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Helicase-dependent amplification is effective in distinguishing Asian ginseng from American ginseng

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ABSTRACT

This study describes for the first time the application of helicase-dependent amplification (HDA) to authenticate botanical species and processed products. Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*) are consumed worldwide as functional food and health remedies. The two herbs share similar morphological appearance but have different pharmacological effects. In this work, a novel isothermal amplification mediated DNA method was applied to authenticate the two ginseng species. Internal control and *P. ginseng* specific primers were designed based on the ribosomal external transcribed spacer (ETS) region. The amplification results were confirmed by real-time monitoring, gel electrophoresis and DNA sequencing. The screened retail samples included dried ginseng root, ginseng powder, ginseng tea granules as well as a four-herb formulation. Our HDA protocol worked well on both purified DNA and crude water extract. In conclusion, HDA is a highly sensitive and specific approach for differentiating Asian ginseng from American ginseng and it has the potential for on-site authentication of herbal products.

1. Introduction

Ginseng is widely consumed around the world as food, health supplements and traditional medicines. Asian ginseng (*Panax ginseng*) which is mainly cultivated in China and South Korea together with American ginseng (*Panax quinquefolius*) which is mainly cultivated in USA and Canada are the most produced and consumed ginseng species (Baeg & So, 2013). This is probably due to the good reputation and increasing scientific evidences for their efficacies (Chen, Chiou, & Zhang, 2008). In general, both materials are tonics to enhance physical functions and protect the body from stress, fatigue and aging. Nevertheless, they are different in nature and pharmacological effects. In the theory of traditional Chinese medicine, Asian ginseng is “warm” and usually used in “yang-deficient” condition to enhance vital energy (“Qi”). In contrast, American ginseng is “cool” and usually used in “yin-deficient” condition to reduce the “internal heat” (Chan et al., 2000). The composition of ginsenosides, the major effective components of ginseng species, is also different in the two herbs. Asian ginseng has a high ratio of Rg1:Rb1 while American ginseng has a low ratio of Rg1:Rb1 (Chen et al., 2008). The ratio of Rf:F11 is another phytochemical marker to distinguish the two species (Leung & Wong, 2010). Despite these facts, according to the morphological observation and DNA analysis, they are still the most closely related ginseng species (Ngan, Shaw, But, & Wang, 1999). It becomes more difficult to distinguish the two herbs after they are processed into commercial products. It has been reported that health products commercially labeled as Asian ginseng were indeed American ginseng (Wallace et al., 2012). To guarantee the quality and efficacy of ginseng products, accurate identification is important. Traditional authentication of herbal materials including morphological and histological inspection is largely dependent on the experience of the examiner and not applicable for processed products. Chemical analysis including HPLC (high performance liquid chromatography), GC (gas chromatography) and MS (mass spectrometry) is independent of the physical form of the examined samples and generally accepted for quality control of food and herbal

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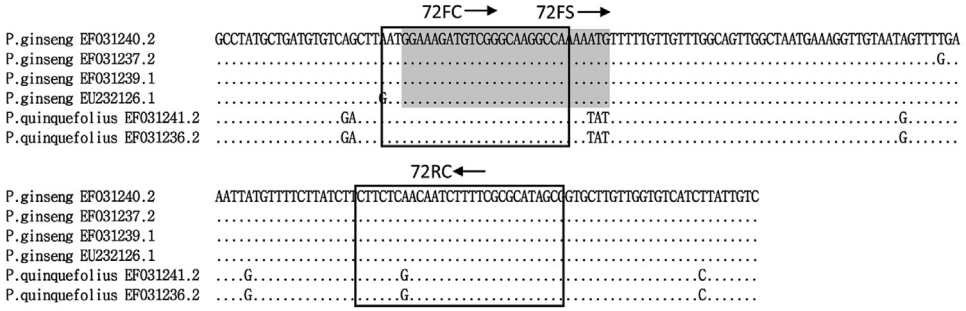
Table 1
The information of tested samples and screening results. "+" means positive amplification in HDA, "−"means negative amplification, "N/A" means not tested. "NICPBP" represents National Institute for the Control of Pharmaceutical and Biological Products (China).

