and their characteristics, highlighting several examples and focusing in the molecular mechanisms underlying these interactions. In the second part, we review the features of these interactions with potential for insect control, including insecticidal toxins, and summarise several strategies and recent developments with agricultural and epidemiological implications.

Types of insect-bacteria interactions

Interactions between insects and bacteria may be symbiotic or pathogenic. The term "symbiosis" was initially coined by Heinrich Anton de Bary in 1879 in his monograph "Die Erscheinung der Symbios" (Strasbourg, 1879) as "the living together of unlike organisms". Most symbioses have a proven biochemical foundation: in some cases one of the partners benefits by the provision of nutrients produced by the other, and in other cases, the waste products produced by one of the partners are recycled by the other. The presence of such associations throughout the evolutionary history of insects is thought to shape the diversity observed in this group of animals. Depending on the fitness effects on the members of the relationship, symbiotic associations can be divided in commensalism, mutualism and parasitism (Moya *et al.*, 2008) Parasitism occurs when one species increases its fitness while the other is harmed by the association. Commensalism occurs when the microbe, while doing no apparent harm, benefits from the host but provides no advantage in return (Dillon *et al.*, 2004). Certain commensal microorganisms could be considered mutualists, with microbes associated with the insect gut being an example: insect gut microbiome contributes to food digestion, produces essential vitamins and keeps out potentially harmful microbes by competing with them for nutrients. Mutualism is a less flexible association where the microbe and insect mutually benefit each other. The terms symbiosis and mutualism are often interchanged.

Pathogenic interactions, although being very different from the perspective of the host, also require a high degree of specialisation and intimate contact with the host. In this respect, many of the molecular mechanisms used by bacterial pathogens and mutualists are similar. Furthermore, the same microorganism can behave differently depending on the fitness of the host and the environmental circumstances, turning from beneficial to detrimental, thus complicating the arbitrary definition of bacterial behaviour. Therefore, the concepts of mutualism and pathogenesis are not clearly differentiated, but rather a matter of balance between the bacteria and the insect host in terms of fitness, reproductive success, feeding and influence of other symbionts. Numerous examples illustrate the idea that most associations might have started as pathogenic and then evolved towards the tolerance of the invader and, providing a

benefit exchange, became mutualistic. Nevertheless, when a pathogen is converted into a mutualist, it is unlikely to re-acquire its pathogenic traits.

In addition to the highly specialised pathogenic and mutualistic interactions, insects can carry microbial pathogens which they actively or passively transmit to other organisms including plants and animals. Although other invertebrates such as nematodes and arthropods (ticks) are effective vectors, many diseases of agricultural and clinical importance are transmitted by insects. In this review we will discuss the potential of insects to act as a reservoir for the evolution of human pathogens.

MUTUALISTIC INTERACTIONS

Insects are the most abundant group of eukaryotes on Earth in terms of species number. Bacterial mutualists impact on more than 10% of insect species, spanning several taxonomic orders, via complementation of the insect diet (Buchner, 1965; Douglas, 1989; Moran *et al.*, 2000; Wernegreen, 2002). Considering the extent of the dependence between the insect and the symbiont and the age of the association, symbionts can be classified in two groups; the obligate primary (P) endosymbionts, which have a long evolutionary history with their hosts and they are required for host survival and fertility, and the facultative secondary (S) symbionts, which have established a more recent association with the host and they have retained their ability to return to a free-living condition (Moya *et al.*, 2008). It is generally assumed that P-endosymbionts evolved from S-symbionts and that a S-symbiont can potentially replace a P-endosymbiont during the evolutionary history of the host. Key features shared by all P-endosymbionts are maternal vertical transmission to the progeny (in the egg) and housing in specialised cells commonly known as bacteriocytes (or mycetocytes), which are occasionaly grouped in an organ-like structure named bacteriome (or mycetome). On the other hand, S-symbionts are usually vertically transmitted although horizontal transmission has been described in few cases; they can also reside in multiple host tissues, such as in cells surrounding the primary bacteriocytes or in their own bacteriocytes (Moya *et al.*, 2008). The majority of symbionts are not culturable outside their host, which renders their study using traditional microbiological, genetic, and physiological methods very difficult. Thus, initial characterisation has depended heavily on microscopical observation. More recently the application of molecular biology techniques have revolutionised the study of endosymbionts. Genome sequencing, heterologous hybridisation and polymerase-chain reaction (PCR), have faciliated not only their identification but have also helped studying their evolution, metabolic potential and phylogenetic relations with other free-living relatives.

Shaping the genome of symbionts

Molecular phylogenies of endosymbionts and their hosts are congruent in showing long-term co-speciation of both partners (Wernegreen, 2002). The long-term association of symbionts with their insect hosts and the specialised functions they fulfill have influenced the rates and patterns of prokaryotic DNA evolution. Most bacterial P-endosymbionts have lost the genes coding for functions that are redundant within the insect environment, maintaining only functions necessary for successful symbiosis, such as synthesis of essential nutrients. In the symbiont, this gene lost due to metabolic

redundancy and the accumulation of mutations in an environment where bacterial horizontal gene transfer is not possible, results in a reduction in genome size, accompanied by an AT bias. In addition to the lack of bacterial DNA exchange, the mutational GC to AT shift could not be re-established due to the loss of DNA replication and repair genes (Wernegreen, 2005). Such bias can pottentially affect the structure and function of the encoded proteins. Indeed, to avoid protein inactivation resulting by both its genome reduction and possibly the action of the host immune system, *Buchnera aphidicola* potentially compensates for such altered protein structures by constitutively expressing the chaperonin GroEL (Wernegreen, 2005). Extreme examples of this genome reduction include *Rickettsia prowazekii*, the etiological agent of epidemic typhus, which parasitises the human body louse, and *B. aphidicola*, the well-studied P-endosymbiont of the pea aphid. Andersson and co-workers have found that the *R. prowazekii* genome encodes only 834 complete open reading frames, 5 fold less than *Escherichia coli* (Andersson *et al.*, 1998; Lang *et al.*, 1997). As in the case of *R. prowazekii*, *Buchnera* spp have shed many of their ancestral genes to adapt to their intracellular lifestyle. The *B. aphidicola* genome is 416 kb with only 362 protein-coding genes and a 6-kb circular plasmid (Perez-Brocal *et al.*, 2006). In this case, the endosymbiont contains less than 15% of the metabolic genes found in the close relative free-living *E. coli* (Perez-Brocal *et al.*, 2006); it synthetises all the amino acids required by the host, apart from tryptophan. This defect can be rescued by secondary symbionts that are indeed able to replace the primary one (Koga *et al.*, 2003), suggesting that the secondary endosymbiont could eventually take the place of the primary if the primary mutated to the point at which it could no longer synthesise most of the essential nutrients required by the host. Even though *Rickettsia* and *Buchnera* are exposed to almost identical environments their metabolic capacities are different and consequently they have developed different interaction with their insect host. The current record for the smallest microbial symbiont genome is the chromosome of *Carsonella ruddii*, a psyllid endosymbiont, which is even smaller: 160 kb in size coding for only 182 proteins (Andersson, 2006; Nakabachi *et al.*, 2006). This minute genome, with the implicit loss of functions, raises the possibility of lateral gene transfer from the endosymbiont to its host, as it occurred in organelles such as mitochondria for which many essential proteins are encoded in the nuclear genome (Andersson, 2006). According to Muller's ratchet principle, which proposes that deleterious mutations accumulate in small populations with no incorporation of new genes, the genomes of essential symbionts which have evolved to such an extreme degree of reduction, have taken a route with no way back to a free-living status and possibly inevitable extinction (Andersson *et al.*, 1996; Muller, 1964). In conclusion, irrespective of whether the relationship is parasitic or symbiotic the cost of survival for the prokaryotes is directly paid in nucleotides (Tamas *et al.*, 2001).

Symbiosis as a mechanism of adaptation and source of phenotypic complexity

Symbionts are maintained because they contribute to the lifestyle of the host. Many insects have a very restricted food source, i.e. blood or plant-sap, lacking essential amino acids or vitamins which can be provided by the symbiont. In turn, the microorganism lives in a protected and favorable environment. Descriptions of new symbionts identified in insects are frequent in the literature. Below, we present in detail two well-studied examples. A broader summary of interactions can be found in Table 1.

Table 1. Summary of a selected number of examples of insect-bacteria interactions (NA, not applicable).

