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Optimal conditions for mycelial growth and nutritional values of the *Auricularia cornea*

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Abstract

Due to its edibility and therapeutic qualities, *Auricularia* Bull. (ear mushroom) is very significant and has a global distribution. A better technique in mushroom cultivation is needed due to the high demand for mushroom consumption and possibly maintaining enough supply throughout the year. In this study, three different *Auricularia cornea* isolates were subjected to four different tests to find the most suitable medium, temperatures, pH and substrates for spawning. The fruiting test and nutritional value analysis were also conducted. The results showed that *A. cornea* grew well on Rice Bran Sucrose Agar (RSA) followed by Malt Extract Agar (MEA) [0.1008 ± 0.0010 to 0.1722 ± 0.0143 g of dried mycelial weight]. The growth of three isolates performed best at a temperature of 25 °C at pH 5–7. Furthermore, the most favorable substrates for *A. cornea* growth were sorghum and paddy grain. However, sawdust (without any supplements) was the least effective. Moreover, the first primordia were observed on 20 ± 3.04, 15 ± 3.13, and 26 ± 1.15 d, respectively. Therefore, these conditions can be considered for *Auricularia* culture from tissue culture and spawning production. The nutritional value analysis showed that the crude protein was 11.22% and 13.14%, fat (0.77% and 1.27%), crude fiber (19.71% and 22.43%) and carbohydrate (72.27% and 70.66%), respectively. Surprisingly, the carbohydrate found in this study was higher than other *Auricularia* spp. (14%–17%) and 2–3 times higher than other edible mushrooms.

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Introduction

Edible mushrooms are often produced industrially and there is a need to provide the best media for preserving and growing cultures and spawn production[1–4], and best temperatures, substrates, conditions and processes for growing the mushrooms[5–11]. The nutritional values of the fruiting bodies of mushrooms of various species have also often been studied[12–16].

Auricularia Bull. is one of the members of the family Auriculariaceae of Basidiomycota. Currently, there are 138 species of *Auricularia* listed in Mycobank (www.MycoBank.org) and it has *A. mesenterica* (Dicks.: Fr.) Pers as a type species[17]. Common names for this genus include jelly fungi, ear mushrooms, and Hed-hoo-noo in Thai[18]. These mushrooms are abundant in tropical, subtropical, and temperate climates[18–20]. Commercially grown *Auricularia* mushrooms include, for example, *A. heimuer* F. Wu, B.K. Cui & Y.C. Dai, and *A. polytricha* (Mont.) Sacc[21–23] which have been reported in China. *Auricularia* spp. also offer nutritional and therapeutic benefits[24–26], for example, *Auricularia cornea* as a pork fat replacement in cooked sausage[27], *A. auricula-judae* (Bull.: Fr.) Queìl. has antioxidant activity[28–33], *A. polytricha* is said to have antibacterial, antihypercholesterolemic, and antioxidant properties[34–36]. Additionally, Trehalose, a substance that may be utilized as a moisturizer in cosmetics, has been found in *Auricularia auricula-judae*[37–39].

Auricularia cornea Ehrenb is widely used for consumption and medicinal purposes[40,41]. The distribution areas of *A. cornea* include Africa, North and South America, Asia, and Europe[42]. The distinguishing characteristics of the species are basidiocarp adhering to the substrate from the corner or center, short stalks, light brown to dark brown and undulate edge, present ridges, and veins, and shorter abhymenial hairs than *A. nigricans*[20,40,42]. This mushroom showed antioxidant activity, reduced alcoholic liver disease (ALD), reduced blood fat, exhibited anticancer activities, and improved immune system[26,41,43–45]. Interestingly, in Thailand, only two species of *Auricularia* mushrooms (*A. auricula-judae* and *A. polytricha)* have been used in commercial cultivation[40]. However, *A. cornea* were shown to have potential in commercial cultivation[20,40].

To date, there have been several reports on optimal conditions for different species of *Auricularia* mushroom[46], but none of them was *A. cornea*. In this study the most suitable medium, temperatures, pH and substrates for spawning of *Auricularia cornea* were studied. Fruiting tests and nutritional value analysis were also conducted.

Materials and methods

Fungal strains

Three different strains of *Auricularia cornea* were used. The first strain MFLUCC18-0346 was previously described[40]. The

other two strains, MFLUCC18-0347, were collected from dead wood in the Mae Suay subdistrict, Chiang Rai, Thailand, while MFLUCC23-0084 was collected from the rubber trunk in the Thasud subdistrict, Chiang Rai, Thailand. The fresh specimens were dried in hot air (40–50 °C) and sealed in Ziplock plastic bags. The strain was isolated by spore isolation and subcultured in PDA medium and incubated at 25 °C for 14 d. The strain collection and dry specimen are deposited in the Mae Fah Luang University Culture Collection (MFLUCC18-0347 and MFLUCC23-0084) and the Mae Fah Luang University Herbarium (MFLU18-0199 and MFLU23-0259).

Macro- and micromorphological character analyses

Morphological characters of three wild strains of Thai *A. cornea* were recorded. Macromorphological characters were described from fresh specimens. The photographs were taken *in situ* and laboratory. Color notation[47] was used. Micromorphological characters were obtained from free-hand sections of dried specimens. The tissues were mounted in H_2O and a 5% aqueous KOH solution and Congo red were used to highlight all structures.

