

Optimal Growth Conditions of *Auricularia cornea* Mycelium Isolated from Para Rubber Plantation

Saowapha Surawut*, Chutapa Kunsook and Na-monrug Khamchatra

Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi, Thailand

E-mail: saowapha.s@rbru.ac.th*, chutapa.k@rbru.ac.th and namonrug.k@rbru.ac.th

*Corresponding author

(Received: 28 January 2026, Revised: 5 June 2026, Accepted: 9 June 2026)

<https://doi.org/10.57260/stc.2026.1465>

Abstract

A survey of para rubber plantations belonging to farmers in Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand, revealed the presence of edible wood ear mushrooms (*Auricularia cornea*). Pure cultures were subsequently isolated from the mycelium of this mushroom and identified using molecular identification. The strains were then preserved in the laboratory for further research. The objective of this research was to determine the factors affecting the growth of the mycelium of *A. cornea* isolated from para rubber plantation. The factors, glucose concentration (2, 2.5, 3, 3.5, 4, 4.5, and 5%), pH (4, 5, 6, 7, 8, and 9), and temperature (25, 30, 35, and 40°C) had been investigated on mycelium growth. The factorial completely randomized design (Factorial CRD) was used to design all the experiments. It was found that this strain grew the greatest on Potato Dextrose Agar (PDA) containing 2% glucose, which had the significantly highest colony diameter (7.51 ± 0.21 cm at 8 days, p -value < 0.05). At pH 6 and 7, the mycelium grew similarly well and condensed mycelium that revealed colonies diameter of 7.55 ± 0.57 and 7.79 ± 0.47 cm at 10 days, respectively (not significantly different). Finally, the mycelium was able to grow the best at 30°C with the significantly highest colony diameter (8.03 ± 0.89 cm at 6 days, p -value < 0.05). The findings of this study revealed that the optimal conditions for *A. cornea* mycelium cultivation are growth on PDA containing 2% glucose, at pH 6 or 7, and incubation at 30°C. Under these optimal conditions, the mycelium of this strain grew rapidly and exhibited dense growth. Therefore, it is suitable for application in cultivating high-quality mycelium before fruiting body cultivation.

Keywords: Optimal conditions, Growth factor, Para rubber plantation, *Auricularia cornea*

Introduction

The para rubber (*Hevea brasiliensis*) plantation is a worldwide agricultural activity, including Thailand. Due to it being a monoculture crop, the farm process and human activity resulted in changes to the biodiversity and function of organisms in this area, including macrofungi diversity (Lan et al., 2020; Li et al., 2018; Monkai et al., 2017). These led the researcher to explore the macrofungi (Mushroom) diversity in the para rubber plantation. Several studies of macrofungi diversity in para rubber plantations have previously been reported (Osemwegie & Okhuoya, 2011; Osemwegie et al., 2010). Some edible macrofungi have been discovered in this area, such as *Ganoderma lucidum*, *Schizophyllum commune*, *Tremella fuciformis*, and *Auricularia auricula* (Osemwegie & Okhuoya, 2011; Osemwegie et al., 2010).

Auricularia is a genus of jelly fungi, wood ear, or Jew's ear mushroom that is classified in the family Auriculariaceae of the Fungi Kingdom, with its edible and medicinal properties such as anti-cancer, anti-microbial, anti-oxidant, anti-diabetic, anti-hypercholesterolemic, and immuno-modulatory (Bandara et al., 2019). The market demand for this macrofungus led to the study of bag cultivation as a commercial cultivation. *Auricularia cornea* was reported in several countries, including Thailand (Bandara et al., 2017; Drewinski et al., 2024; Regis et al., 2024; Surawut et al., 2023), and the high nutritional value of *A. cornea* have been reported. However, the nutritional value widely varies depending on its strain (Walker et al., 2023; Wanich et al., 2024). Therefore, the stain from para rubber plantation of *A. cornea* may offer different nutritional value than previously reported and naturally occurring strains may have local adaptation and potential value for cultivation. The principle of mushroom bag cultivation consists of (i) mycelium isolation, (ii) mycelium spawning, (iii) substrate preparation, and (iv) cultivation to produce mature fruiting bodies (Drewinski et al., 2024; Regis et al., 2024; Thongklang et al., 2020; Wanich et al., 2024). However, the growth of fungal mycelium in optimal conditions results in the effective yield of the fruiting body production. At the step of mycelium isolation, the mushroom cultivator usually uses potato dextrose agar (PDA) for mycelia cultivation because this medium is easy to prepare. It is easily available with cheap ingredients.

