Microorganisms and Biofilms

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Electron and light microscopy of yeast biofilm

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Microbes like bacteria or yeast have different ways of living; they are able to live like planktonic cells or in more dangerous form in adherence to surfaces or interfaces, where are embedded in a matrix of extracellular polymeric substances that they can produce. Biofilm allows protection for the microbial cells from attacks by the immunity system as well as from the effect of antibiotics [1]. Therefore, study of biofilms is important for clinical research. It may help to develop more efficient treatment strategies for biofilm infections. Here we investigate the extracellular matrix of yeast *Candida parapsilosis* and *Candida albicans*.

Yeast and its biofilm have been investigated by scanning electron microscopy (SEM) as well as light microscopy. For surface imaging of the samples both classical and cryo-SEM techniques were employed and compared. For structural characterization also focused ion beam SEM (FIB-SEM) and the cryo-SEM freeze-fracturing technique were applied. FIB-SEM was used for both precise cross section preparations as well as for tomographical acquisition of 3D dataset using the signal of backscattered electrons (BSE) [2]. Freeze fracture provides planar views of the internal organization of membranes or biofilms and thus gives unique structural information.

For in vitro imaging coherence-controlled holographic microscopy (CCHM) [3] is employed; the main advantage remains the quantitative phase contrast imaging for non-invasive label-free live cell. Another light microscopy technique is the two-photon fluorescent confocal microscopy that gives much higher resolution than classical confocal light microscopes. Various labelling are used and compared.

A comprehensive view of the structure of fully hydrated system of yeast cultures living in the polymer matrix is obtained due to using a number of different imaging techniques. The sample preparation is the most critical point and therefore it is important and valuable to compare different microscopic approaches with different sample handling.

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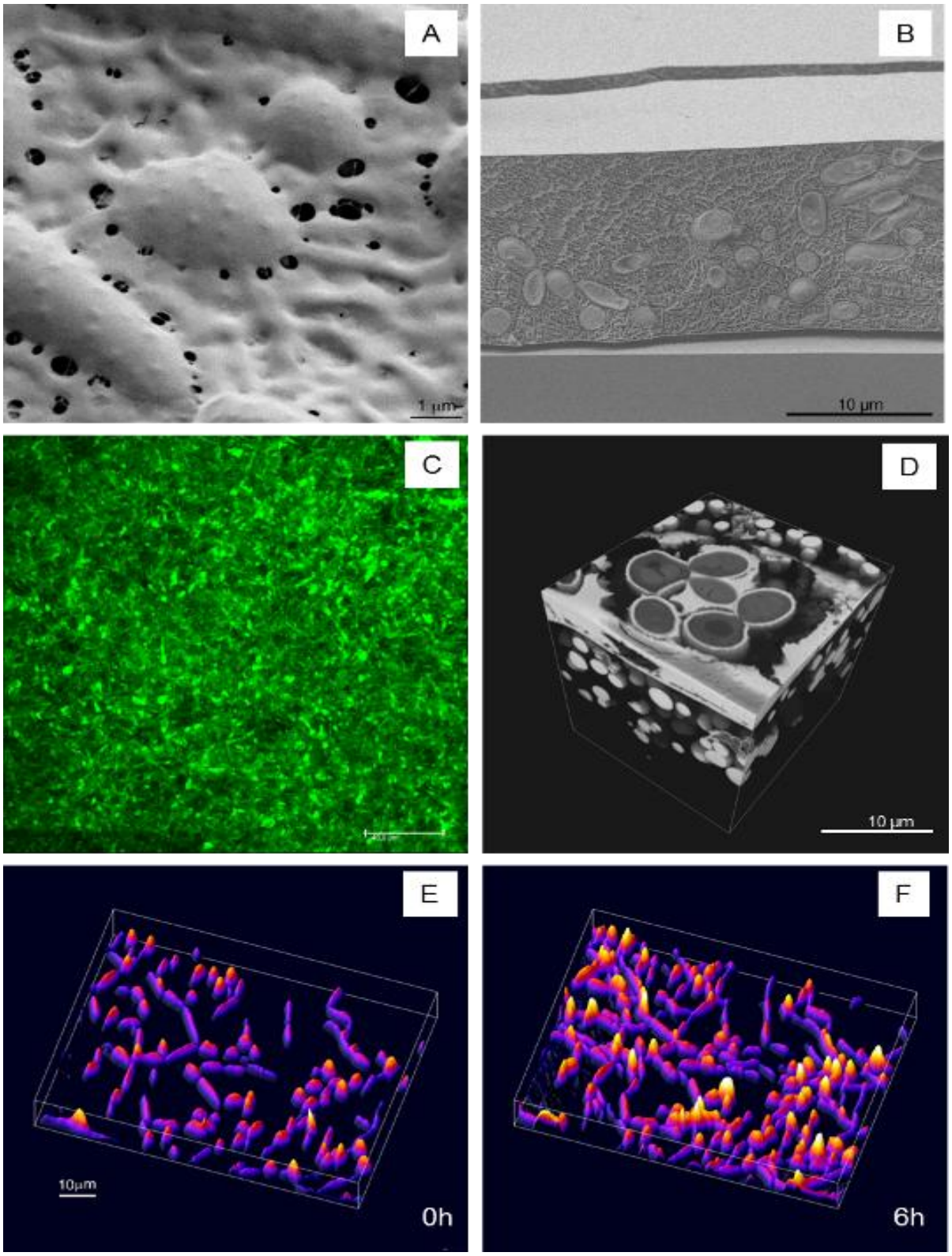

Figure 1. (A) Surface of yeast *C. parapsilosis* grown on the agar (cryo-SEM); (B) Freeze-fracturing and cryo-SEM of grown yeast culture of *C. parapsilosis* on cover glass; (C) Maximal projection of *C. parapsilosis* grown on cover glass with Calcofluor labelling; (D) FIB-SEM tomograph of *C. albicans* after freeze-substitution; (E,F) Series of quantitative phase contrast (CCHM) of growing yeast C. parapsilosis culture at times 0h and 6h.