Journal of Microbiology (2013) Vol. 51, No. 5, pp. 552–557
DOI 10.1007/s12275-013-2666-z

Morphological and Genetic Characteristics of Newly Crossbred Cauliflower Mushroom (*Sparassis latifolia*)

Hong-Duck Sou[1], Rhim Ryoo[1], Sung-Ryul Ryu[2], Kang-Hyeon Ka[1*], Hyun Park[3], and Sung-Hyun Joo[4]

[1]*Division of Wood Chemistry and Microbiology, Korea Forest Research Institute, Seoul 130-712, Republic of Korea*
[2]*Korea Forest Seed and Variety Center, Cheongju-si 380-941, Republic of Korea*
[3]*Division of Research Planning and Coordination, Korea Forest Research Institute, Seoul 130-712, Republic of Korea*
[4]*Department of Forestry, Kyungpook National University, Daegu 702-701, Republic of Korea*

(Received November 8, 2012 / Accepted April 30, 2013)

Cauliflower mushroom (*Sparassis latifolia* or *S. crispa*) is popular for food and medicine. Importance of new varieties of *Sparassis* was raised and studied widely by protection system of UPOV. In this study, 10 crossbred strains of *Sparassis latifolia* that specifically expressed distinctive features during basidiocarp formation and mycelium growth were applied to sawdust medium inoculated with *S. latifolia* mycelia. The 10 crossbred strains were divided into 3 groups on the basis of morphological (size of marginal wave and basidiocarp color) and genetic characteristics. Each phenotype of the parent and crossbred strains represented 3 marginal wave-sizes (large, medium, and small) and 3 color notations (NN155D, 163C, and 8D). Our result suggests that morphological characteristics of cauliflower mushroom can be affected by various environmental and genetic stimuli under artificial conditions such as crossbreed. Also this research showed genetic differences among breeding isolates and their morphological characteristics were correlated with the molecular data within parent and crossed strain.

Keywords: cauliflower mushroom, crossbred strains, basidiocarps, phenotype, RAPD

Introduction

Cauliflower mushroom (*Sparassis latifolia* or *S. crispa*) is an edible and medicinal mushroom that has recently become popular for cultivation in Eastern Asia (China, Japan, and Korea). Various crossbreeding method and new varieties development of cauliflower mushrooms have been interested more and more. Because of protection system promoted by the International Union for the Protection of New Varieties of Plants (UPOV), the importance of new varieties has emerged globally.

Sparassis species were once considered pathogens because they produce a brown rot on conifers and Fagales (Dai *et al*., 2006). However, following preliminary investigations, *S. crispa* was found to have a high β-glucan content (up to 43.6% of the dry weight of the basidiocarps), as measured by the enzyme method of the Japan Food Research Laboratories (Tokyo) (Tada *et al*., 2007). In addition, β-1,3-glucan from the basidiocarp of *S. crispa* was shown to exhibit antitumor activity (Harada *et al*., 2002). In another study, polysaccharide fractions were prepared from cultured *S. crispa*, and the structure and activities of the extracts were examined (Tada *et al*., 2007).

It is frequently found in temperate forests and appears at the stems and stumps of coniferous forest trees such as *Abies holophylla*, *Larix kaempferi*, *Pinus densiflora*, and *P. koraiensis* (Ryu *et al*., 2009). Efficient cultivation methods were developed to produce basidiocarps more easily using coniferous sawdust medium (Park *et al*., 2005, 2011; Ryu *et al*., 2009). These specialized techniques will refer to cultivate other mushroom growing in conifer.

Therefore, additional research needs to establish a stable process of cultivation and to development valuable breeding cauliflower mushrooms. Moreover, concerning new varieties, clear criterion needs to solve a controversy caused by protection law of UPOV Convention. According to these necessities, this study was conducted to produce new superior varieties of *Sparassis*, and tried to understand the morphological and genetic characteristics of *S. latifolia* produced by crossbred strains.

