Vol. 5 Issue- 4, December 2024 (e-ISSN: 2582-8223)

JUST AGRICULTURE
multidisciplinary e-Newsletter

Paratransgenesis for Sustainable Management of Agricultural Insects

Bhumi D Barad, Patel, S. R. and Kachot, A. V.
Department of Entomology, Junagadh Agricultural University, Junagadh, Gujarat

ARTICLE ID: 33

Abstract

Insects are notorious for causing indirect damage to crops through the transmission of diseases, leading to significant agricultural losses. To address this issue, paratransgenesis has emerged as a promising alternative to traditional insecticides. This biotechnological approach involves introducing genetically engineered symbionts into insect pests, which can inhibit proteins or kill the pathogens. Additionally, paratransgenesis has potential applications in enhancing the health of honey bees, which are essential for pollination and overall ecosystem health. This innovative method offers a sustainable solution for pest management and the improvement of pollinator health.

Key words: Paratransgenesis, Transgenesis, Vector, Symbiont, Plant disease

Introduction

Agriculture has always been a susceptible field to pest and diseases, insects are found causing significant damage either directly by harming crops or indirectly by transmitting diseases. Although development of insecticides has been sought out as most reliable solution and is under use for over a century, but this solution is unreliable as issues like resistance, resurgence, residues and environmental concerns keeps arising on long term use of this chemicals. Public health awareness now demands an alternative to existing insect management strategies which are less hazardous and also sustainable. Insects as vectors are the most problematic concern of all agriculturalists as only a few numbers are required to cause tremendous damage to crops hence a different plan of management needs to be drawn for insects as vector which is effective on low populations as well. Additionally, pests like mites and microbial pathogens which are potentially less harmful are found to harm beneficial insects such as honeybees and silkworms. Since insects carry symbionts in their gut that enhance their fitness, these symbionts could be utilized to reduce disease transmission by insect vectors.

Paratransgenesis

In recent years biotechnological tools have gained a lot of lime light in every field of agriculture. Genetically modified insects are amongst them they, are developed for the management of arthropods. Transgenesis and paratransgenesis these two methods are recently developed.

Transgenic insects are insects that have been genetically modified through the insertion of foreign DNA into their genomes. This modification is done to endow them with new traits or abilities that can help in research or pest control applications (Shirk and Bossin, 2000; Scheteliget *et al.*, 2011; Tkachuk *et al.*, 2011). While paratransgenesis is a sophisticated biotechnological strategy designed to control vector-borne diseases by genetically modifying symbiotic organisms that live within disease-carrying vectors. The concept involves engineering bacteria, fungi, or viruses that inhabit the vectors (such as mosquitoes or tsetse flies) to express molecules capable of interfering with pathogen development or transmission.

In this process symbiotic gut bacteria from vectors are isolated sophisticatedly and then genetically modified in vitro to produce compounds that prevent the spread of pathogens. The transformed symbionts are then put back into the host vector, where the expression of designed molecules influences the host's vector competence, or capacity to spread the disease (Durvasula *et al.*,1997; Ward *et al.*, 2001; Elston, *et al.*, 2020).

Paratransgenesis was first explored using the kissing bug *Rhodnius prolixus*, which transmits Chagas disease. Researchers engineered the bacterium *Rhodococcus rhodnii* to produce the antimicrobial peptide Cecropin A, which successfully inhibited the development of *Trypanosoma cruzi* in the insect without harming the host. This work demonstrated the potential of using modified symbionts to control vector-borne diseases.

Following this, the technique was applied to tsetse flies, which spread African sleeping sickness. Scientists modified the common symbiotic bacterium *Sodalis* to produce green fluorescent protein (GFP), and the bacteria spread through the fly population via vertical and trans-stadial transmission. Further studies identified antimicrobial peptides in tsetse flies that can reduce the flies' ability to transmit parasites.

In malaria vectors, researchers have engineered *Escherichia coli* and *Pantoea agglomerans* to produce anti-Plasmodium effectors. Notably, *Pantoea agglomerans* was engineered to reduce the development of *Plasmodium falciparum* and *Plasmodium berghei* in mosquitoes by up to 98% through two strategies: producing antimicrobial peptides and blocking parasite interactions. Researchers have also focused on *Serratia AS1*, which colonizes mosquitoes effectively and can be engineered to inhibit malaria parasites (Beard *et al*., 1992).

Requirements for Paratransgenesis

1. The symbiont bacteria should be present in the insect vector and can be grown in vitro easily.
2. They can be susceptible to the genetic manipulation, such as through transformation with a plasmid containing the desired gene.
3. The engineered symbiont should be stable and colonise all instars during insect development throughout the life-cycle from first instars into adults.
4. The symbiont safe to the humans and animals and should be environment friendly and should not be horizontal transmitted.
5. One or more effector molecules need to be identified and secreted by the recombinant microorganism to achieve the desired inhibitory effect on the parasite-insect vector interaction, without imposing any fitness cost on the modified insect vector.
6. The association between vector and symbiont cannot be attenuated.
7. Field delivery is easily handled.

