Journal of Integrative Agriculture

Functional roles of aphid-associated microbes in multitrophic interactions

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Highlights

- Aphid endosymbionts regulate host nutrition, fitness and resistance to biotic and abiotic stresses.
- Microbes in aphid saliva and honeydew mediate plant defenses.
- Honeydew-associated microbial volatiles can modify the behaviour of natural enemies.

Abstract

Aphids (Hemiptera: Aphididae) are economically important pests of crops worldwide. Although increasing evidence suggests that aphid-associated microorganisms have potential applications in integrated pest management (IPM), our understanding of their roles in multitrophic interactions remains limited. This review synthesizes current knowledge on aphid-associated endosymbionts and secretion-associated microbes, focusing on their influence on aphid physiology, behavior but also on interactions with host plants and natural enemies. Firstly, the functional diversity of endosymbionts, which contribute to improved nutrition, heat tolerance and resistance to pathogens, parasitoids and pesticides was highlighted. Secondly, we examined how microbes from aphid saliva and honeydew can act as molecular cues to induce plant defenses and modify the behaviour of aphidophagous beneficials. Special attention was given to honeydew-associated microbial volatiles (mVOCs), which can modulate the behavior of predators and parasitoids. Finally, emerging biocontrol strategies that leverage symbionts and mVOCs were discussed to propose future directions for integrating microbial ecology into sustainable aphid management.

Keywords: aphid endosymbionts, secretion-associated microbes, plant defense, microbial volatiles, multitrophic interactions

1. Introduction

Aphids are a particularly significant group of insect pests from an agricultural perspective. The family Aphididae encompasses a vast assembly of over 5000 species, each of which specializes in consuming phloem sap through the use of piercing mouthparts (Emden and Harrington 2017). In addition to extracting nutrients, aphids can also transmit plant viruses. Indeed, out of 600 insect-borne viruses, 275 are transmitted by aphids (Emden and Harrington 2017). The honeydew that aphids excrete induces sooty molds (black filamentous saprophytic ascomycetes), which inhibit plant photosynthesis and reduce food production globally. Aphids are estimated to cause annual yield losses ranging from millions to billions of dollars (Dedryver *et al*. 2010).

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Traditional aphid control heavily relies on chemical pesticides, but this approach can lead to several well-known problems, including pest resistance development, harm to non-target organisms, and the presence of chemical residues in agroecosystems, causing ecological disruptions (Hart *et al*. 2003; Pimentel and Burgess 2014). Several of them are progressively ban such as neonicotinoids in Europe. Consequently, concerns about environmental impacts and threats to human health associated with synthetic insecticides have prompted the increased development of integrated pest management (IPM) strategies (Brewer and Goodell 2012). Biological pest control, which employs natural enemies like insect predators and parasitoids, is advocated as a key component of IPM to regulate pest populations.

Over the past two decades, microorganisms have been widely used for pest control in diverse ecosystems (Ruiu 2018), with many promising potential against aphids (Francis *et al*. 2020). While most of these microbials are derived from the environment or plants and are directly toxic to aphids or reduce their fitness, much less is known about the role of aphid-associated microbes in aphid control. In this review, we summarized current knowledge on aphid endosymbionts as well as microbes present in oral (saliva) and digestive (honeydew) excretions, focusing on their effects on facilitating multitrophic interactions among aphids, plants and natural enemies (Fig. 1). The objective of this review is to highlight the importance of aphid endosymbionts and microbes from secretions in complex ecosystems and to explore their potential for developing alternative aphid management strategies.

2. Aphid associated-microbes

Aphid-associated microbes refer to the community of microorganisms that are closely associated with aphids, which are either symbiont or commensal microorganisms associated with the digestive system, inhabiting saliva, gut and honeydew tract.

Almost all aphids possess the obligatory symbiotic *Buchnera*, which are localized within the bacteriocytes of the aphid's body. *Buchnera* are vertically transmitted from the mother to the offspring through the ovaries and they compensate for the nutritional deficiency in their host's diet by providing critical amino acids and/or vitamin co-factors (Douglas 2006). Besides obligate symbiosis, aphids also have nine facultative symbionts, namely, *Hamiltonella defensa* (PABS, T-type) (Russell *et al*. 2003), *Rickettsiella*, *Regiella insecticola* (PAUS, U-type), *Serratia symbiotica* (PASS, R-type), *Fukatsuia symbiotica* (PAXS, X-type), *Rickettsia* (PAR, S-type), *Wolbachia*, *Spiroplasma* and *Arsenophonus* (Guo *et al*. 2017).

