

IFSCC 2025-1137

## **Effects of different culture media and pH on the pigment and antioxidant activity of *Pycnoporus cinnabarinus***

Weilin Chen<sup>\*1</sup>, Chui Li Lim<sup>1</sup>, Yi -Ying Zhan<sup>1</sup>, Chao-Hsun Yang<sup>1</sup>

<sup>1</sup>Cosmetic Science, Providence University, Taichung, Taiwan

### **1. Introduction**

*Pycnoporus cinnabarinus* belongs to the fungal kingdom, specifically within the phylum Basidiomycota, order Polyporales, family Polyporaceae, and genus *Pycnoporus*. This is a small to medium-sized, woody, saprophytic mushroom, typically found growing solitarily or in clusters on dead branches of coniferous and broad-leaved trees. A key characteristic is its bright orange-red fruiting body, which is usually 2.0-11.0 cm wide and lacks rings. The cap surface is orange to red, fading over time, and can be smooth or finely hairy. It has orange flesh and a vermillion pore surface. This fungus is widely distributed in Taiwan, Japan, China, and Europe [1]. *P. cinnabarinus* is a white-rot fungus that causes wood decay by decomposing cellulose, hemicellulose, and lignin [2].

Traditional Chinese medicine literature records that the fruiting body of *P. cinnabarinus* has the functions of clearing damp heat, reducing inflammation and detoxification, and promoting hemostasis. For medicinal purposes, *P. cinnabarinus* and *Pycnoporus sanguineus* were historically classified together, both listed in the Chinese Materia Medica. It was only in recent years that *P. cinnabarinus* was identified as a distinct species from *P. sanguineus*.

Some scholars have conducted research on *P. cinnabarinus* and found that its fruiting body has the ability to reduce inflammation, exhibit anti-oxidant effects, and inhibit vascular proliferation. Using ethanol to extract compounds from the fruit body yields an extract with good anti-inflammatory effects.

According to a study comparing the antioxidant activity of *P. cinnabarinus* extracts obtained with ethanol and water, the ethanol extract demonstrated better antioxidant activity than the water extract. This study [3] also found that phenolic substances are the main antioxidant components in *P. cinnabarinus*.

The red pigments accumulated by *P. cinnabarinus* during growth are mainly cinnabarin, cinnabaric acid, and related compounds, which are antibiotic-like pigments with varying degrees of antitumor and antibacterial activities [4].

*P. cinnabarinus* typically grows in an environment with a pH between 4 and 5. Due to its characteristic ability to produce secondary metabolites (accumulation of pigments), the aim is to adjust the pH to provide *P. cinnabarinus* with a harsher growth environment. The goal is to observe the effect of the pH conditions on the fungus's growth status, as well as on its secondary metabolites and biological activity.

## 2. Materials and Methods

### 2.1 Materials

This study utilized *P. cinnabarinus* BCRC 36234 obtained from BCRC, Taiwan. Key materials included Potato Dextrose Broth/Agar, Sabouraud Dextrose Broth/Agar, Malt Extract Broth/Agar from HiMedia, India. Diaion® HP-20 resin, and analytical reagents (DPPH, ABTS, Folin) was purchased from Sigma Aldrich.

### 2.2 Culture conditions

Cultivation involved:

Solid Culture: *P. cinnabarinus* mycelium was inoculated onto PDA, SDA, and MEA plates at different pH values and incubated at 25°C for 20 days with daily observation

Liquid Culture: A 3-day pre-culture in PDB, SDB, MEB (25°C, 100 rpm) was performed. This was used to inoculate fresh PDB, SDB, MEB adjusted to pH 4-9, cultured at 25°C, 100 rpm for 15 days with daily observation.

### 2.3 Assessment of antioxidant properties

After 15 days, the *P. cinnabarinus* fermentation broth (PcFB) was collected by vacuum filtration. The PcFB was subsequently analyzed for its DPPH and ABTS+ radical scavenging activity, total phenolic content, and reducing power. V-630 UV-Vis spectrophotometer from Jasco (Japan) were used in the antioxidant methodologies [5].

