



# HOKKAIDO UNIVERSITY

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| Title               | Identification, molecular characterization, and application of a novel virus isolated from mosquito larvae in Okushiri Island, Japan [an abstract of dissertation and a summary of dissertation review] |
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## 学位論文内容の要旨

博士の専攻分野名称：博士（農学） 氏 名：Yudistira Wahyu Kurnia

### 学位論文題名

## **Identification, molecular characterization, and application of a novel virus isolated from mosquito larvae in Okushiri Island, Japan**

（北海道奥尻島において蚊幼虫から分離された新規ウイルスの同定、性状解析  
および利用）

Mosquitoes are one of the most important pest insects that have a serious impact on public health; they are a principal vector of serious infectious diseases including malaria, dengue fever, chikungunya, Japanese encephalitis, and yellow fever. Vector control is an essential strategy for reducing the transmission of mosquito-borne diseases. Mosquito-specific viruses with mosquitocidal activity, therefore, may be used as environmentally-friendly biological pesticides for chemical pesticides.

In this study, a small spherical virus, designated Okushiri virus (OKV), was isolated from mosquito larvae and identified as a novel virus belonging to the genus *Negevirus*, a newly proposed insect-specific virus group. Here, an infectious cDNA clone for OKV and an OKV-based foreign gene expression system were constructed as a basis for reverse genetics approach in basic and applied research of negevirus.

### **1. Identification and molecular characterization of Okushiri virus**

A novel virus was isolated from field-collected *Aedes* larvae collected on Okushiri Island, Hokkaido, Japan. This virus, designated Okushiri virus (OKV), replicated in the *Aedes albopictus* cell line C6/36 with severe cytopathic effects and produced a large number of spherical viral particles that were 50-70 nm in diameter and released into the cell culture medium. The OKV had a positive-sense, single-stranded RNA and the genome RNA consisted of 9,704 nucleotides, excluding the poly(A) tail at the 30-terminus, and contained three major open reading frames (ORF1, ORF2, and ORF3). ORF1 encoded a putative protein of approximately 268 kDa that included a methyltransferase domain, FtsJ-like methyltransferase domain, helicase domain, and

RNA-dependent RNA polymerase domain. ORF2 and ORF3 were suggested to encode hypothetical membrane-associated proteins of approximately 45 kDa and 22 kDa, respectively. The genome organization and results of a phylogenetic analysis based on the amino acid sequence predicted from the nucleotide sequence indicated that OKV is a member of a new insect virus group of negevirus with a possible evolutionary relationship to some plant viruses. This is the first study on a novel negevirus isolated from mosquito larvae in Japan.

## **2. Generation of an infectious cDNA clone of Okushiri virus and its derivative capable of expressing an exogenous gene**

To enable genetic manipulation of the OKV genome, an infectious cDNA clone of OKV was constructed. RNA synthesized *in vitro* from pFBOKV, a full-length OKV cDNA, in the presence or absence of cap analogue produced infectious progeny viruses effectively in mosquito C6/36 cells. Subsequently, ORF3 in pFBOKV was replaced with GFP coding sequence to generate a construct designated as pO2GFP. C6/36 cells transfected with pO2GFP-derived RNA successfully expressed GFP, but failed to produce progeny viruses. Co-transfection of C6/36 cells with pO2GFP- and pFBOKV-derived RNA revealed that it is possible to produce infectious pO2GFP-derived progeny viruses by supplying OKV genetic elements and/or gene product deleted in pO2GFP even though the infectivity appeared to be low. This is the first report of construction of a negevirus-based foreign gene expression system. The cDNA clones constructed and biological insights obtained in this study will be powerful tools allowing for reverse genetics of OKV and providing a basis to develop efficient negevirus-based expression vector systems.