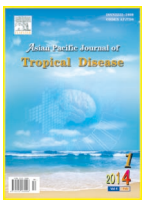
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi:10.1016/S2222-1808(14)60319-4

Wolbachia-a foe for mosquitoes

Nadipinayakanahalli Munikrishnappa Guruprasad[1*], Sushil Kumar Jalali[1], Hosagavi Puttegowda Puttaraju[2]

[1]*National Bureau of Agriculturally Important Insects (NBAII) Post Bag No. 2491, H.A. Farm Post, Bellary Road Bangalore-560024. India*

[2]*Department of Biological Sciences, Bangalore University, Bangalore-560056. India*

PEER REVIEW

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Dr. Kenneth M. Pfarr, Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn-53105, Germany.
Tel: +49-228-287-11476
Fax: +49-228-287-11167
E-mail: pfarr@microbiology-bonn.de

Comments

The *Wolbachia*-based technology will assess a novel control strategy for mosquito control by using Fruit fly virulent Wolbachia. It will also deliver new tools for the accurate assessment of the impact on population age structure in mosquitoes based on *Wolbachia* interventions. To summarize, it is opined that *Wolbachia* provides a biological method to manipulate mosquito population, reduce disease transmission and health burden in humans.
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ABSTRACT

Mosquitoes act as vectors for a wide range of viral and parasitic infectious diseases such as malaria, dengue, Chickungunya, lymphatic filariasis, Japanese encephalitis and West Nile virus in humans as well as in animals. Although a wide range of insecticides are used to control mosquitoes, it has only resulted in development of resistance to such insecticides. The evolution of insecticide resistance and lack of vaccines for many mosquito-borne diseases have made these arthropods highly harmful vectors. Recently, a novel approach to control mosquitoes by transinfection of life shortening maternally transmitted endo-symbiont *Wolbachia* wMelPop strain from fruitfly *Drosophila* into mosquito population has been developed by researchers. The wMelPop strain up-regulated the immune gene expression in mosquitoes thereby reducing the dengue and Chickungunya viral replication in *Aedes aegypti*, and also it significantly reduced the *Plasmodium* level in *Anopheles gambiae*. Here, we discuss the strategy of using *Wolbachia* in control of vector-borne diseases of mosquitoes.

KEYWORDS
Wolbachia, Mosquitoes, Dengue, Chickungunya, Malaria, Vector

1. Introduction

More than half of the world population live under the risk of insect-borne diseases, predominantly those transmitted by mosquitoes. The mosquitoes are vectors of a broad range of harmful viral and parasitic diseases affecting both humans and animals. Mosquitoes are the causative agents of diseases such as malaria, lymphatic filariasis, dengue, chikungunya and West Nile fever. Estimations made by the World Health Organization show that 247 million people in tropical and subtropical regions of the world become ill and about one million people died because of mosquito-borne diseases[1]. So far insecticide-based control programs targeting mosquitoes have been the most successful method of controlling the disease, but it is often expensive and also the effectiveness of such program has been greatly reduced because of the development of insecticide resistance in mosquitoes. This has prompted the need to use more expensive alternative compounds. The massive unjudicial use of chemical insecticides has led to environmental

*Corresponding author: Nadipinayakanahalli Munikrishnappa Guruprasad, National Bureau of Agriculturally Important Insects (NBAII), Post Bag No. 2491, H.A. Farm Post, Bellary Road, Bangalore-560024. India.
E-mail: guruprasadnm@gmail.com

Article history:
Received 18 Nov 2013
Received in revised form 25 Nov, 2nd revised form 3 Dec, 3rd revised form 12 Dec 2013
Accepted 21 Jan 2014
Available online 28 Feb 2014

degradation and biomagnification in ecosystems[2].

