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Analysis of genetic variation of the voltage-gated sodium chanel gene sequence in *Aedes aegypti* NCBI popset (2325355280) using in silico RFLP

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Abstract

Aedes aegypti is a mosquito that belongs to the Culicinae Culicids family, order Diptera, class Insecta. This mosquito has the potential to transmit dengue hemorrhagic fever (DHF). DHF is a disease characterized by sudden fever, bleeding both in the skin and in the body and can cause shock and death. In-silico is a research approach whose use will be discussed with advances in technology and available databases. This approach is very commonly used in medical and other healthcare settings. The method used is to use the NCBI site, screen restriction enzymes with an insilico web and continue with the benchling site. The results obtained were using the NheI enzyme and producing two alleles namely Allele A1 and Allele A2. The A2 allele dominates because it has a fragment percentage of 63,3%.

Introduction,

Aedes aegypti is a mosquito belonging to the Culicinae culicids family, order Diptera, class Insecta. *Aedes aegypti* is closely related to *Anopheles gambiae* and *Drosophila melangester*. This mosquito has the potential to transmit dengue hemorrhagic fever (DHF). DHF is a disease characterized by sudden fever, bleeding both on the skin and on the body and can cause shock and even death. Dengue fever especially attacks children, including babies, although currently the majority of sufferers are increasing (Rahayu *et al.*, 2012). Dengue fever is often found in most tropical and subtropical climates, such as Southeast Asia, Central America, America and the Caribbean. The causative agent of dengue fever is the Dengue virus (DENV) and the natural host is human cells. Dengue virus belongs to the Flaviridae family and the *Flavivirus* genus, and there are 4 serotypes including DEN-1, DEN-2, DEN-3, and DEN-4. Dengue hemorrhagic fever is a severe form of DENV infection identified by loss of plasma (Achyar, 2021).

Adult female *Aedes aegypti* has a blackish brown body. The body size of an adult female *Aedes aegypti* mosquito is 3-4 mm, regardless of the length of her legs. The body and limbs are covered with silver-white striated scales. There are two curved lines on the back of the body vertically to the left and right which are characteristic of this type of mosquito (Ginanjar, 2008). The female mosquito's abdomen is pointed and has cerci that are longer than those of other mosquitoes (Gillot, 2005). Usually, male mosquitoes have a smaller body than females and have nine thick hairs on the antennae of male mosquitoes. Both characteristics can be seen with the naked eye. Recent data from (Nene et al.,2020) The *Aedes aegypti* genome sequence consists of five times 1.38 billion base

pairs larger than the size of the Anopheles gambiae genome. Nearly 50% of the Aedes aegypti genome consists of transposable elements that contribute to a four- to six-fold increase in the average gene length and the relative size of the intergenic regions of *Anopheles gambiae* and *Drosophila melanogaster.*

Figure 1. Taxonomy of *Aedes aegypti* (http://www.itis.gov)

Efforts to control mosquitoes which are vectors of this disease need to be carried out in addition to treating sufferers. This is an important effort to reduce cases caused by mosquitoes. Seventeen binding of pyrethroid insecticides to VGSC causes a phenomenon called knockdown, namely rapid movement followed by paralysis and death. The mechanism for the emergence of resistance to pyrethroids is a change in the structure of VGSC in the mosquito's central nervous system as the main target of pyrethroids, resulting in a decrease in affinity for the insecticide.

Molecularly, in the VGSC gene there is a change in one of the nucleotide bases in the amino acid leucine to phenylalanine which is associated with resistance. VGSC gene mutations cause knockdown resistance (kdr) allele polymorphism, namely the kds, kdr-w and kdr-e alleles. This mutation was first detected in *Anopheles gambiae* from West Africa so it was given the term kdr-w which was caused by changing the amino acid leucine to phenylalanine (TTA to TTT), namely L1014 to 1014F, then the second mutation was called kdr-e or East African which resulted in a change in acid. amino leucine becomes serine (TTA becomes TCA i.e. L1014 becomes 1014S). Mutations in the VGSC gene have also occurred in *Anopheles culifacies* mosquitoes at codon 1014 from India and in Indonesia itself have been reported in *Anopheles sundaicus, Anopheles aconitus, Anopheles subpictus* and *Anopheles vagus* mosquitoes.

