THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF SCIENCE

INSIGHTS INTO THE RARE BIOSPHERE: EXHAUSTIVE ELIMINATION OF TOTAL DNA FROM COMPLEX MICROBIAL COMMUNITIES ENHANCES PCR RESOLUTION OF ALPHA-DIVERSITY.

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Abstract

Use of the Polymerase Chain (PCR) reaction for the amplification of the 16S rRNA gene (rDNA) has allowed the cultivation-independent interrogation of complex microbial communities for taxon richness. There are many well-documented caveats with this technique that suggest universal underrepresentation of total prokaryotic diversity. Here we propose a novel approach aimed at increasing the taxon resolution of PCR by exhaustive amplification inhibition of template DNA from complex communities via stochastic binding of Propidium Monoazide (PMA). PMA is a DNA intercalating molecule capable of forming covalent cross-linkages to organic moieties upon light exposure used to debar template PCR amplification by prohibiting denaturation during PCR cycling. Stochastic intercalating of PMA, as opposed to exhaustive dilution, is shown to disproportionally inactivate DNA from different community members. Using species-specific real-time quantitative PCR on a defined DNA community control we show that high abundance phylotypes are inactivated to a greater extent than low abundance phylotypes by PMA-DNA covalent bond formation. To our knowledge, this is the first study to quantify the effects of template removal, via random chemical binding, on a mock DNA community using species-specific quantitative PCR. We hypothesize that the resulting higher relative proportion of low abundance taxa in the total DNA template mixture enhances amplification of rare biosphere phylotypes. Additionally, this method was applied to a complex environmental DNA sample and, following 16S rDNA PCR amplification and analysis via phylogenetic DNA microarray (PhyloChip), results indicated a 19.5% increase in taxon resolution when compared to a control. Increased

taxon richness observed in the soil sample suggests the amplification of low abundance phylotypes, usually shadowed by the overwhelming presence of genetically homogenous high abundance templates, known as "rare biosphere" members. Exhaustive elimination of predominant phylotypes with PMA is a relatively simple and inexpensive method capable of increasing the resolution of existing techniques interrogating environmental taxon richness and can be readily employed to enhance the phylogenetic breadth of "at-hand" previously surveyed samples.

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Chapter 1

Introduction

Alpha diversity studies are of crucial importance to environmental microbiology. Much attention has been given to the evolutionary and functional ecological role of the “rare biosphere”, defined as an extremely low abundance but insurmountably diverse ubiquitous microbial cohort1. Recently, sans-cultivation high-throughput sequencing technologies have revealed a deeper breath of biological diversity in various habitats than hitherto acknowledged1,2. Low abundance community members, according to the “everything is everywhere hypothesis” serve an opportunistic role and influence community structure via sporadic growth periods and/or lateral gene transfer in times of drastic environmental change3,4,5. The geographical and environmental distribution of microorganisms has been discussed with fervor since the inception of microbiology as a science with opposing viewpoints spawning from environmental determinism against random dispersal hypothesis of micro-biogeography 5. Recovery of viable bacterial cells, using appropriate selection methods, from places where their particular metabolic functions are environmentally prohibited has been documented6,7. Similar observations serve to corroborate the concept of global microbiological dispersal as the driving mechanism behind extensive prokaryotic colonization of every sampled environment on Earth. Environmentally dictated competitive selection is responsible for niche-specific rank abundance distributions with extremely diverse low abundance ranking members putatively serving as reservoirs of genetic variance ensuring minimal probability of local extinction. Increasing the resolution of existing technologies used in censuses of

prokaryotic communities is vital to deciphering the evolutionary and ecological influence of low abundance members on overall microbial community structure and function.

PCR amplification of the small ribosomal subunit gene, a canonical phylogenetic marker, followed by comparison to various ribosomal database systems has been paramount for the systematic interrogation of diverse environments for taxon richness8. Phylogenetic diversity studies generally undertake the laborious task of 16S rDNA clone library construction. Each step necessary to produce a clone library may introduce biases leading to skewed representations of microbial diversity and relative abundance. Other investigations have scrutinized the inconsistencies associated with phylogenetic data derived from comparisons of clone libraries9,10. Implementation of the newly developed 16S rDNA microarray systems (PhyloChip) for taxon richness analysis isolates the bias source to PCR by circumventing additional steps needed for clone library construction. Intrinsic factors in PCR amplification, such as chemical complexity, highly diverse 16S rRNA gene sequence composition, formation of heteroduplexes, heterogeneity of the rrn operon and competition of heterologous templates with identical primer sites, exacerbate inherent reaction kinetic biases4,11-16. Standard PCR methodologies, due to extremely low numerical abundance of rare biosphere members, are inadequate for resolving the diversity associated with this elusive cohort. Rank abundance distribution of microbial species the environment implies a disproportional presence of phylotypes in environmental samples leading to differential amplification of predominant phylotypes and universal underrepresentation of taxonomic breath in nature. Grossly generalizing the plethora of complex factors favoring differential amplification of heterologous

templates, we reason that amplification of low abundance templates is primarily hindered by ratio inequalities to high abundance templates. Inactivation and/or removal of the high abundance phylotypes is therefore, a necessary step for the PCR-based resolution of the cornucopian diversity associated with low abundance “rare biosphere” phylotypes.

