RAPD Analysis of Three Selected Strains of *Hypsizigus marmoreus*

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Abstract: Genomic DNA of three newly-selected strains of *Hypsizigus marmoreus* were amplified with 11 random primers using PCR. The DNA sequence polymorphism of the different strains was analyzed by RAPD so as to confirm the genetic variation of the new strains of *H. marmoreus*.

Key Words: *Hypsizigus marmoreus*, RAPD, gene, analysis

1 Introduction

Nowadays, DNA sequence polymorphism based genetic markers mainly include Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Length Polymorphism (SSLP), Random Amplified Polymorphic DNA (RAPD), Arbitrary Primed PCR (AP- PCR) Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphism (SNP). Of these, RFLP was the first type of genetic marker proposed. The use of RAPD and AP-PCR for genetic mapping and genomic analysis, respectively were described in 1990 by Williams et al.[1] and Welsh & McClelland,[2] respectively The two approaches were actually the same and based on PCR (polymerase chain reaction) amplification. However, RAPD has turned to be an important tool for research work such as germplasm resource identification, classification, genetic diversity analysis and genetic location, as it has the advantages of rapid reaction, easy manipulation and economical efficiency.
Morphological observation and character analysis of the strains of *Hypsizigus marmoreus* selected in this study revealed some variation. However, traits such as size, form, texture, colour and yield, are often influenced by environmental conditions and various human behaviour. These uncertainties can be overcome by genetic markers based on modern biological technology. To assess more accurately the genetic variation among the selected strains of *H. marmoreus*, RAPD analysis was performed with three selected strains, the DNA profiles of which were determined and their genetic correlation confirmed.

2 Materials and Methods

2.1 Strains

Strains S_{11}, S_{14} and S_{15} were selected by the author for this study.

2.2 Test medium

The culture medium consisted of (per litre): glucose 20g, peptone 2g, $MgSO_4$ 0.5g, K_2HPO_4 1g, KH_2PO_4 0.46g, agar 18-20g.

2.3 Primers

All the primers adopted in the study were provided by Shanghai Biotechnology Service Co. Ltd. Eleven out of a total of 40 primers were found to effective in generating amplification products in the preparative tests of

genomic DNA from the *H. marmoreus* strains (Table 1).

Table 1. Nucleotide sequences of the random primers tested

Primer	Nucleotide sequences	Primer	Nucleotide sequences
S6	5 ' TGCTCTGCCC	S42	5 ' GGACCCAACC
S23	5 ' AGTCAGCCAC	S43	5 ' GTCGCCGTCA
S29	5 ' GGGTAACGCC	S95	5 ' ACTGGGACTC
S31	5 ' CAATCGCCGT	S265	5 ' GGCGGATAAG
S37	5 ' GACCGCTTGT	S1327	5 ' ACGCGACAGA
S38	5 ' AGGTGACCGT		

2.4 Culture conditions

Mycelia of *H. marmoreus* were inoculated in to liquid medium and incubated on a rotary shaker at 25°C.

2.5 Extraction of DNA

Genomic DNA was extracted by the CTAB method.[3] Dry, finely ground mycelial samples of *H. marmoreus* (4 gm) were weighed into a conical flask and 2.8 ml preheated extraction solution (0.01 mol/L Tris-HCL, pH 8.0; 2% CTAB; 0.02 mol/L EDTA; 1.4 mol/L NaCl; and 2% mercaptoethanol) was added. The flask was then placed in a water bath at 50°C for 30 min before the addition of 2.8 ml of trichloromethane -iso-pentanol mixture (24: 1). After shaking into a uniform emulsion, the solution was transferred to two centrifuge tubes (10ml) and centrifuged at 3500 rpm for 30min. The supernatant was collected and equilibrated with 4 ml precipitation buffer solution (0.05 mol/L Tris-HCL, pH 8.0; 1% CTAB; 0.01 mol/L EDTA). The solution was then placed at room temperature for 30 min for precipitation to take place, and then centrifuged at 3500 rpm for 15min. The collected precipitate was combined with 2ml of RNA enzyme solution (10 μg/ml in 0.05 mol/L Tris- HCL buffer solution) and placed in a water bath at 37°C for 30min. After centrifugation, 2 volumes of cold anhydrous ethanol were added. The mixture was precipitated for 30 min and then centrifuged at 3500 rpm for 15 min. The precipitation was washed 3x with anhydrous ethanol the DNA fraction retained. The CTAB extraction method has several advantages including a faster experimental procedure, high extraction rate for DNA, and lower requirements for instrumentation and chemicals. The extracted DNA product has a lower protein and polysaccharide content resulting in less interference and higher accuracy. Furthermore, the DNA does not need purification, thereby shortening the experimental procedure.

2.6 DNA amplification

Reaction mixtures for PCR amplification consisted of 2.5μl 10 x PCR buffer; 2.5mM $MgCl_2$, 2 μl; 10 mM dNTP 0.3 μl; 5U/μl Taq polymerase, 0.2 μl; primer (3.3ng/ μl) 2 μl; ddH_2O 15 μl; and template DNA 2 μl (20-50ng). PCR conditions were: 92°C for 5 min, followed by 43 cycles of 94°C for 1 min, 35°C for 2 min, and 72°C for 2 min, then a final extension at 72°C for 10 min.

