Journal of Mushrooms 한국버섯학회지 Research Article

Effect of sawdust on cordycepin production from the medicinal fungus *Pesilomyces tenuipes* in submerged culture

Si Young Ha, Ji Young Jung, and Jae-Kyung Yang*

Department of Environmental Forest Science/Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, 52828, Republic of Korea

ABSTRACT: Cordycepin (3'-deoxyadenosine) is a nucleoside analog known for its diverse range of biological activities. This study investigated the effect of different types of sawdust on the production of the bioactive compound cordycepin. The results of the study showed that different types of wood sawdust affected the biosynthesis of cordycepin and a significant increase was observed when the conventional SDB medium was replaced with 1% NaOH treated pine sawdust. To optimize cordycepin production from *Paecilomyces tenuipes* in a medium containing 1% NaOH-pretreated pine sawdust, we employed Response Surface Methodology (RSM) in its Box-Behnken design (BBD) canonical form. The optimal conditions were determined as follows: a particle size of 109.5111-mesh (140 μm) for 1% NaOH-pretreated pine sawdust, an input weight of 21.1679 g/L, and an incubation time of 73.8423 hours. According to our model, this combination is expected to yield a maximum cordycepin content of 896.1428 μg/mL. Experimental validation of this prediction was performed using the suggested optimal conditions, resulting in an average cordycepin content of 922.6771 μg/mL across three replicates, thus confirming the model's accuracy.

KEYWORDS: Cordycepin, Mycelia, *Paecilomyces tenuipes*, Response surface methodology, Submerged culture

INTRODUCTION

Cordycepin (3-deoxyadenosine), a nucleoside analog, serves as the primary bioactive constituent within Cordyceps, exhibiting a wide array of pharmacological effects, as documented by Tuli *et al*. in 2014. Numerous chemically modified derivatives of cordycepin have displayed potential therapeutic benefits, including anti-cancer properties, as demonstrated by Yoon *et al*. in 2018, anti-inflammatory effects, as observed by Kim *et al*. in 2006, and neuroprotective attributes, as elucidated by Olatunji et al. in 2016. The traditional medicinal use of certain *Cordyceps* species has a rich history in countries such as China, Japan, Korea, and other Oriental nations, owing to their biological and pharmacological activities, primarily attributed to key bioactive constituents like adenosine, cordycepin, and exopolysaccharides, as detailed by Ling *et al*. (2002).

Cordycepin production typically proceeds at a sluggish pace in conventional medium compositions, such as glucose. Hence, natural substances are often introduced into the culture to augment the rate and quantity of cordycepin production, as explored by Mao and Zhong in 2004. An innovative approach to cultivating mycelium employs a blend of solid and liquid media with wood-based materials as the natural substances. This approach is economically advantageous, replacing traditional carbon sources like glucose. Various strategies for cordycepin production from *Cordyceps* have been documented. Shih *et al*. (2007) identified yeast extract as the optimal nitrogen source for cordycepin production in submerged cultures of *Cordyceps militaris* CCRC32219, while Cui and Zhang (2011) highlighted the importance of Mg^{2+} for achieving high cordycepin content. To further optimize cordycepin yield through submerged cultivation of *Cordyceps*, the influences of carbon sources and carbon-to-nitrogen ratios were

J. Mushrooms 2023 September, 21(3):110-117
http://dx.doi.org/10.14480/JM.2023.21.3.110
Print ISSN 1738-0294, Online ISSN 2288-8853

Si Young Ha(Research professor), Ji Young Jung(Research professor), Jae-Kyung Yang(Professor)
*Corresponding author
E-mail : jkyang68@gmail.com
Tel : +82-55-772-1862,, Fax : +82-55-772-1869

Received August 28, 2023
Revised September 18, 2023
Accepted September 21, 2023

systematically explored using central composite design and response surface analysis. This approach yielded a cordycepin production level of about 345 μg/mL, as reported by Mao *et al*. (2005). Nevertheless, submerged cultivation of *Cordyceps* sp. still results in relatively low cordycepin levels, exemplified by the production of only 7.1 mg/L, as disclosed by Hsu *et al*. (2002).

Response Surface Methodology (RSM), a robust statistical and mathematical tool, serves as a valuable resource for discerning influential factors, exploring their interactions, determining optimal conditions, and quantifying the relationships between one or more observed outcomes and critical input variables, all achieved within a constrained number of experimental runs (Zhang et al. 2010; Cui 2010). This approach has demonstrated considerable success across various domains of biotechnology, including the fine-tuning of nutritional parameters (Cui and Zhang 2011; Cui et al. 2016).

