



Production of natural melanin by *Auricularia auricula* and study on its molecular structure



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ARTICLE INFO

Article history:

Received 22 April 2015

Received in revised form 12 June 2015

Accepted 15 June 2015

Available online 16 June 2015

Keywords:

Auricularia auricula

Melanin

Molecular structure

Production characteristics

ABSTRACT

In this study, the production and structure of melanin produced by *Auricularia auricula* have been determined and analyzed in detail. The results showed that the highest mycelial growth rate was observed in low-carbon and carbon-free medium. In low-nitrogen and nitrogen-free medium, melanin yield was very low. Deficiency of tyrosine in medium led to weak secretion of melanin. The inorganic salt could markedly influence mycelia morphology, but did not obviously impact mycelia growth rate and melanin yield. Meanwhile the condensed molecular formula $[\text{C}_{18}(\text{OR})_3\text{H}_7\text{O}_4\text{N}_2]_n$ and structural formula of melanin were concluded based on UV–Vis, HPLC, FTIR, NMR and elemental assay. This is an eumelanin and also a macromolecular polymer of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid. The 6 main components were phenolic hydroxyl, carboxyl, amidogen, carbonyl, methylene, methyne and sulfur. This work testified that nutritional control was very important to promote melanin production, making melanin more affordable as material in food, cosmetics and medicines.

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1. Introduction

Pigments are important constituents in food additives and the demand for pigments has been gradually increasing in recent years (Eriksen, 2008). Natural pigments are considered safe, with pronounced nutritional and therapeutic benefits in comparison to synthetic pigments. Currently, the natural pigments are mostly obtained through extraction of plant materials (Kim et al., 2005; Yolmeh, Habibi Najafi, & Farhoosh, 2014). However, plant cultivation does not guarantee a standardized product, with pigment composition varying from batch to batch. Furthermore, the extraction is a very complicated and time-consuming process, which

severely limits the application of natural pigments on a large scale. Microbial submerged fermentation is considered a promising alternative for the efficient production of natural pigments (Guo, Chen, et al., 2014; Guo, Rao, et al., 2014; Vinarov, Robucheva, Sidorenko, & Dirina, 2003) because it is prone to industrialization.

Melanin is one of the important natural pigments and has been widely and conventionally used in different industrial fields including food, cosmetics, pharmacology, medicines and other fields. The most interesting thing is that melanin can be used as food additives to prevent the rancidity caused by the presence of bacteria by quenching the bacterial quorum sensing (Zhu, He, & Chu, 2011). Melanins are irregular dark brown polymers that are produced in various organisms by fermentative oxidation of nitrogen containing or nitrogen-free diphenols (Aghajanyan et al., 2005). Melanins are considered strong antioxidants and have anti-virus functionality and can effectively protect living organisms from ultraviolet radiation. There are some traditional methods to obtain melanin mainly including the extraction from plant and animal materials, the chemical synthesis based on oxidation of tyrosine and its derivatives, and enzyme catalysis (Eisenman & Casadevall, 2012; Murisier & Beermann, 2006; Vinarov et al., 2003). However, these technologies have shown no potential for large-scale industrial production of melanin due to their high cost and complicated synthesis process. Because of these shortcomings

Abbreviations: A. auricula, *Auricularia auricula*; PDA, potato dextrose agar; CM, complete medium; CFM, carbon-free medium; NFM, nitrogen-free medium; TFM, tyrosine-free medium; SFM, salt-free medium; LCHNM, low-carbon and high-nitrogen medium; LNHCM, low-nitrogen and high-carbon medium; UV–Vis, ultraviolet–visible spectrum; NMR, nuclear magnetic resonance; FTIR, Fourier transform infrared spectroscopy; HPLC, high performance liquid chromatography; SEM, scanning electron microscope.

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many researchers now explore melanin production by bacteria, mycete, or fungi, but these microorganisms often secrete many toxic secondary metabolites. The latter make the produced melanins following these routes difficult to use in food, cosmetics, pharmacology and medicines (Figueiredo-Carvalho, dos Santos, Nosanchuk, Zancope-Oliveira, & Almeida-Paes, 2014; Gonçalves, Lisboa, & Pombeiro-Sponchiado, 2012; Guo, Chen, et al., 2014; Guo, Rao, et al., 2014; Wan et al., 2007). Over the last 50 years, the study of melanin structure has been attracting scientific interest and has become a key research focus in the field of natural pigments. However, the exact structure of melanin is still undefined due to its low solubility in water and most organic solvents. Melanin may exist as three different forms: eumelanins, pheomelanins and allomelanins. Currently, the structure characterization of the first two melanins has been confirmed to a certain extent, but the structure of the third melanin has not yet been determined (Agadzhanian et al., 2011; Aghajanyan et al., 2005; Dong & Yao, 2012; Eisenman & Casadevall, 2012; Khan, Harsha, Giridhar, & Ravishankar, 2011; Stadler & Fournier, 2006; Vinarov et al., 2003; Ye et al., 2014; Zhong, Frases, Wang, Casadevall, & Stark, 2008). The main chemical monomers of eumelanin are 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) instead of benzothiazine and benzothiazole in pheomelanin.