The factors that affect the mycelium growth of *Auricularia* spp. (Bandara et al., 2019; Regis et al., 2024) including *A. cornea* (Walker et al., 2023; Zhang Bo et al., 2017; Zhang et al.,

2018) have been reported. The optimal temperature of *A. cornea* mycelia was 25°C in Thailand (Walker et al., 2023) and 30°C in Brazil (Drewinski et al., 2024), while optimal pH has been reported at 5-7 (Walker et al., 2023). However, different strains of *A. cornea* resulted in different growth performance.

Therefore, the objective of this study was to determine the factors affecting the growth of *A. cornea* mycelium strain from the para rubber plantation. The results of this study could be used to cultivate the mycelium in optimal conditions to obtain effective mycelium before using it to produce fruiting bodies by bag cultivation.

Methodology

Fruiting body samples

Fruiting body of *A. cornea* (collection number WK19) were collected from para rubber plantation located in Wang Takien subdistrict, Khao Saming district, Trat province, eastern Thailand (Latitude 12°24'17" N to 12°30'57" N and longitude 102°25'58" E to 102°33'45" E). The species identification was performed by morphological study and sequence analysis of Internal Transcribed Spacer (ITS) region as previously described (Wanich et al., 2024).

Mycelium preparation

The pure culture of *A. cornea* mycelium was isolated as previously described (Drewinski et al., 2024; Wanich et al., 2024). Briefly, a piece of mycelium was removed from the inside of the fresh fruiting body and transferred to potato dextrose agar (PDA) (Himedia, India). The pure culture mycelium was prepared for the experiment by growing on PDA (containing 2% glucose at pH 5) and incubated at 25°C for 5 days. The factorial completely randomized design (Factorial CRD) was used to design all the experiments.

Effect of glucose on mycelium growth

A 1.0 cm diameter of the cork borer was used to puncture the agar at the edge of the *A. cornea* colony on PDA plate. Then, transfer the agar piece to the center of PDA plate containing glucose concentrations of 2% (normal formula), 2.5, 3, 3.5, 4, 4.5, and 5% (w/v) at pH 5.0 (5 replications for each glucose condition). Subsequently, all plates were incubated at 25°C. The colony diameter was measured every day until full colonization under any conditions (Hoa & Wang, 2015).

Effect of pH on mycelium growth

A 1.0 cm diameter of the cork borer was used to puncture the agar at the edge of the *A. cornea* colony on PDA plate. Then, transfer the agar piece to the center of PDA plate containing optimal glucose concentrations with pH adjusted to 4, 5, 6, 7, 8 and 9 (5 replications for each pH condition). Subsequently, all plates were incubated at 25°C. The colony diameter was measured every day until full colonization under any conditions (Hoa & Wang, 2015).

Effect of temperature on mycelium growth

A 1.0 cm diameter of the cork borer was used to puncture the agar at the edge of the *A. cornea* colony on PDA plate. Then, transfer the agar piece to the center of PDA plate containing optimal glucose concentration and pH value. Subsequently, plates were incubated at 25, 30, 35, and 40 °C (5 replications for each temperature condition). The colony diameter was measured every day until full colonization under any conditions (Hoa & Wang, 2015).

