

Production of Melanin from *Aspergillus fumigatus* as an Avirulence Factor

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Received: 18th October, 19; Revised: 20th November, 19, Accepted: 15th December, 19; Available Online: 25th December, 2019

ABSTRACT

In this quantitative examination of melanin was detected in 41 isolates of *fumigatus*. It was found that the significance of the difference in which the production of melanin ($p < 0.001$) was the range of melanin extracted from *fumigatus* between (1.2 - 3.1 mg/mL). The isolates AFU1, AFU14, AFU29, AFU30, and AFU41 indicated that the maximum production ranged between (2.9-3.1 mg/mL). 2 hours at 100°C. It has similarities with standard melanin characters, and the same chemical characters of melanin extracted from the hymen of *Bjerkandara adusta* have been achieved according to a study (14) meaning there is similarity with standard melanin. The overlay of IR spectra of synthetic melanin extracted from fungal cultures showed a high degree of similarity. Purification of melanin from *A. fumigatus*. Wave numbers shown in 3381, 2927, 1867, 1531, 1404, 1073 and 651 cm⁻¹ The wave range can be traced to the following chemical groups: 3381 cm⁻¹ attributed to OH bonds, 2927 cm⁻¹ to HC or HC = O bonds 1404 (C-CH₃), and 651 cm⁻¹ to (acyclic) CH₂ bonds.

Keywords: FTIR, Melanin, Pigmentation, SDA.

International Journal of Pharmaceutical Quality Assurance (2019); DOI: 10.25258/ijpqa.10.4.16

How to cite this article: Hussein, A.S. and Noman, S.J. (2019). Production of Melanin from *Aspergillus fumigatus* as an Avirulence Factor. International Journal of Pharmaceutical Quality Assurance 10(4): 651-654.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Conidial coloring, resulting in the typical grey-green color, seems to be important for fungal resistance. An important compound in coloring is, which likely enables the fungus to oppose against act the unable to be harmed defense system and increases spore resistance.¹ *Aspergillus fumigatus* conidia have a typical and expected gray-green color due to the removal from a ruling position legal statement under oath of (what colors skin, hair, eyes, etc.) in the conidial cell wall.² It is believed that offers protection against causing reactions from other people or chemicals, oxygen group of similar living things (ROS) produced by phagocytic cells, in that way slowing the rate of phagocytosis of melanized cells by alveolar macrophages and neutrophils.³ Disruption of the first tiny chemical assembly instruction inside of living things in combination with the pathway leads to colorless (white) conidia,⁴ reduces danger/disease in mice and increases the likelihood of being harmed or influenced of conidia to monocytes in vitro.⁵ The color present in the grey-green conidia of *A. fumigatus* is DHN- (what colors skin, hair, eyes, etc.), separated far from others from the lungs of infection, can't fight off disease mice.⁶ The production of what colors skin, hair, eyes, etc.) is one feature of *A. fumigatus*, which adds/gives to its helpful change and to its disease-causing.⁷ The term "(what colors skin, hair, eyes, etc.)" starts from melanos, a Greek word for black.

MATERIALS AND METHODS

Screening of *A. fumigatus* isolates for melanin production

Separated far from others of *A. fumigatus* was grown on SDA plates for 5 days at 37 °C, and conidia were collected as described. Conidia were washed three times with PBS, with a final wash with 1.0 M sorbitol and 0.1 M sodium citrate (pH 5.5). Novozyme cell wall lysing enzymes from was added at 10 mg mL⁻¹ and created and grew overnight at 30°C to create protoplasts. The protoplast was collected by centrifugation, washed three times with PBS, and created and grew in 4.0 M guanidine thiocyanate (denaturant), overnight at room temperature. Dark particles were collected by centrifugation. These were then washed three times with PBS and treated with 1.0 mg/ ml Proteinase K in reaction buffer and created and grew at 37 °C.⁸ Many broken pieces of something destroyed were washed three times with PBS and boiled in 6.0 M HCl for 1.5-hour. After treatment by boiling in acid (what colors skin, hair, eyes, etc.), particles were collected by filtration through Whatman paper No.1 and washed a lot with produced/showed water. Particles were then dialyzed against produced/showed water for 10 days until the acid was completely removed and were then lyophilized appropriate.⁹

Infrared (IR) spectrum of melanin from *A. fumigatus*

For IR spectrum, the pigment was hydrolyzed with 5 mL of 7 N HCl in a sealed glass vial and kept for 2 hours at 100°C.¹⁰

The purified pigments were ground with KBr. The spectrum was recorded in Perkin–Elmer 377 spectrophotometer.¹¹

