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RESEARCH ARTICLE

Development of a Segregating Population with Biological Efficiency in *Agaricus bisporus*

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Abstract

In this study, we made a population with high biological efficiency (BE) to investigate the complex genetic architecture of yield-related traits in *Agaricus bisporus*. MB-013 crossed between bisp 015-p2 and bisp 034-p2, had high BE. Additionally MB-013 was an intervarietal hybrid that intercrosses with *A. bisporus* var. *burnettii*, bisp 015, and *A. bisporus* var. *bisporus*, bisp 034. One hundred and seventy homokaryons were selected using the cleaved amplified polymorphic sequence (CAPS) markers (PIN primer/HaeIII) from 300 single spore isolates (SSIs). One hundred BC_1F_1 hybrids were obtained by crossing the homokaryons of MB-013 with bisp15-p1. The population of 100 BC1F1 hybrids is suitable for analyses of BE.

Keywords: *Agaricus bisporus*, Biological efficiency, Segregatimg population

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Introduction

Agaricus bisporus is one of the most widely consumed mushrooms in the world [1]. In Korea, *A. bisporus* has been one of the most popular edible mushrooms and the total production of *A. bisporus* was 9,732 MT (63 billion won) in 2015 [2].

There are two varieties of *A. bisporus*, i.e., *bisporus* and *burnettii*. *A. bisporus* var. *bisporus* mostly generates two spores with two non-sister nuceli. Around 10~15% of basidia actually produce four spores with haploid nuclei that are compatible with the other haploid nuclei (homokaryons) [3]. *A. bisporus* var. *burnettii* typically has four spores with a single nucleus after meiosis. Among single spore isolates of this variety, approximately 90% were homokayons and 10% were heterokaryons [4, 5]. However, *A. bisporus* var. *burnetti* shows poor features under commercial conditions with respect to pinning, scaling, cap shaped mushroom distribution and yield, compared with those of *A. bisporus* var.*bisporus* [6, 7].

Yield is the most important trait for commercial cultivars in breeding. The genus *Agaricus* is known for its potential to degrade lignocellulosic materials [8, 9]. The bioconversion of the substrate and the biodegradation of lignocelluloses in the substrate,

i.e., biological efficiency (BE) are related to yield. The BE is expressed as the yield of fresh fruiting bodies per 100 g of dry substrate according to Chang et al. [10]. Current studies define it as the percent conversion of dry substrate to dry matter in fruiting bodies [11].

A segregating population is required to perform linkage mapping. In segregating populations, parental lines should be selected based on strains that are significantly and tightly linked to the goal of the mapping study. Additionally, population size is important. The smaller the size of population, the less robust of the linkage map is. Population size has an effect on the detection of linkage, fragmented linkage groups, and locus order [12, 13].

The purpose of this study was to develop a segregating population to perform linkage mapping and quantitative trait locus mapping for traits related to BE.

Materials and Methods

Mushroom strains

The commercial strain *A. bisporus* A15 was obtained from the strain collection of Wageningen UR Plant Breeding. Eight wild strains of *A. bisporus* were obtained from the *Agaricus* Resource Program (ARP) (Table 1) [14].

Table 1. List of *Agaricus bisporus* strains used in this study

Lab name	Cap color	Origin	Location	Number of spores per basisium	ARP
bisp210		USA	near airport, Colorado	2	JPH-1
bisp059		Ukraine		2	IBKF-46
bisp141/03		USA		4	JB-35
bisp103	brown	USA	CA	2	FS-59
bisp034	light brown	USA	San Mateo	2 (3)	FS-20
bisp015	dark brown	USA	Riverside	4	JB-2
bisp170	off-white	UK		2	AMA-3
bisp119/9		USA	California desert	4	119/9
A15	white	commercial	Sylvan		

ARP, *Agaricus* Resource Program.

Preparation of hybrids

Two parental strains (p1 and p2) were isolated as protoclones by established protocols [15] from 9 strains, and strains were intercrossed using a diallel crossing scheme (Fig. 1). Hybrid compatibility was confirmed based on mycelia morphology or molecule markers.

