Journal of Mushrooms 한국버섯학회지 Research Article

Solid media based on *Monochamus alternatus* to growth and physiologically active substance of *Paecilomyces tenuipes* fruiting bodies

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ABSTRACT: *Paecilomyces tenuipes* (*P. tenuipes*) is a fungus cultivated artificially by South Korean researchers, utilizing rice bran as its substrate. The increased demand for this fungus has not been met with successful cultivation methods for fruiting body production in natural environments. Therefore, we tested the effect on the growth of *P. tenuipes* using a Solid media based on pests. In this results, the Solid media based on *M.alternatus* was effective in increasing the growth of *P. tenuipes* and the content of cordycepin. Moreover, we confirmed the conditions for manufacturing a Solid media based on *M.alternatus* for *P. tenuipes* growth. We suggested that the growth-promoting compounds offers valuable insights for optimizing fungal cultivation conditions, thereby enhancing productivity and contributing to a broader understanding of fungal physiology in varying nutritional environments.

KEYWORDS: *Monochamus alternatus*, *Paecilomyces tenuipes*, Cordycepin, Fruiting body, Amino acid

INTRODUCTION

Dong Chong Xia Cao (*Cordyceps*), named for its unique ability to parasitize various insect hosts, primarily invades insects, forming its mycelium within the host or generating spores within the insect's body. This fungus absorbs nutrients from the insect in the winter, Cordyceps absorbs nutrients from the insect, causing the insect's demise. As summer approaches, it undergoes a transformation, producing mushroom structures (Hajek and St. Lengar, 1994). *Cordyceps* belongs to the family Clavicipitaceae within the order Hypocreales, a member of the Pyrenomycetes class of fungi (Miller, 1949). The fruiting bodies of *Cordyceps* originate from the pupae or larvae of insects infected with the entomopathogenic fungus, *Cordyceps*, rendering it pathogenic to insects (Hong *et al*., 2010). Research on *Cordyceps* primarily focuses on understanding its physiological activity and pharmacological efficacy. Previous studies have demonstrated various pharmacological efficacies of *Cordyceps*, including anti-inflammatory (Yang *et al*., 2011), analgesic (Qian *et al*., 2012), promotion of steroidogenesis (Huang *et al*., 2001), immune enhancement (He *et al*., 2001), and anticancer effects (Nakamura *et al*., 2015). In recent years, with substantiated evidence supporting the therapeutic efficacy of *Cordyceps*, there has been an increased demand for this fungus (Feng *et al*., 2018). However, due to the inherent challenges in the natural cultivation of *Cordyceps*, research is actively underway to optimize substrate manufacturing conditions (Pradhan *et al*., 2023) and nutritional parameters, aiming to enhance the quality of the species and increase production yields (Raethong *et al*., 2020; Łysakowska *et al*., 2023).

Nevertheless, China, which has a historical tradition of using *Cordyceps* as a medicinal remedy, has also achieved artificial cultivation after decades of dedicated efforts and numerous attempts. While a select few species such as *Ophiocordyceps sinensis* and *Cordyceps sinensis* (Ghatnur *et al*., 2015; Sun *et al*., 2018) have been successfully cultivated artificially, the reality remains that there is a lack of research

J. Mushrooms 2024 March, 22(1):8-16
http://dx.doi.org/10.14480/JM.2024.22.1.8
Print ISSN 1738-0294, Online ISSN 2288-8853

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Received February 21, 2024
Revised March 18, 2024
Accepted March 25, 2024

and information pertaining to artificial cultivation for many other species (Li *et al*., 2019; Wang *et al*., 2021). *Paecilomyces tenuipes* (*P. tenuipes*) is a fungus cultivated artificially by South Korean researchers, utilizing rice bran as its substrate (Hong *et al*., 2009). While there has been validation of its pharmacological efficacy (Li *et al*., 2020) and toxicity (Nam *et al*., 2001), the current state of affairs is characterized by a lack of established information that enables large-scale production through artificial cultivation. Experimental studies on the artificial cultivation of this fungus are particularly limited compared to other *Cordyceps* species. The untapped potential of *P. tenuipes* is considered significant, as it is one of the fungi that can greatly benefit from an in-depth understanding of its physiological characteristics and growth conditions to enhance its potential utilization. In this study, we aim to cultivate *P. tenuipes* artificially for the following reasons:

