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Growth of *Escherichia coli* in media containing different carbohydrates

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Abstract
This study was conducted in order to evaluate the growth of *Escherichia coli* in minimal media containing different carbohydrates. The experiment was laid out in four different set-ups: (1) glucose only, (2) glucose + sucrose, (3) glucose + lactose, and (4) glucose + mannitol. The optical density (OD) of the inoculated flasks was determined using spectrophotometer set at 600 nm wavelength. The average OD readings at every time point were plotted in Microsoft Excel for the analysis of growth curve.
Results of growth curve revealed the presence of two distinct phases, the log phase and stationary phase. The log phase in set-ups 1, 2 and 3 was comparable and estimated to end after 390 minutes incubation. The log phase in set-up 4 was longer and extended until 600 minutes incubation. Stationary phase in set-ups 1, 2 and 3 started at 420 minutes OD reading, and this could be attributed to the depletion of glucose. Diauxic curves were observed in set-ups 2 and 3. The diauxic curve consists of three stages: (1) exponential cell growth while completely consuming the preferred sugar which is glucose; (2) lag phase; and (3) exponential growth in the second sugar. During diauxic shift, the cells produce the enzymes needed to metabolize the second sugar. The molecular mechanisms involved behind this operating principle include inducer exclusion, local or dedicated transcriptional regulation, global transcriptional regulation, and catabolite repression. No diauxic curve was noticed in set-up 3 and this could be attributed to the simultaneous consumption of glucose and mannitol.

Keywords: bacterial growth, *Escherichia coli*, sugar, Diauxic curve

1. Introduction
The way bacteria use different carbon sources such as glucose, lactose, sucrose and fructose has been a subject of research for a long time [1], in which *Escherichia coli* has been the most commonly used model organism. It is learned from the very start that glucose is the carbon source supporting the fastest growth on this bacterium. Also, glucose is the preferred sugar if bacteria are exposed to a mixture of carbon sources. It seems that *E. coli* uses carbon sources on the basis of "best food served first [2].
The molecular mechanisms involved behind this operating principle include the following: inducer exclusion, local or dedicated transcriptional regulation, global transcriptional regulation and catabolite repression.
According to Chen *et al*. [3], inducer exclusion takes place when the un phosphorylated $EIIA^{glc}$ binds to and stabilizes the resting state of non phospho transferase-sugar transporters, inhibiting the transport of alternative carbon sources. In the local or dedicated transcriptional regulation, it operates at the initiation of gene transcription of sugars catabolic operons. These operons are being subjected to repression by at least one specific regulator, whose depression occurs when the corresponding specific sugar is available and binds to it [4, 5]. Meanwhile, for global transcriptional regulation, the complementary condition for the transcription of sugar catabolic genes is given by the activity of the global regulator CRP (catabolic repressor protein or cAMP regulatory protein). The cAMP-CRP complex is then capable of recruiting RNA polymerase to promoter zones of catabolic operons so their transcription is started if no repressor is present. Hence, a condition for the transcription of catabolic operons is that high cAMP levels are present [6, 7]. Lastly, catabolite repression which is also known as glucose effect [8], and this was derived after observing the repression, when glucose is present, of catabolic enzymes specific for carbon and nitrogen metabolism. This phenomenon was related to cAMP levels that increase when poor carbon sources are present in the environment [9].
The objective of this exercise is to evaluate the growth of *E. coli* in media containing different carbohydrates such as glucose only, glucose + sucrose, glucose + lactose and glucose + mannitol.

2. Materials and Method
2.1. Preparation of culture medium
Minimal medium (M9) composed of salts (0.04 M Na_2HPO_4, 0.02 M KH_2PO_4, 0.009 M NaCl and 0.019 M NH_4Cl) and micronutrients (1 nM $MgSO_4$ and 100 nM $CaCl_2$) was prepared. Each flask containing 200 mL minimal medium was supplemented with different combinations of carbon sources: Set-up 1 = glucose only, Set-up 2 = glucose + sucrose, Set-up 3 = glucose + lactose, and Set-up 4 = glucose + mannitol. The final concentration of glucose was 0.05% whereas the final concentration of the other sugars was 0.025%. Each set-up was replicated two times.

