Video Article

Cryosectioning Yeast Communities for Examining Fluorescence Patterns

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Abstract

Microbes typically live in communities. The spatial organization of cells within a community is believed to impact the survival and function of the community[1]. Optical sectioning techniques, including confocal and two-photon microscopy, have proven useful for observing spatial organization of bacterial and archaeal communities[2,3]. A combination of confocal imaging and physical sectioning of yeast colonies has revealed internal organization of cells[4]. However, direct optical sectioning using confocal or two-photon microscopy has been only able to reach a few cell layers deep into yeast colonies. This limitation is likely because of strong scattering of light from yeast cells[4].

Here, we present a method based on fixing and cryosectioning to obtain spatial distribution of fluorescent cells within *Saccharomyces cerevisiae* communities. We use methanol as the fixative agent to preserve the spatial distribution of cells. Fixed communities are infiltrated with OCT compound, frozen, and cryosectioned in a cryostat. Fluorescence imaging of the sections reveals the internal organization of fluorescent cells within the community.

Examples of yeast communities consisting of strains expressing red and green fluorescent proteins demonstrate the potentials of the cryosectioning method to reveal the spatial distribution of fluorescent cells as well as that of gene expression within yeast colonies[2,3]. Even though our focus has been on *Saccharomyces cerevisiae* communities, the same method can potentially be applied to examine other microbial communities.

Video Link

The video component of this article can be found at http://www.jove.com/video/50101/

Protocol

1. Fixing Yeast Communities

1. Grow yeast communities on a membrane filter (*e.g.*, Millipore MF membrane filter; **Figure 2A**). This protocol corresponds to a typical community of less than $2x10^8$ cells on a 6 mm-diameter membrane filter.
2. Use a piece of Whatman 541 filter paper to cover the bottom of a 1 cm-diameter well (**Figure 2B**). This Whatman paper will be used as a carrier to transfer the community in later stages. Add 1 ml 100% methanol to the well, and leave the well in -20 °C for the temperature to equilibrate.
3. Put liquid N_2 in a dewar. Freeze community in liquid nitrogen by dipping the community for at least 15 sec in liquid N_2 using a tweezer (**Figure 2C**).
4. Transfer frozen community to the well of -20 °C methanol (**Figure 2D**). Ensure that the community is kept horizontal when submerging it in the methanol. Wait 20 min. The MF membrane filter starts to disintegrate in methanol.
5. Transfer community using the carrier Whatman 541 filter paper from the methanol well to a cold Petri dish kept at -20 °C for methanol evaporation (**Figure 2E**). When transferring, keep the community on top of carrier Whatman filter and draw away extra drops of methanol using another piece of thick Whatman paper before putting in the cold Petri dish. This will ensure that evaporation time is consistent from sample to sample.
6. Wait 4-6 hr for evaporation until the carrier Whatman filter looks dry. Insufficient evaporation leaves residual methanol which interferes with sectioning. Over-drying causes cracks and deformation in communities.
7. Take the sample out of -20 °C freezer. Cut away Whatman 541 filter paper not covered by community.
8. Make a rectangular cubic aluminum-foil container which is large enough such that the sample has at least ~2 mm margin from each side (**Figure 2F**). Place the community on the bottom surface of the aluminum-foil container; immediately cover the sample with OCT to 3~4 mm above community height. If important, the orientation of the sample can be adjusted in this step with regard to the sides of the container. Wait 10 min.

9. Place the OCT-embedded sample on a metallic plate on dry ice to freeze. Store the frozen communities at -20 °C or -80 °C freezer.

2. Sectioning Frozen Communities for Fluorescence Imaging

1. Set the temperature of cryosection chamber to -25 °C and leave the samples in the chamber until its temperature reaches the temperature of the chamber.
2. Mount the sample onto a cryo-mount after applying a drop of OCT on the mount. Spread OCT uniformly on the side of sample with exposed Whatman filter paper (bottom of the container) so that OCT thickness is 2-3 mm. Let OCT freeze inside the cryostat chamber before sectioning. Ensuring an OCT margin of 2-3 mm on all sides of the sample helps to preserve the integrity of sections.
3. The sides of the OCT sample block can be used to align the sample. If the sample is not aligned with respect to the blade, sectioning will start from a corner or side of the sample. Adjust the mount to ensure that the blade cuts a perfectly vertical (or horizontal) face of the sample block.
4. Typical sections for yeast communities are ~14 µm-thick. After cutting a section, use a microscope slide kept at room temperature to collect the section, by slowly approaching the slide to the cut section. The section will melt and transfer to the microscope slide. Up to 18 sections can be transferred onto one slide.
5. Store the slides at cold temperatures, preferably -20 °C, before imaging. Loss of signal is minimal when kept for one week at 4 °C. For maximum fluorescence signal, imaging is preferably done immediately after sectioning.

