INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–971/2013/15–3–479–485
http://www.fspublishers.org

Full Length Article

Evaluation of Growth Characteristics and Genetic Diversity of Commercial and Stored Lines of *Hypsizygus marmoreus*

Chengshu Qiu[1,2], Wenjuan Yan[2], Peng Li[1,2], Wangqiu Deng[2], Bin Song[2] and Taihui Li[1,2*]
[1]*School of Bioscience and Bioengineering, South China University of Technology, Guangzhou 510006, P.R. China*
[2]*Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, State Key Laboratory of Applied Microbiology (Ministry - Guangdong Province Jointly Breeding Base) South China, Guangdong Institute of Microbiology, Guangzhou 510070, P.R. China*
*For correspondence: mycolab@263.net; qiuqu200212@gmail.com

Abstract

Hypsizygus marmoreus is an edible mushroom of commercial importance with medicinal properties. Therefore, cultured commercial and stored strains of the fungus were evaluated for various cultivation characteristics (hyphal growth, and size/morphology of fruiting body), and genetic diversity among the strains by inter-simple sequence repeat (ISSR) analysis. We collected 32 *H. marmoreus* strains from different locations in China, and cultured them at the Gumuzhen Bio-technology facility. The cultivation characteristics differed among the main *H. marmoreus* strains. The genomic DNA extracted from *H. marmoreus* was amplified with 18 ISSR primers, which generated 199 bands The average number of amplified bands per primer was 11.10 (mean polymorphism 87.4%). The polymorphism information content (PIC) value for ISSRs ranged from 0.39 to 0.50 (mean PIC value per ISSR 0.48), indicating a medium level of polymorphism among the strains. The analysis of cultivation characteristics and ISSR data showed that there was rich diversity among the strains of *H. marmoreus*. A cluster analysis based on ISSR results classified the 32 of strains of *H. marmoreus* into three main groups. These results showed that ISSR is a useful tool for selecting potential strains and predicting hybrid performance in the outbreeding of mushrooms.

Keywords: Cultivation; Morphology characteristics; Polymorphism information content; Genetic diversity; Marker index

Introduction

Hypsizygus marmoreus (Peck) H.E. Bigelow is a small mushroom widely distributed throughout Europe, Asia, and North America. It grows on dead wood, for example wind-thrown trees or tree stumps and produces fruiting bodies in autumn (Huang, 1990; Akihisa *et al.*, 2005; Bao and You, 2011; Monira *et al.*, 2012). Recently, *H. marmoreus* has become one of the most popular edible and medicinal mushrooms in East Asia, because of its pleasant flavor, and nutritional benefits. However, there is little information about the characteristics of cultivated strains of this fungus, and only limited information about the genetic diversity among lines and cultivars. A reasonable level of genetic diversity is required for sustainable mushroom production because it act as a buffer against the spread of diseases. It is also useful for selecting of genetically and morphologically diverse parents to develop new strains.

The use of molecular markers is an efficient, effective, and rapid way to analyze genetic diversity. Compared with other methods, this method is less affected by environmental factors and by age and physiological condition of the samples, and (Ali *et al.*, 2008; Bohn *et al.*, 1999; Al-Rawashdeh, 2011). Various molecular marker techniques have been used to analyze the genetic diversity of edible mushrooms (Du *et al.*, 2012; Liu *et al.*, 2012; Pawlik *et al.*, 2012; Anderson *et al.*, 2010). For instance, random amplified polymorphic DNA markers were used to study the diversity of *Tricholoma matsutake* (Bao *et al.*, 2007; Nazrul and Bian, 2010a; Tonk *et al.*, 2011). Although PCR-based molecular markers have been widely used in genetic studies, their applications in analyses of edible fungi are limited because of the lack of reference sequencing data. The inter-simple sequence repeat (ISSR) developed by Zietkiewicz *et al.* (1994), is PCR-based method that involves amplification of a DNA segment present at an amplifiable distance between two identical micro-satellite repeat regions oriented in opposite directions. The ISSR method has several advantages over other molecular marker methods; that is, DNA sequence information is not required before amplification, it is inexpensive, simple and highly stable, and it yields abundant genomic information (Bohn *et al.*, 1999; Camacho and Liston, 2001; Patzak, 2001; Behera *et al.*, 2008; Sabou *et al.*, 2010). It can be used to identify species or varieties, to evaluate genetic diversity, in breeding programs, and for genomic finger printing (Bornet and

To cite this paper: Qiu, C., W. Yan, P. Li, W. Deng, B. Song and T. Li, 2013. Evaluation of growth characteristics and genetic diversity of commercial and stored lines of *Hypsizygusmarmoreus. Int. J. Agric. Biol.*, 15: 479–485

