Assessing the Dominant Denitrifying Bacteria in the Mid-Atlantic Bight Sediments

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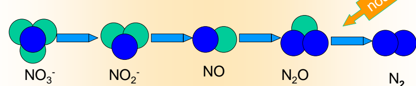
Abstract

Denitrifying bacteria are important in marine environments because they may remove over 50% of the nitrogen input to the ocean (2). In order to elucidate how the oceanic denitrifying ecosystem is structured and maintained, we analyzed 19 sediment samples from the mid-Atlantic Bight (MAB) that had been collected between June and November 1996-1998. The purpose of the project was to identify which denitrifying bacteria dominate communities in oceanic sediments. The denitrifying bacteria were characterized using the nitrous oxide reductase (*nosZ*) gene and Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis. To determine which restriction enzyme provided the best resolution of *nosZ* genes in the MAB, fluorescently labeled PCR product was cut with the enzymes *Mnl1*, *RsaI*, *AluI* and *HaeIII*. MnlI showed the greatest number of well-separated and defined peaks and was used in all further work. A total of 120 different *nosZ* peaks were detected in al samples. To establish the dominant denitrifiers, a frequency analysis was performed on all TRFLP samples. Only those peaks that occurred in 90% or more of the samples were considered dominant. Fifteen peaks were present in high percentage of the TFLP profiles. These peaks represented 51, 66, 82, 113, 146, 149, 175, 195, 222, 259, 313, 353, 428 and 433 bp. A clonal library was created from a single sample, and screening of 50-100 clones is now underway. Six sequences were perform of the peaks of interest and the results were compare with database for identification.

Background

Denitrification converts nitrate into gaseous nitrogen, which is less accessible to the organisms and accumulates in the atmosphere.

Denitrification Pathway



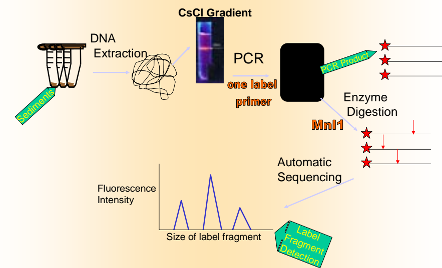
Nitrous oxide reduction is the last step in the denitrification pathway. Facultative anaerobic bacteria perform this process generally under anaerobic conditions. Denitrifying bacteria are important players in the global nitrogen cycle, particularly in coastal waters where high concentrations of nitrogen compounds are introduced through run off and waste inputs. These inputs of nitrogen compounds may have a large impact on algal production and bacterial activity. Additionally,the production of nitrous oxide as an intermediate of the denitrification pathway, is known to contribute to global warming. Understanding which organisms are active denitrifiers can help us understand the effects of nitrogen pollution in the coastal environment. Knowing the organisms that are more abundant and understanding their behavior might also give us tools to modify or create systems to bioremediate polluted sites using these organisms.

Objective

•What are the dominant/most abundant denitrifiers in the Mid -Atlantic bight distributed by temporal and spatial scale?

Methods

Terminal Restriction Fragment Length Polymorphism (TRFLP)



DNA Extraction

- •DNA was extracted from sediment samples collected in LEO-15 site (1)
- •Extractions were performed using a modify Chloroform-Phenol method
- •DNA was cleaned using a CsCl gradient

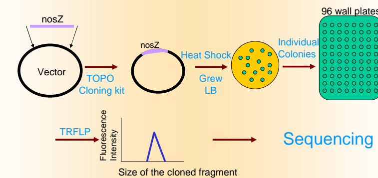
PCR

- •PCR reaction was done using one fluorescent label primer
- •Primer used were ★752F (ACC GAY GGS ACC TAY GAY GG) and 1773R (ATR TCG ATC ARC TGB TCG TT) (3)

Enzyme Digestion

- •Enzyme Mnl1 was used for the digestions

Cloning

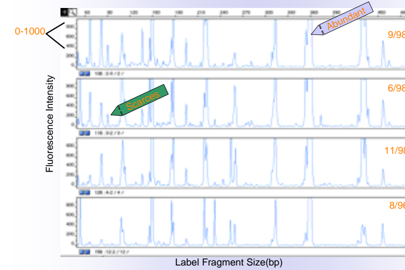


Cloning Protocol

- •TOPO Cloning Kit was used to insert *nosZ* DNA fragment into a vector
- •E.coli chemically competent cells were used, combined with a heat shock procedure, to transform the *nosZ* vector
- •Cells grew on LB media plates and individual colonies were placed on 96 wall plates
- •Clones were screened using TRFLP technique
- •Peaks of interest were sequenced

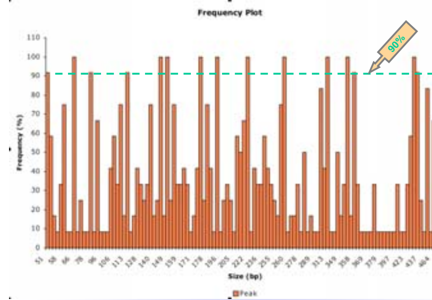
Results

TRFLP



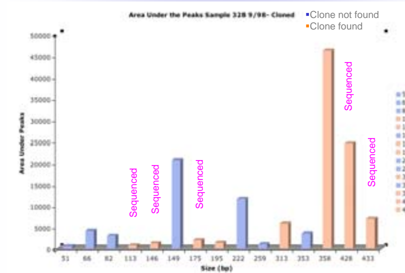
•TRFLP on Station 32 samples: 32B 9/98, 32B 6/98, 32B 11/98 and 32B 8/96

Frequency Plot



- •TRFLP data was translated into Excel and analyzed for how many times peak occurs among the samples
- •Calculated frequency percentage in terms of: times of a peak in samples/# of samples
- •Peaks of interest were defined as those that were present in 90% or more of the samples

Cloned Sample



Sequencing Comparisons

Peak Size	Match	% Match	# of Bases
113	°Uncultured bacterium °Continental Shelf Sediments -USA, New Jersey	90	406
	°Uncultured bacterium °Soil sediments-Germany	86	129
146	°Uncultured bacterium °Continental Shelf Sediments-USA, New Jersey	92	238
175	°Uncultured bacterium °Continental Shelf Sediments-USA, New Jersey	85	214
	°Silicibacter pomeroyi DSS-3 °Alphaproteobacteria- Sagasso Sea	87	178
429	°Uncultured bacterium °Continental Shelf Sediments-USA, New Jersey	94	662
	°Uncultured bacterium °Soil sediments-Germany	85	107
433	°Uncultured bacterium °Continental Shelf Sediments-USA, New Jersey	94	585
	°Uncultured bacterium °Estuary sediment/rock biofilm - Portugal	85	246
	°Silicibacter pomeroyi DSS-3 °Alphaproteobacteria- Sagasso Sea	84	206

•Preliminary sequence identification pending further verification

Conclusion

- TRFLP data showed 15 abundant denitrifiers, defined by frequency of organisms, present in processed samples
- Sequencing and BLAST comparisons showed similarities with other marine organisms found off of the New Jersey coast
- Similarities were also demonstrated over a global scale
- More clones are being screened and sequenced to identify all dominant peaks and verify sequence identification

Reference

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