Initial biofilm formation of *Aureobasidium pullulans*

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Aureobasidium pullulans is a black, non-pathogenic, yeast-like fungus that has been isolated from various environments. It is also important in biotechnology as it produces the extracellular polysaccharide pullulan, which is known to be part of the extracellular polymeric substance (EPS) of the biofilm of *A. pullulans*. However, the formation process and mechanisms of *A. pullulans*' biofilm production are still poorly understood. Therefore, an investigation of the initial biofilm formation of *A. pullulans* is needed. Sixty-two strains of *A. pullulans* isolated from various environments (plants, glaciers, hypersaline water, wooden facades, and indoor environments) were studied. The effects of different nutrient compositions on biofilm production were assessed using crystal violet staining. Subsequently, the alterations in cell morphology and initial biofilm formation of three morphologically distinct strains were investigated using time-lapse fluorescence imaging. The results of crystal violet staining showed differences in biofilm production among the strains. The influence of different nutrients on biofilm production was also observed. Time-lapse observation revealed changes in cell morphology during biofilm formation, which were also nutrient-dependent. These findings provide a foundation for a more in-depth investigation of the formation mechanisms and structure of the biofilm of *A. pullulans*.

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