



Screening of *Auricularia auricula* strains for strong production ability of melanin pigments

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Abstract

Melanin pigments have great application value and development potential in food industry to use as nature functional food colorants. In initial study, twenty-two *Auricularia auricula* strains were screened for stronger production ability of melanin pigments by solid culture. Three *A. auricula* strains (RF201, QD2 and QD6) with higher pigment production capacity were selected for further study through submerged culture supplementing 1 g/L l-tyrosine. The maximal pigment yields of *A. auricula* RF201, QD2 and QD6 were 493.9, 367.6 and 318.5 mg/L, respectively. Among three strains, *A. auricula* RF201 possessed the strongest production ability of melanin pigments. The present study indicated that *A. auricula* RF201 could be used as potential excellent producer of melanin pigments.

Keywords: screening; *Auricularia auricula*; production ability; melanin pigments.

Practical Application: *A. auricula* RF201 can be used as potential excellent producer of nature melanin pigments.

1 Introduction

Melanin pigments are the natural black-brown polymers with high relative molecular weights (Chen et al., 2008). They are formed from the oxypolymerization of phenols through tyrosine oxidases in plant, animal, bacterium and fungus (Dalfard et al., 2006). Melanin pigments obtained from various sources have same physical and chemical characteristics, such as solubility and optical absorption property. Moreover, melanin pigments possess many biological activities, including oxidation resistance (Dong & Yao, 2012; Liu et al., 2011; Tu et al., 2009), anti-aids effect (Zou et al., 2015), and immunoregulatory function (Wu et al., 2008). Therefore, melanin pigments have great application value and development potential in food industry to use as nature functional food colorants.

A. auricula is widely distributed in East and Southeast Asia. Its fruiting body called black fungus in China is used as an edible mushroom for centuries (Sun et al., 2016). *A. auricula* dark-colored fruiting body contains abundant melanin pigment which is popular in China as one of black foods. Melanin pigments are regarded as major bioactive substance in black foods (Tu et al., 2009; Wang et al., 2006). However, extracting processes of melanin pigments from tissues and cells of *A. auricula* fruiting body are costly and cumbersome. Furthermore, the growing cycle of *A. auricula* fruit body on solid culture medium is long (Wu et al., 2006).

A. auricula may also produce melanin pigments by submerged fermentation culture (Zou & Hou, 2017). However, production abilities of melanin pigments are lower for most of *A. auricula* strains. Therefore, it is very important to screen high melanin-producing fungal strain. This research was conducted to screen high melanin-producing fungal strain from twenty-two

A. auricula strains for the potential use as an effective producer of natural melanin pigments.

2 Materials and methods

2.1 Strains

The fungal strains *A. auricula* NKY01, F1, F2, F3, F4, F5, QD1, QD2, QD4, QD6, QD7, QY8582, QY8583, QY8584, RF173, RF201, RF204, RF218, RF230, RF231, RF233 and RF235 were obtained from the Institute of Shanxi Edible Mushroom (Ankang, China). Mycelia kept on PDA slant were inoculated and incubated at 28 °C for 7 days.

2.2 Experimental design

In initial experiment, *A. auricula* strains were screened by solid culture. PDA plate was inoculated with mycelium and incubated at 28 °C for 7 days. Then, 5 mm diameter mycelium disc obtained from PDA plate was transferred to new PDA plate and incubated at 28 °C for 7 days. After that, visual colors of colony center and growth rate of mycelium were measured to screen stronger melanin-producing fungal strains.

A. auricula strains possessed stronger production ability of melanin pigments were further screened by submerged culture. Fungus was transferred to seed medium (potato dextrose broth medium) with 5 mm diameter disc from PDA plate to produce inoculums. Five discs were inoculated to 50 mL seed medium in 250 mL triangular flask and then incubated at 28 °C on a shaking table at 100 rpm for 5 days. Inoculums (8%, v/v) were transferred into 250 mL triangular flask contained 50 mL fermentation medium (potato dextrose broth medium supplementing 1 g/L l-tyrosine as substrate). Afterward, it was cultivated at 28 °C for 8 days at rotation time of 100 rpm. Finally, pigment yield and

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dry weight of mycelium were determined to obtain the strongest melanin-producing fungal strain.

