# Microscopical Examination of Yeast

## Introduction.

In any consideration of the Fungi, it is all too easy to overlook the Yeasts. They may not be the most spectacular of organisms but are good subjects for the microscopist to apply skills in order to see their features clearly.

Yeasts are of considerable economic importance; their activities are vital in, for example, the production of bread, wines, beers, alcohol for industrial purposes and flavourings. Life would be less colourful for many people without these commodities !

There is a wide variety of yeast forms occurring naturally, each with its own characteristics in terms of substrates utilised, temperature requirements, alcohol tolerences, etc. In this article, I shall focus upon bread-making yeast, *Saccharomyces cerevisiae*, which is typical of the group. This 'cultivated' yeast is a single-celled organism (see fig. 1) and is one of the Ascomycetes. Energy is obtained from substrates by a multi-stage biochemical process, the final stage of which can be summarised as:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \uparrow$$

(monosaccharide, e.g. glucose) (ethyl alcohol)

During this "fermentation" process, the yeast cells will grow and reproduce. There is an asexual process called "budding" in which (a) new cell(s) will grow from an existing one before separating as a new individual; in some instances, several cells will be at a stage where they have not yet separated from one another thus forming so-called "pseudohyphae". A sexual mode of reproduction involves conjugation of two cells and the formation of ascospores within what is now described as an ascus.

## Preparation of Specimens for Examination.

The aim is to see :

- dormant yeast cells and some of their sub-cellular structure.
- budding cells. (see fig. 2).
- and, possibly, pseudohyphae.

It is convenient to obtain some dried yeast. This can be stored in a cool place for long periods. It is persuaded into activity as follows.

Mix 1 tsp. dried yeast with 1 tsp granulated sugar (sucrose). Gradually stir in about 240ml (1cup) of luke-warm water. Cover and leave for several hours in a warm place (approx. 30˚C) for the fermentation to become established.

One can compare dormant and budding yeast by taking one specimen of the yeast in sugar solution at the outset and another after some lively fermentation has taken place. With luck, some pseudohyphae will be seen with the budding cells.

*Temporary* mounts are easily prepared. The material to be sampled is stirred and a drop, placed on a clean slide, is covered with a cover-slip.

For *permanent* mounts, it is necessary to spread the drop of liquid over the central area of a clean slide; a glass stirring rod used for transferring the drop is

useful here. The smear is then allowed to dry in air. Fixation of the yeast is effected by passing the slide (with the smear on the upper surface) through a flame twice; this assumes that one is using a Bunsen burner but more exposure to the flame would be needed with, say, a spirit burner, the aim being to get the slide sufficiently hot so that it can only just be tolerated when placed on the back of the hand. Finally, a drop of resin mountant is placed on the smear and covered with a cover-slip. Nb. It is best to use a 0.17 mm thick (or thinner) cover-slip to facilitate examination with an oil-immersion lens.

Improvement of Visibility.

In an aqueous medium, yeast cells show up well but they can be almost invisible when mounted in a resin; this was certainly the case with the "Practamount" I used. There is a great opportunity here for experimentation with the increasing of contrast by staining and optical techniques.

Various stains produce nice effects. With temporary mounts, in particular, a small drop of Tincture of iodine, diluted 50:50 with water and mixed in with an equal amount of the specimen material, is quite effective.

Many stains are effective for permanent mounts. Methylene blue is a good example; a few drops of a 0.5% aqueous solution are placed on an air-dried and 'flamed' smear and then carefully rinsed off with tricking water; mounting in resin follows once the stained smear has thoroughly dried in air.

For a long time, I have hesitated about trying the well-known Gram staining method which is used extensively by Bacteriologists; however, I have now discovered that this is a straightforward technique to apply and my slight modification of the standard method gave pleasing results with yeast. Living cells are Gram positive and stain deep blue but dead cells are Gram negative and show red staining.

A Modified Gram Staining Method for Yeast Cells.

The stains required are: Crystal (or Gentian*) Violet, 0.5% aq.

Tincture of Iodine: Iodine, 1g.
Potassium iodide, 2g.
Water, 300ml

Safranin, 1% (in Cellosolve)

*Same chemical but Gentian Violet contains more impurities.

1. Stir the yeast culture, place a drop on a clean slide and make a smear.
2. Air dry the smear.
3. Fix by 'flaming'.
4. Cover the smear with Crystal (or Gentian) Violet. (1 min.).
5. Rinse by gently running water across the smear over a sink.
6. Cover the smear with Tincture of iodine. (1 min.).
7. Rinse again with water.
8. Apply 95% alcohol over the smear. (approx. 10 sec.). Nb. This would decolourise Gram negative specimens.
9. Rinse again with water.

10. Cover the smear with Safranin (30 – 60 sec.). Nb. This re-stains Gram negative material but Gram positive specimens stay a deep blue colour.
11. Rinse again with water.
12. Air dry.
13. Mount in resin.

Finding unstained cells mounted in resin can be difficult. Closing the field diaphragm, whilst it will reduce resolution of detail, will improve contrast dramatically. Another tip is to make a small mark on the dried smear with a glass-marking pencil; this needs to be where it will be covered by the coverslip so that it will be a feature of the permanent slide upon which one can focus. Phase contrast and dark-ground illumination produced excellent results for me with unstained yeast cells in resin.

With all slides of yeast cells, working through to a x40 (N.A. approx. 0.65) objective reveals the main features that one would wish to see. However, there is an opportunity here to use a x100 (N.A. 1.0+) objective to good advantage.

References

Collins, C.H., 1967. Microbiological Methods. Butterworths, London.
Giltner, W., 1928. General Microbiology. Churchill, London.
Gray, Peter, 1954. The Microtomist's Formulary and Guide. Constable, London.
Levine, S. & Johnstone, L., 1953. The Microscope Book. Sterling, New York.
Peacock, H.A., 1940. Elementary Microtechnique. Arnold, London.
http://www.super-science-fair-projects.com

Acknowledgement.
Brian Wilkinson has been very supportive. I thank him sincerely for his suggestions and helpful advice in connection with the preparation of this article.

Lewis Woolnough