OBJECTIVE: a) Preparation of wet mount and observation of Bakers Yeast (*Saccharomyces cerevisiae*) under light microscope with and without staining

b) Preparation of yeast smear and oibservation under 100X objective lens

Materials:

1. Bright Field Microscope 1
2. Microscope Slide and Cover Slip 1
3. Centrifuge Tube or Small dish 1
4. Centrifuge Tube Rack or small Test Tube Rack 1
5. Methylene Blue (preferably in a dropping bottle, .5% concentration) 1 drop
6. Sample: Yeast dried

Introduction:

Sachharomyces cerevisiae are 5-10 micrometer unicellular, eukaryotic fungus cells also known as Bakers yeast. derives its name from the Latinized Greek meaning "sugar fungus" because it converts sugars and starches into alcohol and carbon dioxide during the fermentation process.

Yeasts cells divide by budding process and this can be observed using wet mount sample preparation under 40X objective lens. Yeast cells are stained with Methylene blue to indicate if the yeast cells are alive or not. Yeast cells that are alive will appear opaque because their enzymes are actively metabolizing or breaking down the methylene blue. Cells that are dead will turn blue because they are unable to metabolize the stain. In addition, the students may be able to see yeast cells on their slides that are undergoing budding- a form of asexual reproduction.

Cells can also be observed under 100X using a yeast smear that is stained with methylene blue for observation at higher magnifications.

Methodology

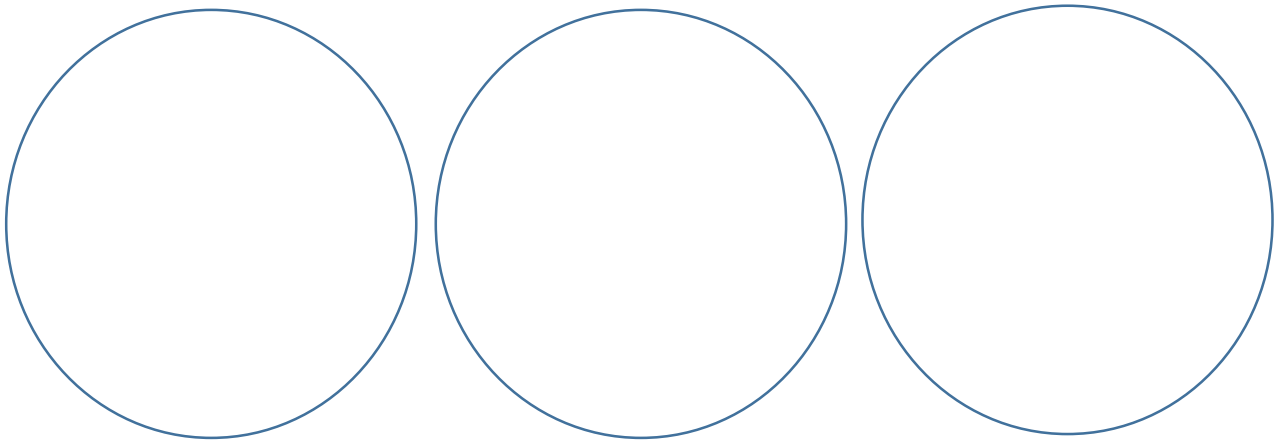
A) Sample Preparation: Weigh 0.5 g of dried yeast powder and add to luke warm water containing 2% w/v glucose. Mix well and incubate for 1-2 h at 37 degree centrigade.

B) Wet Sample Preparation :

1. Take dried yeast cells and add warm water to activate growth of the yeast cells
2. Place 1 drop or 20 micro liters of methylene blue on clean glass slide for observing stained cells. To observe unstained cells, directly place cover slip on drop of yeast by ensuring there are no air bubbles.
3. Place 1 drop of yeast cells on slide containing methylene blue
1. Cover the wet sample preparation with clean glass cover slip. A cover slip should then be placed on the sample before viewing under the microscope.
2. Observe from 4x to 10x to 40 x. Cells will be observable clearly under 40X objective lens.
3. Record observations in practical notebbook
4. C) Observation under 100X:
5. Prepare dry smear of yeast sample using inoculating needle. Red hot flame inoculating needle using spirit lamp prior to using inoculum.
6. Allow smear to air dry

   Place 1 drop of methylene blue stain for 1 minute, wash slide and allow to dry
7. Place 1 drop of immersion oil and observe under 100 x objective lens

Observation:



Wet Unstained (40X) Wet Stained (40X) Stained yeast smear(100X)

1. Observation of unstained wet preparation at 40X showed oval cells in single cells or bunches. Some of the cells could be observed with daughter cell due to budding

Observation of stained wet preparation at 40X showed oval cells in single cells or bunches some stained blue but many were unstained. Unstained cells could be observed with daughter cell due to budding

Observation of methylene blue stained cells at 100X showed magnified oval cells in single cells or bunches. Record observations in practical notebook

Interpretation

Result

Dried yeast underwent activation upon addition to water and started replication by budding. The cells were observed using wet and dried smear preparation under 40X and 100X objective lens under bright field microscope.

Precautions

1. Use clean slide and coverslips for observation of yeast samples

2. Prepare smear carefully using inoculating loop and needle

3. Before and after viewing, clean objective lens of microscope

4. Clean microscope after use properly using lens paper and turn off light source.