Proteome changes of *Pteridium aquilinum* during postharvest storage

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Abstract

Postharvest quality deterioration directly limited the shelf life and edibility of the stem of *P. aquilinum*. Proteome changes in response to quality worsening of the vegetable were investigated using 2-DE and differential proteins were identified by MALDI-TOF/TOF. The results demonstrated several proteins were identified and these proteins were classified into five functional groups based on the protein functions and relevant literatures. Among them, six proteins were related to carbohydrate metabolism, three proteins were connected with to energy and amino acid metabolism, three proteins were involved in cell structure formation, three proteins were associated with stress response and defense and two proteins were engaged in protein synthesis. The biological mechanisms of quality deterioration was shows as follow: (1) The decline of antioxidant capacity would be responsible for accumulation of ROS, and excessive ROS leads to cell oxidation and senescence (2) *P. aquilinum* senescence begins and protein content declines when the vegetable is harvested and stored. (3) Cell structure integrity could be destroyed during postharvest storage.

Keywords: Proteomics; *P. aquilinum*; Quality deterioration; Postharvest storage.

Abbreviation: *P. aquilinum*; MALDI_matrix assisted laser desorption ionization; TOF_time of flight; 2-DE_two dimensional gel electrophoresis; ROS_reactive oxygen species; TCA_the tricarboxylic acid; MDHAR_monodehydroascorbate reductase; Hsp_Heat shock proteins; MDHA_monodehydroascorbate; ADF_actin-depolymerizing factor 10-like

Introduction

The ferns are the sporous plants with fascicular vascular cambia, and about 12000 species are recorded around the world (Xu et al., 2009). Studies conducted in several countries showed that the plant not only invaded crop fields and pastures, but also produced toxic effects (carcinogens and cyanogenic glycosides) that seriously affected human and animal health (da Costa RM et al., 2012). As other plants, the invasive fern *Pteridium aquilinum* can be found in temperate regions of the world where places such as *Arabidopsis thaliana* (Mukherjee et al., 2010), maize (Li et al., 2011), rice (Zhang et al., 2012), fresh-cut *Zizania latifolia* (Luo et al., 2012) and Lotus tuber (Jiang et al., 2012). To our knowledge, studies on biological mechanisms of the vegetable on quality worsening from proteomic angles have not yet been performed up to now. Thus, we used 2-DE technology followed by MALDI-TOF/TOF to identify the differential proteins which may partially explain the molecular mechanisms on quality deterioration. The study may provide useful molecular information for taking effective measures to prolong marketability of a vegetable product in the future.

Result

The functional classification of differential proteins

The results showed that seventeen proteins were identified by MALDI-TOF/TOF and these proteins were involved into five metabolic pathways, including carbohydrate metabolism (6, 35.3%), protein synthesis (2, 11.9%), energy and amino acid metabolism (3, 17.6%), cell structure (3, 17.6%), and stress response and defense (3, 17.6%) based on biological functions of differential proteins and relevant literatures.

The subcellular localization of differential proteins

According to WOLF PSORT database (http://wolfsort.org/), nine proteins were located to two sub-organelles (Table 1),
including mitochondrial (6, 35.3%) and chloroplast (3, 17.6%), while other eight proteins were cytoplasmic proteins (8, 47.1%).

The comparison on experimental Mr/pl and theoretical Mr/pl of differential proteins

As shown in Table 1, the experimental Mr/pl of the identified proteins was in reasonable agreement with the theoretical values of them (Table 1). However, differences between the experimental and theoretical values of Mr/pl for few proteins were noticeable (Table 1). The theoretical Mr of the elongation factor (spot 1) was markedly higher than the experimental value (Table 1). In addition, the theoretical PI (9.39) of prohibitin (spot 4) was far beyond the pH range of IPG strips we used. The discrepancy of Mr/pl might be due to the presence of different isoform, the lack of genomic information for the organisms, the expressions in different organisms and post-translational modification of the same gene expression.