Next-generation sequencing approaches interrogating microbial diversity are also limited by the disproportionate distribution of species in nature. Sequencing efforts, albeit advantageous in terms of providing multiple alternative phylogenetic markers and enhanced qualitative descriptions of novel taxonomic groups, are hampered by the same PCR biases discussed aforetime. Low sequence coverage of numerically unsubstantial organisms and economics of sequencing at depth still pose a major challenge to researchers interrogating microbial species richness and rare phylotypes in the environment. This pertinent issue necessitates the development of a robust method for normalizing high abundance templates derived from abundant “oversampled” organisms by targeted removal from the genetically complex DNA sample phylotype pool. We hypothesized that quantitative removal of high abundance phylotypes will have a positive effect on the first cycles of primer binding during PCR, ultimately leading to the exponential amplification of previously shadowed rare phylotypes.

A simple computer algorithm, modeling random DNA elimination in a complex phylotype cocktail, suggested that high abundance phylotypes were eliminated to a higher extent than numerically uncommon phylotypes. This theoretical computer model observation served as the impetus for the paradoxical notion of seeking an increase in

diversity with decreasing initial DNA template amounts for PCR. To test this model the effects of random DNA inactivation on a defined model DNA community (MDC, Table 1, Figure 1) were quantified using species-specific real-time quantitative PCR (ss RT-qPCR). To render DNA incapable of undergoing the denaturation step of PCR and subsequent amplification, Propidium Monoazide (PMA), an intercalating molecule capable of forming covalent cross-linkages to organic moieties upon light exposure, was used. Existing literature shows that PMA can be successfully employed in inhibiting PCR amplification of a particular fraction of undesirable DNA, such as extracellular DNA in spores, without any apparent hindrance to PCR kinetics17. It is important to highlight that PMA-mediated inactivation of DNA is not a targeted approach per se, but it is a complex stochastic statistical event where the range of phylotypes, n, defined as operational taxonomic units (OTUs) and specific time-dependent molecular binding sequences play a role in expected outcomes.

Initially, we experimentally determined the feasibility of achieving predictable genomic DNA inactivated fractions using PMA. Subsequently, PMA was used to inactivate predetermined percentages of total DNA comprising the MDC. Following photo-activation, PMA treated MDC samples were interrogated for total and species-specific 16S rDNA counts via Real Time-qPCR. Similarly, an environmental soil sample underwent total DNA extraction and was subjected to PMA treatment to inactivate fractions of total DNA. The residual DNA fraction (DNA not inactivated by PMA) was amplified via standard 16S rDNA PCR amplification and amplicons were analyzed for taxonomic breadth via hybridization on PhyloChip 16S rDNA microarray.

Materials and Methods

*PMA treatment of MDC***.** 1µl of 0.025µM PMA working stock was used to treat 4µl of MDC sample (V_f = 5µl). The sample, containing a final PMA concentration of 0.005µM was exposed on ice to a 500W halogen lamp at a distance of 25cm for 4 minutes. Subsequently, total 16S rDNA and species-specific qPCR analyses were performed in triplicates. Note: All procedures involving the use of PMA prior to halogen light exposure were performed in the dark to avoid premature photo-activation.

*PMA treatment of B. pumilus SAFR-032 gemonic DNA***.** Working stock solutions of 5µM, 0.5µM, 0.05µM and 0.005µM PMA were made using an original 200mM stock. 4µl portions of genomic DNA sample were treated with 1µl of the appropriate working PMA stock concentration (V_f = 5µl/sample) to yield final sample concentrations of 1, 0.1, 0.01, 0.001µM PMA. Subsequently samples were exposed on ice to a 500W halogen lamp at a distance of 25cm for 4 minutes. The exact same protocol, sans PMA, was employed to treat the non-PMA treated control dilutions. Each sample was qPCR analyzed in triplicate for total 16S rDNA copy numbers. Note: All procedures involving the use of PMA prior to halogen light exposure were performed in the dark to avoid premature photo-activation.

Soil DNA extraction and PMA treatment. 10.0 g of garden soil underwent total DNA extraction via Soil Max™ Kit from Mo Bio Laboratories, Inc. Quantitative-PCR 16S rDNA analysis, using 1492R and 1369F primers, revealed a total 16S rRNA gene count of approximately 1.55 $X10^7$ copies per µl. Three different concentrations of PMA (0.10,

2.50 and 3.75μM) were used to treat environmental DNA samples. Stock solutions of PMA were used to make five 20 μL aliquots per treatment (Fig.1*)* all of which were then exposed on ice to a 500W halogen lamp at a distance of 25cm for 4 minutes. The exact same protocol was employed to treat the non-PMA treated control dilutions. Note: All procedures involving PMA use prior to halogen light exposure were performed in the dark to avoid premature photo-activation.

DNA pooling for PhyloChip analysis. Following light exposure, all five 20μl aliquots made for each sample (0.75, 0.5, 0.1 μM, 1:10 dilution, 1:2 dilution and untreated control) were pooled to make the final 100μl samples sent for GeneChip® Phylogenetic Microarray analysis at the Lawrence Berkeley Laboratory.

Model DNA Community (MDC) control. Previously isolated DNA from JPL's Biotechnology and Planetary Protection genetic inventory was used to design our model community DNA control. The model is comprised of 16S rDNA from 11 trans-domain microbial constituents (Table 1, Figure 1*).* The relative percentage contribution of each microbial DNA constituent is highly disproportional.