The increased demand for this fungus (Dong *et al*., 2015), driven by the demonstrated efficacies mentioned earlier, has not been met with successful cultivation methods for fruiting body production in natural environments (Zhong and Xiao, 2009). In recent years, numerous studies have been conducted to investigate the optimization of culture conditions for effective artificial cultivation (Wu *et al*., 2024), with a focus on formulating conducive media for growth. These endeavors aim to enhance the efficiency of artificial cultivation methods (Pradhan *et al*., 2024). It has been observed that manipulating the culture conditions, including substrate composition, cultivation environment, and incubation parameters, particularly influences the growth rate and promotes the production of bioactive compounds in species belonging to the *Paecilomyces* genus, such as *lilacinus* (Cabanillas and Barker, 1989; Keiwnick, 2006; Sharma *et al*., 2014), *hepiali* (Chioza and Ohga, 2013), *japonica* (Bae *et al*., 2000), and *fumosoroseus* (Ibrahim and Low, 1993). Indeed, similar to other species within the same genus, *P. tenuipes* exhibited increased biomass and elevated production of bioactive compounds in the optimized medium (Du *et al*., 2012). According to studies conducted by Ha *et al*. (2021), the potential of submerged cultivation as a promising alternative to solid-state fermentation has been emphasized. It has been confirmed that a liquid medium incorporating wood sawdust significantly enhances the mycelial growth of *P. tenuipes* during submerged cultivation. The aforementioned research underscore the value of investigating *P. tenuipes* under various conditions, and such studies can contribute to a comprehensive understanding of the mechanisms regulated the mycelial growth pathways.

Monochamus alternatus (*M. alternatus*) is a key wood-boring insect species associated with various pine tree varieties and serves as a primary vector for the pine wood nematode *Bursaphelenchus xylophilus*, a major pathogen of pine trees (Akbulut and Stamps, 2012). In regions such as Korea, China (Dong *et al*., 2023), and Japan, efforts are underway to eradicate *M. alternatus* as part of measures to protect pine forests. There is a growing need to explore the utilization of captured *M. alternatus* as a valuable resource (Kim et al., 2020). According to the research conducted by Ha *et al*. (2021, 2022) the optimization of a solid substrate utilizing *M. alternatus* has been confirmed as an efficient, environmentally friendly, and viable material for the fruiting body production of *Cordyceps militaris*. As previously indicated, based on the compelling need for utilizing *M. alternatus* as a valuable resource and significant research outcomes, this study has opted to apply this insect to the cultivation of *P. tenuipes.* In the context of insect based substrates for *P. tenuipes*, there is a relative scarcity of research attempts utilizing insects excluding silkworm pupa (Jo *et al.*, 2015) and larvae (Hong *et al*., 2007). This situation is deemed to elevate the significance of the current study compared to research efforts involving other *Cordyceps* species. In the context of insect-based culture medium for *P. tenuipes*, there is a scarcity of research attempts utilizing insects, excluding silkworms, making this study particularly valuable in addressing this research gap.

In this paper, we present the first study utilizing *M. alternatus* for the artificial cultivation and growth characteristics of *P. tenuipes*. Through an evaluation of its potential applications and associated growth features, we aim to contribute new knowledge and insights to the field. In this study, we evaluated the impact of utilizing solid culture with *M. alternatus* and oat based solid culture on the growth characteristics of *P. tenuipes*. The assessment was conducted through the analysis of primordium occurrence, strip length, strip number, and maturation period of the fruiting bodies. The comprehensive approach aims to provide insight into the growth dynamics of *P. tenuipes* under the influence of *M. alternatus* solid culture.

