**Wolbachia genetic similarity in different insect host species: Drosophila melanogaster and Yogyakarta’s (Indonesia) Aedes aegypti as a novel host**

**ANWAR ROVIK, EDWIN WIDYANTO DANIWJAYA, ENDAH SUPRIYATI, AYU RAHAYU, DIAN ARUNI KUMALARWATI, UTARI SARASWATI, ANASTASIA EVI HANDAYANINGSIH, MIFTA PRATIWI RACHMAN, RISKY OKTRIANI, IRIANTI KURNIASARI, DAMIANA SAPTANANDAR, INDAH NURHAYATI, RIZKI SHOLEH, BUDI ARIANTO, WARSITO TANTOWIJOYO, RIRIS ANDONO AHMAD, ADI UTARINI, EGGI ARGUNI**

1 Center of Tropical Medicine, World Mosquito Program Yogyakarta, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jl. Teknika Utara, Sleman 55281, Yogyakarta, Indonesia
2 Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Sunan Kalijaga. Jl. Laksda Adisucipto, Sleman 55281, Yogyakarta, Indonesia
3 Department of Internal Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jl. Farmako, Sleman 55281, Yogyakarta, Indonesia
4 Department of Biotechnology, Universitas Teknologi Sumbawa. Jl. Raya Olat Maras, Sumbawa 84371, West Nusa Tenggara, Indonesia
5 Department of Biochemistry, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jl. Farmako, Sleman 55281, Yogyakarta, Indonesia
6 Department of Biotechnology, Sustainable Agricultural Extension Program, Politeknik Pembangunan Pertanian Malang. Jl. Dr. Cipto 144, Malang 65215, East Java, Indonesia
7 Faculty of Pharmacy, Universitas Sanata Dharma. Jl. Paingan, Depok, Sleman 55281, Yogyakarta, Indonesia
8 Department of Epidemiology, Biostatistics and Population Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jl. Farmako, Sleman 55281, Yogyakarta, Indonesia
9 Department of Health Policy and Management, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jl. Farmako, Sleman 55281, Yogyakarta, Indonesia
10 Department of Child Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jl. Farmako, Sleman 55281, Yogyakarta, Indonesia. Tel./fax: +62-815-7853-1122, *email: eggiarguni@ugm.ac.id

Manuscript received: 19 February 2022. Revision accepted: 20 April 2022.

**Abstract.** Rovik A, Daniwjaya EW, Supriyati E, Rahayu A, Kumalarwati DA, Saraswati U, Handayaningstih AE, Rachman MP, Oktriani R, Kurniasari I, Candrasari DS, Nurhayati I, Sholeh R, Arianto B, Tantowiyo W, Ahmad RA, Utarini A, Arguni E. 2022. Wolbachia genetic similarity in different insect host species: Drosophila melanogaster and Yogyakarta’s (Indonesia) Aedes aegypti as a novel host. Biodiversitas 23: 2321-2328. Wolbachia naturally presents in a large number of insects and other arthropod species. The Wolbachia strain wMel from Drosophila melanogaster has been stably transfected into Aedes aegypti where it stops the mosquito host from being infected with medically important arbovirus like dengue. Consequently, A. aegypti infected with wMel have been released in Indonesia as a public health intervention against dengue. This study genetically compared wMel from Yogya field-caught D. melanogaster and the wMel in stably transfected A. aegypti used for field releases in Yogyakarta, Indonesia. The genetic similarity between wMel Wolbachia was evaluated by sequencing of Wolbachia surface protein (wsp) gene and some polymorphic genomic regions of insertion sites (IS) and variable number tandem repeats (VNTR) loci. The sequence of the Wolbachia surface protein (wsp) gene was 100% identical between hosts. There is no insertion sequence among specimens. The insertion sequence IS-WD1310 was identical between wMel from both hosts and among other strains, as well as the IS-WDS167/7. The VNTR-141 period was identical within wMel from both hosts and among other strains, the VNTR-105 as well. Wolbachia Yogyo field-caught D. melanogaster and Wolbachia strain wMel present in A. aegypti used for bio-control of dengue were genetically identical. These findings provide beneficial understanding to answer the public attention on safety issues, especially on the genetic similarity between Wolbachia strain in the natural and transfected hosts of this novel technology for dengue control.