Quantitative Real-Time PCR. All reported gene numbers were acquired via quantitative real-time polymerase chain reaction on a BioRad CFX96 thermocycler. IQ™SYBR® Green 2X Supermix containing dNTPs, 50U/ml i*Taq*™ DNA polymerase, 6mM $MgCl_2$, SYBR® Green I, 20nM fluorescein real-time PCR reagent was used. Final RXN mix: 10µl of 2X reaction mix, 1µl each of 10-18nM F and R primers, 1µl of sample template

DNA and dH_20 up to 20µl. qPCR protocol: 1) 95.0°C for 3:00, 2) 95.0°C for 0:10, 3) 56.5°C for 0:30 Plate Read, 4) GO TO 2, 39 more times, 5) 95.0°C for 0:10, 6) Melt Curve 65°C to 95°C: Increment 0.5°C for 0:05.

Results

Bacillus Pumillus SAFR-032 Genomic DNA Inactivation.

Previous work had demonstrated the capability of PMA photo-activation for the selective PCR inhibition of extracellular DNA. Here, we first assessed the efficiency of PMA to inactivate desired fractions of a defined genomic DNA sample. Various concentrations of PMA were used to treat SAFR-032 genomic DNA to develop, via species-specific qPCR, a sample-specific standard curve (Figure 2). A 1.0 µM PMA treatment was enough to inactivate >99% of the initial genomic DNA copy numbers, while a tenfold dilution in PMA treatment concentration inactivated only ~63% of the sample.

Total MDC Inactivation

A standard curve showing the gene numbers of specific MDC phylotypes before and after PMA treatment at various concentrations was derived (data not shown). This standard curve allowed an estimation of PMA concentrations necessary to achieve desired fractions of total DNA inactivation in the MDC sample. A 0.005µM PMA treatment was shown to be efficient for the reproducible inactivation of 85-90% of total MCD. MDC samples treated with a 0.005µM PMA treatment for total DNA inactivation were subsequently analyzed via qPCR using universal and species-specific primers (Table 2). The 0.005µM PMA treatment was estimated to achieve 85-90% DNA inactivation.

However, data shows an actual reduction of 70-80% of total DNA comprising the MDC (Figure 3). Species-specific qPCR analysis for *M. formicicum*, the most predominant species in our MDC (Table 2) and *M. luteus*, a low abundance member in the MDC, shows that 75-80% of the initial 16S rDNA copies for the predominant phylotype were inactivated (Figure 4A) while only ~58% of minor contributing member templates were affected (Figure 4B).

PMA Inactivation of Environmental DNA Sample

The environmental sample used for this study was garden soil collected from the Sierra Madre Foothills, located adjacent to the Jet Propulsion Laboratory in La Cañada Flintridge, CA. Total quantification of 16S rRNA gene copies from the environmental garden soil sample was performed via qPCR analysis using universal 16S rDNA primers. Total soil chromosomal DNA quantification resulted in ~1.20×10^{7} 16S rRNA gene copies μl^{-1}. These results fall within the consensus general range of 10^{8}-10^{9} bacterial cells per gram of soil. Subsequently, PMA treatments of 0.75µM, 0.5µM, and 0.1µM were performed on environmental DNA sample aliquots achieving 99%, 86% and 52% total DNA inactivation, respectively (Figure 5).

PhyloChip DNA Microarray

PMA treated environmental DNA samples were sent to the Lawrence Berkeley Laboratory for standard 16S rRNA gene PCR amplification and subsequent massive parallel phylogenetic analysis via PhyloChip DNA microarray. Biodiversity was assessed via DNA microarray hybridization of 16S rRNA gene amplicons (Figure 6).

The control sample showed a total count of 5,011 distinct operational taxonomic units (OTUs) at the 97% identity level. A 1:2 dilution control showed a significant drop in OTUs indicative of dilution ineffectiveness in enhancing resolution of phylogenetic diversity. PMA-mediated inactivation of 52% of total environmental DNA resulted in a 7% drop in sample diversity while 86% inactivation led to a 2% increase in observed OTUs. Remarkably, the inactivation of 99% of total environmental DNA, achieved via 0.75μM PMA treatment, resulted in a significant increase in total OTUs detected by the phylogenetic microarray, increasing the resolution of microbial diversity by 19.5%.

Discussion

There are many caveats associated with studies of species richness employing PCR amplification of 16S rDNA sequences. Chemical complexity of environmental samples and heterologous DNA template composition exacerbate biases leading to the preferential (an often exclusive) amplification of predominant taxons[11,12,14,18-21].

SAFR-032 genomic DNA inactivation from subsequent PCR amplification using photo-activated PMA was confirmed via species specific quantitative PCR establishing the feasibility of using predetermined PMA amounts for inactivating a desired portion of DNA sample. These results validated intact chemical properties of PMA stock solutions, confirmed the photo-activation of covalent bond formation between the PMA-DNA complexes under our experimental set up and corroborated the notion of using small quantities of PMA for inactivating relatively large amounts of genomic DNA.

