Chapter 2

Aims and Objectives
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Human hepatitis B is one of mankind's major infectious diseases for which no effective therapy and treatment currently exists. Out of the 2,000 million people infected worldwide, more than 350 million are chronic carriers. Any attempt to reduce the incidence of hepatitis B on a worldwide scale requires the availability of large quantities of potent, safe and affordable hepatitis B vaccine. Although the vaccine does not cure chronic carriers, it is 95% effective in preventing development of the carrier-state. The World Health Organization had called for inclusion of this vaccine in routine National Immunization Programmes. However, the high cost of the vaccination programmes has impeded introduction of this vaccine in many poorer countries where HBV infection is prevalent, afflicting 5 to 20% of the population.

Hepatitis B vaccines are alum adjuvented highly purified preparations of hepatitis B surface antigen particles (HBsAg). Such particles can either be purified from the plasma of HBV carriers (plasma derived vaccines) or produced in yeast or mammalian cells by recombinant technology (recombinant vaccines). It is a general trend to replace all products originating from animals or humans with those produced by genetic engineering in order to exercise better control over the source of the product. Currently such a particulate form of HBsAg for use as a hepatitis B vaccine is commercially produced in S. cerevisiae and *Pichia pastoris*. However, this vaccine is quite expensive and this has been attributed to the lower expression levels of HBsAg. It has been suggested that higher expression levels may cut down the production cost of this vaccine.

In recent years, the methylotrophic yeast *Pichia pastoris* has emerged as a powerful and inexpensive heterologous system for the production of high levels of functionally active recombinant proteins. High-level expression and efficient assembly of HBsAg has been reported in *Pichia pastoris* by integrating a single copy of the *HBsAg* gene under control of *AOX1* promoter [83]. However, expression levels are significantly lower than those of many other proteins that have been expressed under similar conditions (see Table 1-4).

The major objective of this work is to investigate various strategies that can contribute to maximize HBsAg expression levels in *Pichia pastoris* as a first step towards the production of a low cost hepatitis B vaccine. To fully exploit the potential of the *Pichia* expression system for HBsAg production a systematic investigation of the effects of gene dosage, mode of expression and the nature of *Pichia* host strain will be undertaken.
The following were the specific aims of this study:

(i) Creation and characterization of a series of *Pichia* transformants harboring progressively increasing copies of the HBsAg gene under *AOX1* and *GAP* promoter by *in vivo* and *in vitro* strategies for generation of multicopy clones

(ii) Analysis of single and multicopy *Pichia pastoris* clones of HBsAg by Southern, Northern, Western blots and by HBsAg particle-specific ELISA

(iii) Determination of the influence of gene copy number on HBsAg expression levels by HPLC quantitation

(iv) Evaluation of the ability of recombinant HBsAg to assemble into particulate structures (VLPs) in multicopy *Pichia pastoris* strains

(v) Comparison of induced and constitutive expression of HBsAg

(vi) Assessment of the utility of a protease-deficient host *Pichia* strain to enhance HBsAg yields

(vii) Investigation of the feasibility of secreting HBsAg particles