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# Recombinant *Saccharomyces cerevisiae* serves as novel carrier for oral DNA vaccines in *Carassius auratus*

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ABSTRACT

Oral delivery of DNA vaccines represents a promising vaccinating method for fish. Recombinant yeast has been proved to be a safe carrier for delivering antigen proteins and DNAs to some species *in vivo*. However, whether recombinant yeast can be used to deliver functional DNAs for vaccination to fish is still unknown. In this study, red crucian carp (*Carassius auratus*) was orally administrated with recombinant *Saccharomyces cerevisiae* harboring CMV-EGFP expression cassette. On day 5 post the first vaccination, EGFP expression in the hindgut was detected under fluorescence microscope. To further study whether the delivered gene could induce specific immune responses, the model antigen ovalbumin (OVA) was used as immunogen, and oral administrations were conducted with recombinant *S. cerevisiae* harboring pCMV-OVA mammalian gene expression cassette as gene delivery or pADH1-OVA yeast gene expression cassette as protein delivery. Each administration was performed with three different doses, and the OVA-specific serum antibody was detected in all the experimental groups by western blotting and enzyme-linked immunosorbent assay (ELISA). ELISA assay also revealed that pCMV-OVA group with lower dose (pCMV-OVA-L) and pADH1-OVA group with moderate dose (pADH1-OVA-M) triggered relatively stronger antibody response than the other two doses. Moreover, the antibody level induced by pCMV-OVA-L group was significantly higher than pADH1-OVA-M group at the same serum dilutions. All the results suggested that recombinant yeast can be used as a potential carrier for oral DNA vaccines and would help to develop more practical strategies to control infectious diseases in aquaculture.


## 1. Introduction

Aquaculture has become a major global industry which maintains a rapid growth over the last decades [1]. However, infectious disease outbreaks caused by bacteria, viruses, parasites and fungi are now threatening the sustainable growth of aquaculture [2]. From the economic, environmental and ethical point of view, vaccination is the most appropriate method for the control of disease-causing pathogens [3]. Compared to traditional antigen vaccines (i.e., live attenuated, whole killed and subunit vaccines), DNA vaccines are relatively inexpensive and easy to produce. In addition, they can not only induce both innate and adaptive immunity [4], but also overcome the safety concerns compared to live attenuated vaccines which may reverse to virulent forms [5]. However, DNA vaccines have been often practiced by traditional injection, which is labor intensive and not feasible for large numbers of small fish [3,6]. Oral administration is the most appealing vaccinating method for aquatic species since it is cost effective, simple, effortless, and less stressful to animals of all stages [3,7].

The baker's yeast *Saccharomyces cerevisiae*, historically used as supplement in animal feed, is nonpathogenic, therefore has 'generally recognized as safe' (GRAS) status, and in addition, possesses a strong immunologic adjuvant ability [8,9]. Moreover, *S. cerevisiae* showed a high survival rate in the digestive environment [10]. Thus, *S. cerevisiae* could be a potential delivery vehicle for oral administration of DNA vaccines. Actually, delivery of protein antigens by *S. cerevisiae* has been repeatedly proved to generate adaptive immune response in mice [11,12] and in human [13,14]. Besides, it has been demonstrated that *S. cerevisiae* can transport DNA and mRNA into mammalian antigen presenting cells (APC) *in vitro* [15]. Our previous studies further proved that oral

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administration of *S. cerevisiae* can deliver both protein and DNA into mouse intestine dendritic cells (DCs) and trigger the immune responses [16–19]. In teleost, there is one report claimed that recombinant *S. cerevisiae* expressing infectious pancreatic necrosis virus VP2 viral like particles was used to orally vaccinate rainbow trout and induced a protective immune response [20]. However, whether oral administration of recombinant yeast could present DNAs to fish cells is still unknown.

Based on the studies mentioned above and our previous results obtained from mice and other animals, we proposed the hypothesis that recombinant yeast can be used as *in vivo* gene delivery vehicle to induce immune responses in teleost. In this study, we used the EGFP reporter gene as the target gene to verify the concept that yeast-delivered gene could be expressed in fish intestines, and then we used the well-studied model antigen ovalbumin (OVA) to further assess the production of specific serum antibody during vaccination. This study lays a foundation for developing oral yeast-mediated DNA vaccines in aquaculture.

## 2. Materials and methods

### 2.1. Construction of expression vectors

To establish the yeast-mediated DNA delivery system, CMV promoter and BGH polyA fragments were amplified from pEGFP-C1 and pLenti-EF1α-T2A-EGFP (previously constructed vector in our laboratory) by CMV-F(*Sac*II)/CMV-R(*Bam*HI) and polyA-F(*Xho*I)/polyA-R(*Kpn*I) (Table 1), respectively. The PCR products were digested with the corresponding restriction enzymes and ligated into yeast clone vector pRS426 to yield pRS426-CMV-polyA. Then T2A-EGFP and OVA were separately amplified with primers EGFP-F(*Bam*HI)/EGFP-R(*Xho*I) and OVA-F(*Bam*HI)/OVA-R(*Xho*I) (Table 1) from pLenti-EF1α-T2A-EGFP and chicken genome DNA, respectively. The PCR products were digested with *Bam*HI and *Xho*I, and inserted into corresponding enzyme sites of the pRS426-CMV-polyA to generate pRS426-CMV-EGFP (Fig. 1A) and pRS426-CMV-OVA (Fig. 1C). To construct yeast-mediated protein delivery system, *Kpn*I and *Xho*I enzyme-digested T2A-EGFP and OVA fragments were ligated into pGADT7 to construct pGAD-ADH1-EGFP (Fig. 1B) and pGAD-ADH1-OVA (Fig. 1D), respectively. The fidelity of these four constructs were identified by restriction enzyme *Bam*HI and *Xho*I (Fig. 1, right) and confirmed by sequencing analysis.