Materials and Methods

Strains and crossbreeding

Strains used for crossbreeding were obtained from basidiocarps of *S. latifolia* collected from the Korea National Arboretum (July 18, 2005; leg. H. Park, habit. *Larix kaempferi*, KFRI 700) and Jirisan National Park (July 18, 2007; leg. K.-H. Ka, habit. *L. kaempferi*, KFRI 923) in Korea. Basidiospores were obtained from basidiocarps of KFRI 700 and KFRI 923, and cultured at 23±1°C and 60±5% humidity in the dark to isolate a monokaryotic strain. According to the monokaryotic-monokaryotic (mon-mon) crossing method (Uhart and Albertó, 2009), each monokaryotic isolate was confronted in pairs in Petri dishes using 5-mm-diameter blocks as inocula to breed new strains. Ten new dikaryotic strains, using KFRI 700 and KFRI 923 as parents, were derived from the mon-mon crossing method, which means mating occurred

*For correspondence. E-mail: kasymbio@forest.go.kr; Tel.: +82-2-961-2755; Fax: +82-2-961-2769

between monokaryotic isolates from different strains of the same species. Information of phylogenetic analyses about parent strains (KFRI 700 and KFRI 923) and the crossbred strains (KFRI 1748, KFRI 1749, KFRI 1750, KFRI 1752, KFRI 1753, KFRI 1754, KFRI 1755, KFRI 1756, KFRI 1757, and KFRI 1762) are included in the study of Ryoo *et al.* (2013).

Cultivation and morphological characteristics

Sawdust medium composed of larch (*L. kaempferi*) with the addition of barley flours and sugar (Park *et al.*, 2005) was used for the production of basidiocarps. The density of the sawdust medium was 0.76 g/cm^3, and a hole about 1 cm in diameter and 5 cm in length was made in the middle of the medium for equal inoculation (Park *et al.*, 2011). Each crossbred strain was cultured in liquid potato dextrose broth medium. Mycelia were homogenized and inoculated 72 h later to confirm contamination and were cultured at 23±1°C and 60±5% humidity in the dark, five bottles each. We measured mycelial growth 3 weeks after inoculation. Growth was evaluated from the top of the medium to the bottom where mycelial growth ended, and the bottle was divided into 8 parts. Mycelia grew fully about 8 weeks later in the dark. After the mushroom primordia developed, the culture was moved to a facility maintained at 21±1°C with 95±5% humidity.

Morphological descriptions used color terms and notations from the Royal Horticulture Society color chart, and marginal wave-size was divided by frequency analysis.

Random amplified polymorphic DNA (RAPD) analysis

Genetic variation in the 2 parent strains and 10 crossbred strains was detected using the modified RAPD protocol of Williams *et al.* (1990). Genomic DNAs of KFRI 700, KFRI 923, KFRI 1748, KFRI 1749, KFRI 1750, KFRI 1752, KFRI 1753, KFRI 1754, KFRI 1755, KFRI 1756, KFRI 1757, and KFRI 1762 were extracted by a modified CTAB (cetyltrimethylammonium bromide) buffer method (Lee and Taylor, 1990). These genes were randomly amplified using 20 oligonucleotide primers, listed in Table 3. RAPD reactions were performed for 45 cycles of denaturation for 1 min at 92°C, annealing for 1 min at 35°C, and extension for 2 min at 72°C, with the first denaturation and last extension times extended to 1 min at 72°C and 5 min at 92°C, respectively.

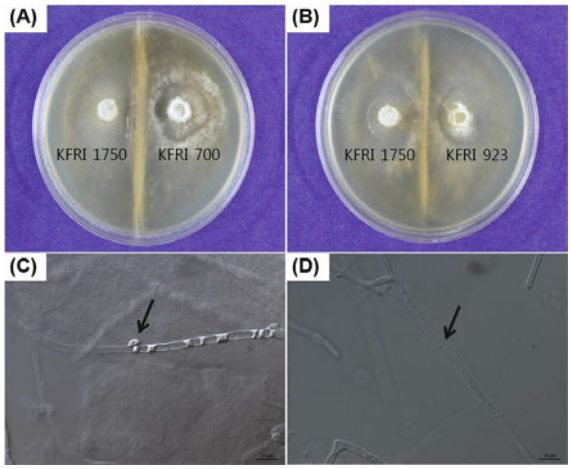

Fig. 1. The result of crossbred between KFRI 700 and KFRI 923. (A, B) Confrontation cultures of *S. latifolia* between crossbred strain (KFRI 1750) and parent strains (KFRI 700 and KFRI 923). (C) Clamp connection of KFRI 1748, (D) Cultivated monokaryon originated by isolate single spore of KFRI 700.

