Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(6): 166-168

Received: 15-08-2015
Accepted: 10-10-2015

Erina Yatmasari
a) Doctoral Program of Health Sciences Department, Faculty of Public Health, Airlangga University, Surabaya, East Java, Indonesia.
b) Parasitology Department, Faculty of Medicine, Hang Tuah University, Surabaya, East Java, Indonesia.

Correspondence:
Erina Yatmasari
a) Doctoral Program of Health Sciences Department, Faculty of Public Health, Airlangga University, Surabaya, East Java, Indonesia.
b) Parasitology Department, Faculty of Medicine, Hang Tuah University, Surabaya, East Java, Indonesia.

Evaluation of the oligonucleotide primers used to detect genetic polymorphism of the *Aedes aegypti* Larval populations in Indonesia

Erina Yatmasari

Abstract
The Randomly Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD-PCR) has been used for molecular studies for determining genetic polymorphism of the *Aedes aegypti* populations. The aim of this study was to evaluate the oligo nucleotide primers which could be used for detecting of genetic variations of the *Aedes aegypti* Larval populations in Indonesia, using RAPD-PCR tecnique. DNA was extracted from individual mosquito larval from two different geographic populations of *Aedes aegypti*, and amplified using single primers of arbitrary oligo nucleotide sequences. There were 20 single primers tested, five produced eight bands successfully.

Keywords: *Aedes aegypti*, genetic polymorphism, RAPD-PCR, Indonesia.

1. Introduction
Detection of genetic variations of *Aedes aegypti* Larval populations, which is important in controlling and eradicating, could be done by molecular methods such as the Randomly Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD-PCR), which was previously mentioned as a method that is cheap, easy, quick and no special skills required [1-3, 6-8].

RAPD-PCR is known as a useful method in analyzing complexes of species. It has several advantages, such as requiring a few genomic DNA sample and has a simple methodology. RAPD-PCR is very useful when there is no prior knowledge of sequencing data, and it does not require hazardous materials. This technique has been used to detect polymorphisms in amplified DNA fragments in many organisms. Polymorphisms have been used as genetic markers in studies involving species diagnostics, population differentiation and DNA fingerprinting.

However, RAPD-PCR results that are visualized with the agarose gel is not always showed as expected. Frequently the oligo primers which were used in previous studies, especially from other countries, could not be used in recent condition of the laboratories [1]. Sometimes requires any manipulations of the results to be observed and documented. In a preliminary study there were twenty RAPD-PCR primers were screened, and five primers were selected based on their reproducibility and clarity.

The aim of this study was to evaluate the oligo nucleotide primers which could be used for further detection of genetic variations of the *Aedes aegypti* larval populations from Indonesia, using RAPD-PCR tecnique, which could be investigated and associated with insecticides resistance, especially larvicides resistance futhermore [4,7]. The oligonucleotide primers which could be suitable to this study, would be highly recommended to be used for advanced studies of the *Aedes aegypti* larval populations in Indonesia.

2. Materials and Methods
This study was conducted in The Laboratory of Parasitology, Faculty of Medicine, Gadjah Mada University of Indonesia.

Aedes aegypti Larval populations were reared from collected mosquito eggs using ovitraps at latitude S 7 °16'24.2508" and longitude E 112 °45'37.6056" of Surabaya, and the laboratory isolate as the control. The collected eggs then were reared until reaching late 3rd or early 4th larval stage.

Genomic DNA was isolated from fresh *Aedes aegypti* Larval stage. The extracting method according to Ballinger-Crabtree *et al.* [8], with a slight modification.

The mixture then was incubated for one hour at -20 °C and spun at 12,000 rpm for 10 minutes, the DNA final pellet which was resuspended in 100 µl of T-10 buffer (10 mM Tris-HCl, pH 8.5) and stored at -20 °C.

The oligo nucleotide primers which were used in this study has been already screened in a preliminary study, according to the previous studies of the polymorphism of the *Aedes aegypti* larval populations in many other countries. There were 20 of 10 bp oligo nucleotide primers (5' to 3') were selected in a preliminary study (TGCCGAGCTG, ACTTCGACAA, TTCCCCCGCT, GGTGCGGGAA, GAGTCTCAGG, ACGGCGTATG, CTCAGTGTCC, GTCCGGAGTG, CAGGCCCTTC, AATCGGGCTG, GTGATCGCAG, CAATCGCCGT, TCGGCGATAG, CAGCACCCAC, TCTGTGCTGG, TTCCGAACCC, GTTGCGATCC, AGGTGACCGT, AGGTGACCGT and GTGACGTAGG) and only five of the primers successfully amplified the DNA of the *Aedes aegypti* late 3rd or early 4th larval stage, produce bands as the results of the RAPD-PCR. The sequences the primers were: 5'GTTGCGATCC'3, 5'AATCGGGCTG'3, 5'AGGTGACCGT'3, 5'GAAACGGGTG'3, and 5'GTGACGTAGG'3, which were chosen to be used in the core study.

