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Note

Examining genetic relationships of Chinese *Pleurotus ostreatus* cultivars by combined RAPD and SRAP markers

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ABSTRACT

Combined randomly amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) were used to assess the genetic diversity of *Pleurotus ostreatus* strains cultivated in China. For the RAPD and SRAP analyses, 479 and 282 polymorphic bands were obtained from 20 *P. ostreatus* strains using 20 and 13 selected primers or primer pairs, respectively. A combined RAPD/SRAP dendrogram grouped the 20 strains into five clades with a coefficient of 0.690. The comparison of RAPD and SRAP was evaluated in the present study. The combined RAPD/SRAP markers provided reliable information regarding the relationships among the *P. ostreatus* strains.

The oyster mushroom *Pleurotus ostreatus* is the second most widely grown edible mushroom in the world (Sanchez 2010), and not only has economic and ecological values, but also has medicinal and biotechnological properties (Irie et al. 2001; Sanchez 2004). Most cultivars of *P. ostreatus* are derived from a limited number of parental strains, resulting in a narrow genetic base. *Pleurotus ostreatus* strains with higher productivity, disease resistance and levels of beneficial metabolitics, such as polysaccharides and lectins, are desired (Fu et al. 2010). An accurate assessment of the genetic diversity of Chinese cultivars will promote the efficient use of elite strains (Paterson et al. 1991).

To date, various molecular markers have been introduced to assess the genetic diversity of several edible and medicinal fungi; these include randomly amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP). Many reports have shown that RAPD and SRAP markers are effective tools in genetic diversity analysis of edible fungi (Calvo-Bado et al. 2001; Lewinsohn et al. 2001; Sun et al. 2006; Tang et al. 2010). Since the principles of RAPD and SRAP aim at amplifying different region of the genome, combined RAPD and SRAP analysis should provide clear discrimination of genotypes (Fu et al. 2010). Tang et al. (2010) successfully combined SRAP and ISSR in the genetic diversity analysis of *Auricularia auricula*. Based on reproductive experiments with isozyme analysis, barrage tests and morphological traits of fruiting body, 153 strains of *P. ostreatus* collected from China were classified into 20 representative groups (unpublished data). To assess the genetic relationship between the 20 strains from each group, combination of RAPD and SRAP marker was used. Twenty strains of *P. ostreatus* (Table 1) were grown on potato dextrose agar (PDA, 20% potato extract, 2% dextrose, 2% agar) plates at 25 °C for 7–10 days. Mycelium was harvested and used for genomic

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Table 1 – Strains of *Pleurotus ostreatus* used in this study.

Strain no.	Original Chinese cultivars' name	Source
JZB2101001	heiping no.1	IPEP-BAAFS
JZB2101002	pingye no.1	IPEP-BAAFS
JZB2101004	pinggu no.4	GY
JZB2101005	ping no.5	HNAU
JZB2101006	jiangdu 156	IPEP-BAAFS
JZB2101007	pinggu 841	IPEP-BAAFS
JZB2101008	ping no.8	HNAU
JZB2101009	xinke no. 9	GY
JZB2101011	pinggu no.11	HNAU
JZB2101012	pinggu no.12	HNAU
JZB2101015	pinggu 9906	GY
JZB2101017	heipinggu	TZ
JZB2101018	pinggu no.81	GY
JZB2101019	pinggu no.82	GY
JZB2101020	xinyu 2000	GY
JZB2101021	heifeng 268	GY
JZB2101022	egypt pinggu	IPEP-BAAFS
JZB2101024	ping no.1	HNAU
JZB2101025	pinggu	FS
JZB2101026	fangshan huihei	FS

Note: *IPEP-BAAFS*: Institute of Plant and Environmental protection, Beijing Academy of Agriculture and Forestry Science, Beijing, China; *GY*: Gaoyou Fungus Research Institute, Gaoyou, Jiangsu, China; *HNAU*: College of Life Sciences, Henan Agricultural University, Zhengzhou, Henan, China; *TZ*: Yongledian, Tongzhou District, Beijing, China; *FS*: Fangshan District, Beijing, China.

Table 2 – Primers for RAPD, SRAP analysis.