DPPH free radical scavenging activity reagent was prepared by, 1 mg 1,1-diphenyl-2-picrylhydrazyl (DPPH) that dissolved in 10 mL of 95% alcohol. 0.1 M Tris-HCl buffer solution with pH 7.4 was used for activity determination. 200 µL of standard or sample was mixed with 800 µL of buffer solution and thoroughly mixed. Then, 1.0 mL of reagent was added, and the mixture was allowed to react in the dark for 20 min. Afterward, the absorbance at 517 nm was measured using a spectrophotometer. Alcohol was used as a blank for reagents, and water was used as a blank for the sample [5].

To prepare the ABTS solution, 7 mM 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2.45 mM potassium persulfate were dissolved in 0.01 M sodium phosphate buffer (pH 7.4) and allowed to react for 24 h. Then diluted with 0.01 M sodium phosphate buffer until the absorbance reached a range of 0.80 to 0.85 at 734 nm. For the assay, 500 µL of standard or sample solution was mixed with 600 µL ABTS solution or buffer (control) and reacted for 10 min [5].

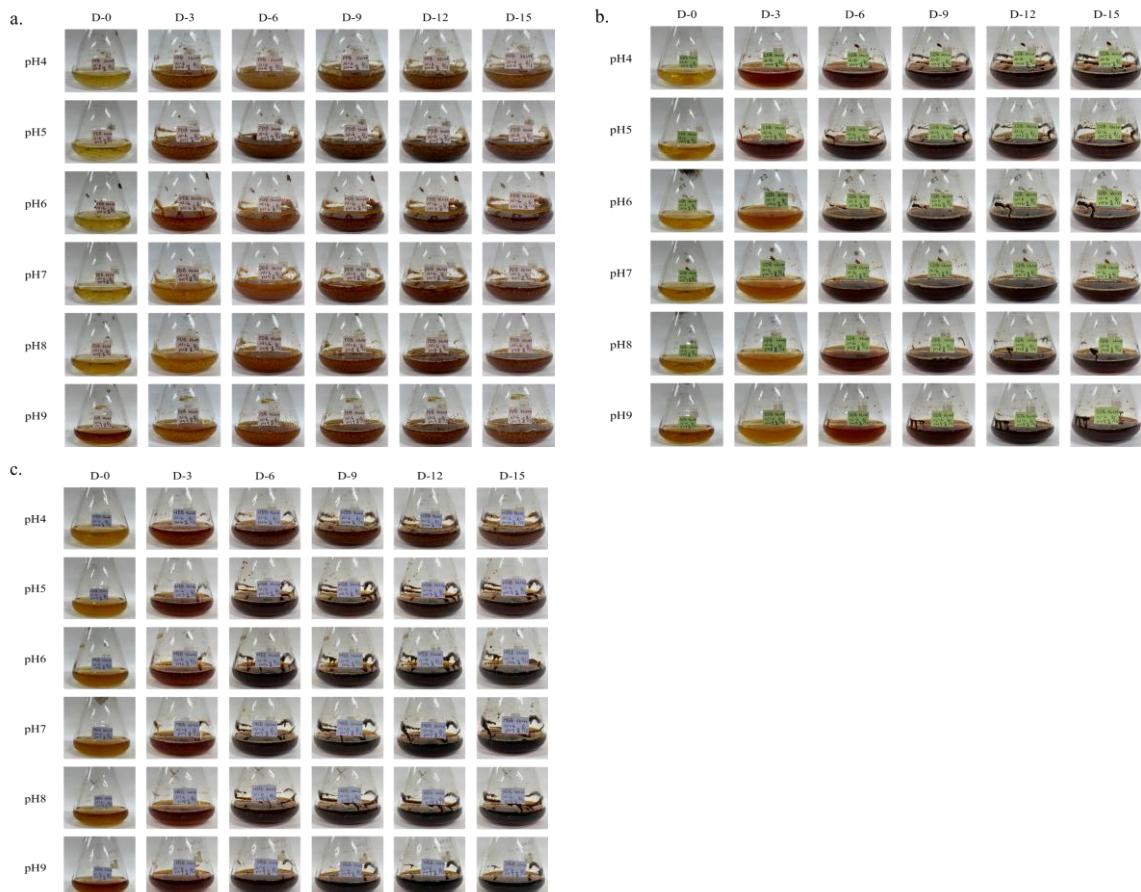
The total phenolic content was determined using the Folin-Ciocalteu Reagent. Firstly, 50 µL of the standard or sample solution was mixed with 2% Na<sub>2</sub>CO<sub>3</sub> for 2 min. Then, 50 µL of 1 N Folin-Ciocalteu Reagent was added, and let the mixture react for 30 min. After the reaction, the absorbance was measured at 765 nm. The total phenolic content of the sample was determined using a calibration curve by gallic acid [5].