DNA extraction, PCR, and sequencing

Dried basidiocarps of *A. cornea* strains MFLUCC18-0347 and MFLUCC23-0084 were used for molecular analysis. The samples were then dried in desiccated at 45 °C and DNA from each sample was extracted with the High Pure PCR Template Preparation Kit (Roche) following the manufacturer's protocol. DNA amplification was performed using primers for ribosomal DNA regions (ITS1/ ITS4)[48]. The brpb2-6F and brpb2-7.1R[49] were used to amplify the region of rpb2 following the PCR conditions described[17]. Sequencing was performed by SolGent Co., Ltd, Yuseong-gu, Daejeon, South Korea.

The sequence data was assembled using BioEdit v. 7.0.9.0[50] and subjected to a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest matches. The sequences of the wild Thai *A. cornea* that were newly obtained for this study were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/submit). Other sequences of this genus (Table 1) were downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/submit). Maximum likelihood analyzes were performed in raxmlGUIv.0.9b2[51] using the GTR + G model of evolution. Phylograms were visualized with the FigTree v1.4.0 program[52] and in Adobe Illustrator CS5 (Version 15.0.0, Adobe, San Jose, CA, USA).

Effect of different media on mycelium growth

In this study, nine different agar-based media were tested. They included Carrot Agar (CA), Corn Meal Agar (CMA), Coconut Water Agar (CW), Malt Extract Agar (MEA), Oat Meal Agar (OMA), Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Rice Bran Sucrose Agar (RSA), and Sweet Potato Agar (SA). The preparations of PSA, CW, and RSA were followed[53]. For PDA, OMA and MEA were followed as described by the manufacturers. The preparation methods for CA were followed as described[54] with an additional 10 g/l sucrose. All media were sterilized at 15 psi for 15 min at 121 °C and then poured into petri dishes (20 ml/plate). A 0.5 cm diameter mycelial plug was cut from the edge of 12-d old colonies grown on PDA and transferred aseptically to the center of each medium plate. For each medium, there were three replicate cultures. The inoculated plates were incubated at 28 °C in dark. The diameters of the colonies were measured and recorded every three days during incubation until the fungal colonies reached the edge. Subsequently, plates with growing mycelium were melted to remove agar with approximately 100 °C water, to extract mushroom mycelia. Each one was dried at 45 °C for 24 h and then weighed[55].

Effect of temperature on mycelial growth

The two-best media were MEA and RSA. They were chosen in subsequent experiments. To evaluate the effect of temperature on mycelial. A 0.5 cm diameter mycelial plug of the *A. cornea* strain MFLUCC18-0346, MFLUCC18-0347, and MFLUCC23-0084 was cut from the edge of 12-day old colonies grown in PDA and aseptically transferred to the center of each medium plate (MEA and RSA) at 20, 25, 30, 40 and 45 °C in three replicates. Mycelial growth was assessed by measuring the colony diameter of the mycelium. The dry mass of the mycelium was recorded by weighing the dried mycelium as previously described[55].

Effect of pH on mycelial growth

The two mediums (MEA and RSA) were adjusted to pH values of 4.0, 5.0, 6.0, 7.0, 8.0 with 1N HCl or 1N NaOH, then sterilized at 15 psi for 15 min at 121 °C and then poured into petri dishes (20 ml/plate). A 0.5 cm diameter mycelial plug of *A. cornea* strain MFLUCC18-0346, MFLUCC18-0347, and MFLUCC23-0084 was cut from the edge of 12 d old colonies grown on PDA and aseptically transferred to the center of each medium plate in three replicates of each treatment and then incubated at 25 °C. Mycelial growth was assessed by measuring the colony diameter of the mycelium. The dry mass of the mycelium was recorded by weighing the dried mycelium as previously described[55].

Effect of cereal grains and agricultural substrate

Rice straw, wheat grain, saw dust, rye grain, paddy grain, and sorghum were tested to determine their suitability for spawn production. Rice straw was cut into 2–3 cm pieces, soaked overnight in water, and boiled for 10 min. For other substrates, they were not chopped, but they still need to be soaked overnight in water and boiled for 10 min. The excess water will be removed by drying the substrate, until the moisture content

Table 1. List of species, specimens, and GenBank accession number of sequences used in this study.

Taxon name	Herbarium code	GenBank accession number (ITS)	GenBank accession number (RPB2)	Country
A. americana	Dai 13636	KM396765	KP729307	China
A. americana	Cui 11657	KT152095	KT152128	China
A. africana	KM133591, type	NR177476	–	Uganda
A. africana	Ryvarden 44929	MH213349	MZ740061	Uganda
A. angiospermarum	Cui 12360	KT152097	KT152130	USA
A. angiospermarum	BJFC 017274, type	NR151847	–	USA

(to be continued)

Table 1. (continued)

