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Proteome of Oriental ginseng *Panax ginseng C. A. Meyer* and the potential to use it as an identification tool

Oriental ginseng (*Panax ginseng C. A. Meyer*) and American ginseng (*Panax quinquefolius*) are two widely used valuable traditional Chinese medicines (TCM). Previously, the identification of ginseng was mainly performed by analyzing the ginsengnosides using high performance liquid chromatography and amplification of polymorphic DNA using polymerase chain reaction. However, these methods cannot be used to distinguish TCM samples which are from different parts (main root, lateral roots, rhizome head and skin) of ginseng and ginseng culture cells from wild-grown ginseng. The present study aimed to identify different species of ginseng, different parts of the same ginseng and cultured cells of ginseng using a proteomic approach. Two-dimensional electrophoresis (2-DE) maps were established from the American ginseng main root, different parts (main root, lateral roots, rhizome head and skins) of Oriental ginseng and Oriental ginseng culture cells. Our results show that the 2-DE maps of different ginseng samples contain sufficient differences to permit easy discrimination. We have also identified common and specific protein spots in the 2-DE maps of different ginseng samples. The use of these "marker proteins" may help to speed up the identification process.

Keywords: *Panax ginseng C. A. Meyer* / *Panax quinquefolius* / Proteome / Traditional Chinese medicine
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1 Introduction

Traditional Chinese medicine (TCM) constitutes an important part of the Chinese traditional medical and pharmaceutical treasure house. *Panax ginseng C. A. Meyer* (Oriental ginseng) and *Panax quinquefolius* (American ginseng) are two of the most widely used TCM. Previous research studies have shown that they have nonorgan specific cancer preventive effects [1], tumor therapeutic activity and stimulatory effects on the immune system [2, 3]. Ginseng has a very complex root system, which consists of the skin, rhizome head, main root and lateral roots. According to the Chinese medical book "Shen nung ben tsao jing" (The Holy Farmer's Materia Medica), different species and parts of ginseng are believed to have different properties and medical values. For example, the main root of *Panax ginseng C. A. Meyer* was known to be more effective in replenishing vital energy than the lateral roots. Moreover, according to Chinese tradition, the Oriental ginseng is classified as a "warm" medicine while the American ginseng is considered to be "cool" in nature. It is therefore of utmost importance to be able to distinguish different species and parts of ginseng effectively. Previously, authentication of ginseng was mainly carried out by HPLC separation of different ginsenosides [4–6] and amplification of polymorphic DNA [7–9]. Presence of different species of ginseng in a mixture can be identified by these methods. However, these methods can neither distinguish the presence of different parts of ginseng in a mixture nor quantify the amount of raw materials present in a mixture. We are therefore interested to investigate whether different parts and species of ginseng can be characterized using a proteomic approach.

The proteome is the total protein complement of a genome [10]. In this project, the proteome of the Oriental ginseng (*Panax ginseng C. A. Meyer*) main root was analyzed by using 2-DE. Proteins of interest were identified and sequenced by Edman *N*-terminal sequencing. We then analyzed and compared the 2-DE maps of the main root, lateral roots, ginseng skin and rhizome head of the Oriental ginseng. Finally, a 2-DE map of the American ginseng main root was also established. Our results indicate that the 2-DE map can be used to differentiate: (1) between Oriental ginseng and American ginseng; (2) between different parts of Oriental ginseng and; (3) between wild-grown Oriental ginseng main root and Oriental ginseng culture cells. Protein markers that are specific to the main root of American ginseng and Oriental ginseng were also identified.

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Abbreviation: TCM, traditional Chinese medicine

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2 Materials and methods

2.1 Sample collection

Fresh 6 years old American ginseng (*Panax quinquefolius)* and Oriental ginseng (*Panax ginseng C. A. Meyer*) were purchased from markets in Los Angeles, USA and Seoul, South Korea respectively. Ginseng was stored at 4°C until use. No ginsengs were stored for more than 1 month. When taking samples from the rhizome head, parts within 0.5 cm from both ends were avoided. As for the skin, we took the most superficial layer and less than 0.2 cm in depth. For the main root, we took the part that was at least 0.5 cm from the surface of the skin and free from lateral roots. Finally, for the lateral root, we took the parts that are free from the main root. Cultured Oriental ginseng cells were initiated from *Panax ginseng C. A. Meyer* main root and kindly provided by Dr. J. Y. Wu, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University. Cultured ginseng cells were collected 14 d after subculturing and stored at –80°C before use.