In the context of this investigation, we employed pine wood sawdust and pretreated sawdust (1% NaOH, water, and ethanol) within the submerged cultivation of *Paecilomyces tenuipes*. Our primary objective was to examine the impact of pine sawdust on the cordycepin content. Furthermore, we endeavored to optimize the culture conditions, encompassing the particle size of pretreated pine sawdust, the input quantity of pretreated pine sawdust, and the incubation duration within a medium containing pretreated pine sawdust. To attain these objectives, we leveraged RSM, specifically employing the Box-Behnken design (BBD), as an instrumental framework to maximize the production of cordycepin.

MATERIALS AND METHODS

Preparation of wood sawdust

Our experimentation involved the utilization of wood sawdust sourced from four distinct plant species: pine (*Pinus densiflora*), Japanese cedar (*Cryptomeria japonica*), oak (*Quercus variabilis* Blume), and tulip tree (*Liriodendron tulipifera*). The selection of these plant species was based on their prevalence in South Korea and their relative underutilization in submerged mycelial growth cultures. We procured samples of these four tree species from the Gyeongsang National University Research Forest in Jinju, South Korea.

Effects of the species of plants used for wood sawdust on cordycepin content of *P. tenuipes*

In this investigation, we sought to evaluate the influence of various plant species on the production of biomass and cordycepin content in *P. tenuipes*. To accomplish this, we conducted individual tests using different wood sawdust types, namely pine (*Pinus densiflora*), Japanese cedar (*Cryptomeria japonica*), oak (*Quercus variabilis* Blume), and tulip tree (*Liriodendron tulipifera*). Throughout these experiments, we maintained all other components of the baseline medium at a consistent level. The *P. tenuipes* strain utilized in this study was procured from the Korean Culture Center of Microorganisms in Seoul, South Korea (donation number: 60304). This strain was routinely cultured on potato dextrose agar (PDA) plates and sub-cultured at three-month intervals. Initially, *P. tenuipes* was cultivated on PDA medium within a petri dish, and subsequently, it was transferred to the seed culture medium by excising a 5-mm diameter agar disk from the PDA plates. Three such disks were employed to inoculate 50 mL of liquid medium. To investigate the impact of the plant species used for sawdust in the submerged culture of *P. tenuipes*, we employed a concentration of 20 g/L of wood sawdust (passing through a 100-mesh sieve) based on its dry weight. As a control, we utilized sabouraud dextrose broth (SDB) medium devoid of wood sawdust. The flasks were then placed in an incubator at 24 ± 2^{o}C with agitation at a rate of 100 rpm for a duration of 5 days. All experiments were carried out in quintuplicate. Upon completion of the cultures, samples retrieved from the flasks underwent centrifugation at $6000 \times g$ for 10 minutes, following which the supernatant was filtered using pre-weighed Whatman filter paper No. 2 (Whatman International Ltd., Maidstone, UK). The mycelium obtained from the centrifugation underwent a thorough wash with distilled water and was subsequently collected via filtration using Whatman filter paper. Cordycepin content and mycelial dry weight were determined after subjecting the mycelium to freeze-drying until a constant dry weight was achieved. Based on the outcomes of these experiments, a series of pretreatment trials was conducted for the plant species exhibiting the highest cordycepin content.

Pretreatment of wood sawdust

For the pretreatment of the 100-mesh wood sawdust,

we employed a combination of water, 1% NaOH, and ethanol. Specifically, 5.0 grams of wood sawdust were immersed in 100 mL of the solvent mixture. This suspension underwent an ultrasonication pretreatment process (JAC-4020; KODO Technical Research Co., Ltd., Hwaseong, South Korea) at 60°C, 20 kHz, and 250 W for a duration of 3 hours. Following the pretreatment of the wood sawdust, the sample was allowed to cool to room temperature, and the solid residues were subsequently recovered via filtration, facilitated by a vacuum pump. The collected solid residues were subjected to thorough rinsing with distilled water until reaching a neutral pH level. Subsequently, they were dried at a controlled temperature of 105 ± 3°C for a minimum duration of 4 hours. Following this drying process, the pretreated sawdust was made ready for utilization in the submerged culture of *P. tenuipes*.

