Introduction

Dengue viruses, of which there are four antigenically distinct serotypes, produce a broad spectrum of disease, ranging from mild febrile illness to a severe hemorrhagic, sometimes fatal, disease. In recent years, dengue virus infection has re-emerged as a significant global public health threat. About 2.5 billion people, in more than a hundred countries are estimated to be at risk of dengue virus infection, with millions of cases occurring annually around the world. There is neither an effective antiviral therapy for its treatment nor a vaccine for its prevention. Available evidence indicates that, immunity against other serotypes (heterotypic immunity) following dengue infection is transient, lasting about a couple of months; on the other hand, resistance to the same serotype (homotypic immunity) is life-long. Thus, it is preferable to develop broad-spectrum immunity against all four serotypes of dengue virus. Efforts are currently on, towards the production of dengue vaccines by traditional methods, using live attenuated strains. But, this strategy is associated with the risk of genetic reversion to virulence. However, recombinant DNA technology can circumvent this potential problem.

The genomic RNA of dengue viruses encode three structural and seven non-structural proteins. Amongst these proteins, the major structural protein known as the envelope (E) protein, has been the focus of intense efforts to develop possible sub-unit dengue vaccine candidates. The dengue virus E protein has been expressed using a variety of different heterologous host systems. For the E protein to serve as a potent antigen, it is desirable to express it in a eukaryotic host to ensure the maintenance of its structural integrity. This work has addressed the possibility of using the methylotrophic yeast, Pichia pastoris, as a host for the expression of recombinant dengue virus type-2 E protein. Further, it has explored the utility of Hepatitis B surface antigen (HBsAg) gene fusion to incorporate the E protein into virus-like particles (VLPs) which may also function as a bivalent immunogen. This thesis describes the design and construction of recombinant dengue virus type-2 E-based genes, their expression in P. pastoris, purification and characterization of the expressed recombinant proteins and finally a preliminary analysis of the immune response they elicit in experimental animals.