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Biological characteristics and cultivation of wild *Auricularia cornea* recorded in Southern, Vietnam

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Abstract To meet the increasingly diverse demand for edible mushrooms and stabilize the supply, it is necessary to domesticate new wild edible mushroom species. Two isolates of *Auricularia cornea* collected from the forest in the Southeast of Vietnam were studied to assess their physiological characteristics and cultivation potential on rubber sawdust. The findings indicated that ABI-F000301 (white strain) and ABI-F000302 (brown strain) exhibited similar morphological traits, confirming their classification as *Auricularia cornea* based on phylogenetic analysis utilizing the ITS marker. Both strains demonstrated robust growth in MCM broth, maintaining a pH between 6 and 7 and thriving at 30ºC. The most effective substrates for mushroom propagation were identified as oat and paddy grain. The mycelial colonization times for these strains in a 1 kg nylon bag were 29.87 ± 1.19 days for the white strain and 31.6 ± 2.03 days for the brown strain. Incorporating rice bran and corn bran at a ratio of 1.5% into the rubber sawdust yielded results of 542.23 ± 121.72 g/kg substrate for ABI-F000301 and 671.41 ± 127.38 g/kg substrate for ABI-F000302. The biological efficiencies achieved were 84.8 ± 19.02% for the white strain and 102.45 ± 19.9% for the brown strain. These results demonstrate that both strains of *Auricularia cornea* possess considerable potential for mass cultivation.

Keywords: Cultivation, Wood ear mushroom, Mycelial growth, Phylogeny, Sawdust

Introduction

Vietnam is a tropical country that offers favorable conditions for agricultural development, resulting in abundant farming by-products. According to the General Statistics Office, the total volume of the country's by-products in 2022 was estimated at nearly 160 million tons, with 56.2%

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(approximately 90 million tons) of post-harvest agricultural by-products from crops.

The rubber tree (*Hevea brasiliensis*) is predominantly grown in certain Highland and South-East provinces. Each year, many old rubber trees were cut down for new planting and the production of rubber wood furniture. This practice generates a significant amount of sawdust waste, ideal for mushroom cultivation in Vietnam, including varieties like *Pleurotus*, *Auricularia*, *Lentinula*, and *Ganoderma* (Nguyen, 2004). *Auricularia*, also known as wood ear mushrooms, ranks among Vietnam's six primary mushroom species and is particularly prevalent in the Southeast region. The country produces between 1,500 and 2,000 dry tons of wood ear mushrooms annually, making up about 10% of the global production (Duc, 2005).

Auricularia Bull., is a significant genus of mushrooms, accounting for around 17% of the world's mushroom production and ranking as the third most cultivated mushroom species after *Lentinula* (22%) and *Pleurotus* (19%) (Royse *et al.,* 2017). *Auricularia* not only provides essential carbohydrates and proteins (Bandara *et al.,* 2019) but also delivers crucial micronutrients often lacking in many diets, such as copper (Cu), selenium (Se), and zinc (Zn) (Carrasco *et al.,* 2018). Additionally, *Auricularia* species have promising potential for developing new therapeutic drugs targeting various human diseases, including cancer treatment (Wu *et al.,* 2014). Their bioactive compounds, particularly polysaccharides, exhibit notable properties such as anti-tumor, antioxidant, anti-proliferative, and immunomodulatory (Ma *et al.,* 2018).

Auricularia cornea is recognized as a medicinal and edible mushroom primarily found in East Asian countries like China and Korea (Chen and Xue, 2018). This mushroom is valued for being low in calories while being rich in essential nutrients, including carbohydrates, proteins, various B vitamins such as riboflavin, niacin, pantothenic acid, and vital minerals like iron, potassium, and phosphorus. Additionally, it contains beneficial polysaccharides. Importantly, *A. cornea* offers antioxidant and antimicrobial properties, making it significant in the health and wellness domains (Khan *et al.,* 2023). Despite its nutritional and therapeutic potential, *A. cornea* remains a relatively new species and has not yet been commercialized in Vietnam. Consequently, there's a pressing need for research on the physiological characteristics of its mycelium. Such studies are crucial for conserving genetic diversity and the potential domestication of wild *A. cornea*, which could lead to its introduction into industrial mushroom cultivation practices. This could pave the way for enhancing the variety and availability of mushrooms in the agricultural sector.

