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Diauxie Elimination by Xylose Inducible Promoters

Abstract

Microorganisms typically prefer to utilize glucose over other sugar carbon sources such as xylose. This is largely regulated through control of gene expression based on the response of regulatory elements to sugars available to the cell. In *E. coli*, the xylose metabolism operon is controlled by both the xylose-inducible XylR activator protein and the cAMP receptor protein (CRP). In this project we attempt to eliminate glucose control over xylose-inducible gene expression in *E. coli* by altering the natural transcriptional control region of the xylose operon. Designs constructed and tested include scrambling the CRP binding site, increasing the strength of the xyl promoter, and overexpressing XylR. Xylose-inducible gene expression that functions independently of glucose regulation provides a useful approach to improving microbial utilization of biomass feedstocks containing mixtures of glucose and xylose.

Introduction

What Is Diauxie?

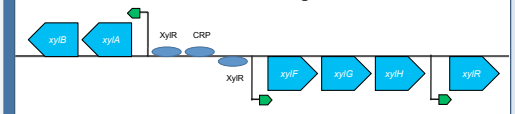
In *E. coli*, the cAMP-receptor protein (CRP) acts as a global regulator in which the cAMP-CRP complex typically increases transcription at catabolic promoters in the absence of the "preferred" catabolite glucose. The result is a phenomenon known as diauxie, in which glucose is preferentially utilized in the presence of other sugars, since expression of catabolic pathways for the other sugars is not fully induced. A consequence of this dual control mechanism is that many bacterial promoters commonly used in biotechnology require the absence of glucose for full transcription activation (e.g., the *lac* and *araBAD* promoters).

Our Project Goals

In wild-type *E. coli* strains, the promoters controlling expression of genes responsible for xylose transport and metabolism are regulated by CRP and the xylose-inducible protein XylR. Our goal in this project is to create and characterize a xylose-inducible but glucose-insensitive gene expression system. This would functionally eliminate a diauxie-type phenotype relating to induction of gene expression from this promoter. In addition to creating a valuable new tool for the part registry, this project has useful applications for biochemical and bioenergy production.

Cellulosic biomass feedstocks targeted for biofuel production or other products contain large percentages of glucose and xylose. In industrial fermentations, cells grown on sugars from cellulosic biomass normally consume glucose as their first carbon source. The gene expression system we are creating would aid in the simultaneous fermentation of mixed sugars, and would have practical applications during the conversion of biomass to ethanol.

Natural Operon



Wild-type *E. coli* xylose metabolism operon

•The left facing ***xylAB*** genes code for xylose isomerase and kinase.
•The other set of genes, ***xylFGH*** are *right* facing and code for active xylose transport proteins.
•A bidirectional operator is necessary because of the opposite directions of transcriptional control for *xylAB* and *xylFGH*.
•**XylR** is located downstream of xylH but is controlled by its own weak promoter and is involved in regulation.

Transcription is initiated when the protein XylR binds to xylose; this complex then binds to the XylR binding site located at both ends of the operator. Full transcriptional activation requires binding of cAMP-CRP to a single CRP binding site. This operator is activated in the presence of xylose and the absence of glucose, since cAMP levels generally vary inversely with the cellular glucose concentration.

References

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Strategies

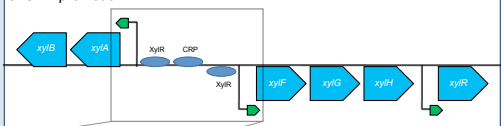
Two approaches to make a glucose-independent, xylose-inducible system:

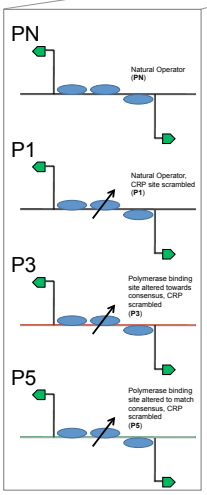
1) Via the protein CRP* (2007 Penn State iGEM Project)

The first approach used a mutated version of the protein CRP called CRP*, which acts as CRP bound to cAMP. This means that our system would always be "turned on" when xylose is present, even if glucose is also present. Problems encountered:
•CRP* is not specific to the xyl operon, it acts as a global regulator.
•Systems turned on or off irregularly, possibly having toxic consequences

2) Selectively engineering the promoter region

This year we focused on constructing and characterizing engineered alterations of the xyl promoter region. We attempted to engineer the promoter region so only XylR transcriptional control remained by:
•scrambling the CRP binding site by random base changes, eliminating CRP binding
•strengthened the RNA polymerase binding sites to compensate for the loss of CRP promotion