Auricularia auricula is a traditional Chinese edible and medicinal mushroom containing many bioactive compounds that have been reported to help in detoxification, the inhibition of bacterial quorum sensing, the lowering of blood pressure and the reduction of blood vessel fat (Ma, Xu, & Feng, 2014; Nguyen, Chen, et al., 2012; Nguyen, Wang, et al., 2012; Reza et al., 2014). *A. auricula* is an organism capable of high secretion of natural edible melanin by submerged fermentation, which can shorten and simplify the production cycle, lead to high production efficiency, and then reduce the cost. Melanin is a secondary metabolite that is generally secreted under restrictive nutrient conditions, and furthermore, melanin accumulation can be also affected by these environmental factors such as aeration and nutrition (Zhang et al., 2015). Therefore, it is important to investigate the effect of environmental conditions on the mycelial growth rate and melanin production by *A. auricula*. It is also significant for improving the melanin production, simplifying and shortening the purification process, and then increasing economic benefit. However, up to now, there are seldom reports on the investigations of the linkage between the mycelial growth rate and melanin production by *A. auricula* under nutrition stress conditions. Furthermore, it is hard to get single component of this melanin to analyze its structure.

In the present study, the growth rate and morphology of mycelia and melanin production from *A. auricula* under different nutritional conditions were determined and analyzed in detail. The chemical structure of melanin has then been systematically investigated using chromatography technologies. This specific structure and molecular formula of natural melanin is significantly different from that of melanin from *Lachnum* YM404 (Ye et al., 2014) and *Catharsius molossus* (Riley, 1997), and is rarely reported at home and abroad. In particular, chemical groups might be connected to each other through various chemical bonds.

2. Materials and methods

2.1. Strain, media, and chemicals

The *A. auricula* strain was obtained from the Fujian General Station of Technology Popularization for Edible Fungi (Fujian, China) and deposited in China Center of Industrial Culture Collection (Serial Number: 1511C0005000004283). This strain

was maintained on PDA slant (potato 200 g/L, glucose 20 g/L and agar 20 g/L) at 28 °C with a periodic transfer. The stock culture was incubated on enriched PDA plate (glucose 20 g/L, yeast extract 5 g/L, peptone 3 g/L, KH₂PO₄ 3 g/L, MgSO₄ 1.5 g/L, VB₁ 0.1 g/L and agar 20 g/L) at 28 °C for 7 days, and then used for culture inoculation. The following media were prepared:

Complete medium (g/L): lactose 10, yeast extract 10, tyrosine 1, CaCl₂ 0.1, NaCl 5;
Carbon-free medium (g/L): yeast extract 10, tyrosine 1, CaCl₂ 0.1, NaCl 5;
Nitrogen-free medium (g/L): lactose 10, tyrosine 1, CaCl₂ 0.1, NaCl 5;
Tyrosine-free medium (g/L): lactose 10, yeast extract 10, CaCl₂ 0.1, NaCl 5;
Salt-free medium (g/L): lactose 10, yeast extract 10, tyrosine 1;
Low-carbon and high-nitrogen medium (g/L): lactose 1, yeast extract 10, tyrosine 1, CaCl₂ 0.1, NaCl 5;
Low-nitrogen and high-carbon medium (g/L): lactose 10, yeast extract 1, tyrosine 1, CaCl₂ 0.1, NaCl 5;
Tyrosine medium (g/L): glucose 1, peptone 3, CaCl₂ 0.1, NaCl 5, tyrosine 1, pH7.0.

All of the chemicals used in this study were of analytical grade and were purchased from Sigma (Sigma-Aldrich, China) unless mentioned otherwise.