**Keywords:** Aedes aegypti, Drosophila melanogaster, genetic similarity, novel host, Wolbachia, wMel

**INTRODUCTION**

Wolbachia is obligate intracellular endosymbiotic bacteria (Calvitti et al. 2010). It maternally transmitted and naturally presents in many insect species (Werren et al. 2008). Wolbachia can manipulate the tissues and reproductive cycles of the host to increase its spread through insect populations (Stevens et al. 2001; Tram et al. 2003). Therefore, Wolbachia infection is estimated to occur naturally in 40-65% of insects, as well as other arthropods and some nematode species (Hilgenboecker et al. 2008; Kumalarwati et al. 2020; Werren et al. 2008; Zug and Hammerstein 2012). In its early discovery, Wolbachia has classified as a strain of one species, Wolbachia pipiens (Calvitti et al. 2010). Currently, Wolbachia is named commonly by their hosts, such as the native wRi strain of Drosophila simulans (isolated in Riverside, California), the native wAlbB strain of Aedes albopictus (Ae. albopictus), the original wPip strain of Culex pipiens (Cx. pipiens), and the wCau strain native to Cdadraulata sp.
Wolbachia has varied effects on the host, including both pathogenic and mutualistic effects. In the host, Wolbachia interferes with its host reproduction resulting in male parthenogenesis, homicide or feminization, sex ratio distortion (Dyson et al. 2002; Hurst et al. 2002), and cytoplasmic incompatibility (Ilinsky and Zakharov 2011). In contrast, Wolbachia also allows mutualistic effects for insect hosts, such as in wasps, Drosophila, and bedbugs (Dedeine et al. 2005; Hosokawa et al. 2010; Sturr and Cline 2002). Aedes aegypti (Ae. aegypti) mosquitoes, the most notable vector for arboviruses transmission, are not naturally infected with Wolbachia. Meanwhile, independent studies have reported that Wolbachia infection is naturally present in other mosquito species, such as Cx. pipiens and Ae. albopictus (Afitzah et al. 2015; Rasgon and Scott 2004; Tsai et al. 2004). Several Wolbachia strains have been successfully artificially transferred into the Aedes mosquito, such as wMelPop (McMeniman et al. 2009), wMel (Walker et al. 2011), wAlbB (Flores et al. 2020; Xi et al. 2005), wRi, wMelICS, wPip (Fraser et al. 2017), wAlbBA, and wAu (Ant et al. 2018).

Wolbachia has applied in various fields, including biotechnology, agriculture, and public health. Wolbachia strain wMelPop in D. melanogaster has been shown to shorten the life of the host. This effect is also seen when wMelPop has been transferred to its novel host, Ae. aegypti (McMeniman et al. 2009). The wMelPop and wMel strains have been shown to reduce dengue (Bian et al. 2010; Blagrove et al. 2012; Ferguson et al. 2015; Flores et al. 2020; Frentiu et al. 2014; Moreira et al. 2009; Walker et al. 2011), Zika (Aliota et al. 2016; Dutra et al. 2016), and chikungunya virus transmission potential of mosquitoes (Blagrove et al. 2013; Moreira et al. 2009; Van den Hurk et al. 2012), as well as the malaria parasite Plasmodium gallinaceum (Moreira et al. 2009) and Plasmodium berghei (Kambris et al. 2010). In some studies, Wolbachia strains that have been transferred from an original host to the new host are very stable. This condition generally occurs when Wolbachia is transferred to a new host, within or between related species in the same genus or family (Dobson et al. 2002; Xi et al. 2005). The success of Wolbachia transfer may depend on the bacteria’s ability to adapt to the new intracellular environment. In other cases, Wolbachia strains exhibit fluctuating infection densities and varying degrees of transovarial transmission. This condition is encountered when the transfer occurs between phylogenetically distant hosts. As a result, the infection is often lost in several generations of the host (Dobson et al. 2002) and has the potential to undergo phenotypic changes (Chrostek and Teixeira 2015).

