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Protocol status: In development
We are still developing and optimizing this protocol

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Dough Rise Assay

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ABSTRACT

This protocol presents an accessible method for actively evaluating dough rise phenotypes in various yeast strains and yeast/bacteria combinations. As dough rise is a pivotal element in bread-making, this approach simplifies assessing the influence of genetic background on dough rise, particularly in non-food science laboratories and educational settings. The process begins by inoculating a sterile dough matrix with yeast and bacteria cultures in a graduated container. The graduations allow for accessible quantification of dough rise over 24 hours. The mixture's height is monitored over time, offering valuable insights into the dynamics of dough rise. This data allows for an active analysis of dough rise dynamics. This protocol is a valuable tool for actively assessing the leavening potential of yeast strains in a controlled and reproducible environment. It actively streamlines the selection of yeast strains that positively impact dough rise, thereby benefiting both the baking industry and sustainability goals through the potential reduction of the environmental impact of bread production.

MATERIALS

Reagents

- YPD media agar
- Synthetic sourdough medium
- ATCC Medium: 0694 Sourdough medium
- MRS media
- All-purpose flour
- Whole-wheat flour
- Distilled water

Equipment

- 15 mL centrifuge tubes with gradation
- Sterilized toothpicks or wooden dowels

PROTOCOL integer ID: 90115

Keywords: sourdough, yeast, fermentation

- 30°C incubator
- 30°C shaker
- Spectrophotometer
- Autoclave
- Centrifuge

Recipes

Yeast Peptone Dextrose Media (YPD) - 1L

- 20 g Bactopeptone
- 10 g yeast extract
- 20 g glucose
- pH 5.0 (HCl)
- 2% agar (for plates)

Synthetic sourdough medium - 1L

- 24 g wheat peptone
- 0.2 g Magnesium sulfate
- 0.05 g Manganese sulfate
- 4 g Monopotassium phosphate
- 4 g Dipotassium phosphate
- 1 mL Tween 80
- 15 g Glucose
- 35 g Maltose
- 0.2 mg Cobalamine
- 0.2 mg Nicotinamide
- 0.2 mg Folic acid
- 0.2 mg Pantothenic acid
- 0.2 mg Pyridoxal-phosphate
- 0.2 mg Thiamine
- pH 4.5 (Citric acid)

ATCC Medium: 0694 Sourdough Medium - 1L

- 20g Maltose
- 3g Yeast extract
- 15 mL Liquid yeast extract
- 0.3g Tween 80
- 6g Trypticase
- pH 5.6 (20% Lactic acid)

deMan, Rogosa, and Sharpe broth (MRS) - 1L

- 10 g Proteose Peptone No. 3
- 10 g Beef extract
- 5 g Yeast extract
- 20 g Dextrose

- 1 g Polysorbate 80
- 2 g Ammonium Citrate
- 5 g Sodium Acetate
- 0.1 g Magnesium Sulfate
- 0.05 g Manganese Sulfate
- 2 g Dipotassium Phosphate

Reducing Microbial Load by Autoclaving Flour

1 Combine unbleached All-Purpose flour and Stone Ground Whole Wheat flour in equal proportions.

2 Autoclave the flour for 20 minutes using the gravity cycle setting.

3 Ensure the container is tightly covered, and store it at room temperature while maintaining cleanliness and hygiene.

Streaking Yeast Cultures on YPD Agar Plates

4 Under sterile conditions, streak yeast to isolate single colonies onto YPD agar plates.

5 Repeat this process for all the yeast strains intended for use.

6 Incubate the plates at a temperature of 30°C for a period of two days or until visible colonies emerge.

7 Subsequently, wrap the plates with parafilm and transfer them to a storage environment at 4°C.

This storage can be maintained for up to two weeks.

Preparation of Yeast Overnight Cultures

8 Add 2 mL of synthetic sourdough medium into a culture tube

9 Utilizing a wooden skewer, select a single colony from the streaked agar plate.

10 Inoculate the sourdough media with the chosen yeast strain.

11 Place the tubes in a shaking incubator set at 30°C for an overnight incubation.

Preparation of Bacteria Overnight Cultures

12 Fill a 15 mL tube with 13 mL of the appropriate bacterial growth medium. L. Plantarum and L. Brevis grow in MRS, while L. Sanfranciscensis thrives in ATCC Medium: 0694 Sourdough Medium.

13 Inoculate the media with bacteria from a glycerol aliquot.

14 Incubate the bacteria under static conditions, allowing them to grow overnight at 30°C. It's important to note that L. Sanfranciscensis may require an extended incubation period of two days or more to reach a sufficiently high concentration for use in the assay.

Measurement of Optical Density (OD600)

15 Utilize a spectrophotometer to measure the optical density (OD600) of each yeast and bacteria overnight culture sample.

Volume Calculation for 0.1 OD per Sample

16 Calculate the volume required to achieve an OD of 0.1 for each sample. Multiply the calculated volume by the number of replicates desired.

Calculation of Water Addition

17 Calculate the amount of water needed to adjust the volume of a single culture to 500 µL.

Combining Yeast and Bacteria

18 If working with a combination of yeast and bacteria, add the volumes required to reach an OD of 0.1 for each species.

19 Calculate the remaining volume needed to reach a total volume of 500 µL.

Preparation of Master Mix for Dough Samples

20 For each individual dough sample, allocate 2 grams of autoclaved flour and 1.5 grams of sterile water.

21 Calculate the cumulative quantities needed for the entire set of samples, including an extra amount for contingencies. This total quantity ensures that all samples can be uniformly prepared.

Mixing the Dough

22 Commence by measuring the designated amount of flour into a suitable vessel, such as a bowl or beaker.

23 Since heat-treating the flour may lead to clumping and dryness, it is advisable to break up the flour particles as needed before proceeding.

24 Gradually add the prescribed amount of sterile water to the flour.

25 Thoroughly mix the flour and water until a consistent and homogenous dough is achieved, ensuring uniformity in preparing the dough samples.

Incorporating Cultures

26 Incorporate 500 µL of the previously prepared culture into each dough sample, ensuring that the cultures are evenly distributed within the dough mixture.

Preparation of the Final Mixture

27 Add 3.5 grams of the 75% hydration dough mixture into each 15 mL Falcon tube.

28 Gently vortex the culture samples briefly to ensure uniformity.

29 In each Falcon tube, add 500 µL of the prepared inoculum.

30 Employ a clean wooden skewer to thoroughly mix the inoculum and the dough within each tube, ensuring a consistent distribution.

Initial Measurement

31 Short spin the centrifuge the Falcon tubes to settle all the dough at the bottom.

32 Measure the initial heights of the mixture within the tubes.

Ongoing Height Measurements

33 At regular intervals, measure the height of the mixture within each tube using the gradations marked on the tube's side.

Record Height Changes

34 Record and document the observed changes in height over time by subtracting the initial dough height from the height measured during subsequent intervals. This data will monitor and analyze the dynamics of the mixture over the specified duration.