Effect of pretreated wood sawdust for cordycepin content in *P. tenuipes* on submerged culture

Previous research has indicated that utilizing a pretreated wood sawdust medium enhances mycelial production, particularly during advanced fungal fermentation stages (Lee *et al*. 2008). Consequently, building upon the findings from preliminary experiments, we selected specific wood sawdust types for further optimization of the culture medium after undergoing pretreatment. To conduct the shake flask culturing, we employed 100-mL Erlenmeyer flasks fitted with a silicone plug, each containing 50 mL of SDB medium. The pH of the medium was initially adjusted to 5.6 and subsequently sterilized at 121°C for 15 minutes. Our investigation focused on evaluating the influence of pretreated sawdust solvents on the submerged culture of *P. tenuipes*. We explored a range of concentrations, varying from 5 to 30 g/L, of pretreated sawdust (comprising 100-mesh, 120-mesh, and 140-mesh particles), with the measurement based on dry weight. As a control, we maintained a culture medium without the inclusion of pretreated sawdust, which consisted solely of SDB medium. The flasks were then subjected to incubation at 24 ± 2°C for a duration of 7 days, with continuous agitation at a rate of 100 rpm. Each experiment was conducted in quintuplicate.

Upon the completion of the cultures, samples obtained from the flasks underwent centrifugation at 6000 × g for 10 minutes. The resulting supernatant was subsequently filtered through pre-weighed Whatman filter paper No. 2 (Whatman International Ltd., Maidstone, UK). The centrifuged mycelium was thoroughly rinsed with distilled water and collected via filtration using Whatman filter paper. The mycelium underwent analysis for cordycepin content and mycelial dry weight after freeze-drying until a consistent dry weight was achieved.

Experimental design using response surface methodology (RSM)

The application of Box-Behnken design has demonstrated its efficacy in optimizing culture conditions for submerged cultivation of Cordyceps spp. (Shih *et al*. 2007). In our current study, we employed a three-level Box-Behnken design (BBD), which allowed us to determine the optimal conditions with a minimal number of experiments, making it a more efficient choice compared to alternative designs (Dong *et al*.

Table 1. Box–Behnken Experimental Design of Medium Containing Pretreated Sawdust for Cordycepin Content of *P. tenuipes* on Submerged Culture

Run	Independent Variables (Actual)			Cordycepin Content (mg/mL)
	X_1	X_2	X_3	Y_1
1	120	10	24	77.5
2	120	20	72	131.8
3	120	20	72	143.8
4	100	20	120	118.7
5	100	0	72	73.6
6	120	20	72	125.6
7	140	20	24	68.5
8	120	20	72	133.7
9	140	10	72	91.5
10	100	20	24	68.2
11	120	20	72	144.9
12	120	10	120	95.9
13	120	30	24	69.1
14	140	30	72	70.6
15	140	20	120	94.9
16	120	30	120	103.2
17	100	10	72	79.0

Independent Variables	Levels		
	-1	0	1
X_1: Pretreated Sawdust Particle Size, Mesh	100	120	140
X_2: Pretreated Sawdust Input Weight, g	10	20	30
X_3: Incubation Time, h	24	72	120

2009). Within the framework of the BBD, we conducted a total of fifteen experiments to systematically investigate the impact of culture conditions on cordycepin production (Y_1) during the submerged cultivation of *P. tenuipes*. The selected factors for examination were particle size (X_1), input weight (X_2), and incubation time (X_3), each characterized at three levels: high (coded as +1), middle (coded as 0), and low (coded as -1) (Table 1). To enhance the reliability of our results, we included three replicates at the central point, which allowed us to assess both process stability and inherent variability. Furthermore, all experiments were conducted in duplicate.

The goodness of fit for the second-order model equation was assessed using the coefficient of determination (R^2), while its statistical significance was confirmed through an F-test. We tested the significance of the regression coefficients using a t-test. For data analysis, we employed the Statistical Analysis System (SAS) Design Expert 11 software (Stat-Ease Inc., Minneapolis, MN, USA).

Determination of cordycepin

Cordycepin content assessment was performed via high-performance liquid chromatography (HPLC) utilizing equipment from YOUNG IN Chromass Co., Ltd., located in Anyang, South Korea. The extraction procedure involved 1 gram of dried mycelium being subjected to extraction using 250 mL of 50% ethanol in an ultrasonic cleaner (JAC-4020; KODO Technical Research Co., Ltd., Hwaseong, South Korea) operating at 20 kHz and 250 W for a duration of 60 minutes. Following extraction, the supernatant was separated through centrifugation at 18,400 × g for 10 minutes and subsequently filtered through a 0.45-μm filter. The resulting filtrate was utilized for the determination of cordycepin content. To quantitatively assess cordycepin, the HPLC system was equipped with a Kinetex 5 μm C18 100A column sourced from Phenomenex, Torrance, CA, USA. The mobile phase consisted of an 85% composition of 0.02 M KH_2PO_4 and 15% methanol, and the flow rate was set at 1.2 mL/min. The injection volume utilized was 20 μL, and detection was achieved using a UV/visible detector (Shimadzu SPA-20A, Kyoto, Japan) operating at 260 nm. Quantification was based on the UV signal response, employing the external standard method, with a standard calibration curve (R^2 = 0.9912174) established using cordycepin standards ranging from 100 to 500 ppm, procured from Sigma-Aldrich Co., St. Louis, MO, USA.

