International Journal of Medicinal Mushrooms, 13(3): 289–298 (2011)

High Genetic Diversity in Wild Culinary-Medicinal Wood Ear Mushroom, *Auricularia polytricha* (Mont.) Sacc., in Tropical China Revealed by ISSR Analysis

Ping Du,[1,3] Bao-Kai Cui,[1] & Yu-Cheng Dai[1,2]*

[1]Institute of Microbiology, P.O. Box 61, Beijing Forestry University, Beijing 100083, China; [2]Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China; [3]Heilongjiang Agricultural Economy Vocational College, Mudanjiang 157041, China

* Address all correspondence to Yu-Cheng Dai, Institute of Microbiology, P.O. Box 61, Beijing Forestry University, Beijing 100083, China. Tel/Fax: +86-10-62336309; E-mail: yuchengd@yahoo.com.

ABSTRACT: The genetic diversity of 40 wild *Auricularia polytricha* strains within ten natural populations in tropical China and five cultivated strains were analyzed by using twelve inter-simple sequence repeat (ISSR) primers. At the species level, the percentage of polymorphic loci (P) = 99.8%, the effective number of alleles (N_e) = 1.3569, Nei's gene diversity (H) = 0.2398, Shannon information index (I) = 0.3896, and total genetic diversity (H_t) = 0.2346 indicate a high level of genetic diversity in wild *A. polytricha*. At the population level of the wild fungus, P = 43.51%, N_e = 1.2675, H = 0.1702, I = 0.2504. However, the genetic diversity of cultivated strains was the lowest of all populations (N_e = 1.1584, H = 0.0940, I = 0.1440). A moderate degree of genetic differentiation (G_{st} = 0.347) among the sampled wild populations was detected based on Nei's gene diversity analysis, suggesting that 65.3% of variation existed within this population. The high genetic variation level within wild populations may mostly result from a low level of gene flow (N_m = 0.9408) and random genetic drift.

KEY WORDS: medicinal mushrooms, *Auricularia polytricha,* gene flow, genetic differentiation, Nei's gene diversity, population, cultivated strains

ABBREVIATIONS: BJFC: the Institute of Microbiology, Beijing Forestry University; **ISSR:** inter-simple sequence repeat; **PCOA:** principal coordinate analysis**; RAPD:** random amplified polymorphic DNA; **SRAP:** sequence-related amplified polymorphism; **UBC:** University of British Columbia

I. INTRODUCTION

Auricularia polytricha (Mont.) Sacc. belongs to Auriculariaceae, higher Basidiomycetes, and is known as wood ear, or red ear.[1] It is distributed worldwide in temperate and tropical regions, occurring on living and dead broad-leaved trees, and decayed stumps or logs.[2,3] *A. polytricha* is a natural edible and medicinal mushroom, and is rich in protein, coarse fiber, carbohydrate, eight kinds of amino acids, polysaccharides, and many kinds of microelements. Its amino acids are essential for humans,[4] and its polysaccharides are important medicinal bioactive metabolites.[5] *A. polytricha* has been reported to have several medicinal functions, such as promoting blood circulation, treating hemorrhoids, and having analgesic and antitumor properties.[6–9] Because of these beneficial characteristics, *A. polytricha* is an excellent edible species, and its consumption has increased year by year. The major cultivated areas of the fungus in China are Sichuan, Henan, and Fujian provinces, and the production of the three provinces was 929,869 tons in 2007. This production was 83.5% of the whole production in China. Although fungus is widely cultivated in China, the strains for cultivation were isolated from limited populations. In fact, natural distribution of *A. polytricha* in China is well known, and it is a common fungus, especially in tropical China. It is important to establish the genetic diversity of *A. polytricha* among natural populations, as this knowledge is needed for breeding of the fungus for the mushroom industry.

High genetic variation may increase the ability of *A. polytricha* to adapt to changing environments and improve breeding programs. In nature, *A. polytricha* has a wide geographical distribution and occurs in a range of habitats, and thus it is possible that the taxon has a rich genetic diversity.

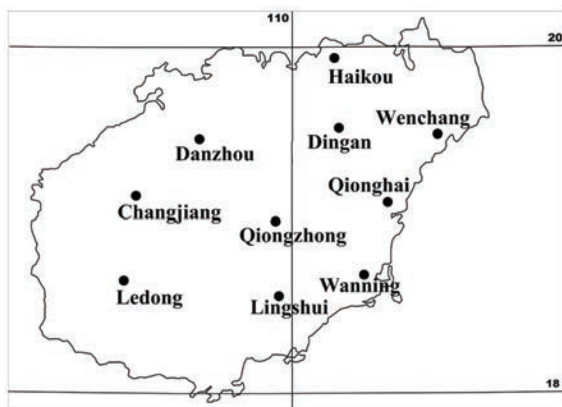

FIGURE 1. Locations of the wild populations of *Auricularia polytricha* sampled in Hainan Province, southern China.