Statistical analysis

The values obtained from the experimental results were analyzed statistically by mean and standard deviation. Comparing the mean by one way-ANOVA with LSD for multiple comparisons. SPSS version 25.0 was used for analysis, and the p -value<0.05 was considered a statistically significant difference.

Results

Effect of glucose on mycelium growth

According to the mushroom cultivator usually used PDA for mycelia cultivation. The addition of glucose, serving as the primary carbon source, may stimulate fungal growth. To determine the effect of glucose on mycelium growth. The mycelium of *A. cornea* was grown on PDA containing 2, 2.5, 3, 3.5, 4, 4.5, and 5% glucose (w/v). The result revealed that *A. cornea* mycelium could grow at glucose concentrations of 2-5% in PDA but showed the best growth at 2% glucose in PDA (Figure 1 and Figure 2), with 7.51 ± 0.21 cm of the colony diameter at 8 days of incubation, which was significantly larger than the other concentrations (p -value<0.05). The incubation period until the mycelia of *A. cornea* grew fully on the culture plate was found in 2% glucose condition at 8 days (Figure 2) followed by the glucose concentration at 2.5, 3, 3.5, 5 and 4.5% with the colony diameter on 8 days was 6.84 ± 0.44 , 6.07 ± 0.21 , 5.55 ± 0.27 , 4.48 ± 0.10 , and 4.47 ± 0.22 cm, respectively.

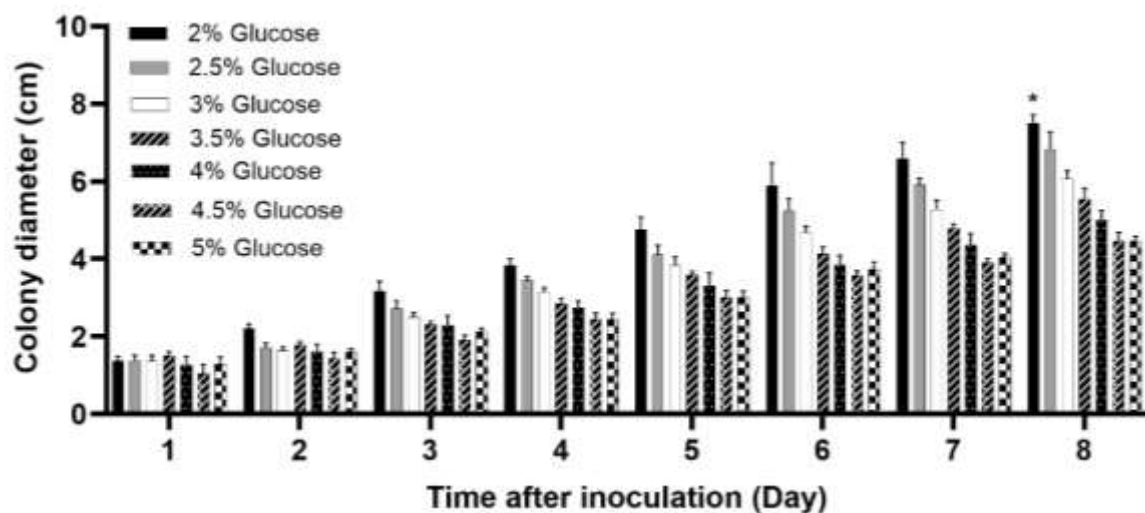


Figure 1 Colony diameter of *A. cornea* on PDA with glucose concentrations of 2, 2.5, 3, 3.5, 4, 4.5, and 5% (w/v) after incubation for 8 days. * Represents a significant difference when compared to other glucose concentrations at 8 days after incubation.

