Enterobacter: ONE BACTERIUM - MULTIPLE FUNCTIONS

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SUMMARY

Insects represent the most successful taxon of eukaryotic life, being able to colonize almost all environments. Microbial symbiomes associated with insects, impact important physiologies, and influence nutritional and immune system status, and ultimately, fitness. A variety of bacterial phyla are commonly present in insect guts, including Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Bacteroidetes, Firmicutes, Clostridia, Spirochetes, Verrucomicrobia, Actinobacteria, and others. Among them, the genus *Enterobacter* has been recognized as a dominant inhabitant of the gut for several important insect species, indicating an essential functional role for this taxon. Here, we review the known *Enterobacter* functional diversity among insects with respect to insect development, host exploitation, reproduction, and interactions with other organisms, in an attempt to provide an overview of the traits that have resulted in their evolutionary success. Many strains of *Enterobacter* species are not simply insect commensals but confer beneficial traits to their hosts that primarily fall into two categories: provision and degradation of nutrients and protection from pathogens.

Key Words: Microbial symbiomes, endosymbiotic bacteria, bacterial functional diversity, mutualisms, commensalisms, pathogenic relationships, nutrient degradation, nutrient provision, pathogen protection

1. INTRODUCTION

Microorganisms are well recognized as essential members of the biosphere. Over billions of years they have evolved into every conceivable niche on the planet. Microorganisms reshaped the oceans and atmosphere and gave rise to conditions conducive to the development of multicellular organisms (Gibbons and Gilbert 2015). Microbial diversity and distribution were mostly inaccessible and underestimated before the advent of molecular fingerprinting and high-throughput sequencing technologies, which now allow us to circumvent culture-based approaches (Fierer and Lennon 2011; Whitman et al. 1998).

Bacteria and archaea estimates continue to rise within an increasing number of environments sequenced using advanced molecular techniques, with the number of existing microbial species predicted to reach millions (Brown et al. 2015; Williams et al. 2017; Zaremba-Niedzwiedzka et al. 2017).

The term symbiosis originates from the Greek word “symbioun” meaning “to live together”; it was defined by Anton de Bary in 1879 as “the living together of two dissimilar organisms, usually in intimate association, and usually to the benefit of at least one partner” (Bary 1879). Symbiotic bacteria are omnipresent in all types of ecosystems, having a significant impact on eukaryotic evolution and diversity (Martin and Schwab 2012; McFall-Ngai 2007; Ruby et al. 2004). Although considerable attention has been given to pathogenic bacteria, pathogens are a minute minority of animal symbionts.

Microbial symbioses are generally categorized as parasitism, commensalism, or mutualism, though some relationships may wander across these defined boundaries depending on evolutionary processes, changes in environmental conditions and/or health state of the host/symbionts (Webster 2014). Host development, defence, nutrient assimilation and disease, in humans and other animals, is influenced by microbial mutualisms, commensalisms and pathogenic relationships (Eren et al. 2015). The development of next-generation DNA sequencing platforms has allowed an in-depth understanding of the composition of the microbial populations inhabiting different hosts and symbiosis research has focused on associations that (i) have economic importance, (ii) have implications for human health, or (iii) offer ecologically fascinating insights (Webster 2014).

It is estimated that the majority of members of the largest class of invertebrates, i.e. Insecta, are involved in some type of symbiosis, with most of these relations being shared with bacteria. Microorganisms can colonize the insect exoskeleton, the gut and haemocoel, and are present within some insect cells. The microbiota may account for 1–10% of the insect biomass, implying that the insect, as well as any other higher organism, can be regarded as a multi-organismal entity (Douglas 2015). Bacterial symbionts are equally prevalent in mammals and insects; however, the bacterial diversity in insect digestive tracts is generally low and rarely exceeds a few tens of species (Colman et al. 2012). Several immunological, physiological and morphological hypotheses have been proposed to explain that fact (Broderick and Lemaitre 2012; Engel and Moran 2013). The lack of adaptive immune function in invertebrates might partly explain this low diversity (McFall-Ngai 2007).

2. INSECT SYMBIONTS

Insect-associated microorganisms cover an immense range of functions and are known to upgrade nutrient-poor diets; aid digestion of recalcitrant food components; protect from predators, parasites, and pathogens; contribute to inter- and intraspecific communication; affect efficiency of disease vectors; and govern mating and reproductive systems (Engel and Moran 2013; Gil and Latorre 2019).

2.1. *Obligate Mutualistic Symbionts*

Insects that depend exclusively on nutritionally restricted diets such as plant sap, vertebrate blood and woody material, commonly possess obligate mutualistic symbionts that are involved in the provision of essential nutrients or the degradation of food materials (Engel and Moran 2013; Engel et al. 2016; Latorre and Manzano-Marín 2017). At least 10% of all insect species depend on obligate nutritional symbioses where bacteria are required to synthesize nutrients that are absent in the diets of their hosts (Wernegreen 2004). Aphids provide an example of such obligate endosymbiosis since all aphids harbour endosymbiotic bacteria (i.e. microorganisms that live inside host cells or tissues) of the genus *Buchnera* in specialized host cells called ‘bacteriocytes’ (Manzano-Marín et al. 2016). Other obligatory endosymbionts include *Wigglesworthia* in tsetse flies (Akman et al. 2002), *Blochmannia* in carpenter ants (Williams and Wernegreen 2010), *Baumannia* in sharpshooters (Wu et al. 2006), *Carsonella* in psyllids (Nakabachi et al. 2006) and *Tremblaya* in mealybugs (Szabó et al. 2017). In a broader definition in the same category, we can include *Candidatus* *Erwinia dacicola* in the olive fruit fly *Bactrocera oleae* (Capuzzo et al. 2005; Ben-Yosef et al. 2014). These intracellular mutualists commonly have the following biological features: (a) they are located inside bacteriocytes, (b) are essential for fitness, (c) are transmitted maternally, and (d) display strict host-symbiont co-evolutionary patterns (Bourtzis and Miller 2008).

2.2. “Facultative” Symbiotic Bacteria

In addition to obligate symbionts, many insects harbour “facultative” symbiotic bacteria, which are not essential for either host survival or reproduction and are typically maintained with a patchy distribution in host populations. Some facultative symbionts, like *Wolbachia*, *Spiroplasma* and *Cardinium*, are famous as reproductive manipulators in insects, affecting host reproduction by inducing male-killing, feminization, parthenogenesis or cytoplasmic incompatibility (Zchori-Fein and Perlman 2004; Doudoumis et al. 2013, 2017; Mateos et al. 2018). It has been speculated that these reproductive manipulators are not only harmful agents but also accelerators of host speciation due to reproductive isolation between infected and uninfected hosts. At the same time, *Wolbachia* has been shown, under certain conditions, to protect their hosts from insect pathogenic viruses as well as to prevent the establishment and transmission of major human pathogens like dengue and chikungunya viruses and the malaria parasite *Plasmodium* (Schmidt et al. 2017; Tan et al. 2017; Ant et al. 2018).

2.3. *Gut Microbiota*

Research conducted mainly in the last ten years, has resulted in tremendous progress in the field of gut microbiota and their impact on host metabolism. In general, gut microorganisms provide several nutritional functions to their hosts, and in return, hosts provide symbiotic microorganisms with a stable, protected, and nutrient-rich environment (Kohl et al. 2014). All animals assemble and maintain a diverse but host-

specific gut microbial community. Aside from the ubiquitous gut microflora in vertebrates, numerous invertebrates harbour endosymbiotic microorganisms inside their body cavity (Feldhaar 2011). It is believed that the gut microbiota can be considered as a bacterial organ, which is integrated into the biological system of the host (Sandeva et al. 2018). Gut microorganisms are also considered endosymbionts (Moya et al. 2008), although they are localized extracellularly within the gut lumen and their persistence within the gut could range from transient visitors to permanent inhabitants (Feldhaar 2011). Gut microorganisms produce a diverse metabolite repertoire from the anaerobic fermentation of undigested dietary components that reach the colon, and from endogenous compounds that are generated by the microorganisms themselves and their hosts (Rooks and Garrett 2016). Increased availability of technologies that profile microbial communities revealed that gut microorganisms are distinct from those of other characterized habitats in the biosphere, which indicates that strong selective forces differentiated gut-dwelling bacteria regardless of their lineage (Ley et al. 2008).

The basic structure of the digestive tract is similar across insects, although they possess a diversity of modifications associated with adaptations to specialized niches and feeding habits, and many of these specializations have evolved for housing gut microorganisms in specific gut compartments (Engel and Moran 2013; Pereira and Berry 2017). A variety of bacterial phyla are commonly present in insect guts, including Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Bacteroidetes, Firmicutes, including *Lactobacillus* and *Bacillus* species, Clostridia, Spirochetes, Verrucomicrobia, Actinobacteria, and others (Colman et al. 2012). The contribution of microorganisms, particularly gut microorganisms, to insect function is highly relevant from several perspectives, relevant to medicine, agriculture, and ecology (Douglas 2015).

2.4. Unveiling the Black Box of Symbiotic Associations

Characterization, exploitation and management of the insect-microbial symbiotic associations can contribute significantly to the control of agricultural pests and disease vectors. Recent advances in “omics”, such as metagenomics, metatranscriptomics, and metaproteomics, have gradually unveiled the black box of symbiotic systems. However, capturing the properties of insect microbiota has been challenging due to the high variability in composition between individuals and closely related species (Pernice et al. 2014). Several biological and ecological factors such as age, genetics and environment have been proposed to explain insect microbiota composition. However, diet is one of the main factors driving variation in the composition of the gut microbiota in vertebrates and invertebrates. In insects, host diet composition has been shown to shape the microbiota composition regardless of taxonomy and geography of the specimens (Colman et al. 2012; Chandler et al. 2011; Yun et al. 2014).

Food itself can be a vector of commensals, and different diets will provide microbial inoculates of different community compositions. Also, one major difference between the human and insect microbiota is that the majority of the insect bacteria seem to be aerobic and therefore capable of digesting food outside the insect. This

function may contribute to explaining why host diet is such a key driver of microbiota composition and explain why bacteria commonly associated with insects are very rare in diverse mammalian species and vice versa (Chandler et al. 2011; Pernice et al. 2014). Some insect-associated microorganisms are related to microbial taxa found in other animals, e.g. Enterobacteriaceae and other Gammaproteobacteria, Firmicutes and Bacteroidetes, but others are absent from any other animal and the external environment (e.g. many protists of the class Parabasalia are found exclusively within termites (Brune and Dietrich 2015)).

3. *ENTEROBACTER* A DOMINANT TAXON OF GUT MICROBIOTA WITH MULTIPLE ROLES

Members of the Enterobacteriaceae family are facultatively anaerobic, Gram-negative rods that are catalase-positive and oxidase-negative (Brenner et al. 1984). Currently, the family comprises 51 genera (Table 1) and 238 species. The number of species displays a wide divergence with a range of 1 to 22 per genus. Twenty-two genera contain only one species, while seven genera have more than ten species (Octavia and Lan 2014).

Table 1. The genera (51) of the family Enterobacteriaceae*

Arsenophonus (1991), *Biostraticola* (2008), *Brenneria* (1999), ***Buchnera*** (1991), *Budvicia* (1985), *Buttiauxella* (1982), *Cedecea* (1981), ***Citrobacter*** (1932), *Cosenzaea* (2011), *Cronobacter* (2008), *Dickeya* (2005), *Edwardsiella* (1965), ***Enterobacter*** (1960), *Erwinia* (1920), ***Escherichia*** (1919), *Ewingella* (1984), *Gibbsiella* (2011), *Hafnia* (1954), ***Klebsiella*** (1885), ***Kluyvera*** (1981), *Leclercia* (1987), *Leminorella* (1985), *Lonsdalea* (2012), *Mangrovibacter* (2010), *Moellerella* (1984), ***Morganella*** (1943), *Obesumbacterium* (1963), ***Pantoea*** (1989), ***Pectobacterium*** (1945), *Phaseolibacter* (2013), *Photorhabdus* (1993), *Plesiomonas* (1962), *Pragia* (1988), ***Proteus*** (1885), ***Providencia*** (1962), *Rahnella* (1981), ***Raoultella*** (2001), *Saccharobacter* (1990), ***Salmonella*** (1990), *Samsonia* (2001), ***Serratia*** (1823), ***Shigella*** (1919), *Shimwellia* (2010), ***Sodalis*** (1999), *Tatumella* (1982), *Thorsellia* (2006), *Trabulsiella* (1992), ***Wigglesworthia*** (1995), ***Xenorhabdus*** (1979), *Yersinia* (1944), and *Yokenella* (1985).

*The year the genus was proposed is listed in parentheses after the genus name; also, the genera of the family that have been characterized in insects are highlighted with bold text

Numerous applications use members of Enterobacteriaceae, including biocontrol in agriculture, production of recombinant proteins and non-protein products, control of infectious diseases, anticancer agents, biowaste recycling, and bioremediation (Watanabe et al. 2000; Zhu et al. 2011; Zhang et al. 2012; Zhao et al. 2012).

