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Tephritidae bacterial symbionts: potentials for pest management

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Abstract

Tephritidae is a large family that includes several fruit and vegetable pests. These organisms usually harbor a variegated bacterial community in their digestive systems. Symbiotic associations of bacteria and fruit flies have been well-studied in the genera *Anastrepha*, *Bactrocera*, *Ceratitis*, and *Rhagoletis*. Molecular and culture-based techniques indicate that many genera of the Enterobacteriaceae family, especially the genera of *Klebsiella*, *Enterobacter*, *Pectobacterium*, *Citrobacter*, *Erwinia*, and *Providencia* constitute the most prevalent populations in the gut of fruit flies. The function of symbiotic bacteria provides a promising strategy for the biological control of insect pests. Gut bacteria can be used for controlling fruit fly through many ways, including attracting as odors, enhancing the success of sterile insect technique, declining the pesticide resistance, mass rearing of parasitoids and so on. New technology and recent research improved our knowledge of the gut bacteria diversity and function, which increased their potential for pest management. In this review, we discussed the diversity of bacteria in the economically important fruit fly and the use of these bacteria for controlling fruit fly populations. All the information is important for strengthening the future research of new strategies developed for insect pest control by the understanding of symbiotic relationships and multitrophic interactions between host plant and insects.

Keywords: fruit fly, tephritids, bacterial symbionts, pest management

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Introduction

Insects are estimated to one of the largest reservoirs of endosymbionts, comprising protozoa, nematodes, viruses, fungi, bacteria, and multicellular parasites (Douglas, 1998; Moran *et al*., 2005; Naaz *et al*., 2016) on earth, and approximately 15% of all insects harbor diverse communities of bacteria (Stouthamer *et al*., 1999; Wernegreen, 2002; Moran *et al*., 2008; Prabhakar *et al*., 2009a). It has been known for the last century that associations between insects and bacteria are very common in nature, and these organisms have co-evolved for more than 250 million years (Petri, 1909). Insect-bacterial interactions between range from pathogenicity to highly sophisticated symbiotic relationships (Wernegreen, 2002; Oliver *et al*., 2003, 2005). Bacteria can be extracellular or intracellular which have significant roles in host insect physiology, the nutrition, as well as reproduction (Dale & Moran, 2006). A notable example is an interaction between insects and gut bacteria, which extend insects by providing nutrients as endosymbiotic bacteria (Douglas *et al*., 2001) and by entering other indigestible substrates, including as lignocellulose-derived organic substances from the soil (Brune, 1998). Symbionts can be classified into two groups: the obligate or primary (P) symbionts and facultative or secondary (S) symbionts. Obligate symbionts have a long evolutionary history with their hosts and are required for host survival and fertility, especially in nutritional function (Baumann, 2005), while facultative symbionts have a much broader array of effects, ranging from mutualism to manipulation of reproduction (Moran *et al*., 2008).

Fruit flies (Diptera: Tephritidae) constitute a large and diverse group of phytophagous species, most of which are frugivores and have an adverse effect on fruit and vegetable crops

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worldwide (Ben-Yosef, 2015). There are nearly 4000 described species from 500 genera of tephritid fruit flies (Qin *et al.*, 2015), of which approximately 1500 are associated with fruit, and more than 250 are considered as economically important (Li *et al.*, 2013) that belongs to five genera: *Anastrepha* Schiner, *Bactrocera* Macquart, *Ceratitis* MacLeay, *Dacus* Fabricius, and *Rhagoletis* Loew (Van Houdt *et al.*, 2010).

Associations between fruit flies and their gut bacterial communities have been well-studied, usually in *Rhagoletis, Ceratitis, Bactrocera*, and *Anastrepha* and (Raghu *et al.*, 2002; Capuzzo *et al.*, 2005; Kounatidis *et al.*, 2009; Prabhakar *et al.*, 2009b, 2013; Naaz *et al.*, 2016). Despite the wide occurrence and economic importance of these organisms as fruit pests, unfortunately a little information is available about the functions of gut symbionts of the fruit fly. But the hypothesis of many researchers is that bacteria not only play an important role of fruit fly survivability but also in attracting different fruit flies towards the infected host fruits (MacCollom *et al.*, 2009; Narit & Anuchit, 2011; Reddy *et al.*, 2014).

It is very uncommon that the fruit fly acts as a vector of human or plant diseases. But there are some researches, as for example, the Mediterranean fruit fly (*Ceratitis capitata*) has the potential capacity to transmit plant disease (Cayol *et al.*, 1994) as well as human pathogens (bacterial) to fruits (Sela *et al.*, 2005). There is another fruit fly, *Drosophila melanogaster* also has the involvement to transmit pathogenic bacteria to postharvest wounded apples (Janisiewicz *et al.*, 1999).

Chemical pesticides (Perry *et al.*, 1998), mass trapping and bait spray (Ras *et al.*, 2017) are commonly used to manage the most invasive fruit fly species, though many pesticides have developed resistance to insect, killing non targeted insects as well as create environmental hazard and mass trapping has lower field performance (Haniotakis, 2005; Kakani *et al.*, 2010; Ras *et al.*, 2017). Therefore, alternative management strategies are now time demanding. In this review, we will provide an overview of the diversity of bacterial communities in economically important fruit flies including their function and the use of bacteria in eco-friendly control strategies for fruit flies.

Development of research on symbiotic bacteria in the fruit fly

The research on symbiotic bacteria and fruit flies began in 1909, after which research progress was slow (fig. 1a) until 2000. Studies performed during that period first focused on bacteria diversity and then on diversity with pest control strategies. However, after 2000, there was a dynamic shift, and from 2000 to 2010, the research focus was on diversity with pest control strategies by using molecular techniques (fig. 1b). After 2010, the research focus has been the function of symbiotic bacteria.

