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We use this protocol and it's working

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Isolation of bacteria and fungi from cheese rind microbiomes

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ABSTRACT

This protocol describes how to harvest and stock bacterial and fungal isolates from cheese rind microbial community samples.

IMAGE ATTRIBUTION

Arcadia Science

Keywords: Bacteria, bacterial, fungus, fungi, microbes, isolate, isolates, cheese, microbial community, community, contaminant, contamination, separate, single, colony, colonies

MATERIALS

- 1.5 mL sterile Eppendorf tubes
- Sterile razor blade
- Sterile 96 well plates (flat bottom)
- Multi-channel pipette
- Solution reservoir (sterile)
- 1 mL and 200 µL sterile pipette tips
- Sterile toothpicks or wooden dowels
- Sterile plastic pestles
- Sterile 2 mL cryotubes
- Sterile 1× phosphate-buffered saline + 20% glycerol
- Sterile 1× phosphate-buffered saline + 0.05% Tween
- Sterile 80% glycerol
- LB broth
- LB agar plates with 100 µg/mL cycloheximide
- PCAMS plates with 50 µg/mL chloramphenicol

Recipe for 1 L of PCAMS without antibiotic:
5 g tryptone
2.5 g yeast extract
1 g dextrose
1 g whole milk powder
10 g sodium chloride
15 g agar
965.5 mL deionized water

Harvesting the cheese rind biofilm

1 If you are starting from a glycerol stock of a previously stored rind community, proceed directly to the dilutions in step 8. If starting from an intact cheese, start at step 2.

> Note
>
> This protocol is focused on obtaining pure bacterial and fungal isolates. For a more detailed protocol on sampling and stocking cheese rind communities (for metatranscriptomics, metagenomics, long-term storage, etc.), see https://www.protocols.io/view/harvesting-and-stocking-cheese-rind-community-samp-ckf2utqe.

2 Holding the cheese in one (gloved) hand and a sterile razor blade in the other, gently scrape the surface of the cheese to remove the rind biofilm. Avoid applying too much pressure, as you will start to dig into the paste. Scrape the rind biofilm into a weigh boat or other container to clean

off the blade as needed.

Note

If the cheese surface contains nooks/crannies, use the corner of the razor blade to gently scrape the rind from these.

3 After removing the desired amount of rind, use a sterile wooden dowel or a pipette tip to homogenize the harvested rind.

Note

If not harvesting the entire rind, scraping from multiple locations may help to capture more of the microbial diversity present.

Resuspension and dilution

4 Add a lentil-sized amount of harvested cheese rind biofilm to 500 µL of sterile 1× phosphate-buffered saline (PBS) + 0.05% Tween in a 1.5 mL microcentrifuge tube.

5 Using a small sterile pestle, gently grind the cheese rind sample until it is completely resuspended in the buffer.

Equipment

Disposable Pellet Pestles NAME

Fisher Scientific BRAND

12-141-364 SKU

https://www.fishersci.com/shop/products/fisherbrand-rnase-free-disposable-pellet-pestles-2/p-4246531 LINK

6 Add another 500 µL of PBS + 0.05% Tween to the suspension.

7 Vortex thoroughly to mix the suspension.

8 Make 10-fold serial dilutions of the cheese rind suspension eight times in PBS + 0.05% Tween to create 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} dilutions.

> Note
>
> You can do these serial dilutions in a sterile 96-well plate. If you have multiple samples, you can use a multichannel pipette after the initial dilution to serially dilute all samples at once. If plating for both bacteria and fungi, you may want a minimum of 250 µL of each dilution.

