TITLE: HUMIC ACID-*Herbaspirillum seropedicae* INTERACTION: EFFECT ON MAIZE ROOT MICROBIOME

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ABSTRACT:

The search for alternative agricultural production technologies has resulted in successful design of microbial inoculants based on endophytic diazotrophic bacteria and humic substances. However, most studies attribute the success of inoculants to their direct effect on plant metabolism and disregard their effect on the equilibrium displacement of microbial communities or microbiomes. This study aimed to analyze, by new generation sequencing (NGS), main changes in bacterial community associated with maize root seedlings inoculated with *Herbaspirillum seropedicae*, combined or not with humic acid. Under growth chamber conditions, maize seeds (*Zea mays* hyb. Dekalb 7815) were surface disinfested, conditioned in Furlani's nutrient solution (low nitrogen, ¼ ionic strength) with the following treatments: a) uninoculated water; b) humic acid extracted from vermicompost (sugarcane filter cake) (50 mg L^{-1}); c) *H. seropedicae* (strain HRC54) grown on DIGYS liquid medium ($2x10^7$ cells mL^{-1}); d) Humic acid + *H. seropedicae*. Total and diazotrophic bacteria counts associated with maize roots were performed respectively on Nutrient Broth solid medium and JNFb semi-solid medium. The total DNA of maize roots was extracted, followed by PCR amplification of the 16S rRNA gene and sequencing on the Ion Torrent PGM System for NGS platform. The number of diazotrophic bacterial cells root-associated was higher for all treatment related to control. The beta diversity, evaluated by the Principal Coordinate Analysis (PCoA), revealed homogeneity within the treatments and differentiation between them, where a higher number of taxa was observed in humic acid + bacterial treatment. Analysis of the alpha diversity, estimated by the Shannon index, showed that there were already bacteria in the control before inoculation of the treatments. Thus, the addition of humic acid, *H. seropedicae* and combination of both increased bacterial diversity in maize roots. The main phyla founded in the roots of the control plants and treated with bacteria and/or humic acid were *Proteobacteria*, *Bacteroidetes* and *Firmicutes*. Within the *Proteobacteria* phylum, the classes *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* were predominant in all treatments. In conclusion, the inoculation of humic acid and *H. seropedicae* interfered in the microbiome modulation of maize roots.

KEYWORDS: biostimulant, diazotrophic bacteria, diversity, Ion Torrent PGM

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