Abstract

This experiment was done to show how the transcription of the *lac* operon can be turned on or off resulting in the expression of a gene or lack thereof. If the *lac* operon is a regulation mechanism and can be turned off by a repressor which binds to the operator and stops transcription, then the operator, or the “switch” determines when enzymes for the metabolic pathway are synthesized, thus controlling transcription. In the experiment, there are three mediums containing *E.coli* and the indicator O-NPG. All three of these mediums were placed in an incubator at 37°C. The lactose treated medium containing *E.coli* was the only medium to show signs of β-galactosidase production. This is due to the addition of lactose to the medium, causing the expression of the lac operon, and production of β-galactosidase.

Introduction­­­

The purpose of the study was to find out how the regulation of gene expression by the lac operon works in *Escherichia coli*. The *lac* operon was one of the first regulation mechanisms studied by scientists. The scientist that first discovered this found that β-galactosidase is the enzyme responsible for breaking down lactose into glucose and galactose. A switch in a segment of DNA was also discovered this is called an operator. This switch determines when the enzymes for the metabolic pathway are synthesized, thus controlling transcription. The operon can be turned off by a repressor which is a protein that binds to the operator and blocks the attachment of RNA polymerase to the promoter, thus preventing the transcription of genes (Zhao, 2010). The only exception of this is if lactose binds to the *lac* repressor at an allosteric site, then the repressor protein changes to the active form that can attach to the operator, turning the operon off (Campbell, 2008). β-galactosidase is found in the *lac* operon. It also includes two other genes that code for enzymes that function in lactose utilization (Campbell, 2008). The regulatory gene codes for an allosteric repressor protein that switches off the *lac* operon by binding to the operator. In this case an inducer is needed to inactivate the repressor (Campbell, 2008).

The inducer of the *lac* operon is allolactose. The absence of allolactose along with the absence of lactose causes the genes of the *lac* operon to be silenced. On the other hand if lactose is present allolactose binds to the *lac* repressor and alters its conformation (Robert, 2010). As a result the repressor cannot attach to the operator. Allolactose induces enzyme synthesis by freeing the *lac* operon from the negative effect of the repressor. Gene regulation is positive whenever a regulatory protein interacts with a genome to turn transcription on (Campbell, 2008).

In this experiment one may discover how the regulation of gene expression by the lac operon works in *Escherichia coli*. It is expected that if the *lac* operon is a regulation mechanism and can be turned off by a repressor which binds to the operator and stops transcription, then the operator, or the “switch” determines when enzymes for the metabolic pathway are synthesized, thus controlling transcription.

Materials and Methods

Twenty-four to thirty-six hours prior to the lab, the instructor inoculated three types of bacterial growth media with *E. coli* bacterial and then put the cultures in the incubator at 37°C. One of the cultures your group was given was grown in a media that had glucose as a carbohydrate source, one of the cultures had lactose as a carbohydrate source, and the third had both lactose and glucose as a carbohydrate source.

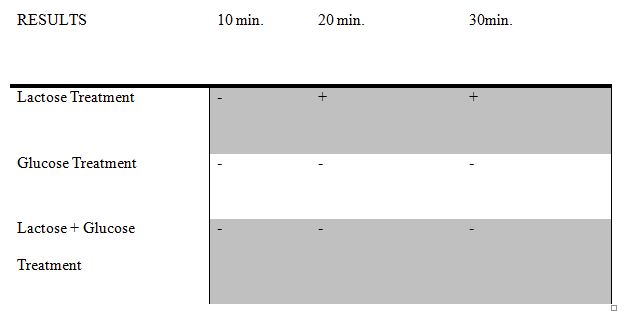
Three test tubes were obtained and labeled “Glucose,” “Lactose,” and “Glucose+Lactose.” A pipette was then utilized to distribute twelve drops of *E. coli* into each test tube in which you labeled. Three different pipettes were used during this step. Ten drops of O-NPG were added to each of the test tubes and mixed thoroughly. Once the tubes have been mixed well, they were placed in the incubator that should be set at 37°C. They were checked every ten minutes check for color changes, and color change was recorded in the table with each test tube media labeled, and each interval checked in which the color was checked at. The presence of color was indicated by writing, (++) for strongly yellow, (+) for weakly yellow, and (-) for not yellow. According to the concentration of yellow you will be able to determine which nutrient caused β-galactosidase to be produced at the greatest rate. The strongest color of yellow indicates the fastest rate, and the weakest color of yellow indicates the slowest rate.

