

# Sequence analysis of the catalytic domain of a *Metarhizium anisopliae* chitinase

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## ABSTRACT

A 375-bp DNA fragment from *Metarhizium anisopliae* strain CG32 genomic DNA was amplified by PCR using chitinase-specific consensus primers. The deduced *M. anisopliae* peptide sequence demonstrated high similarity to the catalytic domains of chitinase genes from *Aphanocladium album* and *Trichoderma harzianum*. However, two introns present in the aligned *A. album* and *T. harzianum* genomic sequences were not detected in the *M. anisopliae* sequence. A comparison was made between this *Metarhizium* chitinase sequence and that of other fungi and bacteria. Possible phylogenetic relationships are discussed.

## INTRODUCTION

The entomopathogenic fungus *Metarhizium anisopliae* is recognised as having significant potential as a mycoinsecticide. This fungus produces a broad range of extracellular enzymes, including protease, chitinase and lipase, that have been suggested by some authors as having a key role in the infection of insects (St. Leger *et al.* 1986; Charnley and St. Leger, 1991) although this is still to be verified. Chitinolytic enzymes are widely distributed in bacteria, fungi, plants and insects and are probably involved in a range of biological interactions and defence systems such as plant defence mechanisms (Jones *et al.*, 1986; Linthorst *et al.*, 1990). Genes encoding chitinases have been cloned from several plants, yeast, insects, bacteria and recently from two filamentous fungi *Aphanocladium album* (Blaiseau and Lafay, 1992)

and *Trichoderma harzianum* (Carsolio *et al.*, 1994; Hayes *et al.*, 1994). To date there are no reports of chitinase sequences from entomopathogenic fungi.

## MATERIAL AND METHODS

A 375-bp fragment from *M. anisopliae* strain CG32 (obtained from the CENARGEN/EMBRAPA - Brazil culture collection) genomic DNA was amplified by PCR using *Taq* DNA polymerase (Boehringer Mannheim) and consensus primers specific to the conserved catalytic domain of several chitinases (kindly donated by Dr. E.E. Deane):

5' GGGAACTCTATGAAGGAATTTCCAACCGGCC

5' CTGAGGGTCAGCTATAGTTACGGCAGCTTTGGGGT

The amplification conditions were an initial 95°C for 6 min, then 30 cycles of 94°C for 30 s for denaturing, followed by 55°C for 30 s for annealing and 1 min 20 s at 72°C for extension. Samples were collected and analyzed in 1.5% (w/v) agarose gels.

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The amplified DNA was ligated into the pGEM-T vector (Promega) and clone pCHM1 selected, and verified by digestion with restriction enzymes. Both DNA strands of the insert were sequenced using the Sequenase II kit (Amersham), using 35S labelled dATP, according to manufacturers instructions. Reactions were resolved on a Flowgen VM4133 vertical electrophoresis apparatus, and gels exposed to X-ray film. Chitinase sequences for comparative purposes were obtained from the GenBank/Swissprot datalibraries. Multiple sequence alignments were generated by the CLUSTAL W program (Thompson *et al.*, 1994) using the Blossum 30 protein weight matrix for peptide alignments. Gaps were not considered in calculations of pairwise percent divergences, and no correction was made for multiple substitutions. A phylogenetic tree was constructed by CLUSTAL W based on the calculated percent divergence table using the Neighbour-joining method (Saitou and Nei, 1987) and plotted using the NJPLOT program of the same package.

## RESULTS AND DISCUSSION

PCR of *M. anisopliae* genomic DNA using degenerate primers specific to the catalytic domain of many chitinases (Henrissat, 1990; Perrakis *et al.*, 1993)

yielded a 375-bp product. This fragment was cloned into the pGEM-T vector, generating clone pCHM1. The DNA sequence of this clone provided conclusive evidence that the amplified fragment was part of a chitinase gene (Figure 1). A remarkable degree of nucleotide homology was obtained between this *M. anisopliae* partial sequence and the published sequences of chitinases from *A. album* (Blaiseau and Lafay, 1992) and *T. harzianum* (Carsolio *et al.*, 1994; Hayes *et al.*, 1994). The *chi1* gene from *A. album* has three short introns (49,53 and 55 bp in length) which occur close together near the N terminus. Introns similar in position though not in sequence are present in *T. harzianum* genomic clone *ech-42* (Carsolio *et al.*, 1994). Corresponding introns were found to be absent from the *Metarhizium* sequence. This result is somewhat unusual, since the majority of cognate genes in filamentous fungi possess introns which are strongly conserved in terms of position, but not necessarily in terms of sequence (Gurr *et al.*, 1987).

