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Genetic analyses of causal genes of albinism (white fruiting body) in *Grifola frondosa*

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Abstract

The tyrosinase 2 gene (*tyr2*) from two compatible strains of *Grifola frondosa*, the albino-type monokaryon strain IM-WM1-25 and the wild-type monokaryon strain IM-BM11-P21, were amplified and characterized (designated $tyr2^{-\Delta 25}$ and $tyr2^{+}$, respectively). A single base deletion in the coding region of $tyr2^{-\Delta 25}$ from IM-WM1-25 was discovered, and this mutation is predicted to cause a frame-shift in translation, yielding inactive protein tyrosinase protein 2 (TYR2). Polymerase chain reaction (PCR) primer pairs were designed to detect normal $tyr2^{+}$ and mutant $tyr2^{-\Delta 25}$, and then the *tyr2* genotype of F1 progenies, which was obtained from basidiospore isolation of IM-BM11-P21 × IM-WM1-25 ($tyr^{+} \times tyr2^{-\Delta 25}$) strain, was analyzed. Back-crossing (F1 progenies × IM-WM1-25) was performed and fruiting body colors of the crossed strains were analyzed. The fruiting bodies of all crossed strains were white and beige, and the corresponding genotypes were $tyr2^{-\Delta 25} \times tyr2^{-\Delta 25}$ and $tyr^{+} \times tyr2^{-\Delta 25}$. These results suggest that the causal gene of the albino mutation is *tyr2* and this study provides a new strategy for the breeding of albino mushrooms belonging to *G. frondosa*.

Keywords: Albino, Linkage, *Grifola frondosa*, Melanin, Tyrosinase

Introduction

Grifola frondosa is a polyporous basidiomycete that grows on decaying wood and an economically important edible mushroom called "Maitake" [1]. Due to recent advances in bottle or plastic bag cultivation technology in Japan, this mushroom is now available in domestic markets throughout the year. In 2016, Japanese annual production of Maitake was 48,523 ton [2]. Maitake is a delicious mushroom but dark brown pigment was extracted from fruiting body into supernatant. Therefore, the meal that used Maitake become black; therefore, Maitake is used for limited cooking.

Generally speaking, the fruiting body of *G. frondosa* consists of a dark brown cap that is colored with visible dark and white stripes. This mushroom exhibits pigmentation from the primordia stage until mature fruiting body formation. In a previous study, the brown pigment in the fruiting body of *G. frondosa* was thought to be melanin, which was absent in the white fruiting body of albino strains. The corresponding genes for the melanin synthesis pathway, such as tyrosinase genes (*tyr1*, *tyr2*), were also identified and characterized. Only *tyr2* transcript levels increased gradually from primordia to the mature fruiting body. However, whether *tyr2* is responsible for melanin formation was not investigated in *G. frondosa* [3]. A similar phenomenon was observed in *Polyporus arcularius*. Brown pigmentation of mycelia in *P. arcularius* occurred only in a dikaryotic strain grown under visible light before the development of primordia and did not occur in a monokaryon strain, even when grown under visible light. Moreover, transcription of tyrosinase 1 gene in *P. arcularius* closely related pigmentation of mycelia and primordia [4].

It is very important for mushroom industry to develop high-yield white color strain of Maitake. In Japan, the white fruiting bodies of *Flammulina velutipes* (Enokitake) [5] and Bunashimeji (Bunapie, Hokuto co., Japan) are very popular, but it is unclear why the fruiting

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bodies of some strains become white. The objective of the present study is to understand the morphological and genetic characteristics of *G. frondosa*. Here, we report the sequence diversity of *tyr2* in both wild-type and albino-type strains, and describe the relationship between *tyr2* and brown phenotypes by back-crossing. This research is one of the attempts to develop useful white strain of Maitake.

Materials and methods

Fungal strains

The wild dikaryotic strain IM-BM11 and the albino dikaryotic strain IM-WM1 were used in this study, and were stocked in Ichimasa Kamaboko Co., Ltd. Basidiospore isolates IM-WM1-16 and IM-WM1-25 were isolated from the fruiting bodies of albino strain IM-WM1. Monokaryotic strain IM-BM11-P21 was derived from regenerated protoplasts of wild-type dikaryotic strain IM-BM11. Hybrid strain IM-BW1 was obtained by crossing IM-WM1-25 with IM-BM11-P21 (Fig. 1).

Method for producing dikaryotic hybrids and cultivation of fruit bodies of dikaryotic hybrid stocks

The dikaryotic stock of this mushroom was prepared by crossing two compatible monokaryotic stocks. The two monokaryotic stocks were placed 4 mm apart in the center of a potato dextrose agar (PDA) plate (Nissui Seiyaku, Tokyo, Japan). After incubation for 7–10 days at 25 °C, the mycelia on the PDA plate at the contact zone between the two parental monokaryotic colonies were inspected under a microscope for the formation of clamp connections as evidence for dikaryotization. The hybrid dikaryotic strain was maintained on a PDA slant.

Cultivation of fruit bodies from the test hybrid stock was carried out on a sawdust substrate. Substrate was prepared by mixing beech sawdust and rice bran at a volumetric ratio of 5:1 and adjusting the moisture content to 65%. Mature fruit bodies were removed with a knife and placed in a Petri dish to obtain spore print.

