P-ISSN 2586-9000
E-ISSN 2586-9027
Homepage : https://tci-thaijo.org/index.php/SciTechAsia
Science & Technology Asia
Vol. 29 No.4 October - December 2024
Page: [247-256]
Original research article

Edible Macrofungi in Plant Genetic Conservation Area, Chanthaburi Province: *Auricularia cornea* strain RSPG5 Fruiting Body Formation and Nutritional Value

Uracha Wanich[1], Chutapa Kunsook[2,3], Pornpimon Kanjanavas[4], Pornpailin Thanyaponl[5], Onanong Boonmee[2,3], Narumon Apinansawat[1], Thassapol Panisan[1], Saowapha Surawut[2,3,*]

[1]*Department of Home economics, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand*
[2]*Department of Biology, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand*
[3]*Research Unit of Excellence in Biodiversity, Plant Genetic Conservation Project, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand*
[4]*Faculty of Nursing, Kasem Bundit University, Bangkok 10510, Thailand*
[5]*Microbiology Program, Department of Biology, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand*

Received 14 February 2024; Received in revised form 27 August 2024
Accepted 13 September 2024; Available online 27 December 2024

ABSTRACT

This study aimed to survey the edible macrofungi found in the Plant Genetic Conservation Area of Chanthaburi province, and to study bag cultivation and nutritional value of the *Auricularia cornea* RSPG5 strain. The molecular identification by internal transcribed spacer (ITS) region analysis revealed that the strains of RSPG1-11 were identified as *Cookeina sulcipes*, *Cookeina tricoloma*, *Termitomyces cylindricus*, *Schizophyllum commune, Auricularia cornea*, *Amauroderma rugosum*, *Dacryopinax spathularia*, *Tremella fuciformis, Pleurotus giganteus, Hohenbuehelia* sp., and *Phallus lutescens,* respectively. *Auricularia cornea* RSPG5 was selected for the investigation of bag cultivation. The average weight of their mature fruiting body was 69.92±24.79 g/bag, yield data was 87.40 g/kg, and biological efficiency was 8.74. The moisture per 100 g was 87.30 g. The

***Corresponding author:** saowapha.s@rbru.ac.th **doi:** 10.14456/scitechasia.2024.85

fruiting body contained 0.63 g of protein, 0.04 g of fat, 0.16 g of ash, 11.88 g of carbohydrate, and 11.66 mg of calcium. The total energy was 50.38 kcal /100 g.

Keywords: *Auricularia cornea*; Bag cultivation; Edible macrofungi; Plant genetic conservation area; Nutritional value

1. Introduction

The Plant Genetic Conservation Area of Rambhai Barni Rajabhat University is a small lowland forest located in the northern part of Chanthaburi Province on the eastern Gulf of Thailand. The forest measures about 7.74 ha and has an average sea level of about 300 m. The forest has a temperature of 28.2-32.2°C with precipitation of 2,000 cm^3 per year. This forest includes diverse habitat types, e.g., swamp forests, tropical rain forests, and freshwater canal areas. There are 127 identified flora species with the predominant trees including *Horsfieldia irya, Anisoptera costata,* and *Aporosa nervosaa.* Fauna such as birds and butterflies include 73 and 23 identified species, respectively. Therefore, this area is a rich natural habitat for living things and is also used for nature education [1].

The macrofungi diversity in the Plant Genetic Conservation Area, Chanthaburi has been explored to establish the database [2]. Forty-one taxa of macrofungi were classified into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera. The edible macrofungi include *Cookeina sulcipes*, *C. tricoloma*, *Amauroderma rugosum*, *Termitomyces* sp., *Schizophylum commune*, *Auricularia cornea*, *Dacryopinax spathularia*, and *Tremella fuciformis*. However, research on the edible macrofungi for its benefits, including cultivation and nutritional value, have not been carried out.

