한국버섯학회지 Journal of Mushrooms Research Article

Varietal characteristics of new white button mushroom 'Seolwon' in *Agaricus bisporus*

Lee Byung-Joo*, Lee Mi-Ae, Kim Yong-Gyun, Lee Kwang-Won, Lee Byung-Eui[1] and Song Ho-Yeon[2]

Crop Research Division, Chungcheongnam-do Agricultural Research & Extension Services, Yesan 340-861, Korea
[1]Industry Academy Cooperation Foundation, Soonchunghyang University, Asan 336-745, Korea
[2]College of Medicine, Soonchunghyang University, Chunan 330-930, Korea

ABSTRACT: Commonly known as the button mushroom, *Agaricus bisporus* is one of the most widely cultivated mushroom species of edible fungi. In the breeding of new button mushroom, Seolwon was developed by crossing two homokaryons. Because of the predominantly pseudohomothallic life cycle, only a small percentage of homokaryotic meiospores are produced, which do not fruit. Homokaryotic cultures derived from these types of single spores produce a vegetative mycelium that contain a variable number of genetically identical nuclei per cell. After crossing two homokaryons, hybrids were cultivated on a small scale and on a commercial scale at a farm. The spawn was made by a commercial spawn producer and the spawned compost by a commercial compost producer. Mycelial growth of Seolwon on CDA was better at 25°C when it was compared with that of Seolgang. The mature cap shape of new strain Seolwon is oblate spheroid and the immature cap shape is round to oblate spheroid. The cap diameter was 39.7 mm on average. In comparison with white strain Seolgang, the strain had a yield that was 11% higher. It produced fruiting bodies which had a higher weight on average per fruiting body and were 9.7% firmer with a good shelf life. Days of fruiting body were 1-2 days later than those of Seolgang. The physical characteristics such as springiness, chewiness, adhesiveness, gumminess were better than that of Seolgang.

KEYWORDS: *Agaricus bisporus*, Button mushroom, Heterokaryon, Homokaryon, Mating, Seolwon

Introduction

Mushrooms are cultivated for the production of food consumption due to its nutritive and medicinal values (Fan *et al*., 2006) including *A. bisporus* (Beelman *et al*., 2003). The button mushroom is one of the most widely cultivated edible mushroom species placing first followed by *Pleurotus ostreatus* and *Lentinus edodes*. It is an edible basidiomycete mushroom native to grasslands in Europe and North America cultivated in more than 70 countries (Cappelli, 1984) increasing its popularity in Eastern Countries such as China and Korea.

A. bisporus is predominantly secondarily homothallic (Khush *et al*., 1995), in which a fertile heterokaryotic mycelium is established from a basidiospore carrying two meiotic nuclei of different mating types. In a number of cases, the two nuclei in the basidiospores are non-sisters and carry compatible mating types with a bipolar mating system comprising multiple alleles. The bisporic spores, therefore, give rise to fertile heterokaryotic progeny (n+n) capable of fruiting bodies (Langton and Elliott, 1980; Kerrigan *et al*., 1993).

A. bisporus was composed of 13 chromosomes that account for a total genomic size of about 34.2 Mbp per haploid genome ranging 1.4 Mbp to 3.65 Mbp and the mating locus MAT was on chromosome I and the cap color locus *PPC1* on chromosome VIII in the construction of a genetic linkage map (Xu *et al*., 1993; Kerrigan *et al*., 1993; Sonnenberg *et al*., 1991, 1996; Foulongne-Oriol *et al*., 2010). The *PPC1* locus was found to be recessive for the white color (Callac *et al*, 1998). Quantitative trait locus mapping of yield-related traits in *A. bisporus* was also identified recently (Foulongne-Oriol *et al*., 2012). *A. bisporus* has certain inherent

J. Mushrooms 2014 June, 12(2):82-87
http://dx.doi.org/10.14480/JM.2014.12.2.82
Print ISSN 1738-0294, Online ISSN 2288-8853

