Songklanakarin J. Sci. Technol.
42 (4), 910-916, Jul. - Aug. 2020

http://www.sjst.psu.ac.th

Original Article

UV-induced mutagenesis in *Volvariella volvacea* to improve mushroom yield

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Received: 22 January 2019; Revised: 22 May 2019; Accepted: 27 May 2019

Abstract

Low biological efficiency is a defect in straw mushroom production. Mycelia of the straw mushroom were exposed to UV radiation to induce mutagenesis and improve the strains of straw mushroom for a high yield. A UV exposure time that yielded a survival rate of about 25% was applied for mutation induction. After UV treatment, 65 putative mutant strains were obtained and seven fast-growing strains were selected for further analysis. Mushroom cultivation experiments revealed that the biological efficiency of four of the new strains was approximately 30% higher than the parental strain. The radial mycelial colony growth rate was assessed on different media and under various temperature and pH conditions for physical characterization. The results indicated that the overall growth rates of the mutant strains were higher than the parental strain. Genetic characterization by random amplification of polymorphic DNA analysis revealed that the genome had been altered in the mutant strains.

Keywords: mutagenesis, straw mushroom, UV, *Volvariella volvacea*, yield

1. Introduction

The straw mushroom, *Volvariella volvacea* (Bull.) Singer, is one of the most important edible mushroom species in tropical countries based on the quantity of mushroom production (Chang, 1996). It is a highly nutritional food source with good flavor properties and this mushroom also has medicinal applications, such as lowering blood pressure, and it has cardioprotective activity (Rathore, Prasad, & Sharma, 2017). The mushroom can grow easily on un-composted substrates such as paddy straw, cotton waste, and water hyacinth and requires a carbon/nitrogen ratio of about 40:60 of cellulosic materials. For cultivation, it needs a high temperature (30–35 ºC) and 80–95% relative humidity (RH). The crop cycle is completed within 20 days. Even though the technique for straw mushroom cultivation has been developed (Ahlawat & Tewari, 2007; Biswas & Layak, 2014), poor biological efficiency (BE) of straw mushroom production is a major issue that needs to be addressed (Ding, Ge, & Buswell, 2006). The straw mushroom yield depends on numerous factors including the environmental conditions, cultivation technique, substrate, and mushroom strain.

New mushroom strains with desirable properties can be obtained by strain selection, hybridization, genetic engineering, and mutation (Kaul, 2002). Hybridization, performed by either cross-breeding or protoplast fusion, is a method of combining different strains to create variability. Mating between monokaryons of two *Ganoderma lucidum* strains gave hybrids with high polysaccharide and triterpene contents (Liu, Ke, Zhang, Liu, & Wu, 2017). Interspecific hybrids of *V. volvacea* × *V. bombycina* and intergeneric hybrid of *V. volvacea* × *Pleurotus florida*, and *P. florida* × *Lentinus squarrosulus* were obtained by protoplast fusion (Chakraborty & Sikdar, 2008; Mallick & Sikdar, 2015; Zhao & Chang, 1997). Genetic engineering involves the direct transfer of a specific gene to generate mushrooms with particular characteristics. The yield of straw mushrooms was improved by transformation of the *Ampullaria crossean* multi-functional cellulase gene (Zhao *et al*., 2010), and the production of king oyster mushroom, *P. eryngii*, increased after overexpression of its own cellulose gene under regulation of a constitutive promoter (Romruen & Bangyee

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khun, 2017). Mutagenesis induced by physical and chemical agents, such as gamma rays, UV light, ethyl methanesulfonate, and methyl methanesulfonate, is commonly used in mushrooms. Several high-yielding mutant strains with morphological and physiological changes caused by mutagenesis have been reported (Djajanegara & Hardoyo, 2009; Lee, Kang, Kim, Lee, & Ro, 2011; Liu, Zhang, Lin, & Guo, 2011; Sermkiattipong & Charoen, 2014; Sharma & Sharma, 2014; Zhi, Fei, Fang, Qiong, & Fang, 2013).

The mutagenesis of *V. volvacea* by UV exposure has previously been used for auxotrophy and to improve the cold tolerance and low-temperature storage time, but not to increase productivity (Liu, Zhang, Lin, & Guo, 2011; Mukherjee & Sengupta, 1986; Zhu *et al*., 2016). Therefore, the mushroom strain improvement by UV for high yield should be investigated. The objective of this study was to establish a new high-yield straw mushroom strain by UV-induced mutagenesis. In this study, the mycelia of the parental strain were treated with UV radiation. The putative mutants were cultured and physiologically and genetically characterized. The new high-yield strains will facilitate the increased commercial production of the straw mushroom as a source of nutritional food with medicinal benefits.