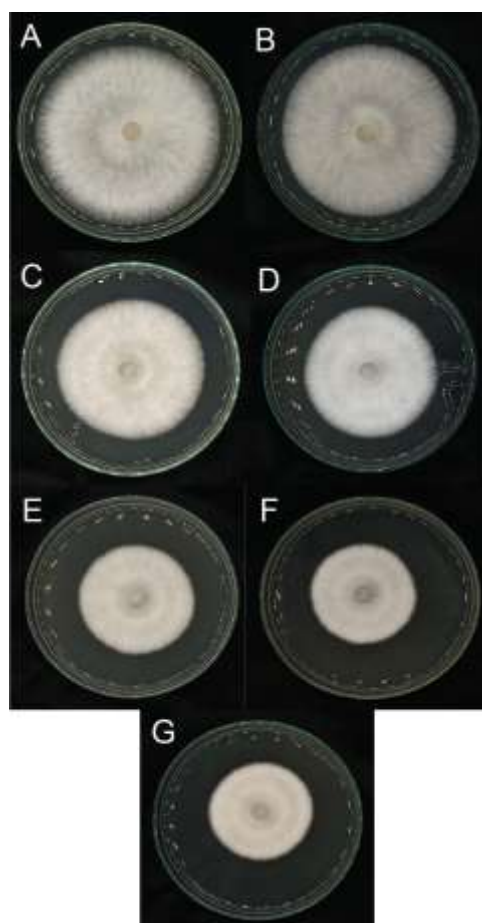


Figure 2 Colony characteristics of *A. cornea* on PDA with glucose concentrations of 2 (A), 2.5 (B), 3 (C), 3.5 (D), 4 (E), 4.5 (F) and 5% (G) (w/v) after 8 days of incubation.

Effect of pH on mycelium growth

To determine the effect of pH on mycelium growth. The mycelium of *A. cornea* was grown on PDA with pH of 4, 5, 6, 7, 8, and 9. The results showed colony diameter of *A. cornea* mycelium was quite similar at pH of 4, 5, 6, 7, 8, and 9 (Figure 3 and Figure 4), with colony diameters of 8.03 ± 0.18 , 7.73 ± 0.48 , 7.55 ± 0.57 , 7.79 ± 0.47 , 6.88 ± 0.34 , and 7.36 ± 0.10 cm, respectively, after incubation for 10 days. At pH 6 and 7, the mycelium grew well and seemed to have high mycelium density when compared to the growth at other pH levels, as shown in Figure 4. However, the colony diameters of mycelium when grown at pH 6 and 7 revealed no significant difference ($p\text{-value} > 0.05$).

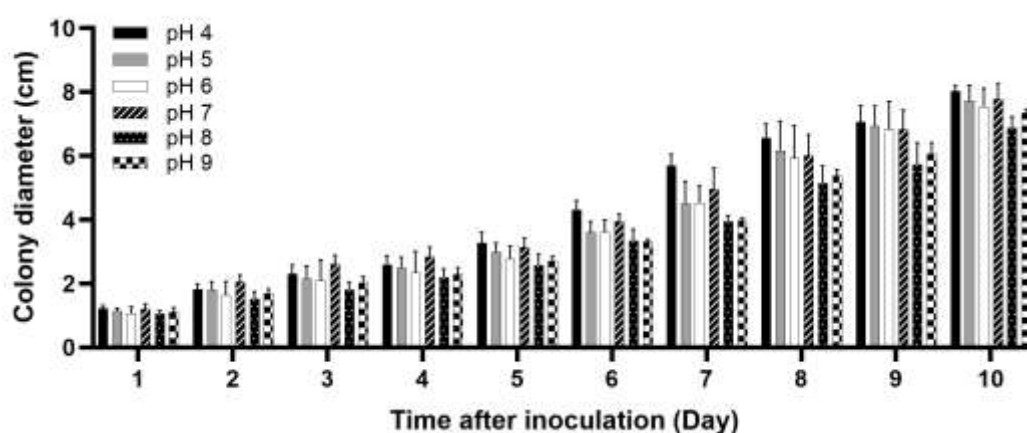


Figure 3 Colony diameter of *A. cornea* on PDA with pH of 4, 5, 6, 7, 8, and 9 after incubation for 10 days.

