ECM 2025 Conference

The 3rd International Electronic Conference on Microbiology

01–03 April 2025 | Online

MDPI

Comparative evaluation of 16S rRNA and housekeeping gene-specific primer pairs for rhizobia and agrobacteria community metagenomics

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INTRODUCTION & AIM

Rhizobia are scattered across 18 genera, in which several taxa are known to have conserved genetic structure across rRNA operons. Recent metagenomic studies have revealed that the **16S rRNA gene** and corresponding variable regions **may have insufficient taxonomic resolution** for the accurate identification of rhizobia and related plant-associated bacteria such as agrobacteria. Therefore, some housekeeping genes, including *gyrB* and *rpoB*, are used as alternative markers to 16S to analyze rhizobia communities. However, the extent to which the targeted genes and their corresponding primers could be suitable in metagenomic studies of all the genera of rhizobia remains elusive. **This work evaluates *in silico* the taxonomic resolution of partial regions of two housekeeping and 16S rRNA genes to differentiate between rhizobia & agrobacteria**.

METHOD

Selected genes, V-regions & primers

Target	Forward sequence (5' to 3')	Reverse sequence (5' to 3')	Size (bp) among taxa
V1-V2	AGAGTTTGATCMTGGCTCAG	CYIACTGCTGCCTCCCGTAG	320-350
V1-V3	AGAGTTTGATCMTGGCTCAG	ATTACCGCGGCTGCTGG	468-523
V3-V4	CCTACGGGNGGCWGCAG	GACTACHVGGGTATCTAATCC	440-465
V3-V5	CCTACGGGNGGCWGCAG	CCGTCAATTYMTTTRAGT	560-585
V4	GTGCCAGCMGCCGCGGTAA	GGACTACHVGGGTWTCTAAT	292
V4-V5	GTGYCAGCMGCCGCGGTAA	CCCCGYCAATTCMTTTRAGT	413
V5-V7	AACMGGATTAGATACCCKG	ACGTCATCCCCACCTTCC	409-417
V6-V9	TAAAACTYAAAKGAATTGACGGGG	TACGGYTACCTTGTTAYGACTT	605-612
V7-V9	YAACGAGCGCAACCC	TACGGYTACCTTGTTAYGACTT	408-415
V1-V9	AGAGTTTGATCMTGGCTCAG	TACGGYTACCTTGTTAYGACTT	1445-1497
"*gyrB*-1"	MGNCCNGSNATGTAYATHGG	ACNCCRTGNARDCCDCCNGA	287-302
"*rpoB*-1"	GATCGARACGCCGGAAGG	TGCATGTTCGARCCCAT	378-384
"*rpoB*-2"	GGYTWYGAAGTNCGHGACGTDCA	TGACGYTGCATGTTBGMRCCCATMA	434-440

Evaluation of taxonomic resolution

Selection of **09 V-regions** (V1 to V9) of **16S** rDNA & universal **primers**

↓

Alignment of full-length 16S sequences for **19 genera of rhizobia & agrobacteria,** with sequences from the type species of each genus

↓

Editing of the alignment of the full-length 16S sequences size to the total number of positions that corresponds to those of each V-region

↓

Each edited dataset was then used to calculate a **pairwise similarity distance** that served **to identify the uniquely distinguishable taxa** for the given V-region (*i.e.* performance) at 97% & 100% cut-offs.

↓

The **performance** of all V-regions **compared** to those of the full-length 16S rRNA gene sequence, in addition to V1-V9 region

The same uniquely distinguishable taxa approach was applied to *rpoB* (two variable regions) and *gyrB* (one region) at ~98% and 100% thresholds to allow for a comparative evaluation.

RESULTS & DISCUSSION

- ✓ **Insufficient resolution confirmed for several 16S V-regions** (including widely used V4, V3-V4, V3-V5, V4-V5, etc.)
- ✓ V5-V7 appeared as the best target, but it has limitation at 97%.
- ✓ **The *rpoB* and *gyrB* markers outcompeted the 16S rDNA** in terms of taxonomic resolution regardless of the threshold (Figure 1), **possibly replacing the use of 16S rDNA V-regions** in the metagenomics of rhizobia and agrobacteria.

