TINY WOLBACHIA BACTERIA COULD SAVE MOMBASA FROM CHIKUNGUNYA AND DENGUE VIRUSES

MOSQUITO DNA EXTRACTION
&
GEL ELECTROPHORESIS

DONE BY:

1. RANIYA SHIRRE
2. AINAAN QUADIR
3. SIEGFRIED KATUMWA
4. NATALIE SIJAH

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SUMMARY

Using the PCR and gel electrophoresis method, we can test the wolbachia bacteria fragments present in mosquito DNA sample, after introducing the wolbachia-infected female mosquito in the wild.

Wolbachia is a safe and natural occurring bacterium which is found in up to 60% of insect species. However it is not found in aedes aegypti hence transferring zika, chikungunya and dengue viruses.

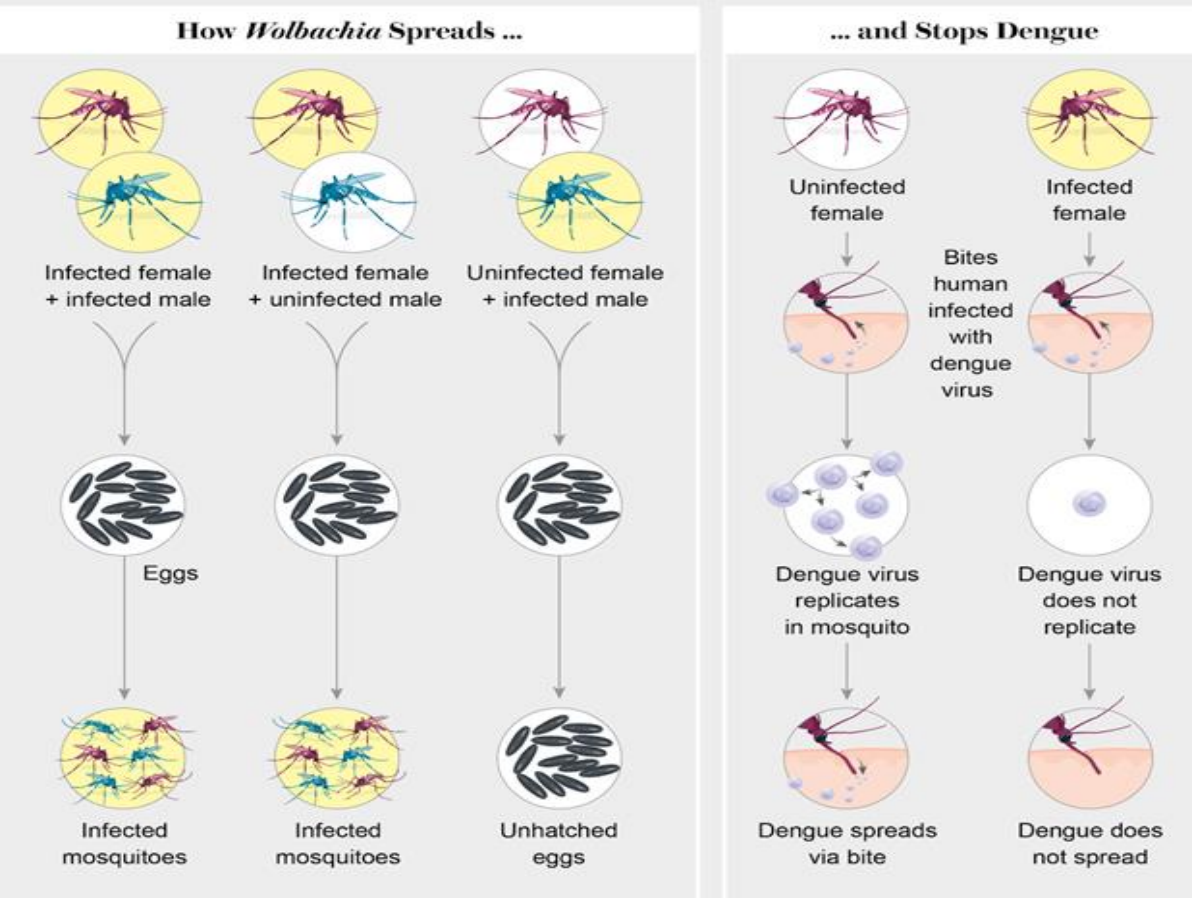
When the wolbachia is present it will consume most of the key molecules, hence making it harder for the growth of these viruses. As result, it will be more difficult for the viruses to be transmitted.

