

Functions and Applications of Intestinal Symbiotic Microorganisms in Insects

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Abstract Insects represent a diverse group of organisms on earth that carry a vast array of symbiotic microorganisms in their intestines. These microorganisms have established a complex and intimate symbiotic relationship with their host insects over an extended period of evolutionary adaptation. The population structure of intestinal symbiotic microorganisms in insects exhibits a high degree of diversity, with notable differences among insect species. Conversely, within the same insect species, the population structure of symbiotic microorganisms in specific intestinal sites demonstrates a certain degree of stability and specificity. These symbiotic microorganisms perform various physiological functions in the host insect. Their biological functions encompass nutrient and material metabolism and immune and protective mechanisms, which have significant effects on host insect longevity, development, and reproductive capacity. These microorganisms have diverse applications, including pest control, bioenergy production, the development of insect resources, *etc.* Recent advancements in high-throughput sequencing technology have revealed a growing diversity and biological properties of these microorganisms. The application of these techniques has significantly contributed to the advancement of research in the field of insect intestinal microbial research, providing researchers with powerful tools for a deeper understanding and utilization of these symbiotic microorganisms. This study reviewed the research progress of insect intestinal microorganisms in three aspects: diversity, biological functions, and applications. The objective was to provide useful references and insights for further research and applications of insect intestinal microorganisms.

Key words Symbiotic microorganisms, Intestinal flora, Population structure, Diversity, High-throughput sequencing

1 Introduction

Insects represent one of the most abundant, evolutionarily oldest, and widely distributed animal groups in nature, with more than one million reported species alone, making them the most diverse species^[1]. In response, insects have also evolved a great variety of intestinal structures and physical and chemical characteristics. The intestinal microorganisms have evolved a multitude of population structures and biological functions over the course of their long-term coevolutionary relationship with their insect hosts. These have a significant impact on the development, reproduction, resistance, immunity, behavior and survival of the insect host^[2].

The initial research on insect microorganisms concentrated on entomopathogenic microorganisms and their potential applications. Nevertheless, with the microecological theory of the animal gastrointestinal tract deepened, researchers began to study the symbiotic relationship between insect-intestinal microorganisms from a microecology perspective. A significant body of research has demonstrated the existence of a diverse array of mutually beneficial symbiotic relationships between microorganisms and host insects. In

some cases, bacteria have been identified as essential for the growth, development, and reproduction of host insects^[3]. The microenvironment in the intestines of insects is complex and variable, involving a range of activities including feeding, digestion, and excretion. These activities affect several life processes, including nutrient metabolism and the structure of the microbial community within insects.

Given the crucial roles and practical applications of insect intestinal microorganisms, the study of these microorganisms has gradually garnered the attention of scholars worldwide. This paper outlines the biological functions and applications of insect intestinal symbiotic flora.

2 Overview of insect intestinal microorganisms

The insect intestine is mainly composed of three parts: the anterior intestine, midintestine, and posterior intestine. The anterior intestine temporarily stores food. The midintestine is the primary site of food digestion and nutrient absorption. The posterior intestine is responsible for eliminating food residues and metabolic wastes and absorbing water and inorganic salts from food residues. The intestine provides a conducive environment for the growth and colonization of microbial flora^[4].

The term "intestinal flora" collectively describes all microorganisms inhabiting the digestive tract of animals^[5]. Insects typically obtain various microorganisms from their surrounding environment and food sources. Following the selection of an intestinal environment, symbiotic bacteria are then obtained. Consequently, the structure of the microbial communities within the intestines of

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insects is significantly influenced by the types of food they consume and the physical and chemical characteristics of their intestinal environment. This co-evolutionary process has led to the colonization of specific intestinal microorganisms in specific intestinal sites of insects^[4].

