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Submission date: 19-Jul-2021 01:03PM (UTC+0800)
Submission ID: 1621432323
File name: of_Dengue_Virus_Type_2_with_Different_Precipitation_Solvents.pdf (406.97K)
Word count: 2901
Character count: 15851

RNA ISOLATION OF DENGUE VIRUS TYPE 2 WITH DIFFERENT PRECIPITATION SOLVENTS

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Received 24 Januaril 2018
Accepted 2 Mei 2018

Abstract

Dengue virus distributed in tropical and subtropical regions in the world. DENV viruses are transmitted between humans primarily by Aedes aegypti and Aedes albopictus mosquitoes and are endemic in most areas in which the vectors occur. Four serotypes of dengue virus are DENV-1, DENV-2, DENV-3 and DENV-4. DENV-2 is comprised of six genotypes. The aim of the research is to compare Methanol, Chloroform, and 2-Isopropanol as the best precipitation solvent of the RNA Isolation of Dengue Virus Type 2. Ethanol precipitation is a commonly used technique for concentrating and de-salting nucleic acids (DNA or RNA) preparations in aqueous solution. RNA isolation by combining Guanidinium thiocyanate and phenol reported has been reported. In this report, we investigated RNA isolation from DENV-2 using QIAamp Mini Kit with 2-Isopropanol, Methanol, Chloroform precipitation solvent. Electrophoregram showed DNA band as the result of RNA isolation with methanol and 2-isopropanol are produced quite well. DNA band of the of RNA isolation with chloroform solvent has the lowest intensity than methanol and 2-isopropanol. This study showed that methanol and 2-isopropanol can used as precipitation solvent for isolating RNA.

Keywords: *DENV-2, 2-isopropanol , Methanol, Chloroform, Ethanol 96%, RNA Isolation*

Introduction

Dengue is a common arbovirus infection transmitted in humans by the mosquito species Aedes (Simmons et al, 2012). Dengue infection is caused by positive-strand RNA viruses in the genus Flavivirus (Johnson et al, 2005). The genomes of and DEN viruses are single-stranded. Infection of the Dengue virus (DENV) remains a serious health problem in tropical and subtropical regions in the world. During the last 11 month of the year 2017, 169,782 suspected dengue cases from all over the island have been reported (WHO,2017). More than 2.5 billion people are currently at risk of DENV infection, with 100 million people being estimated to be infected with DENV annually (Halstead, 2007).

Four serotypes of dengue virus: DENV-1, DENV-2, DENV-3 and DENV-4)are transmitted by the vector mosquitoes such as *Aedesaegypti* and *Aedesalbopictus* (Halstead, 2008). DENV-2 is comprised of six genotypes: Asian I representing strains from Thailand, Asian II representing strains from the Philippines, Cosmopolitan representing strains from South and South East Asia, American representing strains from Central America, South East Asian/American representing strains from South East Asia or from Central and South America, and sylvatic representing strains from West Africa and South East Asia (Weaver, 2009).

The polymerase chain reaction (PCR), in recent years, has numerous applications for the in vitro detection and diagnosis of disease pathogens, and its impact in the field of plant pathology has been the subject of a recent review. The advantages of the PCR technique include high specificity, the theoretical sensitivity to detect a single target molecule in a complex mixture, and high sample throughput. In comparison with serological reagents, PCR primers with any desired degree of selectivity can be synthesized, at a much lower comparable cost than that associated with the development of monoclonal or polyclonal antibodies (Henson, 1993).

Ethanol precipitation is a commonly used technique for concentrating and de-salting nucleic acids (DNA or RNA) preparations in aqueous solution. The basic procedure is that salt and ethanol are added to the aqueous solution, which forces the precipitation of nucleic acid nucleic acids out of solution. Combining Guanidiniumthiocyanate and phenol-chloroform extraction for RNA isolation has been reported (Chomczyns, 1986).The methode describe differs in that it converts the guanidinium-hot phenol method to a single-step extraction which allows isolation of RNA in 4 h and provides both high yield and purity of undegraded RNA preparations. In the present study, a new rapid procedure using 2-Isopropanol, Methanol, Chloroform, extraction for RNA isolation usingQIAamp Mini Kit to get a good quality of RNA is described.

Materials and Methods

Material

Chemical reagents used in this research is Virus dengue Surabaya strains (dengue virus types 2Genbank: KT012509), 2-Isopropanol (Merck, Germany), Methanol (Merck,Germany), Chloroform (Merck, Germany), Ethanol96% (Merck, Germany),RT-PCR Reagen(Invitrogen,Germany), Master Mix PCR (Promega, USA), QIAamp Mini Kit (QIAGEN,United States),TAE buffer (Promega,USA), Agarose1.5% (Promega,USA), Ethidium bromide (Merck, Germany), Primer reverse using TS (Type specific): D1, TS1,TS2, TS3, TS 4 (Sequences are mentioned in **Table 1**)

Table 1. Oligonucleotide primers for RT-PCR and PCR (Lanciotti et al. (1992)).