Branchard, 2001; Joshi *et al.*, 2004; Reddy *et al.*, 2002; Thangavelu *et al.*, 2012; Zhang *et al.*, 2012). Lee *et al.* (2012) and Wang *et al.* (2009) developed sequence-characterized amplified region (SCAR) and amplified fragment length polymorphism (AFLP) marker to identify commercial and wild strains of *H. marmoreus*. However, only a few strains and primers were used. To expand on those results, we cultivated *H. marmoreus* strains under factory production standards to study various cultivation characteristics (growth of hyphae, time required for mycelia to cover the substrate, and the morphology and weight of fruiting-bodies). We also used 18 ISSR primers to analyze the genetic diversity of 32 Chinese strains of *H. marmoreus.* Two indices obtained from the ISSR data, namely, polymorphism information content (PIC) and marker index (MI), were useful to estimate genetic diversity and to distinguish strains of *H. marmoreus.*

Materials and Methods

Fungus Materials

The 32 strains of *H. marmoreus* were collected from the following institutions: nine strains from the Agricultural Culture Collection of China, Beijing, seven strains from Provincial Institutes of Agricultural Science (2 from Fujian, 2 from Hunan, 2 from Sichuuan, 1 from Zhejiang); one strain from Huazhong agriculture university; four strains from Institutes of Edible Fungi and Mushroom (2 from Gaoyou, 1 from Tianda, 1 from Mianyang; one strain from Changbaishan, Jinlin; and five strains from commercial mushroom enterprises of mushroom (three from Shanghai, 1 from Shandong, 1 from Guangdong). Of the 32 strains, 10 were stored strains and 22 were commercial strains (see Supplementary materials). For each strain, mycelia were cultured on potato sucrose agar medium at 25°C before the experiments.

Cultivation of *H. marmoreus*

To culture each strain, the mycelia from a liquid culture were inoculated onto solid substrate (a commercial formulation used at the Gumuzhen Bio-technology facility) in a wide-mouth polypropylene bottle, and grown for approximately 80 d at 20°C. Fruiting was induced by reducing the temperature to 15.6°C in an incubation chamber with 1,700—3,000ppm CO_2 and 96% relative humidity. Each of the strains had 16 replications.

DNA Extraction

Genomic DNA was extracted from fresh mycelia using the Sangon Fungus Genomic DNA Extraction kit (Sangon Biotech Co., Ltd., Shanghai, China). DNA concentration and purity were determined by spectrophotometry (BioSpec-nano, Shimazu, Japan) and electrophoresis on 1.4% agarose gels with known standards.

ISSR PCR

We tested and screened 50 ISSR primers synthesized by Sangon (Sangon Biotech Co., Ltd., Shanghai, China) using 7 DNA samples from *H. marmoreus* strains. The primers tested were those published by the University of British Columbia Canada (UBC set no. 9). We selected 18 ISSR primers capable of producing clear and reproducible bands for amplification of all DNA samples (Table 1).

PCR was performed in a 25 μL reaction mixture containing 30ng template DNA, 1.5 μL 2.5 mM dNTP, 1.5 μL 25 mM $MgCl_2$, 1.5 μL 10×buffer (0.1 mM EDTA, 10 0 mM KCl, 20 mM Tris-HCl in pH8.0), 1 μL 10 mM primers, and 0.5 μL 2.5U of Taq DNA polymerase (Tiangen Biotech, Beijing, China). A blank control and three negative controls were designed to improve the accuracy and reliability of ISSR results. The three negative controls contained DNA from *Coprinus comatus*, lack primers, or lack Taq polymerase. To ensure the specificity of the experiment, only the bands specific to *H. marmoreus* were counted; that is, bands that were also amplified from *C. comatus* were excluded from analyses. Controls without primers or Taq polymerase were used to detect and eliminate reagent contamination.

Amplification was performed using a Biometra Professional The rmocycler (Biometra, GmbH, Germany) with optimized annealing temperatures for individual primers. The thermal cycling conditions were as follows: initial strand separation cycle at 94°C for 10 min, followed by 35 cycles of 1min at 44.1 to 53.1°C (Table 1), extension at 72°C for 2.5 min, and final extensions for 10 min at 72°C. Products were stored at 4°C until analysis.

Amplified products were electrophoresed in 2% agarose gel with 0.5 Tris/Borate/EDTA buffer at 140 V for 2h and then stained with ethidium bromide (0.5 g/mL). Gels with amplification fragments were visualized and photographed under ultraviolet light using a GE Image Quant Digital Imaging System (GE Healthcare Bio-Sciences AB, Sweden). The Normal Rum™ Prestained 250 bp-II DNA ladder was used as a molecular size standard (Generay Biotech. Co., Ltd., Shanghai, China).

