Harvesting and Propagating Wild Yeast for Brewing Beer

Introduction

Wild yeast fermentations are an emerging trend in brewing that can provide a unique sensory experience for the consumer compared to beers brewed with commercial yeast strains. Wild yeast allows brewers to put a local story behind their beers by using strains isolated from the nearby environment. While wild yeasts can impart fruity, spicy, or sour flavors in beers without the addition of adjuncts, the beer's sensory properties, yeast attenuation, and yeast's alcohol tolerance are unpredictable, and evaluations must be conducted to determine the flavor profiles in beers brewed with wild yeast.

Yeast can be isolated by commercial brewing operations and homebrewers alike, and to assist both users we have provided options and directions for those with different available equipment. Some of the more sophisticated equipment may be available to use at a local high school, technical school, college, or university.

To determine the feasibility of using a wild yeast strain in beer, first you must isolate a yeast strain, then observe it under a microscope to confirm it is yeast and not bacteria, and finally, brew with it. Nutrient broth and nutrient agar plates both are needed for this process, with preparation instructions below. Preparations of both materials may be commercially available.

Using a sterile laboratory environment will increase the probability of isolating and propagating wild yeast, but many of these techniques can be performed outside of a laboratory with enough sanitization and use of sterile techniques. Genetic testing is recommended to verify that the isolated organism is yeast and that it is avirulent (not capable of causing disease) to humans. Typically, yeast must be isolated on an agar plate with easily identifiable colonies, which can be achieved using the techniques below.

We can assist with identifying wild yeast isolated using these techniques and can provide support for wild yeast isolation or propagation with either an internal or external laboratory.

Materials Needed to Prepare For and Collect Wild Yeast

Equipment:

- Shaking incubator
- Biosafety cabinet (or Bunsen burners/alcohol lamps)
- Autoclave (or pressure canner)
- Stir plate (with magnetic stir bar)
- Transfer pipettor
- Balance
- pH meter
- Centrifuge
- Water bath
- Incubator
- Forceps and/or scalpel

- Permanent marker
- Centrifuge tube racks
- Refrigerator

Supplies

- distilled water
- light dry malt extract (DME)
- pelleted hops
- lactic acid
- agar
- yeast extract-peptone-dextrose (YPD) broth powder
- 50 ml (~1.7 fl oz) polypropylene centrifuge tubes
- 2 L (~0.52 gallon) borosilicate flask or canning jars with two-piece lids
- sterile inoculating loops
- sterile 100 mm (~3.9 in.) petri dishes
- 25 ml (~0.85 fl oz) pipet
- weigh boats
- 70% ethanol solution
- liquid no-rinse sanitizer

Nutrient Broth Preparation

Figure 1. Preparing a Nutrient Broth to Sterilize in a Home Pressure Canner.

1. Combine the following ingredients in a 2 L (~0.52 gallon) flask:
    1. 1000 ml (~34 fl oz) filtered water
    2. 150 g (~5.3 oz) light DME
    3. 1 g (~0.035 oz) hops
    4. 50 g (~1.8 oz) YPD powder
    5. Magnetic stir bar
2. Place the flask on a stir plate and stir until the ingredients are combined evenly (Figure 1). Note: A stir plate is not needed as long as the ingredients are combined evenly. Heating will help with the homogenization.
3. Measure the pH of the resulting solution.
4. While stirring, slowly add lactic acid to the solution to reach a pH of 4.5.
5. Place the flask in an autoclave on a liquid media cycle (121 °C/250 °F for 15 min). Note: As an alternative to an autoclave, a home pressure canner can be used to sterilize the media using process conditions of 15 PSI for 15 min (Figure 1).
6. Remove the flask from the autoclave after the cycle is run and the media is sufficiently cooled, according to the manufacturer's instructions.
7. Place flask in a water bath to cool to 25 °C (77 °F).
8. Inside a biosafety cabinet, transfer 10 ml (~0.34 fl oz) of broth to sterile, 50-ml (~1.7 fl oz) centrifuge tubes until all broth is used. You may need to agitate the stock container occasionally to distribute sediment. Tighten caps as you go. Note: As an alternative to working in a biosafety cabinet, a Bunsen burner or alcohol lamps can be used to create a sanitary environment. Airborne particles may pose a contamination risk to the media.
9. Place tubes in racks.
10. Store in refrigerator until use, for up to 1 month.