Finally, we assessed the impact of the optimized *M. alternatus* substrate by selecting target substances showing significant differences between the media using Oats and the optimized *M. alternatus* media through GC-MS analysis. By comparing chromatographic profiles on oats-based and *M. alternatus* based media, we sought to identify differentially synthesized metabolites. This elucidation of growth-promoting compounds offers valuable insights for optimizing fungal cultivation conditions, thereby enhancing productivity

and contributing to a broader understanding of fungal physiology in varying nutritional environments.

MATERIALS AND METHODS

Fungal strains

The strain of *P. tenuipes* (DGUM32001) was purchased from the Microbial Culture Center in South Korea. This fungus was maintained on potato dextrose broth (PDB) medium (composed of potato starch from infusion 4 g/L and dextrose 20 g/L) and subsequently transferred for additional experiments after 20 days of cultivation on PDB medium.

Inoculum preparation

A 50 mL aliquot of PDB was aseptically transferred to a 250 mL flask and autoclaved at 121°C for 15 minutes. Each flask was inoculated with three mycelial discs (5 mm) of *P. tenuipes* grown on PDB medium. Inoculated media were statically cultured at 25°C for 10 days. Subsequently, the cultured medium was homogenized at 12,000 g for 5 minutes using a homogenizer (AM-11; Nihonseiki Kaisha Ltd, Tokyo, Japan), and the resulting mycelia were filtered through sterile gauze to remove entangled hyphae.

Host insect

The *M. alternatus* (Japanese pine sawyer), possesses a body length ranging from 20 to 28 mm and has been utilized as a constituent of solid culture medium. *M. alternatus* were obtained from the Forest and Environment Research Institute in Gyeongsangnam-do, South Korea. Original or powdered forms of *M. alternatus*, constituting 25-75%, were added to 20 g of solid culture medium. The powdered form was prepared by grinding for 60 seconds and sieving through a 150 μm mesh screen to ensure uniform size. *M.alternatus* original was used 20 to 30 mm imago. The solid culture medium for *P. tenuipes* was filled into a 300 mL cylindrical plastic bottle (diameter 8 cm, height 12 cm) and sealed with a plastic cap; then, autoclaved at 121°C for 30 minutes.

Solid culture fermentation

To investigate the impact of *M.alternatus* on the formation of fruiting bodies, the influence on cultivated fruiting bodies was examined using oat-based solid culture medium. Oats were chosen based on previous research targeting *P. tenuipes*, where growth and effective substance production were found to be most effective (Chang *et al*., 2022). The total combined weight of the ingredients added to the solid substrate (*P. tenuipes*, *M. alternatus*) was 20 g. The moisture content of the solid culture medium was adjusted to 50-100% during this process. Solid media with moisture contents of 50%, 75%, and 100% were prepared by adding distilled water in amounts of 50, 75, and 100 g to 20 g of solid culture medium, respectively. At this time, 50%, 75%, and 100% mean 50 mL water/100 g *M.alternatus*, 75 mL water/100 g *M.alternatus*, and 100 mL water/100 g *M.alternatus*, respectively. The media were cooled to room temperature, inoculated with 5 mL of seed culture, and cultured at 24°C in the dark for 21 days to promote vegetative growth. The fully colonized solid media were maintained at 20°C. Initially, the cultures received 1,000 lux of light for 12 hours, and then they were kept in the dark to allow the development of spherical fruiting bodies. The presence of heads on the normal part of the fruiting body when the growth of fruiting bodies ceased indicated that the fruiting bodies were no longer growing.

Measurement methods

The number of days taken for the first appearance of primordia in the media was recorded. We recorded the strip number and fruiting body maturation time. Maturity of the fruiting bodies was determined when heads were present on the normal portion of the basidiocarp. Strip lengths were measured using Vernier calipers to provide quantitative data on the growth and development of the solid culture. The mean of strip length for each treatment group was calculated to represent overall growth.

Dry weight measurements were conducted to quantify the biomass of solid cultures. Samples were dried to a constant weight, and the recorded values were used to assess the productivity of each treatment group. The dry weights of the fruiting bodies with the solid culture medium and those dried to a constant weight at 55°C overnight in an oven dryer (OF-12GW, Jeio Tech Co., Ltd., South Korea) after harvesting were recorded.