### 2.2. S. cerevisiae strains and culture conditions

The yeast *S. cerevisiae* strain JMY31 (*MATα, ade2-1; ura3-1; his3-11; trp1-1; leu2-3,112; can1-100*) was cultured in YPD medium. The plasmids for different experiment groups (pRS426-CMV-EGFP, pRS426-CMV-OVA, pGAD-ADH1-EGFP and pGAD-ADH1-OVA) were used respectively to transform JMY31 as described previously [16,21].

### 2.3. Detection of EGFP expression in CIK cells and S. cerevisiae cells

pRS426-CMV-EGFP was used to transfect *Ctenopharyngodon idella* kidney (CIK) cells by FuGENE® HD transfection reagent (Roche, USA) according to the manufacturer's instruction. At 72 h post transfection, photos were taken under fluorescence microscope by the Bio-Rad ChemiDoc XRS (Bio-Rad, USA). In addition, to detect the EGFP expression in yeast, recombinant *S. cerevisiae* transformed with pGAD-ADH1-EGFP as well as with pRS426-CMV-EGFP were examined under fluorescence microscope.

### 2.4. Expression of OVA protein in HEK 293T cells and S. cerevisiae cells

pRS426-CMV-OVA was used to transfect HEK 293T cells. After 72 h, total cellular protein was extracted by RIPA Lysis Buffer (Applygen, China). The protein of recombinant *S. cerevisiae* with pGAD-ADH1-OVA was extracted as previously reported [22]. The protein extraction was used for western blotting analysis according to previous report [16]. The primary and secondary antibodies were mouse anti-HA monoclonal antibody and horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (Abcam, USA), respectively. In addition, the commercial OVA protein (sigma) was subjected to SDS-PAGE to confirm the size of OVA protein.

### 2.5. Preparation of recombinant yeast as feed additives

Different *S. cerevisiae* transformants and the parental strain JMY31 were cultured, harvested and re-suspended in PBS. Then the suspended yeast cells were thoroughly mixed with basal meal powder, and added into feed pellets by a pellet former. The pellets were air-dried at room temperature and then stored at −20 °C.

For the EGFP vaccination group (pCMV-EGFP), the feed contained $1.5 \times 10^8$ yeast cells/g powder. For two control groups, the feed for the JMY31 group included equal number of *S. cerevisiae* cells, and the feed for the PBS group was mixed with PBS. For OVA vaccination, three treatment groups with different yeast cell dosages were set up for both DNA and protein delivery purposes, including low dosage ($1.0 \times 10^8$ yeast cells/g powder), medium dosage ($1.5 \times 10^8$ yeast cells/g powder) and high dosage ($2.0 \times 10^8$ yeast cells/g powder), designated as pCMV-OVA-L, pCMV-OVA-M, pCMV-OVA-H, pADH1-OVA-L, pADH1-OVA-M and pADH1-OVA-H groups for short.

### 2.6. Experimental fish and oral vaccination

Red crucian carp (*Carassius auratus*) about 25 g were obtained from an aquarium market (Shaanxi, China) and then were acclimatized to laboratory conditions for two weeks at 26–28 °C before experimental manipulation.

For delivery of EGFP gene, *C. auratus* were divided randomly into 3 groups (PBS, JMY31 and pCMV-EGFP groups) of 4 individuals.

**Table 1**
Primers for vectors construction.

| Primer name | Sequence (5′ → 3′) | Primer information |
|---|---|---|
| CMV-F(*Sac*II) | TCCccgcggTAGTTATTAATAGTAATCAATTACG | Amplification of CMV promoter |
| CMV-R(*Bam*HI) | CGCggatccAGCTCTGCTTATATAGACCTC | |
| polyA-F(*Xho*I) | CCGctcgagCGCTGATCAGCCTCGACTG | Amplification of polyA |
| polyA-R(*Kpn*I) | CGGggtaccGAGCCCCAGCTGGTTCTTT | |
| EGFP-F(*Bam*HI) | CGCggtaccggatccGGCAGTGGAGAGGGCAGAG | Amplification of T2A-EGFP |
| EGFP-R(*Xho*I) | CCGctcgagTTACTTGTACAGCTCGTCCATG | |
| OVA-F(*Bam*HI) | CGCggtaccggatccATGTACCCATACGACGTTCCAG | Amplification of OVA |
| OVA-R(*Xho*I) | CCGctcgagTTAAGGGGAAACACATCTGCC | |