*Corresponding author
E-mail : byungjoo@korea.kr
Tel : +82-41-635-6061, Fax : +82-41-635-7920
Received April 15, 2014
Revised June 27, 2014
Accepted June 28, 2014

difficulties for a breeding program because uninucleate homokaryotic materials for outcrossing are very laborious and time consuming to obtain (Horgen and Anderson, 2008). Clamp connections also are not produced and this makes the identification of compatible reactions a problem. A minority of basidia produce three or four spores, some of which are homokaryotic and self-sterile on germination (Kerrigan *et al*., 1993). Such homokaryons can be crossed with an appropriate breeding partner resulting in improved strains.

Outcrossing of homokaryons is the method generally used for the development of new strains. However, the dikaryotization of a homokaryon by a heterokaryon called as the Buller phenomenon (Quintanilha, 1937) or di-mon mating (Papazian, 1950) was first demonstrated in *Corprinus lagopus*. This mode of dikaryotization produces a new pair of conjugate nuclei following nuclear migration from the heterokaryon (Xu *et al*., 1996; Clark and Anderson, 2004). By simultaneous inoculation heterokaryotic spores and homokaryon, fruiting bodies were produced via outcrossing in *A. bisporus* (Callac *et al*., 2006). There is also evidence that genetic exchange can occur between heterokaryons. In *Heterobasidion annosum*, parings of heterokaryons yield subcultures that are non parental with respect to somatic incompatibility reactions. Furthermore, in the cultivated button mushroom parings of heterokaryons with other heterokaryons produced recombinant genotypes (Xu *et al*., 1996).

The present study was to introduce new strain by strain improvement program for the commercial production in *A. bisporus*. The new mushroom produced fruiting bodies of a higher weight with a good shelf life including other desirable characteristics.

Materials and Methods

Single basidiospore isolates and homokaryons

Basidiospores were obtained from freshly collected fruiting bodies of *A. bisporus* strains CM020847 and CM020913. They were suspended in sterile distilled water. Suspensions were spread on 9cm Petri dishes with compost dextrose agar (CDA) and incubated at 25°C. The colonies of single spore isolates (SSIs) were transferred onto new Petri dishes of CDA in sterile condition and incubated at 25°C in dark. After 30 days of SSIs incubation, colony diameter and morphology type of each isolates was determined. Based on growth rate, colony morphology, putative homokaryons were selected and fruiting trials were carried out. After crossing between two homokaryons in all possible combinations, a piece of mycelium of paired colonies was taken and placed in Petri dishes containing CDA medium.

Media and culture

Mycelia of *A. bisporus* strains were cultured vegetatively at 25°C on CDA medium. For CDA 20g compost was added to 1L distilled water, boiled for 15 min and 10g dextrose and 15 g agar were added to liquid extract. The volume for 1L was made with distilled water. The medium was sterilized at 121°C for 30 minutes, and then preserved at 4°C. Agar blocks taken from actively growing colonies on CDA plates were inoculated into different culture media for mycelial growth.

Fruiting trial

Mycelial cultures were transferred to 450g of sterile cooked wheat grain, buffered with 5% CaCO3. Colonized grains (40g) were used to inoculate plastic container (57×41×18 cm) containing a complex biologically modified, straw-based substrate. Pasteurized soil was used as the casing material. Case-run, initiation and maturation all occurred in controlled conditions. Two replications were used for each isolate.Heterokaryotic strains were cultivated on a small scale and on a commercial scale at a farm. For this, the spawn was made by a commercial spawn producer and the spawned compost by a commercial compost producer.

Determination of color value

The surface color and L-value of button mushrooms were measured by a Chroma Meter CR-200 (Minolta Camera Co. Japan). Three random locations were measured on the cap of fruiting body and they were compared with the white color values L (brightness)=97, a (greenness)=0 and b (yellowness)=1.95 of white copy paper using ΔE as described by the following equation $\Delta E = \sqrt{(L-L')^2+(a-a')^2+(b-b')^2}$, where ΔE is degree of overall color change in comparison with ideal color values.