2.2. Inoculum preparation and inoculation
Loopful of *E. coli* wild type strain was inoculated in 100 mL minimal medium with 0.1% glucose. The flask was incubated

overnight at 37 °C. Ten millilitre of the overnight culture was transferred to the four set-ups being mentioned above. The growth flasks were incubated at room temperature with vigorous shaking.

2.3. Growth measurement by optical density reading

The optical density (OD) of the inoculated flasks was determined using spectrophotometer set at 600 nm wavelength. An initial OD reading was taken, followed by an hourly interval during the first two readings, and then at 30 minutes interval in the succeeding readings. OD reading was stopped after 750 minutes, wherein a diauxic curve became evident in some of the sugar combinations. The average OD readings at every time point were plotted in Microsoft Excel for the analysis of growth curve.

3. Results and Discussion

OD 600 is used to measure the *E. coli* cell density. The OD 600 value is also an important indicator of the physiological condition of the *E. coli* cells in a given medium after a specific culture period. The OD 600 value determines if the *E. coli* cells may be ready for making competent cells, cell stocks, induction, or for being harvested.

In Figure 1, the prominent growth phases are log phase and stationary phase. The log phase in set-up 1 (glucose), set-up 2 (glucose + sucrose) and set-up 3 (glucose + lactose) lasted until 390 minutes of shaking incubation. For set-up 4 (glucose + mannitol), it had a longer log phase which terminated near 600 minutes of shaking incubation. Highest absorbance reading was also noted in set-up 4. The reduction of OD readings in set-ups 1, 2 and 3 (started 420 minutes) could be due to the depletion of glucose in the medium, and this already signals the start of the stationary phase. At this particular phase, OD readings became erratic. Diauxic curves were observed in set-ups 2 and 3. After the stationary phase, there was an increased OD reading starting at 660 minutes in set-up 2 and 630 minutes in set-up 3.

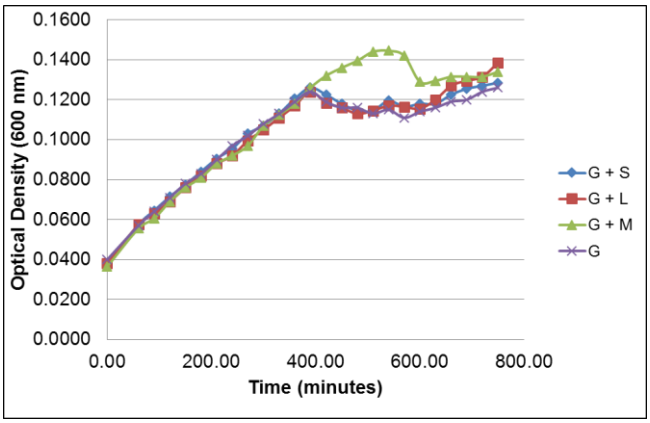

Fig 1: OD reading at every time point of the four sugar combinations (G + S = glucose + sucrose, G + L = glucose + lactose, G + M = glucose + mannitol, G = glucose)

The diauxic curve consists of three stages. First, the cells grow exponentially while they completely consume a preferred sugar such as glucose. They then enter a lag phase, and the third stage consists of exponential growth on the second sugar. Diauxic occurs because organisms use operons or multiple sets of genes to control differently the expression of enzymes needed to metabolize the different nutrients or sugars they encounter. If an organism allocates its energy and other resources (e.g. amino acids) to synthesize enzymes needed to metabolize a sugar that can only support a slower growth rate and not use all or most of its available resources to synthesize the enzymes that metabolize a different sugar providing a faster growth rate, such an organism will be at a reproductive disadvantage compared to those that choose to grow on the faster growth supporting sugar.

