PREVALENCE OF MICROBIOME IN SOIL AND WATER SAMPLES AROUND ANIMAL UNITS USING 16S rRNA SEQUENCING

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I. OBJECTIVES

Food safety is one of the defining food issues for industry, consumers, and policymakers alike. Animal habitat, water source, and soil harbor microorganisms of food safety importance, including foodborne pathogens: *Escherichia coli*, *Salmonella*, *Clostridium*, *Listeria*, and *Campylobacter.* These microbes can cross-contaminate our food system. Therefore, the goal of this project is to use the 16S ribosomal RNA sequencing to examine the prevalence of microbiome in soil and water samples around animal units during the Fall season. The animal units comprise a pig unit, a sheep and goat unit, a dairy farm, a poultry unit, an equine unit, and a cattle ranch around California Polytechnic State University in San Luis Obispo, California.

II. MATERIALS AND METHODS

For this study, soil and water samples (n = 16/Fall—first phase) were aseptically collected around animal units where the collection site (latitude, longitude) was determined using a handheld Global Positioning System. In order to monitor the proper variability of soil and water samples, 3 subsamples were collected from each site (10 to 15 m apart) and later combined into one. Sterilized plastic spoons and tubes were used to collect the soil and water samples, respectively, approximately 10 cm below the surface. Samples were immediately placed in a cooler (≤4°C) and transferred to a laboratory for further processing. DNA was extracted from samples using QIAamp PowerFecal® DNA kit. For the 16S service, 100 ng to 1 µg of genomic DNA per sample was preserved in elution buffer at a minimum concentration of 20 ng/µL. Metagenomic analysis of the microbial population was carried out using the 16S ribosomal RNA gene. Universal polymerase chain reaction primer was designed to target the conserved regions of 16S. Next-generation sequencing (250 bp Paired End, on-average min. of ~10,000–30,000 reads per sample) was used to cluster the sequences into operational taxonomic units, alpha and beta diversity, species classification, and abundance analysis.

III. RESULTS

First-phase results from the Fall season indicated that soil and water samples harbor different microbiome profiles where the phylogenetic relationship of operational taxonomic units was separated by Bray-Curtis distance of more than 0.6. Similarly, the principal component analysis (P = 0.002) demonstrates the clustering of the soil and water fecal groups. More than 30 bacterial phyla were detected in soil and water fecal samples. Among them, relative abundance of Proteobacteria, Bacteroidetes, and Firmicutes group was higher in water samples as compared with soil samples. However, Actinobacteria was the most abundant phylum in soil samples. Similarly, relative abundance of Chloroflexi, Planctomycetes, Acidobacteria, and Verrucomicrobia group was higher in soil samples as compared with water samples.

IV. CONCLUSION

The prevalence of Proteobacteria and Firmicutes in water samples around animal units was high in the Fall season. We are still waiting for the microbial abundance data from other seasons of the year. However, the presence of indicator microorganisms such as coliform, *Salmonella*, and others will be analyzed on above-mentioned samples using similar sequencing technology. Further analysis will demonstrate whether the prevalence of Proteobacteria and Firmicutes around animal units is a good indicator of foodborne microorganisms.

Keywords: 16S ribosomal RNA, microbiome, food safety, Gammaproteobacteria, Firmicutes