RAPD products were run on 1.5% agarose gel with a 100-bp DNA ladder as a size marker. RAPD band matching was performed to calculate the clear band position and molecular base pair value using GeneTools analysis software (Syngene, Synoptics Ltd., UK).

Cluster analysis

To compare morphological and genetic variation in the crossbred strains, each phenotype was treated as a separate locus and scored as present (1) or absent (0) by taxonomical characters (size of marginal wave and color) and band matching points. Relationships among data matrices were constructed using the unweighted pair-group arithmetic average (UPGMA) method in MEGA software version 5.05.

Statistical analyses

Statistical analyses were performed using SPSS (IBM). Analysis of variance was used to analyze experimental data. If the test was significant (P<0.05) and more than 2 replicates were involved, the Duncan test was used for mean comparison.

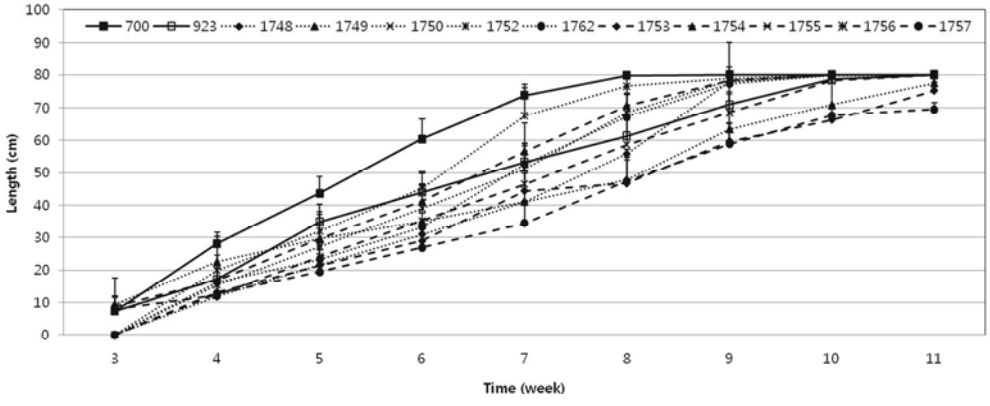

Fig. 2. Mycelial growth of parent and crossbred strains of *S. latifolia* in sawdust medium. KFRI 700 and KFRI 923 are parent strains; others are crossbred strains.

