A Preliminary Study on the Molecular Mechanism of Clustering Variation of *Agarucus bisporus*

Wang Zesheng Chen Meiyuan Liao Jianhua Lu Zhenghui Guo Zhongjie
Fujian Research Institute of Light Industry, Fuzhou, 350005

Abstract: Using the genomic DNA of healthy strains and their clustering variation ones of *A.bisporus* as templates, a large scale of RAPD amplification was performed, and a random primer 014 was found to be able to distinguish the healthy and clustered strains. The DNA fragment relevant to clustering variation was purified and cloned into pUC-T plasmid. Sequencing result showed that the fragment had a size of 735bp, and it encodes the C-terminal 173 amino acids of a certain dehydrogenase. The special probe was prepared with the cloned fragment. The result of Southern blotting suggested the fragment to be a single copy sequence in the genome of *A.bisporus*. At present research on the expression product of the gene related to the cloned fragment is on progress.
Key Words: *Agarucus bisporus*, Clustering variation, Molecular mechanism, RAPD

Agaricus bisporus, commonly known as white button mushroom, has been the most widely cultivation edible fungi all over the world. In nonnatural cultivation, its fruitbodies are normally single and the caps are round. Cluster means that several fruitbodies grow together and share just one stipe, which is an abnormal growing situation of *A.bisporus*. Most of the fruitbodies clustered in the cultivation when using the strains with cluster variation. The caps of clustered fruitbodies huddle together, and quantities of abnormal mushrooms with lower commercial value are produced. Cluster variation was hardly found in China but often abroad especially in America. The earlier study proved that the cluster was due to strains' variation but not to cultivation conditions, and the variation could be remained in asexual reproduction. Because cluster variation only appears in fruitbodies, and shows nothing abnormal in mycelia, its earlier prediction is difficult. The mushroom spawn companies and growing farms abroad lost millions of dollars every year because of the cluster. At present several laboratories all over the world are searching for the internal reasons of cluster variation of *A.bisporus* from different directions such as genomic DNA, RNA, mtDNA and so on. Starting with RAPD of genomic DNA, we carried out a preliminary study on the molecular mechanism of this variation.

1 Materials and Methods

1.1 1.1 **Strains**: Healthy strains of *A.bisporus* AA, GG, I and their clustered isolates A33, A34, A35, A41, A43, G26/1, G26/1/2 and I40 derived from the corresponding clustered fruitbodies through tissue isolation, were supplied by Fujian Mushroom Research & Development Station.

1.2 1.2 **Methods**:

1.2.1 Genomic DNA extraction,PCR and RAPD analysis were as described before (Chen Meiyuan et al.,1998).

1.2.2 Purification and cloning of target bands:The target bands were purified with low melting point agarose gel and linked to pUC-T vector, then transfer into E.coli JM109. The transformants were screened with blue/white method, and verified with double enzyme digestion and PCR.

1.2.3 Probe preparation: Replace dTTP with Dig-dUTP, and prepare the Dig-labeled probe by PCR.

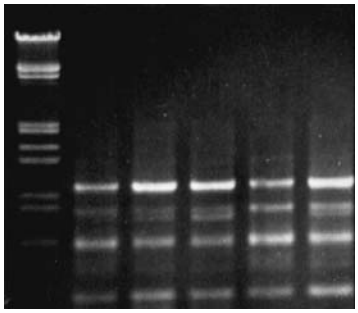
1.2.4 Southern blotting: "Dig DNA Labeling and Detection Kit" was used for Southern hybridization and color development as recommended by the manufacturer (Boehringer Mannheim).

1.2.5 Total RNA extraction and reverse transcription: "Super UNIQ-10 Column Total RNA Extraction Kit" and "First Strand cDNA Synthesis Kit" were used for total RNA extraction and reverse transcription as recommended by the manufacturer (Shanghai Sangon).

2 2 Results and Analysis

2.1 2.1 **RAPD analysis**: Using the genomic DNA of healthy strains AA, GG, I and their clustering variation isolates A35, A41, G26/1/2 and I40 of *A.bisporus* as templates, a big-scale of RAPD with 75 random primers was performed. It was found 4 random primer showing the difference between the healthy and clustered strains at different level, and among them primer 014 was the better. Fig 1 showed the RAPD pattern of healthy and clustered strains amplified by the random primer 014. It can be seen that at about 800bp, the clustered strains produce one more band than the healthy ones.

M 1 2 3 4 5



Lane M: λ DNA/EcoRI+Hind Ⅲ Marker; Lane 1-5: RAPD band patterns of the total DNA of AA,A35,A41, GG and G26/1/2.

