

USE OF ISSR MARKERS FOR STRAIN IDENTIFICATION IN THE BUTTON MUSHROOM, *AGARICUS BISPORUS*

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ABSTRACT

The white button mushroom *Agaricus bisporus* is the most widely cultivated species of edible mushrooms all over the world. Originating from a limited heritage line, commercial strains of *A. bisporus* are supposed to be genetically very similar. Many highly polymorphic molecular markers have been exploited for strain identification in wild and commercial strains of this mushroom. ISSR marker with the whole-genome coverage, accuracy and reproducibility as well as robustness has proved to be a promising marker for genetic diversity analysis of many crops. The objective of this work was to evaluate the potential of ISSR markers for genotype identification in common button mushroom, *A. bisporus*. For this purpose, 18 *A. bisporus* genotypes, including four cultivars, 13 hybrid strains and their single spore progenies along with an indigenous wild strain, were assessed for their similarity using 20 ISSR primers. Out of 20 primers, 10 proved to be discriminative in *A. bisporus*, producing 110 scorable and 76 polymorphic bands. The similarity degree was calculated for each couple of genotypes according to the Jaccard coefficient and grouping was carried out by the UPGMA clustering analysis. ISSR primers successfully identified every single individual; however, high similarity was detected among genotypes. As we expected, the wild genotype Dezful exhibited little relatedness with other genotypes and placed in a separate individual group. Genotypes IM0037 and Dezful with similarity coefficient of 0.44 and genotypes 737 and IM00Ca12 with similarity coefficient of 0.937, were accordingly the least and the most similar genotypes. Our result demonstrates that ISSR markers are powerful enough for detection of polymorphism among closely related genotypes of *A. bisporus*.

Keywords: *Agaricus bisporus*; Genetic Diversity; Molecular marker; Strain identification

INTRODUCTION

Button mushroom (*Agaricus bisporus*), belongs to the genus *Agaricus*, family *Agaricaceae*, Order *Agaricales* and phylum *Basidiomycota*. *Agaricus* is one of the biggest genera of Mushrooms which includes about 300 edible and poisonous species.

One of the most important species of this genus is the white button mushroom which is widely cultivated in most parts of the world. Commercial strains of white button mushroom are classified based on the morphological characters, which are easily affected by the environmental factors. Environmental factors along with the close genetic relation between the isolates, make their isolation and identification difficult and sometimes impossible. In the past two decades, different molecular markers based on nucleic acid polymorphisms, have been exploited in genetic studies of the edible mushrooms.

Castle et al.[1] used RFLP technique for genotyping of *A. brunnescens*, Moore et al. [2] used RAPD for separating cultivars of the button mushroom, Ma and Luo [3] used ITS-RFLP in

genotype identification in the genus *Pleurotus*, Chillali et al. [4] used analysis of ITS and IGS regions for evaluation of genetic diversity in the fungus *Armillaria*, Zhang et al. [5] and Guan et al. [6] used ISSR markers for strain identification in *Lentinula edodes* and isolation of different strains in *A. bisporus*, respectively. Using AFLP, Ghorbani Faal et al. [7] succeeded in partly differentiating closely related hybrid strains of white button mushroom and also obtained some unique bands for a few strains. Variable nature of microsatellite regions, minimum requirements and easy application as well as the reasonable cost, has made ISSR marker a very useful tool for most systematic and ecological evaluations [8]. The aim of this study was to evaluate the ability of ISSR marker, for genetic detection and strain identification of white button mushroom strains, being cultivated in Iran.

MATERIALS AND METHODS

Strains. Eighteen genotypes of *A. bisporus* were used in this study (Table 1).

DNA Extraction. DNA was extracted from 18-20 day-old cultures on compost extract (CE\CYM) liquid medium. Extraction was based on Soltis laboratory protocol [9] with little modifications.

ISSR Analysis. 10 ISSR primers (Table 2) were screened from 20 initial ISSR primers. The amplification reactions was carried out in 20 µl volume containing 30 ng DNA template, 0.5 mM primer, 0.2 mM dNTPs, 2 mM MgCl₂, 1 U Taq DNA polymerase (Genet Bio) and 1x PCR buffer.

Table 1: The 18 *A. bisporus* genotypes used in this study

Strain no.	Strain type	Origin	Strain no.	Strain type	Origin
1	IM002	hybrid strain	10	IM00H15	single spore isolate from commercial strain 737
2	IM003	hybrid strain	11	IM00Ca10	single spore isolate from an unknown commercial strain
3	IM005	hybrid strain	12	IM00Ca12	single spore isolate from an unknown commercial strain
4	IM006	hybrid strain	13	IM00Ca14	single spore isolate from an unknown commercial strain
5	IM008	hybrid strain	14	Dezful	An Iranian wild genotype
6	IM0037	hybrid strain	15	737	commercial strain
7	IM00H2	single spore isolate from commercial strain 737	16	A15	commercial strain
8	IM00H12	single spore isolate from commercial strain 737	17	F60	commercial strain
9	IM00H14	single spore isolate from commercial strain 737	18	2200	commercial strain

The reactions were performed as following: a per- denaturation at 94°C for 3 min, 30 cycles of amplification: 45 sec at 94°C, 1 min at 46-51°C (Table 2), 1 min at 72°C and a final extension at 72°C for 5 min. Amplified products were separated using 1.5% agarose gel and stained by ethidium bromide. DNA ruler 100 bp plus was used as size marker. Band scoring was performed using Total lab software. The similarity degree was calculated for each pair of

genotypes according to the Jaccard coefficient and grouping was carried out by the UPGMA clustering analysis using NTSYS.2 software.

