

Título: DIFFERENCES IN THE EFFICACY OF TWO SOIL DNA EXTRACTION KITS IN BACTERIAL DNA EXTRACTION FROM SOIL SAMPLES

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Resumo:

The soil microbial community has the potential to be used as biological markers of modified ecosystems, thus its characterization can allow the monitoring of anthropogenic actions that can affect the environment. In addition, the study of the soil diversity of microorganisms can lead to a better understanding of the soil ecological interactions and to the obtaining of new microbes and gene sequences with high potential to biotechnological use. In Rondônia, the advancement of livestock on areas of the hydrographic basins of Mutumparaná River, is causing the conversion of the open ombrophilous forest into pasture areas. This process can cause changes in the soil microbiota, affecting nutrient cycling, soil chemical and physical structure and leading to a lower environment resilience capacity. Soil DNA extraction, is a crucial step towards the study of soil microbial diversity and to the obtainment of the soil microbial genomic pool. In this context, this work aimed to analyze the efficiency of two different kits, the UltraClean® Soil DNA Isolation Kit (Mo Bio) and the NucleoSpin® Soil (Macherey-Nagel), in the DNA extraction from soil samples from forest and pasture in areas surrounding the Mutumparaná River basin. The extractions were done following the manufacturer's recommendations, and subsequently, a polymerase chain reaction (PCR) were performed using the *GoTaq® Flexi* DNA Polymerase and the F-968-GC and R-1401 primers pairs, which amplify bacterial 16S rRNA gene sequences. Then, PCR products were separated using the Denaturing Gradient Gel Electrophoresis (DGGE) technique. The DGGE profiles were analyzed via digital image analysis using the BioNumerics software and the bacterial diversity were accessed by the Shannon diversity index calculated by the Past software. Analysis of Variance (ANOVA) on calculated indexes was accomplished using the statistical R-Software. For the same sample of pasture soil the NucleoSpin® Soil provided a Shannon diversity index of 2,46 while the UltraClean® Soil DNA Isolation Kit provide an index of 1,07 (p-value 1,1931e-5). Besides that, the NucleoSpin® Soil provided a Shannon diversity index of 2,43 while the UltraClean® Soil DNA Isolation Kit provide an index of 2,02 (p-value 0,00054263) for the same forest soil sample. The results show that soil DNA extraction with the NucleoSpin® Soil kit provided higher bacterial diversity for both for pasture and forest areas than the UltraClean® Soil DNA Isolation Kit.

Palavras-chaves: bacteria, diversity, DGGE, DNA extraction kits

Agência Fomento: CNPq, CAPES