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Review

Melanin biosynthesis in pathogenic species of *Sporothrix*

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ABSTRACT

Melanins are dark polymers found in the cell wall of pathogenic fungi, including species from the genus *Sporothrix* that are causative agents of sporotrichosis. *In vitro* experiments strongly suggest that these pigments are important for fungal virulence and survival in the host. In *S. schenckii*, melanin biosynthesis occurs via three different common pathways, which generate dihydroxynaphthalene (DHN)-melanin, DOPA-melanin or pyomelanin. Moreover, melanin biosynthesis can be enhanced when the fungus is in contact with some bacteria, such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Melanin pigments have protective effects against antifungals in this genus. New scanning transmission electron tomography data indicates the accumulation of dark pigments in membrane-bound cytoplasmic organelles (melanosomes) in *S. schenckii* yeasts. Here, we provide an up to date of review the biosynthesis and role of melanins and discuss its roles on the cell biology and pathogenesis of *Sporothrix* spp.

1. Introduction

A wide range of organisms, from bacteria to mammals, produce dark pigments referred to as melanins (Plonka and Grabacka, 2006; Vavricka *et al.*, 2010), which provide vital protection against radiation (Plonka and Grabacka, 2006; Vavricka *et al.*, 2010; Wang and Casadevall, 1994). Melanins are polymeric aromatic organic compounds typically brown

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or black, but some melanins can have a yellow to red hue (Henson *et al.*, 1999; Liu and Nizet, 2009). Despite a common role in protecting against radiation, the different forms of melanin found in nature also play specific roles in different species.

The importance of melanin in fungi has been the subject of some reviews (Almeida-Paes *et al.*, 2012; Wang and Casadevall, 1994). Pathogenic and non-pathogenic fungi produce melanin (Henson *et al.*, 1999; Wang and Casadevall, 1994). It has been hypothesized that the pigment is important for fungal survival in the environment, since melanization confers resistance *in vitro* to a wide range of challenges commonly found in fungal habitats, including extreme temperatures, acidity, oxidative conditions, exposure to radiation or heavy metals, and even the presence of natural predators, such as amoeba (Almeida-Paes *et al.*, 2012; Nosanchuk and Casadevall, 2003a).

In pathogenic fungi, the expression of melanin is also associated with virulence (Heinekamp *et al.*, 2012; Jacobson, 2000; Nosanchuk *et al.*, 2002, 2004; Pihet *et al.*, 2009; Taborda *et al.*, 2008). Melanin-producing fungi are more resistant to the conditions imposed by the host-pathogen interaction, in parallel with their increased resistance to harsh environmental conditions (Casadevall *et al.*, 2003; Langfelder *et al.*, 2003). Melanins are cross-linked to the fungal cell wall carbohydrates, and interact with the surrounding molecules, acting as scavengers of free and oxidative radicals. As a consequence, melanins protect the fungus against chemical and physical lysis and prevent antifungal compounds and plant defensins from reaching their targets inside fungal cells (Eisenman and Casadevall, 2012; Nosanchuk and Casadevall, 2003a). Additionally, in several pathogenic fungal species, melanin pigments protect fungi from the mammalian host's innate immune responses, by inhibiting the phagocytosis and killing of fungal cells by host cells (Jacobson, 2000; Nosanchuk and Casadevall, 2003a).

Regardless of their origins, melanins result from the oxidative polymerization of phenolic and indolic compounds (Jacobson, 2000; Plonka and Grabacka 2006; Vavricka *et al.*, 2010). Biochemically, these pigments are negatively charged, hydrophobic, insoluble in organic solvents, bleachable by oxidizing agents and soluble in strong alkali (Almeida-Paes *et al.*, 2012; Vavricka *et al.*, 2010). Analyses by electron paramagnetic resonance showed that melanins contain a stable free radical and suggest that the paramagnetic center of melanins from different fungal species have a similar structure (Cunha *et al.*, 2010; Eisenman and Casadevall, 2012; Pal *et al.*, 2013; Wang *et al.*, 1996). Despite advances in high-end structural techniques such as nuclear magnetic resonance and crystallography, to date no melanin polymer has had its structural organization completely determined, due to polymer size, insolubility, amorphous nature and non-crystalline organization (Eisenman and Casadevall, 2012; Nosanchuk and Casadevall, 2003a).

