HHS Public Access

Author manuscript

Cold Spring Harb Protoc. Author manuscript; available in PMC 2015 May 27.

Published in final edited form as:

Cold Spring Harb Protoc. ; 2015(2): 172–175. doi:10.1101/pdb.prot085076.

Biofilm/Mat Assays for Budding Yeast

Paul J. Cullen[1]

Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York, 14260

Abstract

Many microbial species form biofilms/mats under nutrient-limiting conditions, and fungal pathogens rely on this social behavior for virulence. In budding yeast, mat formation is dependent on the mucinlike flocculin Flo11, which promotes cell-to-cell and cell-to-substrate adhesion in mats. The biofilm/ mat assays described here allow the evaluation of the role of Flo11 in the formation of mats. Cells are grown on surfaces with different degrees of rigidity to assess their expansion and three-dimensional architecture, and the cells are also exposed to plastic surfaces to quantify their adherence. These assays are broadly applicable to studying biofilm/mat formation in microbial species.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Crystal violet (1% w/v in H_2O)

Distilled water, sterile

Yeast strains of interest

The Σ1278b background undergoes biofilm/mat formation (Gimeno et al. 1992). Commonly used laboratory strains have lost the ability to undergo biofilm/mat formation (Liu et al. 1996). A mutant defective for biofilm/mat formation (*e.g.*, flo11Δ) *should be included in the assay as a negative control.*

YEPD agar plates with varying agar concentrations (e.g., 0.3%, 2%, and 4%) <R>

Do not invert 0.3% agar plates.

[1]Correspondence: pjcullen@buffalo.edu.

Equipment

Digital camera

Flat-end toothpicks, sterile

ImageJ software (http://imagej.nih.gov; Schneider et al. 2012)

Incubator set at 30°C

Light microscope with 100× objective

Nitrocellulose filters (circular)

Plastic wrap (e.g., Saran Wrap)

Polystyrene or polypropylene plate (96-well)

Spectrophotometer

METHOD

1. Using a sterile toothpick, transfer cells of the yeast strain of interest to each of the plates listed below. Gently touch the toothpick containing cells to the center of each plate. On a separate set of plates, transfer cells of mutant strain defective for mat formation (a negative control; e.g., flo11Δ).

    - YEPD plate with 0.3% agar

        0.3% agar is optimal to observe colony spreading. On these plates, colonies can show a radial spoke pattern.

    - YEPD plate with 2% agar

        2% agar is optimal to observe mat architecture. It also allows assessment of invasive growth by the platewashing assay (see **The Plate-Washing Assay: A Simple Test for Filamentous Growth in Budding Yeast** [Cullen 2015]).

    - YEPD plate with 4% agar

        4% agar is optimal to observe colony ruffling and z-axis growth (see Fig. 1). The high surface rigidity reduces expansion in the plane of the xy-axis and promotes formation of dense architecturally complex mats that grow upward in the plane of the z-axis.

    - YEPD plate with 4% agar plate with a nitrocellulose filter placed on top

        This plate maximizes complex colony morphology.

2. Maintain the plates in a 30°C incubator in a location where vibrations are minimized. Examine mat expansion visually starting at 24 h and continuing over the course of several weeks.

    Mats can be defined by their FLO11-dependent colony architecture and degree of expansion in the x-, y-, and z-axes by visual inspection (e.g., see Fig. 1).

3. Photograph the biofilms.

   i. Measure the mat areas by photographing the plates and analyzing the photos with ImageJ software (http://imagej.nih.gov; Schneider et al. 2012).

   ii. Examine the ruffled morphology characteristic of mats by photography with a digital camera at 1× to 5× magnification.

4. To separate adherent from nonadherent cells, use an overlay adhesion assay (Reynolds et al. 2008).

   i. Place plastic wrap over the agar plate, and gently pull the plastic wrap off the plate.

      When performing this action, the mat may or may not be removed from the agar. This distinguishes cells that adhere to the plastic from cells that adhere to the agar.

   ii. Photograph both the plate and the wrap.

   iii. (Optional) Remove cells for additional analysis.

5. Quantify the degree of cell adhesion by measuring the adhesion of yeast cells to a plastic surface.

   i. At various times after mat formation has occurred, remove cells using a toothpick. Resuspend in 500 µL of H_2O.

      Always compare the cells of interest with flo11Δ *cells (a negative control).*

   ii. Adjust the optical density of the cells to A_{600} = 2.0.

   iii. Add 100 µL of the cell suspension to a 96-well polystyrene or polypropylene plate.

   iv. Incubate the cells for 4 h at 25°C to allow the cells to settle to the bottom of the wells.

   v. Add 100 µL of 1% crystal violet to each well. Incubate for 20 min at 25°C.

   vi. Wash wells five times with water and photograph adherent cells by with a digital camera or by microscopy at 10×.

      Wild-type cells will adhere to the plastic surface and will be violet in color. In contrast, flo11Δ *cells will not adhere, leaving a transparent plastic surface that is relatively free of cells.*

RELATED INFORMATION

For more discussion on the biology of biofilm/mat formation and applications of the assays described here, see Reynolds and Fink (2001), Blankenship and Mitchell (2006), and Karunanithi et al. (2012).

RECIPES

YEPD Agar Plates

Reagent	Quantity
Bacto-agar (2%)	20 g
YEPD liquid medium	1 L

Add Bacto-agar to YEPD liquid medium in a 2-L flask and autoclave. Fill sterile Petri dishes with 30–40 mL of autoclaved medium.