Statistical Analyses

The data is expressed as the mean ± standard deviation (n = 5). Statistical analyses were conducted at a significance level of 5% using the Statistical Analysis System software (SAS Institute, Inc., Cary, NC, USA). Variations among group means were evaluated through Duncan's multiple-range test employing SAS.

RESULTS AND DISCUSSION

Effect of Various Sawdust Media on Cordycepin Content

Previous research has indicated that acidic components within the culture medium promote mycelial growth and the biosynthesis of metabolites in various ascomycetes and basidiomycetes, including Cordyceps sp. (Leung *et al*. 2006; Liu *et al*. 2011). In this context, we evaluated the influence of submerged culture media on the cordycepin content of *P. tenuipes*, as illustrated in Figure 1. Notably, the standard deviation associated with our results was quite low, indicating a high level of reproducibility among replicates. It is worth mentioning that there are typically two predominant methods for quantifying cordycepin concentration: ultraviolet spectrophotometry and high-performance liquid chromatography (HPLC). For this study, we opted for the HPLC method due to its heightened sensitivity and improved reproducibility, factors that likely contributed to the consistency observed across replicates.

Furthermore, our investigation showcased the impact of different wood sawdust-infused media on the cordycepin content. Specifically, the inclusion of wood sawdust in the medium resulted in a higher cordycepin content compared to the control. It is crucial to acknowledge that a direct comparison of metabolite production across various *Cordyceps* strains from different studies proves challenging, primarily due to variations in nutrient components and culture conditions employed in these studies.

Effect of Pretreatment of Sawdust on Cordycepin Content

The impact of pretreated pine sawdust on the cordycepin content of *P. tenuipes* is visually depicted in Figure 2. Notably, the addition of pine sawdust pretreated with 1%

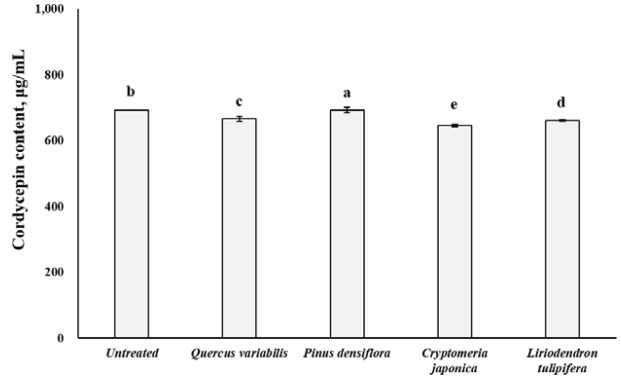

Fig. 1. The effects of various sawdust media on the cordycepin content by *P. tenuipes* in submerged culture. untreated: only SDB medium; pine (*Pinus densiflora*), Japanese cedar (*Cryptomeria japonica*), oak (*Quercus variabilis* Blume), and tulip tree (*Liriodendron tulipifera*); incubation time: 5 d. Each value is expressed as mean ± SE (n = 5). Different letters on the top of the line represent

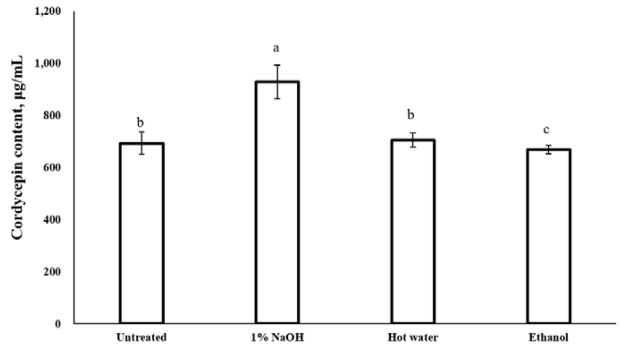

Fig. 2. Effect of pretreatment of pine sawdust (100-mesh, 20g/L) on cordycepin content in submerged culture of *P. tenuipes*. Untreated: The *P. tenuipes* was grown using submerged culture in SDB media; incubation time: 5 d. Each value is expressed as mean ± SE (n = 5). Different letters on the top of the bars represent statistical significance at 5% probability level.