At the time of the diauxic shift, the cells produce the enzymes needed to metabolize the second sugar. For example, in the case of lactose, enzymes produced include lactose permease (which catalyses the transport of lactose into the cell), β-galactosidase (which hydrolyses the intracellular lactose into products that feed into the glycolytic pathway) and lactose transacetylase (which is believed to metabolize toxic thiogalactosides transported by lactose permease) [10]. These peripheral enzymes for lactose are synthesized only if lactose is present in the environment. The genes corresponding to these enzymes are contagious on the DNA and transcribed in tandem, an arrangement referred to as the lac operon [10].

In the absence of lactose, the lac operon is not transcribed because the lac repressor is bound to a specific site on the lac operon called the operator. This prevents RNA polymerase from attaching to the operon and initiating transcription. In the presence of lactose, transcription of lac is triggered because allolactose, a product of β-galactosidase, binds to the repressor, and renders it incapable of binding to the operator. The occurrence of the glucose–lactose diauxie suggests that transcription of lac is somehow repressed in the presence of glucose. Two molecular mechanisms have been proposed to explain this repression: (1) Inducer exclusion and (2) cAMP activaton [10].

Inducer exclusion explains that, in the presence of glucose, enzyme IIAglc (a peripheral enzyme for glucose) is dephosphorylated. The dephosphorylated IIAglc binds to lactose permease and inhibits lactose uptake, thus, reduces the intracellular concentration of allolactose and eventually the transcription rate of the lac operon. It is also suggested that phosphorylated IIA^{glc} activates the enzyme, adenylatecyclase, which catalyses the synthesis of cyclic AMP (cAMP). Since the total concentration of IIA^{glc} remains constant on the rapid time scale of its dephosphorylation, exposure of the cells to glucose causes a decrease in the level of 13 phosphorylated IIA^{glc}, and hence, cAMP. This reduction of the cAMP level forms the basis of yet another mechanism of lac repression [11].

Ptashne and Gann[12] provided a detailed explanation on how activation of cAMP played in glucose-mediated repression of lac operon. According to them, RNA polymerase is not recruited to the lac operon unless a protein called catabolite activator protein (CAP) or cAMP receptor protein (CRP) is bound to a specific site on the lac operon. CAP has low affinity for the CAP site and its affinity only increases when bound to cAMP. In the presence of lactose alone, the cAMP level is high; therefore, CAP becomes cAMP-bound and attaches to the CAP site, and promotes transcription by recruiting RNA polymerase. In the presence of glucose in the culture, there is a decrease in level of cAMP by the mechanism described above. Consequently, CAP, being cAMP-free, fails to bind to the CAP site, and lac transcription is abolished.

No diauxic curve is noticed in set-up 3 (glucose + mannitol). Monod [1, 13] found that in *E. coli*, "A-sugars" namely glucose, fructose, mannitol and mannose have supported comparable maximum specific growth rates, but there was no diauxic lag during growth on a mixture of glucose and any one of the other A-sugars. Subsequent studies have confirmed that in some of these cases, both the substrates are consumed simultaneously. Thus, it is conceivable that simultaneous consumption occurs whenever the ratio of the single-substrate growth rates is close to 1 [1, 13].

4. Conclusion

This exercise was conducted in order to evaluate the growth of *E. coli* in minimal media containing different carbohydrates. Growth curve revealed two distinct growth phases, the log phase and stationary phase. The log phase in set-ups 1, 2 and 3 was comparable and estimated to end after 390 minutes incubation. Meanwhile, log phase of set-up 4 was longer and extended until 600 minutes incubation. Stationary phase in set-ups 1, 2 and 3 started at 420 minutes OD reading and this could be attributed to the start depletion of glucose. Diauxic curves were observed in set-ups 2 and 3. No diauxic curve was noticed in set-up 3. Some studies have confirmed that in some of these cases, both the substrates (glucose and mannitol) are consumed simultaneously.

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