ABBREVIATIONS

aa amino acid
Ab Antibody/antibodies
ADE Antibody Dependent Enhancement
AOX1 Alcohol oxidase 1
AOX1 gene Alcohol oxidase 1 gene
AOX2 Alcohol oxidase 2
AOX2 gene Alcohol oxidase 2 gene
ATCC American Type Culture Collection
AOX1 promoter Alcohol oxidase 1 promoter
APS Ammonium per sulfate
BHK-21 Baby Hamster Kidney cells
BMGY Buffered complex medium containing glycerol
BMMY Buffered complex medium containing methanol
bp Base pairs
CHAPS 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
cm centimetre
CVD ChimeriVax-DENV
DC Dendritic cell
dc-SIGN Dendritic cell-specific intercellular adhesion molecule-3-Grabbing Non-integrin
DENV Dengue Virus
DF Dengue fever
DHF Dengue Hemorrhagic Fever
DME Dengue’s Modified Eagle Medium
DSS Dengue shock syndrome
E Envelope protein
EDIII Envelope domain III
EDIII-T Protein containing EDIIIs of all four serotypes linked in tandem
EDTA Ethylenediaminetetraacetic acid
ELISA Enzyme-linked immunosorbent assay
FCS Fetal Calf Serum
GuHCl Guanidinium-HCl
HBeAg Hepatitis B core antigen
HBeAg gene Hepatitis B core antigen gene
HBV Hepatitis B virus
His Histidine
hr hour(s)
IFN Interferon
IgG Immunoglobulin G
IgM Immunoglobulin M
kDa Kilo Dalton
kb Kilo base pairs
L Litre
LAV Live attenuated virus
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>Luria-Bertani</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>mAb</td>
<td>Monoclonal Antibody</td>
</tr>
<tr>
<td>μF</td>
<td>micro-Farad</td>
</tr>
<tr>
<td>μg</td>
<td>micro-gram</td>
</tr>
<tr>
<td>μl</td>
<td>micro-litre</td>
</tr>
<tr>
<td>μm</td>
<td>micro-metre</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>mg</td>
<td>milli-gram</td>
</tr>
<tr>
<td>ml</td>
<td>milli-litre</td>
</tr>
<tr>
<td>mM</td>
<td>milli-molar</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>ms</td>
<td>milli-second</td>
</tr>
<tr>
<td>MIR</td>
<td>Major Immunodominant Region</td>
</tr>
<tr>
<td>MOI</td>
<td>Multiplicity of Infection</td>
</tr>
<tr>
<td>Mu^+</td>
<td>Methanol utilization plus</td>
</tr>
<tr>
<td>Mu^-</td>
<td>Methanol utilization slow</td>
</tr>
<tr>
<td>Mu^-</td>
<td>Methanol utilization minus</td>
</tr>
<tr>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>NCR</td>
<td>Non-coding region</td>
</tr>
<tr>
<td>ng</td>
<td>nano-gram</td>
</tr>
<tr>
<td>NGC</td>
<td>New Guinea C</td>
</tr>
<tr>
<td>Ni-NTA</td>
<td>Nickel-Nitrioloacetic acid</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>NS</td>
<td>Non-structural</td>
</tr>
<tr>
<td>Ω</td>
<td>Ohm</td>
</tr>
<tr>
<td>OD/OD&lt;sub&gt;600&lt;/sub&gt;</td>
<td>Optical density/ Optical density at 600nm</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PBST</td>
<td>Phosphate buffered saline with Tween 20</td>
</tr>
<tr>
<td>pfu</td>
<td>Plaque forming units</td>
</tr>
<tr>
<td>PRNT</td>
<td>Plaque Reduction Neutralization Test</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducers and activation of transcription</td>
</tr>
<tr>
<td>TEMED</td>
<td>N, N', N'- Tetramethylethlenediamine</td>
</tr>
<tr>
<td>TMB</td>
<td>3,3',5,5'-Tetramethylbenzidine</td>
</tr>
<tr>
<td>TT</td>
<td>Transcription termination sequence</td>
</tr>
<tr>
<td>Tyk</td>
<td>Tyrosine kinase</td>
</tr>
<tr>
<td>VLP</td>
<td>Virus-like-particle</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>YF17D</td>
<td>Yellow Fever virus vaccine</td>
</tr>
<tr>
<td>YPDS</td>
<td>Yeast extract-Peptone-Dextrose-Sorbitol plates</td>
</tr>
<tr>
<td>YPD</td>
<td>Yeast extract-Peptone-Dextrose medium</td>
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Introduction

Dengue is a highly endemic disease of the tropics and is rapidly spreading to the other parts of the world. It is caused by any of the four dengue virus serotypes DENV-1, -2, -3 and -4. Dengue virus belongs to flavivirus genus of flaviviridae family. There is no approved dengue vaccine candidate till now, though there are few vaccine candidates in the clinical phase of development. It is pre-requisite for a vaccine candidate to be safe for human use. Hence, non-replicating subunit vaccine candidates, which are safer than live-attenuated virus, are drawing attention in the field of dengue vaccine development.

Dengue envelope domain III is one of the most critical domains of dengue envelope protein. It is highly accessible on the virion surface and is involved in binding of the virion to the receptor on the host cell surface. This domain also contains virus neutralizing epitopes.

Virus-like-particles (VLPs), as recombinant subunit vaccines, have come into focus with the success of the VLP-based vaccines for Human Papilloma Virus and Hepatitis B Virus. Several viral structural proteins possess an intrinsic ability to assemble into non-infectious, non-replicating structures known as VLPs. VLPs mimic the structure of a virion and thus combine the advantages of whole virus vaccines, with respect to immunogenicity, and recombinant subunit vaccines, with respect to their being non-infectious and safer. One such viral protein, capable of forming VLPs, is Hepatitis B virus core antigen (HBcAg). It has been fused with a variety of antigens, from bacterial, viral and protozoan pathogens, to form chimeric VLPs.

In the present study, dengue envelope domain III has been fused to Hepatitis B core antigen, to further enhance the immunogenicity of envelope domain III. This chimeric protein was expressed in eukaryotic host P. pastoris and purified. The chimeric protein was able to form VLPs. It was evaluated immunologically by immunization in mice. The antiserum, so raised, was found to neutralize dengue virus.