Taxon name	Herbarium code	GenBank accession number (ITS)	GenBank accession number (RPB2)	Country
A. asiatica	BBH895, type	NR169914	–	Thailand
A. asiatica	Dai 16224	KX022011	MZ740045	China
A. auricula-judae	Dai 13210	KM396769	KP729312	France
A. auricula-judae	MT 7	KM396771	KP729314	Czech Republic
A. australiana	MEL 2385783 type	NR176760	–	Australia
A. australiana	HT 190	MZ647503	–	Australia
A. brasiliana	URM 85567 type	NR151845	–	Brazil
A. brasiliana	BDNA 1641	KP729277	–	Brazil
A. camposii	URM 83464	MH213352	MH213428	Brazil
A. camposii	URM 76905, holotype	MH213351	MH213427	Brazil
A. conferta	BJFC 027293, type	NR174873	–	Australia
A. conferta	Dai 18825	MZ647500	MZ740048	Australia
A. cornea	YG-Dr1	MH213353	MH213429	Germany
A. cornea	Dai 12587	KX022012	–	South Africa
A. cornea	Dai 15336	KX022014	KX022074	China
A. cornea	Wu 07	MH213354	MH213430	China
A. cornea	Dai 17352	MH213355	MH213431	Ghana
A. cornea	Lira 663	MH213359	MH213433	Brazil
A. cornea	MFLU13-0403	KX621145	KX661337	Thailand
A. cornea	MFLU16-2104	KX621144	KX661340	Thailand
A. cornea	MFLU 19-0797	MK696312	–	Thailand
A. cornea	**MFLU23-0259**	**OR105042**	**OR119735**	**Thailand**
A. cornea	**MFLU18-0199**	**OR105024**	**OR119734**	**Thailand**
A. delicata	P 14, epitype	MH213364	–	Cameroon
A. fibrillifera	Dai 13598A	KP765615	KX022084	China
A. fibrillifera	F 234519, type	KP765610	–	Papua New Guinea
A. fuscosuccinea	FP102573SP	KX022027	KX022088	USA
A. fuscosuccinea	Dai 17406	MH213366	MH213436	Brazil
A. heimuer	Dai 13503	KM396789	KP729316	China
A. heimuer	Dai 13765, holotype	KM396793	KP729317	China
A. lateralis	Dai 15670, holotype	KX022022	–	China
A. mesenterica	Haikonen 11208	KP729287	KP729323	United Kingdom
A. mesenterica	Miettinen 12680	KP729286	KP729322	Switzerland
A. minutissima	Dai 14881, holotype	KT152104	KT152137	China
A. minutissima	Dai 14880	KT152103	KT152136	China
A. nigricans	Ahti 55718	MH213372	–	Costa Rica
A. nigricans	TJY 93242	KM396803	–	USA
A. novozealandica	PDD 88998	KX022035	–	New Zealand
A. novozealandica	PDD 83897, holotype	KX022034	–	New Zealand
A. orientalis	Dai 14875, type	KP729270	KP729310	China
A. orientalis	Dai 1831	KP729271	KP729311	China
A. pilosa	LWZ 20190421-7, holotype	MZ647506	–	Ethiopia
A. pusio	AK 547	MH213374	MH213443	Australia
A. pusio	Smith 18	MH213375	–	Zambia
A. scissa	TFB 11193, holotype	JX065160	–	Dominican Republic
A. scissa	Ahti 49388	KM396805	KP729324	Dominican Republic
A. sinodelicata	Cui 8596	MH213376	MH213444	China
A. sinodelicata	Dai 13926, holotype	MH213379	–	China
A. subglabra	Dai 17403	MH213382	MH213448	Brazil
A. srilankensis	Dai 19522, holotype	MZ647501	–	Sri Lanka
A. srilankensis	Dai 19575	MZ647502	MZ740058	Sri Lanka
A. submesenterica	Dai 15450, holotype	MH213386	MH213449	China
A. submesenterica	Dai 15451	MZ618942	MZ740059	China
A. thailandica	MFLU 130396, type	KR336690	–	Thailand
A. thailandica	Dai 15080	KP765622	MH213452	China
A. tibetica	Cui 12267, holotype	KT152106	KT152139	China
A. tibetica	Cui 12337	KT152108	KT152141	China
A. tremellosa	Dai 17415	MH213390	MH213455	Brazil
A. tremellosa	AJS 1304	JX065158	–	Mexico
A. villosula	LE 296422, holotype	NR137873	KJ698441	Russia
A. villosula	Hei 1973	MZ618944	MZ740062	China
Exidia qinghaiensis	HMAS 156328, type	NR172805	MW358924	China

Newly generated sequences for this study are indicated in **bold**.

reaches around 60%. Each substrate was transferred to a glass tube (200 × 25 mm), in which each substrate will be uniformly filled to a volume of 88 ml before autoclaving at 121 °C for 20 min[56]. A mycelial plug, approximately 0.5 cm in diameter, was placed aseptically on the surface of each cool substrate in three replicates and incubated at 25 °C. PDA without fungal mycelium was used as a control. The linear growth and colonization rate in glass tubes was measured by the visible progression of mycelia to the substrate every 3 d until it reached the bottom of the glass tube[55].

Spawn production

For spawn production, *Sorghum bicolor* (sorghum) grains were used[7]. After being cleaned and soaked for the entire night, the grains were boiled for 15 min. Bottles containing 100 g of grains were autoclaved at 121 °C for 15 min before being allowed to cool. One-fourth of a PDA plate containing mushroom mycelium was used to inoculate the bottles. For 30 d, the inoculated bottles were incubated at 25 °C in the dark.

Fruiting test

A fruiting test of *A. cornea* was carried out with five replicates. Rubber sawdust was used as the main substrate and mixed (w/w) with 5% of rice bran, 1% of spent brewery grain, 1% of glutinous rice flour, 1% of pumice sulfate and 1% of calcium carbonate. All substrate supplements were mixed manually with 70% moisture. The mixture (800 g) was packed into polypropylene bags and then capped with a plastic ring and lid. Sawdust bags were sterilized at 121 °C for 45 min. After the temperature cooled to 25 °C, 50 g of spawn were inoculated into sawdust bags under aseptic conditions. The bags were incubated at 25 ± 1 °C in the dark, for 60 d. For the fruiting phase, the same temperature and 75%–85% humidity were used. The mushroom yield of each strain and the first primordia was recorded.

