# RAPD Analysis of Species Diversity and Genomic Difference in *Agaricus bisporus*

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## 1 INTRODUCTION

As a secondarily homothallic basidiomycete, *Agaricus bisporus* (Lange) Imbach (= *Agaricus brunescens* Peck) has unusual genetic characteristics, which results in two barriers in the proceeding of genetics and breeding research. First, the predominance of two spored basidia causes most of spores to be heterokaryotic and self-fertile; second, there is no distinguishable physiological morphology between homokaryotic and heterokaryotic hyphae. Both of these two properties make it difficult to obtain homokaryotic sterile spores, which is the basic material in practice of cross-breeding. They also affect the precision of genetical assays in this species.

In order to overcome these natural barriers, genetic markers such as auxotrophic phenotype, recovered fertility, isozyme, RFLP, RAPD etc. were applied into research of genetics and breeding of *A. bisporus* to analyze genetic relationships between different strains, isolate homokaryotic spores, and identify hybrids. Previous studies with different genetic markers had elucidated two special aspects of genetic characteristics of *A. bisporus*, viz.that of nonrandom segregation of the four meiotic products, i.e. non sister nuclei are preferentially incorporated into basidiospores after meiosis II, and that low levels of recombination had been observed.

In this study, we use RAPD to examine (i) the interspecific and intraspecific polymorphism between two different species of *Agaricus*, *A. bisporus* and *A. bitorquis*; (ii) the genetic relationship of the strains of two genetic families of *A. bisporus*, which had been found based on the genetic markers of isozyme; (iii) the differences between RAPD patterns of two types of strains of *A. bisporus* owing different agronomic traits ( high productive type with poor quality, type H *vs.* low productive type with good quality, type G ).

## 2 MATERIALS AND METHODS

### 2.1 *Strains.*

The strains of *A. bisporus* and *A. bitorquis* used in this study are listed as bellow,

*A. bisporus*, type H ( High productive type with poor quality ): 02, 176, 111, 13D, ME, 01; type G ( Low productive type with good quality ): 8213, 8211, C1, FG4, M1#; type S ( Sterile homokaryotic strains ): 5412, 5425, 361-2, As165, 02-1, 110-10; type HG ( hybrids of type H strain and type G strain ): W95-2, 2796, 4607, 2987; wild strain: Fs-3; brown strain: 093.

*A. bitorquis*, 177, TGW, JW8002.

All these strains have been maintained in Fujian Mushroom Research and Development

Station (FMRDS). Mycelial cultures of these strains were grown in solid PDA medium.

**2.2** ***Preparation of genomic DNA.***

Genomic DNA for RAPD reaction and Southern hybridization was isolated using a modified method of Dellaporta et al. (1983).

**2.3** ***RAPD reaction.***

PCR conditions of RAPD were as described (Williams et al.1990). The reaction conditions were: 94°C 1min, 36°C 1min, 72°C 2min, 42 cycles. A portion of each reaction was analyzed on a 1.4% agarose gel.

**2.4** ***Cloning of specific DNA fragment and Dot hybridization.***

Low melting temperature agarose gels were used to isolate and purify the target specific DNA fragment. The purified specific DNA fragment was blunt-ended by klenow fragment and cloned into SmaI site of pUC19. The purified target specific DNA fragment was labeled by Digoxigenin (DIG) and used for dot hybridization as recommended by the manufacturer (Boehringer Mannheim)

**2.5** ***Analysis of data.***

RAPD patterns were analyzed using the method described by Royse and May (1982).

## 3 RESULTS

### 3.1 *RAPD analysis of interspecific and intraspecific polymorphism of Agaricus bisporus and Agaricus bitorquis.*

RAPD analysis was performed for six strains of *A. bisporus*: Fs3, 093, 02, 2796, M1#, 8211 and three strains of *A. bitorquis*: 177, TGW, JW8002. Based on RAPD patterns, statistical data were obtained and a genetic similarity tree among the strains was constructed (Fig.1).

