

Metagenomic-based assessment of bacterial communities in solid and liquid dairy waste

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Figure 1: Dairy barn. Flush system flushes manure down lanes with water.



Figure 2: Flushed liquid dairy waste. This solid-liquid mixer will go to a separator.



Figure 3: Solid-liquid separator. Dairy waste is partitioned to compost piles or lagoons.



Figure 4: Liquid manure stored in an anaerobic lagoon. A primary lagoon transitions to a secondary lagoon.

Figure 5: Cropland receiving the dairy manure.

INTRODUCTION

California is the number one dairy state in the nation. According to the US EPA, 1.4 million dairy cows in 2,700 dairy farms produce 3.2 billion gallons of milk, which is 18% of the national supply. However, these cows also produce about 30 million tons of manure each year – making dairy waste management a prominent issue in California, especially in terms of water and crop quality [1]. From a survey in 2007 of major dairy counties in California, one of the most common manure collection systems was a flushed water or flushed and scraped water system (Fig. 1-2). In a flushed system, this manure is separated into solid and liquid manure (Fig. 3). Solid manure is often piled, composted, and/or distributed to the cropland as bedding, while liquid manure is stored in treatment ponds, or anaerobic lagoons (Fig. 4). Liquid manure also is used as a water source for fields and is prone to contaminating groundwater [2]. A major investigation that has yet to be done is shedding light on the microbial changes from one step in the dairy waste management process to the other and looking for possible functionally transformative microbes in terms of turning excessive manure into viable resources.

The goal of this study is to characterize, using a metagenomic approach, the microbial communities within the liquid and solid dairy manure along the steps of the dairy waste management line in dairy farms around California. The samples went through the following: DNA extraction, PCR at the 16S prokaryotic gene, Illumina high-throughput MiSeq sequencing and bioinformatic analysis. Samples were sorted into a cluster analysis and a heat map, as well as frequency and abundance. In identifying patterns between the liquid and solid dairy manure, this may provide farmers better informed decisions from a microbial approach on how to manage and allocate the animal wastes.

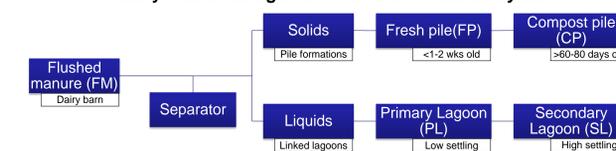
METHODS

Thirty-four samples were collected from flushed dairy manure and dairy wastewater from primary and secondary lagoons in various dairy farms in Orland, Merced, and Tulare counties in California. Whole genomic DNA from each sample was extracted using MO BIO PowerSoil and PowerWater kits. Libraries were prepared by targeting the prokaryotic 16S rRNA region of each sample through PCR amplification and barcode attachment with dual indexing using the 16S Illumina Nextera XT Index kit. The pooled library was sequenced on a MiSeq using PE 300. Bioinformatics pipeline processing was performed by Matthew L. Settles from the Bioinformatics Core at University of California, Davis.

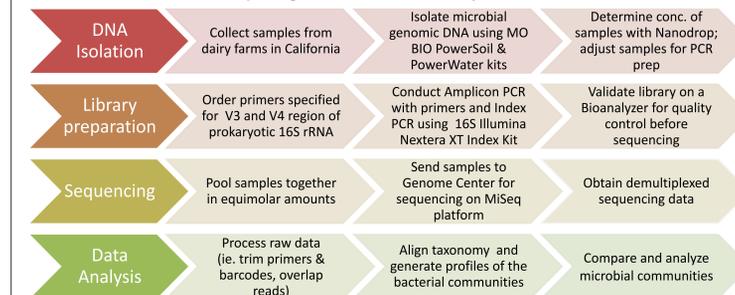
Bioinformatic Pipeline:

1. Barcodes and primers were identified and removed from the sequences.
2. Reads were overlapped to produce single amplicons.
3. Sequences were assigned using the Ribosomal Database Project (RDP) at 50% bootstrap value, possible only up to genus level.
4. Microbial profiles were generated.

