Aug 9th, 10:15 AM - 12:00 PM

Identification of nitrifying bacteria contained in a commercial inoculant using molecular biology techniques

Anthony Harrington  
*University of Nevada, Las Vegas*

John Perry  
*University of Nevada, Las Vegas*

Penny S. Amy  
*University of Nevada, Las Vegas, penny.amy@unlv.edu*

Repository Citation  
http://digitalcommons.library.unlv.edu/cs_urop/2011/aug9/15

This Event is brought to you for free and open access by the Undergraduate Research at University Libraries. It has been accepted for inclusion in Undergraduate Research Opportunities Program (UROP) by an authorized administrator of University Libraries. For more information, please contact marianne.buehler@unlv.edu.
Identification of Nitrifying Bacteria Contained in a Commercial Inoculant Using Molecular Biology Techniques

By Anthony Harrington, John Perry, and Penny S. Amy
University of Nevada, Las Vegas-School of Life Sciences

Introduction

Nitrifying bacteria play an important role in the aquatic and terrestrial nitrogen cycle. Nitrification, one of the processes of the nitrogen cycle, refers to the oxidation of ammonia to nitrite. This process requires two types of chemosynthetic bacteria: ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). These bacteria are essential as they supply nitrate for the growth of plants and aquatic organisms.

Current applications of nitrifiers include: inoculants for aquaria, bioremediators, and nitrogen removal in wastewater treatment plants. Previous studies have shown that Fritz-zyme Turboost 700, a 

Materials and Methods (continued)

Table 1. Sequences of Primers Used for PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>27f</td>
<td>AGAGTTTGATCCTTGCCAG</td>
<td>Bacterial 16s rDNA gene</td>
</tr>
<tr>
<td>1612f</td>
<td>GCTGACCTTGTAGGACCTT</td>
<td>Bacterial 16s rDNA gene</td>
</tr>
<tr>
<td>Nu1022R’</td>
<td>CCGGAATTCAGTCGCTGTTGA</td>
<td>AOB 16s rDNA gene</td>
</tr>
<tr>
<td>NIT3</td>
<td>CCTCGGCCAGCTGCTGCTGCA</td>
<td>Nitrobacter 16s rDNA gene</td>
</tr>
<tr>
<td>Ntpcd85R’</td>
<td>CGGGAAATTCCGGCCCTC</td>
<td>Nitrosospira 16s rDNA gene</td>
</tr>
</tbody>
</table>

Results

Fig. 1 Original Culture NO3 Oxidation.

Fig. 2 NH3 and NO2 Oxidation from subculture.

Fig. 3 Agarose Gel showing Cut Hybrid Plasmids using SAC I Restriction Endonuclease

Conclusion

1. We confirmed the activity of nitrifying bacteria based on NH3 and NO2 oxidation using test strips.
2. Sequencing data showed the presence of Ammonia-oxidizing and Dinitrite-Oxidizing bacteria in Fritz-zyme.
3. Sequencing data also showed the presence of non-nitrifying bacteria from the genera Pseudomonas and Niobe, which could indicate the presence of denitrifying bacteria as well as nitrifying bacteria.

Future Research

- Measure oxidation of NH3 and NO2 using Ion-Selective Electrodes to gain a more accurate measurement of oxidation.
- Find Primers capable of amplifying 16s rDNA from sub-culture samples.
- Possible candidates include EUB338f and EUB338r along with specific 16s rDNA primers.
- Determine if sub-culturing techniques are suitable for isolating pure nitrifiers.

References


Acknowledgements

Dr. Penny S. Amy, Dr. Kurt Regner, John Perry, Diane Yost, Adam Mustafa, and the UNLV Genomics Center.

Mr. Anthony Harrington was the recipient of an award from the NSF Research Experience for Undergraduates (REU) program A Broad View of Environmental Microbiology at the University of Nevada, Las Vegas (IBB 0585233).