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Secondary Bacterial Symbionts in Aphids

Gülay Olcabey Ergin[1]

Niğde Ömer Halisdemir University, Zübeyde Hanım Vocational School of Health Services, Department of Therapy and Rehabilitation, Niğde

Abstract

Aphids are important agricultural pests that feed on plant sap and can reach high population densities in short time, causing plant quality and yield losses. Almost all aphids host symbiont bacteria that allow them to easily adapt to their environment. It is known that the primary symbiont Buchnera aphidicola synthesizes some essential amino acids and vitamins that its host needs, while the secondary symbiont bacteria have a wide range of effects, including protecting the host under stress conditions and resistance against natural enemies. Aphids have become a model system for studying insect-bacteria interactions. To date, secondary bacterial symbionts have been identified in many aphid species, and a significant literature bank has been provided on this interaction in natural populations. Knowing the role and importance of symbiont bacteria in aphid metabolism is very important in the control of these pests. This study reports that secondary symbionts hosted on aphids play important roles in aphid metabolism, therefore bacterial symbionts can be used effectively in controlling the aphids.

Key Words: *Aphid, Biological control, Hamiltonella, Serratia, Symbiont*

Afitlerde İkincil Bakteriyel Simbiyontlar

Özet

Afitler, bitki özsuyu ile beslenen, kısa süre içerisinde yüksek popülasyon yoğunluklarına ulaşarak bitkide kalite ve verim düşüşlerine sebep olan önemli bir tarım zararlısıdır. Hemen hemen bütün afitler bulundukları ortam koşullarına kolayca adapte olmalarını sağlayan simbiyont bakterilere ev sahipliği yapar. Bunlardan birincil simbiyont *Buchnera aphidicola* nın konağının ihtiyacı olan bazı temel amino asitleri ve vitaminleri sentezlediği, ikincil simbiyont bakterilerin ise stres koşulları altında konağını koruma ve doğal düşmanlara karşı dirence kadar çok geniş kapsamlı etkileri olduğu bilinmektedir. Afitler, böcek-bakteri etkileşimlerinin çalışılmasında model bir sistem haline gelmiştir. Bugüne kadar birçok afit türünde sekonder bakteriyel simbiyontlar belirlenmiş ve doğal popülasyonlardaki bu etkileşim hakkında önemli literatür bankası sağlanmıştır. Afit metabolizmasında simbiyont bakterilerin rolü ve öneminin bilinmesi bu zararlılarla mücadelede oldukça önemlidir. Bu çalışmayla afitlerde konaklayan sekonder simbiyontların afit metabolizmasında önemli roller üstlendiği dolayısıyla afitlerle mücadelede bakteriyel simbiyontların etkin olarak kullanılabileceği bildirilmektedir.

Anahtar Kelimeler: *Afit, Biyolojik mücadele, Hamiltonella, Serratia, Simbiyont*

Introduction

It has long been known that the majority of insect species form symbiotic relationships with microorganisms that undertake various functions, including nutrition, protection from pathogens, protection against stress conditions and development of resistance (Buchner, 1965; Gonzalez-Gonzalez et al. 2024). Among insect groups, the most studied group in insect symbiosis are the phloem-feeding aphids (Hemiptera: Aphididae), which have a high ability to adapt to a wide range of environmental conditions, including stress conditions. There is more interest in a few species among aphids, and studies on these species are more common in the literature. Research has mostly focused on *Acyrthosiphon pisum* (Harris), *Sitobion avenae* (Fabricius) and *Aphis fabae* (Scopoli) species from the Aphidinae subfamily, which reveals the need for research on more aphid species (Zytynska et al. 2021; Manzano-Marín et al. 2023; Zepeda-Paulo et al. 2024). Recent studies have revealed the role of symbiotic bacteria in the survival of aphids in harsh conditions (Csorba et al. 2024). Bacterial symbionts may play a role in ecological speciation by directly affecting how aphids use different plant species (Ferrari et al. 2011). All aphids harbour the obligate (primary) symbiont bacterium *Buchnera aphidicola*, which increases its host's chances of survival by synthesizing certain essential amino acids and vitamins that they cannot get in sufficient quantities from the phloem sap. In addition, some aphids also harbor facultative (secondary) symbionts that are not essential for the survival and reproduction of their host but provide various ecological advantages to the host against biotic and abiotic environmental conditions (Heyworth & Ferrari, 2015; Shih et al. 2023).

