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## THE EFFECT OF SAMPLING TECHNIQUES ON THE STUDY OF BACTERIAL DIVERSITY IN *Lotus tenuis* NODULES.

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Bacteria collectively known as rhizobia can form symbiotic relationships with various leguminous plants, inducing the formation of root nodules where the fixation of atmospheric nitrogen (N<sub>2</sub>) takes place. Within the nodules, additional bacterial populations identified as non-rhizobial endophytes are present, though their identity and functional roles remain unexplored. Assessing the bacterial genetic diversity within legume nodules is crucial for devising long-term strategies to enhance the role of these bacteria in agricultural productivity. Research on microbial diversity inside the nodules has been addressed by various sampling methods. In most studies, culture-dependent techniques have been employed, with bacteria being collected from either field-grown plants or legumes cultivated in pots containing soil. However, there have been limited studies investigating whether the isolates derived from nodules from plants under artificial conditions accurately reflect the diversity found in nodules of field grown plants. Our aim was to investigate the microbiome in *Lotus tenuis* nodules collected with two different sampling techniques (field grown and trap plants) using a culture-independent method. The bacterial community profile was accessed through high-throughput amplicon sequencing of the V3-V4 region 16S rRNA gene in the platform DNBSEQ. Raw sequences were filtered and annotated using Mothur v.1.31.2 software. 1.388 OTUs were obtained through the clustering of 97% similarity. Alfa and beta diversity were calculated to identify diversity within and between samples. Differential analysis of taxonomic groups was conducted using linear discriminant analysis (LDA) effect size (LEfSe). The number of sequences per sample was between 127.910 and 130.105. Alpha diversity had no significant differences in both sampling techniques. *Mesorhizobium* (*L. tenuis* canonical symbiont, 97.2-70.0%), *Aminobacter* (14.17-0.0%), *Salmonella* (5.7-0.0%), *Rhizobium* (0.5-0.0%) and *Pantoea* (1.7-0.0%) were the most abundant genera. *Aminobacter* and *Nitrospira* were identified as biomarkers of trap plant experiment using LEfSe analysis, but *Salmonella*, *Pantoea*, and *Pseudomonas*, among others, were identified as biomarkers of field-grown plants (LDA score > 1.3 and P < 0.05). Our results, which show changes in the nodule community depending on the sampling method, have significant implications. This study serves as an initial exploration into whether the bacterial diversity in root nodules of trap plants grown under artificial conditions reflects the population present in field conditions.

Palabras clave: rhizobia - nodules - legumes - amplicon sequencing - sampling methods