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Renato G. Reyes · Fumio Eguchi · Tomoaki Iijima
Miyato Higaki

Physiological considerations for efficient mycelial colonization of Philippine strains of *Volvariella volvacea*

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Abstract The nutritional and physical requirements for the efficient mycelial colonization of *Volvariella volvacea* (Bull. ex. Fr.). Singer were elucidated with the percentage mycelial colonization and density as references. This investigation was limited to the evaluation of two commercial strains (designated Vvc1 and Vvc2) and two wild strains (designated EAAC-0001 and EAAC-0002) of *V. volvacea* from the Philippines with the aim of providing baseline data on their physiological requirements. The four strains of *V. volvacea* had varying preferences for carbon. Vvc1 preferred polysaccharides (starch and cellulose), whereas Vvc2 grew luxuriantly at a relatively rapid rate in sugar alcohol (sorbitol). The two wild strains preferred starch as a carbon source. In terms of nitrogen utilization, soytone, peptone, and glycine supported efficient mycelial colonization of the four strains. The vitamin utilization test revealed that ascorbic acid, calcium pantothenate, and biotin are good sources. The mycelial growth performances of the strains were also evaluated on six dehydrated mycological media. Efficient colonization of Vvc1, Vvc2, and EAAC-0002 with dense mycelial growth was noted in mycological agar. EAAC-0001, on the other hand, grew more efficiently in malt extract agar. The Philippine strains of *V. volvacea* grew luxuriantly when incubated at 35°C and pH 8.0 under dark and sealed conditions. Moreover, the relatively higher moisture content (70%) of the oolong tea leaf formulation favorably stimulated efficient mycelial colonization. Under optimum physiological conditions, Vvc1, Vvc2, and EAAC-0002 were fast-growing strains, whereas EAAC-0001 was a moderately growing type.

Key words Mushroom nutrition · Mushroom physiology · Mycelial growth · *Volvariella volvacea*

R.G. Reyes (✉) · F. Eguchi · T. Iijima · M. Higaki
Department of Foresty, Faculty of Agriculture, Tokyo University of Agriculture, Tokyo 156-8502, Japan
Tel. +81-3-5477-2280; Fax +81-3-5477-2617

Introduction

Volvariella volvacea is the most popular edible mushroom in various parts of the Philippines due to its savory taste, short production time, and acceptability among local consumers. In the wild, it can be found growing on decomposing piles of rice straw and leaf litters of banana plants during the rainy months (May to September). Because of its popularity, this mushroom has been extensively cultivated under artificial conditions on an array of agro-industrial residues, such as cotton wastes, oil palm pericarp, sorghum straw, banana leaves, rice straw, water lilies, and a combination of sugarcane bagasse and cotton waste.[1–3] Even though improved technologies have been introduced in the Philippines, production of this mushroom is still on a semicommercial scale partly because of its instability and irregular mycelial and fruiting performances.[4,5] Our group is currently undertaking studies on the improvement of this mushroom, utilizing both the commercial strains and their wild counterparts as sources of genetic material. Understanding the physical and nutritional requirements of *V. volvacea* is necessary to obtain physiological bases for its improvement.

Materials and methods

Pure cultures of two wild strains of *V. volvacea*, designated EAAC-0001 and EAAC-0002, were obtained from the Culture Collection of the Center for Tropical Mushroom Research and Development at the Central Luzon State University (CTMRD-CLSU). One commercial strain (Vvc1) was given as a gift by Mr. Celso Apigo, the owner of Apigo's Mushroom Farm in Munoz, Nueva Ecija, Philippines. The other commercial strain (Vvc2) was purchased from the Spawn Laboratory of the Bureau of Plant Industry in Manila.