Diversity of bacteria in tephritids

Several studies on tephritids have provided important data on the role of microorganisms in relationships with host plants and in fruit fly biology (Drew, 1987; Drew & Lloyd, 1987; Jang & Nishijima, 1990). The role of extracellular bacteria associated with the digestive tracts of dipteran larvae, pupae, exuviae and/or adults are now understood (Yong *et al.*, 2017b). In this review, we discuss the bacterial diversity identified by culture-dependent and molecular methods (table 1). Before 2002, all researchers identified bacteria in fruit flies by using

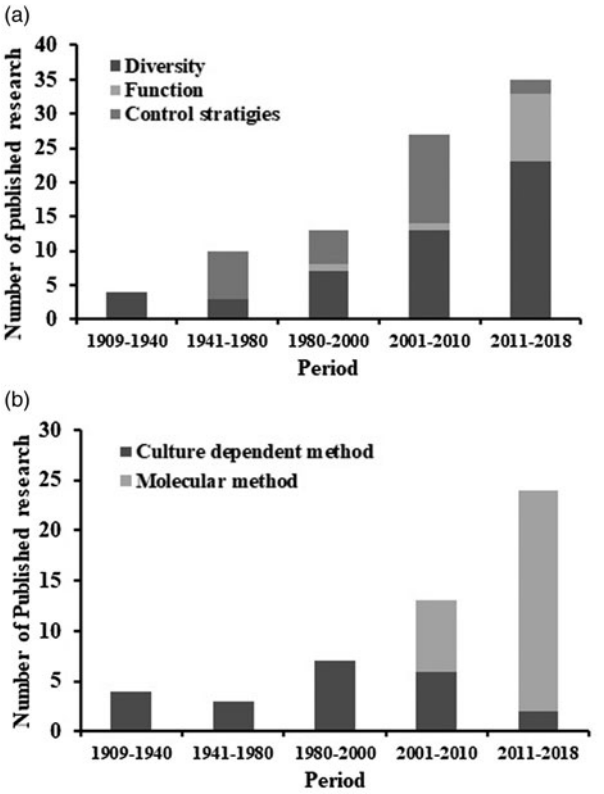


Fig. 1. Development of research on symbiotic bacteria. (a) Based on different research directions and (b) based on different methods.

culture-dependent methods because at that time these were the only methods available for bacterial identification (fig. 1b). Recently, molecular biological techniques have made it possible to study the diversity and taxonomy of native microbial communities. Studies on the bacteria in insects by using molecular techniques have revealed more taxa and larger microbial community structures; including both rare species and unculturable microorganisms (Haynes *et al.*, 2003; Broderick *et al.*, 2004; Egert *et al.*, 2005; Nakajima *et al.*, 2005; Cox & Gilmore, 2007; Miyata *et al.*, 2007). Culture-dependent and molecular method has provided a more holistic of the bacterial community structures in insects (Amann *et al.*, 1995).

Culture-dependent method

Bacterial symbionts were firstly mentioned by Petri in 1909 from the olive fly *Bactrocera oleae* (Dacinae) (Kounatidis *et al.*, 2009; Prabhakar *et al.*, 2009b; Naaz *et al.*, 2016). Petri (1910), found the association of organism *Bacillus (Pseudomonas) savastanoi* Smith (the known cause of olive knot disease) with *B. oleae* and another bacterium *Ascobacterium luteum* in association with *B. oleae*. Thirty-seven species of bacteria were isolated from Tephritidae, and bacterial transport through each stage of the life cycle of *B. oleae* was described; however, the associated bacteria were not identified (Stammer, 1929). In contrast to the earlier report, neither *P. savastanoi* nor *A. luteum* was able to be isolated from eggs or esophageal bulbs of field-collected *B. oleae* adults in the following research (Yamvris

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Table 1. Diversity of bacteria in different organ of fruit fly by using molecular and culture depended methods.

Genus (FF)	Species (FF)	Identified bacteria	Experimented organ	Status of fruit fly	Methods of identification	References
Bactrocera	*B. oleae*	*P. savastanoi*, *A. luteum*	Eggs and esophageal bulb	Field collected	Culture dependent	Petri (1910)
		A. radiobacter, *P. putida* and *S. marcescens*	Esophageal bulb	Field collected	Culture dependent	Belcari *et al.* (2003)
		E. persicina and *E. rhapontici*	Gut	Field collected	Molecular method	Capuzzo *et al.* (2005)
		A. tropicalis	Gut	Both	Molecular method	Kounatidis *et al.* (2009)
	B. dorsalis	*C. freundii*, *E. agglomerans*, *E. cloacae*, *K. oxytoca*, and *Kluyvera* spp.	Gut	Both	Culture dependent	Jang & Nishijima (1990)
		E. sakazakii, *K. oxytoca*, *K. pneumoniae*, *R. terrigena*, and *E. amnigenus*.	Reproductive system	Laboratory reared	Molecular method	Shi *et al.* (2012)
		K. pneumoniae and *Providencia* sp.	Whole body	Both	Molecular method	Liu *et al.* (2016)
		Wolbachia				Sun *et al.* (2007)
	B. cucurbitae	*P. pseudomallei*	Gut	Field collected	Culture dependent	Gupta *et al.* (1982a, b)
		Enterobacter, *Klebsiella*, *Citrobacter*, *Bacillus*, *Providencia*, *Micrococcus*, *Staphylococcus*, *Leclercia*, *and Exiguobacterium*	Mid gut	Field collected	Molecular method	Hadapad *et al.* (2016)
		P. putida, *E. herbicola* (*P. agglomerans*), *C. davisae*, *Arthrobacter* spp., and *X. maltophilia* (*S. maltophilia*)	Gut	Field collected	Culture dependent	Sood & Nath (2002)
		Wolbachia	Gut	Field	Molecular method	Kittayapong *et al.* (2000)
	B. zonata	*Bacillus*, *Enterobacter*, *Klebsiella*, and *Stenotrophomonas*	Gut	Both	Molecular method	Reddy *et al.* (2014)
		Klebsiella, *Microbacterium*, and *Rhodococcus*	Gut	Laboratory reared	Molecular method	Naaz *et al.* (2016)
	B. tau	*P. putida*, *E. herbicola* (*P. agglomerans*), *C. davisae*, *Arthrobacter* spp., and *X. maltophilia* (*S. maltophilia*)	Gut	Field collected	Culture dependent	Sood & Nath (2002)
		K. oxytoca, *Staphylococcus* sp.	Gut	Laboratory reared	Molecular method	Prabhakar *et al.* (2009b)
		Delftia acidovorans, *P. putida*, *Flavobacterium sp.*, *Defluvibacter sp.*, and *Ochrobactrum sp.*	Gut	Laboratory reared	Molecular method	Prabhakar *et al.* (2013)
		Proteus, *Klebsiella*, *Streptobacillus*, *Alcaligenes*, *Haemophilus*, *Erwinia*, *Chromobacterium*, *and Flavobacterium*	Gut	Laboratory reared	Culture dependent	Khan *et al.* (2014)
		Enterobacter, *Providencia* and *Serratia*	Gut	Laboratory reared	Both	Luo *et al.* (2018)
	B. cacuminata	*C. freundii*, *E. cloacae*, *K. oxytoca*, *P. rettgeri*, and *K. pneumoniae* ssp. *ozaenae*	Crop and mid gut	Field collected	Molecular method	Thaochan *et al.* (2010)
	B. tryoni	*C. freundii*, *E. cloacae*, *K. oxytoca*, *P. rettgeri*, *K. pneumoniae* ssp. *Ozaenae*, and *S. marcescens*	Crop and mid gut	Field collected	Molecular method	Thaochan *et al.* (2010)
	B. carambolae	*Erwinia*, *Stenotrophomonas*, and *Chryseobacterium*	Crop and mid gut	Field collected	Molecular method	Yong *et al.* (2017a, b)
	B. minax	*Klebsiella*, *Citrobacter*, *Enterobacter*, and *Serratia*	Gut and reproductive organ	Laboratory reared	Molecular method	Wang *et al.* (2014)
Ceratitis	*C. capitata.*	*K. oxytoca*, *E. agglomerans*	Esophageal bulb	Both	Culture dependent	Marchini *et al.* (2002)
		Klebsiella, *C. freundii*, *Enterobacter spp.*, *Pantoea spp.*, *Pectobacterium spp.*, and *P. stuartii*	Gut	Laboratory reared	Molecular method	Behar *et al.* (2005, 2008)
Rhagoletis	*R. pomonella*	*Pseudomonas (Phytomonas) melophthora*, and *Wolbachia*,	Esophageal bulb	Field collected	Culture dependent	Allen *et al.* (1934); Allen & Riker (1932); Baerwald & Boush (1968); Schuler *et al.* (2013)
		K. oxytoca, *E. cloacae*, and *Wolbachia*	Esophageal bulb	Field collected	Culture dependent	Rossiter *et al.* (1983); Schuler *et al.* (2013)
	R. completa	*Pseudomonas* spp., *Xanthomonas* spp., and *Wolbachia*	Esophageal bulb	Field collected	Culture dependent	Tsiropoulos (1976); Schuler *et al.* (2013)

