
***Coprinus comatus*, a newly domesticated wild nutraceutical mushroom in the Philippines**

Renato G. Reyes^{1*}, Lani Lou Mar A. Lopez¹, Kei Kumakura², Sofronio P. Kalaw¹, Tadahiro Kikukawa³ and Fumio Eguchi³

¹Center for Tropical Mushroom Research and Development, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

²Mush-Tech Co., Ltd., Japan

³Takasaki University of Health and Welfare, Gunma, Japan

Reyes, R.G., Lopez, L.L.M.A., Kumakura, K., Kalaw, S.P., Kikukawa, T. and Eguchi, F. (2009). *Coprinus comatus*, a newly domesticated wild nutraceutical mushroom in the Philippines. *Journal of Agricultural Technology* 5(2): 299-316.

The mycelial growth performance on indigenous culture media and the optimum physical conditions (pH, aeration and illumination) of *C. comatus* as a prelude to its domestication were investigated in this study. In our desire to develop technology for its aseptic cultivation for our immediate plan of using this mushroom for nutraceutical purposes, we have tried growing this mushroom under sterile condition. As such, the amino acid profile and toxicity of *C. comatus* were also elucidated. Results of our investigation revealed that *C. comatus* grown in sealed plates of coconut water gelatin (pH 6.5) produced very dense mycelial growth, 6 days after incubation in the dark. Early initiation of fruiting bodies was observed in bottles containing previously sterilized sawdust (8 parts): rice grit (2 parts) formulation. Matured fruiting bodies were harvested 8 days after inoculation having 18% biological efficiency. *C. comatus* contains more standard (1643 mg/100g sample) than non standard amino acid (465 mg/100g). Eight essential amino acids were detected namely Valine>Leucine>Lysine>Isoleucine>Threonine>Phenylalanine>Tryptophan>Methionine in decreasing order of abundance. Glutamic acid though non-essential is also present in high proportion (441.6 mg/100g). The presence of γ - amino butyric acid (GABA) and Ornithine as non-standard amino acid in addition to its standard amino acid contents make this mushroom a nutraceutical species. Results of the in vitro assay for the inhibition of angiotensin converting enzyme confirmed its importance as antihypertensive natural source of nutraceutical.

Introduction

Though mushroom production in the Philippines is no longer new since its first recorded production in 1930s with *Volvariella volvacea* as the first commercially cultivated species (Clara, 1937), only few are being grown nowadays for commercial purposes. Topping the list is *V. volvacea*, followed

*Corresponding author: Reyes, R.G.; e-mail: boyongreyes@yahoo.com

by different species of *Pleurotus*, *Auricularia*, *Agaricus bisporus* and *Lentinula edodes*. At the turn of the 21st century, *Ganoderma*- based products like tea, capsule, lotion, toothpaste and other forms have been imported from neighbouring countries even though *Ganoderma lucidum* has been found locally growing in the country (Tayamen *et al.*, 2004; Paderes *et al.*, 2004; Reyes and Abella, 2002). In 2008, *Agaricus blazei* - based coffee mixed with *Ganoderma* and other plant-based nutraceuticals was introduced in the local market and was favourably accepted by the Filipino consuming public. The introduction of these imported mushroom-based products into the domestic market has revolutionized the traditional thinking of most Filipinos that mushrooms are not only for culinary but also for nutraceutical purposes as well. It is noticeable that most of these cultivated mushrooms are imported species whose production technologies are also based from the countries of origin. Ironically, the Philippines being a tropical country is rich with mycodiversity which are still in the wild and remained to be harnessed for their nutraceutical potential. (Reyes *et al.*, 1997; 1998; Reyes and Abella, 2002; Reyes *et al.*, 2003; Garcia *et al.*, 2004; Tayamen *et al.*, 2004). If properly harnessed, these species can be used as direct sources of protein and bioactive compounds for the people while generating livelihood, ensuring food security and promoting environmental protection in the countryside. We were successful in developing production technologies for locally growing wild medicinal species such as *Schizophyllum commune* and *Collybia reinakeana* (Reyes *et al.*, 2004, 2006). Wild strain of *S. commune* which is locally known as *kudit* by the Ilocanos, *kudopop* by the Bisayans and *kurakding* by the Bicolanos was rescued from the wilderness of Mt. Nagpale in Abucay, Bataan during our expedition in the area (Garcia *et al.*, 2004). The assistance of the indigenous tribe called Aetas or Baluga in the area facilitated our successful rescue of its cell lines that led to the successful development of production technology. Moreover, *C. reinakeana*, a virtually unknown, giant, wild edible mushroom in the mountainous area of Puncan, Carranglan, Nueva Ecija in the Philippines proliferated the eroded mountain after the powerful earthquake that jolted Luzon in 1990. Its mycelia were successfully isolated that culminated in the development of its production technology (Reyes *et al.*, 2004; Reyes *et al.*, 2000; Reyes *et al.*, 1998). A number of local strains of *V. volvacea* have already been improved (Reyes, 1998a and b; 1999) and generated a more efficient production technology (Reyes 2000). Also, we have surveyed local strains of *Auricularia* (*A. tenuis*, *A. polytricha*, *A. fuscosuccinea* and *A. auricula*) and *Pleurotus cystidiosus* whose commercial counterparts are already available in the domestic market (Musngi *et al.*, 2005; Reyes *et al.*, 2003). These are just some of the rich genetic mushroom resources of the country,

though majority are still in the wilderness and waiting to be picked up, studied and ultimately developed the production technologies for their commercial attributes. One of these wild genetic resources that normally inhabit lawns and gardens or piles of straw is *Coprinus comatus*. In the rural areas of Central Luzon, it is popularly called *kabuteng hapon* since it is being collected only in the afternoon due to its high perishability. Its fruiting body easily matures and opens which ultimately becomes inky due to its autolytic character resulting to the discharge of its basidiospores. The *Volvariella* growers in Central and Northern Luzon called this *kabuteng demonyo* because they considered it as a weed fungus in their mushroom beds. This mushroom initially grows and dominates the substrates which lead to the inability of the mycelia of *V. volvacea* to ramify due to lack of space and limited nutrition. Its fast growing and dominating character coupled with its edibility and popularity prompted us to fully study its biophysiology and functionality.

