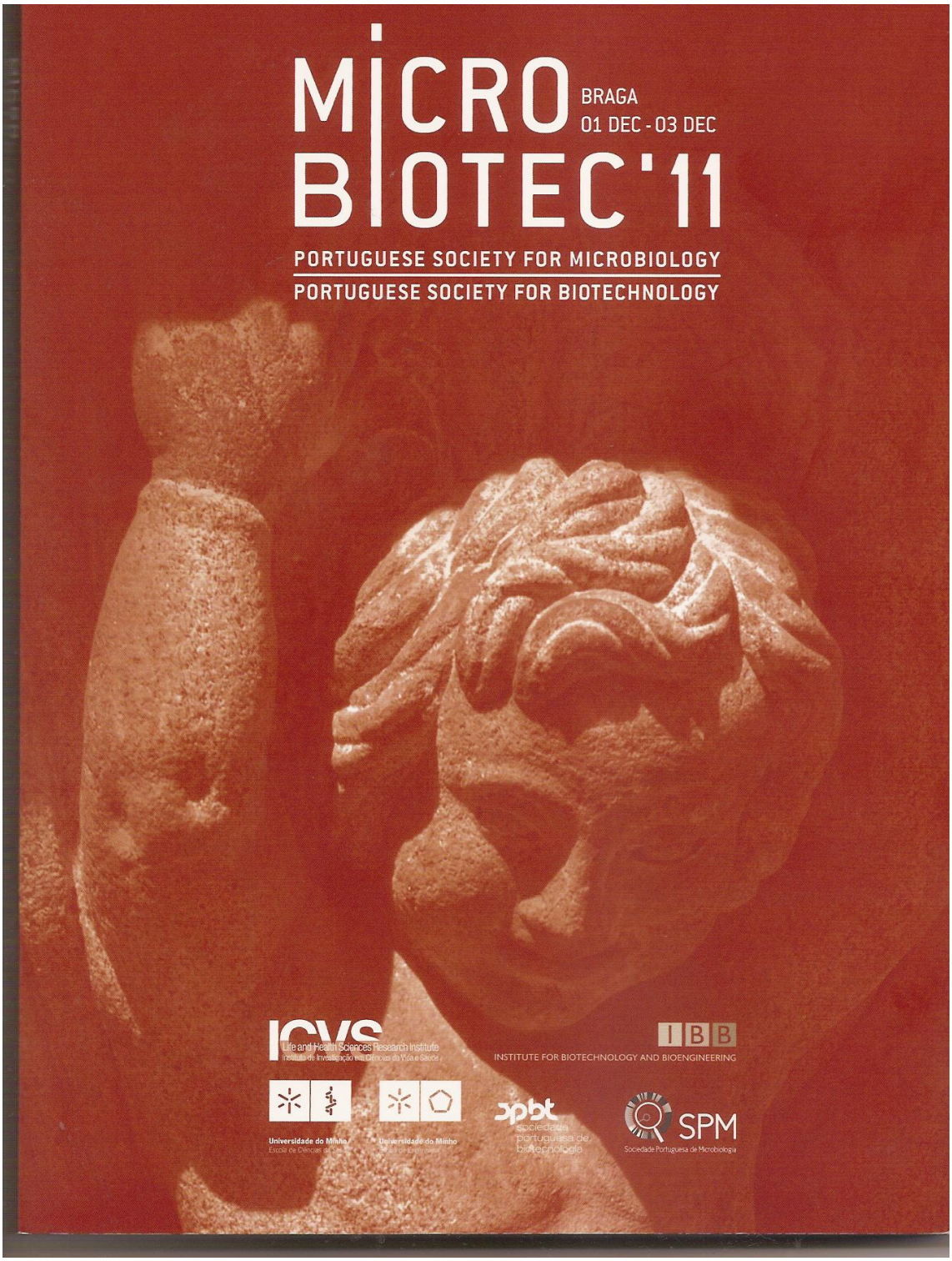

PS2: 66

Characterization of molecular factors from plants pathogen *Phytophthora cinnamomi*

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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. 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 reverse transcriptase PCR, in vivo expression technology, and Bioinformatics tools have been used to study the expression of selected genes from fungi during infection. In this work we intend to integrate the necessary bioinformatics tools that were used in this investigation. These tools include the use of Databases and associated homology programs as Fasta and Clustal, and several programs for sequence analysis and design of experiments such PCR.

PS9: 17

Isolation and characterization of necrosis-inducing phytophthora protein 1 (npp1) gene from plants pathogen *Phytophthora cinnamomi*

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Oomycetes from the genus *Phytophthora* are fungus-like plant pathogens that are devastating for agriculture and natural ecosystems. Due to their particular physiological characteristics, no efficient treatments against diseases caused by these microorganisms are presently available. To develop such treatments, it appears essential to dissect the molecular mechanisms that determine the interaction between *Phytophthora* species and host plants. One of the most widely distributed *Phytophthora* specie, with nearly 1000 host species is *Phytophthora cinnamomi*. Associated with this pathogen is the ink disease of *Castanea Sativa* Mill being one of the most destructive diseases in *C. Sativa* in the northeast of Portugal and the most common symptoms are root necrosis and reduction in root growth, which invariably lead to the trees death. *P. cinnamomi* is able to secrete a novel class of necrosis-inducing proteins, known as Nep1-like proteins (NLPs), more specifically necrosis-inducing *Phytophthora* protein 1 (*npp1*), that causes necrosis on leaf and roots of the plant, leading to the plant death.In order to better evaluate the mechanism of plant necrosis induced by *P. cinnamomi*, the study of factors that affect *npp1* gene expression is extremely important.The *npp1* gene ORF comprises 770 bp encoding a 256 aa protein with a molecular weight of approximately 25 kD. Gene expression *in vitro* in *P. pastoris* (heterologous expression), was studied during growth in different carbon sources, by RT-qPCR. Over expression of our gene in *P. pastoris* was also performed.*In vivo* expression technology has been used to study the expression of *npp1* gene from fungi during infection by RT-PCR. In our work chestnut roots were infected with *P. cinnamomi* and mRNA was extracted at different times of infection to analyze gene expression.These and other results will be presented and discussed.

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PS9: 18