Biological Efficiency (BE) calculation

As the fruiting bodies of *P. tenuipes* were mainly applied after drying, the biological efficiency (BE) values were calculated as follows:

$$\text{BE (\%)} = \left(\frac{Total\ dry\ weight\ of\ fruiting\ bodies}{Total\ substrate\ dry\ weight}\right) \times 100 \quad (1)$$

This metric provided a quantitative measure of the efficiency of fruiting body production relative to substrate utilization.

Measurement of Cordycepin content

The content of cordycepin was quantified in the fruiting bodies grown in the prepared solid culture medium by high-performance liquid chromatography (HPLC) analysis. The HPLC analysis was carried out using an HPLC system (YL9100 plus; YOUNG IN Chromass, Gyeonggi-do, South Korea) equipped with a vacuum degasser, quaternary pump, UV/Vis detector, and analytical software for the detection and analysis of cordycepin. The HPLC conditions utilized in this study were as follows: an Agilent Eclipse Plus C18 column (250 mm × 4.6 mm, 5 μm) was employed, with a mobile phase consisting of methanol and water in a ratio of 20:80 (v/v). The flow rate was set at 1.0 mL/min, with UV detection at 260 nm, and an injection volume of 10 μL. Before injection, the samples underwent filtration through a 0.45 μm membrane filter. Quantitative analysis of cordycepin was conducted by measuring the peak area based on its standard curves. Cordycepin peaks in the samples were identified based on their retention times. The nitrogen content of the solid culture medium derived from *M. alternatus* was determined using Kjeldahl digestion, following the method outlined by Devi and Yadava (2006), and analyzed using a macro elemental analyzer (Vario MACRO cube; ELEMENTAR, Langenselbold, Hesse, Germany).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis was performed using a Shimadzu GC-2010 plus (Tokyo, Japan) GC-MS system. The detector used was the 5975C MSD Detector from Agilent Technologies, and the column used was Rtx-5MS capillary columns (30 × 0.25 mm i.d. × 0.25 μm film thickness, Restek Co., PA, USA).

Helium was used as the carrier gas, and the flow rate was set to 1 mL/min. For the analysis, the temperature settings were as follows: column auxiliary temperature at 280°C, MS source temperature at 230°C, and MS quadrupole temperature at 150°C. The initial oven temperature for the polar metabolite was set to 80°C and maintained for 2 minutes. Subsequently, the temperature was ramped up at a rate of 10°C per minute until it reached 320°C, and then maintained at this maximum temperature for 5 minutes. The injection volume was 1 μL with a split ratio of 1:9. The study utilized oats-based and *M. alternatus* based substrates to create distinct nutritional environments for *P. tenuipes*. Employing GC-MS analysis, we aimed to reveal the metabolic responses of *P. tenuipes* to these diverse growth conditions. Rigorous identification procedures were applied to the GC-MS data for compound identification in the fungal cultures. Metabolite identification was performed using the NIST 11 database and the Wiley 9 mass spectral library.

Statistical analysis

Data are presented as mean ± standard deviation (n = 3). A statistical analysis of the results was performed at a 5% significance level using the SAS software (SAS Institute Inc., 2000). Tukey's significance difference test was employed, using a stringent p-value threshold of < 0.05, facilitating discrimination based on culture differences in abundance or concentration. Comprehensive analysis and interpretation of statistical outcomes provided underscored the reliability of metabolite identification and enhanced the overall integrity of analytical findings.

RESULTS AND DISCUSSION

Infection of *M. alternatus* using *P. tenuipes*

We conducted an experiment to assess the suitability of a Solid media supplemented with *M. alternatus* for the infection of *P. tenuipes*. Only adult individuals of *M. alternatus* were utilized in this study. After collecting *M. alternatus* using traps, the specimens died within 2-3 days, and fungal mycelium growth was observed approximately 7 days after inoculation with *P. tenuipes*. Inoculating 5 mL of fungal mycelium into the insects resulted in excellent infection, with an infection rate of 89.8% (n = 1000), indicating a remarkably high level of efficacy. However, when *P. tenuipes* was used alone, its conidia did not germinate. Consequently, we formulated a solid culture medium by combining oats and *M. alternatus* to promote the growth of *P. tenuipes* fruiting body. The subsequent experiments, described in the following section, were conducted using this medium.