The application of Wolbachia in eliminating dengue has been performed in several countries which are involved in the World Mosquito Program (WMP) initiative, such as Australia, Vietnam, Brazil, Columbia, Vanuatu, Sri Lanka, Fiji, Kiribati, New Caledonia, and Indonesia. The WMP Yogyakarta Indonesia released the wMel-infected Ae. aegypti in 2015 in a small area of Sleman and Bantul Regency (Tantowijoyo et al. 2020), and the City of Yogyakarta in 2016 (Indriani et al. 2020; Utarini et al. 2021). The current study compared the genetic similarity of Wolbachia (Riegler et al. 2005; 2012) from two different hosts: D. melanogaster and Yogya Ae. aegypti as a novel host.

MATERIALS AND METHODS

Backcrossing of wMel-infected Yogya Aedes aegypti

The wMel-infected Ae. aegypti was sourced from the laboratory of WMP Australia (Center for Research and Development of Biomedical and Basic Health Technology, Ministry of Health, Indonesia issued an import recommendation No. YF.01.11/III/4554/2012). The wMel strain was artificially transferred from D. melanogaster to Ae. aegypti by embryonic microinjection (Walker et al. 2011). A wMel-infected Yogya Ae. aegypti was generated by performing backcrossing for a few generations to introgress the local genetic profile into the wMel-infected Ae. aegypti (details provided in Tantowijoyo et al. 2020).

Rearing of wMel-infected Yogya Aedes aegypti

The wMel-infected Yogya Ae. aegypti mosquitoes were reared in a controlled laboratory (details provided in Tantowijoyo et al. 2020).

Rearing of Drosophila melanogaster

The wild-type Drosophila sp. was sourced from the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. The morphologically identified D. melanogaster was reared and maintained in a rearing cage and fed with banana pulp regularly. A total of 50 Drosophila specimens were sampled and preserved with ethanol 80%.

DNA extraction

The samples were individually rinsed with ethanol 96% and aquadest. Then, the DNA samples were extracted following the DNA extraction procedure of the Genejet Genomic DNA Purification Kit (Thermo Scientific). A total of 1 μL of extracted DNA was put on a Nanojet machine (NanoVue) to quantify its concentration and purification. (Kumalawati et al. 2020)

DNA amplification

Wolbachia genetic loci were amplified from genomic DNA by using specific primers. The Wolbachia surface protein (wsp) gene permitted an initial screening of the collected sample. The wsp detection was also used as a quality control for DNA extraction which was amplified using the primers wsp-81F and wsp-691R. The 81F-691R primer set detects the Wolbachia surface protein of length 590-632bp depending on the individual Wolbachia strain (Zhou et al. 1998). Polymerase chain reaction (PCR) tests were run using thermal-cycler machines (BioRad C-1000 Touch). PCR conditions were as follows 95°C, 4min; 34 cycles [95°C, 30s; 50°C, 30s; 72°C, 60s]; 72°C, 10min; 12°C, 5 min (for 81F-691R and IS-WD1310); 95°C, 4min; 34 cycles [95°C, 30s; 50°C, 30s; 72°C, 90s]; 72°C, 10min; 12°C, 5 min (for VNTR-141, VNTR-105, INV [WD0394-WD0541]); 95°C, 4min; 34 cycles [95°C, 30s; 50°C, 30s; 72°C, 120s]; 72°C, 10min; and 12°C, 5 min (for IS-WD5167) (details provided in Riegler et al. 2005; 2012; Zhou et al. 1998).
Visualization of PCR product

PCR products were verified using 1% (w/v) agarose gel electrophoresis (Mupid® eXu submarine electrophoresis system). The electrophoresis was performed at 135 volts for 60 minutes. The resulting bands were visualized using Gel Doc Documentation System (Protein Simple) and compared with DNA Ladder (100bp or 1kb Ladder, Thermo Scientific) to know the estimation size and their possibilities of polymorphism. Sanger sequencing was performed by the 1st Base Pte. Ltd. Company, Singapore.