NaOH to the *P. tenuipes* mycelium yielded the highest cordycepin content, registering at 880 μg/mL. This represented a substantial 120% increase in cordycepin content compared to untreated pine sawdust. Moreover, it was evident that when pine tree sawdust was subjected to pretreatment with 1% NaOH, and the resulting residue was incorporated into the *P. tenuipes* liquid medium, the mycelium mass demonstrated significant growth compared to scenarios involving pretreatment with water and ethanol. As per the findings reported by Lee *et al*. (2008), the residue obtained after alkali extraction of pine tree sawdust indicated the removal of certain phenolic substances, including lignin. This removal process was identified as conducive to the mycelial growth of mushrooms. In alignment with these observations, our study revealed that pretreating pine wood sawdust with 1% NaOH, followed by its addition to a liquid medium, led to the highest levels of both cordycepin content and mycelia dry weight in *P. tenuipes*. A noteworthy aspect of this investigation was the simultaneous enhancement of cordycepin content and mycelium mass in *Cordyceps* mycelium when pine wood sawdust was pretreated with 1% NaOH and incorporated into the liquid medium. Typically, there exists an inverse relationship between cordycepin content and mycelia dry weight in *Cordyceps* mycelium (Hung *et al*. 2009). Therefore, the addition of pine wood sawdust, pretreated with 1% NaOH, to the liquid medium emerged as a beneficial strategy for not only increasing the cordycepin content of *P. tenuipes* mycelia but also augmenting mycelium mass simultaneously.

Effect of Particle Size and Input Weight of 1% NaOH-pretreated Pine Sawdust on Cordycepin Content

The cordycepin content produced by *P. tenuipes* exhibited varying levels in different culture conditions. Specifically, it reached a maximum of 740.13 μg/mL in the untreated culture medium (comprising only SDB medium), 1006.80 μg/mL in the 100-mesh pine sawdust medium, 576.13 μg/mL in the 120-mesh pine sawdust medium, and 564.49 μg/mL in the 140-mesh pine sawdust medium, as demonstrated in Figure 3.

Furthermore, we assessed the effects of the submerged culture medium on cordycepin content in relation to the input weight of sawdust used for *P. tenuipes* cultivation. This investigation revealed that the cordycepin content of *P. tenuipes* achieved maximum values of 751.60 μg/mL, 768.93 μg/mL, 965.56 μg/mL, 1086.80 μg/mL, and 800.44 μg/mL in the untreated medium (consisting solely of SDB medium), as well as media containing 5 g/L, 10 g/L, 20 g/L, and 30 g/L (where "g" represents pine sawdust-based dry weight and "L" signifies SDB medium-based volume), respectively. These findings are illustrated in Figure 4.

It is important to note that conducting a direct comparison of metabolite production among various *Cordyceps* strains from different studies in the literature is challenging due to the limited inclusion of wood sawdust in liquid culture mediums. Previous research by Shukla *et al*. (2002) highlighted the significant influence of sawdust particle size on adsorption rates. Decreasing particle size results in increased surface area,

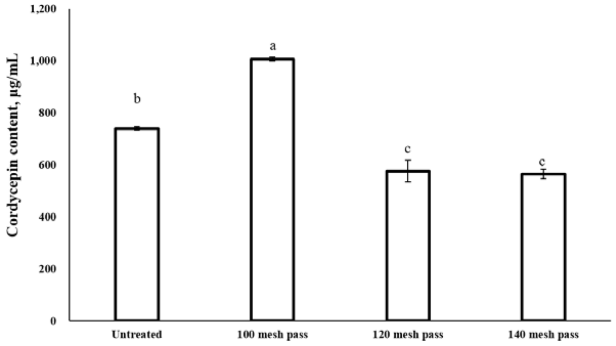

Fig. 3. Effect of particle size of pretreated pine sawdust (20 g/L) on cordycepin content in submerged culture of *P. tenuipes*. Untreated: The *P. tenuipes* was grown using submerged culture in only SDB medium. Used pine sawdust was 1% NaOH-treated pine sawdust; incubation time: 5 d. Each value is expressed as mean ± SE (n = 5). Different letters on the top of the bars represent statistically significant at 5% probability level.