In addition to endosymbionts, aphid saliva and honeydew also contain microbes, although these communities remain less explored. Proteomic analyses of Russian wheat aphid *Diuraphis noxia* saliva have shown a predominance of bacterial-origin proteins, particularly from Enterobacteriaceae and Erwiniaceae (Luna *et al*. 2018). Honeydew, rich in sugars and amino acids, supports microbial colonization and is known to promote the growth of sooty molds. Some studies have also isolated cultivable bacteria from the honeydew of various aphid species (Leroy *et al*. 2011; Fischer *et al*. 2015; Liu *et al*. 2024).

Together, these microbes form a complex network of interactions involving the aphid host, host plants and natural enemies. In the following sections, we discuss how these microbial partners influence aphid biology, modulate plant defense responses and manipulate the behavior of natural enemies, with an emphasis on their potential applications in aphid control strategies.

3. Effect of endosymbionts on their aphid host

It is expected that aphid-symbiont interactions developed between 160 and 280 million years ago (Fukatsu 1994). Due to the co-evolutionary dynamics between symbiotic partners and their hosts, mutualistic relationships emerged during that timeframe. Symbiotic bacteria in the aphid body with various functions. A summary of aphid endosymbionts and their associated functions is presented in Table 1.

3.1 Improvement of aphid nutrition

Aphids derive all their essential nutrition from plant sap, which is low in nitrogen and amino acids (Douglas 2003). As a result, aphids have evolved an obligatory symbiotic *Buchnera*, which are localized within the bacteriocytes of the aphid's body. These endosymbionts are vertically transmitted from the mother to the offspring through the ovaries, and they supplement the host's diet by providing critical amino acids and/or vitamin cofactors (Douglas 2006). In bacteriocytes, host genes encoding glutamine synthetase and glutamate synthase are upregulated, forming a GOGAT (Glutamine synthetase-Glutamate synthase) cycle that assimilates ammonia into glutamate and complements *Buchnera*'s amino acid biosynthetic pathways (Hansen and Moran 2011). The removal of these symbiotic bacteria with antibiotics inhibits the aphids' ability to produce essential amino acids (Douglas *et al*. 2001). Some aphid species from the Lacninae subfamily have evolved co-obligate associations with facultative endosymbiont *S. symbiotica*. Comparative genomics of *Cinara cedri* and *C. tujafilina* revealed that *S. symbiotica* exhibits extensive genome reduction and resides exclusively within bacteriocytes. In these species, the two symbionts show metabolic complementation in amino acid and vitamin biosynthesis, notably, a split tryptophan biosynthetic pathway and riboflavin production rescued by *S. symbiotica* (Manzano-Marín *et al*. 2016, 2017, 2020). These findings indicate a convergent evolutionary process leading to the establishment of co-obligate associations through division of metabolic labor.

Besides their obligate symbiosis with *Buchnera*, aphids engage in facultative symbiotic relationships that offer them a range of lipids and vitamins through biochemical reactions (Guo *et al*. 2017; Pers and Hansen 2021). For example, *S. symbiotica* enhances fatty acid biosynthesis by upregulating fatty acid synthase 1 (*FASN1*) and diacylglycerol-o-acyltransferase 2 (*DGAT2*), contributing to the growth and development of pea aphids *Acyrthosiphon pisum* (Hemiptera: Aphididae) (Zhou *et al*. 2021). The sequencing of *H. defensa*'s genome has revealed its ability to support aphids by providing both necessary and non-essential amino acids, as well as vitamins and coenzymes through its complete glycolytic and tricarboxylic acid cycle pathways (Degnan and Moran 2008).

Obligatory symbiotic bacteria in aphids may have a mutualistic relationship with other facultative symbiotic bacteria and regulate nutrition metabolism for aphids. *Wolbachia* in *Pentalonia nigronervos* (Hemiptera: Aphididae) provides the missing folic acid (vitamin B9) synthesis route and creates a metabolic relay with the obligatory symbiotic bacteria *B. aphidicola* for the cooperative production of riboflavin (vitamin B2) (De Clerck *et al*. 2015). While Manzano-Marín (2020) showed no evidence for *Wolbachia* as a nutritional co-obligate endosymbiont in *P. nigronervosa*, and concluded that the genome-based evidence for such a symbiosis was likely erroneous due to methodological artifacts. These differing perspectives indicate that further exploration in this area remains valuable.