Nutrient content determination

Moisture content for the mushroom samples was determined by the direct oven drying method. The

weight loss after oven drying of each sample of 2 g at 105°C to constant weight was expressed as % moisture content. Crude protein content was determined using the Kjeldahl method (AOAC, 1995). A 0.5 g ground sample from each of the mushroom species was digested in Kjeldahl flask using 98% sulphuric acid after which it was steam-distilled. The resulting distillate was titrated to pink or wine-red colour using 0.01 M hydrochloric acid and the protein percentage was calculated. Crude fat was determined by using the Soxhlet extraction method using petroleum ether as the solvent (AOAC, 1995). Ash content of 1 g powdered sample was determined as the residue of incineration at 550°C in a muffle furnace. Total carbohydrate content was determined by 2 g of each sample in 50 ml distilled water of which 0.2 ml was diluted ten-fold. To 1 ml of the resulting solution and serial dilutions of glucose stock (10 mg/100 ml) solution, 4 ml of anthrone reagent was added and absorbance of the solutions was measured by a spectrophotometer at 620 nm against a blank reagent.

Texture profile analysis

To determine the texture by simulating or imitating the repeated biting or chewing of a sample. Texture profile analysis (TPA) was performed to the cap of mushrooms to evaluate the influence of this processing operation in the textural properties using a texture analyser TA-Plus (Lloyd Instruments Ltd., UK) by two compression cycles between parallel plates performed using a 5 mm diameter plunger with a 5 seconds interval between cycles. Middle area of the cap of the fruit body in the fresh state was the object of probe with 4 analyses. The parameters that have been used were 5 kg force load cell and 2.0 mm/sec test speed. The textural properties of hardness, gunmminess, adhesiveness, springiness, cohesiveness, and chewiness were then calculated.

Results and Discussion

The strain improvement of *A. bisporus* has remained recalcitrant work because of two main reasons: the predominantly homothallic life cycle that mostly leads to produce self-fertile binucleate spores and the lack of morphological features that result in the isolation of homokaryons is very difficult. This type of sexual behavior hampers outcrossing and limits breeding success (Elliott and Langton, 1981). The early strain improvement work was based on selection giving higher yields than the parental strains because the fertile single spores from an individual sporophore differed in growth rate, appearance of the mycelia, sporophore morphology and in productivity. Strain selection based on single spores, multispores or tissue culture may give improvement in the short term but it is unlikely to be as effective as controlled crossing.

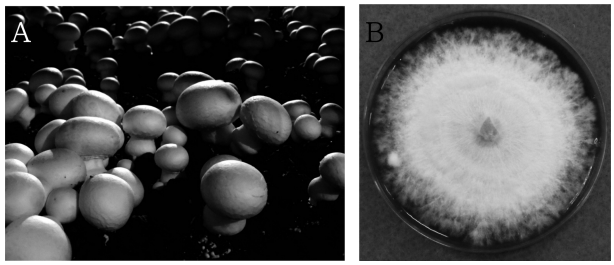

Fig. 1. Fruting bodies (A) and mycelial colony on CDA medium (B) of *A. bisporus* strain Seolwon.

Table 1. Inherent characteristics of *A. bisporus* strains Seolwon compared to Seolgang

Strain	Temperature of mycelial growth (°C)	Temperature of fruiting body (°C)	Color of pileus	Shape of pileus
Seolwon	24-26	16-18	White	Round oblate
Seolgang	23-25	15-17	White	Round oblate

The morphological, agronomical and chemical data of the new mushroom variety should be compared to those obtained from an appropriate variety grown under identical conditions. In this paper, the characteristics of new strain Seolwon was compared to those of Seolgang (Lee *et al*., 2012). The mature pileus of new hybrid strain Seolwon is oblate spheroid in the shape and white in the color (Fig. 1A). Morphological characteristics of mycelial culture showed white in color and feathery in shape on CDA medium (Fig. 1B). Aerial hyphae were well developed in the center and margin of the colony. The cultivation temperature for mycelial growth was similar to that of Seolgang at 25℃ but appropriate temperature for fruiting body formation was a little bit higher (Table 1).