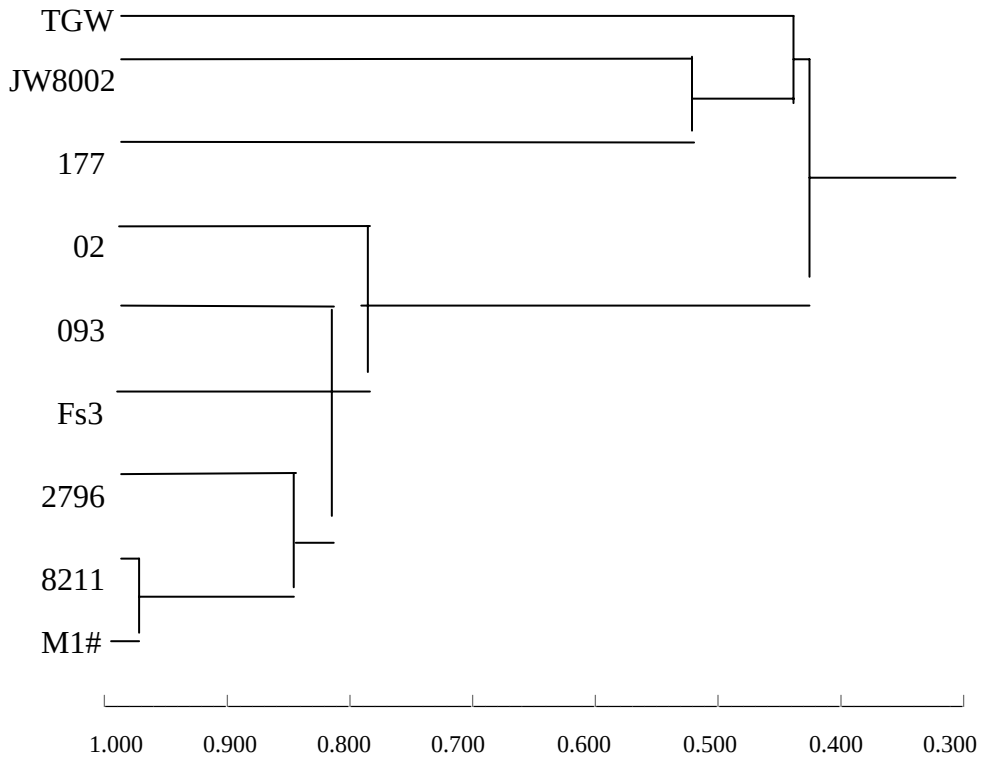
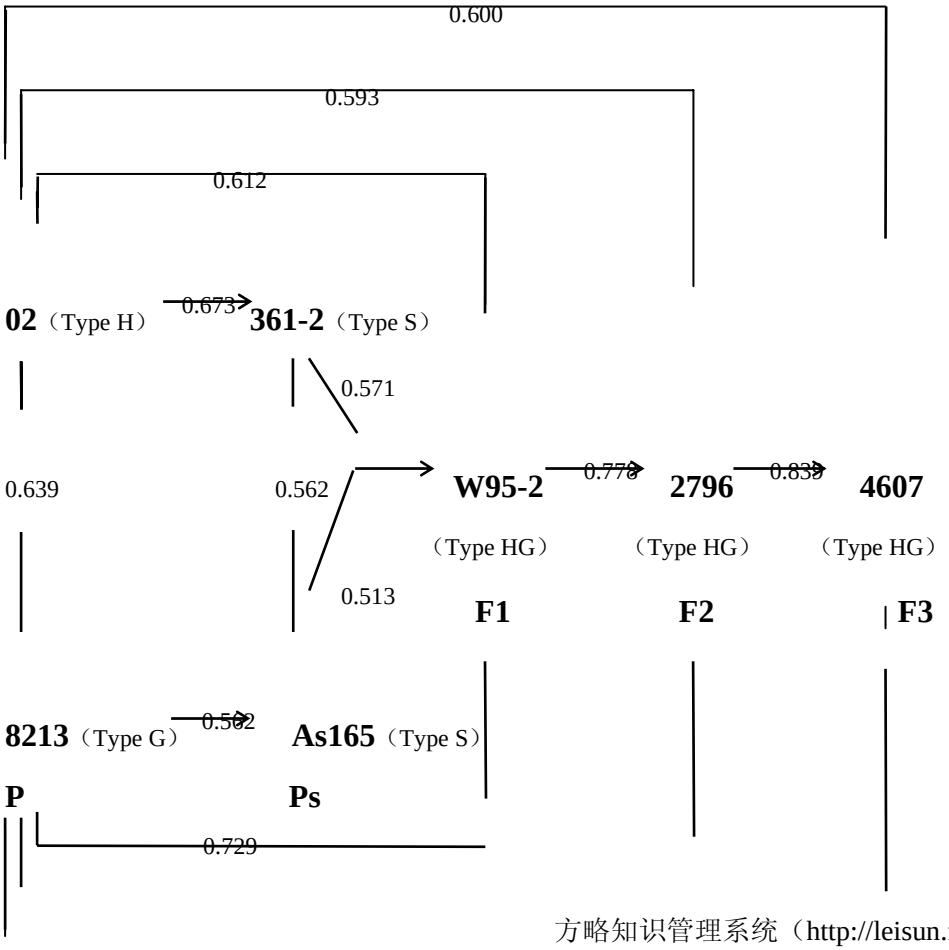


