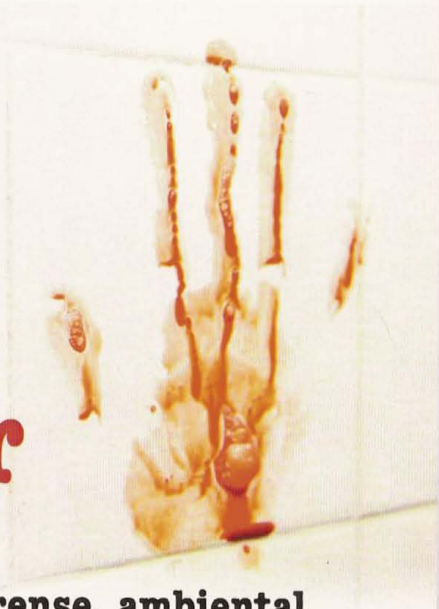
Curso Análise Genética e Molecular



Aplicação nas áreas medicinal, forense, ambiental, agronómica, animal e alimentar

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Uma escola de biociências

Molecular methods for identification of pathogenic factors associated with ink disease of chestnut

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The culture of the chestnut tree is extremely important in the northern region of Portugal, occupying a significant proportion of useful agricultural area. The annual average chestnut production in Portugal can reach 20 000 tons. New plantation areas have increased in the last few decades. However the ink disease caused by the oomycete *Phytophthora cinnamomi* has damage and killed many trees and up to now no concrete solutions have been offered to control the illness. As a consequence, the disease propagation in the orchards of chestnut trees has been causing severe productivity and yield breaks. In addition to the economical losses, the importance of sociological and landscape aspects for the region cannot be neglected.

The oomycetes form a phylogenetically distinct group of eukaryotic micro-organisms that includes some of the most notorious pathogens of plants. Among these, members of the genus *Phytophthora* cause enormous economic losses on crop species as well as environmental damage in natural ecosystems. *Phytophthora cinnamomi* is the most widely distributed *Phytophthora* species, with nearly 1000 host species.

Although they have a filamentous growth habit oomycetes are distantly-related to fungi and possess distinct mechanisms for pathogenicity. Consequently fungicides rarely control them and the few anti-oomycete products are often overcome by resistant pathogen variants. There are no eradication methods available to combat those species.

Oomycetes species can manipulate biochemical and physiological processes in their host plants through a diverse array of virulence or avirulence molecules, known as effectors. In susceptible plants, these effectors promote infection by suppressing defense responses, enhancing susceptibility, or inducing disease symptoms. Alternatively, in resistant plants, effectors are recognized by the products of plant resistance genes, resulting in host cell death and effective defence responses known as the hypersensitive response (HR).

We've identified and characterized some proteins involved in mechanisms of infection by *Phytophthora cinnamomi*: endo-1,3-beta-glucanase (complete cds), exo-glucanase (partial cds); glucanase inhibitor protein (GIP) (complete cds); necrosis-inducing Phytophthora protein 1 (NPP1) (complete cds), transglutaminase, under the projects Identification, characterization and role of molecular factors associated with the mechanisms of infection of Fagaceae species by

Phytophthora cinnamomi, PTDC/AGR-AAM/67628/2006, funding by FCT; Combating by molecular methods to ink-disease of chestnut and other regional cultures, COMBATINTA/SP2.P11/02 - Interreg IIIA, funding by FEDER, among others.

Several technologies, such as reverse transcriptase PCR and in vivo expression technology, have been used to study the expression of selected genes from fungi during infection. Much broader genome-wide approaches, including differential display, cDNA subtractive hybridization, antibody-based strategies and DNA microarrays, have been used to identify infection-associated genes. In vivo transcriptional profiling protocols face a number of technical challenges. For example, most methods have in common the limitation that they are based on the transcript profile of population of cells. This is likely to be less of a problem in in vitro studies of cultures or in more simple models that mimic infections. To understand the true principles of host–fungus interactions, one ultimately needs to investigate the gene expression of cells in the same microenvironment or even on a single-cell basis. Furthermore, we need to look simultaneously at expression profiles of the host, as infections with *P. infestans* and *P. cinnamomi* are the result of an equilibrium between counteracting genetic programs of the host and those of the pathogen.