Wolbachia does not have a negative effect on the environment, animals and humans as it is a natural occurring bacterium.

Once there has been a successful integration of the wolbachia inside the mosquito gene, the specie can be cloned to produce the exact same/identical mosquitoes.

Once a sufficient amount of cloned wolbachia-infected mosquitoes are made, they can be released in small amount to the wild to mate with other female mosquitoes that do not have wolbachia present in them.

The mating would result in producing offsprings that are inherited with the wolbachia bacteria, hence reducing transmission of the viruses.



RESEARCH QUESTION

How can we control the spread of mosquito transmitted disease biologically?

Does wolbachia-infected aedes mosquito spread dengue fever?

Is wolbachia bacteria present in Aedes mosquitoes?

INTRODUCTION

Controlling mosquito borne disease is very an important factor to achieve to be able to increase life expectancy and improve the health quality of the human. One of the ways proven to control mosquito borne diseases is to infect and small breed of mosquito with wolbacia and release the species into the wild. More detailed information on how wolbacia works and is impact can read on the following link: https://www.worldmosquitoprogram.org/en/work/wolbachia-method/how-it-works

Wolbachia is a safe and natural occurring bacterium which is found in up to 60% of insect species. However it is not found in aedes aegypti hence transferring zika, chikungunya and dengue viruses. When the wolbachia is present it will consume most of the key molecules, hence making it harder for the growth of these viruses. As result, it will be more difficult for the viruses to be transmitted. Wolbachia does not have a negative effect on the environment, animals and humans as it is a natural occurring bacterium.

The major causes of these diseases are human carelessness which eventually causes then to become a huge outbreak in no time. Stagnant water is the main site of the mosquito breeding, however we not been keen and letting them be collected in various ways such as in coconut shell or tires increase these risks of disease. The stagnant water not only cause mosquitos but also a very bad stench (smell) and some of the various object where they are collected, the site does not biodegrade.

With the help of Pwani university researcher and professors we were able to analyses the DNA of mosquitos as well as how we could extract the DNA by using much simpler protocol to trace the wolbachia bacteria present.

Using the PCR and gel electrophoresis method, we can test the wolbachia bacteria fragments present in mosquito DNA sample, after introducing the wolbachia-infected female mosquito in the wild.

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What is DNA?

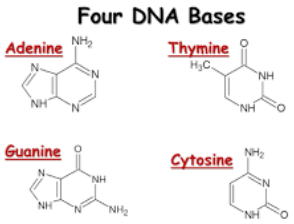
Deoxyribonucleic Acid (DNA): genetic material of all cellular organisms, gigantic molecule which is used to encode genetic information for all life on Earth. DNA is found in the nucleus of the cell inside the chromosomes. DNA contains instruction that make the protein- the building block of the life.

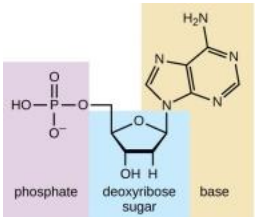
STRUCTURE OF DNA

DNA is a long chain (polymer) of made up of small chemical compounds called nucleotides.

Nucleotides are ring shaped structures composed of:

1. Nitrogenous base
    - Adenine
    - Guanine
    - Cytosine
    - Thymine





2. Deoxyribose sugar

3. Phosphate group

Nucleotides are attached together to form two long strands that spiral to create a structure called a **double helix.** The bases on one strand pair with the bases on another strand. DNA looks like a ladder. The curving sides of the ladder are the sugar-phosphate backbone of the two DNA strands. The rungs are the base pairs.

The spread of diseases by mosquito around the town and the increasing number of the mosquito transmitted diseases had caused us to analyse the type of mosquito in our area and it morphological features to identify the species and the type of diseases spread by it. We used the globe map observer app to identify the morphological features and the type of species in our area.