The reducing ability of PFB was assessed using a modified version of the previously described method. Separately prepare, 1% potassium hexacyanoferrate (III), 10% Trichloroacetic acid 0.1% Iron (III) chloride and 0.2 M potassium phosphate buffer (pH 6.6). 300 µL of buffer and 300 µL of standard or sample solution were reacted with 1% potassium hexacyanoferrate (III) at 50°C for 20 min. Then 10% Trichloroacetic acid was added and vortex for 2 min to mix well. After centrifugation at 3000 rpm for 20 min to remove any precipitates. The supernatant was reacted with 240 µL of 0.1% iron (III) chloride in the dark for 10 min. Finally, the absorbance at 700 nm was measured [5].

## 3. Results

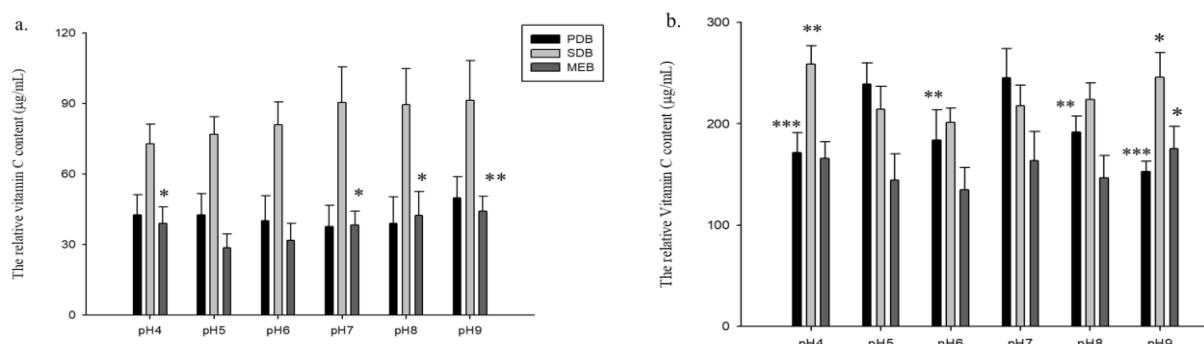
### 3.1 Changes in the PcFB

Figure 1. The effect of different pH values on *P. cinnabarinus* broth culture (a.PDB b.SDB, c.MEB).



Observations from Figure 1 indicate that during liquid cultivation of *P. cinnabarinus*, pigment accumulation occurred most rapidly in the MEB medium. In contrast, pigment accumulation in the PDB medium was slower, and it did not exhibit a consistent trend following pH adjustments.