2.2 Protein extraction

Various parts (Fig. 1) of the ginseng plant or the cultured ginseng cells were cut and weighed before being homogenized 1:1 w/v in extraction buffer containing 8 M urea, 2% CHAPS and 0.28% DTT. The suspension was frozen and thawed three times before centrifugation at 10 000 *g* for 15 min at 4°C. After centrifugation, the supernatant was collected and the protein content was measured by using Bradford assay (Bio-Rad, Hercules, CA, USA). The supernatant was then analyzed by 2-DE.

2.3 First-dimensional gel: IEF

300 μL of sample containing 100 μg of protein in 6 M urea, 2 M thiourea, 2% CHAPS, 0.2% DTT and 6 μL IPG buffer (pH 3–10) was used to rehydrate IPG strips for 16 h in the rehydration cassette for IEF. Electrophoresis was performed using PROTEAN IEF Cell apparatus (Bio-Rad) with Immobiline DryStrips (17 cm, linear pH 4–7 gradient or linear pH 3–10 gradient). Voltage was applied according to the following program: 3 h at 150 V, 1 h at 500 V, 1 h at 1000 V, 2 h at 4000 V and 4 h at 8000 V. A total of about 30 000 Vh was applied.

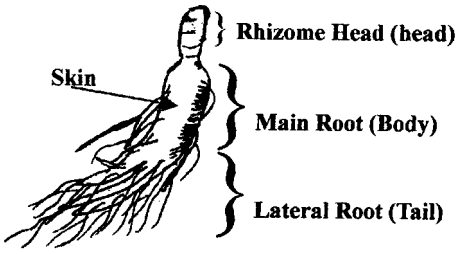

Figure 1. Diagram showing different parts of Oriental ginseng used in the present study.

2.4 Second dimensional gel: SDS-PAGE and image processing

Following IEF, the gel strip was equilibrated with equilibration buffer (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 1% DTT and a trace amount of bromophenol blue) for two minutes. The equilibration buffer was then replaced with fresh equilibration buffer, which contained 1% iodoacetamide instead of DTT. After 10 min, SDS-PAGE was performed using a 15% acrylamide gel running at a constant current of 15 mA/gel until the bromophenol blue dye reached the end of the gel. After electrophoresis, the gel was either stained with a silver stain kit (Bio-Rad) for protein analysis or electro-transferred onto a PVDF membrane for *N*-terminal sequencing.

2.5 Image analysis

Silver stained gels were scanned by an Epson scanner (Perfection 1200U) and the images were analysed by Melanie III (GeneBio, Switzerland) as described in the user manual. Isoelectric points of protein spots identified were estimated by portion as the IPG strips used were of linear gradient.

2.6 *N*-terminal sequencing and database searching

N-terminal sequencing of proteins blotted onto PVDF membrane were performed with a Procise 492 cLC Model 610A protein sequencer (Applied Biosystems, Hong Kong, China) using methodologies described in the instrument manual. Amino acid sequences obtained were searched either against the Protein DataBank (PDB) or SWISS-PROT by BLAST. Settings for querying short sequences for nearly exact matches of peptide were used.

3 Results

3.1 Comparison of 2-DE maps of the main root, lateral roots, rhizome head and skin of Oriental ginseng (*Panax ginseng C. A. Meyer*)

Total protein was extracted from the skin, main root, lateral roots and rhizome head of the Oriental ginseng and 2-DE maps were established by using a pH 3–10 IPG strip in the

first dimension and a 15% SDS-PAGE in the second dimension. 2-DE maps of the Oriental ginseng main root, lateral roots, rhizome head and ginseng skin were shown in Figs. 2–5, respectively. Each figure is a representative of at least six different gels ran with identical samples. Appearance of the protein fingerprints was highly consistent with a variation of less than 5% in the number of spots detected. After analyzing images of the 2-DE maps with Melanie III, different numbers of protein spots were identified in the 2-DE maps of different Oriental ginseng parts. The 2-DE map of the Oriental ginseng main root has the highest number of protein spots (mean = 382 spots), followed by that of the lateral roots (mean = 247 spots) and ginseng skin (mean = 130 spots). The 2-DE map of the rhizome head has the least number of protein spots (mean = 75 spots), which is only one-fifth of that of the main root.

