African Journal of Biotechnology Vol. 10(74), pp. 17023-17029, 23 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1398
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Two-dimensional gel electrophoresis analysis of different parts of *Panax quinquefolius* L. root

Liwei Sun[1]*, Xiujuan Lei[1,2], Rui Ma[1,3], Rui Jiang[1,3], Daqing Zhao[3] and Yingping Wang[2]

[1]Life Science Research Center, Beihua University, Jilin 132013, P. R. China.
[2]Institute of Special Wild Economic Animal and Plant Science, CAAS, Jilin 132109, P. R. China.
[3]Changchun University of Chinese Medicine, Jilin 130117, P. R. China.

Accepted 19 September, 2011

Effective identification of traditional medicine is essential for the development of medical industry. Identification in different parts (rhizome head, lateral root, main root and skin) of *Panax quinquefolius* L. root (American ginseng) is limited by the lack of efficient differentiation methods. In this study, proteomic technologies were used to establish an advanced protocol suitable for the identification of different parts of *P. quinquefolius* L. root. Proteins were extracted from four different parts of a 4-year old *quinquefolius* L. root using optimized sonication and trichloroacetic acid [TCA]/acetone precipitation methods and separated by two-dimensional gel electrophoresis (2-DE). Then 2-DE patterns were matched and analyzed with Image Master 2D Platinum Version 6.0 software. Eight groups of different abundant proteins and 6 specific proteins were inspected (totaling 38 protein spots in all). Further, these proteins were extensively identified by MALDI-TOF-TOF analysis. According to the biological functions, a total of 24 successfully identified proteins could be divided into 5 groups, which were stress response related proteins, energy metabolism related proteins, storage related proteins, hypothesis proteins and unknown proteins. From these results it was concluded that proteomic analysis method was an effective way to identify the different parts of *quinquefolius* L. root. These findings may contribute to further understanding of the physiological mechanisms of *quinquefolius* L.

Key words: Panax quinquefolius L. root, different parts, two-dimensional gel electrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

INTRODUCTION

Panax quinquefolius L. (American ginseng) is a well-known herbal medicine that belongs to the family *Araliaceae*. Previous research has indicated that quinquefolius L. root, which was the mainly used body in traditional Chinese medicine can be divided into four parts (rhizome, lateral root, main root and skin) (Lum et al., 2002) and there were different ginsenosides in these parts which were thought to be the major effective ingredients (Zhang et al., 2008; Qu et al., 2009; Wang et al., 2009a). However, up to now, as a deficiency of effective quality evaluation standard system of *quinquefolius* L., it is still difficult to identify the different parts of an organism that possess the identical genetic type. This seriously influenced the development of quinquefolius L. industry and the exploitation of series products. Hence, how to improve the internal quality evaluation criteria of quinquefolius L. using modern biological technology has become one of the most important problems that need to be resolved.

Proteins are the ultimate expression products of genes. They can clearly reflect the gene expression changes in organisms at protein molecule level. In recent years, following the development of plant proteome and the

*Corresponding author. E-mail: sunliwei1970@yahoo.com. Tel: +86 136 6432 4368.

Abbreviations: 2-DE, Two-dimensional gel electrophoresis; **BSA,** bovine serum albumin; **CBB,** Coomassie brilliant blue; **CHAPS,** **3**-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; **DTT, d-**thiothreitol; **IEF,** isoelectric focusing; **IPG,** immobilized **pH** gradient; **Mw,** molecular weight; **pI,** isoelectric point; **PMSF,** phenylmethanesulfonyl fluoride; **SDS,** sodium dodecyl sulfate; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis; **TCA,** trichloroacetic acid.

improvement of plant genome databases, it is becoming a necessary and efficient way using proteomic analysis to study the dynamic expression of genes and to further explore the scientific identification method of different parts of *quinquefolius* L. root. 2-DE patterns of different parts of *Panax ginseng C. A. Meyer* (Oriental ginseng) and *Panax quinquefolius* L. (American ginseng) main root were first established using 2-DE technology in 2002 (Lum et al., 2002), in which direct extraction method for sample preparations was utilized. Their results showed that proteomes of different ginseng parts were different and could be used to differentiate them. However, despite that the 2-DE pattern of *quinquefolius* L. main root was established, data is still rare concerning all parts of the root. Moreover, because of the existence of different types and contents of interfering substances in every quinquefolius L. root, it is difficult to obtain high-quality proteins from all parts using direct extraction method (as in former report). Therefore, in order to obtain more accurate and comprehensive experimental data, it is necessary to establish an efficient protein extraction method to find specific proteins from different parts which are used as markers to identify the different parts of quinquefolius L. root.

