CONSERVED REGION OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) BASED ON BIOINFORMATICS ANALYSIS

Ika Agus Rini

Jurusan Sains, Fakultas Biologi, Institut Teknologi Sumatera
Lampung Selatan, Indonesia
Korespondensi: ika.rini@bi.itera.ac.id

ABSTRAK

Gajah adalah salah satu hewan yang dilindungi di Indonesia. Endotheliotropic Herpesvirus (EEHV) merupakan salah satu penyakit yang menyebabkan kematian pada gajah. Hingga saat ini, belum banyak penelitian tentang penyakit tersebut, termasuk cara mendeteksi infeksinya. Penelitian ini bertujuan untuk mencari daerah lestari gen U38 dari DNA polimerase berdasarkan sekuens DNA di Gene Bank, sehingga dapat digunakan sebagai primer untuk deteksi dini penyakit yang disebabkan oleh infeksi EEHV pada gajah. Urutan genom EEHV dikumpulkan dari GeneBank, NCBI. Sekuens tersebut dianalisis dengan penyelarasan berganda menggunakan clustalX.2 dan dianalisis lebih lanjut dengan BioEdit. Beberapa daerah lestari yang ditemukan kemudian dianalisis lebih lanjut dengan Blastn untuk mengevaluasi daerah yang akan digunakan sebagai primer. Sebanyak empat belas sekuens didapatkan dari Gen Bank, dua diantaranya tidak dapat dianalisis lebih lanjut. Analisis filogenetik menunjukkan dua isolate dengan nomor KT 832491.1 dan KT 832467.1 memiliki kedekatan sebesar 99% yaitu dari isolate EEHV 3B. isolate yang paling dominan di Asia adalah tipe 1A dan 1B. Pencarian daerah lestari menghasilkan satu sekuens oligonukleotida (5'-GTGTGTATGGATAAGGT-3') yang memenuhi kriteria primer yang baik dan sesuai dengan standar. Sehingga, untuk kedepannya dapat digunakan sebagai kandidat dalam deteksi EEHV pada gajah.

Kata kunci: daerahlestari, eehv, gajah, primer

INTRODUCTION

Elephants are one of the large herbivores that are typical animals in Indonesia. Sumatran elephant (*Elephas maximus sumatranus*) is a protected Indonesian fauna. In Indonesia, Sumatran elephants are protected under Law No. 5 of 1990 concerning Conservation of Biological Resources and their Ecosystems and regulated within government regulations, namely PP 7/1999 concerning Plant Type Mapping and Animals (Bayane and Guiot, 2011). However, elephants experience a decline in population annually with many influencing factors, and one of them is susceptibility to infection.

One of the infectious diseases that has recently attacked elephants is the Elephant Endotheliotropic Herpesvirus (EEHV), which is fatal. The virus can cause acute hemorrhagic disease in Asian and African elephants (*Loxodonta africana*),

resulting in many diseases, reduced reproduction, and death (Garner et al., 2009). In Indonesia, there has not been much research on this virus and how it is handled, especially for the detection of infectious diseases.

EEHV was classified in the family Herpesviridae, subfamily Betaherpesvirinae, genus Proboscivirus, and species Elephantid beta herpesvirus (https://talk.ictvonline.org/taxonomy/). Eight genotypes of EEHV have been identified so far, including EEHV1A, EEHV1B, and EEHV2–71,4. EEHV1A, EEHV1B, EEHV4, and EEHV5 are associated with, and often cause, a severe hemorrhagic disease in Asian elephants, whereas EEHV2, EEHV3, EEHV6, and EEHV7 have been found in African elephants (*Loxodonta africana*) and are generally known to cause non-fatal diseases (Richman et al., 2014). Analyzed by phylogenetic, the virus has a relationship with human cytomegalovirus (HCMV) and three Roseolo viruses in humans (human herpesvirus-6A, -6B, and -7), which are classified into the Betaherpesvirinae subfamily (Dastjerdi et al., 2016).

The target gene for virus detection is DNA polymerase. DNA polymerase is a very sensitive and specific region for detection (Latimer et al., 2011), it also has high specificity for the detection of EEHV in various elephant DNA that has been previously characterized (Stanton et al., 2012). One of the conserved regions that can be used for primer design is core protein from DNA polymerase, the U38 gene. The U38 gene encodes the catalytic subunit of DNA polymerase and has a role in DNA replication (Wilkie et al., 2013).