3.2 Modifications of aphid fitness

Several facultative symbionts found in aphids, such as *Rickettsia*, *Spiroplasma*, and *Wolbachia*, have been reported to impact the reproductive behavior of other arthropod hosts (Simon *et al*. 2002, 2011; Engelstädter and Hurst 2009). These symbionts can induce various reproductive phenotypes, including male killing, feminization, and parthenogenesis, in a wide range of arthropods (Engelstädter and Hurst 2009). It has been reported that *Spiroplasma* infection causes the male-killing phenotype in aphids (Simon *et al*. 2011), while *Rickettsiella* has been found to reduce fecundity in *M. persicae* (Hemiptera: Aphididae) (Gu *et al*. 2023). The presence of *F. symbiotica* imposed a fitness cost on the pea aphid, leading to a decrease of up to 50% in fecundity (Heyworth *et al*. 2020). The insights into these reproductive outcomes may open up new avenues for the development of alternative management strategies.

Moreover, several endosymbiotic bacteria have a range of impacts on the overall fitness of their hosts, and it is important to note that not all these effects are advantageous. For example, *Aphis fabae* (Hemiptera: Aphididae) has a complex relationship with its endosymbiont *H. defensa*. While the latter provides strong protection against parasitoid wasps when present, its absence leads to adverse outcomes such as reduced lifespan and reproductive rate in the aphids (Vorburger *et al*. 2013). Certain cultivable strains of *S. symbiotica* found in the aphid's gut had a significant negative effect on the total number of offspring, adult body mass and reproductive duration (Pons *et al*. 2019b; Perreau *et al*. 2021). In addition to these direct effects, the strains can spread horizontally among aphids through host plants, and when absorbed by plant roots, they can cause new bacterial infections in aphids (Pons *et al*. 2019a). On the other side, Zhou *et al*. (2021) have found that *S. symbiotica* can enhance pea aphid growth by shortening developmental time and increasing body weight, an effect linked to the up-regulation of fatty acid biosynthesis pathways and accumulation of key fatty acids. While experimental inhibition of fatty acid synthase 1 (*FASN1*) and diacylglycerol-o-acyltransferase 2 (*DGAT2*) expression impaired development, fatty acid supplementation restored normal growth, highlighting the nutritional role of *S. symbiotica* in host fitness. Elston *et al*. (2021) engineered a culturable *S. symbiotica* CWBI-2.3T strain, which was shown to significantly reduce the survival rate of aphids. These findings shed light on the multifaceted nature of endosymbiotic relationships and present promising opportunities for future research and potential practical applications in aphid control.

3.3 Morphological modifications

Body color affects species identification, sexual selection, mimicry, aposematism and crypsis (Leonard and Córdoba-Aguilar 2010; Ruxton *et al*. 2018). Natural enemies may recognize their hosts by their body color (Losey *et al*. 1997; Libbrecht *et al*. 2007). Recent studies have revealed that facultative symbionts play an unexpected role in modifying aphid body color. Particularly, *R. viridis* infection in the pea aphid may increase the production of blue-green polycyclic quinones, causing the host to change color from red to green (Tsuchida *et al*. 2014), delivering effector molecules into the host cells (Nikoh *et al*. 2018). This symbiont-dependent color variation might influence the relative risks of predation and parasitism for the aphid, since predators, such as ladybird beetles, prefer to attack red morphs, whereas parasitoids prefer to attack green morphs (Tsuchida *et al*. 2010, 2014).

Aphid wing polyphenism is a critical adaptation strategy for biotic and abiotic environmental changes (Emden and Harrington 2017). Wingless morph for reproduction and winged morph for dispersal. Studies using antibiotics to eradicate *Buchnera* have shown a significant reduction in the production of winged *Sitobion avenae* (Hemiptera: Aphididae) (Zhang *et al*. 2015). However, contrary findings by Hardie and Leckstein (2007) observed no impact on wing development in *Megoura viciae* (Hemiptera: Aphididae) and *A*. *pisum* following antibiotic treatment. These contradictory observations may result from differences in aphid species, antibiotic treatment methods or the efficiency of symbiont removal. Wing morph determination is also highly plastic and can vary with environmental cues such as crowding and host plant quality, which might have differed among studies (Deem *et al*. 2024). A recent study conducted by Kang *et al*. (2022) has provided evidence indicating that the facultative symbiont *S*. *symbiotica* has the ability to impede the temporal determination of the host in wing suppression through the inhibition of apterization, ultimately facilitating the symbiont's transmission. These results offer an innovative viewpoint on the regulation of wing polyphenism in aphids.

