B-galactosidase in e.coli cells | experiment

The experiment for the induction of -galactosidase comprises of two parts. The main aim of the first part was to measure the relative amount of -galactosidase in E. coli cells by using IPTG as the inducer. For the second part, the aim was to observe the outcome of different conditions on the inductions of -galactosidase in E. coli cells.

The lac operon consists of genes that specify the expression of -galactosidase, galactoside permease, and thiogalactoside transacetylase and are labelled Z, Y and A respectively. These three structural genes are translated from a single mRNA. Besides that, another gene that is present nearby the lac operon is a gene that encodes the lac repressor. It is labelled I and it is a protein that causes the inhibition of the synthesis of the three lac proteins. There are two ways of control of the lac operon; negative control and positive control. In negative control, the lac repressor binds to the operator in the absence of an inducer and therefore prevents the transcription of mRNA. The inducer of a lactose system is actually a lactose isomer, 1, 6-allolactose but IPTG is normally used in in vitro studies because it does not get degraded by -galactosidase. However, when the inducer binds to it, the repressor will dissociate from the operator and this allows for the transcription and subsequent translation of the lac enzymes to take place. In the positive control, which is a catabolite repression, this mechanism involves both cyclic AMP (cAMP) and catabolite activator protein (CAP). Both of them will bind together to form a complex which then subsequently binds to a region close to the promoter in the absence of a repressor. This then allows for transcription to occur. (Voet, Voet & Pratt, 2008)

Sample Calculation:

At t= 1 min

Where C= concentration(µmol/L), A= absorbance, E= extinction coefficient, l= pathlength, n= amount(µmol), V= Volume(L)

C = A/El = 0. 003/(21300 x 0. 9) = 0. 156494523 µmol/L

n = CV = 0. 156494523 x 0. 0008 = 0. 00012519561 µmol

n(o-NP) = n(ONPG)

Unit of -galactosidase = 0. 00012519561 µmol /5 minutes = 2. 50391E-05 unit

Units -Galactosidase per mL = 2. 50391E-05 unit/0. 2 mL = 0. 000125196 unit / mL

Graph 1 : Part A (Model results as provided by Anne Galea)

Part A (Own Results)

Sample Calculation:

At t= 2 min

Where C= concentration(µmol/L), A= absorbance, E= extinction coefficient, l= pathlength, n= amount(µmol), V= Volume(L)

C = A/El = 0. 008/(21300 x 0. 9) = 0. 417318727 µmol/L

n = CV = 0. 417318727 x 0. 0008 = 0. 00033385498 µmol

n(o-NP) = n(ONPG)

Unit of -galactosidase = 0. 00033385498 µmol /5 minutes = 6. 6771E-05 unit

Units -Galactosidase per mL = 6. 6771E-05 unit/0. 2 mL = 0. 000333855 unit / mL

Graph 2 : Part A (Own Results)

Part B (Model results as provided by Anne Galea)

A – IPTG

B – Lactose

C – IPTG (5mM) + Glucose

D – IPTG (10mM) + Glucose

E – IPTG + Chloramphenicol

F – IPTG + Rifampicin

G – IPTG + Streptomycin

Time of Induction (mins)

Average Absorbance (A414)

Sample Calculation:

At t= 5 min for A (IPTG)

Where C= concentration(µmol/L), A= absorbance, E= extinction coefficient, l= pathlength, n= amount(µmol), V= Volume(L)

C = A/El = 0. 088/(21300 x 0. 9) = 4. 590506 µmol/L

n = CV = 4. 590506 x 0. 0008 = 0. 0036724048 µmol

n(o-NP) = n(ONPG)

Unit of -galactosidase = 0. 0036724048 µmol /5 minutes = 0. 000734 unit

Units -Galactosidase per mL = 0. 000734 unit/0. 2 mL = 0. 003672 unit / mL

Graph 3 : Part B (Model results as provided by Anne Galea)