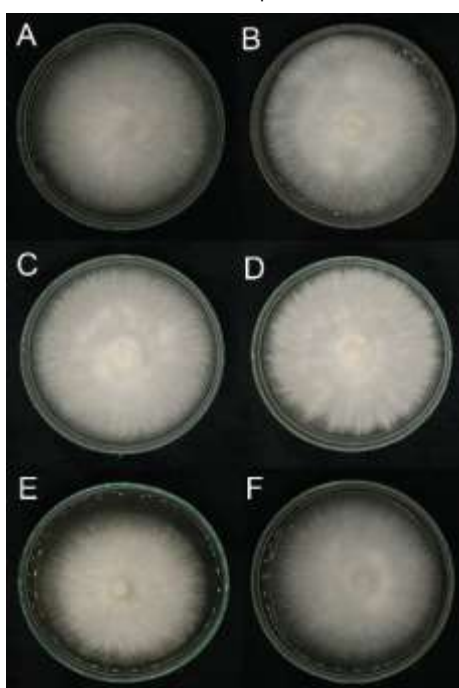


Figure 4 Colony characteristics of *A. cornea* on PDA with pH of 4 (A), 5 (B), 6 (C), 7 (D), 8 (E) and 9 (F) after incubation for 10 days.

Effect of temperature on mycelium growth

To determine the effect of temperature on mycelium growth. The mycelium of *A. cornea* was grown on PDA at 25, 30, 35, and 40°C. The results revealed that it could grow best at 30°C (Figure 5 and Figure 6), with a colony diameter was 8.03 ± 0.89 cm after incubation for 6 days, which was significantly larger than the other temperatures (p -value <0.05). The incubation period until the mycelia of *A. cornea* grew fully on the culture plate was found in 30°C condition at 6 days (Figure 6), followed by the temperature of 35, 25, and 40°C with the colony diameter of 7.18 ± 0.35 , 3.49 ± 0.09 , and 1.00 ± 0.00 cm, respectively. However, even though the temperature of 35°C had a significantly smaller colony diameter than that of 30°C, it was found that the colony characteristics of *A. cornea* mycelium when incubated at 35°C seem to be higher mycelium density, as shown in Figure 6C. It was also found that at 40°C could not cultivate *A. cornea* mycelium at all (the colony diameter was measured as 1 cm, which was the size of the mycelial agar piece placed on the culture plate).

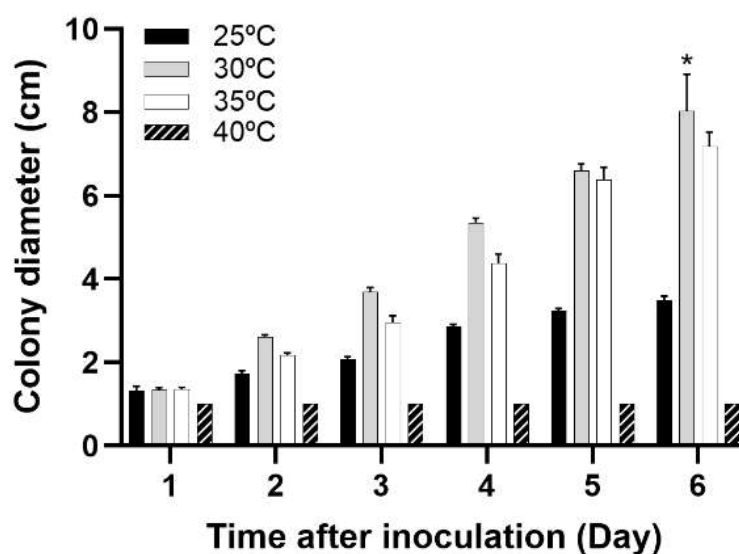


Figure 5 Colony diameter of *A. cornea* on PDA with 2% glucose, pH 6.0, and incubated at 25, 30, 35, and 40°C for 6 days. * Represents a significant difference when compared to other temperatures at 6 days after incubation.