Some efforts have been made to study the genetic diversity of *A. polytricha* by using random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), and sequence-related amplified polymorphism (SRAP) markers.[10,11,1] Most of these studies, however, used cultivated strains, and the diversity at the population level or genetic structure and relationships among wild *A. polytricha* are unknown.

ISSR is a powerful tool for investigating genetic variation within closely related species,[12] and it has been widely used in fungal genetic diversity, population genetics, and strain identification studies.[1,13–18] In the present study, ISSR markers were used to detect variation at the population level in 40 wild strains collected from Hainan Province of southern China and five cultivated strains from Henan, Sichuan, and Hainan provinces. The purpose of this study was to evaluate the levels of genetic variation and relationships among ten wild populations, to compare the relationship between the wild and cultivated strains, and to provide information for further study on cultivation and breeding programs.

II. MATERIALS AND METHODS

A. Samples

Forty natural strains of *Auricularia polytricha* from ten locations (Fig. 1 and Table 1) in Hainan Province, southern China, and five cultivated strains from Henan (41, 42), Sichuan (43, 44) and Hainan (45) provinces were isolated by tissue isolation and are deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC).

TABLE 1. Samples of *Auricularia polytricha* Strains of Different Populations in Hainan Province, Southern China

Population	Strains No.	Locations	Population	Strains No.	Locations
	6	Bawangling Nat. Res.		38	Jianfengling Nat. Res.
	2	Bawangling Nat. Res.	Lingshui	27	Diaoluoshan Nat. Res.
	30	roadside		34	Jiuqujiang
	7	roadside		39	Yelin
Changjiang	37	Bawangling Nat. Res.	Qionghai	29	Yelin
	28	Bawangling Nat. Res.		11	Yelin
	4	Bawangling Nat. Res.		12	Yelin
	5	Bawangling Nat. Res.	Qiongzhong	26	Limushan Nat. Res.
	3	Bawangling Nat. Res.		40	Dafeng Farm
	22	Danzhou, road side		8	Damao
Danzhou	21	roadside	Wanning	31	Damao
	25	Lanyang		9	Damao
	36	Lanyang		10	Damao
	23	roadside		13	Maihao
Dingan	19	roadside		14	Maihao
	33	roadside		16	Maihao
	20	roadside	Wenchang	32	Maihao
Haikou	35	Jinniuling Park		15	Maihao
	24	Jinniuling Park		18	Maihao
Ledong	1	Jianfengling Nat. Res.		17	Maihao

B. DNA Extraction

Strains of each collection were grown in liquid medium, containing 25 mL malt extract dextrose broth and incubated at 26°C for 10 days. The mycelia were harvested and rinsed with distilled water, then dried with filter paper at room temperature. The ZR Fungal/Bacterial DNA Kit™ procedure was used to extract total genomic DNA. The concentration of DNA was estimated by Smart Spec™ Plus (Bio-rad, Hercules, CA, USA) visible spectrophotometer and quality was checked by 0.8% agarose gel electrophoresis.

C. ISSR Amplification and Examination

Forty ISSR primers were designed following the method of University of British Columbia (UBC). Of these, 12 produced clear and reproducible bands, and were selected for the subsequent experiments. The sequences of 12 primers from 5' to 3' were as follows: P2: $GTG(AC)_6$; P3: $GTGACGA(CT)_6$; P10: $(GA)_8C$; P12: $(AG)_8GC$; 818: $C(AC)_7AG$; 823: $T(CT)_7CC$; 826: $A(CA)_7CC$; 834: $(AG)_8$ YT; 836: $A(GA)_7GYA$; 840: $(GA)_8YT$; 841: $G(AG)_7AYC$; 856: $A(CA)_7CYA$.