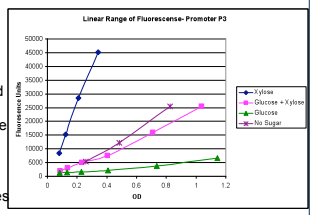


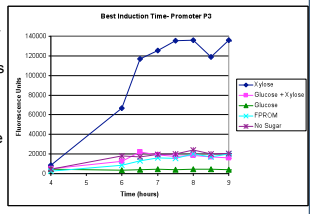
The binding affinity was strengthened by changing a few bases in the polymerase binding region. This was done in steps so that the regions began to resemble a consensus sequence for *E. coli* RNA polymerase binding. Depending on the mechanism(s) of interaction between XylR and RNA polymerase components, too strong of a promoter may result in high levels of constitutive expression (in the absence of xylose), which is undesirable.
PN - This natural promoter is necessary for testing comparisons with altered region promoters.
P1 (weakest) This promoter should show weak RNA polymerase binding and transcription and will help to further elucidate the roles of CRP and XylR in xyl promoter expression.
P3 (medium) The RNA polymerase binding site is a combination of the wild-type and consensus binding sequences.
P5 (strongest) This promoter may be constitutive even in the absence of xylose.

Results

Each test construct (promoter + GFP) was cloned into the pSB1A2 plasmid and transformed into several *E. coli* strains: DH5α, W3110 ΔxylB-G, and W3110 ΔxylB-R. Fluorescence readings were used to measure the levels of induction.

Preliminary induction studies were run to find the optimal induction time and to analyze the linear range for OD versus fluorescence. These graphs are examples of the data we obtained for each of the promoters in DH5α cells grown in LB media. Each promoter gave similar results, and we selected a seven hour induction time and fluorescence testing at 0.2 OD dilution.





In Progress

New Tests

Initially tests were performed in the strain DH5α , which contains the entire xyl operon in the chromosome. Xylose metabolism in this strain thus poses a problem during the characterization of our constructs. Eight new tests are being completed to show the effects of sugar induction, XylR expression, and XylE expression.

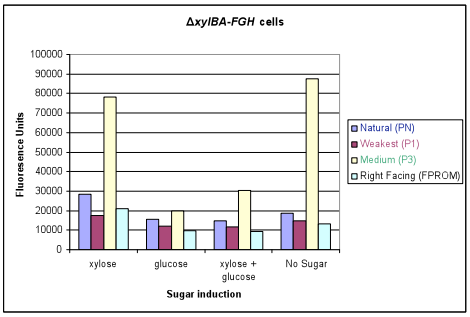
New Constructs

We are currently constructing a plasmid that constitutively express *xylR* and *xylE* genes. Intracellular levels of XylR may be too low to accommodate the high copy plasmid. Native xylose transport genes are also subject to glucose repression, so XylE will be independently expressed at high levels to ensure xylose uptake in the presence of glucose.

New Strains

•Strain W3110 Δ*xylB-G* carries deletion in xylose metabolism (*xylA* and *xylB*) and transport (*xylFGH*) genes.
•Strain W3110 Δ*xylB-R* is similar to the Δ*xylB-G* strain but also does not express XylR, and will serve to further study the role of XylR.

Both of these cell strains will not exhibit any xylose metabolism or transport, and therefore do no deplete xylose available for activation of the test constructs.



The promoters were also tested in the W3110 Δ*xylB-R* strain and graphs of the data are very similar to the results above.

The high fluorescent levels of "No Sugar" may indicate constitutive expression independent of XylR activation. Alternatively, chromosomal XylE transport of xylose is insufficient, hindering activation.

Induction levels in the presence of glucose are low in both strains indicating that glucose repression is still occurring even with the scrambled CRP DNA binding region. The similar results in the different cells (with and without *xylR*), may indicate that there is no interaction between XylR and the RNA polymerase. Our next steps are to retest in minimal media as well as constitutively expressing XylE.

Conclusions

Understanding the behavior of these constructs will provide valuable insights into the natural control region and its dependence on both xylose transport and XylR concentration for induction. These studies will also help us further characterize our engineered promoters, and to find the conditions where glucose/xylose diauxie is eliminated.

One of our hypotheses is that XylR interacts with RNA polymerase to strengthen its binding. Xylose-bound XylR should positively regulate transcription, and the hope is that a strengthened promoter sequence will still require activation by XylR (and not just increase constitutively).

We have also proposed a third strategy involving the xyl transcriptional control region from *Bacillus sp.* In this system XylR (different from the XylR produced by *E. coli*) is known to act as a repressor; xylose binding to XylR derepresses and enables RNA polymerase binding and transcriptional activation.

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