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We use this protocol and it's working

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Harvesting and stocking cheese rind community samples

In 1 collection

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ABSTRACT

This protocol describes how to harvest and stock cheese rind microbial community samples for a variety of purposes, including spatial imaging, metagenomics, metatranscriptomics, microbial isolation/culturing, and bacteriophage extraction. Processing one small cheese wheel for all of these purposes took about 45 minutes in our hands.

IMAGE ATTRIBUTION

Arcadia Science

MATERIALS

Centrifuge able to reach 4 °C
RNAprotect Bacteria Reagent **Qiagen Catalog #76506**
1.5 mL Eppendorf tubes
50 mL conical tubes
Cheese lyre/wire or other cutting utensil that will create a clean cut
Scalpel and sterile blade
Sterile razor blades
Liquid nitrogen OR dry ice–ethanol bath
1× phosphate-buffered saline with 20% glycerol
0.22 µm filter
Cryotubes

Keywords: cheese, microbiome, microbial community, harvest, store, storage, microbes, phage, bacteriophage, DNA, genomic DNA, genome, RNA, metagenomics, metatranscriptomics, scrape, rind, paste, stock, prep, preparation, prepare, spatial imaging, isolation, isolating

Equipment setup

1 Cool a centrifuge that can hold 1.5 mL tubes to 4 °C.

2 Have liquid nitrogen or a dry ice–ethanol bath ready for snap freezing samples.

Sampling for spatial imaging

3 Using a cheese lyre, knife, or other suitable cutting instrument, cut off a corner or other small section of the cheese to reveal a clean profile view of the cheese rind biofilm and cheese paste.

Note

- If desired, remember to take a photo of the intact cheese prior to any sampling.
- You can store the sections of cheese that are cut off (rind and paste) at −80 °C in 50 mL conical tubes for mass spectrometry or other future analyses.
- If it is necessary to sample non-destructively from a larger cheese wheel, you can use a cheese trier to take a small core out of the cheese.

0.5 inch-diameter core
taken from a larger cheese wheel
using a cheese trier

4 Take a scalpel and start from the top of the cheese to cut out a small section of the rind plus paste (around 0.5 in × 0.5 in), taking care not to disturb the structure of the rind. If using a cheese core, remove most of the paste and then section.

Example cheese section

5 Store the cheese sections at −80 °C for later imaging.

Note

In our experience, fresh frozen samples worked better than PFA fixing to maintain cheese rind community structure. PFA fixing of some higher-moisture cheese rinds resulted in the rind biofilm detaching from the paste and/or dissolving.

Rind scraping

6 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.

7 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.

Sampling for metatranscriptomics

8 Add ~200 mg of rind mixture to 1 mL of RNAprotect.

RNAprotect Bacteria Reagent **Qiagen Catalog #76506**

Note

200 mg of rind is about the size of a small garbanzo bean.

9 Thoroughly resuspend the rind in the RNAprotect.

10 Centrifuge at 4 °C for two minutes at 8000 rpm to pellet cells.

Note

Keep the sample cold whenever possible to avoid RNA degradation.

11 Discard the supernatant and snap freeze the sample in liquid nitrogen or a dry ice–ethanol bath.

12 Store the frozen cell pellets at −80 °C until RNA extraction.

Preparing glycerol stocks of full community

13 Prepare a solution of 1× phosphate-buffered saline with 20% glycerol. Filter sterilize with a 0.22 µm filter.

14 Add 2 mL of 1× phosphate-buffered saline with 20% glycerol to a 2 mL cryotube.

15 Add ~200 mg of rind into the 2 mL cryotube containing 2 mL of 1× phosphate-buffered saline with 20% glycerol.

16 Use a sterile wooden dowel to help break up the rind, which may be sticky, into smaller pieces.

Note

Pipetting the mixture with a cut pipette tip can also help to break up the rind.

17 Vortex for at least 20 s to homogenize the mixture.

18 To three new labeled empty cryotubes, distribute 500 µL of the mixture to create a total of four 500 µL glycerol stocks.

Note

- Splitting up the stocks into smaller aliquots can help minimize freeze-thaw cycles.
- Having more than four stock tubes does not hurt; it is always nice to have backups. Adjust to make as many as desired.

19 Store the glycerol stocks at −80 °C. These can be used later for microbial isolation.

Phage and other miscellaneous sample storage

20 If sampling the viral population, add ~200 mg of rind (or more) to a 50 mL conical tube and store at 4 °C until phage extraction.

21 Split the remainder of the harvested rind into 1.5 mL tubes and store at −80 °C for metagenomic DNA extraction and any other desired analyses.

Note

Samples frozen at −80 °C should be suitable for later Hi-C sequencing.