The determination of H$_2$O$_2$ and protein content

The protein content has been on the decline during postharvest storage. The H$_2$O$_2$ content was increasing in the former six days storage, while went down on the ninth day storage. The results showed in Fig 5 and Fig 6.

Discussions

Proteomic changes in *P. aquilinum* are associated with the regulation of different metabolic pathways when the vegetable was harvested and subsequently stored for a few days. However, little is known on the molecular mechanisms of the quality deterioration. V-ATPase is a key player in several aspects of cellular function, including acidification of intracellular organelles and regulation of extra-cellular pH. The protein is indispensable for plant growth under normal conditions due to its role in energizing secondary transport, maintenance of solute homeostasis and facilitating vesicle fusion (Dietz et al., 2001). The expression of V-ATPase was reported to be involved in adapting plants under different stresses such as salinity (Poddia et al., 2013), drought, acid stress, anoxia and cold (Dietz et al., 2001). However, the abundance of V-ATPase was significantly down-regulated in CK3, CK6 and CK9 compared to CK0. Postharvest *P. aquilinum* may lack of the regulatory mechanisms to cope with adverse condition when it encountered multiple stresses during storage. Proteomic studies showed enzymes involved glycolysis in the chloroplast were down-regulated when flag leaves senesces (Zhang et al., 2010). In this experiment, three enzymes of glycolysis, including triosphosphate isomerase (spot 3), phosphoglucomutase family protein isoform 1 (spot 17) and phosphoglycerate mutase (spot 15) in abundance were all down-regulated. It seemed that *P. aquilinum* senescence begins when the vegetable was harvested and subsequently stored. Furthermore, three proteins were associated with TCA cycle including isocitrate dehydrogenase (spot 7), malate dehydrogenase (spot 10) and ATP-Citrate synthase alpha chain protein 2-like (spot 12). The authors considered the down-regulation of these proteins in abundance due to the influence of upstream glycolysis. Three proteins were related to cell structure. Actin 2 (spot 14), an indispensable constituent of the cytoskeleton, played vital functions in cell division, intracellular transport, cell growth, cell to cell communication and organelle positioning. ADF (spot 1) was one of eukaryotic actins regulatory proteins, which accelerated the turnover rate of actins by depolymerizing actin monomers from the pointed ends and severing the filaments (Luo et al., 2012; Zhang et al., 2011). Actins monomers were dissociated and cytoskeleton integrity could be destroyed due to the up-regulation of the protein in abundance during storage. Prohibitins (spot 4) played a crucial role in mitochondrial biogenesis and protection against various stresses and senescence in plant cells (Ahn et al., 2006). Plants are generally subjected to biotic and abiotic stresses during their lifetime and correspondingly evolved a wide range of defense mechanisms to protect themselves. After harvest, *P. aquilinum* will suffer from the main stresses such as microorganism attack, water loss, lack of nutrients supply and mechanical damage. Water loss from plant cells causes a burst of oxidative stress, generating excessive ROS, which results in protein denaturation and lipid peroxidation. Five proteins which are connected with ROS metabolism, protein synthesis and protein fate. MDHAR (spot 3) could deacidity MDHA to ascorbic acid and have a positive effect in eliminating ROS and maintaining reduced power, however ROS producted may be accumulated due to downregulation of MDHAR. Structural damage, function loss and programmed cell death in plant cell may be caused by excessive ROS production (Liang et al. 2014). Glutamate dehydrogenase (spot 6, GDH) plays an important role in providing carbon skeletons for effective functioning of TCA cycle. Lightfoot reported transgenic plants expressing GDH gene are improved in tolerance to, water deficit and pathogen infections (Lightfoot et al., 2007; Pageau et al., 2006). The authors speculated that the up-regulation of GDH in abundance may be responsible for microorganism infection or other stresses during storage. Heat shock proteins function as protein and membrane stabilizers, are elicited by biotic and abiotic stresses (Qin et al., 2009) and also expressed during fruit ripening (Prinsi et al., 2011) and vegetable’s quality deterioration (Jiang et al., 2012). In general, principal Hsps are expressed in large quantities because they participate in facilitating the normal situation of the organism or ameliorate the problems of unsuitable folding and aggregation against biotic and abiotic stresses (Mohamed and Whaibi, 2011; Shi et al., 2014). To our surprise, variation trend of Hsp 101 in abundance was just the opposite of Hsp17.5. It is difficult for the authors to explain the difference. Elongation factor (spot 8 &11) was highly conserved among different species and may be involved in protein synthesis and aging (Riis et al., 1990). As shown in Table 1, the two proteins in abundance were steadily decreased, and the above results indicated protein synthesis could be retarded and the protein content may decrease during storage. The content of H$_2$O$_2$ and protein content were examined (Fig 5, Fig 6) , and the results validated above deduction. As discussed above, *P. aquilinum* inevitably encountered the cell structure disassembly and cell senescence after harvesting. ROS production increased and protein content decreased when the vegetable was harvested and stored. These results may jointly lead to quality deterioration of *P. aquilinum* during postharvest storage.

