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TRICHODERMA KONINGIOPSIS POS7 CARRIES EXPANSIN AND SWOLLENIN CODING GENES

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RESUMO

Introduction: In order to degrade polysaccharides, many enzymes with synergistic action are required. Three main types of enzymes are required for cellulose hydrolysis: endoglucanases (EGs), cellobiohydrolases (CBHs), and β-glucosidases (BGLs). However, to efficiently hydrolyze cellulosic substrates, cellulolytic organisms must first be able to access the cellulose chains that are tightly packed in the form of insoluble microfibrils encased in hemicellulose and lignin. There are evidence that they involve in the amorphogenesis process other biomass-degrading proteins, like the fungal swollenin and expansins. Expansins are known to promote cell wall extension in plants, likely through a mechanism involving the disruption of hydrogen bonding between adjacent sugar chains. Swollenins are expansin-like proteins that act swelling the cellulosic network within plant cell walls. Our group reported that the *Trichoderma koningiopsis* POS7 isolate secretes high levels of cellulolytic-active enzymes. However, the *T. koningiopsis* genes encoding other non-catalytic proteins involved in cellulose hydrolysis have not been yet explored. Objective: Previously, our group annotated 19 cellulases coding genes (seven EGs, ten BGLs and two CBHs). So the focus of this work was to find and annotate the other genes involved in reactions of the whole cellulolytic process made by the fungus *T. koningiopsis* for biomass degradation. Methods: Genomic DNA library construction and draft genome sequencing were performed by Macrogen using the Illumina MiSeq system. These sequences were assembled de novo using the SPAdes software. To predict genes we used two ab initio gene predictor, AUGUSTUS and FgenesH. A database was made with the nucleotide sequences of genes that code for swollenin and expansin proteins in other species of *Trichoderma* and related fungi, as well as also from their available deduced amino acid sequences, which were compared with the annotation files generated by AUGUSTUS and FgenesH software. These sequences were blasted in the NCBI database using the BLASTn and BLASTp tools in order to determine their identity (ie, regions containing sequences of the genes encoding swollenins and expansin proteins). In addition, Integrative Genomics Viewer and Geneious software were used to determine the complete structural regions of each gene of interest. Results: The genome of *T. koningiopsis* POS7 carried two genes encoding for expansin-like proteins and one gene encoding for a swollenin-like protein. One of the expansin gene was located in the minus strand and had 576 bp with three exons, the other expansin gene was located in the plus strand and had 866 bp with one exon. Particularly the swollenin gene was located in the minus strand and had 1.762 bp with six

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exons. The swollenin gene and the first reported expansin gene were located in the same scaffold of one EGs and one CBHs enzymes genes. Conclusions: *T. koningiopsis* POS7 carries in its genome a wide variety of genes encoding proteins involved in cellulose biodegradation that have different action mechanisms, including ones related to amorphogenesis process.

PALAVRAS-CHAVE: amorphogenesis process - annotated genes – cellulase degradation – genome - omics technology

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