2. Materials and Methods

2.1 Mushroom strain

The strain of *V. volvacea* used in this study was from a collection at the Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand. A pure culture of the mushroom was maintained on potato dextrose agar (PDA; potato infusion 200 g/L, dextrose 20 g/L, and agar 15 g/L) at 28 °C and sub-cultured monthly.

2.2 UV-induced mutagenesis and selection of mutants

2.2.1 UV-induced mutagenesis

Mushroom mycelia were cultured on PDA at 30 °C for 5 days. Agar plugs of 0.2 mm diameter were removed from the edge of the actively growing colony and transferred to new PDA agar plates. The plates were then exposed to UV light (100 $\mu J/cm^2$) in a HybriLinker HL-2000 UV Crosslinker (California, USA) for 0–60 min. After UV treatment, the plates were incubated at 30 °C for 48 h in the dark to avoid photoreactivation. A UV dose with a survival rate of approximately 25% was set as the treatment for induction of mutant strains.

2.2.2 Selection of mutants

The surviving isolates were cultured on PDA at 30 °C and the radial growth of the mycelium was measured. Strains with radial mycelial growth rates that were statistically significantly ($P<0.05$) higher than the parental strain were selected for further studies.

2.3 Mushroom cultivation

2.3.1 Spawn substrate preparation

Rice straw was chopped into 2–3-inch long pieces, soaked in tap water for 8 h, and then drained. Rice straw was mixed with dry powdered cow manure at a ratio of 4:1 by volume and then piled up for 3 weeks. The prepared substrate was then placed in a 6.5x12.5 cm plastic bag and sterilized by autoclave at 121 °C for 20 min. Agar plugs of mycelia were inoculated onto the prepared substrate and incubated at 30 °C for 10 days.

2.3.2 Cultivation

The cultivation procedure was adapted from Sermkiattipong and Charoen (2014). Rice straw was chopped into 2–3-inch long pieces, soaked in tap water for 8 h, and then drained. Rice straw, rice bran, and urea were mixed at a ratio of 200:20:1 by dry weight and then packed in a plastic bag for autoclave sterilization. The sterilized rice straw mixture was then placed in an 11x15 inch diameter basket in three layers. Each layer was then inoculated with 5% of the spawn previously prepared. The baskets were placed in a plastic dome maintained at approximately 35–37 °C and 80–90% RH. The substrate was fully covered by mycelia after 5 days and then sprayed with clean water. Mushrooms were formed after 10–12 days. The fresh weight of the straw mushrooms was recorded. The BE was calculated using the following equation (Chang, Lau, & Cho, 1981):

$$\text{BE (\%)} = (\text{fresh weight of mushrooms/dry weight of substrate}) \times 100.$$

2.4 Mycelial growth characteristics of parental and mutant strains

2.4.1 Effect of culture media on mycelial growth characteristics

A 0.6-mm agar plug taken from the edge of the colonies actively growing in PDA (25 °C, 3 days) was inoculated on either PDA, mushroom complete medium (MCM; thiamine-HCl 1 mg/L, KH_2PO_4 1 g/L, $CaCl_2{\cdot}2H_2O$ 0.5 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, $FeSO_4{\cdot}7H_2O$ 10 mg/L, $MnSO_4{\cdot}H_2O$ 1.6 mg/L, $CuSO_4$ 1 mg/L, $NaNO_3$ 2 g/L, starch 40 g/L, fructose 15 g/L, yeast extract 5 g/L, and agar 15 g/L), or malt yeast agar (MYA; malt extract 10 g/L, yeast extract 4 g/L, glucose 4 g/L, and agar 15 g/L) and incubated at 30 °C for 3 days. The radial mycelial growth was measured and used to calculate the radial mycelial growth rate.

2.4.2 Effect of temperature on mycelial growth characteristics

A 0.6-mm agar plug taken from the edge of the colonies actively growing in PDA (25 °C, 3 days) was inoculated on PDA at pH 6.8 and incubated at five different temperatures (25, 30, 35, 37, and 40 °C) for 3 days. The radial mycelial growth was measured and the radial mycelial growth rate was calculated.

2.4.3 Effect of pH on mycelial growth characteristics

A 0.6-mm agar plug taken from the edge of the colonies actively growing in PDA (25 °C, 3 days) was inoculated on PDA at pH values from 5.0 to 8.0 and incubated at 30 °C for 3 days. The radial mycelial growth was measured and the radial mycelial growth rate was calculated.