3.4 Conferring resistance to heat shock

One crucial factor determining the geographic distribution of aphids is their ability to tolerate a range of temperatures. High temperatures can negatively impact aphid survival, fecundity, and development time by reducing the quantity of *Buchnera* cells and bacteriocytes, ultimately decreasing aphid fitness (Chen *et al*. 2000; Montllor *et al*. 2002). Some strains of *Buchnera* exhibit varying degrees of heat resistance. This tolerance is associated with the upregulation of heat-shock proteins (Hsp70) and the GroEL/GroES chaperonin complex, which stabilize protein folding and maintain the integrity of *Buchnera* cells under thermal stress (Fares *et al*. 2002; Dunbar *et al*. 2007). Beyond these intrinsic bacterial responses, aphids also employ additional strategies to safeguard their symbionts from heat stress, such as the absence of the *ibpA* (inclusion body-binding protein A) mutant allele and the presence of specific facultative symbionts (Burke *et al*. 2010; Heyworth *et al*. 2020).

Aphids infected with the facultative symbiont *S*. *symbiotica* demonstrate greater heat tolerance (Chen *et al*. 2000). *S*. *symbiotica* can rescue up to 70% of bacteriocytes and improve aphid fecundity by up to 50% following exposure to varying degrees of heat stress (Montllor *et al*. 2002). Infection with *Regiella* and *F*. *symbiotica* was associated with increased *Buchnera* recovery and offspring production after heat shock (Heyworth *et al*. 2020). *Hamiltonella* can also provide heat protection; however, some studies have shown inconsistent results, indicating that the protective effects may depend on the specific symbiont, host genotype, or their interactions (Heyworth *et al*. 2020). Interestingly, co-infection with *F*. *symbiotica* and *H*. *defensa* can confer high resistance to heat stress, while *H*. *defensa* alone may not provide such protection (Guay *et al*. 2009). It would be intriguing to complement these findings with investigations into the effects of prolonged exposure to elevated temperatures.

3.5 Impacts aphid immunity and protection against natural enemies

Firstly, symbionts may affect aphid immunity and ecological interactions between insects and their natural enemies. Firstly, *Regiella insecticola* functions as a protective symbiont for *M. persicae* against parasitoids (Vorburger *et al*. 2010). Additionally, by carrying a toxin-encoding bacteriophage (APSE) responsible for protection, *H. defensa* and *S. symbiotica* enhance the possibility of pea aphid survival against the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) (Oliver *et al*. 2003, 2005, 2009; Russell and Moran 2006; Doremus and Oliver 2017). Furthermore, *H. defensa* inhibits volatile emissions in aphid-infested plants, thereby reducing the recruitment of parasitic wasps (Frago *et al*. 2017). *H. defensa* can also inhibit salicylic acid (SA) and jasmonic acid (JA) defense pathways and their accumulation in plants, hence influencing the anti-plant defensive responses of aphids (Li *et al*. 2019). Moreover, it has been demonstrated that resistance can be enhanced through the co-infection of different endosymbionts. For example, co-infection with *F. symbiotica* and *H. defensa* provides better resistance to *A. ervi* than *H. defensa* alone (Guay *et al*. 2009). Further investigation is needed to fully understand the precise functions of these combinations and their optimal utilization for aphid control.

Secondly, aphid symbionts have the potential to indirectly protect their aphid hosts against predators. Studies have shown that the survival rate of lady beetles, a predator of aphids, is reduced when they feed on aphids harboring *S. symbiotica*, *H. defensa*, and *Regiella* (Costopoulos *et al*. 2014; Kovacs *et al*. 2017). However, Ramírez-Cáceres *et al*. (2019) reported that *S. avenae* infected with *Regiella* were more susceptible to predation by *Hippodamia variegata* (Coleoptera: Coccinellidae). These discrepancies could be related to predator species specificity or differences in aphid host physiology. The composition of aphid cuticular hydrocarbons and defensive metabolites may differ among symbiont strains, altering predator preference rather than directly changing aphid resistance. Further research is needed to clarify these interactions.

Thirdly, several studies have highlighted the advantageous role of certain symbionts in protecting against pathogens (Scott *et al*. 2008; Teixeira *et al*. 2008; Jaenike *et al*. 2010). For example, it has been reported that infection with *Regiella*, *Rickettsia*, *Rickettsiella* and *Spiroplasma* in pea aphids could reduce mortality and decrease fungal sporulation of *Pandora neoaphidis* on dead aphids. This may indirectly protect nearby aphids, enhancing their resistance to entomopathogenic invasion (Łukasik *et al*. 2013). Parker *et al*. (2013) have demonstrated that the *Regiella* symbiont confers protection against several specialist fungi like *P. neoaphidis* and *Zoophthora occidentalis* but does not exhibit the same protective effect against the generalist fungus *Beauveria bassiana*.