2.3 Visual colors of colony center

Visual colors of colony center were monitored by a Minolta CR-400 colorimeter (Osaka, Japan). Visual colors in Hunter Lab color system were indicated as L^* , a^* and b^* values which respectively represented from darkness to whiteness, from greenness to redness and from blueness to yellowness.

2.4 Growth rate of mycelium

Growth rate of mycelium was determined according to method of Xie et al. (2010). The different colony diameters in vertical direction were monitored with the ruler. The Equation 1 was used to calculate growth rate of mycelium.

$$Y = (X_1 - X_2) / X_3 \quad (1)$$

Where Y was growth rate, X_1 was final colony diameter, X_2 was initial colony diameter (5 mm), and X_3 was incubation time.

2.5 Pigment yield of *A. auricula*

Measurement of pigment yield was performed as described by Zou et al. (2010). Fungal fermentation broths were filtered to eliminate mycelia and impurities. The liquid was adjusted using 2 M HCl to pH 1.5 to precipitate and separate pigment by centrifugation for 15 min at 8000 rpm. Precipitated pigment was respectively cleaned using ethyl acetate and ethanol. Then, melanin pigments were dissolved in 0.02 M NaOH. Optical density of solution contained pigment at 400 nm was determined by Unico-2800 spectrophotometer (Princeton, USA).

2.6 Dry weight of mycelium

Mycelium biomass of *A. auricula* was measured through weighting dry mycelium amount. Fungal fermentation broths were filtered to obtain mycelia, and then mycelia were cleaned with deionized water and dried to achieve constant weight at 110 °C.

2.7 Statistical analysis

Experimental result was showed as mean \pm standard deviation and significant difference ($p < 0.05$) among different strains were analyzed by Fisher's F -test.

3 Results and discussion

3.1 Mycelium growth and pigment secretion of *A. auricula* in PDA plate

Mycelium growth and pigment secretion of twenty-two *A. auricula* strains in PDA plates are shown in Figure 1. All *A. auricula* mycelia used in this experiment grew well in PDA plates. As indicated in Table 1, average growth rate of twenty-two *A. auricula* strains was 5.6 mm/day. *A. auricula* RF230 exhibited the fastest growth rate of 9.3 mm/day. On the contrary, *A. auricula* F3 showed the slowest growth rate (3.9 mm/day). The experimental result showed that there was significant difference ($p < 0.05$) in growth rates among twenty-two *A. auricula* strains. It appeared that different strains from the same fungus obviously had different growth rate of mycelia in PDA culture media, which agreed with results reported by Xie et al. (2010).

Determination results (Table 1) from the colorimeter exhibited that there was significant difference ($p < 0.05$) in L^* , a^* and b^* values of twenty-two *A. auricula* strains in Hunter Lab color system. Visual colors of colony center varied greatly

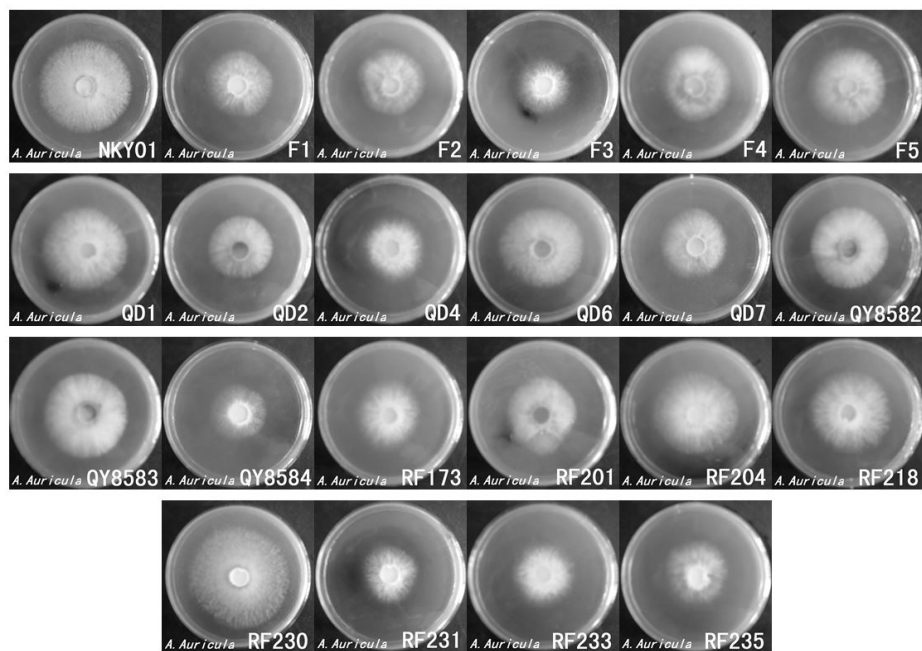


Figure 1. Mycelium growth and pigment secretion of *A. auricula* strains in PDA plates.