When 2-DE maps of different parts of Oriental ginseng were compared, many common protein spots were found. The number of matched protein spots in the 2-DE maps of different Oriental ginseng parts is summarized in Table 1. Among these common protein spots, three of them were found in all 2-DE maps of different Oriental ginseng parts. These three protein spots were marked as C1,

Table 1. Number of matched protein spots in the 2-DE maps of different ginseng samples

Comparison	Parts of ginseng	Number of matched spots
Different parts of Oriental ginseng	OMr ⟺ OLr	104
	OMr ⟺ ORd	33
	OMr ⟺ OSn	50
	OLr ⟺ ORd	42
	OLr ⟺ OSn	48
	ORd ⟺ OSn	5
American ginseng main root and Oriental ginseng main root	OMr ⟺ AMr	90
Wild Oriental ginseng main root and Oriental ginseng culture cells	OMr ⟺ OCc	78

OMr, Oriental ginseng main root; OLr, Oriental ginseng lateral roots; ORd, Oriental ginseng rhizome head; OSn, Oriental ginseng skin; AMr, American ginseng main root; OCc, Oriental ginseng culture cells

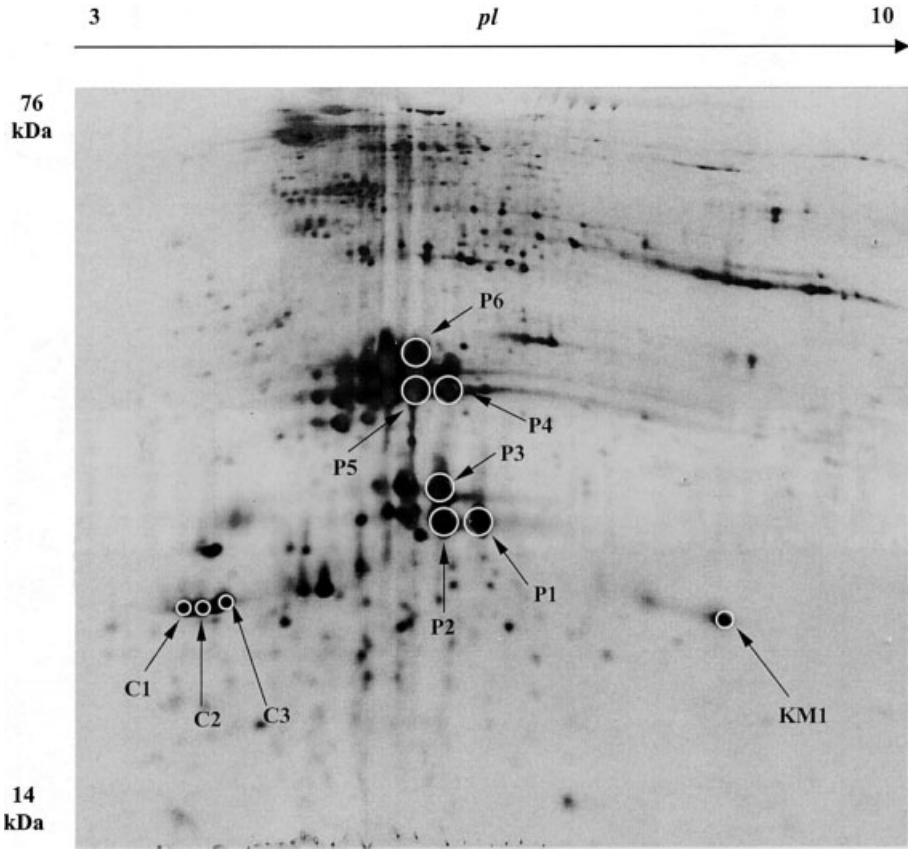

Figure 2. The 2-DE map of the Oriental ginseng main root. Note that protein spots C1-C3 and P1-P6 were common in 2-DE maps of all ginseng samples, while KM1 can be found only in the 2-DE map of Oriental ginseng main root.