The theoretical foundations of our experiments are based on the probabilistic odds of individual random binding events of PMA to DNA. A simple computer model suggested

that, in systems comprised of extremely disproportional phylotype distributions, random PMA binding to DNA (and subsequent photo-catalyzed covalent bond formation leading to inhibition of strand denaturation) would favor the inactivation of numerically predominant phylotype members. MCD analysis using *ss* RT-qPCR before and after PMA treatment was crucial for evaluating the strength of our computational random binding model. Results from the MDC experiment corroborate predictions made by the random phylotype binding computer model. Random inactivation of 70-80% of total MDC constituents by PMA treatment and subsequent photo-catalyzed covalent binding resulted in disproportional PCR inhibition of heterologous DNA templates. Species-specific qPCR analysis revealed that *M. formicicum*, the most predominant phylotype in the MDC, was inactivated to a significantly higher extent than *M. luteus,* a low copy phylotype. These results confirm that non-specific inactivation of heterologous DNA templates results in disproportional inactivation of predominant phylotypes in a defined DNA sample. We speculate that disproportionality of binding is likely a function of skewed taxon distribution.

The next logical step in our investigation was the application of PMA inactivation to an environmental sample. We reasoned that, due to the number and distribution of species in the environment, PMA treatment could result in increased resolution of low abundance members. Following total DNA extraction from a garden soil sample, PMA was used to inactivate various sample fractions from subsequent PCR amplification. Time consuming labor associated with 16S rDNA gene library construction was circumvented by using a DNA microarray (PhyloChip) for phylogenetic analysis. Previous work has

shown that clone library construction and subsequent sequencing are likely to underreport prokaryotic phylogenetic diversity[22,23].

Figures

Figure 1.

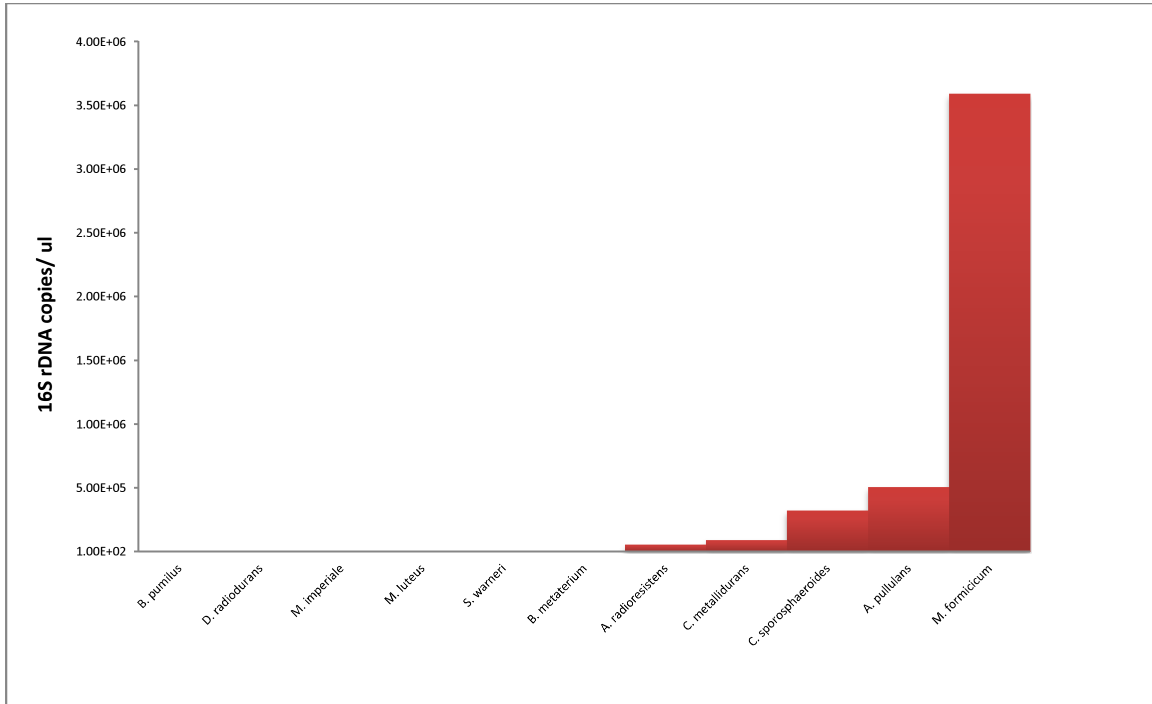


Figure 2.

