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Development of a DNA Microarray for Authentication of Ginseng Drugs Based on 18S rRNA Gene Sequence

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Ginseng drugs, derived from underground parts of *Panax* species (Araliaceae), are the most important group of herbal medicines in the Orient. Previously, the nucleotide sequences of the nuclear 18S rRNA gene of 13 *Panax* taxa were determined, as were the specific polymorphic nucleotides for identification of each species. On the basis of the nucleotide difference, a DNA microarray (PNX array) was developed for the identification of various *Panax* plants and drugs. Thirty-five kinds of specific oligonucleotide were designed and synthesized as probes spotting on a decorated glass slide, which included 33 probes corresponding to the species-specific nucleotide substitutions and 2 probes as positive and negative controls. The species-specific probes were of 23−26 bp in length, in which the substitution nucleotide was located at the central part. Triplicate probes were spotted to warrant accuracy by correcting variation of fluorescent intensity. Partial 18S rRNA gene sequences amplified from *Panax* plants and drugs as well as their derived health foods were fluorescently labeled as targets to hybridize to the PNX array. After hybridization under optimal condition, specific fluorescent patterns were detected for each *Panax* species, and the analyzed results could be indicated as barcode patterns for quick distinction. The developed PNX array provided an objective and reliable method for the authentication of *Panax* plants and drugs as well as their derived health foods.

KEYWORDS: DNA microarray; *Panax*; ginseng drugs; 18S rRNA gene; molecular identification

INTRODUCTION

Ginseng drugs, derived from underground parts of *Panax* species (Araliaceae), are the most important group of herbal medicines in the Orient. The genus *Panax* consists of more than 10 species, which are distributed mainly over the Sino-Japanese floristic region, ranging from northeastern China, Korea, and Japan to Himalayan regions through central and southwestern China, and southward extending to central Vietnam (*1–4*). Two species, *Panax quinquefolius* L. and *Panax trifolius* L., grow in the eastern part of North America (*5*). The underground parts of almost all taxa in this genus are available as medicinal resources in traditional Chinese medicine as well as in folk medicine of many countries. Several of them are highly esteemed medicines, such as ginseng, which has been used as a tonic and adaptogenic agent for more than 2000 years, American ginseng, which has been applied in similar usages as ginseng but is considered to have a cold property, and notoginseng, which is famous for its hemorrhage, disperse extravasated blood effect and potential to cure hepatitis and cardiac disease, etc. (*6, 7*). Due to the tonic effect, the three have also been used as foods for centuries in China, Korea, and other Asian countries. They appear in soups, casserole dishes, etc., used to adjust physical condition and is used to prepare rice porridge for weak persons. In recent years, in response to a growing interest in the health-promoting effects of natural products, various health foods and dietary supplements derived from ginseng drugs have become popular products with increasing demands. Among them, ginseng, American ginseng, notoginseng, and Siberian ginseng are the most common, and a large number of products are available with different forms and dosages.

The ginseng drugs derived from the *Panax* genus are reported to have different bioactivities (*8–10*) and various medicinal values (*11, 12*). The main bioactive constituents of ginseng drugs are triterpene saponins, generally referred to as ginsenosides (*13–15*). Our previous study revealed each ginseng drug had its own characteristic saponin composition (*16*), which provided substantial basis for their varied utilities. Therefore, correct authentication is important for the proper use of each drug according to its chemical and bioactive characteristics.

However, it is difficult to identify *Panax* plants and ginseng drugs due to morphological similarities, especially the medicinally used underground parts. In the case of health foods or

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dietary supplements derived from ginseng drugs, most are in powder, tablet, or capsule forms, and there is no proper index for authentication. Moreover, Siberian ginseng is not derived from the *Panax* genus, but from *Eleutherococcus senticosus* of the same Araliaceae family, which does not contain the same chemical constituents as *Panax*-derived ones. To establish an objective method for identification, we have already investigated and compared nucleotide sequences of nuclear 18S rRNA gene and chloroplast *trn*K gene from 13 *Panax* taxa collected from China, Japan, Vietnam, India, and Nepal, as well as *E. senticosus* from China, and elucidated the marker sequences for authentication of each taxon (*17*). In the present study, we aim to develop a DNA microarray for convenient and objective identification of ginseng drugs as well as their derived health foods based on the species-specific 18S rRNA gene sequences.