The family Enterobacteriaceae is ubiquitous, and many species can exist as free-living in diverse ecological niches, both terrestrial and aquatic, and some are associated with animals, plants, or insects only (Octavia and Lan 2014). For convenience, the members are broadly categorized into three types: (1) those that can cause human infections or are primarily associated with human/animals and the environment, (2) those that are associated with plants or plant pathogens and the environment, and (3) those that are associated with insects or are endosymbionts.

The Enterobacteriaceae (Proteobacteria) are considered as one of the most dominant bacteria families associated with insects (Drew and Lloyd 1987; Behar et al. 2005; Jurkevitch 2011; Rizzi et al. 2013; Wang et al. 2014; Cambon et al. 2018). They gave rise to a variety of symbiotic forms, from the loosely associated commensals, often designated as secondary (S) symbionts, to obligate mutualists, called primary (P) symbionts (Husnik et al. 2011). Many enterobacteria are also opportunistic pathogens.

The genus *Enterobacter* was first described by Hormaeche and Edwards (1960) and was named for the organisms' predominant natural habitat, the intestines of animals (from Greek *enteron*, meaning "intestine"). *Enterobacter* is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria of the family Enterobacteriaceae. The genus is polyphyletic based on the 16S rDNA sequence, with 14 lineages scattered across the 16S rDNA tree.

The taxonomy of *Enterobacter* has a complicated history, with several species transferred to and from this genus. The older species were assigned to the genus based on DNA-DNA hybridization values and phenotypic data; whereas the more recently described taxa rely on 16S rRNA gene- and *rpoB*-sequencing for genus allocation. It has been previously acknowledged that *Enterobacter* contains species which should be transferred to other genera. The polyphyletic nature of the genus makes it difficult to assign novel species to *Enterobacter* unless the strains cluster with the type species (*E. cloacae*) of the genus (Brady et al. 2013).

Members of the *Enterobacter* can be found in soil, water, sewage, vegetable and fruits, plants, terrestrial and aquatic environments. They can be isolated from the intestinal tracts of humans and other animals as commensals, but they are also significant human pathogens (Hoffmann and Roggenkamp 2003; Hoffmann et al. 2005; Davin-Regli and Pagès 2015).

Interestingly, *Enterobacter* spp. have been recognized as inhabitants of the gut of several insect species, suggesting that this genus may play various and vital roles (Vasanthakumar et al. 2006; Geiger et al. 2009; Jiang et al. 2012; Gujjar et al. 2017). In this respect, we reviewed the known *Enterobacter* diversity among insects in relation to the functional role in insect development, host exploitation, reproduction, and interactions with other organisms, in order to recognise the traits that have resulted in their evolutionary success. Many *Enterobacter* strains are not simply insect commensals but confer beneficial traits to their hosts that primarily fall into two categories: provision/degradation of nutrients and protection from pathogens.

3.1. *Enterobacter and Nutrient Bioavailability*

Many insects derive nutritional advantage from persistent associations with microorganisms that variously synthesize essential nutrients or digest and detoxify ingested food (Douglas 2009). These persistent relationships are symbioses, and the species of the genus *Enterobacter* often plays a leading role (Table 2). Endosymbiotic *Enterobacter* species add nutritional supplements to their host diet, most of the times in cooperation with other members of the insect gut microbiota, like *Klebsiella*, *Pectobacterium* or *Citrobacter*. In these interactions, the primary nutrient contributor may switch between the various bacterial species of these genera.

Table 2. Enterobacter species provide nutritional supplements to their host diet

Species	Strain Accession Number	Host		Function	Reference
<i>E. agglomerans</i>	Not available	<i>Coptotermes formosanus</i> Shiraki	Formosan subterranean termite	Nitrogen-fixing	Potrikus And Breznak 1977
<i>E. sp.</i>	Not available	<i>Coptotermes acinaciformis</i> (Froggatt), <i>Coptotermes lacteus</i> (Froggatt), <i>Cryptotermes primus</i> Hill, <i>Heterotermes ferox</i> (Froggatt), <i>Mastotermes darwiniensis</i> Froggatt, <i>Nasutitermes graveolus</i> (Hill), <i>Schedorhinotermes intermedius</i> (Brauer)	Termites	Nitrogen-fixing	Eutick et al. 1978
<i>E. agglomerans</i>	Not available	<i>Dendroctonus terebrans</i> (Olivier)	Bark beetles	Nitrogen-fixing	Bridges 1981
<i>E. sp.</i>		<i>D. frontalis</i> Zimmermann			
<i>E. aerogenes</i>		<i>Ips avulsus</i> (Eichhoff)			
<i>E. cloacae</i>	Not available	<i>Bactrocera tryoni</i> (Froggatt)	Queensland fruit fly	Nitrogen-fixing	Drew and Lloyd 1987; Murphy et al. 1994
<i>E. sp.</i>	Not available	<i>Anoplophora chinensis</i> (Forster)	Wood-boring beetle	Nitrogen-fixing	Rizzi et al. 2013
<i>E. sp.</i>	AB673459 AB673457	<i>Reticulitermes speratus</i> (Kolbe),	Termites	Recycling of uric acid nitrogen	Thong-On et al. 2012
	AB673458 AB673456	<i>Glyptotermes fuscus</i> (Oshima), <i>Cryptotermes domesticus</i> (Haviland)			
<i>E. sp.</i>	AY847157 AY847162	<i>Ceratitidis capitata</i> (Wiedemann)	Mediterranean fruit fly	Nitrogen-fixing	Behar et al. 2005
<i>E. hormaechei</i>	KC759162	<i>Reticulitermes chinensis</i> (Snyder)	Termite	Lignin degradation	Zhou et al. 2017
<i>E. sp.</i>	JQ864378	<i>Reticulitermes chinensis</i> (Snyder)	Termite	Recycling of uric acid nitrogen	Fang et al. 2016
	JX291546				
<i>E. aerogenes</i>	Not available	<i>Mastotermes darwiniensis</i> Froggatt	Termite	Lignin degradation	Kuhnigk et al. 1994
<i>E. cloacae</i>	Not available	<i>Reticulitermes flavipes</i> (Kollar)			

<i>E. aerogenes</i>	Not available	<i>Mastotermes darwiniensis</i> Froggatt	Termites	Hemicellulose degradation	Schäfer et al. 1996
<i>E. sakazaki</i>	Not available	<i>Reticulitermes flavipes</i> (Kollar)			
<i>E. aerogenes</i>	Not available	<i>Coptotermes formosanus</i> Shiraki	Formosan subterranean termite	Cellulose/Hemicellulose degradation	Adams and Boopathy 2005
<i>E. cloacae</i>	Not available				
<i>E. aerogenes</i>	EU305608	<i>Coptotermes curvignathus</i> (Holmgren)	Termite	Lignocellulose degradation	Ramin et al. 2008
<i>E. cloacae</i>	EU305609				
<i>E. cloacae</i>	MF185378	<i>Rhynchophorus ferrugineus</i> (Olivier)	Red palm weevil	Cellulose degradation	Muhammad et al. 2017
<i>E. sp.</i>	JN167548	<i>Rhynchophorus ferrugineus</i> (Olivier)	Red palm weevil	Cellulose/Hemicellulose degradation	Butera et al. 2012
<i>E. sp.</i>	LN829595	Coleoptera	Wood-feeding beetle larvae	Lignocellulose degradation	Manfredi et al. 2015
<i>E. sp.</i>	HM771088	Cerambycidae, Elateridae,	Coleoptera larvae	Cellulose/Hemicellulose degradation	Rojas-Jiménez and Hernández 2015
	HM771065 HM771070 HM771068 HM771104 HM771106 HM771101 HM771061 HM771048 GU827537	Passalidae and Scarabaeidae			
<i>E. sp.</i>	HM235482 HM235485 HM235491 HM235497	<i>Sirex noctilio</i> Fabricius	Wood-feeding wasp	Cellulose degradation	Adams et al. 2011
<i>E. asburiae</i> , <i>E. cloacae</i> , <i>E. sp.</i>	KT957438-43	<i>Plutella xylostella</i> (Linnaeus)	Diamond-back moth	Cellulose/Hemicellulose degradation	Xia et al. 2017
<i>E. sp.</i>	Not available	<i>Diastrammena asynamora</i> (Adelung)	Greenhouse camel cricket	Cellulose/Hemicellulose degradation	Mathews et al. 2019
<i>E. sp.</i>	AB872360-384	<i>Holotrichia parallela</i> (Motschulsky)	Dark black chafer beetle	Cellulose/Hemicellulose degradation	Sheng et al. 2015
<i>E. cloacae</i>	Not available	<i>Coptotermes curvignathus</i> (Holmgren)	Termite	Cellulose degradation	Toczyłowska-Mamińska et al. 2015
<i>E. cloacae</i>	KC512248	<i>Plutella xylostella</i> (Linnaeus)	Diamond-back moth	Trehalose hydrolysis	Adhav et al. 2019

<i>E. cloacae</i>	Not available	<i>Thermobia domestica</i> (Packard)	Firebrat	Water and nutrients	Woodbury and Gries 2013a,b
<i>E. sp.</i>	LJAN01000071	<i>Bactrocera oleae</i> (Rossi)	Olive fruit fly	Amino acids and vitamins	Estes et al. 2018a
	locus_taq:A C520_4801				
<i>E. sp.</i>	KR232642	<i>Ceratitis capitata</i> (Wiedemann)	Mediterranean fruit fly	Probiotic	Augustinos et al. 2015
<i>E. cloacae</i> , <i>E. asburiae</i>	KY810513	<i>Ceratitis capitata</i> (Wiedemann)	Mediterranean fruit fly	Probiotic	Hamden et al. 2013
	KY810514				
<i>E. agglomerans</i>	Not available	<i>Ceratitis capitata</i> (Wiedemann)	Mediterranean fruit fly	Probiotic	Niyazi et al. 2004
<i>E. sp.</i>	Not available	<i>Zeugodacus cucurbitae</i> (Coquillett)	Melon fly	Probiotic	Yao et al. 2017
<i>E. sakazakii</i>	DQ228421-22	<i>Stomoxys calcitrans</i> Linnaeus	Stable fly	Probiotic	Mramba et al. 2007
<i>E. cloacae</i>	Not available	<i>Plutella xylostella</i> (Linnaeus)	Diamond-back moth	Probiotic	Somerville et al. 2019

3.1.1. Nitrogen Fixation

A well-established contribution of *Enterobacter* species to nutrition is nitrogen fixation. Nitrogen fixation is a metabolic capability that is absent from the ancestral eukaryote and has been acquired by multiple eukaryotic groups through symbiosis. Many insects are known to live on low nitrogen diets, and microorganisms have been suggested to provide availability of these essential foodstuffs in various ways (Douglas 2009).

Potrikus and Breznak (1977) identified two *Enterobacter agglomerans* strains from the guts of Formosan subterranean termites (*Coptotermes formosanus*). Nitrogen fixation appears to play an essential role in termite biology by helping them overcome deficiencies related to their nitrogen-poor diet (wood). The process was found to be linked to the termite gut bacteria since antibiotic treatment eliminated the function (Breznak et al. 1973). Strains C-1 and C-2 were characterized with electron microscopy and numerous biochemical assays, including sugar fermentation tests. The nitrogen-fixing ability of the strains was verified with acetylene reduction tests, in both aerobic and anaerobic (100% N₂ or 100% Ar) growing conditions, using different sources of nitrogen (peptone, NH₄Cl, KNO₃). In media lacking peptone, NH₄Cl or KNO₃, nitrogen fixation by both strains occurred only under anaerobic conditions when 100% N₂ was provided. When peptone, NH₄Cl, or KNO₃ was provided in the media, fixation was taking place in both aerobic and anaerobic conditions (Potrikus and Breznak 1977).

Nitrogen fixation by *Enterobacter* species was also identified in the gut of termite species from Australia (in seven out of nine species tested) (Eutick et al. 1978). The *Enterobacter* strains could grow on nitrogen-free media under anaerobic conditions, but also under aerobic conditions in the presence of H₂SO₄ (Eutick et al. 1978).

Some years later, nitrogen-fixing *Enterobacter agglomerans* and *Enterobacter* spp. were isolated from various species of bark beetles, including *Dendroctonus terebrans*, *D. frontalis* and *Ips avulsus* (Bridges 1981). Even though large populations of nitrogen-fixing bacteria were found in the beetles, the *in-situ* assays in larvae did not reveal any acetylene reduction (Bridges 1981).

In the wood-boring beetle *Anoplophora chinensis*, enrichment studies of adult gut homogenates for nitrogen-fixing revealed the presence of four *Enterobacter* strains (Rizzi et al. 2013). The adult insects used in the enrichment cultures were isolated from *Acer saccharinum* L. and *Alnus* host trees (Rizzi et al. 2013).