Nutritional values analyzed

Dry *A. cornea* strain MFLUCC18-0346 and MFLUCC18-0347 were subject to analysis as described, crude protein (AOAC 991.20), crude fat (AOAC 948.15), ash (923.03 and 920.153), moisture content (925.10 and 950.46), crude fiber (internal method TE-CH-122 based on AOAC 978.10), and carbohydrate using method of analysis for nutrition labeling[57]. All analyzes were performed by Central Laboratory (Thailand), Co, Ltd, Chiang Mai Branch.

Statistical analysis

The dry weight of mycelium and the linear growth of mycelium were analyzed with one-way ANOVA and significant differences ($p < 0.05$) and Duncan's multiple range test.

Results

Species confirmation

Auricularia cornea Ehrenb. (Fig. 1.)

Basidiocarp: 0.3–4 cm, attached to the substrate at the corner or center, slimy and thick, dark crenate margin; abhymenial surface dark brown, 7F4 to dark magenta, 13F4; hymenial surface dark ruby, 12F4, there are thick ridges present., dense hair on the upper surface.

Internal features: thickness 1,400–1,470 μm; medulla present; abhymenial hairs, gregarious, hyaline, acute tip, thick-walled, 2–4 μm, hair bases 8–14 μm wide; hair bases, light brown; zona pilosa 165–180 μm; zona compacta 40–70 μm; zona subcompacta superioris 100–130 μm; zona laxa superioris 210–260 μm;

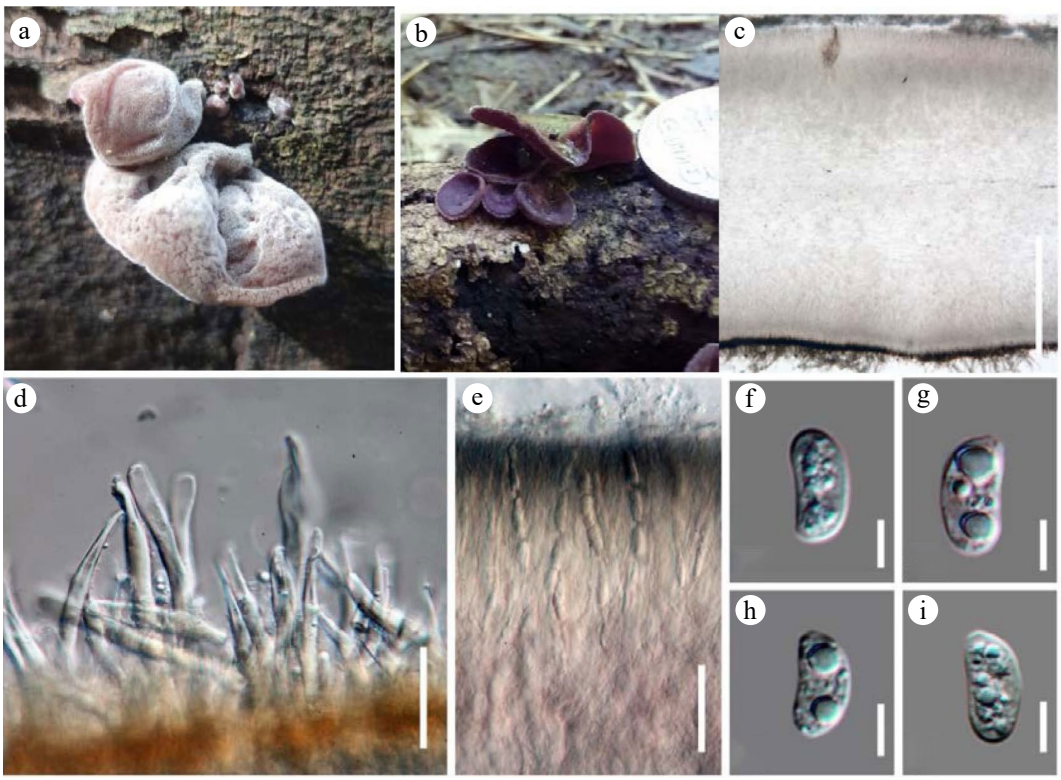

Fig. 1 (a) Basidiocarps of *A. cornea* MFLU23-0259, TW8. (b) Basidiocarps of *A. cornea* MFLU18-0199, LK14. (c) Cross section of the fruit body. (d) Abhymenial hairs. (e) Close-up of a hymenial layer. (f)–(i) Basidiospores. Scale bars: (c) = 500 μm, (d) = 50 μm, (e) = 25 μm, (f)–(i) = 5 μm.

medulla 115–200 μm; zona inferioris 235–265 μm; zona subcompacta inferioris 150–160 μm; hymenium 75–95 μm; basidia 70–80 × 3–5 μm, cylindrical, tapered or blunt ends; basidiospores smooth-walled, allantoid, hyaline, (11.4)12.4–14.8(15.9) × (4.9)5.4–6.4(6.8) μm, $\bar{x}$ ± SD = 13.6 ± 1.2 × 5.9 ± 0.4 μm, Q = 1.8–2.7, $\bar{Q}$ = 2.3

Collections examined: THAILAND, Chiang Rai: Mae Suay, on dead wood, 28 September 2017, Lattana LK14 (MFLU18-0199) and Chiang Rai: Thasud, on rubber dead trunk, 2 September 2021 Arttapon TW8 (MFLU23-0259)

