



Optimal conditions for mycelial growth of medicinal mushrooms belonging to the genus *Hericium*

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Received: 21 October 2021 / Revised: 29 July 2022 / Accepted: 31 July 2022 / Published online: 31 August 2022
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Abstract

Hericium is a well-known genus that comprises edible and medicinal mushrooms with fleshy, distinctive white spines that hang from a tough, unbranched clump, and grows on dying or dead wood. In preparation for the artificial cultivation of these mushrooms in Thailand, an optimization of mycelial growth on different agar culture media, for various conditions (including temperature, pH, cereal grains, and agricultural waste, carbon sources, nitrogen sources, and the ratio of media components) was carried out. For this study, three strains of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, and MFLUCC 21-0020) were favorably grown on OMYA medium, at 25 °C and at a pH of 4–4.5, while one strain of *H. erinaceus* (MFLUCC 21-0021) grew favorably on CDA medium, at 25 °C and pH 5.5. The favorable condition for *H. coralloides* (MFLUCC 21-0050) growth was MYPA medium, at 30 °C and pH 5.5. All five strains presented higher mycelial growth on wheat grain. Carbon and nitrogen sources promoted higher rates using molasses and yeast extract respectively, and a ratio of these media components of 10:1 resulted in higher growth rates. The data presented provide growth requirements that will be useful in the future development of the cultivation of *Hericium* mushrooms.

Keywords Grain spawn · Media components · Medicinal mushroom · pH · Temperature

Introduction

Hericium Pers. is a well-known genus of medicinal mushrooms that belongs to *Hericiaceae*. They are known by different names including bear's head, bearded tooth, hog's head fungus, hóutóugū, lion's mane, monkey's head, old man's

beard, pom pom, white beard, and yamabushitake (Thongbai et al. 2015; Sangtitanu et al. 2020). There are 66 records in the Index Fungorum (<http://www.indexfungorum.org/Names/NAMES.ASP>) and 48 records of taxa in MycoBank (<https://www.mycobank.org/>) and He et al. (2019) record 23 species of *Hericium*. The genus is cosmopolitan but its species occur in higher altitudes in warmer climates, mostly found in North and South America, Europe, Australia, China, Japan, and Asia (Ginns 1985; Boddy et al. 2011; Grumezescu and Holban 2018). In addition, one of the species known from Africa was actually collected around the equator in a Cameroonian mountain range (Jumbam et al. 2019). *Hericium* mushrooms have a serrated basidiome, with members that are classified as white rotters (Hallenberg et al. 2013). The basidiomes of these saprotrophic fungi generally grow with short stalks on a wide range of hardwood, in particular on old or dead broadleaved trees (Mizuno 1999).

Generally, *Hericium erinaceus* has been characterized to have a branched or unbranched hymenophore with structures supporting thorns of various lengths and growing in single or multiple clumps. They have been described as hanging, meaty, at first white, yet becoming yellowish (Ginns 1985), with fragile ice-like spines hanging from scaffolds, and

Section Editor: Martin Rühl

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branched tissues, which mostly grow on dead or rotting wood (Pallua et al. 2012). *Hericium coralloides* are commonly known as comb tooth fungus, and coral tooth fungus (Bisko et al. 2018; Zhang et al. 2019). It has basidiome with peculiar fruiting bodies resembling white coral (Wittstein et al. 2016), and its branches are hanging spines that become brittle and turn a light shade of yellowish-brown when they reach a mature stage (Buchanan 2021).

Hericium has been used to treat various diseases in traditional Chinese medicine (TCM) and has been a valuable source of biologically active compounds (Sullivan et al. 2006; Sliva 2012). The fruiting bodies of *H. erinaceus* have been considered for use as an antioxidant, antitumor immunomodulatory, and anti-inflammatory effects, as well as for the treatment of Alzheimer's disease (Ramberg et al. 2010). The chemical composition of *H. erinaceus* has been shown to have an important biological activity such as hericenones and erinacines that are contained in the fruiting bodies and the mycelium of *H. erinaceus*, respectively (Thongbai et al. 2015). Basidiomes of *H. coralloides* contain coralocins A, B, and C, which were shown to induce nerve growth factor and brain-derived neurotrophic factor expression in humans, and showed moderate cytotoxicity (Wittstein et al. 2016).

In Thailand, *H. erinaceus* commercial mushroom was imported from China, the first cultivation in Chiang Rai, North Thailand (Kalong 2010). Bunroj et al. (2017) reported the cultivation of monkey's head mushroom in the East of Thailand, in which all strains of *Hericium* had adapted and produced fruiting bodies in this region. In addition, the Khun Wang Royal Project Development Center reported that the product of Lion's Mane is an economic crop that increases income for the villagers (unpublished data).

General cultivation parameters of *Hericium* species for fruiting body production are as follows: spawn run at 21–24 °C for 10–14 days, primordia formation at 10–15.6 °C for 3–5 days, and fruiting body development at 18–24 °C for 4–5 days (Stamets 2011). The protein content of fruit bodies was also raised by increasing the rice bran ratio in the growth media (Bunroj et al. 2017). While *Hericium* is known in Thailand, it is considered expensive and only consumed in a niche market. The price to sell and buy depends on the season, 1.5–6 \$ per kg in the winter and 8.5–15 \$ per kg in the summer (The mushroom researchers and growers society of Thailand 2013).

Moreover, there is little information on the cultivation, consumption, and properties of *Hericium* in Thailand. Hence, in the present article, we investigated the effects of media, temperature, pH, cereal grain and agricultural substrate source, carbon and nitrogen sources, and media component ratio on the mycelial growth of five *Hericium* strains in order to find medium additives that can enhance the growth of mycelia and shorten the cultivation time. All data are new records for primary *Hericium* cultivation in Thailand.

