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Differentiating *Panax ginseng* and *Panax quinquefolius* through SDS-PAGE Analysis

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ABSTRACT

Ginseng is a traditional Chinese herb commonly used in the formulation of tonics. There are 2 main varieties: Oriental (*Panax ginseng*) and American ginsengs (*Panax quinquefolius*). Traditional means of authenticating ginseng have become less reliable since they are being processed into various formulations such as tablets, capsules, powder and tea. These products are sometimes also adulterated. This study employed SDS-PAGE as a technique for differentiating between the 2 varieties of ginsengs. Ginseng proteins were separated using a 12% polyacrylamide gel with a three-step electrophoresis: 18 mA for 15 mins, 24 mA for 30 mins and 34 mA for 30 mins. Five bands were identified as potential markers for the ginsengs. Among these, 3 bands at median molecular weights of ~19.47 kDa (interquartile range (IQR) 18.81–20.11 kDa), ~34.42 kDa (IQR 33.75–35.14 kDa) and ~47.35 kDa (IQR 46.16–48.16 kDa) were common to both ginsengs. The band at ~13.23 kDa (IQR 12.78–13.42 kDa) was unique to American ginseng, and the band at ~29.54 (IQR 29.27–29.69 kDa) was unique to Oriental ginseng. This study shows the potential of SDS-PAGE as a proteomic tool in the analysis of ginseng proteins. We hope that these findings can provide an insight to the differences in protein profiles of the 2 ginseng species, and benefit other researchers who are also doing proteomic research on this herb.

Keywords: ginseng proteins, identification, *Panax ginseng*, *Panax quinquefolius*, SDS-PAGE

INTRODUCTION

Ginseng is a common traditional Chinese medicinal herb (Lum *et al.* 2002; Kim *et al.* 2004) used in the formulation of tonics for anti-stress, anti-fatigue and anti-aging purposes (Um *et al.* 2001). Its other therapeutic effects include immune system modulation (Liu *et al.* 1992), stimulation of the central nervous system (Nah *et al.* 1995; Mogil *et al.* 1998), as well as anti-cancer activities (Nakata *et al.* 1998; Yun *et al.* 2001). There are 2 main varieties: *Panax ginseng* C.A. Meyer (Oriental ginseng) and *Panax quinquefolius* L. (American ginseng) (Wu *et al.* 2001; Li *et al.* 2002c).

According to traditional Chinese medicine principles, Oriental ginseng is considered to have the "warm" property, while American ginseng has the "cool" property (Lum *et al.* 2002). With its increasing popularity in the West (Ackloo *et al.* 2000), ginseng products of various forms are commonly found. Examples of such ginseng products include tablets, capsules, shredded slice, powder, and even tea (Um *et al.* 2001; Li *et al.* 2002b). Furthermore, adulteration of ginseng products also occurs (Um *et al.* 2001; Shim *et al.* 2005). This renders traditional means of authenticating ginseng based on morphology and histology to be less reliable (Um *et al.* 2001; Hon *et al.* 2003).