Data Analysis

ISSR amplification products were compared with markers and scored using a binary code (present,1; absent, 0) using Image-master 1D software (Gel-Pro Analyzer, Media Cybernetics, Bethesda, Maryland). Only well-separated bands with a high intensity of polymorphism were selected as markers. Pairwise comparisons were calculated using Jaccard′s coefficient (Jaccard, 1901). The similarity values were used to generate a consensus tree using the un-weighted pair group method with arithmetic mean (UPGMA) algorithm (Nei, 1973). Analyses were performed with NTSYS-pc version 2.1 (Rohlf, 2000). The PIC, MI,

Table 1: ISSR markers used to analyze of genetic diversity of *H. marmoreus*

Primer	Sequence(5′→3′)	AT(°C)	TB	PB	P(%)	PIC	MI	FSR (bp)
UBC807	$(AG)_8$T	46.5	13	12	92.3	0.50	6.50	250-2000
UBC808	$(AG)_8$C	49.7	14	11	78.6	0.43	6.02	450-2200
UBC810	$(GA)_8$T	45.1	12	10	83.3	0.49	5.88	250-2800
UBC811	$(GA)_8$C	45.8	14	11	78.6	0.50	7.00	400-3000
UBC812	$(GA)_8$A	45.8	13	12	92.3	0.49	6.37	250-3000
UBC822	$(TC)_8$A	45.3	13	11	84.6	0.48	6.24	200-3000
UBC823	$(TC)_8$C	45.5	8	8	100.0	0.48	3.84	250-2000
UBC824	$(TC)_8$G	46.5	5	5	100.0	0.48	2.40	300-4000
UBC834	$(AG)_8$(C/T)T	53.1	14	10	71.4	0.47	6.58	200-3000
UBC835	$(AG)_8$(C/T)C	46.3	6	5	83.3	0.50	3.00	500-2000
UBC836	$(AG)_8$(C/T)A	47.9	8	7	87.5	0.49	3.92	100-1500
UBC840	$(GA)_8$(C/T)T	46.3	9	9	100.0	0.50	4.50	250-1200
UBC841	$(GA)_8$(C/T)C	46.1	9	9	100.0	0.50	4.50	250-1500
UBC842	$(GA)_8$(C/T)G	50.9	16	16	100.0	0.50	8.00	200-2200
UBC853	$(TC)_8$(A/G)T	44.6	12	11	91.7	0.39	4.68	250-3000
UBC854	$(TC)_8$(A/G)G	44.1	9	7	77.8	0.50	4.50	250-3000
UBC899	CATGGTGTTGGTCATTGTTCCA	49.8	14	9	52.9	0.43	6.02	200-3000
UBC900	ACTTCCCCACAGGTTAACACA	50.1	10	10	100.0	0.45	4.50	250-2200
Total			199	173				
Minimum			5	5	52.9	0.39	2.40	
Maximum			16	16	100.0	0.50	8.00	
Mean			11.10	9.61	87.46	0.48	5.25	

AT: annealing temperature. TB: total ISSR band. PB: polymorphic bands. P(%): Polymorphism. PIC: polymorphism information content. MI: marker index. FSR: fragment size range

qualitative nature of data, and effective MI were calculated as follows:

PIC values measure the information of a given DNA marker, $PIC_i = 1-\sum(P_{ij})^2$, where P_{ij} is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers (Botstein *et al.*, 1980; Anderson *et al.*, 1993). The MI is the product of the total number of loci per primer (*n*). The MI was calculated for each ISSR primer as MI = PIC × ηβ, where PIC is the mean PIC value, η is the number of bands, and β is the proportion of polymorphism (Powell *et al.*, 1996).

