

MOLECULAR CHARACTERIZATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM SUGARCANE STALK AGAINST RINGSPOT DISEASE OF SUGARCANE (*Epicoccum sorghinum*) IN NEGROS ISLAND REGION, PHILIPPINES

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INTRODUCTION

- Sugarcane (*Saccharum officinarum* L.) is a crucial crop contributing significantly to the economy and industries of the Philippines. However, its productivity is threatened by ringspot disease, caused by *Epicoccum sorghinum*, which negatively affects growth and yield.
- One of the biological controls utilized to control plant diseases is endophytic bacteria. These bacteria directly fight plant pathogens by producing a range of antimicrobial compounds and competing with them for resources and space within the plant (Wang et al., 2019).

OBJECTIVES

- To test isolated endophytic bacteria from sugarcane against ringspot disease using the Dual Culture Assay and Volatile Compound Assay.
- To characterize the species of endophytic bacteria through morpho-cultural characterization and Gram staining analysis;
- To identify the species of endophytic bacteria using molecular characterization through Polymerase Chain Reaction (PCR) and Phylogenetic Analysis.

METHOD

1. Sample Collection

2. Culture Media Preparation

3. Isolation of Plant Pathogens and Endophytic Bacteria

4. Pure Culture Revival and Maintenance

5. DCA and VCA Set-Up

6. Gram Staining

7. Molecular Characterization

8. Data and Phylogenetic Analysis



CONCLUSION

The Dual Culture Assay showed B2-CS and B4-ORS as effective endophytic bacteria against ringspot disease, while the Volatile Compound Assay identified B5-BS as effective. Morphocultural and Gram staining revealed two Gram-positive and three Gram-negative isolates. Molecular analysis identified B2-CS, B4-ORS, and B5-BS as *Burkholderia gladioli*, *Stenotrophomonas rhizophila*, and *Bacillus pumilus*, respectively.

