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

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

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

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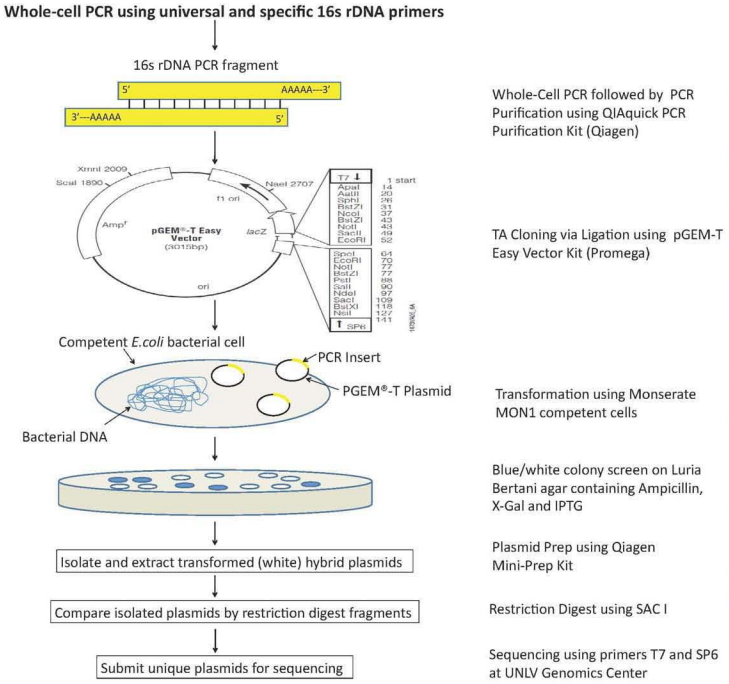
Introduction

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 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 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 that we know exactly what bacteria are present. Using 16s rDNA universal primers and pGEM®-T Easy Vector Cloning Kit (Promega), we amplified the 16s rDNA genes from Fritz-zyme and cloned them into the pGEM®-T Easy *E. coli* vector plasmid. The cloned plasmids were transformed into competent *E. coli* cells and sequenced to identify the bacteria present in each sample. In this study, we determined whether the current enrichment techniques being used are sufficient to eliminate the heterotrophic and spore-forming bacteria present in Fritz-zyme.

Materials and Methods



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

TA Cloning via Ligation using pGEM-T Easy Vector 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 SAC I

Sequencing using primers T7 and SP6 at UNLV Genomics Center

Materials and Methods (continued)

Primer	5'→ 3'	Specificity
27f	AGAGTTTGATCCTGGCTCAG	Bacterial 16s rDNA gene
1492r	ACGGCTACCTTGTTACGACTT	Bacterial 16s rDNA gene
EUB338f	ACTCCTACGGGAGGCAGC	Bacterial 16s rDNA gene
Nso1225r	CGCCAATTGTATTACGTGTGA	AOB 16s rDNA gene
NIT3r	CCTGTGCTCCATGCTCCG	Nitrobacter 16s rDNA gene
Ntspa685r	CGGGAATTCCGCGCTC	Nitrospira 16s rDNA gene

Results

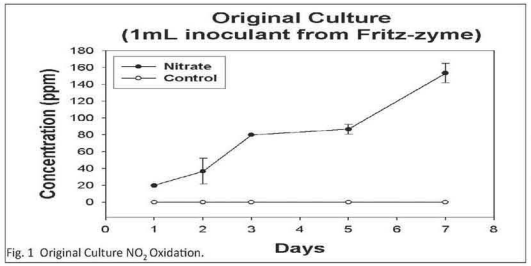


Fig. 1 Original Culture NO_2 Oxidation.

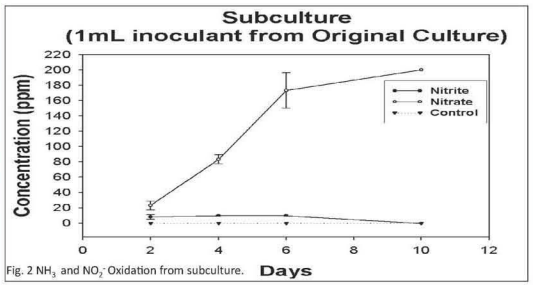


Fig. 2 NH_3 and NO_2^- Oxidation from subculture.

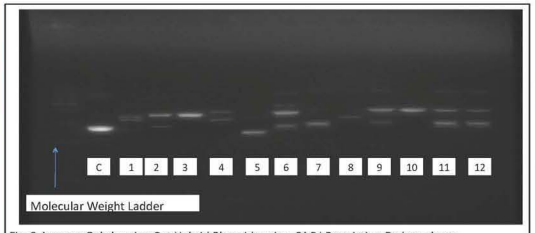


Fig. 3 Agarose Gel showing Cut Hybrid Plasmids using SAC I Restriction Endonuclease

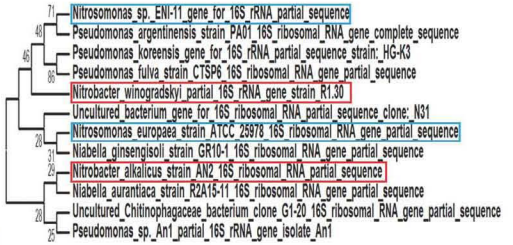


Fig. 4 Phylogenetic tree of cloned inserts created in MEGA 5.0 using results from BLASTn from NCBI Database. Contigs were created in DNA Baser Software. Values next to nodes represent percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates). Blue boxes represent Ammonia-oxidizing bacteria. Red boxes represent Nitrite-oxidizing bacteria.

Conclusion

1. We confirmed the activity of nitrifying bacteria based on NH_3 and NO_2^- 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 ***Niabella***, which could indicate the presence of denitrifying bacteria as well as nitrifying bacteria.

Future Research

- Measure oxidation of NH_3 and NO_2^- 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

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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 (DBI 1005223).