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Table 1. (*Cont.*)

Genus (FF)	Species (FF)	Identified bacteria	Experimented organ	Status of fruit fly	Methods of identification	References
Anastrepha	*A. ludens*	*Listeria*. *Enterobacter*, *Providencia*, *Serratia*, *Staphylococcus* spp., and *Wolbachia*	Gut	Field collected	Culture dependent	Kuzina *et al.* (2001); Schuler *et al.* (2013)
	A. ludens *A. obliqua* *A. serpentina* *A. striata*	*Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Raoultella*, and *Wolbachia*	Gut	Field and lab	Molecular method	Ventura *et al.* (2018); Prezotto *et al.* (2017)

et al., 1970). Two different types of symbiosis were defined in fruit fly: in the first type, bacteria associated with the esophageal bulb (a foregut diverticulum) of the adult fly, and in the second type, the bacteria associated with the blind sacs at the anterior end of the larval midgut (Girolami, 1973). The release of compact masses of bacteria from the esophageal bulb into the midgut was described; however, the identity of the microorganisms was not reported (Girolami, 1983). Nine species of bacteria were isolated from the esophageal bulb of *B. oleae* and eight species from the phylloplane of the host plant (Belcari *et al.*, 2003). Only three species, namely, *Agrobacterium radiobacter*, *Pseudomonas putida*, and *Serratia marcescens*, were isolated from both fruit fly and host plant. It was speculated that *Acetobacter tropicalis* can influence host nutrition and physiology by participating in the glycometabolism of the olive fruit fly (Kounatidis *et al.*, 2009). When it comes to the other *Bactrocera* species, twenty-three strains, characterized as members of the Enterobacteriaceae family were reported from wild adults of *B. dorsalis*; *Pseudomonas pseudomallei* was identified from the adult and larval stages of *B. cucurbitae*, and eight genera named *Proteus*, *Klebsiella*, *Streptobacillus*, *Alcaligenes*, *Haemophilus*, *Erwinia*, *Chromobacterium*, and *Flavobacterium* were identified in *B. tau* (Gupta *et al.*, 1982a, b; Jang & Nishijima, 1990; Khan *et al.*, 2014). Eleven types of bacteria were also isolated when comparing bacterial associated with *B. tau* and *B. cucurbitae*, and, five of these bacteria, namely, *P. putida*, *Erwinia herbicola*, *Cedecea davisae*, *Arthrobacter* spp., and *Xanthomonas maltophilia* (*Stenotrophomonas maltophilia*), were found in both species of fruit fly (Sood & Nath, 2002). All the bacterial isolated belonging to the family Enterobacteriaceae, and the most common species of *Bactrocera* flies were *Citrobacter freundii*, *E. agglomerans*, *E. cloacae*, *K. oxytoca*, and *Kluyvera* spp., and these bacteria were collectively referred to as 'fruit fly type' bacteria (Lloyd *et al.*, 1986; Jang & Nishijima, 1990).

As a very important quarantine pest, the Mediterranean fruit fly *C. capitata*, also harbor bacteria in its esophageal bulb and the bacteria *K. oxytoca* and *E. agglomerans* were identified as the predominant species in wild and laboratory medfly populations, respectively (Marchini *et al.*, 2002). Besides *Pseudomonas (Phytomonas) melophthora* was found to be associated with all stages of *R. pomonella* (Walsh) (Allen & Riker, 1932; Allen *et al.*, 1934; Baerwald & Boush, 1968) another two *R. pomonella* associated bacteria species, *K. oxytoca* and *E. cloacae* were reported to be necessary for normal development in most tephritid species (Rossiter *et al.*, 1983). Fifteen morphologically different bacteria associated with the walnut husk fly *R. completa* (Cresson) were identified (Tsiropoulos, 1976), but only *Pseudomonas* spp. and *Xanthomonas* spp. were present in all stages of the life cycle of the fly. A diverse microbial flora was discovered in the esophageal-bulb of six *Rhagoletis* spp., and *K. oxytoca* was predominant in every species of fly (Howard *et al.*, 1985). Not only bacteria diversity varied in different fruit fly species, but also it is different in new and old colonies of the fruit fly. Out of eighteen identified different gut bacterial species of Mexican fruit flies, *A. ludens*, bacterial species *Aeromonas hydrophila* and *K. pneumoniae* ssp. *pneumoniae* were found only in the old colony whereas, *Providencia stuartii*, *Bacillus cereus*, *P. aeruginosa*, and *Enterobacter sakazakii* from a new colony only (Kuzina *et al.*, 2001).