2.7 Electrophoresis

PCR amplified products (20 μl each) were equilibrated with 4 μl of the above-mentioned buffer. Samples were placed on 0.75% or 2.0% (w/v) agarose gels. DNA/Hind III was used as molecular weight marker (0.1 μg/well). Electrophoresis was performed in 0.5 × TBE buffer at a set voltage of 3V/cm. After the gel was removed,

the reaction products were dyed for 30 min in 0.5 μg/ml ethidium bromide, washed with clean water for 10min, and observed using a UV spectrometer and photographed.

2.8 Data treatment

The molecular weight of every amplified DNA fragment was calculated with GEL software. The similarity coefficients among the strains tested were calculated with Simqual software of the NTSYS-PC program. The clustering was performed using the un-weighted pair-group method with arithmetic clustering (UPGMA), and a genetic relationship dendrogram was constructed.

3 Results and Analysis

3.1 PCR patterns of DNA from *H. marmoreus* strains

The PCR patterns of DNA derived from the three strains of *H. marmoreus* are shown in Figure 1.

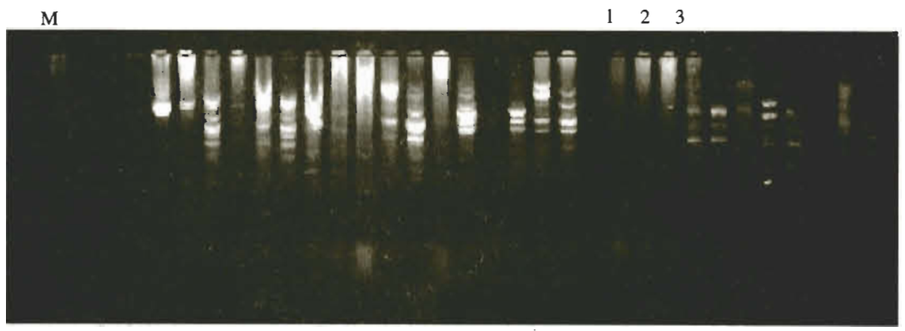

Figure 1. PCR patterns of DNA derived from the three strains of *H. marmoreus*
M: DNA molecular marker; 1. Strain S_{11}; 2. Strain S_{14}; 3.Strain S_{15}.

Of the 40 random primers used in this experiment, 11 primers amplified the required DNA fragments from the genomic DNA of the strains tested. PCR patterns using the 11 primers were derived (2.0% agarose, 3V/cm) in the following sequence: (1) Marker (100 bp ladder), S_{15}· 23; (2) S_1 · 6, S_{14}·6, S_{15}· 6; (3) S_{11}· 37, S_{14}· 37, S_{15}· 37; (4) S_{11}· 1327, S_{14}· 1327, S_{15}· 1327; (5) S_{11}· 42, S_{14}· 32, S_{15}· 42; (6) S_{11}· 265, S_{14}· 265, S_{15}· 265; (7) S_{11}· 29, S_{14}· 29, S_{15}· 29; (8) S_{11}· 31, S_{14}· 31, S_{15}· 31; (9) S_{11}· 95, S_{14}· 95, S_{15}· 95; (10) S_{11}· 43, S_{14}· 43, S_{15}· 43; (11) S_{11}· 38, S_{14}· 38, S_{15}· 38; (12) S_{11}· 23, S_{14}· 23. It was clear that the efficiency of DNA amplification was different with different primers, and the generated bands also differed. Of the 11 primers, the analysis using S_{37}, S_{1327}, S_{42}, S_{29}, S_{31}, S_{265} gave the best results.

3.2 Similarity Coefficient Matrix for the strains tested

A similarity coefficient analysis of the PCR profiles of genomic DNA from the strains tested was undertaken using appropriate software. A similarity coefficient matrix was obtained, the data for which are shown in Table 2.

Table 2. Similarity Coefficient Matrix for the strains tested

Strains tested	No.	1	2	3
S_{11}	1	1.0000		
S_{14}	2	0.6408	1.0000	
S_{15}	3	0.3415	0.2716	1.0000

3.3 Clustering

A statistical analysis of the DNA bands of the three tested strains amplified by the 11 primers was undertaken, and a similarity coefficient dendrogram was constructed (Figure 2). The results indicated that there was some genetic variation among the three strains, and that there was least homogeneity between strains S_{14} and S_{15}, while strains S_{11} and S_{14} showed the closest pair-group clustering. Strains S_{11} and S_{14} could be clustered as a group while strains S_{15} formed a separate group.

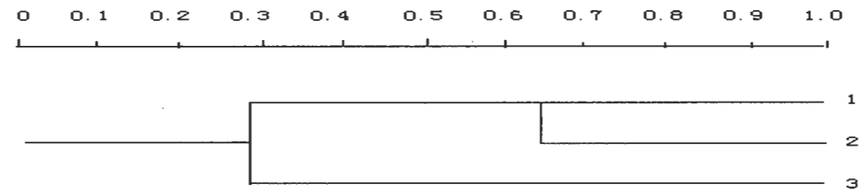

Figure 2. Dendrogram generated from the similarity coefficients of the three *H. marmoreus* strains

4 Discussion

The genomic DNA of three selected *H. marmoreus* strains were examined by RAPD using 11 random primers. Analysis of the resultant PCR patterns indicated that the three strains had different genetic genomes. However, there was a higher degree of similarity between Strains S_{11} and S_{14}, indicating a closer relationship. The fact that variation existed among the strains was also confirmed. Since the basis of RAPD markers is PCR amplification using random primers, a number of fragments were amplified each time. This was inconvenient for analysis of the test results, and for experimental reproducibility. Moreover, the PCR amplified products are usually visualized by electrophoresis on agarose gels which makes automatic analysis ineffective.

References

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