Isolation of basidiospore-derived monokaryotic stocks

Sterilized water (5 ml) was pipetted onto the spore print in the Petri dish, and the plate was vigorously shaken to prepare a spore suspension. The spore densities in the suspensions were determined by counting the number of spores with a hemocytometer under a microscope. The suspension was then diluted to approximately 1×10^4 to 1×10^6 cells/ml. Next, 0.1 ml of the suspension was mixed in a test tube with 2 ml of melted PDA soft agar (agar concentration, 0.7%) medium at 50 °C and then poured onto a PDA plate to prepare a double-layer agar culture. After incubating the cultures at 25 °C for a week, colonies

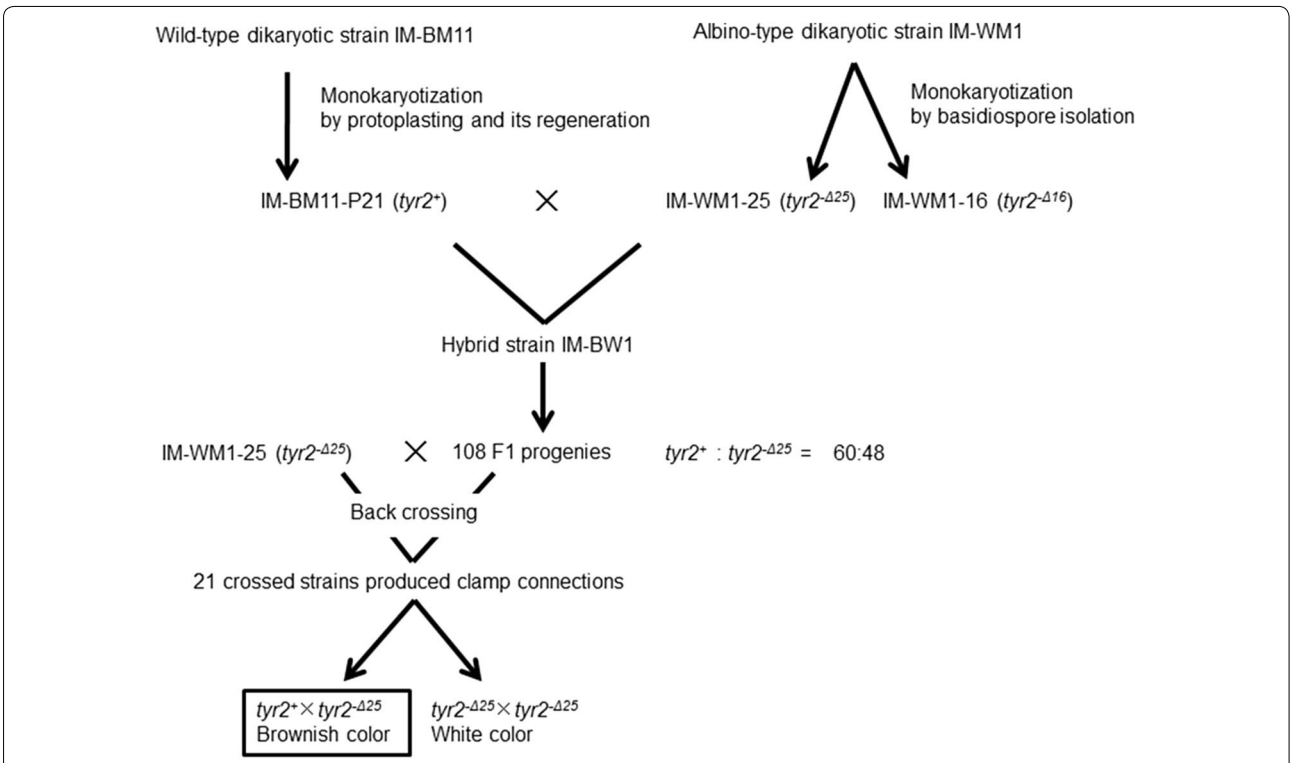

Fig. 1 Diagrams to explain a back-crossing involving strains, genotypes and colors

that appeared on the plate were isolated and transferred onto PDA slants. These slants were incubated for approximately 7 days at 25 °C before being used for crossing experiments. The absence of clamp connections among all basidiospore-derived strains was confirmed under the microscope, and the strains without clamp cell were stocked as monokaryotic strain.

