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Genetic manipulation of endosymbionts to control vector and vector borne diseases

Jay Prakash Gupta, K. P. Shyma, Sanjeev Ranjan, G. K. Gaur, Bharat Bhushan

Indian Veterinary Research Institute,
Izatnagar, Bareilly-243122, UP, India
Corresponding author: Jay prakash gupta, E-mail: jp.prakash01@gmail.com
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Abstract

Vector borne diseases (VBD) are on the rise because of failure of the existing methods of control of vector and vector borne diseases and the climate change. A steep rise of VBDs are due to several factors like selection of insecticide resistant vector population, drug resistant parasite population and lack of effective vaccines against the VBDs. Environmental pollution, public health hazard and insecticide resistant vector population indicate that the insecticides are no longer a sustainable control method of vector and vector-borne diseases. Amongst the various alternative control strategies, symbiont based approach utilizing endosymbionts of arthropod vectors could be explored to control the vector and vector borne diseases. The endosymbiont population of arthropod vectors could be exploited in different ways viz., as a chemotherapeutic target, vaccine target for the control of vectors. Expression of molecules with antiparasitic activity by genetically transformed symbiotic bacteria of disease-transmitting arthropods may serve as a powerful approach to control certain arthropod-borne diseases. Genetic transformation of symbiotic bacteria of the arthropod vector to alter the vector's ability to transmit pathogen is an alternative means of blocking the transmission of VBDs. In Indian scenario, where dengue, chikungunya, malaria and filariosis are prevalent, paratransgenic based approach can be used effectively.
Keywords: Arthropod vectors, Genetically modified endosymbionts, Vector and vector-borne diseases.

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Introduction

Vector borne diseases (VBD) are of global importance because of their economic and health implications in livestock, human and companion animals [1]. Tick and Tick Borne Diseases (TTBDs) affect 80% of the world's cattle population and are widely distributed throughout the world, particularly in the tropics and subtropics [2]; they represent an important proportion of all animal diseases affecting the livelihood of poor farmers in tropical countries. The impact of TTBDs on the livelihood of resource poor farming communities has been ranked high [3]. Cattle ticks are responsible for severe economic losses in both dairy and beef cattle enterprises in tropics [4]. Ticks are responsible for a variety of losses, caused by the direct effect of attachment ('tick worry'), by the injection of toxins, or through the morbidity and mortality associated with the diseases that they transmit. The global cost of Tick and Tick- Born Diseases (TTBDs) has been roughly estimated in the tune of 14000-18000 million US$ per annum of which the control cost in India has been estimated in the tune of 2000 crore rupees per annum [5]. Ticks are also vectors of a variety of pathogens that are implicated in severe pathologies in many mammalian species [6].

Amongst the VBDs of animals, the impact of tick borne diseases viz. theileriosis, babesiosis, anaplasmosis, ehrlichiosis and cowdriosis is ranked high. The traditional methods to control vector and vector borne diseases (VVBDs) are not sufficient enough to curb the menace caused by the VVBD's [7]. The antigenic variation present in most of the vector borne parasites/ pathogens impedes the development of effective vaccine against these VBDs [8,9]. Besides other factors, the global warming has been facilitating the breeding of arthropod vectors and their entry into new geographical areas is likely to disturb the delicate equilibrium and contribute to new epidemics of VBDs [10]. Selection of insecticide resistant vector population and drug resistant vector borne parasite/pathogen population has tremendously contributed to the reoccurrence of VBDs. Due to the reluctance of

multinational national companies to fund insecticide research which is becoming uneconomical, the number of insecticides available for use in field has declined significantly [11] and there is a strong possibility of not having any new insecticide in near future. The continuous and indiscriminate use of the available limited number of insecticides may lead to a stage at which the genotype of entire mosquito and tick populations would turn into homozygous resistant to the available insecticides, a situation that can jeopardize the lives of man and animals globally. The public health preparedness to meet out any such eventualities in future should explore the possibilities of alternative control strategies or the integration of different strategies for the control of VVBDs. Amongst the various alternative control strategies [12], genetically modified symbiont based control strategy is under explored and appears to have great potential to curb the VVBDs in an integrated format.

