Development of Archaeal and Algalytic Bacteria Detection Systems

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I. Introduction

Natural gas (methane) is emerging as a viable power source for many industrial, commercial, and domestic applications. Bio-methane provides a promising replacement for mined natural gas. Methanogenic bacteria produce this bio-methane. These anaerobic bacteria pertain to the Domain Archaea, and are found in extreme environments where few other bacteria survive. They are employed by Up-Flow Anaerobic Sludge Blanket (UASB) reactors in the digestion of wastes to a marketable product (methane). The genome of methanogenic bacteria can be amplified using polymerase chain reaction (PCR), a synthetic DNA replication system. This system employs specific sequences of DNA called primers. The primers employed in this study focused on 16S rRNA amplification providing a fingerprint of the organism's identity. Previous design of these primers was unsuccessful and resulted in non-specific binding.

II. Objective

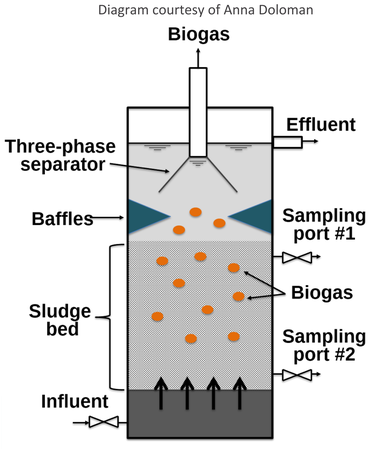
Design a new set of primers and develop a PCR protocol that will allow identification of archaebacteria.

III. Methods

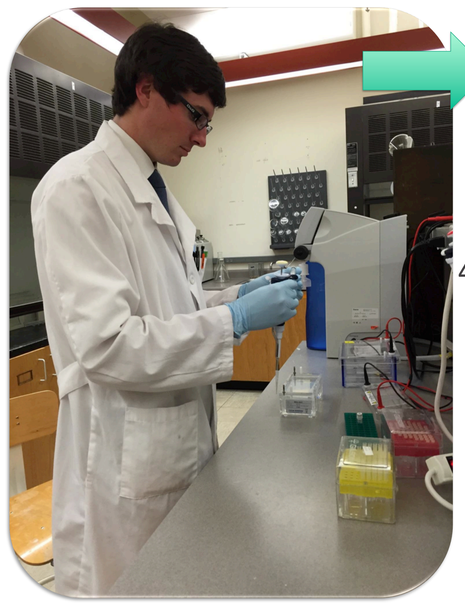
1. Obtain samples containing archaebacteria from methane producing UASB reactor
2. Extract DNA with a commercially available kit (MO BIO PowerSoil® DNA Isolation Kit)
3. Identify archaeal primers through literature search and purchase primers
4. Adjust PCR protocols for optimal amplification
5. Verify amplification of DNA via gel electrophoresis
6. Clone amplified DNA into pCR™4-TOPO® vectors
7. Transform electrocompetent *E. coli* cells with vector
8. Culture transformed cells with vector on antibiotic-containing plates
9. Extract plasmids from selected colonies
10. Use the Basic Local Alignment Search Tool (BLAST) and National Center for Biotechnology Information (NCBI) database to identify DNA sequences

Flow Chart of Methods

UASB Reactor
Diagram courtesy of Anna Doloman

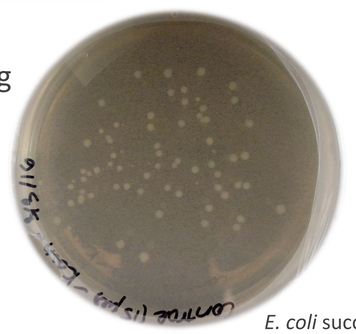


1) Obtain samples and extract DNA



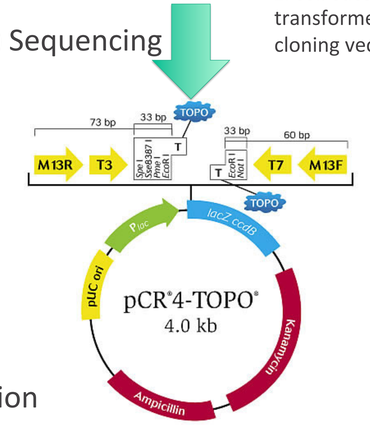
2) PCR, Gel Electrophoresis, and Purification

3) Cloning



E. coli successfully transformed with cloning vector

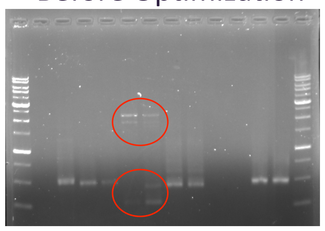
4) Sequencing



Cloning vector

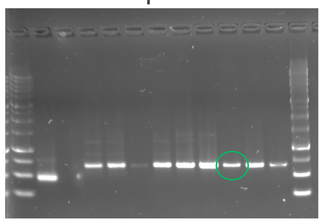
IV. Results

Before Optimization



Gel electrophoresis of isolated DNA products using varied primers:
Primers U519F and ARCH806R
Wells from left to right:
1) DNA Ladder; 2) Negative control; 3,4,5) Universal primer set with DNA template 1; 6,7) Archaeal primer set; 8,9) Universal primer set with DNA template 2; 10,11) Universal primer with no DNA; 12,13) Universal primer with DNA template 3; 14) DNA Ladder

After Optimization



Temperature gradient gel electrophoresis of isolated DNA products:
Primers A571F and UA1204R
Wells from left to right:
1) DNA Ladder; 2) Universal primer set; 3) Negative control; 4) 50°; 5) 51°; 6) 52°; 7) 53°; 8) 54°; 9) 55°; 10) 56°; 11) 57°; 12) 58°; 13) DNA Ladder

BLAST Results

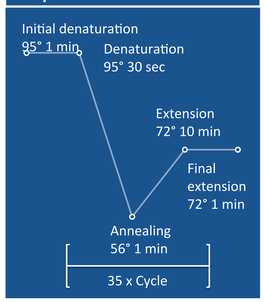
Sample:	Result:	% Match:
1	Uncultured euryarcheote clone	98%
2	Uncultured archaeon clone	99%
3	Uncultured archaeon clone	98%
4	Uncultured *Methanomicrobiales* clone	98%
5	Uncultured euryarcheote clone	99%
6	Uncultured crenarchaeote clone	94%
7	Uncultured *Methanolinea* sp. clone	99%
8	Uncultured archeon clone	94%
9	Uncultured crenarchaeote clone	99%

V. Conclusions

- Obtained clones with archaebacterial 16S rRNA from the environmental samples
- Achieved specific binding of archaea-targeting primers A571F and UA1204R
- Found optimal PCR reaction conditions for archaebacterial primers

PCR Reaction:	
Nuclease Free H_2O	35 µL
10x Taq Buffer	5 µL
$MgCl_2$ (25 mM)	2.5 µL
dNTP (10 µM each)	1 µL
Primers (10 µM)	2 µL each
DNA (29 ng/mL)	2 µL
Taq Polymerase (5 units/µL)	.5 µL

Optimized PCR Conditions:



VI. Further Studies

Apply presented methods to:
- Identify algalytic bacteria
- Optimize bio-methane production

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Cloning Vector Reference: https://www.thermofisher.com/order/catalog/product/K457502

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