

CONSTRUCTION OF A *Pichia pastoris* YEAST STRAIN THAT CARRIES A MUTANT HEPATITIS B SURFACE ANTIGEN GENE

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INTRODUCTION

All subtypes of Hepatitis B virus (HBV) share a common antigenic determinant 'a' which is contained between residues 124-128 of the surface protein (HBsAg) (1) and has a double loop conformation. The second loop is located between residues 139-148 and appears to be conformational (2). This epitope represents the more immunodominant epitope in human vaccinated with either plasma or recombinant derived hepatitis B vaccine (3).

Recently it has been reported a vaccine induce escape mutant of HBV that express an altered 'a' determinant of HBsAg. The alteration consists of a single aminoacid substitution from glycine to arginine at position 145 (4-6).

Here we describe the obtention of a *Pichia pastoris* yeast strain that carries the mHBsAg gene.

MATERIALS AND METHODS

Modification of native HBsAg gene was achieved by site directed mutagenesis using the Oligonucleotide direct *in vitro* mutagenesis system (Amersham, UK). An M13mp18 phage vector that contains the XbaI-EcoRI fragment of native HBsAg gene was used. Transfection procedures were performed in *E.coli* strain JM109. Positive phage plaques were selected in Luria-Bertani (LB) medium containing X-Gal (100 µg/mL), IPTG (20 µg/mL) following by dot blot hybridization and restriction analysis.

Nucleotide sequences were obtained using the Multiwell microtitre plate DNA sequencing system T7 DNA polymerase (Amersham, UK). *P. pastoris* transformation was performed according to Cregg *et al.* (7). Total yeast DNA was purified according to Cryer *et al.* (8). DNA procedures were performed according to Maniatis *et al.* (10).

RESULTS AND DISCUSSION

A fragment of the HBsAg gene, in which the glycine residue at position 145 was substituted by an arginine, was obtained by *in vitro* mutagenesis. The mutation was

confirmed by DNA sequencing. The complete gene was reassembled again in a plasmid vector. After two cloning steps the whole gene was inserted in a yeast integration vector. It was possible to differentiate the native gene from the mutant one not only by DNA sequencing but also by restriction analysis in the final genetic construction.

The final plasmid, named pMF 49, was used to transform the MP-36 *P. pastoris* yeast strain (11). Isolated DNA from ten colonies, analyzed by Southern-blot hybridization using the HBsAg gene as probe, showed that in some of them integration occurred by an homologous recombination event of the expression cassette with yeast genome.

We also performed several studies to evaluate the expression levels of mHBsAg, in comparison with native HBsAg, that showed a substantial alteration in the antigenicity profile of this protein when antibodies against the native protein, were used in conventional immunological ELISA system. Other studies are underway to detect the expression of the mutant protein by recombinant yeast strains.

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