Wolbachia is an intracellular maternally inherited endosymbiotic bacteria found in arthropods. It manipulates its host reproduction in many ways, all of which favour infected females in nature. *Wolbachia* was first discovered in the reproductive tissues of mosquitoes *Culex pipens* by Hertig and Wolbach in 1924 and the species was later named *Wolbachia pipientis*[3,4]. *Wolbachia* infections are widespread within insect species[5-8]. One of the reproductive manipulation induced by *Wolbachia* is cytoplasmic incompatability, which has received considerable attention in biological control of insect vectors and diseases. cytoplasmic incompatability results in the generation of unviable offspring when an uninfected female mates with a *Wolbachia*-infected male[9].

Wolbachia is responsible for inducing a number of reproductive modifications that enables its spread and maintenance in natural populations. *Wolbachia*-induced cytoplasmic incompatability has received considerable attention as a mechanism to control insect vectors and diseases[10,11]. Recently, there has been a substantial increase in *Wolbachia* research related to the interactions of *Wolbachia* with its hosts and its impact on parasite transmission. Fruit fly *Drosophila melanogaster* (*D. melanogaster*) *Wolbachia* strains can invade and sustain themselves in mosquito populations, reduce adult lifespan, affect mosquito reproduction and interfere with pathogen replication. Such endosymbiotic bacterial strains have been introduced in *Aedes aegypti* (*Ae. aegypti*) mosquito populations to reduce their life span, thereby reducing the extrinsic incubation period. The other prospect of exploiting *Wolbachia* is using its ability to interfere with viruses and parasites. *Wolbachia* is known to interact with a wider range of pathogens in transfected mosquitoes including dengue and chikungunya viruses[12]. A recent study showed that *Plasmodium falciparum* development in *Anopheles gambiae* (*An. gambiae*) is suppressed transiently as a result of *Wolbachia* infection. This reproductive parasite is known to indirectly support and up-regulate the insect-host immune system and suppress the pathogen[13]. A major advantage of *Wolbachia*-based control approach for mosquitoes is that cytoplasmic incompatability acts as a self-spreading mechanism for *Wolbachia* to rapidly invade populations from the release of relatively small numbers of individuals. *Wolbachia* provides a biological method to manipulate mosquito populations and reduce disease transmission[14]. Findings have prompted researchers to aid in the control of mosquito-transmitted diseases. It has the benefit of being more environment-friendly than insecticide-based approaches[15,16].

2. *Wolbachia* inhibits dengue and chikungunya virus replication in mosquitoes

Evidence from several recent studies indicates that a strain of life-shortening *Wolbachia* has been detected in the fruit fly *Drosophila*. This virulent *Wolbachia* strain wMelpop is responsible for the shortening of life span in *D. melanogaster*[17]. In *Drosophila,* the wMelPop and another closely related *Wolbachia* strains have the ability of protecting against RNA virus infection by delaying the mortality of flies infected with a range of pathogenic viruses[18,19]. The *Wolbachia* wMelpop infection in *D. melanogaster* induces antiviral response to the *Drosophila* C virus in their hosts, cricket paralysis, Nora and Flock House viruses[20]. These observations in *Drosophila* has made researchers to introduce this bacterial strain into the dengue virus mosquito vector *Ae. aegypti* artificially. The introduction of walbB strain reduces the proliferation of dengue virus when compared with uninfected mosquito population. The *Wolbachia* strain not only reduced the virus replication but also reduced the adult life span. The life-shortening *Wolbachia* exerts its effect by altering the extrinsic incubation period of dengue virus, thereby inhibiting its transmission to new host. Meanwhile life-shortening *Wolbachia* may offer a new technology to control the chikungunya virus as well. These results may offer a potential new method to control vector-borne diseases like dengue and chikungunya virus from *Ae. aegypti*[21].