Isolation of monokaryon via protoplast regeneration

To collect protoplasts from dikaryotic mycelia, an MYG (10-g malt extract, 4-g yeast extract and 4-g glucose) plate (2% agar) was inoculated with the IM-BM11 strain and incubated at 25 °C for 2 weeks. Five agar blocks (2×2 mm^2) cut from the plate were inoculated onto 100-ml Erlenmeyer flasks containing 10-ml MYG liquid medium and incubated statically at 25 °C MYG for 2 weeks. The mycelial mats were filtered through a stainless steel net and washed with buffer (0.6-M mannitol, 0.2-M Na_2HPO_4, 0.1-M citric acid, pH 5.6), suspended in 3-ml buffer containing 2% lywallzyme (Guangdong Institute of Microbiology, Guangdong, China), and incubated at 30 °C for 4 h with gentle shaking at 30-min intervals to release the protoplasts into suspension. Protoplasts were filtered through a 3G1 glass filter into 15-ml polypropylene conical tubes, centrifuged (4 °C, 340×*g* for 5 min) and washed with buffer twice, and suspended again with buffer. Protoplast densities in the suspensions were determined by a hemocytometer under a microscope. The suspension was then diluted to approximately 1×10^4 to 1×10^6 cells/ml. Next, 0.1 ml of the suspension was spread onto regeneration medium [glucose, 20 g; $(NH_4)_2SO_4$, 1.5 g; KH_2PO_4, 1.5 g; $MgSO_4$, 1.0 g; peptone, 2.0 g; yeast extract, 2.0 g; agar, 15 g (pH 5.5); 500 ml of H_2O plus 500 ml of 1.0 M sucrose]. After incubating cultures at 25 °C for a week, the colonies that appeared on the plate were isolated and transferred onto PDA slants. These slants were incubated for approximately 7 days at 25 °C before being used for the crossing experiments.

Genome sequencing and annotation

Genomic deoxyribonucleic acid (DNA) extraction followed the method of Dellaporta et al. [6]. The complete nucleotide sequence of the genomic DNA of albino monokaryotic strain WM1-25 was determined using Illumina HiSeq2000 paired-end technology provided by Hokkaido System Science Co., Ltd. (Sapporo, Hokkaido, Japan). This sequencing run yielded 34,502,348 high-quality filtered reads with 101 bp paired-end sequencing. The genomic sequence was assembled using velvet assembler version 1.1.02 (hash length, 75 bp). The final assembly contained 7354 contigs of total length 35,343,167 bp, with an n50 length of 94,048 bp.

Amplification DNA sequence of *TYR2* from *G. frondosa*

In a previous study, we showed that TYR2 is closely related to the fruiting body color and melanin production [3]. Therefore, we focused on *tyr2* in this study. The DNA sequence of *tyr2* was identified using a tblastn search against the draft genome database of WM1-25 with the tyrosinase protein sequences of *Lentinula edodes* (BAB71736.1). Based on the DNA sequence of *tyr2*, oligonucleotide primers (Table 1) were designed to amplify the entire DNA sequence of *tyr2* from the genomic DNAs of monokaryotic strains IM-WM1-16, IM-WM1-25 and IM-BM11-P21, respectively. All amplified DNA fragments were sequenced directly and used as templates for cycle sequencing. These sequences were then compared using the Clustal X program to identify potential

Table 1 Primers used in this study

Primer	Sequence	Use
TYR2gF	5′-TCTTCATCCTGCTTCCTCTATC-3′	Amplification of full-length genomic clone of *tyr2*
TYR2gR	5′-GCTTGCACCATCGAGACAGCCAA-3′	
Btyr2F	5′-TCATCACTCATTTCCCTGCTGACAC-3′	Used for detection of wild *tyr2* gene (*tyr2*$^+$)
Btyr2R	5′-CCCGTAACGATTTCGCCACTCTC-3′	
Wtyr2F	5′-CATTTTCCTGCTGACACCTT-3′	Used for detection of mutant *tyr2* gene (*tyr2*$^{-\Delta 25}$)
Wtyr2R	5′-CGACGG TGATAATCCAGTCA-3′	
3RTYR2	5′-AGGCGGGCATATGGCTACTG-3′	Used for 3′-RACE
5RTYR2P	5′-(P)CAGATCACTTGCTCC-3′	Used for 5′-RACE (5′-end of this oligonucleotide was phosphorylated)
5RTYR2S1	5′-GCTATTGTACTCACGGAACTGTC-3′	Used for 5′-RACE
5RTYR2A1	5′-AGTCCCCAATATTGACATCATTCC-3′	
5RTYR2S2	5′-CTCACGGAACTGTCCTCTTC-3′	
5RTYR2A2	5′-TTCCAAGGAGTGTATGGCAA-3′	

restriction fragment length polymorphism (RFLP) and mutations.

Amplification complementary DNA (cDNA) sequence of *tyr2*$^{-\Delta 25}$ from *G. frondosa*

Total ribonucleic acid (RNA) was extracted from wild-type monokaryotic strain IM-BM11-P21 using a MagExtractor Kit (Toyobo, Osaka, Japan). The cDNA was synthesized using total RNA as a template by ReverTra Ace qPCR RT Master Mix with gDNA Remover Kit (Toyobo). For amplification of full-length cDNA of *tyr2*$^{-\Delta 25}$, 3′-rapid amplification of cDNA ends (RACE) was performed with a Takara RNA PCR (AMV) version 3.0 kit (Takara Bio, Shiga, Japan) and 5′-RACE with a 5′-Full RACE Core Set (Takara Bio). PCR was carried out according to the kit manufacturer's instructions using the oligonucleotide primers listed in Table 1. Amplified fragments were subcloned into a pMD20 T-vector (Takara bio) and sequenced.

