IDENTIFICATION AND MYCELIAL CULTIVATION OF BLACK LINGZHI MUSHROOM *Amauroderma* COLLECTED IN BU GIA MAP NATIONAL PARK, VIETNAM

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ABSTRACT

Amauroderma and *Ganoderma* are two large genera of fungi belonging to the Ganodermataceae family. Many species of *Amauroderma* have been studied and explored for their utilization as medicinaland functional foods. This study aims to identify and assess the mycelial growth capabilities of two wild species of *Amauroderma*, *A. subresinosum* and *A. rugosum*, collected in Bu Gia Map National Park, Binh Phuoc province, Vietnam. Results conducted from the study on morphological characteristics and nucleotide sequence analysis of the ITS region showed that the *Amauroderma* strain Am16 was quite similar to *Amauroderma subresinosum*, whereas the Am25 one was considered to be highly similar to *Amauroderma rugosum*. In the aspect of the growth potential of these strains, MEA and PDA media at pH of 6.0 and temperatures of 25 - 30°C were reported to be most favorable for mycelial of *A. subresinosum* strain Am16 while PDA medium at pH of 6.0 and temperature of 30°C was suitable for the mycelial of *A. rugosum* strain Am25. The mycelial of *Amauroderma* grows well on rice wheat, and millet in which rice and wheat were the most suitable for cultivating the mycelial of *Amauroderma* strains Am16 and Am25 presented by the high growth rate, from 9.15 ± 0.048 to 8.27 ± 0.386 mm/day and 10.19 ± 0.427 to 10.25 ± 0.315 mm/day and a compact density.

Keywords: *Black Lingzhi, Amauroderma, mycelial, medium, temperature, pH, cereals grain.*

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1. INTRODUCTION

Amauroderma Murril is characterized by annual basidiocarp, usually stipitate, dull to laccate pileus, a di-trimitic hyphal system and globose to ellipsoid basidiospores without a truncated apex, which is double-walled with a hyaline, smooth exospore, thick and rarely smooth endospore wall [1]. *Amauroderma* species such as *A. subresinosum, A. rugosum, A. rude* were considered to have high medicinal value. Many compounds extracted from these species possess high biological activity and have great potential in producing vegetable originated - medicines and functional products [2, 3, 4]. In addition, *A. rugosum* can be used as activated enzymes such as carbohydrate hydrolase, peroxidase, and laccases to decompose organic matter in forests. With this characteristic, *A. rugosum* is applied in industry, biofuel production, and environmental protection [5].

Up to now, limited studies, especially studies on the characteristics of culturing the mycelial and fruiting bodies of *Amauroderma* fungi in the world and Vietnam as well, have been done. Although numerous species of *Amauroderma* have already been discovered, mankind's utilization was under its potential. In Vietnam, several researches into the classification and propagation of the genus *Amauroderma* have been implemented. Le Xuan Tham (2005) [6] and Nguyen *et al.* (2013) [7] focused on the biodiversity of the family *Ganodermataceae*, including the genus of *Amauroderma*. Nguyen and Khanh (2017) reported on the influence of ecological factors on the distribution of the *Amauroderma* genus in the Central Highlands [8]. Tran Thi Phu and Trinh Tam Kiet (2019) discovered many species of *Amauroderma* in the Ngoc Linh Mountain, Quang Nam [9]. Le Xuan Tham (1996) [10] and Ngo Anh *et al.* (2016) [11] successfully cultivated *A. elmerianum* and *A. subresinosum*. Ha Thu Tran *et al.* (2023) determined the optimal conditions for cultivating the mycelial of *A. subresinosum* [12].

Bu Gia Map National Park (NP) is located at 12°8'30" to 12°7'3" North latitude and from 107°3'30" to 107°4'30" East longitude belonging to Bu Gia Map district, Binh Phuoc province, with a buffer zone area of 15,200 ha, including 7,200 ha in Binh Phuoc province and 8,000 ha in Dak Nong province. Considered a transitional area between the Central Highlands and the Southeast, Bu Gia Map National Park possesses a diversified ecosystem with many rare species involving the fungal flora, with 71 species of macrofungi belonging to 13 orders and 25 families. Many species of mushrooms have valuable medicinal and food resources, such as *Termytomyces clypeatus*, *Pleurotus ostreatus*, *Ganoderma lucidum* and *Tremella fuciformis* [13]. Therefore, studying mushroom species in the genus of *Amauroderma* in Bu Gia Map National Park should create excellent opportunities for mankind's purposed utilization in terms of biologically originated medicines production in Vietnam generally and in Binh Phuoc notably.

