CONSTRUCTION OF *Pichia pastoris* STRAINS HARBORING α-GLUCOSIDASE cDNA FROM *Litopenaeus vannamei*

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Introduction. Alpha-glucosidases (EC 3.2.1.20) are glycosyl hydrolases which play a role in the final steps of starch digestion, hydrolyzing alpha-1-4 glucosidic linkages from starch oligosaccharides (1). In contrast to other known glucosidases, no alpha-1-6 glucose link hydrolysis activity has been observed in the α-glucosidase from the shrimp *Litopenaeus vannamei* (1, 2).

In this work, we constructed *Pichia pastoris* recombinant strains that harbor the α-glucosidase cDNA from *L. vannamei.*

Methods. Total RNA was isolated from *L. vannamei* shrimp hepatopancreas and used to synthesized α-glucosidase cDNA by RT-PCR using ThermoScript™ Reverse Transcriptase and Platinum® *Pfx* Polymerase, primers directed to the beginning and the end of the nucleotide sequence encoding the mature protein, with the simultaneous introduction of twelve nucleotides from the 3´ terminus of the alpha-factor prepro-secretion signal sequence from *Saccharomyces cerevisiae*, including a *Sal*I site, and a *Avr*II site at the 5´ and 3´ ends, respectively. The amplified product was cloned into vector pCR2.1TOPO, and subcloned into vector pPIC9 using the artificial *Sal*I and *Avr*II sites at the cDNA and the *Xho*I and *Avr*II sites at the vector pPIC9, to produce the new expression vector pPIC9αglu. The *P. pastoris* host strain, KM71 (*his*4), was transformed with *Sal*I-digested pPIC9αglu. The transformants were selected by histidine auxotrophy, and integration of the expression cassette into the genomes of selected strains was verified by PCR. *Pichia pastoris* recombinant strains were induced in shake-flask cultures with buffered minimal methanol (BMM) medium and RT-PCR assays were carried out to detect α-glucosidase transcripts.

Results. The expression vector pPIC9αglu was constructed harboring *L. vannamei* α-glucosidase cDNA in-frame with the *S. cerevisiae* alpha-factor signal and between the promoter and transcriptional terminator of the *AOX1* gene. We engineered the DNA sequence to produce shrimp α-glucosidase as a mature polypeptide of 899 amino acids in accordance with the sequence of GenBank No. AJ250828 described by Le Chevalier et al. (1) and the signal peptide cleavage site prediction by the SignalP 3.0 Server (3). PCR analysis of the recombinant plasmid, using AOX primers, showed a 3,169-bp product that confirmed the pPIC9αglu construct. Transformation of *P. pastoris* KM71 gave about 50 His^+ transformants. PCR analysis of the genomic DNAs isolated from *P. pastoris* KM71αglu (His^+ transformants) showed a 3,169-bp band that corresponds to the 2,697-bp of the α-glucosidase cDNA with the termination codon, and the 469-bp of the vector pPIC9. These results indicate the absence of the *AOX1* structural gene and the correct integration of the expression cassette into the *P. pastoris* genome, thus confirming the Mut^s phenotype. RT-PCR analysis from methanol induced cells showed a 2,718-bp band from α-glucosidase transcripts indicating the correct transcription of the heterologous gen.

Conclusions. *P. pastoris* recombinant strains harboring the α-glucosidase cDNA from *L. vannamei* were constructed and the functionality of the heterologous gen was demonstrated by specific transcript detection.

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