DEVELOPMENT OF TWO SELECTABLE TRANSFORMATION MARKERS IN ASPERGILLUS NIGER FOR HETEROLOGOUS EXPRESSION

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Introduction. Establishing suitable selection markers for *Aspergillus* began just after the first achievements in transformation; these vectors combine a selectable gene with fungal promoter and terminator sequences. Development of markers such as drug-resistant genes has been delay because ascomycete fungi shows resistance to numerous antimicrobial agents. The most widely used inhibitors for *Aspergillus* are hygromicin and bleomycin (1). Less commonly used selective agents are phosphinothricin and carboxin.
Phosphinothricin inhibits glutamine synthase, causing a rapid accumulation of ammonia that leads to cell death (2). And carboxin prevents mitochondrial respiration by inhibiting the activity of mitochondrial ubiquinone reductase or succinate dehydrogenase (3).
Here we constructed and proved two selectable transformation markers for use in *Aspergillus niger* for later research in biopharmaceutical, vaccine development or environmental applications.

Methods. Two vectors were constructed based primary on the pAN52.1 vector (4) which contains the *A. nidulans gpdA* strong constitutive promoter and *trpC* terminator. One plasmid was constructed changing the *gpdA* promoter with the *pikA* strong constitutive promoter from *A. niger*. Gene markers *bar* and *cbx* were amplified from the vectors pBGWFS7,0 and pBSARSCBX respectly using specific design primers. These genes were cloned in the pAN52.1 and its modification and the insert was determined by enzyme digestion. At that point we obtain the following constructions: *bar* gene under *gpdA* promoter control named pLJANBar and *cbx* gene under *pikA* promoter control named pPika-PAN-Cbx.
Transform of these plasmids in to *A. niger* was done using biolistic technique (5). After screening transformant colonies in medium containing the respective antibiotic, positive transformants were analyzed by PCR using the previously design primers.

Results. On figure 1 it is shown the PCR agarose gel from the pLJANABar tranformants, which present on row 2 the wild type *A niger* SCB2 PCR product, on row 3 the plasmid PCR product with a 560bp band from the *bar* gene and on rows 4-7 the transformants PCR products with the 560bp band, the same as the plasmid.
On figure 2 it is presented the PCR agarose gel from the pPika-PAN-Cbx transformants, that shows on row 2 the PCR product from the wild type, on row 3 the plasmid PCR product that contains a 896bp band from the *cbx* gene, and on rows 4 and 5 the PCR products from the transformants that show the 896bp band identical to the plasmid.

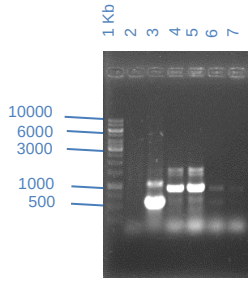

Fig.1 Agarose gel of the PCR products that confirm the insertion of the pLJANBar vector in the wild type *A. niger* SCB2

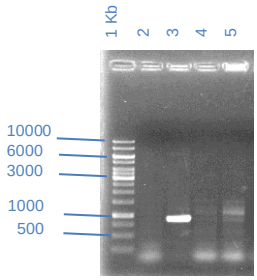

Fig.2 Agarose gel of the PCR products that proves the insertion of the pPika-PAN-Cbx vector in A niger SCB2

Conclusions. A successful insertion of the plasmids pLJANBar and pPika-PAN-Cbx into the wild type *A niger* SCB2 was achieve and confirm by growing the transformant colonies in medium with the appropriate antibiotic and by PCR.

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