The main active ingredients of ginseng are saponins called ginsenosides, and high performance liquid chromatography (HPLC) has been a common technique used in its analysis (Chan *et al.* 2000; Li *et al.* 2000). The main disadvantages of this technique are that the ginsenosides are affected notably by factors such as growth and storage conditions of the plant, as well as post-harvesting processes, and this chemical variation may compromise the botanical identity of the herb. Large quantities of sample are also needed for more accurate analyses (Um *et al.* 2001; Hon *et al.* 2003). Recent studies of sequenced genomes and ribonucleic acids have been a major breakthrough in understanding the blueprint of life (Kopchick *et al.* 2002), hence it is not surprising that genomic methods have also been carried out for the purpose of ginseng authentication. A study by Um *et al.* (Um *et al.* 2001) showed that the 18s ribosomal RNA could be used to authenticate ginseng since it is unique to the ginseng species. However, even though studying of the genome has led us in the understanding of cellular expression, it does not give an insight to the abundance or posttranslational modification of proteins (Fountoulakis 2001; Kopchick *et al.* 2002). In Asia, the quality of ginseng is dependant not only on the species, but also on the origin, morphology and age of the roots (Harkey *et al.* 2001). Genomic methods of authentication do not show the physiological conditions of the ginseng root (Hon *et al.* 2003). Since different species of organisms tend to exhibit different protein profiles which can be used to differentiate them from each other (Chen *et al.* 2002; Martinez *et al.* 2004), variation in their protein profiles enable their genetic variability to be estimated, and specific protein markers can also be screened and identified (Sotelo *et al.* 1993; Marquès *et al.* 2001). The objectives of this study were to identify potential protein markers that were common to these 2 varieties of ginsengs that were commercially sold in Singapore, as well as to differentiate them based on their protein profiles. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used as a means of determining the protein profiles of the Oriental and American ginsengs as it is a high-throughput method capable of analyzing multiple samples (Sotelo *et al.* 1993). Its working principle is based on separation by molecular weights, thus proteins of different masses can be identified (Govorun *et al.* 2002).

MATERIALS AND METHODS

Three grades of Oriental ginseng and 4 grades of American ginseng roots were purchased from a branch of a major local Chinese medicinal chain (Hockhua Ginseng Birdnest Trading Enterprises)

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Table 1 Protein bands identified in Oriental and American ginsengs together with their intensities.

Bands in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)	Molecular weight of proteins (kDa) Median (Interquartile range)	Protein bands in ginseng samples				
		Oriental ginseng		American ginseng		Both kinds of ginseng
		Presence (✓) or absence (×) of band	Intensity of band (%) Median (Interquartile range)	Presence (✓) or absence (×) of band	Intensity of band (%) Median (Interquartile range)	Intensity of bands (%) Median (Interquartlie range)
Band 1	47.35 (46.16 – 48.16)	✓	85.8 (83.6 – 89.3)	✓	78.2 (71.9 – 94.7)	83.2 (77.8 – 92.6)
Band 2	34.42 (33.75 – 35.14)	✓	100.0 (100.0 – 100.0)	✓	85.7 (67.3 – 96.7)	96.7 (85.4 – 100.0)
Band 3	29.54 (29.27 – 29.69)	✓	58.3 (51.9 – 59.9)	×	Not applicable	Not applicable
Band 4	19.47 (18.81 – 20.11)	✓	77.6 (63.9 – 88.3)	✓	82.7 (68.0 – 93.7)	80.2 (67.1 – 88.9)
Band 5	13.23 (12.78 – 13.42)	×	Not applicable	✓	100.0 (90.2 – 100.0)	Not applicable

in Singapore. These ginsengs were categorized into different groups based on increasing order of their prices. The groups with the lowest costs within each ginseng species were represented by group 1 (G1), and the groups with the highest costs of Oriental and American ginsengs were represented by groups 3 (G3) and 4 (G4) respectively. The ginseng roots were ground into fine powder and stored at 4°C before protein extraction. The powders (0.2 g) were incubated with 500 µl of extraction buffer I (0.3% SDS, 50 mM Tris-hydrochloride (Tris-HCl) pH 8.0, and 200 mM dithiothreitol (DTT)) at 100°C for 10 mins, and the resulting solution transferred into ice and incubated with 500 µl of extraction buffer II (deoxyribonuclease I, ribonuclease A, 50 mM Tris-HCl pH 8.0, and 50 mM magnesium chloride solution) for 10 mins. The samples were then centrifuged at 14,500 g for 30 mins and the supernatants were collected and precipitated with 10% trichloroacetic acid solution for an hour at -20°C. The protein pellets were collected and washed with ice-cold acetone at least 4 times to remove contaminants, and stored at 4°C overnight until analysis.