Mycelial growth at different temperatures on CDA medium increased from 15 to 25°C and decreased between 25 and 30°C. In the range of temperature between 15 and 20°C Seolgang was better in mycelial

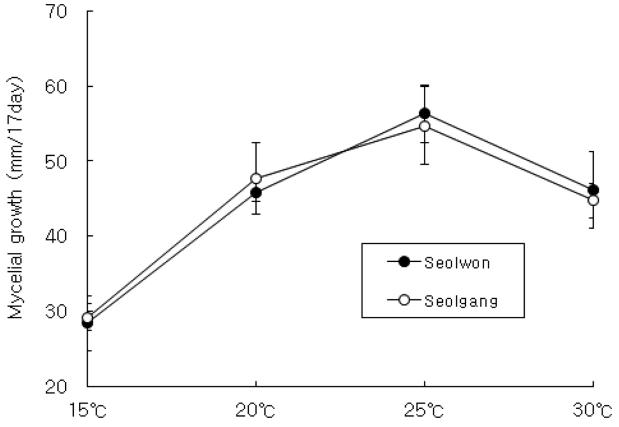

Fig. 2. Mycelial growth of *A. bisporus* strains Seolwon and Seolgang at different temperatures.

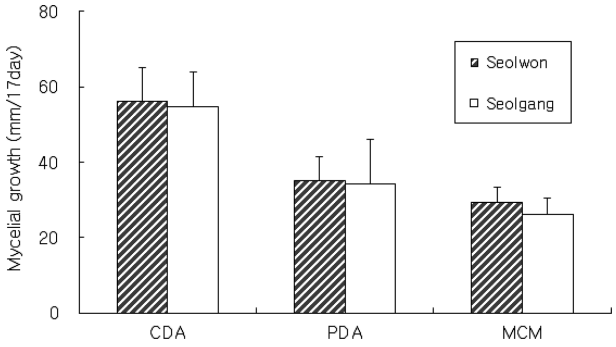

Fig. 3. Mycelial growth of *A. bisporus* strains Seolwon and Seolgang on different media.

growth but Seolwon was better between 25 and 30°C (Fig. 2). Mycelial growth on different culture media was variable in the two strains. The culture media influenced colony growth rate, which ranged from 26.3-56.3 mm/17 days (Fig. 3). Both strains showed higher growth rate on CDA followed by PDA and MCM. Strain Seolwon was higher than Seolgang on CDA, PDA, and MCM. Fruiting of *A. bisporus* depends on a complex set of variables including environmental factors such as carbon dioxide concentration, temperature, humidity and pH and on the nutritional status of the substrate (Flegg and Wood, 1985). The mushroom crop grows in repeating 3- to 5-day cycles called flushes. These flushes are followed by a few days when no mushrooms are available to harvest. The individual flushes tend to produce progressively fewer mushrooms. In commercial practice, three to five flushes are picked before the crop is removed to make room for the next. Most strains of *A. bisporus* are picked before the veil breaks and the stem elongates. In the cultivation strain Seolwon was longer in primordial formation and fruit body development as 28.1 and 7.6 days, respectively (Table 2). As a result, total cultivation period was 35.7 days in Seolwon compared to 34.6 days in Seolgang. Morphological characteristics of fruiting bodies was different between two strains (Table 3). New strain showed 39.7 and 12.5 mm in diameter and thickness of pileus and 22.6 and 14.1 mm in the length and thickness of stipe. When it was compared with Seolgang, Seolwon had bigger and rounder in pileus and thicker and shorter in stipe. Therefore, new hybrid strain Seolwon looked oblate spheroid in the shape. The surface color difference of Seolwon determined by a Chroma meter showed 9.12 compared to 8.33 in Seolgang resulting slightly different ΔE, where ΔE is degree of overall color differences in comparison with color values of L (brightness)=97, a (greenness)=0 and b (yellowness)=1.95 (Table 4). This result shows that Seolwon is slightly whiter compared to Seolgang.

