



## Optimization condition for cultivation of *Agaricus subrufescens* hybrid strains

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### Abstract

*Agaricus subrufescens* is edible mushroom, one of the most important medicinal mushroom with high potential to treat many diseases. Several strains of *A. subrufescens* have been cultivated throughout the world, especially in Brazil. *A. subrufescens* hybrid strain in this study has been bred by the INRA, France. This study investigated the optimum conditions for the cultivation of *A. subrufescens* hybrid strains in Northern Thailand. The suitable conditions for mycelium growth on compost extract agar were established in terms of temperature and pH. The results revealed that the optimum temperature and pH for mycelial growth were 25–30°C and pH 7–9, respectively. Two hybrid strains of *A. subrufescens* CA 918–076 x CA 454–4 and CA 918–076 x CA 487–35 were selected and cultivated on compost media. The ingredients of the compost were consisted of rice straw (200 kg), rice bran (10 kg), urea (2 kg), ammonium sulfate (4 kg), calcium carbonate (2 kg), calcium sulfate (6 kg) and water. The result showed that two hybrid strains, could be cultured using this rice straw compost. Fruiting bodies were produced after 2 months with a yield of 56.92 and 42.30 g /kg of compost, respectively.

**Keywords:** *Agaricus subrufescens*, compost, hybrid strains, mushroom cultivation

### Introduction

*Agaricus subrufescens* Peck. (1893) (synonyms = *A. blazei*, *A. brasiliensis*), is a gilled mushroom belongs family Agaricaceae (Agaricales Basidiomycetes). It is also called almond mushroom due to its almond odor (Firenzuoli et al., 2008; Zied et al., 2012). *A. subrufescens* was first cultivated in the late 1800s in Eastern North America and described by Peck C.H. in 1893 (Kerrigan, 2005). It was firstly isolated in the 1960s in Brazil (Farnet et al., 2013). This is recognized as the main character with chocolate brown basidiospores. This mushroom grows with a hemispherical to convex to plano-convex shape in mature stage. The surface is dry and covered by fibrillose squamulose. It is a saprobe and inhabits rooting leave, it often grows in clusters or scattered occasionally singly on soil. It was discovered in North America, South America and also has been found in Hawaii, Taiwan, Philippines and Thailand (Kerrigan, 2005; Wisitrassameewong et al., 2012). In addition, *A. subrufescens* is popular as a medicinal food having a potential to treat many common diseases such as arteriosclerosis, cancer, diabetes, chronic hepatitis and hyperlipidaemia (Hobbs, 1991) Mizuno et al., 1990; Firenzuoli et al., 2008; Liu et al., 2008; Jumes et al., 2010). It is a popular mushroom market as extensively cultivated in Brazil and in oriental countries such as Japan or China (Farnet et al., 2013). Llarena-Hernandez et al. (2013) could obtain the hybrid strain between the Brazilian and European strains after investigated the hybridization. The hybrid strains were improved in term of yield and quality for developing new cultures under European growing conditions and also a new hybrid named H1X1 was patented in USA (Kerrigan and Wach, 2008). In this study *A. subrufescens* hybrid strains provided by N. Thongklang (Thongklang

et al. 2014) obtained from INRA, France, were optimized and cultivated using compost media prepared in Chiang Rai, Thailand.

## Methodology

### Strains of mushroom

*Agaricus subrufescens* hybrid strains was obtained between Thai strain CA918 and Brazilian CA454, Thai strain CA918 and French strain CA487 (Table1). All strains were maintained on potato dextrose agar (PDA) and transferred to a fresh PDA as master plate for inoculation. The compost extract was prepared by using 800 ml of compost extract and water 200 ml mixed with 10 g of glucose and 20 g of agar.