This study was conducted by preparing genomic DNA of the two populations of the *Aedes aegypti* late 3rd or early 4th larval stage. Amplification was performed in a thermal cycler using 1 cycle at 94°C for 4 min, followed by 45 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min and a final extension at 72°C for 5 min, and stored at 4°C. The amplified products were analyzed on 1.2 % agarose gels run in 1 × TAE buffer, a 100 bp DNA ladder was used as a molecular marker, visualized with an ultraviolet transilluminator and documented.

3. Results

This study found that each of the five oligo nucleotide primers which were used in this study, could be successfully amplified the genomic DNA of two population of the *Aedes aegypti* late 3rd or early 4th larval stage, which the laboratory population produced only one band of each, as the result of being amplified by the sequences of (5' to 3') GTTGCGATCC, AATCGGGCTG and AGGTGACCGT, none of band was produced by the sequences of AGGTGACCGT and GAAACGGGTG. The population from latitude S 7°16'24.2508" and longitude E 112°45'37.6056" of Surabaya produced only one band of each, as the result of being amplified by each of five sequences.

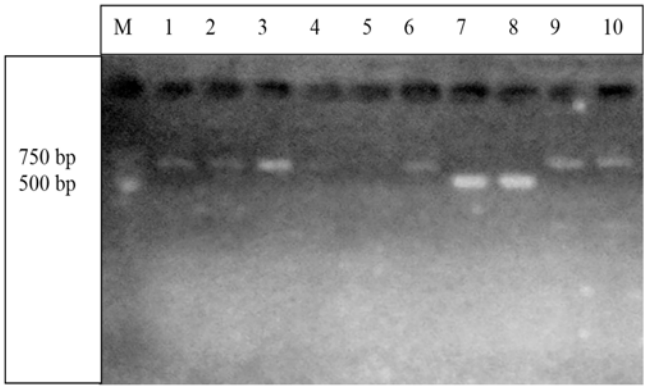

Fig 1: Visualisation of The RAPD PCR Result

The total number of oligo nucleotide primers tested were five and of these five primers produce polymorphic bands successfully. A total of eight bands amplified. Of these six bands were polymorphic and one of both populations were monomorphic. Therefore 75% of the bands showed polymorphism. The five primers used for the RAPD analysis showed the following results:

1) **Primer GTTGCGATCC:** Amplified products of 750 bp respectively showed the presence of a single band of each genomic DNA populations and were monomorphic.
2) **Primer AATCGGGCTG:** It showed the presence of two polymorphic band i.e. 500 bp and 750 bp. The 500 bp fragment was absent in one population i.e. laboratory isolate. The 750 bp fragment was absent in one population i.e. Surabaya.
3) **Primer AGGTGACCGT:** It showed the presence of two polymorphic band i.e. 500 bp and 750 bp. The 500 bp fragment was absent in one population i.e. laboratory isolate. The 750 bp fragment was absent in one population i.e. Surabaya.
4) **Primer GAAACGGGTG:** It showed the presence of single polymorphic band i.e. 750 bp. The 750 bp fragment was absent in one population i.e. laboratory isolate.
5) **Primer GTGACGTAGG:** It showed the presence of single polymorphic band i.e. 750 bp. The 750 bp fragment was absent in one population i.e. laboratory isolate.

The markers GAAACGGGTG (750 bp) and GTGACGTAGG (750 bp) were unique to genomic DNA of the *Aedes aegypti* larval population from Surabaya. Both of the markers were absent only in laboratory isolate. Thus the markers showed polymorphism between both of the populations. This indicates that the populations are genetically distinct.

The RAPD-PCR products from the two genomic DNA of *Aedes aegypti* larval populations were scored as band presence (1) and absence (0). The results was successfully showed the polymorphic bands. Resume of the results were recapitulated as showed in the Table 1.

Table 1: Resume of the monomorphic and polymorphic bands amplified by five oligo nucleotide primers

Sequences (5' to 3')	500 bp	750 bp
GTTGCGATCC	None	Monomorphic
AATCGGGCTG	Polymorphicc	Polymorphicc
AGGTGACCGT	Polymorphicc	Polymorphicc
GAAACGGGTG	None	Polymorphicc
GTGACGTAGG	None	Polymorphicc

The Band Sharing Frequency (BSF) and the genetic distance (d) could be measured according to the data, in order to determine the variety and similarity of both populations. The BSF and d based on the sequences used explained in the Table 2.