Notes: In general, the key criteria used to distinguish *A. cornea* were, Basidiomata usually reddish brown, sometimes white, densely pilose (hairy), crystal present, hyphae with simple septa, basidia 60–75 × 4–6 μm, basidiospores 13.8–16.5 × 4.5–6 μm. We discovered that the internal features of our *A. cornea* differ slightly from the *A. cornea* described[42]. The basidia in our *A. cornea* were larger (70–80 × 3–5 μm) and the basidiospores were smaller (12.4–14.8 × 5.4–6.4 μm). One previous study mentioned that samples with similar macro-morphology are distantly related, while those with slightly different morphology are closely related, but all share micro-morphology and *A. cornea* characteristics[19,42,58–60], so they are treated as *A. cornea*. Similar result was observed in this study, our strains were not hugely different and they also share some characteristics of *A. cornea* (densely pilose and present of crystal). These variations in macro-morphology may be due to a wide distribution in Africa, North and South America, Asia, and Europe[42].

Phylogenetic analyses

Based on phylogenetic analysis comprising 68 fungal accessions including the outgroups. The analysis of maximum likelihood showed that MFLU18-0199 and MFLU23-0259 clustered with the strains of *A. cornea* (Fig. 2). The isolates used in this study are in blue and type species are in bold. Similarly to the findings in a report[42], the phylogenetic analysis (Fig. 2) revealed that both samples (MFLU18-0199 and MFLU23-0259) and other fungal accessions of *A. cornea* constitute a lineage.

Effect of different media, temperature, pH, cereal grains, and agricultural substrates on mycelium growth

Based on the results in Table 2, the three isolates grew the best rice bran sucrose agar (RSA). In addition, the best synthetic medium was malt extract agar (MEA) for the two isolates (MFLUCC18-0346 and MFLUCC18-0347), but not for MFLUCC23-0084. The optimal temperature of three strains of *A. cornea* (MFLUCC18-0346, MFLUCC18-0347 and MFLUCC23-0084) was 25 °C. The statistical analysis indicated that the mycelial dried weight was significantly different, and the highest dried weight was obtained at 25 °C. Furthermore, none of the three isolates was grown at 40 and 45 °C. The most favorable pH range for the mycelial growth of *A. cornea* (MFLUCC18-0346, MFLUCC18-0347, and MFLUCC23-0084) in RSA was pH 5–7, while MEA was pH 5–6. Moreover, the mycelium was fully colonized 27 d after inoculation and the results showed that sorghum and paddy grain were the most favorable for the three isolates of *A. cornea*. On the other hand, sawdust (without any supplements) was the least effective.

Fruiting test

Cultivation of three different strains of *Auricularia cornea* MFLUCC18-0346, MFLUCC18-0347, and MFLUCC23-0084 were carried out with five replicates (Fig. 3). The primordia appeared on 20 ± 3.04, 15 ± 3.13, and 26 ± 1.15 d after fruiting phase, respectively. The average yield of the first flush were 19.40 ± 6.73, 31.00 ± 4.06, and 23.67 ± 8.33 g, respectively (Table 3).

Nutritional values

The crude protein that was analysed from the dry fruiting bodies of *A. cornea* (MFLUCC18-0346 and MFLUCC18-0347) were 11.22% and 13.14%, fat (0.77% and 1.27%), crude fiber (19.71% and 22.43%), carbohydrate (72.27% and 70.66%), and ash (2.90% and 3.37%), respectively (Table 4).

Discussion

China has been cultivating *A. auricula-judae*, often known as black fungus or wood ear mushroom, for around 2,100 years[65]. *A. cornea* and *A. heimuer* are being grown for sale in China, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam[22,66–70]. More than 90% of the world's *A. auricula* production is currently produced in China, making it the leading producer in the world. *A. auricula* (dry goods) produced 674,000 tons in 2018, valued at 37.46 billion Chinese Yuan, and generated 6.15 billion Chinese Yuan in foreign exchange[71].

To date, it can be summarized that *Auricularia* is grown in a variety of culture medium, including Czapek-dox, glucose peptone, malt extract agar (MEA), mesangial cell medium (MCM), potato Dextrose agar (PDA), yeast extract agar (YEA), yeast mannitol agar (YMA) and Leonian medium, all of which have different nutritional profiles and ideal temperature and pH ranges[46,72]. Under ideal temperature (30 °C) and pH 8 conditions, *A. villosula* mycelium can be grown in a mixture of potato juice, sucrose, soybean powder and 0.5% PO_4^{-3} to produce fruit bodies that are extremely comparable to those found in nature[73]. In our study, we found that the three *A. cornea* isolates grew best in RSA (non-commercial media) followed by MEA (commercial). The best pH values range from 5–7 at 25 °C.

Compost and agro-waste products are used in many South-east Asian nations for the low-cost cultivation of *Auricularia* species. For example, *A. auricula-judae* was successfully cultivated in India using compost that was predominantly made of corncobs, rice straw, broadleaf tree sawdust, and cottonseed bran with plaster stone, wheat bran, rice bran, and quick lime as supplemental components[74]. This study found that *A. cornea* grew well on both sorghum and paddy grain. In general, sorghum is widely used to produce spawns. In previous studies, there have been reports on *A. cornea* cultivation using rye grain[61,75] and sorghum[40]. The prices of paddy grain are 0.36–0.42 US$ per kilogram, while the prices of sorghum are 0.61–0.76 US$ per kilogram in Thailand. Interestingly, the addition of agricultural waste was better than simply using sorghum alone[7]. The comparison of paddy grain and sorghum is also summarized (Table 5). The content of lignin found in paddy grain was higher than that of sorghum, while the starch found in paddy grain was 12.64% lower than that of sorghum. Surprisingly, there is only a slightly different in total sugar (1.2% and 1.3%, respectively). Therefore, paddy grain can be considered for *A. cornea* cultivation, not only because of its effectiveness, but also because of its price.