2.1 Diversity of insect intestinal microorganisms The intestinal microorganisms of insects are typically sourced from external environments, resulting in notable variations in their composition and proportions across different insect species and geographic populations. These variations are subject to ongoing changes. The intestinal microbial population structure exhibits considerable diversity among insect species, influenced by a range of factors such as feeding habits, intestinal composition, physiological structure, pH, oxygen partial pressure, intestinal immune system, developmental age, and environmental conditions. However, within the same species, this diversity tends to be more stable^[6]. In specific regions of the insect intestine, the population structure of the intestinal microorganisms is relatively stable due to the relatively closed and stable internal environment. Furthermore, these species of intestinal microorganisms tend to play important physiological roles for the host. For instance, numerous microorganisms are digested by enzymes secreted by the midintestine, providing nutrients to insects. In wood-feeding termites and cockroaches, the symbiotic microorganisms in the posterior intestine assist the host in nitrogen fixation and cellulose degradation^[7].

The insect species that have undergone extensive research on their intestinal bacterial diversity are primarily those belonging to the order Isoptera. Some studies have been conducted on Lepidoptera, Homoptera, Orthoptera, Blattaria, Hymenoptera, Hemiptera, and Coleoptera. The intestinal microbiota of insects encompasses a diverse array of microorganisms, including bacteria, archaea, protozoa, and fungi. The most abundant and diverse microorganisms in the insect intestinal flora are bacteria. The most commonly occurring bacterial groups are Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Spirochaetes, Verrucomicrobi, etc.^[8].

2.2 Dominant autochthonous flora in insect intestine The classification of intestinal microorganisms can be divided into two categories: autochthonous and allochthonous communities, based on the duration of their survival and colonization within the intestine. Autochthonous microorganism colonize and multiply in specific areas of the intestine, such as the posterior intestine of termites and the fermentation chamber of the posterior intestine of grubs. Allochthonous microorganisms do not survive for long periods of time in the intestine of healthy animals^[3]. In insects, the ability of microorganisms to colonize the intestine is of critical importance.

Identification of intestinal bacteria in difference insects has revealed the presence of dominant autochthonous floras. A study of the intestinal bacteria of the termite (Isoptera; Rhinotermitidae) revealed that the dominant bacterial groups present in the termite intestine were those of the Bacteroidetes, Firmicutes, Spirochaetes,

and Proteobacteria phyla. Additionally, the dominant bacterial populations vary by termite species. The predominant bacteria in the midintestine of *Lymantria dispar*, *Helicoverpa armigera*, and *Bombyx mori* are primarily those belonging to the phyla Firmicutes and Proteobacteria^[9]. Among Orthoptera, the dominant bacteria in the intestinal tract of adult *Locusta migratoria* include *Serratia*, *Brachy bacterium*, *Yokenella*, *Enterobacter*, *Microbacterium*, *Citrobacter*, etc.^[10]. The dominant bacterium in the intestine of *Serratia marcescens*, which feeds mainly on plant leaves or stalks, is *Enterobacter*^[3]. The diversity of the intestinal flora of different insect species was examined in a study, and the results revealed that the dominant bacteria were primarily Proteobacteria and Firmicutes^[11].

The dominant autochthonous bacteria vary among different species of insects. For example, Proteobacteria are autochthonous flora in the intestine of aphids, while *Burkholderia* spp. are found to be the dominant bacteria in the intestine of *Riptortus clavatus*, with a proportion of up to 95% in their meso-posterior intestinal cavity. In the Coleoptera, the dominant floras in the intestine of *Melolontha melolontha* belong to Clostridiales. The intestinal bacterial communities in the larvae of *Holotrichia parallela* are primarily affiliated with the phyla Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria^[12]. The Firmicutes and Proteobacteria are the dominant taxa of intestinal bacteria. The dominant intestinal bacteria of *Anoplophora glabripennis* and *Saperda vestita* were studied, revealing notable differences. The intestinal tract of *S. vestita* was almost entirely comprised of Proteobacteria, while the dominant bacteria in the intestinal tract of *A. glabripennis* were found to include Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. The intestinal bacteria of adult *Bactrocera dorsalis* belong to seven different orders or phyla, including γ -proteobacteria, Actinobacteria, α -proteobacteria, δ -proteobacteria, Bacteroidia, Flavobacteria, and Firmicutes. Among them, γ -proteobacteria exceeded 70% of the total number of intestinal bacteria and were the absolute dominant bacteria^[13]. The results of the aforementioned studies on the intestinal microorganisms of different insects indicate that the dominant bacteria present in the intestinal tract of insects vary among species. In most cases, the bacteria are of the Proteobacteria group, which are considered autochthonous to the insects in question.

