18S RIBOSOMAL RNA GENE SEQUENCES OF THREE *PANAX* SPECIES AND THE CORRESPONDING GINSENG DRUGS

Hirotsahi FUSHIMI, a Katsuko KOMATSU, a, b Masaharu ISOBE a and Tsuneyo NAMBA a

Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University, a, b 2630 Sugitani, Toyama 930-01, Japan Faculty of Engineering, Toyama University, b 3190 Gofuku, Toyama 930, Japan

Total DNA was extracted from the fresh underground parts of three *Panax* separate species. The 18S rRNA regions of extracted DNA were amplified by the polymerase chain reaction (PCR) and their sequences were determined. In each species, the sequences were found to be of 1809 base pairs (bps) but with different gene sequences. Different base substitutions were observed at nucleotide positions 497, 499, 501 and 712. The same procedure was performed on commercial samples of Ginseng Radix, Panacis Japonici Rhizoma and American Ginseng. Each sequence completely corresponded with that of each original plant, namely *P. ginseng*, *P. japonicus* and *P. quinquefolius*, respectively. This is the first time that 18S rRNA gene sequencing on *Panax* species was carried out. Previously, Ginseng drugs have been identified mainly by their external and internal structure. Thus this method will be useful in identifying Ginseng drugs at the gene level.

**KEY WORDS** Ginseng; 18S rRNA gene sequence; *Panax ginseng*; *Panax japonicus*; *Panax quinquefolius*

Ginseng drugs are derived from the underground parts of *Panax* species and are very popular medicines in Asian countries. Among them, "Ginseng Radix" is the most famous and is used as a tonic and a remedy for physical fatigue and weak constitution. Recently, for the purpose of authentication of *Panax* species and Ginseng drugs, some *Panax* species and their adulterants have been studied by random amplified polymorphic DNA (RAPD) analysis. 1, 2, 4

In the present paper, we describe for the first time the sequences of the 18S rRNA gene from three *Panax* species together with the corresponding Ginseng drugs. The rRNA gene sequences demonstrate the usefulness of DNA analysis for the identification of *Panax* species and the corresponding crude drugs.

**MATERIALS AND METHODS**

Total DNA was extracted from the fresh underground parts of *Panax ginseng* (cultivated in Nagano Pref., H.F.14 a), *P. japonicus* (collected in Toyama Pref., H.F.13) and *P. quinquefolius* (cultivated in Toyama Pref., H.F.15) by the modified method of Murray and Thompson. 3, 4 The size of the total DNA was determined by 0.6% agarose gel electrophoresis. The DNA was purified with a Gene Clean II kit (BIO 101) and then amplified by PCR using primers derived from the sequence flanking the 18S rRNA gene. 5 Each primer had the following sequences: 18S F: 5’ CAA CCT GGT TGA TCC TGC TGC T 3’ and 18S R: 5’ CTG ATC CTT CTG CAG GTT CAC CTA C 3’. Thermal cycling was carried out as follows: 7 cycles consisting of 3 min at 94°C and 8 min at 65°C followed by 30 cycles of 40 seconds at 94°C and 8 min.

* To whom correspondence should be addressed.

© 1996 Pharmaceutical Society of Japan
at 65°C, and a final extension for 8 min at 65°C. Sequencing of PCR products was performed with the Dye Deoxy Terminator cycle sequencing kit (Applied Biosystems, U.S.A.) following the manufacturer's instructions using a set of sequencing primers."

The outer part of the crude drugs was peeled off to avoid cross-contamination. The same procedure was performed to extract total DNAs of each Ginseng drug, Ginseng Radix (TMPW No. 12017), Panacis Japonici Rhizoma (No. 12022) and American Ginseng (No. 12020) as described above. Then the region of the 18S rRNA gene from each drug was amplified by PCR, followed by sequence analysis under the same conditions as used for the fresh plants.

RESULTS AND DISCUSSION

The average size of total DNAs extracted from each fresh plant was about 20 kb, and DNAs extracted from each crude drug showed a smear in the electrophoretic profile ranging from 0.5 kb to 20 kb (Fig. 1). The same size of DNA fragments (1.8 kb) was amplified using DNAs from either fresh plants or crude drugs, as shown in Fig. 2. The sequence analysis of the rRNA gene from the three Panax species revealed the total length of amplified fragments to be 1809 bps with an average sequence identity of 99.8% to each other. Base substitutions were observed at nucleotide positions 497, 499, 501 and 712 (Fig. 3). The sequences of the 18S rRNA gene from Ginseng Radix, Panacis Japonici Rhizoma and American Ginseng were also composed of 1809 bps and their sequences were identical to those of each original plant, P. ginseng, P. japonicus and P. quinquefolius, respectively.

Fig. 1. Total DNA extracted from Panax species and Ginseng drugs. Total DNA was visualized by ethidium bromide staining under UV.
Lane 1: Panax ginseng; Lane 2: P. japonicus; Lane 3: P. quinquefolius; Lane 4: Ginseng Radix; Lane 5: Panacis Japonici Rhizoma; Lane 6: American Ginseng; Lane 7: 1 kb ladder (GIBCO, U.S.A.); Lane 8: λHindIII (Nippon Gene, Japan).

Fig. 2. PCR products on 18S rRNA gene regions of Panax species and Ginseng drugs. The PCR product was visualized by ethidium bromide staining under UV.
Lane 1: Panax ginseng; Lane 2: P. japonicus; Lane 3: P. quinquefolius; Lane 4: Ginseng Radix; Lane 5: Panacis Japonici Rhizoma; Lane 6: American Ginseng; Lane 7: 1 kb ladder (GIBCO, U.S.A.).
Fig. 3. Comparison of 18S rRNA gene sequences among *Panax* species. Different base substitutions were observed at nucleotide positions 497, 499, 501 and 712.

In the present study, each *Panax* species was found to have unique sequences in the 18S rRNA gene and the base substitutions were concentrated around nucleotide position 500. The result of a homology search against reported sequences of rRNA genes from other species revealed the highest scores of homology (97.4%) with tomato (*Lycopersicon esculentum*) and soybean (*Glycine max*), respectively. Within the 100 highest homologous sequences, frequent base substitutions were observed around the regions of 180-250, 640-700 and 1690-1750 bps. Within these three *Panax* species, however, there were no base substitutions in those regions. Thus the sequence within those regions seemed to be unique to the *Panax* plants investigated so far. Furthermore, the differences in nucleotide sequence around 500 bps found in *Panax* species would be a useful marker to distinguish each species. The present investigation clearly shows that the three *Panax* plants and their corresponding Ginseng drugs can easily be distinguished from each other by their differences in the nucleotide sequence of the rRNA gene and will be useful in identifying the origin of Ginseng drugs.

ACKNOWLEDGMENTS

We thank Prof. Dr. Masao Hattori of the Research Institute for Wakan-Yaku for providing the DNA sequencer and the staff of the Herbal Garden of Toyama Medical and Pharmaceutical University for supplying specimens of *P. quinquefolius*. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, and by a Grant for Research on Aging and Health from the Ministry of Health and Welfare, Japan.

REFERENCES AND NOTES

3) Plant specimens were stored in the Museum of Materia Medica, Analytical Research Center for Ethnomedicines, Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University (TMPW).
9) The specimen number of crude drugs stored in TMPW.
10) The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the following accession numbers: D83275 for *P. ginseng*; D84100 for *P. japonicus*; and D85172 for *P. quinquefolius*.

(Received August 12, 1996; accepted September 15, 1996)