Phylogenetic analysis of wsp gene

Sequence analysis was performed by using BioEdit ver. 7.2 software. The phylogenetic tree was constructed by using a neighbor-joining (NJ) method within the MEGA ver. X software. A 1,000 bootstrap replication was used to construct the evolution distance (Aff Zhah et al. 2015). The wsp sequence was compared to the Wolbachia endosymbiont among different species and hosts, including Wolbachia endosymbiont of Drosophila melanogaster isolate wMel (KX650072), Wolbachia pipiens strain wMel (DQ235407), Wolbachia endosymbiont of Drosophila simulans strain Riverside 1988 (EF423761), Wolbachia endosymbiont of Bemisia tabaci isolate 67.3 (KM404298), Wolbachia endosymbiont of Eurema hecabe isolated from Indonesia (AB278218) and Malaysia (AB278206), Wolbachia endosymbiont of Culex quinquefasciatus isolate Pers6 (MN893364), Wolbachia pipiens strain wMelPop (AF338346), Wolbachia endosymbiont of Drosophila melanogaster isolate Beijing (KU870673), Wolbachia sp. wMel isolate Wuhan (FJ403330), Wolbachia sp. wMel isolate Yunnan (FJ403332), Wolbachia endosymbiont of Drosophila sp. isolate D3G (MN900914), and Wolbachia endosymbiont of Drosophila sp. isolate D3E (MN900913).

Sequence analysis of insertion sites and tandem repeat loci

The sequence from different hosts was aligned within MEGA ver. X software to evaluate the presence or absence of insertion sites, the tandem repeat loci as well. The periodicity or copy number of the tandem repeat sequence was analyzed by using Tandem Repeat Finder ver. 3.1 which is available at (http://tandem.bu.edu/trf/trf.html) (details provided in Riegler et al. 2012). The periodicity of VNTR-141 and VNTR-105 loci were compared to reference sequences including Wolbachia strain wMel (JF797613) and wMel (JF797619).

Ethics approval

This present study was part of the World Mosquito Program (WMP) Yogyakarta (previously known as the Eliminate Dengue Program) at Phase 1. The research protocol has been approved (No. KE/FK/01/EC/2012) by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.

RESULTS AND DISCUSSION

Results

In this study, some polymorphic genomic markers were sequenced to explore the extent of similarity between a wMel strain of Yogyakarta-sourced D. melanogaster and the wMel strain of origin that has been stably transinfected in Ae. aegypti mosquitoes by targeting Wolbachia surface protein (wsp) gene, insertion sites (IS5 family), and variable number tandem repeat (VNTR) loci.

All 50 D. melanogaster and all 50 wMel-infected Yogya Ae. aegypti were infected by Wolbachia as determined by detection of the wsp gene (Figure 1). Figure 2 showed that the wsp gene was 100% identical among tested hosts and reference wsp gene of Wolbachia endosymbiont of Drosophila melanogaster isolate wMel. Agarose gel analysis of insertion sites (IS5 family) amplification products presented identical sizes of expected bands (Figure 3A). It showed that the IS-WD1310 insertion site was absent from all specimens, the IS-WD516/7 insertion site as well (Figure 3B). The agarose gel analysis of tandem repeat loci presented identical sizes of expected bands for the VNTR-105 period and showed a slight difference of the VNTR1-141 period between Wolbachia endosymbiont of D. melanogaster and wMel-infected Yogya Ae. aegypti (Figure 3C-D).

In this study, the analysis results of insertion sites (IS-WD1310) amplification showed that the sequence was identical among tested hosts and reference genome (KX650072). Therefore, all samples lack the IS-WD1310 element based on the size of the sequenced fragment (Table 1). Like IS-WD1310 analysis, the sequenced element of IS-516/7 insertion showed no consistent difference between hosts, D. melanogaster and wMel-infected Ae. aegypti. The amplicon size (including the IS-WD516/7 element) is predicted to be 2,488bp. Primer set targeting the IS-WD516/7 element might be difficult to amplify those sequences. It suggests using additional internal sequencing primers to allow for enough overlapping sequence and properly address the locus.

The analysis results of tandem repeat locus (VNTR-141) amplification showed a gap of 140bp for the sequence of Wolbachia endosymbiont of D. melanogaster. Meanwhile, all sequences of wMel-infected Ae. aegypti match the reference sequence (JF797613) in terms of length. Site with tandem repeats having period sizes from 18bp to 264bp and internal match percentage from 90% to 99%. The sequence periodicity of the VNTR-141 period of Wolbachia sequence was different among host i.e., 6.3 for D. melanogaster and 7.3 for Ae. aegypti specimens (Table 2).