2.2. Influence of nutritional conditions on mycelial growth rate, morphology and melanin production

The colony morphology and mycelia growth rate were studied on various agar media: CM, CFM, NFM, TFM, SFM, LCHNM and LNHCM. The mycelia of *A. auricula* grown on enriched PDA plate were transferred to solid medium by punching out one mycelial tablet ($d = 8$ mm) with a sterilized puncher. These plates were incubated at 28 °C for 7 days. In order to determine the melanin production, flasks (250 mL) were filled with 100 mL of CM, CFM, NFM, TFM, SFM, LCHNM and LNHCM liquid media in advance. The culture inoculation was then transferred to every flask by punching out ten mycelial tablets ($d = 8$ mm) with a sterilized puncher. The cultivation was conducted at 28 °C on a rotary shaker operated at 150 rpm for 8 days. In all experiments, multiple flasks at least in triplicate were run at the same time to ensure reproducibility. The Data Processing System (DPS) statistical software package (version 7.05) was employed to analyze all data and statistical significance of experimental designs.

2.3. Analysis of element composition and molecular structure of melanin

In order to explore the difference between natural melanin from *A. auricula* and other melanins, the element constitution and structure of melanin from *A. auricula* were investigated with Elemental Analyser, HPLC technology, UV–Vis spectroscopy, FTIR spectroscopy and NMR spectroscopy as below.

2.4. Analytical methods

All experiments were performed in triplicate to ensure their reproducibility, and the data presented in “Section 3” represent the mean of three independent experiments.

(1) Melanin extraction, purification and detection.

The fermentation broth of *A. auricula* was collected and squeezed through a nylon mesh to remove mycelia. The pH of

the filtrate was adjusted to 1.5 with 6.0 mol/L hydrochloric acid and stored overnight in a refrigerator at 4 °C. Next, the filtrate was further clarified by centrifugation at 10,000 rpm for 15 min at 4 °C, and the precipitate was washed with deionized water until the pH became neutral. Subsequently, the precipitate was concentrated under reduced pressure, and dried to obtain crude melanin. In order to obtain the pure melanin, the crude melanin was redissolved in 2.0 mol/L NaOH solutions and continuously stirred by magnetic stirrers overnight. The melanin solution was clarified by centrifugation at 10,000 rpm for 15 min. The supernatant was collected, adjusted to pH 1.5 with 7 mol/L HCl and then kept at room temperature for 2 h. The supernatant was centrifuged at 10,000 rpm for 15 min to collect the precipitate. The precipitate was hydrolyzed with 7 mol/L HCl at 100 °C for 2 h, and then collected again by centrifugation at 10,000 rpm for 15 min. The precipitate was washed three times with distilled water to remove chloridion followed by drying at room temperature. The dried melanin was sequentially washed with chloroform, ethyl acetate and absolute ethanol, and dried at room temperature. Subsequently, the dried melanin was redissolved in 2.0 mol/L NaOH solutions, followed by centrifugation for 15 min at 10,000 rpm. The supernatant was adjusted to pH 1.5 and centrifuged at 10,000 rpm for 15 min. The pure melanin was obtained after repeated washing of the precipitate with distilled water and then drying to constant weight in an oven at 60 °C. In addition, in this study, the amount of melanin was determined using a spectrophotometer (Unico MFJ7200, Shanghai, China) at wavelength of 400 nm using 0.1 mol/L NaOH solution as the blank.

(2) Morphology analysis.

SEM was used for the observation of mycelial morphology with the following procedure. Mycelia were firstly washed in a 0.1 M phosphate buffer solution (pH 7.0) for 5 min, and then soaked in 2.5% (v/v) glutaraldehyde solution for approximately 8 h at 4 °C to preserve and stabilize their structure. Next, samples were washed in the buffer solution three times for 15 min once and then fixed for 90 min in a 1.0% osmium tetroxide solution prepared with the buffer solution. After washing three times for 15 min each in the buffer solution, water was removed from the samples by immersion for 10 min each in serially graded solutions of ethanol at concentrations of 50%, 70%, 80%, 90% and 100%. SEM observations were performed with 15 kV acceleration voltage and a beam current of 50 pA using a scanning electron microscope (JEOL, JSM-6380LV, Japan).

(3) Element composition and molecular structure analysis of melanin.

The composition of pure melanin was determined by the method of elemental analysis with an Elemental Analyser Vario EL (Germany).

(4) UV–Vis spectrum.

After the preparation of 50 mg/L melanin solution, the absorption properties of melanin were analyzed with UV–Vis spectroscopy, which was recorded in the wavelength range of 200–1100 nm using 0.1 mol/L NaOH solution as the reference.

(5) FTIR analysis.