Materials and methods

Fungal collections and isolations

Five strains of *Hericium* were used in this experiment. Two collections of commercial *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0020) were obtained from the Thai Royal project shop, Chiang Rai Thailand; one *H. erinaceus* (MFLUCC 21-0019) was obtained from Kunming Institute of Botany, Kunming, China, by plating sterile tissues of the mycelial context onto PDA; and two culture collections of *Hericium* from the Institute of Department Microbial Drugs, Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany, include *H. coralloides* (MFLUCC 21-0050) and *H. erinaceus* (MFLUCC 21-0021) which were isolated from basidiomes provided by the commercial mushroom growing company Pilzgarten GmbH, Fabrikstraße 12, 27389 Helvesiek, Germany, by plating sterile tissues of the mycelial context onto YMG agar. The basidiomes of *H. coralloides* had already been used by Wittstein et al. (2016) as starting material for extraction and isolation of the coralocins A-C and other secondary metabolites.

DNA extraction and PCR amplification

DNA was extracted from the mycelium using the Wizard® Genomic DNA Purification kit (Promega Co., Madison, WI, USA) following the manufacturer's protocols. The internal transcribed spacer (ITS) DNA barcode region (Dentinger et al. 2011; Schoch et al. 2012) was amplified with polymerase chain reaction (PCR) using the primer sets ITS4/ITS5 for ITS (White et al. 1990). The high-purity PCR template preparation kit (ChargeSwitch® PCR Clean-Up Kit, Invitrogen) was used in the PCR procedures. An Eppendorf Mastercycler ep Thermal Cycler (Hauppauge, New York, USA) was used for 50 µl of amplifications, with initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 50 °C for 45 s, and a final extension at 72 °C for 1.30 min. PCR products were sent to Biogenomed (Biogenomed Co., Ltd. Thailand) for purification and sequencing. Raw sequence reads were assembled and edited using the SeqMan program (DNASTAR's lasergene sequence analysis software). Sequences were deposited at the National Center for Biotechnology Information (NCBI) GenBank database.

DNA sequence analysis

DNA sequence analyses inferred from the nuclear ribosomal internal transcribed spacer region (nrITS) confirm the identity of the taxon. All sequences were assembled in

SeqMan™ II expert sequence analysis software (DNASTAR). ITS1 and ITS4 sequences of *H. coralloides* strain MFLUCC 21-0050 and ITS5 and ITS4 of *H. erinaceus* strains MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021 from this study were extracted from the whole ITS amplicon sequence using ITS. Moreover, the ITS1 + ITS2 were blasted against the GenBank database to check for similarities with other sequences derived from *Hericium*. The phylogenetic tree was constructed using maximum likelihood (ML) analyses using the Cipres Science Gateway. The reliability of the tree topology was evaluated by bootstrap analysis of 100 replicates using *Pseudowrightoporia crassihypha* (KM107873) and *Wrightoporiopsis amylohypha* (KM107877) sequences as the outgroup. All obtained sequences were submitted to the GenBank database under the accession numbers and other reference sequences were downloaded from GenBank (Table 1).

Effect of media on mycelial growth

Nine different culture media were used in this study, namely carrot dextrose agar (CDA), corn meal agar (CMA), malt extract agar (MEA), malt yeast peptone agar (MYPA), oat meal agar (OMA), oat meal yeast agar (OMYA), potato dextrose agar (PDA), potato dextrose yeast agar (PDYA), and sabouraud dextrose agar (SDA).

The formulation of the culture media is shown in Table 2. Mycelia discs were cut from the advancing margin of 15 days-old pure cultures and were placed on the center of each medium (5 mm diam); the cultures were sealed with Parafilm and incubated in darkness at 25 °C for 15 days. After incubation, the total yield of mushroom mycelium dried weight was harvested on day 15. The mushroom mycelium and medium were separated by boiling the mycelium culture and which was then dried at 45 °C. The experiment was carried out in triplicates.

Effect of temperature for mycelial growth

The optimal medium for mycelial growth was then used as the basis medium to evaluate the impact of different temperatures (16 °C, 20 °C, 25 °C, 30 °C, and 35 °C) and incubated in darkness. After incubation, mushroom mycelium dried weights were harvested on day 15. The experiment was carried out in triplicate.

Effect of pH for mycelial growth

Liquid media were used to evaluate the optimal pH value. The media were adjusted using an Ohaus st3100 pH meter to a pH of 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 with 1-M NaOH and 1-M HCl. The pH range was measured using a digital pH meter before autoclaving. One hundred

Table 1 Details of the selected taxa of *Hericium* used in the phylogenetic analyses

Species	Isolate/voucher	Country	GenBank
<i>Hericium alpestre</i>	NH 13240	Russia	AF506457
<i>H. americanum</i>	DAOM F-21467	Canada	AF506458
<i>H. coralloides</i>	NH 282	Sweden	AF506459
<i>H. coralloides</i>	FCUG 426	France	JQ716935
<i>H. coralloides</i>	MFLUCC 21-0050	Germany	MZ379513
<i>H. erinaceus</i>	MFLUCC 21-0018	Thailand	MZ342890
<i>H. erinaceus</i>	MFLUCC 21-0020	Thailand	MZ342961
<i>H. erinaceus</i>	MFLUCC 21-0019	China	MZ342907
<i>H. erinaceus</i>	MFLUCC 21-0021	Germany	MZ343154
<i>H. erinaceus</i>	NH 12163	Russia	AF506460
<i>H. erinaceus</i>	SCC 1	India	MT448853
<i>H. erinaceus</i>	HEZY ITS region	China	MW131237
<i>H. erinaceus</i>	HE-01	Thailand	MW672510
<i>H. flagellum</i>	N/A	Poland	MG649451
<i>H. rajchenbergii</i>	FCUG GR1997	Argentina	JX403945
<i>H. rajchenbergii</i>	FCUG GR2041	Argentina	JQ716939
<i>H. yumthangense</i>	BSHC:KD-11-146	India	NR155021
<i>H. yumthangense</i>	Cui 10632	China	MH085971
<i>Pseudowrightoporia crassihypha</i>	Yuan 6247	China	KM107873
<i>Wrightoporiopsis amylohypha</i>	Yuan 3579	China	KM107877