Insect order, common name and *species name*	Bacterial species (group)	Type of interaction	Phenotype
Orthoptera Grassland locusts *Myrmeleotettix palpalis*	*Serratia marcescens* strain HR-3 (γ-proteobacteria)	Pathogen	Paralysis induced by insecticidal metalloprotease (Tao *et al.*, 2006)
Anoplura Human body louse *Pediculus humanus*	*Rickettsia prowazekii* (Andersson *et al.*, 1998) (α-proteobacteria)	Obligate intracellular	NA
Hemiptera Sharpshooters *Homalodisca coagulata*	*Baumannia cicadellinicola* (γ-proteobacteria); *Sulcia muelleri* (Bacteroidetes) (Moran *et al.*, 2005b)	P-endosymbiont	Metabolic complementarity of both symbionts (Wu *et al.*, 2006)
Stinkbugs *Megacopta punctatissima*	*Ishikawaella capsulata* (γ-proteobacteria)	Extracellular symbiont	Gut bacterium vertically transmitted via "symbiont capsule" (Hosokawa *et al.*, 2005; Hosokawa *et al.*, 2006)
Blood sucking bug *Rhodnius prolixus*	*Rhodococcus rhodnii*	Gut symbiont/ commensal	NA
Sap-sucking insects, Aphids *Acyrthosiphon pisum* *Schizaphis graminum* *Baizongia pistaciae* *Cinaria cedri*	*Buchnera aphidicola* BAp (Shigenobu *et al.*, 2000) *Buchnera* BSg (Tamas *et al.*, 2002) *Buchnera* BBp (van Ham *et al.*, 2003) *Buchnera aphidicola* BCc (Perez-Brocal *et al.*, 2006) (γ-proteobacteria)	P-endosymbiont	Amino acid synthesis (Douglas 2006)
Aphids *Acyrthosiphon pisum*	*Hamiltonella defensa* (γ-proteobacteria)	S-symbiont	Confers host defence against natural enemies, parasitic wasps (Oliver *et al.*, 2003)
	Dickeya dadantii (Erwinia chrysanthemi 3937) (γ-proteobacteria)	Specific pathogen	Oral toxicity, encode homologue to *cyt* Bt toxin (Grenier *et al.*, 2006)

Table 1. Contd.

Insect order, common name and *species name*	Bacterial species (group)	Type of interaction	Phenotype
Sap-sucking insects, Psyllids *Pachypsylla venusta*	*Carsonella ruddii* (γ-proteobacteria) (Nakabachi *et al.*, 2006)	Endosymbiont	Essential nutrients, possibly amino acids (Nakabachi *et al.*, 2006)
Sap-sucking insects, Whiteflies *Bemisia tabaci*	*Portiera aleyrodidarum* (γ-proteobacteria)	P-endosymbiont	Probably amino acid synthesis (Thao *et al.*, 2004)
Sap-sucking insects, Mealybugs *Planococcus citri*	*Tremblaya princeps* (β-proteobacteria)	Endosymbiont	Probably amino acid (Baumann *et al.*, 2002)
Neuroptera Antlion *Myrmeleon bore*	*Enterobacter aerogenes* *Bacillus cereus* *Bacillus sphaericus* *Morganella morganii* *Serratia marcescens* *Klebsiella spp*	Temporal association	Pathogens for other insect species prey of the antlion (Nishiwaki *et al.*, 2004; Nishiwaki *et al.*, 2007; Yoshida *et al.*, 2001)
Coleoptera Rice weevil *Sitophilus oryzae*	P-endosymbiont SOPE (γ-proteobacteria)	P-endosymbiont	Vitamin synthesis and influence mitocondrial respiration in the host (Heddi *et al.*, 1998)
Grass grub *Costelytra zealandica*	*Serratia entomophila* & *Serratia proteamaculans* (γ-proteobacteria)	Pathogen (amber disease)	Antifeeding symptoms caused by proteins encoded in the pADAP plasmid (Glare *et al.*, 1993; Jackson *et al.*, 1993)
	Serratia sp	Pathogen	Cytotoxic effect (Tan *et al.*, 2006)
Siphonaptera Human North America flea *Oropsylla montana*	*Yersinia pestis* (γ-proteobacteria) (Parkhill *et al.*, 2001)	Vector	Transmission of mammalian and human pathogen (Perry *et al.*, 1997)

Table 1. Contd.

Insect order, common name and *species name*	Bacterial species (group)	Type of interaction	Phenotype
Diptera Tsetse fly *Glossinia brevipalpis*	*Wigglesworthia glossinidia* (γ-proteobacteria) (Akman *et al.*, 2002)	P-endosymbiont	Essential for fly fertility (Hill *et al.*, 1973)
Tsetse fly *Glossina morsitans*	*Sodalis glossinidius* (γ-proteobacteria) (Toh *et al.*, 2006)	S-symbiont	Proposed to influence trypanosome infection (Welburn *et al.*, 1993)
Tsetse fly *Glossina spp.*	*Wolbachia* (α-proteobacteria)	Symbiont	Cytoplasmic incompatibility (Cheng *et al.*, 2000)
Fruit fly *Drosophila melanogaster*	*Wolbachia pipientis* wMel (α-proteobacteria) (Wu *et al.*, 2004)	Symbiont	Cytoplasmic incompatibility (Stouthamer *et al.*, 1999)
	Pseudomonas entomophila (Vodovar *et al.*, 2006) (γ-proteobacteria)	Pathogen	Oral toxicity (Vodovar *et al.*, 2005)
	Serratia marcescens (Db11) (γ-proteobacteria)	Opportunistic pathogen	Related to insect death (Flyg *et al.*, 1980)
	Erwinia carotovora atroseptica & *E. c. carotovora* (γ-proteobacteria)	Vector	Transmission of plant pathogen (Basset *et al.*, 2000)
Lepidoptera Tobacco horn worm *Manduca sexta* Wax moth *Galleria mellonella* & other species	*Photorhabdus luminescens* (Duchaud *et al.*, 2003) & *Photorhabdus asymbiotica* (http://www.sanger.ac.uk/Projects/P_asymbiotica/) (γ-proteobacteria)	Pathogen	Several toxins with oral and injectable toxicity (ffrench-Constant *et al.*, 2003; ffrench-Constant *et al.*, 2007)
	Xenorhabdus nematophilus (γ-proteobacteria)	Pathogen	Xpt and Xax toxins (Herbert *et al.*, 2007)

Table 1. Contd.

Insect order, common name and *species name*	Bacterial species (group)	Type of interaction	Phenotype
Hymenoptera Carpenter ant *Camponotus floridanus* *Camponotus pennsylvanicus*	*Blochmannia floridanus* (γ-proteobacteria) (Gil *et al.*, 2003) *Blochmannia pennsylvanicus* (γ-proteobacteria) (Degnan *et al.*, 2005)	Non-essential endosymbiont	Improves viability of host pupae (Zientz *et al.*, 2006)
Several insect orders	*Bacillus sphaericus* (Hu *et al.*, 2008) *Bacillus thuringiensis* (Gram-positive)	Opportunistic pathogen	Toxins effective against Lepidoptera, Diptera and Coleoptera (Lecadet *et al.*, 1999; Schnepf *et al.*, 1998)

The tsetse fly: Multiple symbionts have coevolved with the tsetse fly, vector of *Trypanosoma brucei* (and other african trypanosomes) which is the causative agent of sleeping sickness. The fly feeds only on blood, which is rich in proteins but poor in nutrients. The symbionts supply vitamins and other nutrients that can not be synthesised by the fly. At least two known symbionts with different ultrastructural characteristics are known (Aksoy, 2000; Oneill *et al.*, 1993) and they can be found in different tissues within the insect: the primary and secondary endosymbionts are present in the gut tissue, while the third organism was identified in reproductive tissue. The interaction of the fly with the primary P-endosymbiont, *Wigglesworthia glossinidia*, which forms a distinct lineage of γ-Proteobacteria (Chen *et al.*, 1999), can be characterised as an essential symbiosis. Sequencing of *W. glossinidia* genome revealed the presence of several cofactor biosynthetic pathways, including over 60 genes involved in the synthesis of vitamins and nutrients that are necesssay for fly fertility (Akman *et al.*, 2002). The expression profile of these genetic pathways has also been confirmed using microarrays (Akman *et al.*, 2001a). Moreover, a clear reduction in the number of bacterial genes related to the typical free-living state has been reported. Among the most important genes lost is the DNA replication initiation protein, DnaA, which may reflect the dependency of *W. glossinidia* on host genome functions and may be one mechanism by which the host controls symbiont numbers (Akman *et al.*, 2002). Studies of the gene expression in *Wigglesworthia* have been conducted using *E. coli* gene arrays, which have suggested that *Wigglesworthia* could be a facultative anaerobic organism that utilises ammonia as its major source of nitrogen (Akman *et al.*, 2001a). The secondary symbiont of the tsetse fly is the S-symbiont *Sodalis glossinidius* (Akman *et al.*, 2001b). *Sodalis* is harboured both inter- and intracellularly in the tsetse midgut as well as in muscle, fat body, hemolymph, milk gland, and salivary gland tissues of certain species (Cheng *et al.*, 1999). Hemolymph has also been proposed as a possible route for the transmission of *Sodalis* to the progeny (Cheng *et al.*, 1999), which has been possible to culture *in vitro* allowing its biochemical characterisation (Beard *et al.*, 1993; Welburn *et al.*, 1987). *Sodalis*, initially classified as a *Rickettsia*-like organism (RLO), displays high endochitinase activity that has been linked to higher susceptibility to trypanosomal infections of the host (Welburn *et al.*, 1993), which makes this symbiont a suitable target for parasite transmission control. The genome of *Sodalis* has been sequenced (Toh *et al.*, 2006) revealing that more than one third of the coding sequences are pseudogenes. From this unusually high number of pseudogenes, the majority encode homologues to defense or transport and metabolism of carbohydrates, implying that selection on these functions has been relaxed. The genome also contains three symbiotic regions encoding genes similar to type III secretion systems (T3SS) potentially involved in the transmission of the symbiont to the host progeny (Toh *et al.*, 2006). The T3SS is a complex nanomachine that allows bacteria to deliver effectors, that lack other secretion signal sequences and may require chaperones for their delivery, across eukaryotic cellular membranes (Cornelis, 2006). The system is widely distributed among the animal and plant pathogens in the Enterobacteriaceae family, and its effectors interfere with cellular signaling pathways mediating bacterial uptake or entry into eukaryotic cells or phagocytosis evasion for other bacterial species. To date, T3SS components have been identified in the genomes of three facultative endosymbionts, including *Sodalis*, and in two closely related primary symbionts the coleopteran grain weevils, *Sitophilus oryzae* and *S. zeamais*

(reviewed by (Dale *et al.*, 2006). In general, T3SS gene clusters are present in the genomes of insect endosymbionts that have established relatively recent associations with hosts. Interestingly, P-endosymbionts, including *Buchnera*, *Blochmannia*, and *Wigglesworthia*, have no T3SS components in their genomes, although there is genetic evidence which suggests that these species possessed T3SSs during earlier stages of their evolution. The finding that a S-symbiont utilises a T3SS in order to invade host cells suggests that T3SS has been adapted in the context of mutualism to facilitate association between symbiotic bacteria and their hosts.