Secondary symbiotic bacteria of aphids

Insects (Arthropoda: Insecta) owe their being the most diverse and highly successful animals on Earth to their long-term and stable ecological relationships with bacteria. Like other insects, aphids can quickly increase their population density by adapting to almost any environment in a very short time, thanks to the symbionts they contain.There are two types of bacterial endosymbionts that are present in insects: (1) facultative or secondary symbionts and (2) obligate or primary symbionts. *Buchnera aphidicola* is the primary endosymbiont present in almost all aphid species. Facultative bacteria are not essential for aphids, but they perform very important functions. The ecological relationship between these bacteria and aphids is mostly based on defense and immune behaviors. Secondary bacteria identified in aphids; *Serratia symbiotica*, *Hamiltonella defensa*, *Regiella insecticola*, *Erwinia aphidicola*, *Pseudomonas aeruginosa*, *Wolbachia pipientis*, *Rickettsiella* sp., *Rickettsia sp*., *Spiroplasma sp*., *Arsenophonus sp*., *Photorhabdus* sp., *Xenorhabdus* sp., *Candidatus sp.*, *Fukatsuia*, *Sodalis*, *Erwinia haradaeae* and *Bacteroidota* (Oliver et al. 2010; Csorba et al. 2022; Manzano-Marín et al. 2023). In recent years, studies - especially Next Generation Sequencing (NGS) - have shown that the diversity of secondary symbiont bacteria is gradually increasing. For example, a new symbiotic relationship with a bacterium related to Erwinia and Sodalis was reported by Jousselin et al. (2016). Then, the secondary symbiotic bacteria *Pectobacterium* was initially described in aphids by Liu et al. (2023) based on the findings of genetic distance analysis. Zepeda-Paulo et al. (2024) studied the microbiome of the woolly apple aphid *Eriosoma lanigerum* (Hausmann) a serious pest of apple orchards that has not been studied much in aphid-facultative symbiont research. In 16S rRNA gene Illumina sequencing-based taxonomic assignment showed a high representation of reads assigned to a recently recognized bacterial taxon -not previously described in aphids- identified as *Symbiopectobacterium purcellii* (Zepeda-Paulo et al. 2024).
Facultative bacteria are present in different proportions in natural aphid populations, and although they are mostly transmitted vertically from mother to offspring, they can occasionally be transmitted horizontally (Russel et al. 2003; Baumman et al. 2006; Zepeda-Paulo et al. 2024). According to some researchers, temperature can have an impact on symbionts' vertical and horizontal transmission frequencies (Anbutsu et al. 2008; Osaka et al. 2008; Liu et al. 2019).
Bacterial secondary symbionts are known to facultatively colonize their hosts. They do not live there permanently but occur less frequently inside the body of the host (Oliver et al. 2010; Dion et al. 2011). Unlike *B. aphidicola*, which is found in specialized cell groups called bacteriocytes or mycetocytes located adjacent to the ovaries (Braendle et al. 2003), secondary symbionts generally settle freely in secondary bacteriocyte cells, sheath cells around primary bacteriocyte cells (Burke & Moran, 2011) and hemolymph. (Oliver et al. 2010; Dion et al. 2011). For example, *Hamiltonella defensa*, *Serratia symbiotica*, *Regiella insecticola* and *Rickettsiella* are found in the cytoplasm of secondary bacteriocytes and sheath cells and in the hemolymph (in pea aphid) (Fukatsu et al. 2000; Moran et al. 2005). As an another example, earlier research has shown that the endosymbiont *Serratia symbiotica* may colonise the gut (Renoz et al. 2019), bacteriocytes, and hemolymph of *A. pisum* (Harris), among other tissues (Skaljac et al. 2018). According to Skaljac et al. (2019), the movement of *S. symbiotica* into its host plant is facilitated by the colonisation of the aphid's mouthparts, especially the stylets. Sometimes, symbionts can replace each other within the host. For example, after the obligatory symbiont *Buchnera* is eliminated with antibiotics at benign temperatures, *Serratia* in pea aphids moved into the bacteriocytes that *Buchnera* had left behind, allowing the stressed aphid to live and reproduce. However, it has been noted that *Serratia* suppresses *Buchnera* and affects the aphid's performs when these two symbiotic bacteria are present together (Koga et al. 2003). As this example shows, interspecies interactions (competition, mutualism, etc.) between different symbiont bacteria in the same host affect the host quality of life in different ways.

The role of secondary symbionts in aphid metabolism

It is stated that facultative symbionts play important roles such as protecting the host against predators, developing host resistance against biotic and abiotic factors, and varying body color (Koga et al. 2003; Brinza et al. 2009; Zhang et al. 2015). In addition, although it may not be able to synthesize some essential amino acids and vitamins like *Buchnera*, it is reported that it potentially plays a role in aphid nutrition by assisting *Buchnera* (Pérez-Brocal et al. 2006; Burke et al. 2009).For instance, the bacterial community of the Bamboo woolly aphid *Psudoregma bambucicola* (Takahashi) was evaluated in a study by Liu et al. (2023). The three species that dominated the symbiotic community, according to these investigators, were *Wolbachia*, *Pectobacterium*, and *Buchnera*. The capacity of *Pectobacterium* to facilitate *P. bambucicola*'s feeding on hard bamboo stems implies that the two organisms have a symbiotic connection.