Many microorganisms are valuable to the insect for their more comprehensive metabolic capabilities, including their capacity to utilize insect nitrogenous waste compounds (e.g. uric acid), synthesize 'high value' nitrogenous compounds (e.g. essential amino acids) and fix nitrogen (Douglas 2009). Uric acid is another substance utilized as a nitrogen source in insects feeding on nitrogen-poor diets composed of plant material (Potrikus and Breznak 1981). In such a case, four uricolytic *Enterobacter* strains were isolated from the guts of three wood-feeding termite species (*Reticulitermes speratus*, *Glyptotermes fuscus* and *Cryptotermes domesticus*) (Thong-On et al. 2012). Bacteria with uricolytic activity were grown anaerobically on plates containing various concentrations of uric acid. Isolated strains, RsN-1, GfU-1, Cd20b and Cd15a, showed >98% identity with *Enterobacter amnigenus*, *E. aerogenes*, *E. asburiae* and *E. cowanii*, respectively (Thong-On et al. 2012).

Enterobacter cloacae strains isolated from field-collected, and laboratory-reared *Bactrocera tryoni* (Froggatt) fruit flies showed dinitrogenase activity and were able to fix atmospheric nitrogen (Drew and Lloyd 1987; Murphy et al. 1994). Diazotrophic *Enterobacter* strains were also isolated from gut tissue of field-collected Mediterranean fruit flies *Ceratitis capitata* (Behar et al. 2005; Augustinos et al. 2021). However, the most potent nitrogen-fixing effect was produced by *Klebsiella* and *Citrobacter* species. Nitrogen fixation activity was estimated by a variety of methods, including acetylene reduction assays in live flies and bacteria grown on nitrogen-deficient media, amplification of the nitrogenase iron protein gene (*nifH*) from gut extracts and isolated colonies, as well as *in situ* expression and amplification of the *nifH* gene (Behar et al. 2005).

3.1.2. Degradation of Plant Cell Wall Components

Various *Enterobacter* strains are also able to provide nutrients to insects through the degradation of plant cell wall components (lignin, cellulose, hemicellulose) (König et al. 2006). Lignocellulolytic *Enterobacter* species have been identified in plant or wood-feeding insects, like beetles, termites or wasps.

A ligninolytic *Enterobacter* strain was isolated from abdomens of adult *Reticulitermes chinensis* termites that use the plant cell wall polysaccharide in their diet (Zhou et al. 2017). Strain PY12, classified as *E. hormaechei* based on its 16S rRNA sequence, produced a lignin-modifying peroxidase (LiP), a key component in the lignin degradation pathway (Zhou et al. 2017; Janusz et al. 2017). The decolourization of six different dyes determined LiP activity by spectrophotometry. Since the study focused only on one of the ligninolytic enzymes in the pathway, it was not entirely possible to elucidate how strain PY12 contributes to lignin degradation and the possible synergies it develops (Zhou et al. 2017).

Seven *Enterobacter* strains with the ability to degrade lignin and related aromatic compounds were identified in hindguts of various laboratory-reared and field-collected species of termites (Kuhnigk et al. 1994). Degradation was observed under aerobic conditions, while in the absence of oxygen, only slight modifications of the side group of aromatic compounds occurred (Kuhnigk et al. 1994). The strains were characterized as *E. aerogenes* (Km3 and KAn8), *E. cloacae* (Rt5a, Rt5b and Rt5c) and *Enterobacter* sp. (Rt3a and Rt3b). The first two were isolated from *Mastotermes darwiniensis* and the remaining five from *Reticulitermes flavipes* termites. Among the seven strains, Km3 and KAn8 exhibited the most significant degradation potential, with the ability to degrade nine out of 13 substrates tested (Kuhnigk et al. 1994). Apart from degrading lignin, strain Km3 also showed hemicellulose degrading activity (Schäfer et al. 1996). Hemicellulose was also degraded by *Enterobacter sakazakii* strain RA2 that was isolated from hindguts of wild *R. flavipes* termites from France (Schäfer et al. 1996). Interestingly, strain RA2 exhibited all (four out of four) enzyme activities that were tested and were related to polysaccharide degradation. On the other hand, strain Km3 lacked 1,4- β -xylanase activity (Schäfer et al. 1996). In a particular environment, though, bacteria lacking members of the metabolic cascade could act synergistically with other strains present to fully degrade substances.

The Formosan subterranean termite, *C. formosanus* contained *E. aerogenes* and *E. cloacae* strains that effectively utilized xylose as a carbon source (Adams and Boopathy 2005). Two strains isolated from *Coptotermes curvignathus* termites from Malaysia and identified as *E. aerogenes* and *E. cloacae* were able to degrade carboxymethylcellulose (CMC) and cellobiose (Ramin et al. 2008). Other lignocellulolytic strains identified in higher and lower termites belonged to the species *E. aerogenes*, *E. agglomerans* and *E. cloacae* (König et al. 2006; Mannesmann and Piechowski 1989).

3.1.3. Degradation and Biosynthesis of Other Nutrients

In the diamondback moth *Plutella xylostella*, Adhav et al. (2019) characterized, structurally and biochemically, a trehalase from *Enterobacter cloacae* which assists in the hydrolysis of trehalose, a significant energy source in insect metabolism. Firebrat nymphs (*Thermobia domestica*) acquire water and nutrients by consuming *Enterobacter cloacae*, facilitating their growth and survival in the absence of food or water for up to 22 days (Woodbury and Gries 2013a,b).

Genome sequencing of *Enterobacter* sp. OLF (Table 3), isolated from wild California olive fruit flies, revealed genes related to the biosynthesis of amino acids, vitamins and co-factors, degradation pathways, nitrogen metabolism, as well as the production of energy and precursor metabolites (Estes et al. 2018a, 2018b).

This characterization could mean that the strain could potentially supply its host, in cooperation with other symbionts, with amino acids, vitamins or other nutritional compounds missing from the olive fruit diet (Estes et al. 2018a, 2018b).

Table 3. Available genome sequences of *Enterobacter* symbionts from insects

Accession number	<i>Enterobacter</i> Strain	Host	Status	Submission Date
PRJNA420145	<i>E. cancerogenus</i> CR-Eb1	<i>Galleria mellonella</i> (Linnaeus)	complete	2018
PRJNA288712	<i>E. sp. OLF</i>	<i>Bactrocera oleae</i> (Rossi)	complete	2018
PRJNA340971	<i>E. Larv1_ips</i>	<i>Ips typographus</i> (Linnaeus)	draft	2019
PRJNA390046	<i>E. sp. 10-1</i>	Cerambycidae	draft	2017
PRJNA364290	<i>E. JKS000234</i>	Formicidae	draft	2017
PRJNA364289	<i>E. JKS000233</i>	Formicidae	draft	2017
PRJNA179500	<i>E. hormaechei</i> YT2	<i>Tenebrio molitor</i> (Linnaeus)	draft	2012
PRJNA180991	<i>E. hormaechei</i> YT3	<i>Zophobas morio</i> Fabricius	draft	2013
PRJNA180988	<i>E. cancerogenus</i> YZ1	<i>Tenebrio molitor</i> (Linnaeus)	draft	2013
PRJNA169065	<i>E. sp. Ag1</i>	<i>Anopheles gambiae</i> Giles	draft	2012

3.1.4. Probiotic Effects of *Enterobacter*

The most recent contribution of *Enterobacter* strains to insect fitness and nutrition is their emerging role as probiotics in diets of mass-reared *C. capitata* and *Zeugodacus cucurbitae* flies. Such properties can be particularly useful in Sterile Insect Technique (SIT) applications where irradiation treatment takes a heavy toll on insect competitiveness if the dose administered is high. In this regard, strains that were provided as probiotics in diets greatly improved various fitness parameters of the flies including pupal weight, longevity, adult size, flight ability, and adult emergence (Niyazi et al. 2004; Ben Ami et al. 2009; Hamden et al. 2013; Yuval et al. 2013; Augustinos et al. 2015, 2021; Kyritsis et al. 2017; Yao et al. 2017; Cáceres et al. 2019). For example, the provision of *Enterobacter* sp. strain AA26 resulted in increased production of pupae and adults, as well as reduced rearing duration in various developmental stages (from egg to pupa, pupal stage and from egg to adult), particularly for male *C. capitata* flies (Augustinos et al. 2015). However, it did not affect pupal weight, sex ratio, male mating competitiveness, flight ability or life span under food and water deprivation (Augustinos et al. 2015; Kyritsis et al. 2017).

Hamden et al. (2013) on the other hand observed increased pupal weight, male sexual performance and survival rates under food deprivation when they enriched the larval diet of mass-reared *C. capitata* with *Enterobacter* sp. and other beneficial bacteria (*Klebsiella pneumoniae* and *Citrobacter freundii*). In terms of mating competitiveness, irradiated males reared on probiotics achieved more matings and transferred larger quantities of sperm to females. Finally, in addition to pupal weight, probiotics also resulted in increased body size for adult males (head width, abdomen and thorax length) (Hamden et al. 2013). Niyazi et al. observed a diet-dependent probiotic effect on the mate-calling activity, mating success, life expectancy, and survival of mass-reared male *C. capitata* flies (Niyazi et al. 2004). The probiotic effect of *E. agglomerans* and *K. pneumoniae* on each of these parameters differed among the four diets that were tested (two standard adult diets and two enhanced experimental formulations), ranging from significant to non-existent (Niyazi et al. 2004). Differences in the probiotic effect were also observed between the laboratory trials and the field-cage assay (Niyazi et al. 2004).

Similarly, Yao et al. (2017) tested gut-associated *Enterobacter* spp. as probiotics in the larval diet of irradiated laboratory-reared *Z. cucurbitae* flies. Both live and autoclaved bacteria were tested to distinguish between insect-bacteria interactions and plain nutritional value of the probiotic effect. Application of the live bacteria increased female and male pupal weight, various morphological traits of adult flies, including head width and thorax length, as well as survival (Yao et al. 2017). In the case of survival rate, autoclaved bacteria resulted in a greater increase compared to live bacteria (Yao et al. 2017).

Mramba and colleagues studied the effects of *E. sakazakii* on stable fly *Stomoxys calcitrans* development (Mramba et al. 2007). They observed that sterile media did not support any fly development and that the effect was reversed with the addition of *E. sakazakii*. However, an improved effect for fly development was mainly observed in cooperation with other microbial partners in non-sterilized media. The combination of non-sterile media and *E. sakazakii* resulted in a slight increase in the survival of larvae to the pupal stage, in pupal weight and adult emergence and also displayed increased duration of the larval and pupal stages compared to the non-sterile media without the inoculum with *E. sakazakii* (Mramba et al. 2007).

A similar probiotic effect was observed in transgenic diamondback moth larvae (Somerville et al. 2019). The larvae that were grown on an aseptic diet (sterile diet and addition of streptomycin) and were inoculated with streptomycin-resistant *E. cloacae* JJBC exhibited increased pupal weight and production of progeny compared to an aseptic diet without the inoculum (Somerville et al. 2019).

In *Apis mellifera jemenitica* Ruttner, however, the *E. kobei* strain that was examined for its probiotic effect did not manage to reduce mortality of bee larvae infected with *Paenibacillus* spores (Al-Ghamdi et al. 2018).

3.2. *Enterobacter* and Protective Functions

Insects live in close coalition with microorganisms, which immensely influence their ecology and evolution. Microorganisms, such as bacteria, archaea, fungi, protozoa, viruses, can be associated with their insect host permanently or transiently, and such

associations may be beneficial or harmful to the insects' fitness (Gurung et al. 2019). For instance, endosymbionts tend to be dependent on the hosts for obtaining nutrients, whereas they can provide fitness advantages in terms of nutritional components (see above), overcoming host defences, and protection from pathogens, parasites, or environmental stressors (Engel and Moran 2013; Mereghetti et al. 2017).

The insect microbiota are also able to provide protective functions to their hosts, thereby influencing the proliferation of important human or animal pathogens (Table 4), including *Plasmodium*, *Trypanosoma*, dengue, Zika, yellow fever or chikungunya viruses, inside the insect body with a variety of mechanisms, either indirectly, by causing innate responses of the insect immune system or directly, through competition for resources with the pathogen or the production of substances with anti-pathogenic effects (Dong et al. 2009; Moreira et al. 2009; Bian et al. 2010, 2013; Cirimotich et al. 2011b; Walker et al. 2011; Weiss and Aksoy 2011; van den Hurk et al. 2012; Zhang et al. 2013; Dennison et al. 2014; Frentiu et al. 2014; Aliota et al. 2016a, 2016b; Dutra et al. 2016; Tan et al. 2017; Kalappa et al. 2018).