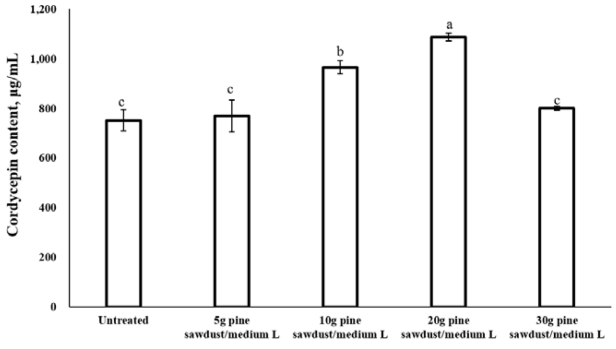

Fig. 4. Effect of particle size of pretreated pine sawdust (100-mesh) on cordycepin content in submerged culture of *P. tenuipes*. Untreated: The *P. tenuipes* was grown using submerged culture in only SDB medium. Used pine sawdust was 1% NaOH-treated pine sawdust; incubation time: 5 d. Each value is expressed as mean ± SE (n = 5). Different letters on the top of the bars represent statistical significance at 5% probability level.

consequently leading to higher adsorption rates at the sawdust surface. Additionally, intraparticle diffusion from the outer surface into the material's pores can also play a critical role. As such, the size of sawdust particles can substantially impact cordycepin content. Nevertheless, further research is warranted to precisely elucidate the relationship between sawdust particle size and cordycepin content.

Incubation Time of *C. militaris* for Cordycepin Production in Medium Containing 1% NaOH-pretreated Pine Sawdust

Figure 5 illustrates the variation in cordycepin content

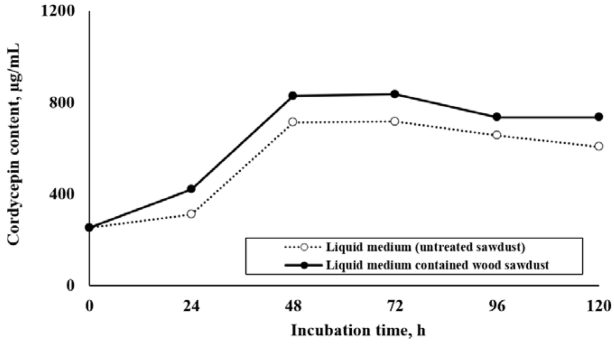

Fig. 5. Effect of incubation time on cordycepin content in submerged culture using medium contained pretreated pine sawdust (100-mesh, 20 g/L) of *P. tenuipes*. The *P. tenuipes* was grown using submerged culture in SDB media; control: only SDB medium. Used pine sawdust was 1% NaOH-treated pine sawdust. Each value is expressed as mean ± SE (n = 5).

during the cultivation period of *P. tenuipes*. Notably, there was a swift increase in cordycepin content after 72 hours of Cordyceps mycelium incubation. This trend was consistent between the control group, which utilized SDB medium exclusively, and the group using a medium containing pine sawdust pretreated with 1% NaOH.

However, as the incubation time continued to extend, there was no substantial further increase in cordycepin content. Consequently, it becomes imperative to determine the optimal incubation duration that yields the maximum cordycepin content.

Optimization of Cordycepin Production in *P. tenuipes* Cultivation Medium Containing 1% NaOH-pretreated Pine Sawdust Using RSM Based on BBD

In this study, we employed Response Surface Methodology (RSM) based on Box-Behnken Design (BBD) to pinpoint the optimal input parameters for 1% NaOH-pretreated pine sawdust within the liquid medium. Our preliminary data analysis had already indicated the substantial impact of several key variables on culture performance concerning cordycepin production. These variables encompassed the input weight, particle size, and incubation time within the shake flask setup.

Figure 6 and Figure 7 presents the matrix associated with the Box-Behnken Design, alongside the observed experimental data, providing a visual representation of our experimental setup and results.

The model's goodness of fit was assessed through the determination coefficient, R^2, which calculated to be

