Video Article

Assay for Adhesion and Agar Invasion in S. cerevisiae

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Abstract

Yeasts are found in natural biofilms, where many microorganisms colonize surfaces. In artificial environments, such as surfaces of man-made objects, biofilms can reduce industrial productivity, destroy structures, and threaten human life. 1-3 On the other hand, harnessing the power of biofilms can help clean the environment and generate sustainable energy. 4-8 The ability of S. cerevisiae to colonize surfaces and participate in complex biofilms was mostly ignored until the rediscovery of the differentiation programs triggered by various signaling pathways and environmental cues in this organism. 9, 10 The continuing interest in using S. cerevisiae as a model organism to understand the interaction and convergence of signaling pathways, such as the Ras-PKA, Kss1 MAPK, and Hog1 osmolarity pathways, quickly placed S. cerevisiae in the junction of biofilm biology and signal transduction research. 11-20 To this end, differentiation of yeast cells into long, adhesive, pseudohyphal filaments became a convenient readout for the activation of signal transduction pathways upon various environmental changes. However, filamentation is a complex collection of phenotypes, which makes assaying for it as if it were a simple phenotype misleading. In the past decade, several assays were successfully adopted from bacterial biofilm studies to yeast research, such as MAT formation assays to measure colony spread on soft agar and crystal violet staining to quantitatively measure cell-surface adherence. 12, 21 However, there has been some confusion in assays developed to qualitatively assess the adhesive and invasive phenotypes of yeast in agar. Here, we present a simple and reliable method for assessing the adhesive and invasive quality of yeast strains with easy-to-understand steps to isolate the adhesion assessment from invasion assessment. Our method, adopted from previous studies, 10, 16 involves growing cells in liquid media and plating on differential nutrient conditions for growth of large spots, which we then wash with water to assess adhesion and rub cells completely off the agar surface to assess invasion into the agar. We eliminate the need for streaking cells onto agar, which affects the invasion of cells into the agar. In general, we observed that haploid strains that invade agar are always adhesive, yet not all adhesive strains can invade agar medium. Our approach can be used in conjunction with other assays to carefully dissect the differentiation steps and requirements of yeast signal transduction, differentiation, quorum sensing, and biofilm formation.

Protocol

1. Put 200ul of growing cultures of interest on synthetic media plates with the required starvation conditions (SC with 2% glucose versus SC with 0.2% glucose, for example) If the density of cultures are too different from each other, adjust cell count per 200ul culture so each drop has roughly the same amount of cells.
2. Make sure to keep records of which drop on the plates is which culture.
3. Keep the plate lid ajar and leave either at room temperature or at 30°C until drops are dry.
4. Seal plates with parafilm and plastic wrap (optional) and leave in 30°C for 3-7 days.
5. Document the **growth** of cells on plates (by scanning, taking a digital picture, etc)
6. Wash cells off of the agar surface with high pressure water (preferably DI water) for about a minute taking care for the agar not to lift and change orientation.
7. Get rid of excess water by tapping plates on paper towels and leaving them open to dry.
8. Once the plates are dry document **adhesion** on each plate (by scanning or taking digital pictures)
9. With a gloved finger, gently rub off cells form the agar surface under running water for about a minute.
10. Dry the plates as before.
11. If using a dissecting scope, remove the needle before placing plates onto the microscope platform.
12. Document **invasion** with 10x or 40x magnification. Make sure to focus on the middle part of each circle, for the edges will be too crowded to see individual cell morphologies. The edges can indicate the level of filamentous growth and can be documented for future reference. Scanning or taking a digital picture of the whole plate can also be helpful.

Discussion

Yeast cells display various differentiation modes according to nutrient availability and environmental conditions, including spore formation under starvation and stress conditions, filamentation under various nutrient stresses, and flocculation. Various yeasts, including S. cerevisiae and C. albicans, can also be found in biofilms formed by a diverse set of microorganisms. Though there is some correlation with filamentation and invasive behavior, it is not clear exactly how filamentation might cause invasion and colonization of surfaces and tissues. Yeast can certainly be found in both vegetative and filamentous forms in biofilms in nature as well as places where they threaten human health, such as catheters and infected human organs. 10-13 In order to understand the signaling pathways utilized by yeasts to infect animals and to participate in harmful and beneficial biofilms, we must develop accessible and reliable assays. Here we have developed an assay, adopted from already existing adhesion and invasion assays available for yeast, which allow us to qualitatively determine the adhesive and invasive phenotypes of yeast strains and mutants in various conditions. The assay presented here eliminates the requirement for streaking yeast cells onto the agar, where the mere action of streaking the agar surface changes the invasive and adhesive qualities of yeast. Digital imaging of especially the invading cells by a microscope allows for semi-quantitative assessment of the degree of invasion and adhesion. Such detection of invasive and adhesive cells is complimentary the single cell agar invasion assay developed by the Sprague lab 9 and can be adapted to do time course experiments.

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