RESULTS AND DISCUSSION

Screening of *A. fumigatus* isolates for melanin production

What colors skin, hair, eyes, etc. was pulled out or taken from something else from *A. fumigatus* conidia of 41 separates far from others after treatment of conidia with a combination of proteolytic, glycolytic enzymes. A hot focused one's effort increases acid resulted in the being completely separate from others of black particles, which was a black color, having to do with measuring things with numbers examining and testing so a decision can be made. An examination from 41 *A. fumigatus* (separates far from others) was found the importance of difference/different version in production ($p \leq 0.001$) as summarized in Table 1 the range of pulled out or taken from something else) from *A. fumigatus* were between (1.2-3.1 mg/mL). The (separates far from others) AFU1, AFU14, AFU29, AFU30, and AFU41 pointed to/showed the maximum production ranged between (2.9-3.1mg/mL) AFU1, AFU41 showed maximum yield of production, pulled out or taken from something else was not able to be dissolved in something in water but (able to be dissolved in something) in 1 M KOH for 2-hour at 100°C. It has a similarity with the standard characters, and the same chemical characters were accomplished or gained with effort for pulled out or taken from something else from hymenia of *Bjerkandara adusta* according to the study of L.H. Hogan, *et al.*¹² which shows a similarity with the standard.

The variances of (what colors skin, hair, eyes, etc.) production may be attributed to few (separates far from others) of *A. fumigatus*, which had few coloring and produce white conidia (Scot, 2006). Wild-type strains of *A. fumigatus* missing color was less disease-causing than strains with green conidia.¹³ Three further poorly studied (tiny chemical assembly instructions inside of living things) participate in the (what colors skin, hair, eyes, etc.) biosynthesis group-related. The *abr1* (tiny chemical assembly instruction inside of living things) codes for (what colors skin, hair, eyes, etc.) (creation/combination) the expression of this (tiny chemical assembly instruction inside of living things) different between strains of *A. fumigatus*, and the ability of strains to produce (what colors skin, hair, eyes, etc.) related to the color of conidia if it yellowish-green and gray conidia, (match up each pair of items in order).¹⁴

The big difference in (what colors skin, hair, eyes, etc.) production in this study may be attributed to the difference in the coloring of the separates far from others; production is one feature of *A. fumigatus* which adds/gives to its helpful change and to its disease-causingity.¹² Their further properties are protection against lysing enzymes, and the ability to provide/discuss something radioprotection⁵ as well as to protect against UV and solar radiation.⁹

Table 1: Ability of *Aspergillus fumigatus* isolates in melanin production.

S. No.	Symbol of isolate	Melanin production mg/mL mean \pm SE
1	AFU1	3.1 \pm 0.1
2	AFU2	1.5 \pm 0.2
3	AFU3	1.2 \pm 0.1
4	AFU4	1.5 \pm 0.2
5	AFU5	2.5 \pm 0.2
6	AFU6	2.4 \pm 0.2
7	AFU7	1.9 \pm 0
8	AFU8	1.7 \pm 0.1
9	AFU9	1.5 \pm 0.2
10	AFU10	1.5 \pm 0.2
11	AFU11	2.0 \pm 0
12	AFU12	2.0 \pm 0.2
13	AFU13	1.9 \pm 0
14	AFU14	3.0 \pm 0
15	AFU15	1.9 \pm 0.05
16	AFU16	2.7 \pm 0.1
17	AFU17	2.7 \pm 0.14
18	AFU18	1.2 \pm 0.1
19	AFU19	1.4 \pm 0.2
20	AFU20	1.9 \pm 0.05
21	AFU21	1.5 \pm 0
22	AFU22	2.8 \pm 0.1
23	AFU23	1.7 \pm 0.087
24	AFU24	2.5 \pm 0.1
25	AFU25	2.3 \pm 0
26	AFU26	1.9 \pm 0.05
27	AFU26	1.8 \pm 0.2
28	AFU28	1.5 \pm 0
29	AFU29	2.9 \pm 0
30	AFU30	3.0 \pm 0.1
31	AFU31	2.0 \pm 0.2
32	AFU32	2.1 \pm 0.6
33	AFU33	2.5 \pm 0.1
34	AFU34	2.8 \pm 0
35	AFU35	2.8 \pm 0.1
36	AFU36	1.7 \pm 0.08
37	AFU37	1.7 \pm 0.1
38	AFU38	1.4 \pm 0.1
39	AFU39	2.4 \pm 0.1
40	AFU40	2.8 \pm 0
41	AFU41	3.1 \pm 0.1

Significantly different. $p < 0.0001$

Infrared IR analysis of melanin extracted from *A. fumigatus*

Infrared analysis IR is thought of as the most information-giving method for (related to what holds something together and makes it strong) analysis of (what colors skin, hair, eyes, etc.).⁹ Therefore, this method was applied to study the color carefully, which piled up after purification, This purified and lyophilized color was compared with the in vitro made/created color both colors were (examined closely so the truth can be found) spectroscopically as potassium bromide disks. The overlay of the IR spectra of (produced by people/not naturally-

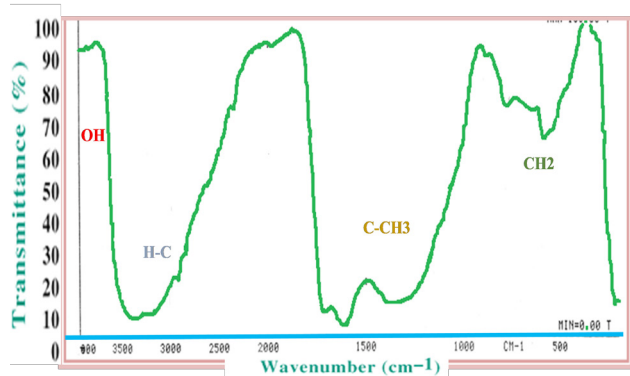


Figure 1: Infrared analysis of Standard melanin.