The importance to carry out this research was to be aware of the most common species of mosquito in our area and how to prevent them to be breed, as well as knowing the type of diseases they spread. We had involved our community in our action to create awareness about the mosquito and the diseases they spread by hosting a forum on 4th of October 2019 at the hall of Shree Swaminarayan Academy where all the parents and researchers were invited to attend and give more information and talk about the diseases. Our research and finding were also presented and a poem known as 'THE FIGHT' proclaimed the message about the species of mosquito and there work.

RESEARCH METHOD

DNA EXTRACTION AND GEL ELECTROPHORESIS

DNA EXTRACTION

We represent a simplified and rapid version of DNA extraction protocol from different mosquito species which is suitable for polymerase chain reaction (PCR) and other molecular biology works. The protocol involves three steps like lysis, solvent extraction and two fold isopropanol precipitation at -20 degree Celsius using 1X STE buffer (50mM NaCl, 50mM Tris-Hcl, 100mM EDTA, pH 8.0).The proposed extraction protocol has an advantage of DNA extraction from mosquitoes using 1X STE buffer at 37ºcelcius which prevents DNA degradation at higher temperature and kept DNA stability in long term storage. The protocol proved by three different mosquito species and removing for potential contamination showed that the protocol yields were of good quantity and quality DNA, typically better than commercial kits .The protocol was evaluated by polymerase chain reaction (PCR)

Reagents

The reagents used to make the STE (Sodium Chloride ,Tries –HCl, EDTA, pH. 8.0) buffer acting as a lysis buffer in this protocol.

- Sodium Chloride
- Tris-Hcl
- Ethylene Diamine Tetraacetic Acid (EDTA)
- Sucrose
- Sodium Dodecyl Sulphate (SDS)
- Triton X
- Proteinase K
- RNAse A solution
- Phenol: Chloroform (1:1)
- Chloroform: Isoamyl alcohol (24:1)
- Isopropanol
- Ethanol
- Tris
- Borate
- Agarose

Equipment

- Incubator
- Centrifuge
- Vortexer
- Freezer (-20 º celcius)
- Nanodrop or Spectrophotometer
- Electrophoresis apparatus

Procedure

1. Wash preserved mosquitoes in sterile distilled water or phosphate buffer saline (PBS) to remove excess alcohol. Fresh mosquitoes can be ground directly.

2.Grind mosquitoes in 1.5 ml eppendorf tube with micropistle in 50-100 µl 1X STE buffer (50mM Nacl, 50mM Tris- HCL,100mM EDTA, pH 8.0) along with 100mM sucrose. Add 1X STE buffer to a total volume of 300-500 µl for a single mosquito and 1 ml for mosquito pool like 4,6,8,10 numbers. Then add 1% SDS, 1% Triton X , 10 µl/ ml RNAse A (20mg/ml), 20 µl/ ml Proteinase K (20mg/ml) and mix it. We used Triton X and Proteinase but did not use RNAse.

3. Lyse for 1 hour 30 minutes at 37º celcius. Gently mix the tube by inverting every 15 minutes.

4. Centrifuge at 12,000g for 10 minutes at 4º celcius .Transfer the supernatant to a fresh tube.

5. Add equal volume of phenol:chloroform(1:1) ,shake the tube well for 5 minutes and centrifuge at 12,000g for 10 minutes at 4º celcius . (This step was skipped to avoid use of Phenol, which is a toxic chemical.)

6.Repeat the above step(5) , then add chloroform:isoamyl alcohol(24:1) and centrifuge at 12,000g for 10 minutes at 4º celcius .