Yeast Extract-Peptone-Dextrose Growth Medium (YEPD)

Reagent	Quantity (for 1 L)	Final concentration (w/v)
Bacto peptone	20 g	2%
Yeast extract	10 g	1%
Dextrose	20 g	2%
H_2O	to 1 L	

Sterilize by autoclaving.

ACKNOWLEDGMENTS

The author thank T. Reynolds for engaging discussions about biofilm/mat form growth. P.J.C. is supported from a U.S. Public Health Service grant (GM098629).

REFERENCES

Blankenship JR, Mitchell AP. How to build a biofilm: A fungal perspective. Curr Opin Microbiol. 2006; 9:588–594. [PubMed: 17055772]

Cullen PJ. The plate-washing assay: A simple test for filamentous growth in budding yeast. Cold Spring Harb Protoc. 2015

Gimeno CJ, Ljungdahl PO, Styles CA, Fink GR. Unipolar cell divisions in the yeast*S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. Cell. 1992; 68:1077–1090. [PubMed: 1547504]

Karunanithi S, Joshi J, Chavel C, Birkaya B, Grell L, Cullen PJ. Regulation of mat responses by a differentiation MAPK pathway in *Saccharomyces cerevisiae*. PLoS ONE. 2012; 7:e32294. [PubMed: 22496730]

Liu H, Styles CA, Fink GR. *Saccharomyces cerevisiae* S288C has a mutation in FLO8, a gene required for filamentous growth. Genetics. 1996; 144:967–978. [PubMed: 8913742]

Reynolds TB, Fink GR. Bakers' yeast, a model for fungal biofilm formation. Science. 2001; 291:878–881. [PubMed: 11157168]

Reynolds TB, Jansen A, Peng X, Fink GR. Mat formation in *Saccharomyces cerevisiae* requires nutrient and pH gradients. Eukaryot Cell. 2008; 7:122–130. [PubMed: 17951523]

Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of image analysis. Nat Methods. 2012; 9:671–675. [PubMed: 22930834]

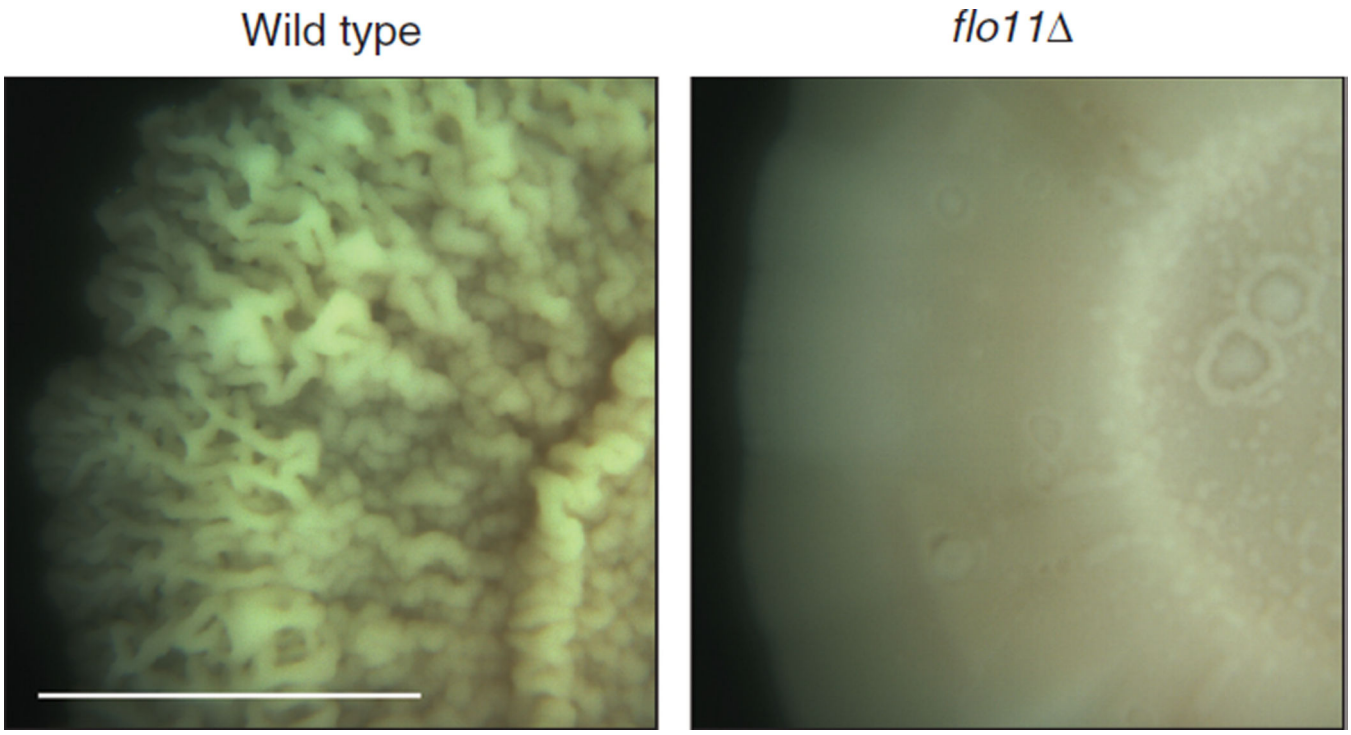

FIGURE 1.
Sample results from a biofilm/mat assay. When grown on medium containing a high agar concentration (YEPD medium containing 4% agar), wild-type cells (*left*) produce a ruffled pattern, whereas *flo11*Δ mutant cells (*right*) do not. Bar, 1 cm. Magnification, 3×.