Constructing genomic libraries for soil metagenomic analysis

Candice N. Farnham[a], Nichole Caprio[a], Shomari Mays[a], Frazier Moore II[a], Jeremy P. Nelson[a], Sarah Sporing[a], Monserrat Serrano, Stephanie Stoelb & Jeanne M. Wages[a]

Abstract

Soil is one of the richest sources for microbial diversity and one of the most challenging of environments from which to successfully culture and study the microbes present. From an agricultural standpoint, soil microbes carry out a number of essential biochemical reactions necessary for plant survival, including nitrogen fixation and phosphorous solubilization. All of the necessary components to support plant life are present in Martian soil; however, they are not present in sufficient quantities to support plant life without heavy supplementation with fertilizers. Using metagenomic analysis, we hope to identify enzymes responsible for increased production of these nutrients and/or enzymes that function in bioremediation. We are using a direct cell lysis and purification approach to isolate genomic DNA (gDNA) from soil microbes and creat genomic libraries. We will use these genomic libraries to screen for individuals that grow on medium supplemented with different forms of nitrogen and phosphorous as well as different concentrations of nitrogen and phosphorous. We will isolate DNA from selected isolates and sequence the DNA to identify enzymes of interest. Those enzymes of interest will be cloned into new isolates to verify the phenotype before proceeding with enzymatic studies.

Introduction

Soil bacteria are incredibly diverse. One gram of soil may contain as many as 4 x 10^7 bacterial cells and may contain anywhere from 2-18,000 unique prokaryotic genomes[1,2]. These microbes carry out a number of essential reactions that support plant life. In addition, recent studies have identified soil microbes that produce new antibiotics[3] and function in bioremediation[4]. The unique composition and properties of soil make it difficult to successfully cultivate many prokaryotes that inhabit soil. Therefore, we are using metagenomic analysis to isolate DNA directly from soil prokaryotes to better survey prokaryotic diversity.

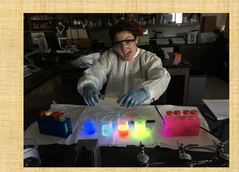
Martian soil contains all of the necessary components to support plant life, but these compounds are not present in sufficient quantities to support plant life without heavy fertilizer use. Fertilizer overuse would contribute to significant perchlorate levels present in run-off that would have to be purified from water for human consumption. Through metagenomic analysis we hope to identify enzymes that may increase nitrogen fixation efficiency to better support plant life and/or enzymes that function in bioremediation to metabolize perchlorates from fertilizer use.

Materials & Methods

Soil Samples: Soil samples were taken from fields at the University of Kentucky, an organic garden and 2 fields from Boston, KY.

Metagenomic DNA isolation: Metagenomic DNA was isolated directly from soil using the PowerSoil™ DNA isolation kit from MoBio. Samples were digested with HindIII, EcoRI and BamHI and DNA quality was analyzed with gel electrophoresis.

Cloning: Metagenomic DNA was digested with BamHI and EcoRI and gel purified using the Qiaquick gel extraction kit. The pUC19 vector was digested with BamHI and EcoRI and gel purified using the Qiaquick gel extraction kit. Metagenomic DNA and pUC19 were ligated using NEB Quick Ligase according to the manufacturer's instructions. Ligations were transformed into OneShot® Mach1™ chemically competent E. coli and selected on LB + Ampicillin plates supplemented with 1.6 mg X-gal. Samples were incubated at 37 °C and assayed for transformation efficiency.



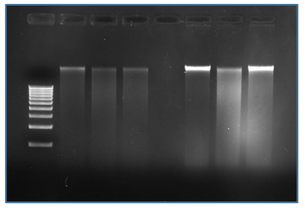

Research Plan & Results

Collect Soil DNA

Soil samples were collected from UK and from an organic garden.

Separation of cells from soil samples
Cell Lysis & DNA purification

Metagenomic DNA



Fragmentation of DNA

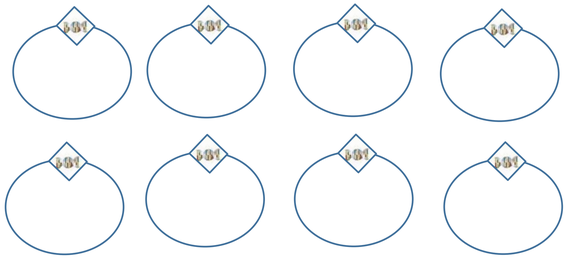
Excision of DNA (2-4 kb)

Metagenomic DNA isolation. DNA was isolated using the MoBio Power Soil DNA isolation kit. Samples were digested with HindIII, BamHI or EcoRI and run on a 1% agarose gel to visualize the DNA.

Linearize Cloning Vector

Ligation

Collect recombinant plasmids for genomic library



Screen library for the ability to grow on altered Nitrogen and Phosphorous

Screen library for bioremediation potential

Conclusions & Future Directions

We have isolated metagenomic from 4 sources in KY. We have tested different restriction enzymes to identify ones that successfully cut metagenomic DNA into 2-5kb fragments. We have purified these fragments and have subcloned this DNA into pUC19 vector. Moving forward, we will scale up our ligations and subcloning to generate genomic libraries. With these libraries in place, we can set up phenotypic screens to identify E. coli that can metabolize alternate sources of nitrogen and phosphorous. In addition, we will identify isolates that can eliminate perchlorates through bioremediation. We will then sequence the metagenomic DNA and identify candidate genes responsible. Candidate genes subsequently will be analyzed to confirm the phenotype.






References Cited

1. Richter, D. D. & Markewitz, D. How deep is soil? Bioscience *45*, 600–609 (1995).
2. Daniel, R. The metagenomics of soil. Nature Reviews *3*, 470-478 (2005).
3. Ling, L. L. et al. A new antibiotic kills pathogens without detectable resistance. Nature *517*, 455-459 (2015).
4. Drewniak, L. & Sklodowska. Arsenic-transforming microbes and their role in biomining processes. Environ Sci Pollut Res Int. *20*, 7728–7739 (2013)

Affiliations

a. Students supported by NASA Kentucky Space Grant Consortium. Grant NNX14AR11A. Subaward 3048112042-15-075