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 that will allow identification of archaebacteria.

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 methanogenic bacteria called primers. These primers were 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.

2. Extract DNA with a commercially available kit (MO BIOPowerSoil® DNA Isolation Kit).

3. Identify archaebacterial 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 containing plates.

Bacteria Detection Systems

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Blindfolds and extract DNA

1) Obtain samples
2) PCR, Gel Electrophoresis, and Purification
3) Cloning
4) Sequencing

Flow Chart of Methods

UASB Reactor

Biogas

Three-phase separator

Effluent

Rattles

Sampling port #1

Sampling port #2

Sludge bed

Influent

3) Cloning

E. coli successfully transformed with cloning vector

4) Sequencing

PCR Reaction:

Nuclease Free H2O: 35 µL
10x Taq Buffer: 5 µL
MgCl2 (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: 95° 1 min Denaturation

Annealing

Extension

95° 30 sec

56° 1 min

72° 10 min

35 x Cycle

Optimized PCR Conditions:

95° 1 min Denaturation

Annealing

Extension

Final extension

35 x Cycle

Plasmid DNA

4.0 kb

pCR®4-TOPO®

Cloning vector

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IV. Results

Before Optimization

After Optimization

V. Conclusions

• Obtained clones with archaebacterial 16S rRNA from the environmental samples

• Achieved specific binding of archaea-targeting primers AS71F and UA1204R

• Found optimal PCR reaction conditions for archaebacterial primers

VI. Further Studies

Apply presented methods to:

• Identify algalytic bacteria

• Optimize bio-methane production

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