Endosymbionts of arthropod vectors: Endosymbiosis is a specific type of symbiosis in which the microbial partner lives within its host and represents the most intimate contact between interacting organisms [13]. Endosymbionts are commonly found in arthropods that always depend on single source of diet that is deficient of some nutrients and the beneficial endosymbionts provide the essential nutrients to arthropods. An endosymbiont can be either intracellular (*Wigglesworthia spp*. in side bacteriome) or extracellular (*Sodalis spp*. in midgut lumen of tsetse fly) organism. Endosymbionts are reported to be present in midgut, haemolymph, fat body and ovaries of the different arthropod vectors.

The arthropod host domesticates the endosymbionts for its own welfare by utilizing the functions that are present in the symbionts but lacking in the host. Scientists classify insect endosymbionts in two broad categories, primary and secondary. Primary endosymbionts have been associated with their insect hosts for many millions of years, they form obligate association and display co-speciation with insect hosts. Primary endosymbionts are essential for the survival of the arthropod host and elimination of the organism has a deleterious effect on the host [14]. Because the host is dependent on symbiosis, endosymbionts are transmitted vertically or maternally to the young ones with no horizontal transmission between hosts. Secondary endosymbionts exhibit a more recently developed association and are not obligate. Secondary endosymbionts are organisms appear to be the resultant of multiple independent infections and horizontal transmission and their contribution to the welfare of arthropod host may not be major or essential. Various authors reported different endosymboints in diverse arthropod vectors. *Wolbachia spp*, a rickettsia which is present in 20-70% of arthropods stated to be a reproductive parasite of arthropods rather than symbiont [15]. These organisms cause a number of interactions with the host and alter the host response that includes cytoplasmic incompatibility (CI), parthenogenesis induction [16] and male killing.

Transmission of symbionts in various arthropod vectors: The different symbionts in the various arthropods are transmitted to their young ones by various ways and means. The predominantly present *Wolbachia* organisms of *Aedes* and *Culex spp.* mosquitoes are transmitted by vertical manner via gametes to their next generations [17]. Though the tsetse flies are larviparous, their young ones get some of the symbiotic organisms through the secretion of milk glands during their intrauterine life [18]. *Rhodococcus rhodhini*, *gut* symbionts of the triatomine bug (*Rhodinus prolixus*) vector of Chagas' disease, transmitted from the adults to the progenies through coprophagy [19]. Understanding of the biology of the transmission of symbionts from the adult vectors to progenies is imperative for the symbiont based control approach to be effectively used in the field conditions.

Endosymbiont-arthropods-pathogen interaction: Perturbation of the interaction between endosymbiont, vector and parasite is the ultimate idea of the symbiont based approach to control VVBDs.

Endosymbiont-Vector interaction: Arthropod vectors benefit from the symbiosis and augment their functional capabilities to facilitate their expansion in to novel niches. The best examble is *Wigglesworthia glossinidia* an intracellular endosymbiont of *Glossina* fly which synthesises plethora of vitamin biosynthetic products that may promote host reproduction. *Wolbachia* organism's biosynthesis of riboflavin and FAD, are essential for the *Brugia malayi* coenzymes and cytochromes.

Endosymbiont-pathogen interaction: The presence of symbionts and the pathogenic organisms together in the vector and the possible role of the interaction between the two in favour of disease transmission had been reported Amongst the bacterial gut flora the *S. marcescens* chitinase has the ability to digest the peritrophic membrane of mosquito [20]. *Plasmodium* ookinetes produce chitinase to invade the midgut epithelium of mosquito.

Vector-Parasite interaction: The competence of the vector to transmit a parasite/pathogen requires a molecule(s) within the vector that interacts with the parasite/pathogen. Proteolytic lectin present in the midgut of tsetse fly stated to have trypanosome transforming activity [21]. Creation of paratransgenic tsetse fly with the phenotype to check the activity of proteolytic lectin of the fly would limit the transmission of African trypanosmiosis.