In this study, based on the fact that *quinquefolius* L. root contains many interfering substances such as polysaccharides, polyphenols, ginsenosides, nucleic acid and so on, it was established that ultrasound combined with TCA/acetone precipitation method could be adopted firstly to acquire high-quality protein samples, followed by the different 2-DE patterns of rhizome, lateral root, main root and skin of *quinquefolius* L. root. Then 2-DE patterns of the four parts were analyzed and compared using Image Master 2D Platinum Version 6.0 software and some characteristic spots in four parts were sought out. In addition, some different expression spots were also found. Finally, these characteristic and different expression spots were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF-TOF) and also an initial research on the classification of the identified proteins was carried out.

MATERIALS AND METHODS

Fresh 4-year old quinquefolius L. roots were purchased in Fusong and Jian, Jilin province. All roots obtained were first mixed together, then each root was dissected into four parts (lateral root, rhizome head, main root and skin) (Lum et al., 2002), washed and then transferred to a mortar. Liquid nitrogen was added immediately and then ground to fine powder using a pestle. The powder was stored at -80°C till protein extraction.

Protein extraction and quantification

1 g of sample powder was suspended in 7 ml ice-cold acetone, containing 10% TCA (w/v) and 0.07% β-mercaptoethanol, and incubated at -20°C for 2 h. Then mixture was centrifuged at 15000 rpm, 4°C for 15 min. The pellet was washed with 7 ml of ice-cold acetone containing 0.07% β-mercaptoethanol and then centrifuged at 15000 rpm, 4°C for 15 min. The washing procedure was repeated twice, after which the final pellet was air-dried at 4°C and redissolved in rehydration buffer (7 M urea, 2 M thiourea, 2% 3-[(3-Cholamidopropyl) dimethylammonio] propanesulfonic acid [CHAPS], 1% phenylmethanesulfonyl fluoride [PMSF] and 1% protein inhibitors). After sonication for 40 min, the mixture was centrifuged at 15000 rpm, 4°C for 10 min, and protein supernatant was transferred to a new tube.

Protein was quantified by Bradford protein assay, with bovine serum albumin (BSA) as a standard. Total protein yield was calculated according to the absorbance at 595 nm (Bradford, 1976). The protein yield was expressed as microgram per gram fresh sample tissue weight (mg g^{-1} fresh wt) and presented as mean ± SD.

Two-dimensional electrophoresis and image analysis

For first-dimensional electrophoresis isoelectric focusing [IEF], the protein supernatant (containing 250 µg of protein per 125 µL) was subsequently loaded onto an immobilized pH gradient (IPG) strip holder and the 7 cm pH 3-10 linear gradient IPG strip (GE Healthcare, USA) was placed onto the holder and then rehydrated for 12 h at 20°C. IEF was carried out on Ettan IPGPhor (Amersham Biosciences, USA) system at 50 µA per strip and focusing was performed under the following conditions: a rapid gradient increase from 30 to 100 V for 1 h, 100 to 500 V for 1 h and then a linear increase from 500 to 1000 V for 2 h, 5000 V for 4000 V.h, and at last a rapid gradient of 5000 V until 6000 V.h. After IEF, the IPG strip was immediately equilibrated for 15 min with an equilibration buffer (75 mM Tris-HCl, pH 8.8, 6 M urea, 2 M thiourea, 30% glycerol, 2% sodium dodecyl sulfate [SDS], 0.002% bromophenol blue, 1% w/v dithiothreitol [DTT] followed for 15 min with the same buffer but containing 2.5% iodoacetamide replaced with 1% DTT. The second dimension sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] was performed on a 10 × 11 cm, 12.5% linear gel in a Hofer MiniVE (Amersham Biosciences, USA) gel system at a current of 5 mA per gel for 15 min and 10 mA for 3 h.

After 2-DE, gel was stained with Coomassie brilliant blue [CBB] R-250. Gel image was digitalized with Image scanner 6.0 (Amersham Biosciences, USA) and analyzed with the Image Master 2D Platinum 6.0 software (Amersham Biosciences, USA) using a semiautomatic method, with manual correction and edition of spot features created by automatic default spot analysis settings. Total numbers of protein spots were determined. Special and differently-expressed protein spots were also detected in four samples.

Protein identification by MALDI-TOF-TOF

Proteins were identified according to the methods described by Maldonado and colleagues (Maldonado et al., 2008).

