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First data obtained by shotgun proteomics from *Nicotiana occidentalis* infected by '*Candidatus* Phytoplasma mali'

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Abstract

The protein content of *Nicotiana occidentalis* infected by the non-cultivable phytopathogenic mollicute '*Candidatus* Phytoplasma mali' strain AT was determined by shotgun proteomics. 102 out of 497 predicted phytoplasma proteins were identified as expressed in shoot tissue. In addition, 940 proteins of *N. occidentalis* were detected. Results demonstrate the successful application of LTQ Orbitrap XL ETD™ mass spectrometer in detecting phytoplasma-specific proteins in protein mixtures. A high portion of proteins with unknown function was identified beside prominent proteins involved in translation. Several of the proteins with unknown function contain a signal peptide suggesting a potential pathogen-host interaction.

Key words: phytoplasma, apple proliferation, proteomics.

Introduction

'*Candidatus* Phytoplasma mali' is the agent associated with apple proliferation disease which represents one of the most economically important phytoplasma diseases in Europe. Infection results in impaired fruit quality and productivity of the apple trees. '*Ca.* P. mali' belongs together with '*Ca.* P. pyri', '*Ca.* P. prunorum' and few other phytoplasmas to the apple proliferation group, which forms a distinct major subclade within the phytoplasmas (Seemüller and Schneider, 2004). The genome sequence of '*Ca.* P. mali' strain AT was determined and highlighted an unusual linear chromosome organisation and a low G+C content of 21.4% (Kube *et al.*, 2008). The condensed genome with a size of 602 kb encodes 497 protein coding genes.

This study aims to evaluate the application of shotgun proteomics to '*Ca.* P. mali' strain AT infected plant material and thereby provide additional information and evidence for the expression of the predicted proteins. The shotgun proteomics approach was successfully used for the identification of proteins assigned to the mulberry dwarf phytoplasma by comparing mass spectra with the proteins of all *Mollicutes* (Ji *et al.*, 2009). Due to the complete genome sequence of '*Ca.* P. mali' a more stringent assignment is possible. Here we present the first experimental derived proteome data of this study.

Materials and methods

Protein isolation from plant material

Nicotiana occidentalis greenhouse plants were inoculated with '*Ca.* P. mali' strain AT as previously described (Kube *et al.*, 2008) at the Julius Kühn Institute Dossenheim. One plant showing distinct symptoms of infection such as little leaves and witches` broom was chosen for the initial experiment. Proteins were isolated from shoots by two different methods in parallel. First approach (I) started by freezing the tissue in liquid nitrogen followed by TissueLyser treatment (Qiagen), suspension in SDS lysis buffer containing protease inhibitors (unpublished) and sonification. For the second approach (II), shoots were disrupted in meshbags (extraction Bags, Bioreba) and proteins isolated using the AllPrep DNA/RNA/Protein Mini Kit (Qiagen) according to the manufacturer's instruction. Proteins were separated in 12.5% SDS-PAGE gel and stained by Coomassie G250 for visualisation. After destaining in 20% methanol, 7.5% acetic acid gel lanes were cut into 16 slices. Reduction and alkylation was performed. Proteins were digested within the gel slices with trypsin (Roche Diagnostics) for 16 h at 37°C in humidified atmosphere. Peptides were extracted and vacuum dried. Afterwards, samples were resuspended in 5% acetonitrile with 2% formic acid.

LC-MS/MS measurement

LC-MS/MS was performed on LTQ Orbitrap XL ETD™ mass spectrometer (Thermo Fisher Scientific) equipped with a nanospray ion source (Thermo Fisher Scientific) and an Agilent 1200 Series HPLC- System (Agilent Technologies). The System was fitted with a self-packed C18 RP column (0.15 mm × 150 mm *PicoFrit,* New Objective; *ReproSil- Pur C18- AQ* Dr. Maisch). Buffers A (2% acetonitrile + 0.1% formic acid) and B (98% acetonitrile + 0.1% formic acid) served as mobile phase and the peptides were eluted via a gradient of 2.7% to 90% buffer B over a period of 150 min with a flow rate of 0.2 µl/min. Full-scan mass spectra were detected with the Orbitrap mass spectrometer. The ten most intense peptides were selected for CID MS/MS scans in the linear ion trap. Each sample has been injected two times.