The full-length cDNA of *tyr2*$^{-\Delta 16}$ and *tyr2*$^{-\Delta 25}$ from monokaryotic strain IM-WM1-16 and IM-WM1-25, respectively, was deduced by alignment of the previously defined *tyr2*$^{+}$ cDNA of IM-BM-11-P21. The location of the initiation and stop codons, and the exons and introns of the gene were determined from the full-length cDNA of *tyr2*$^{+}$ of IM-BM-11-P21. All of the introns started with GT and ended with AG. These cDNA sequences were translated into amino acid sequences by GENETYX, and were then compared to one another using the Clustal X program to identify potential mutations in the *tyr2*$^{-\Delta 16}$ and *tyr2*$^{-\Delta 25}$ amino acid sequence from the albino-type strains.

Determination of genotypes of single-spore isolates

The 6-bp deletion was present at position 1206 bp from start codon (ATG) in *tyr2* albino monokaryotic strains (Fig. 2), as compared with nucleotide sequences of wild-type *tyr2*$^{+}$ from monokaryotic strains. Based on this region, the two primer pairs were designed to produce polymorphic markers for distinguishing the *tyr2* genotype of *G. frondosa* strains. The primer pair Btyr2F and Btyr2R was used for detection of wild-type *tyr2* gene (*tyr2*$^{+}$) isolated from wild-type strain IM-BM11-P21. The primer pair Wtyr2F and Wtyr2R was used for detection of mutant *tyr2* gene (*tyr2*$^{-\Delta 25}$) isolated from albino-type strain IM-WM1-25. PCR was carried out in a 50-µl reaction volume containing 20 ng of extracted genomic DNA, 50 pmol of each primer, 0.2-mM deoxynucleotide triphosphate (dNTP), 1 × PCR buffer and 1.25 U Blend Taq polymerase (Toyobo). Thermal cycling parameters were an initial denaturation step at 95 °C for 3 min followed by 30 cycles of denaturing at 95 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 2 min, and a 10-min final extension at 72 °C. PCR products were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide, and photographed under ultraviolet light.

χ^2 test

Segregation values were calculated, and Chi-squared (χ^2) goodness of fit tests were performed to determine the significance of segregation [i.e., any skewing from the expected Mendelian segregation ratio of 1:1, using Excel (Microsoft)].

Back-crossing

The pedigree diagrams for each strain are shown in Fig. 1. F1 progenies of IM-BW1 were obtained by basidiospore isolation. The 108 monokaryotic isolates were randomly selected for crossing, and then the genotypes of these monokaryotic isolates were confirmed by specific primers. According to the monokaryotic–monokaryotic (mon–mon) crossing method, each monokaryotic isolate was confronted to parental strain IM-WM1-25 (*tyr2*$^{-\Delta 25}$) in Petri dishes using 4-mm-diameter blocks as inoculum to breed new strains. The new dikaryotic strains were derived from the mon–mon crossing method, which means that mating occurred. Each crossbred stain was cultured in sawdust medium to produce fruiting bodies. Morphological descriptions used color terms and notations from the Royal Horticulture Society color chart.

Results

Comparison of *tyr2* in wild-type and albino-type strains

Tyr2 from wild-type monokaryotic strain IM-BM11-P21 is designated *tyr2*$^{+}$, with a nucleotide sequence from the initial ATG to the stop codon of the coding region consisting of 1866 bp and encoding 621 amino acids. The signal peptide sequence may be lacking in *tyr2*$^{+}$, as predicted by SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/), suggesting that it may be an intracellular enzyme.

Two monokaryotic strains IM-WM1-25 and IM-WM1-16 were selected from basidiospores isolated from albino-type dikaryotic strain IM-WM1. The two strains were compatible with IM-BM11-P21 and carried mutant

(See figure on next page.)

Fig. 2 Comparison of the genomic DNA sequences of the tyrosinase 2 gene of *G. frondosa*. B and W represented nucleotide sequences of *tyr2* from brown-colored wild-type monokaryotic strain IM-BM11-P21 and white-colored albino strain IM-WM1-25, respectively. Nucleotide sequences of *tyr2* of albino strain (*tyr2*$^{-\Delta 25}$) that are identical with wild-type strain are indicated by dots. Codons that are containing nucleotide substitution with amino acid changing are underlined and amino acid residues are shown under the codon. The deletion mutation that was the possible main reason for deficiency of tyrosinase activity is boxed. Bold case letters indicate primer positions

