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Role of Bacterial Endosymbionts in Biological Control of Insect Pests

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Abstract

Endosymbionts are microbial or macrobial organisms that will exhibit a symbiotic relationship with other organisms. Most of the endosymbionts are in the form of intracellular or extracellular in nature. Endosymbionts provide nutrients to the organisms that are essential for the survival and development. Apart from providing nutrients, some endosymbionts play a major role in the management of pests. Examples are *Wolbachia*, *Spiroplasma*, *Cardinium* and *Rickettsia.* All these endosymbionts are maternally transmitted *via* transovarial transmission. Their main role is to modify the host reproduction, male killing or host defending. *Xenorhabdus* and *Photorhabdus* are the associated endosymbionts of entomopathogenic nematodes are belonging to the genus *Steinernema* and *Heterohabditis*. The technique which is suitable to modify the gut microbes genetically for the expression of desired effects in insect is Paratransgenesis. The main aim of utilizing the endosymbionts is to reduce the usage of chemical pesticides in the pest management aspects.

Keywords: Endosymbionts, Paratransgenesis, Role, Transmission

Introduction

Symbiosis is a Greek word meaning "living together". It is a close or prolonged association between two or more different organisms. The term was coined by Heinrich Anton Debury in 1879. Symbiont is an organism living in symbiosis or showing closed relationship. The interactive relationship may be in Mutualistic (both organisms receive the benefit), Commensalistic (one organism will get the benefit without killing the other organisms) and Parasitic (one organism will get the benefit by killing the other organism) (Ashwini *et al.*, 2022). There are two types of symbionts. They are Ectosymbionts and Endosymbionts.

(i) **Ectosymbionts** are the organism lives on the body surface of another organism including internal surface such as lining of digestive tubes and ducts of glands. *E.g.*: Hairy darkling beetle (*Lagria villosa*) and their associated symbiont *Burkholderia.* The bacteria lives in the dorsal compartments of *lagria* larvae and protect the larvae from fungal infection.

(ii) **Endosymbionts** are the organism lives in the body or body cells of another organism. Endosymbionts provide nutrients which are required for the development of the hosts. *E.g.*: Aphids and their associated symbiont *Buchnera aphidicola*.

Apart from these some endosymbionts play a major role in controlling the insect pest by attacking their host by manipulating the host reproduction, male killing, affecting the intake of nutritious food, *etc*. *E.g.*: *Wolbachia* kills the males of mosquitoes therefore reducing their breeding population. The nematode killing endosymbionts are in nature like the insect killing endosymbionts. *E.g.*: *Xenorhabdus* and *Photorhabdus*. Some of the genetically improved approaches are introduced for the modifying the characters of insects, *i.e.*, paratransgenesis. This article reviews the bacterial endosymbionts in insects, their transmission, the different species of endosymbionts, their role in various insect pest management.

Endosymbionts

Endosymbionts are the organisms which live inside the insect and nematode host species. *Sodalis glossinidius* was the first insect endosymbiont isolated from Tsetse fly in 1999.

Endosymbionts are of two types. They are Primary and Secondary endosymbionts.

Article History

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1. Primary Endosymbionts

Primary endosymbionts are vertically transmitted from mother to offspring. They are found in some specialized cells called bacteriocyte and they provide essential nutrients to the host. Primary endosymbionts are mutualistic in nature (Table 1).

Table 1: Examples of primary endosymbionts

Sl. No.	Host insects	Symbionts
1.	Aphids	*Buchnera aphidicola*
2.	Psyllids	*Carsonella ruddii*
3.	Whiteflies	*Portiera aleyrodidarum*
4.	Weevils	*Nardonella* spp.
5.	Cowpea pod bug	*Burkholderia* spp.

2. Secondary Endosymbionts

Secondary endosymbionts are horizontally transmitted or *via* the environment. They are commonly found in haemocoel, testes, ovaries, gut, fat body and malphigian tubules of the host. They are the commensal bacteria therefore they invade the host body for their survival and development (Table 2).

Table 2: Examples of secondary endosymbionts

Sl. No.	Host insects	Serratia symbiotica
1.	Aphids	*Fritschea* spp.
2.	Whiteflies	*Arsenophonus triatominarum*
3.	Assassin bug	*Portiera aleyrodidarum*
4.	Mosquitoes, Fruit flies, Honey bees, Spiders and mites	*Wolbachia* spp. *Spiroplasma* spp. *Rickettsia* spp. *Cardinium* spp.