RESULTS AND DISCUSSION

Optimization of protein extraction

Protein extraction is a crucial step for 2-DE analysis (Yao et al., 2006). As a multi-pharmacological traditional medicine, quinquefolius L. root is rich in secondary metabolites such as saponins, polysaccharides, volatile oil and so on. Meanwhile, as a storage organ of this plant, its cells not only have thick cell walls, but also

Table 1. Protein yield and spots number in different parts of *quinquefolius* L. root

Part	Protein yield† (mg g^{-1} fresh wt)	Spots number†
Lateral root	5.64 ± 0.07^{ns}	353 ± 4.9^{ns}
Rhizome head	5.72 ± 0.07^{ns}	398 ± 4.2^{ns}
Main root	4.96 ± 0.04^{ns}	376 ± 0.7^{ns}
Skin	1.96 ± 0.03**	214 ± 6.4**

†Mean ± SD (n=3); ‡ ns, non significant at the 0.05 probability level; **significant at the 0.01 probability level.

contain certain amount of starch, polyphenols, nucleic acids and pigments etc (Jhang et al., 1974). All of these therefore made it difficult to obtain high-quality total proteins if only conventional protein extraction methods were used.

In this study, sonication combined with TCA/acetone precipitation was used to extract protein samples. Since ultrasound is a powerful wave, it can produce shear stress which may break cell wall completely and mince the nucleic acids, polysaccharides and other interfering substances into small fragments, while the optimized TCA/acetone precipitation method could further remove some secondary metabolites and also decrease the protein degradation (Sheoran et al., 2009; Wang et al., 2009b; Zhang et al., 2009). By using them jointly, high-quality protein samples and 2-DE patterns could be achieved. In order to reduce protein degradation, researches on the suitable power and time of ultrasonic treatment were carried out. The data showed that 200 W with 40 min ultrasonic treatment can lead to the highest total protein yield (data not shown).

Comparison of protein yields in different parts of quinquefolius L. root

In this study, Bradford assay was used to determine protein concentration of the different parts of *quinquefolius*. L root, and then protein yields were calculated and compared. The data show that every part resulted in different protein yields (Table 1). Protein content in rhizome head was the highest, estimated to be at 5.72 ± 0.07 mg protein/g fresh rhizome head, followed by lateral root (5.64 ± 0.07 mg protein/g fresh lateral root), main root (4.96 ± 0.04 mg protein/g fresh main root). Skin protein yield was the lowest (1.96 ± 0.03 mg protein/g fresh skin), which was only 34.2% of rhizome head. However, statistical analysis showed that protein yields among the first three had no significant differences ($P>0.05$), but they all had significant differences with skin protein yield ($P<0.01$).

Comparative analysis of the 2-DE patterns in different parts of quinquefolius L. root

Proteins extracted from different parts were analyzed by using 3 to 10 linear IPG strips for the first-dimensional IEF and 12.5% polyacrylamide gels for SDS-PAGE. In this work, one of our objectives was establishing 2-DE patterns, which could be used for searching the most apparent and unique protein spots of each part easily, therefore 7 cm IPG strips was chosen for analysis. The 2-DE patterns of each part were established after being repeated thrice (Figure 1). The results of 2-DE showed that the majority of protein spots in four parts were all focused in the molecular weight [Mw] (15 to 100 kDa), isoelectric point [p*I*] (4.0 to 7.0) range.

2-DE patterns of protein from four parts were matched using Image Master 2D Platinum Software Version 6.0, and data for total number of protein spots resolved in 2-DE in different parts are presented in Table 1. In the four parts, the largest number of total protein spots (398 ± 4.2) was obtained in rhizome head, followed by main root (376 ± 0.7), lateral root (353 ± 4.9) and skin (214 ± 6.4). However, there were no significant differences in total spots number among the first three, although they all resulted in 64.9% more protein spots than skin. With the software analysis and manually editing, 8 groups of proteins were further filtered out that were differentially expressed and 6 specific proteins in four parts (Figures 2 and 3). As shown in the figure, S1 to S8 were 8 groups of proteins that were differentially expressed in four parts in Mw (10 to 25 kDa), pI (4.0 to 7.1) region (Figure 2). Using the 2-DE pattern of lateral root as a reference, the expression abundance in rhizome head, main root and skin changed following this order: S1,S2 down, down, up; S3 up, down, up; S4 down, down, up; S5 unchanged, unchanged, up; S6 up, up, down; S7,S8 up, unchanged, down. Furthermore, S9 to S14 were 6 specific proteins detected in 2-DE patterns of four parts. Among them, S9 (Mw 20 kDa, pI 9.71) was the only spot existing in lateral root, S10 (Mw 20.8 kDa, pI 5.45) was the only spot existing in rhizome head, while S11 to S14 were the only 4 spots found in main root, and their Mw, pI were 24 kDa, 9.5; 23 kDa, 9.5; 18 kDa, 9.5 and 18 kDa, 5.8.