3 Biological functions of insect intestinal microorganisms

3.1 Nutritional and material metabolic functions The long-term synergistic evolution of insects and intestinal microorganisms has resulted in an interdependent symbiotic relationship. The intestinal flora plays a pivotal role in maintaining the normal growth, development, and physiological balance of insects. Research indicates that intestinal symbiotic bacteria can provide nutrients such as amino acids, B vitamins, and sterols to the host, as well as participate in the metabolism and synthesis of substances^[14–15]. For instance, the intestinal symbiotic bacterium *Rhodococcus*

rhodnii provides the host organism, *Rhodnius prolixus*, with vitamin B^[14]. *Homalodisca coagulata* feeds on the sap of plant xylem, which is deficient in amino acids, vitamins and other nutrients. The body contains two specialized symbiotic bacteria, *Baumannia cicadellinica* and *Sulcia muelleri*. *Baumannia cicadellinica* is primarily responsible for the synthesis of vitamins and co-factors, while *Sulcia muelleri* is responsible for the synthesis of amino acids required by the host for the growth and development of *H. coagulata*^[16].

Additionally, insect intestinal microorganisms that produce digestive enzymes assist the host in adapting to specific survival strategies. Bacteria that degrade insoluble macromolecules, such as cellulose and xylan, can be found from the intestines of animals and numerous species of insects. A diverse range of xylanase genes has been identified in the intestinal microbial genomes of termites and Lepidoptera^[17]. The aforementioned evidence indicates that intestinal bacteria are capable of degrading macromolecules. Longicorn intestinal microorganisms can secrete a large amount of highly active cellulases and hemicellulases, which assist the host in the degradation of lignin, cellulose, and hemicellulose in foods^[18]. In addition to the metabolism of nutrients, plants release phytochemicals, which are toxic to insects, to deter insect feeding. Symbiotic microorganisms can also help insects metabolize toxic substances^[19].

3.2 Immune and protective functions Insects are subject to various threats in their natural habitat, including attacks by pathogens, parasites, and predators. It is evident that symbiotic bacteria play a pivotal role in the insect's capacity to combat these threats. A number of studies have indicated that insects treated aseptically are more susceptible to pathogenic bacteria and parasites than controls^[20]. Symbiotic bacterium employs a variety of mechanisms to inhibit the colonization or proliferation of other foreign microorganisms. These include modulation of the activation of the host innate immune system, competitive nutrient depletion, and inhibition of other microorganisms through physical isolation or metabolites^[21]. One of the most extensively researched areas is the impact of symbiotic microorganisms on the immune system. A study comparing the immune gene expression profiles of *Anopheles* mosquitoes with and without symbiotic bacteria found that the presence of symbiotic bacteria activated the immune system of *Anopheles* mosquitoes, resulting in the up-regulation of numerous immune genes, including antimicrobial peptides such as cecropin, defensin, and gambicin^[22]. Consequently, when *Anopheles* mosquitoes are infected with pathogenic microorganisms such as *Plasmodium*, they exhibit heightened immunity, enabling them to suppress the infection^[23]. In the systemic immune response of insects, the Toll and IMD signaling pathways act in concert to regulate the expression of AMPs. In contrast, in insect intestinal immunity, the IMD signaling pathway primarily regulates the expression of AMPs^[24]. The intestinal IMD signaling pathway has been demonstrated to play a pivotal role in intestinal immune defense, as evidenced by the observation that IMD signaling pathway-defi-

cient *Drosophila melanogaster* is more susceptible to pathogenic bacterial infections^[25–26]. Furthermore, research on the intestine of sterile strains of *D. melanogaster* revealed significantly lower expression levels of AMPs than in normal worms. This suggests that host intestinal symbiotic microorganisms may similarly induce the initiation of the IMD signaling pathway^[27].