Control strategies

Vector control: Endosymbionts of arthropod vectors can be targeted and/or manipulated in different ways to control the VVBDs of man and animals. The vector control can be attempted in three different ways by utilizing *Wolbachia* by Chemotherapeutic, Immunological and *Wolbachia* cytoplasmic incompatibility (CI) based approach.

Chemotherapeutic approach: This approach exploits the endosymbionts of arthropods vectors as a chemotherapeutic target with the aim to disturb the symbiosis Sterility was observed in healthy tsetse flies fed with tetracycline (2500µg/ml) due to damage to the mycetome bacterial endosymbionts [14]. Antibiotic (Tetracycline group) against the endosymbionts of nematode parasites when administered to the mammalian host disrupt the symbiosis and lead to growth retardation and/or infertility of the parasite.

Immunological approach: Immunization of animals with the whole killed endosymbionts or purified antigens or recombinant antigens of the endosymbionts would render them immune to tick vectors. The concealed antigens or the mid gut antigens of the blood feeding arthropods like ticks, glossina, mosquitoes are the potential vaccine targets being exploited by the researchers for the control of vectors [22]. Instead of targeting the host (vector) antigens, the endosymbionts could be targeted to disturb the symbiotic relationship between the vector and the symbiont. Following ingestion of the blood from immunized animals, these antibodies together with other components of the immune system such as complement, will destroy the symbionts inside the vector, leading either to death or to disruption of normal gut physiology of the tick and reduce growth and egg-laying ability.

Wolbachia cytoplasmic incompatibility (CI) based approach: Wolbachia infections in arthropods can manipulate reproduction of their hosts in a variety of ways e.g., induced parthenogenesis, male killing, parthenogenesis, and cytoplasmic incompatibility (Cl) [20]. Wolbachia of mosquito species is the extensively studied symbiont of mosquitoes and the same is the widely explored Wolbachia of arthropod vectors. cytoplasmic incompatibility phenomenon of the Wolbachia of mosquito species is presently being explored as a means to control the mosquito population.

Wolbachia cytoplasmic incompatibility (CI): Its is a phenomenon in which mating between Wolbachia infected male insect and female insect of the same species without Wolbachia infection (Unidirectional CI) and mating between insects of the same species with different Wolbachia strain infection (Bidirectional CI), result in embryonic mortality [23]. The reciprocal mating (infected female x uninfected male) and mating between infected individuals are fully compatible.

Mechanism of CI - Although the mechanism of CI has not been elucidated in the molecular level, presently it is explained with two terminologies, modification and rescue. Modification is the process in which the Wolbachia modifies the sperm of the infected male during spermatogenesis by an unknown process. The modified mature sperm is devoid of Wolbachia. If a modified sperm enters an incompatible egg (uninfected or infected with different strain) a delay in break down of nuclear membrane of pronuclei of sperm resulting in mitotic asynchrony [24]. Amongst the incompatible crosses, bidirectional CI could be exploited to control the mosquito population which in turn limits the transmission of mosquito borne diseases.

Vector borne diseases control

Paratransgenesis: Genetic transformation, of commensal or symbiotic bacteria of the arthropod vector is to alter the vector's ability to transmit pathogen. It is an alternative means of blocking the transmission of VBDs. The mid gut bacteria of arthropod vectors can be engineered to express and secrete effector proteins which block the parasite invasion or kill the parasite in the mid gut or hemolymph or reproductive tract. The arthropod vectors that harbor the genetically transformed endosymbionts are called as paratransgenic vector. For this strategy to be used in vector borne disease control, bacteria that survive inside the vector's body have to be identified. The endosymbionts of arthropod vectors can be cultured and genetically transformed to express the effector gene inside the vector in such a way the gene product kills the parasite/pathogen that the vector transmits. Isolation and characterization of the endosymbionts present in midgut, hemolymph and reproductive tracts is the very first step towards the paratransgenic apparoach. In this strategy, the normal symbiont population of the arthropod vector can be replaced with genetically modified symbionts,

