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RESEARCH ARTICLE

Genetic variation and population structure of the mushroom *Pleurotus ferulae* in China inferred from nuclear DNA analysis

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Abstract

To investigate the genetic diversity of an edible fungus *Pleurotus ferulae*, a total of 89 wild samples collected from six geographical locations in the Xinjiang Uygur Autonomous Region of China and two geographical locations in Italy, were analyzed using three DNA fragments including the translation elongation factor (EF1α), the second largest subunit of the RNA polymerase II (RPB2) and the largest subunit of the RNA polymerase II (RPB1). The results indicated relatively abundant genetic variability in the wild resources of *P. ferulae*. The analysis of molecular variance (AMOVA) showed that the vast majority of the genetic variation was found within geographical populations. Both the Chinese populations and the Italian populations of *P. ferulae* displayed a limited genetic differentiation. The degree of differentiation between the Chinese populations and the Italian populations was obviously higher than that between the populations from the same region, and moreover the genetic differentiation among all the tested populations was correlated to the geographical distance. The phylogeny analyses confirmed that samples from China and Italy belonged to another genetic group separated from *Pleurotus eryngii*. They were closely related to each other but were clustered according to their geographical origins, which implied the Chinese populations were highly differentiated from the Italian populations because of distance isolation, and the two populations from different regions might be still in the process of allopatric divergence.

Keywords: genetic variation, population structure, genetic differentiation

1. Introduction

Many fungal species have a wide geographical distribution, and the generalist species distributed at large scale may actually include multiple genetic lineages or cryptic species (Jargeat *et al.* 2010; Douhan *et al.* 2011). Morphological observations usually can not distinguish closely related genetic groups since the fruiting bodies of macro fungi were susceptible to environmental influences. Nevertheless, population genetics and phylogenetic approaches not only

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help to solve the above problems but also provide insights into the population structure and the leading factors of population differentiation (Zheng *et al.* 2015; Cogliati *et al.* 2016; Feng *et al.* 2016).

Pleurotus ferulae, which is commonly called "A Wei Gu", is a major branch of the *Pleurotus eryngii* species complex (Mang and Figliuolo 2010; Rodriguez Estrada *et al.* 2010). However, its taxonomical position has always been controversial. Some researchers regarded it as a separate species, namely *P. ferulae*, while others considered it to be a variety of *P. eryngii*, viz. *P. eryngii* var. *ferulae* (Urbanelli *et al.* 2002; Rodriguez Estrada *et al.* 2010). *P. ferulae* and *P. eryngii* are morphologically similar during the early development stages of the fruiting body. Moreover, their ITS regions lacked the variation (Ro *et al.* 2007). Therefore, *P. ferulae* was usually confounded with *P. eryngii*. However, previous studies have shown that they are different in ecological, physiological, and genetic characteristics (Urbanelli *et al.* 2002; De Gioia *et al.* 2005).

P. ferulae distributed throughout the Mediterranean region and only inhabited in the northwest part of China, such as Ili, Tacheng, and Altay in Xinjiang Uygur Autonomous Region (Chen 1986). Recently, many studies of *P. ferulae* have been focusing on the properties of bioactive substances extracted from mycelia or fruiting bodies (Alam *et al.* 2012; Ding *et al.* 2013), and the cultivation techniques using agricultural and industrial residues (Akyuz and Yildiz 2008; Akyuz and Kirbag 2009, 2010). Therefore, this mushroom is important both economically and ecologically.

So far, most studies of population genetics on fungi were conducted at a local-scale. Meanwhile, mycologists have been continuously exploring the genetic structure of large-scale populations (Taylor *et al.* 2006; Halling *et al.* 2008). Both *Ganoderma applanatum*/*G. australe* complex and *Schizophyllum commune* were widely distributed geographically as a result of the long-distance dispersal, but significant genetic differentiation existed among their geographical populations (James *et al.* 1999; Moncalvo and Buchanan 2008). *P. ferulae* is an edible fungus of exploitation value, but only a few studies were found on genetic diversity of this mushroom. More importantly, those studies were carried out at a local-scale by using the samples only from Mediterranean countries (Lewinsohn *et al.* 2001; Urbanelli *et al.* 2003). The genetic diversity of *P. ferulae* in Xinjiang of China remains poorly understood. The genetic structure and differentiation for large-scale populations of this mushroom have never been reported yet.

In the present study, the genetic diversity of 89 samples of *P. ferulae* from Italy and the Xinjiang Uygur Autonomous Region of China was analyzed using DNA fragments. Some previous studies showed that single-copy protein encoding regions were generally superior to other genes for revealing relationships at various taxonomic levels (Schoch *et al.* 2009), and especially more suitable for diploid species (Xu 2006). Rodriguez Estrada *et al.* (2010) proved that the translation elongation factor (EF1α) and the second largest subunit of the RNA polymerase II (RPB2) were useful for inferring phylogenetic relationships among varieties of *P. eryngii* species complex. In addition, the two DNA fragments have been widely used in the population structure studies (Bergemann *et al.* 2009; Li *et al.* 2010). Brewer and Milgroom (2010) used three nuclear genes of *ITS*/*IGS*, *BTUB* and *EF1α* to reveal that populations of *Erysiphe necator* within the eastern US are geographically differentiated. *ITS*, *BTUB* and *EF1α* were used in comprehensive analyzation to get a discovery that a continuing long-distance gene flow had a pronounced impact on the geographic population structure for the two arctic-alpine lichens, *Flavocetraria cucullata* and *Flavocetraria nivalis* (Geml *et al.* 2010). In this study, we investigated the genetic diversity of *P. ferulae* distributed in China by three nuclear DNA fragments of EF1α, RPB2 and the largest subunit of the RNA polymerase II (RPB1), and explored genetic differentiation between populations from China and Italy. The factors that influence the genetic differentiation were also inferred.

2. Materials and methods

2.1. Sampling

Seventy-eight wild samples of *P. ferulae* were collected from six locations in two regions of Yumin and Tuoli in Xinjiang, China from April to May in 2009 and 2010 (Fig. 1). These sampling regions spanned approximately 110 km from the east to the west and approximately 70 km from the south to the north. In order to avoid sampling the same genotypes several times, we collected all the isolates at least 10 m away from each other. Each of the remaining samples distributed in Italy was a gift from Prof. M. Reverberi of La Sapienza University of Rome, Italy. The sample size and the geographical coordinates for each geographical population were presented in Table 1.

2.2. DNA extraction

Mycelia for DNA extraction were cultured on PDA plates with cellophane at 25°C for two weeks. The total DNA was extracted using a DP305-Plant Genome Extraction Kit (Tiangen, China). The purity and quality of the genomic DNA were determined through spectrophotometry and electrophoresis on 1.0% agarose gel. The DNA solution was stored at –20°C.

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