Effect of form of *M. alternatus* on development of *P. tenuipes* fruiting bodies

In this study, we examined a solid culture medium containing both original and powdered forms of *M. alternatus.* The fungal mycelium was completely colonized within 14 days after inoculation in 300 mL bottles containing 20 g of solid culture medium. Oats, including the original *M. alternatus*, exhibited superior effects in terms of key parameters related to conidial production, including initial germination time (Fig. 1), the number of fruiting body per unit, and conidial maturation time (Table 1).

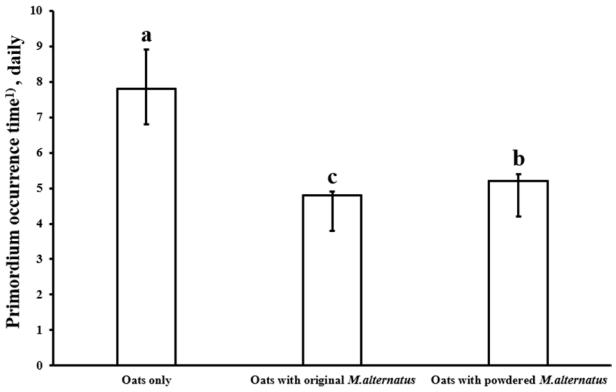

Fig. 1. Effect of solid culture medium on the primordium occurrence time of *P. tenuipes*. Oats with *M. alternatus*, 50:50 (w:w), based on dry weight. Different letters on the top of the line represent statistically significant at 5% probability level. Each value is expressed as mean ± SE (n = 5)
[1)] means first day of strip occurrence.

Table 1. Effects of solid culture medium on the development of *P. tenuipes* fruiting bodies

Solid culture medium	Stipe number (n)	Fruiting body mature time (day) [1)]
Oats only[2)]	15.8 ± 1.5[b]	40.5 ± 1.1[a]
Oats with original[3)] *M. alternatus*	21.3 ± 1.1[a]	30.7 ± 1.1[b]
Oats with powdered *M. alternatus*	16.9 ± 0.2[b]	35.0 ± 1.0[a]

[1)]determined when heads were present on the normal portion of the basidiocarp
[2)]The solid culture medium derived from oats was formulated using 20 g oat medium.
[3)]The medium comprising a blend of oats and *M. alternatus* was experimentally conducted with a weight ratio of 50:50 (w:w)

Effect of ratio of *M. alternatus* supplements on growth of *P. tenuipes* fruiting bodies

The growth of *P. tenuipes* fruiting bodies was evaluated based on stipe length, dry weight of fruiting bodies, and Biological Efficiency (BE) in relation to the added proportion of *M. alternatus*. As described in the preceding section, *M. alternatus* was incorporated in its original form according to the experimental results. In this study, we developed solid-state culture media for fruiting body production through solid fermentation, incorporating various ratios of oats.

The group demonstrating the most efficient growth was the *M. alternatus* : Oats, 70:30 (w/w) group, while the group exhibiting the least efficient growth was the experimental group using only oats (Fig. 2). The dry weight of the fruiting body also exhibited the highest value at about 27.5 g in the 70:30 (w/w) ratio of oats to *M. alternatus*, indicating a about 3 times higher value compared to the experimental group using oats only. The Biological Efficiency (BE) also registered a higher value at 29.5% in the same ratio 70:30 (w/w) compared to other control groups. It demonstrated a 3.47 times higher value compared to the group using oats only. Significant differences in growth were observed in the group using *M. alternatus* and oats in a weight ratio of 7:3 compared to the group using only oats ($p < 0.05$), as well as when compared to other experimental groups.

