Using Functional Genomics and Metagenomics in the Undergraduate Classroom as a Bridge to Research Experiences.
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Abstract

In Fall 2013, a course titled Functional Genomics in Bacteria was taught at CSU San Bernardino. This small, laboratory-focused course allowed students to pursue two distinct group projects in functional genomics and metagenomics. The students participated in all stages of development of both projects. One project focused on identifying elements of the Variovorax paradoxus EPS flagella apparatus by heterologous host complementation. The polar flagellum of this microorganism has been identified biochemically, but the genes were not identified in the genome annotation process. Several non-mobile flagella knockout strains in E. coli were received from the CGSC (New Haven) and strains of ACC deaminase from V. paradoxus EPS were generated in pBAD24 and pBBR1MCS. The construction and evaluation of these libraries was carried out during the quarter, and then followed up by students from the class after the term ended. A second project focused on enriching for Aminocyclopropane-1-carboxylic acid (ACC) deaminase activity from the soil metagenome. ACC deaminase is a key enzyme involved in plant growth promotion by disrupting the ethylene stress hormone synthesis pathway. ACC can also serve as a nitrogen source in culture, as the enzymatic activity yields ammonium and alpha-ketoglutarate. On this basis, an enrichment strategy was devised. Soils were collected and DNA extracted. The DNA was introduced into E. coli using a fosmid library (CopyControl, Epicentre) and the library was validated and sampled. The ACC deaminase activity by growth on medium containing ACC as a sole carbon source. Students in this course learned advanced molecular technologies as well as the use of bioinformatics resources and tools, and were directly involved in the planning and execution of an original, publishable research project. Two of the Fall students involved in this project are currently pursuing graduate degrees, reflecting that “real-world” research experiences in the classroom may stimulate increased participation in STEM careers.

Introduction

Goals of the class:
• Students plan and execute of experiments
• Troubleshoot and evaluate different approaches
• Identify genes in Variovorax paradoxus that can complement defects in Escherichia coli flagella
• Clone genes directly from the environment that encode putative ACC deaminase enzymes

Background

Functional Genomics project
• Variovorax paradoxus is a beta-proteobacterium that has been identified in many contexts, involved in human oral health, plant growth promotion, and degradation of environmental pollutants.
• No flagellar operon (but a T3SS) was identified in V. paradoxus EPS genome. Flagellar loci present in other V. paradoxus strains
• Flagella are present
• Strategy – heterologous host complementation in E. coli mutants (ΔfliA, ΔfliI, ΔfliF, ΔflhB) with partial digest genomic library

ACC Deaminase project
• ACC Deaminase is a key enzyme in PGP activity, modulating plant stress response by interfering with ethylene synthesis.
• Hypothesis – these genes are widespread in bacteria in arid rhizosphere
• Strategy – enrich for acc-deaminase expressing recombinant E. coli carrying fosmid clones from soil metagenomic library in nitrogen limited media

Experimental Procedure

Flagella gene complementation

Experimental Data

Flagella background information

Future Directions

Teaching class again this fall
Follow up on both experiments
• Sequencing to identify productive insertions
• Subcloning to identify specific complementing genes
• Use libraries to identify additional activities
• Write up experiments for publication

Experimental Outcomes

Recombinant E. coli with defects in swarming were partially complemented with plasmids containing Variovorax paradoxus chromosomal DNA using all 4 mutant strains
Recombinant E. coli that grow on M9 with ACC as sole nitrogen source were recovered and passaged
In both cases still need to isolate and identify genes