In planta proteomics and proteogenomics of the biotrophic barley fungal pathogen blumeria f.sp. hordei

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In Plant Proteomics and Proteogenomics of the Biotrophic Barley Fungal Pathogen Blumeria graminis f.sp. hordei

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Overview

Introduction

Whilst there is increasing evidence that the outcome of the interaction between a pathogen and a host is dependent on protein-protein interactions, very little information is available on plant-pathogen interactions. Here a proteomic approach has been employed to supplement the annotation of the recently sequenced genome (www.blugen.org) and to cast light on the biology of the infection process of the economically important barley powdery mildew pathogen Blumeria graminis f.sp. hordei.

Strategy

Proteins from three different Blumeria tissues were isolated from infected barley leaves: 1) conidia (vegetative spores), 2) hyphae (which grow on the leaf surface) and 3) barley leaf epidermis containing haustoria (the "feeding" organs). A gel-LC-MS/MS approach was used: Proteins were separated by SDS-PAGE prior to in-gel tryptic digestion. Proteins were identified using Thermo Scientific LTQ-Orbitrap hybrid mass spectrometers. A proteomic genome database was used to identify and align peptides onto DNA contigs. Validated ORFs were annotated using the SwissProt database for Blast similarity searches and retrieving gene ontology information.

Results and conclusion

In the present study, we describe a strategy which is contributing to the annotation of the recently sequenced genome of Blumeria graminis f.sp. hordei, which is a biotrophic obligate plant pathogenic fungus. Gene ontology analysis reveals differences in the proteome of haustoria. This study is the first systematic in planta proteomic analysis of this economically important obligate plant pathogenic fungus.

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Results

Blumeria proteomes analysed by gel-LC MS/MS.

The vegetative cycle of B. graminis requires exclusive growth on barley. Conidia germinate and form an appressorium required to penetrate into the epidermis. Successful penetration results in haustoria formation and epiphytic hyphal growth. Some hyphal cells will differentiate into sporulating hyphae.

Methods

Experimental

Fig. 1 Sporangulating powdery mildew (B. graminis f.sp. Hordei) on barley. Blumeria cannot grow in vitro. Note the absence of necrotic leaf tissue, which reflects the compatible interaction.

Life cycle

Ungerminated conidia

Fig. 2 The vegetative cycle of B. graminis requires exclusive growth on barley. Conidia germinate and form an appressorium required to penetrate into the epidermis. Successful penetration results in haustoria formation and epiphytic hyphal growth. Some hyphal cells will differentiate into sporulating hyphae.

Gene ontology analysis reveals differences in the proteome of haustoria.

Proteins identified exclusively in haustoria are smaller, secreted and predominately unknown (7/9).

Conclusions

- Successful proteogenomic approach to annotate the genome
- Helps genome assembly
- Reveals gene functionality and novel ORFs
- Characteristics of proteins exclusively detected in haustoria
- Mostly unknown, small and secreted proteins
- Discovery of putative new fungal effectors secreted in the host
- The haustorium is involved in stress, immunity and defence, nutrient uptake and metabolism (monosaccharides).