Table 2 The formulation of the culture media

Ingredients	Amounts (grams/liter)
Carrot dextrose agar (CDA)	
Carrot	200
Dextrose	20
Agar	15
Corn meal agar (CMA)	
Corn meal infusion	2
Tween 80	7 ml
Agar	15
Malt extract agar (MEA)	
Maltose, Technical.	12.75
Dextrin	2.75
Glycerol	2.35
Peptone	0.78
Agar	15
Malt yeast peptone agar (MYPA)	
Malt extract	3
Yeast extract	3
Peptone	5
Dextrose	10
Agar	20
Oat meal agar (OMA)	
Oat meal	60
Agar	12.5
Oat meal yeast agar (OMYA)	
Oat meal	60
Yeast extract	20
Agar	12.5
Potato dextrose agar (PDA)	
Potatoes	200
Dextrose	20
Agar	15
Potato dextrose yeast agar (PDYA)	
Potatoes	200
Dextrose	20
Yeast extract	20
Agar	15
Sabouraud dextrose agar (SDA)	
Sabouraud dextrose (glucose)	40
Sabouraud peptone	10
Sabouraud agar	15

milliliters of the liquid medium found previously to be optimal for growth was inoculated with active mycelia (discs 0.5 mm in diameter) of the different mushroom strains and was incubated in a shaker at 25 °C, 120 rpm for 14 days. The dried mycelial biomass was recorded after 15 days. The experiments were carried out in triplicate.

Effect of different cereal grains and agricultural substrates for mycelial growth

Fifteen substrates were used to determine the best spawn production, eleven types of cereal grains, including *Avena sativa* (oat), *Coix lacryma-jobi* Linn. (millet), *Hordeum vulgare* L. (barley), *Oryza sativa* (brown rice), *Oryza sativa* L. (rice berry), *Oryza sativa* L. ssp. *indica* (rice), *Oryza sativa* var. *glutinosa* (sticky rice), *Sorghum bicolor* (L.) Moench (red sorghum), *Triticum aestivum* L. (wheat), *Vigna radiata* (mung bean), *Zea mays* L. (corn seed), and four types of agricultural wastes including *Cocos nucifera* Linn. (coir), *Morinda coreia* Ham. (bagasse), and *Oryza sativa* L. ssp. *indica* (rice straw, and paddy). Each substrate was washed and soaked overnight (12–14 h), boiled for 10–15 min, and allowed to cool down in order to keep the moisture content at 50–70%. Fifty grams of each medium was filled into test tubes (borosilicate 16×160 mm.), and autoclaved at 121 °C for 15 min (Thongbai et al. 2017). After being left to cool down for 24 h., the media were inoculated with 5 plugs of *Hericium* active mycelium. All media tubes were incubated at room temperature, and mycelial growth length was recorded every 2 days until fully colonized (21 days) by measuring the length of mycelium from the mycelium on the surface of the substrate to the bottom of the media tube test. The experiments were carried out in triplicate.

Effect of carbon sources on mycelial growth

To screen for a favorable carbon source, the following test was performed using basal media supplemented with nine different carbon sources, including dextrose, fructose, glucose, glycine, lactose, maltose, molasses, sucrose, and xylose. The basal medium was composed of 20 g of tested carbon source, 0.05 g of MgSO₄·7H₂O, 0.46 g of KH₂PO₄, 1.0 g of K₂HPO₄, 120 µg of thiamine-HCl, 20 g of agar, and 1,000 ml of distilled water (Hoa and Wang 2015). The basal medium was adjusted to pH 6 before sterilization. After sterilization, an active mushroom mycelial plug (5 mm diam) of each strain was placed at the center of agar plates containing one of ten carbon sources and incubated in the dark for 15 days at 25 °C. After incubation, *Hericium* mycelial growth was recorded and measured. The experiments were carried out in triplicates.

Effect of nitrogen source on mycelial growth

Four different nitrogen-containing mineral salts (ammonium chloride, ammonium nitrate, potassium nitrate, and sodium nitrate) as well as four organic nitrogen sources (malt extract, peptone, urea, and yeast extract) were tested with the basal media supplement. Twenty grams of each

nitrogen source was added to the basal medium and adjusted to pH 6 before sterile. An active mushroom mycelial plug (5 mm in diam) was placed at the center of the agar plates containing each nitrogen source and incubated in darkness for 15 days at 25 °C. After 15 days of incubation, *Hericium* mycelia were recorded and measured. The experiments were carried out in triplicate.

Effect of media component ratio

Mycelial growth was measured in basal media mixed with 2% molasses (w/v) and then continuously added yeast extract. The ratio of media components was adjusted to 1:1, 5:1, 10:1, 1:5, and 1:10 in each medium, adjusted to pH 6 before sterilization. A 5-mm diameter of active mycelium was placed at the center of the agar plate containing the basal medium mixed with molasses and yeast extract and incubated in darkness at 25 °C. After 15 days of incubation, *Hericium* mycelial growth was recorded and measured, and the mycelium was harvested and the yield was measured. The experiments were carried out in triplicates.

Data collection and statistical analysis

Data were collected for the optimal mycelial growth based on culture media, temperature, pH, cereal grains, agricultural substrate, carbon and nitrogen sources, and the ratio of media components. The dry weight was measured. The optimal growth parameter data was carried out using statistical analysis programs with triplicate. Data were compared to obtain a mean separation performed using Duncan's multiple tests ($p < 0.05$) followed by post hoc tests, and expressed in a one-way ANOVA using the SPSS program (Statistics Package for Social Sciences).

Results

According to the BLAST result of ITS1 + ITS2, the taxonomy of all studied strains with *Hericium erinaceus* and *H. coralloides* was confirmed. The ITS sequence of *H. coralloides* (MFLUCC 21-0050) presented high similarity to *H. coralloides* (99.67%) and *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) showed high similarity to *H. erinaceus* (99.53-99.84%) (Table 3). The ITS dataset included 21 sequences of seven *Hericium* species. The amplification of the ITS region showed fragments of approximately 600 base pairs (bp). The topologies of the phylogenetic trees built with maximum likelihood were similar and clearly indicate that the studied specimen is a member of *H. coralloides* clade which shares 93% sequence identity and *H. erinaceus* which shares 82% sequence identity (Fig. 1).

