Malaysian Journal of Microbiology, Vol 14(4) Special Issue 2018, pp. 325-328
DOI: http://dx.doi.org/10.21161/mjm.144185

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Overexpression of recombinant domain III envelope protein of Zika virus

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ABSTRACT

Aims: Zika virus (ZIKV) is a member of the *Flaviviridae* family and is transmitted to humans by mosquitoes. In humans, it causes disease known as Zika fever. The severity of the infection ranged from asymptomatic to mild disease and to infection associated with neurological disorders and congenital anomaly. The common symptoms are maculopapular rash, fever, arthralgia, myalgia, headache and conjunctivitis. The flavivirus genome consists of structural and non-structural proteins. The envelope (E) glycoprotein is the major structural protein which is responsible for virus entry and represents a major target for neutralizing antibodies. The E protein consists of three distinct domains: domain I, domain II and domain III. The domain III (DIII) of the E protein has shown to be useful as antigen for flavivirus serologic diagnosis and immunization in animal model. Hence, the aim of this work is to express the DIII of E protein (EDIII) of ZIKV for immunoreactivity study.
Methodology and results: The EDIII of ZIKV was cloned into pET SUMO cloning vector and transformed into Mach-T1 competent *E. coli* cells. Positive clone was selected, verified and transformed into BL21 (DE3) competent *E. coli* for protein expression. The expression of the recombinant protein was analysed on SDS-PAGE and western blot. The recombinant fusion protein of EDIII/SUMOHIS (rEDIII) was successfully expressed at a molecular weight of approximately 38.2 kDa.
Conclusion, significance and impact of study: The expression of the protein was confirmed by detection with anti-histidine and a flavivirus antiserum, HPR.

Keywords: Recombinant protein, domain III, Zika virus

INTRODUCTION

Zika virus (ZIKV) is a member of the *Flaviviridae* family and is transmitted to humans by mosquitoes (Alera *et al*., 2015). Originating from Africa, the virus was first isolated in the 1970s and until several years ago was only endemic in Africa and Asia (Hayes, 2009). Recently, large outbreaks were reported outside Africa and Asia that include the outbreak in Micronesia, on the island of Yap in 2007 (Duffy *et al*., 2009) and in the southern and western Pacific region including French Polynesia, New Caledonia, Easter Island and Cook Island at the end of 2013 and early 2014 (Musso *et al*., 2014) and the most recent in Brazil in April 2015 (Zanluca *et al*., 2015). In several non-endemic countries including Japan, Germany, Italy, Canada, Australia and the United States, the infection has been diagnosed in returning travelers (Zammarchi *et al*., 2015). Although considered a relatively mild disease, its disease spectrum is currently not well understood. For example, during the outbreak in French Polynesia in 2013, infection with ZIKV was associated with increased incidence of neurological complications including a 20-fold increase of Guillain-Barré syndrome that warrants further studies (Oehler *et al*., 2014).

In Asia, Zika fever has been described sporadically in the Philippines (Alera *et al*., 2015), Cambodia (Heang *et al*., 2012), Thailand (Buathong *et al*., 2015) and Indonesia (Olson *et al*., 1990). Although it had been reported that antibodies against ZIKV were demonstrated in serum samples from patients in peninsular and Borneo in the 50s and 60s (Smithburn, 1969), there is no recent report of Zika fever in Malaysia. However, in May of this year Tappe and colleagues (2015) had reported an acute ZIKV infection in a traveler returning from Borneo, Sabah. Thus, in Borneo, either the virus only rarely infects humans or there is a possibility that the disease is mistaken to other closely related diseases such as dengue and chikungunya. Given that the clinical symptoms of Zika fever are very similar to that of dengue, diagnostic confusion between ZIKV illness and dengue or even chikungunya is possible and has been reported widely (Cook and Holmes, 2006; Caron *et al*., 2012; Roth *et al*, 2015).

Due to this, laboratory confirmation of suspected cases is necessary for proper management of the cases. Generally, diagnosis of ZIKV infection is achieved by reverse transcription polymerase chain reaction (RT-

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538