The pea aphid (*Acyrthosiphon pisum*) is a sap-sucking insect which harbours primary and secondary endosymbionts. The essential P-endosymbiont of pea aphids is *Buchnera.* The insect feeds on plant sap, very rich in carbohydrates but deficient in essential amino acids which are synthesised and provided by the bacterium. The intimate symbiosis between *Buchnera* and aphid is a long-lasting relationship in evolutionary terms, established 50-70 millions years ago (Tamas *et al.*, 2002). Moreover, phylogenetic studies showed that both organisms have undergone cospeciation (Clark *et al.*, 2000). The genome sequences of four *Buchnera* species, associated with different aphids, are available (Perez-Brocal *et al.*, 2006; Shigenobu *et al.*, 2000; Tamas *et al.*, 2002; van Ham *et al.*, 2003). Genome sequence analysis reveals that *Buchnera aphidicola* is unable to synthesise the amino acid tryptophan which can be supplied by a secondary endosymbiont. Indeed, it has been shown that a secondary endosymbiont can completely replace *Buchnera* enabling the host to develop and reproduce normally (Koga *et al.*, 2003). The S-symbiont harboured by the pea aphid is *Hamiltonella defensa*. This association confers defence against natural enemies of the host, namely certain parasitic wasps, by accelerating the death of the parasitic larvae (Moran *et al.*, 2005a; Oliver *et al.*, 2003). *H. defensa* is localised in several tissues and cell types: within the bacteriocytes that harbour *Buchnera*, in some other cell types and also in the hemocoel (Moran *et al.*, 2005a; Sandstrom *et al.*, 2001). Interestingly, this secondary symbiont, *H. defensa*, has an associated bacteriophage called APSE-2. Similar phages, such as APSE-1, have been identified from diverse *H. defensa* strains associated with numerous insect species and they appear to be important in the bacteria lifecycle. All tested phages encode a toxin which interrupts the eukaryotic cell cycle that could help to prevent eukaryotic infections (Moran *et al.*, 2005a). An additional player in the association of aphids with multiple partners is a specific bacterial pathogen, *Dickeya dadantii* (*Erwinia chrysanthemi* 3937). This phytopathogenic bacterium, which causes soft rot diseases on many crops, is highly virulent for the aphid both orally and by septic injury. Its genome encodes genes homologous to *cyt* (encoding spore/crystal toxins in *Bacillus thuringiensis)*. *D. dadantii* seems to be a specific pathogen of the pea aphid as very low pathogenesis was observed against three other insect species (*Drosophila melanogaster*, *Sitophilus oryzae*, and *Spodoptera littoralis*) (Grenier *et al.*, 2006). The pea aphid constitutes a excellent example of complex associations: it can be infected by a phytopathogenic bacteria while it harbours two endosymbionts, the second of which has an integrated phage that can prevent eukaryotic infections. This genetic and metabolic network involving numerous partners highlights the difficulties in dissecting the role of each individual organism, a frequent hurdle in the study of mutualistic associations.

There are many more examples of mutualistic interactions which further illustrate the importance of these associations in the colonisation of specialised niches by the

insects. For instance, sharpshooter bugs feed on plant sap from xylem, which is a very nutrient-poor diet containing mostly inorganic compounds compared to the phloem fluid which is the food source of pea aphids. In consequence, the sharpshooter *Homalodisca coagulata* has two essential endosymbionts with complementary metabolisms, *Baumannia cicadellinicola* synthetises the essential amino acids, as does *Buchnera* for the aphids, while the other (*Sulcia muelleri*) provides vitamins and cofactors (Wu *et al.*, 2006). This example is included in Table 1, along with other discussed in the present review and a selection of additional cases from the extensive literature.

WOLBACHIA

The α-proteobacterium *Wolbachia* was first discovered infecting the ovaries of *Culex pipiens* mosquitoes (Hertig *et al.*, 1924; Iturbe-Ormaetxe *et al.*, 2007) but it is now recognised to be distributed among other invertebrates besides insects, including spiders, mites, terrestrial crustaceans and filarial nematodes (Bandi *et al.*, 2001; Bouchon *et al.*, 1998; Breeuwer *et al.*, 1996; Oh *et al.*, 2000; Rowley *et al.*, 2004; Taylor *et al.*, 1999). The outcome of *Wolbachia*-induced effects on the host is determined by a combination of host genotype, *Wolbachia* strain, host tissue localisation, and interaction with the environment. These factors define the ability of *Wolbachia* to act as a classical mutualist and in other insects as a pathogen, dramatically shortening adult lifespan (McGraw *et al.*, 2004). More than 20% of insects species have been estimated to carry *Wolbachia* strains. This distribution raises up to 75% when considering all arthropods, making *Wolbachia* the most widely spread eubacterium symbiont known to date (Jeyaprakash *et al.*, 2000; Werren *et al.*, 1995). Part of this success is the ability of *Wolbachia* strains to manipulate the reproduction of their arthropod hosts, thus ensuring maternal transmission (Stouthamer *et al.*, 1999). The presence of *Wolbachia* in insects is related to a variety of phenotypes, one of the better studied being cytoplasmic incompatibility (CI) in flies. CI results when infected males mate with non-infected females causing the early events of fertilisation to be disrupted leading to embryonic death (Stouthamer *et al.*, 1999). During an incompatible cross, the sperm enters the egg but its contribution of genetic material to the potential zygote is not successful, so that very few eggs hatch. The infected females have a reproductive advantage as they can successfully mate with both infected and non-infected males, ensuring the rapid spread of *Wolbachia* in the insect population (Aksoy, 2000; Hoffmann *et al.*, 1997). Other host reproductive phenotypes induced by *Wolbachia* are: selective male killing, conversion of genetic males into functional females (feminization) and induction of parthenogenesis (reviewed by (Werren, 1997)). Mutualistic examples of *Wolbachia* in insect reproduction have been described in a parasitoid wasp and a beetle species in which the absence of *Wolbachia* arrested oocyte maturation (Pannebakker *et al.*, 2007; Zchori-Fein *et al.*, 2006). Furthermore, a number of fitness traits in insects such as fertility, longevity and locomotor performance are affected by *Wolbachia* infections with either positive or negative outcomes, depending on the bacterial strain and the host species (Dean, 2006; Dobson *et al.*, 2004; Fleury *et al.*, 2000; Fry *et al.*, 2004; Snook *et al.*, 2000). Pioneering work by Dunning-Hotopp revealed *Wolbachia* DNA fragments that range from nearly the entire *Wolbachia* genome (>1 Mbp) to short (<500 bp) insertions in the nuclear genome of several diverse invertebrate taxa, including fruit flies, wasps and nematodes (Hotopp *et al.*, 2007).

Reverse transcription PCR demonstrated that some of these inserts can be transcribed and that the transcripts contain eukaryotic post-transcriptional signal, which suggests that they have been modified by the host (Hotopp *et al.*, 2007). The importance of lateral gene transfer from *Wolbachia* to their hosts is still unknown. Two *Wolbachia* genomes have been fully sequenced; these are the wMel strain which induces CI in *D. melanogaster* (Wu *et al.*, 2004) and the wBm strain, an obligate mutualist of the filarial nematode *B. malayi* (Foster *et al.*, 2005). Comparative analysis of these genomes has shown that *Wolbachia* have lost multiple metabolic pathways, that their genome contains numerous repetitive DNA elements, and that they potentially express secretion systems which could be important in host-*Wolbachia* interactions (Foster *et al.*, 2005; Wu *et al.*, 2004). Moreover, it has revealed the presence of a variable number of ankyrin (ANK) domains, a rare motif which has not been detected in the majority of known bacterial genomes (Li *et al.*, 2006). The ANK domain is typically a 33-residue L-shaped motif which mediates protein-protein interactions in diverse families of proteins, including cytoskeletal and membrane proteins, transcriptional and developmental regulators, toxins, and CDK (cyclin-dependent kinase) inhibitors (Sedgwick *et al.*, 1999). Comparative analyses of orthologues of these genes from *Wolbachia* strains that are able to infect different insect hosts have shown that they can be highly variable. In addition, the lack or the disruption of these genes correlates with the loss of the CI phenotype (Iturbe-Ormaetxe *et al.*, 2005; Sinkins *et al.*, 2005), suggesting that these genes could facilitate communication with the host and interfere with the reproduction mechanism via CDK1 (Tram *et al.*, 2002). ANK genes have also been found associated with prophage elements (Wu *et al.*, 2004). Numerous prophage genes are present in *Wolbachia* and it is postulated that phages could potentially affect the ecology of *Wolbachia* not only via the ANK genes but also through the regulation of bacterial density which correlates with CI phenotype (Bordenstein *et al.*, 2006). Useful *Wolbachia* genomic information can also be obtained by comparing the genome sequences of closely related intracellular pathogens, which are unable to cause the same phenotypes in the insect host, and also completely unrelated species which have been implicated with reproduction phenotypes. The future sequencing of the arthropod-associated and vertically transmitted bacterial species *Cardinium hertigii*, which induces most phenotypes traditionally associated with *Wolbachia*, such as CI (Gotoh *et al.*, 2007; Hunter *et al.*, 2003) parthenogenesis (Zchori-Fein *et al.*, 2001) and feminisation (Weeks *et al.*, 2001), will provide valuable information about the molecular mechanisms shaping these phenotypes.