Symbiotic microorganisms can also protect their host insects from predation by producing toxic metabolites. For example, the pea aphid's natural enemy, *Aphidius ervi*, lays a single egg on the aphid. The hatched larva then feeds on host tissues, ultimately killing the host. It is noteworthy that the susceptibility of aphids to parasitoidism varies considerably between individuals. Subsequent studies have identified that this variation is attributable to the presence or absence of specific symbiotic bacteria in the aphid, including the symbiotic bacterium *Hamiltonella defensa*, which produces a toxin that directly kills *Asaphes vulgaris* larvae^[28]. Furthermore, symbiotic microorganisms, including leaf-cutting ants, longicorns, carpenter bees, and others, can synthesize a diverse array of detoxifying enzymes that facilitate the metabolism of tannins, terpenes, esters, chlorinated hydrocarbons, and other toxins in their host organisms. Certain intestinal microorganisms are also capable of detoxifying and recycling endogenous toxic substances produced by host insects. For instance, research on aphid intestinal bacteria has demonstrated that symbiotic bacteria within the intestinal tract are capable of transforming significant quantities of ammonia, a byproduct of metabolism, into glutamic acid and aspartic acid. This process serves to recycle ammonia and reduce its concentration, thereby facilitating ammonia detoxification^[29].

3.3 Affecting host insect longevity, developmental duration and reproductive capacity It has been demonstrated that the intestinal flora can also affect the longevity of insects. In *D. melanogaster*, modifying the symbiotic relationship between bacteria and intestinal wall-absorbing cells at different stages of adult life can improve health and extend lifespan^[30]. As *D. melanogaster* ages, the quantity of bacteria in the intestine increases significantly, resulting in inflammation caused by the long-term activation of the stress response gene *FOXO*. This inhibits the activity of a class of molecules called PGRP-SCs (peptidoglycan recognition protein SCs), which modulate the body's immune response to bacteria. Inhibition of PGRP-SC allows for the deregulation of Rel/NFκB, a signaling molecule that plays a pivotal role in initiating an efficacious immune response against intestinal bacteria. The consequence of this immune imbalance is the expansion of the bacterial population and the generation of free radicals. When PGRP-SC expression is increased in intestinal epithelial cells, the bacterial homeostasis is repaired, limiting stem cell proliferation. Consequently, the lifespan of *D. melanogaster* may be extended by enhancing the function of PGRP-SC^[30].

A study on the intestinal symbiotic microorganisms of *Acrosternum hilare* revealed that antibiotic treatment resulted in a notable reduction in the species and quantity of intestinal microor-

ganisms. This was accompanied by a significant prolongation of the developmental duration of the host insect, as well as a notable decline in the survival rate and egg production^[31]. Behar *et al.*^[32] demonstrated that Enterobacteriaceae bacteria in the midintestine of *Ceratitis capitata* significantly influenced the lifespan and developmental duration of the host insect. Furthermore, they observed that mortality rates of *C. capitata* were significantly reduced and lifespan was significantly increased when Enterobacteriaceae bacteria were added to the diet. Perotti *et al.*^[33] demonstrated that after sterilizing the intestinal bacteria of *Hematobia irritans* with antibiotic treatments, the larvae exhibited impaired development into adults when fed only sterile diets. However, the addition of bacteria isolated from the intestine of *H. irritans* to sterile diets resulted in a significant increase in the eclosion rate of up to 74% and a notable enhancement in the pupal weight when compared to the treatment group fed sterile diets. Gavriel *et al.*^[34] examined the impact of antibiotic treatment on the mating behavior of *C. capitata*. Their findings indicated that the removal of intestinal bacteria resulted in a reduction in the mating competitiveness of *C. capitata*. Conversely, the addition of acid-producing bacteria, *Klebsiella oxytoca*, isolated from the intestinal tract of *C. capitata*, led to a significant increase in the mating competitiveness of germ-free insects. This suggests that the absence of intestinal bacteria affects the mating ability of *C. capitata*.

