

Characterization of *envelope-transmembrane* Gene of Jembrana Disease Virus Tabanan 1995 Isolate

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Abstract

The availability of specific and rapid detection methods is essential for monitoring the health status of farmed species, particularly in viral disease as in this case early diagnosis is a critical factor in containing disease outbreaks. Jembrana Disease Virus (JDV) is a lentivirus that causes an acute, severe disease syndrome in infected Bali cattle in Indonesia, resulting in heavy economic losses because of the high mortalities. The virus-host interaction and the modes of transmission are still unknown. The goal of the research was to design a probe candidate of Jembrana Disease Virus based on envelope-transmembrane (*env-tm*) gene to optimize Jembrana disease detection method. The DNA fragment derived from *env-tm* of JDV was used, cloned in pGEX-TM and expressed in *E.coli* DH 5 α . Sequence analysis was conducted with BLAST programs from NCBI. Sequence analyses of the fragments of *env-tm* clone, indicated that it has a very closed genetic relation with 97,68% homology identity. Probe was designed based on the conserved region of *env-tm* using Geneious resulted in JT2 252 bp long. BLAST analyses showed that probes had high specificity to other strains of JDV in Indonesia

Key words : probe, *env-tm*, JDV, specificity, sensitivity.

Introduction

Jembrana disease virus (JDV), a newly recognized bovine lentivirus detected in Indonesia (Chadwick *et al.*, 1995), causes an acute, severe disease in Bali cattle (*Bos javanicus*) and a milder disease syndrome in *Bos taurus*. Infection of Bali cattle with JDV results in a case fatality rate of approximately 20%; the remainder survive with no recurrence of disease (Soesanto *et al.*, 1990).

The disease is atypical of many lentivirus infections: it produces an acute clinical disease persisting for up to 12 days, after a short incubation period of less than 12 days

(Soeharsono *et al.*, 1995). Consistent clinical signs include fever, lethargy and lymphadenopathy. Haematological changes during the acute phase include elevated blood urea concentrations, decreased plasma protein, leucopenia mainly due to a lymphopenia, eosinopenia and thrombocytopenia (Soesanto *et al.*, 1990). Histologically, there is atrophy of follicular (B-cell) areas and marked proliferation of lymphoblastoid cells in parafollicular (T-cell) areas, of lymph nodes and spleen (Dharma *et al.*, 1991). After approximately 5 weeks there is a decrease in the parafollicular proliferation, regeneration of follicular cells, and increased plasma cell formation (Dharma *et al.*, 1991).

Antibody against JDV is not detected in cattle until after recovery from the acute disease, and is not seen in a majority of cattle

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until 11 weeks after infection (Hartaningsih *et al.*, 1994). The histological changes and the delayed antibody response suggests JDV infection results in impairment of humoral immune function, and that the mechanism of recovery from JDV is probably T-cell and not antibody mediated. There is evidence that mortalities are often associated with secondary infections (Dharma *et al.*, 1991), possibly due to immunosuppression during the acute disease syndrome.

The genome of JDV is composed of two linear single stranded RNA viruses. The detection of single-stranded RNA is prone to degradation and until recently the RT and the amplification reaction had to be performed separately. In the absence of any genome sequence data, a sandwich enzyme linked immunosorbent assay (S-ELISA) was developed using antibodies produced in mice.

The drawbacks of the methods is the limited stocks of antibodies, unless they are produced by hybridoma technology and its possibility of bovine immunodeficiency virus (BIV) cross reaction, the other similar virus. This study aims to produce more reliable and sensitive detection methods based on nucleic acid. We will use the conserved *env* sequences from Bali samples to design a high specific probe based on its stable genetic content.

Materials and Methods

Previous cloning of *env-tm* gene in pGEX-2T (Amersham Pharmacia) was PGEX-TM construction which was available in our laboratory and kept frozen in Luria Bertani medium containing glycerol.