Results

Cultivation of *H. marmoreus*

The *H. marmoreus* mycelia were cultivated on a solid substrate in polypropylene bottles. The strains FJNK-1, HN-1, and JSTD could not grow on sawdust. GDGM26173 and HNNK formed only or two very small fruiting bodies at harvest time. The remaining strains grew on solid substrate, produced white mycelia, and produced fruiting bodies during cultivation (Fig. 3). The shortest time for hyphae to cover the fully substrate (HCFS) was 33 d (Fig. 3). After the decrease in temperature to induce fruiting, 27 strains produced fruiting bodies. The color of the fruiting body varied among the strains: dark grey, dark stripe, grey brown with water spots, grey, white, or ivory white (Fig. 1). The pileus ranged from 1 to 60 mm in height, while the stipe range from 20 to 90 mm in height. The fresh weight was 0 to227gper bottle. The *H. marmoreus* strains could be divided into several types according to the fruiting body morphology (color, shape, uniformity). Fig. 1 shows typical and representative

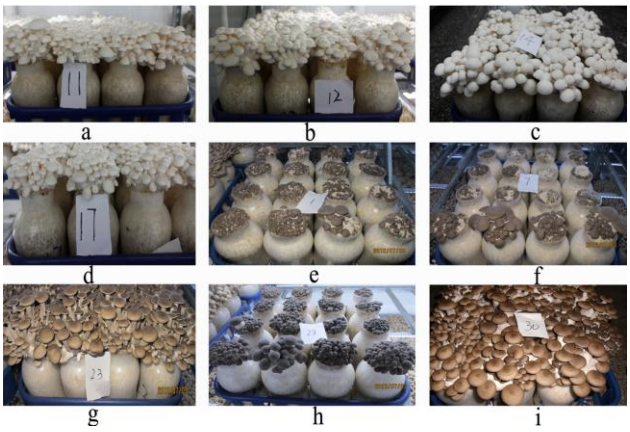

Fig. 1: Fruiting bodies of cultivated strains/partial strains of *H. marmoreus*; **a**, GDGM26169; **b**, GDGM26170; **c**, GDGM26172; **d**, FJNK-2; **e**, ACCC50474; **f**, ACCC51661; **g**, HBYC; **h**, SM-4; **i**, SCNK

morphologies of each type. The fruiting bodies of the white *H. marmoreus* strains were neat and uniform, (Fig. 1), while those of the brown strains showed irregular morphologies (Fig. 1). The yield from the commercial strains was generally higher than that from the stored strains.

ISSR Experiment

After screening, we selected 18 ISSR primers that produced clear bands with good polymorphisms and reproducibility. We optimized annealing temperatures for all the primers before experiments (Table 1). The optimum annealing temperature of selection primers ranged from 44.1 to 53.1°C.

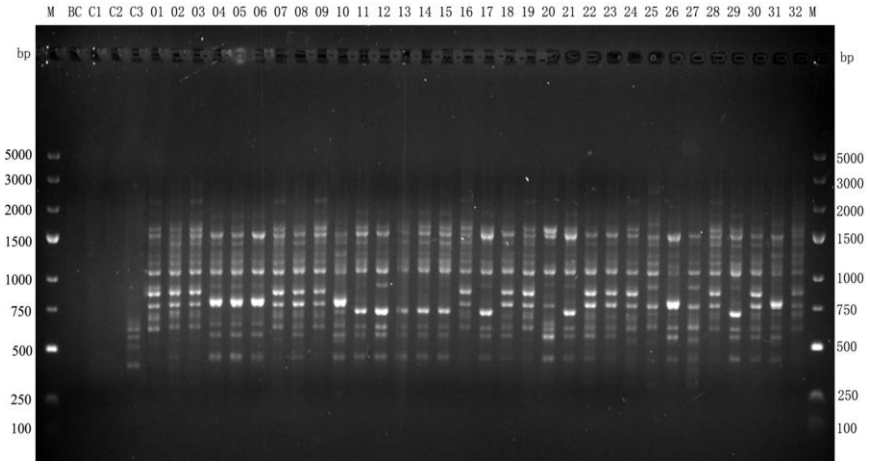

Fig. 2: Band profiles of *Hypsizygus marmoreus* generated by ISSR markers. UBC807. BC, blank control; C1, no primers; C2, no Taq polymerase; C3, *Coprinus comatus*., and Lines 1 to 32 show profiles of *H. marmoreus* (see Supplementary materials)

