Chapter 2

Aims and Objectives
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Dengue virus infection has emerged as a significant global public health threat in the last decade, especially as there is neither an effective antiviral therapy for its treatment nor a vaccine for its prevention. About 40% of the world's population, in more than a hundred countries, are estimated to be at risk of dengue virus infection, with millions of cases occurring worldwide every year. Apart from traditional tissue culture based methods to develop attenuated live virus dengue vaccines, work is in progress in laboratories around the world to use recombinant DNA techniques to design and develop effective dengue vaccines. A major area of vaccine research focuses on the development of dengue virus envelope (E) protein-based subunit vaccines. The envelope protein is a multifunctional protein involved in several key aspects of the dengue virus life cycle. More importantly, it is implicated in receptor-mediated uptake of the virus by susceptible host cells and serves as the primary target of neutralizing antibodies capable of conferring life-long homotypic resistance. Several different heterologous systems have been employed for the expression of native, mutant and chimeric forms of the dengue virus envelope protein.

The major objective of this work has been to evaluate the utility of *P. pastoris* as a host to express the E proteins of DEN viruses. The *P. pastoris*-based expression system has the 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. These features may make the resultant vaccine more affordable to the target population. Since work reported in the literature suggests that dengue E protein may be susceptible to proteolysis in *P. pastoris*, a gene fusion strategy was adopted using the surface antigen of Hepatitis B virus (HBsAg) as the fusion partner. The choice of this fusion partner for the dengue E protein was made on the basis of the following considerations. First, HBsAg can be expressed efficiently in *P. pastoris* using the *AOX1* promoter. Second, recombinant HBsAg expressed in *P. pastoris* is very stable and it spontaneously assembles into ~20nm VLPs, which are highly immunogenic. Third, this inherent capacity of HBsAg to generate particulate
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Structures is likely to be retained in its fusion derivatives. Fourth, large inserts (up to ~16 kb at single locus and ~50 kb at multiple loci) can be easily integrated into the genome of *P. pastoris*, making a tetravalent dengue vaccine design possible, where all the four E proteins can be expressed from a single clone. Fifth, the fusion may serve as a bivalent immunogen capable of eliciting immune response specific to both dengue as well as hepatitis B. Finally, the fusion partner may serve as affinity handle for the purification of the recombinant protein.

This work has focused on DEN-2 virus, as it is the most virulent of all the four serotypes and is more prevalent in our country. The following were the specific aims of this work:

(i) Isolation of the E gene of dengue virus type 2 (*Den2E* gene).

(ii) Design and construction of a chimera consisting of *Den2E* gene fused in-frame with *HBsAg* gene.

(iii) Construction of expression vectors encoding the fusion gene.

(iv) Integration of the fusion gene into the *AOX1* locus of *Pichia pastoris* GS115 genome.

(v) Heterologous expression of the fusion gene in *P. pastoris* by methanol induction.

(vi) Optimization of expression parameters.

(vii) Design and development of a strategy for the purification of the recombinant fusion protein from *P. pastoris* lysates.

(viii) Investigation of the physical status of the recombinant fusion protein (monomers versus VLPs).

(ix) Preliminary analysis of the immune response elicited by the recombinant fusion protein, in experimental animals.