4 Application of research on insect intestinal microorganisms

4.1 Pest control The intestinal microorganisms of insects are intimately linked with the growth and development, nutrient metabolism, and immune protection of their hosts. Consequently, the genetic improvement of these symbiotic bacteria offers a promising avenue for the control of insect pests^[3]. To illustrate this, researchers have demonstrated that the introduction of a toxic protein gene into a symbiotic bacterium of a bug vector insect and subsequent colonization of the vector insect by the symbiotic bacterium can successfully inhibit the ability of the insect to transmit the human disease Chagas disease^[35]. The expression of specific double-stranded RNA (dsRNA) in intestinal symbiotic bacteria results in RNA interference (RNAi) of the corresponding host insect genes, which can lead to pest lethality or population control. For instance, the genetic modification of the dominant symbiotic bacterium of the blood-sucking *Rhodnius prolixus*, *Rhodococcus rhodnii*, to express a specific dsRNA for the vitellogenin gene led to the discovery that the modified symbiotic bacterium, when stably colonized and passed on in the intestine of the stink bug, resulted in a reduction in egg hatchability of 61.5% and a 72.3% reduction in the 1st instar larvae.

Intestinal microorganisms have been shown to possess effective attractant properties against host pests. This can be harnessed for the development of novel attractants for pest control. A strain of *Hansenula anomala*, a yeast-like organism, was isolated from the intestinal tract of *Tetradacus citri*, a major citrus pest. Its fer-

mentation product was found to have a strong luring effect on both females and males of *T. citri*. This was developed as a microbial-derived bait, which proved to be 3.52 times more effective than the traditional bait, a sugar and vinegar solution. Furthermore, the interaction between insect intestinal microorganisms and pathogenic microorganisms can be employed as a means of pest control. Broderick *et al.*^[36] demonstrated that intestinal microorganisms influenced the virulence of *Bacillus thuringiensis* against mosquitoes. When *B. thuringiensis* was present alone, it did not cause individual mortality. However, it could cause the death of pests when it was present alongside other intestinal microorganisms, such as *Escherichia coli* or *Enterobacter*. It is postulated that the presence of intestinal microorganisms may facilitate the breakdown of the insect's own immune barrier, making it more susceptible to the action of *B. thuringiensis*.

4.2 Bioenergy production Phytophagous insects, such as termites, grubs, and wood-feeding cockroaches, feed on lignocellulose and convert the lignocellulose in their food into sugars, thus obtaining the energy needed for growth and development^[37]. Insects employ a combination of their own digestive system and intestinal symbiotic microorganisms to facilitate the digestive metabolism of cellulose. For instance, lower termites depend on symbiotic microorganisms in their intestines to facilitate the digestion and degradation of fibers and the conversion of cellulosic substances in their food into glucose^[38]. In contrast, grubs rely on intestinal microorganisms in the posterior intestinal fermentation chamber to assist in the breakdown of lignocellulose^[39]. A considerable number of strains and genetic resources with lignocellulose-degrading activity have been identified in the intestinal microorganisms of these insects. These resources could be utilized for the bioenergy industry, where they could be used to decompose lignocellulose. A total of 93 cellulolytic and 103 xylanolytic strains have been isolated from the posterior intestinal fermentation chamber of *Holotrichia parallela* larvae (grubs). Comparative screening has been conducted to identify the two most active strains, HP207 and HP455, and to complete the development of their fermentation technology^[40]. Additionally, a gene encoding an endo-xylanase, designated xyn455, and a β -xylosidase, designated xyl455, has also been cloned from *H. parallela*. Bacteria and flagellates with cellulose-hydrolyzing activity have been isolated from insects in the families Tipulidae, Cerambycidae, and Curculionidae. Furthermore, genes related to lignocellulose degradation have been cloned^[41]. The related research provides a foundation for the development of efficient cellulose-degrading bacteria, the isolation and modification of cloned cellulase genes, and the efficient production of bioenergy.