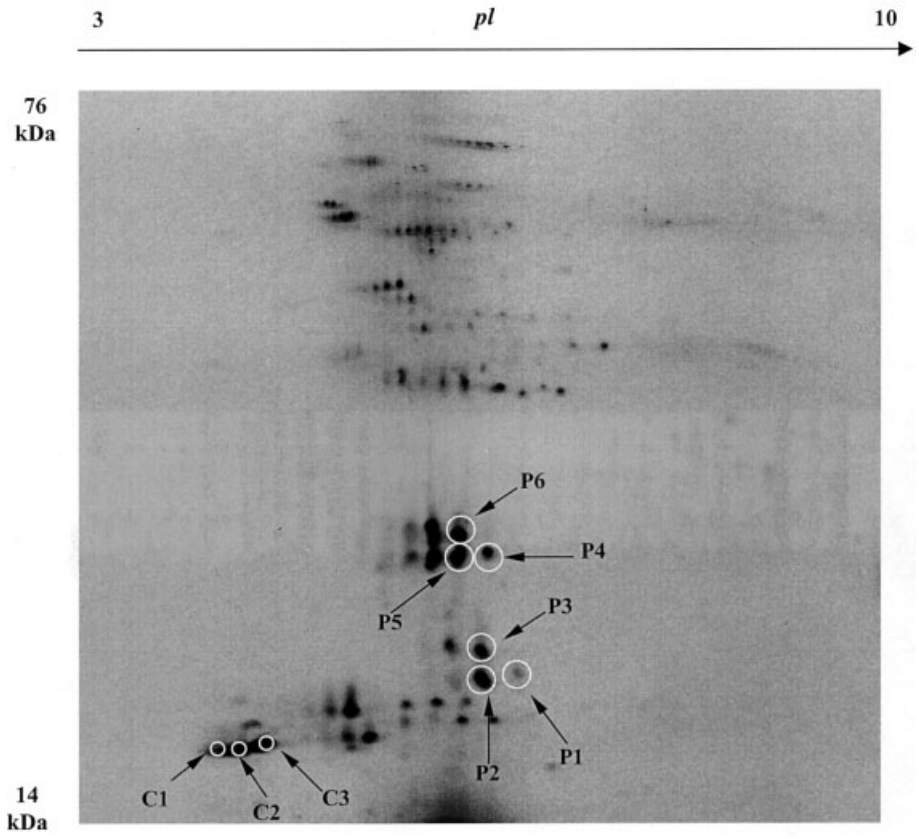

Figure 3. The 2-DE map of the Oriental ginseng lateral roots. Note that protein spots C1-C3 and P1-P6 were common in 2-DE maps of all ginseng samples.

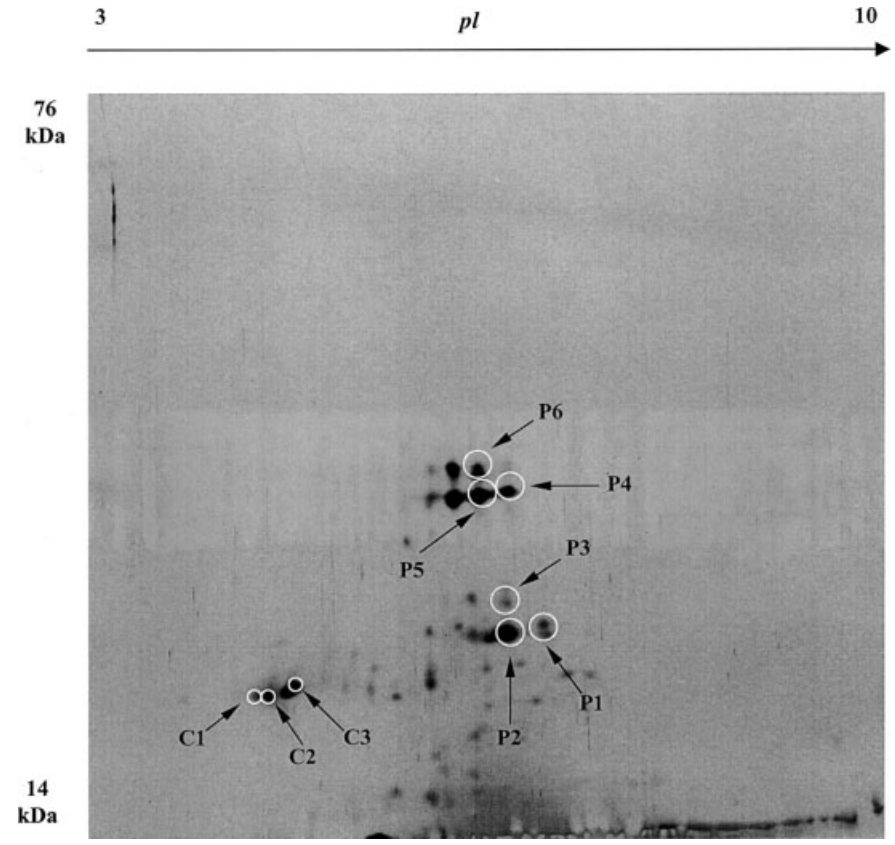

Figure 4. The 2-DE map of the Oriental ginseng rhizome head. Note that protein spots Cl-C3 and P1-P6 were common in 2-DE maps of all ginseng samples.

