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# Isolation and sequence analysis of alpha-tubulin gene from *Phytophthora cinnamomi*

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*Phytophthora* diseases cause widespread economic and environmental losses worldwide. Thousands of plant species are susceptible. In Portugal, *Phytophthora cinnamomi* is responsible for chestnut ink disease. Despite the differences there are a number of key steps common to most infection strategies, including adhesion to the plant surface, plant penetration through the secretion of a diverse range of cell wall-degrading enzymes and hyphal growth. The cell cytoskeleton plays a critical role in these processes. Microtubules are a major constituent of the cell cytoskeleton. They participate in a wide range of cellular functions, such as motility, division, maintenance of cell shape, and intracellular transport. However, microtubule role is variable depending on the organism, cell type and other factors. Tubulin is the major constituent of microtubules and is composed of a heterodimer of two closely related proteins, alpha and beta tubulin. In *S. cerevisiae* cells, the essential *TUB1* gene is the major gene, while the nonessential gene *TUB3* is a minor gene, encoding α-tubulin. The β-tubulin subunit is encoded by the *TUB2* gene. In Magnaporthe grisea both α -and β -tubulins are found as single-copy genes. The oomycetes are, however, phylogenetically quite distinct from the fungi. Analysis of structural, biochemical and molecular characteristics have led to the oomycetes being grouped with the chromophyte algae.

In order to elucidated the role of cytoskeleton in pathogenicity mechanisms of *Phytophthora cinnamomi*, was cloned a gene encoding alpha-tubulin from *P. cinnamomi*. To isolate this gene, the existing Tub1 nucleotide sequences were retrieved from the NCBI GenBank (www.ncbi.nlm.nih.gov/genbank). These sequences were aligned in Clustal and the degenerate primers Tub1 and Tub2 designed. A 1200bp fragment was generated from genomic DNA by PCR and subsequently cloned into pGEM-T vector. To complete the open reading frame it was used the HE-TAIL PCR. The complete ORF was sequenced and submitted in EMBL databases (Accession number AM412177.1). Based on the computational analysis through BioEdit software, *TUB1* has a 1362 bp ORF and encodes a 453 amino acids protein with a molecular weight of 49,911kDa. Phylogenetic analysis of deduced amino acid sequence using FASTA programs from EMBL databases revealed that Tub*1* revealed 99.6% identity with alpha-tubulin of *P. infestans* T30.4 and 98.9% identity with *P. capsici*, but only 68.1 % with alpha-tubulin of *S. cerevisiae*.

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