3. *Wolbachia* and host immune gene up-regulation

Researchers recently found that the presence of wMelPop in mosquitoes up-regulated the host immune gene expression, resulting in inhibition of pathogen replication or multiplication. The up-regulated six immune genes observed in wMelPop infected *An. gambiae* are LRIM1, TEP1, CEC1, DEF1, CTL4 and CLIPB3 as shown by genome analysis[21]. When *Wolbachia* strain wMelPop was transinfected into *An. gambiae*, up-regulation of immune genes LRIM1 and TEP1 has been found, inhibiting the *Plasmodium* development by interfering in the opsonization pathway[22,23]. Fascinatingly, similar results have been seen in the avian malarial parasites *Plasmodium gallinaceum* and *Plasmodium berghei*[24]. The infection of wMelPop strain in mosquito *Ae. aegypti* induces up-regulation of several immune effector molecules, namely, thio-ester containing proteins, C-type lectins, defensin, diptericin, GNBPB1, SPZ1A, cactus and cecropin[25]. The up-regulation of immune effector molecules in *Wolbachia*-infected mosquitoes may be responsible for the resistance to pathogen virus proliferation[26]. Natural *Wolbachia* strains infected in *Culex quinquefasciatus* mosquitoes have shown resistance to West Nile virus[27]. This evidence is more prominent when compared to *Wolbachia* strains infected mosquitoes.

4. *Wolbachia* and malaria parasite (*Plasmodium*) inhibition

Malaria is one of the deadliest infectious disease caused by protozoan parasite *Plasmodium* transmitted by vector

Anopheles mosquitoes. There are estimated 500 million cases of malaria annually and 1 to 3 million deaths primarily associated with *Plasmodium*[28]. When life shortening *Wolbachia* strain wMelPop transinfects into *An. gambiae,* several immune genes are activated, resulting in inhibition of *Plasmodium* parasite development[29]. Several recent studies in *Drosophila* has shown that the immune genes involved in inhibition of *Plasmodium* are LRIM1 and TEP1, and they are likely to inhibit the human malarial parasite *Plasmodium*, if stable infection is transmitted vertically to *Anopheles*[30]. The evidence for pathogen suppression was reported in *Ae. aegypti*, with induced resistance to bacterial pathogen *Pseudomonas aeruginosa*, by reducing the number of *Plasmodium gallinaceum* oocysts by triggering the co-expression of two antimicrobial peptides cecropin A and defensin A, and completely blocking transmission of this avian malaria parasite to native chickens[31]. The density and tissue distribution of *Wolbachia* infections in mosquitoes is an important factor in inhibiting *Plasmodium*[32].

5. Conclusion

The *Wolbachia*-based technology will assess a novel strategy for mosquito control by using virulent *Wolbachia*. It will also deliver new tools for the accurate assessment of the impact on population age structure in mosquitoes based on *Wolbachia* interventions. To summarize, it is opined that *Wolbachia* provides a biological method to manipulate mosquito population and reduce disease transmission and health burden in humans.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are thankful to Department of Biotechnology, Government of India for financial support through DBT-Post Doctoral Research Associateship programme to Dr. N. M. Guruprasad.

Comments

Background

The mosquitoes are vectors of a broad range of harmful viral and parasitic diseases affecting both humans and animals. Mosquitoes are the causative agents of diseases such as malaria, lymphatic filariasis, dengue, chikungunya and West Nile fever. The use of insecticides targeting mosquitoes has been the most successful method, but contentious use of insecticides has led to development of insecticide resistance in mosquitoes. In recent years, potential application of *Wolbachia* endosymbiont to control of mosquito borne diseases has emerged as a more environmental friendly, cost effective biocontrol tool against mosquitoes.

Research frontiers

Fruit fly *D. melanogaster Wolbachia* strains can invade and sustain themselves in mosquito populations, reduce adult lifespan, affect mosquito reproduction and interfere with pathogen replication. Such endosymbiotic bacterial strains have been introduced in *Ae. aegypti* mosquito populations to reduce their life span, thereby reducing the extrinsic incubation period. The other prospect of exploiting *Wolbachia* is using its ability to interfere with viruses and parasites. *Wolbachia* is known to interact with a wider range of pathogens in transfected mosquitoes including dengue and chikungunya viruses. A recent study showed that *Plasmodium falciparum* development in *An. gambiae* is suppressed transiently as a result of *Wolbachia* infection. This reproductive parasite is known to indirectly support and up-regulate the insect-host immune system and suppress the pathogen.