ISSR-PCR amplifications were performed in a Mastercycler PCR System (Eppendorf, Germany) with the cycling program: 1 min at 94°C, fo1lowed by 35 cycles of 30 s at 94°C, 1 min at 51°C (primer P2: 45°C), 90 s at 72°C, and a last extension at 72°C for 10 min. PCR amplification was carried out in 25 μL volumes containing 1X PCR buffer, 25 ng template DNA, 0.9 mM $MgC1_2$, 0.24 mM dNTP mixture, 2 μM primer, 1 U *Taq* DNA polymerase (Promega, Beijing, China), and double-distilled water. Ten microliters of amplification products were electrophoretically separated on 1.5% agarose gels with 1X TAE buffer. A DNA ladder was used as a size standard (D 2000). Gels were stained with ethidium bromide and bands were visualized and photographed under ultraviolet light using the Gel Doc XR system (Bio-rad).

D. Data Analysis

Bands were scored as present (1) or absent (0); the fragments produced by each primer were numbered sequentially and entered into a binomial matrix. ISSR fragments are frequently used without the need to make assumptions regarding Hardy-Weinberg equilibrium.[19] The resulting 0, 1 data matrix was analyzed using POPGENE 32.[20] Percentage of polymorphic loci, Nei's gene diversity (H),[21] Shannon index (I),[22] observed number of alleles (N_a), effective number of alleles (N_e), total gene diversity (H_t), the genetic diversity of individuals relative to their subpopulation (H_s), and genetic differentiation relative to the total population (G_{st}) [23] were calculated. Gene flow estimates (N_m) were calculated as $N_m = 0.5(1–G_{st})/G_{st}$. An unbiased genetic distance (D) matrix[24] between populations was generated (POPGENE 32) and used to create an unweighted pair-group method arithmetic average (UPGMA) dendrogram (MEGA 3.1). Molecular variance (AMOVA) described by Excoffier et al.[25] was analyzed by using ARLEQUIN 3.1.[26] Finally, a principal coordinate analysis (PCOA) was performed based on the genetic similarity matrix of the tested strains using NTSYS pc version 2.10e EIGEN programs.[27]

III. RESULTS AND DISCUSSION

A. Population Genetic Variation

Figure 2 presents examples of ISSR electrophoretic profiles generated by primers 823 and 826. The size of DNA fragments ranged from 100 to 2000 bp; a few bands were more than 2000 bp. At the species level, observed number of alleles (N_a) = 1.9980, effective number of alleles (N_e) = 1.3569, Nei's gene diversity (H) = 0.2398, and Shannon information index (I) = 0.3896 (Table 2) indicate a high level of genetic diversity in wild *A. polytricha*. Twelve primers chosen for analysis produced a total of 499 and 181 ISSR bands in the wild and cultivated strains, respectively, of which 498 and 155 bands were polymorphic, with the percentage of polymorphic loci (P) 99.8% and 85.6%; an average of 41.6 and 12.9 polymorphic bands were amplified per primer. The number of genomic characters observed in the wild strains was distinctly higher than those in cultivated strains, as previous studies have indicated that the genetic variation of wild strains of *Agaricus bisporus* (J.E. Lange) Imbach and *Lentinus edodes* (Berk.) Singer is much higher than that of cultivated strains.[15,28–32]

At the population level, P = 43.51%, N_a = 1.3916, N_e = 1.2675, H = 0.1702, and I = 0.2504. Among the ten natural populations examined in this study, the population from Changjiang had the highest percentage of polymorphic loci (P = 67.13), followed by the Wenchang population (P = 63.13). The Wenchang site, however, supported the