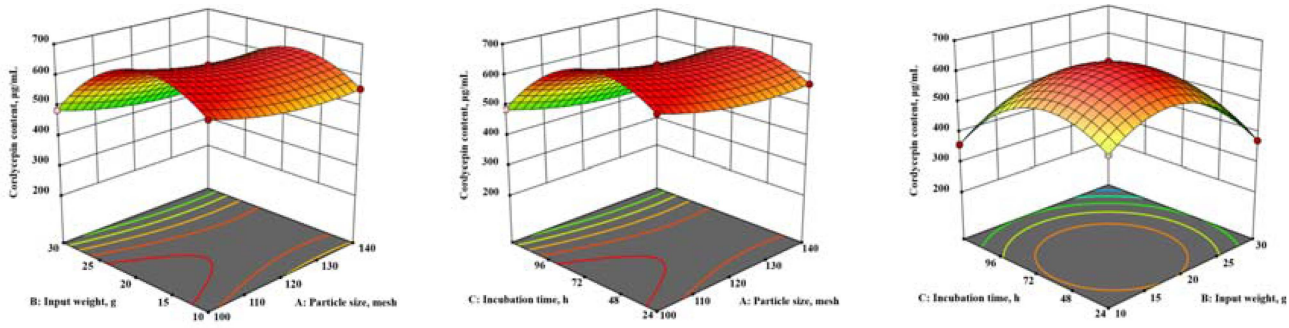

Fig. 6. Contour plots for the effects of 1% NaOH-treated pine sawdust on the cordycepin content

0.981. This high R^2 value implies that approximately 98.1% of the variability in the response can be attributed to this model. Furthermore, the F-values for the overall regression were found to be significant at the 5% significance level, further affirming that the second-order model is a suitable approximation for describing the Response Surface Methodology (RSM) experimental design. The optimal combination of input parameters was determined as follows: 1% NaOH-pretreated pine sawdust particle size of 109.5111-mesh (140 μm), 1% NaOH-pretreated pine sawdust input weight of 21.1679 g/L, and an incubation time of 73.8423 hours. As per the model's predictions, this combination was projected to yield a maximum cordycepin content of 896.1428 μg/mL. To validate this prediction, experiments were conducted using the culture media configured to match the identified optimum conditions. These experiments yielded an average cordycepin content of 922.6771 μg/mL across three replicates. Although the measured value did not precisely align with the value predicted by the response model, it closely approximated the predicted value. By multiple regression analysis on the experimental data, the predicted response for the extraction yield of pectin could be obtained via the second-order polynomial equation; + 1113 - 16 × particle size + 48 × input weight + 6 × incubation time + 0.0001 × particle size × input weight + 0.00002 × particle size × incubation time + 5 × input weight × incubation time + 0.06 × particle size^2 – 1.3 × input weight^2 – 0.05 × incubation time^2.

Historically, cordycepin production during the submerged cultivation of *Cordyceps* spp. had been rather limited. For instance, a mere 7.1 μg/mL of cordycepin was reported for submerged cultivation of *Cordyceps* at the laboratory bioreactor scale (Hsu *et al*. 2002). However, advancements in submerged culture methods,

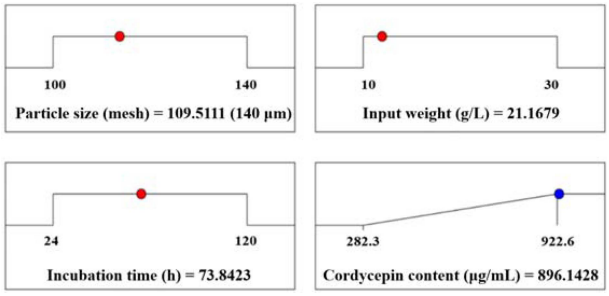

Fig. 7. Regression analysis of the Box–Behnken design experiments

including a two-stage dissolved oxygen control for cultivating *C. militaris* on a commercial scale, as developed by Mao and Zhong (2004), resulted in a significant improvement, with cordycepin levels reaching 188.3 μg/mL. Further efforts to enhance cordycepin production in submerged cultivation, including the manipulation of carbon sources to modulate the carbon/nitrogen ratio, led to cordycepin production levels of 345.4 ± 8.5 μg/mL (Mao *et al*. 2005). Prior to this study, the highest reported cordycepin level was 640 μg/mL, obtained through surface culture utilizing *C. militaris* NBRC 9787 (Masuda *et al*., 2006). Although direct comparisons are challenging due to the scarcity of studies on cordycepin content in *P. tenuipes*, this study contributes valuable data for understanding cordycepin production in *P. tenuipes*.

ACKNOWLEDGEMENTS

This study was carried out with the support of ´R&D Program for Forest Science Technology (Project No. "2023478B10-2325-BC0361382116530001")´ provided by Korea Forest Service (Korea Forestry Promotion Institute).

REFERENCES

Cui JD. 2010. Optimization of medium for phenylalanine ammonia lyase production in E. coli using response surface methodology. Korean Journal of Chemical Engineering 27(1): 174-178.

Cui JD, Zhang BZ. 2011. Comparison of culture methods on exopolysaccharide production in the submerged culture of *Cordyceps militaris* and process optimization. Letters in Applied Microbiology 52(2): 123–128.