3.6 Conferring resistance to pesticides

Aphid resistance to insecticides has become a global concern, as the strong selective pressure exerted by insecticides has led to a progressive increase in pest tolerance across generations, posing serious challenges to crop protection and food security (Bass *et al*. 2014). Accumulating evidence indicates that aphid-associated endosymbionts play a key role in mediating pesticide resistance. For example, higher densities of the primary endosymbiont *B. aphidicola* have been linked to enhanced resistance to imidacloprid and sulfoxaflor in the yellow morph of *A. gossypii* (Guo *et al*. 2020). Similarly, infection with *H. defensa* can increase the resistance of *S. miscanthi* to low concentrations of imidacloprid, acetamiprid, and cyantraniliprole by enhancing detoxification enzyme activities such as *AChE*, *GST*, and *CarE* (Li *et al*. 2021). In contrast, *S. symbiotica* infection appears to increase the susceptibility of *A. pisum* to insecticides, likely due to fitness costs such as reduced fecundity and body weight (Skaljac *et al*. 2018). These contrasting effects highlight that the influence of endosymbionts on insecticide resistance depends on symbiont species or strain, host genotype, and co-infection with other symbionts. Moreover, symbiont infection patterns and densities can be shaped by host plants (Xie *et al*. 2011), potentially leading to changes in resistance or susceptibility among different aphid populations (Leonardo and Muiru 2003; Zhang *et al*. 2016).

Beyond individual endosymbionts, the composition and structural complexity of the entire bacterial community also play an important role in shaping pesticide resistance. Significant regional differences in bacterial community composition have been observed among *S. miscanthi* populations, with large fluctuations in dominant taxa such as *Buchnera*, *Candidatus*, *Regiella*, *Candidatus* and *Hamiltonella*. Higher bacterial diversity was negatively correlated with resistance to avermectin and bifenthrin, whereas increased bacterial network modularity was positively associated with resistance to imidacloprid, avermectin and bifenthrin (Wang *et al*. 2022). These findings indicate that both the diversity and interaction network of the bacterial community contribute to aphid detoxification capacity and adaptability to insecticides.

4. Effect of microbiota from aphid secretions on plant defense response

Aphids can be detected by plants not only through mechanical damage to their tissues, but also through elicitors such as herbivore-associated molecular patterns (HAMPs). HAMPs are commonly discovered in the secretions, saliva, oviposition fluids, and excrement of insect herbivores (Rayapuram and Baldwin 2007).

Aphid saliva contains putative effector proteins that facilitate feeding, as well as some that trigger plant defenses (Mutti *et al*. 2008; Carolan *et al*. 2011). Studies have shown that aphid saliva contains a high proportion of proteins derived from endosymbionts *B. aphidicola* and *S. symbiotica* (Vandermoten *et al*. 2014; Li *et al*. 2022). Saliva proteins GroEL and GroES, derived from *B. aphidicola*, have been identified as effectors that deceive the aphid by triggering the plant's defense response (Chaudhary *et al*. 2014; Li *et al*. 2022). *S. symbiotica* suppresses plant defense systems *via* manipulating the expression of a histidine-rich Ca^{2+}-binding protein-like gene (*ApHRC*) in aphid salivary glands (Pieterse and Dicke 2007; Fürstenberg-Hägg *et al*. 2013; Chaudhary *et al*. 2014; Wang *et al*. 2020).

Honeydew contains compounds that originate from the saliva and guts of aphids and could serve as another elicitor for plant defenses (Schwartzberg *et al*. 2014). Additionally, honeydew serves as an excellent growth medium for microorganisms, and honeydew-associated bacteria may function as microbe-associated molecular patterns (MAMPs), triggering an innate immune response in the plant. It has been reported, brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae) honeydew-associated microbes could elicit rice plant defense responses (Wari *et al*. 2019).

The release of herbivore-induced plant volatiles (HIPVs) to attract natural enemies is recognized as an alternative strategy for indirect plant defense. *S. avenae* pectinase was found to induce volatile emissions in wheat and attracted parasitoids (Liu *et al*. 2009). However, there is limited knowledge regarding the impacts of honeydew and its associated microorganisms on the emission of HIPVs involved in plant defense mechanisms. Further investigation is needed to understand the microbiota of aphids in relation to plant defense mechanisms and determine their potential for effectively suppressing aphid populations.

5. Effect of honeydew-associated microbiota on natural enemies' behavior

Honeydew serves as a source of carbohydrates and amino acids for beneficial insects such as pollinators, predators, and parasitoids (Wäckers *et al*. 2008; Tena *et al*. 2016). Natural enemies that feed on honeydew can increase their effectiveness as biological control agents (Watanabe *et al*. 2014). Moreover, honeydew serves as the host-location cue and oviposition stimulus for parasitoids and predators (Wickremasinghe and Emden 1992; Watanabe *et al*. 2016; Ayelo *et al*. 2022).