MATERIALS AND METHODS

Materials. Plant specimens and crude drug samples including eight *Panax* taxa are the same as used in our previous papers (*17, 18*). The plant specimens were indicated with voucher number, and drug samples were indicated with registration number in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama (TMPW). They are *P. ginseng* C. A. Meyer (PG, voucher K. Komatsu et al., JY18), ginseng (TMPW 18399), *P. japonicus* C. A. Meyer (Japan) (PJJ, voucher S. Zhu 20009), Japanese ginseng (TMPW 18127), *P. quinquefolius* L. (PQ, voucher S. Zhu 01003), American ginseng (TMPW 12020), *P. notoginseng* (Burk.) F. H. Chen (PN, voucher K. Komatsu et al., Y287-1), notoginseng (TMPW 17489), *P. japonicus* C. A. Meyer (China) (PJC, voucher s.leg., Baisanchi-5), *P. japonicus* C. A. Meyer var. *angustifolius* (Seem.) C. Y. Wu et Feng (PJA, voucher H. Fushimi et S. Zhu, 01001), *P. stipuleanatus* H. T. Tsai et K. M. Feng (PS, voucher K. Komatsu et al., Y283-1), and *P. pseudoginseng* Wall. (PP, voucher T. Watanabe, s.n.). Five health food products were included in this study; three were purchased in Japan through the Internet with names of Korean ginseng (powder, TMPW 22794), American ginseng (tablet, TMPW 22801), and notoginseng (capsule, TMPW 22807), and two were obtained in San Diego, CA, with names of ginseng (capsule, TMPW 20346) and American ginseng (capsule, TMPW 22023). In addition, *E. senticosus* (Rupr. et Maxim.) Harm. (voucher S. Zhu 20001) was also tested.

All specimens were stored in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama (TMPW).

DNA Microarray Preparation. The designed oligonucleotide probes were synthesized and then spotted on decorated glass slide according to the arrangement shown in **Figure 2** by Hokkaido System Science Co., Ltd. (Japan).

Isolation of Total DNA, PCR Amplification, and Fluorescent Labeling of Target Sequence. Total DNA was extracted from the dried leaf or underground part of plant specimens and crude drugs as well as health food products by using the DNeasy plant Mini Kit (Qiagen). Amplifications of partial 18S rRNA gene via the Polymerase Chain Reaction (PCR) were performed using 10−100 ng of total DNA as a template in a 50 μL reaction mixture, consisting of 1× Gene Taq universal buffer with 1.5 mM $MgCl_2$, 0.25 mM each of dATP, dCTP, and dGTP, 0.15 mM dTTP, 0.1 mM aminoallyl-dUTP, 0.5 mM of each primer, and 2.0 units of Taq polymerase (Nippon Gene). A pair of primers (18S 5′F 5′-CAA CCT GGT TGA TCC TGC CAG T-3′ and 18S 800R 5′-TGT ATC CAG AGC GTA GGC TTG C-3′) were used. PCR amplifications were carried out in a Thermal Controller (Takara) by cycling conditions of first denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and final extension at 72 °C for 5 min. The $^1/_{10}$ volume of the resulting PCR amplicon was detected by 1.0% agarose gel electrophoresis, and then the remaining part was purified using Millipore montage-PCR column (Millipore). After measurement of DNA concentration, a certain volume of purified solution containing 3−5 ng of PCR amplicon was dried at 80 °C and dissolved in 5 μL of 200 mM sodium dicarbonate solution and together with 5 μL of fluorescent labeling reagent Cy5 was kept at room temperature for 1 h. The labeled target was purified using a QIA quick PCR purification Kit (Qiagen).

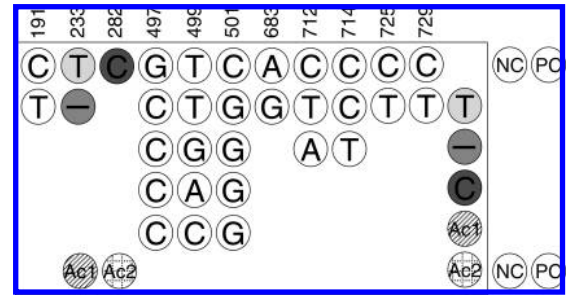

Figure 1. Arrangement of a test version of DNA microarray. Each circle indicates a synthesized oligonucleotide probe spotted on the microarray slide. The substitution nucleotides, as indicated inside the circle, were located at the central part of probes. Hyphen indicates 1 bp deletion at that site. Probes were prepared with similar melting temperature, so that most were of 24−25 bp in length, whereas five probes (shaded by different background in second and third columns) were of 18−20 bp due to high GC contents. Thereby, five 25 bp candidates with obviously higher melting temperature were further prepared to spot in the rightmost column, which are shaded with the same background as their corresponding one in the second and third columns. PC, positive control; NC, negative control; Ac, probe for *Eleutherococcus senticosus*.

Hybridization to DNA Microarray. Hybridization mixture consisted of 6× SSC buffer (150 mM NaCl and 15 mM sodium citrate), 0.2% SDS (sodium dodecyl sulfate), 5× Denhardt's solution, 0.01 mg/mL human cot-1 DNA, and 5 μL of the labeled PCR amplicon. Prior to hybridization, mixture was denatured at 95 °C for 2 min. After centrifugation at 13000 rpm for 5 min, 21 μL of mixture was applied onto the DNA microarray slide, and the microarray slide was placed into a hybridization chamber and then steeped in a 68 °C water bath for 2 h of incubation. After hybridization, the microarray slides were steeped into 2× SSC buffer to remove cover glass, then washed sequentially in 2× SSC buffer containing 0.2% SDS for 5 min twice at 55 °C and for 5 min once at 65 °C, and finally washed in 0.05× SSC buffer for 2 min at room temperature. Three washings at relatively high temperature eliminated uncombined and unspecific adherence of fluorescence-labeled targets to ensure specificity. Finally, the microarray was dried by using a slide centrifuge.