To increase edible mushroom production and commercial grade, ideal growth conditions (temperature, pH), along with nutrient-rich supplementation, are required[76]. The nutritional value of *Auricularia* is known to be affected by the addition of a

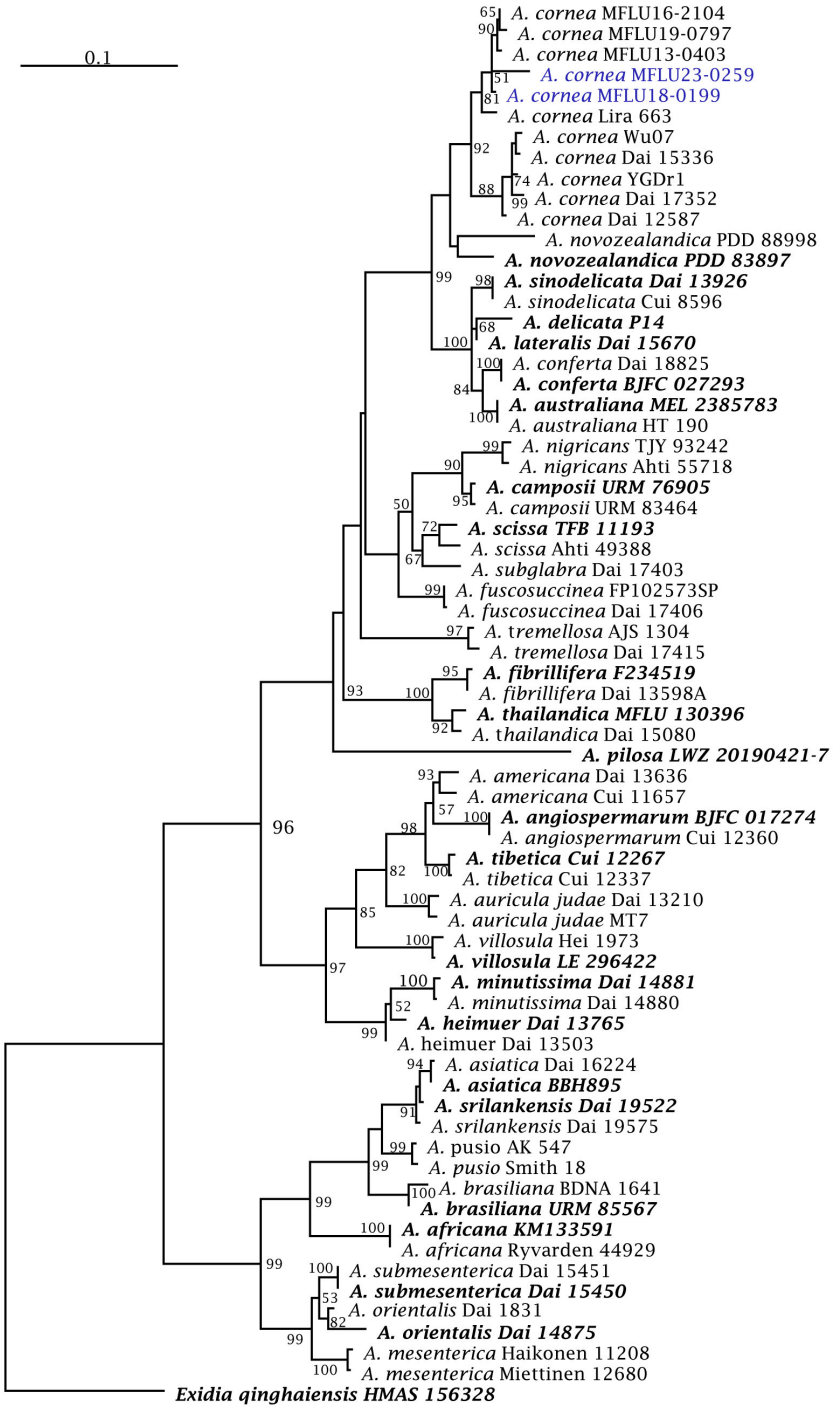

Fig. 2 Maximum Likelihood (ML) tree illustrating the phylogeny of *Auriclaria* based on ITS + RPB2 dataset. The tree is rooted with *Exidia qinghaiensis*.

substrate. For example, compared to other substrates for agro-waste such as rice straw and rice husk, the use of 60% sugar-cane bagasse produced the highest nutrient production (carbohydrates, protein, ash, and fat) from *A. polytricha*[77]. However, in contrast to its Kenyan counterparts raised on sawdust and rice bran, *A. auricula* cultivated on maize cobs and wheat bran showed increased nutritional content (cellulose, proteins, and moisture)[78]. In a previous study demonstrated that the type of medium used for culturing affects the dry biomass weight, moisture content, crude proteins, ash and carbohydrates. For example, yeast extract, rather than the lowering effects of tryptone, beef extract, and peptone, was the nitrogen source that was most beneficial to the growth of *A. polytricha* and dry biomass production[79].

