Bio 212 Genetics Laboratory
Spring 2007

Lab 8: The Lac Operon

Purpose: To study the growth properties and enzyme activities of *E. coli* lactose mutant strains.

Source of laboratory: Dr. Jinnie Garrett, Hamilton College

Reference: Hartl and Jones, Chap. 9 pp. 318-324

Background:

In 1961, Francois Jacob and Jacques Monod proposed a molecular model of gene control in *E. coli*. The operon is a set of closely related genes encoding enzymes or structural proteins which is regulated by molecular signals acting on a DNA element called an operator. The chief advantage of the operon system is its ability to coordinate the expression of several functionally related genes.

The *lac* operon of *E. coli* encodes proteins which catalyze the transport into, and degradation within the cell, of the sugar lactose. This operon has been extensively studied because of the convenient colorimetric assays available for the enzyme involved in lactose breakdown, ß-galactosidase. Synthesis of ß-galactosidase can be induced by addition of IPTG (isopropyl-B-D-thiogalactoside) to a growing culture. IPTG is a nonmetabolizable analog of the normal substrate, lactose. ß-galactoside activity can be observed either on plates containing specific substrates or by enzyme assays. To assay for the enzyme, another analog of lactose, ONPG (orthonitrophenyl ß-D-galactoside) is used. Hydrolysis of this colorless compound results in the production of the intensely yellow nitrophenolate ion.

In this lab, we will use wild-type *E. coli* and two mutant strains that affect components of the *lac* operon. By streaking these strains on various indicator plates and by assaying ß-galactosidase activity after IPTG introduction, you will identify the strains.

The strains we will be using are:

CSH64 - *lac*$^-$ (wild type)

CSH34 - *lac Z*$^-$ (cannot make the enzyme ß-galactosidase, the protein that cleaves lactose into the monosaccharides glucose and galactose)

CSH37 - *lac O*^{c} (has an altered operator region that does not bind lac repressor very well, thus the lac operon is active even in the absence of lactose)

The three types of indicator plates we will be using are:

A. MacConkey lactose plates:

- These plates contain lactose and a pH indicator.
- Colonies able to metabolize lactose (*lac*$^+$) generate lactic acid, acidifying the medium and maintaining a red color.
- Colonies that are unable to metabolize lactose (*lac*$^-$) turn yellowish-white.
- MacConkey plates are very insensitive. Approximately 1000 molecules must be metabolized to generate enough lactic acid to turn the colony red.

B. Xgal plates:

- The compound Xgal (5-bromo-4-chloro-3-indoyl- ß-D-galactoside) is cleaved by the enzyme ß-galactosidase (product of the *lacZ*$^+$ gene) to give galactose and a blue dye.
- Therefore *lacZ*$^+$ colonies turn blue, while *lacZ*$^-$ colonies remain white.
- Xgal plates are extremely sensitive. One molecule of ß-galactosidase is all that is needed to cleave enough Xgal to turn the colony blue. For this reason you will use two types of Xgal plates when you assay the strains.

1. **Xgal/glucose repressor plates**: By adding glucose, cAMP levels will be low and the lactose operon will be repressed provided that the operator region is functional.

2. **Xgal/glycerol IPTG inducer plates**: IPTG (isopropyl-ß-D-thiogalactoside) binds to the repressor and causes the conformational change necessary to expose the operator and induce the lactose operon. IPTG is not dependent on permease to enter the cell and is not cleaved by ß-galactosidase. By adding glycerol to these plates, the cAMP levels will be high, allowing for maximal induction of the lactose operon.

The Xgal plates will answer two questions:

i. Does the strain make ß-galactosidase?

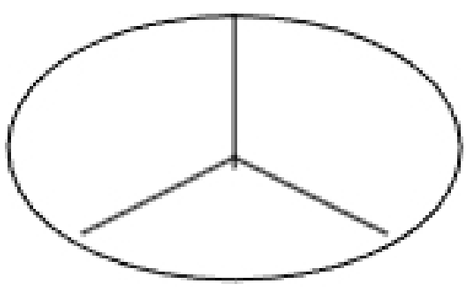
ii. If the strain does make ß-galactosidase, is it constitutively expressed, or is it inducible via IPTG induction?

- Careful analysis of the color of the colonies on Xgal/glucose repressor plates versus Xgal/glycerol IPTG inducer plates will tell you whether the *lac* operon is constitutive or inducible in that strain.
- Remember that even in the wild-type repressed *lac* operon, there will be a few molecules of ß-galactosidase in the cell which will be enough to turn the colony blue. So for each strain, be sure to look at the relative intensity of the blue color in the colonies on repressor versus inducer plates.

Procedure: Part 1: Work in pairs

1. Streak the unknown strains A-2, B-2, C-2 on the sectors of three different indicator plates.
M – MacConkey lactose plates
Xgal$^+$ - Xgal/glucose repressor plates
Xgal$^-$ - Xgal/Glycerol IPTG inducer plates

Divide the plates into 3 sectors and use a sterile toothpick to spread the bacteria on the plates as indicated by the instructor.



2. Invert the plates, and place them in the 37°C incubator overnight.

3. Check all of the plates 24 hours later. You should be able to deduce the identity of the strains at this time. **It is important that you look at your plates at this time. You will miss the differences in the color intensity if you forget to check your plates at the appropriate time.**

Part 2: ß-galactosidase assays: Work in pairs

1. You will be provided with 3 cultures of each of the unknown strains A, B, C – these have been grown in the following media:

1 – minimal glucose
2 – minimal glycerol (no IPTG)
3 – minimal glycerol + IPTG

These cultures have been chilled on ice for 30 minutes.

2. Label 3 large test tubes for each unknown strain in each media, e.g. A-1, A-2, A-3, B-1… (total of 9 test tubes)

3. Add 0.5 ml of freshly grown log-phase cells of the strain you are assaying and 0.5 ml of Z-buffer to each tube.

4. Add one drop of chloroform and one drop of 0.1% SDS to each tube. Vortex vigorously for 10 seconds. The chloroform kills the cells and disrupts the membranes, but does not affect the activity of the enzymes. Incubate the uncapped tubes for 10 min. at 37˚C.

5. Vortex the lysed cells once more, and then add 0.2 ml of ONPG in 0.1M pH 7 phosphate buffer (ONPG is the substrate for the enzyme) to each of the tubes.
6. Place the ONPG-treated tubes at 37˚C for 5 minutes. (If the solution turns yellow immediately, note that in your results.)