Color of fruiting body	Time(D)	Fresh weight (g)	Rank	Strain	Cluster based on ISSR data
Grey brown, marble stripe	54.8±14.8	24.2±4.9	24	ACCC50474	
Dark grey, water spots	54.5±14.8	56.4±29.9	19	ACCC51149	
Grey brown, dark stripe	36.5±4.9	159.2±23.5	5	ACCC51532	A
White	35.0±2.8	52.4±18.5	20	ACCC51661	
Grey brown	>65.0	22.4±7.3	25	ACCC51800	
Dark grey	35.0±2.8	51.6±38.2	21	ACCC51729	
Dark grey,marble stripe	41.0±4.2	136.2±31.8	10	ACCC51533	
White	38.5±7.8	67.8±25.6	15	ACCC51536	B
Dark grey,dark stripe	39.5±7.4	46.1±38.6	22	ACCC51583	
Grey	36.5±4.9	145.9±19.4	7	GDGM26168	
Dark brown		-		HNNK	
Grey brown, water spots	>65.0	166.8±26.3	3	ZJNK.YY	C1
Dark brown, marble stripe	35.0±2.8	41.8±17.5	23	CYQH	
Grey, marble stripe	>65.0	15.2±14.7	26	CBS-1	
*				FJNK-1	
Ivory white	35.0±2.8	63.6±19.0	17	JSGY-2	
Grey, brown stripe	35.0±2.8	61.3±41.7	18	JSGY-1	
Grey brown, water spots	35.0±2.8	186.2±26.2	2	HZND-1	
Grey, water spots	>65.0	157.2±23.1	6	HBYC	C2
*				JSTD	C
Dark brown	38.5±2.1	141.4±30.0	8	SM-4	
Dark brown, water spots	35.0±2.8	65.9±24.6	16	SJZXC	
Grey brown	39.5±6.4	111.9±53.9	12	SCNK	
Ivory white	35.0±2.8	159.7±27.7	4	MYSYB	
White	36.5±4.9	141.4±26.4	8	GDGM26169	
White	36.5±4.9	103.8±29.3	14	GDGM26170	
Ivory white	38.5±7.8	130.8±26.4	11	GDGM26171	
White	54.5±14.8	137.5±34.2	9	GDGM26172	C3
Grey brown, water spots	>65	-		GDGM26173	
Ivory white	38.0±5.4	105.6±25.0	13	FJNK-2	
Ivory white	35.0±2.8	188.1±11.9	1	HN-2	
*				HN-1	

0.85 0.83 0.80 0.78 0.75 0.73 0.70 0.67 0.65 0.62
Coefficient

Fig. 3: Characteristics of *H. marmoreus* strains during cultivation and phylogenetic tree based on ISSR data (Rohlf, 2000)

*No hyphal growth or contamination
- Fruiting body too small to harvest
Time: Time until formation of fruiting bodies
Rank: Rank of the average fresh weight
Values shown are mean ± standard deviation

The 18 selected ISSR primers generated 199 scorable bands, including 173 polymorphic band. The total number of bands amplified by single ISSR primer ranged from 5 to 16 with an average of 11.10. The proportion of polymorphic bands ranged from 52.9% (primer UBC899) to 100% (primers UBC823, UBC824, UBC840, UBC841, UBC842, and UBC900) with an average of 87.46% (Table 1). The size of the detected fragments ranged from 100 to 3000 bp (Table 1; Fig. 2).

The PIC and MI values are shown in Table 1. The PIC value for ISSRs ranged from 0.39 to 0.50 and the mean PIC value per ISSR was 0.48. The MI for ISSRs ranged from 2.40 to 8.00 and the mean MI per ISSR was 5.25.

Polymorphism Analysis

A phylogenetic tree was constructed by UPGMA cluster analysis using 199 ISSR bands from the 32 strains of *H. marmoreus* (Fig. 3). All strains were classified into three groups (at Jaccard′s similarity coefficient of 0.640). Group A consisted of eight storage strains: ACCC50474, ACCC51149, ACCC51532, ACCC51533, ACCC51536, ACCC51661, ACCC51583, and ACCC51800. Group B consisted of five strains: GDGM26168, ZJNKYY, CYQH, HNNK, and CBS-1. The remaining strains were clustered into Group which contained three sub-clusters (at Jaccard's similarity coefficient of 0.675).

Discussion

The strains FJNK-1, HN-1, and JSTD could not grow on sawdust, possible because of lower vigor or poor resistance resulting in no germination, while mycelia of GDGM26173 and HNNK showed growth on sawdust with poor fruiting. This may be due to poor adaptation to the growth conditions such as growth medium or environmental factors resulting in strain degeneration (Kaur *et al.*, 2011; Lee *et al.*, 2011). The HCFS varied among strains. Genetic or environment factors were the key determinant of the HCFs. The HCFS was negatively correlated with the fresh weight of the fruiting body showing that fresh weights of strains with shorter HCFS were greater than those of strains with longer HCFS. Although the HCFS of strains HBYC and ZJNKYY was longer than 65 days, yet the fresh weights of their fruiting bodies were higher than those of most other strains. This may be due to increased vigor as they reached maturity.