Effect of favorable culture media on mycelial growth

The optimal agar media for mycelium growth of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) and *H. coralloides* (MFLUCC 21-0050) are shown in Table 4. *H. erinaceus* strains MFLUCC 21-0018 and MFLUCC 21-0020 were optimal in OMYA; strain MFLUCC 21-0019 was optimal in MYPA, OMYA, and MEA media; and CDA was suitable for *H. erinaceus* strain MFLUCC 21-0021. Moreover, *H. coralloides* strain MFLUCC 21-0050 grew the best on MYPA medium.

Effect of temperature on mycelial growth

The optimal temperature of four strains of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-

Table 3 GenBank BLAST search results of ITS1 + ITS2 sequences of *Hericium* species from this study against GenBank database (I, identity; QC, query cover)

Species	Voucher, GB accession no	Most similar ITS1+ITS2 sequences in GenBank	ITS1	ITS2	ITS1 + ITS2	Original voucher	Locality	Reference
<i>Hericium erinaceus</i>	MFLUCC 21-0018, MZ342890	FJ810143, I = 99.53%, QC = 99%	180/180 (100%)	201/204 (99%)	381/384	dd08026	-	GenBank
<i>H. erinaceus</i>	MFLUCC 21-0020, MZ342961	FJ810143, I = 99.69%, QC = 100%	180/180 (100%)	202/204 (99%)	382/384	dd08026	-	GenBank
<i>H. erinaceus</i>	MFLUCC 21-0019, MZ342907	MT448853, I = 99.83%, QC = 100%	179/180 (99%)	204/204 (100%)	383/384	SCC 1	India	GenBank
<i>H. erinaceus</i>	MFLUCC 21-0021, MZ343154	KT693230, I = 99.84%, QC = 99%	180/180 (100%)	204/204 (100%)	384/384	B2_13319025	USA	Raja et al. 2017
<i>H. coralloides</i>	MFLUCC 21-0050, MZ379513	MZ159723, I = 99.67%, QC = 100%	183/184 (99%)	248/249 (99%)	431/433	K(M):250882	UK	GenBank

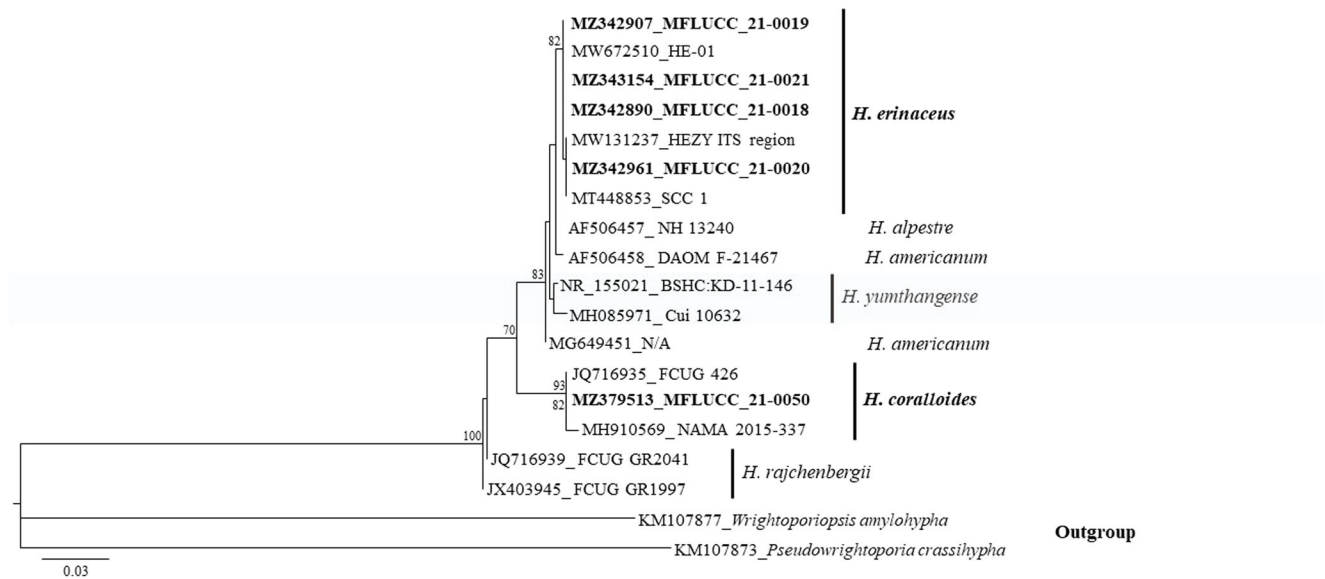


Fig. 1 Maximum likelihood phylogenetic tree inferred from the internal transcribed spacer (ITS), including *Hericium coralloides*, *H. erinaceus*, and the related species. Bootstrap frequencies are equal to or greater than 70% and shown above supported branches

0020, and MFLUCC 21-0021) resulted in a dry weight maximum at 25 °C, while *H. coralloides* (MFLUCC 21-0050) showed a peak of dried weight at 30 °C, and mycelial growth was 16–35 °C. However, the statistical analysis indicated no significant differences in mycelial growth in the temperature range of 16–35 °C (Table 5).

Effect of pH on mycelial growth

The most favorable pH range for mycelial growth of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, and MFLUCC 21-0020) was pH 4–5, while *H. erinaceus* strain

MFLUCC 21-0021 and *H. coralloides* strain MFLUCC 21-0050 grew most abundantly at pH 5.5 (Table 6).

Effect of cereal grain and agricultural substrate

The mycelial growth is shown in Table 7. All *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) were able to grow colonies well on coir, while *H. coralloides* (MFLUCC 21-0050) showed the most abundant colonies on wheat. However, the mycelium characteristics of *Hericium* on coir substrate had the appearance of being thinner than other spawn substrates (Table 8).

