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

KHALIL MALEKZADEH¹, BANAFSHEH JALALZADEH MOGHADDAM SHAHRI¹*, EHSAN MOHSENFARD²

1- Department of Industrial Fungi Biotechnology, ACECR, University campus, Mashhad-Iran –
2- Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad-Iran.

bjalalzadeh@gmail.com

ABSTRACT

The white button mushroom *Agaricus bisporus* is the most widely cultivated specious 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 exploiting 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 *Basidomycota*. *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>
<thead>
<tr>
<th>Strain no.</th>
<th>Strain type</th>
<th>Origin</th>
<th>Strain no.</th>
<th>Strain type</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IM002</td>
<td>hybrid strain</td>
<td>10</td>
<td>IM00H15</td>
<td>single spore isolate from commercial strain 737</td>
</tr>
<tr>
<td>2</td>
<td>IM003</td>
<td>hybrid strain</td>
<td>11</td>
<td>IM00Ca10</td>
<td>single spore isolate from an unknown commercial strain</td>
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<tr>
<td>3</td>
<td>IM005</td>
<td>hybrid strain</td>
<td>12</td>
<td>IM00Ca12</td>
<td>single spore isolate from an unknown commercial strain</td>
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<tr>
<td>4</td>
<td>IM006</td>
<td>hybrid strain</td>
<td>13</td>
<td>IM00Ca14</td>
<td>single spore isolate from an unknown commercial strain</td>
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<td>5</td>
<td>IM008</td>
<td>hybrid strain</td>
<td>14</td>
<td>Dezful</td>
<td>An Iranian wild genotype</td>
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<tr>
<td>6</td>
<td>IM0037</td>
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<td>15</td>
<td>737</td>
<td>commercial strain</td>
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<td>7</td>
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<td>16</td>
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<td>commercial strain</td>
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<td>8</td>
<td>IM00H12</td>
<td>single spore isolate from commercial strain 737</td>
<td>17</td>
<td>F60</td>
<td>commercial strain</td>
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<tr>
<td>9</td>
<td>IM00H14</td>
<td>single spore isolate from commercial strain 737</td>
<td>18</td>
<td>2200</td>
<td>commercial strain</td>
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</table>

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

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5'-3')</th>
<th>$T_a$ (°C)</th>
<th>Total bands</th>
<th>Polymorphic bands</th>
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<th>Total bands</th>
<th>Polymorphic bands</th>
</tr>
</thead>
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<tr>
<td>807</td>
<td>(AG)$_6$T</td>
<td>46</td>
<td>11</td>
<td>6</td>
<td>834</td>
<td>(AG)$_6$YT</td>
<td>49</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>808</td>
<td>(AG)$_6$C</td>
<td>49</td>
<td>13</td>
<td>9</td>
<td>835</td>
<td>(AG)$_6$YC</td>
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<td>(AG)$_6$YA</td>
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<td>(GA)$_6$C</td>
<td>48</td>
<td>10</td>
<td>5</td>
<td>842</td>
<td>(GA)$_6$YG</td>
<td>51</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Ten primers (listed in Table 2) created clear bands suitable for analysis, whereas other primers (805, 812, 840, 843, 844, 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.

![Dendrogram based on ISSR fingerprints for 18 A. bisporus strains](image)

**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.
ACKNOWLEDGEMENTS

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REFERENCES