Protein identification

Identification of the different protein spots (8 groups of differently expressed proteins and 6 specific proteins, totaling 38 protein spots) in four parts were carried out by

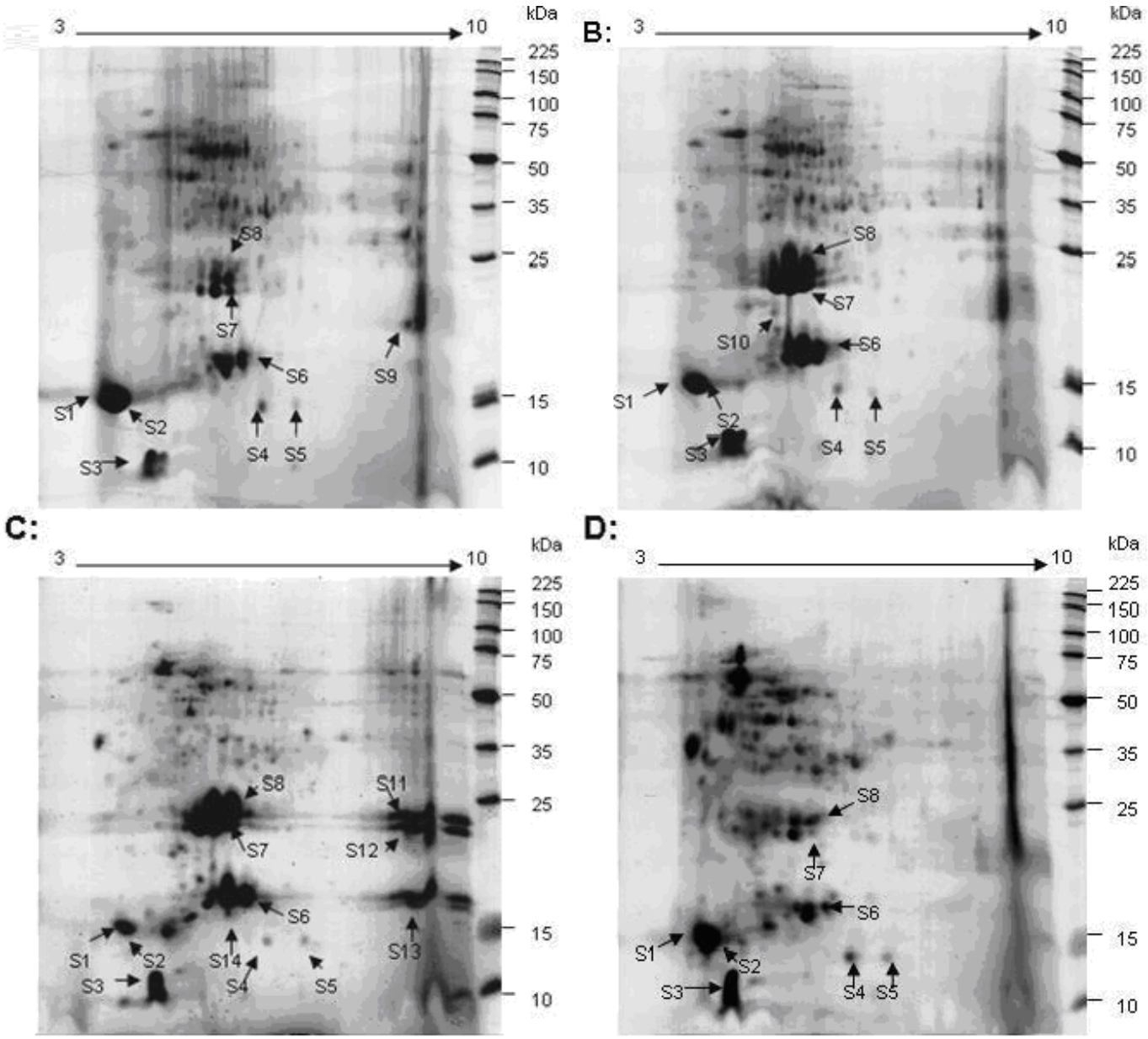

Figure 1. Two dimensional gel electrophoresis protein patterns in different parts of *quinquefolius L.* (A) Lateral root; (B) rhizome head; (C) main root; (D) skin. Proteins (250 µg) were separated in the first isoelectric focusing 3 to 10 pH gradient strips, then in the second dimension using 12.5% polyacrylamide gel electrophoresis. The gels were stained with coomassie brilliant ble R – 250.