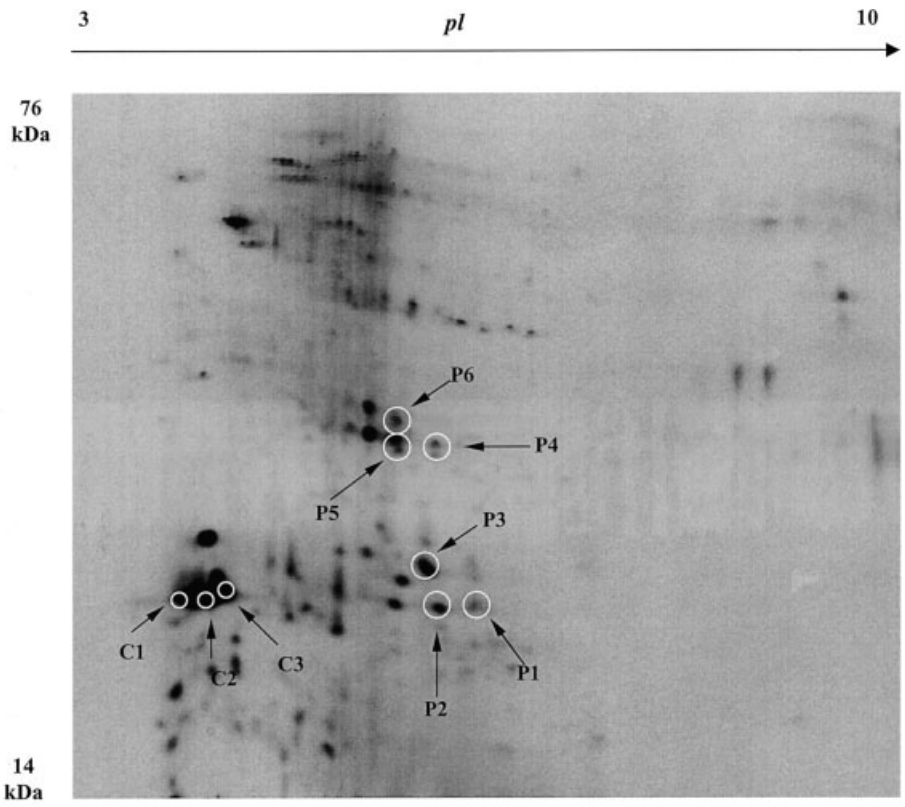

Figure 5. The 2-DE map of the Oriental ginseng skin. Note that protein spots C1-C3 and P1-P6 were common in 2-DE maps of all ginseng samples.

C2 and C3 in Figs. 2–5. C1, C2 and C3 are small acidic proteins with p*I* values of 4.28, 4.35 and 4.44, respectively and a similar mass of 18 kDa. Several other prominent common protein spots were found in the middle of the 2-DE electrophoretogram, with p*I*s ranging from 5.1 to 6.1 and masses of 21–40 kDa. These protein spots are marked as P1–P6 in Figs. 2–5. Although many other common protein spots can be found in the 2-DE maps of different Oriental ginseng parts, the C1–C3 and P1–P6 protein spots are most prominent and well separated. Besides these common protein spots, we were also interested to identify protein spots that were specific in the 2-DE maps of different Oriental ginseng parts. Unfortunately, most of these specific protein spots were of low abundance and cannot be clearly identified. One exception is the protein spot marked as KM1 in Fig. 2. This protein (p*I* = 7.5, mass = 17.5 kDa) was found only in the 2-DE map of the Oriental ginseng main root.

3.2 Comparison of 2-DE maps between American ginseng (*Panax quinquefolius)* main root and Oriental ginseng (*Panax ginseng C. A. Meyer*) main root

To investigate whether 2-DE map can be used to distinguish American and Oriental ginseng, a 2-DE map of the American ginseng main root was generated (Fig. 6). Image analysis with Melanie III showed that the 2-DE map of American ginseng contains 212 protein spots. When compared to a 2-DE map of the Oriental ginseng main root, 90 common protein spots were found. Interestingly, the nine common protein spots, C1–C3 and P1–P6, previously identified to be present in 2-DE maps of all different Oriental ginseng parts, were also common in the 2-DE map of American ginseng main root. Protein spots that were specific in the 2-DE map to the American ginseng main root can also be found. One of the most prominent specific protein spots was marked as AM1, which has a p*I* value of 9.5 and mass of 20 kDa.

3.3 Comparison of 2-DE maps of the main root and the cultured cells of Oriental ginseng (*Panax ginseng C. A. Meyer*)

Cultured ginseng cells were initiated from the main root of Oriental ginseng for the large-scale production of secondary metabolites, such as saponins and ginsengnosides [11–13]. The direct use of cultured ginseng cells in Chinese medicine was not popular because they were believed to be less effective than the wild-grown Oriental ginseng. In the Chinese culture, intact ginseng is usually sold over the counter. However, many prepacked Oriental ginseng powders nowadays are suspected of being manufactured using cultured cells. We are therefore

