Gel-based proteomic analysis of *Botrytis cinerea.* The simplest 1-DE reveals differences in virulence-related protein abundance among strains

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Botrytis cinerea is a phytopathogenic filamentous fungus, which infects more than 200 plant species [1], causing significant yield losses in a number of crops. There are a number of isolates of *B. cinerea* that differ in their virulence against specific crops [2]. In the last few years, *B. cinerea* has been adopted as an important model system in molecular phytopathology fungi studies [1]. In the post-genomics era, Proteomics has became in a powerful tool which can contribute to understand biology, infection strategies, and lyfe cycle of this fungus, to identify virulence factors, and, on the bases of them, to develop crop protection strategies. Up to date only a few proteomics studies have been published on this organism [3-7]. In this work, we present a preliminary gel-based proteomic analysis of mycelium extracts from six different strains of *B. cinerea*. We used, as starting point, one-dimensional electrophoresis (1-DE) and protein identification by MALDI-TOF/TOF. This work is part of an European research project (BOTBANK EUI2008-03686) within Plant-KBBE, intended at develop a collection of mutants of *B. cinerea* and validate the creation of this library with the characterization of the mutant lines whose infectious cycle is affected.

B. cinerea strains used were B05.10 (from Prof. Dr. Paul Tudzynski lab, Münster, Germany), CECT 2100, 2850, 2996 and 20518 (from Spanish Type Culture Collection) and BOLC (provided by Dr. Angel Villegas from IAS, Córdoba, Spain, and isolated from lentil infected plants). Three independent (biological) replicates, each one corresponding to 300 mL flacks containing 100 mL of modified Czapek-Dox minimal medium (2% w/v sucrose, 0.3% w/v $NaNO_3$, 0.1% w/v K_2HPO_4, 0.05% w/v KCl, 0.05% w/v $MgSO_4{\cdot}7H_2O$, pH 5.0) were inoculated with mycelium taken from solid cultures grown on cellophane membrane with potato dextrose agar. The cultures were grown for 6 days at 21ºC with agitation (120 rpm) in darkness. Mycelia were harvested by filtration. The protein precipitation method used was TCA/acetone-phenol/methanol for recalcitrant tissues described in [8]. Fifteen µg of protein were subjected to SDS-PAGE [9], using the Criterion System (Bio-Rad) with precast Criterion Stain Free Gels, Tris-HCl, 4-20% linear gradient (Bio-Rad). The protein band pattern was analyzed using the Image Lab software (Bio-Rad). The bands were cutted out and digested with trypsin. The MS of tryptic peptides was analyzed in a 4800 Proteomics

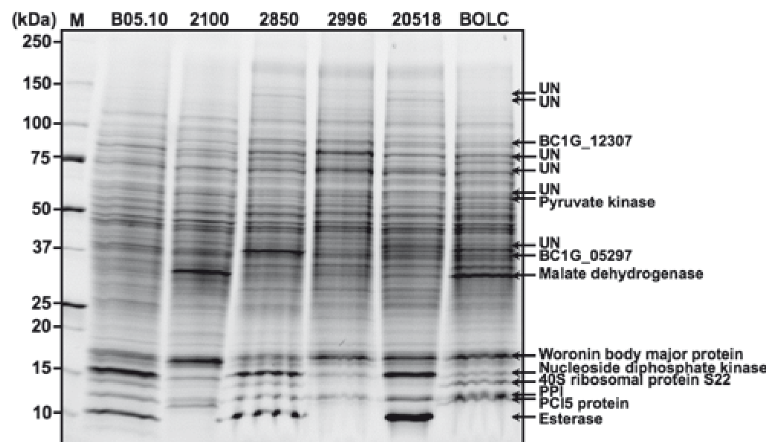

Figure 1. *SDS-PAGE of mycelium protein extracts of six different strains of B. cinerea. Identified proteins are indicated by arrows. UN: unidentified protein.*

Analyzer MALDI–TOF/TOF mass spectrometer (Applied Biosystems). The 3 most abundant peptide ions were subjected to MS/MS analysis. A PMF search and a combined search (+MS/MS) were performed in nrNCBI database of proteins using MASCOT.

Figure 1 shows the protein profile of the mycelium extract from the six strains studied (B05.10, 2100, 2850, 2996, 20518 and BOLC). There were significative qualitative and quantitative differences in the protein profile among strains. Several proteins were identified by MALDI-TOF/TOF MS/MS analysis (Figure 1). Some of them have been reported to be involved in pathogenicity in *B. cinerea* or in other phytopathogenic fungi, such as malate dehydrogenase [5], woronin body major protein [10], peptidyl-prolyl cis-trans isomerase (PPI) [11] and PIC5 protein [12], or implicated in fungal growth and differentiation, such as nucleoside diphosphate kinase [13]. The abundance of these proteins was different among isolated (Figure 1).

Work is now in progress in different directions: i) analysis of spores and culture media in wild-type strains; ii) analysis of mutants, obtained by *Agrobacterium tumefaciens*-mediated transformation (ATMT) and affected in infectious cycle; and iii) use of 2-DE, and LC-based proteomic approaches.

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