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Comprehensive proteome analysis of lettuce latex using multidimensional protein-identification technology

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ABSTRACT

Commercially, lettuce (*Lactuca sativa*) is one of the most important leafy vegetables. Lettuce produces a milky latex of variable chemical compositions within its laticifers. As a step toward understanding the main physiological roles of this latex in higher plants, we embarked on its proteomic analysis. We investigated 587 latex proteins that were identified from the lettuce latex using multidimensional protein-identification technology. A bioinformatics analysis showed that the most frequently encountered proteins in the latex were organellar proteins from plastids and mitochondria, followed by nucleic and cytoplasmic proteins. Functional classification of the identified proteins showed that proteins related to metabolism, cell rescue, defense, and virulence were the most abundant in lettuce latex. Furthermore, numerous resistance proteins of lettuce and viral proteins were present in the latex suggesting for the first time a possible function of the lettuce latex in defense or pathogenesis. To the knowledge of the authors, this is the first large-scale proteome analysis of lettuce latex.

1. Introduction

Commercially, lettuce (*Lactuca sativa*) is one of the most important leafy vegetables. It is the most popular salad vegetable in the world, which is consumed mostly as a fresh product. Lettuce belongs to the family *Asteraceae* and contains nine pairs of chromosomes. Lettuce secretes a colored juice that is often milky called latex. The latex accumulates in specialized continuous tubes called laticifers. Laticifers are specialized cells that can be found in more than 20 plant families including the *Asteraceae* family, the poppy family, Greater Celandine, and rubber trees, among others (Pickard, 2008). Laticifers seem to have polyphyletic origins. Therefore, their classification is based on morphological characteristics and developmental patterns. The primary role of laticifers is to provide a place to deposit numerous metabolites which might be synthesized from several cellular compartments, such as plastids and mitochondria. Although all latex-containing plants produce such kinds of metabolites, increasing studies have demonstrated that the latex in the laticifer is important for defense mechanism against insects. Furthermore, in a recent review paper it was described very well that laticifers might act as a putative storage of excess atmospheric carbon and that they can regulate levels of atmospheric isoprene gas (Hagel et al., 2008). Many of the other latex-containing plants are important to the global economy because poppy is a source of opium and the rubber tree is well known as being a source of natural rubber (Chow et al., 2007; Decker et al., 2000; Nawrot et al., 2007). Furthermore, lettuce latex contains several sesquiterpenoid lactones, phytoalexins lettucenin A and costunolide, which are all directly related to the bitter taste of lettuce (Sessa et al., 2000).

Recently, proteomics has developed into an efficient tool to study the abundance and distribution of proteins in an organism (Chen and Harmon, 2006). In addition, the expression profiles of different tissues have been analyzed and the identification and localization of individual proteins of interest have been accomplished using proteomic approaches. Thus far, two-dimensional gel electrophoresis (2-DE) has been routinely used for protein identification in numerous studies. However, low-abundance proteins and basic proteins normally present in plant tissues cannot be easily separated using the 2-DE approach. A recently developed multidimensional protein-identification technology (MudPIT) is unbiased and is an alternative high-throughput technology that permits the identification of a large number of proteins (Li and Assmann, 2000; Rose et al., 2004; Washburn et al., 2001).

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Recently, several proteomic studies of proteins found in phloem and xylem saps of various plant species have been published. These studies underline the physiological significance of proteomes in plant vascular systems (Aki et al., 2008; Alvarez et al., 2006; Buhtz et al., 2004; Djordjevic et al., 2007; Giavalisco et al., 2006; Kehr et al., 2005). However, only a few proteomic analyses have been carried out on the components of latex in a limited number of plants (including *Papaver somniferum* and *Hevea brasiliensis*) (Chow et al., 2007; Decker et al., 2000; Nessler et al., 1990). This lack of data arises from limited available information regarding the genomic sequence of latex-containing plants. The sticky and viscous nature of latex (arising from oxygen exposure of unidentified chemical components of the latex) makes its study more difficult.

To achieve a detailed understanding of the latex proteome, MudPIT has been used to identify proteins present in lettuce latex. The spectra from lettuce latex obtained using liquid chromatography/tandem mass spectrometry (LC–MS/MS) have been compared against a protein database constructed from the expressed sequence tag (EST) database of lettuce established in the protein databases of the University of California (Davis) (http://cgpdb.ucdavis.edu/database/Database_Description.html) and the National Center for Biotechnology Information (NCBI). In sum, 587 lettuce latex proteins have been identified including a major portion of plastidial and mitochondrial proteins, metabolic pathway proteins, and viral or plant pathogen-related proteins.