Bacteria from fruit flies are not simply envisaged as environmental microorganisms but are in fact symbionts of the host body, where it occupies a specific favorable niche (Dale & Moran, 2006). However, there remains a paucity of information on this subject. Research with modern molecular tools in this particular area is essential for improving our knowledge of fruit fly ecology and for clarification of the functions of these bacteria. Such studies will also be a major step toward understanding bacterium-fruit-fly association.

Molecular characterization

Molecular approaches for microbial detection have led to dramatic changes in the identification of microbial diversity. It is recognized that about 99 percent microbes in the environment cannot be cultured (Amann *et al.*, 1995). However, nucleic-acid-based approaches for the characterization of microbes have provided new information in spite of the limitations of these techniques (Head *et al.*, 1998). Nucleic acid sequencing, particularly those *16S rRNA* gene sequencing, has enabled the identification of the microbiota associated with insects (Brauman *et al.*, 2001; Toth *et al.*, 2001). Random amplification polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis has been used to compare strains of bacteria between insects and within generations. Profiling of the insect gut microbiota is now feasible using methods based on the *16S rRNA* gene (de Vries *et al.*, 2001).

For the first time, five distinct strains of *Wolbachia* were identified (Jamnongluk *et al.*, 2002) in *B. ascita* based on the *wsp* (*Wolbachia surface protein* gene sequence). This report also stated that four of the five *Wolbachia* strains were in the same group as those identified in other tephritid fruit flies, suggesting possible horizontal transfer of *Wolbachia* from other fruit flies into *B. ascita* (Waleron *et al.*, 2002) studied the genotypic characteristics of *Erwinia* was studied by PCR-RFLP analysis (polymerase chain reaction-restriction fragment length polymorphism) of the *recA* gene fragment. The results indicated that PCR-RFLP analysis of the *recA* gene fragment is a useful tool for the identification of species and subspecies of *Erwinia* (Sood & Prabhakar, 2009). RAPD and PCR-RFLP using the *recA* gene and the *rrs* gene were also be used to

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study the genotypic characteristics of gut bacteria of *B. tau*, and it reported that the PCR-RFLP profiles of three symbionts were more accurate than the RAPD-REP-PCR (Sood & Prabhakar, 2009).

Bacteria from several fruit fly species such as *B. zonata*, *B. tau*, *B. cucurbitae*, *B. oleae*, *B. dorsalis*, *B. tryoni*, *B. cacuminata*, *B. carambolae*, and *B. minax* was identified by targeted *16S rRNA* gene sequencing (Illumina MiSeq platform). *Bacillus*, *Enterobacter*, *Klebsiella*, and *Stenotrophomonas* were the dominating bacterial genera, identified from field and laboratory-collected *B. zonata* (Reddy *et al*., 2014). Two most abundant species, i.e. *K. oxytoca* and *Pantoea agglomerans*, belonged to the bacterial family Enterobacteriaceae, and one species, namely, *Staphylococcus* sp., belonged to the family Staphylococcaceae were characterized from *B. tau* (Prabhakar *et al*., 2009b, 2013). But in recently, Luo *et al*. (2018) mentioned three bacteria, namely – *Enterobacter*, *Providencia*, and *Serratia* were the dominant bacteria of both male and female *B. tau*. Twenty-six bacteria belonging to the families Enterobacteriaceae, Micrococcaceae, Bacillaceae, and Staphylococcaceae were isolated and identified from the midgut of the melon fly *B. cucurbitae* whereas, *Enterobacter*, *Bacillus*, *Klebsiella*, *Citrobacter*, and *Providencia* as the dominating genera (Hadapad *et al*., 2016). Symbiotic bacteria of the olive fruit fly (*B. oleae*) consistently yielded a single sequence that exhibited marked similarity with enterobacterial lineages, with closest matches (97%) to *Erwinia persicina* and *E. rhapontici*. The identity of the symbiont was also distinct from *Pseudomonas savastanoi*. A novel species was proposed, by virtue of its unique properties, under the designation '*Candidatus Erwinia dacicola*' (Capuzzo *et al*., 2005; Kounatidis *et al*., 2009).

Till now many researchers worked on oriental fruit fly, *B. dorsalis*. From the gut (Wang *et al*., 2013, 2014), five species from three genera namely, *Raoultella terrigena*, *E. sakazakii*, *Enterobacter amnigenus*, *Klebsiella pneumonia*, and *Klebsiella oxytoca* (Shi *et al*., 2012), were identified. As compared to laboratory-reared population, bacterial diversity was found to higher in the wild populations, and the same result was observed between the samples from late-term invasion regions to early-term invasion regions in China (Liu *et al*., 2016).

Bacterial community varied between crop and midgut and as well as male and female sex of two fruit fly species – *B. tryoni* and *B. cacuminata* (Thaochan *et al*., 2010, 2015). Seventeen bacterial phyla with four major genera, namely, *Enterobacter Klebsiella*, *Serratia*, and *Citrobacter*, were identified from *B. minax* (Wang *et al*., 2013, 2014).

Most of the researchers only used adult fruit flies, but recently some reports (*C. capitata*, *B. dorsalis*, and *B. carambola*) found the bacterial diversity in different developmental stages like egg, larvae, pupae, adult male and female. In *B. dorsalis*, the phylum Proteobacteria was dominant in the immature stages, but Firmicutes dominated in adult stages. In the pupal stage, the genus *Comamonas* was most abundant but completely absent in adults (Andongma *et al*., 2015). *Acetobacter* sp. from Acetobacteraceae was the most abundant in larvae, mainly *Brachybacterium* from Dermabacteraceae, and mainly *Brevibacterium* from Brevibacteriaceae were the most abundant in pupa and bacteria from Enterobacteriaceae were the most abundant in adult *B. dorsalis* (Zhao *et al*., 2018). *Erwinia* was the dominant genus in the larval stage; *Halomonas* was most abundant in adult females; *Stenotrophomonas* was most abundant in adult males; and *Chryseobacterium* was most abundant in the larval and pupal stages of *B. carambola* (Yong *et al*., 2017b). Though the bacterial hosted within the Mediterranean fruit fly differs among instars, life stages (Aharon *et al*., 2013) and host-plant species, most of them belong to the phylum Proteobacteria, including Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria (Malacrinò *et al*., 2018). The most abundant species are *Providencia stuartii*, *Klebsiella* spp., *Pectobacterium* spp. *C. freundii*, *Enterobacter* spp. and *Pantoea* spp. (Behar *et al*., 2005, 2008). The gut of larvae and adults from four species of *Anastrepha* genus, including *A. serpentine*, *Anastrepha ludens*, *A. striata*, and *A. obliqua*, and among which *Klebsiella*, *Enterobacter*, *Raoultella*, *Escherichia* and *Citrobacter* and were confirmed to be the dominant bacteria. Adults had a higher bacterial diversity than larvae, and the adult flies with a broader diet breadth possess a higher bacterial diversity than species with a limited number of hosts (Ventura *et al*., 2018).