Representative Results

Fluorescence images of vertical cross-sections of yeast communities containing red- and green-tagged competitive populations[6] are shown in **Figure 3**. These communities consist of two prototrophic strains of yeast and their competition for shared resources, including space, leads to columnar patterns[7], as displayed in their vertical cross-sections. Resolutions down to a single cell can be resolved in cross-sections. **Figure 3** compares cross-sections of replicate communities obtained with (bottom) and without (top) methanol-fixing, following the protocols in **Figure 1**. For cryosectioning yeast communities without fixing, the protocol is followed starting from step 1.8. The fluorescence signal is preserved in community sections without fixing, but the integrity of the community is compromised. As a result: (1) some cells float away around the edge of the section, and (2) the overall yield (*i.e.*, fraction of sections not perturbed by artifacts of the sectioning procedure) is lower compared to fixed communities.

Figure 4 shows representative bright-field and fluorescence vertical cross-sections of a yeast community. Cells on top of the community form a crown that appears distinct from other cells characterized by stronger scattering in bright-field and brighter fluorescence. These patterns are consistent with reported patterns of differentiation in yeast colonies[4].

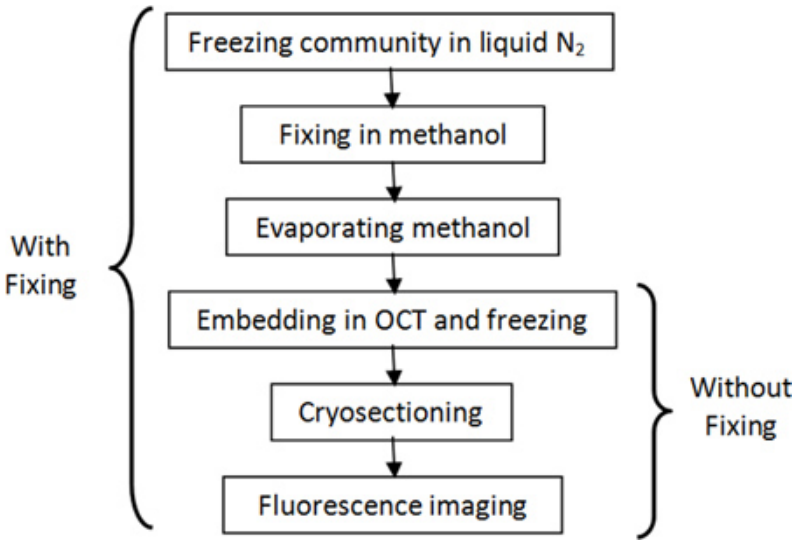

Figure 1. Flowchart of the typical procedure for cryosectioning yeast communities with (left) and without (right) fixing.

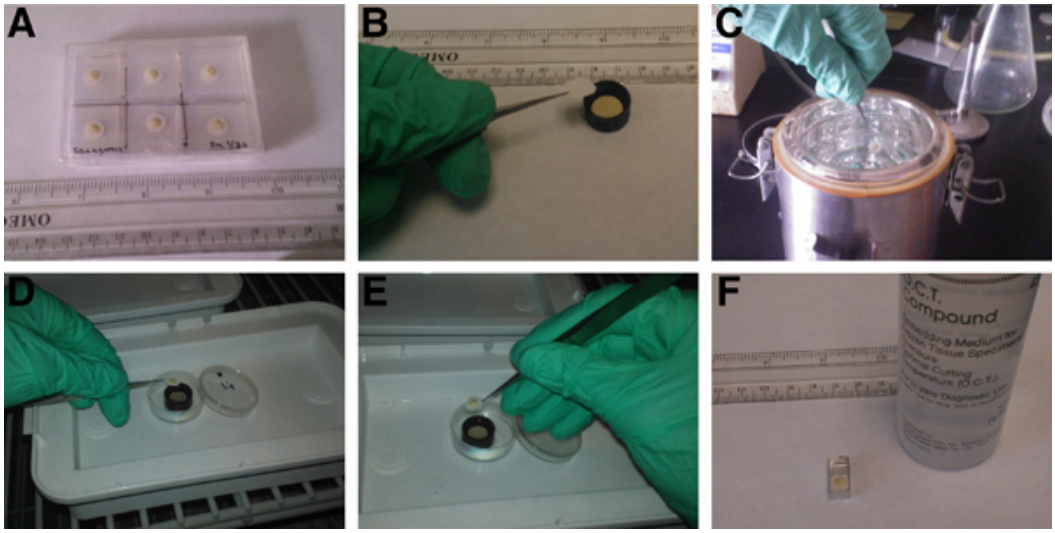

Figure 2. Steps involved in the fixing and freezing of yeast communities are illustrated. (A) Yeast communities are grown on top of 6 mm-diameter disks of MF membrane filter. (B) A piece of Whatman 541 filter paper is put in a well containing methanol to be used as the carrier. (C) Communities are frozen by dipping them in liquid nitrogen for typically ~15 sec. (D) Frozen communities are transferred to -20 °C well of methanol on top of carrier Whatman filter. (E) After fixing, the carrier Whatman filter is used to take communities out of methanol and transfer them to a cold dish for evaporation. (F) The community after evaporation is transferred to a rectangular cubic aluminum-foil container and covered with OCT for final freezing of the block.