MALDI-TOF-TOF. To ensure the probability and accuracy of protein identification, NCBInr database and EST database were all used for identifying proteins by MASCOT (Kim et al., 2003; Nam et al., 2003). A total of 24 protein spots were matched with the correct proteins using NCBInr database. Furthermore, 2 protein spots were matched with the peptide information using EST database of ginseng. In all, the identification rate of protein was 68.4%. The protein identification results are summarized in Tables 2 and 3.

In accordance with their biological functions, the identified proteins can be divided into five different groups (Bevan et al., 1998) (Figure 4), which are stress response related protein group (S1, S2), energy metabolism related protein group (S4, S5), storage related protein group (S7, S10, S12, S13, S14), hypothesis protein group (S9) and unknown protein group (S11). A large proportion (45.4%) of the identified proteins was involved in storage related protein group. These results indicate that *quinquefolius* L. root was the main organ for storing nutrients for plant.

Proteins identification by MS relies on genome sequence information (Nam et al., 2005). Since the full genome of quinquefolius L. has not been sequenced, although, total of 26,000 P. *ginseng* EST sequences are

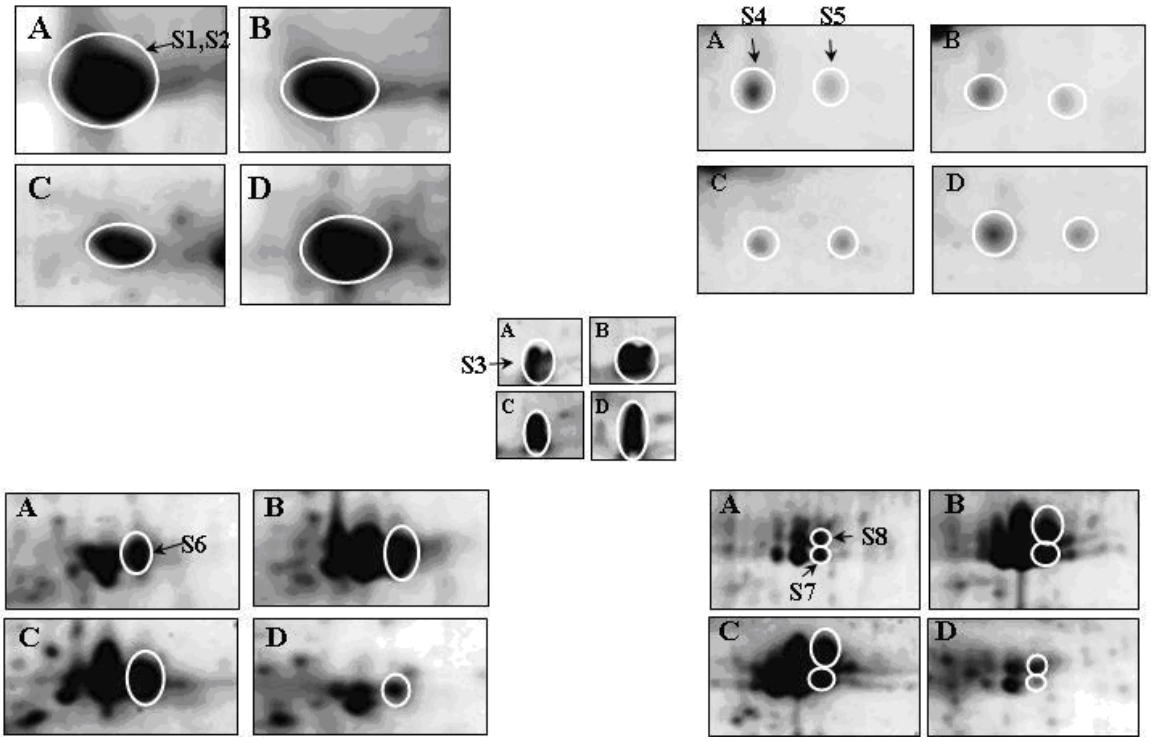

Figure 2. Two-dimensional gel electrophoresis patterns of different abundant protein spots in different parts of *quinquefolius L*. (A) Lateral root; (B) rhizome head; (C) main root; (D) skin.

