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Technical Information Sheet No. 2

A quick method for estimating the percentage of viable cells in a yeast population, using methylene blue staining

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Introduction

It is sometimes necessary to obtain a quick estimate of the percentage of viable cells in a yeast sample. In traditional plate count methods, results are not available until 3 or 4 days after inoculation. A quick staining method can provide an estimate of viability in a few minutes.

Principle

Yeast cells that are viable contain an enzyme that decolourises methylene blue, whereas dead cells do not. When cells from a yeast sample are suspended in the dye, it penetrates into all the cells, but is reduced only by the living cells. It is very simple, therefore, to distinguish between living and dead cells by examining them microscopically: dead cells are stained blue and living cells are unstained.

Procedure

Dissolve 0.01 g methylene blue in 10 ml distilled water. Add 2 g sodium citrate dihydrate and stir until dissolved. Filter through filter paper, making the volume up to 100 ml with distilled water.

Mix equal volumes of yeast sample and methylene blue solution on a microscope slide. (It may be convenient to mix equal quantities of each, using a wire inoculation loop.) The cell concentration should be adjusted so that about 50 yeast cells are present in a microscope field, using a 40 × objective and 10 × or 12.5 × eyepieces.

Approximately 1000 cells should be examined and the percentage of unstained cells of the total recorded. This figure represents the percentage viability of the sample. When counting cells, buds that are less in size than half the parent cell are ignored.

Note. It should be remembered that this method measures the presence of an enzyme in a cell, rather than the ability of the cell to divide. It is possible that the enzyme is present in cells incapable of dividing and the method is thus less accurate than others, such as plate count and slide count methods, which measure the ability of cells to produce daughter cells. It nevertheless provides a very useful rapid indication of the viability of yeast samples.