Till now different bacterial genera, namely – *Pantoea*, *Micrococcus*, *Agrobacterium*, *Xanthomonas*, *Acetobacter*, *Lactobacillus*, *Escherichia*, *Kluyvera*, *Arthrobacter*, *Proteus*, *Bacillus*, *Hafnia*, *Pseudomonas*, *Chryseobacterium*, *Defluvibacter*, *Enterobacter*, *Providencia*,, *Flavobacterium*, *Halomonas*, *Streptococcus*, *Klebsiella*, *Listeria*, *Staphylococcus*, *Ochrobactrum*, *Pectobacterium*, *Serratia*, *Stenotrophomonas*, *Delftia*, *Raoultella*, *Serratia*, *Erwinia* and *Citrobacter* have been isolated, identified and characterized from esophageal bulb, the gut and reproductive organs of laboratory-reared and field-collected tephritid flies, both male and female (Lloyd *et al*., 1986; Drew & Lloyd, 1987; Jang & Nishijima, 1990; Lauzon *et al*., 2000; Zinder & Dworkin, 2000; Bergey *et al*., 2001; Kuzina *et al*., 2001; Marchini *et al*., 2002; Belcari *et al*., 2003; Behar *et al*., 2005, 2009; Capuzzo *et al*., 2005; Sacchetti *et al*., 2008; Kounatidis *et al*., 2009; Prabhakar *et al*., 2009b, 2013; Thaochan *et al*., 2010; Wang *et al*., 2011, 2013, 2014; Reddy *et al*., 2014; Naaz *et al*., 2016; Yong *et al*., 2017b). In general, from many studies, tephritid bacteria mostly belong to the gram-negative type except *Micrococcus*, *Lactobacillus*, *Arthrobacter*, *Bacillus*, *Streptococcus*, *Listeria*, and *Staphylococcus*. Members of the Enterobacteriaceae family and mainly *Klebsiella* and *Enterobacter* are the predominant bacterial species in most of the adults. The bacteria were present mainly in the midgut and to a lesser extent in the salivary glands and reproductive organs (Rani *et al*., 2009; Crotti *et al*., 2010); however, one study explained that the female ovary had the highest bacterial diversity followed by the male testis, and reproductive organs were rich in bacterial community than that of the gut (Wang *et al*., 2013, 2014). Most of the findings supported the intestinal tract from where the highest bacterial diversity was found. This change may be due to the host preference of different tephritids species and also little contribution to the surrounding environment (Michael *et al*., 2008; Colman *et al*., 2012). Bacterial diversity in fruit fly also may varied in different geographic locations (Liu *et al*., 2018).

Wolbachia strains were found from most *Anastrepha* species (Prezotto *et al*., 2017) but in Bactrocera genus, polyphagous species are more likely to harbor compared to stenophagous or monophagous ones (Jamnongluk *et al*., 2002). The genus Ceratitis, only two species were reported in *Wolbachia* (Bourtzis *et al*., 1994). However, four species of *Rhagoletis* (*Rhagoletis cerasi*, *Rhagoletis pomonella*, *Rhagoletis cingulata*, and *Rhagoletis completa)* are the harbour of *Wolbachia* (Schuler *et al*., 2013). Low infection rates of *Wolbachia*, another bacterium with potential for pest management, was detected in four of five Chinese populations of *B. dorsalis* (0.7–3%) (Sun *et al*., 2007). However, no bacteria belonging to the genus *Wolbachia* were detected in one of the Chinese populations,

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which may be attributed to the transient infections that result from spillover events from a yet unknown source, questioning the vertical inheritance of this species (Augustinos *et al.*, 2015; Morrow *et al.*, 2015; Liu *et al.*, 2016)

Regarding the symbiotic bacteria diversity, there was no regularity among different tephritidae species and different development stages. The bacterial diversity is proved to be associated with environment and host plants. Through the molecular method, especially the Pyrosequencing method can reveal more symbiotic bacteria from fruit flies, it also has its disadvantages. The acquisition of pure culture strain using culture-dependent method can lay a solid foundation for studying the function of bacteria.

Function of symbionts in fruit flies

Commonly, fruit flies are associated with rot-inducing, free-living bacteria (mainly those of the Enterobacteriaceae family such as *Enterobacter* spp., *Pantoea and Klebsiella*), which might be enter into the fruit by ovipositor during female oviposition and enhance larval development by providing protein and necessary nutrients (table 2) (Drew & Lloyd, 1991; Lauzon, 2003; Behar *et al.*, 2008, 2009). Regardless of the affinity of fruit flies for the Enterobacteriaceae family, most fruit flies have only a weak alignment with somewhat specific species of bacteria but they can increase their population independently in fruit tissues; outside the insect gut (Girolami, 1973; Behar *et al.*, 2009; Martinez-Sañudo, 2009). Bacteria isolated from adult fruit flies and different tissues of fruit flies can attract egg-laying flies (Wang *et al.*, 2014). Additionally, maximum flies generally tend to keep away from the restrictive situation in green fruit and preferentially lay eggs in ripe fruit (Fletcher, 1987), that offer higher guide for larval development (Greany, 1989; Messina & Jones, 1990; Joachim-Bravo *et al.*, 2001; Rattanapun *et al.*, 2009) and for bacterial proliferation (Behar *et al.*, 2008). There is, however, an exception: the bacteria *Erwinia dacicola* (table 2) in *B. oleae* helps the host fly lay eggs in unripe olive fruit and enables larval resistance to host plant defenses (Ben-Yosef, 2015). The symbionts provide digestive enzymes or vitamins that are absent from the fruit tissues of the host plant and improve digestion efficiency; consummately affects the survival capacity, fecundity with growth and development of host flies (Robacker & Lauzon, 2002). Bacteria isolated from *R. pomonella* can help the host in detoxification of phlorizin from the plant. Genes encoding detoxifying and digestive enzymes were identified in the bacteria associated with *B. oleae*, indicating the potential ability of associated bacteria to help the host fly survive in green olives (Pavlidi *et al.*, 2017). The bacteria can mediate mate selection in *B. dorsalis* by attracting male flies to females harboring the microbiota and by causing the male flies to ejaculate more sperm into the females (Damodaram *et al.*, 2016). Certain bacteria also possess the ability to degrade pesticides (Robertson & Alexander, 1994; Sonia Rodríguez-Cruz, 2006), suggesting that symbiotic bacteria of insects may also contribute to insecticide resistance. The ability of the gut symbiont *Citrobacter* sp. to resist the organophosphate insecticide trichlorfon (table 2) has been confirmed in *B. dorsalis* (Cheng *et al.*, 2017)

