Trichoderma harzianum lipolytic enzymes – a contribution

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Introduction and aims

Trichoderma has been related to the mycoparasitism process due to an extraordinary range of cell-wall degrading enzymes (CWDE): chitinases, β-1,3 and β-1,6 glucanases, celulases and proteases. However, the role of lipases and carboxylesterases in this process is less known, although lipids were up to 3% of fungal CW (Feofilova, 2010). According to Silva et al. (2009) and Lopes et al. (2012), in experiments involving *T. reesei* and *Pythium ultimum*, it seems that lipases are implicated in mycoparasitism and are secreted in a phytopathogen-dependent manner, as they, like most of the CWDE already described, are inducible by substrate. One of the purposes of this work was to find if the lipolytic enzymes of *T. harzianum* were alike homologous enzymes of other *Trichoderma* species. Here we present our contribution to the knowledge of *T. harzianum* T34 lipolytic enzymes (EC 3.1.1).

Material and Methods

From a *T. harzianum* CECT 2413 cDNA library coming from the EU-funded TRICHOEST project and obtained under mycoparasitism, nutritional stress and plant interaction conditions some ESTs were BLAST screened searching for lipolytic enzymes (EC 3.1.1). The nucleotide sequence of two of them were completed from gDNA by HE-TAIL PCR (Michiels et al., 2003) and sequenced. The respective gene ORFs showed significant homology with lipolytic enzymes. However, such genes are not analogous in size nor encode proteins with similar characteristics.

Results and Discussion

Lip1 gene, with a 1677 bp ORF (EMBL database accession AM180877.1), codifies a deduced 56,3 kDa protein of 532 amino acids (B0B099_TRIHA), a carboxylesterase_B_1 (EC 3.1.1.1; PROSITE PS00122, E-value 2,9 e^{-128}), with affinity for short chain fatty acids (C<10) and soluble substrates. *Lip2* gene (AM774154.1) has a 1215 bp ORF and codifies a deduced 44,6 kDa protein of 404 amino acids (B7ZET5_TRIHA), a triacylglycerol lipase (EC 3.1.1.3; PROSITE PS00120) or lipase_classe3 (InterPro IPR002921, Pfam PF01746, E-value de 1,0 e^{-34}), with affinity for long chain fatty acids (C>10) and acting on insoluble substrates.

The two ORFs sequences were submitted to FASTAX program at https://www.ebi.ac.uk/Tools/services for search of similarity, leading to these results:

No similar proteins to Lip1 were described in other species of the genus, seeming that Lip1 is somehow characteristic of *T. harzianum.* Otherwise Lip 2 (B7ZET5_TRIHA) shows 96,8% identity with the proteins A0A1T3C7W4_9HYPO (from *T. guizhouense;* E-value 0.0); 89% with G9MI02_HYPVG (*T. virens;* 3.0E^{-206}); 80,8% with A0A2H2Z8C3_9HYPO (*T. parareesei*; 1.3E^{-200}); 80,6% with A0A024S786_HYPJR (*T. reesei*; 1.0E^{-199}); 77,4% with G9NW28_HYPAI (*T. atroviride* ; 1.4E^{-187}) and 77,4% with A0A2P4ZBE0_9HYPO (*T. gamsii*; 1.4E^{-186}), seeming that very similar lipases are widespread in *Trichoderma* spp. The Clustal Omega Alignment between this deduced proteins are shown in Figure 1.

Lipases_classe 3 have a signature pattern, the sequence [LIV]-{KG}-[LIVFY]-[LIVMST]-G-[HYWV]-**S**-{YAG}-G-[GSTAC] which includes the lipases active center serine. In Lip 2 are present the corresponding sequence VHLIGHSLGG (amino acids 210 a 219, with active serine at position 216). The other two residues that make up the catalytic triad are present at position 282 (the acidic residue which in this case is an aspartic acid), and in position 373 is found the histidine residue. There is also a conserved region located in the N-terminal section, which residues compose the hidrofobic oxyanion hole. In Lip 2 is the sequence IVVAFRGTYSITNTI, in position 130-144. All these features are marked in Figure 1.

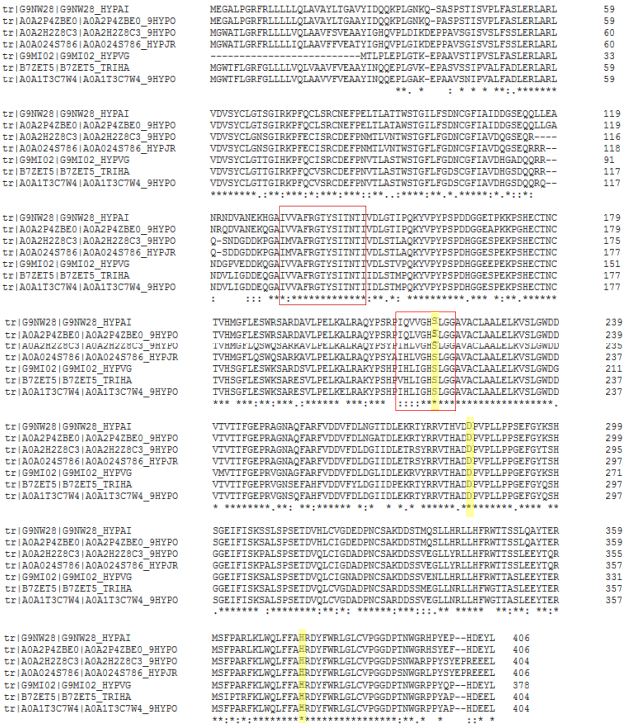

Figure 1 – Clustal Alignment of *T. harzianum* Lip 2 (B7ZET5_TRIHA) and other similar deduced proteins from six *Trichoderma* species (line 1- *T. atroviride* ; 2- *T. gamsii*; 3- *T. parareesei*; 4- *T. reesei*; 5- *T. virens*; 6- *T. harzianum*; 7- *T. guizhouense).* Marked in yellow the position of the residues of lipases catalytic triad (S, D and H); The dark red box surrounds the amino acids of the oxyanion hole, and the red one surrounds the active centre. In the first 21 to 25 positions the amino acids corresponding to the peptide-signal.

Figure 1 highlights the high degree of conservation existing between the lipases class 3 of the different *Trichoderma* species existing in the databases. It is found not only at the level of the amino acids important for the conformation of the protein or for the catalytic activity, but also in the others, it can be expected that its function may be important for species survival.

Phylogenetic analysis (data not shown) reveals greater similarity between B7ZET5_TRIHA *(T. harzianum);* and A0A1T3C7W4_9HYPO *(T. guizhouense);* between G9NW28_HYPAI *(T. atroviride)* and A0A2P4ZBE09HYPO *(T. gamsii),* and between A0A2H2Z8C3_9HYPO *(T. parareesei)* and A0A024S786_HYPJR *(T. reesei).*

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