

## Characterization of olive xylem microbiome community composition by metabarcoding greatly depends on the matrix used to extract DNA and 16S universal bacterial PCR primers

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### Abstract:

Understanding of xylem sap microbiome is becoming of relevant importance for plant health as it could include microbes that may protect against xylem-limited pathogens, such as *Xylella fastidiosa*, and supporting key biological processes. Furthermore, the negative pressure, low oxygen and nutrient content of the xylem sap make it an unique and unexplored microbial environment. In this study, we evaluated the differences obtained in the characterization of the xylem microbiome composition when using xylem sap extracted from xylem vessels using a Scholander pressure chamber or when using macerated fine chips obtained from xylem tissues from 10-year old or 1-year old olive trees. We also compared four different PCR primers pairs targeting 16S rRNA for their efficacy to avoid co-amplification of mitochondria and chloroplast 16S rRNA, as this suppose an important drawback in metabarcoding studies. PCR primers tested included 799F/1062 (V5-V6), 799F/1115 (V5-V6), 967/1391 (V6-V8) and 799F/1193 (V5-V7). Illumina paired-end sequence quality control and chimeric filtering was performed with DADA2 using QIIME2. Taxonomy affiliation into OTUs at 99% was based on Silva reference database. The highest mitochondria and chloroplast amplification was obtained when using xylem chips and 799F/1062 (77.7%) and 967/1391 (99.6%) primers. On the contrary, 799F/1115 and 799F/1193 primers showed the lowest mitochondria (<6.76%) and chloroplasts (<0.02%) amplification, and the highest number of OTUs identified, 245 and 247, respectively. Interestingly, only 81/236 and 27/240 OTUs or 66/144 and 21/149 genera were shared between xylem sap or wood shavings after amplification with 799F/1115 or 799F/1193, respectively. The most abundant bacterial genera (>50% of reads) included *Anoxybacillus*, *Cutibacterium*, *Methylobacterium*, *Pseudomonas*, *Rathayibacter*, *Sphingomonas* and *Spirosoma*; however, their relative importance varied depending of the matrix and primer pairs used. These results will help to optimize analysis of xylem microbiome community composition and more importantly to understand its driving and modifying factors.

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