Research shows that bacteria are scattered into the alimentary tract of female flies, which are then shifted to eggs and then to the fruit by ovipositor; the bacteria are ultimately inherited by the fly's offspring (Shi *et al.*, 2012). Symbiotic bacteria has a great contribution on adult survival (Capuzzo *et al.*, 2005) by providing some essential compound, if, which are rare in host fruit, pectin degradation and nitrogen fixation (Behar *et al.*, 2005). *Gluconobacter*, one of plant pathogenic bacteria was confirmed to affect the hatching rate and growth of *B. dorsalis* and the rot rate of host fruit (He *et al.*, 2017). The precise function of bacterial association of different species of the fruit fly isn't properly understood, and an additional look at is required. With the development of next-generation sequencing method and current biological techniques, more and more attention has been paid to the function of symbiotic bacteria. Genes encoding detoxifying and digestive enzymes have been verified to help host olive fruit fly endure the green olive (Pavlidi *et al.*, 2017)). The function of one single symbiotic bacteria and its transmission in the host fly is still the focus of attention. The function of the key genes from symbiotic bacteria further rises to the hot research topic.

Bacterial symbionts in pest management techniques of tephritids

Recently, symbionts have been used for developing control strategies against pest insect populations because chemical pesticides lead to environmental pollution, risks to human health hazard and chemical resistance and to the death of beneficial insects as well as rapid loss of biodiversity. Bacteria have been seen to be environed in toxic compounds degradation which ingested by host insects, propulsion to insecticide resistance (Eutick *et al.*, 1978; Fukatsu & Hosokawa, 2002). Bacterial odors have been used in pest management strategies as attractants (pheromone traps), bait sprays (Robacker, 2007; Sood *et al.*, 2010; Naaz *et al.*, 2016) and stimulation of insect feeding or oviposition (Drew & Lloyd, 1987; Jang & Nishijima, 1990; Lauzon *et al.*, 2000). Symbiont biology has received increasing attention because insect symbionts can potentially be used to control vector-borne diseases or to suppress insect pests (Crotti *et al.*, 2010).

Bacterial odors as attractants

Various protein bait formulations have been used as attractants for many years in tephritid control programs. The attractancy of protein solutions containing bacteria to tephritids was first reported in 1954, when *B. dorsalis* in Hawaii strongly responded to solutions containing *Proteus* species (Gow, 1954). Fruit-fly-type bacteria were grown on peptone-yeast-extract agar (Drew *et al.*, 1983), and hydrolyzed protein solutions inoculated with these bacteria were seen to strongly attract wild *Bactrocera* species (Drew & Fay, 1988). Furthermore, in the absence of PIB-7 (protein hydrolysate), the response of flies (*B. tryoni*) to the bacteria was significantly high, but relatively lower than the response of flies to bacteria exposed to PIB-7 (Jang & Nishijima, 1990). Volatile compounds, such as 2-butanone, from bacteria were important attractants of dacinae and served as both feeding attractants for females and sex attractants for mature males (Drew, 1987). But, 3-methyl-1-butanol and ammonia released from *Enterobacter agglomerans* attracted only female sex of the Caribbean fruit fly, *Anastrepha suspense* (Epsky *et al.*, 1998). Several volatile compounds, such as ammonia, isoamylamine, trimethylamine, 2,5-dimethylpyrazine, 2-methyl-butylamine, and acetic acid, were presented in cultures of *Staphylococcus aureus* (Robacker & Flath, 1995); 3-methyl-1-butanol, 2,5-dimethylpyrazine, phenethyl alcohol, 3-(methylthio)-1-propanol and 2-methyl-1-propanol were identified from the bacteria *K. pneumonia* (Lee *et al.*, 1995);

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Table 2. Function of the symbiotic bacteria in related to economic fruit fly.

Bacteria	Host fruit fly species	Function (s)	References
Citrobacter sp.	*B. dorsalis*	Helps to resist organophosphate insecticide, trichlorfon.	Cheng *et al.* (2017)
Undetermined species	*B. dorsalis*	Mediate mate-selection	Damodaram *et al.* (2016)
Erwinia dacicola	*B. olea*	Helps host fly to lay eggs in unripe olive fruit and enable larvae resistant to host defense	Ben-Yosef (2015)
E. agglomerans	*R. pomonella*	Help host realize the detoxification of the plant phlorizin	Robacker & Lauzon (2002)
Pantoea, Klebsiella and *Enterobacter* spp.	Unknown	Larval nutrition with protein and essential nutrients	Drew & Lloyd (1991); Lauzon (2003); Behar *et al.* (2008, 2009)
Gluconobacter	*B. dorsalis*	Affect the hatching rate and growth of fruit fly and also the rot of host fruit.	He *et al.* (2017)
K. oxytoca and *P. agglomerans*	*B. tau* and *B. cucurbitae*	Attractancy	Prabhakar *et al.* (2009b)
Klebsiella, *Citrobacter* and *Providencia*	*B. cucurbitae* and *B. dorsalis*	Attractancy	Hadapad *et al.* (2016)
R. terrigena, *K. oxytoca* and *K. pneumoniae*	*B. dorsalis*	Attractancy	Shi *et al.* (2012)
B. cereus	*B. dorsalis*	Attractancy	Wang *et al.* (2013)
E. cloacae and *K. pneumoniae*	*B. zonata*	Attractancy	Reddy *et al.* (2014)
K. oxytoca	*B. zonata*	Attractancy	Naaz *et al.* (2016)
Enterobacter, Providencia	*B. tau*	Attractancy	Luo *et al.* (2018)

butyl isocyanatoacetate, 3-methyl-1-butanol, 2-methyl-1-propanol and 2-phenylethanol, and were found from bacteria, *C. freundii* and *K. oxytoca* (Hadapad *et al.*, 2016).