One of important factors in the quality of mushrooms, the stability of texture can be only maintained very short period of storage, i.e. it is changed quickly after harvest in *A. bisporus* (Nichol 1985). Springiness, chewiness, adhesiveness, cohesiveness and gumminess are considerable characteristics in the texture profile

Table 2. Comparison of cultivation period of *A. bisporus* strains Seolwon and Seolgang

Strain	Period(days)		
	Primordial formation	Development of fruit body	Total
Seolwon	28.1	7.6	35.7
Seolgang	27.4	7.2	34.6

Table 3. Comparison of morphological characteristics of fruiting bodies of *A. bisporus* strains Seolwon and Seolgang

Strain	Pileus		Stipe	
	Diameter (mm)	Thickness (mm)	Length (mm)	Thickness (mm)
Seolwon	39.7	12.5	22.6	14.1
Seolgang	36.1	11.4	24.7	13.9

Table 4. Comparison of color value of the fruiting bodies of *A. bisporus* strains Seolwon and Seolgang

Strain	L[a]	a	b	ΔE
Seolwon	91.04	0.23	8.85	9.12
Seolgang	93.42	0.15	9.47	8.33

[a]L : lightness, a : redness, b : yellowness; ΔE(color difference) = $\sqrt{(L-L')^2+(a-a')^2+(b-b')^2}$, DMRT 5%

analysis of fruit bodies (Table 5). There were some relations between textural properties and quality of fruit body (Beelman *et al*., 1987; Mc Garry and Burton, 1994). Springiness, chewiness, adhesiveness, and gumminess were higher except cohesiveness in Seolwon, suggesting it has better quality in general texture compared to that of Seolgang. Hardness of harvested fruit bodies were considered important to evaluate the quality and shelf life of mushrooms (Mohapatra *et al*. 2010; Singh *et al*., 2010). The weight of fruit body also could be affected by hardness in *A. bisporus*. Seolwon had heavier fruit body with 1.13 kg/Ø5 mm compared to Seolgang with 1.03 kg/Ø5 mm (Table 6). The yield of Seolwon was significantly high with 12.4 kg/m^2 compared to 11.2 kg/m^2. Analyses of correlations between yield components showed that the average weight of fruiting bodies decreased when the yield and the number of mushrooms increased (Foulongne-Oriol *et al*., 2012; Rodie *et alet alet al*. 2000). Given these relationships, the development of high yield producing mushrooms with a high weight may be challenging.

The proximate composition of nutrients compared between Seolwon and Seolgang is shown in Table 7. Seolwon was similar in calorie with 27-28 kcal/100 g and nutrients such as protein, carbohydrate in comparison to Seolgang. In general, these two strains were similar in the nutrient contents. The inorganic elements identified in Seolwon are presented at Table 8. The main contents were Ca, Fe, K, Na, and P. Elements such as K and P were the major elements in most mushrooms including *A. bisporus* (Demirbas, 2001). When Seolwon was compared with Seolwon, most elements were higher in the mineral contents except Mn, Na, and Zn. No heavy metals were detected.

