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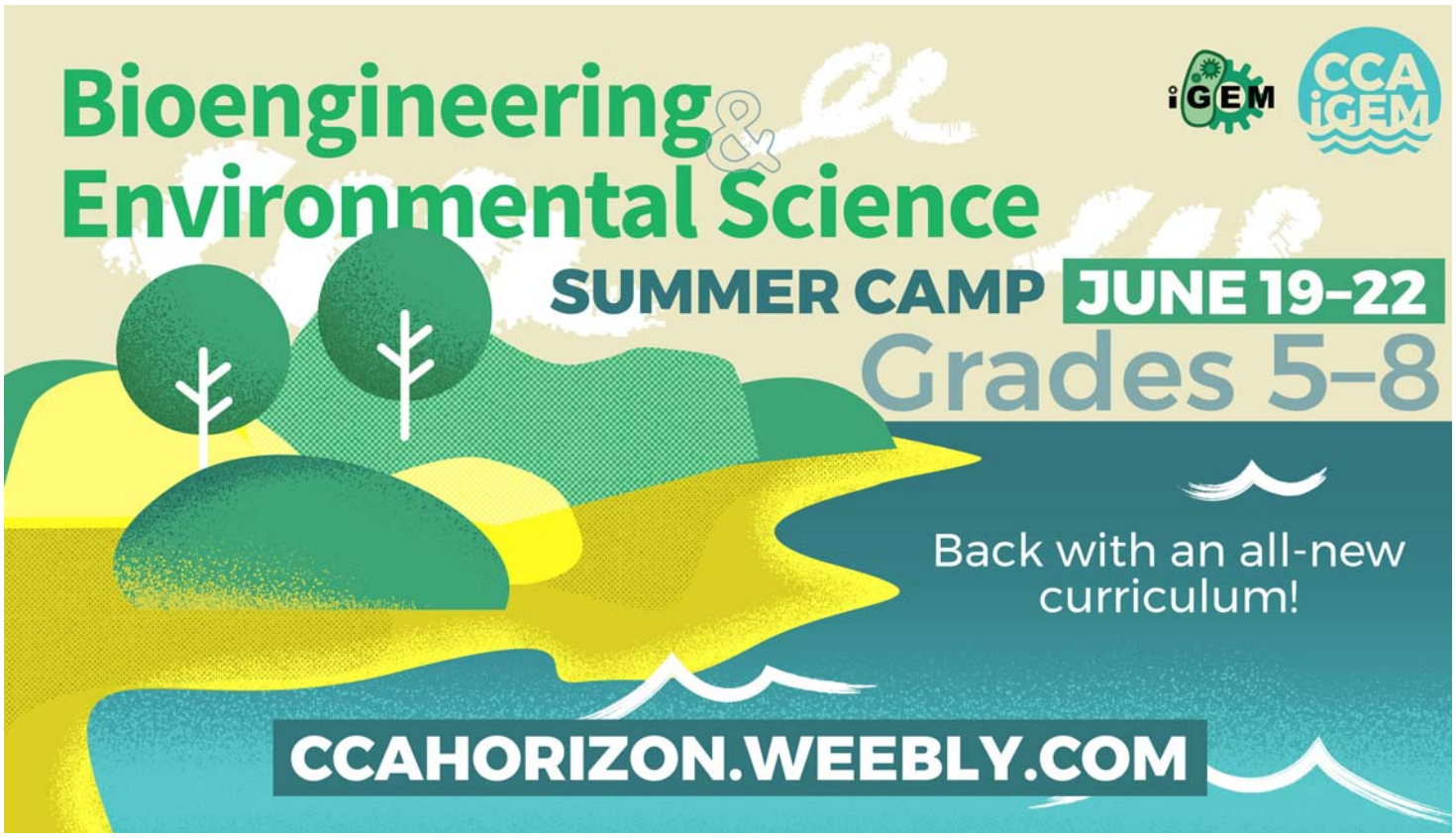
Bioengineering & Environmental Science
iGEM
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SUMMER CAMP JUNE 19–22
Grades 5–8
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CCA San Diego

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Canyon Crest Academy , San Diegiuto Union High School District

imPAHct

Efficient Bacterial System for the Degradation of Polycyclic Aromatic Hydrocarbons in Crude Oil

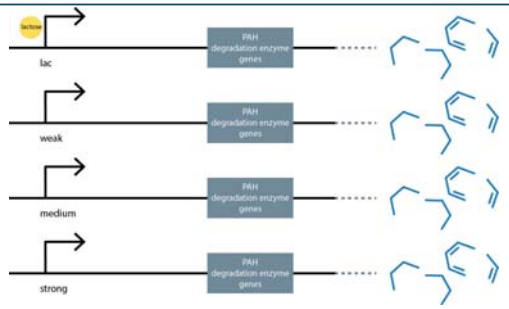
Introduction

Contamination of aquatic and terrestrial environments with crude oils and industrial chemicals is a problem worldwide. Crude oil is composed of **polycyclic aromatic hydrocarbons** (PAHs), compounds that are difficult to degrade and environmentally harmful. It is crucial to find a timely and cost-effective procedure that is commercially viable to catabolize some of the most prevalent, toxic PAHs – **fluorene and phenanthrene**– to innocuous compounds, such as salicylate and phthalate, which are catabolized in bacterial metabolic cycles.