Sawdust is typically used as the primary substrate while growing *Auricularia*[61,70,80]. Our results showed that rubber sawdust is beneficial for three different strains of *A. cornea,* which has similar results as observed in *A. delicata* and *A.*

Table 2. Optimal conditions to grow three different isolates of *A. cornea*.

Name of experiments	Treatments	Fungal isolates		
		MFLUCC18-0346	MFLUCC18-0347	MFLUCC23-0084
Effect of different media (g)	PDA	0.0844 ± 0.0088^{bc}	0.0551 ± 0.0165^{bcd}	0.0389 ± 0.0075^{c}
	PSA	0.0028 ± 0.0015^{d}	0.0394 ± 0.0298^{cd}	0.0256 ± 0.0077^{cd}
	CW	0.0846 ± 0.0037^{bc}	0.0861 ± 0.0170^{bcd}	0.0605 ± 0.0028^{b}
	OMA	0.0378 ± 0.0116^{cd}	0.0634 ± 0.0126^{bcd}	0.0422 ± 0.0068^{bc}
	CMA	0.0196 ± 0.0143^{d}	0.0114 ± 0.0068^{d}	0.0031 ± 0.0009^{d}
	SA	0.0595 ± 0.0113^{bcd}	0.1329 ± 0.0059^{ab}	0.0414 ± 0.0121^{bc}
	RSA	0.1568 ± 0.0310^{a}	0.1722 ± 0.0143^{a}	0.1008 ± 0.0010^{a}
	CA	0.0029 ± 0.0034^{d}	0.0095 ± 0.0053^{d}	0.0073 ± 0.0040^{de}
	MEA	0.1129 ± 0.0495^{ab}	0.1112 ± 0.0722^{abc}	0.0348 ± 0.0144^{c}
Effect of temperature in RSA (g)	20 degrees	0.1341 ± 0.0222^{a}	0.1220 ± 0.0090^{b}	0.0776 ± 0.0148^{a}
	25 degrees	0.1583 ± 0.0237^{a}	0.1881 ± 0.0153^{a}	0.1141 ± 0.0346^{a}
	30 degrees	0.1232 ± 0.0521^{a}	0.1334 ± 0.0140^{b}	0.0103 ± 0.0051^{b}
Effect of temperature in MEA (g)	20 degrees	0.0602 ± 0.0135^{b}	0.0467 ± 0.0080^{b}	0.0578 ± 0.0055^{b}
	25 degrees.	0.1202 ± 0.0168^{a}	0.1242 ± 0.0092^{a}	0.0934 ± 0.0239^{a}
	30 degrees	0.0746 ± 0.0044^{b}	0.0523 ± 0.0056^{b}	0.0219 ± 0.0029^{c}
Effect of different pH in RSA (g)	4	0.0755 ± 0.0079^{c}	0.0753 ± 0.0056^{b}	0.0297 ± 0.0074^{b}
	5	0.0968 ± 0.0103^{bc}	0.1440 ± 0.0123^{a}	0.0373 ± 0.0015^{b}
	6	0.1125 ± 0.0104^{ab}	0.0817 ± 0.0050^{b}	0.0733 ± 0.0067^{a}
	7	0.1344 ± 0.0166^{a}	0.0953 ± 0.0206^{b}	0.0703 ± 0.0016^{a}
	8	0.0403 ± 0.0062^{d}	0.0375 ± 0.0041^{c}	0.0302 ± 0.0013^{b}
Effect of different pH in MEA (g)	4	0.0712 ± 0.0026^{b}	0.0357 ± 0.0050^{b}	0.0285 ± 0.0061^{d}
	5	0.1071 ± 0.0127^{a}	0.0488 ± 0.0152^{b}	0.0611 ± 0.0087^{ab}
	6	0.0706 ± 0.0115^{b}	0.102 ± 0.0237^{a}	0.0742 ± 0.0131^{a}
	7	0.0474 ± 0.0049^{c}	0.0357 ± 0.0049^{b}	0.0419 ± 0.0024^{cd}
	8	0.0460 ± 0.0044^{c}	0.0433 ± 0.0031^{b}	0.0491 ± 0.0050^{bc}
Effect of different substrates of spawn (cm)	Rice straw	15.00 ± 0.00^{bc}	12.00 ± 0.00^{ab}	0.00^{c}
	Wheat grain	11.50 ± 0.00^{d}	9.00 ± 0.00^{b}	3.67 ± 0.62^{b}
	Rye grain	13.67 ± 1.25^{c}	11.67 ± 0.94^{ab}	4.00 ± 0.00^{b}
	Sawdust	6.33 ± 0.94^{e}	5.00 ± 3.56^{c}	0.00 ± 0.00^{c}
	Paddy grain	15.50 ± 0.71^{ab}	15.00 ± 0.82^{a}	5.50 ± 1.78^{ab}
	Sorghum	17.00 ± 0.00^{a}	14.50 ± 0.41^{a}	6.50 ± 1.78^{a}

Values are the means ± SD of the dry weight/ growth in length of mycelial (g/cm). The values of the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$).