Loading buffer (0.09 M Tris-HCl pH 6.8, 20% glycerol, 2% SDS, 0.02% bromophenol blue, and 0.1 M DTT) was pre-warmed at ~66 ± 0.5°C to ensure that the SDS was fully dissolved in the solution. Twelve protein pellets from American ginseng and 9 pellets from Oriental ginseng were mixed with loading buffer (1:1 w/v) and heated at 95°C for 5 mins. SDS-PAGE was then carried out on a 12% polyacrylamide gel using a Mini-PROTEAN 3 Electrophoresis System (Bio-Rad Laboratories Inc., California) with a three-step electrophoresis: 18 mA for 15 mins, 24 mA for 30 mins and 34 mA for 30 mins (Wu *et al.* 1999). The separating gel was washed with de-ionized water for 15 mins and soaked in AMRESCO's Blue-BANdit stain (AMRESCO Inc., Ohio) for an hour. The gel was then soaked in de-ionized water overnight for de-staining (AMRESCO Inc. 1999). The protein bands were scanned at least 5 times using the Quantity One 1-D® analysis software (Bio-Rad Laboratories Inc., California) which calculated the relative mobility of the estimates of proteins based on the migration rate of the standard ladder.

A total of 9 Oriental ginseng and 12 American ginseng samples were analyzed. The Quantity One 1-D® analysis software (Bio-Rad Laboratories Inc., California) and Image-J version 1.42 open-source freeware (National Institutes of Health, Bethesda, Maryland) were used to analyze the molecular weights and intensities of the protein bands. These parameters were expressed in our results as medians and interquartile ranges (IQRs).

RESULTS AND DISCUSSION

Fig. 1 shows the protein profiles of the 2 ginseng species. The protein ladder (PageRuler™ prestained protein ladder, Fermentas Life Sciences, Hanover) was situated at lane 1 and it showed a detection range of 11.00–170.00 kDa, similar to the range described by the manufacturer (Fermentas Life Sciences 2007). Lanes 2 to 4 consisted of representative Oriental ginseng samples grouped from G1 to G3, and lanes 5 to 8 consisted of American ginseng samples grouped from G1 to G4. A summary of the molecular weights and intensities of the protein bands identified in our study is also provided in **Table 1**. From our results, there were 5 bands within the protein ladder range that could be identified in our ginseng samples. Among these bands, there were 3 that were common in both Oriental and American ginsengs: band 1 (median ~47.35 kDa, IQR 46.16–48.16 kDa), band 2 (median ~34.42 kDa, IQR 33.75–35.14 kDa) and band 4 (median ~19.47 kDa, IQR 18.81–20.11 kDa). The other 2 bands at median molecular weights of ~29.54 kDa (IQR 29.27–29.69 kDa, band 3) and ~13.23 kDa (IQR 12.78–13.42 kDa, band 5) were unique to Oriental and American ginsengs, respectively.

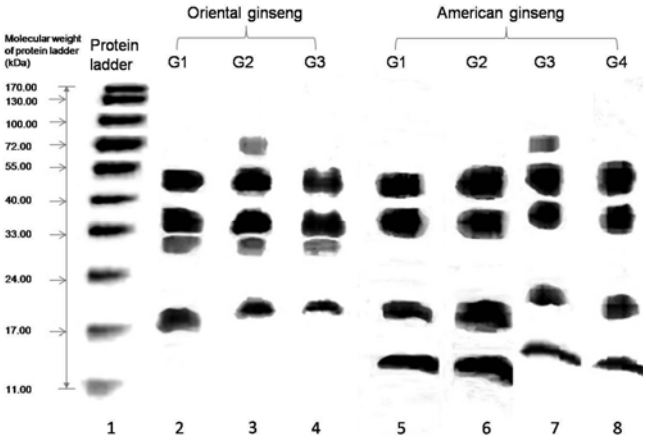

Fig. 1 Protein bands which characterize American and Oriental ginsengs. Lane 1 shows the protein ladder. Lanes 2-4 show the protein profiles of Oriental ginseng samples (G1–G3). Lanes 5-8 show the protein profiles of American ginseng samples (G1–G4).