Detection of Fluorescent Intensity of DNA Microarray and Data Analysis. The hybridized microarray slide was scanned by a ScanArray Lite (Parkard BioChip Technologies, Billerica, MA) at a pixel resolution of 10 μm and the obtained fluorescence image was analyzed by QuantArray software (Parkard BioChip Technologies). The fluorescent intensity was calculated as an average of the three subarrays.

Quantitative Determination of Six Major Ginsenosides in Five Health Foods Claimed As Ginseng. Comparison of ginsenoside contents among ginseng drugs derived from 12 *Panax* taxa has been conducted in our previous study (*16*). In this study, we analyzed the contents of six major ginsenosides in five health foods claimed as ginseng. Sample preparation and analytical conditions are completely the same as in our previous papers (*16, 19*).

RESULTS

On the basis of 18S rRNA gene sequences of eight *Panax* taxa (**Table 1**), we first constructed a test version of DNA microarray, in which five subarrays, each consisting of 42 kinds of oligonucleotide probes, were spotted on a decorated glass slide. Among these eight taxa, 7 are of species-specific sequence in 18S rRNA gene, whereas *P. quinquefolius* showed the same sequence as *P. vietnamensis* and *P. vietnamensis* var. *fuscidiscus*. However, unlike the most frequently used *P. quinquefolius*, drugs derived from *P. vietnamensis* and *P. vietnamensis* var. *fuscidiscus* are avail-

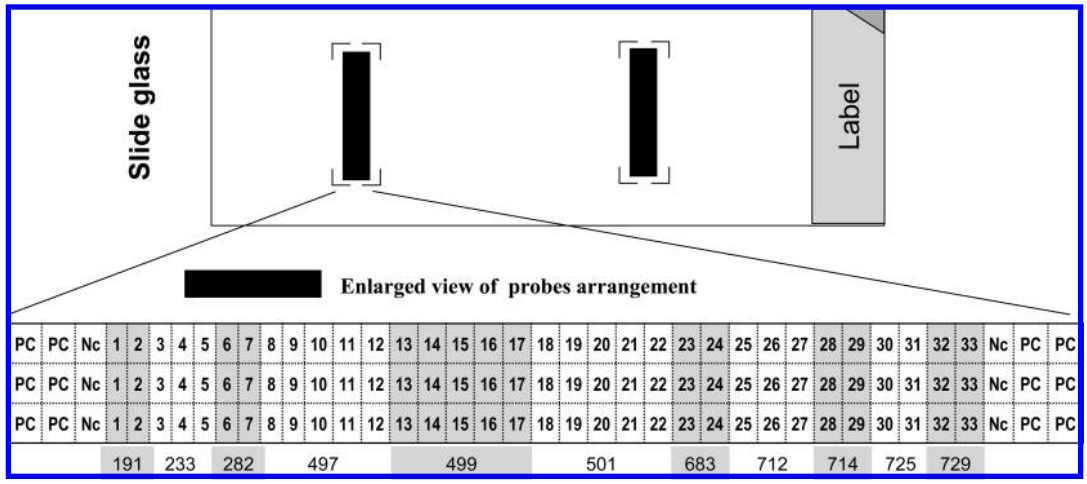

Figure 2. Layout of the designed DNA microarray for identification of *Panax* plants and ginseng drugs. The number in each box is the identity of oligonucleotide probes corresponding to those shown in **Table 2**. Positive control (PC) and negative control (NC) are spotted at both left and right ends. Numerals below boxes indicate the corresponding substitution site in 18S rRNA gene. Two to five probes per site were synthesized on the basis of species-specific nucleotides at that site and then were spotted successively on array slides to form a detecting zone. Triplicates of each probe are spotted in each column.

Table 1. Comparison of 18S Ribosomal RNA Gene Sequences among *Panax* Taxa

	nucleotide positions											
species	191	233	282	497	499	501	683	712	714	725	729	GenBank accession no.
P. ginseng (PG)	C	T	C	G	T	C	A	C	C	C	C	D83275
P. japonicus (Japan) (PJJ)	*	*	*	C	*	G	*	T	*	*	*	D84100
P. quinquefolius (PQ)	*	*	*	C	G	G	*	*	*	*	*	D85172
P. notoginseng (PN)	T	*	*	C	G	G	*	*	*	*	*	D85171
P. japonicus (China) (PJC)	*	*	*	C	*	G	G	*	*	*	*	AB088018
P. japonicus var. *angustifolius* (PJA)	T	*	*	C	*	G	*	*	*	T	*	AB088019
P. pseudoginseng (PP)	*	–	*	C	C	G	*	A	T	*	T	AB088026
P. stipuleanatus (PS)	*	–	*	C	C	G	*	*	*	*	*	AB088025
Eleutherococcus senticosus (OG)	*	–	T	C	A	G	*	*	*	*	*	AB080245