The bag cultivation of macrofungi has previously been described depending on the macrofungi species [3-6]. The 4 main steps for bag cultivation are mycelium isolation, mycelium spawning, substrate preparation, and cultivation to produce mature fruiting bodies. Usually, the edible macrofungi species with the role of saprotroph were studied for macrofungi cultivation because it was not complicated to optimize the conditions on cultivation, such as for *S. commune* [4] and *A. cornea* [3].

A. cornea is a member of jelly fungi (ear mushroom) that is classified in the Kingdom Fungi, Phylum Basidiomycota, Class Agaricomycetes, Order Agaricales, and Family Auriculariaceae. *A. cornea* is edible and has medicinal properties. *A. cornea* was reported as a new record from Thailand with an ear-like structure of the fruiting body that revealed a light brown to dark brown color and the bag cultivation of this fungus had been studied [3].

In Thailand, the *Auricularia* spp. were a favorite for consumption and their inclusion in diverse food processes led to commercial cultivation due to wide market demand. *A. cornea* has been studied for bag cultivation; however, the fruiting body formation depends on substrate formulation and culture conditions.

Therefore, in this study, we report on the edible macrofungi found in the Plant Genetic Conservation Area, Chanthaburi Province, and *A. cornea* as an optional macrofungi that can be cultivated in this area, while also reporting the data of its nutritional value.

2. Materials and Methods

2.1 Collection of the macrofungi

Macrofungi samples were collected from May until July 2023 in the Plant Genetic Conservation Area located in Rambhai Barni Rajabhat University, Chanthaburi. They were classified by their macro-morphological characteristics. These samples were placed in a plastic box, and macrofungal tissues were kept in 100% ethanol at -20ºC for tissue storage and kept in the lysis buffer of the DNA extraction kit (Favorgen, Taiwan) for DNA extraction.

2.2 Molecular identification of the macrofungi

The DNA extraction was performed as described by the manufacturer's method (Favorgen, Taiwan). The internal transcribed spacer (ITS) was used as a target to amplify by PCR with the primers: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTA TTGATATGC-3') [7]. The PCR reaction consisted of 1x PCR master mix (Apsalagen, Thailand), 0.5 µM primers, distilled water (Apsalagen, Thailand), and 10 pg-1 µg of DNA template in 20 µl total volume. PCR amplification was carried out under the following conditions. Initial denaturation at 95°C for 3 min. Denaturation for 35 cycles at 95°C for 30 sec, annealing at 52°C for 30 sec, and extension at 72°C for 1 min. Last, the final extension at 72°C for 10 min. The 2% agarose gel electrophoresis with RedSafe (iNtRON biotechnology, Korea) at 100V for 30 min was used to analyze the PCR product. DNA purification and sequencing were performed by Macrogen (Korea). The percent identities of macrofungi ITS sequences were determined by BLASTn in the GenBank database (https://blast.ncbi. nlm.nih.gov /Blast.cgi) and MycoBank (https://www. mycobank.org/page/Pairwise_alignment). The sequences were analyzed using the Neighbor-Joining method to generate an evolutionary tree [8, 9]. The evolutionary distances were computed using the Maximum Composite Likelihood method [10]. Evolutionary analyses were conducted in MEGA X [11].

2.3 The edibility property and role in ecology

The edibility property and role in ecology of macrofungi were searched in previously published database and FUNGuild (https:// github. com/ UMNFuN/ FUNGuild) [12].

2.4 Isolation of the mycelium

A piece of mycelium was removed from the inside of the fresh fruiting body. Subsequently, it was transferred to potato dextrose agar (PDA) (Himedia, India). The isolated culture was incubated at room temperature and subculture again for pure isolation.

2.5 Spawning of the mycelium

The sorghum grains were soaked in water overnight then boiled for 15 min. Subsequently, 200 g of grains were transferred to a 330 ml glass bottle. These bottles with sorghum grains were sterilized at 121°C for 15 min under pressure by autoclave. The pure culture of mycelia on PDA was cut and transferred to the sorghum seeds in the bottle. The bottles were incubated at room temperature until all seeds were covered with the mycelia.