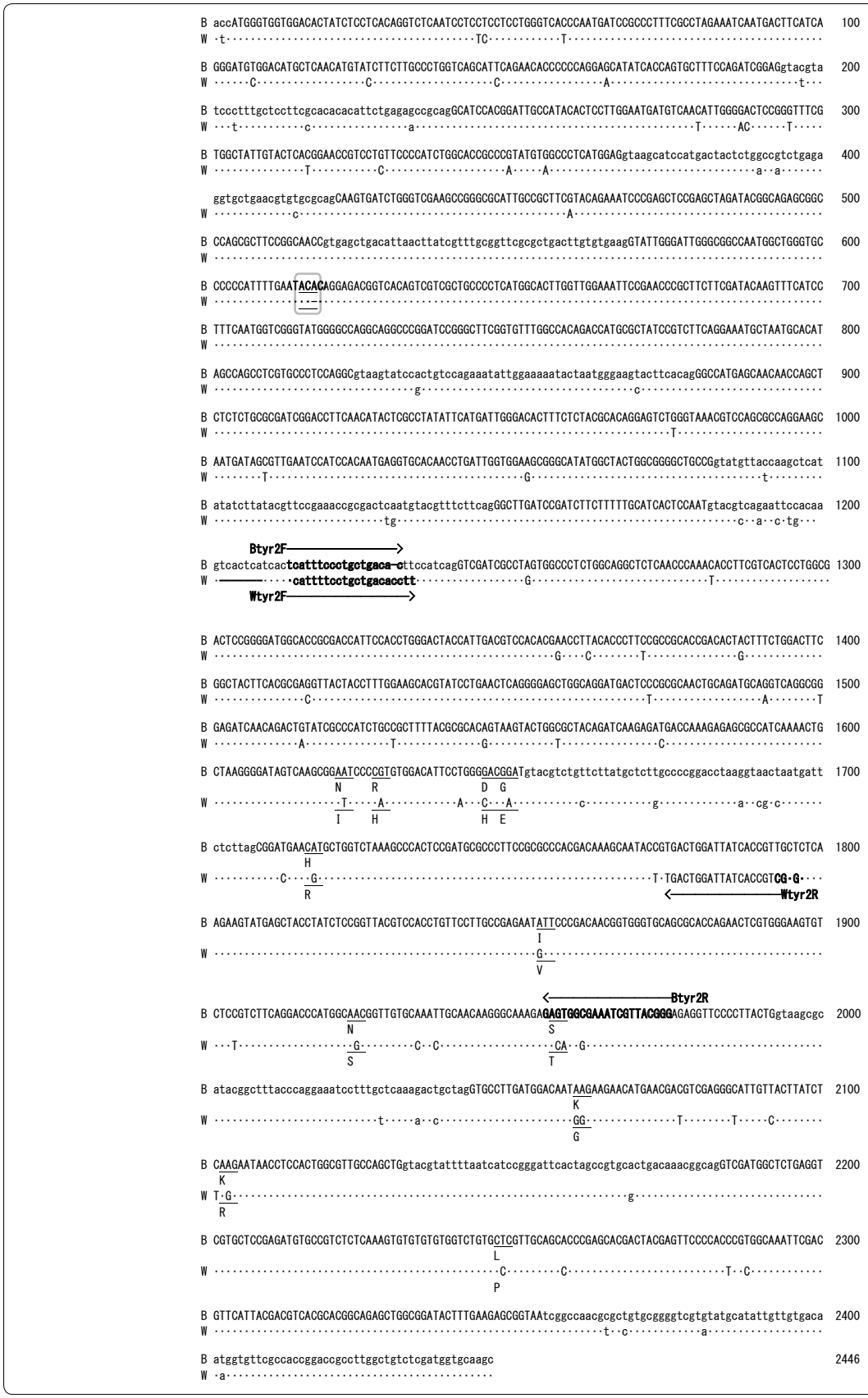
Btyr2F
Wtyr2F
Wtyr2R
Btyr2R

Table 2 Comparison of *TYR2* between wild-type ($tyr2^{+}$) and albino-type ($tyr2^{-\Delta 25}$) monokaryotic strains

Classification	Wild	Albino	
Strain	IM-BM11-P41	IM-WM1-25	IM-WM1-16
Genotype	$tyr2^{+}$	$tyr2^{-\Delta 25}$	$tyr2^{-\Delta 16}$
Nucleotide substitution on the exon (bp)	0	68	92
Amino acid substitution (AAS)	0	33	23
Nucleotide deletion on the exon (bp)	0	1 (No. 4 exon)	0

allelic *tyr2*. The mutant allelic *tyr2* from IM-WM1-25 and IM-WM1-16 were designated $tyr2^{-\Delta 25}$ and $tyr2^{-\Delta 16}$, respectively. The DNA sequences of $tyr2^{-\Delta 25}$ and $tyr2^{-\Delta 16}$ were analyzed and compared with those of $tyr2^{+}$ from IM-BM11-P21 to detect polymorphisms of tyrosinase genes. The results are summarized in Table 2.

In the comparison of nucleotide sequences from the TYR2 coding regions between wild-type monokaryotic strain IM-BM11-P21 and albino-type monokaryotic strain IM-WM1-25, a single base deletion at nucleotide 617 (A) was discovered in the IM-WM1-25. The $tyr2^{-\Delta 25}$ translational reading frame is shifted by a single nucleotide deletion and translation will stop at a TAG stop codon newly generated at amino acid 412 (Fig. 2) by the frame-shift mutation. This stop codon produces a truncated with 53 fewer amino acids than $TYR2^{+}$ protein and $TYR2^{-\Delta 25}$ protein was completely different from $TYR2^{+}$ protein.

In comparisons of the $tyr2^{-\Delta 16}$ coding sequence with the $tyr2^{+}$ coding sequences, we observed that the length did not vary, except for a 23 amino acid substitution (Table 2). $tyr2^{-\Delta 16}$ shared 94% identity with $tyr2^{+}$. Although many missense and silent mutations were observed in $tyr2^{-\Delta 16}$ of IM-WM1-16 isolated from the same parent strain IM-WM1 with IM-WM1-25, relationships between $TYR2^{+}$ function and mutation were not clear in this strain. Therefore, the frame-shift mutation was considered to be a cause of brown color loss and we focused on $tyr2^{-\Delta 25}$ from IM-WM1-25 in the current study.

