Applying proteomic technologies to dissect molecular aspect of phytopathogenic fungi, a *Botrytis cinerea* approach

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Abstract

Two dimensional gel electrophoresis (2-DE) plus mass spectrometry (MS) have been widely used to study the proteome of a good number of tissues, plants, animals or microorganisms. However, few of them have been carried out to study the proteome of phytopathogenic fungi. These organisms are responsible of several plant diseases in different crops around the world, causing very important economic losses to the farmers. There are several reasons to explain the lack of proteomic studies describing fungal plant pathogen proteomes. The main aim of this work is to tackle the strategies to overcome these problems, i.e. by the modification of protein extraction protocols, or using microbiology techniques to decrease the analytical variability.

Main text

Fungal plant pathogens are one of the main responsible of farmer losses. By way of example, the annual consumption of fungicides against phytopathogenic fungus *Botrytis cinerea* (Botrycides) moves about 10% of global fungicide market, which would mean 540 million per year [1]. Moreover, the losses increase with the value of agricultural losses, quantified between 10 and 100 million Euros per year. The losses caused by *B. cinerea* in French vineyards are between 15% and 40% per year, depending on weather conditions. In Holland, *B. cinerea* produces a 20% loss in flower crops, and in Spain 20% - 25% in the strawberry crops. Annual average expenditure on fungicides against *Botrytis*, is around 3 million Euros in tomato crops, 2 million in strawberry and 3 million in grapewine crops (BASF, personal communication). The genus *Botrytis* is an ascomycetes filamentous fungus, which includes a large number of pathogenic species capable of infecting a variety of crops. Some species are able to infect a single crop, such as *B. tulipae, B. squamosa* or *B. fabae*, pathogen to tulips, bean and onions respectively. However, *B. cinerea* is able to attack more than 200 species, including tomatoes, strawberries and grapes, three of the most important crops in Andalusia. This fungus has great flexibility, preferably is able to attack fruit (grapes, strawberries, tomatoes, etc.), flowers, but also is able to attack stems, leaves and seeds. Moreover, it is also capable to attack many different plants (grapevine, tomato, *Arabidopsis*, roses, etc.), in different organs of the same plant (fruits, leaves, petals and other structures of the same plant). Furthermore, the disease may appear directly *"in planta"* or during storage and distribution of fruits. These data show the relevance of this pathogen that is being currently considered as a model organism in all those experimental approaches to the study of plant-pathogen interaction.

The fact that there are only few reports on the proteome of phytopathogenic fungi is mainly due to problems such as the difficulty in obtaining fungal protein extracts or the lack of available fungal protein databases [2]. An effective protein extraction is a key step for obtaining a good resolution avoiding streaking in the 2-DE gels. It will allow us to remove contaminants (i. e. nucleid acid, salts, lipids, pigments) which may disturb the proteins focusing. However, it is especially relevant when the biological source is a filamentous fungus due to its richness in cell wall polysaccharides, and the mechanical resistance of the fungal cell wall. This problem has been overcome by using phenol based protocol [3]. On the other hand, the amount of genomic resources is increasing continuously, and about 25 fungal genome projects are being finished (Broad Institute, MIT, Harvard). This information is crucial to get significant number of positive protein identification hits. In spite of these problems seem to be overcame, there are still some points that become

critical a proteomic approach to filamentous fungal proteome. In spite of other aspects, we are especially interested in two major fields, (i) to decrease the levels of variability founded in these studies, and (ii) to increase the benefit obtained from the large list of identified proteins.

Calculations of analytical and biological variability are directed to quantify the variations associated with the 2-DE experiments and differences in the level of protein expression between independent replicates [4]. The averages of analytical and biological coefficients of variance are used to be calculated [5]. Only those changes consistently present in all the extract replicates, whose quantitative differences are higher than the corresponding biological and analytical variance, should be considered [2]. In spite of the levels obtained in fungal approaches are similar to the obtained variability in plant or microorganism studies [4, 6]. We had used monoconidial isolates in attempt to decrease these levels, but the results remain high. On the other hand, the final results obtained in a proteomic approach use to be a large list of identified proteins. The role of each protein is also discussed for its biological relevance, or used "in further studies". This information is very important and interesting, but if we apply our own and particular "optics", we assume the risk of information losses in other parallel biological process. As an interesting tool is the website PANTHER (Protein ANalysis THrough Evolutionary Relationships; www.pantherdb.org) that allows the classification of identified proteins in different categories according to (i) its biological process or (ii) its molecular function, based on Gene Ontology. This information may highlight some information which is difficult to extract directly from a protein list. In this sense, an algorithm to elucidate the drug target-likeness of a protein has been published [7]. This work could improve the development of new therapeutic targets to fight against several diseases. Unfortunately, at this moment is not feasible to develop similar approach to analyse new candidates for fungicide design (personal communication).

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