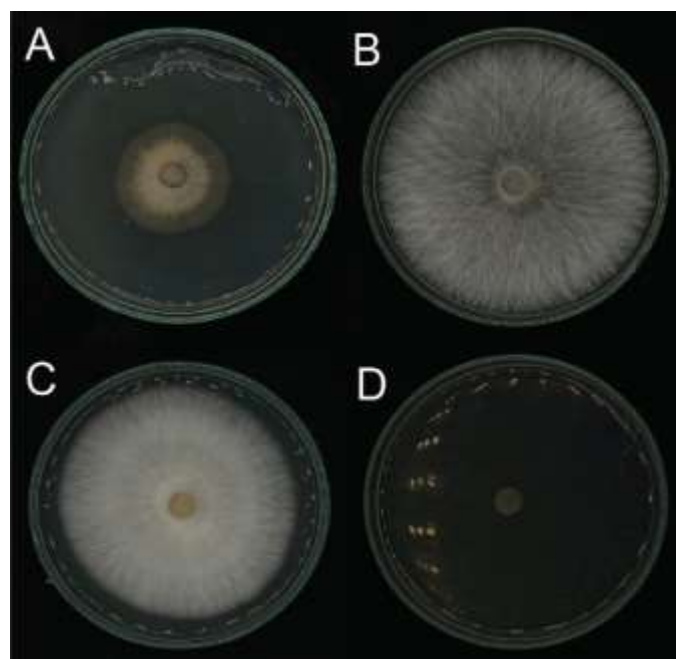


Figure 6 Colony characteristics of *A. cornea* on PDA with 2% glucose concentration, pH 6.0, and incubated at 25°C (A), 30°C (B), 35°C (C), and 40°C (D) for 6 days.

Discussion

To determine the factors affecting the growth of *A. cornea* mycelium strain from the para rubber plantation. The result revealed that *A. cornea* mycelium could grow at glucose concentrations of 2-5% in PDA but showed the best growth at 2% glucose in PDA. The results of this study are consistent with the research of Hoa and Wang (2015) who reported that sugar concentrations of 1-3% (w/v) will cause the fungal hyphae to have the highest colony diameter. However, if the sugar concentration is higher than 3% (w/v), it will affect the change of osmotic pressure within the fungal cells, causing the fungi to be unable to control the balance of substance entry or exit, and the function of the metabolic synthesis process (Hoa & Wang, 2015). The optimal pH for *A. cornea* has previously been described in three different *A. cornea* isolates collected from Chiang Rai province, Thailand (Walker et al., 2023). They found that pH 5-7 was optimal for the growth of *A. cornea* mycelia, which correlated with the result of this study (pH 6-7). In general, fungal hyphae growth is best in acidic to neutral conditions, which is consistent with these studies. In previous studies, *A. cornea* isolates from Chiang Rai province, Thailand were investigated for optimal conditions, not only the optimal pH but also optimal temperature. At 25°C was revealed as the optimal temperature for *A. cornea* mycelia growth (Walker et al., 2023). However, the study of *A. cornea* isolates from Brazil showed optimal temperature at 30°C (Drewinski et al., 2024), which is consistent with

the result of this study (30°C). These studies suggested that different strains of *A. cornea* resulted in different growth performance. The results of this study could be used to cultivate the mycelium in optimal conditions to obtain effective mycelium before using it to produce fruiting bodies by bag cultivation.