ISSR markers have been used to study the genetic diversity of some edible mushrooms (Qin *et al.*, 2006; Nazrul and Bian, 2010b). Previous studies showed that the quality of PCR amplification is mainly affected by the Mg^{2+}concentration and the primer annealing temperature (Bornet and Branchard, 2001). In our study, the optimal annealing temperatures for all primer were lower than those reported for the primers used to analyze *Agaricus bisorus* (Nazrul and Bian, 2010a). The optimal primer annealing temperature may differ among species (Najaphy *et al.*, 2011). The previous reported annealing temperature for ISSR amplification with different primers (Primmer *et al.*, 2005; Najaphy *et al.*, 2011; Nazrul and Bian, 2011) was not able to amplify clear polymorphic bands, or generated smeared banding patterns. The primers in the present study amplified clear, specific, and polymorphic bands at low annealing temperature (Fig. 2).

The PIC value provides an estimate of the discriminatory power of the ISSR marker to detect polymorphism. A low PIC value (0.00-0.25) implied that there is poor genetic diversity among samples, PIC value between 0.25 and 0.5 indicates a medium level of genetic diversity, and high PIC value (>0.50) indicated a high level of genetic diversity (Botstein *et al.*, 1980; Anderson *et al.*, 1993; Smith *et al.*, 1997; Kim and Ward, 2000; Tams *et al.*, 2005; Varshney *et al.*, 2007). The PIC values for markers in the present study ranged from 0.39 to 0.5, with a mean value of 0.48. These findings indicated a medium level of genetic diversity among the studied *H. marmoreus* strains.

The vast majority of white *H. marmoreus* strains were classified into cluster C; thus the color of fruiting body was consistent with ISSR data (Fig. 3).This may indicate that the white strains were more genetically stable than the other strains. The strains with shorter HFCS and higher mean fresh weight were also clustered into cluster C. These findings indicated that two characteristics cultivation of the fungi were correlated with ISSR results. In the ISSR polygenetic tree, most of the stored strains were in one main branch, indicating that the types of strain were also correlated with ISSR results. This may also reflect the greater level of the mutation and genetic recombination among the commercial cultivar (Shnyreva *et al.*, 2004). The characteristics of the cultivated strains and the ISSR results showed that the commercial strains were more genetically diverse than the stored strains, possibly because of high selection pressure and frequent culturing, leading to new genetic recombination. In contrast, there would have been limited opportunities for genetic recombination in the stored strains. Although the stored strains did not show longer HCFS or greater average fresh weight than the commercial strains, some strain showed potential commercial value and could be trialed in commercial production. The lower yields of white *H. marmoreus* than those of other strains suggested that there is higher genetic stability among white strains. The stored strains of ACCC51729 and CBS-1 were classified into the same group as some commercial groups, possibly indicating that they shared an ancestral parent with commercial strains. The ancestors of the commercial cultivars might have come from two provenances: ZJNKYY, CYQH, CBS-1, and HNNK from one provenance, and the other strains from another from another provenance.

In conclusion, the cultivated characteristics during cultivation (hyphal growth rate, color, shape, weight of the fruiting body and fruiting time) varied among *H. marmoreus* strains. The ISSR analyses showed a medium level of genetic diversity among *H. marmoreus* strains. The

results of this study indicated that strains of *H. marmoreus* show abundant diversity in morphological and genetic diversity, and that ISSR is a useful tool for selecting potential strains and for predicting hybrid performance in the outbreeding of mushrooms.

Acknowledgements

The authors would like to thank Dr. Li Li of Huazhong Agricultural University for her assistance in software use and data analysis; to thank Gumuzhen Bio-technology for the cultivated materials, experiment house, and management in mushroom cultivation. This work was financially supported by the Science and Technology Planning Project of Guangdong Province (STPPGD) (Nos. 2009B091300097, 2010A030100015, and 2010A090200011).

References

Akihisa, T., S.G. Franzblau, H. Tokuda, M. Tagata, M.U. Kiya, T.M. Atsuzawa, K. Metori, Y.K. Imra, T. Suzuki and K. Yaukawa, 2005. Antitubercular activity and inhibitory effect on Epstein-Barr virus activation of sterols and polyisoprenepolyols from an edible mushroom, *Hypsizigus marmoreus*. *Biol. Pharm. Bull.,* 28: 1117–1119

Al-Rawashdeh, I.M., 2011. Genetic variability in a medicinal plant *Artemisia judaica* using random amplified polymorphic DNA (RAPD) markers. *Int. J. Agric. Biol.,* 13: 278–282

Ali, M.L., J.F. Rajewski, P.S. Baenziger, K.S. Gill, K.M. Eskridge and I. Dweikat, 2008. Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. *Mol. Breed.,* 21: 497–509