able only in limited local market. Therefore, we included *P. quinquefolius* in the present study. Each subarray was composed of 12 columns of spots (**Figure 1**). In the left 11 columns, two to five probes per column were spotted corresponding to 11 substitution sites in 18S rRNA gene. Most of the oligonucleotide probes were of 24−25 bp in length, with similar melting temperatures (T_m). However, five of them, which had high GC contents, were of 18−20 bp in length, but with relatively high T_m similar to other 24−25 bp probes. Due to this, five candidate oligonucleotides of 25 bp with obviously higher T_m values were further prepared and spotted onto the rightmost column of each subarray to optimize the probes. Targets for hybridization were prepared by Cy5 labeling of partial 18S rRNA gene amplified by PCR from template DNA of various *Panax* species. Trial hybridizations were tested at 55, 60, 65, and 68 °C by using Cy5-labeled targets of *P. ginseng*, *P. quinquefolius*, *P. notoginseng*, *P. japonicus* (Japan), *P. japonicus* (China), and *E. senticosus* (syn. *Acanthopanax senticosus*) as reference. The results showed that high specificity was obtained with increasing stringency of hybridization condition. At 68 °C, nearly expected fluorescent-dot-pattern corresponding to each taxon was observed. However, the spots with short length probes (18−20 bp) yielded very weak signals, whereas the candidate probes (25 bp) showed good performance. In addition, unstable fluorescent intensity was observed from oligonucleotide probes corresponding to substitution sites near the 5′ end of the target sequence, which suggested that the direction of the probe sequence is an important factor related to hybridization performance due to steric hindrance.

Upon the above trials, further improvement has been induced and a DNA microarray for the identification of *Panax* species and ginseng drugs (PNX array) has been designed and produced. As shown in **Table 2**, 35 kinds of specific oligonucleotide were synthesized as probes, including 33 probes corresponding to the species-specific nucleotide substitutions observed at 11 sites (191, 233, 282, 497, 499, 501, 712, 714, 725, and 729 from upstream) in the 18S rRNA gene sequence (**Table 1**), and two probes as positive and negative controls, respectively. The substitution nucleotides were located at the central part of probes, which were 23−26 bp in length (**Table 2**). These probes were spotted on a decorated glass slide according to the arrangement shown in **Figure 2**, where two to five probes per nucleotide site were spotted successively from left to right to form a detection zone for detecting nucleotide substitution at each site (Ex. the 191 zone consisted of 2 probes). Triplicate probes were spotted in each column to warrant accuracy by correcting variation of fluorescent intensity. Signal on/off resulting from a combination of Cy5-labeled target to the corresponding probes was expected to produce a species-specific fluorescent dot pattern, which appeared as an easily detected barcode pattern for each *Panax* taxon (**Figure 3**). By indicating the fluorescent signal by the column number in each zone, for instance, the pattern for ginseng can be recognized as [11111111111] in 11 detecting zones. The positive control was a 27 bp probe complementary to 227−253 positions from