Mode of Transmission

1. Vertical Transmission

Gut microbes are vertically transmitted from generation after generation. They are inherited from their mother to their offspring. *E.g.*: *Plautia stali* (Brown-winged green stink bug) and their Gammaproteobacterium symbiont. The bug possesses symbiont in the gastric caecum of posterior midgut surface. During oviposition, the symbionts are transmitted vertically especially by egg smearing on the surface of the eggs and the young ones will feed their symbionts on the surface of the eggs. They supply required vitamins to the host body.

2. Horizontal Transmission

Gut microbes are horizontally transmitted *via* the environment or by means of contact and they don't inherit from their parents.

E.g.: Termites shed their microorganisms during moulting process and they again acquire them by proctodeal trophallaxis (anus - mouth) (Krishnamoorthy *et al.*, 2020).

Endosymbiotic Bacteria in Insects and Their Role in Biocontrol

1. Wolbachia

Wolbachia is one type of endosymbiont (bacteria) which ultimately infecting the species of arthropod. It is one of the major reproductive parasite and parasitic microbes. They are the cytoplasmic endosymbiont of mites, insects, spiders and some of the filarial nematodes. They are maternally transmitted *via* transovarial transmission. They are first identified by S. Burt Wolbach and Marshall Hertig in 1924 in reproductive tissues of *Culex pipens.* The species was later named as *Wolbachia pipientis* by Hertig in the year of 1936. This bacteria can attack many various types of organs of different organisms, but they are mostly infecting the ovaries and testes of their hosts. *Wolbachia* strains wA1bB was *successfully* infect the *Aedes aegypti* (dengue vector) (Sarwar *et al.*, 2022).

Wolbachia species have the capacity to cause 4 various phenotypes:

• *Male Killing*: During larval development, males of hosts are mostly, which ultimately increases the rate of inborn females.

• *Feminization*: Males which are infected will usually develop as infertile pseudo-females or females. The conversion of genetic males into functional females due to the suppression of androgenic glands. They are most commonly seen in isopods.

• *Parthenogenesis*: In the absence of males, the infected females will reproduce. The best example is the *Trichogramma* sp, with the help of *Wolbachia*, the females have the ability to reproduce in the absence of males. Mostly males are very rare in the case of this *Trichogramma* sp. The reason for the reduction of males is that they have been killed by the very similar strain of *Wolbachia*.

• *Cytoplasmic Incompatability (CI)*: Cytoplasmic incompatibility induces the *Wolbachia*-infected males will ultimately receive the inability to reproduce with *Wolbachia* uninfected females or females attacked by other strain of *Wolbachia*. *Wolbachia*-induced CI results in haploid- diploid species reproduction or diploid species zygotic death and it is one of the reproductive incompatibilities between egg and sperm. This *Wolbachia* strain has the ability to transmit through eggs and not transmitted through sperms. Cytoplasmic incompatibility (CI) occurs normally in two types, they are: (i) unidirectional and (ii) bidirectional. The Unidirectional incompatibility occurs when the males (infected) mated with females (uninfected), which results in embryonic mortality. The bidirectional incompatability normally happens with various strains of *Wolbachia strains* were harboured by the male and female species, which are mutually incompatible in nature. Most of the species are depends on the various *Wolbachia* strains for the reproduction, because they are unable to reproduce in the absence of strains in the host bodies. The species of *Wolbachia* are commonly found in eggs and not in sperm. The infected females have the capacity to spread the infection to their own young ones or offspring. Cytoplasm transfer from the cherry fruit fly (donor) to mediterranean fruit fly (recipient) through micro injection. As a result, unidirectional and bidirectional CI happens and embryonic mortality caused by the transfer of *Wolbachia* from one pest to another pest.

2. Spiroplasma

Spiroplasma is a helical and cell wall-less bacteria. They are commonly found in crustaceans, spiders and insects. They are also maternally transmitted *via* transovarial transmission. Recently found the association of *spiroplasma* with marine and deep-sea invertebrates (jellyfish and sea cucumbers). They are mostly present in the haemolymph or the gut of insect species. Their main role is host reproduction manipulation, male killing or host defending for the endosymbionts. Recent studies strongly suggested that during host-larval development, the expression of phenotype which is male killed is also observed. The male-killing phenotype is also expressed during host larval development.

3. Rickettsia

Rickettsia are commonly known as the bacterial pathogens affecting the humans and are widely transferred by blood sucking insects which are ectoparasites such as fleas, lice and ticks. However, most of the *rickettsial* species are safe endosymbionts, *i.e.*, nonpathogenic endosymbionts with different groups of organisms, such as eukaryotes, protists and arthropods. Numerous pathogenic *Rickettsiae* are vertically transmitted and it can manipulate the host reproduction.