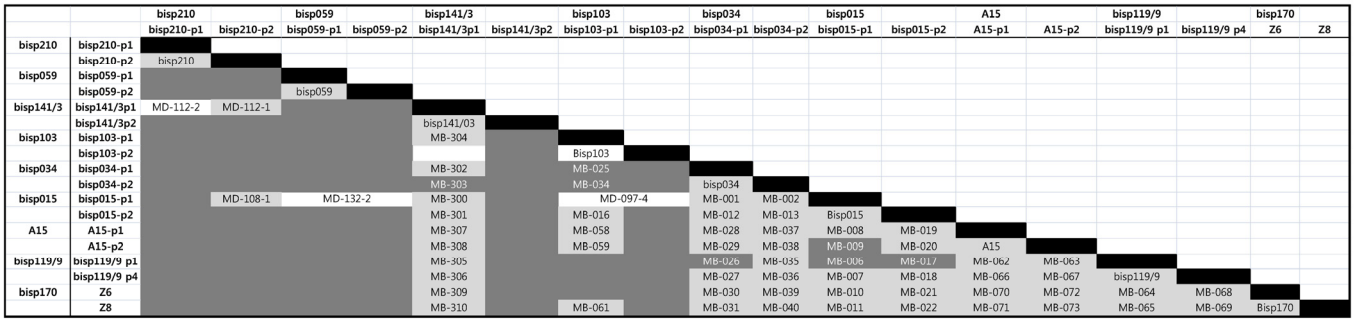

Fig. 1. Diallel matrix of intercrosses with homokaryons of *Agaricus bisporus*. Dark grey box, not crossing; Light grey box, crossing; White box, di-mono crossing.

Spawn preparation and mushroom cultivation

Independent cultivation experiments were performed for each set of 153 hybrids at the mushroom farm of Unifarm in Wageningen UR under a controlled climate. Mushrooms were grown on commercial compost (CNC substrates) and spawned in 0.1 m^2 boxes (40 × 30 × 21 cm) filled with 8 kg of compost. Each individual was grown once in one box [7].

Biological efficiency (BE)

Total weights of all harvested fruiting bodies were measured as the total yield of mushrooms. The BE (mushroom yield per kg of substrate on a dry wt basis) was calculated according to the following formula [10].

$$\text{BE}\,(\%) = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Development of the BC_1F_1 population with CAPS markers

For one hybrid with high BE, over one thousand single spore isolates (SSIs) were obtained to develop a segregating population by microscopy. The mycelia of SSIs were incubated for 7~8 days in malt mops pepton (MMP) media. Then, 15 μL of PCR mix (10× PCR buffer; Qiagen, Germantown, MD, USA), 25 mM $MgCl_2$ (Qiagen), 5 mM dNTP mix (Qiagen), and 5unit of HotStarTaq (Qiagen) was added to each well of a 96 wells PCR plate on ice, and 1 mm^2 of mycelium from each culture were transferred to 50 μL of DNA extraction buffer ($T_{10}E_{0.1}$buffer) in each well of a 96 well plate. The 96 well plates with mycelia were placed in a microwave for 90 sec. The plates were removed from the microwave and directly placed on ice. The mycelia were mixed with the DNA extraction buffer by pipetting up and down and 10 μL of solvent was transferred to the plate containing the PCR mix with three markers followed by PCR. The cleaved amplified polymorphic sequence (CAPS) markers MatA_HD2.1 and MatA_HD1 were in the A-mating type homeodomain region of different *A. bisporus* strains (H97, H37 and Bisp119/9-p4). The PIN50 marker was made following

previous protocols [16, 17]. Amplifications was performed using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) using an initial denaturation step at 95°C for 15 min, 35 cycles consisting of 30 sec at 94°C, 30 sec at the appropriate annealing temperature (Table 2), and 90 sec at 72°C and a final extension step at 72°C for 5 min. The 10 μL of restriction digest master mix (10× restriction buffer, 10 U/μL restriction enzyme) was added to each well of a 96-wells PCR plate on ice. 5 μL of PCR product was added to each well, and the plate was sealed and spun to collect the liquid at the bottom of the wells. After restriction digest, the sample was incubated at 37°C for 2 hr. After incubation, the sample was resolved on 1% (w/v) agarose gels. One hundred selected homokaryons were crossed with the other parental line (bisp15-p1) to develop the BC1F1 population.