In this review, it will be discussed the production and role of melanins in species from the genus *Sporothrix* that are causative agents of human and animal sporotrichosis. It will be focusing on the biosynthesis, structural role, pathogenic importance and evolutionary origins of melanin in *Sporothrix* spp. Additionally, this review includes novel data about melanin biosynthesis in the *Sporothrix* spp., represented by the visualization and 3D reconstruction of membrane-bound organelles containing melanin (and presumably involved in melanin production and storage) by scanning transmission electron tomography.

2. Melanin biosynthesis pathways in the *Sporothrix* spp.

Fungi synthesize melanin from acetyl-CoA or malonyl-CoA, via the activity of a polyketide synthase that forms 1,3,6,8-tetrahydroxynaphthalene [Fig. 1]. Then, a series of reduction and dehydration reactions result in the production of 1,8-dihydroxynaphthalene (DHN), the last intermediate in the pathway before oxidation/polymerization into melanin. This pathway is known as the DHN-melanin pathway and is commonly found in *Ascomycetous* fungi, such as *Magnaporthe grisea*, *Neurospora crassa*, *Aspergillus* spp., *Exophiala dermatitidis*, *Fonsecaea pedrosoi* and *S. schenckii* [Fig. 1].

DHN-melanin was detected in *S. schenckii* conidia and yeast cells (Morris-Jones *et al.*, 2003; Romero-Martinez *et al.*, 2000). Romero-Martinez *et al.* (2000) showed that the DHN-pathway is indeed functional in *S. schenckii*, by treating fungi with tricyclazole (TC), a specific inhibitor of reductases in the DHN-melanin pathway [Fig. 1] (Romero-Martinez *et al.*, 2000). Melanized *S. schenckii* colonies are dark-brown or black, and melanin synthesis defects were identified by colony color changes to reddish-brown or albino. A shunt product of DHN-melanin synthesis, 2-hydroxyjuglone, was detected by chromatography after TC treatment, and its accumulation might have contributed to the reddish-brown colony color in the presence of TC. In the same study, other DHN-melanin intermediates, scytalone and flaviolin, were identified in a reddish-brown mutant by chromatography, suggesting that the underlying mutation affected the DHN-melanin pathway (Romero-Martinez *et al.*, 2000). By transmission electron microscopy (TEM), melanized *S. schenckii* displayed an electron-dense layer surrounding conidia. This cell wall layer is likely to represent melanin accumulation, since it was absent in an UV-made albino mutant (Romero-Martinez *et al.*, 2000). In agreement with this hypothesis, growth of the albino mutant in the presence of scytalone led to reversal of the albino phenotype, with the appearance of the electron-dense granular cell wall layer found in the wild type. This finding suggests that the mutant was defective in a relatively early step in DHN-melanin biosynthesis [Fig. 1], consistent with the albino phenotype (Romero-Martinez *et al.*, 2000).

Recently, the production of DHN-melanin was evaluated in clinical isolates of *Sporothrix* spp. The species *S. brasiliensis* showed higher and faster expression of this insoluble compound than *S. schenckii* under different experimental conditions, and this phenomenon was associated with virulence of this species (Almeida-Paes *et al.*, 2015).

Certain fungi produce melanin from L-3,4-dihydroxyphenylalanine (L-DOPA), via a pathway that has been extensively studied in *Cryptococcus neoformans* [Fig. 1] (Eisenman *et al.*, 2007; Nosanchuk and Casadevall 2003b). DOPA-melanin is produced by the oxidation of L-DOPA, L-tyrosine, catecholamines and derivatives by laccases and tyrosinases,