In general, mushrooms are regarded as functional foods especially in Asia (Chang and Buswell, 1999 and 1996). They also served as excellent sources of protein. For instance, *V. volvacea* contains both standard and non standard amino acids which also exhibited antihypertensive and antidiabetic properties (Eguchi *et al.*, 2008). We also have demonstrated the ability of *S. commune* to produce exopolysaccharides called schizophyllan (Reyes *et al.*, 2009). Schizophyllan is a jelly – like slimy material which is soluble in water. It is utilized in the preparation of skin care products like lotion and creams which acts as viscosifier and as anti-aging, depigmenting and healing agent of the skin (Kim *et al.*, 1999, 2000). Schizophyllan is an active ingredient that can increase skin cell proliferation, collagen biosynthesis and recovery from sunburn. Other commercially cultivated mushrooms like different species of *Pleurotus* and *Auricularia* which are already being commercially cultivated in the country have also been reported to possess functional activities (Eguchi *et al.*, 2008; Jeurink *et al.*, 2008; Zang *et al.*, 2008).

Filipinos are known to be consumers of mushroom but this commodity remained to be a luxury food on the table of an ordinary Filipino family. Its expensive price in the local market and its year round unavailability makes this commodity a special food. Thus, there is a need to harness the economic potential and intensify the production of this commodity in order to satisfy the growing demand. For instance, the Philippines has imported 73 metric tons of mushroom in 2003 despite of its 560 metric tons of production (FAO Statistics, 2004). Thus, with our rich mycological resources coupled with favorable environment, abundance of local substrates for production, a very high market demand and a strong need to ensure food security in the growing population, it is therefore imperative to utilize our wild mycological resources like *C.*

comatus in order to develop production technologies that will lead to their year-round availability for culinary and nutraceutical purposes.

Materials and methods

Source of strain

Immature stages of the fruiting bodies of *C. comatus* were collected from the piles of decomposing rice straw. The mycelia of this mushroom were aseptically rescued following the tissue culture protocol in the laboratory.



Fig. 1. Fruiting bodies of *C. comatus* in naturally composted rice straw.

Preparation of stock culture medium

Coconut water which was derived from newly cracked matured coconut was used as the propagating medium. Twenty grams of white gelatin was shredded and melted in a liter of coconut water. Forty milliliters of the prepared medium was dispensed into newly blanched dried flat bottles. The bottled medium was plugged with cotton roll and sterilized at 15 psi. or 121°C for 20 minutes. This medium was used in tissue culture and subsequently in the maintenance of the pure culture.

Sub-study I. Screening of appropriate indigenous culture media for the efficient mycelial growth of C. comatus

The following locally available culture media were formulated and evaluated for the mycelial growth of *C. comatus*: T₁ - 1 liter decoction from 50 g rice bran; T₂ - 1 liter decoction from 50g rice straw and T₃ - 1 liter coconut water. The three evaluated media were hardened with 2% gelatin prior to sterilization. Triplicate plates per medium were aseptically inoculated with a 10

mm mycelial disc of *C. comatus* and subsequently incubated at room temperature to allow the ramification of its mycelia. Daily mycelial growth was recorded.

Sub-study II. Influence of physical factors on the mycelial growth performance of C. comatus

pH

The most appropriate medium in sub-study I was adjusted to different pH levels: 6.0, 6.5, and 7.0 using 0.1 M NaOH or 0.1 M HCl prior to sterilization. Triplicate plates per medium were aseptically inoculated with a 10 mm mycelial disc of *C. comatus* and subsequently incubated at room temperature to allow the ramification of its mycelia. Daily mycelial growth was recorded.

Aeration

From the most appropriate medium and pH level in sub – study 1, the influence of aeration on the mycelial growth of *C. comatus* was investigated. This was done by sealing individually the triplicate plates with Para film (T₁) and the remaining triplicates were not sealed (T₂). The plates were then incubated at room temperature to allow the proliferation of mycelia.

Light

Six plates containing the most appropriate medium and adjusted to the optimum pH level were inoculated with 10 mm mycelial disc of *C. comatus*. During incubation, the inoculated plates were sealed with Parafilm and divided into two lots: the first triplicate plates (T₁) were incubated in the dark and the remaining three were placed under well lighted condition (T₂).

Sub-study III. Aseptic cultivation of C. comatus

The fruiting body production of *C. comatus* was evaluated on the following formulations: T₁ - 2 parts rice grit and 8 parts sawdust; T₂ – 2 parts rice bran and 8 parts sawdust; T₃ – 1 part rice grit and 1 part rice bran; T₄ – pure rice grit and T₅ – pure rice bran. The formulated substrates were placed in clear bottles and the bottles were individually covered with polypropylene sheets prior to sterilization. The bottled substrates were sterilized at 15 psi, 121C for 45 minutes. After cooling, the previously sterilized bottled substrates were inoculated with a 10 mm mycelial disc of *C. comatus* and subsequently incubated at room temperature.

Sub-study IV. Determination of the nutraceutical profile of C. comatus

Amino acid profile

The determination of the nutritional content of *C. comatus* was confined on its amino acid profile. Both standard and non standard amino acid contents were determined following the standard protocol for amino acid analysis.