Influence of nutritional factors on mycelial colonization

The ability of the mycelia of the four strains to colonize the medium in relation to carbon and nitrogen sources, with vitamins as growth factors, was evaluated and compared for the four strains. Nutritionally complete medium (NCM),[6] consisting of 0.5 g magnesium sulfate, 0.46 g potassium dihydrogen phosphate, 1 g dipotassium hydrogen phosphate, 2 g peptone, 2 g yeast extract, 20 g glucose, and 20 g agar dissolved in 1 l distilled water, was used as maintenance and assay medium. The medium was adjusted to pH 7.5 prior to sterilization.[7] To determine the mycelial response of *V. volvacea* to the various sources of carbon and nitrogen, the NCM was modified by replacing singly the glucose and peptone component with different sources of carbon and nitrogen, respectively. The different sources of vitamins were also incorporated into the modified NCM at 0.012 g/l solution. The modified NCM plate was centrally inoculated with a 5-mm mycelial disk from a 7-day-old culture of *V. volvacea*. The inoculated NCM plates were incubated at 30° ± 2°C.

Carbon sources

Two monosaccharides (glucose, galactose), three disaccharides (maltose, lactose, saccharose), two polysaccharides (soluble starch, cellulose), two sugar alcohols (sorbitol, mannitol), and lignin from softwood were used as carbon sources.

Nitrogen sources

The following sources of nitrogen were evaluated. Four were inorganic (ammonium nitrate, ammonium sulfate, sodium nitrate, potassium nitrate), and ten were organic (casamino acid, L-arginine, L-glutamine, L-asparagine, L-leucine, glycine, peptone, urea, soytone, and casein).

Vitamins as growth factors

The ability of eight vitamins to stimulate mycelial colonization efficiency of the four strains of *V. volvacea* were evaluated and compared. These vitamins comprise nicotinic acid, folic acid, calcium pantothenate, riboflavin, ascorbic acid, biotin, thiamine hydrochloride, and cyanocobalamin.

Dehydrated and commercially available mycological media

The four strains of *V. volvacea* were grown in seven dehydrated mycological media: czapek solution agar (CSA: 30 g saccharose, 2 g sodium nitrate, 1 g dipotassium phosphate, 0.5 g magnesium sulfate, 0.5 g potassium chloride, 0.01 g ferrous sulfate, and 15 g agar in 1 l distilled water); saboraud dextrose agar (SDA: 10 g peptone, 40 g dextrose, and 15 g agar in 1 l distilled water); malt extract agar (MEA: 30 g malt extract and 15 g agar in 1 l distilled water); potato dextrose agar (PDA; Eiken Chemical Co., Japan) (39 g in 1 l distilled water); mycological agar (MA: 10 g bactosoytone, 10 g dextrose, and 15 g agar in 1 l distilled water); saccharose malt extract yeast extract agar (SMYA: 10 g saccharose, 10 g malt extract, 4 g yeast extract, and 15 g agar in 1 l distilled water), and modified NCM (the same composition as in NCM except that glucose was replaced by starch, and 0.012 g each of ascorbic acid, calcium pantothenate, and biotin were added as growth factors). The media were adjusted to pH 7.5 prior to sterilization.

Influence of physical factors on mycelial colonization

Because mycological agar was found to be the most suitable medium for efficient mycelial colonization of the four strains of *V. volvacea*, it was used as both maintenance and assay medium in the following tests of physical parameters except for moisture, which utilized an oolong tea leaf formulation.

pH levels

The pH of the mycological agar was adjusted to varying levels ranging from 4.5 to 9.0 at intervals of 0.5 prior to sterilization.

Temperature levels

Mycological agar (pH 8.0) plates were inoculated separately with a 5-mm mycelial disk from a 7-day-old MA culture of *V. volvacea*. The inoculated plates were incubated at temperatures ranging from 20° to 40°C.

Aeration

Prior to incubation at 35°C, the lid of the previously inoculated plates of *V. volvacea* were twice sealed with Parafilm "M" Laboratory Film (American National Can, Illinois, USA). Unsealed plates served as the control.

Light requirement

A batch of Parafilm-sealed MA (pH 8.0) plate cultures of the four strains were divided into two sets. The first set was incubated under light condition and the other was kept under dark. The temperature at incubation was maintained at 35°C.

Moisture content

The moisture content of the hand-crushed oolong tea leaf formulation, consisting of 3 parts dried oolong tea leaves and 1 part each of rice bran and sawdust, was adjusted to varying levels ranging from more than 40% to less than 75%. Moisture content was determined following the standard procedure for moisture determination.[8]

Results and discussion

The influence of nutritional and physical factors on the mycelial performances of two wild and two commercial strains of *V. volvacea* from the Philippines was assessed based on the ability of the respective mycelia to colonize the substrate over time. The relative mycelial performances of the four strains were compared, with percentage mycelial colonization efficiency and density as references.