For FTIR studies, melanin–KBr pellets were prepared as follows: firstly, KBr was homogenized in an agate mortar and weighed to obtain about 200 mg; secondly, melanin was homogenized in an agate mortar and weighed to about 10 mg; next, weighed KBr and lactase were mixed well in an agate mortar; finally, the melanin–KBr mixture was made into a tablet with a Specachydraulic

pellet press. Melanin–KBr pellets were analyzed with FTIR at wave numbers 4000–500 cm^{-1} . A Nicolet AVATAR360 instrument was utilized in this study. The resolution of the FTIR instrument was 4.0 cm^{-1} , optical path difference (OPD) velocity 0.20 cm s^{-1} , and the data collection interval 1.0 s. The actual spectra were measured as absorbance ratio. Background spectra were measured from clean KBr pellet containing no sample material.

(6) HPLC analysis.

A comparison between pure melanin and standard melanin (Sigma) was carried out with HPLC. Pure melanin and the standard were dissolved in 0.5 mol/L NaOH solution prior to the HPLC analysis. The quantitative method was developed on an Agilent 1100 HPLC system. HPLC detection of melanin was achieved on a Waters SunFire™ C₁₈ column (150 mm × 4.6 mm, 5 μm) coupled with a guard column C₁₈ cartridge (4.0 mm × 3.0 mm). The mobile phase consisted of methanol and 1% acetic acid, which were applied with an isocratic elution for 80 min. Each run was followed by a 10-min equilibration. The flow rate was 0.2 mL/min and the injection volume was 20 μL . The detection wavelength was set at 280 nm and the column temperature was set at 25 °C.

(7) NMR analysis.

The ¹H, ¹³C and 2D NMR spectra were recorded on a Bruker AVANCE III 600 spectrometer using TMS (Tetramethylsilane) as an internal standard, and the chemical shifts were recorded as δ values.

3. Results and discussion

3.1. Effect of nutritional conditions

The growth rate of mycelia and melanin production from *A. auricula* were determined and analyzed under different nutritional conditions. The results showed that nutritional constituents affect significantly not only the mycelial growth rate, but also melanin production. As listed in Table 1, the mycelial growth rate is highest in CFM and LCHNM, and lowest in NFM. In all tested media for submerged fermentation, the CM supports the highest production of melanin (2.22 g/L), but the mycelium dry weight is relatively low. Melanin production also reached 2.08 g/L in SFM. When *A. auricula* was cultivated in NFM, the mycelia formed small colonies easily and were very slim and fragile. Moreover, the production of melanin is the lowest, being only 0.01 g/L, because the lack of nitrogen in the medium inhibited the mycelial growth and tyrosinase synthesis, which are key steps in melanin secretion. In general, melanin synthesis is mainly a result of oxidative polymerization of phenolic compounds by two main polyphenol oxidases: (1) tyrosinase that has monophenol monooxygenase and *o*-diphenol:oxygen-oxidoreductase activities and (2) laccase that has *p*-diphenol:oxygen-oxidoreductase activity (Aghajanyan et al., 2005). In this study, there is positive correlation between tyrosine concentration and melanin production, and nearly no melanin was generated in TFM, which also demonstrated the tyrosinase-catalyzed reactions using tyrosine as substrate in *A. auricula*. At the same time, the tyrosine did not affect mycelial morphology. The production of melanin was 0.85 g/L in CFM. When LCHNM was adopted, the melanin production (1.78 g/L) was slightly lower than the value given by CM, but the mycelia had the best growth rate and the margin of colony growth was regular. All these results showed that the growth and metabolism of *A. auricula* are in close proximity with the carbon concentration. The low carbon concentration had a pronounced effect on the growth, but was unfavorable for melanin synthesis because the carbon source not only supplies cellular energy need but also

Table 1Mycelial growth rate and melanin production of *A. auricula* under different media.

| Media | CM | CFM | NFM | TFM | SFM | LCHNM | LNHCM |
|---|-------------------|------------------|------------------|------------------|------------------|------------------|-------------------|
| Average growth rate (cm/d) ^a | 0.77 ± 0.04 cd | 1.09 ± 0.0 6a | 0.41 ± 0.0 1e | 0.71 ± 0.0 2d | 0.83 ± 0.0 3c | 1.00 ± 0.0 6b | 0.76 ± 0.05 cd |
| Melanin production (g/L) ^a | 2.22 ± 0.07 a | 0.85 ± 0.0 d | 0.01 ± 0.0 f | 0.03 ± 0.0 f | 2.03 ± 0.0 b | 1.78 ± 0.0 c | 0.35 ± 0.02 e |

^a Different letters in the same column mean significant difference at 0.05 levels.