The purpose of this study is to find conserved regions of the U38 gene in EEHV DNA polymerase from DNA sequence in Gene Bank. The regions can be further utilized in designing primers for early detection of diseases caused by EEHV infection in elephants. In addition, this study can also be preliminary data in designing detection kits for EEHV infections in elephants, especially in Indonesia. So that the next detection can be done quickly before death occurs.

METHODS

Materials

EEHV genome sequences were collected from GenBank https://www.ncbi.nlm.nih.gov/nuccore/?term=EEHV database, with the key search is "nucleotide" and "EEHV."

Methods

Sequence Alignment of EEHV Genome

All sequences that have been downloaded from the database in the form of a fasta format were then analyzed by multiple sequences alignmentusing ClustalX.2 to find out the conserved region. The alignment result was read using BioEdit.

Primer Design

The selection of the template regions was made by database similarity searching using BLASTn (Lipman and Pearson, 1985). NetPrimer was used to evaluate the ability of the selected regions to be used as oligonucleotide primers. The primary design used primer 3 Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and was configured with the Primary Blast.

RESULTS AND DISCUSSION

EEHV genome searching for U38 gene

Based on the NCBI GenBank database https://www.ncbi.nlm.nih.gov/, 26 sequences were obtained which have complete documented sequences of the U38 gene. These sequences were from all genotypes of EEHV. The search results were then performed further analysis with multiple sequence alignment and the phylogenetic tree used MEGA 7.

Sequence alignment and conserved region

Fourteen conserved regions were obtained from multiple sequences analysis using ClustalX.2. Two of the 14 sequences can not be analyzed. The sequences obtained from GenBank were also analyzed for their relationship using MEGA 7 (Kumar et al., 2016). Phylogenetic (fig. 1) results show that sequences with accession numbers KT832491.1 and KT832467.1 have a very close relationship, and the last evolutionary time, both sequences are isolates of EEHV 3B.

Figure 1. Analysis of phylogenetic tree from U38 sequences that download from a gene bank.

Based on the results, the dominant EEHV types are type 1A and 1B. Elephant endotheliotropic herpesvirus type 1 (EEHV1) is the most important causative agent of an acute fatal hemorrhagic disease in Asian elephants (*Elephas maximus*) (Takehana et al., 2019). EEHV1, which has subtypes 1A and 1B, was recognized as the most common type to cause fatal HD in Asian elephants (Stanton et al., 2012; Wilkie et al., 2013). EEHV1 is found in all cases of infection in Asian elephants (Latimer et al., 2011).

Evaluation of primer candidates

From Net Primer analysis results of the seven regions, only 5'-GTGTGTATGGATAAGGT-3' met the criteria to be used as an oligonucleotide primer, with a NetPrimer rating of 100 (maximum).

Tabel 1. Conserved region selected on template

Nucleotide seq.	Base Position	BLASTn result according to EEHV type
5'-TTTTGTGCACACGATGT-3'	412-428	1A, 5
5'-TGGATTTTGAAATTTTTGAACACT-3'	770-793	1A, 1B, 5
5'-TGGTATTCTCTGAAAAC-3'	847-863	1A, 1B, 5
5'-AGACAAACCAGTAAAGA-3'	874-890	5
5'-GTGTGTATGGATAAGGT-3'	1420-1436	1A, 1B
5'-CACAAACTTTTTTGTAAA-3'	1594-1611	1A
5'-TAGCTGCGGCTAGAA-3'	1715-1729	1B
5'-TATGGATTTACCGGTGT-3'	2143-2159	1A
5'-GATTTAACGATTGGA-3'	2255-2269	1A
5'-AATTTTGTAGCGCACATAAC-3'	2419-2438	1A, 1B, 5
5'-TACGAAAACAGAGTG-3'	2764-2778	1A, 1B, 5
5'-ATATTTCCAGAGTTTATA-3'	3067-3084	1A

The purpose of multiple sequence alignments is to find the highest similarity of sequences that have been obtained from the GenBank. Fourteen of the conserved region were identified in this study and evaluated with the NetPrimer to find out the best primer criteria. They must not have potential secondary structures such as hairpins or dimmers; have a GC content of 45-60%; have a Tm between 52-58°C; their 5' ends stability has to be greater than the stability of their 3' ends; be 17-25 nucleotides in length (Dieffenbach et al., 1995; Ye et al., 2012).

CONCLUSION

Based on the similarity in sequence, 14 conserved regions were found in the U38 gene of EEHV. From alignment of the conserved region, only one oligonucleotide (5'-GTGTGTATGGATAAGGT-3') has met the criteria for good primers, thus can be used as a template for the primer to detect EEHV. From this study, one of those primer candidates can be used as oligonucleotides to detect EEHV in elephants.

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