Influence of nutritional factors on mycelial colonization

The commercial and wild strains of *V. volvacea* exhibited varying degrees of mycelial colonization efficiency when grown on different sources of carbon (Table 1). Among the carbon sources, lactose, galactose, and lignin did not support efficient colonization of the four strains. Although glucose was considered the most utilizable form of carbon for the metabolism of many microorganisms including mushrooms, this monosaccharide was not as suitable as a complex form, such as starch, for efficient colonization of the two wild strains of *V. volvacea*. Moreover, the two commercial strains had varying carbon requirements. The growth of Vvc2 was more luxuriant and rapid in sorbitol. Though rapid in saccharose, the mycelial growth of Vvc1 was not as dense as in polysaccharides and sugar alcohol. Polysaccharides and sugar alcohol favorably influenced the colonization efficiency of the four strains compared to monosaccharides (galactose and glucose) and disaccharides (maltose and lactose). The results on the carbon utilization of the four strains of *V. volvacea* both agreed and contradicted previous observations by other researchers. For instance, starch was found by Ofuso-Asiedu et al.[9] to be the most suitable source of carbon for *V. volvacea* isolates, whereas Chakravarty and Mallick[10] recorded more growth when their *V. volvacea* isolates were grown in glucose than in starch. Variation on the utilization of carbon by isolates of *V. volvacea* from different geographical regions may further support the observation about their adaptability to a number of agroindustrial residues in nature such as sugarcane bagasse, rice straw, water hyacinth, cotton wastes, and others.[1]

With regard to nitrogen utilization, soytone, peptone, and glycine supported the efficient mycelial colonization of the four strains (Table 2). Among the three best sorces of nitrogen, soytone and peptone produced very dense mycelial growth for all the strains. *V. volvacea* is similar to *V. esculenta* in terms of nitrogen utilization.[11] It was also observed that Vvc1 and EAAC-0002 were almost comparable in that they utilized a wide range of nitrogen sources. Vvc2 and EAAC-0001, on the other hand, were limited to utilizing certain sources of nitrogen. Differences in the utilization of nutrients by the different strains maybe attributed to the different cultural methods employed when cultivating the commercial strains and the natural growing substrates of the wild species. Prior to this investigation, Vvc1 was commercially cultivated using rice straw as the main medium, whereas Vvc2 was grown in banana leaves.

Among the eight evaluated vitamins, ascorbic acid, calcium pantothenate, and biotin supported mycelial colonization of the four strains (Table 3). The response of Vvc1 was similar to that of EAAC-0002 in having a wide range of vitamin requirements. The mycelial colonization of Vvc2, on the other hand was observed to be efficient only in ascorbic acid-supplemented plates. Vitamin utilization by these four strains are quite different from that of the Puerto Rican isolates.[7]

The mycelial growth performances of the four strains were evaluated on a number of dehydrated mycological media (Table 4). A modified nutritionally complete me-

Table 1. Influence of varying sources of carbon on the mycelial colonization of different strains of *Volvariella volvacea*