In general, the intensities of the protein bands were higher for American ginseng than Oriental ginseng (**Table 1**), with the median intensities of the common bands ranging from 80.2% (IQR 67.1–88.9%) to 96.7% (IQR 85.4–100.0%). The protein bands with the lowest intensities in Oriental and American ginsengs were observed for band 3 (median 58.3%, IQR 51.9–59.9%) and band 1 (median 78.2%, IQR 71.9–94.7%), respectively. We postulated that the varying intensities of the protein bands for the different ginsengs could be indicative of the varying amounts of protein extracted from the samples. Jiang *et al.* had demonstrated that ammonium sulfate and trichloroacetic acid precipitation methods could result in higher protein yields compared to other protein extraction techniques (Jiang *et al.* 2004). A prolonged incubation of our ginseng samples with trichloroacetic acid could have improved the amount of extracted protein, and possibly produced darker bands for our samples. Furthermore, the lower resolution of band 4 of our samples could be due to separate proteins of different molecular weights being identified as a single "blurred" band in our results. A recent study using 2-dimensional gel electrophoresis (2-DE) had identified the bands within the same range as 2 separate proteins of molecular weights 18 and 20 kDa (Lum *et al.* 2002). The former band at 18 kDa was identified by the authors as ribonucleases I and II of Oriental ginseng, but the latter band at 20 kDa was unidentified. However, our results were consistent with another study using the same SDS-PAGE technique, in which a common single band was identified at the 20 to 21 kDa range (Wu *et al.* 1999). This discrepancy could be due to the different analysis techniques, since 2-DE could separate overlapping protein bands according to their isoelectric

points (Friedman *et al.* 2009). Nevertheless, SDS-PAGE could identify the presence of protein within this band range for both types of ginsengs, but further confirmatory analysis of the identity of the ginseng proteins could be carried out in future studies with other analytical techniques, such as mass spectrometry (MS) (Li *et al.* 2009). In addition, the prefractionation process described by Kim *et al.* (2003) could also be utilized to improve the resolutions of the 5 ginseng protein bands in future.

There were several bands that were observed at the ~64.55 kDa (IQR 64.00–67.56 kDa) and ~62.63 kDa (IQR 62.51–65.15 kDa) ranges in some of our Oriental and American ginseng samples respectively. However, these bands were not consistent in our results. Additionally, another band at ~108.41 kDa was also observed in 1 of the Oriental ginseng samples. These bands could be indicative of the ginseng stress-related proteins, since it has been suggested that proteomic methods could detect the responses of ginsengs to different environmental conditions (Hon *et al.* 2003; Nam *et al.* 2003). We hypothesized that these bands were not observed throughout all our ginseng samples, probably because the different grades of ginsengs that were bought from the retailer were grown or cultivated in different environments. The amount of constituents in ginseng roots could have been affected by different growing environments and cultivation conditions (Schooley 1998; Li *et al.* 2002a). However, we could not confirm our hypothesis due to the small sample sizes of both kinds of ginseng roots in our study. Future work could focus on these bands with larger numbers of Oriental and American ginseng root samples that are obtained from different growing environments and cultivation conditions.

