Proteomic approach to *Botrytis cinerea* survival structures

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Abstract

The ascomycete *Botrytis cinerea* is a phytopathogenic fungus infecting a high number of crops, causing significant yield losses in these crops in Andalusia (Spain). In the last few years, *B. cinerea* has been adopted as an important model system in molecular phytopathology. Several approaches have been applied to this fungus to unravel its mechanisms of infection. These studies have revealed the complexity and wide variety of infection strategies used by *B. cinerea*. This study presents an initial approach to characterize the proteins content of two principal structures of resistance: conidia and sclerotia, which constitutes the principal inoculum of the fungus in the fields. After 2-DE, the majority of the spots were found from 5 to 8 of pI values, presenting a Mr from 14 to 105 kDa. After protein identification will be done, our main aim is to find those proteins involved in primary plant lesion.

Main text

Botrytis cinerea Pers. Fr. is a phytopathogenic ascomycete that causes significant yield losses in a substantial number of crops. In the last years, many research advances regarding the infection process developed by this pathogen have been made. Lately, proteomics technologies have allowed the efficient characterization and identification of a large number of proteins from *B. cinerea* [1-3]. However, a special interest is focused in those proteins involved in the initial steps of plant infection process due to these proteins are excellent candidates for fungicide design or targets to design diagnosis techniques.

Conidial suspensions (1 x 10^8 cond/mL) were obtained from 15 days *B. cinerea* 2100 (Spanish type culture collection) malt agar plates following Carbu *et al* [4]. Sclerotia were obtained from *B. cinerea* strain 992.1-89 (University of Cádiz culture collection) maintained during 30 days in malt agar, at 22ºC to obtain a stock of well maturated sclerotia. Both, conidia and sclerotia, were homogenized by using FastPrep Instrument (Q-BIOgene, Valencia, USA), 12 cycles of 30 second and 6.0 of speed. Protein extraction was developed following Fernandez-Acero *et al* [2]. Protein concentration was determined by using the RC-DC Protein Assay (Bio-Rad). 2-DE was developed following Fernandez-Acero *et al* [2] using Immobilized pH gradient (IPG) strips (Bio-Rad, 7 cm,) 3-10 or 5-8 linear pH gradient (Figure 1).

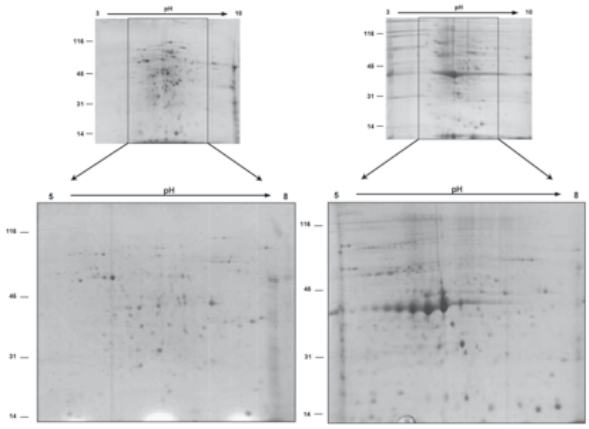

Figure 1. *2-DE gel from the first proteomic approach to conidia (left) and sclerotium (right), showing the protein separation in IPG 3-10 (up) and de definitive IPG 5-8 (down).*

Obtained proteins from ungerminated conidial suspension showed that most of the stained spots were allocated between pI 5 and 8. The distribution of the spots attending to its molecular weight (Mr) was found from 14.7 to 105.7 kDa. Those spots presented in all the three replicates were used to calculate the analytical variability of the experiment. One hundred and eight spots were used obtaining % CV average of 42.05 %, which is similar to our previous experiments. Two dimensional gels from Sclerotia present more diverse content. In spite that both structures show similar pI distribution, the molecular weight was between 3.1 to 158.8 kDa, showing a wider distribution. The obtained % CV

result was 44.61% by using 205 protein spots. This report shows the first proteomic approach to *B. cinerea* conidial germination, representing its initial step. Moreover, our initial results with sclerotia seem to coincide with the previous studies with *Sclerotinia sclerotiorum* [5] presenting a mayor protein with 34-36 kDa and 3 isoforms with a pI of 6.2, 6 and 5.8. Our gels show 6 isoforms with a Mr about 41 kDa, and a pI of 5.23, 5.51, 5.65, 5.81, 5.94 and 6.19 as a mayor component. In spite of the observed differences and waiting the results from MALDI TOF/TOF, we assume that our protein is the same, a Ssp1 protein involved in sclerotia development.

References

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Perfil serológico de la respuesta de anticuerpos frente al inmunoma de *Candida* en el pronóstico de las candidiasis invasivas

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Las candidiasis invasivas son infecciones de notable morbilidad y mortalidad, en pacientes severamente inmunocomprometidos y enfermos críticos [1]. El hallazgo de nuevos biomarcadores de pronóstico para estas infecciones oportunistas puede suponer un nuevo camino para tomar decisiones terapéuticas más precisas e individualizadas que podrían a su vez mejorar la evolución clínica de estos pacientes. Para esta búsqueda, pueden ser útiles técnicas globales de alto rendimiento tales como la inmunoproteómica clásica o el análisis del proteoma serológico (SERPA) [2]. Diferentes estrategias SERPA (basadas en electroforesis bidimensional seguida de "western-blotting" y espectrometría de masas) han permitido el descubrimiento de varios biomarcadores clínicos y dianas terapéuticas para estas micosis oportunistas [2-5].

Con el fin de identificar y validar un nuevo método de pronóstico para las candidiasis invasivas, se combinó la tecnología SERPA con análisis bioinformáticos para examinar, en un estadio precoz de infección, los perfiles serológicos de la respuesta de anticuerpos frente al inmunoma de *Candida* en 45 pacientes con candidiasis invasivas que tuvieron un desenlace fatal o favorable.

Análisis de conglomerados jerárquicos bidimensionales y del componente principal de los patrones de reactividad de anticuerpos séricos frente a 31 proteínas inmunogénicas de *Candida* separaron con precisión pacientes con estas micosis oportunistas en dos grupos con pronósticos diferentes (aquellos que sobrevivieron de los que fallecieron durante el proceso infeccioso). Estos subgrupos fueron independientes de las características demográficas