**Table 1:** Cultivars used in this study

Code in this study	Origin code	Origin	Type
Hybrid	Thai and Brazilian	CA918-075 X CA454-4	T1xB
Hybrid	Thai and Brazilian	CA918-076 X CA454-4	T2xB
Hybrid	Thai and French	CA918-075 X CA487-35	T1xF
Hybrid	Thai and French	CA918-076 X CA487-35	T2xF
Thai wild strain	Parental of Thai strain	CA918	T

## Optimization condition for growing mycelium

### Effect of temperature

The mycelial growth of the four hybrid strains and parental strain of *A. subrufescens* were studied on a different range of temperature. All pure culture isolates were grown on PDA, then a 0.5 mm-diameter plug from the edge of growing mycelium was transferred to compost extract media in triplicate and incubated at 16, 20, 25, 30 and 35°C. Mycelial growth was evaluated by determination of dry weight and colony radius every 2 days for 10 days. For determination of dry weight, the agar-mycelial was removed by placed in a beaker, and then the medium was melted and washed out with hot water.

### Effect of pH

The optimal pH for mycelial growth were determined by using compost extracted agar. The initial pH of the media was adjusted separately by 1N HCl or 1N NaOH to pH 4, 5, 6, 7, 8 and 9 prior autoclave at 121°C for 15 minutes. A 0.5 mm-diameter plug of mycelium was inoculated on centric of plate. All culture media were incubated at 30°C. Mycelial growth was evaluated by determination of dry weight and colony radius every 2 days for 10 days. For determination of mycelium dry weight, the agar-mycelial was removed and placed in a beaker, and then the medium was melted and washed out with hot water.

### Spawn production

The sorghum were washed, cleaned and soaked in distilled water for 24 hours, then washed 1-2 times before boiled for 15 minutes. After draining the excess water, the grains were sterilized, cooled down to room temperature, then inoculated with the mycelium and incubated at 25°C for 14 days or until completely colonized.

### Mushroom cultivation

The compost media base on rice straw as main substrate was mixed with calcium carbonate, urea, rice bran, di ammonium phosphate, gypsum and water, followed by pasteurization for 6 hours by maintaining the temperature at 45–50°C. Allow the compost to cool down to room

temperature before applying the spawn 20 g of colonized grain/kg compost. A 5 kg of the mixture was placed in plastic tray (35x25x20 cm). The inoculated compost was incubated at 25°C with relative humidity at 91–95 % for the beginning of colonization. The completely colonized compost was then covered with the pasteurized casing with 2.5 inch layer. The number and fresh weight of fruiting bodies were recorded after casing. The experiments were performed with five replicated per strain and cultivation condition.

#### Statistical analysis

The mycelial growth and mycelium dry weight were verified by calculating the average of triplicate measurements of the diameter in centimeter (cm) of mycelium growth and milligram (mg) of mycelium dry weight using statistical analysis and significant differences were determined by Tukey's test with a significance level  $p \leq 0.05$  followed by post-hoc test in a one-way ANOVA analysis using SPSS version 11.5 program.