Table 2. Sequence of Oligonucleotide Probes Printed on Glass Slide

no.	probe	sequence (5′−3′)[a]	length (bp)	GC %
1	PNX-18S-191-1	GCATCCCTTCCA**g**AAGTCGGGGGTTT	25	0.56
2	PNX-18S-191-2	GCATCCCTTCCA**a**AAGTCGGGGTTT	25	0.52
3	PNX-18S-233-1	AGCAACGGGCAG**aa**GCCCGCGTCGA	25	0.68
4	PNX-18S-233-2	AGCAACGGGCAG**a**GCCCGCGTCGA	24	0.71
5	PNX-18S-233-3	AGCAACG**a**GCA**ta**GCCCGCGTCGA	24	0.63
6	PNX-18S-282-1	GTCGCCGGCACGA**g**GGCCGTGCGATC	26	0.77
7	PNX-18S-282-2	GTCGCCGGCACGA**a**GGCCGTGCGATC	26	0.73
8	PNX-18S-497-1	ACAATACCGGGCT**g**At**T**cAGTCTGGT	26	0.50
9	PNX-18S-497-2	CAATACCGGGCT**cAtTg**AGTCTGGT	25	0.52
10	PNX-18S-497-3	CAATACCGGGCT**cAgTg**AGTCTGGT	25	0.56
11	PNX-18S-497-4	CAATACCGGGCT**cAaTg**AGTCTGGT	25	0.52
12	PNX-18S-497-5	CAATACCGGGCT**cAcTg**AGTCTGG	24	0.58
13	PNX-18S-499-1	AATACCGGGCT**gAtTc**AGTCTGGTAA	26	0.46
14	PNX-18S-499-2	ATACCGGGCT**cAtTg**AGTCTGGTAA	25	0.48
15	PNX-18S-499-3	ATACCGGGCT**cAgTg**AGTCTGGTAA	25	0.52
16	PNX-18S-499-4	ATACCGGGCT**cAaTg**AGTCTGGTAA	25	0.48
17	PNX-18S-499-5	ATACCGGGCT**cAcTg**AGTCTGGTAA	25	0.52
18	PNX-18S-501-1	TACCGGGCT**gAtTc**AGTCTGGTAATT	26	0.48
19	PNX-18S-501-2	ACCGGGCT**cAtTg**AGTCTGGTAATT	25	0.48
20	PNX-18S-501-3	TACCGGGCT**cAgTg**AGTCTGGTAATT	26	0.50
21	PNX-18S-501-4	ACCGGGCT**cAaTg**AGTCTGGTAATT	25	0.48
22	PNX-18S-501-5	ACCGGGCT**cAcTg**AGTCTGGTAATT	25	0.52
23	PNX-18S-683-1	GGTGTGCACCG**a**TCGTCTCGTCC	23	0.65
24	PNX-18S-683-2	GGTGTGCACCG**g**TCGTCTCGTCC	23	0.70
25	PNX-18S-712-1	CGGCGATGCG**cTc**CTGTCCTTAA	23	0.61
26	PNX-18S-712-2	CGGCGATGCG**tTc**CTGTCCTTAA	23	0.57
27	PNX-18S-712-3	CCGGCGATGCG**aTt**CTGTCCTTAA	24	0.54
28	PNX-18S-714-1	CGGCGATGCG**cTc**CTGTCCTTAACT	25	0.60
29	PNX-18S-714-2	CGGCGATGCG**aTt**CTGTCCTTAACT	25	0.52
30	PNX-18S-725-1	TCCTGTCCTTAA**c**TGGCCGGGTCGT	25	0.60
31	PNX-18S-725-2	TCCTGTCCTTAA**t**TGGCCGGGTCGT	25	0.56
32	PNX-18S-729-1	TCCTTAACTGG**c**CGGGTCGTGCCT	24	0.63
33	PNX-18S-729-2	TCCTTAACTGG**t**CGGGTCGTGCCT	24	0.58
34	positive control	atcatcg cagcaacggg cagaagcccg	27	0.63
35	negative control	AGTCAGCCAGTCAGGCACTTCGATA	25	0.52

[a] Nucleotide substitutions are uncapitalized and indicated in boldface.

upstream of 18S rRNA gene sequence, which is a highly variable region in the 18S rRNA gene and in which high homology within *Panax* taxa was confirmed. Thereby, the positive control printed on both sides served as an indicator of the outline of each array. Signals at these spots indicated success in fluorescent labeling as well as hybridization of the *Panax*-derived target to microarray. The negative control was a 25 bp probe with randomly selected nucleotides, which was confirmed to be nonhomologous to the target sequence. A microarray slide enabled two samples to be simultaneously assayed.

Scanned images of the PNX array hybridized with fluorescent-labeled targets from *P. ginseng*, *P. quinquefolius*, and *P. japonicus* (Japan) were confirmed to be the same as expected as shown in **Figure 3**. Subsequently, crude drug samples were tested in the same way. As shown in **Figure 4**, by using a Cy5-labeled partial 18S rRNA gene sequence of ginseng as target, signals were observed in the first columns of all detection zones for the 11 substitution positions, respectively, which yielded a pattern presented as [11111111111] specific to *P. ginseng* (**Figure 4A**). When using Cy5-labeled target from American ginseng, the third columns in zones for detecting nucleotide differences at positions 497, 499, and 501 produced obvious fluorescent signals, indicating a specific pattern [11133311111] for *P. quinquefolius* (**Figure 4B**). In the case of Cy5-labeled target of Japanese ginseng, fluorescent signals were observed in the second columns in zones for detecting substitutions at positions 497, 499, 501, and 712, and almost no signal was obtained in both columns in zone for position 714. Thereby, a specific pattern for *P. japonicus* (Japan) was observed as [11122212011] (**Figure 4C**). In addition, **Figure 4D** shows the fluorescent profile of *P. pseudoginseng*, which possessed considerable nucleotide difference from other *Panax* species (**Table 1**). Fluorescent signals were obtained in the fifth column in zones for detecting positions 497, 499, and 501. Of the zones for detecting nucleotides at other positions, signals were observed at the second, third, second, and second columns of positions 233, 712, 714, and 729, respectively. Thereby, a completely different profile [12155513202] was observed for *P. pseudoginseng*. Correspondingly, specific fluorescent profiles have been confirmed as expected for other *Panax* species.