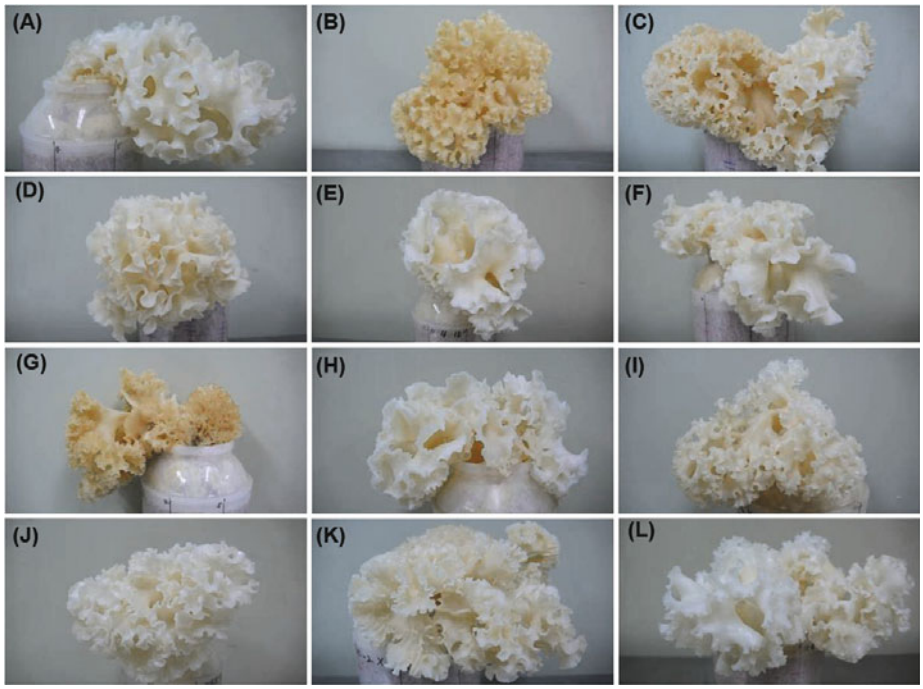

Fig. 3. Twelve basidiocarps of parent and crossbred strains of *S. latifolia*. (A) and (B) are parent strains, and (C-L) are crossbred strains. Each basidiocarp has individual color and morphological characteristics. A, KFRI 700; B, KFRI 923; C, KFRI 1748; D, KFRI 1749; E, KFRI 1750; F, KFRI 1752; G, KFRI 1762; H, KFRI 1753; I, KFRI 1754; J, KFRI 1755; K, KFRI 1756; L, KFRI 1757.

Results

Characteristics of crossbred strains

Crossbreeding: 10 crossbred strains were screened by clamp connections within the contact zone and on the obverse sides of donor inoculum blocks under a microscope at 1,000× magnification (Uhart and Albertó, 2009). Mycelial from 10 crossbred strains produced clamp connections, and confrontation cultures were made between the crossbred and parent strains (Fig. 1). The presence of clamp connections meant that a dikaryon was formed and the 2 confronted mycelia had different specificities. In addition, success of the confrontation cultures represented success of the crossbreeding. These 2 parent strains and the 10 crossbred strains each produced basidiocarps with their characteristics (Fig. 3).

Mycelial growth: Mycelia began to grow after 3 weeks on the sawdust media of inoculation and were fully grown after 8–11 weeks. Parent and crossbred strains grew on sawdust medium at a similar rate. KFRI 700, a parent strain, grew the fastest, followed by crossbred strains KFRI 1752 and KFRI 1754 (Fig. 2). Mycelial growth was slow and required a longer cultivating period in cauliflower mushrooms than in other cultivated mushrooms. For example, although growth depends on the strain, KFRI 700 required 60 days for incubation and 42 days for harvest (Ryu *et al.*, 2009).

Basidiocarps: All of the parent and crossbred strains produced basidiocarps. Comparison of basidiocarps is focused on taxonomical characteristics. Marginal wave, color, and clamp connection are the most commonly used morphological characters for comparing basidiocarps of cauliflower mushrooms (Desjardin *et al.*, 2004; Dai *et al.*, 2006). KFRI 700 and KFRI 923, the parent strains, showed definite differences in marginal wave and color, although they were confirmed as the same species by taxonomical characteristics. Marginal wave-size was divided into 3 groups, large (>7 mm), medium (4–7 mm), and small (<4 mm), by frequency analysis. KFRI 700 was white (NN155D) and had a large marginal wave, but KFRI 923 was yellowish brown (163C) and had a small marginal wave. Basidiocarps of the 10 crossbred strains were influenced by the 2 parent strains. Four crossbred strains, KFRI 1749, KFRI 1752, KFRI 1755, and KFRI 1757, were white (NN155D), and KFRI 1748 and KFRI 1762 were yellowish brown (163C). In addition, 4 crossbred strains, KFRI 1750, KFRI 1753, KFRI 1754, and KFRI 1756, were cream-colored (8D), which was between white (NN155D) and yellowish brown (163C). Morphologically, KFRI 1749, KFRI 1752, and KFRI 1753 had large marginal waves, like KFRI 700. However, KFRI 1754, KFRI 1755, KFRI 1756,

Table 1. Characteristics of *Sparassis* strains used in this study

Strain No.	Type	Size of marginal wave[a]	Color[b]
KFRI 700	Parent	L	NN155D (white)
KFRI 923	Parent	S	163C (yellowish brown)
KFRI 1748	Crossbreed	M	163C (yellowish brown)
KFRI 1749	Crossbreed	L	NN155D (white)
KFRI 1750	Crossbreed	M	8D (cream)
KFRI 1752	Crossbreed	L	NN155D (white)
KFRI 1762	Crossbreed	S	163C (yellowish brown)
KFRI 1753	Crossbreed	L	8D (cream)
KFRI 1754	Crossbreed	S	8D (cream)
KFRI 1755	Crossbreed	S	NN155D (white)
KFRI 1756	Crossbreed	S	8D (cream)
KFRI 1757	Crossbreed	S	NN155D (white)

[a] L, large (>7 mm); M, medium (4–7 mm); S, small (<4 mm).
Three groups (L, M, and S) were divided by frequency analysis using SPSS.
[b] Royal Horticulture Society (RHS) color chart.
RHS NN155D (white group), white; RHS 8D (yellow group), cream; RHS 163C (grayed-orange group), yellowish brown.