Primer	Sequence	Genome Position	Size,inbp, of amplified DNA product (primers)
D1	5'-TCAATATGCTGAAACGCGCGAGAAACCG-3'	134-161	511
TS1	5'-CGTCTCAGTGATCCGGGGG-3'	568-586	482(D1 and TS1)
TS2	5'-CGCCACAAGGGCCATGAACAG-3'	232-252	119(D1 and TS2)
TS3	5'-TAACATCATCATCATGAGACAGAGC-3'	400-421	290(D1 and TS3)
TS4	5'-CTCTGTTGTCTTAAACAAGAGA-3'	506-527	392(D1 and TS4)

Methods

RNA extraction

Total RNA was extracted from PositiveDENV-2 in Verocells using QIAamp Mini Kit.RNA extraction using various solvent at the precipitation step: 2-Isopropanol, Methanol, Chloroform , and Ethanol 96%. RNA measurements were quantitatively performed using nanodrop spectrophotometry with an polysaccharides absorb most UV light at λ230 nm and protein at λ280 nm.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

RT-PCR was carried out in order to transcribed RNA to cDNA using a primer set described by Lanciotti et al. (1992). RT-PCR process used concentration from the reagent. Mix dNTP, primer, NFW, and RNA then centrifuged for 1 min. For the next step, put the mixture in Thermocycle with temperature 65°C for 5 min. Then, make master mix that consist of FS Buffer, RNAse Out, DTT and superscript. The mixture was centrifuged at 8,000 x g for 1 min. The first master mix from

Thermocycle mixed with the second master mix, centrifuged for 1 min and put it down to Thermocycle at 50°C for 60 min, and then continued for temperature 85°C for 5 min.

PCR (polymerase chain reaction)

PCR reactions were allowed to proceed in thermocycler programmed to incubate for 1 hour at 42°C and then to proceed with 35 cycles of denaturation (94°C, 30 s), primer annealing (55°C, 1 min), and primer extension (72°C, 2 min) described by Lanciotti et al. (1992).

Electrophoresis

Electrophoresis was used electrophoresis gel 1.5%and Ethidium bromide as dye. Master mix in this research had been colored, so loading die and TAE didn't necessary. Some steps of electrophoresis process were mixing 1 μL marker, 6 μL TAE buffer, and 3 μL DNA. The gel was run for 30 min at 100 volt and stained with ethidium bromide. The bands were visualized on an ultraviolet trans-illuminator (Sharp, 1973)

Result and Discussion

The DNA DENV-2 isolation results were demonstrated on electrophoregram (Figure 1), almost all variations with various solvent modifications DNA bands give good intensity, except for the DNA bands on line 2. The DNA bands of line 1 was the result of marker.

The DNA bands of line 2 was the result of RNA isolation with chloroform solvent. In the 4thline of DNA bands the isolation result obtained a DNA band with a lower intensity than 3 others. RNA is a polar molecule caused by the presence of phosphate groups in the skeleton and chloroform can't be mixed with water and its ability to deproteinization based on the ability of denatured polypeptide chains to enter or mobilize into intermediate phase chloroform - water. High protein concentrations in the intermediate phase can cause the precipitation protein. While lipids and other organic compounds will be separated on the chloroform layer. Effective deproteinization processes depend on the magnitude of the phase between water and chloroform. This process can be performed by forming an emulsion from water and chloroform. This can only be done by shaking out or centrifuging strongly because chloroform can't be mixed with water (Hery, 2014).

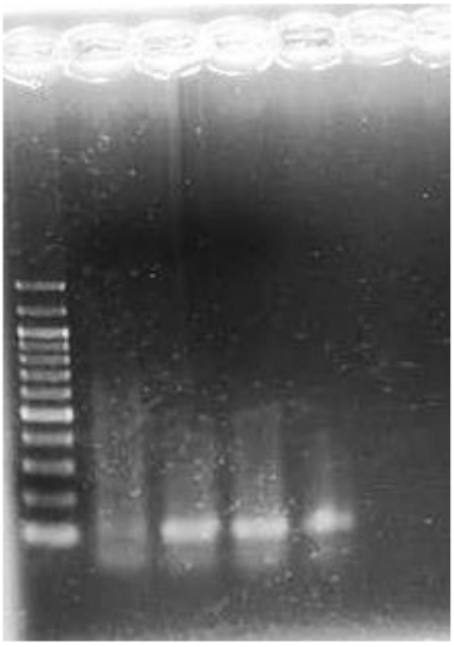
The DNA bands of line 3 were result of DNA isolation with 2-isopropanol solvent which is produced quite well.Line shows a DNA band that has an intensity almost equal to methanol and ethanol band. The addition of 2-isopropanol related to the principle of precipitation. First; the addition of 2-isopropanol is to decrease the solubility of nucleic acids in water. This is because the polar water molecule surrounds the RNA molecule in the aqueous solution. The positive dipole load of water interacts with a negative charge on the RNA phosphodiester group. This interaction increases the solubility of RNA in water (Surzycki, 2000). 2-Isopropanol can be mixed with water, but less polar than water. 2-Isopropanol molecules can't interact with polar groups of nucleic acids so that 2-isopropanol is a weak solvent for nucleic acids; second, the addition of isopropanol will remove the water molecules in the RNA solution so that the RNA will be precipitated; third, the use of cold isopropanol will decrease the activity of water molecules thus facilitating the precipitation of RNA.some salts are less soluble in isopropanol (compared with in ethanol) and will be more likely to be precipitated together with RNA.