Steps for paratransgenesis:

For the successful development of paratransgenic insects, the following steps must be carefully undertaken (Fig. 1).

1. **Isolation of Suitable Microorganisms:** First step in paratransgenesis is to identification of symbiotic microorganisms associated with the vector species. These symbionts play a crucial role in the vector's biology and can be genetically modified to express specific proteins.
2. **Identification of Effector Proteins:** Identify proteins that prevent the vector from transmitting the pathogen. These proteins could be toxic to the pathogen or interfere with its transmission.
3. **Transformation of the Symbiont:**The genes coding for the identified effector proteins are introduced into the symbiont. This genetic modification allows the symbiont to express these proteins.
4. **Effective Delivery of the Transformed Symbiont:** The transformed symbiont (also known as the "transformant") is reintroduced into the insect host (vector).

5. **Spread Within and Between Host Populations:** For the long-term disease control, the genetic modified symbiont spread within the vector population.

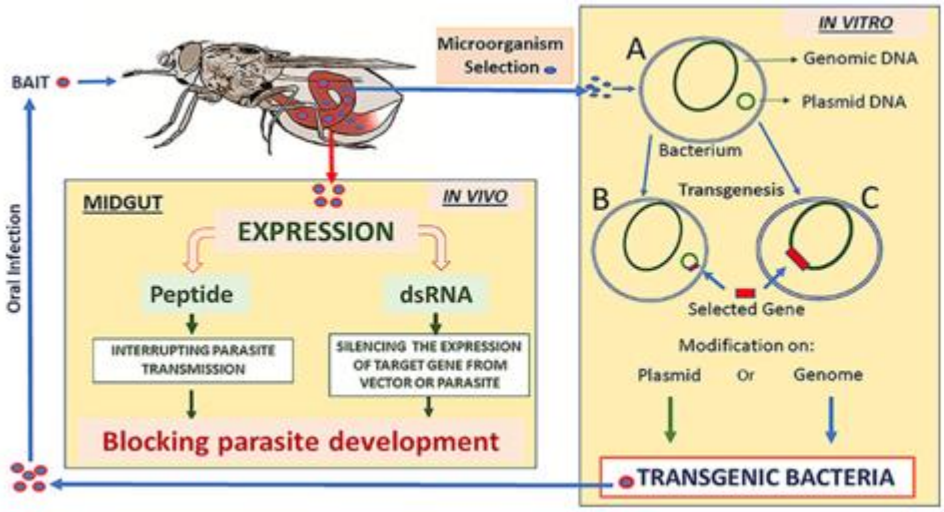

Fig. 1: Process for the development of paratransgenic insects

Similar to its use in vector control, paratransgenesis can be employed to engineer bacteria that reside in the digestive systems or other parts of agricultural pests. For example, genetically modified bacteria can produce toxins or antimicrobial peptides that are toxic to pests or disrupt their reproduction. In agriculture, paratransgenesis was first developed for the control of xylem dwelling bacterial pathogen, *Xylella fastidiosa* which causes Pierce's disease (PD) of grapevines and is transmitted by Glassy-Winged Sharpshooter (GWSS), *Homalodisca vitripennis.* An obligatory bacterial symbiont *Pantoeaagglomerans*, was used as a paratransgenic agent. Two antimicrobial peptides, melittin and scorpine-like molecules (SLM) engineered into the *P. agglomerans* and when this engineered symbiont colonized into the GWSS. Paratransgenic GWSS fail to transmit pathogen to the grape plants. For long term effect in the field calcium-alginate microparticles based strategy can be used. These microparticles provide a physical barrier between the bacteria and the outer environment to decrease environmental contamination as well as provide protection against desiccation and UV radiation (Arora *et al.*, 2018). Cellulose digesting protozoa present in termite gut, which was killed by the bacteria. Engineered Bacteria, *Trabulsiellaodontotermitis* inserted into the Formosan subterranean termite which can survive for 48 hr and kill the protozoans (Tikhe*et al.*, 2017). Gut associated bacteria *Serratia symbiotica* CWBI is a culturable bacteria present in black bean aphid and it can be genetically engineered using the various techniques and colonizes into the guts of aphid (Elston*et al.*, 2021).

