Cloning and Expression Analysis of the Galactose Oxidase Genes from *Fusarium austroamericanum* and *Fusarium subglutinans*

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Galactose oxidase is a copper enzyme that has several applications as galactose concentration determination and cancer detection. The enzyme is secreted by a few fungi species including *Fusarium austroamericanum* (*Gibberella zeae*) and *Fusarium subglutinans* (*Gibberella fujikuroi*). The galactose oxidase is coded by three known genes in *Fusarium* species. The galactose oxidase *gaoA* gene from *F. austroamericanum* was the only one already used for recombinant studies. The recombinant *F. austroamericanum* GaoA protein has a low catalytic efficiency and is produced in a low level in *Escherichia coli*. Considering that it would be interesting to study the galactose oxidase homologous genes in *F. austroamericanum* and *F. subglutinans*, the present work had as objectives to clone, to sequence, and to express these genes in *E. coli* and to analyze their endogenous expression. Pairs of primers were designed to amplify the region that codes for the probable mature form of the *G. zeae* *gaoA*, *gaoB*, and *gaoC* and *F. verticilloides* (a *F. subglutinans* related species) *gaoA* and *gaoB* genes respective enzymes. The amplified DNA fragments from the *F. austroamericanum* or *F. subglutinans* genomic DNA were cloned in the pET101/D-TOPO® (Invitrogen) plasmid and transformed in *E. coli* BL21 Star™(DE3). The endogenous transcription was studied using RT-PCR. As results, both studied *gaoA* genes were expressed in a low level. The endogenous transcription of the *gaoA* and *gaoB* genes in *F. austroamericanum* and of the *gaoB* gene in *F. subglutinans* was evidenced. The sequence of the cloned *F. austroamericanum* *gaoB* and *F. subglutinans* *gaoA* genes were obtained.


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