In addition, identification of health foods derived from five ginseng drugs in the forms of powder, tablet, and capsule has been conducted by using the PNX array. **Figure 4E** shows the identification result of a health food in the form of a capsule, claimed as notoginseng. The signal pattern was observed as [21133311111], which is the specific pattern of *P. notoginseng* due to *P. notoginseng*'s having specific nucleotides at positions 191, 497, 499, and 501. The result indicated this product was prepared from *P. notoginseng*, fitted with its label. In the same ways, another four health food products were identified as two *P. ginseng* and two *P. quinquefolius*, respectively. The partial 18S rRNA gene sequences of these five have been determined, and the results demonstrated the specificity of the developed PNX array. Furthermore, the contents of six major ginsenosides in these five health foods were determined and compared with our previous data (*16*). The obtained chemical constituent patterns were consistent with the molecular identification results, that is, the five products presented characteristic chromatograms (**Figure 5**) and constituent compositions (**Table 3**) corresponding to their original species. Notoginseng showed a simple HPLC chromatogram with three marked peaks of ginsenosides Rg_1, Rb_1, and Rd, together with a flat baseline ranging from 8 to 30 min (**Figure 5A**). Korean ginseng and ginseng presented a relatively complex chromatogram (**Figure 5B**) and had the lowest contents of total ginsenosides, which was quite lower than the other two (**Table 3**). American ginseng was distinguished by an obviously high content of ginsenoside Re. However, the two investigated products in the present study were somewhat different from our previous results; significantly high amounts of ginsenosides Rc and Rd were detected (**Table 3**).

DISCUSSION

The accurate identification of herbal drugs is a prerequisite of clinical application as well as chemical and pharmacological research. Conventional identification methods, including sensory assessment, external features discrimination, and anatomical comparison of internal structures, as well as chemical evaluation (*20*), is based on various phenotypic characters, which are inevitably affected by the growth stage and environment of medicinal plants. Moreover, morphology-based identification depends heavily on the experiences and subjective judgment of operators. In contrast, genotype-based identification, which is not influenced by the above factors, can provide objective resolution. During the past two decades, a variety of molecular methods have been tried for authentication of medicinal plants and herbal drugs, such as random amplified polymorphic DNA (RAPD), arbitrarily primed Polymerase Chain Reaction (AP-PCR), amplification fragment length polymorphism (AFLP), directed amplification of minisatellite region DNA (DAMD), restriction fragment length polymorphism (RFLP), sequence-characterized amplified region (SCAR), direct sequencing of certain DNA regions, PCR-RFLP, amplification refractory mutation system (ARMS) and others (*18, 21–24*). However, a

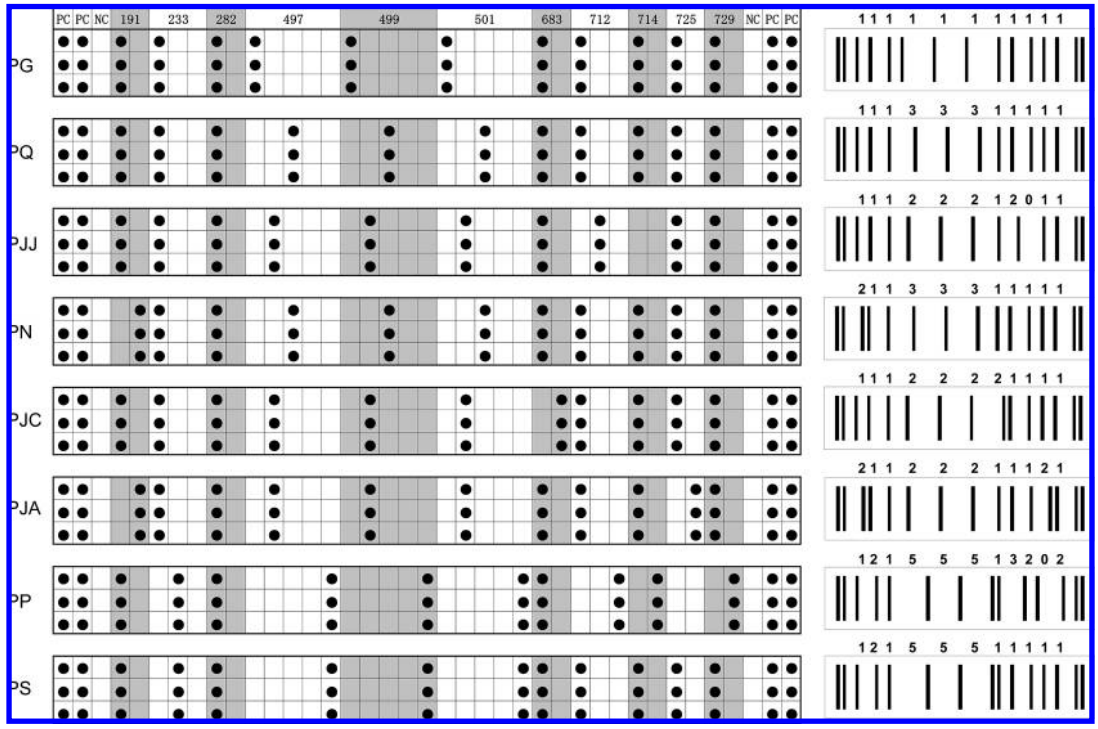

Figure 3. Expected fluorescent patterns of *Panax* species when hybridized to PNX array. Numerals above the box indicate the corresponding substitution position in 18S rRNA gene; two to five probes form a detecting zone for each position separated by white or gray background. Fluorescent signals are presented as the column number in each zone. Finally, fluorescent patterns of *Panax* species can be indicated by barcode (right panel) for easy detection. Positive control is doubly printed on both left and right ends to serve as an indicator of the outline of each array. PC, positive control; NC, negative control.