Table 4. *Enterobacter* species can provide protective functions to their hosts, aiding them to cope with abiotic and biotic stress

<i>Enterobacter</i> Species	Strain Accession Number	Host	
<i>Enterobacter</i> sp.	JF690924	<i>Anopheles arabiensis</i> Patton	Mosquito
<i>Enterobacter cloacae</i>	Not available	<i>Anopheles stephensi</i> Liston	Mosquito
<i>Enterobacter</i> sp.	JQ680715	<i>Anopheles gambiae</i> Giles	Mosquito
<i>Enterobacter cloacae</i> , <i>Enterobacter</i> <i>amnigenus</i>	Not available	<i>Anopheles albimanus</i> Wiedemann	Mosquito
<i>Enterobacter ludwigii</i>	MF084966 MF084975	<i>Delia antiqua</i> (Meigen)	Onion fly
<i>Enterobacter</i> sp.	EU693561 EU693573	<i>Leptinotarsa decemlineata</i> Say	Colorado potato beetle
<i>Enterobacter ludwigii</i> , <i>Enterobacter asburiae</i>	KX398658 KX398648 KX398650 KX398654 KX398657	<i>Helicoverpa zea</i> (Boddie)	Corn earworm
<i>Enterobacter agglomerans</i>	Not available	<i>Rhagoletis pomonella</i> (Walsh)	Apple maggot fly
<i>Enterobacter asburiae</i> , <i>Enterobacter cloacae</i> , <i>Enterobacter</i> sp.	KT957438 KT957439 KT957440 KT957441 KT957442 KT957443	<i>Plutella xylostella</i> (Linnaeus)	Diamondback moth
<i>Enterobacter</i> sp.	KX117074	<i>Leptinotarsa juncta</i> Germar	False potato beetle
<i>Enterobacter</i> sp.	JX296530 KC977257	<i>Leptinotarsa decemlineata</i> Say	Colorado potato beetle
<i>Enterobacter cloacae</i>	KM878728 KM886372 KM878717	<i>Coptotermes formosanus</i> Shiraki	Formosan subterranean termite
<i>Enterobacter cloacae</i>	Not available	<i>Glyphodes duplicalis</i> Inoue et al.	Mulberry pyralid
<i>Enterobacter amnigenus</i>	Not available	<i>Anopheles dirus</i> Peyton & Harrison	Mosquito

These effects continuously draw attention as a means of controlling vector-based infectious diseases, like malaria or dengue virus, that are transmitted by *Anopheles* and *Aedes* mosquito vectors (Cirimotich et al. 2011a; Jayakrishnan et al. 2018; Saldaña et al. 2017).

3.2.1. *Enterobacter* Anti-pathogenic Effects in Mosquitoes

Among various bacterial strains that show anti-pathogenic effects, one of the best characterized is *Enterobacter* strain *Esp_Z*, which was isolated from the midgut of wild *Anopheles arabiensis* mosquitoes in Zambian populations. The *Enterobacter* strain inhibited *Plasmodium* development in the midgut by 98%, 99% and 99% before the ookinete, oocyst, and sporozoite stage respectively (Cirimotich et al. 2011c). This response proved to be independent of the mosquito immune system since anti-*Plasmodium* genes, that are usually triggered during *Plasmodium* infections, showed similar activity in mosquitoes infected with *Esp_Z* or with non-inhibitory bacteria.

Additionally, silencing of anti-*Plasmodium* genes with RNAi in mosquitoes infected with *Esp_Z* did not result in *Plasmodium* oocyst development (Cirimotich et al. 2011c). The response also proved to be independent of bacterial retention of mosquito factors that are essential for *Plasmodium* development like, xanthurenic acid, iron and substances involved in fatty acid metabolism. Moreover, the inhibition effect was dose-dependent, both *in vitro* and *in vivo* conditions, with 10^4 bacterial cells providing near-complete protection against parasite infection and coinciding with active bacteria replication (Cirimotich et al. 2011c).

In view of the above, the inhibition activity was therefore hypothesized to be the result of anti-pathogenic substances. Indeed, the *Enterobacter* strain was found to produce *Plasmodium*-killing (Luckhart et al. 1998; Kumar et al. 2003; Peterson et al. 2007; Molina-Cruz et al. 2008) reactive oxygen species (Cirimotich et al. 2011c). These molecules were not detected in non-inhibitory bacteria, and their effect was neutralized by antioxidants in *Esp_Z*-containing insects, such as vitamin C and glutathione (Cirimotich et al. 2011c).

On the other hand, Eappen et al. (2013) identified *Enterobacter cloacae* strains that inhibited *Plasmodium* development by activating a component of the immune system of *Anopheles stephensi* mosquitoes. More specifically, *E. cloacae* was able to induce the expression of a serine protease inhibitor (SRPN6) in the mosquito midgut. The effect was linked to bacteria that were acquired through feeding and were exposed to the luminal side of the midgut epithelium, whereas bacteria injected in the haemocoel and exposed to the basal surface of the midgut epithelium were incapable of SRPN6 induction (Eappen et al. 2013). Unlike strain *Esp_Z*, the *E. cloacae* strains did not interfere with *Plasmodium* ookinete formation, but with the differentiation of ookinetes into oocysts after they traversed the midgut epithelium. Silencing of the SRPN6 gene resulted in an increase in the number of *Plasmodium* oocysts and increased prevalence of infection, implying that additional immune components are likely to participate in the inhibition process by *E. cloacae* (Eappen et al. 2013).

In field-collected *Anopheles gambiae* mosquitoes from Cameroon, *Enterobacter* spp. bacteria isolated from their midgut reduced *Plasmodium falciparum* oocyst intensity and prevalence by 35% and 15%, respectively. However, the reduction in infection was lower when parasite exposure was higher (Tchioffo et al. 2013).

Enterobacter bacteria that were isolated from the midgut of field-collected *Anopheles albimanus* mosquitoes in Mexico suppressed *Plasmodium vivax* infection in the insectary and field-collected samples (Gonzalez-Ceron et al. 2003). After seven days of treatment, *E. cloacae* and *E. amnigenus* 2 reduced *Plasmodium* infection by 17 and 53 times compared to control mosquitoes, respectively. Additionally, *E. cloacae* infected mosquitoes showed 2.5 times lower *Plasmodium* oocyst density than the controls (Gonzalez-Ceron et al. 2003).

3.2.2. *Enterobacter* Effects in Herbivorous Insects

Two *Enterobacter ludwigii* strains (B424 and B539) that were part of the natural gut microbiota of the onion fly *Delia antiqua* collected from garlic fields in China, showed inhibitory effects against *Beauveria bassiana* (Balsamo), an entomopathogenic fungus that is used as a biocontrol agent in pest management applications (Valero-Jiménez et al. 2014; Zhou et al. 2019). The vital role of the bacterial symbionts of *D. antiqua* on the larvicidal potential of *B. bassiana*, was determined by first examining axenic (bacteria-free) and non-axenic larvae infected with the fungus (Zhou et al. 2019). In this case, treatment with the pathogen did not affect the survival of non-axenic larvae but indicated a significant reduction in the survival of axenic larvae. Furthermore, axenic larvae inoculated with microbiota were characterized by significantly higher survival rates than non-inoculated axenic larvae, when both were infected with the fungus (Zhou et al. 2019). Following the above tests, symbiotic bacteria were isolated from the surface and guts of the onion fly, and certain strains were tested for their anti-fungal effect. The *Enterobacter* strains strongly inhibited (ca. 70-99%) conidia germination and the hyphal growth of the entomopathogen fungus *B. bassiana*. Additionally, as expected from the previous tests, inoculation of axenic larvae with strain B424 significantly increased survival rate against the fungal infection (Zhou et al. 2019).

Similar observations were made with the Colorado potato beetle *Leptinotarsa decemlineata* fourth-instar larvae that were field-collected in Maryland and Virginia, USA (Blackburn et al. 2008). *Enterobacter* sp. strains 2B1C and 2B2D that were isolated from larval faecal fluid were found to inhibit two entomopathogens, *Photorhabdus temperata* Fischer-Le Saux et al. and the fungus *B. bassiana*. *P. temperata* is a bacterial symbiont of the entomopathogenic nematode *Heterorhabditis marelatus* Liu & Berry which can be used to suppress the Colorado potato beetle. However, in the presence of the *Enterobacter* strains the nematode fails to complete its reproductive cycle in the beetle, possibly due to the inhibition of *P. temperata* (Armer et al. 2004). The *in vitro* tests were based on comparing on PP3 agar plates (Bacto® Proteose Peptone) the growth of *P. temperata* and *B. bassiana* conidia in the presence of each *Enterobacter* strain. Both strains reduced the growth of *P. temperata* by approximately 33% while at the same time, strain 2B1C and 2B2D reduced fungal growth by almost 80% and 42.5% respectively (Blackburn et al. 2008).

Enterobacter strains have also been recognized as essential factors in shaping interactions between herbivorous insects and plants, by either activating or suppressing plant defences. In such a case of activation, laboratory-reared *Helicoverpa zea* larvae inoculated orally with an *Enterobacter ludwigii* strain were found to activate tomato plant *Solanum lycopersicum* defences (Wang et al. 2017). The strain was isolated from oral secretions, the regurgitant, of field-collected *H. zea* larvae in Rock Springs, Pennsylvania, USA. The *E. ludwigii* strain along with an *E. asburiae* strain were found to increase the activity of glucose oxidase (GOX), a molecular signal that induces plant defences in response to herbivory, in the labial glands of laboratory-reared larvae (Wang et al. 2017). Even though direct application of both strains on wounded tomato plants resulted in suppression of polyphenol oxidase (PPO) activity, a plant defence component which is regulated by jasmonic acid (JA), when tomato plants were damaged by *E. ludwigii*-inoculated larvae, they showed an increase in JA-regulated genes (proteinase inhibitor II (*Pin2*), cysteine proteinase inhibitor (*CysPI*) and polyphenol oxidase F (PPOF)), and a suppression in salicylic acid (SA) response (pathogenesis-related protein 1 (P4), *Pr1a* (P4)). Suppression of JA-mediated response proved to be dose-dependent, with high bacteria concentrations producing significant results when applied directly on the plant.

In the diamondback moth, two *Enterobacter* species, *E. asburiae* and *E. cloacae*, encoded all the genes related to the aerobic pathway for catechol degradation and could significantly degrade phenol *in vitro* (Xia et al. 2017). These compounds are important plant defence compounds of *Brassica* plants as they are toxic to insects (Xia et al. 2017).

In a contrary case of suppressing plant defences, in antibiotic-treated laboratory-reared false potato beetle (*Leptinotarsa juncta*) larvae, *Enterobacter* inoculation reduced JA-regulated gene expression (PPO) in tomato leaves (non-preferred host, *S. lycopersicum*), but did not have any effect on horsenettle (preferred host, *Solanum carolinense* Linnaeus) (Wang et al. 2016). Damage to leaves by untreated false potato beetle larvae or application of their oral secretions to wounded leaves resulted in reduced PPO activity in both plants compared to antibiotic-treated larvae (Wang et al. 2016). Additionally, direct application of *Enterobacter* on wounded leaves suppressed JA-regulated PPO and *CysPI* expression and increased SA-regulated *Pr1* expression in tomato, but not in horsenettle (Wang et al. 2016).

Similarly, Chung et al. (2013) observed that damage caused to tomato plants by antibiotic-treated Colorado potato beetle larvae increased JA-regulated gene expression (*CysPI* and *PPOF/B*) and a decrease in the SA defence response (*Pr1* (P4)) compared to untreated larvae. Additionally, lower PPO activity was observed when oral secretions of untreated larvae were applied on mechanically wounded plants. These observations suggested that bacteria in oral secretions of the Colorado potato beetle suppressed the JA-mediated defence mechanism. Indeed, an *Enterobacter* strain that was isolated from larval oral secretions suppressed JA-mediated antiherbivore defence response (PPO) in tomato plants when it was inoculated in antibiotic-treated Colorado potato beetle larvae (Chung et al. 2013).

4. BACTERIAL PARATRANSGENESIS WITH *ENTEROBACTER*

As *Enterobacter* strains form extremely stable relations with insects by constituting a prevalent portion of their gut microbiota, they can function as vectors for the introduction of functional genes that could be useful for multiple applications, including pest population control (Wilke and Marrelli 2015).

Such a system was examined in *Enterobacter cloacae* species isolated from the guts of Formosan subterranean termites in Hawaii (Husseneder and Grace 2005). The bacteria were transformed with a recombinant plasmid containing genes encoding ampicillin resistance and green fluorescent protein (GFP) and were fed to termite workers from three colonies (Husseneder and Grace 2005). The infection was established rapidly, with 90–100% of termite workers containing transformed bacteria 12 h after feeding, which persisted in the population for up to 11 weeks. Moreover, fluorescent microscopy revealed that the ingested bacteria expressed the GFP gene in the termite gut. Transformed bacteria were efficiently transferred through a colony, with infection rates reaching 80–100% after six days, even when the initial ratio between infected and uninfected termites was low. Recombinant bacteria were also transferred into the soil by infected termites but declined rapidly within four to five weeks (Husseneder and Grace 2005).

