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Impact of metagenomic DNA extraction procedures on the identifiable endophytic bacterial diversity in *Sorghum bicolor* (L. Moench)

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Highlights

Plant DNA extraction protocols affect diversity of retrieved endophytic bacteria.

Plant mDNA yield and purity are also affected by DNA extraction protocol used.

Sorghum-associated endophytic bacterial communities are diverse.

Sorghum root and stem endophytic communities are significantly different.

Sorghum-associated endophytic bacteria have potential for plant growth promotion.

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Abstract

Culture-independent studies rely on the quantity and quality of the extracted environmental metagenomic DNA (mDNA). To fully access the plant tissue microbiome, the extracted plant mDNA should allow optimal PCR applications and the genetic content must be representative of the total microbial diversity. In this study, we evaluated the endophytic bacterial diversity retrieved using different mDNA extraction procedures.

Metagenomic DNA from sorghum (*Sorghum bicolor* L. Moench) stem and root tissues were extracted using two classical DNA extraction protocols (CTAB- and SDS-based) and five commercial kits. The mDNA yields and quality as well as the reproducibility were compared. 16S rRNA gene terminal restriction fragment length polymorphism (t-RFLP) was used to assess the impact on endophytic bacterial community structures observed. Generally, the classical protocols obtained high mDNA yields from sorghum tissues; however, they were less reproducible than the commercial kits. Commercial kits retrieved higher quality mDNA, but with lower endophytic bacterial diversities compared to classical protocols. The SDS-based protocol enabled access to the highest sorghum endophytic diversities. Therefore, "SDS-extracted" sorghum root and stem microbiome diversities were analysed via 454 pyrosequencing, and this revealed that the two tissues harbour significantly different endophytic communities. Nevertheless, both communities are dominated by agriculturally important genera such as *Microbacterium*, *Agrobacterium*, *Sphingobacterium*, *Herbaspirillum*, *Erwinia*, *Pseudomonas* and *Stenotrophomonas*; which have previously been shown to play a role in plant growth promotion. This study shows that DNA extraction protocols introduce biases in culture-independent studies of environmental microbial communities by influencing the mDNA quality, which impacts the microbial diversity analyses and evaluation. Using the broad-spectrum SDS-based DNA extraction protocol allows the recovery of the most diverse endophytic communities associated with sorghum tissues and, as such, establishes a reliable basis for future study of endophytic communities.

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Keywords

Metagenomic DNA extraction; Endophytic bacteria; Sorghum root and stem; t-RFLP; Pyrosequencing

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