4.3 Insect resource development The study of the physiological relationship between insect intestinal microorganisms and insect nutrition can facilitate the development and utilization of economic insect resources, as well as improving the rearing of insects^[42]. Sun Xueqi *et al.*^[43] isolated and identified the intestinal aerobic microorganisms of silkworms. They selected five strains of

intestinal aerobic microorganisms and another strain to make microecological preparations, which were then added to silkworm food. Their findings indicated that the morbidity of silkworms was significantly reduced, and the total cocoon quantity and cocoon layer quantity were also greatly increased. Zhang Li^[44] isolated and identified the main flora of intestinal microorganisms of *Tenebrio molitor*, and created several dominant strains of the isolated strains into microecological preparations, which were then fed to *T. molitor* larvae. It was observed that the weight of *T. molitor* larvae fed *Curtobacterium* sp. exhibited a notable increase, indicating that intestinal microorganisms exerted a positive influence on the growth and development of *T. molitor*. Additionally, it was discovered that the fermentation products of intestinal microorganisms in the food waste biodegradation resource insect, *Hermetia illucens*, exhibited a pronounced trapping effect on its adult oviposition. Furthermore, the key bacterial complex (BSF-4) was identified, which has been utilized as a microecological preparation for the expansion and large-scale production of *H. illucens*^[45].

4.4 Governance of environmental issues A multitude of plastic products have become ubiquitous in all aspects of modern life. However, the disposal of waste plastic in the natural environment is a significant challenge, as it is difficult to degrade. The most common methods of disposal are landfills and incineration, both of which have the potential to cause severe environmental pollution^[46]. Various studies have identified a plethora of insects that depend on the intestinal symbiotic microbial environment to degrade plastics. For instance, *Bacillus* sp., *Bacillus aeruginosa* and *Microbacterium oxydans* in the intestine of *Galleria mellonella* have the capacity to degrade polyethylene. *Bacillus anthracis*, *Bacillus* sp., and *Stenotrophomonas* in the intestine of *T. molitor*, as well as strains belonging to Enterobacteriaceae, Spiroplasma, and Enterococcaceae in the intestinal symbiotic microorganisms of *T. obscurus*, have the capacity to degrade polystyrene^[46]. Heavy metal pollution represents another significant environmental concern. The preliminary findings indicated that *Comamonas* sp., belonging to *Comamonas*, Comamonadaceae, exhibited robust tolerance to heavy metals, including copper and nickel, from the intestinal tract of *Propillocerus akamusi* larvae. The findings indicate that a greater number of potential insect intestinal symbiotic microbial species with the capacity to address environmental pollution issues will likely be identified in the future^[47].

5 Conclusions and prospects

Insects, as the most diverse species, have evolved a wide range of biological functions through long-term synergistic evolution of intestinal symbiotic microorganisms and their host insects. The comprehensive examination of insect intestinal microorganisms has led to the identification of an increasing number of species and their physiological functions within the host. These findings have been applied in a range of significant fields, including agriculture, energy, and environmental protection. The current body of research on insect intestinal symbiotic bacteria has focused on a limited

number of species, including the model insect *D. melanogaster*, a few agricultural pests, human disease vectors, and resource insects. In the future, it would be beneficial to expand this research to include the intestinal symbiotic microorganisms of more agricultural and forestry pests. In intestinal symbiotic microorganisms, a few dominant species often play key roles. moving forward, there is a need to focus on the few dominant autochthonous populations to discover key genes with more diverse biological functions or specific biological functions for the host insects. Insects represent the most diverse group of animals, and their ability to adapt to a wide range of extreme environments is linked to the functional diversity of intestinal symbiotic microorganisms and the rich microbial flora and their functional genetic resources contained in the intestine. These resources are yet to be fully explored, offering significant potential for future applications.

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