Related reports

Wolbachia is an intracellular maternally inherited endosymbiotic bacteria found in arthropods. It manipulates its host reproduction in many ways, all of which favour infected females in nature. *Wolbachia* was first discovered in the reproductive tissues of mosquitoes *Culex pipens* by Hertig and Wolbach in 1924 and the species was later named *Wolbachia pipientis*. *Wolbachia* infections are widespread within insect species . One of the reproductive manipulation induced by *Wolbachia* is cytoplasmic incompatability, which has received considerable attention in biological control of insect vectors and diseases (Brelsfoard and Dobson 2011).

Innovations & breakthroughs

The transinfection of life shortening *Wolbachia* from fruit fly, invades mosquitoes by reducing the adult life span and triggering immune responses, thereby inhibiting pathogen replication.

Applications

This review paper has given more emphasis on the use of *Wolbachia* endosymbiont, which is benefit of being more environmental friendly, cost effective than insecticide based approaches in biocontrol of mosquito borne disease.

Peer review

The *Wolbachia*-based technology will assess a novel control strategy for mosquito control by using fruit fly virulent *Wolbachia*. It will also deliver new tools for the accurate assessment of the impact on population age structure in mosquitoes based on *Wolbachia* interventions. To summarize, it is opined that *Wolbachia* provides a

biological method to manipulate mosquito population and reduce disease transmission and health burden in humans.

References

[1] World Health Organization. World malaria report. Geneva: World Health Organization; 2010. [Online] Available from: http://www.who.int/malaria/publications/atoz/9789241564106/en/index.html. [Accessed on 14th August, 2013]

[2] Service MW. *Mosquito ecology: field sampling methods*. 2nd ed. London: Chapman & Hall; 1993.

[3] Hertig M, Wolbach SB. Studies on rickettsia-like microorganisms in insects. *J Med Res* 1924; **44**(3): 329-374.

[4] Hertig M. The rickettsia, *Wolbachia pipiens* (gen. et sp.n.) and associated inclusions of the mosquito, *Culex pipiens*. *Parasitology* 1936; **28**(4): 453-486.

[5] Werren JH, Jaenike J. *Wolbachia* and cytoplasmic incompatibility in mycophagous *Drosophila* and their relatives. *Heredity (Edinb)* 1995; **75**(Pt 3): 320-326.

[6] Jeyaprakash A, Hoy MA. Long PCR improves *Wolbachia* DNA amplification: wsp sequence found in 76% of sixty-three arthropod species. *Insect Mol Biol* 2000; **9**(4): 393-405.

[7] Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. How many species are infected with *Wolbachia*?-a statistical analysis of current data. *FEMS Microbiol Lett* 2008; **281**(2): 215-220.

[8] Sumithra, Guruprasad NM, Puttaraju HP. A comparative analysis of long PCR and standard PCR technique in detecting the *Wolbachia* endosymbiont. *Curr Trends Biotechnol Pharm* 2012; **6**(4): 472-478.

[9] Guruprasad NM, Mouton L, Puttaraju HP. Effect of antibiotic, temperature curing of *Wolbachia* and seasonal variation on the reproductive fitness of the Uzifly *Exorista sorbillans* (Diptera: Tachinidae). *Symbiosis* 2011; **54**(3): 151-158.

[10] Saridaki A, Bourtzis K. *Wolbachia*-induced reproductive parasitism and applications. *Entomol Hell* 2009; **18**: 3-16.

[11] Guruprasad NM, Jalali SK, Puttaraju HP. *Wolbachia* and its prospectives in biological control of insect pests and diseases vectors. *Appl Entomol Zool* 2013; doi: 10.1007/s13355-013-0178-2.

[12] Brelsfoard CL, Dobson SL. An update on the utility of *Wolbachia* for controlling insect vectors and disease transmission. *Asian Pac J Mol Biol Biotechnol* 2011; **19**(3): 85-92.