Materials and Methods

Plant material

*P. aquilinum* was harvested at vegetative stage from the “SanTai hill”, situated in Chizhou city, Anhui province, China and immediately transported to experimental laboratory. The individuals of the *P. aquilinum* (CK0) were selected for uniform size (20 cm in length) and color and the absence of visual defects. The stems of the vegetable were
Table 1. Identification of 17 differential proteins from *P. aquilinum* during postharvest storage.

<table>
<thead>
<tr>
<th>Spot no</th>
<th>Protein name</th>
<th>Accession no/species</th>
<th>Sequence coverage (%)</th>
<th>Matched peptide sequence</th>
<th>Theor/exp Mr(kDa)/pl</th>
<th>Protein subcellular localization</th>
<th>Score/threshold</th>
<th>Spot % volume variations (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Energy and amino acid metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>V-ATPase subunit A</td>
<td>gi</td>
<td>137460 Daucus carota</td>
<td>11</td>
<td>YSNSDTVVYVGCGER</td>
<td>69.1/70.1</td>
<td>cytoplasm</td>
<td>509/50</td>
</tr>
<tr>
<td>6</td>
<td>glutamate dehydrogenase</td>
<td>gi</td>
<td>4150965 Asparagus officinalis</td>
<td>8</td>
<td>FHGYSPAIVTGKPVDLGGLGR</td>
<td>44.5/44.9</td>
<td>mitochondrial</td>
<td>168/50</td>
</tr>
<tr>
<td>5</td>
<td>methionine synthase protein</td>
<td>gi</td>
<td>18483235 Sorghum bicolor</td>
<td>6</td>
<td>YGAGIGPQYDIHSPR</td>
<td>84.1/90.2</td>
<td>mitochondrial</td>
<td>371/48</td>
</tr>
<tr>
<td>3</td>
<td>cytosolic triosephosphate isomerase</td>
<td>gi</td>
<td>310768790 Pteris vittata</td>
<td>20</td>
<td>FFVGGNWK VACPGETLEQR</td>
<td>27.3/27.3</td>
<td>chloroplast</td>
<td>316/49</td>
</tr>
<tr>
<td>17</td>
<td>phosphoglucomutase family protein isoform 1</td>
<td>gi</td>
<td>508709149 Theobroma cacao</td>
<td>6</td>
<td>FLFEDGSR QHCHAT4YALSQGLK</td>
<td>63.5/70.9</td>
<td>chloroplast</td>
<td>235/49</td>
</tr>
<tr>
<td>15</td>
<td>2,3-bisphosphoglycerate-independent phosphoglycerate mutase 1</td>
<td>gi</td>
<td>18391066 Arabidopsis thaliana</td>
<td>11</td>
<td>ALEYEDDFKDR AVGPIVDGDAVTFNR</td>
<td>60.8/64.7</td>
<td>chloroplast</td>
<td>386/49</td>
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<tr>
<td>12</td>
<td>PREDICTED: ATP-Citrate synthase alpha chain protein 2-like</td>
<td>gi</td>
<td>357155751 Brachypodium distachyon</td>
<td>5</td>
<td>WGNVEFPLPFGR</td>
<td>47.1/49.4</td>
<td>mitochondrial</td>
<td>107/49</td>
</tr>
<tr>
<td>7</td>
<td>isocitrate dehydrogenase</td>
<td>gi</td>
<td>19171610 Cucus sativus</td>
<td>16</td>
<td>HAFGDQYR TIEAAADGTTR</td>
<td>46.4/47.4</td>