Carbon source	Mycelial colonization efficiency by strain, mean (%) ± SD			
	Vvc1	Vvc2	EAAC-0001	EAAC-0002
Monosaccharides				
Glucose	43.66 ± 2.82 (++++)	59.22 ± 3.05 (++++)	35.22 ± 1.08 (++++)	67.00 ± 2.71 (++++)
Galactose	24.89 ± 2.05 (+++)	23.78 ± 2.31 (+++)	31.67 ± 1.52 (+++)	52.89 ± 2.33 (+++)
Disaccharides				
Maltose	48.67 ± 3.94 (++++)	58.67 ± 1.39 (++++)	43.00 ± 2.24 (++++)	73.56 ± 4.75 (++++)
Lactose	22.78 ± 4.23 (+++)	15.44 ± 0.61 (+++)	22.78 ± 1.11 (+++)	36.11 ± 2.43 (+++)
Saccharose	62.56 ± 3.68 (+++)	73.55 ± 5.07 (+++)	40.78 ± 2.14 (+++)	92.00 ± 3.16 (+++)
Polysaccharides				
Starch (soluble)	54.44 ± 4.08 (++++)	76.33 ± 1.19 (++++)	54.88 ± 0.99 (++++)	95.22 ± 1.34 (++++)
Cellulose	55.44 ± 6.9 (++++)	64.67 ± 2.47 (++++)	53.44 ± 1.68 (+++)	84.22 ± 4.8 (+++)
Sugar alcohol				
Mannitol	53.33 ± 8.21 (++++)	80.00 ± 2.22 (++++)	41.33 ± 1.60 (++++)	90.00 ± 0.88 (+++)
Sorbitol	52.33 ± 2.47 (++++)	82.33 ± 2.09 (++++)	41.22 ± 2.49 (++++)	88.78 ± 3.39 (+++)
Lignin	39.03 ± 0.23 (+++)	43.55 ± 1.45 (+++)	33.33 ± 1.57 (+++)	59.78 ± 2.10 (+++)
Control (without carbon source)	44.66 ± 4.85 (+++)	69.22 ± 1.95 (+++)	51.44 ± 2.10 (+++)	85.44 ± 3.00 (+++)

Data presented were based from the average of five replicate plates 3 days after incubation at 30°C. Mycelial density was described as: +, very thin; ++, thin; +++, dense; and ++++, very dense. Mycelial colonization efficiency was expressed as the percentage of recorded mycelial growth at a specific time to the total diameter of the plate

Table 2. Influence of varying sources of nitrogen on the mycelial colonization of different strains of *Volvariella volvacea*

Nitrogen source	Mycelial colonization efficiency, by strain (%), mean ± SD			
	Vvc1	Vvc2	EAAC-0001	EAAC-0002
Inorganic				
Ammonium nitrate	52.55 ± 5.16 (++++)	69.11 ± 2.46 (++++)	32.67 ± 1.99 (++++)	80.00 ± 1.05 (++++)
Ammonium sulfate	47.07 ± 2.27 (++++)	73.33 ± 5.18 (++++)	34.89 ± 1.62 (++++)	78.89 ± 7.56 (++++)
Sodium nitrate	57.66 ± 5.86 (++++)	72.56 ± 0.99 (++++)	32.33 ± 3.93 (++++)	83.89 ± 4.80 (++++)
Potassium nitrate	39.67 ± 6.47 (++++)	74.22 ± 4.38 (++++)	39.00 ± 3.17 (++++)	83.67 ± 8.58 (++++)
Organic				
Soytone	87.62 ± 5.11 (++++)	75.08 ± 3.03 (++++)	48.15 ± 2.97 (++++)	99.26 ± 4.89 (++++)
Casamino acid	48.67 ± 8.44 (++++)	72.22 ± 1.61 (++++)	33.56 ± 3.98 (++++)	71.22 ± 1.96 (++++)
L-Arginine	41.77 ± 2.98 (+++)	68.33 ± 6.86 (++++)	29.78 ± 2.57 (++++)	63.67 ± 1.59 (++++)
L-Glutamine	49.11 ± 2.47 (++++)	73.56 ± 5.40 (++++)	36.00 ± 2.94 (++++)	78.00 ± 4.26 (++++)
L-Asparagine	43.55 ± 2.82 (+++)	74.44 ± 1.01 (++++)	36.11 ± 2.22 (++++)	82.04 ± 1.47 (++++)
L-Leucine	39.00 ± 3.25 (+++)	59.44 ± 7.46 (++++)	32.44 ± 4.13 (++++)	36.11 ± 7.24 (++++)
Glycine	57.22 ± 9.58 (+++)	77.33 ± 5.83 (++++)	39.56 ± 3.50 (++++)	86.11 ± 3.51 (++++)
Peptone	57.66 ± 7.03 (++++)	77.78 ± 4.91 (++++)	38.22 ± 4.09 (++++)	88.66 ± 1.05 (++++)
Urea	17.00 ± 0.44 (+++)	26.56 ± 3.54 (++++)	33.00 ± 2.66 (++++)	52.89 ± 0.54 (++++)
Casein	53.44 ± 4.12 (+++)	67.00 ± 1.15 (++++)	29.22 ± 3.70 (++++)	84.89 ± 6.57 (++++)
Control (without nitrogen source)	52.00 ± 4.89 (+++)	74.78 ± 6.5 (+++)	36.89 ± 3.05 (+++)	78.22 ± 7.91 (+++)