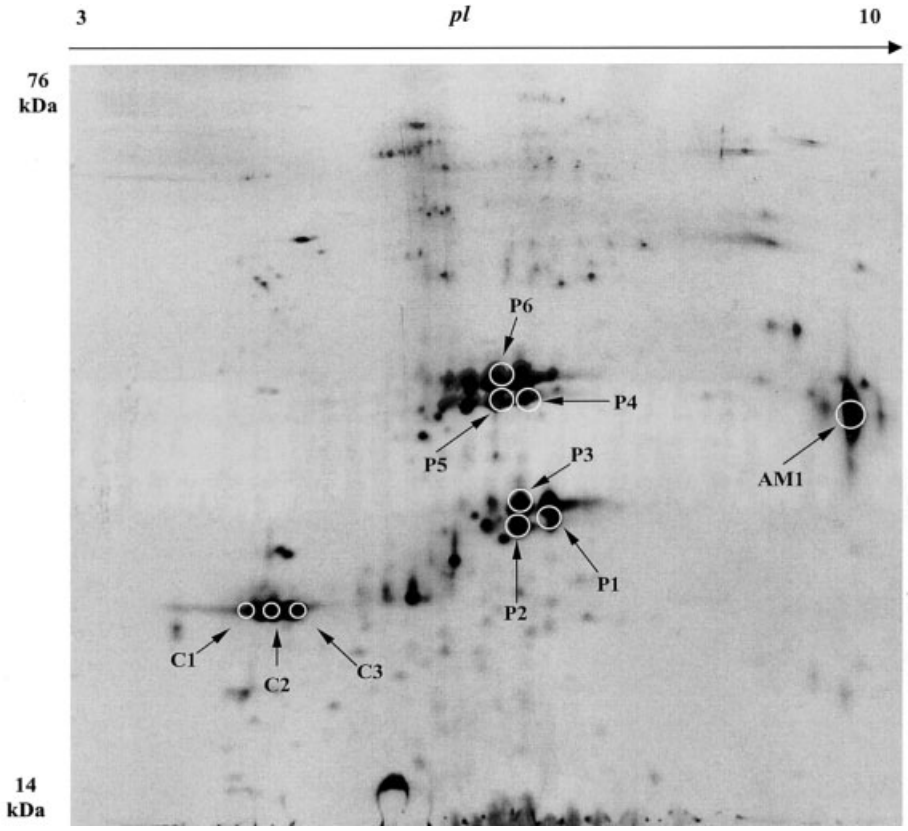

Figure 6. The 2-DE map of the American ginseng main root. Note that protein spots C1-C3 and Pl-P6 were common in 2-DE maps of all ginseng samples, while AM1 can be found only in the 2-DE map of American ginseng main root.

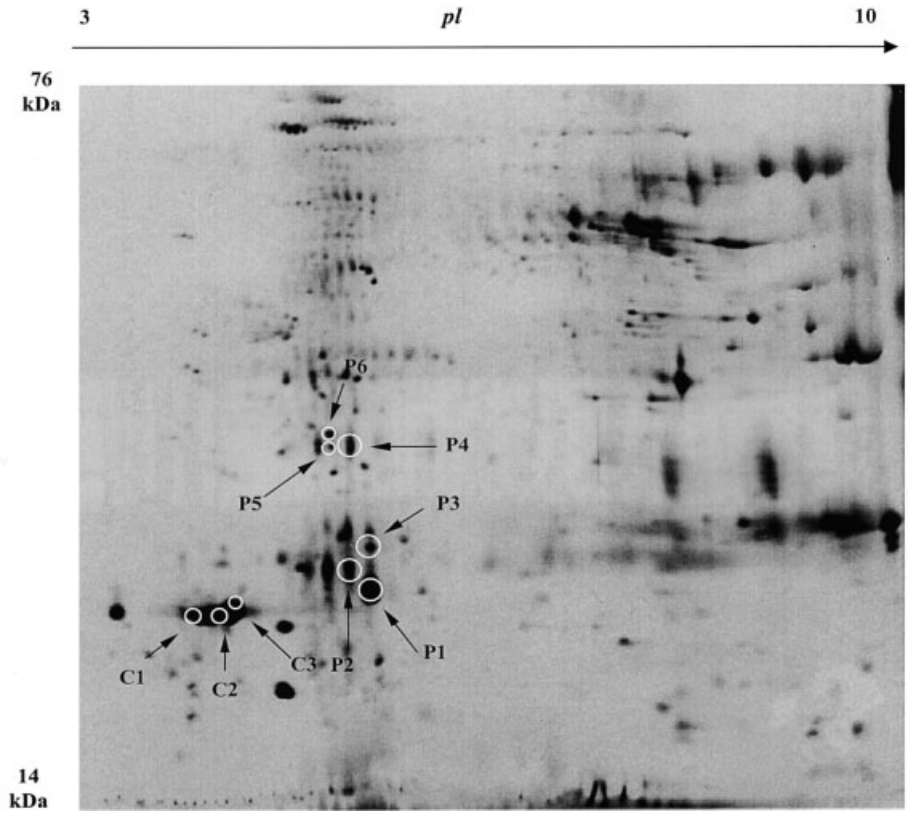

Figure 7. The 2-DE map of the Oriental ginseng main root culture cells. Note that the pattern of protein spots was very different from the wild Oriental ginseng main root. Protein spots C1-C3 and P1-P6 were common in 2-DE maps of all ginseng samples, although P1-P6 were less prominent when compared to other samples.

interested to establish a 2-DE map for cultured Oriental ginseng cells and comparing it to a map for the wild-grown Oriental ginseng main root. The 2-DE map of the cultured Oriental ginseng cells is shown in Fig. 7.