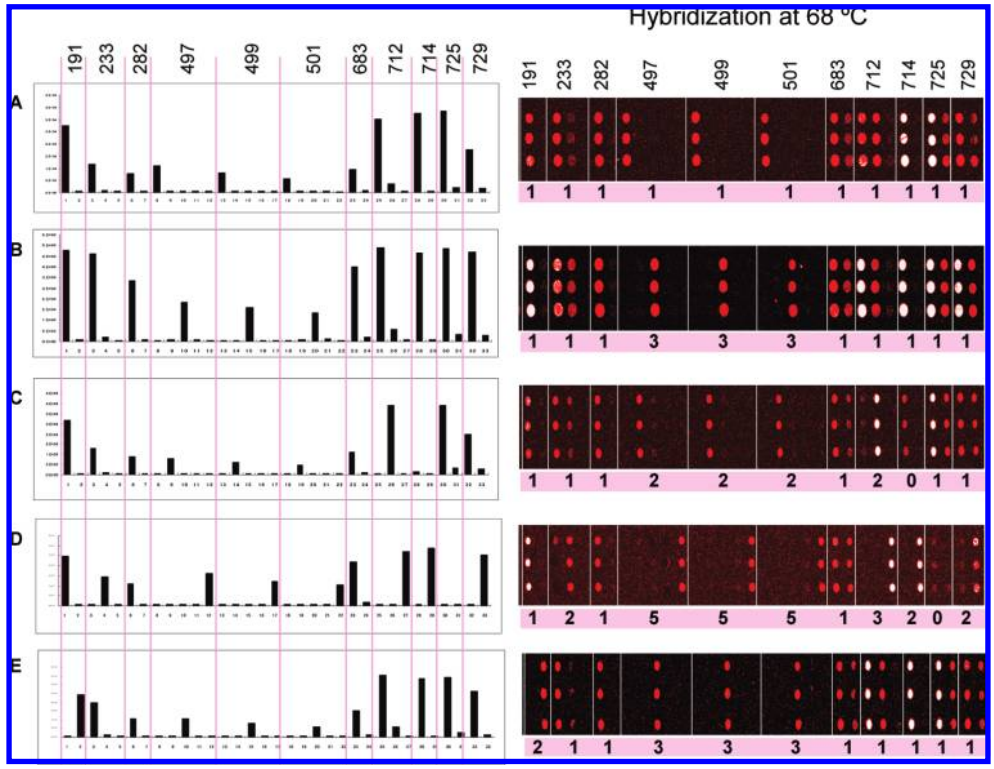

Figure 4. Scanned images (right) and signal intensity plots (left) of PNX array hybridized with fluorescent-labeled targets from *Panax* species and ginseng drugs: (**A**) *P. ginseng* or ginseng; (**B**) *P. quinquefolius* or American ginseng; (**C**) *P. japonicus* or Japanese ginseng; (**D**) *P. pseudoginseng*; (**E**) a health food claimed as notoginseng derived from *P. notoginseng*. Three spots in each column are the same probe printed in triplicate.

high degree of DNA degradation in herbal drugs makes the former five methods difficult to apply.

Microarray, which enables tens of thousands of DNA probes to be spotted on a small solid matrix, is a powerful tool for various research and diagnostic purposes. It has been extensively used in genome-wide gene expression profiling, gene function analysis, genotyping, and SNP detection for the identification of pathogenic bacteria, microsporidian species, etc. (*25*–*27*). In