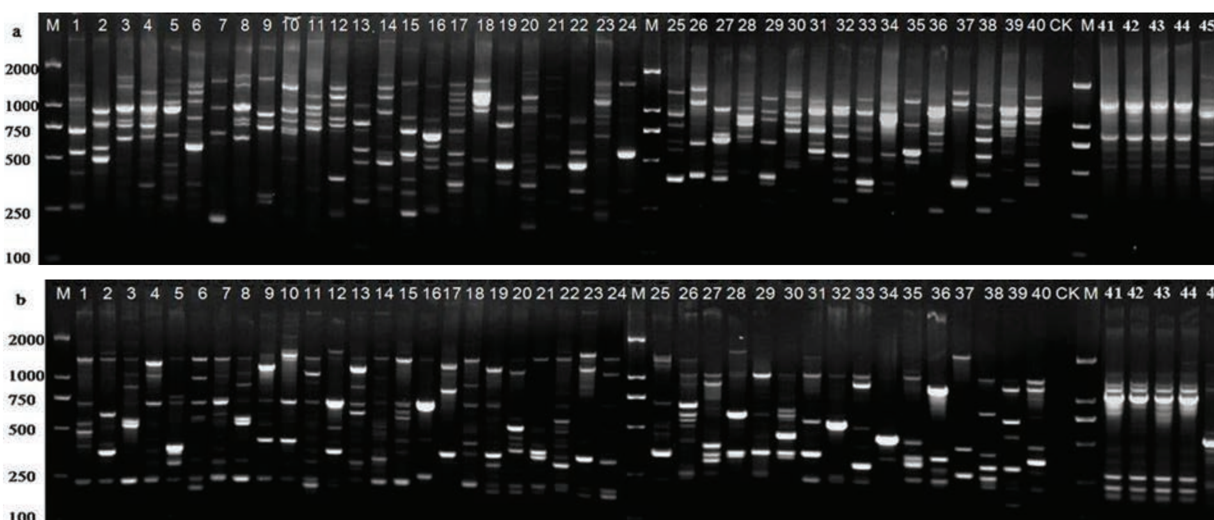

FIGURE 2. ISSR profiles of 45 *Auricularia polytricha* strains using primers 823 (a), 826 (b). Lane M: D 2000 marker. Lane CK: control.

highest level of genetic diversity (H = 0.2150, I = 0.3273), followed by Changjiang site (H = 0.2081, I = 0.3232), while Qiongzhong had the lowest level of variation (P = 23.45%, H = 0.1172, I = 0.1625). Only a single strain was collected from the Lingshui site, and thus it could not be evaluated. Although the number of individuals was the same among the Haikou, Ledong, and Qiongzhong sites, the Ledong site exhibited the highest genetic diversity among the three populations, indicating that genetic diversity of *A. polytricha* from this nature reserve was higher than that from the roadside. Humans are more active at the roadside than in nature reserves, and logging transportation is frequent along the roadside. therefore, the wild *A. polytricha* on different logs may come from a few original strains, and this seems to be the main reason for low genetic diversity of the fungus from the roadside.

Although the five cultivated strains were from three provinces, based on present analysis their genetic diversity is the lowest among the studied populations (N_a = 1.2972, N_e = 1.1584, H = 0.0940, I = 0.1440) if we consider them as one population.

The total genetic diversity (H_t = 0.2346) was mostly attributed to diversity within populations

TABLE 2. Genetic Diversity Statistics for All Loci within Populations of *Auricularia polytricha* in Hainan Province

Population	Polymorphic loci No.	Percentage of polymorphic loci (*P*)	Nei's diversity (*H*)	Shannon index (*I*)
Changjiang	335	67.13	0.2081 ± 0.1725	0.3232 ± 0.2504
Danzhou	229	45.89	0.1861 ± 0.2056	0.2727 ± 0.2989
Dingan	230	46.09	0.1841 ± 0.2021	0.2710 ± 0.2955
Haikou	121	24.25	0.1212 ± 0.2145	0.1681 ± 0.2974
Ledong	131	26.25	0.1313 ± 0.2202	0.1820 ± 0.3053
Lingshui	—	—	—	—
Qionghai	265	53.11	0.1966 ± 0.1926	0.2945 ± 0.2831
Qiongzhong	117	23.45	0.1172 ± 0.2120	0.1625 ± 0.2940
Wanning	211	42.28	0.1721 ± 0.2044	0.2519 ± 0.2970
Wenchang	315	63.13	0.2150 ± 0.1830	0.3273 ± 0.2663
Mean	217	43.51	0.1702 ± 0.2008	0.2504 ± 0.2875
Total	498	99.8	0.2398 ± 0.1281	0.3896 ± 0.1656

TABLE 3. Analysis of Molecular Variance (AMOVA) for 40 *Auricularia polytricha* Samples from Ten Populations

Source of variation	Degrees of freedom	Sum of squares	Variance components	*P* value*	Percent of total variance
Among populations	9	579.713	1.02889 Va	<0.001	1.67
Within populations	30	1813.712	60.45706 Vb	<0.001	98.33
Total	39	2393.425	61.48595		

* *P* value was calculated by a permutation procedure based on 1023 replicates.