Fig. 1 The tree of genetic similarities among the strains of *Agaricus bisporus* and *Agaricus bitorquis.*

From the data in Fig.1, a relatively high degree of genetic similarity is concluded to among the strains of *A. bisporus*; the average S value among this species is 0.804. Meanwhile, there is obvious intraspecific variability in another species of *Agaricus*, *A. bitorquis*, the average genetic similarity is 0.429. The sources of the tested strains of *A. bisporus* were chosen widely different with view to obtain more common research significance, the genetic variation among all these strains is still very low. Similar results had been reported by Castle et al.(1987,1988) and Royse et al.(1982), where RFLP and isozyme were taken as genetic markers respectively. Though the fruitbodies of these two related species look rather similar, our results show that the interspecific consanguinity between these two species was not as near as expected, the average value of genetic similarity is 0.404. Among three strains of *A. bitorquis*, strain JW8002 is the strain which is the most related to the strains of *A. bisporus*, while the high yield strain, 02, has the shortest genetic distance to *A. bitorquis* among the strains of *A. bisporus*.

### 3.2 *RAPD analysis of two genetic families of Agaricus bisporus.*

Nine random primers were used in the analysis. From the RAPD patterns, the genetic maps of these two families were presented(Fig 2, 3).



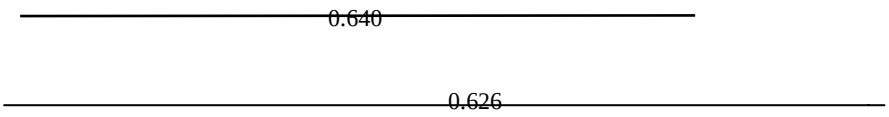



Fig. 2 The constitution of the genetic family with the parental strains 02 and 8213 and the degree of genetic similarity between different individual strain ( P, Ps, and F stand for heterokaryotic parental strain, homokaryotic parental strain and the filial generations )

