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Opportunism and Conversations in the Environment

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Opportunism and Conversations in the Environment

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Trichoderma harzianum Lip1 gene

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The nucleotide sequence of *T. harzianum Lip1* gene can be accessed in EMBL database (AM180877.1), including the 5' upstream and the 3' downstream regions. *Lip1* open reading frame (ORF) has 1667 bp. However, according to the predictive analysis of introns made in the application AUGUSTUS (Stanke et al., 2008) restricted to fungi, and based on the comparison with sequences of *Fusarium graminearum* genes, its entire nucleotide sequence is not converted into amino acids, having an intron of 44 bp detected at positions 1576 to 1619 of the ORF. The protein encoded by *T. harzianum Lip1* (Lip1) has a carboxylesterase type-B signature, with a serine active site (PROSITE PS00122) (Sigrist et al., 2002). As in lipases and serine proteases, the catalytic triad of esterases is formed by three amino acids: a serine, a glutamic or aspartic acid, and a histidine. Sequence around the serine-containing active center serine is well preserved, and is used as a signature pattern: F-[GR]-G-x(4)-[LIVM]-x-[LIV]-x-G-x-S-[STAG]-G. As secondary pattern was selected a conserved region located at the N-terminal region, which contains a cysteine involved in a disulfide bond, the sequence is [EDA]-[DG]-C-L-[YTF]-[LIVT]-DNS]-[LIV]-[LIVFYW]-x-[PQR]. In Lip 1 are present the sequences FGGDPDKVTLWGFSAG, and EDCLTLNVQRP, in the the amino acid positions 216-231 and 115-125. The serine at the active center of Lip 1 corresponds to residue 229, with a relative position similar to that existing in other lipases and a primary structure coincident with the consensus G-x-S-x-G, described as an active center of lipases. The other components of the catalytic triad are the residues E361 and H474. The *oxyanion hole,* critical for catalysis, is located in residues 134-144. The three-dimensional structural prediction made on the Phyre2 server (Kelley & Sternberg, 2009), based on the homology of Lip1 with the crystallized protein 4BE4 of the fungus *Ophiostoma picea* in closed conformation (Gutiérrez-Fernández et al., 2014), evidences the Lip1 "lid" region, constituted by an α-helix (residues 99-107) flanked by two "loops" that end in a disulfide bridge (Cys 83-Cys117). *Lip1* was cloned in *Pichia pastoris*, and lipolytic activities of transformants were evaluated. The presence of homologous genes was searched in the genomes of *T. atroviride, T. reesei and T. virens* by Southern Blot, but hybridization only occurred in *T. harzianum*.

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