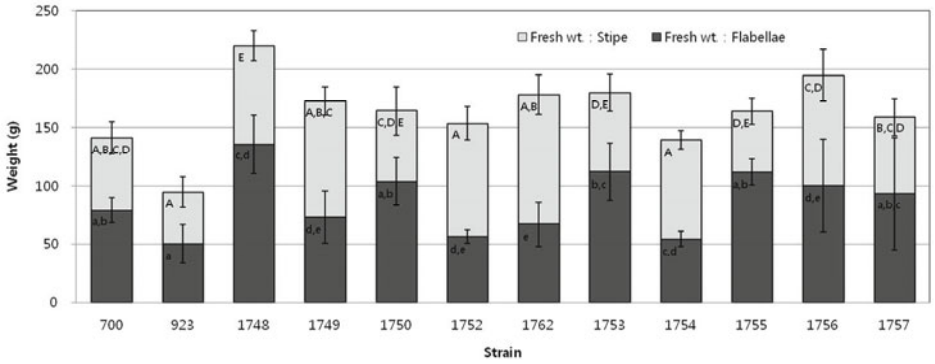

Fig. 4. Fresh weights of basidiocarps (stipes and flabellae) from parent and crossbred strains. Crossbred strains are more productive than parent strains. Means followed by the same letters across bars are significantly different at P=0.01 by Duncan's multiple range test.

KFRI 1757, and KFRI 1762 had small marginal waves, like KFRI 923. KFRI 1748 and KFRI 1750 had medium marginal waves (Table 1 and Fig. 3).

Productivity: We tried to compare productivity in the parent and crossbred strains by fresh weight, dry weight, and ratio of flabellae to stipe. Crossbred strains, except KFRI 1754, were more productive than parent strain KFRI 700. KFRI 1748, KFRI 1753, and KFRI 1756 were the most productive, and flabellae weighed 135.6 g in KFRI 1748 and 112 g in KFRI 1753. However, the stipe of KFRI 1762 weighed 111.1 g and was the heaviest among the crossbred strains. KFRI 1748 and KFRI 1756 represented 220.2 g and 194.8 g of total fresh weight, respectively. Total fresh weights of these 2 strains weighed 50–100 g more than total fresh weights of KFRI 700 and KFRI 923 (Fig. 4). Dry weight was 6–7% of fresh weight and was proportionate. Flabellae of KFRI 1784 weighed 9.2 g, and the stipe of KFRI 1756 weighed 5.9 g. In addition, total dry weights of KFRI 1756 and KFRI 1784 weighed 6–8 g more than total dry weights of KFRI 700 and KFRI 923. Stipe and flabellae weights were similar when they were fresh, but flabellae weighed 2–3 times more than stipes when they were dried. The fresh weight ratio of flabellae to stipe in KFRI 1755 was 2.1, and flabellae total weight was higher than stipe total weight. KFRI 1750 had a dry weight ratio of flabellae to stipe of 3.4. Both the parent strains and the crossbred strains showed a fresh weight ratio of 1.2. However, the dry weight ratios of parent and crossbred strains were 2.5 and 2.0, respectively.

Molecular characteristics

UPGMA dendrogram based on RAPD analysis and morphological phenotype: Phylogenetic position of parent isolates, KFRI 700 and KFRI 923, were proved by Ryoo *et al.* (2013). In ITS rDNA, evolutionary analyses of genetic variation among 2 parent strain and 10 crossbred isolates were conducted in MEGA 5.05 (Tamura *et al.*, 2011). The number of base substitutions per site from between their sequences was shown (Table 2). Analyses were conducted using the Jukes-Cantor model (Jukes and Cantor, 1969). The analysis involved 12 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 578 positions in the final dataset.