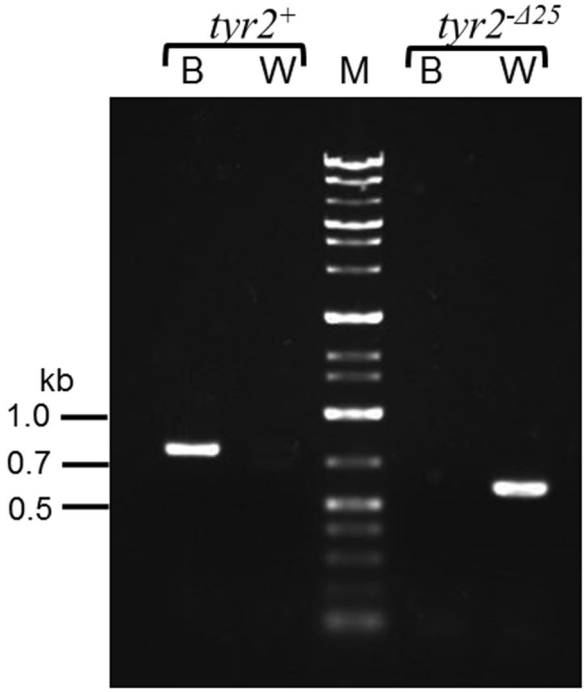

Fig. 3 PCR amplification of $tyr2^{+}$ and $tyr2^{-\Delta 25}$ genes from IM-BM11-P21 and IM-WM1-25 strains, respectively. The specific primer pairs Btyr2F/Btyr2R (for $tyr2^{+}$) and Wtyr2F/Wtyr2R (for $tyr2^{-\Delta 25}$) were used for amplification of genomic DNA. B and W represented PCR product of $tyr2^{+}$ from brown-colored wild-type monokaryotic strain IM-BM11-P21 (770 bp) and that of $tyr2^{-\Delta 25}$ from white-colored albino strain IM-WM1-25 (582 bp), respectively. 100 bp DNA Step Ladder markers (Nippon gene, Toyama, Japan) was used for molecular size calibration (lane M)

Linkage analysis and DNA polymorphisms

To reveal genetic relationships between the F1 progenies and *tyr2* genotype, and to develop efficient breedingmethod, linkage analysis was carried out. A total of 108 F1 progenies of IM-BW1 (IM-BM11-P21 × IM-WM1-25) were randomly isolated, designated IM-BW1-1 to IM-BW1-108. Genotypes of all F1 progenies were determined using the specific primer pairs Wtyr2F/Wtyr2R and Btyr2F/Btyr2R, respectively. In all $tyr2^{+}$ genotype monokaryotic DNA, the detected band was around 770 bp, but it was around 582 bp in the $tyr2^{-\Delta 25}$ genotype (Fig. 3). We found that 60 monokaryotic strains showed the $tyr2^{+}$ genotype, while the other 48 monokaryotic strains showed the $tyr2^{-\Delta 25}$ genotype. To determine whether the clone region is linked to genotype, we tested whether the ratio of the genotype in progeny fit a theoretical 1:1 ratio using χ^2 tests. Because $\chi^2 = 1.333$ is less than $\chi^2_{0.05} = 3.841$, $p > 0.05$, we concluded that the cloned region fits a 1:1 ratio. This DNA polymorphic marker showed a significant segregation that was used for further analysis.

Table 3 Characteristics of strains used in this study

Strains	Type	*tyr2* genotype	Color (×IM-WM1-25)	Biomass
IM-BM11-P21	Parent	+	Dark brown	
IM-BW1-8	F1	+	Brownish	479 g
IM-BW1-9	F1	+	Brownish	349.8 g
IM-BW1-13	F1	–	White	420.7 g
IM-BW1-36	F1	–	White	516.8 g
IM-BW1-38	F1	–	White	402.4 g
IM-BW1-39	F1	+	Brownish	226.2 g
IM-BW1-54	F1	–	White	548.1 g
IM-BW1-73	F1	–	White	304.7 g
IM-BW1-82	F1	+	Brownish	396.6 g
IM-BW1-83	F1	+	Brownish	487.9 g
IM-BW1-103	F1	+	Brownish	353 g
IM-BW1-104	F1	+	Brownish	336.8 g
IM-BW1-108	F1	+	Brownish	529.8 g

