Industrial microbiology

Laboratory exercises 2016/2017

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Exercise: **Determination of growth inhibition of yeast *Saccharomyces cerevisiae***

1) Theoretical background

a) Saccharomyces cerevisiae

Saccharomyces cerevisiae belongs to the two main phyla of the Fungi kingdom, *Ascomycota* and *Basidiomycota*. *S. cerevisiae* cells (Figure 1) are round spherical forms, in the range of 5 to 12 μm, having a cellular core and cytoplasmic organelles such as mitochondria, ribosomes, and endoplasmic reticulum.

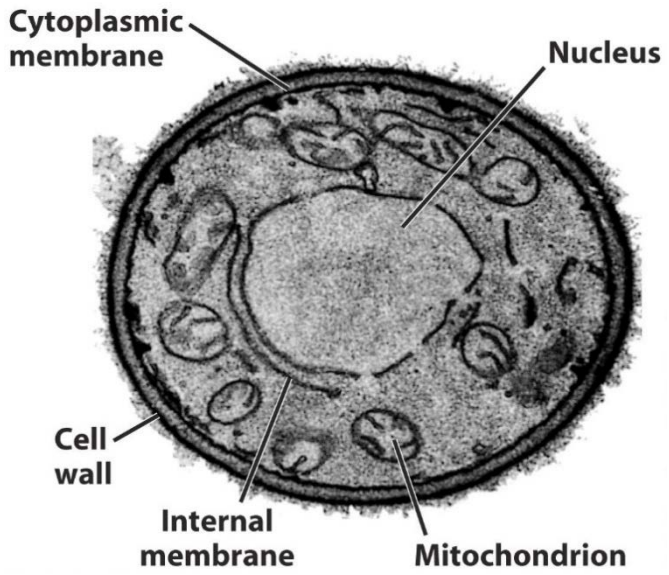

Figure 1: Cross-section of the *S. cerevisiae* cell

Selected strains are used in the alcohol fermentation of wine and beer and in the production of fermented foods (bakers' yeast). The importance of yeast in fermentation was explained for the first time by Louis Pasteur, a French chemist and bacteriologist. *S. cerevisiae* is able to grow at pH 1.6. In the presence of oxygen, sugars can be oxidized with the mitochondrial respiratory system. They are able to convert sugar into ethanol and CO_2.

b) Write the definition of inhibition and list the most common inhibitors that inhibit the growth of microorganisms:

c) Inoculation of *S. cerevisiae*

Inoculation is performed in a sterile environment with an aseptic technique. All microbiological examinations, from sampling to the final identification of microbes, must be performed aseptically. Therefore, we work with the infectious samples or cultures of microorganisms as follows; 1. disable access to unwanted micro-organisms that would contaminate our cultures and interfere with the experimental results; 2. prevent the spread of micro-organisms, because infection of ourselves or others could occur.

- For aseptic work, it is essential that glassware, accessories for microbiological applications, equipment and microbial culture media are sterile. Only by using an aseptic technique will the results of the tests be correct, so it is important that we work near the flame of the gas burner, the inoculation loop and the glassware during the work and that the working surface is regularly cleaned with disinfectants.
- Even with careful consideration of aseptic work rules, it is possible to contaminate the environment or the culture.

Microbiology is divided into different areas such as clinical microbiology, with identification of pathogens, and sanitary microbiology with water and food control. Depending on the working areas, different techniques are used in different microbiological laboratories.

2) Protocol

Preparation of the *S. cerevisiae* liquid medium*:*

5 g meat peptone

3 g yeast extract

3 g malt extract

10 g D-(+) glucose

1 L H_2O

Transfer the culture of *S. cerevisiae* with the inoculation loop to the liquid medium, using aseptic technique.

Prepare 5 Erlenmeyer flasks with *S. cerevisiae* liquid medium containing:

Erlenmeyer flask 1	/ (control)
Erlenmeyer flask 2 and 3	*S. cerevisiae*
Erlenmeyer flask 4 and 5	*S. cerevisiae* + 5 mL of inhibitor

Selected inhibitors: **ethanol and acetic acid**

Incubate the inoculated microorganism in the liquid medium at 30°C with constant shaking at 400 rpm.

Pipette 1 mL of the sample from each Erlenmeyer flask at measured time intervals (t = 0, 15, 30, 45, 60, 90, 120, 150, 180, 240 min) to measure their absorbance on the UV-Vis spectrophotometer at a wavelength of 600 nm. Write down the absorbance in the table and calculate the degree of inhibition according to the equation:

$$Inhibition\ (\%) = \frac{\left(abs_{average}\ S.cerevisiae - \ abs_{average} inhibitor\right)}{abs\ _{average}\ S.cerevisiae} * 100$$

3) Results and discussion

Present results in a table and write observations.

Draw diagram: inhibition of yeast growth (%) vs. time.

References

Xu, X., Lambrecht, A.D., Xiao, W. (2014). Yeast Survival and Growth Assays. In: Xiao W. (eds) Yeast Protocols. Methods in Molecular Biology (Methods and Protocols), vol 1163. Humana Press, New York.

Feldmann H. (2012). Yeast: Molecular and Cell Biology, 2nd Edition, Wiley-VCH Verlag GmbH & Co. KGaA.