Materials and methods

Fungal strains

Two wild strains of *Auricularia cornea*, designated as ABI-F000301 (white) and ABI-F000302 (brown), were collected from the Dong Nai Culture and Nature Reserve in the southeastern region of Vietnam. When the fresh specimens were taken to the laboratory, some were isolated by fungal tissue, grown in PDA medium, and kept at 25°C for 10 days. The remaining specimens were dried at 50 - 60ºC and stored in ziplock plastic bags. Both the strain collection and the dried specimens are maintained at the Applied Biotechnology Institute (ABI).

DNA extraction, PCR, and sequencing

DNA was extracted from the mycelium of two strains, ABI-F000301 and ABI-F000302. The extracted DNA was then purified, precipitated, dried, and dissolved in a buffer for further analysis. To amplify the internal transcribed regions (ITS) of the ribosomal DNA (rDNA) region, the primer pair ITS1/ITS4 (White *et al.,* 1990) was employed. The PCR procedures utilized a high-purity PCR template preparation kit (MyTaqTMHS Mix, Bioline). After amplification, the PCR products were purified and sequenced at First Base Company in Kuala Lumpur, Malaysia. The resulting sequences were subsequently deposited in the GenBank database of the National Center for Biotechnology Information (NCBI).

DNA sequence analysis

The sequence data was edited and assembled using ATCG software (www.genetyx.co.jp), and a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was conducted to identify matches with high-similarity species. Sequences were then aligned and manually adjusted with Aliview 1.28 (Larsson, 2014). A maximum likelihood (ML) phylogenetic tree was constructed using MEGA X, applying rapid bootstrap analysis with 1000 replicates under the T93+G model (Kumar *et al.,* 2018). The study included *Elmerina dimidiata* (JQ764664) and *E. efibulata* (MZ618495) as outgroups.

Effect of different media on mycelial growth

In this study, six types of liquid media were tested: Malt Extract Broth (MEB), Potato Dextrose Broth (PDB), Malt Yeast Broth (MYB), Mushroom

Complete Medium (MCM), Minimum Medium (MM), and Ohta Medium. A 0.5-cm-diameter mushroom piece was cut from the edge of 7-day-old colonies grown on PDA and carefully transferred into flasks with 20 ml of medium. The flasks were then kept at 30ºC and left in the dark until the mycelium filled them up. The growth rate was calculated by the dried mycelial biomass weight. The experiment was repeated three times.

Effect of temperature on mycelial growth

The optimal liquid medium for mycelial growth was then used as the basal medium to evaluate the effects of different temperatures (5ºC, 10ºC, 15ºC, 20ºC, 25ºC, 30ºC, 35ºC) and incubated in the dark. After incubation, the dry weight of the mushrooms was harvested on day 10. The experiment was performed three times.

Effect of pH on mycelial growth

The basal medium was used and adjusted with 1M NaOH and 1M HCl to evaluate the optimal pH for mycelial growth. Media with pH levels ranging from 3 to 6 were autoclaved, while those at pH levels 7 to 9 were aseptically filtered before mushroom inoculation. The dried biomass was collected on the 10^{th} day of culture for analysis. This experiment was carried out in triplicate.

Effect of different cereal grains on mushroom propagation

Six types of cereals were evaluated as potential substrates for mushroom propagation: *Avena sativa* (oat), *Coix lacryma-jobi* Linn. (millet), *Hordeum vulgare* L. (barley), *Oryza sativa* L. ssp. indica (paddy), *Sorghum bicolor* (red sorghum), and *Triticum aestivum* L. (wheat). Each substrate was thoroughly washed, soaked overnight, boiled for 10 to 15 minutes, and then cooled to maintain a moisture content of 50 to 70%. Fifty grams of each cereal were placed in test tubes (200 × 25 mm) and autoclaved at 121ºC for 15 minutes (Thongbai *et al.,* 2017). After allowing the tubes to cool for 24 hours, each medium was inoculated with a 10-mm-diameter piece of mushroom. All culture tubes were incubated at 30ºC, and mushroom propagation was monitored every two days until the mycelium filled the test tubes, which occurred 20 days after inoculation. The entire experiment was conducted in triplicate.