Dairy Waste Management Flowchart: Flushed System

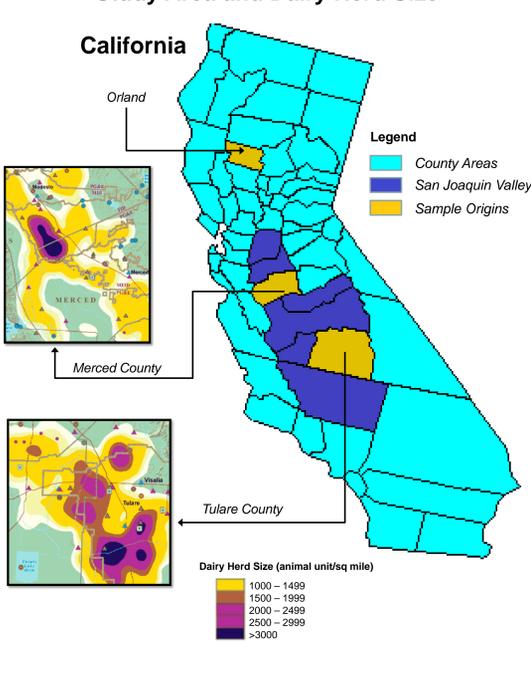


Method for Acquiring Bacterial Community Profiles Flowchart



RESULTS

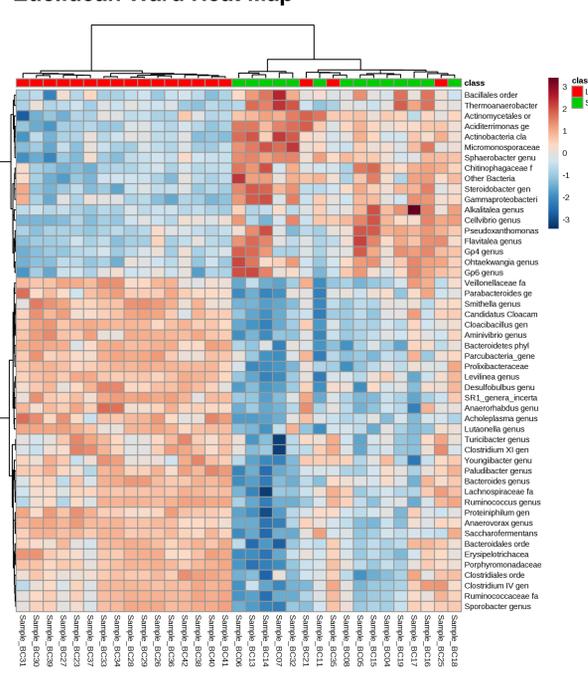
Study Area and Dairy Herd Size



1818 taxa were found throughout all the samples prior to filtering. During filtering, bacteria with $\geq 1\%$ in at least two samples or $\geq 5\%$ in 1 sample of the 7.9 million reads in RDP classification were categorized for a class. The bacteria that did not meet the threshold were aggregated into one class, called "Other Bacteria". As a result, the total taxa were reduced to 128.

The heat map for the 128 taxa, arranged by abundances of taxa corresponding to the samples, revealed an apparent distinct segregation in the microbial communities between solid and liquid samples.