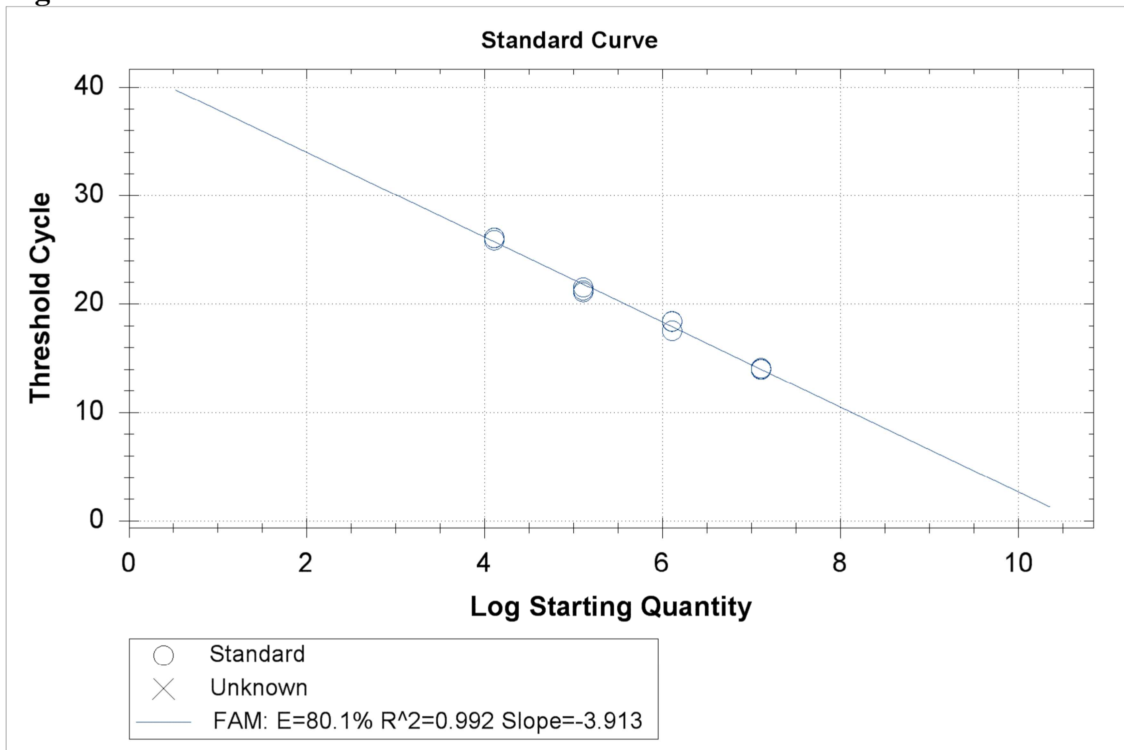


Figure 3.

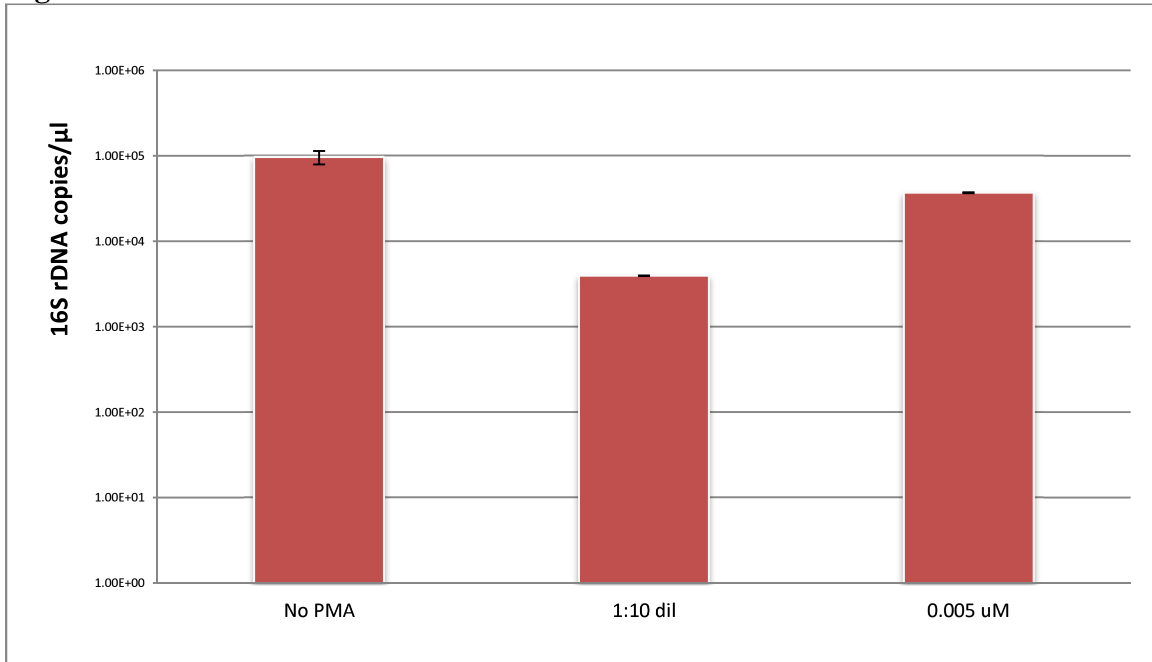


Figure 4.

