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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 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, biofilters, and nitrogen removal in wastewater treatment plants. Previous studies have shown that Fritz-zyme Turbosaint 700, a commercial freshwater inoculant, has been successfully used in a semi-hydroponic system, i.e., aquaponics. In our laboratory, preliminary data have shown that Fritz-zyme contains more than the specific nitrifying bacteria. In order to determine an optimal consortium for zeoponics, it is necessary to determine whether the current enrichment techniques being used are sufficient to eliminate the heterotrophic bacteria present in Fritz-zyme.

Materials and Methods (continued)

<table>
<thead>
<tr>
<th>Primer</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Z7F</td>
<td>Bacterial 16s rDNA gene</td>
</tr>
<tr>
<td>1692r</td>
<td>Bacterial 16s rDNA gene</td>
</tr>
<tr>
<td>EU3.38F</td>
<td>Bacterial 16s rDNA gene</td>
</tr>
<tr>
<td>Neo1225r</td>
<td>AOB 16s rDNA gene</td>
</tr>
<tr>
<td>NIT3r</td>
<td>Nitrite-oxidizing bacteria</td>
</tr>
<tr>
<td>Ntp4865r</td>
<td>Nitrosospira 16s rDNA gene</td>
</tr>
</tbody>
</table>

Results

Fig. 1 Original Culture NO3 Oxidation.

![Fig. 2 NH3 and NO2 Oxidation from sub-culture](image)

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 Nitrite-Oxidizing bacteria in Fritz-zyme.
3. Sequencing data also showed the presence of non-nitrifying bacteria from the genera Pseudomonas and Niobe.

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 EU3.38F and EU3.38R along with specific 16s rDNA primers.
- Determine if sub-culturing techniques are suitable for isolating pure nitrifiers.

References


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

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