ACKNOWLEDGEMENT

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RESULTS & DISCUSSION

ANTAGONISTIC TEST

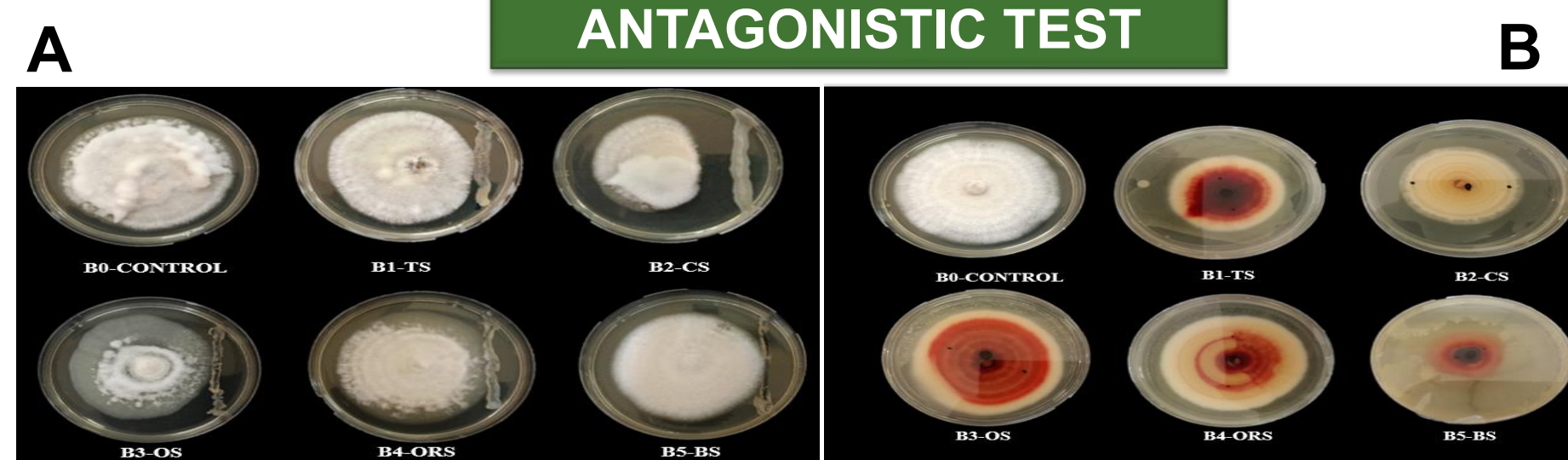


Figure 1. Antagonistic test of different isolates of endophytic bacteria against *Epicoccum sorghinum*: (A) Dual Culture Assay; (B) Volatile Compound Assay

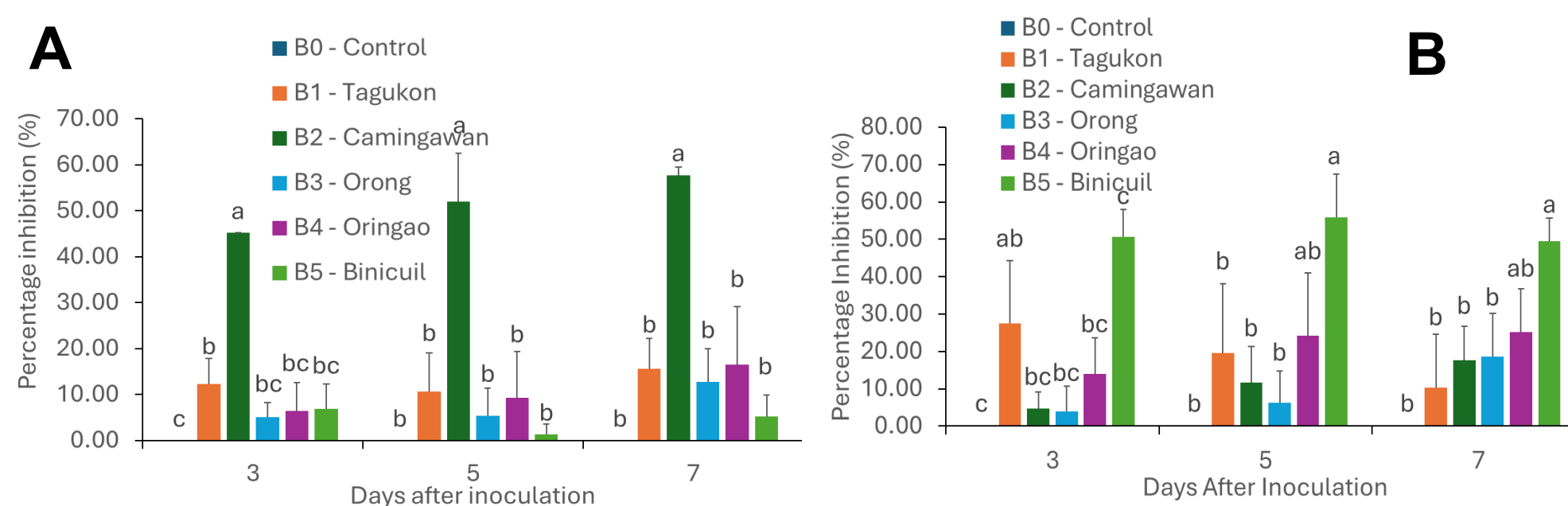


Figure 2. Percentage Inhibition of *Epicoccum sorghinum* treated with endophytic bacteria. (A) Using the Dual Culture Assay; (B) Using Volatile Compound Assay

- The results revealed that *Burkholderia gladioli* (Camingawan) exhibited the highest inhibition in the DCA (57.79%), followed by *Stenotrophomonas rhizophila* (Oringao) (16.55%)
- In VCA, *Bacillus pumilus* (Binicuil) was the most effective, showing a 49.56% inhibition rate.