<td>mitochondrial</td>
<td>386/49</td>
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49
<table>
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<tr>
<th></th>
<th>Protein Name and Description</th>
<th>Accession Number</th>
<th>Alignment Score</th>
<th>Identity</th>
<th>Similarity</th>
<th>Organism</th>
<th>Structure</th>
<th>Datasets</th>
<th>Heatmap</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>malate dehydrogenase, cytoplasmic</td>
<td>gi</td>
<td>1133373* Medicago sativa</td>
<td>9</td>
<td>VLVTGAAGQIGYALVPMIAR</td>
<td>35.9/41.3</td>
<td>6.39/6.48</td>
<td>mitochondrial</td>
<td>129/49</td>
</tr>
<tr>
<td>14</td>
<td>Actin 2</td>
<td>gi</td>
<td>3746938* Anemia phyllitidis</td>
<td>25</td>
<td>GYSFTTAER SEYDESQPSIVHR IWHHTFYNELR SYELPGQVITIAGAR VAPEEHVLLTEAPLNPK TTGIVLSDGVTVHPYEGYALPHAILR</td>
<td>41.8/44.2</td>
<td>5.31/5.55</td>
<td>cytoplasm</td>
<td>574/49</td>
</tr>
<tr>
<td>1</td>
<td>PREDICTED: actin-depolymerizing factor 10-like (ADF)</td>
<td>gi</td>
<td>460406249* Solanum lycopersicum</td>
<td>8</td>
<td>IFFVAWSPETAR</td>
<td>49.0/14.6</td>
<td>6.57/5.72</td>
<td>cytoplasm</td>
<td>64/49</td>
</tr>
<tr>
<td>4</td>
<td>prohibitin</td>
<td>gi</td>
<td>384245685* Coccomyxa subellipsoidea</td>
<td>8</td>
<td>EFNILVDIAIHTLSFGTEFTK AKEFNILVDIAIHTLSFGTEFTK</td>
<td>31.0/32.1</td>
<td>9.39/7.27</td>
<td>mitochondrial</td>
<td>177/49</td>
</tr>
<tr>
<td>13</td>
<td>monodehydroascorbate reductase</td>
<td>gi</td>
<td>113472842* Rheum australe</td>
<td>8</td>
<td>AYLFPegaar LPGFHTCgsgger</td>
<td>47.4/45.6</td>
<td>5.63/5.95</td>
<td>cytoplasm</td>
<td>180/49</td>
</tr>
<tr>
<td>2</td>
<td>heat shock protein 17.5 (sHsp 17.5)</td>
<td>gi</td>
<td>186694325* Malus domestica</td>
<td>22</td>
<td>IDQVKAAMENGVLTVPK</td>
<td>17.5/13.4</td>
<td>6.20/6.42</td>
<td>cytoplasm</td>
<td>188/49</td>
</tr>
<tr>
<td>9</td>
<td>101kDa heat shock protein (Hsp101)</td>
<td>gi</td>
<td>11561808* Triticum aestivum</td>
<td>10</td>
<td>RPYSVLFDEVek AIAEIQLFDDENLLVR VILFIDEHLVGLGAGR FQQVYVVAESVADTISLR</td>
<td>101.3/115.0</td>
<td>5.84/6.70</td>
<td>cytoplasm</td>
<td>402/49</td>
</tr>
<tr>
<td>11</td>
<td>elongation factor 1-gamma</td>
<td>gi</td>
<td>351722398* Glycine max</td>
<td>4</td>
<td>VPVLETPDGPIFESNAIAR</td>
<td>47.9/55.5</td>
<td>6.30/6.05</td>
<td>cytoplasm</td>
<td>150/50</td>
</tr>
</tbody>
</table>
Fig 1. Identification of seventeen protein spots was performed by 2-DE and MALDI-TOF-TOF analysis. The numbers with arrows indicated the differential expressed protein spots.
Fig 2. Representative 2-DE maps of *P. aquilinum* at different storage time.