Fruiting test

Rubber sawdust served as the primary substrate and was mixed (w/w) with 1.5% rice bran, 1.5% corn bran, and 1% calcium carbonate. All substrate

supplements were combined manually to achieve a moisture content of 65%. The mixture (1 kg) was packed into polypropylene bags and then sealed with plastic rings and cotton plugs. After sterilizing the sawdust bags at 100ºC for 8 hours, they were allowed to cool to room temperature. At this point, 50 grams of paddy grain spawn were inoculated into each aseptic bag. Thirty nylon spawn bags for each strain were prepared and incubated at 28ºC in the dark. The mycelial growth rate in each 1 kg nylon bag was recorded every 5 days. Once the spawn run was complete, slits were made in the bags to promote fruiting. The temperature for fruiting stage was 28 ± 2ºC, humidity 80 - 90%. Each flush's mushroom yield was recorded in the spawn bags and calculated as the total weight of fresh mushrooms per kilogram of substrate (Royse, 2010; Llarena-Hernández *et al.,* 2011; Thongklang *et al.,* 2014). The calculation used to determine the biological efficiency was: Biological efficiency (%) = Yield of fresh fruiting bodies (g)/dry weight of substrate (g) × 100 (Liang *et al.,* 2019). Cultivation testing was conducted with three replicates.

Statistical analysis

Data were analyzed using Duncan's multiple range test ($p < 0.05$) and post hoc tests to obtain a mean separation. The analysis was conducted using one-way ANOVA through the SPSS program.

Results

Phylogenetic analyses

The final ITS dataset consisted of 52 *Auricularia* sequences, including the outgroup. The completed alignment comprised 572 characters, and the resulting maximum likelihood (ML) tree is shown in Figure 1. The isolates used in this study are highlighted in bold. Phylogenetic analysis indicated that these isolates (ABI-F000301 and ABI-F000302) clustered with other *A. cornea* fungal accessions, forming a distinct lineage.

Effect of different factors on mycelial growth

The results presented in Table 2 indicate that the optimal medium for the two isolates, ABF101 and ABF102, was MCM. These strains preferred a temperature range between 25 and 30ºC, with the maximum dry weight achieved at 30ºC. The most favorable pH level for the growth of ABI-F000301 mycelium was pH 6, while strain ABI-F000302 showed optimal growth at pH

7. Additionally, strain ABI-F000301 thrived on oats and barley, whereas strain ABI-F000302 was most abundant when grown on paddy grain.