In this study, the analysis results of tandem repeat locus (VNTR-105) amplification showed no difference in the number of repeats across all samples, Wolbachia endosymbiont of D. melanogaster and wMel-infected Ae. aegypti. There is no change in repeat number based on the reference sequence (JF797619). Site with tandem repeats having period sizes from 80bp to 291bp and internal match percentage from 97% to 98%. The sequence periodicity of the VNTR-105 period of Wolbachia sequence from both hosts was identical i.e., 4x1 + 2x0.5 for Ae. aegypti and D.
melanogaster specimens (Table 2). It showed that the structure of the VNTR-105 period is less conserved than the VNTR-141 period.

Discussion

Wolbachia infection has been studied widely in the context of evolution, biology, and ecology, and its application in various fields, such as health, agriculture, and vector control. Several studies (e.g. Bing et al. 2014; McMeniman et al. 2009; Morrow et al. 2014) have transferred Wolbachia across species and genera within and between insect orders, both single and multiple hosts; for example, Dobson et al. (2002) found that infection of Wolbachia strain wRi was maintained stably in the host cells of Drosophila, Spodoptera, and Aedes mosquitoes. Stable transfection has been successfully achieved for several species of mosquitoes including Ae. aegypti, Ae. albopictus, Ae. polynesiensis, Cx. pipiens, An. stephensi, and An. gambiae (Bian et al. 2013; McMeniman et al. 2009; Hoffmann et al. 2011; Jin et al. 2009; Walker et al. 2011; Xi et al. 2005). Those studies aimed to determine the ability of Wolbachia to increase host immunity against various pathogenic infections in a different host.

Wolbachia have been reported to suppress viruses from a range of RNA virus families. In laboratory research, Wolbachia-infected Ae. aegypti can inhibit the replication of the dengue virus in the mosquito's body (Bian et al. 2010; Ferguson et al. 2015; Flores et al. 2020; Moreira et al. 2009). In some field studies, Wolbachia-infected Aedes mosquitoes are shown to reduce the dengue cases e.g., Nazni et al. (2019), Ryan et al. (2019), Tantowijoyo et al. (2020), Indriani et al. (2020), and Utarini et al. (2021). During field-application of Wolbachia-infected Ae. aegypti, various safety issues might be raised by the public or communities, including the possibilities of genetic changes of Ae. aegypti in their natural habitat.

Several studies reported the possibilities of genotypic and phenotypic changes of Wolbachia in a transfected host e.g., McMeniman et al. (2008), Schneider et al. (2013), and Woolfit et al. (2013). The genetic similarity of Wolbachia between original species and local infected Ae. aegypti mosquito was important to increase self-evidence to convey this novel technology to local people residing in the release area in Yogyakarta, and hopefully when implemented to other areas throughout Indonesia. Therefore, monitoring of genetic changes is important to be conducted.

The Wolbachia surface protein (wsp) has been one of the most widely used for Wolbachia identification and systematics. It is well-known that there are 11 supergroups of Wolbachia which have been designated based on their ftsZ, wsp, and 16S rRNA genes (Riegler et al. 2012; Zhao et al. 2021). Zhou et al. (1998) proposed that the wsp sequence similarity among Wolbachia strains should be greater than 97.5% identical. In this study, the analysis of sequence similarity showed that Wolbachia was 100% identical among tested hosts and reference wsp gene of Wolbachia endosymbiont of D. melanogaster isolate wMel (KX650072) (Figure 2). It showed that Wolbachia native to Yogya D. melanogaster has no difference with wMel strain in a novel host, Yogya Ae. aegypti.

![Figure 1. Initial screening of collected samples for DNA quality assessment](Image)

**Table 1.** Sequence analysis of insertion sites in different Wolbachia hosts using MEGA ver. X software

<table>
<thead>
<tr>
<th>Host</th>
<th>Wolbachia strain</th>
<th>Marker</th>
<th>Presence/Absence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila melanogaster</td>
<td>wMel</td>
<td>IS-WD1310</td>
<td>Absent</td>
</tr>
<tr>
<td>Yogya Ae. aegypti</td>
<td>wMel</td>
<td>IS-WD516/7</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**Table 2.** Sequence analysis of tandem repeat loci in different Wolbachia hosts using a Tandem Repeat Finder ver. 3.1.