2.6 Substrate formulation and bag cultivation

Sawdust from rubber trees (100 kg) was mixed with rice bran (3 kg), $CaSO_4 \cdot 2H_2O$ (1 kg) or gypsum, $CaMg(CO_3)_2$ or dolomite (0.5 kg), and $MgSO_4.7H_2O$ (50g) with 50% (w/w) moisture content on a wet mixed substrate by water. The mixed substrate (800g) was filled in plastic bags (16.50 cm wide and 28.00 cm long) and then capped with a plastic ring and lid to set the bottleneck. These bags were sterilized at 121 °C for 20 min in an autoclave and were cooled overnight. The 5g of mycelia spawn were inoculated in the sterilized bags at the bottleneck and incubated at room temperature.

2.7 Nutritional value of harvested fruiting body

The moisture, ash, protein, fat, calcium, and iron content of the fruiting body were determined using the Association of Official Analytical Chemists (AOAC, 2019) criteria. The carbohydrate content was determined by calculation of the following equation: Carbohydrate = 100 – (protein + fat + moisture + ash) [13]. The energy value of the fruiting body was determined by multiplying the protein content by 4, carbohydrate content by

4, and fat content by 9 [14]. The data are presented as average values.

3. Results and Discussion

3.1 Molecular identification of macro-fungi, edible properties, and role in an ecology

Eleven samples of macrofungi were collected from the Plant Genetic Conservation Area, Chanthaburi province in 2023, and their strains were designated as shown (Table 1 and Fig. 1). They were classified by macromorphology that divided them into 5 groups: (i) cup or disc-like fungi (RSPG1 and RSPG2), (ii) gilled fungi (RSPG3, RSPG4, RSPG9, and RSPG10), (iii) jelly fungi (RSPG5, RSPG7, and RSPG8), (iv) polypores and bracket fungi (RSPG6), and (v) stinkhorn fungi (RSPG11).

Table 1. Macrofungi samples in this study, their strain, and grouping by their micromorphology.

Strain	Group
RSPG1	Cup or disclike fungi
RSPG2	Cup or disclike fungi
RSPG3	Gilled fungi
RSPG4	Gilled fungi
RSPG5	Jelly fungi
RSPG6	Polypores and bracket fungi
RSPG7	Jelly fungi
RSPG8	Jelly fungi
RSPG9	Gilled fungi
RSPG10	Gilled fungi
RSPG11	Stinkhorn fungi

The molecular identification by BLASTn (Table 2) and phylogenetic tree analysis of ITS sequence revealed in Fig. 2.

Fig. 1. Edible macrofungi found in Plant Genetic Conservation Area, Chanthaburi province. A: RSPG1, B: RSPG2, C: RSPG3, D: RSPG4, E: RSPG5, F: RSPG6, G: RSPG7, H: RSPG8, I: RSPG9, J: RSPG10, and K: RSPG11.