($H_s = 0.1532$) and a moderate degree of genetic differentiation among populations ($G_{st} = 0.347$), suggesting that 65.3% of the variation existed within populations. Wright [33] believed that for some loci, the rates of gene flow could offset population differentiation ($N_m > 1$), whereas for others random genetic drift could result in population divergence ($N_m < 1$). In this study, the indirect estimate of gene flow based on genetic distance was lower ($N_m = 0.9408$), indicating that there were low levels of gene exchange among wild *A. polytricha* and a high level of divergence among populations. The level of gene flow via dispersal of sexual basidiospores and fruiting bodies is constrained by the low migratory capabilities of carriers. In the present study, it has been basically concluded that geographical isolation was probably responsible for the lower gene flow. Some evidence for higher gene flow has been found in many basidiomycetous mushrooms, such as *Thelephora ganbajun* M. Zang ($N_m = 3.128$) and *Agaricus bisporus*, suggesting that human-aided dispersal was responsible for such long-distance gene flow.[34–38]

In this study, AMOVA analysis (Table 3) indicated that only 1.67% of the variance existed among populations and most of the variation (98.33%) was found within populations, which was lower than that in the result of POPGENE. Dalgleish and Jacobson[39] also reported that 86% of the total variation was accounted for within *Morchella esculenta* (L.) Pers. sites. The estimated genetic differentiation and gene flow suggests that the high level of genetic variation within populations of *A. polytricha* in Hainan Province is due to the combined effects of limited gene flow and random genetic drift.

B. Population Genetic Distances and Relationship

The genetic distance (*D*) matrix among the ten populations (Table 4) varied from 0.0287 (the smallest genetic variation, between Changjiang and Qionghai) to 0.1908 (the greatest difference, between Ledong and Lingshui). The UPGMA dendrogram (Fig. 3) based on Nei's genetic distances (1978) among the ten populations showed that all populations could be classified into two groups with a genetic distance value of 0.04. Group I de-

TABLE 4. Nei's (1978) Original Measures of Genetic Identity (Above Diagonal) and Genetic Distance (Below Diagonal) between Populations of *Auricularia polytricha*

Population	Changjiang	Danzhou	Dingan	Haikou	Ledong	Lingshui	QiongHai	Qiongzhong	Wanning	Wenchang
Changjiang		0.9587	0.9532	0.9388	0.9522	0.8650	0.9717	0.9473	0.9653	0.9618
Danzhou	0.0422		0.9614	0.9404	0.9300	0.8520	0.9615	0.9264	0.9393	0.96
Dingan	0.0479	0.0393		0.9526	0.9299	0.8519	0.9509	0.9195	0.9405	0.9709
Haikou	0.0632	0.0614	0.0485		0.9205	0.8437	0.9306	0.8983	0.9109	0.9372
Ledong	0.0490	0.0726	0.0727	0.0828		0.8263	0.9346	0.9212	0.9170	0.9350
Lingshui	0.1451	0.1601	0.1602	0.1699	0.1908		0.8650	0.8760	0.8446	0.8550
QiongHai	0.0287	0.0393	0.0504	0.0719	0.0676	0.1450		0.9523	0.9508	0.9643
Qiongzhong	0.0541	0.0765	0.0839	0.1072	0.0821	0.1324	0.0488		0.9141	0.9235
Wanning	0.0353	0.0626	0.0614	0.0934	0.0867	0.1689	0.0504	0.0899		0.9514
Wenchang	0.0389	0.0408	0.0295	0.0649	0.0672	0.1567	0.0363	0.0796	0.0498	

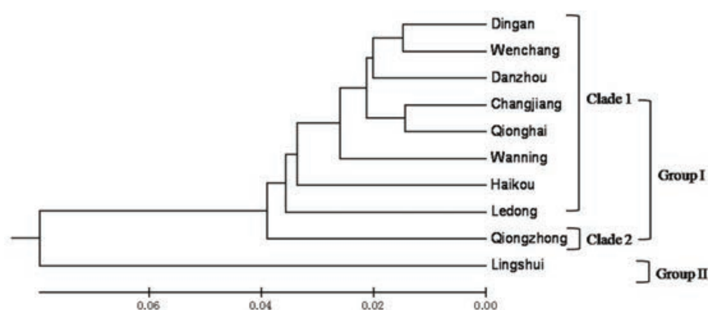

FIGURE 3. UPGMA dendrogram based on the genetic distances among ten wild populations of *Auricularia polytricha.*

lineated two clades, and eight populations consisted of clade 1, demonstrating that the eight populations were closely related; furthermore, clade 2 composed a single population (Qiongzhong). And group II comprised Lingshui alone. Cluster analysis revealed that genetic distance of populations is not correlated with the geographical distance.