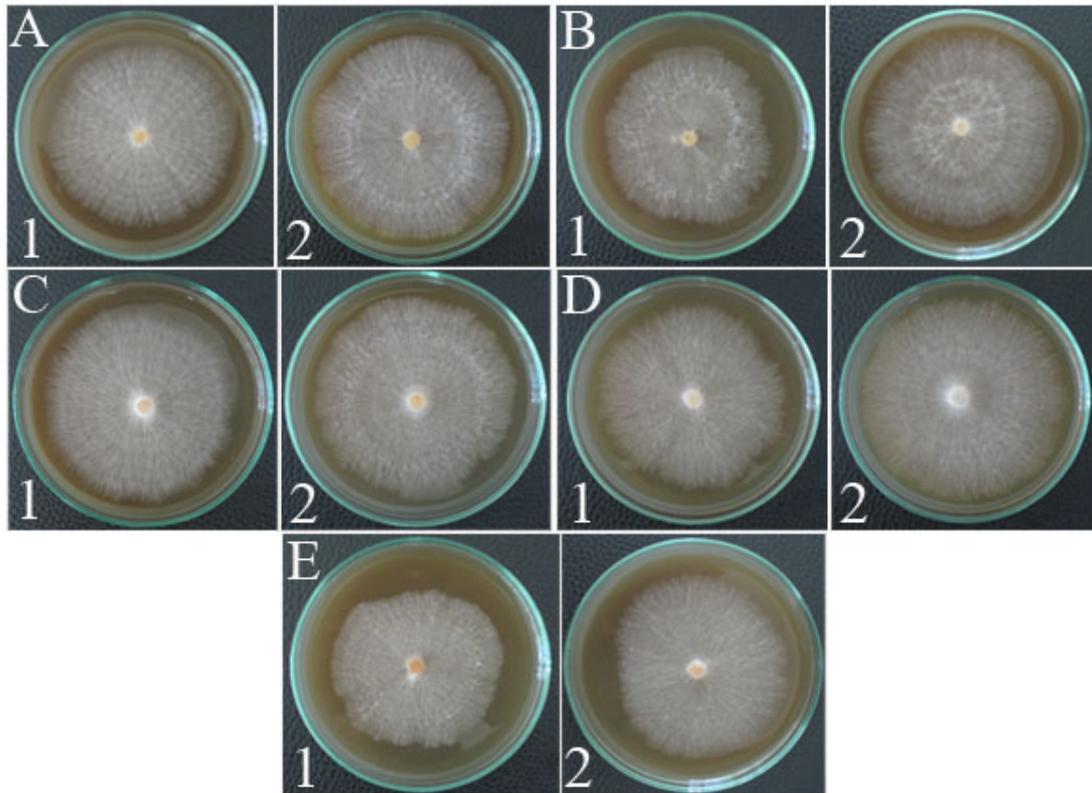
### Results

#### The effect of temperature

*Agaricus subrufescens* was grown on compost extracted agar and inoculated in the range temperatures at 16, 20, 25, 30 and 35°C. In this study, the optimal temperature for mycelial growth was found at 25 and 30°C (Table1). The results from this study corresponded to the previous studies by Colauto et al. (2008) who reported optimal temperature for this strain ranged between 28°C and 31°C. While Quimi et al. (1990) reported that the optimal temperature range from 28°C to 30°C and also Zied et al. (2011) reported the optimal temperature of *A. subrufescens* was between 25 to 29°C.

#### The effect of pH

The mycelial growth was evaluated in the culture media with initial pH values from 4 to 9. The result from this study showed that optimal pH for mycelial growth was range between 7 to 9 for all *A. subrufescens* hybrid strain except Thai wild strain that could grow all tested pH range. Its growth was not different between the pH. However, the growth of Thai wild type strain was lower than the hybrid strain. The optimal pH of *Agaricus bisporus* on compost cultivation was reported as pH 7.5 by Gerrits (1988) and Rinker (1993) and pH 7 to 7.5 for casing soil for *A. subrufescens* (Calvalcante et al., 2008). This result indicated that neutral pH range was optimal pH for *A. subrufescens* hybrid strain.



**Figure 1:** Mycelium growth on compost extracted media when incubated at 25°C (1) and 30°C (2) for 10 days. A) strain T1xB, B) strain T2xB, C) strain T1xF, D) strain T2xF and E) strain T.

**Table 2:** Average mycelial growth rate of *A. subrufescens* strains cultivated on compost extract agar under various condition for 10 days.