Fig.1 RAPD pattern of healthy and clustered strains of *Agaricus bisporus* amplified by the random primer 014

2.2 2.2 **Purification and cloning of the specific DNA fragment relevant to cluster**: The target specific DNA fragment showing the cluster variation was purified and cloned into pUC-T vector. The transformants were obtained by blue/white screening. After determined by PCR with the primer 014 and double enzyme digestion with EcoRI+BamHI, the transformant No.14 was proved to have cloned the cluster special DNA fragment successfully (Fig.2).

1 2 3 4 5 6 M

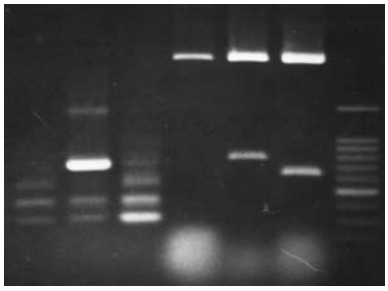


Fig.2 Determination of the clonants by PCR (with the primer 014) and double-enzyme (EcoRI+BamHI) digestion

Lane 1-3: PCR band patterns of plasmid pUC-T, clonant No.14 and No.2; Lane 4-6: Double-enzyme digestion patterns of plasmid pUC-T, clonant No.14 and No.2; Lane M: 100bp DNA ladder

2.3 2.3 **Probe preparation and Southern blotting**: Using EcoRI digested No.14 plasmid as template, the specific DNA probe was prepared by PCR with primer 014. Southern blotting of total DNA of AA, A34 with No.14 probe proved the cloned DNA fragment to be a single copy sequence in the genome of *A.bisporus*(Fig.3), which offered the possibility of further study.

1 2 3 4 5 6

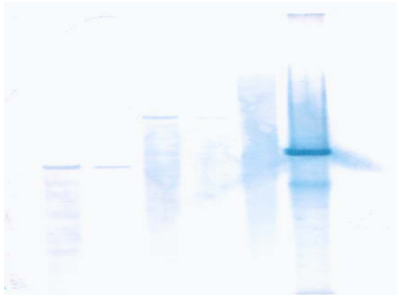


Fig.3 Southern blotting of genomic DNA of *A.bisporus* with No.14 probe

Lane 1-6: A34 total DNA/EcoRI; AA total DNA/EcoRI; A34 total DNA/BamHI; AA total DNA/BamHI; AA total DNA CK; No.14 plasmid/ BamHI

2.4 2.4 **Sequence analysis of cloned DNA fragment**: Sequencing result showed that the cloned DNA fragment had a size of 735bp (including the primer sequence). The sequence was reverse-complemented into its mRNA sequence. The result of GenBank searching showed that from the new 5' end, the sequence encodes a partial open reading frame (ORF) for the c-terminal 173 amino acids

of a certain dehydrogenase. There are two or three frameshifts in the coding sequence: one at about position 210-215, one between positions 400-460 (where there is a 20 AA non-aligning insertion in *A.bisporus* with respect to other fungal sequences), and probably also one at about position 35. The remaining 215 bases downstream of the ORF show no significant homology to any sequence in GenBank.

2.5 2.5 **Expression product analysis of relevant gene**: To find out the difference of expression of the dehydrogenase gene containing the cloned sequence, total RNAs were extracted from the healthy or clustered fruitbodies and reverse transcribed into cDNA. The full-length mRNA sequence of the dehydrogenase from *Schizosaccharomyces pombe* was obtained by Genbank searching. According to this sequence and the 735bp special sequence, some PCR primers covering the mRNA sequence were designed and synthesized, and PCR amplifications were performed using the synthesized cDNA. At present some interesting DNA fragments were obtained and their cloning and sequencing were on progress.

3 Discussion

3.1 Through a big-scale of RAPD, we screened the primer 014 which could well distinguish the healthy and clustered strains of *A.bisporus*, so a DNA level marker was offered for early prediction of cluster variation of *A.bisporus*. But it's not time for its application to production because the RAPD marker was unstable. We have to test much more cluster strains to confirm the relativity between the marker and the cluster variation.The final aim of this study is to find a stable and reliable marker for early prediction, and clarify the molecular mechanism of cluster variation, but it's obvious there is still a long way.

3.2 The cloned sequence encodes the c-terminal 173 amino acids of a certain dehydrogenase. This enzyme is a nuclear-encoded protein of the mitochondrial matrix and it is important in cellular respiration. Very serious genetic diseases are associated with genetic deficiencies in this enzyme. Our findings suggest the possibility that one aspect of clustering might involve respiratory pathways. However, this would be a speculative hypothesis needing much further investigation.

3.3 At present we are studying on the expression product of the dehydrogenase containing the cloned sequence relevant to cluster variation from mRNA. If necessary, we'll construct the cDNA library of the healthy strain, find the target gene by hybridization and sequence it, then design the specific primers and amplify the gene in cluster strains for comparison.