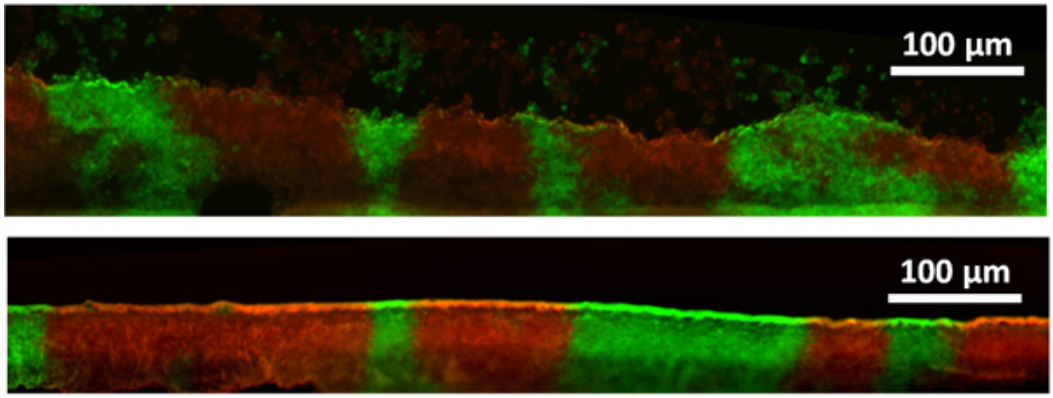

Figure 3. Representative fluorescence images of vertical cross-section of replicate yeast communities grown under identical conditions are shown without (top) and with (bottom) methanol-fixing. The community consists of two prototrophic strains of S. cerevisiae tagged with mCherry and yEGFP fluorescent proteins[7]. Without fixing, cells are not bound together and may float away or rearrange during sectioning. Methanol-fixing keeps community cells together, but causes shrinkage as observed in the difference between community heights (% average).

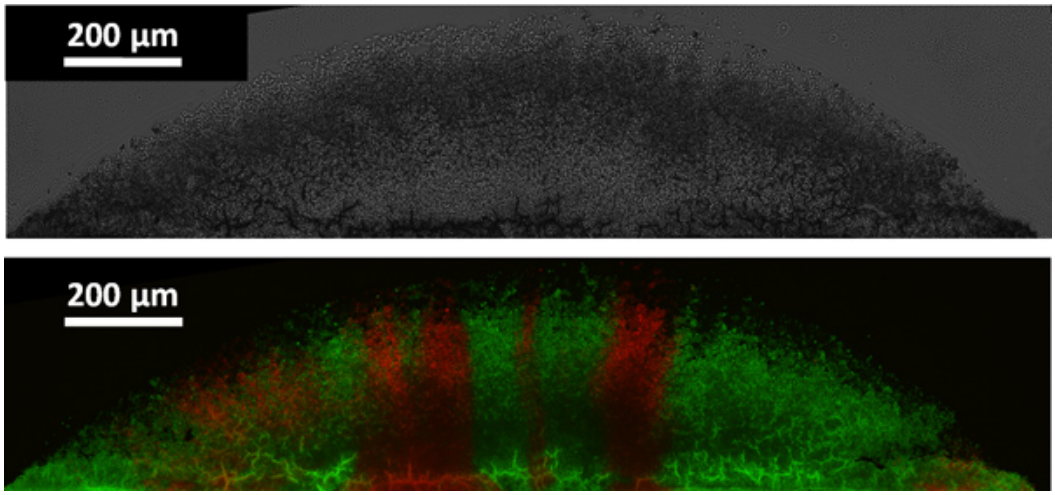

Figure 4. Representative bright-field (top) and fluorescence (bottom) images of vertical cross-section of a yeast community are shown. The community consists of two prototrophic strains of S. cerevisiae tagged with dsRed and YFP fluorescent proteins[7].

Discussion

The procedure presented here offers a way to inspect the internal structure of yeast communities. The methanol fixing step makes the yeast colony rigid[8] and preserves the integrity of the colony; however, it also causes shrinkage[8] that should be taken into account in interpreting the results. We typically see around 30% shrinkage in height of yeast colonies, compared to colonies directly embedded in OCT without fixing[9].

To avoid shrinkage, an alternative approach for cryosectioning is to directly embed the communities in OCT and freeze them. Fluorescence is preserved in the method based on direct embedding, but the drawback is that the cells within each section are not bound together. As a result, some of the cells at the edges of the section float away, and the sections are more likely to suffer disturbances in the sectioning process (**Figure 4**). Although not focused on here, bright-field images of cross-sections of yeast communities may also give us useful information about the state of cells within the community[9]. Whether or not the property of interest is affected by the fixing step has to be examined on a case-by-case basis.

Disclosures

No conflicts of interest declared.

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