Assay for ACE inhibitory activity.

Functional components of the *C. comatus* were obtained through hot water extraction following the procedure of with some modification. The active components of the milled mushroom samples (20g) were extracted in 600 ml hot water at 80 - 90°C in a water bath for 2 hrs. The milled mushroom were separated from the extract by filtration using filter paper no. 2 (Toyo Co., Japan). The filtrate was frozen by dipping in cold methanol and vacuum dried in a freeze drier (VA-500F Freeze Drier Taitec Co., Japan). The dry weight of the harvested freeze dried extract was noted and the percentage yield of extract was recorded.

The procedure of Kumakura *et al.* (2008) in assessing the inhibition of angiotensin converting enzyme (ACE) was adopted with minor modification. Five mg of mushroom hot water extract was mixed in 1 ml 100 mM Borate buffer, pH 8.3 in order to make a 5% concentration. The mixture was filtered through a 0.45 µm nylon syringe filter (Whatman, Inc. USA). Ten µl of the sample was mixed in 20 µl 60 mU/ml ACE (from rabbit lung) and 30 µl 1M NaCl. The mixed samples were pre incubated at 37°C for 5 minutes in a water bath. After the 5 minute - pre incubation, 60 µl 6mM Hippuric acid-Histidine-Leucine (Hip-His-Leu) was added to the mixture and incubated in water bath at 37°C for 60 minutes. Hip-His-Leu served as substrate of the enzyme. To stop the reaction, 60 µl 1N HCl was added. The supernatant was filtered through a 0.45 µm nylon syringe (Millex®, LH, Japan). The filtrate was subjected to reversed phase HPLC with the following conditions: injected dose, 20 µl 1V; analytical column, C18 (GL Sciences, Inc. Japan); Mobile phase, methanol:10 mM KH₂PO₄ at 1:1 and adjusted to pH 3.0 using phosphoric acid; flow rate, 0.5 ml/min. HA and hippuryl-L-histidyl-L-leucine (HHL) were detected at 228 nm. In addition to the mushroom samples, the vial containing ACE + NaCl in buffer was also incubated and served as negative control. For positive control, the reaction of the vial containing ACE + NaCl in buffer was immediately terminated by 1 N HCl prior to incubation.

Sub-study V. Safety analysis of C. comatus

The protocol of Eguchi *et al.* (2008) on toxicity test of mushroom and Inatomi *et al.* (2006) on single oral dose toxicity was adopted with minor modification.

Acclimatization of mice

Prior to oral administration of the hot water extract, the 4 week old female Crlj:CD1 (ICR) mice were acclimatized for 1 week in a highly restricted animal room with ad libitum feeding of pelleted feeds (Oriental Yeast Co. Ltd. Chiba, Japan) and water. After the acclimatization stage, the mice were starved with feeds for 24 hrs. Only water was supplied ad libitum to the mice during this period.

Oral administration of hot water extract

One ml of the hot water extract of *C. comatus* with the following dosages: 100 mg/kg, 250mg/kg; 500 mg/kg and 1000 mg/kg body weight was orally administered to the mice. For the control, set of 5 mice were only administered with distilled water. The behavior of the mice was observed 30 minutes, 1 hr, 3, 6, 12, 24, 48 and 36 hrs after administration. Pre-weighed powdered feeds (Oriental Yeast Co. Ltd. Chiba, Japan) and water were only provided until 3 hrs thereafter.

Results and discussion

Taxonomic position and characteristic of *C. comatus* in its natural habitat *Coprinus comatus* (O.F. Müll.) Persoon belongs to Kingdom Fungi, Subkingdom Dikarya, Phylum Basidiomycota, Subphylum Agaricomycotina, Class Agaricomycetes, Order Agaricales and Family Agaricaceae. This mushroom naturally grows on decomposing piles of rice straw and in *V. volvacea* beds when old stocked rice straw which has been enriched with nitrogenous fertilizer was used as a growing medium. It produces long, conical shaped whitish cap that produced scaly like structures on its surface. The white stipe which is smooth and fibrous with a hollow cavity has a thin ring. It elongates easily and allows the expansion of the cap. Underneath the cap are the closely arranged gills which are freely attached from the stem. These very crowded gills appear white at first, turning pinkish and ultimately become black. Once the cap has expanded, it easily deteriorates and becomes inky and deliquescent resulting to the dispersal of basidiospores.

Growth on indigenous culture media

The luxuriance and rapidity of growth of a certain mushroom partly depend on the appropriate culture medium used in its cultivation in the laboratory. Since we are developing a practical technology that is suitable under Philippine condition, we evaluated three indigenous culture media whose components can easily be accessed in the locality. These are rice bran, rice straw and coconut water. As depicted in Table 1, coconut water favored the efficient colonization of the mycelia of *C. comatus* within 8 days of incubation (Table 2) producing a uniform, cottony and vigorous mycelial growth with zonation pattern (Fig. 2).

Table 1. Mycelial growth of *Coprinus comatus* in indigenous culture media.

Incubation (day)	Rice bran decoction gelatin	Rice straw decoction gelatin	Coconut water gelatin
1	23.72 ^a	21.30 ^a	19.54 ^a
2	35.89 ^b	41.48 ^a	29.89 ^c
3	46.91 ^b	59.47 ^a	42.32 ^b
4	56.85 ^b	77.13 ^a	51.61 ^b
5	64.95 ^b	91.56 ^a	61.07 ^b
6	69.94 ^b	97.66 ^a	70.74 ^b
7	74.39 ^c	98.60 ^a	81.62 ^b
8	81.22 ^b	98.60 ^a	98.60 ^a
9	87.20 ^b	98.60 ^a	98.60 ^a
10	93.25 ^b	98.60 ^a	98.60 ^a
11	98.60 ^a	98.60 ^a	98.60 ^a
Mean	66.63 ^b	80.14 ^a	68.29 ^b

Individual value which refers to the incubation in day (within column) having the same letter superscript are not significantly different from each other at 5% level of significance. Mean values within row having the same letter superscript are not significantly different at 5% level of significance.