Table 2: Ten selected ISSR primers and the polymorphic bands generated by each

Primer name	Sequence (5'-3')	T _a (°C)	Total bands	Polymorphic bands	Primer name	Sequence (5'-3')	T _a (°C)	Total bands	Polymorphic bands
807	(AG) ₈ T	46	11	6	834	(AG) ₈ YT	49	11	6
808	(AG) ₈ C	49	13	9	835	(AG) ₈ YC	51	8	6
809	(AG) ₈ G	49	10	3	836	(AG) ₈ YA	49	7	6
810	(GA) ₈ T	47	7	5	841	(GA) ₈ YC	50	18	15
811	(GA) ₈ C	48	10	5	842	(GA) ₈ YG	51	15	15

RESULTS AND DISCUSSION

Ten primers (listed in Table 2) created clear bands suitable for analysis, whereas other primers (805, 812, 840, 843, 844, 845, 858, P8, P38 and P39) either produced no band or non scorable ones. These 10 primers totally produced 110 scorable bands, among which 76 were polymorphic. The ability of primers to detect polymorphism among different genotypes was different. Primer 842 with 15 polymorphic bands out of 15 (Figure 1) and primer 841 with 15 polymorphic bands out of 18 comprised the most discriminative primers.

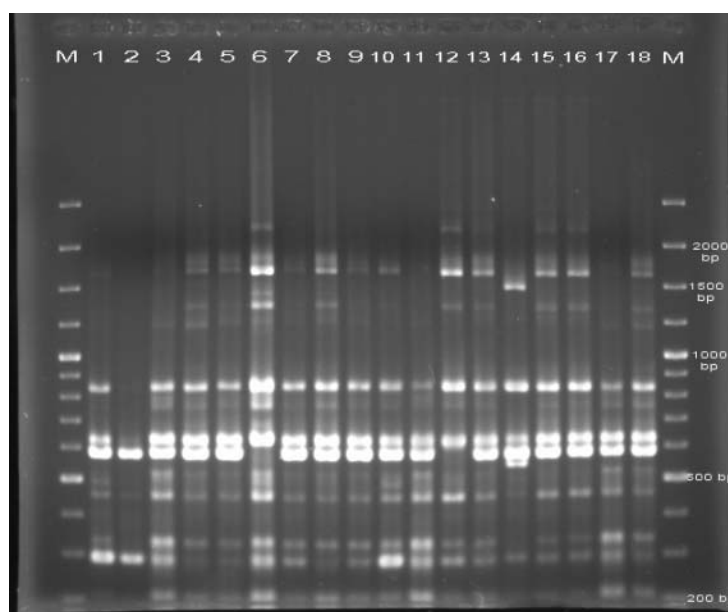


Figure 1: ISSR amplified pattern by primer 842, M: size marker 100 bp plus, Lanes 1-18: Genotypes IM002, IM003, IM005, IM006, IM008, IM0037, IM00H2, IM00H12, IM00H14, IM00H15, IM00Ca10, IM00Ca12, IM00Ca14, Dezful, 737, A15, F60 and 2200.

Based on the sequence of these primers, it seems that GA and AG motives have higher proportion and better distribution in the genome of *A. bisporus*. This is consistent with the results of Guan et al., too [6].

According to the similarity matrix, genetic similarity of sample pairs varied between 0.448 and 0.938. Genotypes 737 and IM00Ca12 with similarity coefficient of 0.937, and genotypes IM0037 and Dezful with similarity coefficient of 0.44 were the most and the least

similar genotypes, respectively (Figure 2). Genotype Dezful which is a wild genotype revealed a completely different banding profile with all 10 selected primers and stood quite distinct from other genotypes in the final cluster.

