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Genotyping of *Ganoderma* species by improved random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analysis

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ABSTRACT

Species of *Ganoderma* are used in traditional medicines. An improved random amplified polymorphic DNA (RAPD) analysis, where the RAMP time is prolonged, has been used to characterize the genetic variation in some well known species of *Ganoderma*. The DNA materials were collected from ten *Ganoderma* strains, amplified with randomly selected 24 RAPD primers and evaluated by agarose gel electrophoresis. A cluster dendrogram was constructed for genetic analysis on the basis of amplification results. The improved RAPD amplified DNA with consistent and clear banding patterns. A total of 316 bands were found with 93% polymorphism. There was a significant genetic distance between the different strains of *Ganoderma*, with an index of similarity coefficient in the range of 0.52–0.74. The inter-simple sequence repeat (ISSR) analysis of the *Ganoderma* DNA samples showed similar trend results to the RAPD analysis with 0.49–0.81 similarity coefficients. This study reports the high level of genetic differences between different species or strains of a single species of *Ganoderma* and confirms the significance of the improved RAPD method in genetic characterization of organisms. Therefore, the improved RAPD combined with ISSR techniques might be used for the genetic characterization of organisms.

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1. Introduction

In traditional medicinal practices, *Ganoderma* mushroom species have been used for more than two thousand years. These medicinal mushrooms are popularly known as their Chinese name '*língzhī*' or Japanese name 'Reishi'. Although *Ganoderma lucidum* is the most popular for its medicinal importance, other species of *Ganoderma* also possess significant health beneficial properties. A number of studies have reported the health beneficial effects or medicinal importance of different *Ganoderma* species against a number of life-threatening diseases, including cancer, cardiovascular disease, diabetes, immunodeficiency, liver disease, and neurodegenration (Hajjaj et al., 2005; Ma et al., 2011; Chu et al., 2012; Tie et al., 2012; Xiao et al., 2012; Jin et al., 2012; Boh, 2013; Shi et al., 2013; Wang et al., 2013; Wu et al., 2013; Yue et al., 2013). Also these mushrooms have potential antioxidant and antimicrobial activities (Ofodile et al., 2005; Li and Wang, 2006; Shi et al., 2013; Heleno et al., 2013). Because of their significant medicinal importance, *Ganoderma* mushrooms lay on the mainstream of Chinese traditional medicine, and they are listed as recognized traditional medicines in American Herbal Pharmacopeia (http://www.herbal-ahp.org/documents/BRM-CRS%20List/ahp-brm-list_5_2013.pdf). The medicinal properties of *Ganoderma* are due to their chemical composition, specifically polysaccharides. However, nutritional and chemical composition of mushrooms are affected by several environmental factors, including differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis, time interval between harvest and measurement methods (Khan et al., 2008). Also different genetic characteristics have significant influence. The variations in the geography and environment in China have produced vast number of biodiversity, including noticeable variations within genus and species. *Ganoderma* species are grown wildly and commercially all over China. There are so many variations in their size, shape, color and odor within these species. These might be due to their variable genetic characteristics. The genetic studies of these medicinal mushrooms are not so many. Some earlier studies have interpreted the gene phylogeny and molecular characterization of *Ganoderma* (Moncalvo et al., 1995; Sokol et al., 1999). Later specific genes in *Ganoderma* species have been characterized, but very few studies were performed for the measurement of genetic distance and diversity between intra or inter species of *Ganoderma*. In this study, we have collected the DNA materials from different *Ganoderma* species and strains, and aimed to characterize them genetically by using DNA fingerprinting.

Random amplified polymorphic DNA (RAPD) analysis, along with some other molecular techniques like amplified fragment length polymorphism (AFLP) analysis, simple sequence repeat (SSR) analysis, inter-simple sequence repeat (ISSR) analysis have been used for the genetic characterization of different animals, plants, fungi and other organisms (Agarwal et al., 2008; Shizanto et al., 2009; Zhang et al., 2012; Liu et al., 2012). At present, RAPD analysis is not only used for genetic characterization of different organisms, but also used for identification and the study of genetic diversity in synthetic or newly found species, which have importance agriculturally and industrially (Bhat et al., 2012; Shakeel et al., 2013). As a molecular marker, RAPD has gained popularity because of some advantages, for example, DNA sequence information is not needed in this technique; fewer requirements of template DNA; no hazardous contamination; and cheap technology. However, there remain some disadvantages too, including low production and poor reproducibility. Interestingly, the resolution and production can be greatly increased by an improved RAPD technique, named RAMP-PCR, where ramp time of traditional RAPD-PCR is increased from annealing to extension, i.e., ramp time from the step of annealing to extension is elongated (Fu et al., 2000, 2013). For the genetic characterization of some species of *Ganoderma* from different origins, we have employed our developed RAMP-PCR in this study. To validate the improved RAPD results, we have further analyzed the ISSR of tested *Ganoderma* samples.

2. Materials and methods

2.1. Experimental reagents

The RAPD primers were purchased from SBS Genetech Corporation (Table 1). 2 × PCR Taq Mastermix was purchased from TianGen Biotech Co. Ltd (Beijing, China). DNA Markers were purchased from Takara Biotechnology (Dalian) Co. Ltd in China. The potato dextrose broth (PDB) medium, used for fungal growth were prepared as follows: 200 g of diced potatoes were

Table 1
Sources of RAPD *Ganoderma* samples.

No.	Accession name	Sources of deposit	Deposit no.
001	*Ganoderma gibbosum* (Blumii et Nees) Patouillard	Guangdong Culture Collection Center	GIM5.6
002	*Ganoderma tropicum* (Jungh.) Bres.	Guangdong Culture Collection Center	GIM5.289
003	*Ganoderma applanatum* (Pers.ex Wullr) Pat	Guangdong Culture Collection Center	GIM5.282
004	*Ganoderma australe* (Fr.) Pat	Guangdong Culture Collection Center	GIM5.288
005	*Ganoderma sinense*	Institute of Microbiology of Chinese Academy of Sciences	CGMCC5.0069
006	*Ganoderma* sp	Institute of edible Fungi of Fujian academy of Agricultural Sciences	ACCC51329
007	*Ganoderma lucidum* (Curtis) P. Karst	Institute of Edible Fungi of Fujian Academy of Agricultural Sciences	CFCC85862
008	*Ganoderma lucidium*	Institute of Microbiology of Chinese Academy of Sciences	CGMCC5.0026
009	*Ganoderma neojaponicum* Imazeki	Beijing Agricultural University	CFCC87599
010	*Ganoderma lucidium* (Leysser Fr.) Karst.	Institute of Wensheng Edible Fungi in Shantou	GIM5.250SL