provides the carbon framework for melanin in the biosynthesis of melanin by *A. auricula*. The morphology of mycelia was affected by inorganic salt but there were no obvious effects on mycelial growth rate and melanin production. Although the mycelia grown in SFM were thick and spotlessly white, the growth rate was slower than that in CM and the margin of colony growth was irregular, which was due to the fact that some inorganic salts can inhibit the mycelium growth of *A. auricula* and affect its morphology. The research on *Hypsizygus marmoreus* reported that NaCl could stimulate mycelium growth and CaCl₂ and other inorganic salts inhibited the growth to varying degrees (Sun, Li, Ruan, Zhang, & Hu, 2014). As shown in the SEM image of Fig. 1, mycelia cultivated in SFM were sturdy and wrinkled. This may be because the osmotic pressure imbalance caused by saline ions results in changes in mycelial morphology of *A. auricula*. The above results suggest that it is very important to select nutritional conditions to improve melanin production and reduce the cost during submerged fermentation of *A. auricula*.

3.2. Elemental analysis

To better characterize the chemical composition of melanin from *A. auricula*, HPLC analysis of two melanins were firstly performed using a diode-array detection system. Fig. 2 shows the chromatograms obtained from *A. auricula* melanin (A) and the

synthetic standard (B). The melanin chromatogram profile is a single symmetrical elution peak and the retention times were 7.388 and 7.601 min, respectively. Furthermore, the peak pattern for melanin from *A. auricula* is similar to that of non-natural melanin synthesized by the oxidation of tyrosine by HPLC, which indicates that the melanin from *A. auricula* may contain eumelanin and have a similar structure as the standard melanin. However, the elemental analysis showed that the melanin from *A. auricula* mainly contained C, H, N, O and S elements as listed in Table 2. The content of S element was obviously lower than that in *Lachnum* YM404 (14.83), while the contents of H, N and O elements were higher (2.8, 6.48, 14.8) (Ye et al., 2014), indicating that contents of various elements in different melanins were various. According to the classification method chosen for melanin, the results from just elemental composition would mean that the melanin from *A. auricula* should be classified as a phaeomelanin because of the presence of S. In addition, compared to the S content of eumelanin (0.09%) and phaeomelanin (9.78%), the S content of melanin from *A. auricula* was 0.94%. This demonstrated that phaeomelanin is also a main constituent of melanin produced by *A. auricula*. Furthermore, phaeomelanins contain N and S, their synthesis pathway is similar with that of eumelanins, and cysteine or glutathione may be involved. Therefore, based on above, it is preliminarily concluded that the natural melanin from *A. auricula* chiefly consists of eumelanins and phaeomelanins.

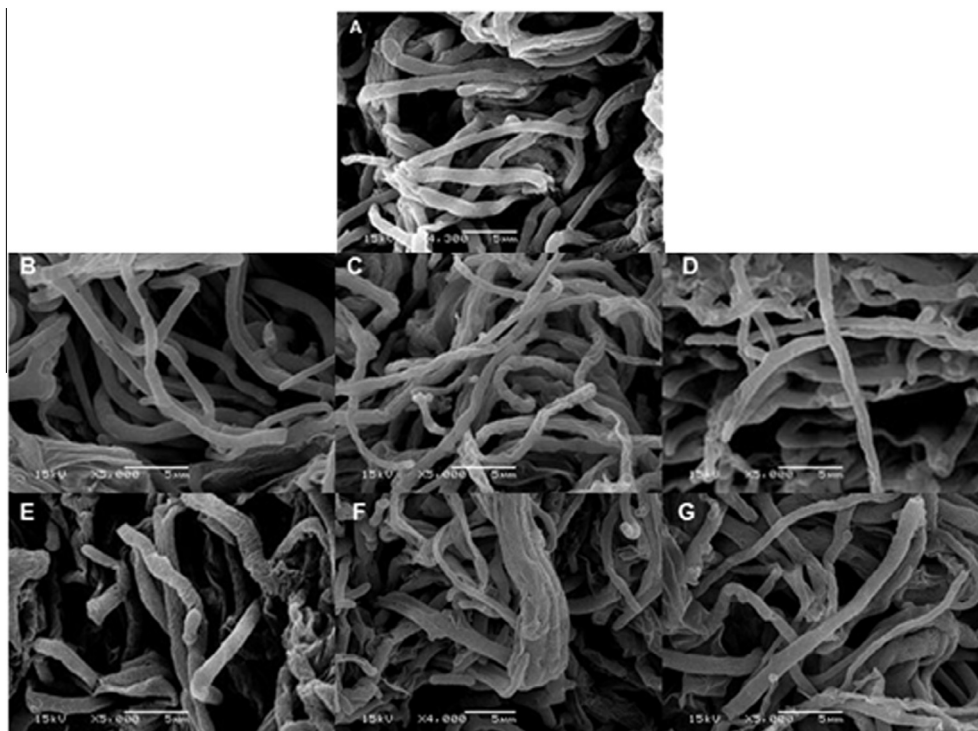


Fig. 1. Mycelium morphology of *A. auricula* in different nutritional media. (A): CM; (B): CFM; (C): NFM; (D): TFM; (E): SFM; (F): LCHNM; (G): LNHCM.

