

2. Aims and Objectives

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Dengue is a globally widespread, arthropod-borne infection, caused by any of the four Dengue virus serotypes 1, 2, 3 and 4 (DENV-1, -2, -3, -4). This disease is endemic in many areas and 50 million people are estimated to be affected annually worldwide, according to WHO report. This disease has been on a rise since the last decade and has become a global public health threat. Currently, there is neither an approved vaccine nor antiviral therapy against dengue. Apart from attenuated live virus dengue vaccines, a number of approaches like inactivated viruses, subunit vaccines, DNA vaccines, cloned engineered viruses, chimeric viruses using yellow fever vaccine and attenuated dengue viruses are being developed. Though, attenuated DENV vaccine candidates are in the advanced phase, currently, a lot of emphasis is being laid on subunit vaccine development because of the safety concerns over attenuated DENV vaccines. As a result, Envelope domain III (EDIII) has come into focus as a potential candidate for subunit vaccine development. EDIII is responsible for recognition of host cell receptor and generation of neutralizing antibodies. Thus, this study focuses on utilization of this domain for the development of a potential dengue vaccine candidate.

In order to further enhance the immunogenicity of this domain, it can be presented on the surface of a virus-like-particle (VLP). In this study, Hepatitis B core antigen (HBcAg) was used as a carrier for the presentation of DENV-2 Envelope Domain III (EDIII-2) on the surface of its VLP. The choice of this fusion partner, for presentation of EDIII-2, was made on the basis of the following considerations. First, HBcAg spontaneously assembles into ~30nm VLPs. Second, this inherent capacity of HBcAg to generate VLPs is likely to be retained in its chimeric form also. Third, HBcAg VLPs generate B-cell, T-cell dependent and independent immune responses, and hence, are highly immunogenic. Fourth, the epitope of interest can be introduced in the Major Immunodominant Region (MIR) of HBcAg, which is its most exposed region, in order to optimally expose the epitope on the surface of the VLP for better immunogenicity. Fifth, it can be expressed efficiently in *P. pastoris*, under the control of *AOX1* promoter.

The major objective of this work has been to present EDIII-2 on the surface of HBcAg VLP, utilizing *P. pastoris* as a host for its expression. The selection of *P. pastoris*, as a

host, offers advantages of both prokaryotic (high expression levels, easy scale-up, inexpensive growth media) and eukaryotic (capacity to carry out most of the post-translational modifications) expression systems.

The following were the specific aims of this study:

- a) Design and construction of *HBcAg* gene, optimized for expression in *P. pastoris*.
- b) Design and construction of a chimeric *HBcAg-EDIII-2* gene, optimized for expression in *P. pastoris*.
- c) Cloning of genes in a suitable, integrative, *P. pastoris* expression vector.
- d) Integration of gene into the genome of *Pichia pastoris*, KM71H.
- e) Heterologous expression of HBcAg and HBcAg-EDIII-2 in *P. pastoris*, by methanol-based induction.
- f) Optimization of expression conditions for HBcAg-EDIII-2 protein.
- g) Purification of HBcAg and HBcAg-EDIII-2 proteins from *P. pastoris*.
- h) Functional characterization of HBcAg-EDIII-2 protein.
- i) Electron-microscopic evaluation of the proteins for VLP formation.
- j) Analysis of the immune response elicited, by the recombinant proteins, in experimental animals.