Part B (Own Results)

A – IPTG

B – Lactose

C – IPTG (5mM) + Glucose

D – IPTG (10mM) + Glucose

E – IPTG + Chloramphenicol

Time of Induction (mins)

Average Absorbance (A414)

Sample Calculation:

At t= 5 min for A (IPTG)

Where C= concentration(µmol/L), A= absorbance, E= extinction coefficient, l= pathlength, n= amount(µmol), V= Volume(L)

C = A/El = 0. 046/(21300 x 0. 9) = 2. 399583 µmol/L

n = CV = 2. 399583 x 0. 0008 = 0. 0019196664 µmol

n(o-NP) = n(ONPG)

Unit of -galactosidase = 0. 0019196664 µmol /5 minutes 0. 000384 unit

Units -Galactosidase per mL = 0. 000384 unit/0. 2 mL = 0. 00192 unit / mL

Graph 4 : Part B (Own Results)

Discussion

From the results, it has been shown that among all the different conditions that the induction of -galactosidase had been carried under, the one with only IPTG being added to it seemed to show the highest amount of units of -galactosidase per mL being present. However, the other conditions showed similar results as the their units of -galactosidase per mL were almost the same. The induction time that this experiment was 45 minutes to ensure that an appropriate amount of -galactosidase would be present for analysis. Moreover, in order to ensure that each tube had the designated time of induction, CTAB was used. The role of CTAB is to ensure that once E. coli is drawn from the flask, and pipetted into the tube, it is killed. This is to make sure that the E. coli does not continue the translation for -galactosidase. Furthermore, CTAB also causes the E. coli cell to lyse and therefore releasing its contents which in this case is the -galactosidase. By observing the Graph 3, it was shown that lactose does not induce as much -galactosidase compared to IPTG. During induction, lactose will turn into it’s isomer 1, 6-allolactose and induces -galactosidase. However, compared to IPTG,

allolactose would undergo degradation by -galactosidase and IPTG would not. (Voet et al., 2008) Therefore, the units of -galactosidase per mL would be lower with lactose compared to IPTG because as time passes, less lactose would be present and hence there would be less inducers present to carry out the induction of -galactosidase whereas IPTG does not undergo degradation by -galactosidase and the amount of IPTG is constant throughout induction. In the induction where the condition was with IPTG and glucose, the results obtained were low. When glucose is present, the concentration of cAMP decreases. This leads to the absence of the CAP-cAMP complex and therefore does not permit the transcription process to be carried out. This is because without the CAP-cAMP complex, RNA polymerase is unable to bind to the promoter. (Voet et al., 2008) Since transcription is unable to take place, -galactosidase is unable to be produced. Based on the Graph 3, inhibition carried out by chloramphenicol, rifampicin, and streptomycin all showed similar curves on the graph. However, each one of them causes inhibition by different methods. Chloramphenicol has properties which causes the inhibition of synthesis of proteins in bacterial cells. This is due to the fact that it prevents the uptake of free amino acids by tRNA. Moreover, it also interferes with the transfer of amino acids from their carrier proteins to the ribosome and this prevents the -galactosidase enzyme to be synthesised. (Rendit & Ochoa, 1962) Rifampicin, however, causes inhibition by preventing the initiation of RNA polymerase on the deoxyribonucleic acid (DNA) but does not affecting the elongation. This is done by binding the RNA polymerase enzyme irreversibly to the DNA which prevents the transcription of the DNA to form an mRNA strand which is used to translate the -galactosidase enzyme. (Reid & Speyer, 1970) Streptomycin instead causes

inhibition by inducing the ribosome to intentionally misread mRNA. This generally causes the inhibition of growth in susceptible cells. (Voet et al., 2008) However, in this case, it prevents the translation of the enzyme -galactosidase. Therefore, from all these three ways on inhibition, it is proven that the processes of transcription and translation are needed in order for the induction of -galactosidase to take place.