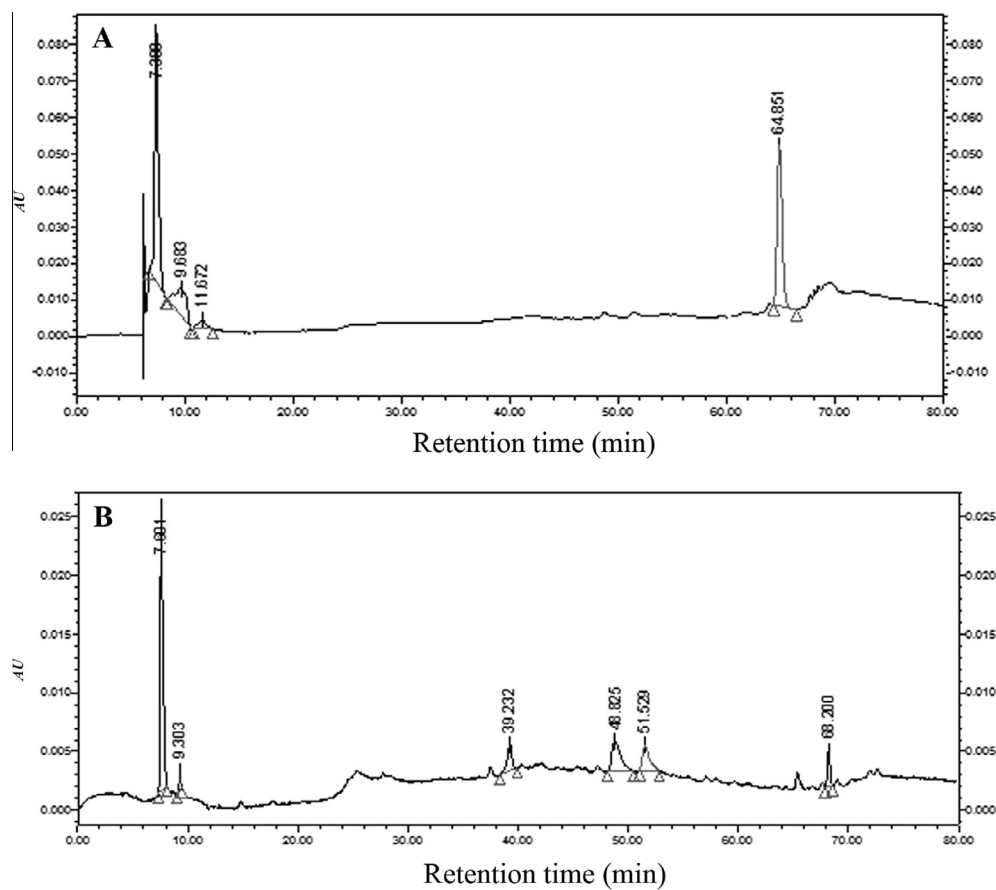


Fig. 2. HPLC chromatogram of melanin. (A): Melanin from *A. auricula*; (B): standard melanin.

Table 2
Elemental composition of melanin.

| Samples | Content (%) | | | | |
|---------------------------------|-------------|------|------|------|---------------------|
| | C | H | N | S | O |
| Melanin from <i>A. auricula</i> | 44.29 | 4.63 | 8.49 | 0.94 | 41.65 [*] |
| Synthetic eumelanins | 56.45 | 3.15 | 8.49 | 0.09 | 31.82 ^{**} |
| Phaeomelanins | 46.24 | 4.46 | 9.36 | 9.78 | 30.16 ^{**} |

^{*} The content of oxygen element was calculated from the equation: O% = 100% – C% – H% – N% – S%.

^{**} These data were derived from Ito's research.

3.3. UV-visible light absorption spectrum

The natural melanin isolated from fermentation broth of *A. auricula* had a maximum absorbance in the UV region and its optical density gradually decreased with an increase of wavelength (Fig. 3A), which is in accordance with other research (Riley, 1997). However, it is different from other reports on the melanin isolated from the fruiting body of *A. auricula* (Sun, Zou, Xu, Su, & Hu, 2010). The maximum absorbance wavelength of melanin from the fruiting body was at 215 nm, which may be a result of the difference of pH between solutions and melanin source causing minor change of the natural melanin structure. In addition, no obvious absorption peaks at 260 and 280 nm were observed, suggesting that there were no nucleic acid, lipids and other proteins in the melanin isolated from *A. auricula*. However, in the UV range of wavelengths, the melanin absorption spectrum represents the decreasing line with a plateau in the range of 270–280 nm, which may be the result of aromatic amino acid presence in melanin

molecule. In general, the most significant feature of this melanin is the intensive absorption over a wide wavelength range by high conjugate regions. In HPLC analysis of melanin from *A. auricula*, the peak response value is high, which demonstrates the presence of many conjugate groups.

