Gene Regulation Using The Lac Essay, Research Paper

Gene Regulation Using the Lac Operon

Abstract

Gene regulation in cells has been studied extensively in both eukaryotic and prokaryotic organisms. Gene control is employed as a way of saving the cell energy by only producing those enzymes it needs. In eukaryotes, genes are regulated so as maintain levels of cell specificity. In this experiment, the restriction of the production of the -galactosidase enzyme in the prokaryote Escherichia coli can be achieved by employing the lac operon. This operon is controlled by the presence or absence of lactose in the medium. Six different media were prepared and incubated in 20-minute increments for 100 minutes. At each interval, a small amount of sample was removed and tested for enzyme activity and growth as dictated by optical density. The results were plotted graphically and the outcome for each culture was compared with the outcome expected according to the medium type. Based on the results, the regulation of -galactosidase production in varied media by the lac operon was successfully achieved.

Introduction

In prokaryotic organisms, structural genes are organized in clusters that are controlled from a single regulatory site. The lac operon, the gene cluster involved in the metabolism of lactose in E.coli, has been studied over and over again and provides a model for the study of gene regulation. The metabolism of lactose calls for two enzymes. Permease, aiding in the transport of lactose into the cell, and -galactosidase, in charge of cleaving lactose to produce glucose and galactose, are encoded by two consecutive genes Z and Y respectively. Although not required for the metabolism, a third gene, gene A, codes for the enzyme transacetylase. All three of these genes are transcribed onto a strand of mRNA termed polycistronic (multi-gene). By regulating the production of this mRNA, the synthesis of the Z, Y, and A genes can be controlled.

An additional gene, the I gene, is located near the A, Z, and Y genes and codes for a regulator protein that is capable of blocking the expression of the polycistronic genes. This repressor binds to the DNA near the point where transcription of the polycistronic mRNA begins. This binding site is termed the O-site or operator. Innate properties of the repressor allow it to only bind to those sites on the DNA near the genes that it is to control. In binding to the operator, the repressor allows transcription to be initiated by RNA polymerases bound at the P or promoter site. The complete POZYA segment is termed the operon.

The repressor molecule itself is the focus of this system and contains two different binding sites. One recognizes the specific operator sequence for the lac operon and the other is a lactose binding site. As mentioned, when the repressor binds to the operator (Lac- environment), the transcription of the -galactosidase and permease (as well as transacetylase) is prevented. However, when lactose binds to the repressor (Lac+ environment), the repressor undergoes a conformational change. This change in shape releases the repressor from the operator site. The RNA polymerase can then proceed to transcribe the polycistronic mRNA. The result is the successful production of -galactosidase and permease.

In this experiment, the principles of the lac operon are used to study the synthesis of -galactosidase in E.coli in different media. Six different cultures were used, each of which were incubated in 20-minute intervals. At the 0, 20, 40, 60, 80, and 100 minute mark, a small sample was removed from the culture and involved in three different tests involving optical density. With these results, the activity of the -galactosidase and the growth level of the polycistronic gene could be determined. Those cultures containing lactose in some amount will have slight to rapid growth. Those cultures without lactose, those with broth, will not experience growth. According to the way the lac operon system works, enzyme activity and gene growth can be predicted based on the media and lactose content of the culture.

Methods

Consult the lab manual pgs. 56-57 for lab protocol.

Results

(see attached sheets for the following)

Table 1: Spectrophotometric Data

Table 2: -Galactosidase Activity

Graph 1: Time vs. Enzyme Activity

Graph 2: Time vs. Optical density (620nm)

Discussion

The first culture contained Lac- grown on nutrient broth and resuspended in 1% lactose. Due to the mutation in the -galactose gene, no enzyme activity should be expected and slight growth due to the presence of lactose should be seen. When referring to graph 1, a negligible amount of activity is observed. When referring to graph 2, a decrease at 20 minutes followed by a negligible increase in growth starting at 40 minutes is observed. The second culture contained Lac- grown on nutrient broth and resuspended in 0.8% nutrient broth solution. No enzyme activity and no growth were expected. With the exception of a slight peak in enzyme activity at 40 minutes, these expectations are met. The third culture contained Lac+ grown with lactose and resuspended in the lactose solution. An increase in enzyme activity and an increase in growth due to the presence of lactose were expected. The results on both graphs fit this expectation perfectly. The fourth culture contained Lac+ grown with lactose and resuspended in the nutrient broth solution. A decrease in enzyme activity and a slowed growth due to the addition of nutrient broth were expected. While the growth peak in graph 2 shows no drastic increase or decrease, graph 1 shows a substantial decrease in enzyme activity. The fifth culture contained Lac+ grown on nutrient broth and resuspended in the lactose solution. An increase in enzyme activity and growth should be expected due to the addition of lactose to the mixture. These trends can be seen quite well in both graphs. The sixth and final culture contained Lac+ grown on nutrient broth and resuspended in nutrient broth solution. It should be obvious that no enzyme activity and slight growth should occur. Aside from a sharp enzyme peak at 60 minutes, these predictions appear true.

Based on what is known about gene restriction using the lac operon, the cultures in this experiment demonstrated projected behavior. Five out of six of the cultures used exhibited enzyme activities and growth rates characteristic of the medium they contained. Any errors may be attributed to improper, inaccurate, or non-sterile techniques, errors in making the initial sample, or timing inconsistencies. Aside from minimal glitches, this experiment successfully demonstrated how the lac operon works to regulate the gene expression of the gene coding for the -galactosidase enzyme.

Sources

1.) Genetics Lab Manual, Spring 2000: pgs. 56-57.

2.) Griffiths, Anthony J.F., Miller, Jeffery H., Suzuki, David T., Lewontin, Richard C., Gelbart, William M. An Introduction to Genetic Analysis. 1996. W.H. Freeman and Company, NY: pgs. 546-547.