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Optimization of confocal microscopy for visualization and quantification of yeast biofilm

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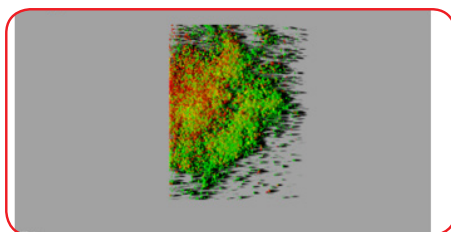
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Introduction & Aim: Due to the resistance of biofilm to conventional cleaning processes, an industrial biofilm, especially in dairy industry, could cause extensive problems in final products in case of insufficient sanitation process. Our work focuses on visualization and quantification of yeasts biofilm using confocal microscopy. Commercial dyeing kits are optimized for pathogenic bacteria; therefore, the dyeing protocol for yeasts must have been created. The yeasts were isolated as industrial contaminants from cheese and saline solution and were identified as *Yarrowia lipolytica* and *Debaryomyces hansenii* using MALDI-TOF.

Material & Methods: μ CLEAR[®] Chimney well plate was used for yeasts cultivation and the cultivation time, washing, dyeing and visualization of yeasts biofilm was optimized. LIVE/DEAD[®] BacLight[™] Bacterial Viability and Counting Kit was used for dyeing and the biofilm visualization was performed by a confocal laser scanning microscopy with a rotating disc, a ten-fold magnification and a 1 μ m z-step. For the living, dead and damaged cells, biomass volume and total biofilm structure were determined using Imaris software.

Results: The cultivation time was set to 24 hours with fresh broth well washing after 2 hours which better corresponds with real conditions. While living cells predominated in *D. hansenii* biofilm, the damaged cells prevailed in the *Y. lipolytica* biofilm, which may have been related to the different aging rates of the biofilm of both strains. The dyeing time was shortened to 60 minutes to reduce the toxic effect of propidium iodide.

Conclusion: New approaches for visualization and quantification of biofilm are needed for better understanding of the biofilm forming and persistence. We have shown that visualization of yeast biofilm is one of the ways to study these biofilms. These findings can lead to monitoring the effectiveness of sanitation solutions and to designing better cleaning processes.



Biography

Jana Smolova is a PhD candidate working under many research works related to microbiology and biotechnology. She has been working on biofilms with different methods and techniques to get the best results with the latest development of biofilms. She has interest in biotechnological experiments based on microbes.

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