Anderson, N.O., A. Younis and Y. Sun, 2010. Intersimple sequence repeats distinguish genetic differences in Easter Lily 'Nellie White' clonal ramets within and among bulb growers over years. *J. Amer. Soc. Hortic. Sci.,* 135: 445–455

Anderson, J.A., G.A. Churchill, J.E. Autrique, S.D. Tanksley and M.E. Sorrells, 1993. Optimizing parental selection for genetic linkage maps. *Genome,* 36: 181–186

Bao, D.P., A. Koike, F. Yao, K. Yamanaka, T. Aimi and Y. Kitamoto, 2007. Analyses of the genetic diversity of Matsutake isolates collected from different ecological environments in Asia. *J. Wood Sci.,* 53: 344–350

Bao, H.H. and S.G. You, 2011. Molecular characteristics of water-soluble extracts from *Hypsizigus marmoreus* and their *in vitro* growth inhibition of various cancer cell lines and immunomodulatory function in raw 264.7 cells. *Biosci. Biotechnol. Biochem.,* 75: 891–898

Behera, T.K., A.B. Gaikward, A.K. Singh and J.E. Staub, 2008. Relative efficiency of DNA markers (RAPD, ISSR and AFLP) in detecting genetic diversity of bitter gourd (*Momordica charantia* L.). *J. Sci. Food Agric.,* 88: 733–737

Bohn, M., H.F. Utz and A.E. Melchinger, 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. *Crop Sci.,* 39: 228–237

Bornet, B. and M. Branchard, 2001. Nonanchored inter simple sequence repeat (ISSR) markers reproducible and specific tools for genome fingerprinting. *Plant Mol. Biol. Rep.,* 19: 209–215

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Amer. J. Hum. Genet.,* 32: 314–331

Camacho, J.F. and A. Liston, 2001. Population structrue and genetic diversity of *Botrychinm pumicola* (Ophioglossaceae) based on inter-simple sequence repeats (ISSR). *Amer. J. Bot.,* 88: 1065–1070

Du, P., B.K. Cui and Y.C. Dai, 2012. Assessment of genetic diversity among wild *Auricularia polytricha* populations in China using ISSR markers. *Cryptogamie Mycol.,* 33: 191–201

Huang, N.L., 1990. *Hypysizygus marmoreus* and its culture technique. *Edible Fungi,* 12: 23–24

Jaccard, P., 1901. Etude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bull. Soc. Vaud. Sci. Nat.,* 37: 547–579

Joshi, K., P. Chavan, D. Warude and B. Patwardhan, 2004. Molecular markers in herbal drug technology. *Curr. Sci. Ind.,* 87: 157–165

Kaur, L., S. Dhanda, H.S. Sodhi, S. Kapoor and P.K. Khanna, 2011. Storage and preservation of temperate mushroom cultures, *Agaricus bisporus* and *Pleurotus florida*. *Ind. J. Microbiol.,* 51: 234–238

Kim, H.S. and R.W. Ward, 2000. Patterns of RFLP-based genetic diversity in germplasm pools of common wheat with different geographical or breeding program origins. *Euphytica,* 115: 197–208

Lee, C.Y., J.E. Park, J. Lee, J.K. Kim and H.S. Ro, 2012. Development of new strains and related SCAR markers for an edible mushroom, *Hypsizygus marmoreus*. *FEMS Microbiol. Lett.,* 327: 54–59

Lee, S.R., W.J. Joo, Y.U. Baek, I. Kim, K.O. Chay, S.H. Cho, S.J. Lee and S.O. Kang, 2011. Effect of light and reductones on differentiation of *Pleurotus ostreatus*. *J. Microbiol.,* 49: 71–77

Liu, J.Y., Z.H. Ying, F. Liu, X.R. Liu and B.G. Xie, 2012. Evaluation of the use of SCAR markers for screening genetic diversity of *Lentinula edodes* strains. *Curr. Microbiol.,* 64: 317–325

Monira, S., A. Haque, A. Muhit, N. Sarker, A. Alam, A. Rahman and P. Khondkar, 2012. Antimicrobial, antioxidant and cytotoxic properties of *Hypsizygus tessulatus* cultivated in Bangladesh. *Res. J. Med. Plant,* 6: 300–308

Najaphy, A., R.A. Parchin and E. Farshadfar, 2011. Evaluation of genetic diversity in wheat cultivars and breeding lines using inter simple sequence repeat markers. *Biotechnol. Biotechnol. EQ.,* 25: 2634–2638

Nazrul, M. and Y.B. Bian, 2011. Differentiation of homokaryons and heterokaryons of *Agaricus bisporus* with inter-simple sequence repeat markers. *Res. Microbiol.,* 166: 226–236