2.5 Random amplification of polymorphic DNA analysis

2.5.1 DNA extraction

DNA was extracted according to the method described by Lee and Taylor (1990). Fungal mycelia were ground in liquid nitrogen using a mortar. Subsequently, 30 mg of ground mycelia were mixed well with 400 µL of lysis buffer (50 nM Tris-HCl, pH 7.2, 50 mM EDTA, 3% SDS, and 1% 2-mercaptoethanol). Then, 1.5 µL of 20 mg/mL proteinase K was added, mixed well, and incubated at 55 °C for 1 h. Next, 1.5 µL of 10 mg/mL of RNaseA was added, mixed well, and incubated at 37 °C for 15 min. Chloroform:Tris-EDTA-saturated phenol (400 µL) was added, mixed well, and centrifuged at 10,000*g* for 15 min. Then, 350 µL of the supernatant was transferred to a new tube. A 10-µL aliquot of 3 M sodium acetate and 200 µL of isopropanol were added, mixed well, and centrifuged at 10,000*g* for 5 min. The supernatant was discarded and then the DNA pellet was washed with 1 mL of 70% ethanol and centrifuged at 10,000*g* for 5 min. After discarding the supernatant, the DNA pellet was dried in air and resuspended in TE-buffer (2 M Tris-HCl, 1 M glacial acetic acid, and 50 mM EDTA). The DNA extracts were stored in a freezer at –20 °C until required.

2.5.2 Random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR)

The polymerase chain reactions (PCRs) were carried out in 50-µL volumes containing 100 ng of DNA, 1X Taq buffer, 0.2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM primer (A10: 5′-GTGATCGCAG-3′, A11: 5′-CAATCGCCGT-3′, and B08: 5′-GTCCACACGG-3′), and 1.25 units of Taq DNA polymerase (Thermo Fisher Scientific, Lithuania). Amplifications were performed in an Applied Biosystems GeneAmp PCR System 9700 programmed for one cycle of initial denaturation at 94 °C for 5 min, 45 cycles of denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min, extension at 72 °C for 2 min, and then a final extension at 72 °C for 7 min. The PCR products were then stored at 4 °C until used. The PCR products (10 µL) were separated on 1.5% agarose gel and GeneRuler 100 bp Plus DNA (Thermo Fisher Scientific, Lithuania) was used as a molecular weight marker. Gels were stained with ethidium bromide and visualized under UV illumination. The RAPD-PCR was performed twice, and only markers that were reproducibly amplified were scored as present or absent.

2.6 Statistical analysis

The experiments were performed in triplicate. Analysis of variance (ANOVA) and Tukey's Test ($P<0.05$) were applied to the results using SPSS software.

3. Results

3.1 Effect of UV radiation on survival of mushroom mycelia

UV radiation causes lethality in a time-dependent manner in straw mushroom mycelia. The mycelia were not killed with UV exposure times of less than 20 min. After 25–40 min of exposure, the survival rate was over 75%. After 45 min of UV exposure, the survival rate dramatically decreased to less than 25%. The median lethal dose (LD_{50}) was 42.97 min (Figure 1). A UV exposure period of 45 min yielding a 23% survival rate was set as the condition for mutation induction.

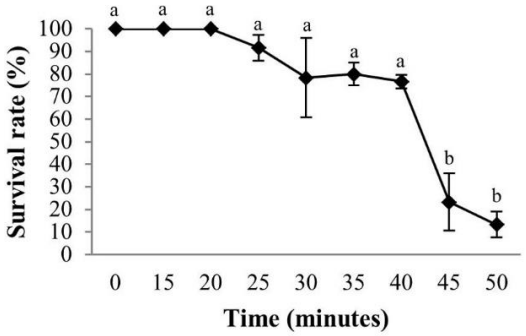

Figure 1. Effect of UV radiation on the survival rate of straw mushroom mycelia. The plotted values are the means of triplicate experiments and the vertical bars indicate the standard deviations of the means. There are no significant differences between the points marked with the same letter ($P<0.05$).

3.2 Selection of mutant strains

To perform UV-induced mutagenesis, 200 agar plugs of straw mushroom mycelia were treated with UV radiation for 45 min. Of these, 65 isolates (32.5%) survived. For mutant strain selection, the colonial radial growth rate of the mutant strains was compared with the parental strain. Only seven strains exhibited a growth rate that was significantly higher than the parental strain ($P<0.05$) and were thus selected for further studies.