2. MATERIAL AND METHOD

2.1. Mushroom collections and isolations

Mushroom specimens collected at Bu Gia Map National Park include Am16 (GPS: 12°08'53.9"N 107°10'30.3"E) and Am25 (GPS: 12°08'55.1"N 107°10'28.4"E) were processed and isolated by vegetative tissue on PDA (Potato Dextrose Agar) medium and then preserved at 10°C in the Mushroom Research and Development Center.

2.2. Morphological study

Morphological characteristics, size, color, and GPS were recorded when collecting fungal specimens in the wild. The specimens were dried at 45°C [14]. Macroscopic morphological characteristics were described on the basis of dry specimens and observed under an optical microscope according to the method of Li *et al.* (2014) [15]. Microscopic characteristics such as hyphae and basidiospores were observed and photographed on Cotton Blue and Melzer reagent-stained slides. The following abbreviations were also used in the report of the study: IKI: Melzer reagent, IKI-: non-amyloid and dextrinoid; KOH: 5% potassium hydroxide; CB: Cotton Blue. Measurements were performed by microscope of up to 1000 times magnifications with the assistance of Piximetre version 5.2 software.

2.3. Molecular biological analysis

2.3.1. DNA extraction, PCR amplification and Sequencing

The total DNA of two *Amauroderma* specimens was extracted from the mycelial according to the modified method of Winnepenninckx *et al.* (1993) [16]. The ITS gene region was amplified using the primer pair TS1/ITS4 (5'-TCCGTAGGTGAACCTGCGG-3' and

5'- TCCTCCGCTTATTGATATGC-3'). The total volume of one reaction was 25 µL, including 5.0 µL reaction buffer, 0.5 µL DNA polymerase, 0.5 µL forward primer ITS1, 0.5 µL reverse primer ITS4, 15.5 µL distilled water, and 3.0 µL template DNA. The cycle of each PCR reaction: 98°C for 2 minutes, continued by 35 cycles of 98°C for 15 seconds, 55°C for 30 seconds, 72°C for 60 seconds, and ended with 72°C for 7 minutes before keeping the sample at 4°C. PCR products were electrophoresed on 1.2% agarose gel, then purified with an ExoSAP-IT kit and sequenced at 1st Base Company, Malaysia.

2.3.2. Phylogenetic analysis

The obtained nucleotide sequence data were compared, arranged and edited using Chromas 2.6.6 and BLAST tools on Genbank to identify homologous species. The phylogenetic tree was constructed using the maximum likelihood method and the MEGA X tool with a bootstrap value of 1000 replicates [17]. The reference ITS sequences were nucleotide sequences of species/varieties in the same genus retrieved from Genbank based on the publications of Costa-Rezende *et al.* (2017) and Sun *et al.* (2020) [18, 19].

2.4. Effect of media on mycelial growth

The *Amauroderma* mycelial (Am16, Am25) were cultivated in four different media MEA (Malt Extract Agar, including (g/L): Peptone 3.0, malt extract 30, agar 15, distilled water 1000 ml); PDA (Potatoes Dextrose Agar, including (g/L): potato 200, D-glucose 20, agar 20, distilled water 1000 ml); SDA (Sabouraud Dextrose Agar, including (g/L): D-glucose 20, peptone 10, agar 15, distilled water 1000 ml); CZ (Czapek dox, including (g/L): Sucrose 30, $MgSO_4.7H_2O$ 0.5, K_2HPO_4 1.0, KH_2PO_4 1.0, $NaNO_3$ 3.0, KCl 0.5, $FeSO_4.7H_2O$ 0.01, agar 20, distilled water 1000 ml). The experiment was repeated three times.

2.5. Effect of pH on mycelial growth

The optimal medium for culturing was used to assess the appropriate pH value for the mycelial growth of two strains of *Amauroderma* (Am16, Am25). 1M NaOH and 1M HCl solution were used to adjust the pH value of the culture medium before sterilization with 5, 6, 7 and 8. Three replications were implemented in this trial.

2.6. Effect of temperature on mycelial growth

The optimal medium and pH value continued to be used to investigate the appropriate temperature for the mycelial growth of *Amauroderma* mushrooms. The mycelial of strains Am16 and Am25 were tested at 20, 25, 30 and 35°C to determine the most suitable temperature. Three replications were implemented in this trial.

