

Sequencing of 16S rRNA's regions in the study of the diversity of contaminants and pathogenic microbes in biofertilizer

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Abstract:

The biofertilizers produced from the anaerobic biodigestion aim the adequate fermentation to decrease of pathogenic microorganisms and indicators of environmental pollution. Therefore, the purpose of this study was to evaluate the presence of contaminants and pathogenic microorganisms in biofertilizers. For this, two biofertilizers were produced by anaerobic biodigestion with caprine waste (BC) and ovine waste (BO). Samples of the biofertilizer were submitted to the total DNA extraction and used in PCR (Polymerase Chain Reaction) containing universal primers for prokaryotes. The PCR products were purified using magnetic beads and they were submitted to a second PCR for the bond of Index adapters, and they were purified one more time. The samples were united in a single pool, which was submitted to agarose gel electrophoresis followed by purification. The purified pool from the agarose gel was quantified by real time PCR. Then, 10 pM of DNA was sequenced in MiSeq equipment (Illumina®). The sequences were analyzed using the Mothur software. 6573 sequences were obtained from microorganisms belonging to Gammaproteobacteria class that includes several groups of pathogens, with 63.65% of the sequences present in the BO and 36.35% in BC. The presence of a large number of sequences (5814) belonging to groups of infectious bacteria can be highlighted. These infectious bacteria can cause plant diseases, animal diseases and human diseases, as an example of the orders: Aeromonadales (Succinivibrionaceae family), Alteromonadales (Alteromonadaceae family) Chromatiales (Chromatiaceae and Ectothiorhodospiraceae family) Oceanospirillales (Oceanospirillaceae family) Xanthomonadales (Xanthomonadaceae family) Thiotrichales (Piscirickettsiaceae family) Pseudomonadales (Moraxellaceae and Pseudomonadaceae family). It was also found 72 sequences from the Actinobacteria Class which includes some beneficial microbial groups with biological importance for the soil, but also includes other groups that are able to cause diseases such as the Corynebacteriales orders (Nocardiaceae family), Micrococcales (Cellulomonadaceae family) Propionibacteriales (Nocardioidaceae family), present in both biofertilizers with 55.5% of the sequences in the BO and 44.5% in BC. As follows, the present study indicates the necessity of efficiency in the use of biofertilizers, and the use of proper dosage looking for a balance between vegetative growth and the lowest risk of environmental contamination.

Keywords: waste, pathogens, environmental, goats, sheep.

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