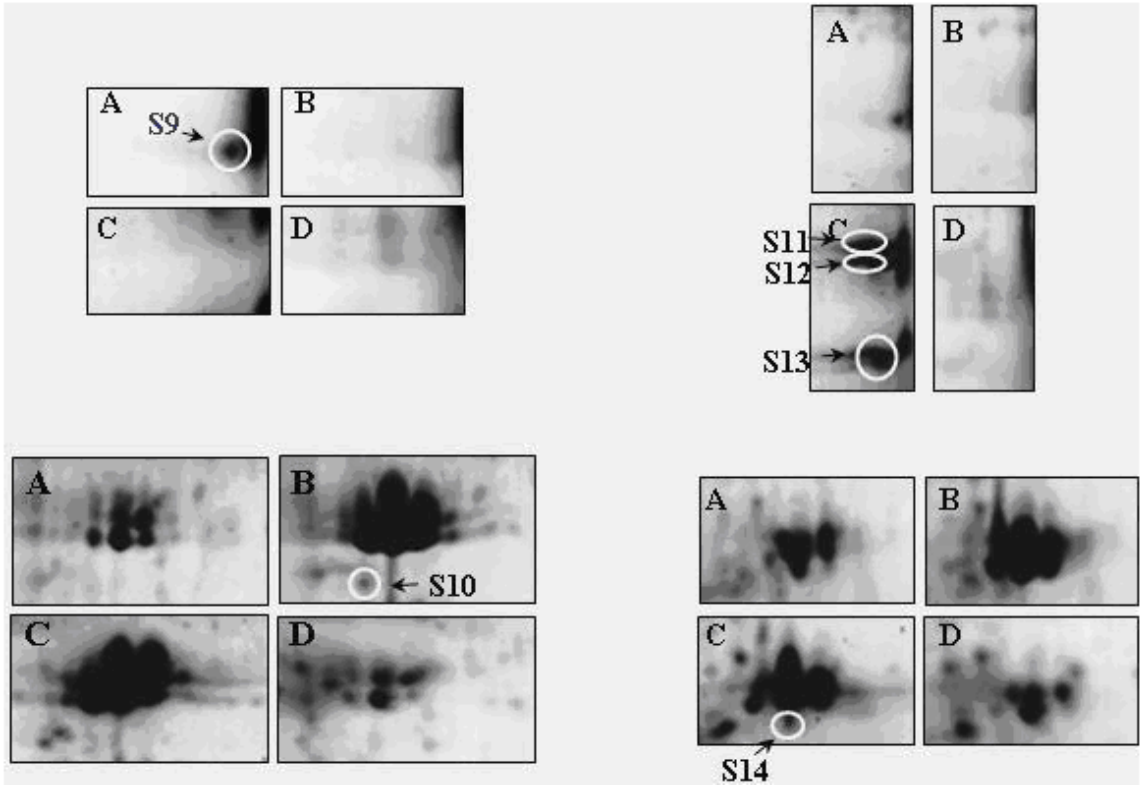

Figure 3. Two-dimensional gel electrophoresis patterns of specific protein spots in different parts of *quinquefolius L*. (A) Lateral root; (B) Rhizome head; (C) main root; (D) skin.

Table 2. Protein identification through MALDI-TOF-TOF and NCBInr database.

Spot	Molecular weight (Mw) /Isoelectric point (p*I*) (Predicted)/(Observed)	Score	Sequence coverage (%)	Accession number (NCBInr)	Species	Protein name
S1	(16.4/4.41)/ (16.5/4.37)	350	75.2	P80889	*Panax ginseng*	Ribonuclease1
S2	(16.5/4.56)/ (16.7/4.51)	259	68	P80890	*Panax ginseng*	Ribonuclease2
S4	(16.2/6.32)/ (15.9/6.41)	259	23.6	AAZ85394	*Solanum lycopersicum*	Cytosolic nucleoside diphosphate kinase
S5	(15.7/7.04)/ (15.7/7.07)	178	239	CAA53073	*Solanum lycopersicum*	Nucleoside diphosphate kinase
S7	(27.3/5.87)/(24.7/6.0)	164	29.8	P83618	*Panax ginseng*	Ribonuclease-like storage protein
S10	(27.3/5.87)/(20.8/5.45)	80	29.4	P83618	*Panax ginseng*	Ribonuclease-like storage protein
S12	(27.3/5.87)/(23.7/9.81)	151	34.9	P83618	*Panax ginseng*	Ribonuclease-like storage protein
S13	(27.3/5.87)/(18.2/9.89)	104	29.4	P83618	*Panax ginseng*	Ribonuclease-like storage protein
S14	(27.3/5.87)/(17.6/5.87)	117	22.3	P83618	*Panax ginseng*	Ribonuclease-like storage protein

Table 3. Protein identification through MALDI-TOF-TOF and EST database.