Two predominant symbionts, *K. oxytoca* and *P. agglomerans* (reported by Prabhakar *et al.*, 2009b), of *B. tau* exhibit attractancy toward two species of fruit fly, namely, *B. cucurbitae*, and *B. tau* (Sood *et al.*, 2010). But, in recent research, *Enterobacter cloacae Enterobacter* sp., and *Providencia alcalifaciens* attract more adult both male and female of *B. tau* (Luo *et al.*, 2018).

Metabolites from *K. oxytoca* attracted more adult of *B. dorsalis* in field condition whereas other two bacteria *R. terrigena* and *K. pneumoniae* had the capacity to attract (Shi *et al.*, 2012). However, it is showed that metabolites from *B. cereus* allured the highest number of *B. dorsalis* adults in the laboratory and in the field (Wang *et al.*, 2013). On the other hand, bacterial suspension from *Citrobacter, Klebsiella*, and *Providencia* attracted a significantly higher number of females compared to males of *B. cucurbitae* and *B. dorsalis* (Hadapad *et al.*, 2016).

The bacteria *E. cloacae* and *K. pneumoniae* exhibited the highest attractancy toward all age groups of *B. zonata* (Reddy *et al.*, 2014). However, *K. oxytoca* showed the highest attractancy toward adult female *B. zonata* compared to the metabolites produced by other bacteria, and compared to the combinations of these metabolites used in the field bioassay (Naaz *et al.*, 2016). An interesting finding from Naaz *et al.* (2016) was that the individual bacterial metabolites attracted more male adult flies of *B. zonata* compare to metabolites from a combination of different bacteria. Female flies harboring bacteria showed a higher ability to attract males than the female flies whose microbiota had been abolished by antibiotic treatment (Damodaram *et al.*, 2016).

The enchantment of fruit fly adults to intestine bacteria is especially because of the presence of chemical compounds along with ammonia, alcohols, ketones, sulfur-containing compounds, several amines, pyrazines, phenols and acetic acid (DeMilo *et al.*, 1996; Robacker *et al.*, 1998; Liscia *et al.*, 2013; Hadapad *et al.*, 2016). But sometimes, combinations of attractive chemicals are not attractive (Robacker *et al.*, 2004). The response of fruit flies to specific bacteria pointed out that, a system of bacterial attraction for fruit flies possibly exists in nature and also may contribute the fruit fly behaviour.

Potential application of symbionts in fruit fly control

Manipulation of symbionts for the control of tephritids was first proposed in 1929–30. For killing of symbiotic bacteria, foliar application of copper carbonate was suggested firstly and at a later stage, this preparation was improved by adding sugar (Baker *et al.*, 1944; Fytizas & Tzanakakis, 1966a, b). Treatment of the larvae of the melon fruit fly *B. cucurbitae* with the antibiotics oxytetracycline and sulfanilamide destroyed the symbiotic microorganisms in the mycetocytes of the midgut region and eventually led to reduced larval survival rates (Chinnarajan *et al.*, 1972). These two approaches (copper and antibiotics) were the first steps toward this application; however, these approaches failed to be commercialized because of the low efficacy of copper carbonate and the residual hazards of streptomycin and other antibiotic treatments. Then, copper was reported to play an important role as symbioticide in the elimination of fruit-fly-associated bacteria and was thus helpful in managing the first- and second-instar larvae of olive fruit flies (Belcari & Bobbio, 1999).

Endosymbiotic bacteria of the genus *Wolbachia* induce cytoplasmic incompatibility, thelytokous parthenogenesis, male-killing or feminization in their hosts, and thus, these bacteria may be useful in IPM (Jamnongluk *et al.*, 2002). As a primary symbiont, *Wolbachia* has been used to control an important malaria vector, *Anopheles stephensi* (Bian *et al.*, 2013). However, *Wolbachia* has not been applied in tephritid fruit flies by now. The most important obstacle is the low infection of *Wolbachia*. How to resolve this problem determines whether we can use *Wolbachia* for tephritid fruit fly management. Finding effective Wolbachia strains with a high infection rate

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from genetically close species, and introducing it into the target tephritidae fruit fly through genetic modification can be an effective solution (Bian *et al.*, 2013). Foliar application of the fruit fly symbiont *K. oxytoca* as bait in combination with insecticides under field conditions resulted in a significant reduction in fruit fly infestation (65.46%) relative to the untreated control (79.56%) (Sood *et al.*, 2010). Gut bacteria of Mediterranean fruit fly are related to the management of this fruit fly (Ben Ami *et al.*, 2010; Gavriel *et al.*, 2011).

Recently one of the strategies for controlling these invasive pest species is sterile insect technique (SIT). Gut symbiotic bacteria, *E. agglomerans* and *K. pneumoniae* increase mating performance, calling activity, or life expectancy of mass-reared male of *C. capitata* (Niyazi *et al.*, 2004). But gamma irradiation influences the gut microbial community by reduction of *Klebsiella* sp. and increase the *Pseudomonas* sp. abundance that phenotypically affects the performance of the sterile male (Ben Ami *et al.*, 2010). However, increasing of *K. oxytoca* led to stable colonization and decreasing the abundance of *Pseudomonas* spp., accelerate mating competitiveness compared with wild males. Furthermore, bacteria belonging to the family Enterobacteriaceae, in which one of the members is *K. oxytoca*, extended the longevity of the fly (Behar *et al.*, 2008). *Enterococcus phoeniculicola*, another isolated gut bacteria, can promote the larval and pupae developmental, while *Lactobacillus lactis* showed negative effects on *B. dorsalis* development (Khaeso *et al.*, 2018). This approach can be used proudly to prolong the survival capacity of the sterile male insect, that can be improved the success rate of SIT programs. On the other hand, commensal bacteria influence many aspects of an organism's behavior. In *B. dorsalis*, the fly microbiota can mediate mate selection. Compared to female flies whose bacteria had been abolished by antibiotic treatment, male flies were attracted to and ejaculated more sperm into females harboring the microbiota (Damodaram *et al.*, 2016). The effect of commensal bacteria on the mate-selection behavior of *B. dorsalis* could be useful for devising control operations against this devastating pest (Yuval *et al.*, 2013).

