

Biosynthesis and physiochemical characterization of melanin from *Fonsecaea monophora*

Chen Ying-Dan, Lu Chun, Li Mei-Rong, Ma Han, Lu Rong-Biao, Ying Song-Chao, Zheng Zhi-Xin, Li Hai-Yi, Feng Pei-Ying*

(Department of Dermatology and Venereology, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou,

510630, China)

BACKGROUND

Chromoblastomycosis (CBM) is a chronic (sub)cutaneous melanized fungal infection characterized by verrucose lesions and dark-colored, thick-walled muriform cells in the infected tissue. *Fonsecaea* species is one of the recurrent causative agents of the disorder which containing five clinically relevant cryptic species i.e. *Fonsecaea pedrosoi*, *F. monophora*, *F. nubica*, *F. multimorphosa* and *F. pugnacious*. In Southern China, *F. monophora* (75%) and *F. nubica* (25%) are the prevalent causative agents of CBM. Melanin is an insoluble compound which is mainly deposited in the cell walls of melanized fungi and plays an important role in virulence and pathogenicity. To better understanding the pathogenesis of CBM, we study the melanin physiochemical characterization and melanin synthesis pathway of *F. monophora*.

METHODS & RESULTS

- Strain CBS 122845 originated from an 81-year-old male as a meristematic mutant of the filamentous fungus *F. monophora* was used in this study.
- Pure melanin mass was extracted from the *F. monophora* isolate using the cell wall crude extract method. Then we used Ultraviolet (Uv), Fourier transformed infrared (FT-IR) and electron paramagnetic resonance (EPR) spectra assay to evaluate the physiochemical characterization of melanin (Figure 1). Furthermore, we observed the pigment production of the colonies on different mediums (PDA, PDA with L-DOPA, PDA with DOPA-melanin inhibitors and PDA with DHN-melanin inhibitors), and the quantity of melanin produced on different above media were measured using BioTek Eon microplate reader.

- The melanin extracted from *F. monophora* shared similar physiochemical and spectroscopic properties with the synthetic L-DOPA melanin. The formation of melanin pigment statistically significant increased on the L-DOPA medium, and decreased on the DOPA-melanin inhibitor medium (sodium azide) and DHN-melanin inhibitors medium (phthalide and tricyclazole) compare to their blank contrast medium (Figure 2).

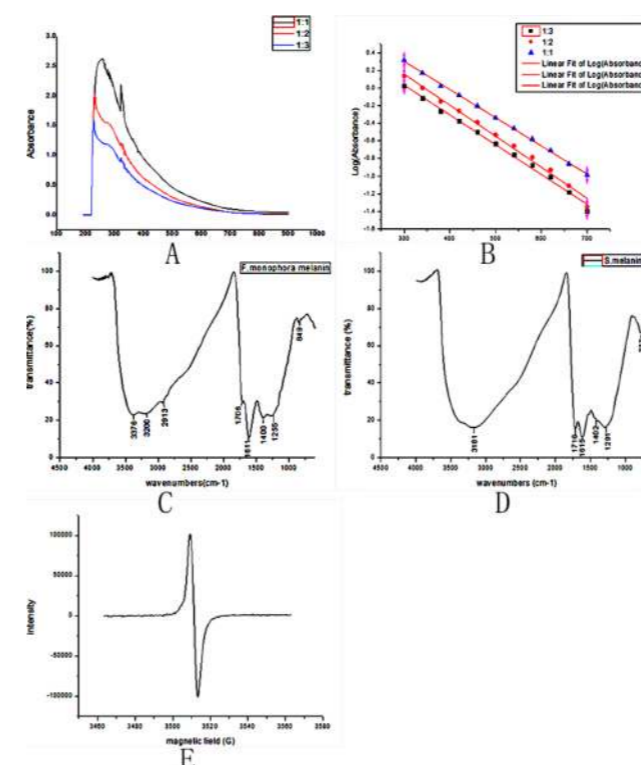


Figure 1: UV - visible spectroscopic properties of melanin pigment showing maximum absorption peak between 200 and 300 nm (A). Log absorbance of three different concentrations of melanin solution between 300 and 700nm wavelength (B). FT - IR spectrum (C,D) and EPR spectral analysis of the melanin pigment from *F. monophora* (E).

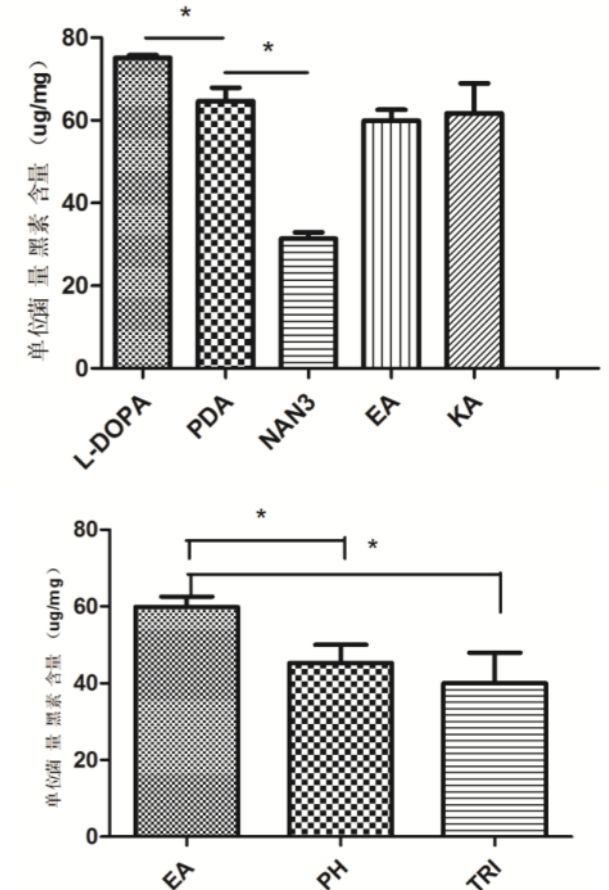


Figure 2: Quantitative Statistical analysis of extracted melanin shows that L-DOPA increase the production of melanin while the sodium azide obviously inhibit the synthesis of melanin, and both the DHN melanin inhibitors Phthalide and tricyclazole inhibit the synthesis of melanin in *F. monophora*.

CONCLUSION & DISCUSSION

- The melanin produced by *F. monophora* is mainly DOPA melanin, and it may synthesize simultaneously by DOPA-melanin and DHN-melanin pathway.
- The melanin synthesis pathway of *F. monophora* is different with that of *F. pedrosoi*, which only having DHN-melanin pathway.

Corresponding author: Feng Peiying, Email: fpying77@gmail.com
 This work was partly funded by the National Science Foundation of China (No. 81401650 to Peiying Feng).