Table 2. List of primers used for the development of the BC_1F_1 population

Marker		Sequence	Ta (°C)
PIN150	Forward	CAATCTCAAGCTTGCCTGG	58
	Reverse	AGGTGACATGTCAGAAGCGC	
MatA_HD2.1	Forward	TGGCAACTAAAACAGGTATG	50
	Reverse	TATCGAGGGAGGACCAACT	
MatA_HD1	Forward	CYTCCCTTCCTCATACACA	50
	Reverse	GCCATCGRTCTGGGTATTA	

Results and Discussion

BE of hybrids

One hundred fifty-three hybrids were obtained with eighteen protoclones from nine strains. Five strains were *A. bisporus* var. *bisporus* and bisp 141/03, bisp015 and bisp119/9 were *A. bisporus* var. *burnettii*. After intercrossing, 64 lines were used to investigate mycelial morphology and examine markers, and 60 hybrids were cultivated for mushroom production to measure BE (Fig. 1). The average BE of hybrids ranged from 0.096 in MB001 (crossed between bisp 015-p1 and bisp 034-p1) to 0.394 in MB-310 (crossing

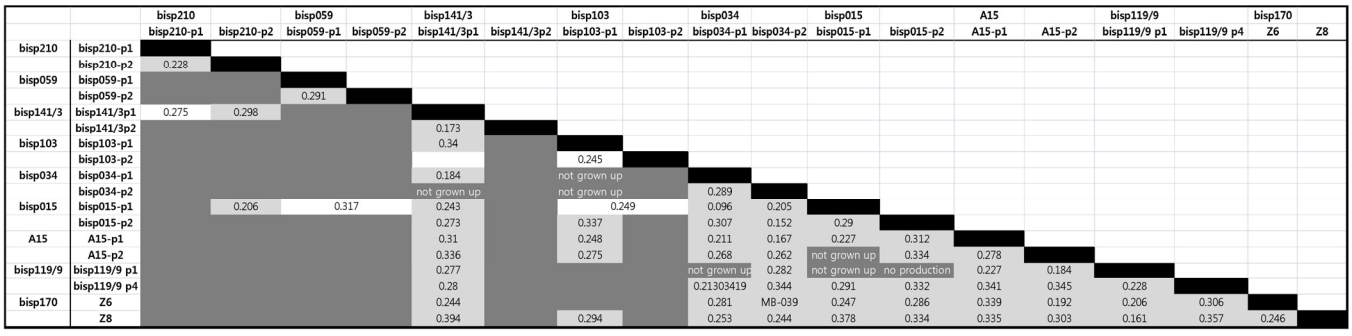

Fig. 2. Biological efficiency of hybrids with homokaryons of *Agaricus bisporus*.

between 141/3p1 and Z8) (Fig. 2). The average BE in each homokaryon was calculated as the mean of the BE of all hybrids with the same homokaryons. The values for homokaryons ranged from 0.223 in bisp 119/9p1 to 0.304 in bisp 119/9 p4. The homokaryons were successfully crossed from 6 to 11 times. The crossing frequency of bisp 141/3p1, A15-p1, and Z8 was the highest, and that of bisp 103-p1 and bisp 119/9p1 was the lowest among homokaryons (Table 3). *A. bisporus* var. *bisporus* was not different from *A. bisporus* var. *burnettii* with respect to the frequency of crossing. Some hybrids were not cultivated because the parental lines were not crossed [6]. MB-013 is an intervarietal hybrid resulting from the crossing between with bisp 034-p2 and bisp 015-p2. The hybrid had a BE of 0.152 and was selected to construct an F_1 population.

Table 3. Average BE and frequency of successful intercrossing for each homokaryon of *Agaricus bisporus*

	Mean BE	Successful cross
bisp141/3p1	0.278	11
bisp103-p1	0.29	6
bisp034-p1	0.234	9
bisp034-p2	0.243	8
bisp015-p1	0.247	8
bisp015-p2	0.296	10
A15-p1	0.277	11
A15-p2	0.278	10
bisp119/9 p1	0.223	6
bisp119/9 p4	0.304	10
Z6	0.261	9
Z8	0.3	11

BE, biological efficiency.