Table 1. Growth rate of mycelium and visual colors of colony center of *A. auricula* strains.

Strains	Growth rate (mm/day)	Visual colors		
		L^*	a^*	b^*
NKY01	7.9±0.1 ^b	58.9±0.2 ^c	0.6±0.1 ⁿ	19.7±0.1 ^d
F1	4.9±0.2 ^j	58.2±0.4 ^d	3.7±0.1 ^h	20.3±0.6 ^d
F2	5.4±0.1 ^h	52.9±0.2 ^h	6.8±0.2 ^d	23.4±0.5 ^b
F3	3.9±0.2 ^l	57.5±0.3 ^e	5.4±0.1 ^f	25.1±0.2 ^a
F4	5.5±0.1 ^g	49.4±0.2 ^k	7.1±0.2 ^d	21.3±0.5 ^c
F5	6.2±0.1 ^e	51.3±1.4 ^j	5.1±0.5 ^f	19.4±1.4 ^d
QD1	6.8±0.2 ^c	50.9±0.3 ^j	4.4±0.5 ^g	17.5±0.7 ^e
QD2	4.8±0.1 ^j	37.3±0.7 ^o	15.4±0.6 ^a	19.5±1.6 ^d
QD4	5.1±0.1 ⁱ	54.5±0.3 ^g	3.7±0.2 ^h	14.9±0.5 ^g
QD6	6.9±0.2 ^c	40.3±0.4 ⁿ	4.4±0.2 ^g	12.5±0.3 ^j
QD7	5.4±0.1 ^h	53.5±0.5 ^h	1.3±0.1 ^m	11.9±0.4 ^j
QY8582	6.1±0.1 ^f	45.4±1.1 ^m	10.3±0.9 ^b	25.1±0.2 ^a
QY8583	6.5±0.1 ^d	47.3±1.1 ^l	8.9±0.5 ^c	25.3±0.4 ^a
QY8584	4.1±0.2 ^l	59.1±0.1 ^c	2.9±0.1 ⁱ	20.3±0.1 ^d
RF173	5.5±0.1 ^g	52.6±0.1 ⁱ	3.9±0.1 ^h	17.9±0.1 ^e
RF201	5.5±0.1 ^g	36.5±0.2 ^o	6.2±0.1 ^e	13.3±0.1 ⁱ
RF204	6.9±0.2 ^c	57.3±0.2 ^e	1.6±0.1 ^l	15.8±0.3 ^f
RF218	6.6±0.2 ^d	52.4±0.6 ⁱ	5.1±0.5 ^f	19.4±1.2 ^d
RF230	9.3±0.2 ^a	59.9±0.2 ^a	-0.8±0.1 ^o	13.7±0.1 ^h
RF231	4.2±0.1 ^k	59.4±0.3 ^b	5.1±0.1 ^f	25.1±0.3 ^a
RF233	5.1±0.1 ⁱ	56.9±0.1 ^f	2.1±0.1 ^j	15.7±0.2 ^f
RF235	4.8±0.1 ^j	58.9±0.4 ^c	1.9±0.1 ^k	19.5±0.4 ^d

Data in same column with different superscript letter are significantly different at $p < 0.05$.

among different strains. *A. auricula* RF201 and QD6 exhibited lower L^* values (36.5 and 40.3), a^* values (6.2 and 4.4) and b^* values (13.3 and 12.5), indicating that their colony centers were dark with a little red and yellow colored. *A. auricula* QD2 possessed lower L^* value (37.3) and the largest a^* value (15.4), which implied that its colony center was dark red. *A. auricula* F3, QY8582, QY8583 and RF231 showed larger b^* values (25.1, 25.1, 25.3 and 25.1), suggesting that their colony centers were yellow.