Paratransgenesis

Paratransgenesis is a technique to modify the gut microbes genetically for the expression of desired effects in insects. This approach is mostly suitable for the microorganisms which can be transformed, culturable and can be readily introducible into the various hosts of insects. The **s**ymbionts are transformed by maternally or *via* coprophagy across an insect population. Recent research progresses are mainly focusing on some of the pests of crops and diseases of humans (Schmitz *et al.*, 2012).

Example

Rhodococcus rhodnii is the gut symbiont of the *Rhodnius prolixus* (triatomine bug), the effector molecules such as cecropin A and related pore-forming molecules are expressed due to the genetic modification of some of the genes of *Rhodococcus rhodnii*. These effector molecules have the capacity to act against the *Trypanosoma cruzi*, a protozoan, which is the causative agent for the Chagas disease. By the inoculation of egg shells or seeding of engineered symbiont onto the food with faeces, these endosymbiont can be introduced into the progeny of insects.

Endosymbiotic Bacteria in Nematodes

Entomopathogenic nematodes are belonging to the genus *Steinernema* and *Heterohabditis* and their associated endosymbionts are *Xenorhabdus* and *Photorhabdus.* The infective stage of EPN is IJ3 (Infective Juvenile 3) stage (Dauer stage). *Photorhabdus* bacteria is the endosymbiont of *Heterohabditis* makes colony in the anterior portion of intestine behind the basal bulb and they eliminates from the mouth (anterior) region. *Xenorhabdus* bacteria is the *Steinernema* endosymbiont colonize in intestine's bilobed vesicle and they liberates from anus (posterior) region (Yuksel, 2022).

Mode of Action

The juveniles enter into the host body through spiracles or other natural openings. After entering into the host body, the EPN releases the symbionts inside the host haemocoel. The cells of bacteria will duplicate and generate several toxic compounds. Insect host dies (24-48 hours) soon after infection. Nematodes feed on the cadaver and reproduce inside the host. Nutrients of the cadaver get depleted in the host. Then the nematodes will leave the dead insect body and will search for the new insect host species.

Role of EPNs Endosymbionts in Biocontrol

1. Insecticidal Activity

• Bacterial suspension (5×10^4 CFU ml^{-1}) of *Xenorhabdus nematophila* showed 100% larval mortality in 3rd instar larvae of *Pieris brassicae*.

• At 96 h, treatment of *P. luminescens* (10^8 cells ml^{-1}) showed higher adult mortality (60.67%) in spider mite (*Tetranychus urticae*).

2. Nematicidal Activity

• *Photorhabdus* spp. is lethal to the J2 and adults of *M. incognita* and *Bursaphelenchus xylophilus* (J4) (Table 3).

• *X. budapestensis* SN84, metabolite (Rhabdopeptide) exhibited the properties of nematode killing in J2 of *M. incognita* (Table 4).

Table 3: Bioactive complexes produced by *Photorhabdus*

Sl. No.	Bacterial species	Bioactive complexes/ secondary metabolite	Biological asset
1.	*Photorhabdus luminescens*	Anthraquinone	Insecticidal
2.	*P. luminescens*	Rhabduscin	Insecticidal
3.	*P. temperata*	Anthraquinone (1,3-dimethoxy-8-hydroxy-9,10-anthraquinone and 3-methoxychrysazine)	Insecticidal
4.	*P. temperata*	Benzaldehyde	Antimycotic
5.	*P. asymbiotica*	Glidobactin/Cepafungin I	Insecticidal
6.	*Photorhabdus* spp.	Galtox	Insecticidal

Table 4: Bioactive complexes produced by *Xenorhabdus*

Sl. No.	Bacterial species	Bioactive complexes/ secondary metabolite	Biological asset
1.	*X. bovienii*	Xenocyloins	Insecticidal
2.	*X. bovienii*	Amicoumacin	Antibacterial, insecticidal, antifungal
3.	*X. budapestensis*	Fabclavine	Antibacterial, antifungal, antiprotozoal
4.	*X. khoisanae* strain SB10	Xenocoumacin	Antimicrobial
5.	*X. nematophila*	Rhabducin	Insecticidal
6.	*X. szentirmaii*	Xenofuranones A and B	Insecticidal

Conclusion

The main aim is targeting the endosymbionts for biological control and reducing the usage of chemical pesticides. Endosymbiotic bacteria and their secondary metabolites, toxic substances and bioactive compounds need to be explored and should be optimized in the future. The transmission of endosymbionts is *via* vertically or horizontally. Sometimes transmission is also by the environment. The various endosymbionts are used in the management of various insect pests. The endosymbionts can be effectively utilized and it can be genetically modified to produce desired effects on the insect host. After studying the effects of endosymbionts and it can be formulated in various ways for the pest management.

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