Although HPLC and MS techniques have traditionally been commonly used in the analysis of traditional Chinese medicines, such as ginseng, there are several disadvantages associated with these techniques (Yap *et al.* 2005). For example, indirect detection methods have to be carried out for HPLC analysis if there are no standards available, and the use of methanol, a toxic chemical for extraction in HPLC analyses, is not suitable for food processing. Conversely, MS techniques can be too costly for use in routine analyses, and is also time-consuming due to the need for chemical derivatization. On the other hand, even though genomic methods, such as restriction fragment length polymorphism, polymerase chain reaction, randomly amplified polymorphic DNA and DNA sequencing techniques, among others, are gaining recognition in ginseng analysis, limitations of these methods include the lack of reproducibility, the need for prior genetic knowledge for some analysis techniques, and the need for separate enzymatic steps for amplification and restriction processes (Yap *et al.* 2005). In contrast, SDS-PAGE has been used to analyze proteins from cancer cells and bacteria (Cash 2009; Li *et al.* 2009), but its use in analyzing herbal medicines is still not widespread. The main advantage of proteomic techniques, such as SDS-PAGE, is their high-throughput capability, which enables the processing of multiple samples simultaneously in each single run (Sotelo *et al.* 1993). Moreover, these technologies can also ultimately bridge the gap between genotype and phenotype in the biological sciences (Goodacre *et al.* 2004; Nam *et al.* 2005). Albeit the fact that there is a lack of availability of the full genome sequence of ginseng, and this may limit the proteomic research done on this herb, our results could, nonetheless, still discriminate between the 2 ginseng species through the use of SDS-PAGE analysis.

LIMITATION AND RATIONALE OF STUDY

The main limitation of our study was the small sample sizes of both the Oriental and American ginsengs. However, we did not want to repeat the many analytical studies that had already been done on ginseng, particularly those involving HPLC and MS techniques (Yap *et al.* 2005). As our institution was just newly set-up in Singapore during the period of this study, we were not able to gain access to these equipments. Thus, our research was meant as a thought-provoking idea on how ginseng protein analysis could be carried out in the laboratory without the help of such complicated and expensive equipments. Additionally, we wanted to provide an alternative viewpoint to other researchers as to how different types of ginsengs could be differentiated based on their protein profiles.

A quick search carried out in Pubmed (July 2011) using "ginseng" and "HPLC" or "mass spectrometry" as keywords revealed 376 and 200 studies respectively for each of these techniques. On the other hand, our search in Pubmed using the keywords "ginseng" and "SDS-PAGE" revealed only 33 articles, of which none of them were related to the analysis of ginseng proteins from commercial ginseng roots sold in medicinal halls. In fact, we only managed to find 2 articles that were closely related to our study (Wu *et al.* 1999; Nam *et al.* 2005). The paper by Nam and colleagues (Nam *et al.* 2005) was a review of genomic and proteomic techniques on only 1 species of ginseng (*Panax ginseng* C.A. Meyer, also known as Asian/Oriental ginseng), while the other paper by Wu and colleagues (Wu *et al.* 1999) managed to identify several protein bands that were common to 3 species of ginsengs (*Panax ginseng*, *Panax quinquefolius* and *Panax notoginseng*). Our study was different from the latter study by Wu *et al.* in that we observed 3 bands (bands 1, 2 and 4) that were common to both *Panax ginseng* and *Panax quinquefolius*, which were not previously identified by them. Furthermore, our results showed that band 3 (median 29.54 kDa, IQR 29.27–29.69 kDa) was unique to Oriental ginseng, and this was also not reported by Wu and colleagues. As such, we believe that this study not only achieved its aim of identifying potential protein markers of ginseng (i.e. the *Panax* genus), but also managed to discriminate the 2 varieties (*Panax ginseng* and *Panax quinquefolius*) by their protein profiles. We hope that these results can contribute to, and complement, the existing body of knowledge in research involving proteomic analysis of ginsengs.

CONCLUSION

This study has shown that American and Oriental ginsengs can be differentiated by their protein profiles based on SDS-PAGE analysis. We have managed to identify 5 protein bands that are present in the ginsengs, 2 of which can be used to distinguish between them. Our study shows the potential of SDS-PAGE as a proteomic tool in the analysis of ginseng proteins, particularly in an environment where access to sophisticated and expensive equipments is limited. We hope that our findings can provide an insight to the differences in protein profiles of the 2 ginseng varieties, and benefit other researchers who are also doing proteomic research on this herb.

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