Development of Archaeal and Algalytic Bacteria Detection Systems

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 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

Flow Chart of Methods

UASB Reactor

Three-phase separator

Effluent

Biogas

Sampling port 1

Sampling port 2

Biogas

Sludge bed

Influent

Baffles

Biogas

4) Sequencing

Cloning

E. coli successfully transformed with cloning vector

PCR, Gel Electrophoresis, and Purification

pCR™4-TOPO®

4.0 kb

3) Cloning

Dual cloning

Cloning vector

BLAST Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result</th>
<th>% Match</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uncultured euryarchaeote clone</td>
<td>98%</td>
</tr>
<tr>
<td>2</td>
<td>Uncultured archaeon clone</td>
<td>99%</td>
</tr>
<tr>
<td>3</td>
<td>Uncultured archaeon clone</td>
<td>98%</td>
</tr>
<tr>
<td>4</td>
<td>Uncultured Methanomicrobiales clone</td>
<td>98%</td>
</tr>
<tr>
<td>5</td>
<td>Uncultured euryarchaeote clone</td>
<td>99%</td>
</tr>
<tr>
<td>6</td>
<td>Uncultured crenarchaeote clone</td>
<td>94%</td>
</tr>
<tr>
<td>7</td>
<td>Uncultured Methanolinea sp. clone</td>
<td>99%</td>
</tr>
<tr>
<td>8</td>
<td>Uncultured archaeon clone</td>
<td>94%</td>
</tr>
<tr>
<td>9</td>
<td>Uncultured crenarchaeote clone</td>
<td>99%</td>
</tr>
</tbody>
</table>

IV. Results

Before Optimization

Gel electrophoresis of isolated DNA products using varied primers:
- Primers A571F and UA1204R
- 10x DNA Ladder
- Universal primer set
- 3, 4, 5 Universal primer set with DNA template 2
- 10, 11 Universal primer with no DNA
- 12, 13 Universal primer with DNA template 2
- 14 DNA Ladder

After Optimization

Temperature gradient gel electrophoresis of isolated DNA products:
- Primers A571F and UA1204R
- 10x DNA Ladder
- Universal primer set
- 1, 2, 3 Universal primer set with DNA template 1
- 10, 11 Universal primer with no DNA
- 12, 13 Universal primer with DNA template 2
- 14 DNA Ladder

V. Conclusions

- Obtained clones with archaebacterial 16S rRNA from the environmental samples
- Achieved specific binding of archaebacteria-targeting primers A571F 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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Cloning Vector Reference: https://www.thermofisher.com/order/catalog/product/K457502

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