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

School of Biological Sciences, The BioCentre and Department of Chemistry

University of Reading

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

Laurence V. Bindschedler[1] | Tim Burgis[2] | Davinia J.S. Mills[1] | Jenny Ho[3] | Pietro D. Spanu[2] | Rainer Cramer[1]

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 *in planta* proteomes of biotrophic plant pathogens. Here a proteogenomic approach has been employed i) to supplement the annotation of the recently sequenced genome (www.BluGen.org) and ii) 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 trypsin digestion. Peptides were separated by RP-nLC coupled to a 3D ion trap or an orbitrap mass spectrometer. A Blumeria genomic database was used to identify and align peptides onto DNA contigs. Validated ORFs were annotated using the SwissProt database for Blast similarity searches and recovering gene ontology information.

Results and conclusion

In the present study, we describe a strategy which is contributing to the genome annotation and to the manual curation of the genomic data. Over 800 proteins were identified, some of which are not associated with an EST sequence, and thus represent novel experimental evidence for the existence of expressed genes. Therefore, in addition to validating experimentally *ab initio* predicted genes derived from the related fungi *Sclerotinia sclerotiorum* and *Leptosphaeria maculans*, this proteogenomic approach has led to the discovery of unpredicted expressed and translated open reading frames.

This study highlights some of the changes in the Blumeria proteome during infection, development, and disease. Gene ontology analysis reveals organ specificity for some biological functions, in particular for the haustorium, the specialised feeding and plant-fungus interacting organ of fungal biotrophs. Of note is a high proportion of proteins identified in haustoria that are involved in stress responses. This suggests that haustoria are organs which have to cope with reactive oxygen species, for example hydrogen peroxide, produced by the barley cells in response to infection. Most of the proteins detected only in the haustoria are predicted to be secreted proteins, which may play a role as "effectors" required for the establishment of the disease. This research provides an unique insight in the development of powdery mildew disease such as the discovery of novel secreted fungal effectors which may influence the pathogen virulence and the plant susceptibility during the disease, and thus is a step towards understanding the molecular interactions between the two players.

Novel aspect

This study is the first systematic *in planta* proteomic analysis of this economically important obligate plant pathogenic fungus.

Affiliations

[1]University of Reading, United Kingdom
email to: l.v.bindschedler@reading.ac.uk
[2]Imperial College London, United Kingdom
[3]Thermo Scientific, Hemel Hempstead, United Kingdom

Introduction

The pathogen : Barley powdery mildew.

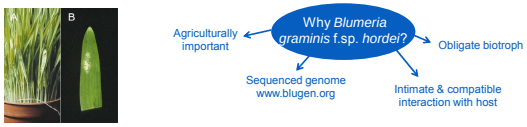

Fig. 1 Sporulating powdery mildew (*B. graminis f.sp. Hordei*) on barley. Blumeria cannot grow *in vitro*. Note the absence of necrotic leaf tissue, which reflects the compatible interaction.

Life cycle

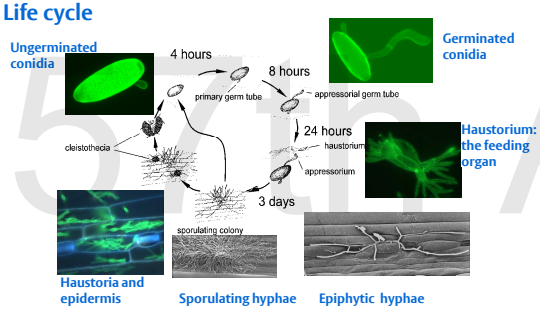

Fig. 2 The vegetative life cycle of B. graminis requires exclusive growth on barley. Conidia germinate and form an appressorium required to penetrate into the epidermis. Successful penetration results in haustoria formation and epiphytic hyphal growth. Some hyphae will differentiate into sporulating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

Methods

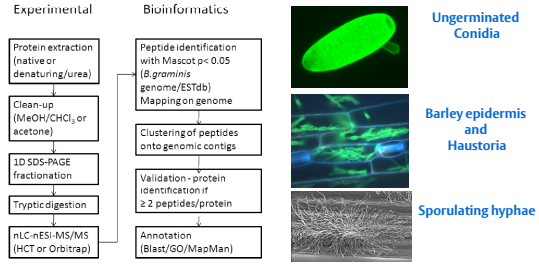

Fig. 3 Workflow to study the proteome of B. graminis: Gel-LC-MS/MS analysis was performed on an ion trap and orbitrap. Proteins were identified from conidia, sporulating hyphae and barley epidermis containing haustoria.

