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Symbiosis: It's important nutritional and non-nutritional roles in insects

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Abstract

The nutrition and survival of insects are regulated by tiny microbes inhabiting in close association with them, known as symbionts, either ectosymbionts or endosymbionts. Endosymbionts may be again categorised into 2 types such as extra-cellular and intracellular symbionts. These may be described as primary/ P-type/obligatory and secondary/S- type/facultative symbionts. Depending on their role in survival, reproduction/ defence, they are useful in nutritional and non-nutritional roles in insects. This behaviour of endosymbionts can be further manipulated to develop para-transgenic insects, which are modified genetically. Due to developed biotechnological and biochemical studies, the detailed study on endosymbionts has been carried out and this knowledge can be further helpful in pest management approaches.

Keywords: Endosymbionts, nutritional management, reproduction, survival, symbionts

Introduction

The abundance and wider adaptability of insects has made the survival of this tiny creatures over range of habitats possible. One of the reasons, attributing to its success is to efficiently utilise the nutrient deficient food. In this context, some organisms inhabiting inside the insect/its nesting structures are sharing mutualistic association, that lead to enormous success of the insects [1]. Many of the insects have been reported to feed on nutritionally deficient diets, that are being supplied by the inhabitant microorganisms. Therefore, the sympatric evolution of the microorganisms along with its host insects had always been an interesting topic to carry out research [2]. Either these symbionts are directly helpful in supplementing the metabolic needs or indirectly helpful through providing defence to the host insects [3-5]. Symbiosis, the beneficial association between insects and different micro-organisms has been coined first time by Anton de Bary in 1879 [6]. Thus symbiosis, a potential interaction lies somewhere in between parasitism and mutualism [7, 8]. Many micro flora are directly beneficial to insects by helping them to digest low nutrient diets [9-11], such as phloem sap, blood of animals etc [12-15]. Symbiosis may be of 2 types, i.e. ectosymbiosis (if the symbiont lives inside the nest of host insect but outside its body) and endosymbiosis (inside the host insect body). Again, the endosymbionts can be differentiated into 2 types, such as extracellular symbionts i.e. symbionts inhabiting inside the gut or any other organ without any specialized cells and intracellular symbionts, where the symbionts live in close association with the host in specialized cells [16, 17]. After, the separation of symbionts from the insects, it becomes difficult to survive, so their identification and further research on their development becomes a daunting task, but the study of symbionts had gained a great momentum in recent years due to development of different molecular techniques.

Classification of symbionts

Table 1: Classification of symbionts depending on the location of endosymbionts

Ectosymbiosis	Endosymbiosis
i. Ectosymbiosis (if the symbiont lives inside the nest of host insect but outside its body) ii. e.g. Ambrosia Beetles-Subfamilies Scolytinae and Platypodinae; Ant Subfamily Myrmicinae-Attine; Termites Subfamily Macrotermitinae.	i. Endosymbiosis (if the symbiont lives inside the host insect body) ii. Endosymbionts can be differentiated into 2 types a. Extracellular symbionts i.e. symbionts inhabiting inside the gut or any other organ without any specialized cells. e.g. <i>Sodalis</i> , <i>Nocardia</i> b. Intracellular symbionts, where the symbionts live in close association with the host in specialized cells. e.g. <i>Buchnera</i> , <i>Wigglesworthia</i>

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Table 2: Classification of symbionts depending on the association with the host

Primary endosymbiont and	Secondary endosymbiont
i. Primary endosymbionts are associated with the host from many million years ago	i. Secondary endosymbionts exhibit a more recently developed association
ii. e.g. Pea aphid, <i>Acyrtosiphon pisum</i> and <i>Buchnera</i> , tsetse fly, <i>Glossina morsitans</i> and <i>Wigglesworthia glossinidia brevipalpis</i> and endosymbiotic protists in lower termites	ii. e.g. Pea aphid <i>Acyrtosiphon pisum</i> and <i>Ragiella insecticola</i> , <i>Hamiltonella defensa</i> and <i>Serratia symbiotica</i>