	Primers	Sequences (5′→3′)	Reference
RAPD primers	S22	TGCCGAGCTG	Sangon Co. Ltd (Shanghai, China)
	S24	AATCGGGCTG	Sangon Co. Ltd (Shanghai, China)
	S27	GAAACGGGTG	Sangon Co. Ltd (Shanghai, China)
	S28	GTGACGTAGG	Sangon Co. Ltd (Shanghai, China)
	S36	AGCCAGCGAA	Sangon Co. Ltd (Shanghai, China)
	S39	CAAACGTCGG	Sangon Co. Ltd (Shanghai, China)
	S43	GTCGCCGTCA	Sangon Co. Ltd (Shanghai, China)
	S64	CCGCATCTAC	Sangon Co. Ltd (Shanghai, China)
	S72	TGTCATCCCC	Sangon Co. Ltd (Shanghai, China)
	S75	GACGGATCAG	Sangon Co. Ltd (Shanghai, China)
	S78	TGAGTGGGTG	Sangon Co. Ltd (Shanghai, China)
	S79	GTTGCCAGCC	Sangon Co. Ltd (Shanghai, China)
	S126	GGGAATTCGG	Sangon Co. Ltd (Shanghai, China)
	S159	ACGGCGTATG	Sangon Co. Ltd (Shanghai, China)
	S242	CTGAGGTCTC	Sangon Co. Ltd (Shanghai, China)
	S370	GTGCAACGTG	Sangon Co. Ltd (Shanghai, China)
	S484	AGTGCGCTGA	Sangon Co. Ltd (Shanghai, China)
	S485	CCGCGTCTTG	Sangon Co. Ltd (Shanghai, China)
	S2056	CTGGTGCTCA	Sangon Co. Ltd (Shanghai, China)
	S2059	ACAAGCGCGA	Sangon Co. Ltd (Shanghai, China)
SRAP primer pairs	me1/em3	TGAGTCCAAACCGGATA/GACTGCGTACGAATTGAC	Sangon Co. Ltd (Shanghai, China)
	me1/em4	TGAGTCCAAACCGGATA/GACTGCGTACGAATTTGA	Sangon Co. Ltd (Shanghai, China)
	me1/em10	TGAGTCCAAACCGGATA/GACTGCGTACGAATTTAG	Sangon Co. Ltd (Shanghai, China)
	me1/em13	TGAGTCCAAACCGGATA/GACTGCGTACGAATTGGT	Sangon Co. Ltd (Shanghai, China)
	me1/em16	TGAGTCCAAACCGGATA/GACTGCGTACGAATTCGG	Sangon Co. Ltd (Shanghai, China)
	me1/em17	TGAGTCCAAACCGGATA/GACTGCGTACGAATTCCA	Sangon Co. Ltd (Shanghai, China)
	me2/em5	TGAGTCCAAACCGGAGC/GACTGCGTACGAATTAAC	Sangon Co. Ltd (Shanghai, China)
	me2/em6	TGAGTCCAAACCGGAGC/GACTGCGTACGAATTGCA	Sangon Co. Ltd (Shanghai, China)
	me2/em17	TGAGTCCAAACCGGAGC/GACTGCGTACGAATTCCA	Sangon Co. Ltd (Shanghai, China)
	me3/em2	TGAGTCCAAACCGGAAT/GACTGCGTACGAATTTGC	Sangon Co. Ltd (Shanghai, China)
	me3/em3	TGAGTCCAAACCGGAAT/GACTGCGTACGAATTGAC	Sangon Co. Ltd (Shanghai, China)
	me3/em6	TGAGTCCAAACCGGAAT/GACTGCGTACGAATTGCA	Sangon Co. Ltd (Shanghai, China)
	me3/em7	TGAGTCCAAACCGGAAT/GACTGCGTACGAATTATG	Sangon Co. Ltd (Shanghai, China)

DNA extraction using DNeasy mini Plant DNA extraction kit (Qiagen, Germany).