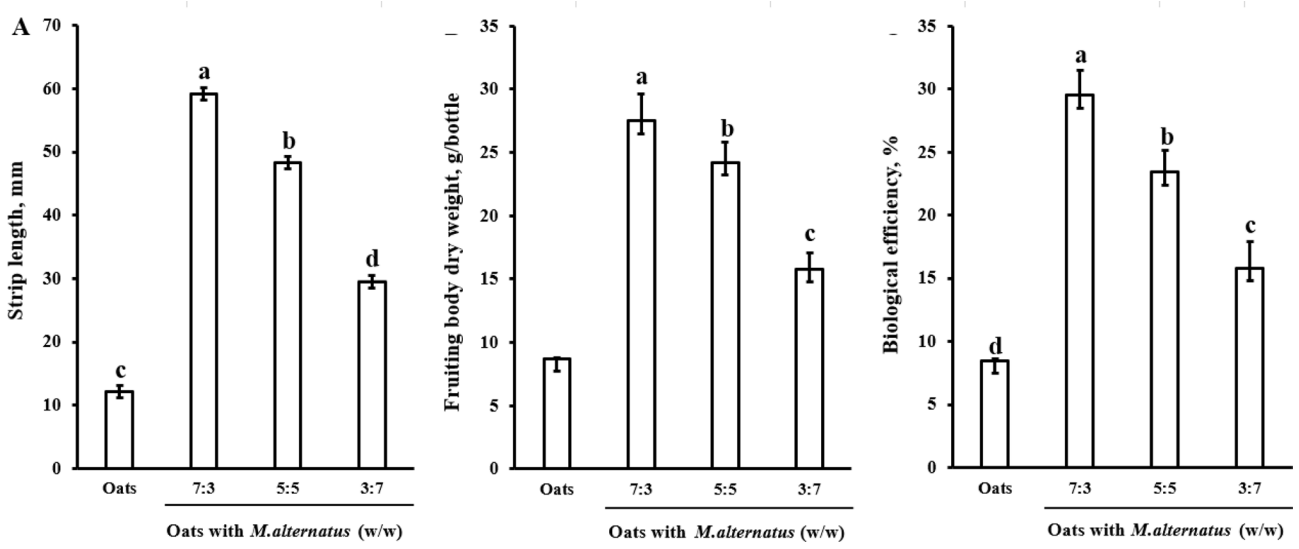

Fig. 2. The effects of solid culture medium concluding the various ratio of *M. alternatus* supplements on the growth of *P. tenuipes* **(A)** proportion of stipe length (mm); **(B)** fruiting body dry weight (g/bottle); **(C)** biological efficiency (%). Each value is expressed as mean ± SE (n=5). Different columns represent statistically significant at 5% probability level.

Table 2. Growth of fruiting bodies of *P. tenuipes* according to the water content of the solid culture medium including the *M. alternatus*

Solid culture medium	Water content (%)	Strip length (mm)	Fruiting body dry weight (g/bottle)	BE (%)[1)]
Oats	50	N.P[3)]	N.P	N.P
Oats	75	N.P	N.P	N.P
Oats	100	14.8 ± 1.7[b]	9.3 ± 0.1[b]	8.1 ± 0.1[c]
Oats with *M.alternatus*[2)]	50	N.P	N.P	N.P
Oats with *M.alternatus*[2)]	75	10.7 ± 1.1[c]	10.0 ± 0.1[b]	13.5 ± 1.0[b]
Oats with *M.alternatus*[2)]	100	50.1 ± 1.0[a]	23.7 ± 0.1[a]	19.6 ± 1.0[a]

[1)] Biological efficiency (%), fruiting body weight/medium weight×100.
[2)] Oats with *M. alternatus* 70:30 (w:w), based on dry weight.
[3)] Not primordium.
The Duncan test was conducted with the vertical axis of the table as the reference.
Each value is expressed as mean ± SE (n=5). Different columns represent statistically significant at 5% probability level.

