Elicitor-induced β-glucan contents in fruit body of cauliflower mushroom (Sparassis latifolia)

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ABSTRACT
Strain KFRI 723 was used for the cultivation of fruit body to promote the production of β-glucan induced by the elicitor using physical stimulation in the superior cultivated fruit body. Three different elicitor treatments on the physical stimulation (UV, temperature, and harvest time) were treated on the primodia of fruit body. Elicitor controlled by UV light has an effect on the change of the β-glucan contents in flabellae and stipe of cauliflower mushroom, both parts have the highest concentration of β-glucan in strains irradiated by UV for 10 min: 41.36±2.96% of flabellae and 42.16±2.90% of stipe. Both flabellae and stipe represented β-glucan contents in order of low (10±1°C) - mid (21±1°C) - high temperature (25±1°C). In the different harvest time, the β-glucan contents of flabellae and stipe show high contents at 21st and 26th day and decreased since 31st day of harvesting.

Introduction
Sparassis species (cauliflower mushroom) are edible/medicinal mushrooms commonly found throughout the temperate regions of northern hemisphere including Eastern Asia (China, Japan, and Korea), growing at the base of coniferous trees (Abies holophylla, Larix kaempferi, Pinus densiflora, and P. koraiensis) or on oaks (Desjardin et al. 2004; Ryoo et al. 2013). The mushroom has been cultivated in Korea, Japan, China, Germany, and the US by using sawdust-based media of several conifers, oaks, alder, cottonwood, aspen, and elm (Ryu et al. 2009; Stamets 2000). Various kinds of cultivation methods were developed to produce the mushroom more efficiently by using coniferous sawdust-based media (Park et al. 2005; Park et al. 2011; Ryu et al. 2009; Sou et al. 2013).

The incidence of cancer is gradually increasing, and the spectrum of cancer-prone is changing annually. The immunomodulating substance, biological response modifier (BRM), and biotherapy, including immunotherapy, are important for the treatment of cancer. Some β-glucans are well-known BRMs; the mushrooms that they are derived from are widely distributed in nature and are used as medicines and food (Harada et al. 2006; Matsuoka et al. 1997; Wasser 2002). Lentil from Lentinus edodes and Sonifilan from Schizophyllum commune have been clinically used for cancer therapy in Japan (Fujimoto et al. 1983; Harada et al. 2006; Tada et al. 1997). Sparassis latifolia is a mushroom more efficiently by using coniferous sawdust-based media (Park et al. 2005; Park et al. 2011; Ryu et al. 2009; Sou et al. 2013).

KEYWORDS
Sparassis latifolia; β-glucan; ultraviolet radiation; temperature; harvest time; yield

Materials and methods
Characteristics of Spawn
The fungus used for this study (Strain KFRI 723) was isolated from the basidiospores of fruit body collected from Korea National Arboretum in 2006. The basidiocarp was grown at the bottom part of Pinus koraiensis. The culture collection is preserved at the Korea Forest Research Institute (herbarium KFI), the Republic of
Korea as an official strain. This strain KFRI 723 used in this study was clearly identified by taxonomical and molecular characteristics as *S. latifolia* (Ryoo et al. 2013; Sou et al. 2013).

**Cultivation of Cauliflower Mushroom**

A sawdust-based medium was used for the cultivation of cauliflower mushroom as reported by Park et al. (2005). The sawdust of larch (*Larix kaempferi*), barley flour, and sugar were mixed in the ratio of 80:20:3 (w/w/w) and the moisture content was adjusted to approximately 65%. The mixture was bottled in a 500-mL incubation bottle with the density of 0.76 g/cm³. We made a 1.5 cm in diameter hole with a depth of 5 cm in the center of the medium. The bottles were autoclaved for 90 min at 121°C. The inoculum was cultured in potato dextrose broth (PDB) for 3 week to prepare it for use as a spawn. The mycelia were homogenized and used for inoculation after keeping 72 h to confirm discontamination.

We inoculated 10 mL of liquid inoculum of *S. latifolia* into each bottle, and the amount of inoculum was 13 ± 1 mg in dry weight. The bottles were incubated in a dark room at an ambient temperature of 21 ± 1°C, with 60 ± 5% relative humidity. It took about 2 month to fill a whole bottle with mycelium under the dark incubation.