Table 4 Dry weight of mycelial growth on different culture media for 15 days (gram)

Culture media	<i>H. erinaceus</i>				<i>H. coralloides</i>
	MFLUCC 21-0018	MFLUCC 21-0019	MFLUCC 21-0020	MFLUCC 21-0021	MFLUCC 21-0050
CDA	0.1354 ± 0.0254 ^{ab}	0.0462 ± 0.0086 ^{cd}	0.0774 ± 0.0131 ^{bc}	0.1118 ± 0.0055 ^a	0.0873 ± 0.0005 ^b
CMA	0.0440 ± 0.0046 ^{ef}	0.0458 ± 0.0180 ^{cd}	0.0399 ± 0.0091 ^{de}	0.0373 ± 0.0068 ^{de}	0.0324 ± 0.0066 ^d
MEA	0.0739 ± 0.0089 ^{de}	0.0807 ± 0.0138 ^a	0.0573 ± 0.0289 ^{cd}	0.0547 ± 0.0076 ^c	0.0724 ± 0.0073 ^{bc}
MYP A	0.0761 ± 0.0049 ^d	0.0835 ± 0.0042 ^a	0.0769 ± 0.0067 ^{bc}	0.0526 ± 0.0025 ^{cd}	0.1119 ± 0.0180 ^a
OMA	0.1144 ± 0.0060 ^{bc}	0.0675 ± 0.0141 ^{abc}	0.0951 ± 0.0230 ^{ab}	0.0872 ± 0.0122 ^b	0.0872 ± 0.0105 ^b
OMY A	0.1496 ± 0.0340 ^a	0.0814 ± 0.0159 ^a	0.1085 ± 0.0199 ^a	0.0641 ± 0.0043 ^c	0.7786 ± 0.0024 ^b
PDA	0.0637 ± 0.0093 ^{d^{ef}}	0.0513 ± 0.0041 ^{bcd}	0.0254 ± 0.0009 ^e	0.0527 ± 0.0166 ^{cd}	0.0382 ± 0.0013 ^d
PDY A	0.0869 ± 0.0214 ^{cd}	0.0695 ± 0.0078 ^{ab}	0.0756 ± 0.0020 ^{bc}	0.0515 ± 0.0137 ^{cd}	0.0397 ± 0.0039 ^d
SDA	0.0364 ± 0.0069 ^f	0.0401 ± 0.0154 ^d	0.0601 ± 0.0161 ^{cd}	0.0226 ± 0.0034 ^e	0.0580 ± 0.0106 ^c

Notes. Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$)

Table 5 Dry weight of mycelial growth on different temperatures after 15 days of incubation (gram)

Temperature (°C)	<i>H. erinaceus</i>				<i>H. coralloides</i>
	MFLUCC 21-0018	MFLUCC 21-0019	MFLUCC 21-0020	MFLUCC 21-0021	MFLUCC 21-0050
16	0.0729 ± 0.0286 ^a	0.2009 ± 0.0146 ^b	0.0660 ± 0.0067 ^b	0.1076 ± 0.0314 ^a	0.0739 ± 0.0234 ^a
20	0.0547 ± 0.0320 ^a	0.1870 ± 0.0276 ^{bc}	0.0980 ± 0.0120 ^{ab}	0.1126 ± 0.0533 ^a	0.0803 ± 0.0036 ^a
25	0.1316 ± 0.0781 ^a	0.2522 ± 0.0178 ^a	0.1403 ± 0.0447 ^a	0.1285 ± 0.0586 ^a	0.0522 ± 0.0060 ^b
30	0.0644 ± 0.0185 ^a	0.1354 ± 0.0399 ^d	0.0704 ± 0.0166 ^b	0.0823 ± 0.0088 ^a	0.0880 ± 0.0161 ^a
35	0.0925 ± 0.0127 ^a	0.1424 ± 0.0184 ^{cd}	0.1050 ± 0.0472 ^{ab}	0.1089 ± 0.0049 ^a	0.0842 ± 0.0047 ^a

Notes. Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$)

Effect of carbon sources on mycelial growth

Nine different carbon sources were tested for promoting mycelial growth of all *H. erinaceus* strains (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) and *H. coralloides* (MFLUCC 21-0050) was higher on the basal medium supplemented with molasses (Table 9).

Effect of nitrogen sources on mycelial growth

The nitrogen source for mycelial growth of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) and *H. coralloides* strain MFLUCC 21-0050 was higher on the basal medium supplemented with yeast extract (Table 10).

Effect of media components ratio

A test for the ratio of media components suitable for favorable growth of *H. erinaceus* and *H. coralloides* was observed using

the basal culture medium, which was adjusted to a ratio of molasses and yeast extract of 1:1, 5:1, 10:1, 1:5, and 1:10, respectively. The most favorable media component ratio was 10:1 for both species as measured by peaks of the dry weight of mycelial growth (Table 10, Fig. 2).

Discussion

Commercial mushrooms such as *Auricularia*, *Flammulina*, and *Lentinula* have been remarkably popular in the world market (Chang and Wasser 2018). In Thailand, these mushrooms are consumed, yet the price is not sufficiently high for export to the world market. *Hericium* has been consumed in a niche market and would likely reach a broader market if cultivation could be made more efficient.

Several studies have investigated the mycelial growth of *Hericium*, including *H. abietis*, *H. alpestre* (currently valid name: *H. flagellum*), *H. americanum*, *H. coralloides*, *H. erinaceus*, and *H. laciniatum* growth on PDA (Han et al.