2.7. Effect of cereal grains substrates on mycelial growth

Four types of cereal grains, including rice, sorghum, wheat, and millet, were used to evaluate the mycelial growth ability of the fungal strains Am16 and Am25. Cereal grains were first soaked in clean water to remove impure parts and then boiled for 15 minutes. After cooling, cereal grains were mixed with 1.0% $CaCO_3$ powder, put into 200 x 25 mm test tubes (50 grams of substrate each), and sterilized at 121°C for 60 minutes. Each test tube was inoculated with a piece of Am16 or Am25 spawn (10 x 10 mm) and incubated at 25°C under dark conditions [20]. Three replications were implemented in this trial.

2.8. Data collections

The mycelial growth rate, mycelial biomass, and mycelial density indicated the mycelial growth ability of the black Lingzhi strain. The mycelial growth rate (mm/day) was calculated using the method described by Nguyen *et al.* (2019) [21]. Mycelial biomass (grams) was measured by the Wannasawang *et al.* (2023) method [20]. The mycelial was separated from the culture medium by boiling and rinsing hot water, then dried to a

constant weight (13% moisture content). Mycelial density was observed and evaluated at four levels: Compact (C), somewhat compact (SC), somewhat thin (ST) and thin (T) [22].

2.9. Data analyse

The research results were statistically treated using Excel 2019 and GraphPad Prism 9.1 software. The differences between treatments were assessed by one-way ANOVA analysis of variance and multiple group comparisons using Tukey's test at a significant level of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Morphological study

Specimen Am16:

Black Lingzhi - *Amauroderma subresinosum* (Murril) Conner, 1983

= *Fomes subresinosum* Murril, 1908

= *Ganoderma subresinosum* (Murril) Humphrey, 1938

= *Trachyderma subresinosum* (Murril) Imazeki, 1952

The basidiocarp is semi-circular, sessile, flat, wooden hard, about 8 x 20 cm dimension. The pileu surface is dark brown, dull with concentric furrows and many rough wrinkles. The pileu margin is obtuse, shiny black, split into the pileu. The context is creamy white, up to 1.0 cm thick at the base and woody hard. The hymenium surface is dark brown, unstratified, up to 1.0 cm thick. The pore surface is grey, pores circular or polygonal, 3 - 4 per mm (Figure 1a, b, c). The pileipellis composed of apical cells that clavate close together, and are yellowish-brown.

Basidiospores are ovoid to ellipsoid, non-truncated shape, pale yellow color, double-walled structure and about 14.5 - 18.2 × 9.5 - 12.1 µm dimension. The hyphal system is trimitic, generative hyphae thin-walled, branched, 2.0 - 4.0 µm; skeletal hyphae thick-walled, branched, nearly solid, 2.0 - 3.0 µm; binding hyphae thick-walled, branched, 1.5 µm (Figure 1d, e).

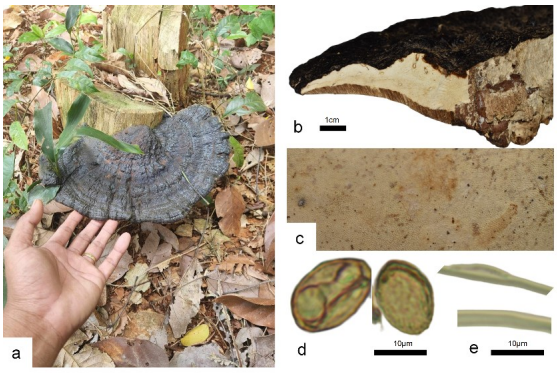

Figure 1. Morphological characteristics of specimen Am16

Notes: a: Basidiocarp; b: Context and tubes layer; c: Pore surface; d: Basidiospores, e: Hyphal system.

Specimen of Am25:

Black Lingzhi - *Amauroderma rogusum* (Blum & T. Nees) Torrend, 1920

= *Amauroderma amoiense* J.D. Zhao & L.W. Hsu, 1983

= *Amauroderma* wuzhishanense Zhao & X.Q. Zhang, 1987.

The basidiocarp is nearly circular and convex, stipitate, about 4.0 x 5.0 cm. The stip is cylindrical and centered; its surface is gray and covered with tomentose. The pileu surface is dark brown, gray to purple-brown, dull, concentric furrows, and consists of many rough wrinkles. The pileu margin is obtuse, shiny black, and split into small ones. The context is light brown, up to 0.8 cm thick at the base, and wood hard. The hymenium surface is dark brown, unstratified, and up to 1.0 cm thick. The pore surface is gray when fresh, color changing to blood red when bruised, then quickly darkening; pores circular or polygonal, 5 - 6 per mm (Figure 2a, b, c). The pileipellis is composed of apical cells that clavate close together and are yellowish-brown.