	Growth rate (cm of mycelial radius)*				
	T1xB	T2xB	T1xF	T2xF	T
<b>Temperatures</b>					
(°C)					
16	2.37 <sup>c</sup>	1.83 <sup>c</sup>	2.87 <sup>c</sup>	2.57 <sup>c</sup>	2.03 <sup>c</sup>
20	3.0 <sup>b</sup>	2.53 <sup>b</sup>	4.00 <sup>b</sup>	3.90 <sup>b</sup>	2.73 <sup>b</sup>
25	7.37 <sup>a</sup>	7.23 <sup>a</sup>	7.33 <sup>a</sup>	6.57 <sup>a</sup>	6.47 <sup>a</sup>
30	7.50 <sup>a</sup>	7.37 <sup>a</sup>	7.77 <sup>a</sup>	7.00 <sup>a</sup>	6.27 <sup>a</sup>
35	1.47 <sup>d</sup>	1.57 <sup>d</sup>	1.43 <sup>d</sup>	1.33 <sup>d</sup>	0
<b>pH</b>					
4	4.23 <sup>c</sup>	4.47 <sup>c</sup>	4.77 <sup>b</sup>	3.93 <sup>b</sup>	4.73 <sup>a</sup>
5	5.03 <sup>bc</sup>	4.63 <sup>c</sup>	4.83 <sup>b</sup>	3.97 <sup>b</sup>	4.77 <sup>a</sup>
6	5.10 <sup>b</sup>	5.40 <sup>b</sup>	6.07 <sup>a</sup>	6.17 <sup>a</sup>	4.93 <sup>a</sup>
7	5.83 <sup>ab</sup>	5.97 <sup>ab</sup>	7.03 <sup>a</sup>	6.63 <sup>a</sup>	4.90 <sup>a</sup>
8	5.93 <sup>a</sup>	6.00 <sup>a</sup>	6.77 <sup>a</sup>	6.50 <sup>a</sup>	4.83 <sup>a</sup>
9	6.53 <sup>a</sup>	5.87 <sup>ab</sup>	6.05 <sup>a</sup>	6.43 <sup>a</sup>	4.87 <sup>a</sup>

\* Within a column, values followed by the same letter are not different at  $p \leq 0.05$  by Tukey's test.

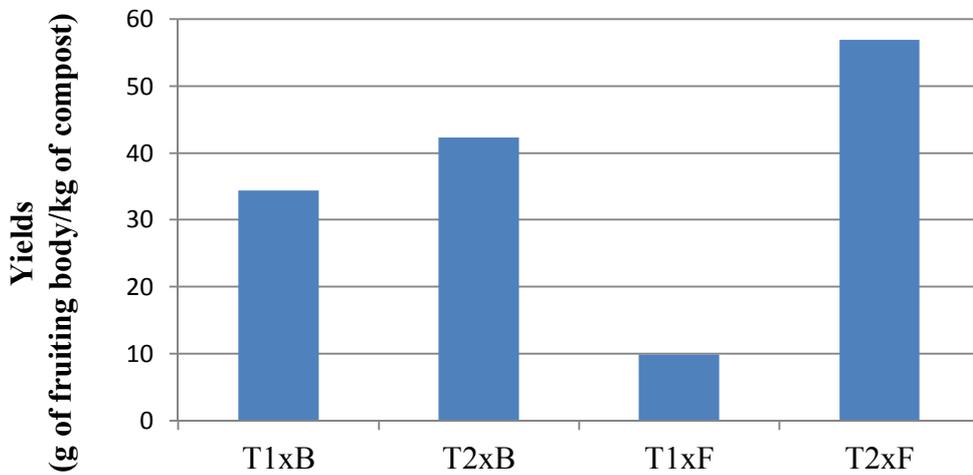
**Table 3:** Dry weight of mycelium grown on compost extract agar under various condition for 10 days.

	Dry weight (mg)*				
	T1xB	T2xB	T1xF	T2xF	T
<b>Temperature</b>					
(°C)					
16	3.07 <sup>d</sup>	3.87 <sup>b</sup>	10.10 <sup>b</sup>	7.47 <sup>d</sup>	8.63 <sup>bc</sup>
20	9.23 <sup>c</sup>	7.27 <sup>b</sup>	15.77 <sup>b</sup>	14.73 <sup>b</sup>	12.27 <sup>b</sup>
25	32.57 <sup>b</sup>	51.23 <sup>a</sup>	68.03 <sup>b</sup>	32.30 <sup>b</sup>	40.59 <sup>a</sup>
30	48.67 <sup>a</sup>	54.80 <sup>a</sup>	85.67 <sup>a</sup>	56.33 <sup>a</sup>	49.37 <sup>a</sup>
35	4.33 <sup>d</sup>	3.50 <sup>b</sup>	3.00 <sup>d</sup>	5.10 <sup>d</sup>	0
<b>pH</b>					
4	14.60 <sup>b</sup>	14.57 <sup>c</sup>	15.57 <sup>b</sup>	11.73 <sup>c</sup>	15.63 <sup>a</sup>
5	16.63 <sup>b</sup>	15.90 <sup>c</sup>	20.10 <sup>b</sup>	12.53 <sup>c</sup>	15.07 <sup>a</sup>
6	17.17 <sup>b</sup>	27.60 <sup>ab</sup>	76.27 <sup>a</sup>	29.70 <sup>b</sup>	17.70 <sup>a</sup>
7	24.53 <sup>a</sup>	30.17 <sup>a</sup>	77.03 <sup>a</sup>	39.50 <sup>a</sup>	18.43 <sup>a</sup>
8	23.37 <sup>a</sup>	25.13 <sup>b</sup>	76.07 <sup>a</sup>	38.30 <sup>ab</sup>	18.33 <sup>a</sup>
9	27.67 <sup>a</sup>	27.86 <sup>a</sup>	75.37 <sup>a</sup>	38.50 <sup>a</sup>	17.43 <sup>a</sup>