3.4. FTIR analysis

FTIR analysis of crude and pure melanins was also performed. As shown in Fig. 3B, pure melanin from *A. auricula* has strong characteristic absorption at 3399 and 1633 cm^{-1} and broad absorption at 3300–3500 cm^{-1} corresponding to the stretching vibration of the –OH and –NH₂ groups. Melanin also exhibited an absorption in C–H at 2925 cm^{-1} and the characteristic band at 1633.71 cm^{-1} (C=C, –COO[–], C=O), 1075.44 cm^{-1} (C–O) phenols or carboxylic groups, which were characteristic absorption peaks for fungal melanin. The transmission pattern of melanin similarly showed the functional groups present in melanin. The FTIR showed that the two spectroscopic curves were fundamentally identical. In the FTIR spectrum of pure melanin, some narrow and sharp peaks appeared which may be caused by some fragments produced by the destruction in the acid precipitation step. However, compared to the FTIR spectrum of the standard, the molecular structure of melanin from *A. auricula* shows no obvious differences.

3.5. NMR analysis

In order to further confirm the molecular structure of melanin from *A. auricula*, NMR analysis of pure melanins were carried out. The 1D NMR spectrum of melanin in D₂O displayed signals in both

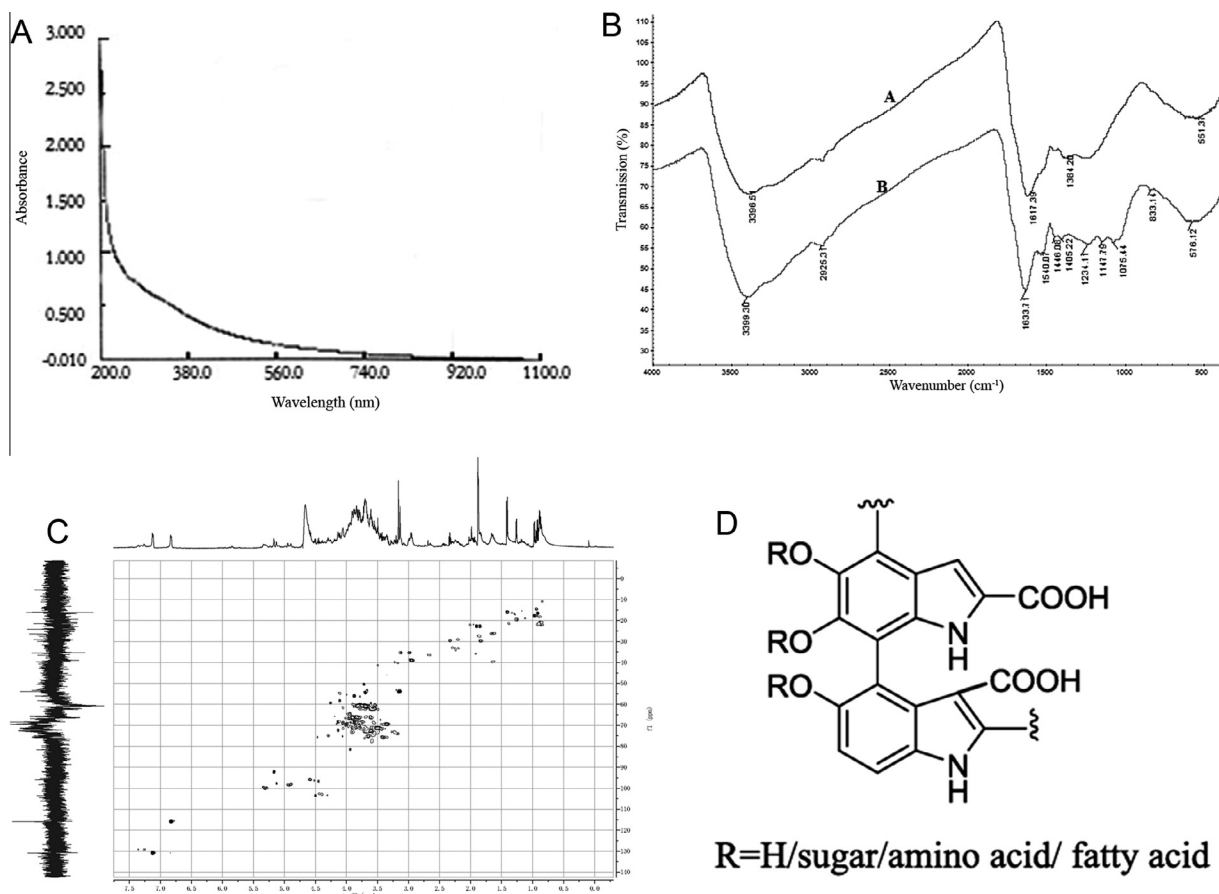


Fig. 3. Chromatographic and structural analysis of natural melanin extracted from *A. auricula* (A): UV-Vis spectrum of melanin; (B): FTIR spectroscopy of melanin (A) crude melanin, (B) pure melanin; (C): the HSQC spectrum of melanin in D₂O; (D): the proposed partial polymer chain structure of melanin.

the aliphatic and aromatic regions, and was ascribable to methyls, methylenes, methynyls, aromatic protons, according to the ¹H–¹³C HSQC correlations (Fig. 3C). In the upfield of ¹H NMR spectrum at δ_H 0.5 ppm to δ_H 2.5 ppm the peaks can be assigned to CH₃, CH₂ groups of alkyl fragments with corresponding to δ_C 15 ppm to δ_C 40 ppm in the ¹³C NMR spectrum. Those aliphatic carbons were due to proteinaceous material (Guo, Chen, et al., 2014; Guo, Rao, et al., 2014). Peaks from δ_H 3.5 ppm to δ_H 4.5 ppm in the ¹H NMR spectrum can be assigned to protons on carbons attached to oxygen or nitrogen atoms. Besides, several signals displayed in the anomeric region at δ_H 4.7–5.4 ppm. Those protons in the oxygen and anomeric regions indicated the existence of carbohydrates although some signals are overlapped. In the aromatic regions of ¹H NMR spectra, the single signal at δ_H 8.29 (s) ppm indicated the existence of pyrrole-CH group of a carboxyl substituted indole (Jalmi, Bodke, Wahidullah, & Raghukumar, 2012) meanwhile the doublet peaks