Though fastest colonization was observed in rice straw decoction gelatin compared to rice bran and coconut water, the mycelial density was observed to be thin and scanty which implies that the nutrients present in the medium might not be sufficient to nourish a vigorous and luxuriant mycelial growth. Early fructification was noted however in rice bran decoction gelatin. The superiority of coconut water as a culture medium for the efficient mycelial growth of *C. comatus* further reaffirms our previous findings regarding its suitability as a culture medium for the propagation of mycelia of most wild mushrooms we have cultivated including *Ganoderma lucidum*, *Auricularia polytricha* and *Schizophyllum commune* (Reyes *et al.*, 2009; Bulseco *et al.*, 2005; Garcia *et al.*, 2004; Tayamen *et al.*, 2004; Reyes *et al.*, 1992, 1993). Coconut water is an

ideal medium due to its nutrient content that can fulfill the nutritional needs of mushroom. It was reported to contain 0.2 g fats, 0.3 g protein, 5 g sugar, 310 mg potassium, 1.1 mg iron per 100 gram (Snowdon 2003). Though the natural substrate of *C. comatus* is rice straw, it is ironic to note that it only produced very scanty mycelial growth. The non aggressiveness of the mycelia of *C. comatus* in this substrate may be attributed to improper formulation of the rice-straw-based medium. Thus there is a need to reinvestigate the appropriate formulation of rice straw-based medium. Rice bran on the other hand, stimulated early fructification. Early fructification while in pure culture is not desirable in culture maintenance as it triggers contamination. Rice bran is also commonly used as a culture medium which the mushroom growers found it practical and economical to use. However, in this investigation, there is a need to reevaluate the formulation to come up with the appropriate concoction for the mycelial growth of *C. comatus*.

Table 2. Incubation period of mycelial growth of *Coprinus comatus* in different culture media.

Culture Media	Incubation Period (day)	Mycelial Density
Rice bran decoction gelatin	11 ^c	thick, cottony with early fructification
Rice straw decoction gelatin	7 ^a	thin
Coconut water gelatin	8 ^b	very thick, cottony, with circular zonation pattern

Means within column having the same letter superscript are not significantly different from each other at 5% level of significance

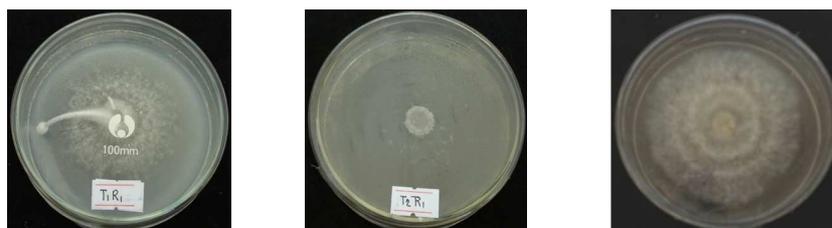


Fig. 2. Cultural characteristics of *C. comatus* in different indigenous culture media. Note: T₁R₁ (rice bran decoction gelatin), T₂R₂ (rice straw decoction gelatin), T₃R₁ (coconut water gelatin).

Physical requirements for the efficient mycelial growth of C. comatus

Since coconut water gelatin was the best medium in the previous study, it was used as the assay medium in this investigation. Though mycelial growth of *C. comatus* was also characterized to be cottony, vigorous and very

dense in pH 6.0 and 7.0, it was observed that pH 6.5 significantly stimulated the mycelial growth of *C. comatus* which colonized the plate containing coconut water gelatin 2 days ahead of the two pH levels (Table 3 and Fig. 3 and 4). We have noticed that its favorable response to pH 6.0 makes it different from other leaf litter inhabiting mushrooms like *V. volvacea* (Reyes *et al.*, 1998) and *C. reinakeana* (Reyes *et al.*, 1997) where the optimum pH is between pH 7.0 to 8.0. Moreover, Chaiyama *et al.* (2007) reported that the optimum pH for *C. comatus* is at pH 6 which does not conform to our findings. This implies that different strains of *C. comatus* may have varying cultural requirements for growth including the pH of the substrate.

Table 3. Influence of pH of coconut water gelatin on the incubation period and mycelial density of *C. comatus*.

pH level	Incubation period (day)	Mycelial density
6.0	8 ^b	Very thick and cottony
6.5	6 ^a	Very thick and cottony
7.0	8 ^b	Very thick and cottony

Means having the same letter superscript are not significantly different from each other at 5% probability level by DMRT.

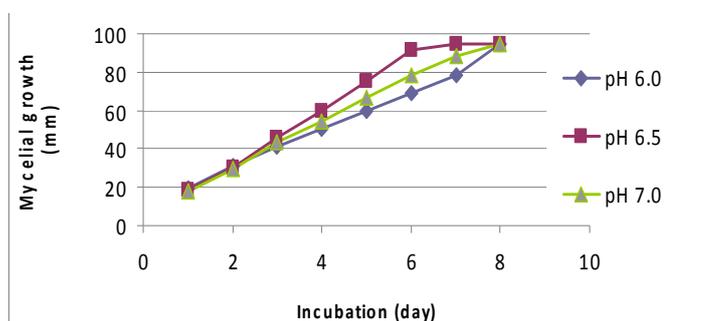


Fig. 3. Influence of pH of the culture media on the mycelial growth of *Coprinus comatus*.