Honeydew-associated microbial volatiles can then manipulate the behaviors of their natural enemies. For example, *Staphylococcus sciuri* isolated from pea aphid honeydew attracted and strongly stimulated oviposition of *Episyrphus balteatus* (Diptera: Syrphidae) through microbial volatile organic compounds (mVOCs) (Leroy *et al*. 2011). Cues associated with mealybug honeydew-associated bacterial volatiles allowed *Anagyrus dactylopii* (Hymenoptera: Encyrtidae) to locate their hosts (Fand *et al*. 2020). Bacteria-containing honeydew from *S. miscanthi* and its mVOCs significantly attracted the aphid parasitoid *A. gifuensis* and *Harmonia axyridis* (Liu *et al*. 2024, 2025). mVOCs can activate olfactory receptor neurons expressing odorant-binding proteins (OBPs) in *H. axyridis*, influencing host-searching behavior (Liu *et al*. 2025). This huge diversity of mVOCs originating from honeydew microbiota will open up new avenues for non-chemical pest management strategies.

Interestingly, the function of honeydew-associated microbial volatiles is also observed in the ant-aphid mutualism (Xu and Chen 2021). The volatile compounds released by *S. xylosus* and *S. sciuri*, which were obtained from the honeydew excreted by mutualistic *A. fabae* and non-mutualistic *A. pisum*, respectively, were found to attract ants. In exchange for honeydew, some of these ants protect aphids from natural enemies (Fischer *et al*. 2015, 2017). Ants exhibited a stronger preference for the volatiles emitted by *S. xylosus* compared to those emitted by *S. sciuri*. This observation suggests that these bacteria may play a role in guiding ants in distinguishing between mutualistic and non-mutualistic aphids (Fischer *et al*. 2015, 2017). However, it is worth noting that under specific conditions, ants have been known to prey on aphids (Oliver *et al*. 2012). The development of honeydew-associated microbial volatiles for aphid control may thus be influenced by the complex relationship among natural enemies, ants, and aphids. Consequently, it is essential to conduct a comprehensive evaluation encompassing both positive and negative aspects.

6. Conclusions and Perspectives

Due to the widespread dissemination of microorganisms, aphids are often exposed to them and are in constant co-evolution with them. The relative ubiquity of aphid-microbial associations likely indicates that the effects of microbes on aphid traits, but also in plant-aphid-natural enemy interactions are more prevalent than currently recognized, and often overlooked role in aphid biological control. Therefore, we have provided a synthesis of actual literature about the effects of endosymbiont on aphid trait, and aphid secretion bacteria on plant response and the ecological roles of honeydew mVOCs towards natural enemies.

We hope that this review could increase awareness of the available strategies for microbial biological control of aphids and inspire research for their further development and widespread use.

Currently, the intentional use of aphid endosymbionts into biological control remains largely limited to laboratory studies. A major limitation is that most endosymbionts cannot be cultured outside their hosts, making large-scale production and field application unfeasible. Experimental manipulation in the laboratory has provided valuable insights into the roles of endosymbionts. For example, antibiotics can be used to selectively eliminate endosymbionts in aphids, allowing assessment of their effects on host physiology, behavior, resistance to natural enemies and pesticides. Another promising laboratory strategy involves transferring endosymbionts from one insect species to another that does not naturally host these microbes, which can induce significant physiological and behavioral changes in the recipient insects. For instance, when *Wolbachia*, an organism isolated from *Drosophila*, was injected into *Aedes aegypti* Linnaeus (Diptera: Culicidae) embryos, it dramatically reduced virus load and mosquito transmission (Fraser *et al*. 2017). In addition to inhibiting viral transmission, *Wolbachia* infection causes cytoplasmic incompatibility, resulting in reproductive disruption and population reduction (Ferguson *et al*. 2015; Joshi *et al*. 2017). Another strategy involves disabling reproduction and controlling pest populations by dispersing sterile male insects or *Wolbachia*-infected incompatible females into the environment. These tactics have limitations, though. The mass production of sterile insects is often challenging and expensive (Martinez *et al*. 2015). Some defensive symbionts could also pose problems for biological control due to their role in promoting high resistance in aphids (Vorburger 2014). Future research should focus on experimentally verifying the functions of obligate endosymbionts using approaches such as selective elimination *via* antibiotics, RNAi-mediated gene silencing, or cross-host transplantation.

Research on a few herbivore species has demonstrated that the perception of herbivory involves a multifaceted process that incorporates several cues arising from secretions and their associated microbial communities. However, limited research has addressed how these MAMPs activate plant defense responses at the molecular level. Future studies should utilize genomic, proteomic, and metabolomic approaches in aphid models to identify additional microorganisms or microbial effectors capable of eliciting plant resistance. Laboratory-based functional assays, combined with validation under greenhouse and field conditions, will be essential to translate these findings into practical crop protection strategies.