We have designed a novel methodology for the degradation of multiple PAHs by aggregation into E. coli of the most important **catabolic genes** from multiple catabolic pathways upstream of common innocuous intermediates. This methodology allows **broad spectrum transformation** of PAHs within an oil environment into safer residues. We have conducted a series of biotransformation experiments to measure the efficacy of the newly engineered strains containing phenanthrene and fluorene degradation synthetic pathway, demonstrating the bacteria's ability to harness the PAHs as a **carbon source** and ultimately degrade the compounds.

These bacteria have **commercial applications** and can be incorporated in oil spill remediation and bioreactor use, achieving detoxification through combinatorial genetic bioremediation.

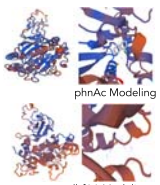
Inducible and Constitutive Promoter Preparation



The optimized sequences were ligated and transformed under an inducible promoter for controlled experiments and a constitutive promoter for general enzymatic expression. We cloned two fragments in varying permutations to identify discrepancies in expression originating from the organization of the sequences and to troubleshoot for potential toxicity issues caused by buildup of intermediates.

We tested our bacteria's ability to degrade fluorene and phenanthrene by growing colonies in minimal media with fluorene or phenanthrene as the carbon sources. We also tested the growth of our bacteria using crude oil drilled from Pennsylvania, Ecuador, and Saudi Arabia.

Modeling



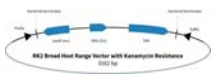
phnAc Modeling

dbfA1 Modeling

We analyzed the dioxygenases that initiate both the phenanthrene and fluorene pathways that lead into the reaction chains. The following are models using SWISS_MODEL based on crystallized alignments. Structure of the potential active site is shown with a mononuclear iron (orange sphere).

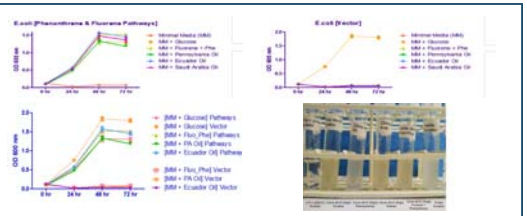
Broad Host Vector

pSB3K3 → BBa_K2491030



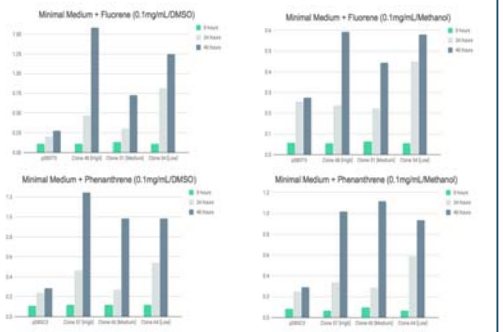
Consists of an origin of replication, oriV, and the plasmid replication initiator, TrfA protein. The copy number of RK2 is about 4-7 per cell in E. coli, 3 in Pseudomonas aeruginosa, and 4-7 in Agrobacterium. Teams may now have an additional broad range, low-copy system within a wide variety of bacterial strains.

Degradation of Crude Oil Evaluation



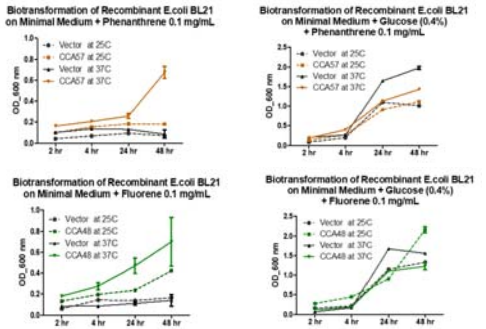
Oil derived from Pennsylvania, Ecuador, and Saudi Arabia was introduced to our phenanthrene and fluorene pathways in a two-plasmid system or a control vector. The results with and without glucose are displayed.

Efficiency of Varying Promoter Strengths



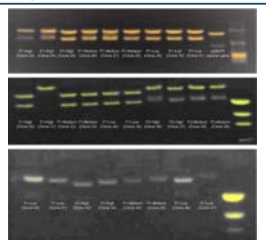
Growth of recombinant E. coli BL21DE3 cultures harboring a control plasmid or fluorene (top)/phenanthrene (bottom) pathway under three differently strengthened constitutive promoters. Data points represent value averages of duplicate of OD at 600 nm taken over time: 2 independent colonies per clone.

Recombinant E Coli. Growth via PAH and Glucose Inoculation in Varying Temperatures



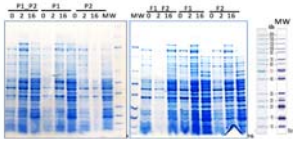
The best clones were selected (Clone 57 - high) and (Clone 48 - high) and temperature was measured as an effect on growth rates against a control vector in phenanthrene and fluorene respectively.