Fig. 4. Cultural characteristics of *C. comatus* in coconut water gelatin with varying levels of pH, 6 days after incubation.

Aeration is a limiting factor in the mycelial growth of *C. comatus*. We have observed that mycelial growth was promoted in plates sealed with parafilm than those plates which were not sealed during the entire incubation (Fig. 5). The sealed plate cultures produced very dense, cottony mycelia with complete ramification 6 days after incubation (Fig. 6) which was 2 days earlier than those plate cultures which were not sealed with parafilm. Though illumination influences the fructification of mushroom in general, its effect on the mycelial ramification of *C. comatus* was also determined. We have observed that illumination did not significantly stimulate the mycelial performance (Fig. 7). *C. comatus* when incubated under dark produced very dense, cottony mycelia 6 days after incubation which was 3 days earlier than when incubated under illumination (Fig. 8). This important finding further reaffirms our observation about the specific location of this mushroom in its natural habitat. Since it is usually found inside the pile of the composted rice straw, the village people who collect this mushroom have to lift the upper layer in order to get the fruiting bodies.

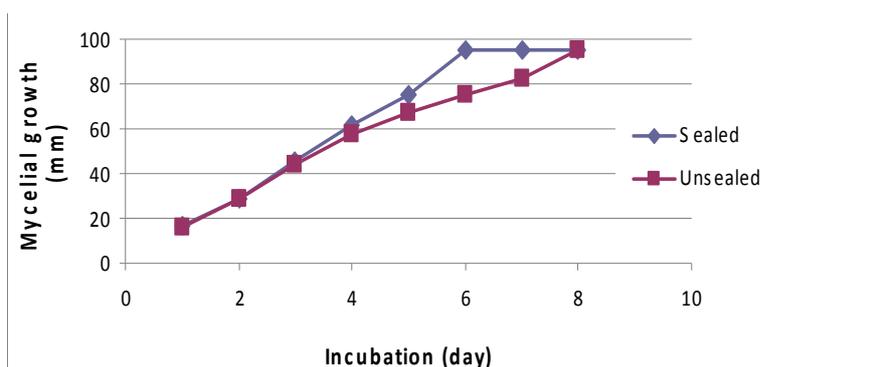


Fig. 5. Influence of aeration on the mycelial growth of *Coprinus comatus*.

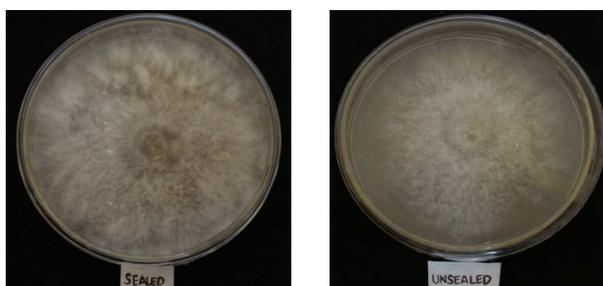


Fig. 6. Cultural characteristics of *Coprinus comatus* as influenced by aeration during incubation.

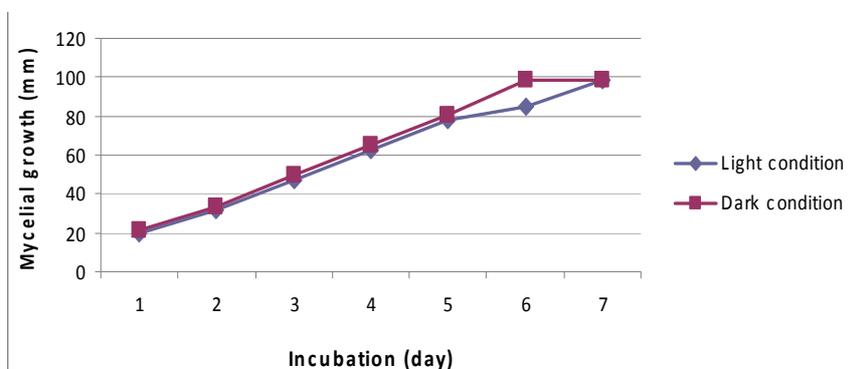


Fig. 7. Influence of illumination on the mycelial growth of *Coprinus comatus*.



Fig. 8. Cultural characteristic of *Coprinus comatus* when incubated in the dark and illuminated condition.

Aseptic cultivation of fruiting bodies

Having understood the physical requirements for growth of *C. comatus*, we evaluated five substrate formulations for its fruiting body production under aseptic condition. We have noticed that sawdust (8 parts) when enriched with rice grit (2 parts) lead to early fructification (Fig. 9). Fruiting bodies were formed in this formulation 8 days after inoculation compared to a formulation consisting of 8 parts sawdust with 2 parts of rice bran which took 17 days before fruiting bodies were formed. It is ironic to note that though *C. comatus* is associated with rice straw in its natural habitat, formation of fruiting bodies was not observed in the formulation of rice grit and rice bran either when combined or used singly. Though relatively lower compared to other mushrooms, *C. comatus* had 18% biological efficiency in sawdust – rice grit combination and 14% biological efficiency in sawdust-rice bran formulation. Despite its lower biological efficiency compared to other commercially cultivated mushrooms, *C. comatus* is considered as the fastest growing mushroom grown under artificial condition. *Pleurotus sajor caju* for instance takes 30 days before it can convert the rice

straw based- substrate into fruiting bodies with 24% biological efficiency (Villaceran *et al.*, 2006; Aquino *et al.*, 2008). Similarly, *V. volvacea* usually produce fruiting bodies 14 days after spawning in rice straw with biological efficiency which ranges from 12 to 20%.