C. Individual Genetic Distances and Relationship

The genetic distance (*D*) coefficients among 40 wild strains was also computed using MEGA 3.1 and ranged from 0.148 with the least dissimilarity between strains 28 and 30 (strains from the Changjiang site showing a close relationship) to 0.309 with the greatest dissimilarity between strains 5 (Changjiang) and 16 (Wenchang); 14 (Wenchang) and 23 (Dingan); 14 and 25 (Danzhou). However, the genetic distances were from 0.023 (41 and 42, 43, 44) to 0.159 (45 and 42, 43, 44) among five cultivated strains, so the cultivated strains seem to be closely related genetically, especially in the strains from Henan (41, 42) and Sichuan (43, 44) provinces, which are the two major cultivated areas of *A. polytricha* in China. The cultivated strains in the two provinces were almost from the same primary strain for cultivation. The Hainan cultivated strain is distinctly different from Henan and Sichuan cultivated strains (Fig. 2), and it was closer to the wild strain 38 (D: 0.114) than any other cultivated strains (D: 0.136, 0.159). This means Hainan cultivated strain may come from the local wild strain, or otherwise the wild *A. polytricha* came from cultivated strain. So the dispersal of spores from cultivation to the environment could increase the gene flow of the fungus among the wild population. The genetic variation of wild *A. polytricha* was also much higher than that of some other mushrooms, such as *Pleurotus eryngii* (DC.) Quél (*D*: 0.178–0.262)[40] and *Agaricus bisporus* (*D*: 0.036–0.280),[32] but seems much lower than a closely related species *Auricularia auricula-judae* (Bull.) Quél. (*D*: 0.09–0.66)[17] and *Lentinus edodes* (*D*: 0.01–0.38).[15]

C. Cluster Analysis

The dendrogram constructed by UPGMA based on the genetic distance matrix of 45 *Auricularia polytricha* strains showed four distinct groups with a distance coefficient value of 0.125 (Fig. 4): Group I and Group II comprising three strains, respectively; Group III including five cultivated strains and Group IV for the remaining strains. The structure of the tree is very similar to the one from NTSYS-pc analysis (figure not shown). Clustering analysis of individuals revealed that some strains from different populations intermixed in the dendrogram, whereas individuals from the same population did not group together, suggesting that no geographical pattern occurred in the population genetic variation.

A principal coordinate analysis (PCOA) (Fig. 5) was basically consonant with the clustering data of Fig. 4, and the Hainan cultivated strain was clustered with wild strains. This indicated that strain 45 has a unique genotype compared with other cultivated strains.

People in Hainan usually use cultivated mushrooms as food, and few wild *Auricularia polytricha* resources were collected. It was reported that different *A. polytricha* strains vary in not only their output, cultivation cycle, shape, and taste, but also in their pharmacological effects.[1] The evaluation

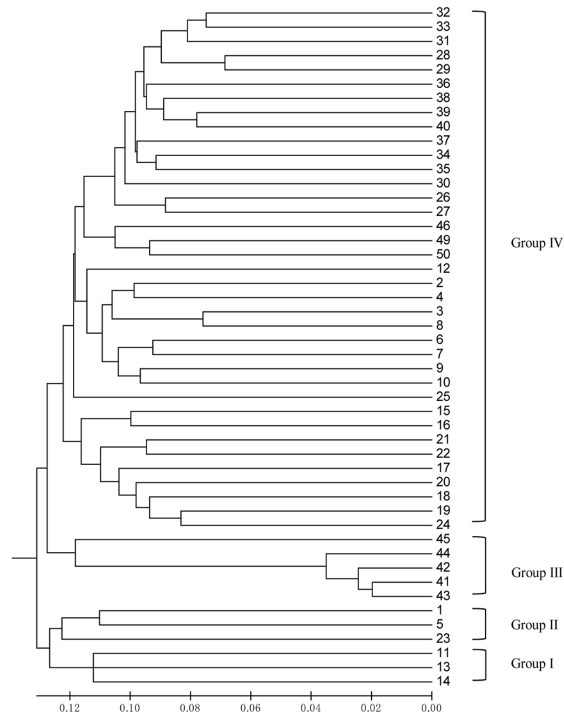

FIGURE 4. UPGMA dendrogram based on the genetic distances of 45 *Auricularia polytricha* strains.

of genetic diversity would promote the efficient use of genetic variations in the breeding and pharmacological programs.