External Symbionts

1. Fungus-Growing Insects

Insects from orders Isoptera, Hymenoptera and Coleoptera had developed an ability to utilise abundant resources that were previously inaccessible by cultivating special species of certain fungi, further some of which has evolved as important pests in different forest ecosystems [18]. A group of beetles belong to Scolytinae and Platypodinae known as, Ambrosia beetles [19] which bore long galleries in woods had been reported to have body compartments that are shallow and simple/complex invaginations associated with specialized glandular cells to carry the beneficial fungi [20]. Thus, the term *mycangia*, for example, has been applied to structures such as the double slots in thorax of *Dentroctonus frontalis* Zimmermann [21], scores on the head of *Scolytus ventralis* LeConte [22], and paths in feathery arrows of *Pityoborus* spp. [23]. Furthermore, some authors also have termed it as pseudo-mycangia which have no link with the glandular structures. The inter-relationship between the fungus and beetles have been observed to be mutualistic as the fungus weakens the plant facilitating the beetle feeding process and the insect is helpful in transmission and spread of the fungus [24-26]. As the beetles are dependent on wood for their food and survival, but it is a poor source of vitamins, sterols and other nutrients, and fungi convert this nutritionally deficient food into more digestible forms [20].

Ant Subfamily Myrmicinae -Attine

These ants construct chambers using leaves and flower fragments and cultivate the fungi. Similar to beetles, they also utilise the fungi to access the nutrient deficient diet and similarly the fungi also gain transport and spread by the ants.

Termites Subfamily Macrotermitinae

Among over 2,600 species of termites, only the subfamily Macrotermitinae family Termitidae, with approximately 330 known species, developed a symbiotic relationship with fungi of the genus *Termitomyces*, and became most important decomposer of the world [27, 28]. The woods are rich source of lignin, which is difficult to be digested by the termites, thus the fungi enable the termites to digest the lignin present in plants.

2. Internal Symbionts

Different insects have reported to modify their organ structures to harbour the internal symbionts [10, 29-31]. The bacterial flora inhabiting inside the gut are mostly gram-negative and coliform [17, 32].

Protozoa

In 1923, the cellulose-based food of termites was related to a mutualism association with intestinal protozoa have been identified by American zoologist L. R. Cleveland [33, 34]. These protozoans are helpful in digestion of cellulose in termites which otherwise, is undigestible by the termites itself [33-36]. Although basal termites present endogenous cellulases,

enzymes from the symbiont are needed to support the metabolism of the host [37]. This explains why basal termites, although producing endogenous enzymes, are dependent on protozoan for survival on a diet of cellulose.

Primary or Essential Symbionts

There are 10% of the insects which harbour intracellular symbionts for their survival and development. Primary symbionts may be defined as, symbionts that are essential for the reproduction and survival of the host and they reside in specialised cells inside the insects [38]. These cells are known as mycetocytes/ bacteriocytes. As the earliest discovery of these symbionts dates back was a fungus so the terms mycetocytes develop [38, 39]. Thus, the cells harbouring bacteria as primary or P- type endosymbionts can also properly be named as bacteriocytes [1]. Examples of obligate intracellular symbionts are *Buchnera* in aphids, *Wigglesworthia* in *Glossina* flies [40], *Blochmannia* in ants [41-43], *Carsonella* in psyllids [44], and *Blattabacterium* in cockroaches [45]. These bacteria live exclusively within host cells and are vertically transmitted to descendants.

Buchnera

The nutritionally deficient phloem sap is the major food source for the Hemiptera, in Sternorrhyncha, and in Auchenorrhyncha [46]. Thus, they need endosymbionts for supplementing their nutrition. *Buchnera aphidicola* is a gram-negative protobacteria that dominates aphid microbiota and represents over 90% of all microbial cells in the insect tissues. This bacterium lives inside large polyploidy cells called bacteriocytes, which are grouped into structures called bacteriomes, located adjacent to ovarioles. *Buchnera* within each *Buchnera* cell are separated from cytoplasmic contents by a membrane originating from the host cell called symbiosome membrane [47]. These bacteria are transferred, vertically, directly from female to offspring during the blastoderm [48, 49]. The phloem sap although a richer source of sugar, lipids yet deficient in amino acids. The relationship between the essential and nonessential amino acids is around 1:4 to 1:20 in the phloem. This relationship is considered low when compared with the ratio of 1:1 in animal proteins; consequently, the essential amino acid content in phloem sap is insufficient to support the growth and development of aphids [50].

Wigglesworthia

The tsetse fly *Glossina* spp. (Diptera: Glossinidae), an important vector of protozoa that causes sleeping sickness in humans feed exclusively on blood during different developmental stages. Thus, the nutritional deficiencies are need to be supplemented by the symbionts. In this regard, *Wigglesworthia* has the ability to synthesize various vitamins, including biotin, thiazole, lipoic acid, FAD (riboflavin, B2), folate, pantothenate, thiamine (B1), pirodixina (B6), protoheme iron, and nicotianamine [51] has been reported to supplement nutritionally the insect. *Wigglesworthia* lives in

bacteriocytes in the hind gut/proctodeum and are reported to be transmitted to the larva via secretion of milk glands [52].