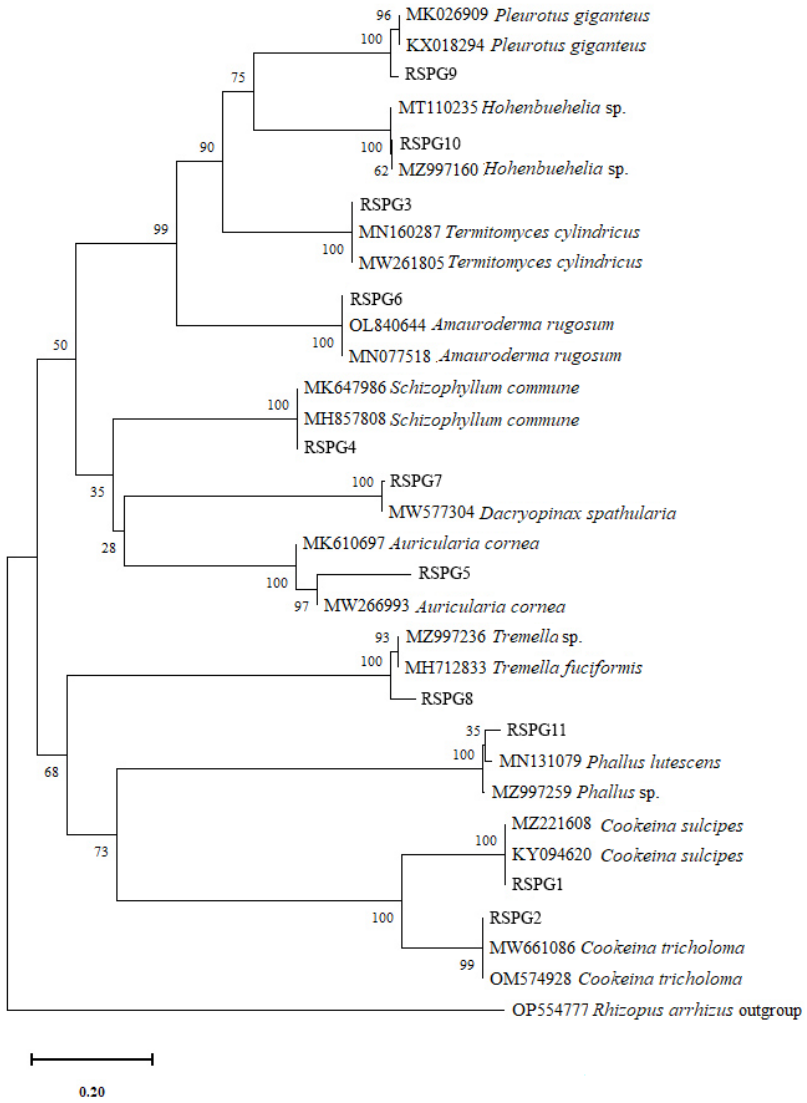

Fig. 2. Phylogenetic tree based on ITS sequences of edible macrofungi in this study.

Table 2. BLASTn results of macrofungi collected from the Plant Genetic Conservation Area.

Strain	Best match (Accession No.)	
	ITS	Similarity (%)
RSPG1	*Cookeina sulcipes* (KY094620)	100
RSPG2	*Cookeina tricholoma* (OM574928)	100
RSPG3	*Termitomyces cylindricus* (MN160287)	100
RSPG4	*Schizophyllum commune* (MK647986)	100
RSPG5	*Auricularia cornea* (MK610697)	100
RSPG6	*Amauroderma rugosum* (OL840644)	100
RSPG7	*Dacryopinax spathularia* (MW577304)	99.77
RSPG8	*Tremella fuciformis* (MH712833)	99.51
RSPG9	*Pleurotus giganteus* (MK026909)	99.53
RSPG10	*Hohenbuehelia* sp. (MZ997160)	100
RSPG11	*Phallus lutescens* (MN131079)	97.18

Strains RSPG 1-11 were identified as *Cookeina sulcipes*, *Cookeina tricoloma*, *Termitomyces cylindricus*, *Schizophyllum commune, Auricularia cornea*, *Amauroderma rugosum*, *Dacryopinax spathularia*, *Tremella fuciformis, Pleurotus giganteus, Hohenbuehelia* sp., and *Phallus lutescens,* (Table 2 and Fig. 2). These macrofungi play the role of saprotrophs in their ecology ecology, while *T. cylindricus* plays the role of symbiotroph.

In a previous study, the diversity of macrofungi in the Plant Genetic Conservation Area, Chanthaburi province in 2021 was reported. Forty-one taxa of macrofungi were classified into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera with *Microporus xanthopus* as the most frequent species. Moreover, the edible macrofungi included *C. sulcipes*, *C. tricoloma*, *A. rugosum*, *Termitomyces* sp., *S. commune*, *A. cornea*, *D. spathularia,* and *T. fuciformis* [2]. In the present study, additional edible macrofungi have been reported including *Pleurotus giganteus, Hohenbuehelia* sp. , and *Phallus lutescens*.

