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From adhesion to function: Multiscale imaging of *Aureobasidium pullulans* for living coating development

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Aureobasidium pullulans is an emerging candidate for engineered living coatings, yet its early adhesion and surface colonization dynamics remain insufficiently characterized, particularly across substrates relevant to material applications. Current knowledge of fungal-based coatings largely emphasizes functional performance, while the mechanistic understanding of initial attachment and spatial organization remains limited. We implemented a multimodal microscopy workflow combining digital microscopy, fluorescence imaging, and quantitative fluorescence analysis to investigate temporal colonization on plastic coverslips and pine wood. On plastic, dispersed initial attachment rapidly evolved into continuous surface coverage. Quantitative fluorescence revealed a significant increase in signal from Day 1 to Day 3 (25,328 to 42,510 RFU; $P = 5.4 \times 10^{-4}$), followed by a marked decrease at Day 6 (10,555 RFU). Microscopic observations indicate that this decline reflects reduced dye penetration within a compact, melanized extracellular matrix rather than a reduction in biomass. In contrast, colonization on wood followed fiber orientation and developed into cohesive, spatially structured communities embedded within surface irregularities. These substrate-dependent growth patterns highlight both fungal adaptive responses and methodological constraints when quantifying biomass on porous, heterogeneous materials. Overall, this study provides mechanistic insight into fungal–material interactions and establishes a reproducible analytical framework to support the rational development of fungal-based living coatings.

KEYWORDS: *Aureobasidium pullulans*; surface colonization; plastic; pine wood; living coating

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