In another example, *Enterobacter cloacae* strain WBMH-3-CM^r was transformed with plasmids containing the ice nucleation gene *inaA* of *Erwinia ananas* IN10 and were ingested by larvae of mulberry pyralid *Glyphodes duplicalis* (Watanabe et al. 2000). Ice nucleation genes increase the supercooling points and reduce the tolerance of overwintering insects to cold, resulting in death by freezing. The supercooling points of mulberry pyralid larvae treated with the transgenic *E. cloacae* strain were higher than *E. ananas*-treated larvae, and their mortality rate reached 64.3% after 2 h exposure at -5°C and eventually increased to 95.2–100% after 6 and 18 h of exposure (Watanabe et al. 2000).

In *Anopheles dirus* larvae, *Enterobacter amnigenus* bacteria isolated from their gut tissue were transformed with the mosquito-larvicidal toxin of *Bacillus sphaericus* 2297 (Khampang et al. 2001). The fragment encoding the binary toxin was inserted into various plasmids, under different promoters in order to improve expression levels. *E. amnigenus* carrying a recombinant plasmid with the native *B. sphaericus* promoter exhibited the highest toxicity among the plasmids that were tested and proved to be 20 times more effective than *B. sphaericus* 2297 (Khampang et al. 2001).

5. INSECTICIDAL ACTIVITY OF *ENTEROBACTER*

Apart from their beneficial impact on host fitness as members of the insect gut microbiota, *Enterobacter* strains may also exhibit insecticidal activity even against their hosts, functioning as biocontrol agents in pest management applications (Table 5).

The shift in the behaviour of otherwise beneficial symbionts could be due to changes in the composition or location of the insect gut microbiota. Several examples of entomopathogenic *Enterobacter* strains have been documented. Among them, *E. cloacae* strain SL11 caused 30-73% mortality, depending on bacteria concentration, when fed to larvae of its host *Spodoptera litura* (Thakur et al. 2015). The strain also had a negative impact on essential fitness parameters of *S. litura* progeny, including development from larva to adult, growth rate, life span, morphology and reproduction. The pathogenic *E. cloacae* strain dominated the gut microbiota in infected insects, caused a reduction in the number of haemocytes and produced immune responses of phenoloxidase and lysozyme (Thakur et al. 2015).

Table 5. *Enterobacter* species providing insecticidal activity

<i>Enterobacter</i> Species	Strain Accession Number	Host		Function	Reference
<i>Enterobacter cloacae</i>	KP058541	<i>Spodoptera litura</i> (Fabricius)	Tobacco cutworm	Insecticidal effect	Thakur et al. 2015
<i>Enterobacter hormaechei</i> , <i>Enterobacter</i> sp.	GU906249 GU906251	<i>Rhynchites bacchus</i> (Linnaeus)	Leafroller weevil beetles	Insecticidal effect	Gokce et al. 2010
<i>Enterobacter cloacae</i>	Not available	<i>Bemisia argentifolii</i> Bellows & Perring	Silverleaf whitefly	Insecticidal effect	Davidson et al. 2000
<i>Enterobacter cancerogenus</i>	Not available	<i>Oberea linearis</i> (Linnaeus)	Hazelnut and walnut twig borer	Insecticidal effect	Bahar and Demirbağ 2007
<i>Enterobacter</i> sp.	KM979225	<i>Cimbex quadrimaculatus</i> (Müller)	Sawfly	Insecticidal effect	Cakici et al. 2015
<i>Enterobacter</i> sp.	JQ066777	<i>Spodoptera littoralis</i> (Boisduval)	Cotton leafworm	Insecticidal effect	Cakici et al. 2015
<i>Enterobacter aerogenes</i>	Not available	<i>Euproctis chrysorrhoea</i> (Linnaeus)	Brown-tail moth	Insecticidal effect	Yaman et al. 2000
<i>Enterobacter aerogenes</i>	AF306521	<i>Myrmeleon bore</i> (Tjeder)	Antlion	Insecticidal effect	Yoshida et al. 2001

Enterobacter strains with pathogenic effect against their host cause mortality in variable degree. In leafroller weevil beetles *Rhynchites bacchus* two *Enterobacter* strains (*E. hormaechei* Rb3 and *Enterobacter* sp. Rb5) caused 13% mortality (Gokce et al. 2010) in the silverleaf whitefly, *Bemisia argentifolii*, *E. cloacae* WFA73 produced 34% adult *B. argentifolii* mortality after 24 h and 75% after 48 h (Davidson et al. 2000). *E. cancerogenus* OI11 caused 15% mortality in larvae of the beetle *Oberea linearis* ten days after treatment (Bahar and Demirbağ 2007).

The larvicidal effect of *Enterobacter* sp. CQ4 resulted in 58% mortality within ten days of third instar larvae of *Cimbex quadrimaculatus* (Çakici et al. 2015). In the cotton leafworm *Spodoptera littoralis*, *Enterobacter* sp. SL4 showed low larvicidal activity (17%) against third instar larvae, ten days after treatment (Çakici et al. 2014). Finally, an *Enterobacter aerogenes* isolate from the brown-tail moth *Euproctis chrysorrhoea* caused 45% mortality in third/fourth instar larvae (Yaman et al. 2000).

Yoshida et al. (2001) identified a mechanism of insecticidal activity based on a paralysing toxin that was produced by *Enterobacter aerogenes* bacteria contained in the saliva of *Myrmeleon bore* larvae. The 63 kDa protein assists larvae in capturing their prey and shows homology to the heat shock protein GroEL of *Escherichia coli*. The two proteins share similar sequences that contain only a few differences, in 11 residues and the carboxy terminus. A series of individual mutations revealed crucial residues for toxicity. Specific substitutions resulted in the loss of insecticidal activity of the *E. aerogenes* protein or the development of toxic effect by the otherwise harmless GroEL chaperone (Yoshida et al. 2001).

6. ENTEROBACTER GENOMICS

The number of available genome sequences from the *Enterobacter* taxon has not reached a threshold where comparative genomics can drive hypotheses and experiments. Recent progress in the genomics era resulted in one complete genome sequence of *Enterobacter cancerogenus* CR-Eb1 isolated from the larval gut of the greater wax moth *Galleria mellonella* (Chung et al. 2018). Also, there are several draft genome sequences of *Enterobacter* strains available from other insects (Table 3). In order to fully utilize the powerful analysis of comparative genomics and draft genomes from *Enterobacter* strains, more genomic and transcriptomic data are required from *Enterobacter* strains covering diverse functional roles. The availability of these genomes will be able to assist us in understanding their functional roles by defining their differences and similarities in gene content (Khamis et al. 2019).

The availability of *Enterobacter* sequenced bacterial genomes will also allow a more profound understanding of their organizational features that are related to fundamental cellular processes such as coordinated gene expression, chromosome replication and cell division. Genomic data will provide the means to characterize the fluidity of bacterial chromosomes, including genome rearrangements that imperil the selective features of chromosome order. Furthermore, a high-density microarray can be developed for the analysis of expression and genome content in a wide variety of *Enterobacter* strains, both sequenced and not sequenced.

Comparative genomics of more complete *Enterobacter* genomes will also allow studying the integration process from free-living to endosymbiont. Usually, symbiotic bacteria undergo drastic genetic, phenotypic, and biochemical changes, which can be detected by comparison with free-living relatives (Lo et al. 2016). Genome reduction, in some cases, is extremely strong, and it has generated the smallest bacterial genomes found to date; gene contents are so limited that their status as cellular entities is questionable (Latorre and Manzano-Marín 2017). It would be exciting to further improve our understanding of *Enterobacter* species' diversity in terms of their evolutionary history.

7. CONCLUSIONS

More than a hundred years of biological research has demonstrated the importance of microorganisms in the health and disease of higher organisms. Similarly, insects have symbiotic interactions that enable them to exploit unusually limited nutritional resources. In particular, recent findings suggest that symbiotic associations between insects and *Enterobacter* species may be beneficial to host fitness because of their various abilities to hydrolyse and ferment carbohydrates, catalyse nitrogen fixation, and produce vitamins and pheromones.

Also, the dominance of *Enterobacter* indicates an essential role in the protection of the insect host, or its nutritional resources, against parasitoids or predators, and also in terms of the interference in the transmission of malaria and other vector-borne diseases.

This review summarized our current knowledge of the relationship between *Enterobacter* and insect hosts. Characterization, exploitation and management of the insect-*Enterobacter* symbiotic associations can significantly contribute to and support integrated pest management applications for the control of agricultural pests and disease vectors.

To further decipher the *Enterobacter*-based symbiotic interactions we propose a systems biology approach in which, *in silico* predictions based on genomic analyses and phylogenetic information will be assessed by transcriptomic, proteomic, and metabolomic analyses. This information is considered essential for the success of downstream field applications.

8. REFERENCES

- Adams, L., and R. Boopathy. 2005. Isolation and characterization of enteric bacteria from the hindgut of Formosan termite. *Bioresource Technology* 96: 1592–1598.
- Adams, A. S., M. S. Jordan, S. M. Adams, G. Suen, L. A. Goodwin, K. W. Davenport, C. R. Currie, and K. F. Raffa. 2011. Cellulose-degrading bacteria associated with the invasive woodwasp *Sirex noctilio*. *The International Society for Microbial Ecology (ISME) Journal* 5: 1323–1331.
- Adhav, A., S. Harne, A. Bhide, A. Giri, P. Gayathri, and R. Joshi. 2019. Mechanistic insights into enzymatic catalysis by trehalase from the insect gut endosymbiont *Enterobacter cloacae*. *The Federation of European Biochemical Societies (FEBS) Journal* 286: 1700–1716.
- Akman, L., A. Yamashita, H. Watanabe, K. Oshima, T. Shiba, M. Hattori, and S. Aksoy. 2002. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genetics* 32: 402–407.
- Al-Ghamdi, A., K. Ali Khan, M. Javed Ansari, S. B. Almasaudi, and S. Al-Kahtani. 2018. Effect of gut bacterial isolates from *Apis mellifera jemenitica* on *Paenibacillus* larvae infected bee larvae. *Saudi Journal of Biological Sciences* 25: 383–387.
- Aliota, M. T., S. A. Peinado, I. D. Velez, and J. E. Osorio. 2016a. The wMel strain of *Wolbachia* reduces transmission of Zika virus by *Aedes aegypti*. *Scientific Reports* 6: 28792.
- Aliota, M. T., E. C. Walker, A. Uribe Yepes, I. Dario Velez, B. M. Christensen, and J. E. Osorio. 2016b. The wMel strain of *Wolbachia* reduces transmission of Chikungunya virus in *Aedes aegypti*. *PLoS Neglected Tropical Diseases* 10.
- Ant, T. H., C. S. Herd, V. Geoghegan, A. A. Hoffmann, and S. P. Sinkins. 2018. The *Wolbachia* strain wAu provides highly efficient virus transmission blocking in *Aedes aegypti*. *PLoS Pathogens* 14: e1006815.
- Armer, C. A., R. E. Berry, G. L. Reed, and S. J. Jepsen. 2004. Colorado potato beetle control by application of the entomopathogenic nematode *Heterorhabditis marelata* and potato plant alkaloid manipulation. *Entomologia Experimentalis et Applicata* 111: 47–58.