Based on their clear banding profiles for the tested strains, 20 out of 200 RAPD primers (Table 2) were selected to detect polymorphic RAPD bands among the strains. The PCR mix for RAPD analysis consisted of 1× PCR buffer (Mg^{2+} plused, Takara, Japan), 0.2 mmol/l dNTP, 160 pmol of primer, 1 U *Taq* polymerase (Takara, Japan), 50 ng of template DNA, and sterile, deionized water to make the volume up to 50 μl. DNA amplification was performed using the following parameters: 45 cycles of 94 °C for 60 s, 34 °C for 60 s and 72 °C for 60 s (Barracosa et al. 2008). For SRAP analysis, 13 out of 153 primer pairs (Table 2) were selected to produce clear banding profiles for the tested strains. The PCR mix for SRAP analysis was the same as for RAPD except 80 pmol of each primer was used. The SRAP amplification program was as follows: 3 min initial denaturation at 94 °C; 5 cycles consisting of 94 °C for 60 s, 35 °C for 60 s and 72 °C for 90 s; 30 cycles consisting of 94 °C for 60 s, 50 °C for 60 s and 72 °C for 90 s; and a final 8 min extension at 72 °C (Fu et al. 2010). Amplification products of RAPD and SRAP were fractionated on a 2.0% (w/w) agarose (Oxoid, Madrid, Spain) gel. Polymorphic DNA bands were documented as either present (1) or absent (0). The genetic similarity coefficients between the strains of *P. ostreatus* were calculated by the NTSYSpc 2.1e (Rohlf 2000). The dendrogram was constructed based on the coefficients obtained by the unweighted pair group method with arithmetic averaging algorithm (UPGMA) (Xiao et al. 2010).

Of the 505 amplified RAPD bands, 479 were polymorphic, with an average of 24 polymorphic fragments per primer. Percentage polymorphism ranged from 82.6% (S23 and S2059) to a maximum of 100% (S24, S64, S126, S242, S370 and S2056), with an average of 94.85% (Table 3). A dendrogram based on RAPD data was constructed by UPGMA and the 20 strains were grouped into six main clades (I–VI in Fig. 1) with a coefficient of 0.685. Of the 300 amplified SRAP bands, 282 were polymorphic, with an average of 21.7 polymorphic fragments per primer pair. Percentage polymorphism ranged from 86.4%

Table 3 – Polymorphism obtained by RAPD and SRAP analysis in 20 *Pleurotus ostreatus* strains.

	Primer/primer combination	Total bands	Number of bands in each strain[a]	Polymorphic bands	Percentage of polymorphism
RAPD	S22	26	3–15 (11.6)	25	96.2
	S24	29	1–17 (10.6)	29	100.0
	S27	25	7–15 (11.3)	24	96.0
	S28	23	3–15 (9.7)	21	91.3
	S36	23	7–15 (10.8)	19	82.6
	S39	23	6–14 (9.7)	22	95.7
	S43	21	8–16 (11.8)	20	95.2
	S64	24	7–16 (11.9)	24	100.0
	S72	21	3–11 (7.1)	20	95.2
	S75	33	9–14 (12.4)	30	90.9
	S78	25	6–14 (10.4)	24	96.0
	S79	30	16–21 (18.9)	25	83.3
	S126	29	2–18 (12.1)	29	100.0
	S159	20	6–14 (11.2)	17	85.0
	S242	22	4–16 (7.6)	22	100.0
	S370	22	3–14 (9.0)	22	100.0
	S484	34	6–17 (11.5)	32	94.1
	S485	26	7–19 (14.0)	25	96.2
	S2056	26	8–16 (11.9)	26	100.0
	S2059	23	10–18 (13.1)	19	82.6
	Total	505	177–257 (227.1)	479	
	Average	25.3	8.9–12.9 (11.4)	24.0	94.85
SRAP	me1/em3	28	3–17 (11.3)	28	100.0
	me1/em4	20	4–12 (9.0)	18	90.0
	me1/em10	19	5–11 (7.6)	18	94.7
	me1/em13	25	7–16 (9.7)	23	92.0
	me1/em16	22	6–12 (8.8)	19	86.4
	me1/em17	22	5–14 (9.3)	20	90.9
	me2/em5	22	7–12 (9.4)	22	100.0
	me2/em6	24	3–14 (9.7)	23	95.8
	me2/em17	25	5–13 (9.1)	24	96.0
	me3/em2	26	7–16 (12.1)	24	92.3
	me3/em3	23	5–15 (9.0)	22	95.7
	me3/em6	23	6–15 (10.9)	20	87.0
	me3/em7	21	3–13 (8.7)	21	100.0
	Total	300	99–145 (124.3)	282	
	Average	23.08	7.6–11.2 (9.6)	21.7	94.0

a Number in parentheses indicates the average value.