[13] Pinto SB, Mariconti M, Bazzocchi C, Bandi C, Sinkins SP. *Wolbachia* surface protein induces innate immune responses in mosquito cells. *BMC Microbiol* 2012; **12**(Suppl 1): S11.

[14] Iturbe-Ormaetxe I, Walker T, O'Neill SL. *Wolbachia* and the biological control of mosquito-borne disease. *EMBO Rep* 2011; doi: 10.1038/embor.2011.84.

[15] Walker T, Moreira LA. Can *Wolbachia* be used to control malaria? *Mem Inst Oswaldo Cruz* 2011; **106**(Suppl 1): S212-S217.

[16] Rasgon JL. Using predictive models to optimize *Wolbachia*-based strategies for vector-borne disease control. In: Aksoy S, editor. *Transgenesis and the management of vector-borne disease*. New York: Springer; 2008, p. 1-11.

[17] Min KT, Benzer S. *Wolbachia*, normally a symbionts of *Drosophila*, can be virulent, causing degeneration and early death. *Proc Natl Acad Sci U S A* 1997; **94**(20): 10792-10796.

[18] Hedges LM, Brownlie JC, O'Neill SL, Johnson KN. *Wolbachia* and virus protection in insects. *Science* 2008; **322**(5905): 702.

[19] Teixeira L, Ferreira A, Ashburner M. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol* 2008; **6**(12): 2.

[20] Osborne SE, Leong YS, O'Neill SL, Johnson KN. Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathog* 2009; **5**(11): 1000656.

[21] Moreira LA, Itrube-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. *Cell* 2009; **139**(7): 1268-1278.

[22] Blandin S, Shiao SH, Moita LF, Janse CJ, Waters AP, Kafatos FC, et al. Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell* 2004; **116**(5): 661-670.

[23] Povelones M, Waterhouse RM, Kafatos FC, Christophides GK. Leucine-rich repeat protein complex activates mosquito complement in defense against *Plasmodium* parasites. *Science* 2009; **324**(5924): 258-261.

[24] Kambris Z, Blagborough AM, Pinto SB, Blagrove MS, Godfray HC, Sinden RE, et al. *Wolbachia* stimulates immune gene expression and inhibits *Plasmodium* development in *Anopheles gambiae*. *PLoS Pathog* 2010; **6**(10): 1001143.

[25] Moreia LA, Saig E, Turley AP, Ribeiro JM, O'Neill SL, McGraw EA. Human probing behavior of *Aedes aegypti* when infected with a life shortening of *Wolbachia*. *PLoS Negl Trop Dis* 2009; **3**(12): 568.

[26] Bian G, Xu Y, Lu P, Xie Y, Xi Z. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathog* 2010; **6**(4): 1000833.

[27] Glaser RL, Meola MA. The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. *PLoS One* 2010; **5**(8): 11977.

[28] World Health Organization. World malaria report. Geneva: World Health Organization; 2008. [Online] Available from: http://www.who.int/malaria/publications/atoz/9789241563697/en/index.html. [Accessed on 16 August, 2013]

[29] Kambris Z, Cook PE, Phuc HK, Sinkins SP. Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* 2009; **326**(5949): 134-136.

[30] Hughes GL, Ren X, Ramirez JL, Sakamoto JM, Bailey JA, Jedlicka AE, et al. *Wolbachia* infections in *Anopheles gambiae* cells: trnscriptomic characterization of a novel host-symbiont interaction. *PLoS Pathog* 2011; **7**(2): 1001296.

[31] Kokoza V, Ahmed A, Woon Shin S, Okafor N, Zou Z, Raikhel AS. Blocking of *Plasmodium* transmission by cooperative action of cecropin A and defensin A in transgenic *Aedes aegypti* mosquitoes. *Proc Natl Acad Sci* 2010; **107**(18): 8111-8116.

[32] Jin CY, Ren XX, Rasgon JL. The virulent *Wolbachia* strain wMelPop efficiently establishes somatic infections in the malaria vector *Anopheles gambiae*. *Appl Environ Microbiol* 2009; **75**(10): 3373-3376.