3.3 Mushroom productivity of the parental and mutant strains

The selected mutant strains were identified as UV32, UV34, UV35, UV43, UV44, UV45, and UV46. These strains were investigated for mushroom productivity. Laboratory-scale cultivation of the mushrooms indicated that four of the seven mutant strains, namely UV34, UV35, UV43, and UV44, gave BE values that were significantly higher than the parental strain ($P<0.05$) (Figure 2). The BE values of the UV34, UV35, UV43, and UV44 strains were 13.12±0.35%, 13.19±0.22%, 13.24±0.28%, and 13.22±0.35%, respectively, while that of the parental strain was 10.41±0.95%.

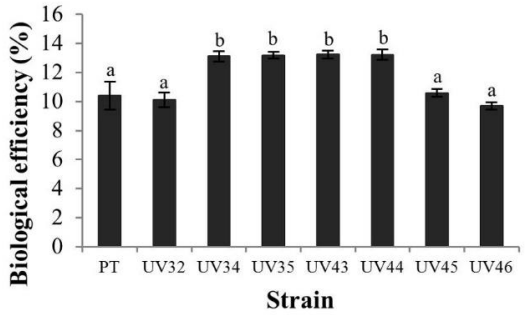

Figure 2. Biological efficiency of parental (PT) and mutant strains. The plotted values are the means of triplicate experiments and the vertical bars indicate the standard deviations of the means. There are no significant differences between the points marked with the same letter (P<0.05).

3.4 Mycelial growth characteristics of parental and mutant strains

The mycelial growth characteristics of the straw mushroom strains were studied on different culture media and under various temperatures and pH conditions. The parental strain grew slower than the mutant strains under all of the tested parameters. The mutant strains exhibited the best mycelial growth on MCM, followed by PDA and then MYA. There were no significant differences in the growth of the parental strain on the three tested media (P<0.05) (Table 1). All of the tested strains showed good growth at 35 °C and 37 °C, moderate growth at 25 °C and 30 °C, and poor growth at 40 °C. At 30–37 °C, the growth of the mutant strains was statistically significantly higher than the parental strain (P<0.05). The optimum growth temperatures for all strains were 35 and 37 °C (Table 2). The mutants showed the best growth at pH 7.5–8.0. At all of the tested pH conditions, the mutant strains grew significantly faster than the parental strain (P<0.05) (Table 3).

3.5 RAPD analysis

RAPD analysis was performed to verify that a mutation had occurred. A total of 40 random 10-mer primers were used for the RAPD analysis, but only three primers, i.e. A10, A11, and B08, yielded reproducible polymorphic amplification DNA fragments (Figure 3). Each primer amplified one to three positions. From these primers, 42 DNA fragments with sizes that ranged from 800 to 2500 bp were obtained. In this study, the DNA fragments with low amplified intensity were considered to be different from those with high amplified intensity because a single base substitution mutation caused by UV radiation may affect the priming efficiency of the primers for the DNA templates. The parental strain and the mutated strains, i.e. UV32, UV34, UV35, UV45, and UV46, represented the same genotype according to the RAPD analysis. Interestingly, the profiles of the RAPD-PCR products present in the parental strain were different when the genomic DNA from mushroom strains UV43 or UV44 was used as the template. This indicated that these strains were genetically different from the parental strain.

Table 1. Effect of culture media on the radial mycelial growth rate of parental (PT) and mutant strains.

Strain	Culture media		
	PDA	MCM	MYA
PT	7.29±1.44 abc	7.52±0.66 abc	2.52±0.71 ab
UV32	13.54±0.64 efg	21.35±2.84 l	5.48±1.26 ab
UV34	11.98±0.91 ef	17.83±2.49 ijk	4.79±0.32 ab
UV35	12.67±0.55 efg	21.23±1.48 kl	7.71±0.22 abcd
UV43	14.08±0.44 fgh	20.54±1.80 jkl	13.81±0.94 fgh
UV44	11.81±0.80 ef	15.44±1.80 ghi	4.46±0.38 a
UV45	11.04±1.43 def	17.10±0.97 hij	8.06±0.58 bcd
UV46	10.15±0.75 cde	20.00±2.14 jkl	7.40±0.44 abc

The values presented are the means and standard deviations of triplicate experiments. There are no significant differences between the points marked with the same letter (P<0.05).
PDA=potato dextrose, MCM=mushroom complete medium, MYA= malt yeast agar.