Genetic diversity at the population/individual/species level can be analyzed based on different marker profiles, both molecular and protein. The concept of using DNA sequence polymorphisms as genetic markers was developed with the emergence of restriction fragment length polymorphism (RFLP) markers (Adhikari *et al.,* 2017). *Random Fragment Lenght Polymorphism* (RFLP) analysis is a technique commonly used to detect genetic variations at the DNA sequence level. RFLP detection is carried out by comparing group profiles created after cutting DNA with different object/person

restriction enzymes (Zulfahmi, 2013). Individuals of the same species show polymorphism as a result of insertions/deletions (known as InDels), point mutations, translocations, duplications, and inversions and polymorphisms are thought to influence the transcription and splicing processes of genes (Badriyya, 2020).

In-silico is a term to describe "performance on a computer or through computer simulation". The term in-silico was first used by the public in 1989 at the "Cellular Automata: Theory and Application" workshop in Los Alamos, New Mexico. Pedro Miramontes, a mathematician from the National Autonomous University of Mexico, presented his results on "Physiochemical Constraints of DNA and RNA, Cellular Automata, and Molecular Evolution". In his explanation, Miramontes uses the term "in silice" to characterize biological research that uses computers entirely. In-silico is a research approach whose use will be discussed with advances in technology and available databases. This approach is very commonly used in the medical and other health care fields. Although this research is relatively new, technological advances and information databases are already available. This approach is very commonly used in the medical and other health care fields. Although in-silico studies are relatively new, their use is already very widespread. The availability of publicly available, open, and free databases (DNA, RNA, etc.) offers researchers the opportunity to use this information to solve medical problems and advance medical and health sciences.

In-silico PCR is also called electronic PCR or virtual PCR because it is computationally amplified using bioinformatic software. The in silico PCR results for each DNA template were consistent with the Primer BLAST results. The importance of carrying out in silico PCR before carrying out experiments in the laboratory is to prevent errors in primer design so that the annealed primer is not specific to the target gene only (Achyar, 2021).

Materials and Methods

Materials

The voltage-gated sodium channel-like target gene sequence used in this in-silico test was obtained from NCBI fasta with Popset number: 2325355280 sent by Mashlawi, A.M at https://www.ncbi.nlm.nih.gov/popset/2325355280. In the Popset there are 53 gene sequences coding for restriction enzyme candidate screening that will be used in in-silico RFLP on the site http://insilico.ehu.es/restriction/. This tool will compare the restriction patterns of many DNA sequences tested as well as the restriction enzymes that cut them.

Methods

On the site http://insilico.ehu.es/restriction/, select "Compare reconstruct pattern of many sequences". This gene sequence has been downloaded in fasta format beforehand, entered in the existing column. In the next step, the results of the alignment of each sequence will be seen and the same sequences will be discarded to facilitate the analysis process. Next, select the option "only restriction enzymes with known bases (no N, R, Y...)" to get restriction enzymes with good results. Then select the option "Get the list restriction enzyme" to get the restriction enzyme results that will be used in the next stage, namely *Restriction-Fragment Length Polymorphism* (RFLP) *in-silico* or virtual restriction carried out using tools on the site https://www.benchling.com/. This site is free, but you must register first using your email or Google account. The initial stage carried out was to import 30 voltage-gated sodium channel-like gene sequences that had been downloaded from NCBI into the project folder on the Benchling website. Next, make the cut by clicking the scissors symbol in the right corner of the screen. Then select “find enzyme” and select the name of the restriction enzyme that was previously determined during screening. Next, click the "run digest" menu to carry out restrictions. The electrophorogram image is obtained by clicking on the "virtual digest" menu.

Results and Discussion

Restriction Enzyme Candidate Screening

Screening for restriction enzyme candidates is carried out on the website (http://insilico.ehu.es/restriction/) by means of before entering the fasta sequence in *in-silico,* we first take the fasta on the NCBI website. The NCBI PopSet used for this restriction enzyme is PopSet: 2325355280. Where the organism is *Aedes aegypti* with a target gene voltage-gated sodium channel-like gene. When you have finished screening restriction enzymes, select one of the enzymes that has a variety of cutting edges in each sequence sample. The results of restriction enzyme screening from the Voltage-Gated Sodium Chanel gene sequence in *Aedes aegypti* can be seen in Figure 2.

