BIL 151
Enzymes: A Sample Experiment with NaCl
Part II. Hypothesis Formulation and Experimental Prep

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Today's lab provides an example of how to design your own experiment.
We will examine how catalase functions at different ion concentrations of sodium chloride (NaCl).
For your own original team project, you will choose some factor other than salt tolerance.
You should use the methods and thought processes outlined below to develop your own project.

1. Building the Hypothesis

OBSERVATIONS provide a springboard for new ideas.
Our **literature search** found previous research on the effects of salinity on peroxidase:

- Increased NaCl concentration has been shown to increase peroxidase activity in the halophytic archaen, *Halobacterium halobium* (Brown-Peterson 1993).
- Many plants show an increase in peroxidase activity when experiencing salt stress. In one study on rice (*Oryza sativa*), salt stress was induced by immersing the plant's roots in 0.1M NaCl for 48 hours (Swapna 2003).
- Wild-type *Saccharomyces cereviseae* does have some degree of salt tolerance. Concentrations of 0.3M NaCl induced stress responses after 45 minutes of exposure (Lewis 1995).

Based on this information, we ask the **QUESTION:**

> "How would an increase in NaCl concentration (from 0M to 0.3M) affect the rate of H_2O_2 catalysis by yeast (*Saccharomyces cereviseae*)?"

We now rephrase our question in the form of a testable **HYPOTHESIS**.
Should your hypothesis be one-tailed or two-tailed? Not sure? Watch this tutorial:

https://www.youtube.com/watch?v=enQDKXI2PdA

Previous research indicates that increased NaCl concentration increases catalase activity.
Hence, our **overall hypothesis** could reasonably be **one-tailed**:

> "The rate of oxygen production due to yeast (*Saccharomyces cereviseae*) peroxidase catabolysis of hydrogen peroxide will be significantly **higher** in a 0.3M NaCl solution than in a 0M NaCl solution."

We can **justify our hypothesis** with the following **reasoning**.

1. Although the exact structure of peroxidases varies across different taxa (bacteria, fungi, plants), all peroxidases have the same basic four-heme structure (Brown-Peterson 1993).
2. Since
   a. peroxidase structure is similar among taxa and
   b. multiple taxa display the same increase in peroxidase activity when exposed to salt,
   c. peroxidase activity in *Saccharomyces cereviseae* should follow this same trend.
3. Previous studies observed increased catalase activity (stress response) only after the model organism was incubated in NaCl solution for some time. Therefore, we will do the same. (Swapna 2003, Lewis 1995)

The overall hypothesis must now be "remodeled" into mutually exclusive **experimental hypotheses**.

You will be comparing rates of catalase reaction in two groups of yeast:

- **Treatment group** will be incubated in NaCl in sodium phosphate buffer.
- **Control group** will be incubated plain sodium phosphate buffer.

Given this information, you should be able to devise null and alternative experimental hypotheses. Enter them here:

H_O: _____

_____

H_A: _____

_____

Finally, we must define outcome(s) that would allow us to reject or fail to reject our hypotheses. We **PREDICT** that,

"If increased ion concentration increases peroxidase activity, then the observed maximum rate of oxygen production will be higher for yeast in the 0.3M NaCl solution than for yeast in the 0M NaCl solution." (Reject H_o)

"If increased ion concentration does not affect peroxidase activity, then the observed maximum rate of oxygen production will not differ between yeast in the 0.3M NaCl solution and yeast in the 0M NaCl solution." (Fail to reject H_o)

2. Experimental Design – Measuring Reagents in Advance

Before starting an experiment, **always develop a solid plan to follow** because…

Poor preparation → ERRORS → Throw out data → Wail and flail → Start over

Before you begin collecting data:

- Finish your protocol design completely
- Calculate all quantities of substances you will need to prepare your reagents

Each of three control trials will require

10mL of stock yeast suspension
20mL of stock H_2O_2 solution
x (to be calculated by you) mL of phosphate buffer to keep reaction volume constant
x (to be calculated by you) mL of DI water to keep reaction volume constant

Each of your three treatment trials will require

10mL of stock yeast suspension
20mL of stock H_2O_2 solution
x (to be calculated by you) mL of 1.0M NaCl solution
x (to be calculated by you) mL of phosphate buffer to keep reaction volume constant
x (to be calculated by you) mL of DI water to keep reaction volume constant

Given this information, fill in the TOTAL volumes (mL) will you need of:

Yeast suspension: _____mL
H_2O_2 solution: _____mL
NaCl solution: _____mL
Phosphate buffer solution: _____mL
DI water: _____mL

To ensure consistency between treatment and control samples, how much of each of these reagents should you use in each **control** sample? (HINT: Look back at the quantities needed for **treatment** runs)

_____**mL of yeast in phosphate buffer**
_____**mL of phosphate buffer**
_____**mL of deionized (DI) water**
_____**mL of H_2O_2 stock solution**

An incubation period of 30 minutes is necessary to allow yeast to absorb and respond to the NaCl. Should you also incubate your control samples for 30 minutes? Yes or no? _____ (Why?)

3. Learn to Calculate Concentrations and Mix Reagents

To practice making solutions of particular concentrations, complete the following three exercises. Examples have been **color coded** to make it easier to see which variable corresponds to which. **Solve for x**, giving both **number** and **units**.