The extent to which bacterial symbionts are spread among diverse species and orders in the insect world and how many symbionts can one individual insect harbour is difficult to estimate. However, recent studies show that the diversity and incidence of insect bacterial symbionts may have been underestimated. One reason for this could be a bias among the insect species sampled, followed by a restricted analysis and identification of symbionts, often looking only for a particular species group. In a particular case study with ladybird beetles the authors collected a large number of individuals (over 2100) from 21 species and used PCR to detect *Wolbachia, Spiroplasma*, *Rickettsia* and *Flavobacteria*, all bacterial species known to cause male killing in insects. The majority of symbionts detected (around 65) were *Spiroplasma* and *Rickettsia* which have not been extensively studied before, suggesting that these groups can be as common as *Wolbachia* in insect populations (Weinert *et al.*, 2007).

PATHOGENIC INTERACTIONS

Among the bacterial species that are able to cause disease in insects some of them are opportunistic, such as *Bacillus thuringiensis*, while others, as in the case of *Photorhabdus luminescens,* are very specific and engage in a complex lifecycle which involves being vectored by nematodes. In any case, the display of potent virulence mechanisms, including a variety of toxins, and the development of strategies to evade the host immune system are required for the success of the infection. Several examples from the genera *Serratia, Pseudomonas*, *Xenorhabdus, Photorhabdus* and *Bacillus* are discussed below.

Serratia marcescens

S. marcescens is the most frequently reported entomopathogen for a wide variety of invertebrate hosts, from insects to nematodes, and is an opportunistic pathogen in other organisms such as plants and humans (Bulla, 1975; Grimont *et al.*, 1978). *S. marcescens* Db11 was isolated from moribund *Drosophila* flies in the laboratory (Flyg *et al.*, 1980). It is proposed that *Serratia* displays different virulence programs depending on the mode of infection. Infection of *Drosophila* with *Serratia* via the oral route kills the flies within 4 to 8 days. During oral infection this pathogen persists in the gut where it triggers local antimicrobial peptide (AMP) expression and crosses the intestinal barrier to reach the hemocoel without eliciting a systemic immune response, making it a desirable/useful model for the study of enteric infections *in vivo* (Nehme *et al.*, 2007). It has been suggested that the survival of flies that have been infected orally with *S. marcescens* could be attributed to the local expression of AMP in the gut and the ability of insect hemocytes to recognise and engulf bacterial cells. Indeed *S. marcescens*-infected flies, deprived of the important phagocytosis receptor Eater, succumb to infection an average 2 days earlier than wild-type flies (Kocks *et al.*, 2005). Although injuries are probably not a frequent source of entry of pathogens in *Drosophila*, a septic injury model of *S. marcescens* has been developed by direct injection of bacterial suspension into the insect hemocoel, and showed that even a small number of *S. marcescens* cells are able to kill *Drosophila* within 30 h (Flyg *et al.*, 1980) (Kurz *et al.*, 2003), possibly by escaping immune defences (i.e. downregulation or late expression of AMP) due to low release of peptidoglycan into the hemolymph (Lemaitre *et al.*, 2007).

S. marcescens is not the only entomopathogenic member of the genus *Serratia*. The species *S. entomophila* and *S. proteamaculans,* containing the pADAP plasmid, are responsible for the amber disease in the grass grub *Costelytra zealandica*. The bacteria colonise the gut, later passing to the hemocoel after a long infection period, a process which can partially be attributed to the expression of the *sep* and *afp* genes. Both loci are encoded in the plasmid and show similarity with the insecticidal toxin complex genes and the virulence cassettes from *Photorhabdus* species (Glare *et al.*, 1993; Hurst *et al.*, 2000; Jackson *et al.*, 2001; Jackson *et al.*, 1993). Other strains of *Serratia* spp., free from pADAP plasmid, caused mortality in the *C. zealandica* larvae, suggesting that *Serratia* toxicity against insects can also be independent of the disease-causing plasmid (Tan *et al.*, 2006).

Pseudomonas entomophila

P. entomophila are Gram-negative γ-proteobacteria which were initially isolated from a *Drosophila* fly in Guadeloupe (Vodovar *et al.*, 2005). *P. entomophila* is able to kill larvae of several insect species (Vodovar *et al.*, 2005). Upon ingestion, *P. entomophila* induces a strong immune response (both local and systemic activation of the immune deficiency pathway (Imd) for Gram-negative bacteria recognition) that has no effect on the bacteria remaining in the gut lumen, which results in food-uptake blockage and killing larvae and adults by starvation. Transcription analyses of orally infected flies revealed that *P. entomophila* infection alters the expression of genes associated with host gut physiology (Vodovar *et al.*, 2005). Its persistence in *Drosophila* larvae is a multifactorial process. Among the important virulence factors mediating persistence of *P. entomophila* in the gut is the abundant protease AprA, which degrades AMPs produced by gut epithelia (Liehl *et al.*, 2006). The genome sequence of *P. entomophila* has been recently published revealing a great number of potential virulence factors such as toxins, proteases, lipases, hemolysins, proteins involved in alginate synthesis and secondary metabolites that may compensate for the lack of a T3SS in the insect infection proccess (Vodovar *et al.*, 2006).

Xenorhabdus and Photorhabdus

The entomopathogenic bacteria *Xenorhabdus* and *Photorhabdus* (Gram-negative γ-proteobacteria) establish symbiotic associations with the entomopathogenic nematode (EPN) species *Steinernematidae* and *Heterorhabditidae* respectively, which deliver the bacteria into the insect hemocoel. The lifecycle of both *Xenorhabdus* and *Photorhabdus* involves a symbiotic phase in association with the nematode and a pathogenic phase during insect infection. Although both bacteria can be cultured in laboratory conditions, they appear to live associated with their symbiotic nematodes in the soil and not in a free-living state. The bacteria colonise the intestine of the nematode in the infective juvenile stage (IJ) which actively seeks insect prey. The IJ enters the insect through its natural openings (mouth, anus or spiracles) and releases the bacteria in the host circulating hemolymph. A single IJ is enough to establish a successful infection in the insect (Forst *et al.*, 2001). Once in the hemocoel, the bacteria replicate (Daborn *et al.*, 2001) and deliver toxins that rapidly kill the insect. The bacteria initially colonise the inner membrane of the gut and subsequently spread within the insect colonising the fat body and other tissues (30-42 h post infection) (Silva *et al.*, 2002). During these stages, the bacteria release several toxins and exoenzymes that play a role in insect death and bioconversion of the insect body. Bioconversion of the cadaver is essential for bacteria replication and, in turn, for successful reproduction of the nematodes. After several cycles of growth and reproduction, the insect cadaver is exhausted and the infective juveniles re-acquire the bacteria and leave the host to colonise new insects (ffrench-Constant *et al.*, 2003; Herbert *et al.*, 2007).

The success of these entomopathogenic bacteria in killing and bioconverting the insect host is partly due to the evasion of the host immune system and also to the display of an armoury of toxins. The genome of *P. luminescens* encodes a strikingly high number and diversity of virulence factors including toxins, hemolysins, adhesins, proteases and antibiotic-synthesis genes. This redundancy could be partly explained by

its ability to kill a broader number of host species (compared to other entomopathogens) and perhaps also by an increased promiscuity reflected in a greater exchange of genetic material with other pathogenic bacteria. The first toxins described in *Photorhabdus* were the Tc: toxin complex (Waterfield *et al.*, 2001). There are three types of *tc* genes: "A" genes encode for an active toxin and the "B" and "C" genes encode the potentiators that enhance the insecticidal activity of the first one. Several loci encode different type and numbers of *tc* homologues depending on the *Photorhabdus* species. Their demonstrated oral toxicity make them suitable for agricultural applications, and this aspect will be expanded in the corresponding section. Other insecticidal toxins from *Photorhabdus* are: *mcf1* which is apoptotic to insect and mammalian cell lines (Daborn *et al.*, 2002; Dowling *et al.*, 2004; Dowling *et al.*, 2007), PVC (*Photorhabdus* virulence cassettes) similar to bacteriocins (Yang *et al.*, 2006) and the *pirAB* toxins, similar to insect hormones (Waterfield *et al.*, 2005b). A number of toxin-encoding genes have been also described from *Xenorhabdus* species, namely *xpt* and *xaxAB* (Sergeant *et al.*, 2003; Vigneux, 2007). The *xpt* genes are homologues of the *Photorhabdus tc*, while the *xaxAB* were discovered in *Xenorhabdus* as an apoptotic toxin, which *Photorhabdus* ortholog is *phlA* (Vigneux, 2007). The future publication of the *X. nematophilus* genome sequence will enable genome-wide comparisons of the two related genera (Latreille *et al.*, 2007). Such analysis will shed light on the similarities and differences between these two parallel entomopathogenic lifestyles.