Spot	Peptide tag	Score	Database	Accession number (EST)	Protein name
S9	NNCPYTVWAAASPGGGR TNCFDGSGR	81 72	EST	ES554665	Predicted protein
S11	CSEALLKQTDYFR	72	EST	EW712053	Unnamed protein

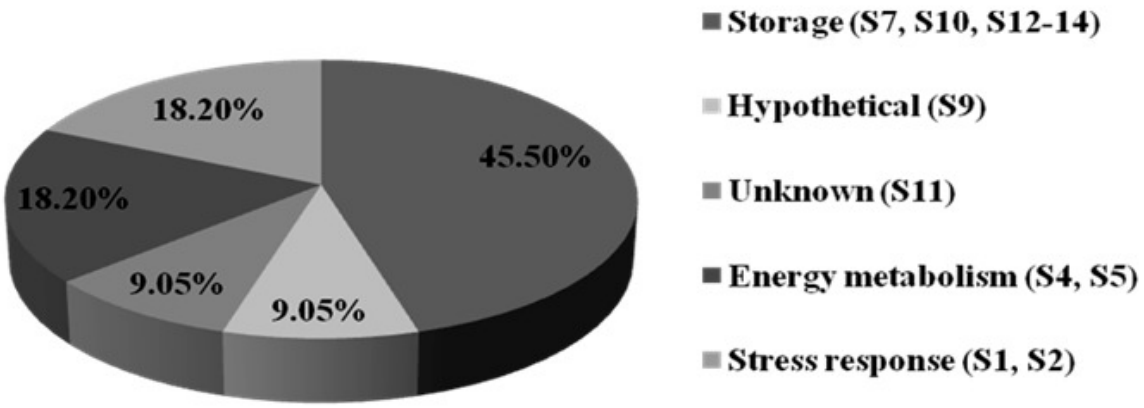

Figure 4. Classification of the identified proteins.

available at http://plant.pdrc.re.kr:7777/index.html, it is still difficult to use these related databases for the identification of some specific proteins, especially the proteins with low or high pI and Mw in quinquefolius L. root. Comprehensive identification of proteins in every part is however in progress.

Conclusion

Quinquefolius L. root is a widely used traditional medicine. There are different kinds of ginsenosides and pharmacological effects in different parts, so it is necessary to establish an effective identification method

of *quinquefolius* L. root. It is an organ rich in variety of compounds influencing protein extraction and resolution of 2-DE. In this case, a combination of optimal sonication and TCA/acetone precipitation methods was used to extract proteins and obtain 2-DE patterns. Our results showed that although protein yields or 2-DE patterns had differences in four parts of *quinquefolius* L. root, 8 groups of different abundant proteins and 6 specific proteins were searched out (a total of 38 protein spots) and identified. Using these proteins as characteristic markers therefore, every part of *quinquefolius* L. root could be easily distinguished. Proteomic analysis may contribute to further understanding of the physiological mechanisms of *quinquefolius* L. It is expected that this work can also provide methodology to the proteomics and identification study of other traditional medicine species.

ACKNOWLEDGEMENTS

This work was finally supported by National Key Technology R&G Program (No.2007BAI38B02), National Natural Science Foundation of China (nsfc81041091), Jinlin Provincial Scientific and Technologic Development Project (No.20096042), and the Foundation for the Development of Science and Technology of Jinlin City (No.200813).

REFERENCES

Ana Maldonado M, Sira Echevarría-Zomeño, Sylvia Jean-Baptiste, Martha Hernández, Jesús Jorrín-Novo V (2008). Evaluation of three different protocols of protein extraction for Arabidopsis thaliana leaf proteome analysis by two dimensional electrophoresis. J. Proteomics. 71(4): 461-472.