- Augustinos, A. A., G. A. Kyritsis, N. T. Papadopoulos, A. M. M. Abd-Alla, C. Cáceres, and K. Bourtzis. 2015. Exploitation of the medfly gut microbiota for the enhancement of Sterile Insect Technique: Use of *Enterobacter* sp. in larval diet-based probiotic applications. *PLoS One* 10: e0136459.
- Augustinos, A. A., G. A. Kyritsis, C. Cáceres, and K. Bourtzis. 2021. Insect symbiosis in support of the Sterile Insect Technique, pp. 605–630. *In* V. A. Dyck, J. Hendrichs, and A. S. Robinson (eds.), *Sterile Insect Technique – Principles and practice in Area-Wide Integrated Pest Management*. Second Edition. CRC Press, Boca Raton, Florida, USA.
- Bahar, A., and Z. Demirbağ. 2007. Isolation of pathogenic bacteria from *Oberea linearis* (Coleoptera: Cerambycidae). *Biologia* 62: 13–18.
- Bary, A. de. 1879. Die Erscheinung der Symbiose: Vortrag. Karl J. Trübner Verlag, Strassburg, Germany.
- Behar, A., B. Yuval, and E. Jurkevitch. 2005. Enterobacteria-mediated nitrogen fixation in natural populations of the fruit fly *Ceratitis capitata*. *Molecular Ecology* 14: 2637–2643.
- Ben Ami, E., B. Yuval, and E. Jurkevitch. 2009. Manipulation of the microbiota of mass-reared Mediterranean fruit flies *Ceratitis capitata* (Diptera: Tephritidae) improves sterile male sexual performance. *The International Society for Microbial Ecology (ISME) Journal* 4: 28.
- Ben-Yosef, M., Z. Pasternak, E. Jurkevitch, and B. Yuval. 2014. Symbiotic bacteria enable olive flies (*Bactrocera oleae*) to exploit intractable sources of nitrogen. *Journal of Evolutionary Biology* 27: 2695–2705.
- Bian, G., Y. Xu, P. Lu, Y. Xie, and Z. Xi. 2010. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathogens* 6: 1–10.
- Bian, G., D. Joshi, Y. Dong, P. Lu, G. Zhou, X. Pan, Y. Xu, G. Dimopoulos, and Z. Xi. 2013. *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. *Science* 340: 748–751.
- Blackburn, M. B., D. E. Gundersen-Rindal, D. C. Weber, P. A. W. Martin, and R. R. Farrar. 2008. Enteric bacteria of field-collected Colorado potato beetle larvae inhibit growth of the entomopathogens *Photorhabdus temperata* and *Beauveria bassiana*. *Biological Control* 46: 434–441.
- Bourtzis, K., and T. A. Miller (eds.). 2008. *Insect symbiosis*. CRC Press, Boca Raton, Florida, USA. 324 pp.
- Brady, C., I. Cleenwerck, S. Venter, T. Coutinho, and P. De Vos. 2013. Taxonomic evaluation of the genus *Enterobacter* based on Multilocus Sequence Analysis (MLSA): Proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radincitans*, *E. oryzae* and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. *Systematic and Applied Microbiology* 36: 309–319.
- Brenner, D. J., G. R. Fanning, and J. K. Leete Knutson. 1984. Attempts to classify herbicola group-*Enterobacter agglomerans* strains by deoxyribonucleic acid hybridization and phenotypic tests. *International Journal of Systematic Bacteriology* 34: 45–55.
- Breznak, J. A., W. J. Brill, J. W. Mertins, and H. C. Coppel. 1973. Nitrogen fixation in termites. *Nature* 244: 577.
- Bridges, J. R. 1981. Nitrogen-fixing bacteria associated with bark beetles. *Microbial Ecology* 7: 131–137.
- Broderick, N. A., and B. Lemaître. 2012. Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* 3: 307–321.
- Brown, C. T., L. A. Hug, B. C. Thomas, I. Sharon, C. J. Castelle, A. Singh, M. J. Wilkins, K. C. Wrighton, K. H. Williams, and J. B. Banfield. 2015. Unusual biology across a group comprising more than 15% of domain Bacteria. *Nature* 523: 208–211.
- Brune, A., and C. Dietrich. 2015. The gut microbiota of termites: Digesting the diversity in the light of ecology and evolution. *Annual Review of Microbiology* 69: 145–166.
- Butera, G., C. Ferraro, S. Colazza, G. Alonzo, and P. Quatrini. 2012. The culturable bacterial community of frass produced by larvae of *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) in the Canary island date palm. *Letters in Applied Microbiology* 54: 530–536.

- Cáceres, C., G. Tsiamis, B. Yuval, E. Jurkevitch, and K. Bourtzis. 2019. Joint FAO/IAEA coordinated research project on “Use of symbiotic bacteria to reduce mass-rearing costs and increase mating success in selected fruit pests in support of SIT application”. BMC Microbiology 19: 284.
- Çakici, F. O., A. Sevün, Z. Demirbağ, and İ. Demir. 2014. Investigating internal bacteria of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae and some *Bacillus* strains as biocontrol agents. Turkish Journal of Agriculture and Forestry 38: 99–110.
- Çakici, F. O., I. Ozgen, H. Bolu, Z. Erbas, Z. Demirbağ, and I. Demir. 2015. Highly effective bacterial agents against *Cimbex quadrimaculatus* (Hymenoptera: Cimbicidae): Isolation of bacteria and their insecticidal activities. World Journal of Microbiol Biotechnology 31: 59–67.
- Cambon, M., J.-C. Ogier, A. Lanois, J.-B. Ferdy, and S. Gaudriault. 2018. Changes in rearing conditions rapidly modify gut microbiota structure in *Tenebrio molitor* larvae. bioRxiv: 423178.
- Capuzzo, C., G. Firrao, L. Mazzon, A. Squartini, and V. Girolami. 2005. ‘*Candidatus* Erwinia dacicola’, a coevolved symbiotic bacterium of the olive fly *Bactrocera oleae* (Gmelin). International Journal of Systematic and Evolutionary Microbiology 55: 1641–1647.
- Chandler, J. A., J. M. Lang, S. Bhatnagar, J. A. Eisen, and A. Kopp. 2011. Bacterial communities of diverse *Drosophila* species: Ecological context of a host–microbe model system. PLoS Genetics 7: e1002272.
- Chung, J., H. Jeong, and C.-M. Ryu. 2018. Complete genome sequences of *Enterobacter cancerogenus* CR-Eb1 and Enterococcus sp. strain CR-Ec1, isolated from the larval gut of the greater wax moth, *Galleria mellonella*. Genome Announcements 6: e00044-18.
- Chung, S. H., C. Rosa, E. D. Scully, M. Peiffer, J. F. Tooker, K. Hoover, D. S. Luthe, and G. W. Felton. 2013. Herbivore exploits orally secreted bacteria to suppress plant defenses. Proceedings of the National Academy of Sciences of the USA 110: 15728–15733.
- Cirimotich, C. M., A. M. Clayton, and G. Dimopoulos. 2011a. Low- and high-tech approaches to control *Plasmodium* parasite transmission by *Anopheles* mosquitoes. Journal of Tropical Medicine 2011: 891342.
- Cirimotich, C. M., J. L. Ramirez, and G. Dimopoulos. 2011b. Native microbiota shape insect vector competence for human pathogens. Cell Host Microbe 10: 307–310.
- Cirimotich, C. M., Y. Dong, A. M. Clayton, S. L. Sandiford, J. A. Souza-Neto, M. Mulenga, and D. Dimopoulos. 2011c. Natural microbe-mediated refractoriness to *Plasmodium* infection in *Anopheles gambiae*. Science 332: 855–858.
- Colman, D. R., E. C. Toolson, and C. D. Takacs-Vesbach. 2012. Do diet and taxonomy influence insect gut bacterial communities? Molecular Ecology 21: 5124–5137.
- Davidson, E. W., R. C. Rosell, and D. L. Hendrix. 2000. Culturable bacteria associated with the whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae). The Florida Entomologist 83: 159–171.
- Davin-Regli, A., and J.-M. Pages. 2015. *Enterobacter aerogenes* and *Enterobacter cloacae*; versatile bacterial pathogens confronting antibiotic treatment. Frontiers in Microbiology 6: 392.
- Dennison, N. J., N. Jupatanakul, and G. Dimopoulos. 2014. The mosquito microbiota influences vector competence for human pathogens. Current Opinion in Insect Science 3: 6–13.
- Dong, Y., F. Manfredini, and G. Dimopoulos. 2009. Implication of the mosquito midgut microbiota in the defense against malaria parasites. PLoS Pathogens 5: e1000423.
- Douglas, A. E. 2009. The microbial dimension in insect nutritional ecology. Functional Ecology 23: 38–47.
- Douglas, A. E. 2015. Multiorganismal insects: Diversity and function of resident microorganisms. Annual Review of Entomology 60: 17–34.
- Doudoumis, V., A. M. M. Abd-Alla, G. Tsiamis, C. Brelsfoard, S. Aksoy, and K. Bourtzis. 2013. Tsetse-*Wolbachia* symbiosis: Comes of age and has great potential for pest and disease control. Journal of Invertebrate Pathology 112: S94-S103.
- Doudoumis, V., F. Blow, A. Saridaki, A. Augustinos, N. A. Dyer, I. Goodhead, P. Solano, J.-B. Rayaisse, P. Takac, S. Mekonnen, A. G. Parker, A. M. M. Abd-Alla, A. Darby, K. Bourtzis, and G. Tsiamis. 2017. Challenging the *Wigglesworthia*, *Sodalis*, *Wolbachia* symbiosis dogma in tsetse flies: *Spiroplasma* is present in both laboratory and natural populations. Scientific Reports 7: 4699.
- Drew, R. A. I., and A. C. Lloyd. 1987. Relationship of fruit flies (Diptera: Tephritidae) and their bacteria to host plants. Annals of the Entomological Society of America 80: 629–636.
- Dutra, H. L. C., M. N. Rocha, F. B. S. Dias, S. B. Mansur, E. P. Caragata, and L. A. Moreira. 2016. *Wolbachia* blocks currently circulating zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. Cell Host & Microbe 19: 771–774.

- Eappen, A. G., R. C. Smith, and M. Jacobs-Lorena. 2013. *Enterobacter*-activated mosquito immune responses to *Plasmodium* involve activation of SRPN6 in *Anopheles stephensi*. PLoS One 8: e62937.
- Engel, P., and N. A. Moran. 2013. The gut microbiota of insects – Diversity in structure and function. Federation of European Microbiological Societies (FEMS) Microbiology Reviews 37: 699–735.
- Engel, P., W. K. Kwong, Q. McFrederick, K. E. Anderson, S. M. Barribeau, J. A. Chandler, R. Scott Cornman, J. Dainat, J. R. de Miranda, V. Doublet, O. Emery, J. D. Evans, L. Farinelli, M. L. Flenniken, F. Granberg, J. A. Grasis, L. Gauthier, J. Hayer, H. Koch, S. Kocher, V. G. Martinson, N. Moran, M. Munoz-Torres, I. Newton, R. J. Paxton, E. Powell, B. M. Sadd, P. Schmid-Hempel, S. J. Song, R. S. Schwarz, D. vanEngelsdorp, and B. Dainat. 2016. The bee microbiome: Impact on bee health and model for evolution and ecology of host-microbe interactions. mBio 7: e02164-15.
- Eren, A. M., M. L. Sogin, H. G. Morrison, J. H. Vineis, J. C. Fisher, R. J. Newton, and S. L. McLellan. 2015. A single genus in the gut microbiome reflects host preference and specificity. The International Society for Microbial Ecology (ISME) Journal 9: 90–100.
- Estes, A. M., D. J. Hearn, S. Agrawal, E. A. Pierson, and J. C. Dunning Hotopp. 2018a. Comparative genomics of the *Erwinia* and *Enterobacter* olive fly endosymbionts. Scientific Reports 8: 15936.
- Estes, A. M., D. J. Hearn, S. Nadendla, E. A. Pierson, and J. C. Dunning Hotopp. 2018b. Draft genome sequence of *Enterobacter* sp. strain OLF, a colonizer of olive flies. Microbiology Resource Announcements 7: e01068-18.
- Eutick, M. L., R. W. O'Brien, and M. Slaytor. 1978. Bacteria from the gut of Australian termites. Applied Environmental Microbiology 35: 823–828.
- Fang, H., W. Chen, B. Wang, X. Li, S.-J. Liu, and H. Yang. 2016. Cultivation and characterization of symbiotic bacteria from the gut of *Reticulitermes chinensis*. Applied Environmental Biotechnology 1: 3–12.
- Feldhaar, H. 2011. Bacterial symbionts as mediators of ecologically important traits of insect hosts. Ecological Entomology 36: 533–543.
- Fierer, N., and J. T. Lennon. 2011. The generation and maintenance of diversity in microbial communities. American Journal of Botany 98: 439–448.
- Frentiu, F. D., T. Zakir, T. Walker, J. Popovici, A. T. Pyke, A. van den Hurk, E. A. McGraw, and S. L. O'Neill. 2014. Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with Wolbachia. PLoS Neglected Tropical Diseases 8: e2688.
- Geiger, A., M.-L. Fardeau, P. Grebaut, G. Vatunga, T. Joséando, S. Herder, G. Cuny, P. Truc, and B. Ollivier. 2009. First isolation of *Enterobacter*, *Enterococcus*, and *Acinetobacter* spp. as inhabitants of the tsetse fly (*Glossina palpalis palpalis*) midgut. Infection, Genetics and Evolution 9: 1364–1370.
- Gibbons, S. M., and J. A. Gilbert. 2015. Microbial diversity - exploration of natural ecosystems and microbiomes. Current Opinion in Genetics & Development 35: 66–72.
- Gil, R., and A. Latorre. 2019. Unity makes strength: A review on mutualistic symbiosis in representative insect clades. Life (Basel) 9: e21.
- Gokce, C., A. Sevim, Z. Demirbağ, and I. Demir. 2010. Isolation, characterization and pathogenicity of bacteria from *Rhynchites bacchus* (Coleoptera: Rhynchitidae). Biocontrol Science and Technology 20: 973–982.
- Gonzalez-Ceron, L., F. Santillan, M. H. Rodriguez, D. Mendez, and J. E. Hernandez-Avila. 2003. Bacteria in midguts of field-collected *Anopheles albimanus* block *Plasmodium vivax* sporogonic development. Journal of Medical Entomology 40: 371–374.
- Gujjar, N. R., S. Govindan, A. Verghese, S. Subramaniam, and R. More. 2017. Diversity of the culturable gut bacterial communities associated with the fruit flies *Bactrocera dorsalis* and *Bactrocera cucurbitae* (Diptera: Tephritidae). Phytoparasitica 45: 453–460.
- Gurung, K., B. Wertheim, and J. F. Salles. 2019. The microbiome of pest insects: It is not just bacteria. Entomologia Experimentalis et Applicata 167: 156–170.
- Hamden, H., M. N. Guerfali, S. Fadhl, M. Saidi, and C. Chevrier. 2013. Fitness improvement of mass-reared sterile males of *Ceratitis capitata* (Vienna 8 strain) (Diptera: Tephritidae) after gut enrichment with probiotics. Journal of Economic Entomology 106: 641–647.
- Hoffmann, H., and A. Roggenkamp. 2003. Population genetics of the nomen species *Enterobacter cloacae*. Applied Environmental Microbiology 69: 5306–5318.
- Hoffmann, H., S. Stindl, A. Stumpf, A. Mehlen, D. Monget, J. Heesemann, K. H. Schleifer, and A. Roggenkamp. 2005. Description of *Enterobacter ludwigii* sp. nov., a novel *Enterobacter* species of clinical relevance. Systematic and Applied Microbiology 28: 206–212.