The DNA bands of line 4 was result of RNA isolation with ethanol 96%. In this experiment, ethanol as a control. Line shows a DNA band that has an intensity almost equal to methanol and 2-isopropanol band. As a polar solvent, the water molecule has a partial negative charge around its oxygen atom, and a partial positive charge around its hydrogen atom. Therefore, negatively charged RNA

can interact with water molecules, and dissolve in them. Salt serves to neutralize the charge on the framework of sugar phosphate. Commonly used salt is sodium acetate. In solution, sodium acetate dissociates into sodium ion (Na^+) and ion $[CH3COO]^-$. The monovalent cationin this case (Na^+) sodium ion neutralize the negative charge on the phosphate group ($PO4^{3-}$) of RNA, thus making the molecule less soluble in water. However, the addition of salt does not necessarily cause the precipitation of RNA from the solution. The interactions between the ions in the solution are influenced by the Coulumb Styles which are heavily dependent on the solvent dielectric constant. Water as a solvent has a high dielectric constant that makes sodium ions and RNA phosphate groups difficult to interact. In contrast, ethanol has a much lower dielectric constant than water. The addition of ethanol will lower the dielectric constant of the solution to facilitate the interaction of sodium ions and RNA phosphate groups. The charge neutralization of the phosphate group makes the RNA less hydrophilic and eventually precipitated or out of the solution (Oswald, 2007).

The DNA bands of strip 5 was result of RNA isolation with methanol . Line shows a DNA band that has an intensity almost equal to line ethanol and 2-isopropanol band**.** Mostly Methanol is used for extraction various polar compounds but certain group of non polar compounds are fairly soluble in methanol if not readily soluble. The concentration in the extract may be lower than if a non-polar solvent was used because methanol dissolves a larger portion of polar compounds and the solubility of non-polar compounds may be reduced Methanol is commonly used because it is relatively inexpensive, lots of compounds dissolve in it, relatively free of regulation compared to ethanol, easily evaporated (Ahamed,2017).

For the next know RNA measurements were quantitatively performed using nanodrop spectrophotometry with an absorbance ratio of 260/280 and 260/230. Absorbance data showed in **Table 2**. The principle off nanodrop spectrophotometric work pure RNA capable of absorbing ultraviolet light due to the presence of purine pyrimidine bases.



1 2 3 4 5

Figure 1. Electrophoregram of DNA DENV-2 from RNA extraction method from various solvents. Line (1) Marker, (2) Chloroform, (3) 2-isopropanol, (4) Ethanol 96%, (5) Methanol.

Table 2. The data result of Nanodrop Spectrophotometry for RNA sample

Sample	A260/280 nm	A260/A230 nm
DENV2 - Methanol	3.27	0.32
DENV2 – Chloroform	327	0.09
DENV2 – Isopropanol	3.19	0.89
DENV2- Ethanol 96%,	3.25	0.49

The presence of contaminants can also be known through spectrophotometer. According to Rapley and Heptinstall

(1998). Polysaccharides absorb most UV light at λ230 nm and protein at λ280 nm. The level of purity of RNA can be known by measuring the amount of sample absorbance at λ230 nm, λ260 nm, and λ280 nm, then measure large comparison (ratio) A260/A280 and A260 against A230 (Amanda,2015). Pure RNA isolate has an A260/280 ratio of 2.0±0.1. A low A260/A280 ratio indicates protein contamination. From the test results can be known at A260/280 nm much samples have a ratio of ore thean 2.0, it can be concluded that the RNA isolate is not contaminated with proteins. As for the test results on A260/230 showed that no sample less than 1.5 which means the results of RNA still contain othe contaminants, pure RNA isolate has an A260/A230 ratio 2.0-2.4 (Farrell,2005).

Conclusion

In conclusion, we here in reported the isolation of DENV-2 strains with Methanol, Chloroform, 2-Isopropanol precipitation solvents. Modification of RNA isolation methods from DENV-2 is expected to produce a good quality RNA. Good RNA quality can be seen with the high intensity of the resulting DNA band and the low intensity of the smear. Thus, from this study reported that methanol and 2-isopropanol precipitation solvent give the best resultas precipitation solvent.

Acknowledgments

We thank the following colleagues at the Dengue Study Group, Institute of Tropical Disease Universitas Airlangga, Department of Chemistry, Faculty of Science and Technology UniversitasAirlangga and Japan Initiative for Global Research network on Infectious Disease (J-GRID).

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