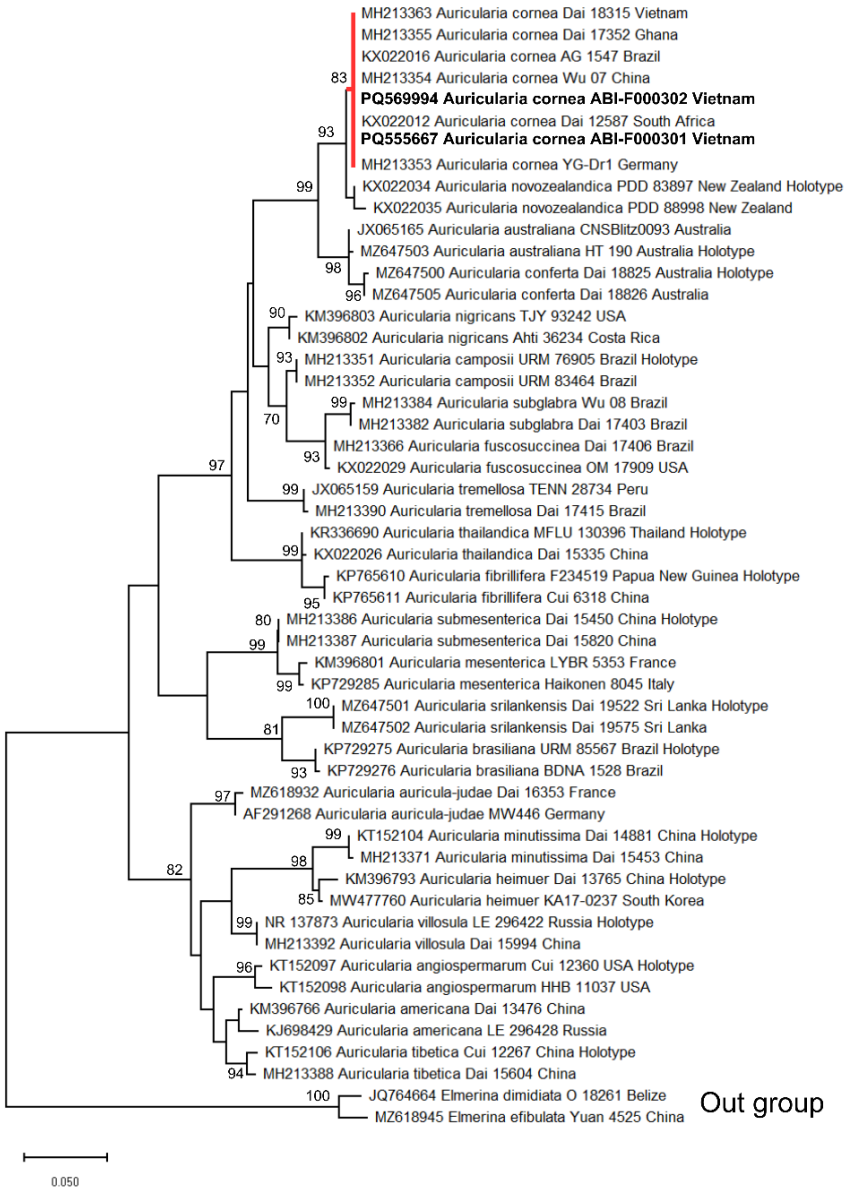

Figure 1. Maximum likelihood phylogenetic tree inferred from the internal transcribed spacer (ITS) of *A. cornea*. Bootstrap frequencies are equal to or greater than 70% and are shown above-supported branches

Table 2. Optimal conditions for mycelial growth of two isolates of *A. cornea*

Experiment	Treatment	Isolate strain	
		ABI-F000301 (white)	ABI-F000302 (brown)
Effect of different media (g)	PDB	0.0660gh ± 0.0117	0.0659gh ± 0.0094
	MYB	0.0724hi ± 0.0136	0.0825jk ± 0.0085
	MEB	0.0792ij ± 0.0115	0.0918l± 0.0191
	MCM	0.0893kl ± 0.0103	0.0962l ± 0.0130
	MM	0.0342bc ± 0.0043	0.0516ef ± 0.0087
	Ohta	0.0110a ± 0.0012	0.0046a ± 0.0008
Effect of different temperature (g)	5ºC	0.0017ab ± 0.0003	0.0010a ± 0.0001
	10ºC	0.0028abc ± 0.0004	0.0028abc ± 0.0004
	15ºC	0.0074d ± 0.0013	0.0072cd ± 0.0014
	20ºC	0.0281e ± 0.0049	0.0281e ± 0.0025
	25ºC	0.0911h ± 0.0065	0.0993i ± 0.0079
	30ºC	0.1033j ± 0.0071	0.1042j ± 0.0068
	35ºC	0.0045abcd ± 0.0006	0.0059bcd ± 0.0011
Effect of different pH (g)	3	0.0009a ± 0.0002	0.0009a ± 0.0002
	4	0.0320c ± 0.0049	0.0475b ± 0.0037
	5	0.0632e ± 0.0046	0.0730c ± 0.0074
	6	0.0882f ± 0.0074	0.0958e ± 0.0116
	7	0.0423d ± 0.0059	0.0618f ± 0.0078
	8	0.0316c ± 0.0055	0.0469d ± 0.0080
	9	0.0088b ± 0.0019	0.0170c ± 0.0025
Effect of different cereal grains (cm)	Red sorghum	13.68a ± 0.72	11.95b ± 0.42
	Millet	13.55a ± 0.47	11.91b ± 1.14
	Paddy grain	13.50a ± 0.55	13.36d ± 0.60
	Barley	14.18b ± 0.40	12.36bc ± 0.78bc
	Wheat bran	13.45a ± 0.47	10.36a ± 1.32
	Oat	14.45b ± 0.35	12.77cd ± 0.75