Plasmid preparation

Plasmid were extracted using alkaline lysis method. Briefly, bacterial pellets were dissolved in 0.3 ml buffer I (50 mM Tris-HCl, pH 7.5, 10 mM EDTA and incubated for 5 min at room temperature. 0.3 ml of buffer 2 (0.2 M NaOH, 1% SDS) was then added and the suspension mixed without vortexing. Finally

0.3 ml of buffer 3 (2.55 M potassium acetate, pH 4.8) was added and the solution then mixed. Solution centrifuged in a minicentrifuge for 15 min at maximal speed (13.000 rpm) at room temperature. the supernatant (0.8 ml) was then precipitated by addition of 5 isopropanol and centrifugation. The pellet was then washed with 70% ethanol then slightly dried. It was then dissolved in 50 μ L 10 mM Tris-HCl pH 7.5.

Amplification of plasmid insert

Plasmid insert were analysed by colony PCR amplification using upstream primer ATAGGATCCATGGCCGTGGGGATGGT-CATAT and downstream primer CAGCG-GATCCTCCAAGCTACGTGTC. Plasmid PCR was carried out in 50 μ l reaction containing 5 ng pGEX-TM, PCR DIG labelling mix (200 μ M dNTP, 1 μ l digoxigenine-11-dUTP), 1-5 unit Taq DNA polymerase (1 μ l), and 50 pmol every primer gen *env-tm*. Amplification was performed using BioRad thermal cycler according to the following protocol : 25 cycles of 95°C for 5 min, 55°C for 1 min and 72°C for 2 min

Computer analysis

The nucleotide sequence were used to search for homologous sequences in nucleic acid databases using BLASTN. Geneious was also used for sequence similarities and probe design.

Results and Discussion

Figure 1A showed isolation of *env-tm* gene from *E coli* DH5 α . Previous cloning of *env-tm* gene in pGEX-2T allowed us to isolate *env-tm* gene easily and rapidly. It confirmed that clone pGEX-TM gave a fragment of about 6.0 kb, consist of 4.9 kb pGEX-2T and 1.1 kb *env-tm* gene.

The *env-tm* gene insertion analysis was also done by PCR method using specific primer to amplify *env-tm* fragment. PCR analysis gave a spesific fragment of around 1.1 kb showed by figure 1B. The result

showed us that *env-tm* was exist. This result also showed that insert can be analysis by PCR method using specific primer.

Sequencing analysis showed point mutation on 619-621 compared to Tabanan 1987 (Chadwick *et al.*, 1995). CAA (619-621 position) mutated to CAG, both of codon are glutamin genetic code (amino acid number 207). It can be happened because of viral genomic evolution or genetic variation on Tabanan 1995 isolat had been used. Error due to sequencing had a minimum value because sequencing result had same data with other sequencing result obtained from pcDNA-TM clone that inserted independently (Kusumawati *et al.*, in press). Sequence analysis was conducted with BLAST programs (Altschul *et al.*, 1997) from NCBI showed in Figure 2. *env-tm* gene inserted in pGEX-TM had high homology identity of around 90% up to 100%, *E value* 0,0., perfectly . A high level of nucleotide conservation (97-100%) was observed in *gag* sequences from samples taken in Bali and Sumatra, indicating that the source of JDV in

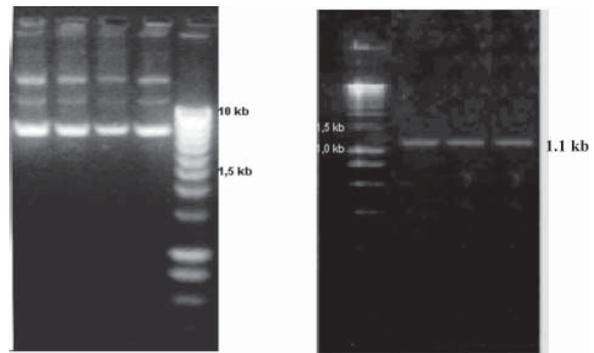


Figure 1. (A). pGEX-TM 6.0 kb DNA plasmid recombinant electrophoresis. (B). *env-tm* gene PCR from linearized plasmid. Marker was indicated.

