ISSN 0316-4357

THE GENETICS SOCIETY OF CANADA

BULLETIN

LA SOCIÉTÉ DE GÉNÉTIQUE DU CANADA

Volume 35, Number 4, December 2004 Volume 35, Numero 4, Decembre, 2004

http://life.biology.mcmaster.ca/GSC/

"Mendel crossed peas with enthusiasm"

Hi, I'm Matt Young, a Ph.D. student in Microbiology at the University of Manitoba. I am currently studying the role of the carboxyl terminal extension of the mitochondrial DNA polymerase (Mip1p) in mitochondrial DNA replication/maintenance in baker's yeast (*Saccharomyces cerevisiae*). Aside from the obvious great uses of yeast (e.g. wine, beer, and bread), its fermentative power makes it is an ideal organism for studies of mitochondrial DNA replication as it survives without mtDNA when using this metabolic mode. This allows me to analyze mutations that reduce respiratory competence or damage mitochondrial DNA; these studies would not be possible in obligately aerobic organisms.

Quick Measurement of Glucose Concentration in *Saccharomyces cerevisiae* Cultures.

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Studies of the facultative anaerobic ascomycete, *Saccharomyces cerevisiae,* typically require cultivation on or in glucose-containing media (usually 2% YPD, 1% yeast extract, 2% peptone, and 2% dextrose/glucose). In our lab, we study the effect of mutations on respiratory metabolism. If yeast cells run out of glucose, a shift from fermentative metabolism to aerobic metabolism occurs whereby cells survive by consuming nonfermentable carbon sources such as ethanol, glycerol, and other by products of glucose fermentation [(1)]. Physiologically, this diauxic shift begins selecting for cells which only respire making it difficult to assess any mutation's effect on respiration. Therefore, monitoring the concentration of glucose in yeast cultures is vital for observing true effects of mutations on respiration, i.e. loss or decrease of respiratory competence. When the concentration of glucose falls below 0.2%, cells begin the shift to respiratory metabolism [(1)]; therefore, in our experiments, this threshold cannot be reached.

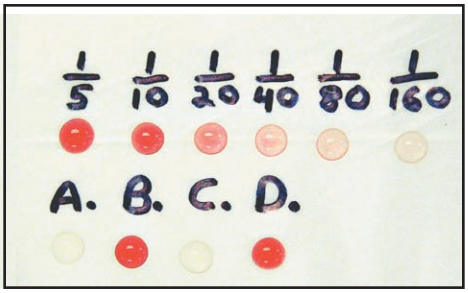

Fig. 1. *Quantitation of Glucose in S. cerevisiae cultures grown in 10% YPD broth. One hundred microlitre drops of Trinder reagent (0.5 mM 4-Aminoantipyrine, 20 mM p-hydroxybenzene sulfonate, 15,000 U/L glucose oxidase from Aspergillus niger, 10,000 U/L horseradish peroxidase, pH 7) were spotted for each standard and control onto PARAFILM®. Next, 1 µl drops of either (i) glucose standards or (ii) supernatants obtained from yeast cultures were mixed by pipetting up and down with the Trinder drops. The reaction was allowed to incubate at room temperature for 10 minutes and then analyzed visually. A, B, C, and D are supernatants obtained from different isolated cultures of Saccharomyces cerevisiae grown overnight at 30°C. A and C cultures were grown for 22.5 hours whereas B and D cultures were grown for 17 hours. Glucose standards were made by diluting 10% YPD broth in water at the indicated dilutions in (i).*

Our 'Quick measurement of glucose concentration' method is a variant of an older SIGMA-DIAGNOSTICS® (currently Sigma-Aldrich) method [(2)]. It is based on the principle that glucose can be oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. In the presence of peroxidase, hydrogen peroxide can react with 4-aminoantipyrine and p-hydroxybenzene sulfonate to form a dye (quinoneimine) which is a brilliant red colour. The intensity of this colour is proportional to the glucose present (2).

The critical threshold for glucose in Fig. 1 **(i)** is the 1/40 dilution representing 0.25% glucose concentration in the media. Therefore, when studying mutations affecting respiration,

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we do not want our cultures to slip under this mark. As seen in Fig 1 **(ii), B** and **D** cultures would still be adequate for further experimentation whereas **A** and **C** would already be in a state which selects for respiring cells. In comparison, if one was to carry out the same experiment spectrophotometrically at 505 nm according to the SIGMA-DIAGNOSTICS® manual [(2)] and calculate the concentration in each sample, the results would indicate that **A** contains 0% glucose, **B** 2.4%, **C** 0%, and **D** 2.5%. These results are in agreement with "eyeballing" the PARAFILM® as shown above (Fig. 1) but this measurement would take significantly longer to obtain.

In summary, methods of glucose determination can be tedious, time consuming, and require additional materials not provided by the manufacturer (e.g. a spectrophotometer and cuvets). In comparison, the quick measurement of glucose concentration in *S. cerevisiae* cultures method requires only the Trinder reagent, a pipette, a marker, and some PARAFILM®.

Acknowledgements

This work was supported by an NSERC (the Natural Sciences and Engineering Research Council) to Deborah A. Court and a UMGF (University of Manitoba Graduate Fellowship) to Matthew J. Young.

1. Maris, F.M., A.L.K. Assumpcao, D. Bonatto, M. Brendel, and J.A.P. Henriques (2001) Diauxic shift-induced stress resistance against hydroperoxides in Saccharomyces cerevisiae is not an adaptive stress response and does not depend on functional mitochondria. Curr. Genet. 39(3): 137-149.
2. Sigma Diagnostics (1990). Glucose (Trinder) Manual for Quantitative, Enzymatic Determination of Glucose in Serum or Plasma at 505 nm (Procedure No. 315).

REPORT ON DISCUSSIONS OF RELATIONSHIP WITH THE CANADIAN FEDERATION OF BIOLOGICAL SOCIETIES

A report from the ad hoc committee, as presented verbally at the Annual General Meeting, Toronto, June 2004. Presenter: John Bell

Background: The President asked John Bell and Art Hilliker to investigate the relationship with CFBS at the Annual Meeting in Halifax and report at the following Annual Meeting in Toronto. Conversations were held with Bruce Sells (Executive Director of the CFBS) and various CFBS briefs and documents were examined. The committee "met" several times by e-mail, phone and in person.
Summary: The relationship with CFBS whereby the GSC collects dues on behalf of CFBS, although seemingly revenue-neutral, is not truly so. There are expenses billed to the GSC for the collection of CFBS dues, and the committee suggested that the Executive might want to consider ways to recover these costs from the members or from CFBS itself. Of greater concern, however, was a feeling by the ad hoc committee that more members might be recruited if there was not the added expense of the CFBS dues, or that our fees could be raised slightly if the CFBS expense was not an impediment. Apart from withdrawing from CFBS, there might be 'partial options'. One such option would be for GSC to withdraw and individual members could still join CFBS via the Life Science Committee. These fees might be collected by the GSC as a courtesy to our members who elected to join CFBS via this venue. As well, CFBS might be open to discussions of alternative arrangements. The committee did feel that withdrawal from the CFBS should involve a referendum, since the membership of GSC voted to join CFBS initially via a referendum. John Bell then moved that the President write a letter formally requesting withdrawal from CFBS. Those attending the Annual Meeting supported this motion.

Editor's note: See the September, 2004, issue of the Bulletin for the CFBS stance.