Table 2: Resume of the Band Sharing Frequencies and Distance of Both two Batch of the *Aedes aegypti* Larval Stage

Sequence	F (BSF) = Nxy : (Nx + Ny)	d (genetic distance) = 1 – F
5'GTTGCGATCC'3	1	0
5'AATCGGGCTG'3	0	1
5'AGGTGACCGT'3	0	1
5'GAAACGGGTG'3	0	1
5'GTGACGTAGG'3	0	1
All five sequencess	0.25	0.75

Note: Nx = Bands of the laboratory population amplified with each sequences

Ny = Bands of the 7°16'24.2508" latitude and 112°45'37.6056" longitude of Surabaya population amplified with each sequences

Nxy=Total of monomorphic bands of both of the populations

4. Discussion

It is important to know the amount of genetic variability and similarity of the vectors. Variation in vector competence between different *Aedes aegypti* populations had been already demontrated in previous studies. Two *Aedes aegypti* larval populations genotypes with different geographic origins have been chosen for this study. Geographical differences have been attributed by some authors to the existence of different genetic structures of mosquitoes vectors.

Twenty oligo nucleotide primers were used for genetic variability detection of *Aedes aegypti* larval populations in preliminary study. From those primers, only five have produced bands successfully. In the core study, the five primers showed polymorphism between both of the *Aedes aegypti* larval populations. According to many studies, the polymorphism levels varied according to the geographic origin of the populations the primers used.

The preparation of DNA extracts were used, affecting the clarity and sharpness visualization RAPD-PCR results. Preliminary studies, showed that RAPD-PCR results were more clear and vivid the visualization, using population DNA of the individual, who are members of the population, than the population DNA. By using five significant primers, results revealed that the bands ranged from 500 bp to 750 bp.

This study found that the five oligo nucleotide primers which were used in previous overseas studies as evaluated before, are recommended to use in future study of the polymorphism of the *Aedes aegypti* late 3rd or early 4th larval populations in Indonesia, using RAPD-PCR technique furthermore.

Based on finding from specific studies about RAPD-PCR technique, that state of a few disadvantages of the RAPD-PCR technique, such as any smears or unclear of the results, a slight modify based on the laboratory condition should be held any time, and has to record or well documented. In order to improve the method to detect the polymorphism of the *Aedes aegypti* larval populations from different geographic origin, finally it would be published in order to reach a better examination later.

The primer (5' to 3') GAAACGGGTG and GTGACGTAGG amplified 750 bp Surabaya specific bands. It was showed that the diversity of the species was attributed to wide spead (geographical diversity) and domestication of *Aedes aegypti* larval stage from wild populations.

The number of RAPD-PCR oligo nucleotide primers used in this study may be sufficient to permit an estimation of genetic diversity and similarity of the *Aedes aegypti* larval populations. However, there were any weak points in this study, such as limited distance and number of populations, as they were only about 200-300 km apart during the performance of this study. In future, additional populations, sampling sites and individuals will be necessary to decrease these weak points and increase the efficiency and accuracy of this study.

5. Conclusion

It is suggested that the five primers of RAPD-PCR which were used in this study, could be applied for detecting genetic variability of *Aedes aegypti* Larva populations from Indonesia in future studies**.** Further studies involving more populations to be conducted to get more accurate and precise information.

6. References

1. Dos Santos Paduan K, Araújo JP Jr, Rubella PEM. Genetic variability in geographical populations of *Aedes aegypti* (Diptera, Culicidae) in Brazil elucidated by molecular markers. Genetic and Molecular Biology 2006; 29(2):391-3.
2. Julio NB, Chiappero MB, Rossi HJ, Dueñas JCR, Gardenal CN. Genetic structure of *Aedes aegypti* in the city of Córdoba (Argentina), a recently reinfested area. Mem Inst Oswaldo Cruz Rio de Janeiro 2009; 104(4):626-7.
3. Hiragi C, Simoes K, Martins E, Queiros P, Lima L, Monnerat R. Genetic variability in *Aedes aegypti* (L.) (Diptera Culicidae) populations using RAPD markers. Neotropical Entomology 2009; 38(4):542-3.
4. Neekhra B, Pandey D, Jain SK. RAPD-PCR Based Marker Approach for the Genetic Differentiation of Two Species of Cockroach (Order-Dictyoptera), Journal of Life Sciences. 2012; 6:1328-30.
5. Kaura T, Kumar R, Chaudhry S. Genome fingerprinting of two populations of Aedes (Stegomyia) albopictus Skuse (Asian Tiger) using random primers (Diptera Culicidae) Journal of Applied and Natural Science. 2013; 5(1):95-6.
6. Jain SK, Neekhra B, Pande D, Jain K. Marker System in Insect Study A Review Indian Journal of Biotechnology. 2010; 9:7-10.
7. Tde F Patarro, Guirado MM, Ravazzi LM, HEM de C Bicudo. Genetic structure of Aedes aegypti populations determined using pairwise comparisons. Genetic and Molecollar Research 2013; 12(3):3776-7.
8. Ballinger-Crabtree ME, Black WC IV, Miller AR. Use of Genetic Polymorphism Detected by The Random-Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) for Differetiation and Identification of *Aedes aegypti* Subspecies and Populations Am J Trop Med Hyg. 1992; 47(6):893-5. sss