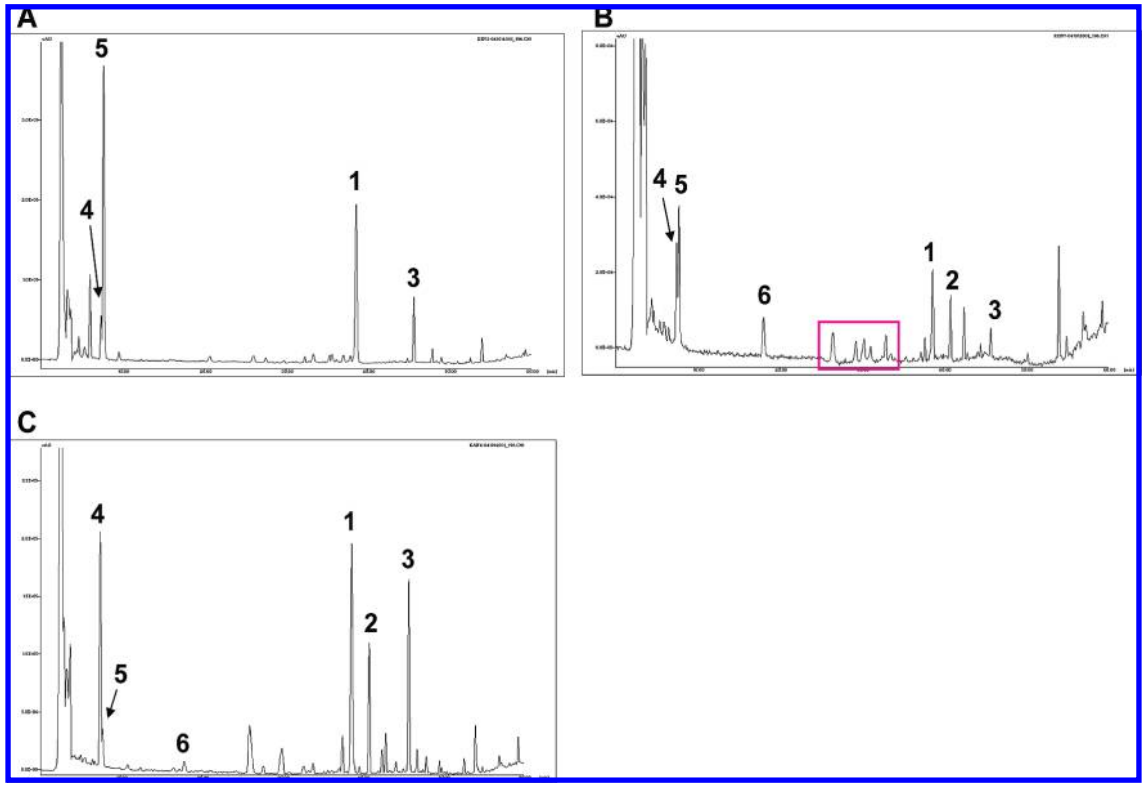

Figure 5. HPLC chromatograms of three health foods claimed as ginseng: (**A**) notoginseng; (**B**) Korean ginseng (peaks within 23−32 min are mainly assignable to malonyl-ginsenosides); (**C**) American ginseng. Six ginsenosides have been quantitatively measured, which are **1**, ginsenoside Rb_1; **2**, ginsenoside Rc; **3**, ginsenoside Rd; **4**, ginsenoside Re; **5**, ginsenoside Rg_1; **6**, ginsenoside Ro.

Table 3. Contents (Milligrams per Gram) of Six Major Ginsenosides in Five Health Foods Claimed as Korean Ginseng, Ginseng, American Ginseng, and Notoginseng

	G-Rb_1	G-Rc	G-Rd	G-Re	G-Rg_1	G-Ro	total
Korean ginseng	2.13	1.72	0.55	1.78	2.27	1.93	10.38
ginseng	5.17	3.54	2.41	3.91	3.51	3.92	22.46
P. ginseng (10 samples)[a]	1.13−5.22	0.48−2.42	0.1−0.47	0.70−1.29	1.21−4.46	0.64−3.03	
American ginseng	19.88	7.56	9.41	13.16	1.82	1.67	53.50
American ginseng	28.86	5.36	8.12	14.62	1.55	3.91	62.41
P. quinquefolius (8 samples)[a]	12.55−21.29	1.05−1.78	1.36−1.90	6.27−10.59	0.32−1.58	2.99−3.57	
notoginseng	19.44	trace	4.39	3.44	25.74	ND	53.01
P. notoginseng (8 samples)[a]	17.95−27.24	trace	4.57−6.31	1.72−3.61	27.85−37.96	ND	

[a] Partial results of our previous study (*16*) on 47 crude drug samples derived from 12 *Panax* taxa, indicating the range of content in samples.

the field of pharmacognostic studies, so far, few trials have been reported by applying this new technology for medicinal plant authentication. Tsoi et al. (*28*) designed six oligonucleotide probes involved in a microarray based on the 26S rRNA gene to identify six *Fritillaria* species. Carle et al. (*29*) prepared a 19-probe-containing microarray based on the 5S rRNA gene sequence to identify 19 toxic traditional Chinese medicinal plants. On the other hand, Zhang et al. (*30*) printed PCR products of the ITS region originating from 16 *Dendrobium* species on glass slides to develop a microarray for the identification of the 16 *Dendrobium* species. However, all three studies applied only one probe for each species, which may leave uncertainty in the identification when targets from closely related species were used.

In the present study, we developed a DNA microarray (PNX array) involving multiple species-specific oligonucleotide probes to produce distinct fluorescent patterns for the identification of *Panax* species and ginseng drugs. The PNX array provides a unique barcode fluorescent pattern for each *Panax* species, enabling an effective authentication. Our results showed this approach could offer accurate authentication of eight *Panax* species as well as their derived ginseng drugs, including the three most popular ones, ginseng, American ginseng, and notoginseng. Especially useful is its ability to unequivocally identify the related health foods even in powder, tablet, and capsule forms.

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