Twenty oligonucleotide primers were used to amplify the segments of genomic DNA in the 2 parent strains and 10 crossbred strains of *S. latifolia*. These twenty primers showed significant band profiles and high possibilities of screening each strain. The sizes of these polymorphic fragments were about 0.182–2.924 kb. DNA band patterns were replicated by at least 2 PCR amplifications.

The 2 parent strains and 10 crossbred strains were divided into 3 groups based on the UPGMA dendrogram of the RAPD marker. In the UPGMA dendrogram by genetic variation, the first group included KFRI 923, KFRI 1754, KFRI 1756, KFRI 1755, KFRI 1757, and KFRI 1762. The second group included KFRI 700, KFRI 1749, KFRI 1752, and KFRI 1753, and the third group included KFRI 1748 and KFRI 1750 (Fig. 5A). Fig. 5B also shows the UPGMA dendrogram based on phenotype (size of marginal wave and color). The

Table 2. Estimates of evolutionary divergence between ITS rDNA sequences of 2 parent and 10 crossbred strain of *Sparassis*

Species	KFRI 700	KFRI 923	KFRI 1748	KFRI 1749	KFRI 1750	KFRI 1752	KFRI 1753	KFRI 1754	KFRI 1755	KFRI 1756	KFRI 1762
KFRI 700											
KFRI 923	0.002										
KFRI 1748	0.002	0.000									
KFRI 1749	0.000	0.002	0.002								
KFRI 1750	0.002	0.000	0.000	0.002							
KFRI 1752	0.000	0.002	0.002	0.000	0.002						
KFRI 1753	0.000	0.002	0.002	0.000	0.002	0.000					
KFRI 1754	0.002	0.000	0.000	0.002	0.000	0.002	0.002				
KFRI 1755	0.002	0.000	0.000	0.002	0.000	0.002	0.002	0.000			
KFRI 1756	0.002	0.000	0.000	0.002	0.000	0.002	0.002	0.000	0.000		
KFRI 1757	0.005	0.003	0.003	0.005	0.003	0.005	0.005	0.003	0.003	0.003	
KFRI 1762	0.000	0.002	0.002	0.000	0.002	0.000	0.000	0.002	0.002	0.002	0.005

Table 3. Sequences of RAPD primer pairs

RAPD primer	Sequence (5′→3′)	GC percentage (%)
OPA S1	GTT TCG CTC C	60
OPA S2	TGA TCC CTG G	60
OPA S3	CAT CCC CCT G	70
OPA S4	GGA CTG GAG T	60
OPA S5	TGC GCC CTT C	70
OPA S6	TGC TCT GCC C	70
OPA S7	GGT GAC GCA G	70
OPA S8	GTC CAC ACG G	70
OPA S9	TGG GGG ACT C	70
OPA S10	CTG CTG GGA C	70
OPA S11	GTA GAC CCG T	60
OPA S12	CCT TGA CGC A	60
OPA S13	TTC CCC CGC T	70
OPA S14	TCC GCT CTG G	70
OPA S15	GGA GGG TGT T	60
OPA S16	TTT GCC CGG A	60
OPA S17	AGG GAA CGA G	60
OPA S18	CCA CAG CAG T	60
OPA S19	ACC CCC GAA G	70
OPA S20	GGA CCC TTA C	60