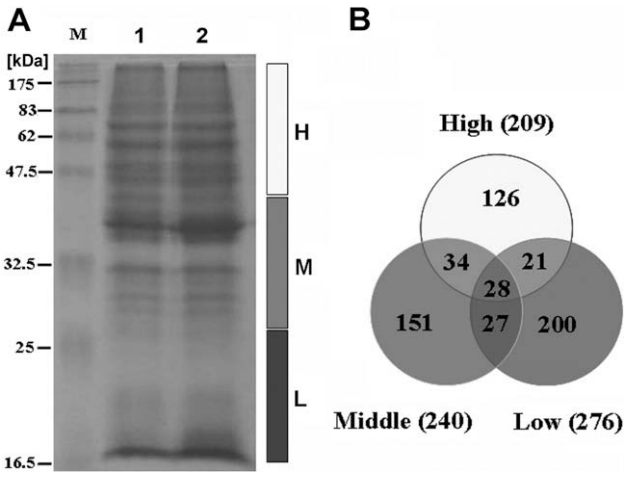

Fig. 1. SDS–PAGE of latex proteins from lettuce (*Lactuca sativa*). (A) SDS–PAGE analysis of the latex proteins from lettuce. Lanes: molecular marker, 1 (first experiment), 2 (second experiment); the molecular masses are indicated on the left. The one-dimensional gel of the latex proteins was excised into three sections; H (high), M (middle), and L (low). (B) Venn diagram of proteins identified in each gel section. A total of 587 proteins were identified by MudPIT analysis, and 138 proteins were found in at least two gel sections. Among them, 28 proteins were detected in all gel sections.

2. Results and discussion

2.1. Protein identification by MS/MS analysis

Protein identification inside lettuce latex by MS/MS analysis is limited due to its lack of genome-sequence information. Because protein identification relies exclusively on protein homology to other plant species it does not always give reliable data (Habermann et al., 2004). However, several previous studies have successfully applied the EST database of a plant species to protein identification by MS/MS analysis (Kim et al., 2003; Sheoran et al., 2007). In lettuce, more than 68,000 ESTs from various tissues (under The Compositae Genome Project) have been constructed from two distinct lettuce genotypes: cultivated lettuce (*Lactuca sativa L. cv. Salinas*) and wild lettuce (*Lactuca serriola L.*).

The isolated lettuce latex proteins were separated by electrophoresis using one-dimensional gel electrophoresis (1-DE) (Fig. 1A). Proteins on gels were stained for 30 min in Coomassie brilliant blue (CBB)-staining buffer. To enrich the number of proteins identified, the 1-DE gel was separated into high, middle, and low sections and then analyzed using the LC–MS/MS technique (Fig. 1A). MS/MS spectra obtained from the three gel sections were used to search for sequence similarity within a protein database constructed from the lettuce EST database and the lettuce protein database obtained from the NCBI. In total, 725 lettuce latex proteins from the three gel sections were identified in the lettuce EST database of the Compositae Genome Project Database (CGPDB) (596 proteins and 1,013 peptides) and the NCBI (129 proteins and 196 peptides) (Table S1 and Fig. 1B). After eliminating redundant proteins from the three gel sections, integration of all datasets indicated that 587 unique proteins (704 peptides) were lettuce latex proteins (Table S2). Interestingly, when the protein lists from the two different databases were compared, only one protein (deoxyhypusine synthase) was found to be common among them. This enzyme is involved in the posttranslational activation of the eukaryotic initiation factor 5A (eIF5A) (Ober and Hartmann, 1999). This result suggests that use of the lettuce EST database is a successful method for lettuce proteome analysis. In addition, it proves the effectiveness of using multiple databases for protein identification by MS/MS analysis.

The identified proteins had molecular masses ranging from 4.7 to 367 kDa (Table S2). Interestingly, 459 proteins (78%) had molecular masses between 20 and 30 kDa, indicating the presence of a large number of small molecules in lettuce latex. Organelles include several enzymes that permit them to synthesize many types of small molecules. Therefore, a large number of organellar proteins, such as plastidial and mitochondrial proteins, were detected in our analysis. The previously described latex proteome of the opium poppy had shown that the major latex proteins (comprising 50% of the latex proteins) had molecular masses of about 20 kDa (Decker et al., 2000; Nessler et al., 1990).

The isoelectric points (p*I*s) of identified latex proteins varied from 5.8 to 13 (Table S2). Compared to the number of acidic proteins (p*I* < 6), the number of basic proteins (p*I* > 8) was much greater and constituted 72% of the total proteins. Latex proteome from opium poppy contained either mostly acidic or neutral proteins. Specifically, only five proteins among the 98 proteins identified were basic (with p*I*s in the range of 8–9) in the opium study (Decker et al., 2000; Nessler et al., 1990). This result dramatically contrasts with the current data. The p*I* distribution in the lettuce proteome showed a normalized pattern within the p*I* range of 4–13 with a peak at the basic p*I* of 9–10. This result clearly shows the advantage of MudPIT compared to the classical 2D-gel analysis in the detection of basic proteins.

2.2. Functional classification of lettuce latex proteins

To categorize the latex proteins into functional classes, all latex proteins were converted into their respective *Arabidopsis* gene-identification (AGI) numbers derived from the *Arabidopsis* information resource (http://www.arabidopsis.org/) by sequence similarity (Table S2). The identified lettuce latex proteins were classified into functional classes using FunCat (Ruepp et al., 2004). More than half of the identified proteins consisted of five major protein classes: metabolism-related proteins (20%), cell rescue, defense, and virulence proteins (12%), proteins with binding function (10%), cellular transport proteins (8%), and proteins that control protein fate (8%).