In conclusion, this study revealed a relatively high genetic diversity among wild *A. polytricha* within populations in Hainan, so it has a strong ability to adapt to changing environmental conditions. The genetic diversity of cultivated strains was the lowest among the studied populations. The dispersal of spores from cultivation to the environment could affect the gene flow of the local wild population. The information on the genetic relat-

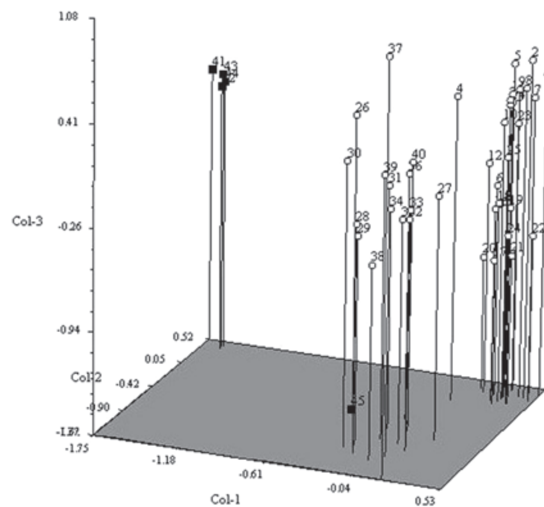

FIGURE 5. Relationships among the 45 *Auricularia polytricha* strains visualized by principal coordinate analysis (PCOA) of ISSR-based genetic similarities. Coordinates 1, 2, and 3 are indicated. ■ = cultivated strains and ○ = wild strains.

edness among the strains with different genetic bases will be useful for future breeding programs, for example, the selection of parent strains of *A. polytricha*. Genetic depression is a common phenomenon in cultivated strains of *A. polytricha*, and based on the present study genetic diversity could be improved when more wild strains with good characteristics are introduced as breeding material for cultivation.

ACKNOWLEDGMENTS

We thank Prof. Kevin D. Hyde (Mae Fah Luang University, Thailand), who improved the manuscript. The research was supported by the Fundamental Research Funds for the Central Universities (No. BLYJ200901) and the National Natural Science Foundation of China (Project No. 30910103907).

REFERENCES

1. Yu MY, Ma B, Luo X, Zheng LY, Xu XY, Yang ZR. Molecular diversity of Auricularia polytricha revealed by inter-simple sequence repeat and sequence-related amplified polymorphism markers. Curr Microbiol. 2008;56:240–45.
2. Imazeki R, Hongo T. Colored illustrations of fungi of Japan (Japanese). II, Plate 57. Osaka: Hoikusya, 1965, p. 172.
3. Reichard BM, Evaristo AA, Apolonia LL, Renato GR. Four species of wild Auricularia in Central Luzon, Philippines as sources of cell lines for researchers and mushroom growers. J Agricultural Tech. 2005;1:279–300.
4. Cheung PCK. The hypocholesterolemic effect of two edible mushrooms: Auricularia auricula (tree-ear) and Tremella fuciformis (white jelly-leaf) in hypercholesterolemic rats. Nutr Res. 1996;16:1721–25.
5. Kim HW. Toxic components of Auricularia polytricha. Arch Pharm Res. 1993;16:36–42.
6. Liu B. The Chinese medicinal fungi. Taiyuan: Shanxi People's Press; 1984.
7. Ying JZ, Mao XL, Ma QM. Icons of medicinal fungi from China (Chinese). Beijing: Science Press; 1987.
8. Sheu F, Chien PJ, Chien AL. Isolation and characterization of an immunomodulatory protein (APP) from the Jew's Ear mushroom Auricularia polytricha. Food Chem. 2004;87:593–600.
9. Dai YC, Yang ZL, Cui BK, Yu CJ, Zhou LW. Species diversity and utilization of medicinal mushrooms and fungi in China. Int J Med Mushr. 2009;11:287–302.
10. Zhang D, Zheng YL, Gao JW, Wu W, Chen H. Studies on germplasm resources of Auricularia polytricha by inter-simple sequence repeat (ISSR). J Mountain Sci Suppl. 2006;24:142–48.
11. Zhang D, Zheng YL, Wang B. Studies on germplasm resources of Auricularia polytricha by random amplified polymorphic DNA. Biotechnol Bull. 2007;18:117–23.
12. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 1994;20:176–83.
13. Kazuhisa T, Teruyuki M, Kozaburd H, Yukitaka FN. Genetic diversity and strain-typing in cultivated strains of Lentinula edodes (the shii-take mushroom) in Japan by AFLP analysis. Mycol Res. 2002;106:34–39.
14. Gryta H, Carriconde F, Charcosset JY. Population dynamics of the ectomycorrhizal fungal species Tricholoma populinum and Tricholoma scalpturatum associated with black poplar under differing environmental conditions. Environ Microbiol. 2006;8:773–86.
15. Zhang RY, Huang CY, Zheng SY, Zhang JX, Ng TB, Jiang RB, Zuo XM, Wang HX. Strain-typing of Lentinula edodes in China with inter-simple sequence repeat markers. Appl Microbiol Biot. 2007;74:140–45.
16. Su HY, Wang L, Liu LD, Chi XY, Zhang YX. Use of inter-simple sequence repeat markers to develop strain-specific SCAR markers for Flammulina velutipes. J Appl Genet. 2008;49:233–35.
17. Fu LZ, Zhang HY, Wu XQ, Li HB, Wei HL, Wu QQ, Wang LA. Evaluation of genetic diversity in Lentinula edodes strains using RAPD, ISSR and SRAP markers. World J Microb Biot. 2010;26:709–16.
18. Tang LH, Xiao Y, Li L, Guo Q, Bian YB. Analysis of genetic diversity among Chinese Auricularia auricula cultivars using combined ISSR and SRAP markers. Curr Microbiol. 2010;61:132–40.
19. Fontaine C, Lovett PN, Sanou H, Maley J, Bouvet JM. Genetic diversity of the shea tree (Vitellaria paradoxa), detected by RAPD and chloroplast microsatellite madders. Heredity. 2004;93:639–48.
20. Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan JM. PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32, Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada; 2000.
21. Nei M. Analysis of gene diversity in subdivided populations. Proc Nat Acad Sci USA. 1973;70:3321–23.
22. Lewontin RC. Testing the theory of natural selection. Nature. 1972;236:181–82.
23. Nei M. Molecular evolutionary genetics. New York: Columbia University Press; 1987.
24. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 1978;89:583–90.
25. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992;131:479–91.
26. Excoffier L, Laval G, Schneider S. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinform Online. 2005;1:47–50.
27. Rohlf FJ. NTSYS-PC numerical taxonomy and multivariate analysis system, version 2.10. New York: Exeter Software; 2000.
28. Loftus MG, Moore D, Elliott TJ. DNA polymorphism in commercial and wild strains of the cultivated mushroom