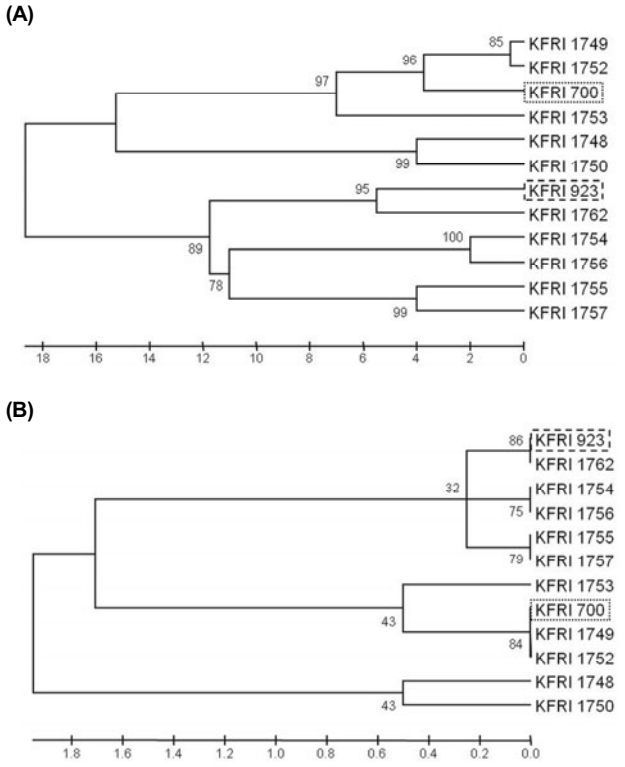

Fig. 5. RAPD dendrograms of *S. latifolia* based on the nucleotide sequence of genomic DNA using the maximum composite likelihood method with 1,000 bootstrapping by unweighted pair-group arithmetic average. (A) RAPD dendrogram of 2 parent strains (KFRI 700 and KFRI 923) and 10 crossbred strains of *S. latifolia* based on the nucleotide sequence of genomic DNA from cultured basidiocarps. (B) RAPD dendrogram of 2 parent strains (KFRI 700 and KFRI 923) and 10 crossbred strains of *S. latifolia* based on phenotype (size of marginal wave and color).

morphological characteristics (phenotype) used to classify the genus *Sparassis* included size of marginal wave and color of basidiocarp. Both dendrograms were divided into 3 groups, and the 3 groups were divided by marginal wave-size. These results indicate that basidiocarp color is important but that marginal wave is more important for classifying the genus *Sparassis*.

Discussion

Cauliflower mushrooms reflect various morphological and physiological characteristics according to changes in environmental and medium conditions. However, this study was conducted to verify the diverse characteristics of basidiocarps resulting from crossbreeding, not from changes in environmental conditions.

In eastern Asia, short-log cultivation and sawdust cultivation were developed in the 2000s, and several patents have been obtained for the artificial cultivation of cauliflower mushrooms. Research has been conducted not only on basidiocarps but also on mycelia and metabolites of *Sparassis* species. Previous studies investigated the optimal methods for culturing mycelia of *Sparassis* species and extracting the antineoplastic constituent (Harada *et al*., 2002; Kurosumi *et al*., 2006) and sparassol, ScI, and ScII, which are antifungal metabolites produced by *Sparassis* species (Woodward *et al*., 1993). Worldwide, people became interested in breeding, cultivating, and using new varieties of plants based on the UPOV Convention, which has expanded considerably in recent years (Jordens, 2005), and tried to produce new varieties of plants in every field. For these reasons, development of new varieties has become very important.

As shown in the RAPD dendrogram using UPGMA (Fig. 5B), the 2 parent and 10 crossbred strains were divided into 3 groups based on the basidiocarp characteristics of marginal wave-size and color. According to Burdsall and Miller (1988a and 1988b) and Wang *et al.* (2004), *Sparassis* species are variable in basidiocarp macromorphology, and classified by shape and color. Marginal wave-size makes up the largest part in macromorpholgy in *Sparassis*. Also in test guideline (TG) of *Sparassis crispa* for the protection of new varieties in Japan, marginal wave-size and color were one of the most important items in new varieties. We could suggest that, therefore, basidiocarp marginal wave-size and color play a vital role in classifying cauliflower mushrooms and that marginal wave and color are the most important production and physiological characteristics to consider in cauliflower mushroom crossbreeding for new varieties.

The cauliflower mushroom basidiocarp is divided into 2 parts, the flabellae and the stipe. In the market, flabellae are more valuable than stipes for food. However, this study showed that stipes and flabellae also have different production characteristics. We assumed that these characteristics may also affect the physiology between flabellae and stipes, although results are not yet definitive; for example, stipes and flabellae have different β-glucan contents. Thus, additional studies of the characteristics of flabellae and stipes are needed, and uses for both structures should be investigated.

Therefore, diverse and specialized strains of cauliflower

mushrooms should be studied and crossbred not only to increase productivity as food but also to improve medicinal functions.

Acknowledgements

This work was supported by project (FP 0801-2009) of Korea Forest Research Institute.

References

Burdsall, H.H. Jr. and Miller, O.K. Jr. 1988a. Type studies and nomenclatural considerations in the genus *Sparassis*. *Mycotaxon* **31**, 199–206.

