pepA GENE DELETION IN A FUNGAL STRAIN OF *Aspergillus niger* TO IMPROVE THE YIELDS OF AN HETEROLOGOUS PROTEIN OF INDUSTRIAL INTEREST

María José Mosqueira[1], Adán Chávez[3], Fernando, García[2],Juan Ortíz[3], Sergio Sánchez[1]; 1.Instituto de Investigaciones Biomédicas UNAM, México, D.F., A.P. 70228, C.P. 04510; 2. UNAM, Centro de Física Aplicada y Tecnología Avanzada, Boulevard Juriquilla No. 3001, Juriquilla, Querétaro, México. C.P. 76230; 3. ALTECSA S.A de C.V. Avenida 15 de Agosto Lote 3 y 4. Cd. Industrial Xicohtencatl II. Huamantla , Tlaxcala. C.P. 90500; adanchavez1@hotmail.com

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Introduction. Filamentous fungi play an important role in the biotechnology industry due to the capacity of producing high yields of enzymes and diverse metabolites, including antibiotics, organic acids, pigments and other food additives (1). In particular, the genus *Aspergillus* has been a promising system in the expression of heterologous proteins (2). Nevertheless, at an industrial level, one of the biggest problems that heterologous protein production has to face in expression systems such as *A. niger* and other filamentous fungi is the proteolytic activity of extracellular proteases (3). The proteolytic spectrum of *A. niger* has been analyzed and it has been found a predominance of acid proteases, which include two aspartyl proteases: the aspergillopepsin A (PEPA) and the aspergillopepsin B; and two serine carboxypeptidases: the aspergillopeosin F and the aspergillopepsin G; from which PEPA constitutes 84% of the acid proteolytic activity of this fungi (4).

The objective of this work is to develop an *A. niger* mutant strain deficient in aspergillopepsin A (*pepA* disrupted), through the deletion of the gene *pepA,* to increase the yields in the production of a specific heterologous protein of industrial interest.

Methods. As deletion or disruption of the gene required homologous recombination of the introduced DNA, a construct was designed in which the selectable marker *pyrG* was flanked by the flanking sequences of the gene *pepA* (300 bp each side). Two transformation techniques were used: 1) Transformation by electroporation of germinated conidia, which involves exposing the conidia to short duration high voltage electrical pulses in the presence of the exogenous DNA (5); and 2) Transformation of protoplasts prepared by young mycelia, which involves the preparation of protoplasts followed by exogenous DNA uptake mediated by $CaCl_2$/PEG treatment. In both techniques, two kinds of transformation were tried: 1) *pepA* disruption, and 2) *pepA* disruption and co-transformation with the sequence of the specific heterologous protein. The transformants were selected and purified by culture media lacking uridine and uracile following three rounds of reisolation from single colonies in the absence of these compounds. Ostensible transformants genomes were analyzed, amplification of the genes *pyrG* and *pepA* was carried out to test out disruption.

Results. Selective culture media resulted in several colonies ostensibly transformed. The purification by the three round reisolation of single colonies allowed a better selection of the strains of both techniques. In the case of electroporation transformants, just one colony (strain 1) survived the three rounds, growing slowly in the media lacking uridine (3-4 days after culture). For protoplasts transformants, two colonies (strains 2 and 3) survived the third round, growing fastly and producing a large amount of conidia. The genome analysis was carried out (See Fig.1).

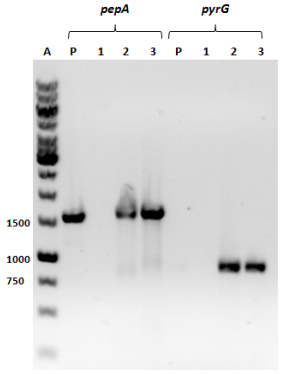

Fig.1 Amplification of the genes *pepA* and *pyrG* of three of the *A. niger* strains resulted of both transformations. P, parental strain; 1, ostensible *pepA* disruptant (Electroporation); 2, ostensible *pepA* disruptant co-transformed with the heterologous protein sequence (Transformation of protoplasts); 3, ostensible *pepA* disruptant co-transformed with the heterologous protein sequence (Transformation of protoplasts). *pyrG* ~850 bp, *pepA* ~1520 bp.

Strain 1 seemed to be *pepA* disrupted. However it did not amplify the selectable marker in spite of growing in the absence of uridine. Both, strains 2 and 3 are not *pepA* disrupted, but their genome contains *pyrG* gene, maybe due to an illegitimate recombination.

Conclusions. An *A. niger* mutant strain deficient in aspergillopepsin A (*pepA* disrupted) was obtained by transformation by electroporation. None co-transformed strains were *pepA* disrupted.

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