at δ_H 7.02 (d, J = 7.5 Hz) and 6.73 ppm (d, J = 7.5 Hz) suggested the benzene-CH group of indole, and the two aromatic protons were *ortho* according to the coupling constant. This partial structure was supported by ¹H–¹H COSY correlations. The above comprehensive information of 1D and 2D NMR spectrum data suggested that the melanin isolated from *A. auricula* is an eumelanin, which is a macromolecular polymer of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid, associated with some carbohydrates and proteinaceous matters (Bhavin, Novruz, Alan, & Clifford, 2003; Katritzky, Akhmedov, Denisenko, & Denisko, 2002). Combined with the other analysis results, the condensed molecular formula of this melanin was speculated as [C₁₈(OR)₃H₇O₄N₂]_n and the structure formula was

concluded in Fig. 3D. The information obtained should be of value for the more efficient production of melanin and therefore for its application in food, cosmetics, pharmacology, medicines and other fields.

4. Conclusions

In this study, the growth rate and morphology of mycelia, production characteristics and molecular structure of melanin from *A. auricula* have been determined and analyzed in detail. The results showed that different nutritional conditions have significant effect on mycelial morphology and melanin production. This melanin is an eumelanin and also a macromolecular polymer of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid, chiefly containing phenolic hydroxyl, carboxyl, amidogen, carbonyl, methylene, methyne and sulfur. At the same time, its condensed molecular formula ([C₁₈(OR)₃H₇O₄N₂]_n) and structural formula were also concluded. This is the first known report describing the molecular structure of melanin produced by *A. auricula*.

Notes

The authors declare no competing financial interest.

Acknowledgments

This work was financially supported by the Natural Science Foundation of China (31201669, 41276135 and 31172010),

Natural Science Foundation of Fujian Province for Distinguished Young Scholars (2014J06010), the major platform for the development of edible fungus industry technology in Fujian Province (2014N21010008), “Wu Xin” Project of Agriculture of Fujian Provincial Development and Reform Commission of China ([2012]931), Research Funds for Distinguished Young Scientists in Fujian Agriculture and Forestry University (xjq201209), Minjiang Scholars (2013A13) and Program for New Century Excellent Talents in University (NCET-13-1031). Special thanks to Robert K. Thomas FRS from University of Oxford for his contribution in improving the manuscript.

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