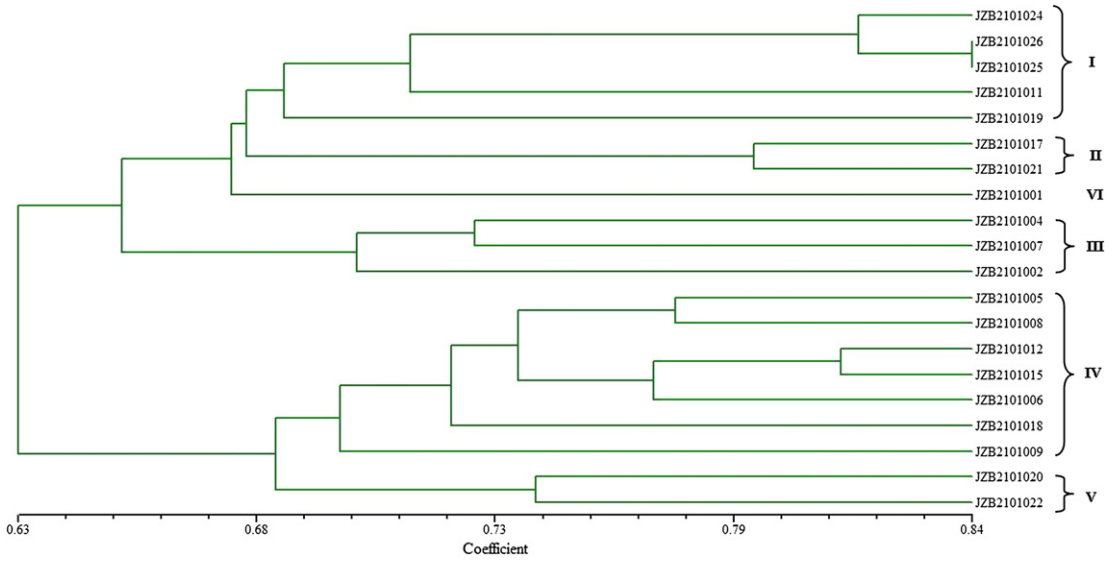

Fig. 1 – UPGMA dendrogram of 20 *Pleurotus ostreatus* strains constructed by using genetic similarity analysis based on molecular profiles revealed by RAPD analysis.

(me1/em16 primer pair) to a maximum of 100% (me1/em3, me2/em5 and me3/em7 primer pair), with an average of 94% (Table 3). A dendrogram constructed by UPGMA on the basis of the distance matrix by SPAP analysis showed that 20 strains fell into five main clades with a coefficient of 0.690 (Fig. 2). A comprehensive dendrogram was constructed on the basis of combined RAPD/SRAP data. Five putative clades among the 20 strains of *P. ostreatus* were obtained with a coefficient of 0.690 (Fig. 3). The structure of the tree was more similar to the one from RAPD analysis than the one from SRAP analysis. The most significant difference observed was that clade II clustered with clade I and III in the RAPD analysis and combined RAPD/SRAP analysis, while clade II clustered with clade IV and V in SRAP analysis.

Discrepancies were observed from some strains according to different methods. JZB2101001 clustered into clade II in SRAP and combined RAPD/SRAP analysis (Figs. 2 and 3), while it formed a single clade VI in RAPD analysis (Fig. 1). JZB2101020 formed a single clade based on SRAP analysis (clade V in Fig. 2), but clustered with JZB2101022 in the RAPD and combined RAPD/SRAP analysis (clade V in Figs. 1 and 3). Comparing the RAPD with the SRAP analysis, strain JZB2101011 and JZB2101019 fell into different clades in SRAP analysis (Fig. 2), while they clustered together with JZB2101024, JZB2101025, JZB2101026 and formed clade I in the RAPD analysis and combined RAPD/SRAP analysis (Figs. 1 and 3). In addition, JZB2101005, JZB2101012 and JZB2101015 had a close relationship with JZB2101009 in SRAP analysis