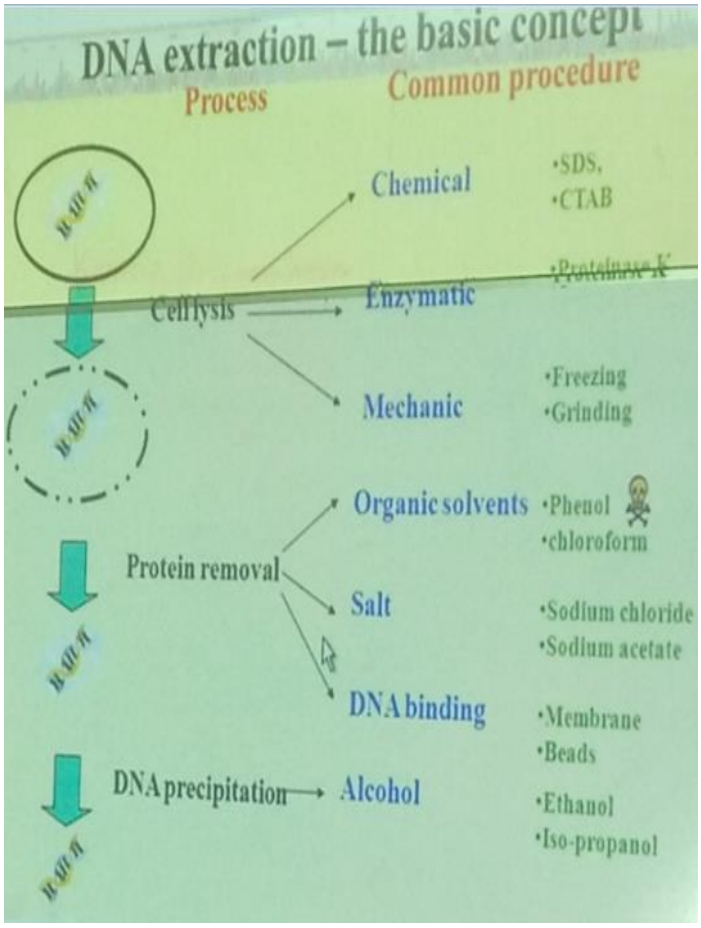
7.Transfer the very clear supernatant to a fresh tube , add two fold volume cold isopropanol and keep it for 1 hour at -20º celcius .

8.Centrifuge at 12,000g for 30 minutes at 4º celcius and then remove the supernatant .

9. Wash the pellet with 70% ethanol .

10. Keep the pellet at 37 º celcius for 10 minutes.

11. Dissolve the dry pellet in nuclease free water or TE buffer (PH. 8.0). Store DNA at 4 º celcius or -20 º celcius.



TIMINGS

This protocol consists of three phases like (1) Incubation phase (2) Washing /Extraction phase (3) Precipitation phase.

(1)The incubation phase is a lysis step where maximum cells were disrupted. This phase limits for 2 hours.

(2)Washing /Extraction phase is a removal of lipids, proteins and salt complexes from mosquito materials. This phase limits for 40 minutes.

(3)Precipitation phase is the DNA precipitation in isopropanol organic solution happened at -20º celcius .This phase limits for 1 hour.

The end of DNA isolation is a 70% ethanol washing step contribute to removal of proteins , Then /dissolved in nuclease free water or TE buffer (PH. 8.0).

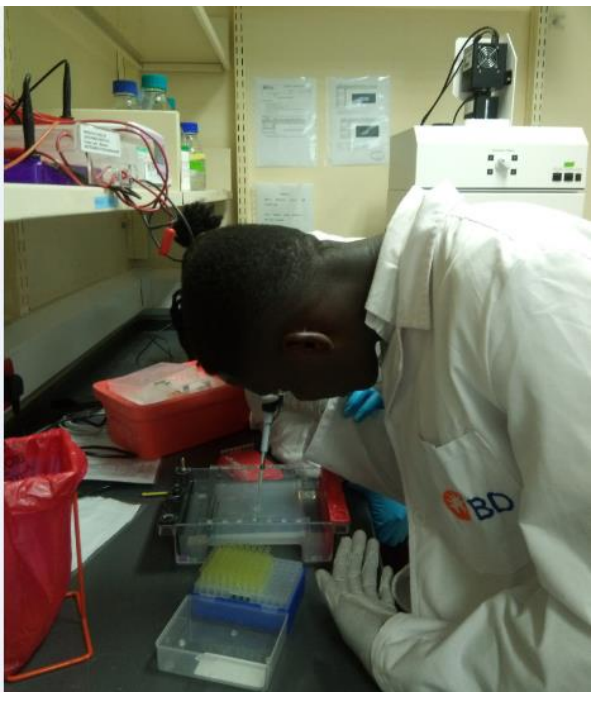
The total DNA isolation protocol completed within 4 hours 30 minutes for a single mosquito but two to three days in case of other published protocols