See Table 1 for explanations

Table 3. Influence of vitamins on the mycelial colonization of different strains of *Volvariella volvacea*

Vitamin	Mycelial colonization efficiency, by strain (%), mean ± SD			
	Vvc1	Vvc2	EAAC-0001	EAAC-0002
Cyanocobalamin	72.55 ± 2.85 (+++)	63.33 ± 5.32 (++++)	49.44 ± 4.42 (++++)	86.78 ± 1.49 (++++)
Thiamine hydrochloride	54.66 ± 2.50 (+++)	64.22 ± 3.35 (++++)	46.44 ± 3.86 (++++)	88.00 ± 1.91 (++++)
Biotin	65.67 ± 4.40 (+++)	63.78 ± 1.32 (++++)	52.56 ± 5.16 (++++)	91.66 ± 0.79 (++++)
Ascorbic acid	71.33 ± 6.47 (+++)	72.22 ± 2.68 (++++)	52.89 ± 2.68 (++++)	97.11 ± 0.73 (++++)
Riboflavin	64.89 ± 4.20 (+++)	64.00 ± 2.64 (++++)	50.00 ± 3.84 (++++)	93.00 ± 1.90 (+++)
Calcium pantothenate	72.22 ± 0.78 (++++)	62.78 ± 2.29 (++++)	52.78 ± 3.51 (++++)	92.22 ± 1.30 (++++)
Folic acid	64.11 ± 5.96 (++++)	66.11 ± 2.45 (++++)	49.78 ± 3.46 (++++)	92.33 ± 1.20 (++++)
Nicotinic acid	65.89 ± 2.44 (++++)	65.22 ± 2.98 (++++)	50.00 ± 1.52 (++++)	91.00 ± 1.77 (++++)
Control (without vitamins)	62.00 ± 3.30 (+++)	67.44 ± 3.95 (+++)	50.33 ± 2.79 (++++)	88.67 ± 2.10 (+++)

Data presented were based on the average of five replicate plates 2 days after incubation at 30°C. Mycelial density was described as Table 1

Table 4. Influence of varying types of dehydrated culture media on the mycelial colonization of different strains of *Volvariella volvacea*

Dehydrated culture medium	Mycelial colonization efficiency, by strain (%), mean ± SD			
	Vvc1	Vvc2	EAAC-0001	EAAC-0002
Saboraud dextrose agar (SDA)	29.00 ± 3.71 (++++)	53.33 ± 1.15 (++++)	26.11 ± 1.25 (+++)	60.11 ± 1.38 (+++)
Czapeks solution agar (CSA)	53.66 ± 3.50 (+)	83.15 ± 7.11 (+)	43.44 ± 1.59 (+)	88.67 ± 2.53 (+)
Potato dextrose agar (PDA)	44.44 ± 1.36 (++++)	60.22 ± 4.49 (++++)	34.01 ± 2.52 (+++)	72.55 ± 1.74 (+++)
Saccharose malt and yeast extract agar (SMYA)	43.44 ± 2.37 (++)	55.44 ± 6.51 (+++)	46.11 ± 3.14 (++++)	90.33 ± 2.41 (+++)
Mycological agar (MA)	64.03 ± 6.37 (++++)	89.55 ± 3.08 (++++)	43.00 ± 2.21 (++++)	97.78 ± 1.36 (++++)
Malt extract agar (MEA)	51.11 ± 6.17 (+++)	69.78 ± 5.40 (+++)	48.55 ± 4.82 (++++)	86.22 ± 6.41 (+++)
Nutritionally complete medium (NCM) – control	72.89 ± 7.32 (+++)	90.14 ± 7.16 (++++)	61.11 ± 5.39 (++++)	98.67 ± 0.93 (+++)