**Physical Stimulation as an Elicitation (UV, Temperature, and Harvest time)**

For the treatment of UV irradiation, the cultivating bottles with primodia of cauliflower mushroom were exposed to UV lamp in every 10 minutes from 10 min to 60 min. The wavelength of UV light was 254 nm based on UV-C which included in germicidal ultraviolet. The bottles stimulated by UV light were translocated into the cultivation facility maintained at 21 ± 1°C with 95 ± 5% humidity for the development of fruiting bodies.

For the temperature stimulations, the cultivating bottles with primodia of cauliflower mushroom received three kinds of temperature regimes for 24 h; low temperature (10 ± 1°C), mid temperature (21 ± 1°C; control), and high temperature (25 ± 1°C). After the treatments, the cultivation bottles were translocated into the cultivation facility maintained at 21 ± 1°C with 95 ± 5% humidity for the development of fruiting bodies.

We tried to examine the effect of harvest time on the content of β-glucan to compare with results from elicitors such as UV and temperature. For the treatment of harvesting time, the matured fruit body was harvested in five different periods. The mushrooms were harvested at different maturity with 5 days interval for 21, 26, 31, 36, and 41 days of staying in the fruiting facility. Each mushroom of the cultivation bottle harvested from all kinds of treatment was divided into flabella and stipe for the yield of cultivated basidiocarps and the assessment of the β-glucan contents of each part. Every treatment had five replicates.

**Measurement of β-glucan Content in Cauliflower Mushroom**

Contents of total glucans and α-glucans were determined in the polysaccharide extracts using the Mushroom and Yeast β-glucan Assay Procedure K-YBGL 09/2009 (Megazyme Int.). The quantity of β-glucan was calculated by subtracting the amount of α-glucan from the total glucan contents (Kozarski et al. 2011; Park et al. 2014).

There is a difference in rate between the chemical characteristics depending on the physiological and morphological parts of mushroom (Zied and Pardo-Giménez 2017). Thus, in this study, we measured the content of β-glucan depending on the morphological part (flavella and stipe).

**Statistical Analyses**

Statistical analyses were performed using SPSS (IBM). Analysis of variance (ANOVA) and independent T-test were used to analyze the experimental data. If the test was significant (*P* < 0.05), the Duncan test was used for the comparison of mean.

**Results**

**Yield of Cauliflower Mushroom Induced by Elicitation**

The average yield of cauliflower mushroom was similar to those from previous studies (Park et al. 2005; Park et al. 2011; Ryu et al. 2009); 141 ± 14.17 g as the fresh weight of total, which was about 37% of production efficiency.

From the treatment of UV irradiation, the yield of control and that of 60 min irrigation showed the highest yield of flavella; 65.99 ± 10.99 g and 63.28 ± 9.15 g, respectively. The yield of flavella decreased continuously from that of control to those of exposure for 40 min; 65.99 ± 10.99 g for control and 52.45 ± 17.27 g for 40 min exposure. However, the mushroom yield showed reversal trend from the exposure of UV for 50 min and 60 min. The yield of each bottle exposed for 50 min and 60 min showed relatively higher production than that of bottle exposed for 40 min; 54.28 ± 18.14 g for 50 min and 63.28 ± 9.15 g for 60 min.

By the way, the UV treatment showed reversed trend to the yield of stipe that shown for the yield of flavella. The yield of stipe were increased by the exposure time at first; 49.76 ± 15.93 g for control, 52.81 ± 14.67 g for 10 min, 53.56 ± 15.48 g of for 20 min and 59.35 ± 16.51 g for 30 min as the highest. But the yield showed decreasing pattern from the exposure of UV for 40 min to 60 min; 57 ± 13.94 g for 40 min, 53.8 ± 16.17 g for 50 min and 50.75 ± 8.23 g for 60 min (Figure 1). However, according to the statistical analyses, both fresh weights of flabellae and stipe of
S. latifolia by UV light treatment were not significantly different.

From the treatment of temperature, both yield of flabella and that of stipe were decreased as the stimulating temperature increased; 79.68 ± 24.35 g of flabella and 45.88 ± 21.05 g of stipe in low temperature (10 ± 1 °C), 78.39 ± 25.13 g of flabella and 43.70 ± 20.64 g of stipe in mid temperature (21 ± 1 °C; control), 69.09 ± 13.14 g of flabella and 21.33 ± 7.63 g of stipe in high temperature (25 ± 1 °C). Especially, the higher temperature than the general cultivation method resulted in statistically significant decrease in yield (Figure 2).