Characteristics of crossbred strains

A classical genetic technique (back-crossing) was used to analyze the characteristics of fruiting body color in *G. frondosa*. The parental IM-WM1-25 ($tyr2^{-\Delta25}$) was crossed with each of the 108 F1 progenies. The 108 crossbred strains were screened based on the presence of clamp connections under a microscope. Only 21 monokaryotic strains, IM-BW1-6, 8, 9, 13, 29, 36, 38, 39, 54, 55, 58, 66, 69, 73, 80, 82, 83, 103, 104, 105 and 108, produced clamp connections with IM-WM1-25. The IM-WM1-25 × IM-BW1-6, 29, 55, 58, 66, 69, 80 and 105 did not produce basidiocarps. Comparison of basidiocarps focused on color. The genotype of *tyr*2 and color of basidiocarps of backcross strains are shown in Table 3 and Fig. 4. Two colors were observed in the F_0 and subsequent generations. The colors could be classified as white and beige, and the corresponding genotypes were $tyr2^{-\Delta25} \times tyr2^{-\Delta25}$ and $tyr2^{+} \times tyr2^{-\Delta25}$. We believe that $tyr2^{-}$ may be a recessive gene and defective *tyr2* genes in the both alleles were required for albinism. Therefore, we could identify the $tyr2^{-\Delta16}$ mutation, but $tyr2^{-\Delta16}$ might be also a defective mutant gene. We attempted to compare productivity in crossbred strains based on fresh weight. IM-BW1-54 ($tyr2^{-\Delta25}$) × IM-WM1-25 ($tyr2^{-\Delta25}$) weighed 548.1 g and was the heaviest among the crossbred strains, while IM-BW1-39 (tyr^{+}) × IM-WM1-25 ($tyr2^{-\Delta25}$) weighed 226.2 g, which was lower than other crossbred strains.

Discussion

In a previous study, we demonstrated that the brown pigments of the *G. frondosa* fruiting body are mainly melanin [3]. *G. frondosa* produces melanin via the L-3,4-dihydroxyphenylalanine (L-DOPA) melanin biosynthetic pathway, and tyrosinase is a key enzyme for melanization in this process. Therefore, the color of the fruit body of the mushroom is primarily determined by a single tyrosinase gene. In the Agaricales mushroom, the color of the fruiting body is also related to polyphenol oxidases, such as tyrosinase and laccase. The mechanisms of mushroom browning have been investigated extensively in *Agaricus bisporus* [7]. Browning in this species is mainly due to melanin [8], and tyrosinase seems to be the principal enzyme in its synthesis [9]. In *Pholiota microspora*, tyrosinase gene (*tyr*) and laccase gene 9 expression were markedly increased during the primordia and fruiting body stage [10]. These phenomena indicate that the content of melanin in the fruiting body may be determined by the complementary activity of more than two types of phenol oxidase in the Agaricales mushroom. The brown pigment was produced in primordia stage of wild-type strain but was not produced in albino strain throughout its all developmental stage (data not shown). Brown pigment production was not necessary for fruiting body development. Melanin was not necessary for *Rosellinia necatrix* pathogenesis but is involved in survival through morphogenesis [11]. Therefore, in *G. frondosa*, Brown pigment production might be involved in survival such as protection of DNA from damage of ultra violet in the nature.

tyr2 was the first candidate gene to be tested in *G. frondosa*, and the results of this study confirmed that color of the fruiting body was affected by *tyr2*. In addition, approximately 200 *tyr* mutations have been described in humans (http://albinismdb.med.umn.edu/) [12]. A frame-shift mutation generating a premature stop codon (TGA_{491}) resulting in a truncated TYR protein that is shortened by 21 amino acids has been reported in humans. This mutation is found in the putative transmembrane region in exon 5 of the *tyr* gene (between nucleotides 1420 and 1500) and results in the elimination of the carboxyl-terminal portion. In *G. frondosa*, the detected mutation occurs in exon 4 of the *tyr*2 gene (nucleotide 1236), generating a premature stop codon, and as in humans, the carboxyl-terminal portion is also eliminated. This region contains a short amino acid sequence (serine–histidine–leucine) that acts as a targeting signal for the transport of several peroxisomal enzymes into peroxisomes. Our data demonstrate that a frame-shift mutation is present in the coding region of the *tyr2* gene. This mutation would disrupt the reading frame of wild-type *tyr2*. Disruption of translation appears to be responsible for the absence of melanin synthesis in *G. frondosa*. Therefore, understanding this mutation should facilitate a more detailed explanation of the mechanisms of melanin synthesis in *G. frondosa*.