Agaricus bisporus. Theoret Appl Genet. 1988;76:712–18.
29. Kerrigan RW. Evidence of genetic divergence in two populations of Agaricus bisporus. Mycol Res. 1990;94:721–33.
30. Chiu SW, Ma AM, Lin FC, Moore D. Genetic homogeneity of cultivated strains of shiitake (Lentinula edodes) used in China as revealed by the polymerase chain reaction. Mycol Res. 1996;100:1393–99.
31. Sonnenberg ASM. Genetics and breeding of Agaricus bisporus. Mushr Sci. 2000;15:25–39.
32. Moore AJ, Challen MP, Warner PJ, Elliott TJ. RAPD discrimination of Agaricus bisporus mushroom cultivars. Appl Microbiol Biot. 2001;55:742–49.
33. Wright S. Evolution in Mendelian population. Genetics. 1931;16:97–159.
34. Sha T, Xu JP, Palanichamy MG, Zhang HB, Li T, Zhao ZW, Zhang YP. Genetic diversity of the endemic gourmet mushroom Thelephora ganbajun from southwestern China. Microbiol. 2008;154:3460–68.
35. James TY, Porter D, Hamrick, JL, Vilgalys R. Evidence for limited intercontinental gene flow in the cosmopolitan mushroom, Schizophyllum commune. Evolution. 1999;53:1665–77.
36. Xu J, Cheng M, Tan Q, Pan Y. Molecular population genetics of basidiomycete fungi. In J. Xu (ed.): Evolutionary genetics of fungi. Norwich, UK: Horizon Scientific Press; 2005.
37. Xu J, Kerrigan RW, Callac P, Horgen PA, Anderson JB. Genetic structure of natural populations of Agaricus bisporus, the commercial button mushroom. J Hered. 1997;88:482–88.
38. Xu J, Kerrigan RW, Sonnenberg AS, Callac P, Horgen PA, Anderson JB. Mitochondrial DNA variation in natural populations of the mushroom Agaricus bisporus. Mol Ecol. 1998;7:19–33.
39. Dalgleish HJ, Jacobson KM. A first assessment of genetic variation among Morchella esculenta (Morel) populations. J Hered. 2005;96:396–403.
40. Georgios IZ, Giuseppe V, Kalliopi P. Genetic polymorphism and taxonomic infrastructure of the Pleurotus eryngii species complex as determined by RAPD analysis, isozyme profiles and ecomorphological characters. Microbiology. 2001;147:3183–94.
41. Shun SJ, Gan W, Lin SQ, Zhu J, Xie BG, Lin ZB. Analysis of genetic diversity in Ganoderma population with a novel molecular marker SRAP. Appl Microbiol Biot. 2006;72:537–43.