Paratransgenesis can be used to combating the varroa mite, which are a significant threat to bee colonies and also vector for the DWV and *Israeli Acute Paralysis Virus* (IAPV). The Lactic acid Bacterial community, particularly *Lactobacillus* genus having great potential for paratransgenesis in honey bee. Transforming *L. kunkeei* and using it as a vector to promote the health of honey bees and aid in functional genetics research-related activities (Rangberg*et al.*, 2012). In 2020, Leonard *et al.* successfully engineered *Snodgrassellaalvi*, a symbiotic bacterium found in honey bees, to induce an RNA interference (RNAi) immune response. The modified *S. alvi* was able to stably recolonize bees, producing double-stranded RNA that activated RNAi, leading to the suppression of specific host genes. This resulted in changes to the bees' physiology, behaviour, and growth. Additionally, the engineered *S. alvi* was capable of killing parasitic Varroa mites by triggering the mites' RNAi response. This symbiont-mediated RNAi technique offers a valuable tool for studying bee functional genomics and holds potential for enhancing bee health. Research has explored using paratransgenesis to control bollworms, which are major pests of cotton crops. By modifying symbiotic bacteria to produce toxins that affect bollworms, it is possible to reduce their population and the resulting crop damage.

Challenges:

Selecting the appropriate symbiont is crucial due to the wide range of insect pests in agriculture. Effectively spreading transgenic bacteria across a diverse pest population is challenging along with its acceptance in farming community. Ensuring long-term effectiveness is essential for sustainable pest management. In India, this approach is still in the developmental stage, with a focus on biosafety, regulatory compliance and understanding the long-term impact of such technologies and proper infrastructure and framework planning this technology can surely be utilised to overcome the lag of developing pests and incompetent chemical control measures.

References

Beard, C. B., Mason, P. W., Aksoy, S., Tesh, R. B., & Richards, F. F. (1992). Transformation of an insect symbiont and expression of a foreign gene in the Chagas' disease vector Rhodniusprolixus. The American Journal of Tropical Medicine and Hygiene, 46, 195–200.

Durvasula, R. V., Gumbs, A., Panackal, A., Kruglov, O., Aksoy, S., Merrifield, R. B. and Beard, C. B. (1997). Prevention of insect-borne disease: an approach using transgenic symbiotic bacteria. Proceedings of the National Academy of Sciences, 94(7), 3274-3278.

Elston KM, Perreau JMaeda GP, Moran NA, Barrick JE. (2021). Engineering a Culturable Serratia symbiotica Strain for Aphid Paratransgenesis. *Appl Environ Microbiol.*, 87.

Elston, K.M., Perreau, J., Maeda, G.P., Moran, N. and Barrick, J.E. (2020). Engineering a Culturable *Serratia symbiotica* Strain for Aphid Paratransgenesis. *Applied and Environmental Microbiology*, 87.

Leonard, S. P., Powell, J. E., Perutka, J., Geng, P., Heckmann, L. C., Horak, R. D., Davies, B. W., Ellington, A. D., Barrick, J. E. and Moran, N. A. (2020). Engineered symbionts activate honey bee immunity and limit pathogens. *Science* (New York, N.Y.), 367(6477), 573–576.

Rangberg, A., Diep, D. B., Rudi, K. and Amdam, G. V. (2012). Paratransgenesis: an approach to improve colony health and molecular insight in honey bees (*Apis mellifera*). *Integrative and comparative biology*, *52*(1), 89–99.

Ratcliffe, N.A., Furtado Pacheco, J.P., Dyson, P. Castro, H. C. Gonzalez, M. S.,Azambuja P. and. Mello,C. B. (2022). Overview of paratransgenesis as a strategy to control pathogen transmission by insect vectors. *Parasites Vectors*, 15, 1-31.

Schetelig, M.F., Götschel, F., Viktorinová, I., Handler, A.M. and Wimmer E. A. (2011). Recombination technologies for enhanced transgene stability in bioengineered insects. *Genetica*, 139, 71–78 (2011).

Shirk, P. D. and Bossin, H. (2000). Development of piggyBac transposon-derived gene vectors and their utilization for transgenic insects.

Tikhe, C. V., Sethi, A., Delatte, J. and Husseneder, C. (2017). Isolation and assessment of gut bacteria from the Formosan subterranean termite, *Coptotermesformosanus* (Isoptera: Rhinotermitidae), for paratransgenesis research and application. *Insect Science*, 24 (1), 93-102.

Tkachuk, A.P., Kim, M.V., Savitskiy, V. and Savitskiy, M. (2011). The prospects of using transgenic insects in biological programs. Biology Bulletin Reviews, 1, 265-278.

Ward, T. W., Jenkins, M. S., Afanasiev, B. N., Edwards, M., Duda, B. A., Suchman, E., Jacobs-Lorena, M., Beaty, B. J., Carlson. O. (2001). *Aedes aegypti* transducing densovirus pathogenesis and expression in Aedes aegypti and Anopheles gambiae larvae. *Insect molecular biology*, 10(5), 397-405.