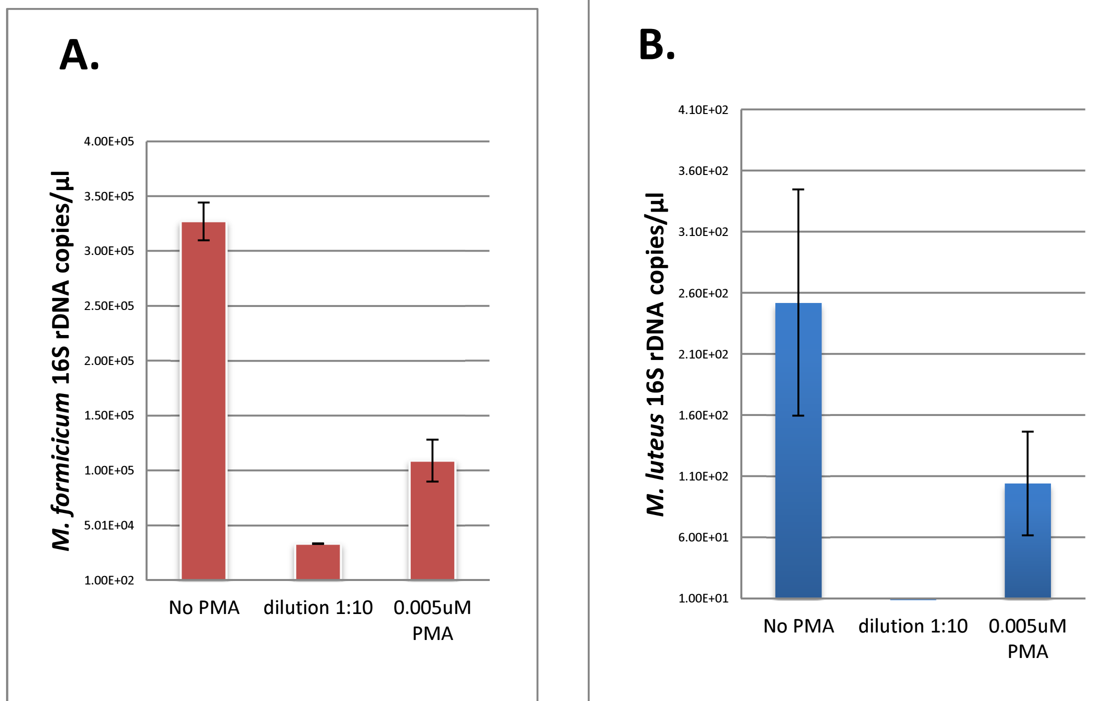


Figure 5.

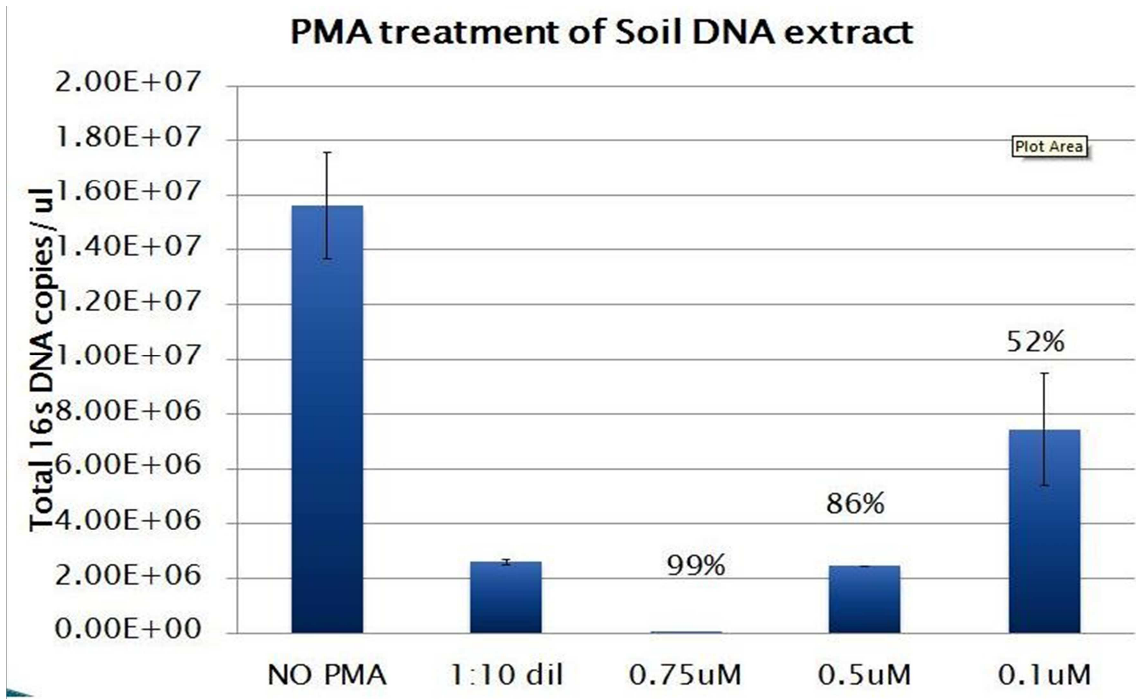
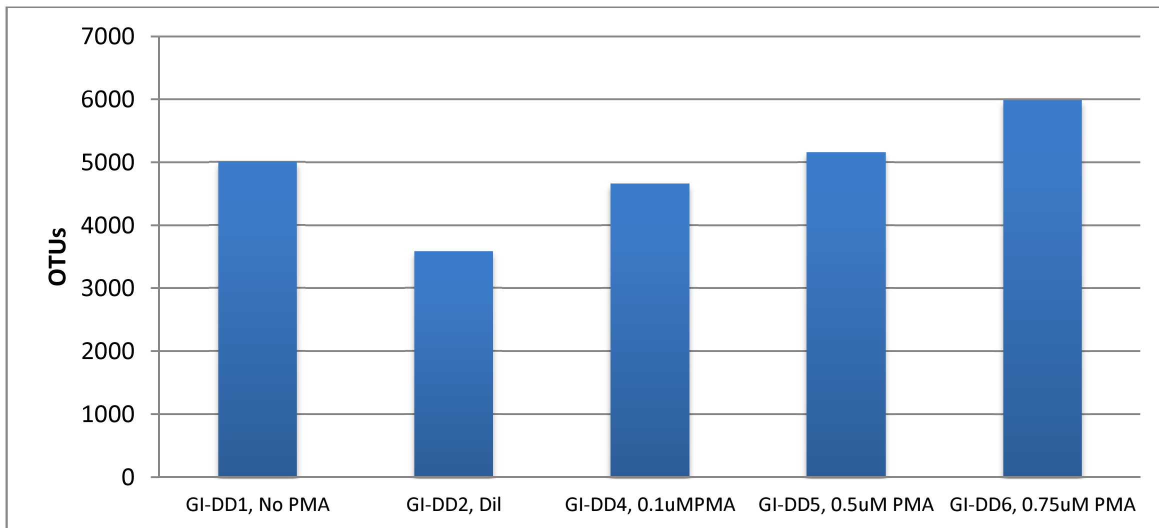


Figure 6.