Aphids are among the poikilothermic insects that are most susceptible to the negative effects of high temperatures. Genetic diversity among aphids and the specificity of the symbiotic bacterial species they host may contribute to differences in adaptive capacity and tolerance to heat stress (Burke et al. 2010; Brisson et al. 2010; Montllor et al. 2002).As temperatures rise, aphids face numerous challenges that can disrupt their normal functioning and threaten their survival. For example; disruption of aphid metabolism, cellular damage in aphids, protein denaturation, oxidative stress, and disruption of cellular membranes and indirectly negatively impacts upon aphid physiology, compromising key functions such as feeding, reproduction, and immune responses (Csorba et al. 2024). Bacterial symbionts play important roles in the ability of aphids to cope with heat stress. Majeed et al. (2022) have reported that *Buchnera aphidicola*, the primary symbiont of aphids, contributes to the synthesis of heat shock proteins (HSPs). Symbionts increase the resilience of insects to heat stress by promoting the synthesis of HSPs.

Many studies have emphasized that the most common endosymbiont bacterium, *Serratia symbiotica* (Jousselin et al. 2016), after the obligate symbiont *B. aphidicola*, increases the success of its host under heat stress and contributes to aphid defense (Burke et al. 2009). *Serratia* may also affect other trophic relationships, as demonstrated by Wang et al. 2024, by influencing the physiology and behaviour of major predators in order to promote host aphid defence. The results of their study demonstrate that the facultative symbiont *Serratia* enhances aphid fitness by disrupting with ladybeetle larvae's predation approach.

Studies have emphasized that symbiont bacteria are directly involved in the symbiont-aphid-parasitoid/predator interaction. For example, in the presence of facultative symbionts, the primary wasp parasitoid *Aphidius ervi* Haliday is less effective on the pea aphid *A. pisum* (Harris) (in the presence of predators) (Purkiss et al. 2022). On the other hand, according to Inchauregui et al. (2023), the facultative symbiont *R. insecticola* in the body of *A. pisum* (Harris) does not provide defense against the pathogenic fungus *Batkoa apiculate*.

Facultative symbionts protect aphids against pathogen attacks. It has been reported that an X-type facultative bacterium, a member of Enterobacteriaceae, protects aphids against the fungal pathogen *Pandora neoaphidis* and increases their resistance to the parasitoid *Aphidius ervi* Haliday (Heyworth and Ferrari, 2015). It is reported in the literature that *Hamiltonella defensa*, one of the secondary symbionts, contains a lysogenic bacteriophage that protects its host against the parasitic wasp *Aphidius ervi* Haliday, and *Regiella insecticola* provides resistance to some fungal pathogens (Jousselin et al. 2016). Additionally, it has been demonstrated, also in a lab setting, that when *R.insecticola* is present in the body of *Aphis fabae* Scopoli, the percentage of nymphs parasitized by the main wasp parasitoid *Aphidius colemani* (Dalman) is significantly lower than when the bacterium is absent from the aphid control group (Vorburger et al. 2010). Various abiotic factors such as temperature and drought can alter the course of the symbiont-aphid-parasitoid/predator interaction. Studies conducted under field conditions indicate that the resistance of *H. defensa* to primary wasp parasitoids in the Black Bean aphid, *Aphis fabae* Scopoli, may vary depending on the temperature regime to which the previous aphid generation was exposed. It has been reported by Gimmi et al. (2023) that the symbiont *H. defensa* increases the resistance of aphids to heat; furthermore, under such stress conditions, the defense capacity of the aphid against parasitoids may decrease. The English grain aphid *Sitobion avenae* (Fabricius) possesses the facultative symbiotic bacteria *H. defensa* and *R. insecticola*, which have been shown to delay the infection process with pathogenic fungal species *Beauveria bassiana* and *Metarhizium bruneum*. However, it has been reported that the absence of these symbionts causes a decrease in the fecundity of aphids infected with pathogenic fungi (Ali et al. 2022).

Result

The facultative symbionts play a major role in increasing the fitness of the host aphid by controlling physiological responses in the host as well as by disturbing the predators' strategies of predation. In order to get a deeper understanding of the diversity and function of endosymbionts across a variety of host plant species, this work highlights the need of investigating symbionts using the entire microbiome of insect hosts as well as numerous aphid samples. Also, there may be opportunities to develop strategies for integrated pest management based on the biological control of aphids by focusing on their symbionts.

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