Nutrient Agar Plate Preparation

1. Combine the following ingredients in a 2 L (~0.52 gal) flask:
    1. 1000 ml (~34 fl oz) filtered water
    2. 150 g (~5.3 oz) light DME
    3. 1 g (~0.035 oz) hops
    4. 50 g (~1.8 oz) YPD powder
    5. Magnetic stir bar
2. Place the flask on a stir plate and stir until the ingredients are combined evenly. Note: A stir plate is not needed as long as the ingredients are combined evenly. Heating will help with the homogenization. Complete hydration is required for proper preparation of agar prior to sterilization, so we recommend a stir plate for this process.
3. Immerse the pH meter in the broth (according to manufacturer's instructions) to measure the pH of the resulting solution at room temperature (20–25 °C or 68–77 °F).
4. While stirring, slowly add the lactic acid to the solution to reach a pH of 4.5. If a stir plate is used, swirl the flask intermittently between additions until a stable pH reading is achieved, and then add more lactic acid if necessary.
5. Lightly cover the flask with aluminum foil.
6. Place the flask in an autoclave on a liquid media cycle (121 °C/250 °F for 15 min). Note: As an alternative to an autoclave, a home pressure canner can be used to sterilize the media using process conditions of 15 PSI for 15 min. If this process is used, standard canning jars with two-piece lids can be used to contain the liquid media.
7. Remove the flask from the autoclave.
8. Place flask in a water bath to cool to 54 °C (130 °F).

9. Inside a biosafety cabinet, pour the solution into sterile petri dishes. You may need to agitate the flask occasionally to distribute sediment. Note: As an alternative to working in a biosafety cabinet, a Bunsen burner or alcohol lamps can be used to create a sanitary environment. Before preparing plates, wipe down surfaces with a 70% ethanol solution and allow to dry.
10. Lightly cover with lids and allow petri dishes to cool and solidify. Note: If you are not using a biosafety cabinet, cover the dishes before extinguishing the flame from the Bunsen burner/alcohol lamp.
11. Store inverted in a refrigerator until use, for up to 1 month. Discard plates if any growth occurs.

Harvesting Wild Yeast

Figure 2. Collecting a Flower Sample to Evaluate for the Presence of Wild Yeast.

1. Collect a sample of your choice from outside using a sterile scalpel and forceps (if needed; Figure 2). Examples of materials to collect include tree bark, flowers, and fruits. Note: Tools may be sterilized with 70% ethanol, liquid no-rinse sanitizer, or by placing them in boiling water for 10 min.
2. Place the sample in a broth tube and seal the tube.
3. Place the tube into a shaking incubator at 30 °C (86 °F) at a moderate speed (e.g., 225

rpm) for 18–24 hr. Note: As an alternative to a shaking incubator, the tubes can be held at room temperature and swirled occasionally to promote oxygen absorption into the media. However, the yeast may take longer to grow using this method, and may need to incubate as long as 48 hr.

4. Obtain a sterile loop and perform a T-streak on a nutrient agar plate.
    1. Draw a T on the underside of the plate (not the lid) with a permanent marker.
    2. Obtain a loopful of culture from the tube with a sterile inoculating loop and streak in the top section of the plate (Figure 3, Step 1).
    3. Using a new, sterile loop, start the streak in the top section that has already been streaked, and continue the streak in the right section (Figure 3, Step 2).
    4. Repeat this process with a new, sterile loop, this time starting in the right section