Tables

Table 1.

	Microbes	Strains	Phylum	% contrib.	Total 16S RNA gene Copy Number/ µl
1	*Methanobacterium formicicum*	DSM 1535	Euryarchaeota (Archaea)	78.04	3.59E+06
2	*Aureobasidium pullulans*	28v1	Ascomycota (Eukarya)	11.00	5.06E+05
3	*Clostridium sporosphaeroides*	DSM 1294	Firmicutes	7.00	3.22E+05
4	*Cupriavidus metallidurans*	CH34	(β−)proteobacteria	2.00	9.20E+04
5	*Acinetobacter radioresistens*	50v1	(γ−)proteobacteria	1.21	5.52E+04
6	*Bacillus megaterium*	KL-197	Firmicutes	0.50	2.30E+04
7	*Staphylococcus warneri*	82-4	Firmicutes	0.25	1.15E+04
8	*Micrococcus luteus*	ATCC 4698	Firmicutes	0.01	4.60E+02
9	*Microbacterium imperiale*	47v1	Actinobacteria	0.01	4.60E+02
10	*Deinococcus radiodurans*	ATCC 13939	Deinococcus-Thermus	0.01	4.60E+02
11	*Bacillus pumilus*	SAFR-032	Firmicutes	0.01	4.60E+02
			Total copies/µl	100%	**4.60E+06**

Table 2.

Primers	Target	Name	Sequence (5' to 3')
Universal	16S	27f	AGA GTT TGA TCM TGG CTC AG
		1392r	ACG GGC GGT GTG TRC
Species specific	*M. formicicum*	ssMFf	ATT GCT GGA GAT ACT ATT
		ssMFr	GGG ATT ATA GGA TTT CAC
Species specific	*M. luteus*	ssMLf	TAA CCT GCC CTT AAC TCT
		ssMLr	AAA CCG ATA AAT CTT TCC AA
Species specific	*B. pumilus*	600f	TGA AGC ACT TGA GAA ATT
		980r	TGC TGC AAA GAA AAT

Bibliography

1. Sogin, M.L. et al. Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences of the United States of America* **103**, 12115-20 (2006).

2. Venter, J.C. et al. Sequencing of the Sargasso Sea. *Science* **304**, 66-74 (2004).

3. Falkowski, P.G., Fenchel, T. & Delong, E.F. The microbial engines that drive Earth's biogeochemical cycles. *Science (New York, N.Y.)* **320**, 1034-9 (2008).

4. Bent, S.J. & Forney, L.J. The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity. *The ISME journal* **2**, 689-95 (2008).

5. O'Malley, M. a The nineteenth century roots of "everything is everywhere". *Nature reviews. Microbiology* **5**, 647-51 (2007).

6. Isaksen, M. Thermophilic sulfate-reducing bacteria in cold marine sediment. *FEMS Microbiology Ecology* **14**, 1-8 (1994).

7. Bianchi, M., Marty, D., Teyssie, J.-L. & Fowler, S. Strictly aerobic and anaerobic bacteria associated with sinking particulate matter and zooplankton fecal pellets. *Marine Ecology Progress Series* **88**, 55-60 (1992).

8. Cole, J.R. et al. The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. *Nucleic acids research* **33**, D294-6 (2005).

9. Cottrell, M.T., Kirchman, D.L. & Kirchman, D.L. Community Composition of Marine Bacterioplankton Determined by 16S rRNA Gene Clone Libraries and Fluorescence In Situ Hybridization Community Composition of Marine Bacterioplankton Determined by 16S rRNA Gene Clone Libraries and Fluorescence In Situ Hybridization. *Society* (2000).doi:10.1128/AEM.66.12.5116-5122.2000.

11. Suzuki, M.T. & Giovannoni, S.J. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Applied and environmental microbiology* **62**, 625-30 (1996).

12. Wang, G.C. & Wang, Y. Frequency of formation of chimeric molecules as a consequence of PCR coamplification of 16S rRNA genes from mixed bacterial genomes. *Applied and environmental microbiology* **63**, 4645-50 (1997).

13. Chandler, D.P., Fredrickson, J.K. & Brockman, F.J. Effect of PCR template concentration on the composition and distribution of total community 16S rDNA clone libraries. *Molecular ecology* **6**, 475-82 (1997).

14. Becker, S., Böger, P., Oehlmann, R. & Ernst, a PCR bias in ecological analysis: a case study for quantitative Taq nuclease assays in analyses of microbial communities. *Applied and environmental microbiology* **66**, 4945-53 (2000).

15. Acinas, S.G., Marcelino, L.A., Klepac-ceraj, V. & Polz, M.F. Divergence and Redundancy of 16S rRNA Sequences in Genomes with Multiple rrn Operons. *Society* **186**, 2629-2635 (2004).

16. Bustin, S. a & Nolan, T. Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. *Journal of biomolecular techniques : JBT* **15**, 155-66 (2004).