All *A. auricula* mycelia in this experiment secreted pigment during growth, whereas content of melanin pigments in PDA plate was positive correlated with L^* value in Hunter Lab color system (Fan et al., 2008). Therefore, according to L^* value in colony center, three *A. auricula* strains (RF201, QD2 and QD6) with higher pigment production capacity were selected for further study through submerged culture supplementing 1 g/L l-tyrosine.

3.2 Pigment production and mycelium biomass of *A. auricula* in fermentation broth

The pigment production kinetic curves of *A. auricula* strains RF201, QD2 and QD6 in fermentation broth supplementing 1 g/L l-tyrosine are shown in Figure 2. As expected, the pigment yield quickly enhanced when incubation time was extended but it slowly reduced later. The maximal pigment yields were obtained on 6 or 7 days of incubation. Lagunas-Muñoz et al. (2006) reported that recombinant *Escherichia coli* W3110 could produce 6 g/L melanin pigment on 3 days of incubation. However, *E. coli* was one of the major foodborne pathogens which could

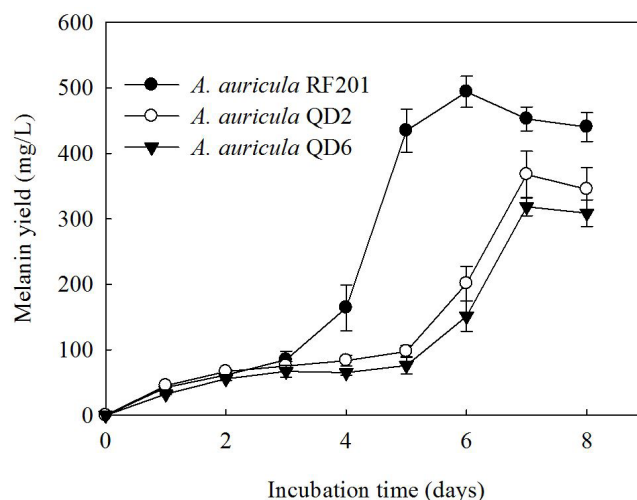


Figure 2. Pigment production kinetic curve of *A. auricula* strains in fermentation broth.

produce shiga toxin and its propagation might result in serious human diseases (Santos & Stephanopoulos, 2008). Moreover, other hidden trouble and potential fatal danger might exist in metabolites of *E. coli* (Vu-Khac & Cornick, 2008). *A. auricula* was a nontoxic macro-fungus and had been used as an edible mushroom for centuries in China. Melanin pigments produced by *A. auricula* were safe food colorants and could be applied to food processing.

Table 2. Pigment yield and dry mycelium weight of *A. auricula* strains.

Strains	Incubation time (days)	Pigment yield (mg/L)	Dry weight of mycelium (g/L)
RF201	6	493.9±23.7 ^a	8.1±0.4 ^a
QD2	7	367.6±35.8 ^b	6.3±0.4 ^b
QD6	7	318.5±13.9 ^c	6.8±0.8 ^b

Data in same column with different superscript letter are significantly different at $p < 0.05$.

As shown in Table 2, the maximal pigment yield of *A. auricula* RF201, QD2 and QD6 were 493.9, 367.6 and 318.5 mg/L on 6, 7 and 7 days of incubation, respectively. Among three strains, *A. auricula* RF201 possessed the strongest ($p < 0.05$) production ability of melanin pigments in the shortest incubation time. Meanwhile, dry weight of mycelium (8.1 g/L) of *A. auricula* RF201 was the highest ($p < 0.05$) than those of other two *A. auricula* strains, which indicated that *A. auricula* RF201 possessed stronger growth and metabolism ability. The present study suggested that *A. auricula* RF201 could be used as potential excellent producer of melanin pigments.

4 Conclusions

Twenty-two *A. auricula* strains were screened for the strongest production ability of melanin pigments by solid culture and submerged culture. Comparing visual colors of colony center and growth rate of mycelium, *A. auricula* strains RF201, QD2 and QD6 were selected for further study. In fermentation broths, the maximal pigment yields of *A. auricula* RF201, QD2 and QD6 were 493.9, 367.6 and 318.5 mg/L, respectively. The present study suggested that *A. auricula* RF201 was the strongest melanin-producing fungal strain for the potential use as an effective producer of natural melanin pigments.

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