Fig. 9. Fruiting bodies of *Coprinus comatus* in a formulation consisting of 8 parts sawdust and 2 parts rice grit.

Nutriceutical profile of Coprinus comatus

The nutraceutical analysis of *C. comatus* was initially confined on the elucidation of its standard and non-standard amino acid including its mineral content (Table 4). *C. comatus* contains more standard than the non-standard amino acid. Our analysis showed that it contains 8 of the essential amino acids namely Valine>Leucine>Lysine>Isoleucine>Threonine>Phenylalanine>Tryptophan>Methionine in decreasing order of abundance. Its glutamic acid content is relatively high which suggests that *C. comatus* is a very good flavoring ingredients in food preparation. Most of the non-standard amino acids were not detected. But it is surprising to note that *C. comatus* possesses Ornithine and γ -amino butyric acid (GABA). These two non-standard amino acids are important in establishing the nutraceutical status of this mushroom. GABA is the chief inhibitory neurotransmitter and plays an important role in regulating neuronal excitability throughout the central nervous system of mammals. In humans, GABA is also directly responsible for the regulation of muscle tone (Watanabe *et al.*, 2002) thus produces relaxing, anti-anxiety and anti-convulsive effect (Foster and Kemp, 2006). Ornithine specifically L-Ornithine as a non-essential amino acid and an important intermediate in the urea cycle functions along with Arginine in removing ammonia out of the body. These two amino acids are also known to assist in the formation of nitric oxide which plays an important role in vascular function particularly on blood flow. Thus, Ornithine not only aids in detoxification pathways but also supports the

production of nitric oxide which aids in the maintenance of a healthy cardiovascular system. Moreover, the presence of Ornithine in the sampled mushrooms suggests the possibility of using this mushroom in antidiabetic purposes which was previously demonstrated by Eguchi *et al.* (2008).

Table 4. Amino acid profile of *Coprinus comatus* grown under aseptic condition.

Amino acid content (mg/100 g)			
Standard		Non-standard	
Aspartic acid, Asp (D)	70.6	Phosphoserine	112.5
Threonine, Thr (T)	61.7	Taurine	N.D.
Serine, Ser (S)	76.2	Phosphoethanolamine	N.D.
Asparagine, Asn (N)	39.6	Urea	111.2
Glutamic acid, Glu (E)	441.6	Sarcosine	N.D.
Glutamine, Gln (Q)	57.4	α -amino adipic acid	6.4
Glycine, Gly (G)	55.2	Citrulline	N.D.
Alanine, Ala (A)	222.8	α - aminobutyric acid	1.9
Valine, Val (V)	94.4	Cys-Thionine	9.2
Cysteine, Cys (C)	22.3	β -Alanine	3.5
Methionine, Met (M)	5.3	β -aminobutyric acid	N.D.
Isoleucine, Ile (I)	63.0	γ - aminobutyric acid (GABA)	41.9
Leucine, Leu (L)	80.6	Monoethanolamine	17.8
Tyrosine, Tyr (Y)	61.2	Ammonia	124.5
Phenylalanine, Phe (F)	59.6	Hydroxylysine, Hyl	N.D.
Histidine, His (H)	31.0	Ornithine, Orn	36.1
Lysine, Lys (K)	64.8	1-Methyl Histidine	N.D.
Tryptophan, Trp (W)	18.2	3-Methyl Histidine	N.D.
Arginine, Arg (R)	57.5	Anserine	N.D.
Proline, Pro (P)	60.0	Carnosine	N.D.
		Hydroxyproline, Hyp	N.D.
Total	1643.0	Total	465

N.D. = not detected

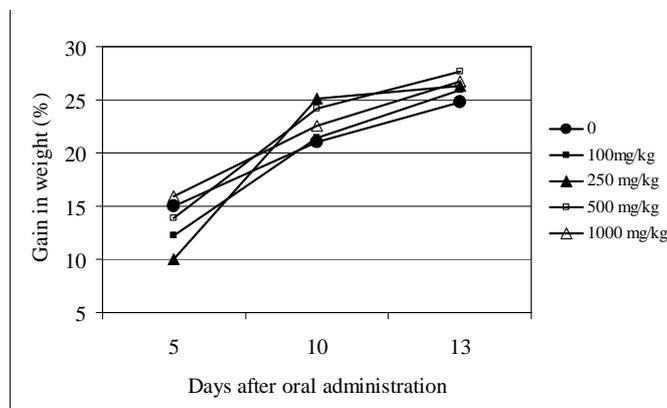


Fig. 10. Influence of different doses (mg/kg) of the hot water extract of *C. comatus* on the gain in weight (g) of Crlj : CD1 (ICR) mice.

The hot water extract of *C. comatus* has the ability to inhibit the angiotensin converting enzyme by more than 30%. This observation is almost the same with the other wild mushrooms from the Philippines that we have evaluated (data not shown). Angiotensin converting enzyme is the enzyme that converts inactive angiotensin I to angiotensin II which exhibits antihypertensive activity. This enzyme which is located in the endothelial lining of the vasculature of lungs is a Zinc – containing exopeptidase that has the capacity to cleave dipeptides from the C-terminal of various oligopeptides (Curtis *et al.*, 1978; Yang *et al.*, 1971). It can convert the inactive angiotensin I, an inactive decapeptide, to a potent vasopressor octapeptide called angiotensin II and at the same time inactivates bradykinin which exhibits vasodilating function (Ukeda *et al.*, 1991). Other mushrooms were also reported to have this kind of activity. The different post harvest processing of *Ganoderma lucidum* for instance was confirmed by Kumakura (2008) to inhibit the enzyme that converts angiotensin I to II. Though the active component of the hot water extract of *C. comatus* was not yet elucidated in our study, it is suggested that its polysaccharides, proteins or peptides might be involved in its ability to inhibit the conversion as previously reported by other researchers in other edible mushrooms (Choi *et al.*, 2001; Hagiwara *et al.*, 2005). *C. comatus* is not toxic to mice as revealed in Fig. 10. The different dosages of the hot water extract of *C. comatus* had positive influence on the weight of mice. Marked difference in gain in weight was noted in mice that received hot water extract from 500 to 1000 mg/kg, 13 days after oral administration having 22% gain in weight compared to the control set of mice and those that received 100 mg/kg and 250 mg/kg extract which registered 20% gain. Mice did not show any abnormal

external manifestation of toxicity like loose feces and discomfort as a result of the intake. This observation suggests that *C. comatus* is an edible mushroom.