Despite the potency of protein toxins, in lab-reared insects *Xenorhabdus* and *Photorhabdus* are unable to kill insects that have been pre-infected with non-pathogenic bacteria. Although it is possible that insects will be naturally immune primed, these experiments were performed by injecting high numbers of bacteria directly into the insect circulating system and not via the natural route of infection mediated by the entomopathogenic nematodes (Eleftherianos *et al.*, 2006a; Gotz *et al.*, 1981). *Photorhabdus* trigger the expression of microbial recognition proteins, such as hemolin, immulectin 2 and peptidoglycan recognition protein which shows that the bacteria are recognised by the host and raises the possibility that the host immune response has to be somehow controlled by the bacteria in order to kill the insect (Eleftherianos *et al.*, 2006b). Gram-negative bacteria are able to escape destruction mediated by humoral factors, by secreting outer membrane proteases or by chemically modifying LPS lipid A with 4-aminoarabinose moieties (Peschel, 2002). In *Salmonella*, these modifications are mediated by the *pmrHFIJKLM* operon, which is controlled by the PhoPQ two-component regulatory system (Gunn *et al.*, 1998a; Gunn *et al.*, 1998b; Guo *et al.*, 1997). A homologue of the *pmr* operon also exists in *P. luminescens* (*pbgPE*) and has been shown to be controlled by PhoPQ and also mediate modifications in LPS (Duchaud *et al.*, 2003; ffrench-Constant *et al.*, 2003). Mutations of either *phoP* or *pbgE3*, the *prmK* homologue, increased sensitivity of *P. luminescens* to AMPs and eliminated virulence in an insect model (Duchaud *et al.*, 2003; ffrench-Constant *et al.*, 2003). In contrast, *Xenorhabdus phoPQ* mutants show only sensitivity against AMPs, but not loss of virulence, suggesting that LPS modification is not required for adaptive resistance (Goodrich-Blair *et al.*, 2007). Interestingly, *X. nematophilus* suppresses the expression of AMPs by an unknown mechanism (Ji *et al.*, 2004; Park, 2007), which appears to be controlled by the master regulator Lrp (Cowles *et al.*, 2007). Both *Xenorhabdus* and *Photorhabdus* are able to inhibit phenoloxidase activation in a variety of insects (da Silva *et al.*, 2000; Dunphy *et al.*, 1991; Eleftherianos *et al.*, 2007).

Recently, Eleftherianos and co-workers have shown that *Photorhabdus* produces a small-molecule antibiotic stilbene (ST) that acts as an inhibitor of phenoloxidase (PO) in the insect host *M. sexta*. Inactivation of the *Photorhabdus* gene *stlA*, which encodes an enzyme that produces cinnamic acid, a key precursor for production of ST, eliminates ST production and PO inhibitory activity. Genetic complementation of the mutant and external supply of cinnamic acid can restore the ability to evade both melanisation and nodulation (Eleftherianos *et al.*, 2007).

Photorhabdus and *Xenorhabdus* also evade insect immunocompetent cells. Hemocytes from insects infected with *X. nematophilus* appear apoptotic (Cho *et al.*, 2004). Interestingly, three distinct phenethylamide compounds have been isolated *X. nematophilus* culture supernatants (Paik *et al.*, 2001). One of these compounds has been shown to induce apoptosis in human tumor cells (Hwang *et al.*, 2003). Both bacterial species have also been shown to interfere with the cellular responses by hijacking the the non-self recognition pathway by producing unidentified organic compounds which inhibit the activity of secretory phospholipase A2 (PLA2), an enzyme which is responsible for the biosynthesis of eicosanoids that are important in non-self signalling, hemocyte aggregation and nodulation (Park *et al.*, 2003a; Park *et al.*, 2003b; Park *et al.*, 2004). In addition *P. luminescens* encodes a T3SS with at least one effector, LopT, which is a homologue of the YopT effector secreted by *Yersinia* species upon calcium depletion. LopT has been shown to be secreted by the heterologous T3SS of *Yersinia* as the factors that induce type III secretion in *Photorhabdus* are still unknown (Brugirard-Ricaud, 2004). Interestingly, LopT expression is induced in the insect environment and a T3SS structural component appears necessary for suppression of nodule formation (Brugirard-Ricaud, 2004). *In silico* analyses of the unclosed *X. bovienii* and *X. nematophilus* genomes have shown that none of the species contain a T3SS or a LopT homologue (Goodrich-Blair *et al.*, 2007). Nevertheless, *Xenorhabdus* have a functional flagellar T3SS, which has been shown to secrete at least one toxic effector, a lipase, (Park *et al.*, 2006), and partially contribute to virulence (Goodrich-Blair *et al.*, 2007).

Bacillus cereus group and Bacillus sphaericus

The *Bacillus cereus* group of organisms contains the Gram-positive spore-forming bacteria *Bacillus thuringiensis, Bacillus anthracis*, *Bacillus cereus* (*sensu stricto*), *Bacillus mycoides*, *Bacillus pseudomycoides* and *Bacillus weihenstephanensis* (Rasko *et al.*, 2004). Although *B. cereus* and *B. anthracis* are human pathogens, *B. thuringiensis* is regarded as an insect pathogen commonly used as a crop protection agent against insects from the orders of Lepidoptera, Diptera and Coleoptera (Lecadet *et al.*, 1999; Schnepf *et al.*, 1998), and as such it will be described in this section. The ecology of *B. thuringiensis* is still an enigma: it is a ubiquitous soil microorganism, but it can also be found in other environmental niches, including phylloplane and the insect host intestinal system, rarely causing natural epizootic episodes (Jensen *et al.*, 2003). On the other hand *B. anthracis* and *B. cereus* spores are found in the soil. *B. cereus* can be occassionally found in the insect gut not only as spores but also as growing vegetative cells (Helgason et al., 2000; Margulis et al., 1998). Tabaniid flies (horse and deer flies of the *Tabanus* and *Chrysops* genera) have also been reported to disseminate anthrax and to excrete *B. anthracis* spores in their faeces after initial

feeding on animals infected with anthrax (Khrisna Rao *et al.*, 1958). Despite their direct/indirect association with insects neither *B. cereus* nor *B. anthracis* are considered insect pathogens. The huge differences in pathogenicity and virulence properties exhibited by these two species can be attributed to plasmid acquisition, these plasmids encode important virulence factors and insecticidal toxins. *B. anthracis* contains two plasmids, pXO1 and pXO2, which are necessary for full toxicity in humans (Ezzell *et al.*, 1999; Welkos, 1991; Welkos *et al.*, 1993). These plasmids carry genes responsible for the production of the anthrax toxin (encoded by genes *pagA*, *lef* and *cya*) as well as for genes implicated in *B. anthracis* antiphagocytic activity (*capB*, *capC*, *capA* and *capD*) (Welkos, 1991; Welkos et al., 1993). Although some pXO1-like plasmids have also been isolated from *B. cereus* strains, there is genetic evidence that these plasmids lack virulence genes (Rasko *et al.*, 2004; Read *et al.*, 2003). On the other hand, pXO1 and pXO2 plasmids are not present in *B. thuringiensis*, from which a wide range of smaller cryptic plasmids encoding the insecticidal protoxin termed δ-endotoxin have been isolated (Gonzalez *et al.*, 1981).

While lacking δ-endotoxin genes, the *B. cereus* genome still carries a variety of genes whose products are implicated in the disintegration of insect tissues, and it produces an immune inhibitor protein (InhA) that specifically hydrolyses cecropins and attacins in the immune hemolymph of *Hyalophora cecropia in vitro* (Dalhammar *et al.*, 1984; Edlund *et al.*, 1976; Fedhila *et al.*, 2003). These observations suggest that the natural habitat for the common ancestor of the *B. cereus* group might be the insect gut (see also section related to vectoring) (Waterfield *et al.*, 2004).

δ-endotoxin are expressed during sporulation and they constitute up to 25% of the dry weight of the sporulated cells (Agaisse *et al.*, 1995). δ-endotoxins are divided in two multigenic families: the crystal (cry) and cytolytic (cyt). The crystal toxins appear active against specific insect orders including Lepidoptera, Diptera, Hymenoptera and Coleoptera while the cytolytic toxins are toxic to Diptera (Bravo et al., 2007; Gonzalez et al., 1981; Hofte et al., 1989). The highly alkaline pH observed in the lepidopteran midgut in conjugation with midgut proteases mediates the activation of the protoxin and disruption of the gut epithelium by binding to receptors in the epithelial cells. It has been proposed that after ephithelial disruption, feeding cessation occurs leading to starvation and finally death of the insect. An alternative mode of function for the crystal toxins suggests that the spores initially located in the lumen of the gut had access to the hemocoel to germinate creating a systemic infection and subsequent death of the insect. However, recent work by Broderick and co-workers demonstrated that the disease-causing agents are enterobacteria that normally reside in the insect midgut (Broderick *et al.*, 2006).

In addition to δ-endotoxins, *B. thuringiensis* produces the vegetative insecticidal proteins (Vip) which are synthetised and secreted during vegetative growth. Vip1 and Vip2 constitute a binary toxin that is highly active against the western corn rootworm (*Diabrotica virgifera*) but not Lepidoptera (Han *et al.*, 1999). On the other hand, Vip3 proved to be highly insecticidal to several lepidopteran pests (Estruch *et al.*, 1996).