Fig 3. Magnified views of some differential proteins marked in Fig. 1. Arrows and numbers indicated the differential spots at different storage time.

Fig 4. The categorization of metabolism pathways of identified proteins according to their putative functions. The percentage and the number of protein of each group were indicated.
protein content was determined according to Bradford method (Bradford, 1976).

2-DE and staining

Protein extracts (1.2mg) were subjected to 17cm pH 5-8 IPG strips (Bio-Rad). The iso-electric focusing was performed on a PROTEAN IEF system (Bio-Rad) at 50v for 13h, at 100v, 200v, 500v and 1000v for 1h, respectively and at 4000v, 8000v in linear for 2h, respectively and then run at 8000v to until 70 kVh at 20°C. After isoelectric focusing, the strips were equilibrated by gentle stirring for 15 min with 2% dithiothreitol in equilibration buffer (50mM Tris-HCl, pH8.8, 6M urea, 20% glycerol, and 2% SDS). A second equilibration was carried out for 15 min in the same equilibration buffer containing 2.5% iodoacetamide. Following equilibration, the strips were run in 12% polyacrylamide gels using the Ettan DALT Twelve vertical set (GE Healthcare) for 1 h at 1 W/gel, followed by 15w/gel until the bromophenol blue reached the gel bottom. After SDS-PAGE, gels were stained with 0.12% colloidal Cooamassie brilliant blue G250 overnight.

Image analysis and data

The gels were scanned at 300 dpi with a Versdoc 3000 scanner (Bio-Rad) and image analysis was performed using PDQuest analysis software. The protein spots were checked manually and added or deleted if necessary. Protein spots were considered valid according to their presence in at least three spots of 2-DE gels for each sample. Protein spot intensities were normalized as a percentage of the total volume in all the spots present in the whole gel. Protein spots with at least 2-fold increase/decrease and statistically significant (Student’s t-test, p<0.05) of were considered as differential proteins.

Protein identification

For MALDI-TOF/TOF analysis, protein spots showing changes statistically significant (p<0.05) and a 2-fold threshold were excised from the gels and digested by addition of trypsin (Promega, USA). The resulting peptides were analyzed by a 4800 MALDI-TOF/TOF proteomic analyzer (Applied Biosystems, USA). Proteins were identified by searching based on the NCBInr (non-redundant) database of plants using the MASCOT search engine v3.5 (Matrix Science Ltd, London). Only the best matches with higher score were accepted when the result showed more than one eligible result.

The determination of H2O2 content

Two gramme of bracken stem was homogenized with 6 mL of chilled 100% acetone and then centrifuged at 10000 g for 20 min at 4°C. The supernatant was collected immediately for H2O2 analysis according to the method of Patterson (Patterson et al., 1984). H2O2 content was expressed as μmol /gFW.

Conclusions

Proteome analysis had provided extensive information for understanding the biological mechanisms of quality deterioration after harvesting: (1) The decline of antioxidant capacity would be responsible for accumulation of ROS, and excessive ROS leads to cell oxidation and senescence (2) P. aquilinum senescence begins and protein content declines when the vegetable is harvested and stored for a few days (3) Cell structure integrity could be destroyed during postharvest storage. For better understanding the above results, molecular biological analysis and other sub-cellular proteomic studies should be performed in the future.

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References