In general, the adequate harvesting time was considered as 31st day after putting the cultivation bottles into the fruiting facilities with considering the spore dispersion (Ryu et al. 2009). However, for the treatment of harvesting time, the yield showed different tendency between flabella and stipe. The yield of flabella was increased as the harvesting time delayed; 31.72 ± 10.10 g at 21st day, 49.51 ± 13.43 g at 26th day, 45.33 ± 7.79 g at 31st day, 57.71 ± 9.44 g at 36th day and 59.87 ± 15.65 g at 41st day. But, the yield of stipe showed the peak at 26th day of harvest; 61.97 ± 10.44 g and the productivity was decreased after 31st day of harvest; 39.14 ± 10.29 g at 31st day, 46.08 ± 8.61 g at 36th day and 39.80 ± 10.17 g at 41st day (Figure 3).

It meant that the weight of stipe did not increase after the conventional harvesting time while that of flabella increased continuously.

**Contents of β-glucan Induced by Elicitation**

The β-glucan contents of cauliflower mushroom for the control were 36.07 ± 4.08% in flabella and 37.96 ± 2.67% in stipe. The β-glucan content of stipe was higher than that of flabella among all bottles harvested at conventional time (31st day) in this experiment. But, the β-glucan content of stipe was lower than that of flabella when the harvesting was done in unconventional time.

Elicitation by UV light had an effect on the β-glucan contents in cauliflower mushroom. Both parts (flabella and stipe) showed the highest concentration of β-glucan in the bottles irradiated by UV for 10 min; 41.36 ± 2.96% of flabella and 42.16 ± 2.90% of stipe. But the exposure longer than 20 min to UV light showed similar β-glucan contents compared to that of control; 36.19 ± 3.66% of flabella and 36.28 ± 5.36% of stipe in 20 min, 34.01 ± 5.17% of flabella and 36.80 ± 6.38% of stipe in 30 min, 36.39 ± 4.68% of flabella and 36.29 ± 5.36% of stipe in 40 min, 36.55 ± 4.59% of flabella and 36.96 ± 5.34% of stipe in 50 min and

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Figure 1. Fresh weights of basidiocarps (flabella and stipe) of S. latifolia by UV light treatment. Data are means ± standard error, n = 5. The values were not significantly different at P = 0.05 by Duncan’s multiple range tests.

Figure 2. Fresh weights of basidiocarps (flabella and stipe) of S. latifolia by temperature treatment. Data are means ± stand error, n = 5. Means followed by the same letters across bars are significantly different at P = 0.05 by Duncan’s multiple range tests.

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37.08 ± 4.23% of flabella and 38.30 ± 2.76% of stipe in 60 min (Figure 4).

The mushrooms treated by mid temperature (21 ± 1°C; control) for 24 h showed the highest β-glucan contents among the temperature elicitation. The β-glucan contents of flabella were 40.39 ± 7.10%, 37.76 ± 3.95% and 32.77 ± 2.73% for the treatment of low-temperature, mid-temperature and high-temperature, respectively. The β-glucan contents of stipe also showed similar tendency to those of flabella; 41.72 ± 7.74%, 38.49 ± 5.31% and 34.38 ± 3.62% for the treatment of low-temperature, mid-temperature and high-temperature, respectively (Figure 5).

In the different harvest time, the β-glucan contents of flabella showed the highest at 21st day (46.26 ± 3.44%) and at 26th day (47.72 ± 4.90%) and started to decrease from 31st day of harvesting; 43.42 ± 4.08% at 31st day, 42.81 ± 6.12% at 36th day and 39.04 ± 5.66% at 41st day. So we could conclude that the β-glucan contents of flabella showed a dilution phenomenon. The stipe also showed the same tendency of β-glucan contents to that in flabella. The β-glucan content of stipe showed the highest at 21st day (46.50 ± 5.47%) and at 26th day (47.80 ± 3.86%) and then the content was decreased as 36.28 ± 3.11% at 31st day, 35.30 ± 2.57% at 36th day and 34.49 ± 1.85% at 41st day (Figure 6).