Bevan M, Bancroft I, Bent E, Love K, Goodman H, Dean C, Bergkamp R, Dirkse W, Van Staveren M, Stiekema W, Drost L, Ridley P, Hudson SA, Patel K, Murphy G, Piffanelli P, Wedler H, Wedler E, Wambutt R, Weitzenegger T, Pohl TM, Terryn N, Gielen J, Villarroel R, De Clerck R, Van Montagu M, Lecharny A, Auborg S, Gy I, Kreis M, Lao N, Kavanagh T, Hempel S, Kotter P, Entian KD, Rieger M, Schaeffer M, Funk B, Mueller-Auer S, Silvey M, James R, Montfort A, Pons A, Puigdomenech P, Douka A, Voukelatou E, Milioni D, Hatzopoulos P, Piravandi E, Obermaier B, Hilbert H, Düsterhöft A, Moores T, Jones JD, Eneva T, Palme K, Benes V, Rechman S, Ansorge W, Cooke R, Berger C, Delseny M, Voet M, Volckaert G, Mewes HW, Klosterman S, Schueller C, Chalwatzis N. (1998). Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of Arabidopsis thaliana. Nature. 391(6666):485-488.

Bradford M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Chem. 72: 248-254.

Jhang JJ, Staba EJ, Kim JY (1974). American and Korean ginseng tissue cultures: Growth, chemical analysis, and plantlet production. *In Virtro* Cell Dev-PL. 9(4): 253-259.

Kim SI, Kim JY, Kim EA, Kwon KH, Kim KW, Cho K, Lee JH, Nam MH, Yang DC, Yoo JS, Park YM (2003). Proteome analysis of hairy root from Panax ginseng C. A. Meyer using peptide fingerprinting, internal sequencing and expressed sequence tag data. Proteomics, 3(12): 2379-2392.

Lum JH, Fung KL, Cheung PY, Wong MS, Lee CH, Kwok FS, Leung MC, Hui PK, Lo SC (2002). Proteome of Oriental ginseng Panax ginseng C. A. Meyer and the potential to use it as an identification tool. Proteomics, 2(9): 1123-1130.

Nam MH, Heo EJ, Kim JY, Kim SI, Kwon KH, Seo JB, Kwon KH, Seo JB, Kwon O, Yoo JS, Park YM (2003). Proteome analysis of the responses of Panax ginseng C. A. Meyer leaves to high light: Use of electrospray ionization quadrupole-time of flight mass spectrometry and expressed sequence tag data. Proteomics, 3(12): 2351-2367.

Nam MH, Kim SI, Liu JR, Yang DC, Lim YP, Kwon KH, Yoo JS, Park YM (2005). Proteomic analysis of Korean ginseng (Panax ginseng Meyer C.A.). J. Chromatogr. B. 815(1-2): 147-155.

Qu C, Bai Y, Jin X, Yutang W, Kun Z, Jingyan Y, Hanqi Z (2009). Study on ginsenosides in different parts and ages of Panax quinquefolius L. Food Chem. 115(1): 340-346.

Sheoran IS, Ross ARS, Olson DJH, Sawhney VK. (2009). Compatibility of plant protein extraction methods with mass spectrometry for proteome analysis. Plant Sci. 176(1):99-104.

Wang C-Z, Jing-Tian X, Anna F, Han H, Aung HH, Hui H, Mehendale SR, Tong-Chuan H, Wei D, Chun-Su Y (2009a). Antiproliferative Effects of Different Plant Parts of Panax notoginseng on SW480 Human Colorectal Cancer Cells. Phytother. Res. 23: 6-13.

Wang D-Z, Lin L, Leo Lai C, Hua-Sheng H (2009b). Comparative studies of four protein preparation methods for proteomic study of the dinoflagellate Alexandrium sp. using two dimensional electrophoresis. Harmful Algae, 8(5): 685-691.

Yao Y, Yi-Wei Y, Jin-Yuan L (2006). An efficient protein preparation for proteomic anaylsis of developing cotton fibers by 2-DE. Electrophoresis, 27(22): 4559-4569.

Zhang H-F, Xiao-Hua Y, Li-Dong Z, Ying W (2009). Ultrasonic-assisted extraction of epimedin C from fresh leaves of Epimedium and extraction mechanism. Innov. Food. Sci. Emerg. 10(1): 54-60.

Zhang K, Xiao W, Lan D, Juan LI, Chen-ling QU, Li-gang C, Hai-yan J, Han-qi Z (2008). Determination of Seven Major Ginsenosides in Different Parts of Panax quinquefolius L. (American Giseng) with Different Ages. Chem. Res. Chinese. U. 24: 707-711.