Results

Blumeria proteomes analysed by gel-LC MS/MS.

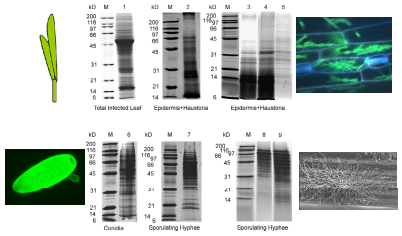

Fig. 4 Proteins from conidia, sporulating hyphae and barley epidermis containing haustoria were extracted in denaturing conditions and separated by SDS-PAGE prior to in-gel digestion and analysis on an ion trap or orbitrap mass spectrometer.

A proteogenomic approach.

A proteogenomic approach has been used to supplement the genomic annotation, the identification of novel proteins and the manual curation of the Blumeria genome, which is characterised by a high content (70%) of repetitive DNA (www.BluGen.org).

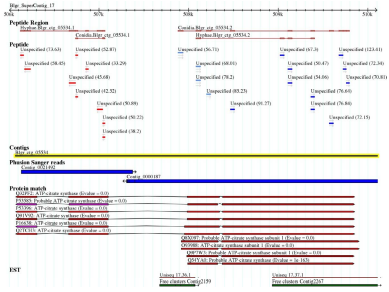

Fig. 5 Identified peptides were aligned on the *Blumeria* genome draft and grouped into proteins/ ORFs. This allowed the identification of new unpredictable ORFs as well as detecting mis-assembled contigs as the example shows on the left (screenshot from the Blumeria Gbrowser: www.Blugen.org).

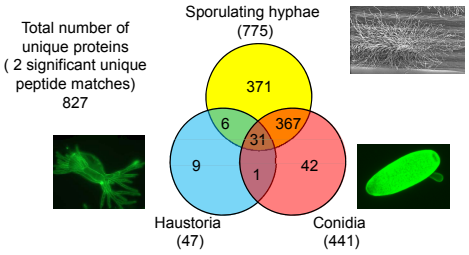

Fig. 6 Identified proteins in conidia, sporulating hyphae and haustoria.

Gene ontology analysis reveals differences in the proteome of haustoria.

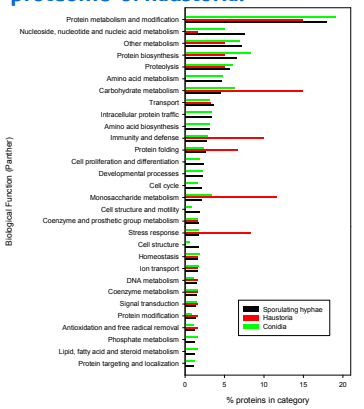

Fig. 7 GO (Panther) protein classification of proteins identified in conidia, haustoria and sporulating hyphae. The haustorium proteome was characterised by a higher proportion of proteins involved in carbohydrate (monosaccharide) metabolism, immunity and defence as well as stress response, including detoxification of ROS (i.e. catalase).

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

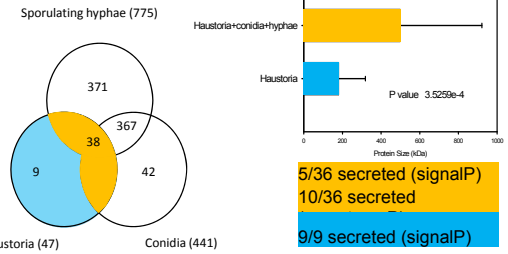

Fig. 8 Proteins exclusively detected in haustoria (blue) are secreted and are significantly smaller than proteins identified in other tissues (orange).

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).