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Adhesion and biofilm formation of *Aureobasidium pullulans* on wood analyzed by multi-modal microscopy

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Aureobasidium pullulans is a highly adaptable and polymorphic black yeast belonging to the phylum Ascomycota (Zalar et al., 2008). It is commonly found in diverse ecological niches, including soil, plant surfaces, wood, air, and even in extreme environments such as saline waters and cold climates (Andrews et al., 2002; Botić et al., 2014; Gunde-Cimerman et al., 2000; Wang and Pecoraro, 2021). Its metabolic versatility enables the production of various compounds, most notably pullulan, an extracellular polysaccharide with numerous industrial applications in the food, pharmaceutical, and biodegradable packaging industries (Prasongsuk et al., 2018). Previous studies have shown that *A. pullulans* have a broad spectrum of recombinant and polyextremotolerant genes that contribute to its remarkable resistance to environmental stress (Gostinčar et al., 2019).

To investigate the adhesion capacity of *A. pullulans* to wood, we analysed three strains isolated from a wooden façade (IN-007), dried olives (IN-515), and glacial ice with sediment (IN-517) on pine wood samples. Initial morphological assessment by light microscopy (LEICA DM 2700 M) revealed several morphological forms of strains, including yeast-like cells, pseudohyphae, hyphae, and chlamydospores. After inoculating *A. pullulans* strains onto pine wood, adhesion to the wood was assessed after washing with distilled water. Each strain was monitored for seven days at 25 °C and 75-80% relative humidity. Optical digital microscopy (Keyence VHX-6000) and fluorescence microscopy (EVOS M7000, ThermoFisher Scientific) were used following Calcofluor White staining. Results showed a clear increase in fungal density and surface coverage over time.

These findings confirm the strong adhesion and biofilm-forming ability of *A. pullulans* on wood surfaces paving the way for the development of quantitative assays using specific fluorescent dyes to evaluate fungal adhesion. Furthermore, this work provides a foundation for the development of protective biologically inspired living coatings to enhance the durability and performance of various façade materials in architectural applications.

Keywords: *Aureobasidium pullulans*, adhesion, biofilm, wood surface

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