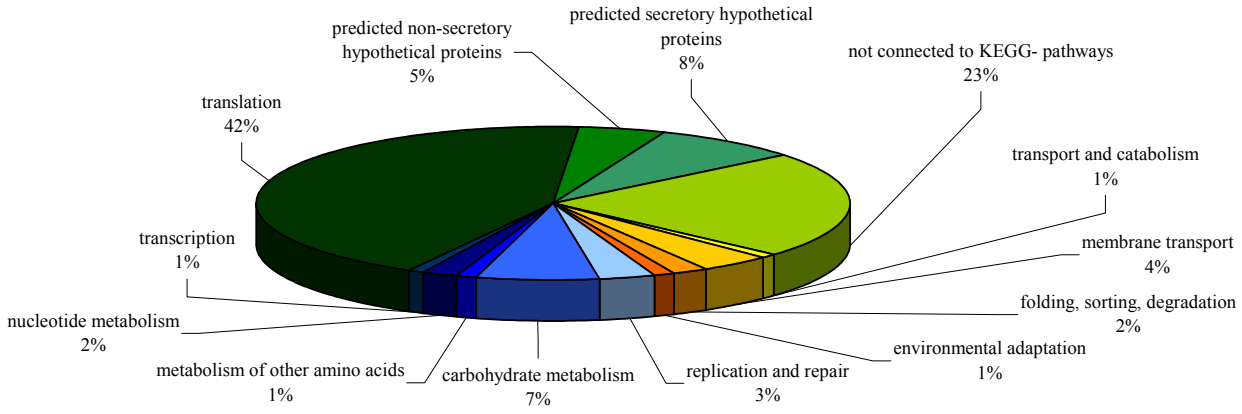

Figure 1. Functional categories of the 102 identified '*Ca*. P. mali' strain AT proteins according to their connection to KEGG pathways. Proteins with assigned function but no connection to KEGG were grouped in "not connected to KEGG pathways" whereas those with no assigned function were grouped in "predicted secretory/non-secretory hypothetical proteins".
(In colour at www.bulletinofinsectology.org)

Protein identification

Protein identification has been carried out using the Andromeda search engine (Cox *et al.*, 2011) in the MaxQuant V1.1.1.25 environment (Cox *et al.*, 2008) with a FDR at peptide and protein level of 1% and a maximum mass deviation for the fragment ions of 0.5 Da. A target-decoy database was constructed comprising 9002 *N. occidentalis* (http://www.ncbi.nlm.nih.gov/) and 497 '*Ca.* P. mali' protein sequences (CU469464). In addition, 13,080 open reading frames (ORFs) with a minimum length of 20 amino acids were calculated from the "*Ca.* P. mali" strain AT genome sequence and also implemented in the database. A set of common contaminant proteins (http://www.maxquant.org/) like keratines has been included. A maximum of three missed cleavages was allowed for the protease trypsin. Oxidation of methionine and acetylation of the N-terminus have been applied as variable modifications. Carbamidomethylation was set as fixed modification. A protein hit has been considered as valid, if the protein was identified by at least two peptides, of which one had to be unique.

Results and discussion

Within these first experiments 102 proteins of '*Ca*. P. mali' and 940 *N. occidentalis* were identified. While the plant derived proteins are still under investigation, a preliminary analysis of the '*Ca.* P. mali' proteins was performed. Genes assigned to the KEGG (Kanehisa *et al.*, 2010) based functional category translation (figure 1) dominate the overall number of proteins. The nearly complete set of ribosomal proteins dominates this category. However, proteins of unknown function represent the third largest group of the identified proteins. A high portion of predicted secretory proteins without assigned function is remarkable and indicates the lack of information in phytoplasmas. As supposed the immunodominant membrane protein Imp was identified.

First results clearly indicate that the proteomic shotgun approach is successful applicable for the identification of expressed '*Ca*. P. mali' proteins in plants. Analysis of the plant-derived proteins and of additional tissue from other *N. occidentalis* plants representing biological replicates is in progress.

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