Generic name	Species name	Sample code	Sample form	Collection place	Internal control	Specific HDA
Asian ginseng	*P. ginseng*	T3118	Dried root	Northeast, China	+	+
Asian ginseng	*P. ginseng*	T3119	Dried root	Northeast, China	+	+
Asian ginseng	*P. ginseng*	T3120	Dried root	Jilin, China	+	+
Asian ginseng	*P. ginseng*	T2921	Dried root	Russia	+	+
Asian ginseng	*P. ginseng*	T2928	Dried root	Russia	+	+
Asian ginseng	*P. ginseng*	T2870	Powder	Local store	+	+
Asian ginseng	*P. ginseng*	T3365	Dried root	Jilin, China	+	+
American ginseng	*P. quinquefolius*	T1745	Dried root	Local store	+	−
American ginseng	*P. quinquefolius*	T1746	Dried root	America	+	−
American ginseng	*P. quinquefolius*	T1747	Dried root	America	+	−
American ginseng	*P. quinquefolius*	T2878	Dried root	Local store	+	−
American ginseng	*P. quinquefolius*	T2876	Powder	Local store	+	−
American ginseng	*P. quinquefolius*	T2879	Dried root	Local store	+	−
American ginseng	*P. quinquefolius*	T2874	Tea granule	Local store	+	−
American ginseng	*P. quinquefolius*	T2884	Tea granule	Local store	+	−
Notoginseng	*P. notoginseng*	T186	Standard material	NICPBP	−	N/A
Notoginseng	*P. notoginseng*	T1105	Dried root	China	−	N/A
Purple jasmine	*M. jalapa*	T1824	Specimen	Jiangxi, China	−	N/A
Purple jasmine	*M. jalapa*	T1840	Specimen	Sichuan, China	−	N/A
Pokeberry	*Ph. acinosa*	T3127	Dried root	China	−	N/A
Pokeberry	*Ph. acinosa*	T3165	Dried root	Hunan, China	−	N/A

medicine. However, the chemical profiles of botanical materials vary by the growth environment, harvest time, storage conditions and processing procedures (Shaw, Ngan, But, & Wang, 2002). These analyses are also complex and require expensive equipment which is not ideal for field application. DNA is relatively constant and has become a popular complementary tool for authentication. Regarding ginseng, DNA authentication has been applied to single herbal products (dried roots, powder, capsule, tea and red ginseng extract) (Wang et al., 2011) and various marketed herbal medicine preparations (Shim et al., 2005). Our group has previously applied arbitrarily-primed PCR (Cheung, Kwan, But, & Shaw, 1994), RAPD (random amplified polymorphic DNA) (Shaw & But, 1995), DALP (direct amplified length polymorphism) (Ha, Yau, But, Wang, & Shaw, 2001), SCAR (sequence-characterized amplification region) (Wang, Ha, Ngan, But, & Shaw, 2001) and AFLP (amplified fragment length polymorphism) (Ha, Shaw, Liu, Yau, & Wang, 2002) to authenticate ginseng species. Most of these approaches require DNA amplification by polymerase chain reaction (PCR) and a thermal cycler. To streamline the amplification process for on-site authentication, we tested the feasibility of using HDA isothermal amplification to authenticate ginseng. HDA is a novel isothermal amplification technique (Vincent, Xu, & Kong, 2004). Its reaction scheme is similar to PCR, but the template DNA is unwound by a helicase instead of heat. To our knowledge, this is the first report of

a



b

Primer	Sequence (5'>3')	Type	Internal control	Specific HDA
72FC	AATGGAAAGATGTCGGGCAAGGCCA	Common forward	√	
72RC	GCTATGCGCGAAAAGATTGTTGAGAAG	Common reverse	√	√
72FS	GGAAAGATGTCGGGCAAGGCCAAAAATG	Specific forward		√

Fig. 1. Primer design for internal control and specific HDA. (a) ETS sequences of *P. ginseng* and *P. quinquefolius* were retrieved from NCBI and aligned. The internal control primers (72FC/72RC) were designed based on the conserved sequence as indicated in empty boxes. *P. ginseng* specific primer (72FS) was designed according to the polymorphic region as shown in the grey box. Arrows represent the direction of 5′ to 3′ amplification in HDA. (b) Information of HDA primers. 72FC/72RC primers were used for internal control; while 72FS/72RC primers were used for specific HDA.