<table>
<thead>
<tr>
<th>Host</th>
<th>Periodicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VNTR-141</td>
</tr>
<tr>
<td>wMel (JF97613)</td>
<td>7.3</td>
</tr>
<tr>
<td>wMel (JF97619)</td>
<td>7.2</td>
</tr>
<tr>
<td>Drosophila melanogaster isolate 1</td>
<td>6.3</td>
</tr>
<tr>
<td>Drosophila melanogaster isolate 2</td>
<td>6.3</td>
</tr>
<tr>
<td>Drosophila melanogaster isolate 3</td>
<td>6.3</td>
</tr>
<tr>
<td>Drosophila melanogaster isolate 4</td>
<td>6.3</td>
</tr>
<tr>
<td>Drosophila melanogaster isolate 5</td>
<td>6.3</td>
</tr>
<tr>
<td>Aedes aegypti isolate 1</td>
<td>7.2</td>
</tr>
<tr>
<td>Aedes aegypti isolate 2</td>
<td>7.2</td>
</tr>
<tr>
<td>Aedes aegypti isolate 3</td>
<td>7.2</td>
</tr>
<tr>
<td>Aedes aegypti isolate 4</td>
<td>7.3</td>
</tr>
<tr>
<td>Aedes aegypti isolate 5</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Note: "wMel reference sequence (containing tandem repeat loci). n/a: not applicable
The current study evaluated some polymorphic markers as published by Riegl et al. (2005; 2012), even if the inversion period was not evaluated in this study. Wu et al. (2004) has submitted a complete genome map of Wolbachia strain wMel. The Wolbachia genome shows a very high proportion of insertion sites of the transposable element, the VNTR loci, and genes encoding ankyrin return domain. The results of this study showed that insertion sites (IS-WD5167 and WD1310) were absent in the sequenced fragment in both Wolbachia hosts i.e., D. melanogaster and Ae. aegypti. The proportion of tandem repeat periods seems to vary among isolates. The analysis of VNTR-141 loci matches the reference, even if a gap of 140bp sequence was found for Wolbachia endosymbiont of D. melanogaster. Riegl et al. (2012) reported the periodicity of 141bp element in wMel genome is 7.3 (consisting of the internal 15bp direct repeat A, a 23bp hairpin with a 9bp palindromic stem, an 18bp insertion, and the internal 15bp direct repeat B). Meanwhile, the periodicity of 105bp element in wMel full genome is 4x1 + 2x0.5 (containing four complete 105bp periods and two periods with 25bp internal deletions). The analysis of the VNTR-105 loci matches the reference in terms of length.

Figure 2. Phylogenetic tree of Wolbachia endosymbiont of Drosophila melanogaster and wMel-infected Yogya Aedes aegypti based on the wsp gene sequence. Wolbachia endosymbiont of Drosophila melanogaster and wMel-infected Yogya Aedes aegypti were placed in the same clade with wMel reference sequence (KX650072), as well as Wolbachia strain wMelPop and wMel.
In conclusion, this study showed no difference in the genetic of Wolbachia among tested hosts and wMel reference genome (NC002978). It means that Wolbachia from Yogya D. melanogaster and Wolbachia strain wMel present in Ae. aegypti used for bio-control of dengue were genetically identical. This result supported previous studies which found that there are no or only slight genetic differences among Wolbachia strains in the natural host, transfected or novel host, and field-caught specimens. The key attribute of Wolbachia is that the World Mosquito Program is basing its intervention on its demonstrated ability to interfere with the replication of dengue in Wolbachia-infected Aedes mosquitoes. Thus, this information of genetic similarity will be a positive response for community safety concerns, not only for the concern of capability of reducing dengue transmission but more importantly, that Wolbachia strain that being transfected into mosquito is similar to those in the natural organism. Questions such as the persistence of the virus blocking capacity of Wolbachia after generations in the natural population are essential to be answered in the future.

ACKNOWLEDGEMENTS

The authors thank all of World Mosquito Program (WMP) Yogya team and Tahija Foundation for the financial support for the activities of WMP Yogya, Indonesia.

REFERENCES