Bacterial isolation

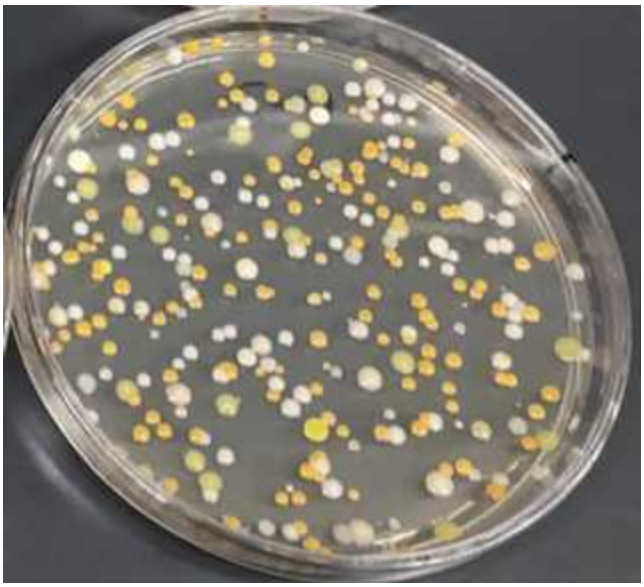
9 Use four LB + 100 µg/mL cycloheximide agar plates to plate 100 µL each of the 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} dilutions from step 8.

> Note
>
> When working with new communities, it may take some trial and error to identify the correct dilution to plate for getting single colonies. These recommendations are based on our past experience.
>
> Cycloheximide is an antifungal. It is not always effective against *Galactomyces*. If trying to remove *Galactomyces* fungi, you can add 21.6 µg/mL natamycin.

10 Leave the plates at room temperature until you can see individual bacterial colonies on at least one of the dilution plates.

Note

In our experience with cheese communities, this may take around 3–5 days. Over time, pigments will start to develop in some colonies, which will make identification of different morphologies easier. Leaving the plates exposed to light (e.g. on the bench top) can help encourage pigment production.



Bacterial colonies from a cheese rind community

11 Using a sterile toothpick or other sterile instrument, gently touch a single individual colony and quadrant-streak onto a fresh LB + 100 µg/mL cycloheximide agar plate.

12 Leave at room temperature until single colonies appear.

Note

You should store plates inside a plastic bag with a moistened paper towel to prevent them from drying out.

Using a sterile toothpick or other sterile instrument, gently touch a single pure individual colony

13 and inoculate into 2 mL LB broth.

14 Shake the culture at 200 rpm at room temperature until cloudy.

15 Add 250 µL of culture to 750 µL of sterile 80% glycerol in a labeled 2 mL cryotube, invert to mix well, and immediately store tube at −80 °C.

16 Repeat this process until all desired colonies from the original community plate have been stocked.

Note

We usually try to get at least one representative from each unique morphology, but this will depend on your downstream purpose.

Fungal isolation

17 Use four PCAMS + 50 µg/mL chloramphenicol agar plates to plate 100 µL each of the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions from step 8.

Note

When working with new communities, it may take some trial and error to identify the correct dilution to plate for getting single colonies. These recommendations are based on our past experience.

Chloramphenicol is an antibacterial drug.

18 Leave the plates at room temperature until you can see individual fungal colonies on at least one of the dilution plates.

Note

In our experience with cheese communities, this may take around 3–7 days for fungi. Morphologies may become more distinguishable over time as more color develops.

Fungal colonies from a cheese rind community

19 Using a sterile toothpick or other sterile instrument, gently touch a single individual colony and quadrant streak onto a fresh PCAMS + 50 µg/mL chloramphenicol agar plate.

20 Repeat as many times as needed onto a new plate to get a pure single colony.

21 From a pure single colony, use a sterile toothpick to pick up fungal spores and then streak the spores in horizontal lines back and forth across a new full plate. Then turn the plate 90° and streak in horizontal lines back and forth across the plate. The goal is to spread the fungus evenly over the surface of the plate.

Note

You should store plates inside a plastic bag with a moistened paper towel to prevent them from drying out.

22 Once the fungus is grown and sporulated (~5–7 days), add 1 mL of sterile PBS with 20% glycerol to the plate. Use a cell scraper to gently scrape the surface of the plate to collect the fungal spores into the liquid.

23 Use a 1 mL pipette to collect the liquid with the resuspended spores. Vortex to mix thoroughly.

24 Transfer the liquid to a labeled 2 mL cryotube and store at −80 °C.

25 Repeat this process until all desired colonies from the original community plate have been stocked.

> **Note**
>
> We usually try to get at least one representative from each unique morphology, but this will depend on your downstream purpose.