Cloning Verification



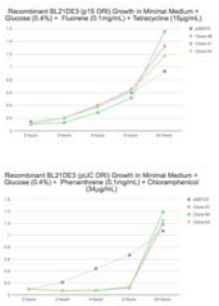
Verification of cloned inserts for both pathways - P1 and P2 for phenanthrene + F1 and F2 for Fluorene.

Protein Verification



The catabolic genes were cloned under the control of a modified T7 promoter expressed in E. coli BL21(DE3). Aliquots of the cultures were taken at time 0, 2, and 16 hours after induction from constructs containing the full pathway (P1_P2 and F1_F2) or fragments (P1, P2, F1, or F2) and were analyzed by SDS-PAGE gels. Differences in band patterns between time points can be attributed to synthesis of recombinant proteins.

Efficiency of Toxicity Reduction



In order to examine the role of toxicity and metabolic burden of PAH pathways, glucose was added to correct for limited growth due to a lack of a carbon source. Clone 57, 60, and 64 are high, medium, and low strength promoters, respectively. Temperature was set at 37°C for optimal growth.

Constructs

Research Summary

1. Microorganisms identified through genomic search
2. Operons were designed as synthetic genes.
3. Catabolic pathways were cloned into a RK2 plasmid.

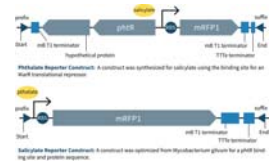
Fluorene and Phenanthrene Mining

Each PAH degradation sequence was split into two parts and then combined to make a polycistronic sequence. We used RBSs between the open reading frames of the pathway.

Conclusion

- **High-strength constitutive promoters** are deemed the most effective - as seen by the higher growth rates in bacteria under CCA-48 and CCA-57 in phenanthrene and fluorene.
- Pathways have a significant impact on bacteria survival/growth rate through use as a carbon source. They **limit toxicity** within the environment. The middle-strength constitutive promoter creates the least toxic environment.
- Higher temperatures demonstrate **better degradation rates of PAH**s and growth rates of bacteria. Therefore, wastewater should be heated before treatment.
- PAHs are used effectively as a **carbon source** by our synthetic bacteria, signifying the completion of the pathway to pyruvate.
- PAH synthetic pathways can of PAH levels within their environments. **effectively minimize toxicity**
- PAHs within bacteria containing pathways can be implemented as a carbon source almost as effectively as a simple sugar like glucose, signifying high output rate and pathway efficiency.

Future Directions



Implementation of a RFP quantifiable reporter plasmid designed to test the products of our synthetic pathway for confirmation of product formation and PAH degradation.

Further studies on efficiency in multiple strains using RK2.

Human Practices

Silver Human Practices//Public Engagement

CCA iGEM Summer Camp: generate funds and inspire a passion for synthetic biology in young scientists

STEM Days: expose concepts of our research to wider audiences

Community Survey: gauge public opinion and engage with people in the field related to our research

Bioethics Seminar: discuss ethics of our genetically engineered bacteria in the environment

Synthetic Biology Kits: further introduce younger audiences to biology

Lab Fellows: followed safety protocols and always wore PPE

Gold Human Practices

Argonne National Laboratory: influenced our applied design with addition of sponge

Synthetic Genomics: introduced novel method to evaporate the crystals from PAHs

BP Biosciences Center: combined heavy mechanical cleanup with microbial approach

Roger Prince: discussion regarding the mechanics of our degradation pathway

Beach Cleanup: guided us to use crude oil samples and really develop our project for application in a real type of ecosystem.

Collaborations

iGEM Goes Green (an initiative by TU Dresden): to minimize the carbon footprint of our project (planted a fig tree on our campus)

University of Bristol: discussed the construction of our wiki.

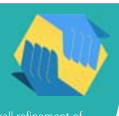
University of Nebraska-Lincoln: Made adjustments to their document "Safety Cases and Their Use in iGEM Competitions Feedback" that helped other iGEM teams.

Nazarbayev University, Astana, Kazakhstan: Helped broaden the scope of our project through their insight and advice.

Acknowledgments

All experiments were designed and performed by CCA_San_Diego iGEM team.

Mr. Ariel Haas, Dr. Gerstin, and Ms. Erin Eddingfield, our school affiliates.
Adriane Barros, our iGEM mentor.
Joanne Couvrette and the rest of the CCA Foundation.
All the CCA clubs, companies, and individuals who guided or helped us in the overall refinement of our iGEM team's human practice initiatives or lab work.



- *Developed:* **an innovative approach** for gene augmentation to target degradation of multiple toxic compounds in crude oil
- *Experimentally validated:* our synthetic catabolic pathways for fluorene and phenanthrene **can degrade crude oils** of multiple sources
- *Conceived:* a **shuttle system** to transfer genes in different bacteria
- *Developed:* **modeling of first enzyme** of the catabolic pathway to identify amino acid residues responsible for specificity to PAHs
- *Conceived:* **an absorption system** that amplified the bioremediation system
- *Established:* **a simple approach** for detection of degradation of polycyclic aromatic hydrocarbons