GENE ENCODING A TANNASE IN *Aspergillus niger* AND PRODUCTION OF THE ENZYME IN *Pichia pastoris* USING A SYNTHETIC GENE

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Introduction. Tannases (tannin acyl hydrolase, EC. 3.1.1.20) hydrolyze ester bonds in gallotannins, complex tannins, and gallic acid esters. These enzymes are used in food and beverage processing; however, its practical use is limited, because of insufficient large-scale production.

In the present work, we sequenced the gene encoding *Aspergillus niger* GH1 tannase, which has attractive properties for industrial use (1), and engineered the methylotrophic yeast *Pichia pastoris* with a synthetic gene to produce and secrete the enzyme.

Methods. The gene encoding *A. niger* GH1 tannase was synthesized by polymerase chain reaction (PCR) using primers designed from *A. niger* CBS 513.88, and cloned into the vector pGEM-T. Nucleotide sequences from five plasmids from different *Escherichia coli* colonies were determined at the Instituto de Fisiología Celular (UNAM), using T7, SP6, and two internal primers. Sequences were aligned using the Contig Assembly Program (CAP) module of the BioEdit v7.0.8.0 program. The consensus sequence and deduced amino acid sequence were compared with sequences at databases using BLAST tools. A synthetic gene encoding the *A. niger* GH1 tannase (*antgs*) was designed based on *P. pastoris*-preferred codons, and AT-rich stretches with silent mutations were removed. A nucleotide sequence with *P. pastoris*-preferred codons encoding the alpha-factor prepro-secretion signal sequence from *Saccharomyces cerevisiae*, including the *Bam*HI site, and a *Avr*II site were introduced at the 5´ and 3´ ends, respectively. The designed nucleotide sequence was synthesized, cloned into vector pUC57, and subcloned into vector pPIC9 using the *Bam*HI and *Avr*II sites. The *P. pastoris* recombinant strains were constructed by transformation of the *P. pastoris* host strain, KM71 (*his4*), with *Sal*I-digested pPIC9*antgs*. The transformants were selected by histidine auxotrophy, and the integration of the expression cassette into the genomes of the selected strains was verified by PCR. *P. pastoris* recombinant strains were tested to select an overproducer strain for tannase, using microcultures in buffered minimal glycerol (BMG) and buffered minimal methanol (BMM) media. Tannase production from the selected strain was verified in shake-flask cultures, also in BMG and BMM media, analyzing the cell-free culture medium by SDS-PAGE and for tannase activity.

Results. The sequence of the mature tannase gene had a length of 1,686 bp and encodes a protein of 562 amino acids. Nucleotide and amino acid sequences showed identities of 93% and 98%, respectively, with A. *niger* CBS 513.88 tannase. The synthetic gene had a full length of 1,961 bp. Transformation of *P. pastoris* KM71 gave 15 His^+ transformants. PCR analysis of the genomic DNA from transformants showed a 2,158-bp band, which confirmed the correct integration of the expression cassette into the *P. pastoris* genome. The *P. pastoris* recombinant strains showed tannase activity in the cell-free culture medium from BMM cultures, and the enzyme was detected by SDS-PAGE.

Conclusions. The nucleotide and amino acid sequence from *A. niger* GH1 tannase was described and the production of the enzyme was demonstrated in the *P. pastoris* expression system.

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References.

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