

Paratransgenesis: A Promising Tool for Controlling the Vector Borne Disease

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Paratransgenesis is an innovative biotechnological tool that uses genetically modified symbiotic microorganisms for the regulation of insect-borne and agricultural diseases. Through the utilization of natural microbiota of vectors, paratransgenesis helps in the transfer of effector molecules and RNA interference molecules to target pests. Application of this technology is seen in the suppression of the spread of malaria and Chagas disease, as well as increasing immunity in honeybees. Engineered symbionts are made to colonize vectors successfully and propagate using various pathways and generations. This approach minimizes ecological disturbance and offers specific long-term regulation. Paratransgenesis overcomes the harmful effects of conventional pesticides, including non-target effects and resistance. Its safety and efficacy are supported by successful laboratory and semi-field case studies. This technology promises to revolutionize integrated pest management and disease control worldwide.

Introduction

Vector-borne diseases (VBDs) affect nearly one-sixth of the global population, accounting for 17 per cent of communicable diseases and placing 80 per cent of people at risk. Major VBDs include malaria, dengue, chikungunya, leishmaniasis, chagas disease, schistosomiasis and African trypanosomiasis with over one billion cases and causing about one million deaths annually. India bears a substantial share of this burden, with 698 million people at risk of malaria, contributing 79% of Southeast Asia's cases in 2022. The country also accounts for 34% of global dengue cases, while chikungunya, kala-azar and lymphatic filariasis continue to pose major health challenges (Kumar *et al.*, 2024). Vector control remains the cornerstone of prevention, relying on integrated vector management (IVM) that combines biological, chemical, and environmental measures. However, challenges such as insecticide resistance, poor implementation and adaptive vector behaviour necessitate innovative, sustainable and community-driven solutions. Since many VBDs lack effective vaccines or treatments, insecticide-based control is critical but has its own limitation. Emerging genetic strategies such as transgenesis and paratransgenesis offer promising alternatives. These innovative vector control methods utilize genetically modified organisms (GMOs) but operate through distinct mechanisms (Fig. 1). Transgenesis modifies the insect genome to reduce the population of vectors. While paratransgenesis, this innovative method involves the genetic modification of the native microbiome of insect

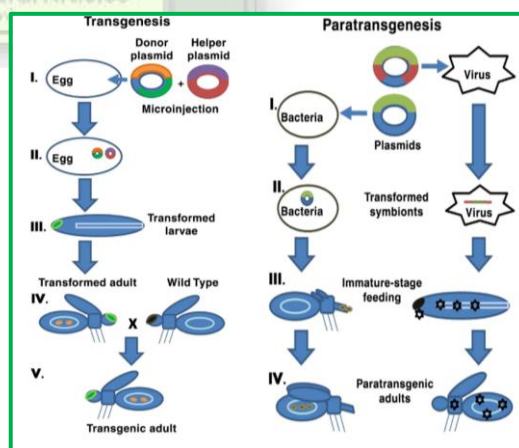


Fig. 1. Difference in method and mode of action between Transgenesis and Paratransgenesis

vectors, including mutualistic symbiotic, commensal bacteria, fungi and viruses to produce anti-pathogen factors that inhibit disease-causing agents (Ratcliffe *et al.*, 2022).

Requirements for successful paratransgenesis (Fig. 2)

(Ratcliffe *et al.*, 2022) include:

- Presence of a culturable symbiont or commensal microorganism (bacterium, fungus or virus) within the insect vector that can be genetically manipulated and co-localizes with the pathogen in host tissues.
- The microorganism should persist across all developmental stages, ideally exhibiting transstadial transmission from larvae to adults.
- It must be non-pathogenic to humans and animals and capable of colonizing multiple vector species or strains.
- The genetic modification should not compromise microbial fitness, stability or normal functionality within the host.
- Identified effector molecules must be secreted effectively by the recombinant microorganism to inhibit pathogen-vector interactions without imposing fitness costs on the vector.
- An efficient method for introducing and disseminating the recombinant microorganism into wild vector populations is essential
- Regulatory and community approval must be obtained, supported by comprehensive environmental risk assessments to address biosafety concerns and minimize horizontal gene transfer risks.