Acknowledgement

The support of Japan Student Services Organization (JASSO) while the senior author is a postdoctoral fellow at Takasaki University of Health and Welfare is greatly recognized.

References

- Aquino, A.R.N., Nital, P.S., Bellere, A.D., Kalaw, S.P. and Reyes, R.G. (2008). *Pleurotus* Growers' Best Practice in Central Luzon: To Wrap or not to Wrap. Proc. 10th Annual Scientific Meeting of the Mycological Society of the Philippines. Benguet State University, La Trinidad, Benguet. pp. 28.
- Bulsecos, M.G.M., Abella, E.A. and Reyes, R.G. (2005). Morphogenesis of *Schizophyllum commune*, a wild edible mushroom of Mt. Nagpale, Abucay, Bataan, Philippines. *Journal of Nature Studies* 4(1): 20-28.
- Chaiyama, V., Petcharat, V., Kritsaneepaiboon, P. (2007). Some morphological and physiological aspects in the cultivation of *Coprinus comatus* (O.F. Mull.) Gray. *Songklanakarin Journal of Science and Technology* 29(2): 261-274.
- Chang, S.T. and Buswell, J.A. (1999). *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Aphyllophoromycetidae) mushrooming medicinal mushroom. *International Journal of Medicinal Mushrooms* 1: 139-146.
- Chang, S.T. and Buswell, J.A. (1996). Mushroom nutraceuticals. *World Journal of Microbiology and Biotechnology* 12: 473-476.
- Choi, H.S., Cho, H.Y., Yang, H.C., Ra, K.S. and Suh, H.J. (2001). Angiotensin I converting enzyme inhibitor from *Grifola frondosa*. *Food Research International* 34: 177-182.
- Clara, F.M. (1937). Culture of edible mushroom in the Philippines. *Philippine Journal of Agriculture* 8: 225-232.
- Curtiss, C., Chon, J.N., Vrobel, T and Francious, J.A. (1978). Role of the rennin-angiotensin system in the systemic vasoconstriction of chronic congestive heart failure. *Circulation*. 58: 763-770.
- Eguchi, F., Matsuoka, H., Kikukawa, T., Watanabe, Y. and Matsuda, T. (2008). Safety analysis of a *Flammulina velutipes* extract (chitoglucan) by a 90-day subchronic toxicity test and a genetic toxicity test. *Pharmacometrics* 75(1/2): 25-31.
- Eguchi, F., Yoshimoto, H., Reyes, R.G., Kikukawa, T. and Matsuda, T. (2008). The change of component and functionality in the growth stage of *Volvariella volvacea*. Proc. The 5th Meeting of East Asia for Collaboration on Edible Fungi. September 17-20, 2008, Kyushu University Research and International Exchange Plaza, Fukuoka, Japan. pp. 89.
- FAO Statistics. (2004).
- Foster A.C., Kemp, J.A. (2006). Glutamate- and GABA-based CNS therapeutics. *Current Opinion in Pharmacology* 6(1): 7-17.
- Garcia, B.L., Reyes, R.G., Santos, J.C. and Abella, E.A. (2004). Domestication of wild edible mushrooms as non – timber forest products resources among the Aetas of Mt. Nagpale, Abucay, Bataan: *Schizophyllum commune* and *Mycena* sp.. *The Journal of Tropical Biology* 3: 51-53.