The identification of the symbiont *Citrobacter* sp., which plays a key role in the degradation of trichlorfon, leading to insecticide resistance opened new avenues of research for the control of the most economically important fruit fly *Bactrocera dorsalis* (Cheng *et al.*, 2017). On the other hand, the symbiotic bacteria modified with toxin genes can be used in the management of fruit flies (Sood & Nath, 2005). Fluorescence *in situ* hybridization showed that the bacteria was aggregated in rectal pads, and can be vertically transmitted via egg surface contamination. The important biological role of these bacteria in the fitness of host insects provide us broader application prospects of this pesticide – resisting bacteria for pest management (Guo *et al.*, 2017).

Symbiotic bacteria play a potential role in the rearing of parasitoids as host detection (Leroy *et al.*, 2011), breakdown host defense mechanism as well as quality improvement. This area is not well studied in tephritids, but the findings from other host-parasitoid systems (Thibout *et al.*, 1993; Hoffmeister & Gienapp, 1999; Paredes *et al.*, 2016) may be pertinent for the mass-rearing and applications of parasitoids on tephritids. For instance, chemicals from the host feces often influence host detection. As for example, parasitoid was *Halticoptera laevigata* and its host *Myoleja lucida* (Hoffmeister & Gienapp, 1999). This interaction was shown to be mediated by microbes in moth parasitoid *Diadromus pulchellus* (Wesmael) (Thibout *et al.*, 1993). Symbiotic bacteria possess a direct or indirect effect on defensive mechanism against parasites and pathogens (Oliver *et al.*, 2014). As for example, bacterial species, *Serratia symbiotica* (Oliver *et al.*, 2003), *Candidatus* Hamiltonella defensa (Schmid *et al.*, 2012) and *Regiella insecticola* (Vorburger *et al.*, 2010), found from the aphids have great contribution as a protective symbiont. However, several studies suggest that *Spiroplasma* is a defensive symbiotic bacterium of Drosophila (Xie *et al.*, 2015; Paredes *et al.*, 2016). These types of protective bacteria may exist in tephritids, used for the rearing of parasitoids but several studies are required. On the other hand, host bacteria not only involve in host immunity but also create a suitable environment of parasitoids rearing as well as improve the quality such as size, fecundity and longevity of parasitoids. An elegant example of parasitoid, *D. longicaudata* with host, *Anastrepha ludens* (Eben *et al.*, 2000; Cicero *et al.*, 2012).

The attractancy of gut bacterial symbionts to fruit fly species has low-field efficacy (Sood *et al.*, 2010). Therefore, bacterial attractancy alone is not an effective control measure. Maybe extracellular extracts can be used as odors instead of bacteria itself in the future. Bacterial attractancy can be used only for pest monitoring and surveillance. Attractancy, SIT and parasitoids application can be complementary approaches; the use of natural predators (parasitoids) is generally more efficient in case of is higher host intensity, whereas in low host densities, SIT is generally more efficient based on theoretical models as well as field studies (Barclay, 1987; Bloem *et al.*, 1998). These three methods can be integrated as components of Integrated Pest Management (IPM) strategies.

Conclusions and research gap

Bacterial endosymbionts are reported to be common in insects, especially in fruit flies. Molecular tools are essential to improve our knowledge of fruit fly ecology. Recently, next-generation sequencing of PCR-generated amplicons (*16S rRNA* gene) has been successfully employed (Lemos *et al.*, 2014; Wang *et al.*, 2014; Cheng *et al.*, 2017; Yong *et al.*, 2017a, b), and compare to traditional method of microbiota identification, these high-throughput technologies have shown a higher diversity of microbiota. However, the short sequences used for high-throughput sequencing only identify bacteria at the genus level, which restricts access to detailed molecular information. Elucidation of the functions of the bacteria in and for the hosts will be a major step toward understanding bacterium-fruit-fly associations. The *in vivo* and *in vitro* experiments on cultivable bacteria make it easier to determine the functions of symbiotic bacteria (Cheng *et al.*, 2017). However, the uncultivable nature of many symbionts makes the identification of bacterial function more challenging. In recent years, antibiotics have been very useful to abolish or modulate the targeted gut bacteria, which makes up for the limitations of non-cultivability (Damodaram *et al.*, 2016). However, this method also has disadvantages; non-lethal and toxic concentrations of antibiotics can influence the results of the experiment. It is important to focus on the selection of correct negative controls in order to eliminate the influence of the antibiotic on the experimental results. Perhaps in the future bacteriophages can be used instead of antibiotics to eliminate bacteria (Louradour *et al.*, 2017). While for the cultivable gut bacteria, identification of their clear localization and transmission pathway between intra- and inter-species will also be useful for the function study and for the future pest management technologies (Guo *et al.*, 2017).

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In this review, we described several families and genera of bacteria that are associated with 17 economically important species of the fruit fly; however, there are more than 250 species that are economically significance (Li *et al*., 2013). To date, the diversity of bacteria in many economically important species of fruit fly is unknown. The members from Enterobacteriaceae are often considered to be the dominant bacteria of several tephritids, such as *Anastrepha, Dacus*, *Bactrocera* and *Ceratitis*. Bacteria of this family (*E. herbicola, E. cloacae and K. oxytoca*) are believed to mediate interactions between adult fruit flies and the larval host plant. To date, few specific functions of symbiotic bacteria have been identified, but most of the functions remain unknown.

Symbiotic bacteria have great attraction potential. This property is useful for fruit fly monitoring and surveillance, but for fruit fly control, bacterial attractancy should be combined with SIT and the use of parasitoids. However, there is little known about the mass rearing of parasitoids of fruit flies and about the associated microbiota. In addition, an in-depth study on the functions of the symbiotic bacteria of fruit flies can provide a new target for novel control techniques based on sterilization. Many interesting and meaningful scientific questions deserve our further study. How to get the extraction with high attracting ability from symbionts? How to find suitable *Wolbachia* strains and use them for fruit fly management after genetic modification? What is the function of some single strains and the mix function of several strains? What is the molecular mechanism of the function of symbiotic bacteria? Which genes control those functions? What we can get from the genome and transcriptome of symbiotic bacteria? All these further studies on the bacteria of fruit flies will also help develop the use of bacteria as an environmentally friendly method to control infestations of fruit and vegetable pests.

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