The macrofungi species that have previously been reported as edible mushrooms but have no evidence of commercial cultivation consist of *C. sulcipes, C. tricoloma, T. cylindricus, A. rugosum,* and *D. spathularia.* However, nutritional components, bioactive compounds, and medicinal properties have been reported. *C. sulcipes* possesses a high protein and phosphorus content, and a low content of lipids [15], and the β-D-glucans of *C. tricoloma* showed significant inhibition of neurogenic pain [16]. Meanwhile, the genus of *Termitomyces* is widely consumed, including *T. cylindricus*. This species was recently reported in Thailand [17]. Additionally, *A. rugosum* [18, 19] and *D. spathularia* [20, 21] revealed nutritional value, antioxidant, anti-inflammatory activity, and produced bioactive compounds such as long- chain glycolipids used as food additives and have the benefit of having very low oral bioavailability, rapid elimination, and are mainly excreted in the feces.

In this study, some edible macrofungi have previously been studied for cultivation and growth for commercial use such as *S. commune* [4], *T. fuciformis* [22, 23], *A. cornea* [3], and *P. giganteus* [24]. The medicinal properties of these macrofungi have been reported [25-27], such as the peptides produced by *S. commune* showing cellular antioxidant activity in the context of a human intestinal cancer cell line [28].

Hohenbuehelia sp. was found in this study. Some species of *Hohenbuehelia* were reported as edible mushrooms but with very low culinary value [29]. However, the *Hohenbuehelia* could not be identified at the species level in this study. This suggests that the sequence analysis of additional genes may be required, such as the sequence analysis of 3 loci including ITS, the D1/D2 variable region of the large subunit gene (LSU), and the translation elongation factor (TEF1) [30].

In addition, some species from the *Phallus* genus have been reported as edible. However, some other species are considered poisonous, such as *P. multicolor*. Further, *P. lutescens* have been reported as a new species in China. The local people in southern China will eat the fruiting bodies of *P. lutescens* having a white or yellowish color, but there have been no reported cases of poisoning caused by this species. Therefore, *P. lutescens* might be a newly recognized edible fungus [31].

Among these edible macrofungi found in this study, *Auricularia cornea* RSPG5 was of the most interesting and was selected for further study due to its edibility, medicinal properties, nutritional value, cultivation evidence, and wide market demand.

3.2 Mycelia isolation and spawning

The mycelia of *A. cornea* RSPG5 could be isolated as a pure culture on PDA (Fig. 3A) and their spawning (Fig. 3B)

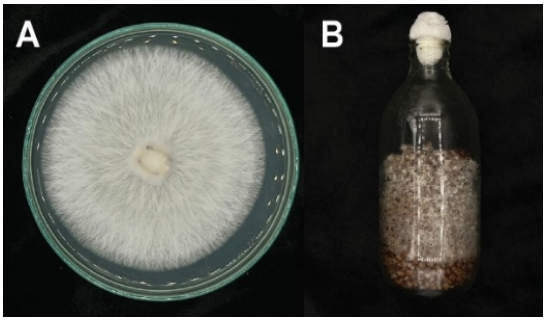

Fig. 3. The mycelia of *A. cornea* RSPG5 on PDA (A) and their spawning (B).

