## Insight Into Anaerobic Soil Disinfestation Through The Lens Of Molecular Biology

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Anaerobic soil disinfestation (ASD), also referred to as biological soil disinfestation, is a potential alternative to chemical fumigation for controlling soilborne pathogens. ASD is a novel approach for controlling a broad-spectrum of plant pathogens while being environmentally safe. Previous research has shown that application of ASD controls soilborne plant pathogens as effectively as methyl bromide (MeBr) fumigation. Vegetable yields of ASD treated fields were comparable to, or exceeded those treated with MeBr in a Florida raised-bed vegetable production study. The fundamental basis of ASD consists of creating an environment which is conducive for facultative and obligate anaerobes to grow. Previous research has indicated that secondary metabolites generated by facultative and obligate anaerobic bacterial communities commonly found in soil are one of the factors that aids in controlling soilborne pathogens. Our current approach for application of ASD in the field consists of incorporating composted chicken manure with an easily-decomposable soil amendment, such as molasses, to stimulate soil microbial activity. These soil amendments are incorporated into raised beds or the upper 15 to 20 cm of soil in flat applications. Solarization and soil saturation are then applied to create conditions favorable for anaerobic bacteria. Tarping the soil with plastic clear polyethylene mulch and saturating the topsoil limit the resupply of oxygen.

Soil bacterial populations are essential for an effective ASD treatment; however, the identities of the important members of these communities remain unknown. In a successful ASD application it is assumed that different bacterial species contribute to oxygen-depletion and the production of organic acids. Having a better understanding of the microbial dynamics of ASD will provide insight into creating a more efficient system. The objective of the current study is to determine the bacterial species which are important in generating anaerobic activity. Soil samples were collected from multiple ASD-treated fields. The pH of one of the treated fields was taken every 2 to 3 days and the soil pH level dropped as low as 4.3, while the average pH of the untreated control remained stable at approximately 6.0. The decrease of soil pH correlated with a shift in bacterial populations. Through length heterogeneity PCR (LH-PCR), a bacterial population shift can be detected between pretreated and ASD post-treatment soil. Using the bacterial universal primer set, V2 and V3, a region of the 16s rRNA of the DNA extract from soil samples was amplified. From two field trials, three bacterial species of interest have been detected. A correlation has been observed between the lowering of soil pH and the increase of these components of the population over time. These three "species" dominate the post-treatment population, comprising as much as 67% of the total population, while accounting for 16% or less of the pretreatment population. Cloning and next generation sequencing will aid in determining the identity of these species.