Basidiospores are globose, non-truncated, pale yellow, double-walled, about 9.8 - 11.9 x 8.2 - 9.5 µm. The hyphal system is trimitic; generative hyphae thin-walled, branched, 1.5 - 2.0 µm; skeletal hyphae thick-walled, branched, nearly solid, 2.0 - 3.0 µm; binding hyphae thick-walled, branched, 2.0 µm (Figure 2d, e).

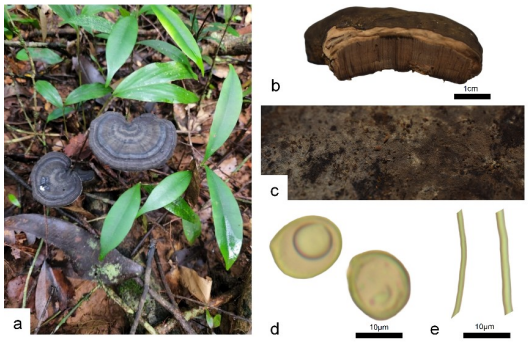

Figure 2. Morphological characteristics of specimen Am25

Notes: a: Basidiocarp; b: Context and tubes layer; c: Pore surface ; d: Basidiospores, e: Hyphal system.

Results obtained from the study on macroscopic and microscopic morphological showed that specimen Am16 is similar to *Amauroderma subresinosum* and specimen Am25 is similar to *A. rugosum*, described in the document of Happuarachchi *et al.* (2018) [23] characterized by basidiocarp annual, sessile, weakly laccate, woody; pileu surface dark brown, radially rugose, concentrically sulcate, about 16 - 20 × 11 - 13 cm; margin obtuse, irregular, yellowish-brown; context up to 2.0 cm thick, light orange; hymenium indistinctly stratos; pore surface greyish orange; pores circular, 3 - 5 per mm; basidiospores are ellipsoid to elongate, pale orange to greyish orange. The basidiocarp of *A. rugosum* is stipitate, weakly laccate, corky, about 2.5 - 3.5 × 2.0 - 2.5 cm; margin blunt or wavy; context up to 8 mm thick, light brown; hymenium up to 1.2 cm long; pore surface is brownish orange to bruising brown; pores circular, 3 - 5 per mm; basidiospores is subglobose, brownish yellow, about 12.1 - 13.1 × 10.2 - 11.2 µm; pileipellis is a hymeniderm with clavate like cells, brownish orange; hyphal system trimitic; generative hyphae, hyaline, thin-walled with clamp connections; skeletal hyphae, thick-walled, nearly solid, sometimes branched, orange white; binding hyphae, thick-walled, branched, nearly solid, orange white.

3.2. Molecular biological analysis

Table 1. Results of ITS region sequencing and Blast on NCBI of 2 samples Am16 và Am25

Samples	Lenght of sequenced ITS region (bp)	BLASTn results on NCBI		Species identified
		Accession	Percent. identity (%)	
Am16	675	MK119823 MZ354871 FJ154784 MH106885 MF680429	100 100 99.83 100 99.68	*Amauroderma subresinosum*
Am25	557	KJ531665 KJ531664 KU219982 MZ354884 MZ354887	98.63 98.63 99.48 99.44 99.44	*Amauroderma rugosum*

Totally purified total DNA was used as a template for the PCR reaction to amplify the ITS gene area with the ITS1/ITS4 primer pair. Analysis of ITS sequencing data showed that the nucleotide sequence of sample Am16 was 675 bp long and sample Am25 was 557 bp long. Based on the results of the comparison and searching for similar ITS sequences in the NCBI database resulting from the BLASTn tool, the nucleotide sequence of the ITS region of sample Am16 has a high level of genetic similarity (99.68 to 100%) with the species *A. subresinosum* with reference codes MK119823, MZ354871, FJ154784, MH106885, MF680429. In contrast, the ITS region of sample Am25 has a high level of similarity (98.63 to 99.44%) with the reference sequences of the species *A. rugosum* KJ531665, KJ531664, KU219982, MZ354884, MZ354887 (Table 1).

