Although still often considered as simple unicellular organisms, in natural settings yeast cells tend to organize into intricate multicellular communities. Due to specific mechanisms only feasible at the population level, their capacity for social behavior is advantageous for their survival in a harmful environment. Feral *Saccharomyces cerevisiae* strains form complex structured colonies, which display many properties typical of natural biofilms causing (among others) serious infections in the human body. In our recent paper, we looked inside a growing colony using two-photon confocal microscopy. This allowed us to elucidate its three-dimensional colony architecture and some mechanisms responsible for community protection. Moreover, we showed how particular protective mechanisms complement each other during colony development and how each of them contributes to its defense against attacks from the environment. Our findings broaden current understanding of microbial multicellularity in general and also shed new light on the enormous resistance of yeast biofilms.

Microbial multicellular communities can be found in various (even extreme) environments in the wild. Yeast cells can form diverse structures when attached to solid surfaces (e.g., biofilms, colonies), when growing at a liquid/air interface (e.g., cell films on the surface of sherry wine that are called “flors”) or when they mutually interact in a liquid environment and form cell clumps called “flocs”. Each of these structures possesses some level of internal cell organization and complexity connected with the formation of differentiated cell subpopulations and also possesses a significant resistance to environmental impacts. Pathogenic yeasts (i.e., of Candida sp.) can colonise various surfaces within the human body, including host tissues and artificial medical devices, and form biofilms that resist otherwise effective drug therapy. Biofilms are thus very difficult to eliminate and serve as a source of serious systemic infections. The questions of how yeast multicellular populations orchestrate their development and how they achieve their environmental protection are therefore also important in terms of medical care. However, as it is difficult to grow artificial biofilms in the laboratory that have properties similar to those of fully developed natural biofilms, many aspects of biofilm formation are still rather elusive.

Single cells of feral *S. cerevisiae* strains plated on solid medium retain the ability to develop into structured colonies with typical “fluffy” morphology within a couple of days of growth. As shown below, such colonies share many properties with natural biofilms, and we therefore call them biofilm colonies. The biofilm colony model enabled us to discover the spatiotemporal localization of specific cell subpopulations with different functions and determine their impact on the protection and survival of the whole colony.

After relatively few cell divisions occur and a simple mound colony is formed, particular cell subpopulations begin to diverge and play different roles. Cells at the colony base form elongated cell chains called pseudohyphae. These filamentous structures invade the agar medium, anchoring the structure to the solid substrate. Cells in peripheral layers surrounding the entire colony (including subsurface parts) are
equipped with drug-efflux pumps (Pdr5p and Snq2p) localized to the plasma membrane, the expression of which is controlled by Pdr1p together with another, as yet unidentified transcription factor (Fig. 1). These proteins that belong to the family of pleiotropic drug resistance membrane transporters are capable of removing various (including toxic) substances from the cells and protect them (and thus also the whole colony) against external attacks. It has been demonstrated that various drug-efflux pumps play a role in yeast biofilm resistance against extracellularly added toxic compounds. However, this has usually been based on the overall change in behavior of mutated strains or expression differences between biofilms and planktonic cells, without more detailed information on the transporter’s function over the course of community development. In addition to the presence of these pumps, cells at the surface layers of the aerial colony part enter the stationary phase and thus become more resistant to potential environmental stress (Fig. 1). Meanwhile, cells in internal colony areas start to produce extracellular polymeric matrix (ECM; Fig. 1) of unknown composition and thus become fully embedded in this matrix. The ECM apparently adopts the role of a protective barrier, because it blocks the penetration of even harmful compounds. ECM is one of the defining components of many yeast multicellular communities including biofilms. Despite the sequestration potential of the ECM in clinical biofilms being implied, its contribution to biofilm resistance is unclear and sometimes even doubted. As a colony develops, the area of cells embedded in ECM expands (Fig. 1); in later stages, the ECM encloses almost all colony cells. Complementarily, the layer of cells containing functional drug-efflux pumps surrounding the colony becomes thinner as the transporters are degraded (Fig. 1) and almost completely disappears in an older, fully developed colony. Only the tips of the pseudohyphae in the agar not covered with ECM still maintain functional drug efflux pumps on the membrane, thus enabling the active defense of these exposed cells.

Figure 1. Internal structure of colony of feral Saccharomyces cerevisiae strain. Thirty-six h-old (left) and 72 h-old (right) colony. Boxes in vertical colony cross-sections summarize structure and function of cell subpopulations in upper aerial and bottom subsurface colony parts; the localization of dividing, non-dividing and stationary cells is depicted, as well as cells with active drug efflux pumps Pdr5p and Snq2p. The presence of ECM is marked with black line hatching. Flo11p-dependent fibers interconnect cells in both aerial and subsurface colony parts.
Moreover, is accompanied by changes in gene expression. Cells within such a colony appear fluffy. In addition to ECM that may provide stability to the 3-D colony structure, the flexibility and undulation of the surface colony layer could be dependent on the presence of fibrous interconnections between the cells. These interconnections are observed throughout the entire colony, including subsurface filamentous cells invading the agar (Fig. 1). Cell-cell and cell-substrate adhesion is another feature typical of multicellular communities and is often ascribed as a function of cell wall adhesive proteins, including the FLO family of Saccharomyces cerevisiae. From mutants in individual FLO genes, only those lacking FLO11 are unable to develop a 3D colony architecture and they form smooth and flat colonies. Δflo11 colonies also lack intercellular connections, suggesting that Flo11p has a unique function in the formation of fibrous cell-cell interconnections in biofilm colonies. In plentiful and stable laboratory conditions, colonies no longer need the traits described above. They are therefore switched off and energy, otherwise consumed in the ECM, could be used in a more profitable way. Thus, laboratory and domesticated strains (those arising after passages of feral strains on complex media) form non-adhesive, smooth flat colonies expanding predominantly in the horizontal direction.

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