Table 5. Comparison of texture profile on pileus of the fruiting bodies of *A. bisporus* strains Seolwon and Seolgang

Strain	Springiness (mm)	Chewiness (mJ)	Adhesiveness (N)	Cohesiveness (g.s)	Gumminess (N)
Seolwon	0.914	46.7	0.192	0.153	48.1
Seolgang	0.896	45.4	0.188	0.161	47.5

Table 6. Comparison of hardness, weight, and yield of the fruiting bodies of *A. bisporus* strains Seolwon and Seolgang

Strain	Hardness (kg/Ø5 mm)	Individual weight (g/ea)	Yield (kg/m^2)	Yield index
Seolwon	1.13	11.4	12.4a[a]	111
Seolgang	1.03	10.6	11.2b	100

[a]LSD(5%)=1.0495

References

AOAC. 1995. Official Methods of Analysis (16th ed.). Arlington VA, USA: Association of Official Analytical Chemists.

Beelman RB, Okereke A, Guthrie B. 1987. Evaluation of textural changes related to postharvest quality and shelf life of fresh mushroom. In Development in Crop Science Vol. 10, Cultivating Edible Fungi, p251-258, ed. Wuest PJ, Royse DJ, Beelman RB. Elsevire, Amsterdam.

Beelman RB, Royse DJ, Chikthimmah N. 2003. Bioactive components in button mushroom *Agaricus bisporus* (J. Lge) Imbach (*Agaricomycetideae*) of nutritional, medicinal, and biological importance. *Int J Med Mushrooms.* 5:321-337.

Callac P, Moquet F, Imbernon M, Guedes-Lafargue MR, Mamoun M, Olivier JM. 1998. Evidence for *PPC1*, a determinant of the pilei-pellis color of *Agaricus bisporus* fruitbodies. *Fungal Genet Biol.* 23:181-188.

Callac P, Spataro C, Caille A, Imbernon M. 2006. Evidence for outcrossing via the Buller phenomenon in a substrate simultaneously inoculated with spores and mycelium of *Agaricus bisporus*. *Appl Environ Microbiol.* 72:2366-2372.

Cappelli A. 1984. Fungi Europaei: *Agaricus*. Giovanna Biella. Saronno, Italy

Clark TA, Anderson JB. 2004. Dikaryons of the basidiomycete

Table 7. Comparison of proximate constituents of nutrients in the fruiting bodies of *A. bisporus* strains Seolwon and Seolgang

Strain	Calorie (kcal/100g)	Moisture (%)	Crude protein (%)	Crude lipid (%)	Carbohydrate (%)	Ash (%)
Seolwon	27.95	92.26	5.31	0.07	1.52	0.91
Seolgang	27.34	92.23	5.24	0.06	1.46	0.87

Table 8. Inorganic elements content in the fruiting bodies of *A. bisporus* strains Seolwon and Seolgang

Strain	Concentration (mg%)								
	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
Seolwon	8.02	0.23	1.61	501.4	0.29	0.07	7.84	113.6	0.09
Seolgang	7.34	0.12	1.54	431.6	0.24	0.12	8.17	107.9	0.14

fungus *Schizophyllum commune*: evolution in long-term culture. *Genetics.* 167:1663-1675.

Demirbas A. 2001. Concentrations of 21 metals in 18 species of mushrooms growing in the East Black Sea region. *Food Chem.* 75:453-457.

Elliott TJ, Langton FA. 1981. Strain improvement in the cultivated mushroom *Agaricus bisporus*. *Euphytica.* 30:175-182.

Fan L, Pan H, Scoccol AT, Pandey A, Soccol CR. 2006. Advances in mushroom research in the last decade. *Food Technol Biotechnol.* 44:303-311.

Flegg PB, Wood DA. 1985. Growth and fruiting. In The Biology and Technology of the Cultivated Mushroom, p141-177, ed. Flegg PB, Spencer DM, Wood DA. Wiley & Sons, Chichester.

Foulongne-Oriol M, Rodier A, Rousseau T, Savoie JM. 2012. Quantitative trait locus mapping of yield-related components and oligogenic control of the cap color of the button mushroom, *Agaricus bisporus*. *Appl Environ Microbiol.* 78:2422-2434.