17. Nocker, A., Sossa-Fernandez, P., Burr, M.D. & Camper, A.K. Use of propidium monoazide for live/dead distinction in microbial ecology. *Applied and environmental microbiology* **73**, 5111-7 (2007).

18. Egert, M. & Friedrich, M.W. Formation of Pseudo-Terminal Restriction Fragments , a PCR-Related Bias Affecting Terminal Restriction Fragment Length Polymorphism Analysis of Microbial Community Structure. *Society* **69**, 2555-2562 (2003).

19. Lueders, T. & Friedrich, M.W. Evaluation of PCR Amplification Bias by Terminal Restriction Fragment Length Polymorphism Analysis of Small-Subunit rRNA and mcrA Genes by Using Defined Template Mixtures of Methanogenic Pure Cultures and Soil DNA Extracts. *Society* **69**, 320-326 (2003).

20. Qiu, X. et al. Evaluation of PCR-Generated Chimeras , Mutations , and Heteroduplexes with 16S rRNA Gene-Based Cloning. *Society* **67**, 880-887 (2001).

21. Suzuki, M., Rappe, M. & Giovannoni, S. Kinetic bias in estimates of coastal picoplankton community structure obtained by measurements of small-subunit rRNA gene PCR amplicon length heterogeneity. *Applied and environmental microbiology* **64**, 4522-9 (1998).

22. La Duc, M.T. et al. Comprehensive census of bacteria in clean rooms by using DNA microarray and cloning methods. *Applied and environmental microbiology* **75**, 6559-67 (2009).

23. Yergeau, E. et al. Environmental microarray analyses of Antarctic soil microbial communities. *The ISME journal* **3**, 340-51 (2009).

Academic Vita

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The Pennsylvania State University, Schreyer Honors College
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Awards/Special Recognition/Honors:

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Selected to join Phi Beta Kappa Honor Society (Spring 2011 – Present)

Research/Professional Experience:

Biology, Deep-Sea Ecology, Penn State: Res. Asst. (Spring 2010 – Present)

- Traveled through Gulf of Mexico to perform analysis of deep sea imagery
- Used ArcMap/ArcView Geographic Information Systems to digitize mosaics
- Performed analysis of collected data through overlay statistics
  - Chi Square Test
  - P-value significance
- Examined associations and determined correlations

National Aeronautics and Space Administration, Biotechnology and Planetary Protection, California Institute of Technology (Spring 2011)

- Focused on preventing forward and backward contamination of planets in space missions
- Aimed to relate extremophiles on Earth to potential life on Mars and Europa
- Studied survivability of bacterial spores under various extraterrestrial conditions
- Examined resistance of spores to gamma radiation
- Developed new technologies for discovering deep microbial diversity
- Connected findings to opportunities on other planets

Unilever Home & Personal Care, North American Regional Claims R&D, Trumbull, CT (Summer 2011)

- Aimed to improve assessments of skin cleansing efficacy
- Developed new quantification techniques to improve measurements
- Tested various soils, substrates, and quantification method
- Designed and performed a 20 subject clinical study
- Harnessed consumer and technical data for the advancement of marketable claims

Immunology and Infectious Diseases, Penn State: Res. Asst. (Spring 2009 – Fall 2009)

- Performed small and large scale DNA plasmid preparations
- Performed PCR in order to increase amount of sample DNA
- Ran DNA samples through gel electrophoresis and analyzed results with molecular weight markers
- Used DNA samples to grow mumps viral particles into colonies
- Performed mammalian cell culture to use as experimental sample
- Transfected mammalian cells with mumps viral particles
- Observed behavior of transfected cells and analyzed results

Center for Cancer Pharmacology, University of Pennsylvania (Summer 2009)

Identifying Biomarkers of Cancer and Pre-term Birth by Mass Spectrometry

- Cultured mammalian lung (NHBE) and placental (BeWo) cells in a specific medium
    - Cultured in both unlabeled (KSFM or F-12K) and heavy labeled (SILAC) media
    - Self-prepared all media used for cell cultures
- Concentrated proteins and checked concentration with Bradford Assays
- Performed tryptic digestions on cells to extract proteins in a secretome
- Prepared protein samples for SCX–HPLC with DTT, IAA, and Trypsin
- Performed SCX – HPLC on digested samples
- Lyophilized samples and prepared for and performed Mass Spectrometry
- Collected spectral data and analyzed with MASCOT and SEQUEST
- Interpreted data through pattern matching with PERL programming

Maize Genetics Laboratory, Penn State: Res. Asst. (2006 – 2008)

- Performed genetic experiments by crossing elite germplasm with mutant plants
- Performed phenotypic and PCR based genotypic identification of F_2 segregating populations
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Volunteer Activities/Special Projects/Outreach:

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Raised money for P.A.W.S. and Make-A-Wish charity (2007)
Volunteered at the American Cancer Society (January 2007)
Volunteered in the Toys for Tots charity (December 2006)
Participated in Pennsylvania Governor's School for the Agricultural Sciences (Summer 2007)
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Giant Foods: Cashier (November 2007 – May 2009)

The Pennsylvania State University, Dept. of Mathematics: Grading Asst. (Fall 2009 – Fall 2010)

The Pennsylvania State University, Dept. of Biology: Teaching Assistant (Fall 2009 – Present)

Extracurricular Activities:

Active member of GlobeMed at Penn State (Fall 2009 – Present)
Active member of the Penn State Ski Club (Fall 2009 – Present)