The phylogenetic tree of two samples (Am16, Am25) and 22 ITS sequences of the same species on Genbank constructed by the Maximum likelihood method that applied the MEGA X tool with a bootstrap value of 1000 was shown in Figure 3. Specimen Am16 collected at Bu Gia Map National Park with sequences of the species *A. subresinosum* (FJ154784, MZ354871, MK119823, MH106885, MF680429, ON202920) formed a separate evolutionary branch closely related to each other with a bootstrap value of 100%. The Am25 sample, together with the sequences of *A. rugosum* (KU219982, KJ531664, MZ354887, MZ354884, KJ531675, KJ531665, MK077646), formed another clade, with a bootstrap value of 100%.

According to morphological and ITS nucleotide sequence analyses, it is obvious that the black Lighzhi mushroom strains Am16 and Am25 collected at Bu Gia Map National Park have the same origins as *A. subresinosum* and *A. rugosum*, as reported by lots of scientists all over the world. In the deep study of microscopic characteristics, Costa-Rezense *et al.* (2017) [18] and Sun & *et al.* (2020, 2022b) [19, 24] stated that *A. subresinosum* was different from other species of the genus presented by fibrous form, pale white color and basidiospores of the ellipsoid to ovoid shape without a truncated apex. *A. rugosum* has macroscopic and microscopic morphological characteristics similar to *Amauroderma s.str*. However, the pore surface of *A. rugosum* is distinguished by a rapid reaction to turn blood-red when bruised. Following the opinion of Steyeart (1972) [25], Sun *et al.* classified *A. subresinosum* into the *Magoderma* genus together with *M. infundibuliforme*; *A. rugosum* into the new genus *Sanguinoderma* together with *S. rude, S. guangdongense, S. infundibulare, S. longistipitum, S. melanocarpum, S. microsporu, S. tricolor*. The genera *Magoderma* and *Sanguinoderma* have been officially updated on the Mycobank and Index Fungorum databases.

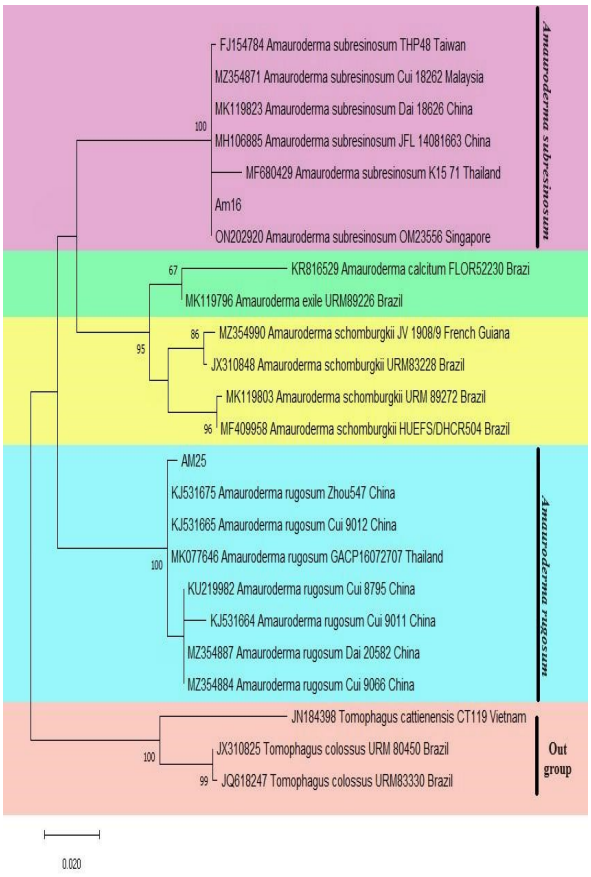

Figure 3. Maximum likelihood phylogenetic tree of strains Am16 and Am25 based on ITS region nucleotide sequences. Bootstrap frequencies are greater than 50%.

3.3. Effect of media on mycelial growth

Table 2. Mycelial growth rate and mycelial biomass of two *Amauroderma* strains on media

Media	*Amauroderma* Am16			*Amauroderma* Am25		
	Mycelial growth rate (mm/day)	Mycelial biomass (gram)	Mycelial density	Mycelial growth rate (mm/day)	Mycelial biomass (gram)	Mycelial density
MEA	$6.26^{a} \pm 0.119$	$0.191^{a} \pm 0.0034$	SC	$4.78^{c} \pm 0.097$	$0.141^{c} \pm 0.0060$	C
PDA	$6.52^{a} \pm 0.159$	$0.222^{a} \pm 0.0084$	SC	$6.64^{a} \pm 0.090$	$0.244^{a} \pm 0.0031$	SC
SDA	$5.59^{b} \pm 0.175$	$0.172^{b} \pm 0.0030$	SC	$5.62^{b} \pm 0.103$	$0.166^{b} \pm 0.0123$	C
CZ	$1.33^{c} \pm 0.429$	$0.010^{c} \pm 0.0019$	T	0.0^{d}	0.0^{d}	-

Note: Values followed by different letters in the same columns indicated the significant differences at $P < 0.05$ after Ducan method.

