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Short communication

Assembly of bacteriophage Qβ virus-like particles in yeast *Saccharomyces cerevisiae* and *Pichia pastoris*

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Abstract

Recombinant bacteriophage Qβ coat protein (CP), which has been proposed as a promising carrier of foreign epitopes via their incorporation either by gene engineering techniques or by chemical coupling, efficiently self-assembles into virus-like particles (VLPs) when expressed in *Escherichia coli*. Here, we demonstrate expression and self-assembly of Qβ CP in yeast *Saccharomyces cerevisiae* and *Pichia pastoris*. Production reached 3–4 mg/1 g of wet cells for *S. cerevisiae* and 4–6 mg for *P. pastoris*, which was about 15–20% and 20–30% of the *E. coli* expression level, respectively. Qβ VLPs were easily purified by size-exclusion chromatography in both cases and contained nucleic acid, shown by native agarose gel electrophoresis. The obtained particles were highly immunogenic in mice and the resulting sera recognized both *E. coli*- and yeast-derived Qβ VLPs equally well.

Keywords: Bacteriophage Qβ; Coat protein; Expression; Virus-like particles; Yeast

1. Introduction

The recombinant virus-like particles (VLPs) formed by coat protein (CP) of RNA-bacteriophage fr (Borisova et al., 1987; Kozlovskaya et al., 1988; Pushko et al., 1993) or MS2 (Mastico et al., 1993) of the *Levivirus* genus in the *Leviviridae* family were among the first proposed icosahedron carriers for the presentation of foreign short-sized epitopes on their surface. More recently, MS2 and fr VLPs were found to tolerate longer, 24 and 52 amino acid (aa) insertions, respectively, with retained self-assembly (Heal et al., 1999; Voronkova et al., 2002).

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High-level expression of the CP gene of bacteriophage Qβ from *Allolevivirus* genus of the *Leviviridae* family, which causes the formation of Qβ VLPs, was obtained in *Escherichia coli* (Kozlovska et al., 1993). The native Qβ particle (with quasi $T = 3$ symmetry) contains 180 copies of 133 aa-long CP enveloping a complex of single-stranded genomic RNA and maturation protein, and several molecules of a prolonged CP, 324 aa-long A1 protein, a natural read-through product of the UGA termination codon of the CP gene (Weber and Konigsberg, 1975). The A1 extension was considered a promising target site for foreign insertions, since it contained elements typical for spike-like structures exposed on the surface of particles (Kozlovska et al., 1996). In the presence of the wild-type CP as a helper, the formation of mosaic Qβ capsids with hepatitis B virus (HBV) preS1 or human immunodeficiency virus 1 gp120 epitopes of different length inserted at the A1 extension was demonstrated (Kozlovska et al., 1996; Vasiljeva et al., 1998). Non-mosaic Qβ CP derived VLPs (without A1 extension) have been used as a template for chemical coupling of desired peptides to surface-exposed lysine residues (Storni et al., 2004). Short CpG oligonucleotides, the most promising vaccine adjuvants known to date (for review, Krieg, 2004), were packaged successfully in vitro into chemically-engineered particles resulting in increased VLP immunogenicity and protection of CpG from nucleases (Storni et al., 2004; Schwarz et al., 2005).

For vaccine development, expression systems beside that of *E. coli* are desirable to avoid contamination of bacterial endotoxins. Yeast is an attractive eukaryotic microorganism presenting such an alternative. Up to now, a number of structural genes from mammalian viruses have been expressed in yeast resulting in the formation of VLPs (Valenzuela et al., 1982; Miyanohara et al., 1986; Kniskern et al., 1986; Cregg et al., 1987; Jacobs et al., 1989; Janowitz et al., 1991; Hofmann et al., 1995, 1996; Sasnauskas et al., 2002; Samuel et al., 2002; Slibinskas et al., 2004). The yeast expression system has been used successfully to produce the first licensed HBV vaccine (McAleer et al., 1984). In this work, we demonstrate the formation of recombinant RNA phage VLPs in two different yeasts to open, therefore, a way for further development of a yeast-derived phage VLP technology.

2. Cloning and expression of Qβ CP gene in yeast *S. cerevisiae* and *P. pastoris*

The Qβ CP-encoding gene was PCR-amplified from *E. coli* expression plasmid pQβ10 (Kozlovska et al., 1993) with the forward primer 5′-TT *TCT AGA* ACA **ATG** GCA AAA TTA GAG ACT G-3′ and the reverse primer 5′-T T*AC TAG* ***T*****TA** ATA CGC TGG GTT CAG C-3′ (start and termination codons are shown in bold). For expression in *S. cerevisiae*, the PCR fragment was digested with *Xba*I/*Spe*I (restriction sites are in italics) and cloned under the control of the galactose-inducible promoter in *Xba*I-treated vector pFX7 carrying the formaldehyde resistance gene (Samuel et al., 2002). The resulting pFX-Qβ plasmid was used to transform *S. cerevisiae* strains AH22 *MATa leu2 his4*, and *S. cerevisiae* DC5 *MATa leu2 his3*, as well as the wild-type strain *S. cerevisiae* FH4C, as described earlier (Sasnauskas et al., 2002; Ražanskienė et al., 2004). The transformed clones were selected on agarized YEPD medium supplemented with 3–10 mmol formaldehyde and cultivated as described by Sasnauskas et al. (1999). Transformants were incubated at 30 °C on a shaker in flasks containing YEPD medium supplemented with 3–5 mmol formaldehyde for 20–24 h until optical density OD_{590} reached 6–8. For induction, galactose was added to 3% in medium and cultivation was continued for another 20–24 h, with final OD_{590} 12–14. Production of Qβ CP was slightly better in the *S. cerevisiae* AH22 and *S. cerevisiae* FH4C strains as shown by SDS-PAGE and immunoblotting (Fig. 1A and B). This finding was confirmed by Ouchterlony double radial immunodiffusion with rabbit polyclonal anti-Qβ antibody, where lysate from the DC5 strain formed visible immunoprecipitation lines at dilution up to 1:64, but lysates from the *S. cerevisiae* AH22 and *S. cerevisiae* FH4C strains—up to 1:128 (see Fig. 1B).

For expression of the Qβ CP gene in *P. pastoris*, the Qβ CP-encoding PCR fragment was cloned by blunt-end ligation into *Sna*BI-treated vector pPIC3.5K (Invitrogen, Groningen, The Netherlands), under the control of the *AOX1* promoter. The resulting pPIC-Qβ plasmid after linearization with *Ecl*136II was used for transformation of the *P. pastoris* GS115 *his4* strain by electroporation (Bio-Rad, Gene Pulser), according to Cregg and Russell (1998). Mut^+His^+ transformants were selected on the minimal agar medium (0.67%