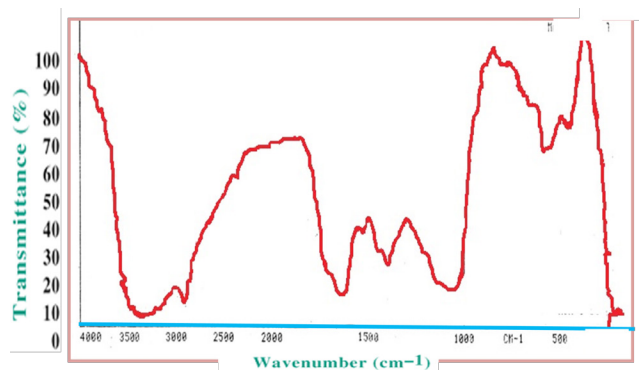


Figure 2: Infrared analysis of melanin extracted from *A. fumigatus*.

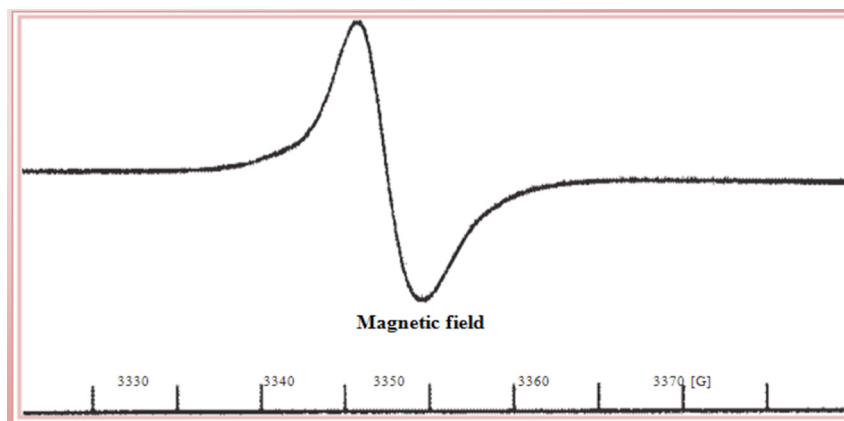


Figure 3: ESR spectroscopy of melanin particles extracted from *Aspergillus fumigatus*.

occurring) (what colors skin, hair, eyes, etc.) (pulled out or taken from something else) from fungal cultures showed a high degree of thing that's almost the same as another thing Purified melanin from *A. fumigatus* exhibited wave numbers at 3381, 2927, 1867, 1531, 1404, 1073 and 651 cm^{-1} The waveband can be ascribed to the following chemical groups: 3381 cm^{-1} was attributed to OH bonds, 2927 cm^{-1} to H-C or H-C=O bonds 1404 (C-CH₃), and 651 cm^{-1} to (acyclic) CH₂ bonds as shown in Figure 1 and 2.

All the 41 isolates of *A. fumigatus* included in this study had the ability to produce melanin and appeared the same spectra when analysis by IR as shown in Figure 2.

Electron spin resonance (ESR) spectroscopy analysis of melanin extracted from *A. fumigatus*.

Electron spin rich sound/important quality ESR spectroscopy of the black particles collected from *A. fumigatus* conidia produced a signal a strong sign of a stable body-damaging chemical population agreeing with/matching up with/regularly working with the standard (what colors skin, hair, eyes, etc.) (Figure 1).¹⁷

Electron spin rich sound/important quality ESR spectroscopy has been used to study and define (what colors skin, hair, eyes, etc.)s based on the properties of unpaired electrons present in (what colors skin, hair, eyes, etc.) (2) by using a Gunn diode as a microwave source. A total of 2 mg freeze-dried material was used in each case (analysis was carried out in silica cuvette).

The spectrum was just like the signal created by the pulled out or taken from something else before that/before now from *Cryptococcus neoformans* and *Aspergillus niger*,¹⁵ *Paracoccidioides brasiliensis*, *Histoplasma capsulatum* (3) and *Sporothrix schenckii*,⁷ and represents the first biophysical confirmation of the production of (what colors skin, hair, eyes, etc.) by *A. fumigatus*, which (pulled out or taken from something else) from 41 (separates far from others) of *A.* (kills bugs by using poisonous gas) included in this study gave the same spectra when analysis by ESR as shown in Figure 3.

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