Another member of the insecticidal toxin producing species of the *Bacillus* genus is the soil-occuring *B. sphaericus*, which is part of the *B. subtilis* group (Hu *et al.*, 2008). *B. sphaericus* is toxic against mosquito larvae. The mosquitocidal properties are due to the action of two types of toxins: the highly active binary toxins BinA/BinB within spore crystals and the Mtx toxins (de Maagd *et al.*, 2003). Upon ingestion

by the target insect, the binary toxins are solubilised and proteolytically activated, bind specific receptors and form pores in target cells disrupting the mosquito gut epithelium (Darboux *et al.*, 2001; Davidson *et al.*, 2001). The Mtx1/2/3 family of toxins are produced during vegetative growth and their low activity might be due to low levels of production in addition to degradation during sporulation. Interestingly, purified Mtx1 showed very high toxicity against mosquito larvae (Thanabalu *et al.*, 1995). Mtx proteins can act synergistically with other mosquitocidal toxins (Wirth *et al.*, 2007).

INSECTS AS VECTORS FOR A NUMBER OF DISEASE-CAUSING BACTERIA

Insects are the primary or intermediate hosts or carriers of animal and plant diseases. Pathogens that are capable of being transmitted by insects include protozoa, bacteria, viruses, and helminths such as tapeworms, flukes, and roundworms. In this review we will refer to one remarkable example of insects as vectors of bacteria-associated disease. Given the numerical superiority of invertebrate-associated bacteria as well as the frequent association of insects with humans it is postulated that bacterial horizontal flux occurs from the insect associated to the vertebrate-associated bacteria (Waterfield *et al.*, 2004). This suggests that insects have the potential of acting as reservoirs for emerging human pathogens. In fact, insects have been implicated in the spread of the world's most deadly bacterial species *Yersinia pestis,* agent of bubonic plague.

Yersiniae are Gram-negative rods which belong to the family of Enterobacteriaceae. They consist of 11 species with three of them pathogenic to humans: the enteropathogenic *Y. pseudotuberculosis* and *Y. enterocolitica*, which are widely found in the environment, and the blood borne *Y. pestis* (Wren, 2003) which is the only vectored by insects. *Y. pestis* is transmitted by the flea; the bacteria interfere with the valvular function of the proventriculus, which guards the entrance to the midgut and controls the normal flux of blood during meals. The formation of a biofilm at the proventriculus by *Y. pestis* blocks the foregut and accumulates infected blood to the esophagus, resulting in regurgitation of blood to the bite site during feeding (Hinnebusch, 2005). Although this transmission model has been generally accepted, the long incubation period prior to a short window of infection implied by this model can not explain the rapid spread of epidemic. Transmission studies using the primary vector to humans in North America, *Oropsylla montana*, which rarely becomes blocked, as a model have revealed that the flea is immediately infectious and remains efficient during the early phase of infection (4 days post-infection) and even longer because the fleas do not suffer block-induced mortality (Eisen *et al.*, 2006).

Although *Y. pseudotuberculosis* and *Y. pestis* share 98% sequence identity, the outcome of the infection caused by each species is spectacularly different (Chain *et al.*, 2004). Pioneering studies by Achtman and co-workers have established that *Y. pestis* has evolved from *Y. pseudotuberculosis* just 1,500-20,000 years ago (Achtman *et al.*, 1999). A crucial step in *Y. pestis* evolutionary jump from an enteric pathogen to a flea-transmitted systemic vertebrate pathogen was the acquisition of two plasmids: the pPla and pMT1. pPla contains the plasminogen activator Pla that aids pathogen propagation in the mammalian host (Sodeinde *et al.*, 1988) by facilitating the spreading of *Y. pestis* after subcutaneous injection into the host. pMT1 encodes the putative murine toxin Ymt and the F1 capsule. Although capsule deficient mutants are still able to establish infections to African green monkeys (*Cercopithecus aethiops*) (Davis *et*

al., 1996), *Y. pestis* strains that lack the entire pMT1 plasmid are unable to colonise fleas (Hinnebusch *et al.*, 1998). Ymt has been shown to act as an intracellular phospholipase D (Hinnebusch *et al.*, 2000) and is required for the survival of *Y. pestis* in the flea midgut compartment, but not in the proventriculus compartment (Hinnebusch *et al.*, 2002). Moreover the chromosomally unstable haemin storage (*hms*) locus that encodes outer-surface proteins is also required for flea-borne transmission as it is necessary for infection of the proventriculus (Hinnebusch *et al.*, 1996). Deletion of the *hms* locus in *Y. pestis* results in changes in blood-feeding behaviour and less efficient transmission of plague (Hinnebusch *et al.*, 1996).

Genes that have been implicated in insect parasitism have also been detected in all three human pathogenic *Yersinia* species. These genes are homologues of the *tc* insecticidal-toxin complexes found in entomopathogenic Enterobacteriaceae such as *P. luminescens, S. entomophila* and *X. nematophilus* (ffrench-Constant *et al.*, 2007; Hurst *et al.*, 2000; Hurst *et al.*, 2007b; Joo Lee *et al.*, 2004; Sulakvelidze, 2000; Waterfield *et al.*, 2007; Waterfield *et al.*, 2001). Close inspection of the *Y. pestis* genome shows that some of the *tc* homologues are not intact, however recent microarray analysis have revealed that these toxin complexes are conserved and potentially functional in *Y. pseudotuberculosis* and *Y. enterocolitica* (Bresolin *et al.*, 2006; Erickson *et al.*, 2007; Hinchliffe *et al.*, 2003; Stabler *et al.*, 2003). *tc* homologues of *tcaA, tcaB, tcaC* and *tccC* are activated at low growth temperatures in *Y. enterocolitica* strains of biovars 2, 3 and 4 (Bresolin *et al.*, 2006). The insecticidal activity of *Yersinia* Tc is a matter of controversy. Whole-cell extracts of *Y. enterocolitica* strain W22703 cultivated at 10°C, but not at 30°C, led to insect mortality when fed to *M. sexta* larvae, in contrast to an insertional *tcaA* mutant (Bresolin *et al.*, 2006). However, the *Y. pestis* and *Y. pseudotuberculosis* TcaAB and TcaC-TccC purified proteins are non-toxic to fleas and, moreover, *Y. pseudotuberculosis* mutants deleted of *tc* genes retained acute toxicity (Erickson *et al.*, 2007). This controversy could be explained by differences in the midgut physiology between fleas and lepidopteran larvae (Dow, 1992) as well as differences in the sequences of the *tc* genes between the different species. However it is important to note that high fitness costs faced by *Y. pestis* within the flea could have eliminated the enterotoxicity of *tc* genes and redefined their function as molecules that contribute to the establishment of a transmissible infection in the flea by either stabilising biofilm formation or playing a role in combating as-yet unknown flea antibacterial effectors present in the midgut proventriculus (Erickson *et al.*, 2007).

Insect physiology and bacterial virulence

Insect physiology and susceptibility to infectious agents are highly affected by stress-inducing factors, including food deprivation, temperature, mechanical forces and chemical poisoning, as well as by the presence of other bacteria or toxins, that compromise immune defenses. A fine balance in the maintenance of the insect gut microflora is crucial to the optimal physiology in the insect (Ashburner *et al.*, 2005). The following two examples illustrate how the excess of gut microbes or changes in the conditions can result in pathogenicity.

Erwinia carotovora 15 (Ecc15), the phytopathogenic bacteria that cause soft rot in fruits, is a Gram-negative bacterial strain that was initially identified for its ability to persist in *Drosophila* gut and to elicit local and systemic immune responses following oral infection (Basset *et al.*, 2000; Tzou *et al.*, 2000). The genetic element

contributing to the persistence of *Erwinia* in the fly gut is the *Erwinia* virulence factor (*evf*), a novel gene with no homology in the databases. Enterobacteria expressing *evf* show improved survival in *Drosophila* gut and trigger a systemic immune response (Basset *et al.*, 2003). Recently, Acosta-Muniz and co-workers demonstrated that in fact, *evf* does not counteract immunity, as *Erwinia evf* mutants do not persist in immune-deficient *Drosophila*. Furthermore, *evf* is not a toxin and it is not required for survival in midgut organ cultures (Acosta Muniz *et al.*, 2007). Interestingly, bacteria expressing *evf* allow persistence *in trans* of bacteria lacking *evf* indicating that this gene promotes the accumulation of Gram-negative bacteria in the anterior midgut by affecting gut physiology (Acosta Muniz *et al.*, 2007).

An example of how the insect's physiological status can define a new pathogenic situation is the contribution of gut flora to the virulence of *B. thuringiensis*. The δ-endotoxins form pores in the membranes of epithelial cells causing disruption of the insect gut epithelium. The final cause of insect death was thought to be the spread of *B. thuringiensis,* once it gained access to the hemocoel after epithelial disruption, accompained by feeding cessation leading to starvation. However, the highly potent crystal toxin is not pathogenic by itself but only in conjunction with the Gram-negative enterobacteria inhabiting the insect gut (Broderick *et al.*, 2006). Reestablishment of an *Enterobacter* sp. that normally resides in the midgut microbial community, restores *B. thuringiensis*-mediated killing. On the other hand the enteric bacteria alone did not induce mortality in the absence of the Bt toxin that permeabilises the gut epithelium allowing their access to the hemocoel. These findings together with the observation that *B. thuringiensis* is unable to multiply in the insect hemolymph while enterobacteria can replicate in it, suggest that the Bt toxin is disrupting the gut epithelium, providing the opportunistic enteric bacteria with free access to the more favorable environment of the hemocoel, where they replicate, leading to septicemia and death of the insect host (Broderick *et al.*, 2006).

