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Quantification of fungal biomass in *Penicillium brevicompactum* biofilms by image analysis

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Abstract

Biofilms are microbial communities consisting of microorganisms surrounded by an extracellular polymeric matrix attached to a surface. The filamentous fungi (ff) are excellent colonizers of surfaces, forming biofilms in potable water distribution systems and are directly linked to problems of quality control and public health. The mycological culture-dependent techniques are indirect and time consuming, been of great importance the implementation and improvement of methodologies that involves characteristics such as sensitivity, selectivity, reproducibility and shorter time of analysis. The measurement of biomass in a biofilm is required for numerous activities related to the study and prediction of biofilm behavior, including, for example, making comparisons between different disinfectants or antimicrobial agents for control of biofilm.
The aim of this work is the development of a new technique for measuring fungal biomass in biofilms using image analysis.
Penicillium brevicompactum (MUM 05.17) was chosen as a fungal model as it is the most commonly ff isolated from tap water (Gonçalves *et. al.*, 2006). The biofilm formation was made with slide glasses in contact with sterilized tap water into a Petri dish under 25 ^{o}C during 10-14 days. The biofilm growth was confirmed when small dots of mycelium were observed on the surface of the slide. Calcofluor White M2R was used as staining for the visualization of fungal cells walls. After staining the microscope slides with biofilms of *P. brevicompactum* were observed under an Axioskope epifluorescent microscope (Carl Zeiss, Germany) using UV light equipped with 40x/0.30 and 10x/0.65 objectives. The images were acquired with a color camera Zeiss AxioCam HRc using the software Zeiss Axiovision to calculate the hyphal length. The others measures were made based on the value of fungal density and average diameter of 5µm as described by Gaspar *et. al.* (2001).
P. brevicompactum was able to grow in water and form biofilms on glass slides under the conditions described here. Calcofluor staining technique was easy to perform with quick fungal wall detection. The images generated were adequate to assess the measurements using the software Zeiss Axiovision. From the data generated and to validate this image analysis technique fungal dry-weight and ergosterol content are now used.

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