AGAROSE GEL ELECTROPHORESIS

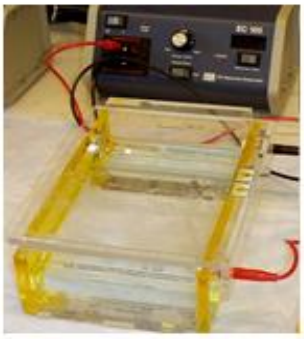
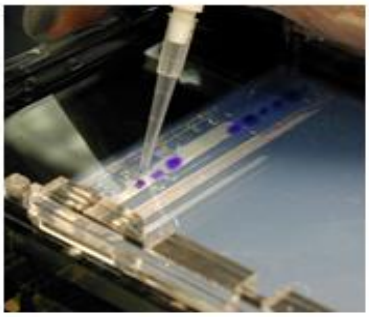
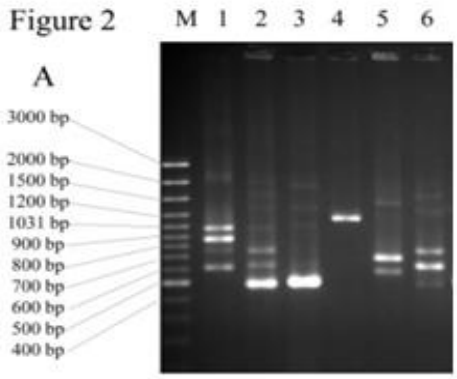
Basic principle:

DNA is negatively charged and can be separated by applying an electric field to move the negatively charged molecules through an agarose matrix. Agarose is a carbohydrate which forms a gel with large pores that allows DNA molecules to move through it when an electric field is applied. Shorter/smaller molecules move faster than larger/longer ones because the smaller molecules move more easily through the pores of the gel.

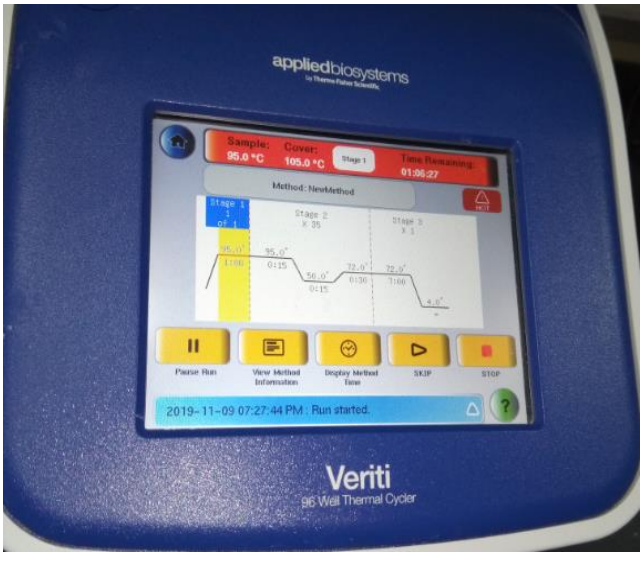
By placing the molecules in wells in the gel and applying an electric field, the molecules will move through the matrix at different rates, **depending on their size or mass.** DNA being **negatively** charged, will migrate toward the **anode** (positive electrode).Dyes used to make the DNA visible after electrophoresis



PROCEDURE:

A: Horizontal agarose gel electrophoresis equipment showing the gel tank and agarose gel in it. 	B: Loading agarose gel with DNA samples in loading buffer containing blue dye to help us see how far the DNA has moved along the gel. 
C: Visualization of bands after gel electrophoresis. The image of the gel is captured using a camera. Lane M is the molecular weight marker (DNA fragments of known size), while lanes 1 to 6 are DNA samples run on agarose gel.	Figure 2 