Applications

In depth knowledge of the molecular mechanisms underlying insect-bacteria interactions and their phenotypic manifestation in the host has led to the development of methods for insect control. Management strategies of insect vectors and pests include a variety of approaches. The disruption of essential symbionts of insects can have a high impact, along with the manipulation of microbes involved in essential insect activities, such as vectoring ability, metabolic requirements or the resistance to natural enemies. From the point of view of entomopathogenic bacteria a wide variety of toxins are under study to contribute to insect control. Studies on the molecular biology and physiology of insects have unveiled protein mechanisms that allow the expression of a specific peptide to target an insect population. In the following section we describe several examples of techniques for insect control, some of them currently in use and other with future potential.

MANIPULATION OF ENDOSYMBIONTS

Essential insect symbionts provide a desirable target for the control of insect pests that rely upon them, such as aphids. The complete elimination of endosymbionts *in vitro* is possible using antibiotics that reduce the lifespan of the insect and can sup-

press the population within a few days or weeks. Nevertheless, the use of antibiotics for field pest management is not a viable option, and finding new active compounds against endosymbionts will be required (Douglas, 2007). An alternative strategy is the manipulation of insect-fitness traits through symbionts. Paratransgenesis was defined as the modification of an insect phenotype by genetically transforming its associated microorganisms (Ashburner *et al.*, 1998). The requirements for a successful paratransgenic control strategy are: i) an obligate symbiotic relationship between bacteria and vector, ii) the feasibility of culturing and genetically modifying the bacteria without compromising its viability, iii) the stability of the transgene that should encode an effective antiparasite molecule expressed by the bacteria, and finally iv) the availability of efficient means to distribute the bacteria among the vector population (Riehle *et al.*, 2005). Several vectoring insects have been targeted in order to suppress the transmission of a parasite by means of modifying their symbionts. One of such example is the transmission of Chagas disease. The disease agent, *Trypanosoma cruzi,* is transmitted by the blood-sucking bug *Rhodnius prolixus* which harbour gut symbiont *Rhodococcus rhodnii*. By genetically modifying the endosymbiont to express antitrypanosomal genes in the insect gut, the paratransgenic insects become incapable of vectoring the disease (Ben Beard *et al.*, 2002). In the analogous case of the *Trypanosoma brucei* vector *Glossina*, control of the vectorial capacity of the tsetse fly has been achieved by transforming the secondary symbiont *Sodalis glossinidius* to express *Glossina* attacin, an antimicrobial peptide effective against protozoa. Tsetse flies which were fed the modified *S. glossinidius* had fewer parasites after being fed with a trypanosoma blood meal (Aksoy, 2003). The use of gut bacteria to control malaria transmission by *Anopheles* mosquitoes has also been proposed and is currently under study (Riehle *et al.*, 2005).

The paratransgenic approach has the advantage of being very specifically targeted, both in terms of the type of molecule used and the site of expression. However, one major challenge is to link the effector gene with an efficient driver mechanism to ensure spread in the insect population. In a manner analogous to *Wolbachia* (discussed below), novel genetic tools are available to efficiently drive the gene of interest in a targeted population. Selfish genetic elements (that spread in the population at the expense of their host) such as certain transposable elements, can be engineered to drive systems for the expression of genes that block disease transmission (Sinkins *et al.*, 2006). In principle, one or several mechanisms could be used to intervene at different stages of transmission, or even simultaneously combined to maximise success (Sinkins *et al.*, 2006). The implementation of driver-system strategies depends upon the feasibility of transforming the vector to introduce the modified selfish genetic element. Germline transformation in mosquitos using transposable elements has been successful in *Aedes aegypti*, the yellow fever vector (Coates *et al.*, 1998) and in two malaria vectors: *Anopheles stephensi* and *Anopheles gambiae* (Catteruccia *et al.*, 2000; Grossman *et al.*, 2001). Despite the achievements in generating refractory mosquitos (unable to transmit a parasite) and the promising possibilities of using gene-driver systems, the actual efficiency of disease reduction upon introduction of modified vectors in the wild is an unresolved question.

Using Wolbachia to influence mosquito and fly populations

Understanding *Wolbachia* biology allows the potential use of this symbiont to disrupt pest and vector populations (Douglas, 2007). As mentioned earlier, *Wolbachia* has been proposed as driver system for mosquito genetic replacement, enabling the expression

of transgenes that block parasite transmission in a self-sustained manner (Sinkins, 2004). A vital aspect for using *Wolbachia* in this way is the stability of the transgene, as lateral gene transfer from symbiont to host has been documented to affect at least four insect species (Hotopp *et al.*, 2007). The second application of *Wolbachia* for diptera control is the use of the CI phenotype (causing failure of egg hatch) to disrupt insect populations. CI induced by *Wolbachia* is a mechanism amenable to manipulation since different infection levels induce various patterns among insect populations. Briefly, unidirectional CI is seen between infected and uninfected populations (only uninfected females are not compatible with infected males), bidirectional CI is observed between populations that are infected with different *Wolbachia* strains (any cross between populations is not compatible), and bidirectionally incompatible CI occurs when *Wolbachia* strains are combined in superinfected insect individuals (superinfected males will be incompatible with females infected with only one strain) (Sinkins *et al.*, 2006). In *A. aegypti* mosquitoes, vector of dengue and yellow fever, embryos were infected with *Wolbachia* wAlbB by microinjection. Stable infections were established which gave high rates of CI, disrupting egg hatching in the following generation. In laboratory tests, where an uninfected population was seeded with infected females, wAlbB *Wolbachia* was able to rapidly spread into the *A. aegypti* population, reaching infection fixation within seven generations (Xi *et al.*, 2005b). The use of microinjection for *Wolbachia* transmission has also been successful for *Aedes albopictus*, although wild populations are naturally infected. In this particular case, population replacement strategies will require an incompatible *Wolbachia* strain or the generation of a superinfected mosquito population (Xi *et al.*, 2005a). An additional example of *Wolbachia* being used as a tool for control or reduction of insect populations, is its application to an agriculturally important pest that affects a great number of fruit varieties. *Wolbachia* has been tested in the laboratory to control medfly (*Ceratitis capitata*). The wild population of medflies is not infected with *Wolbachia*. In the assay, *C. capitata* males were transfected using a related fly species as donor. CI in the newly infected flies had a 100% success rate. Laboratory trials were conducted seeding a population of uninfected males and females with different numbers of infected males. It was shown that a ratio of 50 infected males per female was sufficient to obtain less than 1 hatched eggs. This result demonstrates that CI caused by *Wolbachia* infection could be an effective tool for the population control of this pest (Zabalou *et al.*, 2004).

INSECTICIDAL TOXINS FROM GRAM-NEGATIVE BACTERIA

A straightforward method of insect control is the use of bacterial entomopathogens through the manipulation of their toxins, which could ultimately be delivered in transgenic crops. There is a growing number of insect pathogens unveiled among Gram-negative bacteria. However, *P. luminescens* constitutes the most plausible alternative to the commonly used Gram-positive *B. thuringiensis*.

A recent unexpected discovery was the fact that the bacterial chaperon GroEL from the antlion-associated *Enterobacter aerogenes* has a paralysing toxic effect on other insects such as cockroaches. Several amino acid changes in the *E. aerogenes* compared to *E. coli* GroEL sequence were responsible for the paralysing function (potentially mediated by binding particular receptors in the host) (Yoshida *et al.*,

2001). Other insecticidal toxins have been found from several bacterial species associated with the antlion *Myrmeleon bore*. These bacteria are injected by the *M. bore* larvae into their preys in order to infect and kill them before feeding on their fluids (Nishiwaki *et al.*, 2007).

In *Serratia marcescens* a purified metalloprotease has been shown to cause toxicity in locusts (Tao *et al.*, 2007). Although the specific mechanism has not yet been elucidated, the proteolytic activity of this enzyme could be involved in maturation of other proteins, direct toxin activity or degradation of hosts connective tissues (Bowen *et al.*, 2003). Further research will be needed to assess the toxicity towards other insects, and to test heterologous expression as a first step toward future transgenic crops protected from locust pests.

Serratia entomophila and *Serratia proteamaculans* possess two sets of toxins encoded in the pADAP plasmid that contribute to amber disease in *Costelytra zealandica,* the New Zealand grass grub. The first cluster, *sepABC,* are responsible for the amber coloration and the gut clearance symptoms, and the *afp* genes cause cessation of feeding in the larvae. The SepABC toxins in *S. entomophila* have similarity to *Photorhabdus* Tc. Conversely to Tc toxins, where TcdA can work as a separate toxin, SepABC need to be coexpressed to cause amber disease. *In vitro* assays showed that the amber phenotype reverted after 7-14 days post treatement indicating that the toxins need to be continously expressed to cause disease (Hurst *et al.*, 2007b). The antifeeding phenotype in *C. zealandica* was conferred by the *afp* (antifeeding prophage) genes also encoded in the pADAP plasmid (Hurst *et al.*, 2007a; Hurst *et al.*, 2007b). Afp expression caused cessation of feeding within 2 days and larvae mortality at 11 days when the dose was increased. Afp structure was similar to phage tail-like bacteriocin and the contracted and extended forms were visualised. A toxin delivery role, working as micro-injectors, has been proposed for the Afp, and its PVC orthologues in *Photorhabdus* (Hurst *et al.*, 2007a; Yang *et al.*, 2006). Amber disease is highly host specific and affects only larvae of the *C. zealandica* species. However, insights into these toxin types and their mode of action, in particular the bacteriocin-like Afp, may generate potential applications for other insect species in the future.