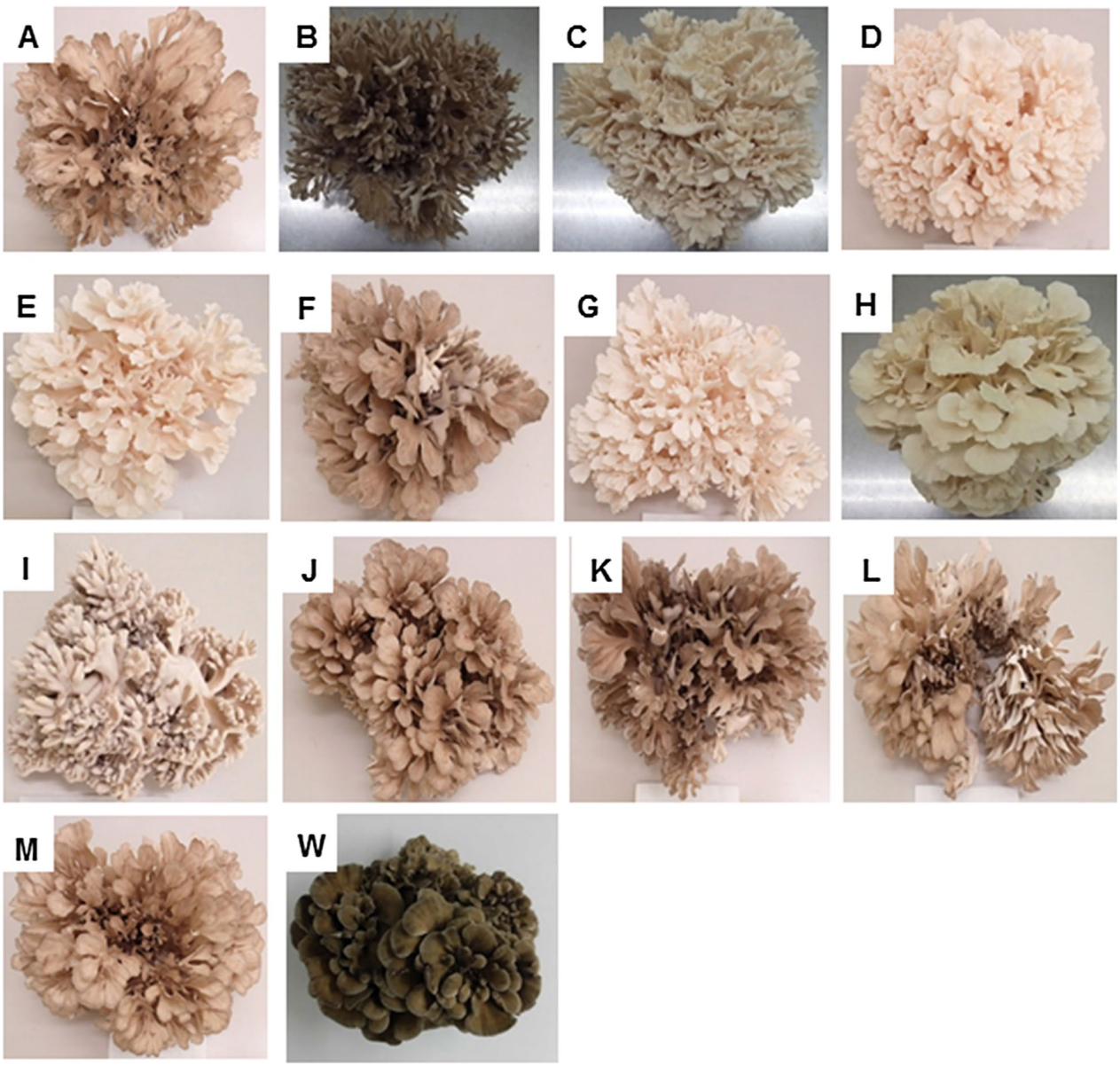

Fig. 4 Thirteen fruiting body of crossbred strains of *G. frondosa*. Each fruiting body has individual color and morphological characteristics. **A** IM-BW1-8; **B** IM-BW1-9; **C** IM-BW1-13; **D** IM-BW1-36; **E** IM-BW1-38; **F** IM-BW1-39; **G** IM-BW1-54; **H** IM-BW1-73; **I** IM-BW1-82; **J** IM-BW1-83; **K** IM-BW1-103; **L** IM-BW1-104; **M** IM-BW1-108, **W** wild-type dikaryotic strain IM-BM1

Previously, marker-assisted breeding provides ways to improve breeding efficiency [13, 14]. In this study, we demonstrated that dikaryotic strains carrying homozygous $tyr2^{-\Delta 25}$ were produced white-colored fruiting body. Therefore, the detection procedure of genotype of $tyr2^{-\Delta 25}$ among basidiospore isolates using PCR procedure developed in this study can strongly contribute efficient breeding for useful white strains.

Conclusions

A single base deletion in the coding region of $tyr2^{-\Delta 25}$ from IM-WM1-25 was discovered, and this mutation is predicted to cause a frame-shift in translation, yielding inactive protein TYR2. Oligonucleotide primer pairs were designed to detect $tyr2^{-\Delta 25}$ and tyr^{+} by PCR amplification. The fruiting bodies of all crossed strains were white and beige, and the corresponding genotypes were $tyr2^{-\Delta 25} \times tyr2^{-\Delta 25}$ and $tyr^{+} \times tyr2^{-\Delta 25}$. These results suggest that the causal gene of the albino mutation is *tyr2*

and this study provides a new marker-assisted selection method for the albino-type monokaryon.

Abbreviations
AAS: amino acid substitutions; cDNA: complementary DNA; DNA: deoxyribonucleic acid; dNTP: deoxynucleotide triphosphate; L-DOPA: L-3,4-dihydroxyphenylalanine; MYG: malt extract–yeast extract–glucose; PCR: polymerase chain reaction; RNA: ribonucleic acid; RACE: rapid amplification of cDNA ends; PDA: potato dextrose agar; *tyr*: tyrosinase gene; RFLP: restriction fragment length polymorphism; TYR: tyrosinase protein.

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Not applicable.

Authors' contributions
NK cultivated fruiting body of crossed strains and analyzed color of fruiting body. MH analyzed nucleotide sequence of *tyr2* genes of wild and albino strain. FC detected genotype of wild and mutant *tyr2* gene by PCR. NS analyzed mating type of selected strains under the microscope. TY performed a part of cultivation experiments using various substrate and edited the manuscript. TA was a major contributor in experimental design and writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data obtained or analyzed during this study are included in this article.

Ethics approval and consent to participate
Not applicable.

Consent for publication
All of authors have read and approved to submit it to Journal of Wood Science.

Competing interests
The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

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