- Hormaeche, E., and P. R. Edwards. 1960. A proposed genus *Enterobacter*. International Bulletin of Bacteriological Nomenclature and Taxonomy 10: 71–74.
- Husník, F., T. Chrudinský, and V. Hypša. 2011. Multiple origins of endosymbiosis within the Enterobacteriaceae (γ -Proteobacteria): Convergence of complex phylogenetic approaches. BMC Biology 9: 87.
- Husseneder, C., and J. K. Grace. 2005. Genetically engineered termite gut bacteria (*Enterobacter cloacae*) deliver and spread foreign genes in termite colonies. Applied Microbiology and Biotechnology 68: 360–367.
- Janusz, G., A. Pawlik, J. Sulej, U. Świdarska-Burek, A. Jarosz-Wilkolazka, and A. Paszczyński. 2017. Lignin degradation: Microorganisms, enzymes involved, genomes analysis and evolution. Federation of European Microbiological Societies (FEMS) Microbiology Reviews 41: 941–962.
- Jayakrishnan, L., A. V. Sudhikumar, and E. M. Aneesh. 2018. Role of gut inhabitants on vectorial capacity of mosquitoes. Journal of Vector Borne Diseases 55: 69.
- Jiang, J., C. Alvarez, P. Kukutla, W. Yu, and J. Xu. 2012. Draft genome sequences of *Enterobacter* sp. isolate Ag1 from the midgut of the malaria mosquito *Anopheles gambiae*. Journal of Bacteriology 194: 5481–5481.
- Jurkevitch, E. 2011. Riding the Trojan horse: Combating pest insects with their own symbionts. Microbial Biotechnology 4: 620–627.
- Kalappa, D. M., P. A. Subramani, S. K. Basavanna, S. K. Ghosh, V. Sundaramurthy, S. Uragayala, S. Tiwari, A. R. Anvikar, and N. Valecha. 2018. Influence of midgut microbiota in *Anopheles stephensi* on *Plasmodium berghei* infections. Malaria Journal 17: 385.
- Khamis, F. M., P. O. Mireji, F. L. O. Ombura, A. R. Malacrida, E. O. Awuoch, M. Rono, S. A. Mohamed, C. M. Tanga, and S. Ekesi. 2019. Species-specific transcriptional profiles of the gut and gut microbiome of *Ceratitidis quiliicii* and *Ceratitidis rosa sensu stricto*. Scientific Reports 9: 18355.
- Khampang, P., P. Luxananil, S. Tanapongpipat, W. Chungjatupornchai, and S. Panyim. 2001. Recombinant *Enterobacter amnigenus* highly toxic to *Anopheles dirus* mosquito larvae. Current Microbiology 43: 448–451.
- Kohl, K. D., J. Amaya, C. A. Passemment, M. D. Dearing, and M. D. McCue. 2014. Unique and shared responses of the gut microbiota to prolonged fasting: A comparative study across five classes of vertebrate hosts. Federation of European Microbiological Societies (FEMS) Microbiology Ecology 90: 883–894.
- König, H., J. Fröhlich, and H. Hertel. 2006. Diversity and lignocellulolytic activities of cultured microorganisms, pp. 271–301. In H. König, and A. Varma (eds.), Intestinal microorganisms of termites and other invertebrates. Springer, Berlin-Heidelberg, Germany.
- Kuhnigk, T., E.-M. Borst, A. Ritter, P. Kämpfer, A. Graf, H. Hertel, and H. König. 1994. Degradation of lignin monomers by the hindgut flora of xylophagous termites. Systematic and Applied Microbiology 17: 76–85.
- Kumar, S., G. K. Christophides, R. Cantera, B. Charles, Y. S. Han, S. Meister, G. Dimopoulos, F. C. Kafatos, and C. Barilla-Mury. 2003. The role of reactive oxygen species on *Plasmodium melanotic* encapsulation in *Anopheles gambiae*. Proceedings National Academy of Sciences of the USA 100: 14139–14144.
- Kyritsis, G. A., A. A. Augustinos, C. Cáceres, and K. Bourtzis. 2017. Medfly gut microbiota and enhancement of the Sterile Insect Technique: Similarities and differences of *Klebsiella oxytoca* and *Enterobacter* sp. AA26 probiotics during the larval and adult stages of the VIENNA 8D53+genetic sexing strain. Frontiers in Microbiology 8: 2064.
- Latorre, A., and A. Manzano-Marín. 2017. Dissecting genome reduction and trait loss in insect endosymbionts. Annals of the New York Academy of Sciences 1389: 52–75.
- Lauson, C. R., S. E. Potter, and R. J. Prokopy. 2003. Degradation and detoxification of the dihydrochalcone phloridzin by *Enterobacter agglomerans*, a bacterium associated with the apple pest, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). Environmental Entomology 32: 953–962.
- Ley, R. E., C. A. Lozupone, M. Hamady, R. Knight, and J. I. Gordon. 2008. Worlds within worlds: Evolution of the vertebrate gut microbiota. Nature Reviews Microbiology 6: 776–788.
- Lo, W.-S., Y.-Y. Huang, and C.-H. Kuo. 2016. Winding paths to simplicity: Genome evolution in facultative insect symbionts. Federation of European Microbiological Societies (FEMS) Microbiology Reviews 40: 855–874.
- Luckhart, S., Y. Vodovotz, L. Cui, and R. Rosenberg. 1998. The mosquito *Anopheles stephensi* limits malaria parasite development with inducible synthesis of nitric oxide. Proceedings of the National Academy of Sciences of the USA 95: 5700–5705.

- Manfredi, A. P., N. I. Perotti, and M. A. Martínez. 2015.** Cellulose degrading bacteria isolated from industrial samples and the gut of native insects from Northwest of Argentina. *Journal of Basic Microbiology* 55: 1384–1393.
- Mannesmann, R., and B. Piechowski. 1989.** Verteilung von Gärkammerbakterien einiger Termitenarten. *Materials and Organisms* 24: 161–177.
- Manzano-Marin, A., J.-C. Simon, and A. Latorre. 2016.** Reinventing the wheel and making it round again: Evolutionary convergence in *Buchnera-Serratia* symbiotic consortia between the distantly related Lachninae aphids *Tuberolachnus salignus* and *Cinara cedri*. *Genome Biology and Evolution* 8: 1440–1458.
- Martin, B., and E. Schwab. 2012.** Current usage of symbiosis and associated terminology. *International Journal of Biology* 5: 32.
- Matcos, M., H. Martinez, S. B. Lanzavecchia, C. Conte, K. Guillen, B. M. Moran-Aceves, J. Toledo, P. Liedo, E. D. Asimakis, V. Doudoumis, G. A. Kyritsis, N. T. Papadopoulos, A. A. Avgoustinos, D. F. Segura, G. Tsiamis, and K. Bourtzis. 2018.** *Wolbachia pipientis* associated to tephritid fruit fly pests: From basic research to applications. *bioRxiv*: 358333.
- Mathews, S. L., M. J. Epps, R. K. Blackburn, M. B. Goshe, A. M. Grunden, and R. R. Dunn. 2019.** Public questions spur the discovery of new bacterial species associated with lignin bioconversion of industrial waste. *Royal Society Open Science* 6: 180748.
- McFall-Ngai, M. 2007.** Adaptive immunity: Care for the community. *Nature* 445: 153.
- Mereghetti, V., B. Chouaia, and M. Montagna. 2017.** New insights into the microbiota of moth pests. *International Journal of Molecular Sciences* 8: 2450.
- Molina-Cruz, A., R. J. DeJong, B. Charles, L. Gupta, S. Kumar, G. Jaramillo-Gutierrez, and C. Barillas-Mury. 2008.** Reactive oxygen species modulate *Anopheles gambiae* immunity against bacteria and *Plasmodium*. *The Journal of Biological Chemistry* 283: 3217–3223.
- Moreira, L. A., I. Iturbe-Ormaetxe, J. A. Jeffery, G. Lu, A. T. Pyke, L. M. Hedges, B. C. Rocha, S. Hall-Mendelin, A. Day, M. Riegler, L. E. Hugo, K. N. Johnson, B. H. Kay, E. A. McGraw, A. F. van den Hurk, P. A. Ryan, and S. L. O'Neill. 2009.** A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* 139: 1268–1278.
- Moya, A., J. Peretó, R. Gil, and A. Latorre. 2008.** Learning how to live together: Genomic insights into prokaryote–animal symbioses. *Nature Reviews Genetics* 9: 218–229.
- Mramba, F., A. B. Broce, and L. Zurek. 2007.** Vector competence of stable flies, *Stomoxys calcitrans* L. (Diptera: Muscidae), for *Enterobacter sakazakii*. *Journal of Vector Ecology* 32: 134–139.
- Muhammad, A., Y. Fang, Y. Hou, and Z. Shi. 2017.** The gut entomotype of red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae) and their effect on host nutrition metabolism. *Frontiers in Microbiology* 8: 2291.
- Murphy, K. M., D. S. Teakle, and I. C. MacRae. 1994.** Kinetics of colonization of adult Queensland fruit flies (*Bactrocera tryoni*) by dinitrogen-fixing alimentary tract bacteria. *Applied Environmental Microbiology* 60: 2508–2517.
- Nakabachi, A., A. Yamashita, H. Toh, H. Ishikawa, H. E. Dunbar, N. A. Moran, and M. Hattori. 2006.** The 160-kilobase genome of the bacterial endosymbiont *Carsonella*. *Science* 314: 267.
- Niyazi, N., C. R. Lauzon, and T. E. Shelly. 2004.** Effect of probiotic adult diets on fitness components of sterile male Mediterranean fruit flies (Diptera: Tephritidae) under laboratory and field cage conditions. *Journal of Economic Entomology* 97: 1570–1580.
- Octavia, S., and R. Lan. 2014.** The family Enterobacteriaceae, pp. 225–286. *In* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson (eds.), *The Prokaryotes: Gammaproteobacteria*. Springer, Berlin-Heidelberg, Germany.
- Pereira, F. C., and D. Berry. 2017.** Microbial nutrient niches in the gut. *Environmental Microbiology* 19: 1366–1378.
- Pernice, M., S. J. Simpson, and F. Ponton. 2014.** Towards an integrated understanding of gut microbiota using insects as model systems. *Journal of Insect Physiology* 69: 12–18.
- Peterson, T. M. L., A. J. Gow, and S. Luckhart. 2007.** Nitric oxide metabolites induced in *Anopheles stephensi* control malaria parasite infection. *Free Radical Biology and Medicine* 42: 132–142.
- Potrikus, C. J., and J. A. Breznak. 1977.** Nitrogen-fixing *Enterobacter agglomerans* isolated from guts of wood-eating termites. *Applied Environmental Microbiology* 33: 392–399.
- Potrikus, C. J., and J. A. Breznak. 1981.** Gut bacteria recycle uric acid nitrogen in termites: A strategy for nutrient conservation. *Proceedings of the National Academy of Sciences of the USA* 78: 4601–4605.

