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

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Introduction. Invertases (EC. 3.2.1.26) hydrolyze sucrose, producing glucose and fructose, and are used in candies and fructooligosaccharides (FOS) production.

In this work, we sequenced the gene encoding invertase from *Aspergillus niger* GH1, which has been shown to have a high specific activity, and used a synthetic gene to produce the enzyme in the methylotrophic yeast *Pichia pastoris*.

Methods. The gene encoding *A. niger* GH1 invertase was synthesized by PCR, using primers designed from *A. niger* IBT10sb β-fructofuranosidase gene, and cloned into the vector pGEM-T. Nucleotide sequences from four 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 of databases using BLAST tools. A synthetic gene encoding the *A. niger* GH1 invertase (*InvGS*) was designed based on *P. pastoris*-preferred codons, and AT-rich stretches with silent mutations were removed. Twelve nucleotides from the 3´ terminus of the alpha-factor prepro-secretion signal sequence from *Saccharomyces cerevisiae*, including the XhoI site and a NotI 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 XhoI site and NotI site. *Pichia pastoris* recombinant strains were constructed by transformation of the *P. pastoris* host strain, KM71 (*his4*), with SalI-digested pPIC9InvGS. 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 tested to select an overproducer strain for invertase, using microcultures in buffered minimal glycerol (BMG) and buffered minimal methanol (BMM) media. Invertase 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 invertase activity.

Results. The sequence of the invertase gene had a length of 1,770 bp and encodes a protein of 590 amino acids with a putative signal sequence of 15 amino acids. The invertase sequence presented sequence identities of 99% and 97% with invertases from *A. kawachi* IFO 4308 and *A. niger* B60, respectively (1, 2). The invertase synthetic gene had a full length of 1,755 bp. Transformation of *P. pastoris* KM71 gave 12 His^+ transformants. PCR analysis of the genomic DNA from transformants showed a 2,194-bp band which confirmed the correct integration of the expression cassette into the *P. pastoris* genome. The *P. pastoris* recombinant strains showed invertase activity in the cell-free culture medium from BMM cultures, and the enzyme was detected by SDS-PAGE.

Conclusions. The nucleotide and amino acid sequences for *A. niger* GH1 invertase were described and the production of the enzyme was demonstrated in the *P. pastoris* expression system.

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