Nazrul, M.I. and Y.B. Bian, 2010a. ISSR as new markers for identification of homokaryotic protoclones of *Agaricus bisporus*. *Curr. Microbiol.,* 60: 92–98

Nazrul, M.I. and Y.B. Bian, 2010b. Efficiency of RAPD and ISSR markers in differentiation of homo- and heterokaryotic protoclones of *Agaricus bisporus*. *J. Microbiol. Biotechnol.,* 20: 683–692

Nei, M., 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA,* 70: 3321–3323

Patzak, J., 2001. Comparison of RAPD, STS, ISSR and AFLP molecular methods used for assessment of genetic diversity in hop (*Humulus lupulus* L.). *Euphytica,* 121: 9–18

Pawlik, A., G. Janusz, J. Koszerny, W. Malek and J. Rogalski, 2012. Genetic diversity of the edible mushroom *Pleurotus sp.* by amplified fragment length polymorphism. *Curr. Microbiol.,* 65: 438–445

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski, 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite)markers for germplasm analysis. *Mol. Breed.,* 2: 225–238

Primmer, C.R., J.N. Painter, M.T. Koskinen, J.U. Palo and J. Merilä, 2005. Factors affecting avian cross-species microsatellite amplification. *J. Avian Biol.,* 36: 346–360

Qin, L.H., Q. Tan, M.J. Chen and Y.J. Pan, 2006. Use of intersimple sequencerepeatsmarkers to develop strain - specific SCAR markers for *Lentinula edodes*. *FEMS Microbiol. Lett.,* 257: 112–116

Reddy, M.P., N. Sarla and E.A. Siddiq, 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica,* 128: 9–17

Rohlf, F.J., 2000. *Numerical Taxonomy and Multivariate Analysis System Version 2.1*, pp:1–35. Applied Biostatistics Inc., New York, USA

Sabou, L., F. Brhada, I.T. Alami and A.F. Maltouf, 2010. Genetic Diversity of Moroccan *Lupinus* Germplasm Investigated using ISSR and AFLP Markers. *Int. J. Agric. Biol.,* 12: 26–32

Shnyreva, A.V., I.S. Belokon, M.M. Belokon and I.P. Altukhov, 2004. Interspecific genetic variability of the oyster mushroom *Pleurotus ostreatus* as revealed by allozyme gene analysis. *Genetika,* 40: 1068–1080

Smith, J.S.C., E.C.L. Chin, H. Shu, O.S. Smith, S.J. Wall, M.L. Senior, S.E. Mitchell and S.K.J. Ziegle, 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays L.*) comparison with data from RFLPs and pedigree. *Theor. Appl. Genet.,* 95: 163–173

Tams, S.H., A.E. Melchinger and E. Bauer, 2005. Genetic similarity among European winter triticale elite germplasms assessed with AFLP and comparisons with SSR and pedigree data. *Plant Breed.,* 124: 154–160

Thangavelu, R., K.M. Kumar, P.G. Devi and M.M. Mustaffa, 2012. Genetic diversity of *Fusarium oxysporum* f.sp. *cubense* isolates (Foc) of India by inter simple sequence repeats (ISSR) analysis. *Mol. Biotechnol.,* 51: 203–211

Tonk, F.A., R.R.A. Giachino, C. Sonmez, S. Yuce, E. Bayram, I. Telci and M.A. Furan, 2011. Characterization of various *Hypericum perforatum* clones by hypericin and RAPD analyses. *Int. J. Agric. Biol.,* 13: 31–34

Varshney, R.K., K. Chabane, P.S. Hendre, R.K. Aggarwal and A. Graner, 2007. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Sci.,* 173: 638–649

Wang, L., X.H. Hu, Z.Y. Feng and Y.J. Pan, 2009. Development of AFLP markers and phylogenetic analysis in *Hypsizygus marmoreus*. *J. Gen. Appl. Microbiol.,* 55: 9–17

Zhang, Q.S., B.L. Xu, L.D. Liu, Q.Q. Yuan, H.X. Dong, X.H. Cheng and D.L. Lin, 2012. Analysis of genetic diversity among Chinese *Pleurotus citrinopileatus* Singer cultivars using two molecular marker systems (ISSRs and SRAPs) and morphological traits. *World J. Microbiol. Biotechnol.,* 28: 2237–2248

Zietkiewicz, E., A. Kafalskl and D. Labuda, 1994. Genome fingerprinting by simple sequence repeats (SSR) - anchored polymerase chain reaction amplification. *Genomics,* 20: 176–183

(Received 28 September 2012; Accepted 17 December 2012)