

**Title: DIFFERENTIAL PHYLOGENETIC, GENOMIC AND PHYSIOLOGICAL PROFILES OF PSEUDOMONADS FROM SUGARCANE RHIZOSPHERE AND BULK SOIL**

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**Abstract:**

The sugarcane rhizosphere microbiome is composed by a huge bacterial diversity which supports the plant growth and development. *Pseudomonas* spp. is an example of bacterial genus known to perform a lot of beneficial functions. Thus, the knowledge of its biology and association with plant roots is very important. Therefore, this study aimed to understand the ecology of *Pseudomonas* inhabiting the rhizosphere compared to the ones living in bulk soil. We used two approaches to investigate these differences. In the first, we used molecular analyzes (quantitation by qPCR and sequencing using Illumina MiSeq) to assess the rhizospheric selection on the *Pseudomonas* genus community. Secondly, we isolated several *Pseudomonas* strains and analyzed its phylogenetic (16S rRNA gene sequencing), physiological (BIOLOG) and genomic (BOX-PCR) profiles. The rhizosphere environment showed significant ( $P=0.002$ ) higher numbers of bacteria belonging to the *Pseudomonas* genus compared to bulk soil ( $8.7 \times 10^5$  against  $2.5 \times 10^4$  copies of 16S rRNA gene per soil gram). The QIIME analysis of *Pseudomonas* reads also showed higher OTU numbers in rhizosphere than in bulk soil, and the multivariate analysis (NMDS) indicated a different *Pseudomonas* community structure between rhizosphere and bulk soil, confirmed by ANOSIM ( $P=0.002/ r^2=0.74$ ). The isolation with *Pseudomonas* agar base resulted in 34 strains, which were classified in 3 different 16S rRNA gene groups according to the Genbank database (*P. fluorescens*/ *P. koreensis*; *P. plecoglossicidal* *P. monteilii*; and *P. putida*). The genomic and physiological profiles showed an incongruence when compared to the phylogenetic profile by 16S rRNA. The BOX profile pointed 7 groups, whereas the BIOLLOG analysis showed at least 10 groups of different profiles, dividing strains with the same taxonomic classification in separated genomic and metabolic profiles. There is a clear rhizosphere selection effect upon the *Pseudomonads* community and the culture dependent analyzes suggest that it may exist different selective pressures acting also on the the bacterial genomes, even in strains of the same species. We selected 6 strains for genetic marking by transformation with PNKBOR plasmid and the next step will be to inoculate these strains in a microcosm experiment with sugarcane plants trying to observe genomic rearrangements. Our hypothesis is that the rhizosphere selection on the *Pseudomonads* community also influences the genomic evolution of individuals.

**Keywords:** Natural selection; microbial genetics; soil microbiology; PGPR; NGS.

**Funding Agency:** CAPES