Identification of Nitrifying Bacteria Contained in a Commercial Inoculant Using Molecular Biology Techniques

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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 nitrate. This process requires two types of chemoautotrophic 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, biofertilizers, and nitrogen removal in wastewater treatment plants. Previous studies have shown that Fritz-zyme Turbostart 700, a commercial freshwater inoculant, has been successfully used in a semi-hydroponic system, i.e., zeoponics. In order to determine an optimal consortium for zeoponics, it is necessary to know exactly what bacteria are present.

Materials and Methods

Whole-cell PCR using universal and specific 16s rDNA primers

Whole-Cell PCR followed by PCR Purification using QIAquick PCR Purification Kit (Qiagen)

TA Cloning via Ligation using pGEM-T Easy Vector Cloning Kit (Promega)

Transformation using Monserate MON1 competent cells

Blue/white colony screen on Luria Bertani agar containing Ampicillin, X-Gal and IPTG

Plasmid Prep using Qiagen Mini Prep Kit

Restriction Digest using SAC1

Sequencing using primers T7 and SP6 at UNLV Genomics Center

Materials and Methods (continued)

<table>
<thead>
<tr>
<th>Primer</th>
<th>5’ → 3’</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>27F</td>
<td>AGAGTTTGTATCCAGCTCTAGAG</td>
<td>Bacterial 16s rDNA gene</td>
</tr>
<tr>
<td>16S18r</td>
<td>AGCTTACCCCTGTTGACTT</td>
<td>Bacterial 16s rDNA gene</td>
</tr>
<tr>
<td>EUB338F</td>
<td>ACCTCTAAGAGAAGGACG</td>
<td>Bacterial 16s rDNA gene</td>
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<tr>
<td>Nit3r</td>
<td>CGGATGCTATGCTTGA</td>
<td>Nitrobacter 16s rDNA gene</td>
</tr>
<tr>
<td>Nsp458r</td>
<td>CGGAATTCCGGG</td>
<td>Nitrosospira 16s rDNA gene</td>
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Results

Conclusion

Future Research

References

Acknowledgements

• We confirmed the activity of nitrifying bacteria based on NH4 and NO3 oxidation using test strips.
• Sequencing data showed the presence of Ammonia-oxidizing and Nitrite-Oxidizing bacteria in Fritz-zyme.
• Sequencing data also showed the presence of non-nitrifying bacteria from the genera Pseudomonas and Niabella, which could indicate the presence of denitrifying bacteria as well as nitrifying bacteria.

Measure oxidation of NH4 and NO3 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.


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