Problem: What solid mass (g) of Chemical Z is needed to make a specific volume of specific concentration of Chemical Z?
Use this formula:

[desired volume (L)] x [desired concentration (moles/L] x [MW of Chemical Z (g/mole)] = g of Chemical Z needed (←this is your unknown)

Example: To make 200mL (= 0.2L) of 0.5M NaCl (molecular weight = 58.4 g/mole) solution, calculate:

(0.2 L) x (0.5 mole/L) x (58.4g/mole) = _____g of NaCl

Solve and fill in the blank! How many g of NaCl must you add to 0.2L of water to make a 0.5M solution?

What solid mass of Chemical Z must I add to my yeast suspension to bring it to a desired concentration? Use this formula:

[desired concentration (moles/L)] x [(desired total volume (L)] x (MW of Chemical Z) = g of Chemical Z needed (←this is your unknown)

For example, if you wish to bring 10mL of yeast suspension from 0M NaCl to 0.3M:

(0.3 mole/L) x (0.01L) x (58.4 grams/mole) = _____ g of NaCl

Solve and fill in the blank! How many g of NaCl must you add to 0.01L of yeast suspension to make a 0.3M NaCl solution?

What volume (L) of known molarity NaCl stock solution must be added to yeast suspension to attain a desired NaCl molarity of a desired volume of yeast suspension? Use this formula:

[stock NaCl solution conc. (moles/L)] x [stock NaCl solution volume (L)] (←this is your unknown) =
[desired NaCl concentration in yeast suspension) x (desired final volume of yeast suspension)

For example, if you have a stock solution of 1M NaCl and wish to achieve 0.3M NaCl in 15mL (= 0.015L) of yeast suspension, solve for **x**:

(1 mole/L) x (x) = (0.3mole/L) x (0.015L) x = _____

Solve and fill in the blank! How many L of 1M NaCl are needed to make 15mL of 0.3M NaCl/yeast?

***IMPORTANT:* If your initial yeast suspension volume (10mL) and the value of x do not add up to the desired final volume (15mL), you must add deionized (DI) water to your suspension to achieve the desired final volume. This must be done for every experimental run to be sure volumes are constant.**
In the example above, x = 4.5mL
Your starting volume of yeast suspension is 10mL.
Your desired reaction volume (which must be constant across all experimental runs) is 15mL.
To make up the difference, you must add 0.5 DI water.

4. Prepare your Samples

Inventory your workstation to be sure you have everything you need.
Wash any used/dirty items with deionized (DI) water and drain on a paper towel.

Label measuring vessels (syringes, graduated cylinders, etc.) with the reagent they will contain.
Use labeling tape and your Sharpie/lab marker.

Never measure reagents in beakers or flasks. They are not accurate.
Only graduated cylinders or syringes are sufficiently accurate for measuring liquid reagents.

1. Label six beakers:
   - Treatment 1
   - Treatment 2
   - Treatment 3
   - Control 1
   - Control 2
   - Control 3
2. Stock yeast suspension is on the tray at the center of your lab table.
   - **Decant** enough stock suspension into your team's beakers to allow for measurement error, but not much more than you need. Don't waste!

Always mix suspensions thoroughly before use.
This ensures consistent reactant concentration throughout the liquid.

3. Add 10 mL of yeast suspension to each Treatment and Control beaker, using the labeled syringe.

DO NOT CONTAMINATE STOCK OR OTHER SOLUTIONS!
Always use appropriately labeled measuring tools and glassware correctly.
If you contaminate any solutions or suspensions, your experiments (and possibly those of your fellow lab students) will NOT WORK.

4a. Treatment Sample Preparation:

4. Add **4.5 mL of 1M NaCl** and **0.5 mL of DI** water to the **Treatment 1 beaker**.
5. **Record the time on the beaker label.**
6. **Set a timer or alarm** on your phone for **30 minutes**.
   (When your alarm goes off, it's time to use Treatment 1 for an experimental run.)

WAIT FIVE MINUTES.

7. Repeat steps 4, 5, and 6 to prepare **Treatment 2**.

WAIT ANOTHER FIVE MINUTES.

8. Repeat steps 4, 5, and 6 to prepare **Treatment 3**.

(The five minute intervals will allow you to stagger the times the samples are ready to run.)

4b. Control Sample Preparation:

1. Add **4.5 mL of phosphate buffer** and **0.5 mL of DI water** to the **Control 1 beaker**.
   (Why add buffer and DI water? Why not just use the 10mL of yeast suspension alone?)
2. **Record the time on the beaker label.**
3. **Set a timer or alarm** on your phone for **30 minutes**.
   (When your alarm goes off, it's time to use Control 1 for an experimental run.)

WAIT FIVE MINUTES.

5. Repeat steps 1, 2, and 3 to prepare **Control 2**.

WAIT ANOTHER FIVE MINUTES.

6. Repeat steps 1, 2, and 3 to prepare **Control 3**.

REMEMBER: All experimental groups—treatment or control—must be incubated for the same amount of time. As you set up your solutions, take this into account. Be sure each sample has been incubated for the correct amount of time before you begin its experimental run.

You are now ready to proceed to the actual experiment.

Literature Cited

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Lewis JG, Learmonth RP, Watson K. 1995. Induction of heat, freezing and salt tolerance by heat and salt shock in *Saccharomyces cerevisiae*. Microbiology 141:687-694.

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Swapna TS. 2003. Salt stress Induced Changes on Enzyme Activities during Different Development Stages of Rice (*Oryza sativa* Linn.). Indian Journal of Biotechnology 2:251-258.