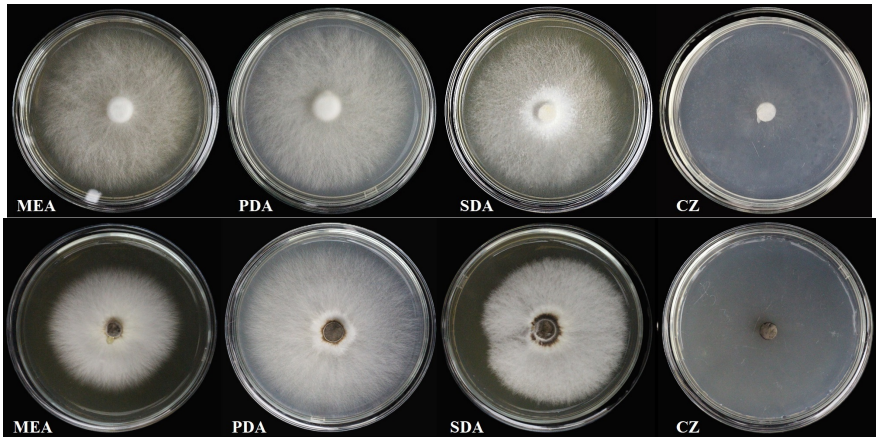

Figure 4. The mycelial of strains Am16 and Am25, after five days of incubating on different media

Data in table 2 and figure 4 showed that *Amauroderma* strain Am16 grows best on MEA and PDA medium indicated by high mycelial growth rate, from 6.20 ± 0.150 to 6.52 ± 0.159 mm/day; large mycelial biomass, from 0.191 ± 0.0034 to 0.222 ± 0.0084 grams, and compact mycelial density (C). In contrast, PDA media was considered suitable for mycelial strain Am25, 6.64 ± 0.090 mm/day, 0.244 ± 0.0031 grams, and somewhat compact density (SC), respectively. On the CZ medium, the *Amauroderma* mycelial (Am16, Am25) grew weakly, even no success obtained.

The medium is essential in fungal culture because it provides the nutrients for mycelial growth. Research results showed that the medium affects 78% of the growth rate of mycelial. Up to now, Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapek dox Agar (CDA), Yeast Malt Extract Agar (YMEA), Corn Meal Agar (CMA), Sabouraud Dextrose Agar (SDA), Malt Yeast Extract (MYE). The two media types, PDA and MEA, bring maximum efficiency due to their rich, complex composition for the proper growth of fungi [26]. This study also identified PDA and MEA as two media types suitable for developing *Amauroderma* mycelial, the most ideal of which is PDA.

3.4. Effect of pH on mycelial growth

As mentioned above, PDA medium is the optimal medium to investigate the mycelial growth ability of *Amauroderma* strains (Am16, Am25) at different pH values in which pH of 5.0 - 6.0 was considered to be favorable and pH 0f 6.0 gave the best result indicated by high growth rate (6.55 ± 0.131 mm/day), mycelial biomass (0.226 ± 0.0078

mm/day), and somewhat compact density (SC) whereas pH of 6.0 - 7.0 was suited to the mycelial of the strain Am25, the growth rate of 6.68 ± 0.127 mm/day, mycelial biomass of 0.247 ± 0.0036 grams, somewhat compact density were recorded at pH of 6.0 (Table 3, figure 5).

Table 3. Mycelial growth rate and mycelial biomass of *Amauroderma* strains at different pH values

pH	*Amauroderma* Am16			*Amauroderma* Am25		
	Mycelium growth rate (mm/day)	Mycelial biomass (gram)	Mycelial density	Mycelium growth rate (mm/day)	Mycelial biomass (gram)	Mycelial density
5	$6.19^{b} \pm 0.142$	$0.187^{b} \pm 0.0098$	SC	$4.49^{d} \pm 0.180$	$0.100^{d} \pm 0.0088$	SC
6	$6.55^{a} \pm 0.119$	$0.226^{a} \pm 0.0078$	SC	$6.68^{a} \pm 0.127$	$0.247^{a} \pm 0.0036$	SC
7	$5.18^{c} \pm 0.110$	$0.119^{c} \pm 0.0041$	SC	$6.25^{b} \pm 0.104$	$0.197^{b} \pm 0.0089$	SC
8	$4.08^{d} \pm 0.171$	$0.098^{d} \pm 0.0060$	C	$5.10^{c} \pm 0.112$	$0.143^{c} \pm 0.0070$	SC

Note: Values followed by different letters in the same columns indicated the significant differences at P < 0.05 after Ducan method.