Image analysis by Melanie III found 371 different protein spots in its 2-DE map. Although the number of protein spots is similar to that of the main root of wild-grown Oriental ginseng (382 protein spots), the pattern of the 2-DE map of cultured ginseng cell is very different from that of main root of wild-grown Oriental ginseng. The proteome of cultured ginseng cells contains a much higher number of alkaline proteins as revealed in the 2-DE map. Further analysis shows that among these 371 protein spots, only 78 of them could be matched with that of the 2-DE map of wild-grown Oriental ginseng main root. The three common protein spots (C1–C3), which were present in all parts of wild Oriental ginseng and American ginseng, can also be found in the 2-DE map of the cultured ginseng cells. However, another series of conserved protein spots P1–P6, were less prominent in the 2-DE map of culture Oriental ginseng cell.

3.4 Analysis of the common protein spots C1–C3 and P1–P6 in the 2-DE map of Oriental ginseng main root by *N*-terminal sequencing

During the analysis of the 2-DE proteome maps of different Oriental ginseng parts (main root, lateral roots, ginseng skin and rhizome head), American ginseng main root and cultured cells of Oriental ginseng, we identified several common protein spots, marked as C1–C3 and P1–P6. To further characterize these protein spots, they were electrotransferred onto a PVDF membrane, stained with Coomassie blue and subjected to *N*-terminal (Edman degradation) sequencing. The results are summarized in Table 2. Protein spots C1 and C2 were found to be ribonuclease I and II (GenBank accession number P80889 and P80890), respectively. However, C3 was *N*-terminally blocked and the sequence could not be determined. Interestingly, the *N*-terminal sequence of protein spots P1–P6 were found to be the same (SDYPKAMFAKRXQ XPA). BLAST searching with this stretch of amino acids did not reveal any matched protein in the database.

Table 2. Identification of the common protein spots C1–C3 and P1–P6 b *N*-terminal sequencing

Protein spots	*N*-terminal sequence	Matching protein in database
C1	VEATSTVPAQKLYAGL	Ribonuclease I of *Panax ginseng* (GenBank accession no. P80889)
C2	VQKTETQAISPVPAEKLFIG	Ribonuclease II of *Panax ginseng* (GenBank accession no. P80890)
C3	*N*-terminal blocked	–
P1	SDYPKAMFAKRXQXPA[a)]	No match in database
P2	SDYPKAMFAKR	
P3	SDYPKAMF	
P4	SDYPXAMFAK[a)]	
P5	SDYPXAMFAXR[a)]	
P6	DYPKAMFAKR	

a) X stands for ambiguities in the sequence

4 Discussion

Ginseng is one of the most valuable and widely used TCMs. In the present study, we investigated whether the proteomic approach could be used to identify different species, or even different parts of the same species of ginseng. As the proteome of an organism is very dynamic, we believed that different species and different parts of ginseng would have very different proteomes. Therefore, the 2-DE maps could be used to distinguish different species and parts of ginseng.

Firstly, we established and compared the 2-DE maps of different parts (the main root, lateral roots, rhizome head and ginseng skin) of the Oriental ginseng (*Panax ginseng C. A. Meyer)*. Although the 2-DE maps of different Oriental ginseng parts have many common protein spots, their patterns were sufficiently different from each other such that they could be recognized easily.

We then investigated the 2-DE maps of the American ginseng (*Panax quinquefolius)* main root and the cultured cells of Oriental ginseng. Again, the patterns of their 2-DE maps were very different and could be easily differentiated. Interestingly, several common protein spots (C1–C3) have been identified in all the 2-DE maps investigated. We confirmed the identities of C1 and C2 as ribonuclease I and II, respectively. However, C3 was *N*-terminally blocked and its identity required further investigation by other methods, such as mass spectrometry. Another series of common protein spots, P1–P6, were also found in all 2-DE maps of ginseng, although they were less prominent in the Oriental ginseng culture cells. All of these six proteins have the same *N*-terminal sequence SDYPKAMFAKRXQXPA. A BLAST search revealed no similar protein in the database. Further, despite P1–P6 protein spots being different in mass and p*I* values, they clustered together in the 2-DE map. Whether these proteins were generated by partial

degradation, protein modification or are different isoforms remains to be determined. Indeed, many proteins have been reported to have different post-translational modifications under different environments [14–17]. This may account for the observation that protein spots P1–P6 were less prominent in the Oriental ginseng culture cells.