Sumatra was most likely to have originated from Bali. JDV appears to be remarkably stable genetically and has undergone minor genetic changes over a period of nearly 20 years in Bali despite becoming endemic in the cattle population of the island (Desport *et al.*, 2007). Homology analysis using ClustalW modification and homology tree that formed from the alignment showed in Figure 2.

Sequence analyses of the fragments of *env-tm* clone, indicated that it has a

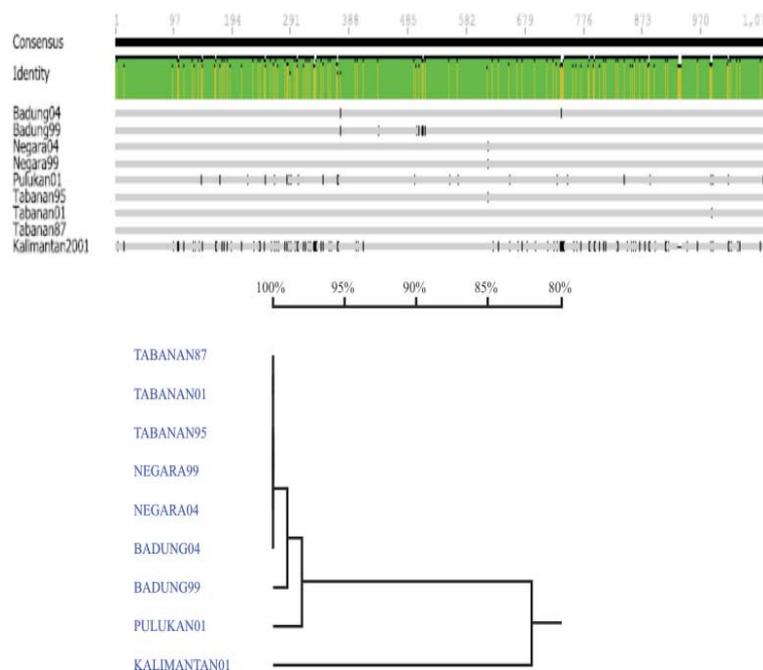


Figure 2. Homology analysis using ClustalW modification and homology tree that formed from the alignment .

very closed genetic relation with 97,68% homology identity with any others strain in Indonesia.

Probe a known DNA or RNA fragment (or a collection of different known fragments) which is used in a hybridization assay to identify closely related DNA or RNA sequences within a complex, poorly understood mixture of nucleic acids (the *target*). In standard hybridization assays, the probe is labeled, but in reverse hybridization assays the target is labeled (Reue,1998). In standard nucleic acid hybridization assays

the probe is labeled in some way. Nucleic acid probes may be made as single-stranded or double-stranded molecules, but the working probe must be in the form of single strands. Conventional DNA probes are isolated by cell-based DNA cloning or by PCR. In the former case, the starting DNA may range in size from 0.1 kb to hundreds of kilobases in length and is usually (but not always) originally double-stranded. PCR-derived DNA probes have often been less than 10 kb long and are usually, but not always, originally double-stranded.

