Preliminary proteomic analysis of pear leaves in response to pear decline phytoplasma infection

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Abstract

Pear decline phytoplasma, ‘Candidatus Phytoplasma pyri’, belongs to the apple proliferation (AP) group and causes serious diseases in Pyrus communis fruiting cultivars in many areas around the world. It induces two types of symptoms, depending on the rootstock: ‘slow’ decline occurs on trees with tolerant or resistant rootstocks; ‘quick’ decline occurs on trees with sensitive rootstocks. The most common symptoms are leaf curl and a premature reddening and loss of foliage in the autumn. To better understand the pathogen-stress response of pear (Pyrus communis L.) to pear decline phytoplasma, we have initiated a comparative proteomic analysis of infected and healthy pear leaves. The proteins so far identified are mainly involved in carbohydrate metabolism and photosynthesis.

Key words: LC/ES/MSMS; host-pathogen interactions; stress response; plant pathogens.

Introduction

Phytoplasmas are small (0.2-0.8 µm), wall-less, pleomorphic prokaryotes responsible for numerous economically important plant diseases. They are characterized by a very small genome and are obligate parasites of plants and some insects that act as vectors (Lee et al., 2007). So, these organisms remain un-culturable by conventional methods. Phyto- plasma pyri, belongs to the apple proliferation (AP) group and causes serious diseases in Pyrus communis fruiting cultivars in many areas around the world. It induces two types of symptoms, depending on the rootstock: ‘slow’ decline occurs on trees with tolerant or resistant rootstocks; ‘quick’ decline occurs on trees with sensitive rootstocks. The most common symptoms are leaf curl and a premature reddening and loss of foliage in the autumn. To better understand the pathogen-stress response of pear (Pyrus communis L.) to pear decline phytoplasma, we have initiated a comparative proteomic analysis of infected and healthy pear leaves. The proteins so far identified are mainly involved in carbohydrate metabolism and photosynthesis.

Materials and methods

Pears (Pyrus communis L.) were harvested in the orchard of the Fruit Tree Research Institute in Caserta, Italy. Pooled leaves from healthy and PD-infected pear samples were immediately frozen in liquid nitrogen and ground thoroughly with a prechilled mortar and pestle. The powder obtained was suspended in 10% TCA/acetone. After centrifugation (16,000 g for 3 min), the pellets were washed first with 80% methanol/0.1 M ammonium acetate, and then with 80% acetone. Protein pellets were vacuum-dried at room temperature, and then suspended in 1:1 phenol (pH 8)/SDS buffer. After a thorough mixing (1 hr), the phenol phases were collected and precipitated by methanol/ammonium acetate. The pellets were then washed once with 100% methanol and once with 80% acetone. The resulting pellets were finally suspended in SDS sample buffer. Protein was quantified using the 2-D Quant kit, following the manufacturers’ instructions.

Purified proteins from both healthy and infected samples were run on SDS-polyacrylamide gel. Gels were visualized by Coomassie or silver nitrate staining. Protein bands showing significant changes in abundance between healthy and infected samples were selected and excised for protein identification by in-gel trypsin digestion.
**Table 1. Proteins identified by LC/ESMSMS.**

<table>
<thead>
<tr>
<th>Acc. n° NCBI and identified protein</th>
<th>Biological function</th>
<th>Acc. n° NCBI and identified protein</th>
<th>Biological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>gi/131385 Oxygen-evolving enhancer protein 1, chloroplastic</td>
<td>Photosynthesis</td>
<td>gi/194708200 Actin</td>
<td>Structural</td>
</tr>
<tr>
<td>gi/7525028 Photosystem II D2 protein</td>
<td>Photosynthesis</td>
<td>gi/115800 Chlorophyll a-b binding protein 3, chloroplastic</td>
<td>Photosynthesis</td>
</tr>
<tr>
<td>gi/81301580 Cytochrome f</td>
<td>Photosynthesis</td>
<td>gi/403160 Ribulose 1,5-bisphosphate carboxylase small s.u.</td>
<td>Photosynthesis</td>
</tr>
<tr>
<td>gi/17981607 Sorbitol 6-phosphate dehydrogenase</td>
<td>Carbohydrate metabolism</td>
<td>gi/28630975 Photosystem II D1 protein</td>
<td>Photosynthesis</td>
</tr>
<tr>
<td>gi/5031279 Porin</td>
<td>Transport</td>
<td>gi/27311547 Unknown protein</td>
<td>Unknown</td>
</tr>
<tr>
<td>gi/119905 Ferredoxin-NADP reductase, leaf isoyzme, chloroplastic</td>
<td>Photosynthesis</td>
<td>gi/136057 Triosephosphate isomerase, cytosolic</td>
<td>Carbohydrate metabolism</td>
</tr>
<tr>
<td>gi/119656699 Photosystem II 32 KDa protein</td>
<td>Photosynthesis</td>
<td>gi/8272386 Endo-chitinase class III</td>
<td>Carbohydrate metabolism</td>
</tr>
<tr>
<td>gi/19184 Type 1 CP29 polypeptide</td>
<td>Photosynthesis</td>
<td>gi/16175 Adenylate translocator</td>
<td>Carrier</td>
</tr>
<tr>
<td>gi/2254440674 Hypothetical protein</td>
<td>Unknown</td>
<td>gi/132270 Rubber elongation factor</td>
<td>Metabolism</td>
</tr>
</tbody>
</table>

The peptide fragments obtained were then subjected to LC/ESMSMS analysis. Database searching, peptide mass fingerprinting (PMF), and MS/MS were performed using MASCOT 2.1 against the NCBI non-redundant *Viridiplantae*-specified protein sequences.

**Results and discussion**

Proteomic analysis represents a useful tool to gain insight into the plant host responses to stresses. To investigate the effects of PD phytoplasma on the pear protein profile SDS-PAGE on leaf proteins from infected and healthy plants showed differentially expressed protein bands that were excised from the gel and analyzed by LC/ESMSMS. The accession numbers and names of the identified proteins are listed in table 1. Among the 18 proteins identified, two were annotated as unknown, the others are involved in diverse processes including photosynthesis, carbohydrate metabolism, and metabolite transport.

These findings support the data deriving from physiological and biochemical analyses showing that infection with phytoplasmas is associated with increase in soluble carbohydrate and starch content, and decrease in the photosynthesis rate, carboxylation efficiency, and pigment content of leaves (Xianling et al., 2009). In a study on gene expression profile of PD infected periwinkles, genes involved in plant defense/stress responses, protein metabolism and transport, transcriptional regulation, vesicle trafficking, and carbohydrate metabolism were identified (De Luca et al., 2011). Proteomic analysis showed that the expression of many proteins changed during phytoplasma infection. These changes may alter many physiological and biochemical processes, and result in diverse and severe symptoms in infected plants.

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**References**


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