Table 6 Dry weight of mycelial growth on pH optimal condition after 2 weeks of incubation (gram)

pH	<i>H. erinaceus</i>				<i>H. coralloides</i>
	MFLUCC 21-0018	MFLUCC 21-0019	MFLUCC 21-0020	MFLUCC 21-0021	MFLUCC 21-0050
4	1.0110 ± 0.0770 ^a	0.8665 ± 0.2710 ^a	1.0252 ± 0.0904 ^{ab}	0.2388 ± 0.0116 ^{abc}	0.3021 ± 0.0231 ^b
4.5	0.9382 ± 0.0716 ^{ab}	0.9266 ± 0.0526 ^a	1.1797 ± 0.6005 ^a	0.2425 ± 0.0328 ^{abc}	0.3100 ± 0.0617 ^b
5	0.7612 ± 0.1538 ^c	0.5509 ± 0.0962 ^{ab}	0.6795 ± 0.0409 ^{abc}	0.2427 ± 0.0051 ^{abc}	0.2840 ± 0.2160 ^b
5.5	0.7450 ± 0.0503 ^c	0.4466 ± 0.0442 ^{bc}	1.0019 ± 0.2113 ^{ab}	0.2722 ± 0.0143 ^a	0.5256 ± 0.0542 ^a
6	0.8302 ± 0.0847 ^{bc}	0.3396 ± 0.2757 ^{bcd}	0.9706 ± 0.1192 ^{ab}	0.2545 ± 0.0431 ^{ab}	0.0159 ± 0.0112 ^c
6.5	0.5558 ± 0.0166 ^d	0.1299 ± 0.2116 ^{cd}	0.9243 ± 0.1836 ^{abc}	0.2534 ± 0.0139 ^{ab}	0.0047 ± 0.0011 ^c
7	0.3889 ± 0.0574 ^e	0.2084 ± 0.3270 ^{bcd}	0.8770 ± 0.2493 ^{abc}	0.1731 ± 0.0527 ^d	0.0311 ± 0.0241 ^c
7.5	0.7568 ± 0.0257 ^c	0.0106 ± 0.0063 ^d	0.3659 ± 0.3725 ^c	0.2028 ± 0.0202 ^{bcd}	0.0316 ± 0.0210 ^c
8	0.5105 ± 0.0426 ^{de}	0.3139 ± 0.3180 ^{bcd}	0.4724 ± 0.4144 ^{bc}	0.1915 ± 0.0097 ^{cd}	0.0109 ± 0.0056 ^c

Notes. Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$)

Table 7 Effect of different substrates of spawn on mycelium of *Hericium* growth after 21 days of incubation (centimeters)

Substrate media	<i>H. erinaceus</i>				<i>H. coralloides</i>
	MFLUCC 21-0018	MFLUCC 21-0019	MFLUCC 21-0020	MFLUCC 21-0021	MFLUCC 21-0050
Bagasse	3.1222 ± 0.6598 ^{bcd}	2.4666 ± 1.7306 ^{def}	1.0444 ± 0.1602 ^f	4.6987 ± 0.4309 ^{ab}	5.3062 ± 0.0588 ^f
Barley	3.7827 ± 0.1342 ^{bcd}	1.9211 ± 0.3224 ^{efg}	2.6876 ± 0.4223 ^d	3.1666 ± 0.4522 ^d	3.6247 ± 0.2075 ^g
Brown rice	4.6166 ± 0.3482 ^{abc}	3.8951 ± 0.7033 ^{abc}	4.4987 ± 0.2808 ^b	4.5518 ± 0.2537 ^b	5.2259 ± 0.2118 ^f
Coir	5.4741 ± 0.0669 ^a	5.0543 ± 0.4159 ^a	5.2592 ± 0.1201 ^a	5.2592 ± 0.1091 ^a	7.0086 ± 0.3483 ^{bc}
Corn	0 ^e	1.6382 ± 0.4443 ^{fg}	1.6580 ± 0.6804 ^c	2.0061 ± 0.3997 ^f	1.3172 ± 0.4042 ⁱ
Millet	2.7321 ± 0.8136 ^d	1.9382 ± 0.1955 ^{efg}	2.7617 ± 0.2093 ^d	2.5012 ± 0.3038 ^{ef}	2.8098 ± 0.5613 ^h
Mung bean	0.3666 ± 0.6351 ^e	0.9333 ± 0.1855 ^{gh}	0.9790 ± 0.0396 ^f	0.7358 ± 0.4138 ^g	0 ^j
Oat	0.4442 ± 0.1681 ^e	0 ^h	0 ^g	0 ^h	1.2889 ± 0.0694 ⁱ
Paddy	0.9382 ± 1.3735 ^e	0 ^h	0 ^g	0.9481 ± 0.0648 ^g	3.4099 ± 0.3109 ^g
Rice	2.9148 ± 2.4376 ^d	2.8629 ± 0.0582 ^{cde}	3.0506 ± 0.0466 ^d	3.8012 ± 0.1619 ^c	6.5321 ± 0.0815 ^{cd}
Rice berry	3.7469 ± 0.9967 ^{bcd}	4.7738 ± 0.6923 ^a	3.7321 ± 0.4277 ^c	4.8679 ± 0.4493 ^{ab}	7.2802 ± 0.1557 ^b
Rice straw	0.7481 ± 0.3429 ^e	0 ^h	0 ^g	0 ^h	1.0790 ± 0.0182 ⁱ
Sticky rice	3.0444 ± 0.4627 ^{cd}	3.4605 ± 0.2851 ^{bcd}	4.3086 ± 0.3623 ^b	2.7370 ± 0.4361 ^{de}	5.9049 ± 0.3702 ^c
Sorghum	4.0617 ± 0.1408 ^{abcd}	3.5747 ± 1.2822 ^{bcd}	2.4913 ± 0.5111 ^d	3.8086 ± 0.6654 ^c	6.2815 ± 0.0971 ^{de}
Wheat	4.7012 ± 0.0407 ^{ab}	4.2670 ± 0.4419 ^{ab}	3.9864 ± 0.2830 ^{bc}	4.6531 ± 0.4704 ^{ab}	7.8442 ± 0.5311 ^a

Notes. Values are the means ± SD of length of mycelial growth (centimeters). Values in the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$)

2005). Figlas et al. (2007) suggested the growth of the mycelium of *H. erinaceus* on the MYPA medium at 25 °C. According to Julian et al. (2018), PDA was appropriate for *H. erinaceus* and SDA was suitable for *H. coralloides*. However, Bich et al. (2018) reported PDA supplemented with fresh mushroom extract was the most suitable medium for mycelial growth of *H. erinaceus*. In this study, the mycelial cultures of the different strains of *Hericium* species were studied in various culture conditions. The results revealed that OMYA and CDA were suitable for *H. erinaceus*, while

MYPA was suitable for the growth of *H. coralloides*. These data indicate that the optimal culture media and the nutrient requirements for mycelial growth differ, depending on the *Hericium* strain used.