Xenorhabdus and *Photorhabdus* toxins mainly affect coleopteran and lepidopteran larvae, although some of the toxins have a broader spectrum of action, including Diptera (Duchaud *et al.*, 2003). Among the most potent toxins produced by *Xenorhabdus* are the "toxin complex" (Tc/Xpt) (Sergeant *et al.*, 2003) and the XaxAB cytotoxin. Both of them are orally toxic to insects when expressed in *E. coli* (Sergeant *et al.*, 2003; Vigneux, 2007). The XaxAB cytotoxin, from a novel family of binary toxins, has apoptotic activity both in insect and mammalian cells which constitutes an obstacle for its potential use in insect control. *xaxAB* homologues have been found in a variety of pathogens including *Photorhabdus*, *P. entomophila*, *Y. enterocolitica* and *Proteus mirabilis*, and, surprisingly, even in the plant pathogen *Pseudomonas syringae* (Vigneux, 2007). The *xpt* genes from *X. nematophilus,* unlike the *Photorhabdus tc*, are co-localised and co-expressed. Insecticidal activity was tested by cloning and expressing individual and combined *xpt* in *E. coli*. All combined, *xptA1, xptA2, xptB1* and *xptC1,* were effective against three commercially important lepidopteran species. *Pieris brassicae* and *Pieris rapae* were more specifically targeted by the combination of *xptA1,xptB1* and *xptC1*, while *xptA2*, *xptB1* and *xptC1* were involved in toxicity to *Heliothis virescens* (Sergeant *et al.*, 2003). The Tc toxins were originally identi-

fied as high molecular weight insecticidal complexes present in the supernatant of *P. luminescens* subsp. *akhurstii* strain W14 (Bowen *et al.*, 1998a; Bowen *et al.*, 1998b). The *tc* genes from *Photorhabdus* are found in variable copy number and genome location in the different species. Four *tc* loci are found in *P. luminescens (tca, tcb, tcc* and *tcd)*, each containing genes encoding for different modules: the "A" genes encode toxins and "BC" potentiators (i.e. *tcdA*, *tcdB*, and *tccC*). Recent experiments suggest that "B" genes also encode toxins and "C" a protein involved in processing/ modification of the "B" toxin, effectively synergising the "A" toxins. Furthermore, a "BC" pair can cross-synergise an "A" toxin encoded elsewhere in the genome (Waterfield *et al.*, 2005a). Tc toxins have oral and injectable activity against *Manduca sexta* (Waterfield *et al.*, 2001). The oral activity against caterpillars makes them valuable alternatives to Bt for the development of transgenic crops (Waterfield *et al.*, 2005a). Indeed, the toxin protein TcdA from *P. luminescens* was expressed in *Arabidopsis thaliana*. The transgene conferred protection against first instar larvae of *M. sexta*, but mortality caused by the toxin decreased for more developed larvae (Liu *et al.*, 2003). One possible improvement would be the incorporation of the "BC" potentiator genes into the transgenic plant to boost toxicity. Homologues of the *tc* genes are present in other Gram-negative bacteria, some of which interact with insects (*X. nematophilus, S. entomophila* and *Y. pestis*) and others with not known insect association (*P. syringae* and *Fibrobacter succinogenes*) (ffrench-Constant *et al.*, 2003; Waterfield *et al.*, 2001). Recently, the identification of Tc toxins in the Gram-positive *Paenibacillus* led to the suggestion that *tc* homologues may be more widespread that initially thought (ffrench-Constant *et al.*, 2007). The experimental confirmation that more than one "A" toxin can be cross-potentiated by an heterologously expressed "BC" pair (even from a different organism of origin) opens the door to multiple combinations for specific insect control (ffrench-Constant *et al.*, 2007).

ENTOMOPATHOGENIC NEMATODES

Insects can became infected via entomopathogenic nematodes (EPN). EPN fulfill two important roles in the infective cycle: the transmission of pathogenic bacteria from host to host and the delivery of the microorganisms in the hemocoel ensuring an effective infection. EPNs have been used in Europe since 1993 as a reliable method of insect control (Shapiro-Ilan *et al.*, 2002). IJ can be grown *in vitro* in artificial media and then used in spray formulations or in the irrigation systems. Nematodes in the IJ stage seek actively their insect larvae prey, increasing the efficiency of action. Some of the pests successfully targeted with EPN are: artichoke plume moth, black vine weevil and black cutworm (Shapiro-Ilan *et al.*, 2002). The limitations of EPN in insect control are the lack of effect against foliar pests and the discontinuous protection to crops, as EPN are effective for 4 weeks after application, and they require moist soil conditions (Chattopadhyay *et al.*, 2004).

BT TOXINS

The insecticidal proteins from *B. thuringiensis* (Bt) and *B. sphaericus* (Bsp) have been extensively studied. Formulations containing sporulated *B. thuringiensis* have

been used for decades to control pests in agriculture, and more recently sprays of *B. sphaericus* have been applied to dipteran vectors (de Maagd *et al.*, 2003). Several Bt toxins have been successfully expressed in transgenic plants leading to insect-resistant crops (de Maagd *et al.*, 2003). The most widely used are the pore-forming Cyt and Cry δ-endotoxins. Synergistic effect of both types of toxins, Cyt and Cry, has been reported (Bravo *et al.*, 2007). In addition, synergy with other molecules from the host, such as the binding protein cadherin in the tobacco hornworm *Manduca sexta* has been described. Heterologous expression of a peptide from *M. sexta* cadherin in *E. coli* increased Cry1A toxicity (Chen *et al.*, 2007). Indeed, mutations in cadherin are linked with resistance in some lepidopteran pests (Gahan *et al.*, 2001; Morin *et al.*, 2003; Xu *et al.*, 2003). A related study showed that native Cry1A proteins required cadherin to form oligomers, but not the modified Cry1A proteins lacking one alpha-helix. These results indicate that cadherin enhances toxicity by facilitating the formation of Cry1A oligomers and that modified toxins may provide a solution to overcome insect resistance (Bravo *et al.*, 2007). The development of molecular techniques for recombination of known Cry proteins in order to obtain novel insecticidal toxins has been recently reviewed (Kaur, 2006). In addition, the search for new *B. thuringiensis* strains that provide toxin variants is ongoing (Balaraman, 2005).

The development of transgenic crops was a major advance in the substitution of chemical insecticides. A first generation of Bt crops expressing modified toxins against lepidopteran pests was available since 1996: Bt cotton (Bt toxin Cry1Ac) and Bt corn (Bt toxin Cry1Ab). Many more transgenic varieties, expresing different toxins, are available today including rice (High *et al.*, 2004; Huang *et al.*, 2005). This first-generation of Bt crops relied on the "high dose plus refugia" strategy, consisting in the introduction of non-transgenic plants alongside Bt crops to promote the survival of susceptible pests. The crossing of susceptible and resistant insects dilutes the resistant alleles in the insect population. This refuge strategy has contributed to delay insect resistance (Tabashnik *et al.*, 2008). Despite the fact that no cases of insect-resistance have been reported in the field (Ali *et al.*, 2007), improvements in Bt crops will be needed to avoid them in the future. Second and third generation of transgenic plants expressing two or more traits such as pest or disease resistance, and having inducible or tissue-specific toxin expression, are being developed (Christou *et al.*, 2006). The gene pyramiding approach, consisting in the combination of two toxins that act independently (i.e. binding different receptors) engineered in one crop was applied to cotton plants in 2003 (Moar *et al.*, 2007).

Besides insect-resistance to transgenic plants, other common concern about the use of Bt crops is the potential interference with non-targeted arthropods in the field. Several studies have addressed the question, one of them looking at the impact on biological control. The abundance of natural enemies of pests and their biocontrol functions were assessed in Bt crops versus non-Bt crops and no significant different was found (Romeis *et al.*, 2006). It is suggested that the reduction of chemical pesticides resulting from the use of *Bt*-transgenic varieties could enhance the biological control component in the field. Finally, there is an international initiative to assess the impact of insect-resistant transgenic crops on non-targeted arthropods (Romeis *et al.*, 2008). The initiative involves a consensus of methods and guidelines that will help evaluating the global impact of transgenic crops. In conclusion, a wide variety of approaches are available for insect control and some others are being successfully

developed. Evaluation of the impact of current strategies and further studies on new alternatives will provide tailored methods to ensure more targeted and environmentally friendly aproaches for integrated pest management.

Concluding remarks

Technological advances in genomics and proteomics are shedding light on the highly dynamic associations of insects with bacteria. These techniques along with computational analyses and mathematical modelling will enable a more comprehensive understanding of these complex interactions. Furthermore, several insect species are used as models to study human pathogens, owing to the similarities between vertebrate and invertebrate innate immunity.

The diversity and versatility of insect-bacteria interactions points to an enormous potential regarding the mechanisms for the modulation and control of insect populations with medical and agricultural implications. Further studies will be required to elucidate the details but the combination of currently used techniques and new approaches should make possible to tailor strategies for pest management.

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