Burdsall, H.H. Jr. and Miller, O.K. Jr. 1988b. Neotypification of *Sparassis crispa*. *Mycotaxon* **31**, 591–593.

Dai, Y.C., Wang, Z., Binder, M., and Hibbett, D.S. 2006. Phylogeny and a new species of *Sparassis* (Polyporales, Basidiomycota): evidence from mitochondrial *atp*6, nuclear rDNA and *rpb*2 genes. *Mycologia* **98**, 584–592.

Desjardin, D.E., Wang, Z., Binder, M., and Hibbett, D.S. 2004. *Sparassis cystidiosa* sp. nov. from Thailand is described using morphological and molecular data. *Mycologia* **96**, 1010–1014.

Harada, T., Miura, N.N., Adachi, Y., Nakajima, M., Yadomae, T., and Ohno, N. 2002. IFN-γ introduction by SCG, 1, 3-β-D-glucan from *Sparassis crispa*, in DBA/2 mice *in vitro*. *J. Interferon Cytokine Res*. **22**, 1227–1239.

Jordens, R. 2005. Progress of plant variety protection based on the International Convention for the Protection of New Varieties of Plants (UPOV Convention). *World Patent Informat*. **27**, 232–243.

Jukes, T.H. and Cantor, C.R. 1969. Evolution of protein molecules. *In* Munro, H.N. (ed.), Mammalian Protein Metabolism, pp. 21–132, Academic Press, New York, N.Y., USA.

Kurosumi, A., Kobayasi, F., Mtui, G., and Nakamura, Y. 2006. Development of optimal culture method of *Sparassis crispa* mycelia and a new extraction method of antineoplastic constituent. *Biochem. Engineer. J*. **30**, 109–113.

Lee, S.B. and Taylor, J.W. 1990. Isolation of DNA from fungal mycelia and single spores, pp. 282–287. *In* Innis, M.A., Gelfand, D.H., Sninsky, J., and White, T.J. (eds.). PCR protocols. Academic Press. San Diego, California, USA.

Park, H., Lee, B.H., Oh, D.S., Ka, K.H., Bak, W.C., and Lee, H.J. 2005. Cultivation of cauliflower mushroom (*Sparassis crispa*) using coniferous sawdust-based media with barley flours. *Kor. J. Forest Energy* **24**, 31–36.

Park, H., Ryu, S.R., and Ka, K.H. 2011. Cultivation of *Sparassis crispa* on several kinds of medium density and particle size of sawdust-based medium made of *Larix kaempferi*. *Mokchae Konghak* **39**, 68–74.

Ryoo, R., Sou, H.D., Ka, K.H., and Park, H. 2013. Phylogenetic relationships of Korean *Sparassis latifolia* based on morphological and ITS rDNA characteristics. *J. Microbiol*. **51**, 43–48.

Ryu, S.R., Ka, K.H., Park, H., Bak, W.C., and Lee, B.H. 2009. Cultivation characteristics of *Sparassis crispa* strains using sawdust medium of *Larix kaempferi*. *Kor. J. Mycol*. **37**, 49–54.

Tada, R., Harada, T., Nagi-Miura, N., Adachi, Y., Nakajima, M., Yadomae, T., and Ohno, N. 2007. NMR characterization of the structure of a β-(1→3)-D-glucan isolate from cultured fruit bodies of *Sparassis crispa*. *Carbohydrate Res*. **342**, 2611–2618.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution.

Uhart, M. and Albertó, E. 2009. Mating tests in *Agrocybe cylindracea* sensu lato. Recognition of *Agrocybe wrightii* as a novel species. *Mycol. Progress* **8**, 337–349.

Wang, Z., Binder, M., Dai, Y.C., and Hibbett, D.S. 2004. Phylogenetic relationships of *Sparassis* inferred from nuclear and mitochondrial ribosomal DNA and RNA polymerase sequences. *Mycologia* **96**, 1015–1029.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **18**, 6531–6535.

Woodward, S., Sultan, H.Y., Barrett, D.K., and Pearce, R.B. 1993. Two new antifungal metabolites produced by *Sparassis crispa* in culture and in decayed trees. *J. Gen. Microbiol*. **139**, 153–159.