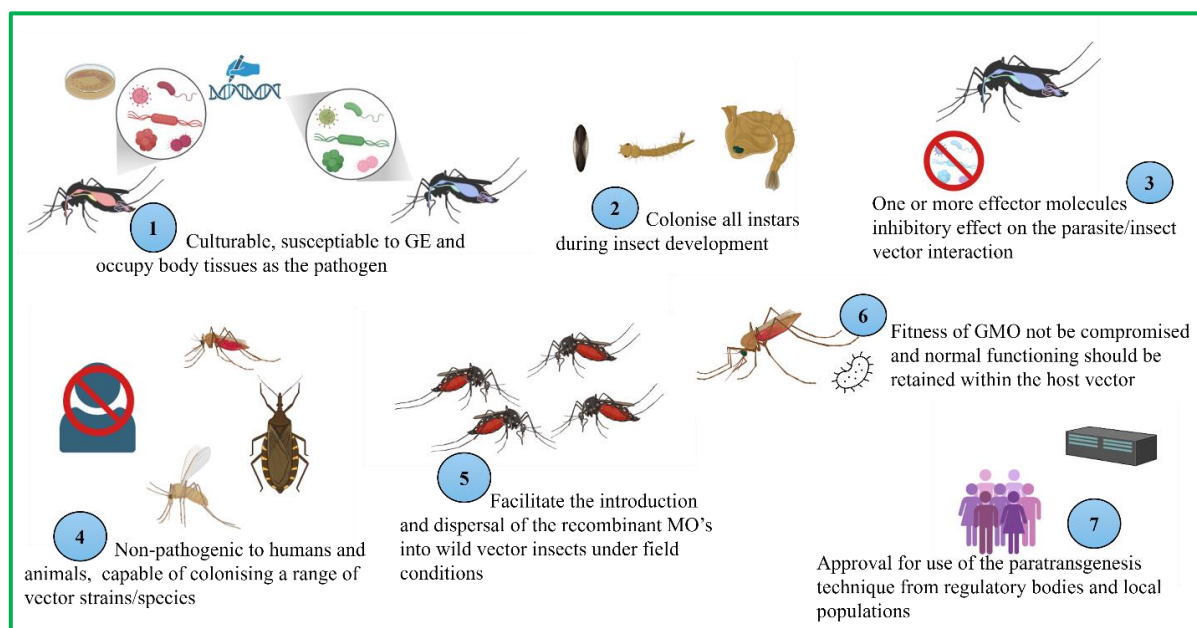


Fig. 2. Requirements of successful paratransgenesis strategy

Application of Paratransgenesis

A. Medical entomology

VBDs pose a major threat to human health, being transmitted by arthropods such as mosquitoes, ticks and flies. Dengue and chikungunya, both spread by *Aedes* mosquitoes, cause severe viral infections characterized by high fever, rash, and intense joint pain, with dengue sometimes leading to life-threatening complications. Leishmaniasis, transmitted by sandflies and caused by *Leishmania* parasites, affects both skin and internal organs, while Chagas disease, spread by triatomine bugs, can result in chronic cardiac and gastrointestinal disorders if untreated.

In mosquitoes, diverse microbiomes including bacteria like *Asaia*, *Pantoea*, *Serratia*, *Enterobacter* and *Pseudomonas* play crucial roles in physiology and immunity. Harnessing these bacteria for paratransgenesis has shown remarkable progress. For example, genetically modified *Pantoea agglomerans* expressing effector molecules such as Scorpine, EPIP4 and

Shiva 1 significantly inhibited *Plasmodium falciparum* development in *Anopheles* mosquitoes, achieving over 95% reduction in parasite load. Similarly, modified *Serratia* AS1 demonstrated the ability to colonize mosquito midguts, reproductive organs, and transmit venereally, horizontally and vertically across generations, effectively delivering effector molecules that reduced *P. falciparum* infection by more than 90 per cent. These findings underscore the potential of stable microbial symbionts in achieving long-term suppression of malaria transmission (Wang et al., 2012).

Beyond protein effectors, paratransgenesis has also been applied to deliver double-stranded RNA (dsRNA) for gene silencing. For instance, *Rhodococcus rhodnii*, an endosymbiont of kissing bugs, was engineered to deliver dsRNA targeting the RHBP gene, crucial for egg development. This resulted in reduced gene expression and significantly fewer eggs, highlighting its potential in controlling Chagas disease vectors. Furthermore, studies with *mCherry*-labelled *Serratia* have shown that genetically modified symbionts can spread through multiple routes including contact with host skin, larval habitats and mating ensuring efficient transmission and persistence in ticks (*Haemaphysalis longicornis*), sandflies (*Phlebotomus papatasi*) and mosquitoes (*Anopheles stephensi* and *Culex* spp.) (Koosha et al., 2018).

Once an effective paratransgenic microorganism is developed, it can be introduced into wild populations through sugar baits, larval breeding sites or release of colonized insects. These microorganisms then propagate naturally across generations, providing long-term disease suppression with minimal ecological disruption.

B. Agricultural entomology

Paratransgenesis has demonstrated significant potential in agricultural pest management. Insects such as aphids, whiteflies, leafhoppers, thrips and termites serve as key vectors for plant and structural diseases, leading to substantial economic losses globally.