3.3 Cultivation for fruiting body production

The mature fruiting bodies of *A. cornea* RSPG5 were harvested (Fig. 4). Average weight was 69.92±24.79 g/bag, yield data was 87.40 g/kg, and biological efficiency (B.E) was 8.74 (Table 3). In Thailand, the *A. cornea* wild strain was studied for cultivation. The average weight in each flushed was 40 g/bag, yield data was 50.42 g/kg, and B.E. was 12.07 [3]. This study revealed that *A. cornea* RSPG5 seemed to produce more fruiting bodies than previously described in another wild strain. The factors that may affect this result were substrate formulation and climate. This study used different compositions of substrate formulation for bag cultivation and the substrates were easily available in the local area. Another factor in bag cultivation was the local climate that may have better supported the growth and development of the fungal fruiting bodies. Eastern Thailand has a tropical climate with rain being more abundant resulting in higher humidity, especially during the rainy season, during which the bag cultivation of this study took place.

Fig. 4. Bag cultivation of *A. cornea* RSPG5.

Table 3. Average weight, yield data, and biological efficiency (B. E.) of *A. cornea* RSPG5.

Average weight (g/bag),	Yield data (g/kg)	Biological efficiency (B.E.)
69.92±24.79	87.40	8.74

3.4 Nutritional value of harvested fruiting body

The nutritional values of the fruiting body from *A. cornea* RSPG5 is shown in Table 4. The moisture of *A. cornea* RSPG5 was 87.30 g water per 100 g fruiting body. The fruiting body contained 0.63 g of protein, 0.04 g of fat, 0.16 g of ash, 11.88 g of carbohydrate, and 11.66 mg of calcium. The total energy was 50.38 kcal/100 g. Generally, the nutritional value per 100 g of fresh *A. auricula-judae* contains 50 Kcal of energy, 10.90 g of carbohydrate, 14 g protein, 0.10 g of fat, 1.8 g of dietary fiber, 60 mg of calcium, 6.1 mg of iron, 0.04 mg of thiamine, 0.71 mg of riboflavin, 2.80 mg of niacin, 21 mg of vitamin C, and 87.1 g of water [32]. However, the nutritional value is influenced by the substrate formulation and culture conditions as previously described [33-36].

Table 4. Nutritional value of between *A. auricula-judae* and *A. cornea* RSPG5.

Nutrition Component	Nutrient content per 100 g of fresh	
	A. auricula-judae	***A. cornea* RSPG5**
Moisture (g)	87.10	87.30
Protein (Nx4.38) (g)	14	0.63
Fat (g)	0.10	0.04
Ash (g)	-	0.16
Carbohydrates (g)	10.9	11.88
Calcium (mg)	60	11.66
Energy (kcal)	50	50.38

4. Conclusion

The edible macrofungi found in the Plant Genetic Conservation Area of Chanthaburi province contained *C. sulcipes*, *C. tricoloma*, *T. cylindricus*, *S. commune, A. cornea*, *A. rugosum*, *D. spathularia T. fuciformis, P. giganteus, Hohenbuehelia* sp., and *P. lutescens. A. cornea* RSPG5 was selected for the investigation of bag cultivation and nutritional value. The average weight of their mature fruiting body was 69.92±24.79 g/bag, yield data was 87.40 g/kg, and biological efficiency was 8.74. Additionally, the moisture per 100 g was 87.30 g, meanwhile, the fruiting body contained 0.63 g of protein, 0.04 g of fat, 0.16 g of ash, 11.88 g of carbohydrate, and 11.66 mg of calcium with a total energy of 50.38 kcal/100 g. This study suggested that *A. cornea* RSPG5 can produce fruiting bodies in bag cultivation and could be used for commercial cultivation in this area. *A. cornea* RSPG5, along with the other edible macrofungi reported in this study should be further investigated for bag cultivation, nutrition value, and food processing.

Acknowledgments

This work was financially supported by Plant Genetic Conservation Project Under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG) in the fiscal year 2023 and Rambhai Barni Rajabhat University and Thailand Science Research and Innovation (Grant number 1104/2566). We would like to thank Nittaya Aemphithak, Pimchanok Wongkongdee, Thanisorn Noytamto, Thanyaphong Watthanasak, Kanyarat Charonram and Phuttarawadee Poonkla for the preparation of materials for the field trip, macrofungi samplings, and laboratory study.

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