The deduced peptide sequence indicated that the *M. anisopliae* enzyme conformed to the general description of class II chitinases present in fungi, certain plants and bacteria (Perrakis *et al.*, 1993). Homology between the sequences of *M. anisopliae*, *A. album* and *T. harzianum* was striking at the peptide level (Figure 2), which indicates that this group should be recognised as a distinct sub-class within class II chitinases. Significantly, however, phylogenetic analysis of the aligned

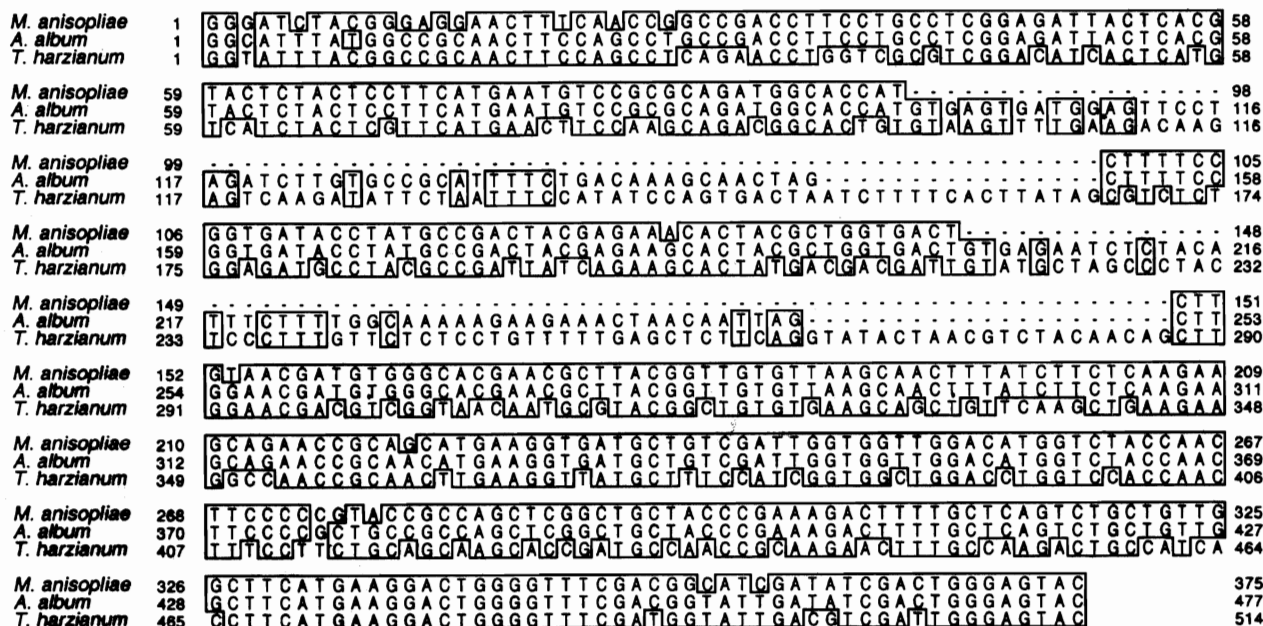


Figure 1 - Alignment of the chitinase-specific nucleotide sequences of *chiA* from *Metarhizium anisopliae* (sequence deposited in Genbank/EMBL/DDJB libraries under accession number X89212: *M. anisopliae* DNA for *chiA* gene), *chi1* from *Aphanocladium album* (Blaiseau and Lafay, 1992), and *ech-42* from *Trichoderma harzianum* (Carsolio *et al.*, 1994). Identical residues are boxed, and gaps necessary for alignments represented by dashes. Numbering begins arbitrarily from the first aligned nucleotide.



## RESUMO

O fragmento de 375 bp foi amplificado por PCR, a partir do DNA genômico da linhagem CG32 de *Metarhizium anisopliae*, usando primers específicos para quitinase. A sequência peptídica deduzida do fragmento do gene de quitinase de *M. anisopliae* demonstrou alta similaridade, a nível de domínio catalítico, com os genes de quitinase de *Aphanocladium album* e *Trichoderma harzianum*. Entretanto, dois introns presentes nas sequências genômicas de *A. album* e *T. harzianum* não foram detectados na sequência de *M. anisopliae*. A relação filogenética entre as sequências dos genes de quitinase de *Metarhizium* e outros fungos e bactérias é discutida.

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