ABSTRACT

Dengue is one of the most important mosquito-borne viral disease of humans. Dengue epidemics have been occurring annually since 2006 in Pakistan involving more than 40,000 dengue cases along with more than 500 deaths till 2015. Furthermore, there is a great risk of dengue, chikungunya and other mosquito borne diseases due to favorable climate for vector breeding. Lack of effective vaccine and antiviral drugs along with increased problem in insecticide resistance and their toxic effects on environment make it urgent to find the novel alternate approaches for dengue control in Pakistan. The current study documents the possibility of the control of major dengue vector *Aedes aegypti* using maternally transmitted bacteria of the genus *Wolbachia*. These are obligate, intracellular symbionts, frequently found in more than 65% insects but not naturally found in *Ae. aegypti*. In the recent decades, *Wolbachia* has got much importance due to its potential of altering population of their hosts by cytoplasmic incompatibility (CI), pathogen propagation inhibition and shortening of the life span in mosquito vectors. The ability of *Wolbachia* to spread into mosquito vector population along with inhibition in viral propagation justifies the need to use *Wolbachia* as a biological agent for the control of dengue. The current study involves detection of various native strains of *Wolbachia* from local five insect species; *Aedes albopictus*, *Bemisia tabaci*, *Culex quinquefaciatus*, *Drosophila melanogaster* and *D. simulans* collected from Punjab, Pakistan. However, six insect species (*Ae. aegypti*, *Anopheles subpictus*, *An. stephensi*, *Cx. tritaeniorhynchus*, *Microtermes mycophagus* and *Periplaneta americana*) were found negative for *Wolbachia*. Native seven *Wolbachia* strains; *w*AlbA/*w*AlbB, *w*Tab, *w*Pip, *w*Mel / *w*MelPop and *w*Riv were molecularly characterized using polymerase chain reaction (PCR) by targeting the *Wolbachia* surface protein (*wsp*), 16S rRNA and filamenting temperature-sensitive mutant Z (*ftsZ*) genes. Secondly, *w*AlbB *Wolbachia* isolated from wild collected *Ae. albopictus* was successfully induced in laboratory reared local dengue vector *Ae. aegypti* via embryonic microinjection. The stable vertical transmission of *w*AlbB in *Ae. aegypti* population was achieved within eight generations. In addition, the same native strain *w*AlbB induced strong inhibition of dengue viruses in newly developed wc2 *Ae. aegypti* cell line in density dependent manner. No impact of *w*AlbB on general fitness, fecundity, body size and mating competitiveness of new host *Ae. aegypti* was observed under

laboratory and semi-field conditions. However, *Wolbachia* presented strong CI effect as zero egg hatching in crosses between *Wolbachia* infected males and wild uninfected females. In addition, there was significant ($P < 0.0001$) decrease in life span of *Wolbachia* infected females under starvation conditions as compared to uninfected to females. In conclusion locally collected *Wolbachia* strain *w*AlbB has a great potential to block dengue viruses transmission to humans and suppress *Ae. aegypti* vector population in natural field conditions. The strain could be used as a biological tool for dengue control in Pakistan.