Cui J, Cui L, Jia S, Su Z, Zhang, S. 2016. Hybrid cross-linked lipase aggregates with magnetic nanoparticles: A robust and recyclable biocatalysis for the epoxidation of oleic acid. Agricultural and Food Chemistry 64: 7179-7187.

Dong CH, Xie XQ, Wang XL, Zhan Y, Yao YJ. 2009. Application of Box-Behnken design in optimisation for polysaccharides extraction from cultured mycelium of *Cordyceps sinensis*. Food and Bioproducts Processing 87(2): 139-144.

Hsu TH, Shiao LH, Hsieh C, Chang DM. 2002. A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom Dong Chong Xia Cao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. Food Chemistry 78(4): 463-469.

Hung TL, Keawsompong S, Hanh VT, Sivichai S, Hywel-Jones N. 2009. Effect of temperature on cordycepin production in *Cordyceps militaris*. Thai Journal of Agricultural Science 42(4): 219-225.

Kim HG, Shrestha B, Lim SY, Yoon DH, Chang WC, Shin DJ, Han SK, Park SM, Park JH, Park HI. 2006. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-KB through Akt and p38 inhibition in RAW 264.7 macrophage cells. European Journal of Pharmacology 545(2-3): 192-199.

Kim SW, Hwang HJ, Xu CP, Sung JM, Choi JW, Yum JW. 2003. Optimization of submerged culture process for the production of biomass and exo-polysaccharides by *Cordyceps militaris* C738. Journal of Applied Microbiology 94(1): 120-126.

Lee WY, Ahn JK, Park EJ, Ka KH. 2008. Effect of wood xylem flour in liquid culture on mycelial biomass of Lentinus lepideus and *Lentinus edodes*. The Korean Journal of Mycology 36(1): 45-50.

Leung PH, Zhang QX, Wu JY. 2006. Mycelium cultivation, chemical composition and antitumour activity of a *Tolypocladium* sp. fungus isolated from wild *Cordyceps sinensis*. Journal of Applied Microbiology 101(2): 275-283.

Ling JY, Sun YJ, Zhang H, Peng L, Zhang CK. 2002. Measurement of cordycepin and adenosine in stroma of *Cordyceps* sp. by capillary zone electrophoresis (CZE). Journal of Bioscience and Bioengineering 94(4): 371-374.

Liu Z, Li P, Zhao D, Tang H, Guo J. 2011. Anti-inflammation effects of *Cordyceps sinensis* mycelium in focal cerebral ischemic injury rats. Inflammation 34(6): 639-644.

Mao XB, Zhong JJ. 2004. Hyperproduction of cordycepin by two-stage dissolved oxygen control in submerged cultivation of medicinal mushroom *Cordyceps militaris* in bioreactors. Biotechnology Progress 20(5): 1408-1413.

Mao XB, Eksiwong T, Chauvatcharin S, Zhong JJ. 2005. Optimization of carbon source and carbon / nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. Process Biochemistry 40(5): 1667-1672.

Masuda M, Urabe E, Sakurai A, Sakakibara M. 2006. Production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. Enzyme and Microbial Technology 39(4): 341-646.

Olatunji OJ, Feng Y, Olatunji OO, Tang J, Ouyang Z, Su Z. 2016. Cordycepin protects PC12 cells against 6-hydroxydopamine induced neurotoxicity via its antioxidant properties. Biomedicine & Pharmacotherapy 81: 7-14.

Shih IL, Tsai KL, Hsieh C. 2007. Effects of culture conditions on the mycelial growth and bioactive metabolite production in submerged culture of *Cordyceps militaris*. Biochemical Engineering Journal 33(3): 193-201.

Shukla A, Zhang YH, Dubey P, Margrave JL, Shukla SS. 2002. The role of sawdust in the removal of unwanted materials from water. Journal of Hazardous Materials 95(1-2): 137-152.

Tuli HS, Sandhu SS, Sharma AK. 2014. Pharmacological and therapeutic potential of *Cordyceps* with special reference to Cordycepin. 3 Biotech 4: 1-12.

Yoon SY, Park SJ, Park YJ. 2018. The anticancer properties of Cordycepin and their underlying mechanisms. International Journal of Molecular Sciences 19(10): Article number 3027.

Zhang BZ, Gui JD, Zhao GX, Jia SR. 2010. Modeling and optimization of phenylalanine ammonia lyase stabilization in recombinant Escherichia coli for the continuous synthesis of L-phenylalanine on the statistical-based experimental design. Journal of Agricultural and Food Chemistry 58: 2795-2800.