### 3.2 Antioxidative effect of PcFB



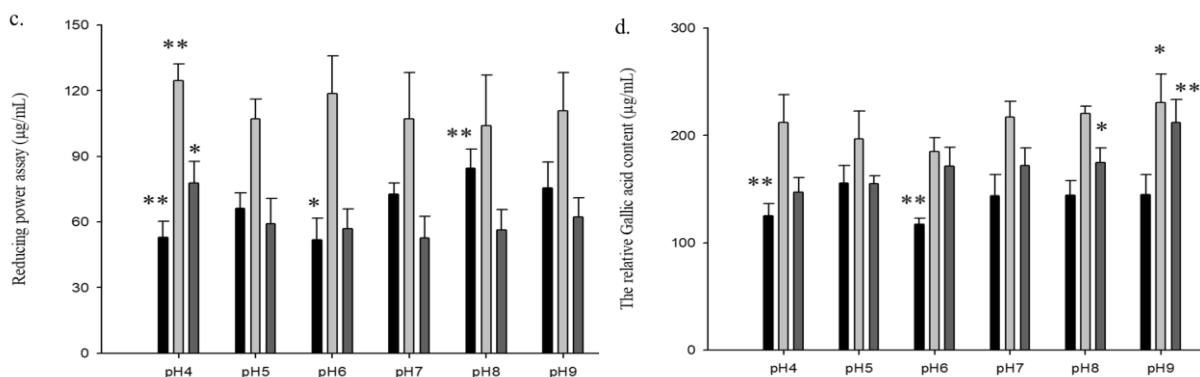


Figure 2. The effect of different pH values cultured on *P. cinnabarinus* at the antioxidant activity of PcFB. a. DPPH radical scavenging assay, b. ABTS<sup>+</sup> radical scavenging activity, c. Reducing power assay, d. Total phenolic content assay. Data are presented as the mean  $\pm$  SE from triplicate experiments. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

Figure 2 presents the antioxidant activity results. All experiments were performed in triplicate. Significant differences were determined by comparing the results at various adjusted pH values against those of the original medium pH (pH 5). Figure 2a shows that SDB medium at pH 9 yielded the highest relative Vitamin C equivalent content (91.34 µg/mL); however, no significant difference was observed compared to the unadjusted pH 5 control across the tested pH range for SDB. Conversely, for the MEB medium, DPPH scavenging activity showed a significant increase at all adjusted pH values compared to pH 5. Figure 2b indicates that in PDB medium, ABTS scavenging activity significantly decreased across all adjusted pH values compared to pH 5. In SDB medium, activity at pH 4 (258.95 µg/mL) and pH 9 (245.76 µg/mL) showed a significant increase compared to the unadjusted pH 5 control. As shown in Figure 2c, the reducing power activity in SDB medium at pH 4 (124.59 µg/mL) was significantly increased compared to the pH 5 control. Figure 2d reveals that the total phenolic content in PDB medium decreased following pH adjustments compared to pH 5. For SDB medium, only the pH 9 condition showed a significant increase (230.64 µg/mL) relative to pH 5. In MEB medium, significant increases were observed at pH 8 (172.82 µg/mL) and pH 9 (212.20 µg/mL) compared to the unadjusted pH 5 control.

### 3.3 Production of pigment from PcFB

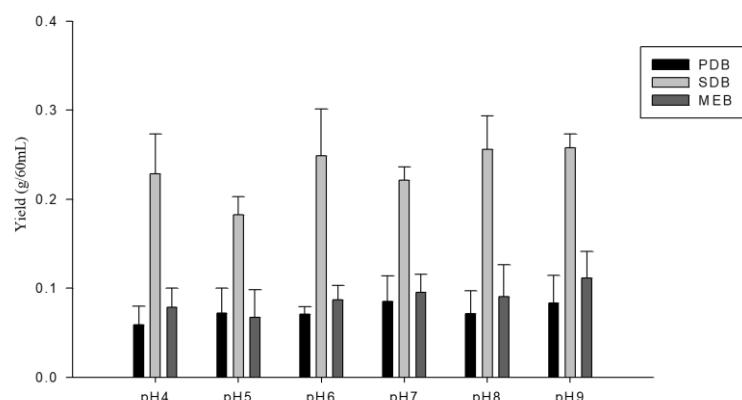


Figure 3. Effects of different pH values culture conditions on the pigment production of *P. cinnabarinus* in the PcFB.