Additionally, *Auricularia* spp. or wood ear mushrooms are multipurpose in cooking and offer beneficial nutrients. Their nutritional value varies depending on the species, but they generally consist of 79.9–93.2% carbohydrates, 6.5–13% crude protein, 3.5–12.5% crude fiber, and 0.48–4.5% fat (% dry weight). The main components are polysaccharides, which make up as much as 66.1% of the total composition, including mannan, glucan, pectin, chitin, and cellulose. Furthermore, some species of wood ear mushrooms contain 18 different amino acids, covering all the essential amino acids necessary for the body. Glutamic acid is the most abundant of these. In addition, wood ear mushrooms also contain important minerals. This includes essential elements such as calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), and iron (Fe). Wood ear mushrooms have also been found to contain vitamins A, D, and K. Furthermore, wood ear mushrooms have been reported to contain bioactive compounds such as polysaccharides and melanin, which have antioxidant, anti-tumor, and metabolic regulation properties (Liu et al., 2025). Therefore, the fruiting body of *A. cornea* isolated from para rubber plantation strain may produce different nutritional content and bioactive compounds that may be used as high nutritional food and for medicinal use.

Conclusion and Suggestions

This research studied the factors affecting the growth of mycelium of *Auricularia cornea* strains isolated from para rubber plantation, where located in Wang Takien subdistrict, Khao Saming district, Trat province, Thailand. The results found that the optimal conditions for *A. cornea* mycelium cultivation are growth on PDA containing 2% glucose, at pH 6 and 7, and incubation at 30°C. It was found that *A. cornea* mycelium under the appropriate cultivation conditions grew rapidly and seemed to be dense mycelium. These results could be used to cultivate the mycelium of *A. cornea* strain from the para rubber plantation in the laboratory to obtain the effective mycelium before performing the spawning culture of this fungus and then growing it to produce its fruiting body. Additionally, further study should also examine the nutritional value and bioactive properties of fruiting bodies produced from this locally adapted strain in order to support their use as both edible and medicinal mushrooms.

Acknowledgement

This study was supported by the Rambhai Barni Rajabhat University Research Fund (Grant number 2216/2567) and Plant Genetic Conservation Project under the Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, Rambhai Barni Rajabhat University in the fiscal year 2024/2025. The authors would like to thank Trat Rubber Co-operative Limited for coordinating with para rubber farmers or land owners to permit to enter the area.

References

- Bandara, A., Rapior, S., Mortimer, P., Kakumyan, P., Kd, H., & Xu, J. (2019). A review of the polysaccharide, protein and selected nutrient content of *Auricularia*, and their potential pharmacological value. *Mycosphere*, 10(1), 579–607.
<https://doi.org/10.5943/mycosphere/10/1/10>
- Bandara, A. R., Karunarathna, S. C., Phillips, A. J. L., Mortimer, P. E., Xu, J. C., Kakumyan, P., & Hyde, K. D. (2017). Diversity of *Auricularia* (Auriculariaceae, Auriculariales) in Thailand. *Phytotaxa*, 292(1), 19-34. <https://doi.org/10.11646/phytotaxa.292.1.2>
- Drewinski, M. P., Zied, D. C., Gomes, E. P. C., & Menolli, N., Jr. (2024). Cultivation of a wild strain of wood ear *Auricularia comea* from Brazil. *Current Microbiology*, 81, 390.
<https://doi.org/10.1007/s00284-024-03920-5>
- Hoa, H. T., & Wang, C. L. (2015). The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, 43(1), 14-23. <https://doi.org/10.5941/myco.2015.43.1.14>
- Lan, G., Wu, Z., Yang, C., Sun, R., Chen, B., & Zhang, X. (2020). Tropical rainforest conversion into rubber plantations results in changes in soil fungal composition, but underling mechanisms of community assembly remain unchanged. *Geoderma*, 375, 114505. <https://doi.org/10.1016/j.geoderma.2020.114505>
- Li, H., Guo, J., Karunarathna, S. C., Ye, L., Xu, J., Hyde, K. D., & Mortimer, P. E. (2018). Native forests have a higher diversity of macrofungi than comparable plantation forests in the Greater Mekong Subregion. *Forests*, 9(7), 1-14. <https://doi.org/10.3390/f9070402>
- Liu, M., Jiang, W., Huang, K., Li, L., Meng, Q., You, X., Pu, K., Cheng, M., Gao, Z., Qi, J., & Li, M. (2025). Sustainable cultivation and functional bioactive compounds of *Auricularia* Mushrooms: Advances, challenges, and future prospects. *Biology*, 14(11), 1555.
<https://www.mdpi.com/2079-7737/14/11/1555>

