How to Perturb Yeast: A Series of Experiments Investigating Yeast Growth and Protein Composition

Pamela L. Connerly

Natural Science and Mathematics Division, Indiana University East,
2325 Chester Blvd., Richmond, IN 47374-1289
Phone: 765-973-8609

pconnerl@indiana.edu

Abstract: As part of an upper level Cell Biology course, a three-week series of laboratory exercises was conducted investigating the growth and protein composition of yeast. Five teams of two to four students each first conducted a 10-hour growth curve on a culture of *Saccharomyces cerevisiae* strain S288C, then prepared crude protein extracts and assayed them using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, students were asked to design an experimental condition they believed would change the growth and/or protein composition of the yeast. Conditions tested included heat shock, nutrient limitation, temperature change, and UV treatment. The growth and protein composition assays were repeated after the student-determined treatment. This activity allowed each team of students to take control of the experimental aspect of the project, although each group utilized the same laboratory procedures.

Introduction

The laboratory component of an upper-level Cell Biology course serves many purposes including reinforcing the lecture material, giving students exposure to and experience with modern laboratory equipment and techniques, developing students' critical thinking, group work and writing skills. I generally strive to present students with a variety of laboratory exercises, some more contained (completed within a single lab period), and some which stretch over multiple weeks and incorporate multiple concepts. For the series of laboratory experiments described in this poster, my goals were (1) to give the students an introduction to the use of baker's yeast, *Saccharomyces cerevisiae*, as a model organism, (2) to engage students in a project in which they personally contributed to the design, (3) to introduce the concept of growth curves, (4) to introduce students to protein analysis using SDS-PAGE, and (5) to encourage students to synthesize data from multiple experiments to reach a common conclusion.

S. cerevisiae is a very common and easy-to-use model eukaryote. I attempted to emphasize throughout the lecture portion of the course that much of what we know about the function of the cell comes from work using *S. cerevisiae* and other model organisms. Working hands-on in the lab with this organism should give students a more tangible understanding of those experiments. I find that students

become more invested in their work when they have contributed to the design of an experiment. However, due to time and resource limitation, one must generally ensure that all students are performing basically the same experiments. This series of experiments allowed students to make decisions about how to perturb the growth of yeast, while all groups completed the same two analyses to measure effects on the yeast – growth curves and SDS-PAGE of crude cell lysates. Growth curves are a typically method used to measure the growth rate and general health of a many strains of microorganisms. With a doubling time of approximately 90 minutes, a 10 hour growth curve was sufficient to get an estimate of the log phase growth rate of the culture under various conditions. SDS-PAGE allows separation of proteins by molecular weight such that protein profiles of yeast cells maintained in different conditions can be compared. Finally, students were asked to write a formal laboratory report in the style of a journal article including Introduction, Methods, Results, Discussion and Reference sections. Reports were written individually, although groups did work together on data analysis.

Procedure

The lab exercises were conducted over a three-week period, and students were required to commit time outside of lab as well. The activities used for each lab period are briefly described below.

Lab Period One

The process of using spectrophotometric analysis of optical density at 600 nm (OD_{600}) as a measure of cell concentration was introduced. Students prepared a standard curve comparing OD_{600} with measured cell concentration, as determined by visual counts made using a hemocytometer and microscope. From this analysis students were able to define a ratio relating concentration in cells/mL to OD_{600} units. Different groups arrived at slightly different values fairly similar to those reported in the literature. The process for measuring yeast culture growth by OD_{600} over a 10 hour period was discussed and students were asked to schedule time outside of class for their measurements. At each timepoint, students collected a 1 mL aliquot of the growing culture and moved it to 4°C. At the end of the 10 hour growth period students measured OD_{600} of all collected samples and additionally collected 1 mL of the final culture in a microcentrifuge tube for protein analysis. The cells were spun down in a microcentrifuge, the supernatant was removed, and the cell pellet was frozen at -20°C for later analysis.

Lab Period Two

Students prepared crude cell lysates from the frozen yeast pellet by resuspending the frozen pellet in Laemmli Sample Buffer, and heating the sample at 95°C for 10 minutes using a heat block. The cell suspension was centrifuged and the supernatant was transferred to a fresh ultracentrifuge tube to serve as a source of sample to be loaded onto SDS-PAGE gels. As SDS-PAGE gels were prepared and run, students designed treatments they thought would change the growth and/or protein expression pattern of the yeast. Student ideas included UV treatment, heat shock, nutrient changes, and temperature changes. Students were required to develop a protocol to conduct their alteration from the original growth conditions. Students again collected OD_{600} growth data over a 10-hour period and froze an aliquot of cells at the end of the growth. Depending on the complexity of the experimental design, most students additionally re-ran the original growth conditions as a control. Experiments and growth curves were again conducted outside of class time.

Lab Period Three

Students prepared crude cell lysates from their second frozen yeast pellet and ran another round of SDS-PAGE. Gels were collected, stained with Coomassie blue, and dried between layers of cellophane by the instructor. Dried Coomassie blue stained gels were later returned to the students. Further class time was devoted to data analysis.

Student Designed Experiments

Students chose a variety of topics to examine during their second round of growth curves and SDS-PAGE analysis of protein composition. One group examined the effects of ultraviolet (UV) irradiation by exposing a small aliquot of yeast cells to brief UV irradiation from a hand-held UV source, then allowed the cells to grow over a 10-hour growth period under normal conditions. A second group changed the sugar source in the rich yeast media (YPD: 10% yeast extract, 20% peptone, 20% dextrose) from dextrose (glucose) to maltose. Another group also modified the media by reducing either the dextrose or peptone to 50% of the original recipe concentration. Two additional groups investigated the effects of temperature alteration. One group changed the incubation temperature from 30°C to 35°C for the entire growth period, and one group heat shocked the cells at 42°C for 5-15 minutes prior to the 10-hour growth period.

Lab Reports

Students were asked to individually write formal laboratory reports about this series of labs. Reports were graded based on the quality of each section of the paper including the abstract, introduction, methods, results, discussion, and references. Groups worked together on data analysis such as generating doubling times from yeast growth data and drawing conclusions about SDS-PAGE protein profiles.

Conclusions

This series of laboratory experiments was a successful method to include student-inquiry in a lab exercise utilizing limited protocols (growth curves and protein gels). Students took ownership of the project, coming to the laboratory outside of scheduled hours to complete their growth curves and to observe their SDS-PAGE gels. The main problem with the series of experiments was that changes in total protein composition were not detected with any of the treatments. While this was disappointing to the students, it did foster good conversation and instruction about the large number of proteins present in even simple yeast cells, the difficulty in observing small variations in protein concentration, and the difficulty in detecting proteins present in low concentration. Students enjoyed the experience of pouring, loading, and running SDS-PAGE as well as the opportunity to conduct "real" experiments.

About the Author

Pamela L. Connerly earned her Ph.D. in Biochemistry and Molecular Biology from The University of Chicago. She is currently an Assistant Professor of Biology at Indiana University East in Richmond, Indiana, where she teaches Biological Concepts, Cell Biology, Genetics, and Introduction to Biotechnology. She conducts research focusing on the process of endocytosis in the methylotrophic budding yeast *Pichia pastoris*.