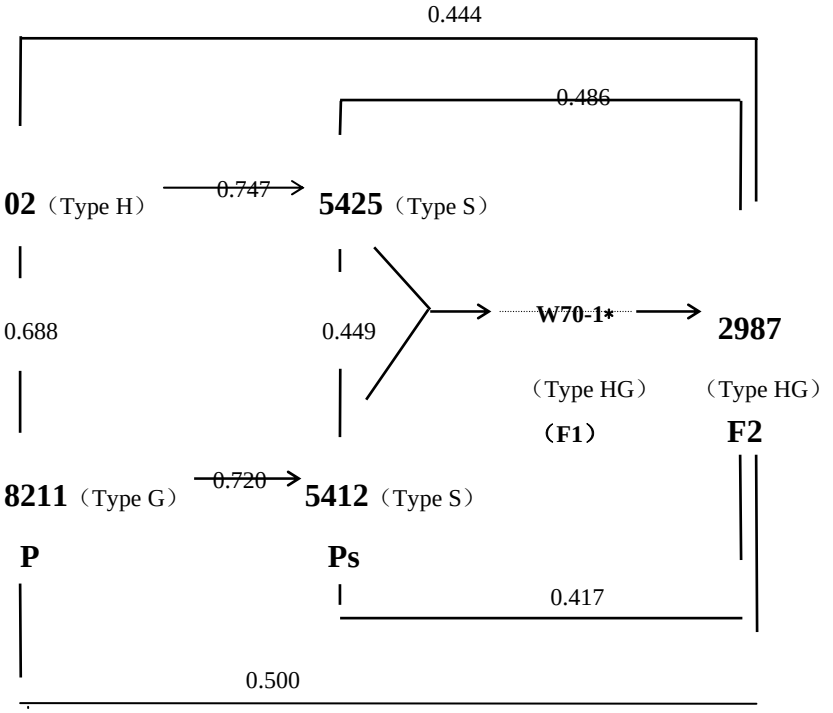



Fig. 3 The constitution of the genetic family with the parental strains 02 and 8211and the degree of genetic similarity between different individual strains (the meaning of the symbols are the same as Fig 2)

*: This strain had not been taken into the research for having not been preserved

In the genetic family shown in Fig 2, the conclusion can be drawn that as the number of genetic generations, the genetic distance between progenies and the original parents from which hybrids are derived increases. Similar phenomena can be seen in the family shown in Fig 3. Meanwhile, all hybrid progenies appear more similar to its type G parental strains than to type H parental strains, because genetic similarity between hybrid progenies and its type G parental strain was generally higher. This result correlates to the observation in cultivation that hybrid progenies and the type G parental strains have more similar agronomic traits, such as thicker veil tissue, smaller pink gill and more rounded cap.

### 3.3 *Cloning of specific DNA fragment displaced by RAPD and RFLP analysis.*

Using 20 random primers, RAPD differential display was carried out between two types of *A. bisporus* strain with complementary phenotype in cultivation, one is type G (low quantitative with good quality) including the strains 8213, 8211, C1, M1#, FG4; another is type H (highly quantitative with poor quality) including the strains 02, 176, 111, 13D, 01. After screening, it was found that the amplification patterns with OPU17 were different between the two types of strain (Fig 4). There is a specific DNA fragment with size about 2,000bp appeared only in type G strains. A specific DNA fragment named $G17_{2000}$ was extracted and purified using low melting temperature agarose gel ( Fig 5 ). Purified DNA was labeled by DIG and used as probe in the following experiment.

The specific DNA fragment $G17_{2000}$ was cloned into vector pUC19 which had been linearized by the restriction enzyme SmaI. The recombinant plasmid was identified by restriction enzyme digestion and dot hybridization ( Fig. 6 ).

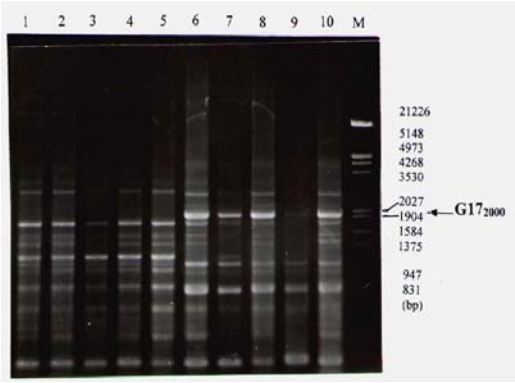



Fig. 4 Amplification pattern with random primer OPU17.