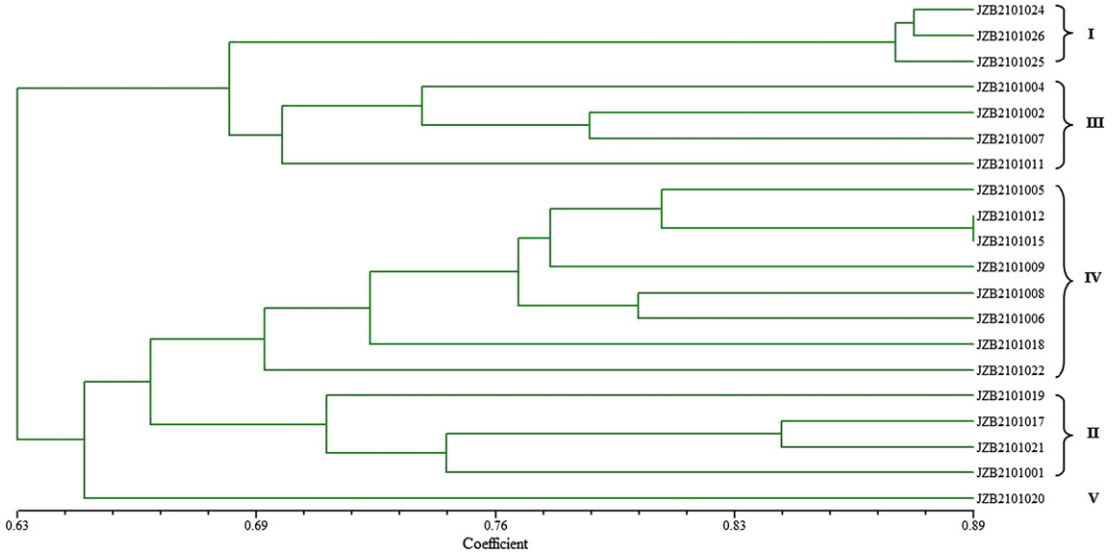

Fig. 2 – UPGMA dendrogram of 20 *Pleurotus ostreatus* strains constructed by using genetic similarity analysis based on molecular profiles revealed by SRAP analysis. Clades I–V correspond to clades I–V in Fig. 1.

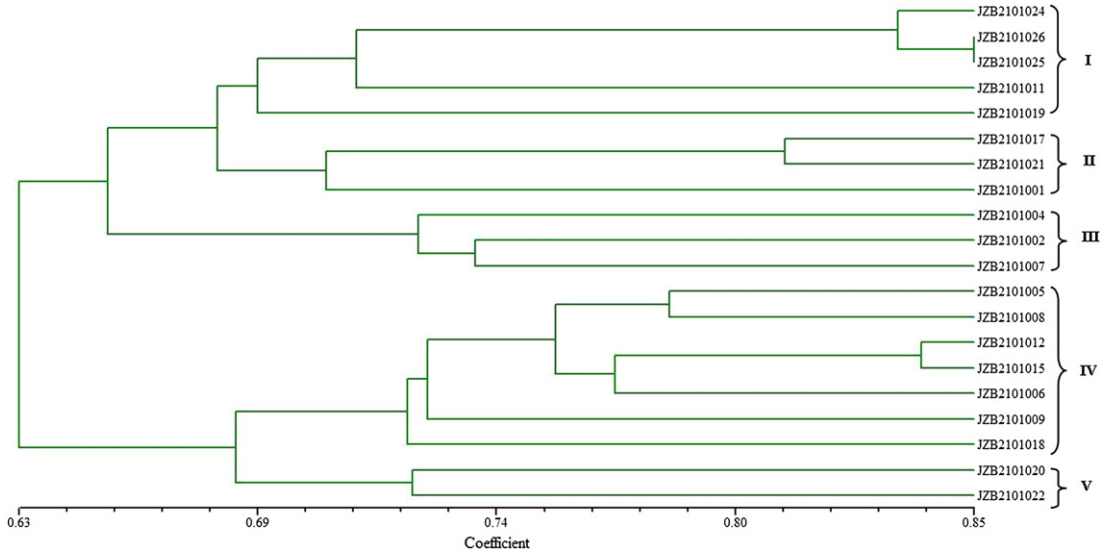

Fig. 3 – UPGMA dendrogram of 20 *Pleurotus ostreatus* strains constructed by using genetic similarity analysis based on molecular profiles revealed by combined RAPD/SRAP analysis. Clades I–V correspond to clades I–V in Fig. 1.

(Fig. 2), however this sub-clade was not present in the combined RAPD/SRAP analysis (Fig. 3).

In conclusion, based on the analysis of combined RAPD and SRAP marker, 20 strains of Chinese *P. ostreatus* cultivars were grouped into 5 clades and showed significant genetic diversity between them. The information regarding the genetic diversity of the strains will be useful for selecting resources to use in strain improvement by cross-breeding with the aim to avoid inbreeding depression.

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