\* Within a column, values followed by the same letter are not different at  $p \leq 0.05$  by Tukey’s test.

**Mushroom cultivation**

In this studied, *A. subrufescens* strains are presented in Table 1 were used for cultivation on rice straw based material of compost media. The result is showed in Figure 2 that the Thai – France strain (T2xF) gave the highest yield of fruiting bodies with 56.92 g /kg compost. The strain was produced the fruiting bodies after casing 18 days in the controlled conditions.



*A. subrufescens* hybrid strains.

**Figure 2:** Comparison of biomass production in each strain.

## Discussion

The studies aimed to compare the suitable condition for mycelial growth of different *A. subrufescens* hybrid strains including the effect of temperature and pH for mycelial development on the compost substrate. Five strains of *A. subrufescens* in this study showed optimal temperature between 25–30°C for mycelial growth which was relatively to previous reported by Chang (2008) at 23–37°C, Colauto et al. (2008) 28–31°C, Huang (1997) 20–33°C, Mendonca et al. (2005) 25–28°C, Neves et al. (2005) 25–30°C, Quimi et al. (1990) 28–30°C, Mendonca et al. (2005) 25–30°C, and Zied et al. (2011) 25–29°C. Therefore, the suitable temperature for mycelium growth should be maintained from 25–30 °C.

All strains used in this study were able to grow at pH 6–9. Park (2001) reported optimal pH for the substrate reaction range between 6–7, high acid or basic condition inhibited mycelium growth. Kopytowski Filho et al. (2008) and Cavalcante et al. (2008) reported that the optimal pH for compost substrate and casing layer was pH 7.0–7.5. It is suggested that neutral pH was the suitable pH for these mushroom strains.

Five strains of *A. subrufescens* were cultivated in Northern Thailand by using rice straw as main substrate mixed with the ingredients. The result showed the strain T2xF gave the highest yield at the temperature 25°C with 91–95 % relative humidity after casing layer for 18 days. This mushroom species were studied in many countries such as in French by Llarene-Hernandez et al. (2013) who found the fruiting and biomass production of wild European strains (France and Brazilian strains) with 157.5 and 11.5 g/kg substrate after casing for 30 days at  $23 \pm 0.5^\circ\text{C}$  with  $98 \pm 2.0$  % relative humidity. The mushroom requires high temperature and high humidity to produce fruiting bodies particularly in Brazil (Dias et al., 2004; Mantovani et al., 2007; Dias et al., 2010). The humidity during the formation of primordia was at 80–90%, while 75–80% was the relative humidity for development of fruiting bodies (Iwade and Mizuno 1997; Stamets 2000). Chang (2008) reported humidity at 70–85% was optimum for formation of fruiting bodies. The hybrid strains of *A. subrufescens* showed a potential to grow in Northern Thailand because the optimal conditions for growing of this stains were temperature range from 25–30°C with 91–95 % relative humidity that related with the conditions in Northern Thailand. The standard condition including temperature, humidity and pH of substrate and casing were important factor for mushroom cultivation.

## Conclusions

The results from this study demonstrated that *A. subrufescens* can grow at the temperature range between 25–30°C and initial pH range between 6–9. Fruiting body of these strains can be produced by using rice straw compost prepared in Chiang rai, Thailand. However, the yield of the production should be improved to reach a commercial scale.

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