In the expanding field of chemical ecology, systematic investigation of mVOCs on the behavior of natural enemies is required. Rigorous laboratory and field experiments should aim to identify mVOCs with robust ecological functions and develop formulations that ensure stability and prolonged release. To facilitate field application, researchers should design slow-release devices that are practical, cost-effective, and capable of efficient volatile dispersal. Ultimately, integrating mVOC-based approaches with traditional aphid control methods, including biological control and IPM, will maximize their effectiveness and feasibility in agricultural systems.

Before integrating and deploying microorganisms for aphid biocontrol on a large scale, careful consideration of formulation stability, delivery methods, and non-target impacts is required. Techniques such as seed or growth medium inoculation may reduce application frequency while maintaining efficacy

(Ansari *et al*. 2008; Keyser *et al*. 2014). Development of microbial insecticides should consider whether the active component is the microbe itself, its spores or bioactive metabolites and tailor formulations accordingly to ensure adaptability in diverse agricultural contexts. A phased implementation pathway, comprising laboratory screening, greenhouse verification, small-scale field trials, and integration into IPM will be critical to align microbial biocontrol strategies with practical crop management needs.

As high throughput sequencing and omics technologies continue to advance and become more widely available, researchers are now able to perform thorough investigations of the microbiomes in a variety of agroecosystems, including those that contain insects, plants, and other natural resources. This marks the dawn of an exciting era, characterized by the discovery of novel microbes and microbiota activities that can be harnessed for the control of insect pests such as aphids. In addition, new developments in nanotechnology, gene editing, and microbial engineering have enabled scientists to improve methods for obtaining bioactive substances from uncultured bacteria and applying them to pest management. These innovative techniques are anticipated to provide significant advantages for managing pests, particularly aphids on a broad range of crops.

In summary, aphid endosymbionts and secretion-associated microbes play crucial roles in shaping aphid physiology, behavior, and interactions with plants and natural enemies. Understanding these microbial effects provides promising avenues for biocontrol, including the potential modulation of aphidicide resistance and adaptation to extreme conditions such as heat stress, natural enemy and pesticide pressure. Future research should integrate molecular, metabolomic, and behavioral approaches to uncover functional mechanisms, identify microbial effectors, and assess their ecological impacts. Moreover, translating laboratory discoveries to practical applications, through targeted microbial inoculations, mVOC-mediated attraction of natural enemies, and integration into IPM strategies can facilitate sustainable and environmentally friendly aphid management. By combining mechanistic insights with phased greenhouse and field validation, these strategies offer a forward-looking roadmap for effective microbial-based pest control.

Acknowledgements

This work was supported by the China Postdoctoral Science Foundation (2025M783836), the Qingdao Postdoctoral Research Project, China (QDBSH20250102110), the Major Special Projects for Green Pest Control, China (110202201017(LS-01)) and the Agricultural Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences (ASTIP-TRIC04). We would like to thank all individuals who contributed to this work, whether through their valuable insights or support throughout the research process.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Ethical statements

All applicable international, national and institutional guidelines for the care and use of animals were followed.