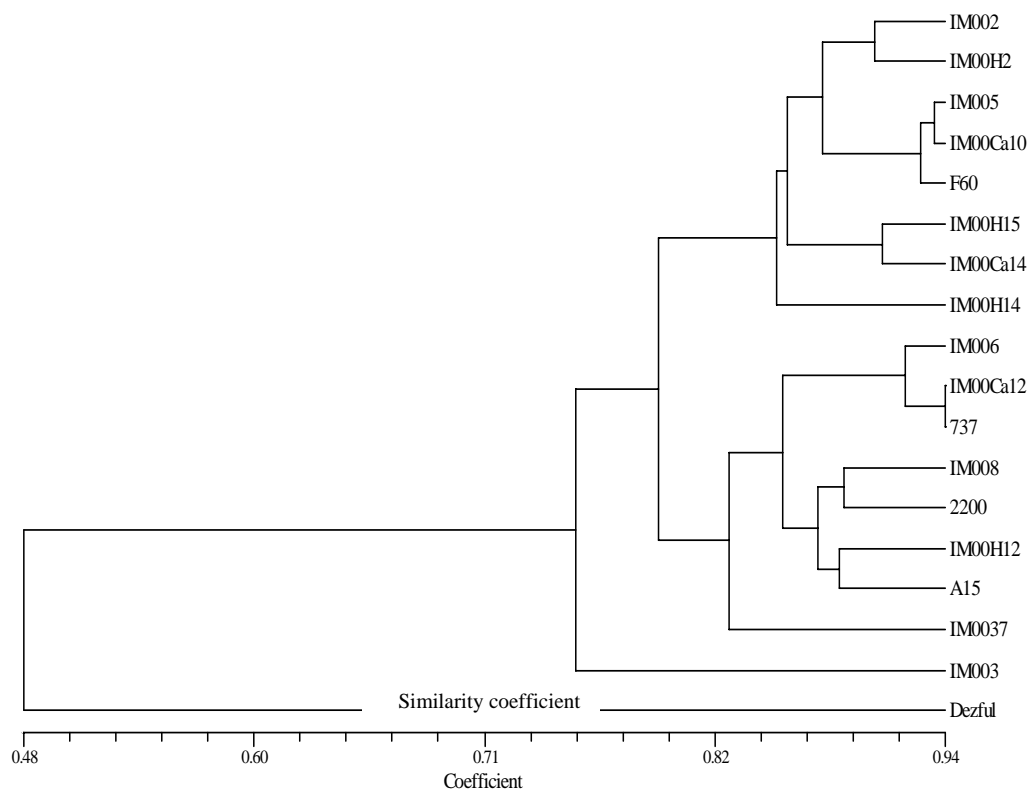


Figure 2: Dendrogram based on ISSR fingerprints for 18 *A. bisporus* strains

Based on the similarity coefficient and the relating dendrogram, strains IM00Ca10, IM00Ca12 and IM00Ca14, all three single spores of an unknown Canadian strain, proved to be very similar to each other and the commercial strain 737. This result led us to this hypothesis that these might have been originated from a single strain, 737. Moore et al. [2] had also described that commercial *A. bisporus* cultivars from different companies reflected a lack of genetic diversities.

In a work by Guan et al.[6], 12 main strains of *A. bisporus* were collected from different provinces in China and tested by six ISSR primers. Although all the strains were successfully differentiated, the results showed a high similarity coefficient between the strains, implying that they might originate from a single maternal strain, U1.

The results of our study also showed high similarity between the commercial strains of *A. bisporus* which are currently cultivated in Iran. At the mean time, ISSR markers provided a good resolution ability to detect small differences.

CONCLUSIONS

Overall, it can be concluded that ISSR markers are useful tools to assess genetic variations among closely related strains of *A. bisporus*. Furthermore, primers containing repetitive sequences of AG or GA will be more favorable for genetic identification and strain detection in this species.

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REFERENCES

- [1] Castle AJ. et al. (1987). Restriction fragment length polymorphisms in the mushrooms *Agaricus brunnescens* and *Agaricus bitorquis*. *Appl Environ Microbiol.* 53:816–822.
- [2] Moore AJ. et al. (2001). RAPD discrimination of *Agaricus bisporus* mushroom cultivars. *Appl Microbiol Biotechnol*, 55: 742–749.
- [3] Ma FY. & Luo XC. (2002). PCR-based restriction analysis of internal transcribed spacers of nuclear ribosomal DNA in the genus *Pleurotus*. *Mycosystema.* 21:356–362.
- [4] Chillali M. et al. (1998). Variation in the ITS and IGS regions of ribosomal DNA among the biological species of European *Armillaria*. *Mycol. Res.* 102(5): 533-540.
- [5] Zhang R., et al. (2007). Strain-typing of *Lentinula edodes* in China with inter simple sequence repeat markers. *Appl Microbiol Biotechnol.* 74: 140–145.
- [6] Guan XJ. et al. (2008). Differentiation of commercial strains of *Agaricus* species in China with inter-simple sequence repeat marker. *World J Microbiol Biotechnol.* 24:1617–1622.
- [7] Ghorbani Faal, P. et al. (2009). Preparation of AFLP Mediated-Molecular Certificate for 12 Bred Strains of the Button Mushroom, *Agaricus bisporus*. *J Plant Protec.* 23(1): 58-67.
- [8] Reddy MP. et al. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Eyphytica.* 128: 9-17.
- [9] Soltis Lab CTAB DNA Extraction Protocol. (2002). The Soltis Lab, Florida Museum of Natural History, on line at: <http://www.flmnh.ufl.edu/soltislab>.