AccII,Bsh1236I,BspFNI,BstFNI,BstUI,MvnI CG'CG		435 458	435 458	435 458	435 458	435 458	435 458	435 458	437 460	437 460	436 459	276 451 474	276 451 474	276 451 474	274 449 472
AfaI,RsaI GT'AC		272 346	167 272 346	272 346	272 346	272 346	272 346	272 346	274 348	274 348	273 347				
AhlI,BcuI,SpeI A'CTAG_T		202	202	202	202	202	202	202	202	202					
AluI,Alu8I AG'CT											293				
Alw44I,ApaLI,VneI G'TGCA_C									164		164				
AspLEI,BstHHI,CfoI,HhaI G_CG'C		166 460	460	166 460	166 460	166 460	166 460	166 460	462	166 462	461	166 278 476	166 278 476	166 278 476	164 276 474
AsuNHI,BspOI,NheI G'CTAG_C		251	251	251	251	251	251	251	253	253	252				
BalI,MlsI,MluNI,MscI,Msp20I TGG'CCA		84	84	84	84	84	84	84	84	84	84	84	84	84	82
BfaI,FspBI,MaeI,XspI C'TA_G		105 203 252	105 203 252	105 203 252	105 203 241 252	105 203 241 252	105 203 241 252	105 203 241 252	105 203 243 254	105 203 243 254	105 242 253	105 270	105 270	105 270	103 268
BfuCI,Bsp143I,BssMI,BstMBI,DpnII,Kzo9I,MboI,NdeII,Sau3AI 'GATC_		22 221 430 472	22 221 430 472	22 221 430 473	22 221 306 430 472	22 221 306 430 472	22 221 306 430 472	22 221 430 472	22 173 223 308 432 474	22 173 223 432 474	22 222 431 473	22 446 488	22 446 488	22 446 488	22 444 486
BglII A'GATC_T									173	173					
BmtI G_CTAG'C		255	255	255	255	255	255	255	257	257	256				
BpvUI,MvrI,PvuI,Ple19I CG_AT'CG									311						
BseX3I,BstZI,EagI,Ecl1XI,Eco52I C'GGCC_G		409	409	409	409	409	409	409	411	411	410	425	425	425	423
BshFI,BsnI,BspANI,BsuRI,HaeIII,PhoI GG'CC		84 411	84 411	84 411	84 411	84 411	84 411	84 411	84 413	84 413	84 412	84 427	84 427	84 427	82 425

Figure 2. Restriction enzyme screening

RFLP *In-silico*

This *in-silico* restriction is carried out by logging into the benchling website. This is done by importing 30 sequences into the database, after which you will see information about the enzyme that cuts at what nucleotide position and produces what size fragments and you will also see ladder information from these sequences.

The restriction enzyme used is the NheI enzyme because this enzyme has cutting variations in each sequence sample. The results of *in-silico* RFLP using the NheI restriction enzyme can be seen in Figure 3.

Figure 3. Restriction electrophorogram using the NheI enzyme

Restriction using the NheI enzyme on the Voltage-Gated Sodium Chanel gene sequence in *Aedes aegypti* using 30 gene sequences produced two different gene variations, namely the A1 allele has a band fragment measuring 500 bp and the A2 allele has a band fragment measuring 250 bp. In the fragment obtained there was only 1 band which indicated that there was no cutting by the restriction enzyme along the fragment so it remained intact.

Table 1. Analysis using the *in-silico* RFLP method on *Aedes aegypti*

Enzim Restriksi	Situs Pengenalan Restriksi	Ukuran Fragmen (bp)	Alel	Jumlah Kehadiran Fragmen (N = 30)	Presentase Kehadiran Fragmen (%)	Frekuensi Alel
NheI	G’CTAG_C	500 bp	A1	11	36,7%	0,367
		250 bp	A2	19	63,3%	0,633

Based on Table 1, using the NheI restriction enzyme which has a G'CTAG_C recognition site, we obtained two alleles, namely A1 and A2 with two different fragment sizes, where the A1 allele has an RNA fragment of 500 bp and the A2 allele has a fragment size of 250 bp. Of the 30 sequence fragments used, 11 sequences have a fragment length of 500 bp and 19 sequences have a fragment length of 250 bp. The fragment percentages of the two alleles are also different, where the A1 allele has a percentage of 36.7% and the A2 allele has a percentage of 63.3%. The allele frequency of the two alleles is also different, the A1 allele is 0.367 and the A2 allele is 0.633. The A2 allele dominates the population in PopSet 2325355280 compared to the A1 allele.

The percentage of genetic variation shows the level of genetic diversity of an organism which also shows the tolerance range of the organism to adapt for its survival. Organisms that have high diversity will be easier to adapt than organisms that have low diversity.

Conclusion

Based on the results obtained in the process of screening restriction enzymes and carrying out RFLP in-silico, it was found that the enzyme used in the RFLP process was the NheI enzyme. Using this enzyme produces two fragment alleles, namely Allele A1 and Allele A2. Allele A1 has a fragment measuring 500 bp and Allele A2 has a fragment size of 250 bp. Allele A2 is more dominant than Allele A1 because it has a presentation of 63.3%.

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