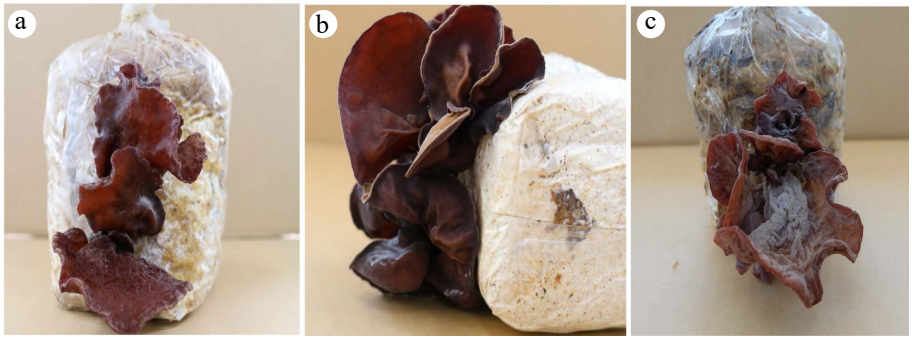

Fig. 3 (a) Cultivated basidiocarps of *A. cornea* (MFLUCC18-0346) day 35 after fruiting phase. (b) Cultivated basidiocarps of *A. cornea* (MFLUCC18-0347) on day 28 after the fruiting phase and (c) Cultivated basidiocarps of *A. cornea* (MFLUCC23-0084) day 35 after the fruiting phase.

Table 3. Comparison of mushroom yield in the first flush.

Content	MFLUCC 18-0346	MFLUCC 18-0347	MFLUCC 23-0084
First primordia after fruiting phase (d)	20 ± 3.04^{b}	15 ± 3.13^{a}	26 ± 1.15^{c}
Average yield of the first flush (g)	19.40 ± 6.73^{a}	31.00 ± 4.06^{b}	23.67 ± 8.33^{ab}

Values are the means ± SD of the first primordia/average yield. The values of the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$).

cornea[40,81]. *Auricularia* species can be grown on a variety of substrates, including agricultural waste. The fact that several of them produced higher yields than sawdust alone makes this a significant discovery as a substitute method of mushroom cultivation. For instance, the B.E. of *A. polytricha* grow in sawdust + oil palm frond + spent grain and sawdust + empty fruit bunch + spent grain were 288.9% and 260.7%, respectively[70], whereas in sawdust alone was 105.9%. Moreover, *A. polytricha* cultivated in sawdust + panicum repens stalk produced higher

Table 4. Nutritional values of *Auricularia cornea* (MFLUCC18-0346 and MFLUCC18-0347) compared with other mushrooms.

Mushrooms	Ash (%)	Carbohydrate (%)	Crude Fiber (%)	Fat (%)	Protein (%)	Countries	References
Auricularia cornea MFLUCC18-0346	2.90	72.27	19.71	0.77	11.22	Thailand	This study
A. cornea MFLUCC18-0347	3.37	70.66	22.43	1.27	13.14	Thailand	This study
A. thailandica	4.3	no data	4.62	2.93	12.99	Thailand	[61]
A. auricula	9.21	59.73	7.72	12.18	11.16	Thailand	[62]
A. auricula-judae	2.82	60.23	21.07	0.13	9.06	Thailand	[63]
Auricularia sp.	6.36	no data	28.55	2.71	8.54	Sri Lanka	[64]
Lentinula edodes	6.51	23.63	26.78	0.98	39.48	Thailand	[63]
Pleurotus sajor-caju	7.73	30.26	28.40	1.50	28.74	Thailand	[63]
Volvariella volvacea	10.38	24.35	22.84	0.52	39.20	Thailand	[63]

Table 5. Comparison of the nutritional content of paddy grain and sorghum.

Parameters	Paddy grain	Sorghum
Crude protein (%)	8.5	10.6
Crude fiber (%)	11	2.8
Crude fat (%)	2.5	3.3
Starch (%)	64.3	73.6
Lignin (%)	5.5	1.1
Total sugar (%)	1.2	1.3

These data are retrieved from www.feedtables.com.

yields than sawdust alone, with B.E. values of 148.12% and 99.49%, respectively[79]. Additionally, maize cobs and wheat bran were suggested as acceptable substrate for growing the *A. auricular* brown and black strains of mushrooms in Kenya[77]. The productivity (B.E.) of Thai *A. cornea* fruiting trials in sawdust substrate was poor (72.46% ± 11.23%) in the previous study[40]. As a result, more research will be done to create the ideal environment for the cultivation of the Thai *A. cornea* utilizing local agricultural waste in the laboratory and in the industry.

Asian cuisine frequently uses *Auricularia* spp. (Hed Hoo Noo) as one of the components. The nutritional values of two strains of *Auricularia* (*A. cornea* MFLUCC18-0346 and *A. cornea* MFLUCC18-0347) were analyzed and compared to those of other *Auricularia* and other known edible mushrooms. However, similar results were obtained with crude protein, fat, and fiber, except for carbohydrate. Surprisingly, the carbohydrate found in this study was higher than those from other *Auricularia* spp. (14%–17%) and 2–3 times higher than other edible mushrooms. It should be noted that in the fruiting body of *A. auricula-judae*, polysaccharides are one of the most vital active ingredients, promoting antioxidant, immunomodulatory, anticancer, anticoagulant, antifatigue, and other properties[82,83]. Therefore, potential medical applications of these two strains of mushrooms must be carried out.

Author contributions

The author confirms contribution to the paper as follows: study concept and design: Thongklang N; data collection: Walker A, Wannasawang N, Taliam W; samples collection: Keokanngeun L, Walker A; analysis and interpretation of results: Walker A, Luangharn T, Thongklang N; draft manuscript preparation: Walker A, Thongklang N. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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