* All data were illustrated as mean ± standard deviation; Means (each column) followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple-range test; The alphabet lower case letters set were separated in each column.

Mycelial growth and basidiocarp production

Table 3 presents the cultivation results of the two isolates ABI-F000301 (white strain) and ABI-F000302 (brown strain) on the rubber sawdust substrate. It took the mycelium of *A. cornea* ABI-F000301 and ABI-F000302 to fully colonize 1 kg sawdust nylon bag at 29.87 ± 1.19 and 31.6 ± 2.03 days, respectively. The duration from full spawn until maturation of basidiocarps in the first harvest was 33.13 ± 3.74 and 32.2 ± 5.76 days. The average yield of these strains ABI-F000301 and ABI-F000302 was obtained at 542.23 and 671.41 g/kg substrate and biological efficiency was 84.8 ± 19.02 and 102.45 ±19.9%.

Table 3. Ability to cultivate ABI-F000301 and ABI-F000302 strains of *A. cornea*

Content	ABI-F000301 (white strain)	ABI-F000302 (brown strain)
Time of mycelial running fully bags (days)	29.87 ± 1.19	31.6 ± 2.03
Time of first harvest (days)	33.13 ± 3.74	32.2 ± 5.76
Average mushroom yield (g)/kg fresh substrate	542.23 ± 121.72	671.41± 127.38
Biological efficiency BE (%)	84.80 ± 19.02	102.45 ± 19.90

Figure 2. Basidiocarp of *A. cornea strains*, ABI-F000301 strain (white) and ABI-F000302 (brown)

Discussion

Auricularia species can be cultivated under various conditions, enabling large-scale production globally (Bandara *et al.,* 2019). Although this genus ranks fourth among commonly grown mushrooms, scientific research, and production have rapidly increased due to its culinary and medicinal benefits (Regis and Geösel, 2024).

Numerous studies have examined the mycelial growth of *Auricularia* species. For instance, three Thai strains of *A. cornea* (MFLUCC18-0346, MFLUCC18-0347, and MFLUCC23-0084) showed optimal growth in a natural medium called RSA (Rice Bran Sucrose Agar) with a pH range of 5 to 7. Additionally, a synthetic medium, MEA (Malt Extract Agar), was suitable for strains MFLUCC18-0346 and MFLUCC18-0347, but not for MFLUCC23-0084, with an ideal pH of 5 to 6. The optimal temperature for these Thai A. cornea strains' growth was 25ºC (Walker *et al.,* 2023). The mycelium of *A.*

auricula-judae thrived in PDA and MCM media at temperatures between 25 and 30ºC, with a pH range of 6 to 9 (Jo *et al.,* 2014). *A. villosula* can be cultured in a mixture of potato juice, sucrose, soybean flour, and 0.5% phosphate (PO_4^{-3}) at 30°C and a pH 8 for biomass production (Zhang *et al.,* 2018b). Our results indicate that the MCM medium is ideal for the mycelial biomass growth of strains ABI-F000301 and ABI-F000302, with the highest dried biomass achieved at 30ºC. This finding aligns with Drewinski's results, which showed that the optimal temperature for *A. cornea* mycelium growth (a wild strain from Brazil) was also 30ºC (Drewinski *et al.,* 2024). Various wood-ear mushrooms prefer this temperature range. Furthermore, the suitable pH value for the mycelial growth of these strains was pH 6, consistent with the study on Thai *A. cornea* (Walker *et al*., 2023). These data suggest that the optimal culture medium, temperature, and pH conditions are conducive to the growth of the studied organisms (Xu and Yun, 2003).

