A Synthetic Biology Approach to Bioremediation of Tailings Ponds

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Introduction

Tailings ponds containing toxic by-products are a common environmental problem for any industrial mining operation and have existed as long as the processes themselves. Alberta’s oil reserves make up the second largest proven reserves of oil in the world1 and are in the form of tar sands, a sand, clay, water, and bitumen mixture2. The bitumen is separated from the sticky tar sand by washing with hot water where the heated water acquires toxins; the resulting mixture is then stored in the form of toxic ponds. Tailings ponds contain many harmful chemicals such as aromatic acids, catechol, and heavy metals. Catechol is a common intermediate in degradation pathways of toxins present in tailing ponds and is often utilized for funneling these pathways into metabolizable compounds, as a result catechol can be used to create a broader, more efficient remediation process. Here, we are targeting catechol for degradation into a common metabolic intermediate that can be fed into the Krebs Cycle by using yxIE from Pseudomonas putida that degrades the aromatic catechol-2,3-dioxgenase (yIE). To reduce cross-talk and increase the system’s efficiency yxIE will be targeted into micromercompartments, formed from an engineered Aquifex aeolicus protein, lumenase synthase (LS). This complex can then be puriﬁed and applied to the tailings for catechol degradation. By funneling other pathways through catechol we can develop efﬁcient methods for deacremination of tailings ponds.

Chemical Degradation

We are using catechol as the general entry point for toxin degradation by using yxIE, which is able to degrade catechol into 2-hydroxyacetone and salicylic acid (2-HS), a bright yellow substance that can be metabolized by the cell. This will allow for the degradation of the products of yxIE pathways that degrade toxins such as aromatic acids into catechol. This will increase both the efﬁciency of bioremediation and the types of compounds that can be degraded.

Figure 1. Initial kinetic analysis of yxIE following the production of 2-HS. The production of 2-HS by different concentrations of S30 (cell lysate) extract followed at 375 nm. Green and red lines are 2 μM, and 4 μM of S30 cell extract, respectively, from cells expressing yxIE. The blue line is 2 μM, control S30 cell extract. These measurements provide critical information for future metabolic control analysis and is a prerequisite for any metabolic engineering for the use of catechol as a general entry point into the bacterial metabolism.

Table of Contents

Chemical Degradation

Compartmentalization

The compartment we are using is composed of a single protein (lumenase synthase from Aquifex aeolicus) that forms an isocitrate dehydrogenase by assembling 60, 120, or 180 monomers3. Previous studies have shown that by selectively mutating ﬁve of the interior amino acids of the compartment to guanidylate, and by attracting a positively charged tet arginine tail (R4) to the C-terminus of the protein, that the tagged protein can be selectively targeted into the compartment. Based on this approach we will be using these features to selectively target yxIE into the compartment.

DNA Degradation

To reduce the risk of genetic propagation we will degrade the DNA of the organism rendering it unable to replicate. In 2007, UC Berkeley submitted a Biobrick containing an inducible endonuclease BanFl gene that when induced will degrade all DNA in the cell. UC Berkeley demonstrated that expression of this protein does not hinder the function of already translated proteins which is important for our team due to our project relying on functioning proteins.

Future Directions

• Compartmentalization
  • Assamble co-expressing tagged CFP and YFP constructs and characterize
  • Assemble a micromercompartment test construct in Figure 28 and characterize
  • Optimize LS micromercompartment localization conditions

• Catechol Degradation
  • Assemble an yxIE construct with the tag for LS micromercompartment localization
  • Optimize catechol degradation by determining the effects of factors such as yxIT
  • Determine methods for application to tailings ponds

• Computational simulation is necessary for future metabolic control analysis and will be based on the 2-HS data as the general entry point into bacterial metabolism

Submitted Parts

Characterized Parts

Judging Criteria

+ Had a fun summer, now we are here!
+ Completed and submitted the iGEM 2010 judging form
detailed and shared the team project description
+ Presented a poster and presentation
+ DNA submitted for our new Biobrick parts
+ Demonstrated the function of new Biobrick parts
+ Characterized existing Biobrick parts
+ Detailed our Human Practices work
+ Collaborated with teams such as Calgary for parts characterization
+ Addressed the iGEM safety questions
+ All work demonstrated was done by the Lethbridge 2010 iGEM undergraduates
+ Ask us about the Artsmith part of our project, it demonstrates another approach for using scientific methods and data

References

Thanks to Nathan Puhl Hamad Sadeghi, and Biochemistry Teaching lab and coordinators, the Department of Chemistry and Biochemistry and the Faculty of Arts and Science.

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Acknowledgements