

GEL-FREE/LABEL-FREE ANALYSIS TO STUDY THE PROTEOME OF BOTRYTIS CINEREA

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Botrytis cinerea is a necrotrophic plant pathogenic fungus, which infects more than 200 plant species, and causes significant economic losses both pre- and post-harvest in numerous crops (Elad *et al.*, 2004; Williamson *et al.*, 2007). Due to development of a wide number of strategies and technologies, in the last years, mass spectrometry (MS)-based Proteomics has become in a successful tool that can contribute to understand biology of plant pathogenic fungi, to identify virulence factors, and, on the basis of them, to develop crop protection strategies (reviewed in Gonzalez-Fernandez *et al.*, 2010). Within the BOTBANK project, we are using such approach to characterize and validate a collection of mutants of *B. cinerea* whose infectious cycle is affected. In this work, we present a preliminary gel-free/label-free proteomic analysis to study the proteome of *B. cinerea*, by comparing two wild-type strains with different virulence.

Mycelium protein extracts from B05.10 and T4 strains were obtained by TCA/acetone-phenol/methanol method (Wang *et al.*, 2006; Maldonado *et al.*, 2008) with some modifications. Total protein extracts were digested with trypsin. LC-MS^E analysis was performed in a SYNAPT HDMS mass spectrometer (Waters) interfaced with a NanoAcquity UPLC System (Waters).

The use of gel-free/label-free has allowed us the identification of 226 protein species in B05.10 and 172 in T4, and the quantification of proteins that differ in the two strains. Some of these proteins are involved in pathogenicity mechanisms or implicated in fungal growth and differentiation, providing us relevant information about the biology and infection strategies of this fungus.

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