Proteomics has previously been used to identify different morphologically similar microorganisms, including bacteria, yeast and fungi [18, 19]. Recently, it has also been used to characterize transgenic plants [20, 21]. Compared to the traditional DNA typing approach, proteomics could potentially identify different samples with the same genome, such as different parts of an organism. Our results in the present study clearly showed that proteomes of different Oriental ginseng parts were different and could be used to distinguish them. In the course of our investigation, we have also identified several common and specific protein spots in the 2-DE maps of different parts and species of ginseng. These common and specific proteins might be used to generate antibodies, which could be used to set up an ELISA assay and speed up the identification process. For example, as elaborated earlier, ribonuclease I and II are present in all the 2-DE electrophoretograms. Therefore, with the use of antibodies, an ELISA test can be set-up to measure the amount of ribonucleases present in a mixture to gauge the amount of raw materials of ginseng present in a mixture.

5 Concluding remarks

In conclusion, the present study shows that different species and even different parts of the same species of ginseng could be identified using the proteomic approach.

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6 References

[1] Yun, T. K., Choi, S. Y., *Int. J. Epidemiol.* 1998, *27*, 359–364.
[2] Chen, X. G., Lei, X. H., Fu, Z., Li, Y., *J. Ethnopharmacol.* 1998, *60*, 71–78.
[3] Shin, H. R., Kim, J. Y., Yun, T. K., Morgan, G., Vainio, H., *Cancer Causes Control* 2000, *11*, 565–576.
[4] Fuzzati, N., Gabetta, B., Jayakar, K., Pace, R., Peterlongo, F., *J. Chromatogr. A.* 1999, *854*, 69–79.
[5] Lee, T. M., Der Marderosian, A., *J. Pharm. Sci.* 1981, *70*, 89–91.
[6] Li, W., Gu, C., Zhang, H., Awang, D. V. *et al.*, *Anal. Chem*. 2000, *72*, 5417–5422.
[7] Tochika-Komatsu, Y., Asaka, I., Ii, I., *Biol. Pharm. Bull*. 2001, *24*, 1210–1213.
[8] Um, J. Y., Chung, H. S., Kim, M. S., Na, H. J. *et al.*, *Biol. Pharm. Bull.* 2001, *24*, 872–875.
[9] Mihalov, J. J., Marderosian, A. D., Pierce, J. C., *J. Agric. Food Chem.* 2000, *48*, 37–44.
[10] Wilkins, M. R., Sanchez, J. C., Gooley, A. A., Appel, R. D. *et al.*, *Biotechnol. Genet. Eng. Rev.* 1996, *13*, 19–50.
[11] Zheng, G. Z., Wang, S. L., Gan, F. Y., Liang, Z., *Chin. J. Biotechnol.* 1989, *5*, 47–53.
[12] Furuya, T., Kojima, H., Syono, K., Ishii, T., Uotani, K., *Chem. Pharm. Bull. (Tokyo)* 1973, *21*, 98–101.
[13] Yip, T. T., Lau, C. N., Kong, Y. C., Yung, K. H. *et al.*, *Am. J. Chin. Med.* 1985, *13*, 89–92.
[14] Echevarria, C., Yidal, J., Le Marechal, P., Brulfert, J. *et al.*, *Biochem. Biophys. Res. Commun*. 1988, *155*, 145–150.
[15] Manjunath, S., Williams, A. J., Bailey-Serres, J., *Plant J*. 1999, *19*, 21–30.
[16] Green, L. S., Karr, D. B., Emerich, D. W., *Arch. Microbiol*. 1998, *169*, 145–151.
[17] Huber, J. L., Huber, S. C., Nielsen, T. H., *Arch. Biochem. Biophys*. 1989, *270*, 681–690.
[18] VanBogelen, R. A., Schiller, E. E., Thomas, J. D., Neidhardt, F. C., *Electrophoresis* 1999, *20*, 2149–2159.
[19] Niimi, M., Cannon, R. D., Monk, B. C., *Electrophoresis* 1999, *20*, 2299–2308.
[20] Thiellement, H., Bahrman, N., Damerval, C., Plomion, C. *et al.*, *Electrophoresis* 1999, *20*, 2013–2026.
[21] Ziv, M., de Vienne, D., *Plant Mol. Biol*. 2000, *44*, 575–580.