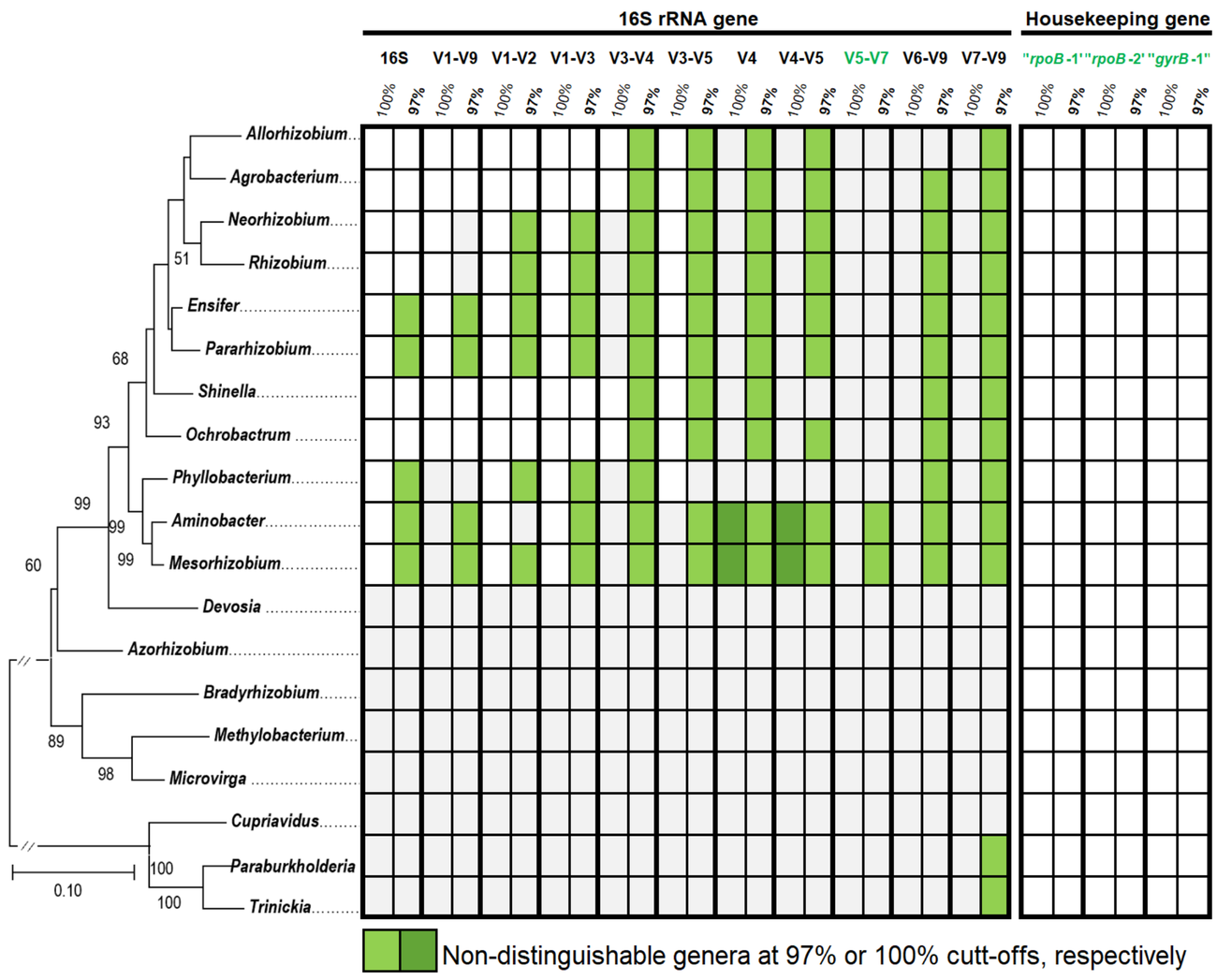

Fig. 1. Maximum Likelihood (ML) phylogenetic tree based on the full-size sequence of the 16S rRNA gene (1564 positions, TN93+G+I model, 1000 replicates, Bootstrap values ≥ 50% indicated, scale bar = number of substitutions per site. A white box indicates that a taxon can be uniquely distinguished with the given V-region and gene length and clustering method, while **a green shaded box** indicates that **a taxon is merged** with at least one other taxon.

CONCLUSION

***rpoB* and *gyrB* gene markers have higher resolution and could replace 16S rDNA in metagenomics of rhizobia & agrobacteria.**

FUTURE WORK / REFERENCES

A limit to our performance appraisal is that the approach is not tested yet with biological samples. Future work will do the test.

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