resulting in population of arthropod vectors refractory to the particular vector borne parasite/pathogen. This strategy has shown promise in controlling the transmission of *Trypanosoma cruzi* by *Rhodnius prolixus.* The genetically transformed *Rhodococcus rhodnii* was delivered into the asymbiotic first instar nymph orally in such a manner to express an antimicrobial peptide L-cecropin A, inside the gut lumen which conferred resistance status to the paratransgenics [25]. Wolbachia might be used to prevent the transovarian transmission of arboviruses viz., dengue virus, La crosse virus, rift valley fever virus. With the paratransgenic approach, African trypanosomosis can be controlled using the tsetse symbiont. Here the genes are not inserted to tsetse chromosome, but instead to the tsetse symbiont, Sodalis. Sodalis is well suited for paratransgenesis because a) it resides in the gut in close proximity to pathogenic trypanosomes, b) a system for culturing Sodalis in vitro has been developed [26] and can be used in conjunction with standard molecular biology techniques to insert and express foreign genes of interest in this bacterium [27] c) Sodalis is highly resistant to many trypanocidal peptides, [28,29] d) recombinant Sodalis (recSodalis) can be reintroduced into tsetse by thoracic microinjection and passed on to future progeny where they successfully express the marker gene product [30] and e) its genome is completely sequenced and annotated, and this information will serve as a valuable resource that can be exploited to improve the efficiency of this expression system. Because these commensal bacteria live naturally in close proximity to where trypanosomes develop and replicate in the tsetse midgut, expression of trypanocidal products in Sodalis has the potential to block parasite development in the fly.

Though this technology is useful in many aspects, it has some demerits viz., socio-ethical issues, fitness of the genetically modified (GM) symbiont, alteration of ecosystem, horizontal gene transfer among compatible bacteria, transfer of GM symbiont to non-target arthropods and subsequently getting into the food chain, it can not be kept aside of our list of arsenals to fight against VBDs. The endosymbionts of arthropod are having small genome and/or more psuedogenes which indicates the long term co-evolution with loss of considerable size of their genome and functional genes by conserving the genes essential to adapt to the arthropod microenvironment. This genome erosion and adaptive degeneration phenomenon questions the stability of the transgene/ effector gene in long run. The advancements in the biotechnology will find safer solutions to the aforementioned problems in near future.

Future prospects

Whether transformed symbionts, can replace non-transformed in natural insect populations, can't be said with certainty, despite of the early success with the transformation of insect vector symbionts. Symbionts perhaps have no fitness load on insect hosts and are capable of being transmitted via transovarian transmission or lateral transmission. Thus, a strong gene drive system can potentiate the effectiveness of paratransgenesis. An example of such gene drive system can be *Wolbachia* Endosymbionts [31]. Although there has been a great advances in the development of stable lines of genetically modified disease vectors [32-34], there exists many challenges in to the application of transgenesis to control vector-borne diseases outside the laboratory. Future studies mimicking field conditions likely will uncover the importance of fitness to the establishment of transgenic mosquitoes in natural habitats. India which suffers lot due to dengue, chikungunya, malaria and filariosis need a programme on *Wolbachia* CI and paratransgenic based approach to encounter afore-mentioned VBDs. The main constraints to the establishment of an efficient transgenic vector approach are the scarcity of a transgenes that effectively reduce pathogen load. Again further studies utilizing QTL mapping, reverse genetics, gene knockdown, or other techniques to identify traits associated with vector competence can reveal candidate genes that, when targeted, may effectively block pathogen development and transmission.

Conclusion

A variety of very effective methods have been employed for suppressing arthropod vector populations, including the application of biological control agents and the elimination of breeding sites, with a continuing and heavy reliance on the use of chemical insecticides. However, the development of insecticide resistance by vector insects, the cost of developing and registering new insecticidal compounds, and the increase in legislation to combat the detrimental effects of insecticidal residues on the environment, have emphasized the need to assess alternative strategies for vector control which are cost effective and safer. Endosymbionts of the arthropod vector are identified as a potential source for the control of VVBDs and symbiont based approaches are considered as cost effective. Bioprospecting of molecules involved

in the interaction between vector, symbiont and parasite/pathogen will offer more targets to check the transmission of VBDs through paratransgenic approach

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