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The Role of Filamentous Fungi in the Inter-Kingdom Complex Association of Water Biofilms

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The provision of safe drinking water (DW) is a top priority issue in any civilized society. Biofilms in drinking water distribution systems (DWDS) are responsible for several undesirable effects in the water quality. One the main drawbacks is their potential to protect pathogenic microorganisms from stress conditions. In such microcosms, microbial diversity leads to a variety of complex relationships involving interspecies and intraspecies interactions. However, most of the available information was obtained from studies with bacteria [1]. Very few reports on filamentous fungal biofilms can be found in the literature, probably because these microorganisms cannot fit completely within restrictive biofilm definitions based on bacteria [2]. In fact, the term "water biofilm" is rarely applied to filamentous fungi. However, fungi are especially adapted to growth on surfaces, as evidenced by their absorptive nutrition mode, their secretion of extracellular enzymes to digest complex molecules, and apical hyphal growth. Fungi are therefore excellent candidates for biofilm formation but this aspect is still poorly understood. Therefore, the understanding on the mechanisms underlying DW multispecies biofilm formation and behaviour is of utmost importance in order to develop more efficient control strategies. This includes the knowledge on the role of cell-surface properties, inter-kingdom interactions and chlorine effects on initial adhesion and biofilm control.

Members of the AMG group found in a recent study that *Penicillium expansum*, a filamentous fungi isolated from Braga DWDS, grows as a complex, multicellular biofilm in 48 h. Similarly to bacterial biofilms, it was possible to identify the different phases of filamentous fungi biofilm formation (induction, exponential, stationary, and sloughing off). Microscopic analysis allowed identifying several steps: the involvement of conidia on initial adhesion (4 h), germling (8 h), initial monolayer (12 h), a monolayer of intertwined hyphae (24 h), mycelial development, hyphal layering and bundling, and development of the mature biofilms ($\geq$ 48 h). It was possible to apply several quantitative methods, previously used with bacterial biofilms, to study filamentous fungal biofilms. The metabolic activity and biomass of the fungal biofilms were shown to increase over time and a correlation between metabolism, biofilm mass and hyphal development was found. Additionally, the association of *P. expansum* with *Acinetobacter calcoaceticus*, a DW-isolated bacterium, increased resistance of the inter-kingdom biofilm to chlorine.

The overall results clear proposes that it seems fundamental to gain deeper insights into the mechanisms promoting multi-species biofilm ontogenesis in DWDS, including inter-kingdom (prokaryotes and eukaryotes), and the role of fungal-bacteria association on resistance to disinfection.

References

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