Results

O-NPG was added to each medium containing *E.coli* and as a result the mediums developed a yellow pigment, or no yellow pigment would be present. The first medium, the lactose treatment showed no yellow pigment at the ten minute interval, but at the twenty and thirty minute intervals a weak, yellow pigment was recorded. The second medium, the glucose treatment showed no signs of yellow pigment at ten, twenty, or thirty minute intervals. The third medium, the lactose + glucose treatment had the same results as the glucose treatment.

After the experiment was completed the lactose medium containing *E.coli* was the only medium in which a yellow pigment could be seen. The yellow pigment was first noticed at the twenty-minute interval, and was present at the thirty-minute interval as well. The other two treatments consisted of: a glucose treatment, and a lactose + glucose treatment. Coincidentally a yellow pigment was not seen in either of the mediums that contained glucose.

**The Result of O-NPG on Each Medium Containing *E.Coli***



Discussion

In the experiment the lactose treated medium containing *E.coli* was the only medium to show signs of a yellow pigment. This is due to the fact that lactose acts as an inducer to the *lac* operon. This means that when lactose is present in the lac operon the LacI repression is inactivated which allows the expression of the gene *lac* Z, to produce β-galactosidase (Zhao, 2010). Lactose caused β-galactosidase to be produced at the greatest rate. Evidence of this statement is shown in the results where the lactose treated medium was the only medium to show a yellow pigment during the thirty minute time frame. This all correlates with Robert’s research on the lac operon. The only thing that doesn’t correspond with Robert’s data was the fact that the lactose + glucose treatment did not show signs of the gene being expressed as lactose did. As stated above lactose is the inducer of the lac operon in the experiment meaning whenever it is present the gene should be expressed.

The question is, “Why would the lactose treated medium have a yellow pigment, but the other lactose treatment including glucose not?” This means that the presence of glucose must be restricting the pigment or blocking/stopping something that would lead to the process of the pigment being noticeable. This could also just be an unexpected part of the experiment in which part of the experiment was interrupted or altered. It is strange that there would be a yellow pigment recorded for the lactose treatment, but no for the other lactose treatment containing glucose. This was not the case with the lactose + glucose treated medium. An explanation for this unexpected result could be that part of the experiment was inconsistent, such as: the opening and closing of the incubator door, the reaction of β-galactosidase being slower and not having sufficient time for the gene to show, or it could have been a result of not mixing the O-NPG well with the mixture.

The hypothesis, “In this experiment one may discover how the regulation of gene expression by the lac operon works in *Escherichia coli*. It is expected that if the *lac* operon is a regulation mechanism and can be turned off by a repressor which binds to the operator and stops transcription, then the operator, or the “switch” determines when enzymes for the metabolic pathway are synthesized, thus controlling transcription.” can be tested in another experiment by boiling the lactose treated medium to denature the repressor. This will let us see if β-galactosidase is still produced, and if the O-NPG indicator is still expressed when lactose is not present.

The experiment supported the hypothesis because the *lac* operon controlled whether or not the gene would be expressed when *Escherichia coli* was added to lactose treated medium. When lactose was present the gene was expressed and when lactose was not present the gene was not expressed. The reason for this is that the operon can be turned off by a repressor which is a protein that binds to the operator and blocks the attachment of RNA polymerase to the promoter, thus preventing the transcription of genes (Campbell, 2008). The expression of this operon is regulated by the LacI repressor protein. The protein inhibits transcription from the P *lac* promoter. This inhibition is relieved by inducers such as: allolactose and/or non-metabolizable lactose analogues, such as thiomethyl-b-galactoside (TMG). (Robert, 2010).

References

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