RESULTS



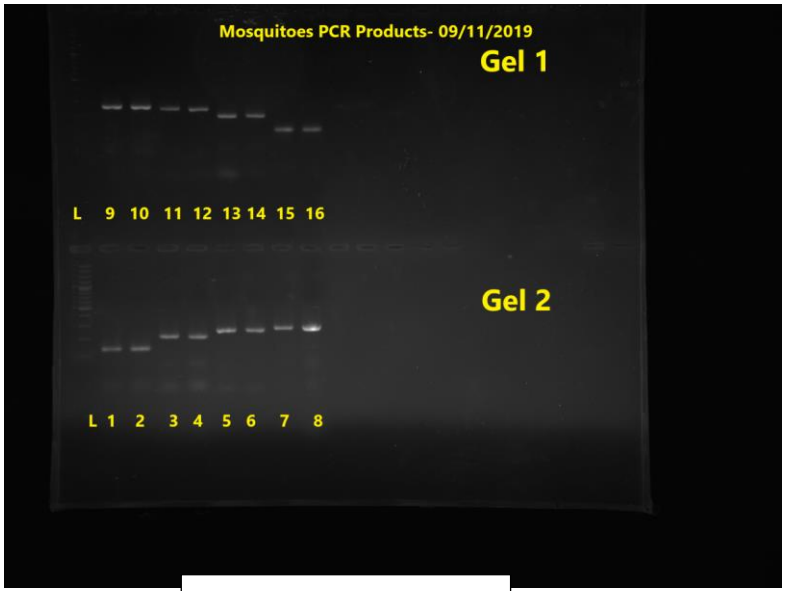


Figure 1

In the figure 1 above which had two separate gels used for electrophoresis show that the different number of movement of DNA fragments hence show the size of different fragments present in the mosquito DNA samples?

In lane 1 and 2 in gel 2 shows that they have smallest fragments of DNA as they moved the furthest and in lane 15 and 16 in gel 1 also had the smallest fragments of DNA present on their respective wells.

In lane 7, 8,9,10 respectively in gel 1 and gel 2 have the largest DNA fragments hence they did not move further away from their wells.

DISCUSSION

Using the PCR and gel electrophoresis method we can introduce the wolbacia bacteria into mosquito DNA hence reducing the transfer of the viruses. Wolbacia is a safe and natural occurring bacterium which is found in up to 60% of species. However it is not found in aedes aegypti hence transferring zika, chikungunya and dengue viruses.

The first way is introducing wolbacia in the mosquito immune system makes it harder for the viruses to survive in the body hence making it hard for the mosquitoes to be infected with the viruses about.

The second way is by competing wolbacia against the viruses for key molecules like cholesterol. Both the viruses and the wolbacia require cholesterol to survive inside the mosquito. When the wolbacia is present it consume most of the key molecules hence making it harder for the growth of these viruses as result it will be more difficult for the viruses to be transmitted.

Wolbacia does not have a negative effect on the environment, animals and humans as it is a natural occurring bacterium.

Once there has been a successful integration of the wolbacia inside the mosquito gene the specie can be cloned to produce the exact same/identical mosquitoes. Once sufficient amount of cloned wolbacia infected mosquitoes are made, they can be released in small amount to the wild to mate with other female mosquitos that do not have wolbacia present in them. The mating would result in producing offspring that are inherited with the wolbacia bacteria hence reduces transmission of the viruses.

CONCLUSION

Agarose gel electrophoresis it used to obtain DNA fragment which are greater than 50 base pairs.

The entire procedure of PCR and gel electrophoresis took place in a sterile environment, Pwani University laboratory, to avoid any contamination.

The gel electrophoresis helped in obtaining different mosquito DNA fragment and showed the amount of base pair in a fragment depending on its movement from the negative to the positive side.

This is a genetic engineering process hence can be used for analyzing the fragment, and its content to modify by isolating certain proteins which aid the mosquito to transfer viruses.

RECOMMENDATION

Agarose is used to separate big fragments of DNA as it has big pores hence it is not recommended to us agarose if you want to derive small DNA fragment as the pieces will just pass through the large pores along with the large DNA fragments.

If you want to obtain small DNA fragments then it is recommended to use SDS-PAGE (SODIUM DODECYL SULFATE –POLYACRYLAMIDE GEL ELCTROPHORESIS). Polyacrylamide is the substance that the gel is made out of. SDS is the chemical agent that denatures protein disrupting any non-covalent interaction they may have.

In conclusion, SDS-PAGE is better option for deriving smaller DNA fragment and proteins while agarose is used to derive large DNA fragments.

APPENDICES

- GLOBE observer app
- https://protocolexchange.researchsquare.com/article/nprot-2566/v1
- https://www.worldmosquitoprogram.org/en/work/wolbachia-method/how-it-works
- https://www.worldmosquitoprogram.org/en/learn/faqs#