Cereal grains serve as substrates for mushroom propagation before cultivation. Table 2 demonstrated that oat and barley grains are most suitable for the propagation of the white strain ABI-F000301, whereas paddy grain is appropriate for the brown strain ABI-F000302. According to Pyra *et al.* (2016), paddy grain, maize grains, and rubber sawdust were identified as the best substrates for *Auricularia* mycelial propagation, requiring a minimum of 16 and 18 days, respectively, in the grains (Pyra *et al.,* 2016). Thus, paddy was selected as the raw material for mushroom propagation due to its effectiveness and lower cost than other grains. This finding aligns with the research conducted by Walker (Walker *et al*., 2023).

Mushrooms are nutritious and can be cultivated using bio-waste, agricultural, or agro-industrial residues (Atila, 2017; Sánchez, 2010). *Auricularia* species, in particular, possess extracellular enzymes that can effectively degrade lignocellulose-based substrates (Adenipekun *et al.,* 2015; Lu and Tang, 1986; Ma *et al.,* 2011). Cellulose and lignin are crucial for both mycelial growth and the production of basidiocarps in *Auricularia* (Adenipekun *et al.,* 2015; Irawati *et al.,* 2012), leading to the frequent use of sawdust as a primary substrate (Abd Razak *et al.,* 2013, Bandara *et al.* 2017, Liang *et al.,* 2019). Moreover, research indicates that mycelial growth is notably slower in high-nitrogen substrates than those with lower nitrogen content (Abd Razak, 2013; Yang *et al.,* 2013). Bandara's findings showed that *A. thailandica* demonstrated optimal mycelial growth on sawdust that contained relatively low nitrogen levels (Bandara *et al.,* 2017). Rubber sawdust, which is high in cellulose and lignin (40.11% and 24.15%, respectively) and has a low nitrogen content of 0.19% (Nam *et al.,* 2020), appears to be advantageous for growing several strains of Thai *A. cornea* (Walker *et al*., 2023) and shows similar results

in *A. delicata* (Wang *et al*., 2016). Thus, rubber wood sawdust is an economically viable substrate for cultivating wood ear mushrooms in Vietnam.

Table 3 indicates that the time required for the two strains of *A. cornea*, ABI-F000301, and ABI-F000302, to colonize the spawn bags fully was 30 to 32 days, respectively. After colonization, it took 63 to 64 days to harvest the mushrooms for the first time since inoculation. The biological efficiency of these strains was found to be 84.80% for the white strain (ABI-F000301) and 102.45% for the brown strain (ABI-F000302). In contrast, according to Khan (Khan *et al.,* 2023), the quickest time to harvest a wild strain AC13 of *A. cornea* was 80 days; however, the biological efficiency of wild strains is typically low, with AC24 reaching only 53%. Thongklang noted that the primordia of *A. cornea* appeared on day 76, resulting in a biological efficiency of 72.46% (Thongklang *et al.,* 2020). For another white strain of *A. cornea*, Bandara found that it took 53 days to colonize the spawn bags, yielding a biological efficiency of just 16.5% (Bandara *et al.,* 2020). Notably, a recent study in Brazil using *Eucalyptus* sawdust substrate for *A. cornea* achieved a biological efficiency of 106.9% (Drewinsk *et al.,* 2024). Despite being supplemented with only 1.5% rice bran and corn bran each in a rubber sawdust substrate, the two strains of *A. cornea*, ABI-F000301 and ABI-F000302, demonstrated efficient cultivation. This suggests that *A. cornea* could be optimized for production, providing a stable economic opportunity for farmers engaged in mushroom cultivation.

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