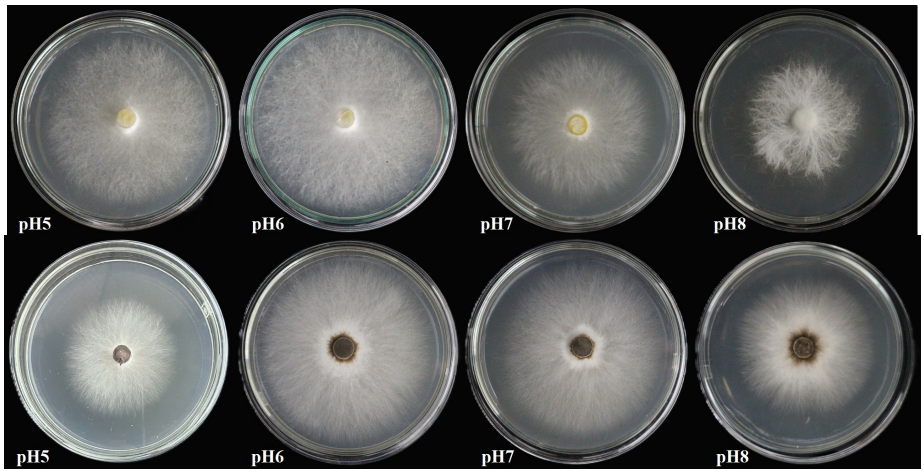

Figure 5. The mycelial growth of strains Am16 and Am25, after 5 days of incubating at different pH values

The pH of the environment affects the functional differentiation and the ability of the cell membrane to absorb essential nutrients. For wood-growing fungi, the optimal pH ranges from 3.0 (solid acid) to 9.0 (strong alkaline), such as *Ganoderma australe* pH 7.0 - 8.0, *G. lucidum* pH 5.0 - 12.0, *Hericium erinaceus* pH 6.0 and *Trametes versicolor* pH 6.0 - 7.0 [26]. In this study, the *A. subresinosum* strain Am16 is suitable for pH 5.0 - 6.0, optimal pH 6.0; the *A. rugosum* strain Am25 is ideal for pH 6.0 - 7.0, optimal pH 6.0. The results of this study are consistent with the studies of Gaijuan *et al.* (2015) [27] and Ha Thu Tran *et al.* (2023) [12] on *A. subrugosum* and *A. subresinosum* species.

3.5. Effect of temperature on mycelial growth

The suitable temperature for the mycelial of *Amauroderma* strains Am16 and Am25 to grow is 25 - 30°C. At 35°C, the growth of mycelial of *Amauroderma* is badly affected. It is obvious that the mycelial of strain Am16 had the best growth at a temperature range of 25 - 30°C, presented by a high growth rate (6.67 ± 0.106 to 6.72 ± 0.069 mm/day), big mycelial biomass (0.209 ± 0.0049 to 0.219 ± 0.0070 grams) and somewhat compact density (Table 4, figure 6). Meanwhile, the strain

Am25 showed the most optimal growth ability at 30°C, with a growth rate of 6.89 ± 0.071 mm/day, mycelial biomass of 0.309 ± 0.0081 grams, and a compact mycelial density (Table 4, Figure 6).

Table 4. The mycelial growth rate and biomass of *Amauroderma* strains at different temperatures

Temperature (°C)	*Amauroderma* Am16			*Amauroderma* Am25		
	Mycelium growth rate (mm/day)	Mycelial biomass (gram)	Mycelial density	Mycelium growth rate (mm/day)	Mycelial biomass (gram)	Mycelial density
20	$4.36^{b} \pm 0.150$	$0.104^{b} \pm 0.0069$	SC	$4.34^{c} \pm 0.079$	$0.083^{c} \pm 0.0031$	ST
25	$6.54^{a} \pm 0.106$	$0.225^{a} \pm 0.0044$	SC	$6.67^{b} \pm 0.069$	$0.246^{b} \pm 0.0072$	SC
30	$6.72^{a} \pm 0.069$	$0.230^{a} \pm 0.0062$	SC	$6.89^{a} \pm 0.071$	$0.309^{a} \pm 0.0081$	C
35	-	-	-	-	-	-

Note: Values followed by different letters in the same columns indicated the significant differences at $P < 0.05$ after Ducan method.