M: DNA/HindIII marker, lanes 1-10 are PCR products of strain 02, 176, 111, 13D, 01, 8213, 8211, M1#, C1, FG4, respectively.

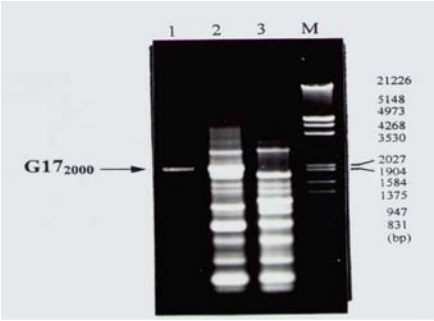



Fig. 5 Purification of target DNA fragment $G17_{2000}$.

M: λDNA/HindIII+EcoRI marker;

1: PCR product of strain 02 with OPU17;

2: PCR product of strain 8213 with OPU17;

3: Purified DNA fragment $G17_{2000}$.

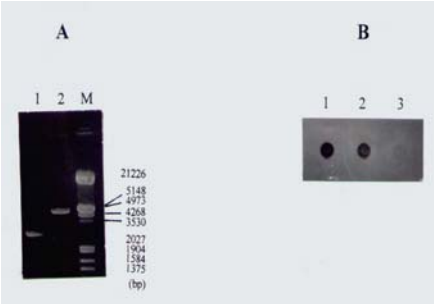



Fig.6 A: Identification of recombination plasmid with $G17_{2000}$.

M: λDNA/HindIII +EcoRI;  1: pUC19 plasmid/SacI;
2: recombination plasmid/SacI.
B: Identification of recombination plasmid with $G17_{2000}$ by Dot hybridization.
1: positive control; 2: recombination plasmid;
3: negative control.

## 4 DISCUSSION

In our study of RAPD analysis of interspecific and intraspecific polymorphism of *Agaricus bisporus* and *Agaricus bitorquis*, limited genetic diversity was found in *A. bisporus*, all degree of genetic similarity being higher than 0.75, even though the studied strains with different phenotypes cover a great range of sources. It indicates that because no aboriginal strain of *A. bisporus* exists in China, most strains maintained in our plasm bank or used in different cultivation areas are derived from a few ancestors introduced from abroad at the beginning of this century.

The genetic relationship between *A. bisporus* and *A. bitorquis* was not directly evaluate by RFLP in Caste et al.'s(1987) study. Our result show a low degree of interspecific genetic similarity (0.404), suggesting the difficulty to perform cross-breeding between these two related species.

To improve the quality of the plasm of *A. bisporus*, the following three ways of cross-breeding are theoretically feasible, (i) commercial strain × wild strain, for there are more genetic polymorphism and some agronomic traits of commercial interest in wild strains which generally have disease or pest resistance and can grow well in adverse environment; (ii) conservative two spores strain × newly discovered tetraspore strain, for the trait of 4-spore will facilitate crossing, breaking out the barrier set by the secondarily homothallism; (iii) *A. bisporus* × *A. bitorquis*, though it may be the most difficult way, the economically advantageous traits of thermal and mycovirus resistance of *A. bitorquis* are still appealing to breeders to try this uneasy way. In fact, a preliminary study had been done in 1991 by us trying to fuse their protoplasts (Wang et al.1990).

One of the most important applications of RAPD is that it is a useful tool in differential display at genomic DNA level to search for specific DNA fragment tightly linked with the trait of interest. we compared the RAPD patterns of two different kind of strains of *A. bisporus* with complementary agronomic phenotypes, one is high yield but poor quality strain, type H and another is low yield but good quality strain, type G. We finally obtained a 2,000bp DNA fragment, $G17_{2000}$ which is specific in type G strains. Sequencing $G17_{2000}$ is on going in order to find more specific DNA probes or PCR primers for the internal sequences of $G17_{2000}$ to distinguish the type G and H strains more effectively.

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