- Hagiwara, S., Takahashi, M., Shen, Y., Kaihou, S., Tomiyama, T., Yazawa, M., Tamai, Y., Sin, Y., Kazusaka, A. and Terazawa, M. (2005). A phytochemical in the edible tamogitake mushroom (*Pleurotus cornucopiae*), D- mannitol, inhibits ACE activity and lowers the blood pressure of spontaneously hypertensive rats. *Bioscience, Biotechnology and Biochemistry* 69(8): 1603-1605.
- Inatomi, S., Kobayashi, H. and Sasaki, M. (2006). An acute single oral dose toxicity study of a compression extract of *Agaricus blazei* Murrill fruit body in rats. *Pharmaceutics*. 70(3/4): 93-95.
- Jeurink, P.V., Noguera, C.L., Savelkoul, H.F., Wichers, H.J. (2008). Immunomodulatory capacity of fungal proteins on the cytokine production of human peripheral blood mononuclear cells. *International Journal of Immunopharmacology* 8(8): 1124-33.
- Kim, M.S., Park, K.M., Chang, I.S., Kang, H.H. and Sim, Y.C. (2000). β -1,6-branched β -1,3-glucans in skin care. *Allured Cosmetic and Toiletries Magazine* 115: 79-86.
- Kim, J.S., Kim, M.S., Lee, D.C., Lee, S.G., So, S., Kim, Y.T., Park, B.H. and Park, K.M. (1999). Production of β -1,6-branched β -1,3-glucan useful as skin anti-aging, depigmenting and healing agent. JP 11313667.
- Kumakura, K., Kumakura, H., Ogura, M. and Eguchi, F. (2008). Pharmacological effects of *Ganoderma lucidum* collected from ume (Japanese apricot) trees. *Journal of Wood Science* 54: 502-508.
- Musngi, R.B., Abella, E.A., Lalap, A.L. and Reyes, R.G. (2005). Four species of wild *Auricularia* in Central Luzon, Philippines as sources of cell lines for researchers and mushroom growers. *Journal of Agricultural Technology, an international journal of agriculture* 1(2): 279-299.
- Paderes, C.S., Abella, E.A. and Reyes, R.G. (2004). Population biology of *Ganoderma* sp. in the CLSU main campus. *The Journal of Tropical Biology* 3: 47-48.
- Reyes, R.G. (2000). Indoor cultivation of paddy straw mushroom, *Volvariella volvacea*, in crates. *Mycologist* 14(4): 174-176.
- Reyes, R.G. and Abella, E.A. (2002). Two species of *Ganoderma* at Central Luzon State University. *The Journal of Tropical Biology* 1: 5-8.
- Reyes, R.G., Abella, E.A., Eguchi, F., Iijima, T. and Higaki, M. (2004). Physiology of *Collybia reinakeana*, a wild, endemic, edible mushroom from Puncan forest, Carranglan, Nueva Ecija, Philippines. *The Journal of Tropical Biology* 3: 11-19.
- Reyes, R.G., Abella, E.A. and Gatdula, G.G.E. (2006). *Schizophyllum commune*, a wild edible mushroom in the Philippines, as source of ethnomycological nutraceuticals. *Journal of Nature Studies* 5(1): 135-139.
- Reyes, R.G., Abella, E.A. and Manaloto, A.R. (1992). Use of indigenous culture media for *Pleurotus sajor-caju*. *CLSU Scientific Journal* 13(1): 34-40.
- Reyes, R.G., Abella, E.A. and T.H. Quimio. (2003). Wild Macrofungi of the Central Luzon State University. *The Journal of Tropical Biology* 2: 8-11.
- Reyes, R.G., Eguchi, F., Iijima, T. and Higaki, M. (1998). Influence of medium composition and plant growth regulators on the mycelial growth of *Collybia reinakeana*. *Journal of Agricultural Sciences - Tokyo University of Agriculture* 43: 43-50.
- Reyes, R.G., Eguchi, F., Iijima, T. and Higaki, M. (1998a). Physiological considerations for the efficient colonization of *fukurotake*, *Volvariella volvacea*. *Journal of Wood Science* 44: 408-413.
- Reyes, R.G., Eguchi, F., Iijima, T. and Higaki, M. (1998b). Regeneration of protoplasts from the hyphal strands of, *Volvariella volvacea*. *Journal of Wood Science* 44: 401-407.
- Reyes, R.G., Eguchi, F., Iijima, T. and Higaki, M. (1999). Protoplast hybrids of Philippine strains of *Volvariella volvacea*. *Communication in Applied Cell Biology* 16: 13-27.

- Reyes, R.G., Eguchi, F., Iijima, T. and Higaki, M. (1997). *Collybia reinakeana*, a wild edible mushroom from the forest of Puncan, Nueva Ecija, Philippines. *Mushroom Science and Biotechnology* 15(2): 99-102.
- Reyes, R.G., Encarnacion, A.D. and Abella, E.A. (1993). Utilization of selected agro-industrial wastes for mushroom (*Pleurotus sajor-caju*) production. *CLSU Scientific Journal* 14(1): 9-23.
- Reyes, R.G., Grassel, W. and Rau, U. (2009). Coconut water as a novel culture medium for the biotechnological production of schizophyllan. *Journal of Nature Studies*. 7(2).
- Snowdon, W., Osborn, T., Aarberslsberg, B. and Schultz, J. (2003). Coconut and its role in health. Secretariat of the Pacific Community. Cataloguing-in-publication data. 23pp.
- Tayamen, M.J.T., Reyes, R.G., Floresca, E.J. and Abella, E.A. (2004). Domestication of wild edible mushrooms as non – timber forest products among the Aetas of Mt. Nagpale, Abucay, Bataan: *Ganoderma* sp. and *Auricularia polytricha*. *The Journal of Tropical Biology* 3: 49-51.
- Ukeda, H., Matsuda, H., Kuroda, H., Osajima, K., Matsufuji, H. and Osajima, Y. (1991). Preparation and separation of angiotensin I converting enzyme inhibitory peptides. *Nippon Nogeikagaku Kaishi* 65: 1223-1228.
- Villaceran, A.B.Jr., Kalaw, S.P., Nitural, P.S., Abella, E.A. and Reyes, R.G. (2006). Cultivation of Thai and Japanese Strains of *Pleurotus sajor-caju* on rice straw - based *Volvariella volvacea* mushroom spent and composted rice straw in Central Luzon Region, Philippines. *International Journal of Agricultural Technology* 2(1): 69-75.
- Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T. and Hayasaki, H. (2002). GABA and GABA receptors in the central nervous system and other organs. *International Review of Cytology* 213: 1-47.
- Yang, H.Y.T., Erdos, E.G. and Levin, Y. (1971). Characterization of a dipeptide hydrolase (Kininase II: angiotensin I converting enzyme). *Journal of Pharmacology and Experimental Therapeutics* 177: 291-300.
- Zhang, Z.M., Wu, W.W. and Li, G.K. (2008). A GC-MS study of the volatile organic composition of straw and oyster mushrooms during maturity and its relation to antioxidant activity. *Journal of Chromatography Science* 46(8): 690-696.

(Received 3 August 2009; accepted 14 October 2009)