Temperature is the main abiotic factor affecting the growth and development of mushroom mycelia through the respiration and biosynthesis processes, as well as the assimilation and transport of sugar and nitrogen. The adaptation of mushroom strain to temperature is generally related to genetic characteristics and climatic conditions of the sites collected [26]. A temperature range of 20°C to 30°C considered favorable for basidiomycetes was reported in most studies concerned. It is also in the same direction for *Amauroderma* strains Am16 and Am25 in this research.

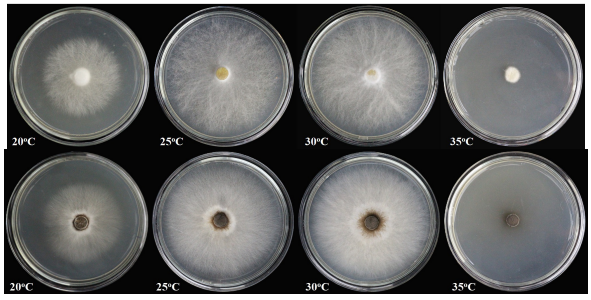

Figure 6. The mycelial growth of strains Am16 and Am25, after 5 days of incubating at different temperatures

3.5. Effect of cereal grains substrates on mycelial growth

Rice, wheat, and barley could be used as suitable cereal substrates for the propagation of *Amauroderma* mycelia (Am16, Am25) better than millet (Figure 7). Among which rice and wheat substrates gave the best performance for the growth of Am16 and Am25 strains as shown by the best growth rate, reaching 9.15 ± 0.048 to 8.27 ± 0.386 mm/day and 10.19 ± 0.427 to 10.25 ± 0.315 mm/day, respectively (Figure 7a), and compact density (Figure 7b - c).

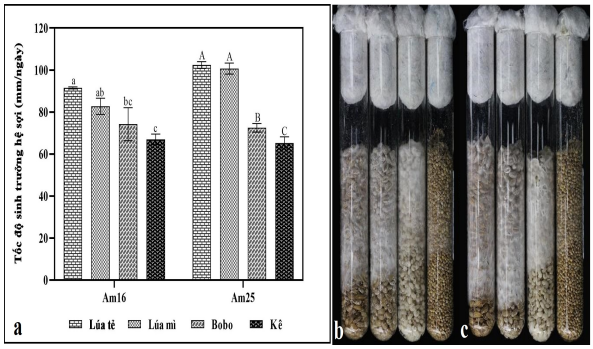

Figure 7. The growth of mycelium of strains Am16 and Am25 on cereal grain substrates

Notes: a- Growth rate; b- Mycelial of strain Am16; c- Mycelial of strain Am25.

Cereal grains are commonly used as media for mushroom spawn production because of their rich nutritional content and not clumped structure, which allows the spawn to be evenly distributed in the substrate [28], of which rice and wheat were

considered suitable for *A. subresinosum* strain Am16 and *A. rugosum* strain Am25. In fact, wheat grains were most popularly used in this field because they act as a carbohydrate reserver that provides sufficient nutrients for the growth of mycelium [28], whereas rice grains were suitably used for the mycelial multiplication of some mushrooms, such as *Pleurotus sajor caju* [29] and *Ganoderma lucidum* [30].

4. CONCLUSION

Morphological characteristics of *Amauroderma* strains Am16 and Am25 collected at Bu Gia Map National Park, Binh Phuoc province, were similar to two species *A. subresinosum* and *A. rugosum* in which train Am16 has the same origin with *A. subresinosum*, (96.68 to 100% identity) while strain Am25 is involved in *A. rugosum* (98.63 to 99.44% identity) based on ITS region nucleotide sequence analysis and phylogenetic tree. MEA and

PDA media at pH of 6.0 and temperature of 25-30°C were regarded the most suitable for the growth of mycelial of *A. subrsinosum* strain Am16 whereas *A. rugosum* strain Am25 grows optimally on PDA one at pH of 6.0 and 30°C temperature.

The substrate of cereal grains, including rice and wheat, can be suitably used to produce mushroom spawn of *Amauroderma* strains Am16 and Am25.

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