Varying temperatures showed that the mycelium growth of *Hericium* was similar at 16–35 °C. This result was in agreement with the results reported by Han et al. (2005) and Imtiaj et al. (2008) which reported an extended range of temperature for the growth of *Hericium* mycelium at 20–30 °C. However, Bich et al. (2018) reported that the optimum temperature for

Table 8 Effect of carbon source of basal media on the mycelial growth of *Hericium* strains (gram)

Carbon source	<i>H. erinaceus</i>				<i>H. coralloides</i>
	MFLUCC 21-0018	MFLUCC 21-0020	MFLUCC 21-0019	MFLUCC 21-0021	MFLUCC 21-0050
Dextrose	0.0093 ± 0.0005 ^b	0.0302 ± 0.0093 ^b	0.0053 ± 0.0009 ^b	0.0113 ± 0.0020 ^b	0.0054 ± 0.0012 ^b
Fructose	0.0053 ± 0.0012 ^b	0.0455 ± 0.0151 ^b	0.0057 ± 0.0003 ^b	0.0051 ± 0.0007 ^b	0.0106 ± 0.0037 ^b
Glucose	0.0184 ± 0.0052 ^b	0.0213 ± 0.0123 ^b	0.0186 ± 0.0054 ^b	0.0173 ± 0.0013 ^b	0.0042 ± 0.0001 ^b
Glycine	0.0032 ± 0.0010 ^b	0.0055 ± 0.0019 ^b	0.0057 ± 0.0011 ^b	0.0022 ± 0.0004 ^b	0.0039 ± 0.0033 ^b
Lactose	0.0027 ± 0.0001 ^b	0.0108 ± 0.0072 ^b	0.0036 ± 0.0013 ^b	0.0020 ± 0.0004 ^b	0.0105 ± 0.0047 ^b
Maltose	0.0054 ± 0.0005 ^b	0.0108 ± 0.0045 ^b	0.0046 ± 0.0018 ^b	0.0037 ± 0.0006 ^b	0.0160 ± 0.0057 ^b
Molasse	0.2097 ± 0.0654 ^a	0.3384 ± 0.1088 ^a	0.1983 ± 0.0387 ^a	0.0930 ± 0.0300 ^a	0.0453 ± 0.0208 ^a
Sucrose	0.0060 ± 0.0019 ^b	0.0054 ± 0.0022 ^b	0.0041 ± 0.0011 ^b	0.0071 ± 0.0025 ^b	0.0146 ± 0.0041 ^b
Xylose	0.0042 ± 0.0006 ^b	0.0044 ± 0.0005 ^b	0.0037 ± 0.0032 ^b	0.0037 ± 0.0014 ^b	0.0149 ± 0.0097 ^b

Notes. Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$)

Table 9 Effect of nitrogen source of basal media on the mycelial growth of *Hericium* strains (gram)

Nitrogen source	<i>H. erinaceus</i>				<i>H. coralloides</i>
	MFLUCC 21-0018	MFLUCC 21-0020	MFLUCC 21-0019	MFLUCC 21-0021	MFLUCC 21-0050
NH ₄ Cl	0.0060 ± 0.0007 ^{cd}	0.0054 ± 0.0015 ^d	0.0055 ± 0.0025 ^{cd}	0.0025 ± 0.0001 ^c	0.0033 ± 0.0008 ^{cd}
NH ₄ NO ₃	0.0026 ± 0.0011 ^{cd}	0.0030 ± 0.0014 ^d	0.0051 ± 0.0023 ^{cd}	0.0034 ± 0.0006 ^c	0.0033 ± 0.0008 ^{cd}
Malt extract	0.0314 ± 0.0091 ^b	0.0394 ± 0.0162 ^b	0.0153 ± 0.0037 ^b	0.0216 ± 0.0081 ^b	0.0112 ± 0.0006 ^b
Peptone	0.0078 ± 0.0014 ^c	0.0200 ± 0.0039 ^c	0.0053 ± 0.0027 ^{cd}	0.0063 ± 0.0022 ^c	0.0158 ± 0.0056 ^b
KNO ₃	0.0031 ± 0.0005 ^{cd}	0.0061 ± 0.0029 ^d	0.0039 ± 0.0015 ^{cde}	0.0030 ± 0.0001 ^c	0.0105 ± 0.0086 ^{bc}
NaNO ₃	0.0024 ± 0.0037 ^{cd}	0.0010 ± 0.0003 ^d	0.0013 ± 0.0002 ^{de}	0.0011 ± 0.0002 ^c	0.0027 ± 0.0026 ^d
Urea	0.0016 ± 0.0006 ^{cd}	0.0015 ± 0.0009 ^d	0.0064 ± 0.0021 ^c	0.0034 ± 0.0024 ^c	0.0024 ± 0.0015 ^d
Yeast extract	0.0936 ± 0.0051 ^a	0.0746 ± 0.0157 ^a	0.0500 ± 0.0045 ^a	0.0864 ± 0.0172 ^a	0.0942 ± 0.0055 ^a

Notes. Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$)

vegetative growth of *Hericium* mushrooms to be 25 °C. This study recommends a temperature of 25 °C for *H. erinaceus* while the growth of *H. coralloides* may occur at a variety of temperatures.

The pH values most suitable for the mycelial growth of *H. erinaceus* and *H. coralloides* were in the range of pH 4-5.5. This result was similar to the report by Boddy et al. (2011), which reported *H. cirrhatum*, *H. coralloides*, and *H. erinaceus* optimum growth at pH 5.5 and Intiaj et al. (2008) presented the most favorable growth at pH 6. Moreover, the pH range for mycelial growth of medicinal mushrooms such as *Phlebopus portentosus* included suitable growth at pH 4 (Thongklang et al. 2011) and Shim et al. (2005) revealed that pH 7 was the optimum for the growth of *Macrolepiota procera*.