Foulongne-Oriol M, Spataro C, Cathalot V, Monllor S, Savoie JM. 2010. An expanded genetic linkage map of an intervarietal *Agaricus bisporus* var. *bisporus* x *A. bisporus* var. *burnettii* hybrid based on AFLP, SSR and CAPS markers sheds light on the recombination behaviour of the species. *Fungal Genet Biol.* 47:226-236.

Horgen PA, Anderson JB. 2008. Edible mushrooms. In Biotechnology of Filamentous Fungi, p447-462, ed. Finkelstein DB, Ball C. Butterworth-Heinemann, Stoneham, USA.

Kerrigan RW, Royer JC, Baller LM, Kohli Y, Horgen PA, Anderson JB. 1993. Meiotic behavior and linkage relationships in the secondarily homothallic fungus *Agaricus bisporus*. *Genetics.* 133:225-236.

Khush RS, Watch MP, Horgen PA. 1995. Molecular strategy for *Agaricus* breeding. In The Mycota, Vol. II. Genetics and Biotechnology, p321-337, Kuck, U. Springer-Verlag, Heidellberg, Berlin.

Langton FA, Elliott TJ. 1980. Genetics of secondarily homothallic basidiomycetes. *Heredity.* 45:99-106.

Lee BJ, Lee MA Kim YG, Lee KW, Lim YP, Lee BE, Song HY. 2012. Strain improvement in the white button mushroom 'Seolgang' and its varietal characteristics in *Agaricus bisporus*. *J Mushroom Sci Prod.* 10:151-159.

Mc Garry A, Burton KS. 1994. Mechanical properties of the mushroom, *Agaricus bisporus*. *Mycol Res.* 98:241-245.

Mohapatra D, Bira ZM, Kerry JP, Frias JM, Rodrigues FA. 2010. Postharvest hardness and color evolution of white button mushrooms (*Agaricus bisporus*). *J Food Sci.* 75:146-152.

Nichol R. 1985. Post-harvest physiology and storage. In The Biology and Technology of Cultivated Mushroom, p195-210, ed. Flegg PB, Spencer DM, Wood DA. John Wiley and Sons Inc., New Jersey.

Papazian HP. 1950. Physiology of the incompatibility factors in *Schizophullum commune*. *Bot Gaz.* 112:143-163.

Quintanilha A. 1937. Contribution a l'tude gntique du phnomne de Buller. *Compt Rend Acad Sci Paris.* 205:745-747.

Rodier A, Devesse C, Rousseau T, Vedie R, Imbernon M, Olivier JM. 2000. Breeding brown hybrids of button mushroom (*Agaricus bisporus*) from a factorial cross. *Mushroom Sci.* 15:289-297.

Singh P, Langowski HC, Wani AA, Saengerlaub S. 2010. Review Recent advances in extending the shelf life of fresh *Agaricus* mushrooms: a review. *J Sci Food Agric.* 90:1393-1402.

Sonnenberg ASM, Groot PWJ, Schaap RJ, Baars JJP, Visser J, Van Griensven LJLD. 1996. Isolation of expressed sequence tags of *Agaricus bisporus* and their assignment to chromosomes. *Appl Environ Microbiol.* 62:4542-4547.

Sonnenberg ASM, Hollander KD, Van de Munckhof APJ, Van Griensven LJID. 1991. Chromosome separation and assignment of DNA probes in *Agaricus bisporus*. In Genetics and Breeding of *Agaricus*, p57-61, ed. Van Griensven LJID. Pudoc, Wageningen, The Netherlands.

Xu J, Horgen PA, Anderson JB. 1996. Somatic recombination in the cultivated mushroom *Agaricus bisporus*. *Mycol Res.* 100:188-192.

Xu J, Kerrigan RW, Horgen PA, Anderson JB. 1993. Localization of the mating type gene in *Agaricus bisporus*. *Appl Environ Microbiol.* 59:3044-3049.