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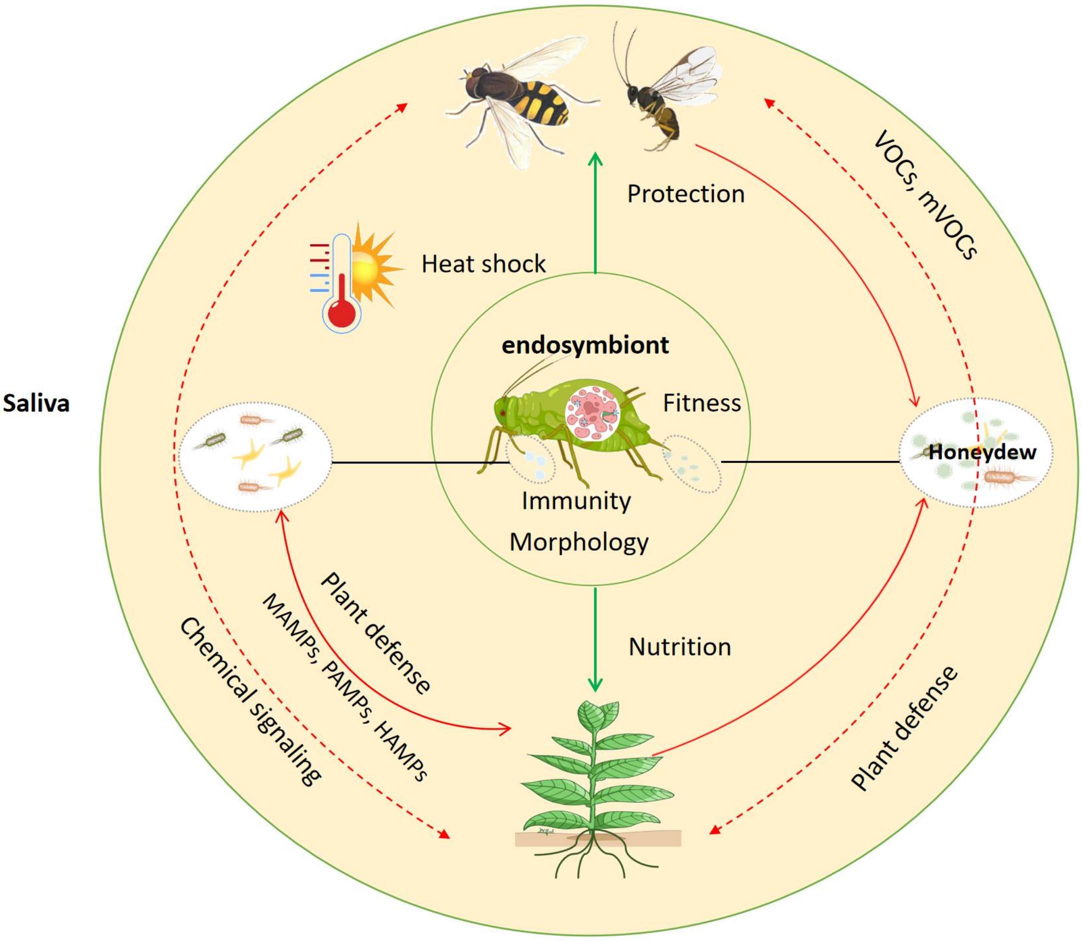

Fig. 1 Multitrophic interactions mediated by aphid-associated microbiota between plants, aphids and natural enemies. Solid and dashed arrows represent direct interactions and indirect interactions, respectively. The green and red arrows correspond to positive effects and negative effects on aphids, respectively.VOCs, volatile organic compounds; mVOCs, microbial volatile organic compounds.

Table 1 Diversity and ecological functions of endosymbionts in aphids

Symbionts	Other names	Classification	Functions	Reference
Buchnera aphidicola		Gammaproteobacteria	Nutrition supply	Douglas (1998)
Hamiltonella defensa	PABS, T-type	Gammaproteobacteria	Nutrition supply Parasitoid resistance Heat shock resistance Increase the body mass and fecundity Longevity Defensive behavior	Oliver *et al.* (2003); Oliver *et al.* (2005); Russell and Moran (2006); Doremus and Oliver (2017)
Rickettsiella		Gammaproteobacteria	Change body color Fungi resistance Reduced longevity Reduced fecundity	Tsuchida *et al.* (2010); Łukasik *et al.* (2013); Gu *et al.* (2023)
Regiella insecticola	PAUS, U-type	ammaproteobacteria	Fungi resistance Parasitoids resistance Enhance host plant fitness Change body color	Scarborough *et al.* (2005); Leonardo and Mondor (2006); Vorburger *et al.* (2010); Tsuchida *et al.* (2011)
Serratia symbiotica	PASS, R-type	Gammaproteobacteria	Heat shock resistance Parasitoid resistance Nutrition supply Slight pathogenic on aphids	Chen *et al.* (2000); Koga *et al.* (2003, 2007); Russell and Moran (2006); Doremus and Oliver (2017); Pons *et al.* (2019b)
Fukatsuia symbiotica	PAXS, X-type	Gammaproteobacteria	Parasitoids resistance Heat shock resistance Fungi resistance	Guay *et al.* (2009); Heyworth and Ferrari (2015); Donald *et al.* (2016)
Rickettsia	PAR, S-type	Alphaproteobacteria	Heat shock resistance Fungi resistance Host plant fitness	Chen *et al.* (2000); Simon *et al.* (2007); Łukasik *et al.* (2013)
Wolbachia		Alphaproteobacteria	Asexual reproduction Nutrition supply	De Clerck *et al.* (2014, 2015)
Spiroplasma		Mollicutes	Fungi resistance Male-killer Growth, reproduction and longevity	Fukatsu *et al.* (2001); Simon *et al.* (2011); Łukasik *et al.* (2013)
Arsenophonus		Gammaproteobacteria	Host plant specialization	Wagner *et al.* (2015)