- Monkai, J., Hyde, K. D., Xu, J., & Mortimer, P. E. (2017). Diversity and ecology of soil fungal communities in rubber plantations. *Fungal biology reviews*, 31(1), 1-11. <https://doi.org/10.1016/j.fbr.2016.08.003>
- Osemwegie, O., & Okhuoya, J. (2011). Diversity and abundance of macrofungi in rubber agroforests in southwestern Nigeria. *Nordic Journal of Botany*, 29(1), 119-128. <https://doi.org/10.1111/j.1756-1051.2010.00717.x>
- Osemwegie, O., Okhuoya, J., Oghenekaro, A., & Evueh, G. (2010). Macrofungi community in rubber plantations and a forest of Edo State, Nigeria. *Journal of Applied Sciences*, 10(5), 391-398. <https://doi.org/10.3923/jas.2010.391.398>
- Regis, J., Geösel, A., & Geösel, R. M. (2024). Cultivation of *Auricularia* species: a review of the history, health benefits, principles, practices, environmental conditions, research methods, and recent trends. *Sydowia*, 76, 21-33. <https://doi.org/10.12905/0380.sydowia76-2024-0021>
- Surawut, S., Kunsook, C., Nak-eiam, S., Khamchatra, N.-m., Bhudharak, S., Phontharod, W., Boonmee, O., Yasawong, M., & Kanjanavas, P. (2023). Biodiversity and functional distribution of macrofungi from Plant Genetic Conservation Area, Chanthaburi province, Thailand. *Current Applied Science and Technology*, 23(5), 1-19. <https://doi.org/10.55003/cast.2023.05.23.003>
- Thongklang, N., Keokanngun, L., Taliam, W., Hyde, K.D. (2020). Cultivation of a wild strain of *Auricularia cornea* from Thailand. *Current Research in Environmental & Applied Mycology*, 10(1), 120-130. <https://doi.org/10.5943/cream/10/1/13>
- Walker, A., Wannasawang, N., Taliam, W., Keokanngun, L., Luangharn, T., & Thongklang, N. (2023). Optimal conditions for mycelial growth and nutritional values of the *Auricularia cornea*. *Studies in Fungi*, 8(19), 1-10. <https://www.maxapress.com/article/doi/10.48130/SIF-2023-0019>
- Wanich, U., Kunsook, C., Kanjanavas, P., Thanyaponl, P., Boonmee, O., Apinansawat, N., Panisan, T., & Surawut, S. (2024). Edible macrofungi in Plant Genetic Conservation Area, Chanthaburi province: *Auricularia cornea* strain RSPG5 fruiting body formation and nutritional value. *Science & Technology Asia*, 29(4), 247-256. <https://ph02.tci-thaijo.org/index.php/SciTechAsia/article/view/252797>

- Zhang Bo, Z. B., Miao RenYun, M. R., Zhou Jie, Z. J., Huang ZhongQian, H. Z., Li XiaoLin, L. X., & Tan Wei, T. W. (2017). Effects of cultivating substrates with different nitrogen sources on agronomic traits, quality and production efficiency of *Auricularia cornea*. *Journal of Southern Agriculture*, 48(12), 2210-2217. <http://nfnyxb.xml-journal.net/en/article/doi/10.3969/j.issn.2095-1191.2017.12.15>
- Zhang, J., Li, X., & Yin, Y. (2018). A method for measuring the degree of fermentation of the edible mushroom cultivation substrate. *Natural Resources*, 9(11), 355-360. <https://doi.org/10.4236/nr.2018.911022>