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The genus Trichoderma is cosmopolitan in soils, wood decomposition and plant material. Species of Trichoderma are often dominant components of the soil microflora in various habitats. This is due to different metabolic capacity and competitiveness in nature.

The genus Trichoderma are frequently used in biological control because of its antagonist ability of phytopathogenic fungi. The mechanisms employed by Trichoderma spp. to antagonize other fungi are competition (for space and / or nutrients), antibiotics and microparasites, while in the latter case, involved lytic enzymes such as proteases, glucanases, chitinases and lipases. Some of these proteins have a large agricultural potential, especially as active components of new formulations of fungicides.

Trichoderma harzianum Riba (Ascomycota, Hypocreales, Hypocreaceae) is a filamentous fungus, assexual, common isolated of tropical soil of plant material, rhizosphere ecosystems and decomposing organic material a ratio of 10^7-10^9 spores per gram of soil (Figure 1).

**Isolation and analysis of lip2 gene from Trichoderma harzianum**

**Material and Methods**

Total genomic DNA was isolated from strain CECT 2413 of T. harzianum to proceed a TAIL-PCR.

The sequences obtained from TAIL-PCR were cloned in pGEM®-T vector.

In order to determine protein expression, the ORF of the lip2 gene was cloned in vector pET-28a (+). The expression was induced for 8h and 16h with 100 mM IPTG in LB medium and expression was assessed by SDS-PAGE.

**Aims**

Characterize at molecular level of the lip2 gene by the cloning on pET-28a (+) vector and evaluation of his expression by SDS-PAGE.

**Genomic DNA was isolated from T. harzianum**

**Cloning on pGEM®-T**

**Sequencing**

**Expression by SDS-PAGE**

**Conclusions**

- HE-TAIL PCR is an efficient method to amplify unknown genomic DNA sequences adjacent to short known regions.
- Cloning to lip2 in vector bacterial expression (pET28a (+)), followed by induction, led to heterologous expression of the protein, confirmed by SDS-PAGE.
- The protein is found in the supernatant, meaning it is excreted into the culture medium.
- It is assumed that this protein, for its likely extracellular location, may contribute in combination with other enzymes widely described in Trichoderma harzianum the mechanism of biological control of plant pathogens.

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