See Table 1 for explanations

dium served as control. Although varying degrees of mycelial performances were noted in MA, results showed that the *V. volvacea* strains, with the exception of EAAC-0001, grew luxuriantly in MA with very dense mycelial growth. Though producing very dense growth in MA, EAAC-0001 growth was relatively more rapid in MEA than in MA. The mycelial performances of the three strains in MA were almost comparable to that in the control medium. The efficiency of MA to stimulate mycelial colonization may be due to its soytone content, as soytone was found to enhance the mycelial colonization efficiency of *V. volvacea*. SMYA and PDA also stimulated the growth performances of the

strains. Stimulation in these media was limited only in terms of mycelial colonization efficiency. The mycelial density ranged from dense to very dense. Czapeks solution agar, on the other hand, did not support efficient mycelial performance of the four strains.

Influence of physical factors on mycelial colonization

Aside from nutritional factors, physical factors influence the efficient mycelial colonization of *V. volvacea*. Temperature, pH, light, aeration, and moisture were examined in this experiment. The significant results are presented in Table 5.

The four strains of *V. volvacea* from the Philippines had relatively the same sensitivity to temperature. Efficient mycelial colonization was noted when cultures were incubated at 35°C. No growth was obtained in cultures maintained at 15°C. Slow colonization at 20°C and a declining trend at 40°C were also recorded. This observation confirms that strains from the Philippines are of the tropical type and are relatively similar to isolates from Thailand,[1] Indonesia, and Hong Kong.[12,13]

Like temperature, the pH of the medium is crucial to the growth of *V. volvacea*. It influences not only the activities of the enzyme but also that of the vitamins and the entry of organic acids; thus an increase in the pH of the medium produces changes in the permeability and other surface characteristics of the growing structures.[14] The four strains tolerated a wide pH range (i.e., pH 4.5–9.0). Strains were highly efficient in terms of colonizing the medium when grown in a slightly alkaline condition (pH 8.0). Growth started to decelerate from pH 8.5 to 9.0. Similarly, although growth was evident in cultures that were acidic to slightly acidic (pH 4.5–6.0), the colonization efficiency of the four strains in this range was not remarkably high. The pH responses of these strains were similar to those of the strains reported by Torres-Lopez and Hepperly.[7]

Aeration also played an important role on the mycelial colonization efficiency of *V. volvacea*. Although this setup was limited in terms of the determination of oxygen and carbon dioxide, it was observed that plate cultures of the four strains, when double-sealed with Parafilm, had very dense mycelial growth with abundant aerial mycelia compared to the unsealed plates. Though the ability of the two wild strains to colonize the media in both set-ups was not significantly different, the two strains produced very dense mycelial growth in sealed plates compared to that in the unsealed set. Similarly, the two commercial strains grew rapidly with very dense growth in sealed plates. This observation provides an explanation for the common practice of the growers to seal the mushroom bed with plastic sheets during incubation. It might be that sealing provides not only the proper temperature and humidity but also the necessary gaseous requirements of the growing mycelia. This response

Table 5. Influence of physical factors on the mycelial colonization of different strains of *Volvariella volvacea*

