

Growth advantage associated with robust pattern formation in expanding yeast colonies

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Short Abstract — Pattern formation occurs at all levels of biological organization, from microbes to higher eukaryotes. It is often unclear whether specific patterns form robustly in response to various environments and whether such patterns confer selective advantage. The adhesin gene *FLO11* directs pattern formation in growing yeast colonies, enabling fast colony expansion on abiotic surfaces in specific environmental conditions. We observed robust *FLO11*-directed pattern formation upon various environmental conditions. Pattern formation was associated with faster colony expansion in all environments tested. Moreover, fluorescently labeled *FLO11* wild type cells out-competed their *FLO11*-mutant counterparts when mixed inoculates expanded into colonies on agar surface.

Keywords — pattern formation, *FLO11*, competition, yeast

I. INTRODUCTION

Adhesin-mediated multicellular processes could contribute to adherence of pathogenic fungi to medical devices, causing fungal drug resistance, and leading to increased infection and mortality rates^[1]. The adhesin gene *FLO11* in baker's yeast, *Saccharomyces cerevisiae*, is responsible for adherence to inert surfaces and for pattern formation during mat expansion on semi-solid agar surfaces in specific environmental conditions^[2]. There is evidence that patterned biofilm structure can mediate mass transport and deposition^[3]. However, it is unclear whether pattern formation in *S. cerevisiae* occurs in a wide variety of environmental conditions, and whether the complex structure confers a growth advantage when *FLO11* wild type and mutant cells compete during colony expansion. In the absence of a competitive advantage for either strain, mixed isogenic cell populations expand on plates in sectors of approximately constant arc-degree^[4]. Deviations from this pattern indicate a competitive advantage of one of the strains. We sought to answer these questions by computational analysis of *FLO11* wild type and mutant yeast colonies grown alone and in competition.

II. METHODS AND RESULTS

We plated haploid *S. cerevisiae* TBR1 (Σ 1278b, mata, FLO11 wt, tryp) and TBR5 (Σ 1278b, mata, Δ FLO11, tryp) cells in the center of cell culture plates with various agar and sugar concentrations. We incubated these plates at 30 °C and

imaged them with a BioRad imager daily along the time course. We measured three characteristics of growing colonies by automated image analysis using the Image Processing Toolbox in Matlab: (i) the area of the yeast colony (ii) non-circularity measured by perimeter²/area (P2A); and (iii) patterning features using conformal mapping^[5].

Both colony size and irregularity decreased monotonously as agar concentrations increased from 1.5% to 6.0%. By contrast, colony size had a non-monotone dependence on sugar concentrations, with medium sugar levels (1.0%) promoting colony expansion at the highest rate, compared to sugar levels of 0.5% or 2.0%. Importantly, *FLO11* wild type colonies expanded faster in all environments than their *FLO11* mutant counterparts. Corresponding to faster colony expansion, *FLO11*-directed pattern formation occurred robustly in response to a wide range of agar, galactose and glucose levels. Specific pattern features were sensitive to the environment. To further investigate whether *FLO11*-directed pattern formation conveyed competitive advantage during colony expansion in agar plates, we labeled *FLO11* wild type and mutant cells by expressing yEGFP and mCherry from the *pGALI* promoter, using the histidine auxotroph marker. Cultures of mixture of *FLO11* wild type and mutant labeled by either GFP or mCherry at different ratio were inoculated to the center of the agar plate. Growing colonies consisted of red and green sectors. However, contrary to results with two fluorescently labeled, but otherwise isogenic bacterial colonies^[4], we observed boundaries of Δ *FLO11* sectors curving inward relative to *FLO11* wild type sectors. This indicates competitive advantage of *FLO11* wild type cells during colony expansion when mixed with Δ *FLO11* cells. Reverse labeling further confirmed the effect.

III. CONCLUSION

FLO11-directed pattern formation occurred robustly in a wide range of agar and sugar concentrations, and conferred advantage to yeast colonies, both when expanding individually or in direct competition with their *FLO11*-mutant counterparts.

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