4. Discussion

Even though the straw mushroom can grow on a wide range of cellulosic substrates, it is limited by its low BE (Ding, Ge, & Buswell, 2006). Several factors influence the productivity of straw mushrooms, including the strain. To overcome this problem, new high-yield strains have been generated using various techniques. For example, a multi-

Table 2. Effects of temperature on the radial mycelial growth rates of parental (PT) and mutant strains.

Strain	25 °C	30 °C	35 °C	37 °C	40 °C
PT	6.29±0.82 d	7.29±1.44 d	16.94±0.73 i	18.65±1.30 ij	2.33±0.00 a
UV32	7.67±0.41 de	13.54±0.64 gh	25.69±0.81 no	26.17±0.52 no	3.29±0.20 ab
UV34	6.92±0.67 d	11.98±0.91 fgh	23.15±0.55 klm	22.90±0.86 kl	3.00±0.00 a
UV35	7.69±0.57 de	12.67±0.55 fgh	26.29±0.08 no	25.46±0.20 mno	3.71±0.17 abc
UV43	7.58±0.61 d	14.08±0.44 h	27.00±0.57 o	27.13±1.19 o	3.46±0.17 ab
UV44	6.77±1.08 d	11.81±0.80 fgh	21.50±1.43 k	21.29±2.59 k	2.77±0.16 a
UV45	6.17±0.92 cd	11.04±1.43 fg	25.48±0.57 mno	24.33±1.16 lmn	3.54±0.16 ab
UV46	5.56±0.48 bcd	10.15±0.75 ef	21.13±1.68 jk	21.77±1.28 k	3.17±0.15 ab

The values are the means and standard deviations of triplicate experiments. There are no significant differences between the points marked with the same letter (P<0.05).

Table 3. Effects of pH on the radial mycelial growth rates of parental (PT) and mutant strains.

Strain	5.5	6.0	6.5	7.0	7.5	8.0
PT	7.42±1.09 Ab	6.88±0.65 A	7.38±0.93 ab	9.23±0.52 abcdef	8.46±0.44 abc	10.10±0.86 bcdefgh
UV32	12.00±0.62 fghijklmno	11.79±0.38 efghijklm	11.08±0.30 cdefghij	14.29±0.43 lmnopq	13.10±1.10 ijklmnop	18.52±0.81 tuvw
UV34	11.06±0.76 cdefghij	10.08±0.54 bcdefgh	9.54±0.63 abcdef	13.96±0.45 jklmnopq	11.31±2.43 cdefghijk	14.88±1.39 opqr
UV35	10.71±2.15 cdefghi	10.40±1.30 cdefghi	15.50±0.07 pqrs	14.00±1.36 klmnopq	18.23±0.81 stuv	21.35±1.71 Wx
UV43	12.52±0.30 ghijklmno	14.08±0.56 klmnopq	14.75±0.46 nopqr	17.31±0.56 rstu	20.17±1.23 uvwx	22.92±1.38 X
UV44	12.52±1.19 ghijklmno	7.23±0.57 Ab	8.65±2.21 abcd	11.92±0.65 fghijklmn	12.69±1.60 hijklmnop	14.29±0.48 lmnopq
UV45	9.48±0.69 abcdef	9.63±0.98 abcdefg	14.17±0.53 klmnopq	13.23±0.93 ijklmnop	18.46±0.89 tuvw	19.15±0.65 tuvw
UV46	11.75±0.50 efghijklm	8.96±0.53 abcde	11.50±0.97 defghijkl	14.65±0.79 mnopqr	16.56±0.63 qrst	20.71±1.09 vwx

The values are the means and standard deviations of triplicate experiments. There are no significant differences between the points marked with the same letter (P<0.05).

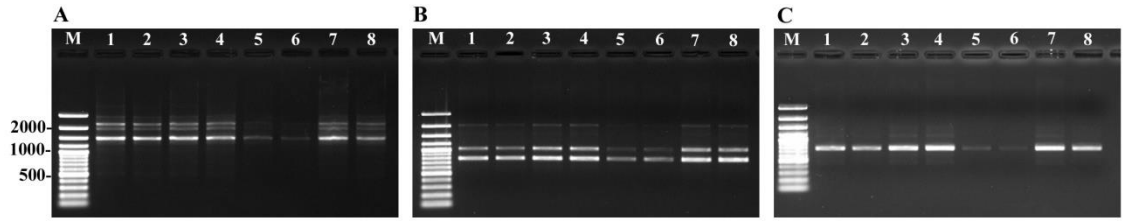

Figure 3. Random amplification of polymorphic DNA analysis of the seven mutant straw mushroom strains using different primers: (A) Primer A10, (B) primer A11, and (C) primer B08. Lane M: DNA molecular weight marker; Lane 1: Parental strain; Lane 2: UV32; Lane 3: UV34; Lane 4: UV35; Lane 5: UV43; Lane 6: UV44; Lane7: UV45; Lane 8: UV46.