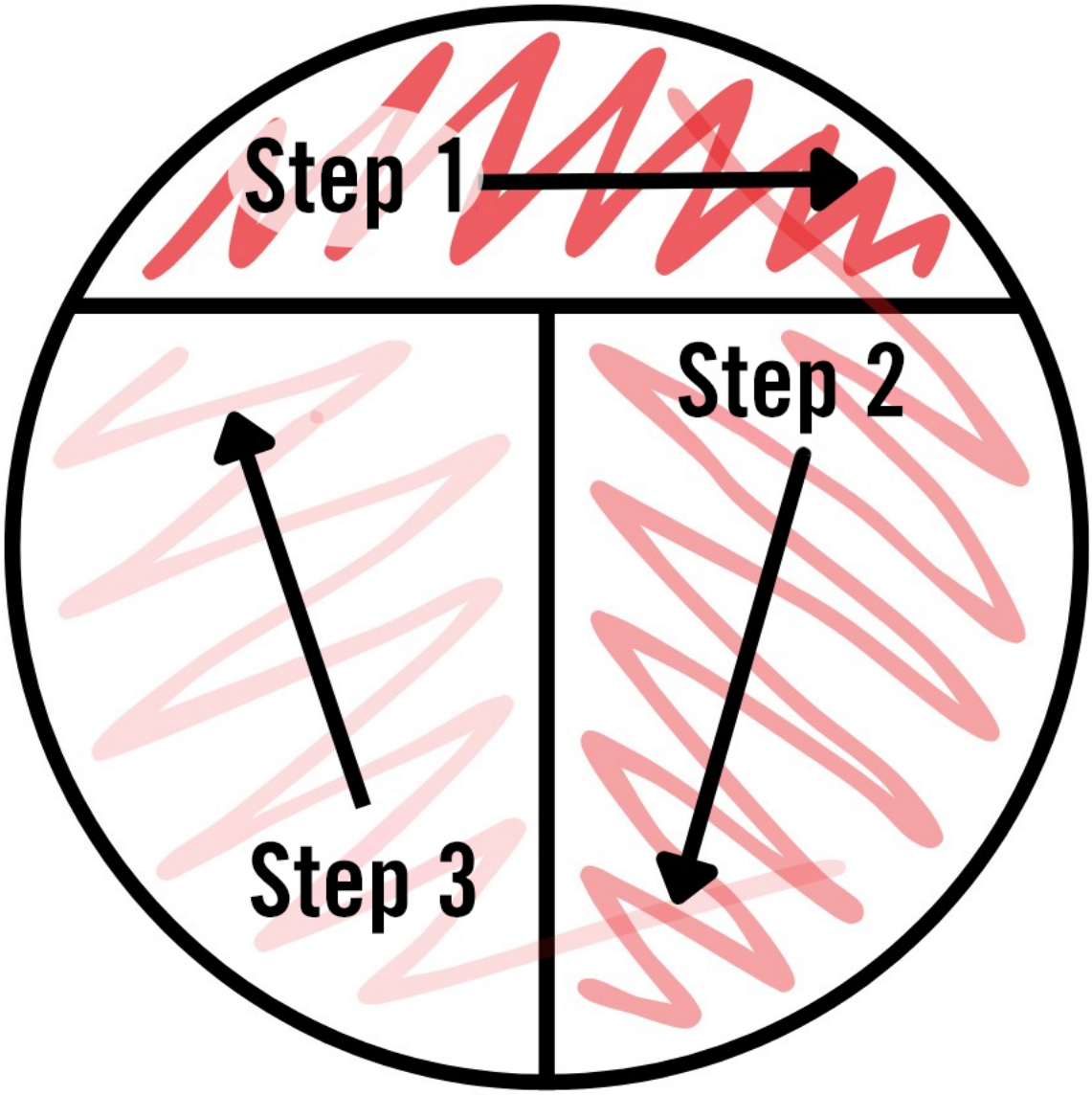

Figure 3. Example of the streaking pattern for a T-streak.

5. Place plates inverted (lid side down, agar side up) in an incubator at 30 °C (86 °F) for up to 3 days (72 hr). If no growth occurs, discard plates after 4 days. Note: As an alternative to using an incubator, the plates can be left at room temperature until colony growth is observed. If no growth is observed, discard plates after 1 week.
6. Retrieve plates and observe the morphology (form and structure) of the colony growth. There may be more than one size, shape, and color of colony present. Figure 4 illustrates some common morphologies that can be observed.

7. How do I know if I have a yeast colony?
    1. If prepared properly, the media should select for yeast cultures and inhibit bacterial growth because of the low pH and antimicrobials present in hops.
    2. Yeast colonies typically are round, creamy, and opaque. They usually are white, but also may be pink. Other organisms also may be able to grow, such as bacteria (usually appear slimy and shiny) or molds (usually appear fuzzy). We also recommend observing colonies under a microscope and sending petri dishes with individual colonies to be genetically sequenced to confirm that they are yeast. We can assist with identifying wild yeast that you may isolate.

Figure 4. Examples of Culture Growth on Nutrient Agar Plates. Shown are (A) Yeast and mold growth, (B) bacteria growth, and (C) mixed yeast growth with pink and white colonies.