Effect of solid culture medium water content on growth of *P. tenuipes* fruiting bodies

In the aforementioned section, we identified that the weight ratio of *M. alternatus* to oats at 70:30 (w/w) provided the most efficient growth conditions for *P. tenuipes.* Therefore, we aimed to compare the growth based on fixed weight ratios of the added ingredients in the Solid media and their corresponding efficiencies. In this study, we evaluated the growth variation of *P. tenuipes* fruiting bodies on Solid media with different moisture content levels (50%, 75%, and 100%). Moisture content was employed to assess growth in terms of stipe length, fruiting body dry weight, and BE values, which were significantly higher in the 100% moisture content medium ($p < 0.05$). In the 50% moisture content medium, neither the oats-only medium nor the 70:30 (w/w) medium with *M. alternatus* and oats showed initiation of primordium occurrence. In the 75% moisture content medium, primordium occurred in the 70:30 (w/w) medium with *M. alternatus* and oats; however, the medium using only oats did not exhibit primordium growth. *M. alternatus* appeared to positively influence not only the growth rate of *P. tenuipes* but also its initiation. The stipe length, fruiting body dry weight, and BE were recorded as 10.7 ± 1.1 mm, 10.0 ± 0.1 g/bottle, and 13.5 ± 1.0%, respectively. In the 100 % moisture content medium, both the medium with *M. alternatus* and oats at a 70:30 (w/w) ratio and the medium using only oats exhibited mycelial initiation. In the medium with *M. alternatus* and oats at a 70:30 (w/w) ratio, the stipe length, fruiting body dry weight, and BE were recorded as 50.1 mm, 23.7 g/bottle, and 19.6 %, respectively. In the medium using only oats, the stipe length was 14.8 mm, fruiting body dry weight was 9.3 g/bottle, and BE was 8.1 %. When comparing the two experimental groups, the medium mixed with *M. alternatus* and oats showed 3.39 times, 2.55 times, and 2.42 times higher values for each parameter compared to the medium using only oats. Through the conducted experiments, we confirmed that an increase in moisture content resulted in an enhancement of growth efficiency. Additionally, it was observed that *M. alternatus* has a positive impact on mycelial initiation.

Analysis of cordycepin content according to moisture content management

As mentioned in the preceding section, measurements were not possible for oats only medium at moisture content levels of 50% and 70% due to the absence of germination. Similarly, measurements were not feasible for the experimental group with a 50% moisture content in the medium containing both oats and *M. alternauts* for the same reason. In the grain medium, at a moisture content of 100%, the cordycepin content was found to be 0.9 μg/g. In the grain medium and the medium mixed with *M. alternatus*, the cordycepin content at moisture levels of 75% and 100% was recorded as 100.9 μg/g and 111.9 μg/g, respectively. When comparing the data in Table 2, it was observed that the cordycepin values did not demonstrate a mathematical relationship with stipe length, mycelial dry weight, or BE values.

Given the absence of a mathematical proportional relationship between growth indicators and cordycepin content, it is reasonable that *M. alternatus*, irrespective of growth efficiency, may have acted as a catalyst for cordycepin

Table 3. Effects of water content of *M. alternatus* supplemented media on the cordycepin content of fruiting bodies during solid state fermentation

Solid culture medium	Water content (%)	Cordycepin content (μg/g)
Oats	50	Not primordium [2)]
Oats	75	Not primordium [2)]
Oats	100	0.9 ± 0.0[c]
Oats with *M.alternatus* [1)]	50	Not primordium [2)]
Oats with *M.alternatus* [1)]	75	100.9 ± 3.1[b]
Oats with *M.alternatus* [1)]	100	111.9 ± 1.7[a]

[1)] Oats with *M. alternatus* 70:30 (w:w), based on dry weight.
[2)] Not primordium.
The Duncan test was conducted with the vertical axis of the table as the reference.
Each value is expressed as mean ± SE (n=5). Different columns represent statistically significant at 5% probability level.

synthesis during the growth process of *P. tenuipes*. Although there was no evident mathematical relationship between the growth of *P. tenuipes* and the cordycepin content, it was observed that with the addition of *M. alternatus* and an increase in moisture content, there was a trend of simultaneous increase in the growth rate of *P. tenuipes* and cordycepin content.