functional cellulose gene from *Ampullaria crossean* was transformed into the straw mushroom by polyethylene glycol (PEG)-mediated protoplast transformation. The new strains containing the foreign gene showed 12–20% higher BE than the untransformed strain (Zhao *et al*., 2010). Three new strains with 20–40% higher BE than the parental strain were obtained by exposing straw mushroom mycelia to gamma radiation (Sermkiattipong & Charoen, 2014).

UV radiation has been used in *Pleurotus* spp. to produce mutant strains with high BE but, to the best of our knowledge, not in straw mushrooms (Beejan & Nowbuth, 2009; Sharma & Sharma, 2014). UV-induced mutagenesis has only been applied in mushrooms to develop auxotrophic strains or strains with improved cold tolerance and low-temperature storage times (Mukherjee & Sengupta, 1986; Liu, Zhang, Lin, & Guo, 2011; Zhu *et al.*, 2016). Hence, it is important for UV-induced mutagenesis to be applied to straw mushrooms to generate alternative high-yielding strains for cultivation.

The UV exposure time is crucial to the mutation efficiency. Liu, Zhang, Lin, and Guo (2011) suggested that a UV exposure time that generates a survival rate of approximately 25–35% is suitable for inducing mutations in straw mushrooms. Conditions with lower survival rates are harmful to the mushrooms and there is a lower likelihood of obtaining mutants, while conditions with higher survival rates result in less genetic variability. In this study, a UV exposure time of 45 min gave a 23% survival rate. Therefore, 45 min was used as the condition for mutation induction. After the UV treatment, 65 putative mutant strains were obtained. Only seven of the mutant strains were faster growing than the parental strain. Of these seven strains, the UV34, UV35, UV43, and UV44 strains exhibited approximately 30% higher BE values than the parental strain. The results indicated no correlation between mycelial growth and mushroom production.

UV irradiation induces crosslinking of two neighboring pyrimidines, especially thymine, forming pyrimidine dimers in one strand of DNA. This subsequently causes errors in DNA replication. Zhu *et al.* (2016) suggested that changes in the fungal genome resulting from mutagenesis may lead to one or more signal transduction pathways being activated or deactivated, thus increasing or decreasing the efficiency of certain metabolic pathways. In this study, mutations caused by UV irradiation lead to physiological alterations. The mutants demonstrated higher growth rates in different media and at the temperatures and pH conditions tested. Furthermore, higher BE values than the parental strain were observed.

Molecular markers can be used to describe genetic variations in DNA resulting from mutation or alteration of nucleotide(s) in a genome. The RAPD analysis clearly indicated that the genomes of UV43 and UV44 differed from

the genome of the parental strain. The presence of no or lightly amplified DNA fragments in both mutant strains indicated that a nucleotide base substitution mutation occurred as a result of UV radiation. This reduced the priming efficiency of the primers for the DNA templates. Although RAPD analysis showed 100% similarity for strains UV43 and UV44, they presented different growth characteristics. The UV43 strain grew significantly faster than the UV44 strain in different media and temperature or pH conditions tested. However, under the conditions used in this study, RAPD-PCR analysis was not able to distinguish any variation among the mutants. Therefore, other genetic marker techniques, such as restriction fragment length polymorphism, amplified fragment length polymorphism, and simple sequence repeats, should be applied to elucidate the variation among these strains.

5. Conclusions

Seven new high-yield strains of straw mushroom were generated by UV-induced mutagenesis. All of the new strains grew faster than the parental strain in different media and under various temperature and pH conditions. The BE values of four of the seven high-yield strains were higher than the parental strain. RAPD analysis revealed that two of the high-yield strains were genetically different from the parental strain.

This study clearly demonstrated that mutants created by UV-induced mutagenesis have improved growth and cultivation characteristics compared to those of the parental strain. These mutants will facilitate the commercial production of straw mushrooms but will also be useful for characterization of the metabolic pathways involved in mycelial growth and fruitification.

Acknowledgements

This work was financially supported by Silpakorn University Research and Development Institute (Grant No. SURDI 60/01/17).

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