1. Obtain a single colony with a sterile inoculating loop and inoculate a fresh tube of broth.
2. Place the tube into shaking incubator at 30 °C (86 °F) and 225 rpm for 18–24 hr. Note: As an alternative to a shaking incubator, the tubes can be held at room temperature and swirled occasionally to promote oxygen absorption into the media. However, the yeast may take longer to grow using this method, and may need to incubate as long as 48 hr.
3. Obtain a sterile inoculating loop and transfer a loopful of this culture into a fresh tube of broth.
4. Seal tube and place into shaking incubator at 30 °C (86 °F) and 225 rpm for 18–24 hr. Note: As an alternative to a shaking incubator, the tubes can be held at room temperature and swirled occasionally to promote oxygen absorption into the media. However, the yeast may take longer to grow using this method, and may need to incubate as long as 48 hr.
5. Observe the resulting culture for aroma.

Scaling Up

If the isolated wild yeast is producing desirable aromas during culturing, it may be appropriate to scale up. Start with a light wort base [1.050 original gravity (OG); see steps below to prepare wort] and prepare enough for brewing a small batch of beer (2 L or ~1/2 gallon). We recommend using a wort that is amber or lighter in color and with relatively low alpha acid hop additions, so the characteristics of the yeast can stand out.

Prepare the wort as if brewing a beer: Sanitize a container with an airlock, add the wort, and pour in the liquid culture of the wild yeast. Allow it to ferment at room temperature (25 °C or 77

°F) until the gravity is no longer changing. Examine the fermentation for aroma, appearance, gravity, and pH (Figure 5).

Figure 5. Sensory panel evaluating the flavor and aromas of beer produced with wild yeast.

Scale-Up Supplies Needed (in addition to the above):

- 250 ml (~8.5 oz) Erlenmeyer flasks with screw-cap lids
- kettle capable of holding 21 L (~5.5 gal) of boiling liquid
- heat source to boil kettle
- chiller and source of cold water
- hydrometer
- 2 L fermentation vessels (~1/2-gallon mason jars are suggested)
- lids fitted with airlocks for containers
- liquid no-rinse sanitizer

Yeast Scale-Up

1. Follow the steps for “Harvesting Wild Yeast.”
2. Sanitize Erlenmeyer flasks with ethanol or liquid no-rinse sanitizer.
3. Prepare nutrient broth using steps for “Nutrient Broth Preparation.” Prepare enough for 100 ml (~3.4 fl oz) per yeast strain.
4. Transfer 100 ml (~3.4 fl oz) of the nutrient broth into one Erlenmeyer flask per yeast strain that is being scaled up.
5. Inoculate nutrient broth with 10 ml (~0.34 fl oz) of the culture you prepared in the “Harvesting Wild Yeast” steps.

6. Seal the flask with an airlock or loose foil. Note: If an airlock is used, fill it with 70% ethanol or a liquid sanitizing agent.
7. Allow yeast to propagate in the starter culture for 24 hr at room temperature, shaking the flask occasionally.

Wort Preparation

Makes 19 L or ~5 gallons.

1. Add 2.5 kg (~5.5 lb) of light DME to 21 L (~5.5 gal) of water in a clean kettle.
2. Bring the kettle to a boil (Figure 6).
3. Boil for 15 min.
4. Add 28 g (~1 oz) of low alpha acid hops (e.g., Fuggles) and 100 g (~3.5 oz) YPD broth.
5. Cool to 21 °C (~77 °F).
6. Obtain a sample for measuring original gravity (~1.050).
7. Divide into even portions between sanitized fermentation containers.
8. Pitch 100 ml (~3.4 fl oz) yeast culture, using one culture for each container.
9. Close lids to containers and fill airlocks with ethanol or liquid sanitizer.

Figure 6. Homebrew Equipment to Brew Wort for Scaling Up Wild Yeast Fermentations.

Summary

The techniques described here outline the steps required to isolate and propagate wild yeast for brewing beer. Once suitable yeast strains have been identified, they can be stored on agar

plates for up to 6 months in the refrigerator by growing a liquid culture in the growth medium (instructions above) and performing a streak on a fresh agar plate. These steps should be repeated every 6 months to keep the yeast fresh and viable for brewing.

The most important considerations are to maintain a sanitary environment and to properly prepare the media to increase the chances of isolating wild yeast. We highly recommend using genetic sequencing to verify that these organisms are not harmful to humans. This additional step can also help inform the utility of the strain for brewing beer if previous research has identified it as a viable brewing strain.