Euclidean Ward Heat Map



Comparison between Solid and Liquid Samples



Top 15 Genera in Solid (Out of 14 samples)					Top 15 Genera in Liquid (Out of 20 samples)				
Names	Level	Freq	Abd (avg)	Std d	Names	Level	Freq	Abd (avg)	Std d
<i>Acinetobacter</i> genus	genus	14	7.9%	11.7%	<i>Clostridium XI</i> genus	genus	20	4.4%	4.2%
<i>Psychrobacter</i> genus	genus	14	1.5%	3.1%	<i>Cloacibacillus</i> genus	genus	20	3.4%	3.0%
<i>Pseudoxanthomonas</i> genus	genus	14	1.5%	2.4%	<i>Petrimonas</i> genus	genus	20	3.2%	4.9%
<i>Corynebacterium</i> genus	genus	14	1.4%	3.0%	<i>Proteiniphilum</i> genus	genus	20	1.8%	1.7%
<i>Saccharibacteria_genera_incertae_sedis</i> genus	genus	14	1.4%	2.4%	<i>Candidatus Cloacomonas</i> genus	genus	20	1.5%	1.6%
<i>Flavobacterium</i> genus	genus	14	1.1%	1.2%	<i>Anaerovorax</i> genus	genus	20	1.4%	1.2%
<i>Luteimonas</i> genus	genus	14	1.0%	0.8%	<i>Bacteroides</i> genus	genus	20	1.3%	1.3%
<i>Bacillus</i> genus	genus	14	0.7%	1.4%	<i>Turicibacter</i> genus	genus	20	1.1%	0.9%
<i>Pseudomonas</i> genus	genus	14	0.7%	0.8%	<i>Levilinea</i> genus	genus	20	0.8%	0.6%
<i>Clostridium XI</i> genus	genus	14	0.6%	0.7%	<i>Clostridium XIVa</i> genus	genus	20	0.8%	0.6%
<i>Youribacter</i> genus	genus	14	0.6%	0.9%	<i>Pseudomonas</i> genus	genus	20	0.8%	2.3%
<i>Palaeobacter</i> genus	genus	14	0.6%	1.0%	<i>Clostridium sensu stricto</i> genus	genus	20	0.5%	0.5%
<i>Bacteroides</i> genus	genus	14	0.6%	0.9%	<i>Ruminococcus</i> genus	genus	20	0.5%	0.4%
<i>Lachnospiraceae</i> fa	genus	14	0.6%	0.5%	<i>Anaerorhabdus</i> genus	genus	20	0.4%	0.7%
<i>Ruminococcus</i> genus	genus	14	0.6%	0.5%	<i>Dietzia</i> genus	genus	20	0.2%	0.4%
<i>Proteiniphilum</i> gen	genus	14	0.6%	1.0%					
<i>Anaerotruncus</i> genus	genus	14	0.6%	0.5%					
<i>Saccharofermentans</i>	genus	14	0.6%	1.0%					
<i>Bacteroidales</i> order	genus	14	0.6%	1.0%					
<i>Erythrobacter</i> genus	genus	14	0.6%	1.0%					
<i>Porphyromonadaceae</i>	genus	14	0.6%	1.0%					
<i>Clostridiales</i> order	genus	14	0.6%	1.0%					
<i>Clostridium XI</i> gen	genus	14	0.6%	1.0%					
<i>Ruminococcaceae</i> fa	genus	14	0.6%	1.0%					
<i>Ruminococcus</i> gen	genus	14	0.6%	1.0%					
<i>Sporobacter</i> genus	genus	14	0.6%	1.0%					

Bacteria were sorted by frequency of appearance in each category, then by average abundance with a corresponding standard deviation. Only the top 15 genera are shown. Highlighted genera are bacteria that both categories have in common.

Pathogens of Interest (Out of 34 Samples)				
Names	Level	Frequency	Abundance (avg)	Standard dev.
<i>Escherichia/Shigella</i>	genus	20	0.0155%	0.0223%
<i>Listeria</i>	genus	3	0.000175%	0.000879%
<i>Salmonella</i>	genus	7	0.000722%	0.00327%

Out of 34 samples, the presence of 3 well-known pathogens was observed by obtaining the numbers of appearances within the sample set and the average abundance in all the samples.

CONCLUSIONS AND FUTURE WORK

This metagenomic-based study assessed the abundance and variation of microbial species that exist along the dairy waste management line in various dairy farms in California. Here we have found that there is a clear distinction in the microbial composition between solid samples collected from manure piles and liquid samples from manure lagoons. For example, the top 15 genera between the two sample types greatly differed in microbes, which can be attributed to the different environments. In addition, 0.0155% of *E. coli* and other major known food-borne pathogens such as *Salmonella* (0.000175%) or *Listeria* (0.000722%) were found in relatively low levels – consistent with previous findings [3]. Although other potential pathogens might indicate potential pathogens such as *Acinetobacter*, *Clostridium XI* or *Bacillus*, some also contain species that are potentially beneficial for soil health and viable for other industrial uses. Other genera found, such as *Comanomas* genus that is involved in degradation of highly toxic and carcinogenic phenol compounds [4], contain novel microbes that are not well-understood, but may be useful for reducing environmental contaminants. Further work needs to be done on determining microbes on a more species-specific level using qPCR to determine the presence of other beneficial microbes such as those within the genus *Pseudomonas*, which contains known nitrogen fixers. The ongoing research presents an opportunity to inform farmers on the effects of various manure management practices on bacterial communities in dairy waste. A comprehensive survey of microbial communities found in animal wastes could help improve the methods of storing and disposing the wastes. It could allow to find ways to limit the presence of pathogenic bacteria and permit use of animal wastes as a biofertilizer/biostimulant for crop production.

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