Physical factor	Mycelial colonization efficiency, by strain (%), mean ± SD			
	Vvc1	Vvc2	EAAC-0001	EAAC-0002
Temperature (°C)				
15	0	0	0	0
20	9.11 ± 1.09 (+)	10.89 ± 0.44 (+)	10.00 ± 0 (+)	17.89 ± 1.33 (+)
25	25.55 ± 2.51 (+++)	20.00 ± 1.57 (+++)	26.44 ± 0.76 (++)	41.94 ± 0.92 (++)
30	100.00 ± 0.00 (++++)	74.89 ± 4.19 (++++)	60.33 ± 2.62 (++++)	100.00 ± 0.00 (++++)
35	100.00 ± 4.04 (++++)	94.89 ± 1.19 (++++)	64.89 ± 3.69 (++++)	100.00 ± 0.00 (++++)
40	62.55 ± 3.95 (++)	35.67 ± 3.85 (++)	44.44 ± 5.59 (++)	69.44 ± 2.94 (++)
pH				
4.5	22.80 ± 1.03 (+++)	35.78 ± 1.99 (+++)	32.00 ± 1.78 (+++)	40.33 ± 1.08 (+++)
5.0	33.78 ± 4.35 (+++)	49.11 ± 3.11 (+++)	41.44 ± 2.28 (+++)	56.44 ± 2.41 (+++)
5.5	43.00 ± 4.19 (+++)	56.11 ± 1.11 (+++)	40.44 ± 1.33 (+++)	63.20 ± 3.94 (++++)
6.0	45.44 ± 2.10 (++++)	57.78 ± 1.88 (++++)	43.00 ± 3.23 (+++)	71.78 ± 3.07 (++++)
6.5	50.89 ± 2.68 (++++)	67.67 ± 4.62 (++++)	46.44 ± 2.41 (++++)	83.70 ± 3.04 (++++)
7.0	57.22 ± 1.76 (++++)	85.89 ± 3.72 (++++)	44.33 ± 2.73 (++++)	90.00 ± 3.09 (++++)
7.5	63.89 ± 3.47 (++++)	89.44 ± 3.81 (++++)	49.67 ± 4.63 (++++)	93.33 ± 2.91 (++++)
8.0	71.44 ± 2.06 (++++)	89.88 ± 2.82 (++++)	54.44 ± 4.63 (++++)	95.76 ± 2.41 (++++)
8.5	67.67 ± 3.73 (++++)	87.00 ± 1.34 (++++)	52.22 ± 5.97 (++++)	95.22 ± 1.15 (++++)
9.0	63.33 ± 2.34 (++++)	81.11 ± 4.98 (++++)	51.93 ± 2.70 (++++)	95.18 ± 0.85 (++++)
Moisture (%)				
42.38	11.89 ± 2.47 (+)	10.67 ± 1.66 (++)	13.89 ± 1.36 (+++)	12.44 ± 6.90 (+++)
55.98	37.22 ± 5.55 (++++)	22.89 ± 3.34 (++++)	39.33 ± 3.15 (++++)	49.11 ± 5.05 (++++)
65.83	52.89 ± 1.63 (++++)	39.67 ± 3.79 (++++)	52.00 ± 2.50 (++++)	66.44 ± 2.69 (++++)
69.31	53.33 ± 3.27 (++++)	44.78 ± 1.14 (++++)	61.00 ± 3.34 (++++)	74.55 ± 3.76 (++++)
72.30	60.23 ± 4.45 (++++)	46.44 ± 2.93 (++++)	66.44 ± 4.17 (++++)	86.22 ± 1.73 (++++)
Illumination				
Under dark	57.00 ± 2.27 (++++)	54.00 ± 1.84 (++++)	25.00 ± 1.76 (++++)	98.45 ± 0.54 (++++)
Under light	61.44 ± 2.09 (+++)	69.78 ± 2.45 (+++)	41.33 ± 1.94 (+++)	100.00 ± 0.00 (+++)
Aeration				
Unsealed	82.11 ± 11.02 (+++)	79.89 ± 1.07 (+++)	58.55 ± 3.69 (+++)	98.22 ± 5.82 (+++)
Sealed	90.11 ± 2.66 (++++)	95.00 ± 2.49 (++++)	58.19 ± 3.88 (++++)	98.89 ± 4.10 (++++)

See Table 1 for explanations

of *V. volvacea* is similar to that of *Ganoderma lucidum*[15] and our wild isolates of *Collybia reinakeana*.

Light was found to influence the mycelial colonization efficiency of the four strains. The positive effect of light was limited to the ability of the mycelia to colonize the medium, as cultures maintained under dark produced very dense growth with abundant aerial mycelia. It has been reported that light is responsible for the initiation of fruit body formation[16] and is an inducer of the mycelial growth of *Agaricus blazei*.[17]

The mycelial colonization efficiency of the four strains of *V. volvacea* was greatly influenced by increasing levels of moisture in the oolong tea leaf formulation. The efficiency of the mycelia to colonize the medium was less at 42% moisture. Luxuriant mycelial growth with efficient colonization was noted in substrates having a moisture content of more than 70%. This finding was in conformity with the previous reports by other researchers regarding the moisture requirement of this mushroom.[12,18]

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