Blochmannia

This obligatory endosymbiont was reported to be associated with ants of the genera *Polyrhachis*, *Colobopsis*, and *Camponotus* [41, 42, 53-55], but it has been exclusively studied in *Camponotus*, a genus specialized in plant secretions and exudates from aphids [56]. Aphids, that feed on nutritionally deficient diets secrete out sugar rich honey dew, that are eaten by these ants, thus sharing a close relationship with the aphids [56]. Even though, the honey dew is nutritionally rich in amino acids, yet the intracellular symbionts, *Blochmannia* genome retains genes that allow the biosynthesis of all nine essential amino acids and fatty acids, suggesting that the bacterium has a role in ant nutrition [57].

***Sitophilus Oryzae* Primary endosymbiont (SOPE)**

Sitophilus (Coleoptera: Curculionidae), rice weevil larvae feed mainly on albumen cereals, which have nutritional deficiencies such as pantothenic acid, biotin, and riboflavin, aromatic amino acids, phenylalanine, and tyrosine [58]. The primary intracellular symbiont of the genus *Sitophilus* (Coleoptera: Curculionidae) was called SOPE (*Sitophilus oryzae* primary endosymbiont) [59], a gram-negative bacterium found in bacteriomes in larvae and in the ovaries of adults. The symbionts are present in an apical bacteriome in ovaries and thereby transmit the symbiont to progeny.

Secondary Symbionts

Other than, providing insects with sufficient nutrition, these endosymbionts also have some secondary yet important role in insects such as temperature tolerance [60-62], and increased resistance against development of parasitoids in aphids [63]. Furthermore, it can be said that these facultative symbionts represent an intermediate between a free-living style of obligatory symbiosis, in which vertical transmission of microorganisms occur from parents to offspring and the parasite, in which optional mode transmission has typically been associated with virulence [64]. Many Heteropteran families such as Alydidae, Phyllorhoridae, Acanthosomatidae, Scutelleridae, Plataspidae, Pentatomidae, Coreidae, and Parastrachiidae [32, 65-71] have certain modifications in the digestive tracts called as caeca or bacterial crypts that houses large number of bacterial symbionts. The vertical transmission of the endosymbionts has been proposed and elaborated by Buchner in 1965 [48] that the symbionts present on the egg masses were vertically transmitted from the females and acquired orally by the first instar. After which, they reach the gastric caeca and stay there. The genus *Triatoma* (Hemiptera), because of haematophagy, deficient in nutrients is dependent on symbiotic bacteria that are transmitted within the population via coprophagy. The first microorganism identified was the bacteria symbiont *Rhodococcus rhodnii*, an actinomycete, discovered in *Rhodnius prolixus* Stal [72]. Thus, the endosymbionts supplement their host nutritionally with amino acids and vitamins B complex [48, 73]. Any deprivation of the symbionts will lead to damage to the host, such as sterility, reduced growth, and lower longevity [73].

3. Non-nutritional Symbiotic Interactions

Other than, the aforementioned roles of endosymbionts of the primary and secondary endosymbionts in nutrition,

reproduction and defence some other roles also have been discovered. Out of which, *Wolbachia*, a protobacteria that infects the reproductive organs of many arthropods is important. It may be transmitted horizontally and vertically by maternal transference [74, 75]. Hosts infected with *Wolbachia* may suffer reproductive incompatibility, parthenogenesis, and feminization [1, 76]. *Wolbachia* is generally not necessary for host survival, but in some hosts, it acts as an obligate symbiont [77]. Recent estimates reported that 66% of insect species are infected with *Wolbachia* [78]. Another microorganism called CFB or CLO was discovered and also causes reproductive disturbances to hosts [79]. These microorganisms have essential role in the speciation of many arthropods [80], yet, apparently their presence does not guarantee a better performance of the host. Studies on the mechanisms of *Wolbachia* genome provided new information on how the integration occurs and the host-symbiont consequences [4].

Conclusion

The studies on diverse symbionts including ectosymbionts and endosymbionts are useful in deciphering their critical role in insect life cycle, survival and reproduction. Thus, this information can be useful in genetically modifying the internal inhabitants through para-transgenesis and thus this evolved approach can be mass utilised by scientists to develop an eco-friendly method to control pest population non-chemically.

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