The most suitable agricultural substrate and cereal grain for mycelium growth of *H. erinaceus* which showed the best vegetative mycelium growth are coir and wheat, respectively, while the substrates suitable for the mycelium growth of *H. coralloides* are wheat and rice. This result was similar to those of Siwulski and Sobieralski (2005), who reported the highest

yields of *H. erinaceus* strains CS 91 and DSM 11325 on wheat bran. In addition, Hoa and Wang (2015) reported that brown rice was the most favorable to the mycelial growth of *Pleurotus ostreatus* and *P. cystidiosus*.

For the effect of carbon and nitrogen sources on mycelial growth, *H. erinaceus* and *H. coralloides* had the most favorable growth on molasses and yeast extract, respectively, which is in agreement with Hoa and Wang (2015), recording molasses as a good carbon source for *Pleurotus ostreatus* and *P. cystidiosus*. According to Shim et al. (2005), maltose was the best for mycelial growth. Moreover, Wiriya et al. (2014) reported that sucrose was the best carbon source for mycelial growth. So both aforementioned studies showed that disaccharides were better than monosaccharides; however, molasses contains a surplus of 43% sugars (Jamir et al. 2021). Besides, Thai and Keawsompong (2019) showed that yeast extract was the most suitable for *Tricholoma crassum* and Gbolagade et al. (2006) found that yeast extract enhanced the greatest mycelial growth of *Lentinus subnudus*. Wiriya et al. (2014) also reported that organic nitrogen sources were the best to promote mycelial growth. However, molasses and

Table 10 Effect of media components ratio on the mycelial growth of *Hericium* strains (gram)

Ratio	<i>H. erinaceus</i>				<i>H. coralloides</i>
	MFLUCC 21-0018	MFLUCC 21-0020	MFLUCC 21-0019	MFLUCC 21-0021	MFLUCC 21-0050
1:1	0.0836 ± 0.0424 ^c	0.0543 ± 0.0085 ^b	0.0327 ± 0.0135 ^b	0.1252 ± 0.0117 ^a	0.1510 ± 0.0313 ^a
1:5	0.0856 ± 0.0156 ^c	0.1081 ± 0.0464 ^b	0.0564 ± 0.0152 ^b	0.1087 ± 0.0129 ^a	0.1344 ± 0.0268 ^a
1:10	0.0732 ± 0.0192 ^c	0.0626 ± 0.0064 ^b	0.0334 ± 0.0071 ^b	0.0451 ± 0.0098 ^b	0.0557 ± 0.0017 ^c
5:1	0.1833 ± 0.0232 ^b	0.0963 ± 0.0308 ^b	0.0370 ± 0.0023 ^b	0.1166 ± 0.0166 ^a	0.0942 ± 0.0086 ^b
10:1	0.2349 ± 0.0310 ^a	0.3547 ± 0.0621 ^a	0.1133 ± 0.0260 ^a	0.1141 ± 0.0083 ^a	0.1537 ± 0.0198 ^a

Notes. Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$)

Fig. 2 Morphology of mycelium of different *Hericium* strains on basal media with varying the ratios of media components after 15 days of incubation



yeast extract were the complex media. Palmonari et al. (2020) reported that molasses is a by-product of sugar extract, and Ramadhani et al. (2022) said that sugar was widely known as a carbon source. In addition, yeast extract was estimated to contain 40% organic carbon (Holwerda et al. 2012), while Tomé (2021) reported yeast extract content of nitrogenous compounds at 45 to 70%, which included 80% of protein nitrogen and 10–12% of nucleic acid nitrogen. Additionally, molasses served as a carbon source, and yeast extract served as a nitrogen source. The ratio of media components of 10:1 was the best for the mycelial growth of *H. erinaceus* and *H. coralloides*. This result was similar that of et al. (2005), who reported for *Macrolepiota procera* an optimum carbon to nitrogen ratio ($\text{NaNO}_3/\text{D-glucose}$) of 10:1

Conclusion

In this study, *Hericium erinaceus* strains MFLUCC 21-0018, MFLUCC 21-0019, and MFLUCC 21-0020 showed the most favorable growth on OMYA at a pH range of 4–4.5 at 25 °C. For spawn tests, coir was demonstrated to be optimal. *H. erinaceus* strain MFLUCC 21-0021 had the most favorable

growth on CDA and a pH range of 4–5.5 at 25 °C. Coir grains were similarly optimal for the spawn test, while *H. coralloides* strain MFLUCC 21-0050 showed favorable growth on MYPA for pH 5.5 at 30 °C and wheat grain for spawn tests. For the carbon and nitrogen sources, the best growth rates for all five strains were obtained using molasses and yeast extract respectively and the ratio of media components was 10:1 for the best mycelial growth. Further experiments with diverse strains of these two species need to be conducted to improve productivity and biological efficiency. In addition, all of these experiments can be used to develop suitable methods for inducing their mushroom product formation on artificial media composed of agricultural by-products. However, these improved techniques can be used to enhance the mycelial production of *Hericium*, and the fungi may eventually be used for processing food products with high efficacy, and compounds that are useful against nervous system diseases. One of the next goals of our studies is to investigate how effective production of basidiomes can be accomplished. Given the fact that these fungi originate from temperate climate zones and fruit in nature in autumn, it may be necessary to start production at higher altitudes where the temperatures are not so high.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11557-022-01829-6>.

Acknowledgements We would like to thank Mr. Adam Kaplan for editing the English in the paper.

Authors contribution D. G., N. T., and T. L. conceptualized and initiated this review with the help of K. D. H. and M. S. wrote the first draft of the manuscript together. D. G. wrote the first draft of the phylogenetic part and designed Figs. 1 and 2. All other authors have read, provided useful edits, and agreed to the published version of the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by a grant from Research and researcher for industries (PHD6210018/2562).

Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

Competing interests The authors declare no competing interests.

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