BC_1F_1 population

Three hundred SSIs were collected in the MB-013 line from the crossing between bisp 034-p2 and bisp 015-p2 to select over 100 of homokaryons, because one parental strain, bisp 015, was tetrasporic, *A. bisprorus* var. *burnettii*. After intercrossing *A. bisporus* var. *bisporus* with *A. bisporrus* var. *brunettii*, the first-generation hybrids were homokaryotic tetrasporic strains, dominant trait as observed by Kerrigan et al. [5]. Mycelia of SSIs of MB-013 were subjected to direct PCR with PIN 150 and the PCR product was digested PCR with HaeIII (Fig. 3) as a CAPS marker [18]. One hundred and seventy homokaryons were selected in the F_1 population. The PIN 150 marker was designed based on the gene sequences located on chromosome I and near the mating (MAT) gene were tested to screen putative homokaryotic protoclones [16, 17]. Therefore, the marker has the potential to

discriminate between heterokaryons and homokaryons. The 100 selected homokaryons were crossed with bisp 015-p1 to construct the BC_1F_1 population (Fig. 4). The selected F_1 population will be re-sequenced including bisp 034-p2 and bisp 015-p2, to perform linkage mapping. Furthermore, mushrooms of the BC_1F_1 populations will be used to calculate BE and for quantitative trait locus (QTL) mapping.

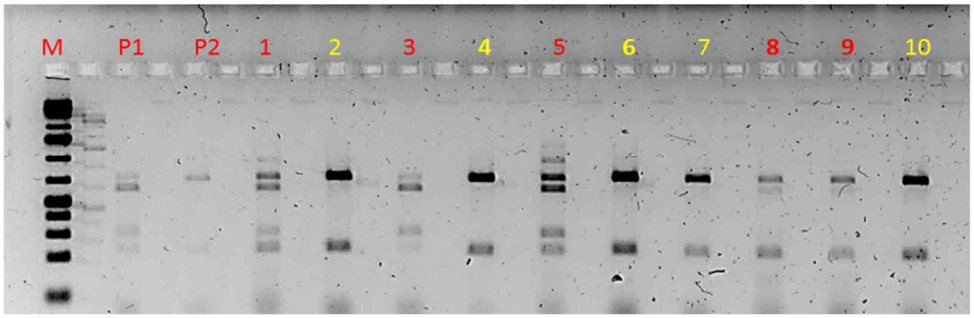

Fig. 3. CAPS marker (PIN150 and HaeIII) analysis of SSIs in the MB013 line to select 100 homokaryons of *Agaricus bisporus*. CAPS, cleaved amplified polymorphic sequence; SSIS, single spore isolates; M, 1 kb marker; P1, bisp015-p2; P2, bisp034-p2; 1~10, single spore isolates from MB013.

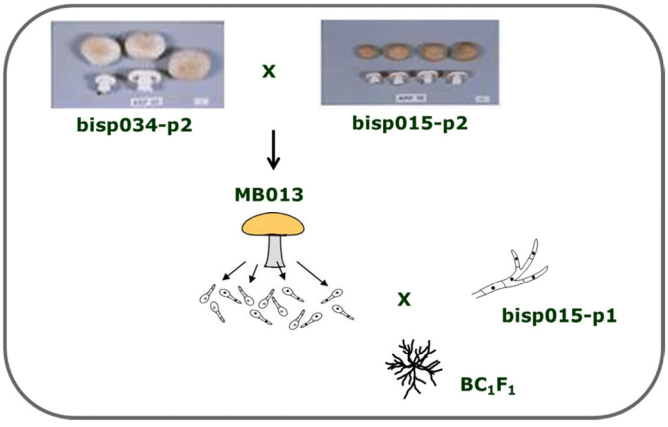

Fig. 4. Crossing scheme for homokaryons of the MB013 line (F1) with bisp15-p1 to construct the BC_1F_1 population.

Acknowledgments

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