Expression of melanin pigments is associated with virulence in pathogenic fungi of the Sporothrix schenckii complex by conferring resistance to antifungal drugs and to host immune system. Also, Highly melanized S. schenckii and other fungi has been reported as being more virulent against mouse models. This is a strong motivation for the study of the structural characteristics around melanin production and its role in the cell biology of pathogenic fungi.

We studied by scanning electron microscopy and STEM tomography the contribution of melanin to cell wall structure and the presence of this pigment in organelles. Analyses of the surface morphology of mycelia by scanning electron microscopy showed that heavily melanized areas of fungal colonies of S. brasiliensis have a matrix-like material covering and connecting conidia and parts of hyphae (figure 1A). On S. schenckii samples such structure was not observed (figure 1B).

STEM tomography of 300 nm thick slices of resin embed S. schenckii yeasts showed an electron-dense layer localized in the cell wall of the fungus and electron-dense content inside cytoplasmic organelles. In transmission electron microscopy studies, melanin is usually easily observed as an electron-dense material in the cell or tissue. Segmentation based on threshold levels indicated that the electron-dense layer and the electron-dense content of cytoplasmic organelles have gray values on a same specific range. We hypothesized that such electron-dense material observed in both organelles and on the cell wall is melanin, and that melanin in S. schenckii could be produced in melanosomes and exported to cell wall, as observed in other pathogenic fungi.

This study and further observation of melanized and non-melanized fungi may shed light on how melanin participates to cell wall assembly in melanized S. schenckii.

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Fig. 1: Extracellular matrix associated to conidia (arrowhead) and linking conidia and hyphae (arrow) in S. brasiliensis (A). In B, S. schenckii showed no extracellular matrix covering cell features. Bars = 1 µm.

Fig. 2: A, B and C shows slices after STEM tomography of S. schenckii and WBP reconstruction, spaced 50 nm in depth. Electron dense material is observed in organelles (arrow) and in an external layer of the cell wall (arrowhead). Segmentation shows cell membranes (D), electron dense material (Melanin, E) and their association (F), bar = 400nm.