Figure 3 presents a comparison of pigment yields. Consistent with the observations noted in Figure 1, the quantitative results in Figure 3 confirm that PDB medium resulted in the lowest pigment accumulation among the three media tested. Notably, pigment accumulation in MEB medium commenced as early as the third day. By day 15, visual inspection indicated that the color intensity of the MEB medium was comparable to that of the SDB medium. However, a crucial finding during the pigment extraction process was that the pigment from the MEB culture failed to bind effectively to the resin beads employed in the procedure. This binding issue resulted in a significant discrepancy in the quantified pigment yield, showing a much lower value for MEB compared to SDB, despite their similar visual color intensity in the culture.

#### 4. Discussion

Research by Téllez-Téllez Maura found that the optimal pH for pigment production differs between *Pycnoporus* strains (pH 5.5 for HEMIM-80 and pH 6.5 for HEMIM-55). Additionally, HEMIM-80 exhibited broader tolerance, producing pigment across all tested pH values, whereas HEMIM-55 failed to produce pigment at basic pH levels. This highlights that pH control is crucial and must be customized for the specific strain used in biotechnological pigment production [6]. In parallel, our research also showed significant yield differences depending on the medium, with PDB resulting in the lowest yield. Critically, pigment in MEB medium, despite appearing visually intense, yielded low quantified values due to poor binding during the resin extraction process. This contrasts with SDB medium, which allowed for effective extraction. Consequently, both pH regulation and appropriate media selection are essential for optimizing and reliably measuring pigment yields from *Pycnoporus*.

#### 5. Conclusion

SDB medium resulted in the highest quantifiable pigment yield, while MEB, despite rapid visual color development, yielded less pigment quantitatively due to extraction inefficiencies. PDB consistently produced the lowest pigment levels. Antioxidant activities were highly dependent on both medium and pH: MEB significantly enhanced DPPH scavenging and total phenolics at alkaline pH (8-9), whereas SDB showed increased ABTS<sup>+</sup> activity (pH 4, 9), reducing power (pH 4), and phenolics (pH 9) under specific conditions. PDB generally exhibited reduced antioxidant potential after pH adjustments.

#### 6. References

- [1] Herpoël, I., Moukha, S., Lesage-Meessen, L., Sigoillot, J. C., & Asther, M. (2000). Selection of *Pycnoporus cinnabarinus* strains for laccase productio1n. FEMS Microbiology Letters, 183(2), 301–3026.
- [2] Watanabe, T., Katayama, S., Enoki, M., Honda, Y., & Kuwahara, M. (2003). New metabolites, pycnoporons A, B and C, produced by white rot fungus *Pycnoporus cinnabarinus*. Mycoscience, 44(2), 111-115.
- [3] Hui Huang, Jian-Jiang Zhong. (2008). Antioxidant activities of crude extracts and phenolic compounds of *Pycnoporus cinnabarinus* mycelia. Food Chemistry, Volume 107, Issue 3, pages 1136-1142.
- [4] Field, J. A., et al. (1995). Significance of fungi in bioremediation of contaminated soils and sediments. *Antonie Van Leeuwenhoek*, 67(2), 147-156.
- [5] Chen, W.L., Hsu, J.C., Lim, C.L., Chen, C.Y., & Yang, C.H. (2020). Expression of the Thermobifida fusca  $\beta$ -1,3-glucanase in *Yarrowia lipolytica* and its application in hydrolysis of  $\beta$ -1,3-glucan from four kinds of Polyporaceae. *Processes*, 9, 56–77.

- [6] Mendoza G. M.<sup>1,2</sup>, Hernández N. R. M.<sup>1,2</sup>, Elba C. V. V.<sup>3</sup>, Quiroz D. M.<sup>4</sup>, Acosta-Urdapilleta Ma. de L.<sup>1</sup>, Díaz G. G.<sup>5</sup>, Téllez-T. M.<sup>1</sup>.(2014). Effect of pH on the radial growth rate and pigment production of two strains of Pycnoporus.194-198.