- Ramin, M., A. R. Alimon, N. Abdullah, J. M. Panandam, and K. Sijam. 2008. Isolation and identification of three species of bacteria from the termite *Coptotermes curvignathus* (Holmgren) present in the vicinity of Universiti Putra Malaysia. *Research Journal of Microbiology* 3: 288–292.
- Rizzi, A., E. Crotti, L. Borruso, C. Jucker, D. Lupi, M. Colombo, and D. Daffonchio. 2013. Characterization of the bacterial community associated with larvae and adults of *Anoplophora chinensis* collected in Italy by culture and culture-independent methods. *BioMed Research International* 2013: 420287.
- Rojas-Jiménez, K., and M. Hernández. 2015. Isolation of fungi and bacteria associated with the guts of tropical wood-feeding Coleoptera and determination of their lignocellulolytic activities. *International Journal of Microbiology* 2015: 285018.
- Rooks, M. G., and W. S. Garrett. 2016. Gut microbiota, metabolites and host immunity. *Nature Reviews Immunology* 16: 341–352.
- Ruby, E., B. Henderson, and M. McFall-Ngai. 2004. We get by with a little help from our (little) friends. *Science* 303: 1305–1307.
- Saldaña, M. A., S. Hegde, and G. L. Hughes. 2017. Microbial control of arthropod-borne disease. *Memórias do Instituto Oswaldo Cruz* 112: 81–93.
- Sandeva, R., G. Sandeva, B. Chakarova, and A. Dimitrova. 2018. Gut microbiota - Our new important organ. *Albanian Journal of Agricultural Sciences (Proceedings of ICOALS 2018)*: 175–181.
- Schäfer, A., R. Konrad, T. Kuhnigk, P. Kämpfer, H. Hertel, and H. König. 1996. Hemicellulose-degrading bacteria and yeasts from the termite gut. *Journal of Applied Bacteriology* 80: 471–478.
- Schmidt, T. L., N. H. Barton, G. Rašić, A. P. Turley, B. L. Montgomery, I. Iturbe-Ormaetxe, P. E. Cook, P. A. Ryan, S. A. Ritchie, A. A. Hoffmann, S. L. O'Neill, and M. Turelli. 2017. Local introduction and heterogeneous spatial spread of dengue-suppressing *Wolbachia* through an urban population of *Aedes aegypti*. *PLoS Biology* 15: 2001894.
- Sheng, P., Y. Li, S. D. G. Marshall, and H. Zhang. 2015. High genetic diversity of microbial cellulase and hemicellulase genes in the hindgut of *Holotrichia parallela* larvae. *International Journal of Molecular Science* 16: 16545–16559.
- Somerville, J., L. Zhou, and B. Raymond. 2019. Aseptic rearing and infection with gut bacteria improve the fitness of transgenic diamondback moth, *Plutella xylostella*. *Insects* 10: 89.
- Szabó, G., F. Schulz, E. R. Toenshoff, J.-M. Volland, O. M. Finkel, S. Belkin, and M. Horn. 2017. Convergent patterns in the evolution of mealybug symbioses involving different intrabacterial symbionts. *The International Society for Microbial Ecology (ISME) Journal* 11: 715–726.
- Tan, C. H., P. J. Wong, M. I. Li, H. Yang, L. C. Ng, and S. L. O'Neill. 2017. wMel limits Zika and chikungunya virus infection in a Singapore *Wolbachia*-introgressed *Ae. aegypti* strain, wMel-Sg. *PLoS Neglected Tropical Diseases* 11: e. 0005496.
- Tchioffo, M. T., A. Boissière, T. S. Churcher, L. Abate, G. Gimonneau, S. E. Nsango, P. H. Awono-Ambéné, R. Christen, A. Berry, and I. Morlais. 2013. Modulation of malaria infection in *Anopheles gambiae* mosquitoes exposed to natural midgut bacteria. *PLoS One* 8: e81663.
- Thakur, A., P. Dhammi, H. S. Saini, and S. Kaur. 2015. Pathogenicity of bacteria isolated from gut of *Spodoptera litura* (Lepidoptera: Noctuidae) and fitness costs of insect associated with consumption of bacteria. *Journal of Invertebrate Pathology* 127: 38–46.
- Thong-On, A., K. Suzuki, S. Noda, J. Inoue, S. Kajiwarra, and M. Ohkuma. 2012. Isolation and characterization of anaerobic bacteria for symbiotic recycling of uric acid nitrogen in the gut of various termites. *Microbes and Environments* 27: 186–192.
- Toczyłowska-Mamińska, R., K. Szymona, H. Madej, W. W. Zhen, A. Bala, W. Brutkowski, K. Krajewski, P. S. H'ng, and M. L. Maminski. 2015. Cellulolytic and electrogenic activity of *Enterobacter cloacae* in mediatorless microbial fuel cell. *Applied Energy* 160: 88–93.
- Valero-Jiménez, C. A., A. J. Debets, J. A. van Kan, S. E. Schoustra, W. Takken, B. J. Zwaan, and C. J. M. Koenraadt. 2014. Natural variation in virulence of the entomopathogenic fungus *Beauveria bassiana* against malaria mosquitoes. *Malaria Journal* 13: 479.
- van den Hurk, A. F., S. Hall-Mendelin, A. T. Pyke, F. D. Frentiu, K. McElroy, A. Day, S. Higgs, and S. L. O'Neill. 2012. Impact of *Wolbachia* on infection with chikungunya and yellow fever viruses in the mosquito vector *Aedes aegypti*. *PLoS Neglected Tropical Diseases* 6: e1892.
- Vasanthakumar, A., I. Delalibera, J. Handelsman, K. D. Klepzig, P. D. Schloss, and K. F. Raffa. 2006. Characterization of gut-associated bacteria in larvae and adults of the southern pine beetle, *Dendroctonus frontalis* Zimmermann. *Environmental Entomology* 35: 1710–1717.

- Walker, T., P. H. Johnson, L. A. Moreira, I. Iturbe-Ormaetxe, F. D. Frentiu, C. J. McMeniman, Y. S. Leong, Y. Dong, J. Axford, P. Kriesner, A. L. Lloyd, S. A. Ritchie, S. L. O'Neill, and A. A. Hoffmann. 2011. The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 476: 450–455.
- Wang, H., L. Jin, T. Peng, H. Zhang, Q. Chen, and Y. Hua. 2014. Identification of cultivable bacteria in the intestinal tract of *Bactrocera dorsalis* from three different populations and determination of their attractive potential. *Pest Management Science* 70: 80–87.
- Wang, J., S. H. Chung, M. Peiffer, C. Rosa, K. Hoover, R. Zeng, and G. W. Felton. 2016. Herbivore oral secreted bacteria trigger distinct defense responses in preferred and non-preferred host plants. *Journal of Chemical Ecology* 42: 463–474.
- Wang, J., M. Peiffer, K. Hoover, C. Rosa, R. Zeng, and G. W. Felton. 2017. *Helicoverpa zea* gut-associated bacteria indirectly induce defenses in tomato by triggering a salivary elicitor(s). *New Phytologist* 214: 1294–1306.
- Watanabe, K., K. Abe, and M. Sato. 2000. Biological control of an insect pest by gut-colonizing *Enterobacter cloacae* transformed with ice nucleation gene. *Journal of Applied Microbiology* 88: 90–97.
- Webster, N. S. 2014. Cooperation, communication, and co-evolution: Grand challenges in microbial symbiosis research. *Frontiers in Microbiology* 5: 164.
- Weiss, B., and S. Aksoy. 2011. Microbiome influences on insect host vector competence. *Trends in Parasitology* 27: 514–522.
- Wernegreen, J. J. 2004. Endosymbiosis: Lessons in conflict resolution. *PLoS Biology* 2: e68.
- Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences of the USA* 95: 6578–6583.
- Wilke, A. B. B., and M. T. Marrelli. 2015. Paratransgenesis: A promising new strategy for mosquito vector control. *Parasites & Vectors* 8: 342.
- Williams, L. E., and J. J. Wernegreen. 2010. Unprecedented loss of ammonia assimilation capability in a urease-encoding bacterial mutualist. *BMC Genomics* 11: 687.
- Williams, T. A., G. J. Szöllösi, A. Spang, P. G. Foster, S. E. Heaps, B. Boussau, T. J. G. Ettema, and T. M. Embley. 2017. Integrative modeling of gene and genome evolution roots the archaeal tree of life. *Proceedings of the National Academy of Sciences of the USA* 114: E4602–E4611.
- Woodbury, N., and G. Gries. 2013a. How firebrats (Thysanura: Lepismatidae) detect and nutritionally benefit from their microbial symbionts *Enterobacter cloacae* and *Mycotypha microspora*. *Environmental Entomology* 42: 860–867.
- Woodbury, N., and G. Gries. 2013b. Firebrats, *Thermobia domestica*, aggregate in response to the microbes *Enterobacter cloacae* and *Mycotypha microspora*. *Entomologia Experimentalis et Applicata* 147: 154–159.
- Wu, D., S. C. Daugherty, S. E. Van Aken, G. H. Pai, K. L. Watkins, H. Khouri, L. J. Tallon, J. M. Zaborsky, H. E. Dunbar, P. L. Tran, N. A. Moran, and J. A. Eisen. 2006. Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. *PLoS Biology* 4: e188.
- Xia, X., G. M. Gurr, L. Vasseur, D. Zheng, H. Zhong, B. Qin, J. Lin, Y. Wang, F.-Q. Song, Y. Li, H. Lin, and M. You. 2017. Metagenomic sequencing of diamondback moth gut microbiome unveils key holobiont adaptations for herbivory. *Frontiers in Microbiology* 8: 663.
- Yaman, M., Z. Demirbağ, and A. O. Beldüz. 2000. Isolation and insecticidal effects of some bacteria from *Euproctis chrysorrhoea* L. (Lepidoptera: Lymantriidae). *Acta Microbiologica Polonica* 49: 217–224.
- Yao, M., H. Zhang, P. Cai, X. Gu, D. Wang, and Q. Ji. 2017. Enhanced fitness of a *Bactrocera cucurbitae* genetic sexing strain based on the addition of gut-isolated probiotics (*Enterobacter* spec.) to the larval diet. *Entomologia Experimentalis et Applicata* 162: 197–203.
- Yoshida, N., K. Oeda, E. Watanabe, T. Mikami, Y. Fukita, K. Nishimura, K. Komai, and K. Matsuda. 2001. Protein function: Chaperonin turned insect toxin. *Nature* 411(6833): 44.
- Yun, J.-H., S. W. Roh, T. W. Whon, M. J. Jung, M.-S. Kim, D.-S. Park, C. Yoon, Y.-D. Nam, Y.-J. Kim, J.-H. Choi, J.-Y. Kim, N.-R. Shin, S.-H. Kim, W.-J. Lee, and J.-W. Bae. 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied Environmental Microbiology* 80: 5254–5264.
- Yuval, B., E. Ben-Ami, A. Behar, M. Ben-Yosef, and E. Jurkevitch. 2013. The Mediterranean fruit fly and its bacteria – potential for improving Sterile Insect Technique operations. *Journal of Applied Entomology* 137: 39–42.

- Zaremba-Niedzwiedzka, K., E. F. Caceres, J. H. Saw, D. Bäckström, L. Juzokaite, E. Vancaester, K. W. Seitz, K. Anantharaman, P. Stamawski, K. U. Kjeldsen, M. B. Sott, T. Nunoura, J. F. Banfield, A. Schramm, B. J. Baker, A. Spang, and T. J. Ettema. 2017. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* 541: 353–358.
- Zchori-Fein, E., and S. J. Perlman. 2004. Distribution of the bacterial symbiont *Cardinium* in arthropods. *Molecular Ecology* 13: 2009–2016.
- Zhang, H., W. Mu, Z. Hou, X. Wu, W. Zhao, X. Zhang, H. Pan, and S. Zhang. 2012. Biodegradation of nicosulfuron by the bacterium *Serratia marcescens* N80. *Journal of Environmental Science Health B*. 47: 153–160.
- Zhang, G., M. Hussain, S. L. O'Neill, and S. Asgari. 2013. *Wolbachia* uses a host microRNA to regulate transcripts of a methyltransferase, contributing to dengue virus inhibition in *Aedes aegypti*. *Proceedings of the National Academy of Sciences of the USA* 110: 10276–10281.
- Zhao, C., N. Tang, Y. Wu, Y. Zhang, Z. Wu, W. Li, X. Qin, J. Zhao, and G. Zhang. 2012. First reported fatal *Morganella morganii* infections in chickens. *Veterinary Microbiology* 156: 452–455.
- Zhou, H., W. Guo, B. Xu, Z. Teng, D. Tao, Y. Lou, and Y. Gao. 2017. Screening and identification of lignin-degrading bacteria in termite gut and the construction of LiP-expressing recombinant *Lactococcus lactis*. *Microbial Pathogenesis* 112: 63–69.
- Zhou, F., X. Wu, L. Xu, S. Guo, G. Chen, and X. Zhang. 2019. Repressed *Beauveria bassiana* infections in *Delia antiqua* due to associated microbiota. *Pest Management Science* 75: 170–179.
- Zhu, B., M.-M. Lou, G.-L. Xie, G.-F. Wang, Q. Zhou, F. Wang, Y. Fang, T. Su, B. Lu, and Y.-P. Duan. 2011. *Enterobacter mori* sp. nov., associated with bacterial wilt on *Morus alba* L. *International Journal of Systematic and Evolutionary Microbiology* 61: 2769–2774.