Changes in Color and Morphology by Elicitation

In addition, the elicitation of UV light influenced the color of each mushroom as well as the contents of β-glucan. The color of primodium and ultimately that of fruiting body were changed to yellowish by the irradiated of UV (Figure 7). But, the UV irradiation did not affect to the morphological characteristics of fruit body (Figure 4; Figure 7).

Discussion

Most mushrooms reflect diverse characteristics of environmental or artificial conditions. These conditions or treatments sometimes form unexpected characteristics rather than its natural characteristics. In mushroom, the yield and levels of β-glucan of fruit body are influenced by environmental factors (Yang and Liau 1998). This study tried to increase the content of β-glucan in cauliflower mushroom through easy and inexpensive method without any noxiously chemical treatment, such as UV irradiation, temperature stimulation and different harvesting period.

β-glucan is one of the major cell wall components in fungi. Thus proper stimulus on cell wall may induce the formation of β-glucan. According to the former study, pulsed UV light and UV-B irradiation effectively
increased the contents of vitamin D\textsubscript{2} without affecting the quality of fruit body of *Pleurotus ostreatus* (Koyyalamudi et al. 2011; Lee and Yim 2011). Another result of study also shows that UV irradiation is the generator of vitamin D\textsubscript{2} in 4 species of edible mushrooms (*Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus bisporus* and *Pleurotus cystidus*). In the study, 4 species of mushrooms were irradiated with Ultraviolet-A (UV-A; wavelength 315–400 nm), Ultraviolet-B (UV-B; wavelength 290–315 nm), and Ultraviolet-C (UV-C; wavelength 190–290 nm) (Jasinghe and Perera 2006).

In addition, another study reported the influence of storage temperature and UV-irradiation on the ergosterol content in 9 edible mushrooms (*Agaricus bisporus*, *Boletus edulis*, *Calocybe gambosa*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hygrophorus marzuolus*, *Lactarius deliciosus*, *Lentinus edodes* and *Pleurotus ostreatus*) (Villares et al. 2014).

Temperature is one of the most important environment factors in mushroom development. In general, low temperature treatment stimulates mycelium to cease vegetative growth and produce fruitbodies. Thus, when fruitbodies develops, it is known that the physicochemical and morphological changes of mushroom appear.

In this study, we tried to suggest an elicitor through physical stimulation using UV light, cultivation temperature or harvest time to enhance the contents of β-glucan in cauliflower mushroom (*S. latifolia*). From the three kinds of treatments, the optimum condition for high β-glucan contents of cultured strain (*S. latifolia*) was verified as 10 min of UV expose time, 21°C of stimulating temperature, and earlier harvesting (about a week) than conventional harvesting (Figure 4; Figure 5).

The contents of β-glucan in both of flabella and stipe decreased after 31st day of harvest period (Figure 6). According to the former study of cultivation characteristics of KFRI 723, the harvest period of KFRI 723 was 31st day (Ryu et al. 2009). We could recognize that the conventional harvesting timing is suitable for maximizing the contents of β-glucan in the cultivation of cauliflower mushroom, although we could not find the clear correlation between the yield and β-glucan content of fruitbodies. Considering the mature fruit body disperses the spores, we may need to find the relationships between the contents of β-glucan and spore dispersal in fruit body.

Most fungi are exposed to many kinds of stimulation naturally or artificially. Especially, *S. latifolia* susceptibility react to those of conditions. It has been revealed that not only the contents of β-glucan but also the production of methyl orsellinate and sparassol are being changed by host plants (Kim et al. 2013).
Therefore, the physicochemical characteristics of cell wall stimulated by the elicitor may give useful information on further research to improve the level of yield and β-glucan in cultivation of mushrooms. The correlation between the stimulation and the physiological reaction of fungi should be studied, which may provide good directions for both of the economical and medicinal advantages on fungal research field.

Conclusion

This study was conducted to promote the production of β-glucan induced by the elicitor using physical stimulation in the cultivated fruit body of cauliflower mushroom (S. latifolia). KFRI 723 was cultivated on sawdust-based medium, and three different elicitor treatments (UV, temperature, harvest time) were treated on the primodia of fruit body. As a result, both of flabellae and stipe parts have the highest concentration of β-glucan in strains irradiated by UV for 10 min. Also, both parts represented β-glucan contents in order of low- mid-high temperature. β-glucan contents was high at 21st and 26th day, and decreased since 31st day of harvesting.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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