One of the earliest successful applications was in the glassy-winged sharpshooter (*Homalodisca vitripennis*), the vector of *Xylella fastidiosa* subsp. *fastidiosa*, responsible for Pierce's disease in grapevines. *Pantoea agglomerans*, a natural gut symbiont, was genetically engineered to express antimicrobial peptides such as Melittin and Scorpine-like Molecule. When grape stems coated with the modified bacteria were fed upon by the sharpshooters, the pathogen load within the insect's gut declined sharply. The SLM-expressing strain was particularly effective, reducing infection rates of *X. fastidiosa* to below 20%, compared to 40–80% in controls. This demonstrated a sustainable, symbiont-mediated suppression of pathogen transmission and highlighted paratransgenesis as a viable tool for protecting grapevine health and wine industry (Arora et al., 2020).

In aphids, which transmit numerous plant viruses, *Serratia symbiotica* strain CWBI-2.3T was genetically modified to express GFP to study colonization and host tolerance. The bacterium successfully colonized the midgut of *Acyrtosiphon pisum* with stable populations ($\sim 10^8$ CFUs) and remained tissue specific. While most aphid species tolerated the symbiont, *Aphis craccivora* showed reduced survival, emphasizing the importance of host-symbiont specificity in designing paratransgenic systems. These findings underscore the feasibility of stable colonization without compromising host survival as a critical requirement for field application (Elston et al., 2021).

Another innovative approach involved RNA interference (RNAi) mediated through bacterial symbionts in thrips (*Frankliniella occidentalis*). The isolate BFo2 was engineered to produce dsRNA targeting the α -tubulin gene, essential for cell division and intracellular transport. Thrips feeding on dsTub-expressing bacteria exhibited significantly higher mortality, especially in the first larval stage (L1) and displayed marked suppression of α -tubulin transcript levels (Whitten et al., 2016).

C. Urban pest management (termites)

The concept has also been extended to structural pests such as the Formosan subterranean termites (*Coptotermes formosanus*), which depends on gut protozoans for cellulose digestion. *Kluyveromyces lactis* yeast was genetically engineered to produce ligand-hecate peptides,

which selectively bound and lysed these protozoa. Termites that ingested cellulose baits coated with the modified yeast exhibited protozoan depletion, reduced size, and early mortality due to starvation. This experiment illustrated the feasibility of symbiont-targeted biocontrol as a non-chemical termite management approach.

Complementary studies using the termite gut bacterium *Trabulsiella odontotermis* further confirmed the potential of microbial colonization for paratransgenesis. Genetically modified strains expressing GFP and kanamycin resistance (Kmr:Tn7) successfully colonized termite guts for up to 36 days and were horizontally transmitted between colony members. The persistence and transmissibility of these engineered symbionts make them excellent candidates for developing long-lasting, colony-wide control systems (Tikhe *et al.*, 2016).

D. Apiculture

Honeybees (*Apis mellifera*) are crucial pollinators that sustain both agricultural productivity and biodiversity. However, modern apiculture faces severe challenges from parasites such as *Varroa destructor* mites, and viral and fungal pathogens, including the Deformed Wing Virus (DWV) and *Nosema* spp., which collectively contribute to global colony losses. In recent years, paratransgenesis has emerged as a promising biotechnology to enhance honeybee health by manipulating their natural gut microbiota.

Honeybees possess a stable and specialized gut microbiome composed of bacterial species such as *Snodgrassella alvi*, *Gilliamella apicola*, *Bifidobacterium* and *Lactobacillus*, which aid digestion, nutrient absorption, and immune defense. Among these, *S. alvi* one of the core symbiont inhabiting the ileum has proven particularly suitable for genetic modification and long-term colonization, making it an ideal candidate for paratransgenic applications.

In a breakthrough study, Leonard *et al.* (2020) engineered *S. alvi* to express double-stranded RNA (dsRNA) targeting DWV genome. Honeybees colonized with this modified bacterium showed significantly higher survival rates after DWV exposure, particularly when treated with the construct pDS-DWV2, which effectively silenced viral replication. Importantly, the modified bacteria colonized the bee gut without affecting normal physiology, confirming the safety and compatibility of this approach.

Further extending this strategy, *S. alvi* was engineered to produce dsRNA molecules targeting multiple essential genes in the *Varroa* mite, a major ectoparasite of honeybees. When mites were exposed to bees harboring the dsRNA-producing *S. alvi* (pDS-VAR), their survival rate declined sharply, demonstrating that paratransgenic delivery of RNA interference (RNAi) can disrupt mite gene expression and reduce parasitic loads.

Conclusion

Paratransgenesis is an innovative and sustainable biotechnology that leverages genetically modified symbiotic microorganisms to control insect pests, manage vector-borne diseases, and enhance the health of beneficial species such as pollinators. By engineering symbionts to produce effector molecules, including antimicrobial peptides or double-stranded RNA, this approach disrupts pathogen transmission and impairs pest survival without relying on chemical pesticides. Delivery through oral feeding, baiting, or environmental introduction allows stable colonization and transmission within and across generations of target populations. By minimizing environmental impact, preserving non-target and beneficial organisms and reducing the risk of resistance, paratransgenesis represents a precise, eco-friendly, and transformative tool for integrated pest management, sustainable agriculture and pollinator conservation worldwide.

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