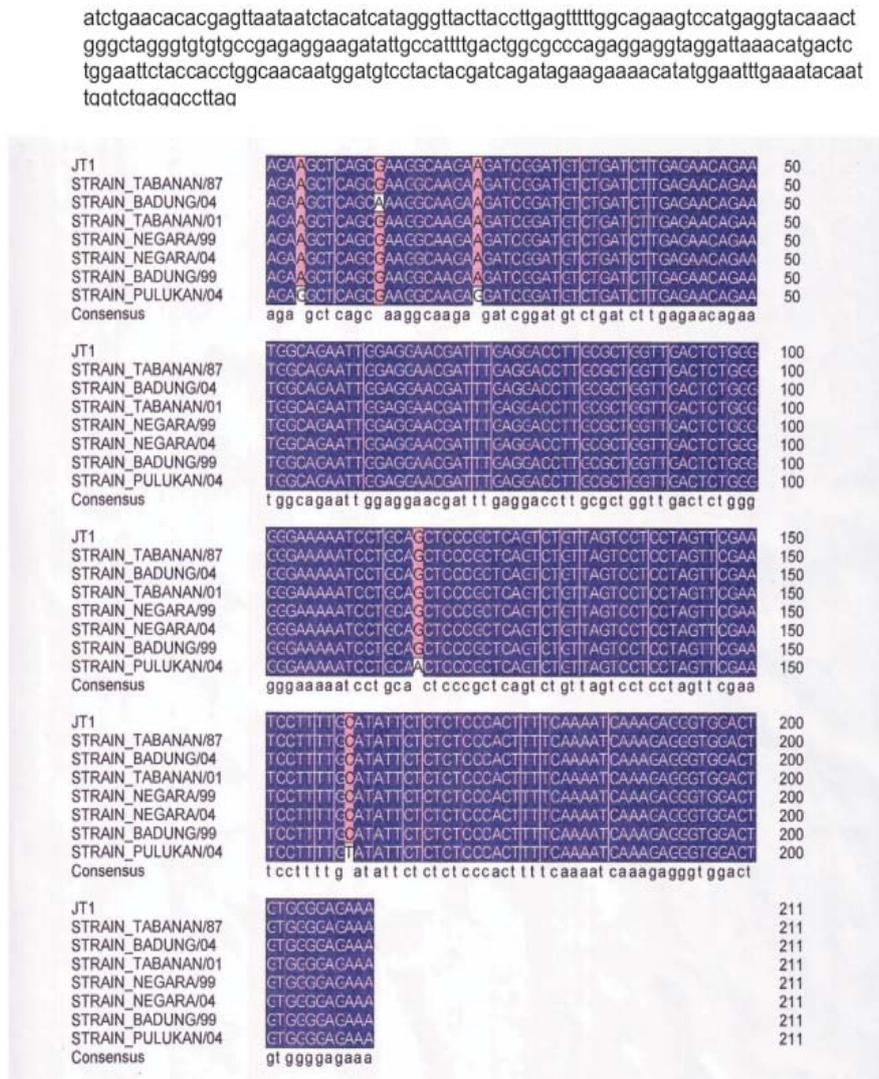


Figure 3. Alignment result of probe with other strain in Indonesia

Conventional DNA probes are usually labeled by incorporating labeled dNTPs during an *in vitro* DNA synthesis reaction.

From bioinformatics database and BLAST analysis, it could be identified conserved sequence region of *env-tm* located between 1442 and 1693 of JDV genome (252 base pair). Those conserved sequence has a potency for nucleic acid probe candidate. Probe can be designed using bioinformatic database and BLAST analysis. BLAST can be used to determine similarity between probe sequence and targeted organisms (Pruitt *et al.*, 2005).

BLASTN analysis showed that probe candidate can be used to detect *env-tm* JDV strain Tabanan 1987, Tabanan 2001, Negara 1999, Negara 2004, Badung 2004, and Badung 1999. *nv-tm* gen JDV strain that complement with the *probe* having homology identity up to 99,89% (Figure 3).

As the conclusion, sequence analysis was conducted with BLAST programs from NCBI. Sequence analyses of the fragments of *env-tm* clone, indicated that it has a very closed genetic relation with 97, 68% homology identity. Probe was designed based on the conserved region of *env-tm* located between 1442 and 1693 of JDV genome using Geneious resulted in JT2 252 base pair long. Those conserved sequence has a good potential for nucleic acid probe candidate.

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