GC-MS analysis of *M. alternatus* and oats medium and oats only medium

The phytochemical composition of the plant was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The composition of the medium, optimized through a series of preceding experiments, was structured with a weight ratio of 70:30 (w/w) between *M. alternatus* and oats, and a moisture content of 100%. The subject of the experiment was *P. tenuipes*, which was cultivated in this prepared medium. As a control group, *P. tenuipes* fruiting bodies grown solely on an oats-only medium with a moisture content of 100% were utilized. The target compounds selected for this study include the amino acids, valine, threonine, pyroglutamic acid, serine, homoserine, proline, 2-aminobutyric acid, γ-aminobutyric acid, and ornithine.

As presented in Fig. 3, all target compounds exhibit a significant increase when cultured in media containing *M. alternatus* mycelia, indicating substantial growth in the fungal mycelium. Especially, valine demonstrated a 17.21-fold increase, while pyroglutamic acid, homoserine, and threonine exhibited significant growth with fold increases of 11.62, 10.25, and 10.14, respectively. Yu et al. (2022) identified significant metabolic pathways linked to protein synthesis, including the histidine metabolism, glycine, and threonine metabolism, and valine, and isoleucine biosynthesis.

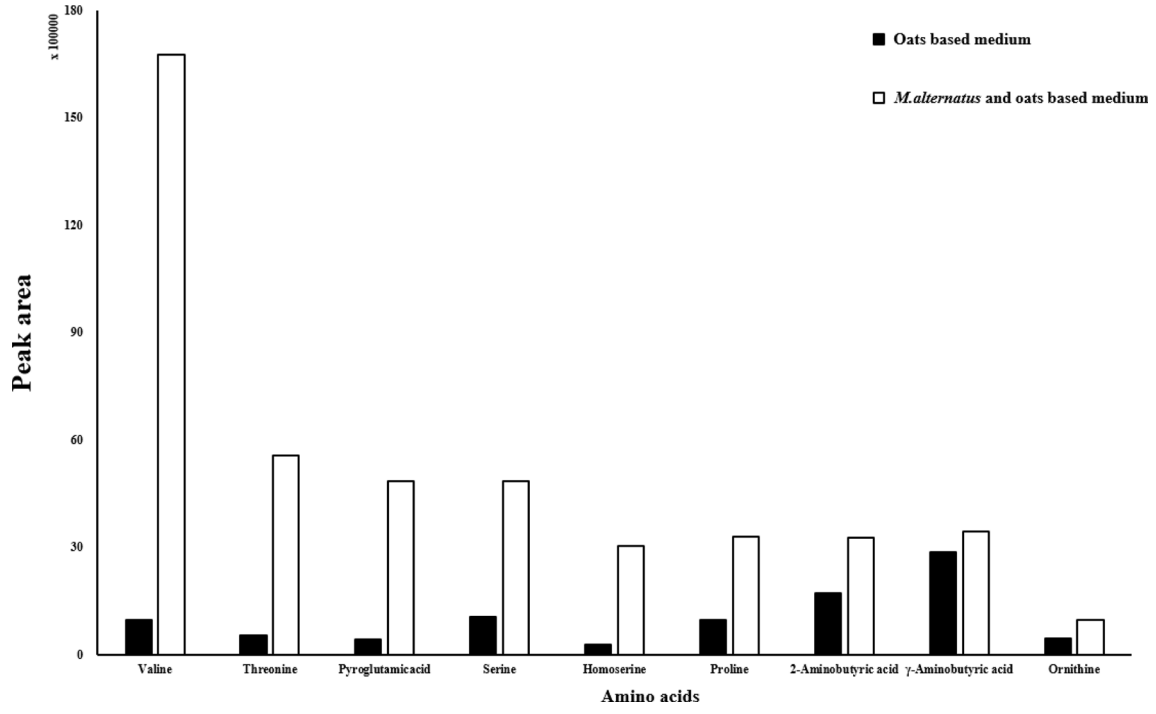

Fig. 3. The effects of amino acids among metabolites produced by *P. tenuipes* at oats based medium and *M. alternatus* and oats based medium.

ACKNOWLEDGEMENTS

This study was carried out with the support of ´R&D Program for Forest Science Technology (Project No. "2023478B10-2425-BC0361382116530002")´ provided by Korea Forest Service (Korea Forestry Promotion Institute).

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