CHARACTERIZATION OF ENDOPHYTES

Bacteria code	Colony color	Elevation	Margin	Opacity	Consistency	Surface
B1-TS	Cream	Raised	Entire	Translucent	Butyrous	Smooth
B2-CS	Pale yellow	Raised	Entire	Translucent	Butyrous	Rough
B3-OS	Pale yellow	Raised	Erose	Translucent	Mucoid	Wrinkle
B4-ORS	White	Convex	Erose	Translucent	Mucoid	Rough
B5-BS	Cream	Convex	filamentous	Translucent	Butyrous	Smooth

Bacteria code	Gram Staining	Shape	Form
B1-TS	+	Bacillus	Spindle
B2-CS	-	Bacillus	Spindle
B3-OS	-	Bacillus	Spindle
B4-ORS	-	Coccus	Punctiform
B5-BS	+	Bacillus	Spindle

- Endophytic Bacteria (B1-TS) or Tagukon and B5-BS or Binicuil are gram-positive, while B2-CS, B3-OS and B4-ORS are gram-negative. While in shape, form, and other cultural characteristics, variations were observed.

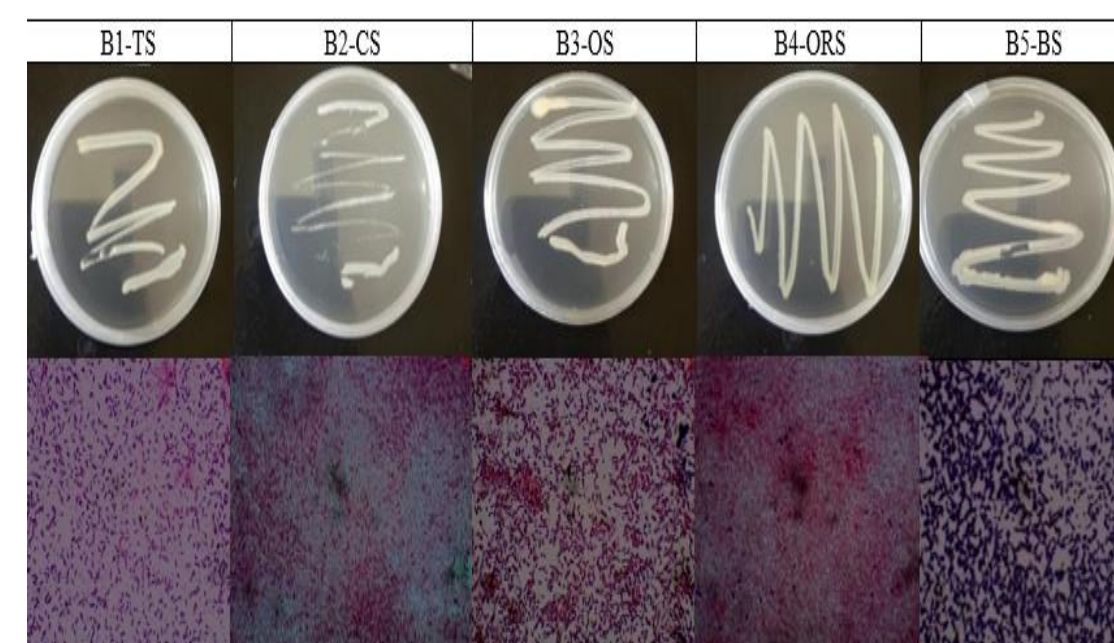


Figure 3. Morpho-cultural and gram staining analysis of five (5) endophytes.

IDENTIFICATION OF ENDOPHYTES

DNA sequencing identified B2-CS as *Burkholderia gladioli*, B4-ORS as *Stenotrophomonas rhizophila*, and B5-BS as *Bacillus pumilus*. The analysis also confirmed that the sugarcane ringspot pathogen used was *Epicoccum sorghinum*.

PHYLOGENETIC ANALYSIS

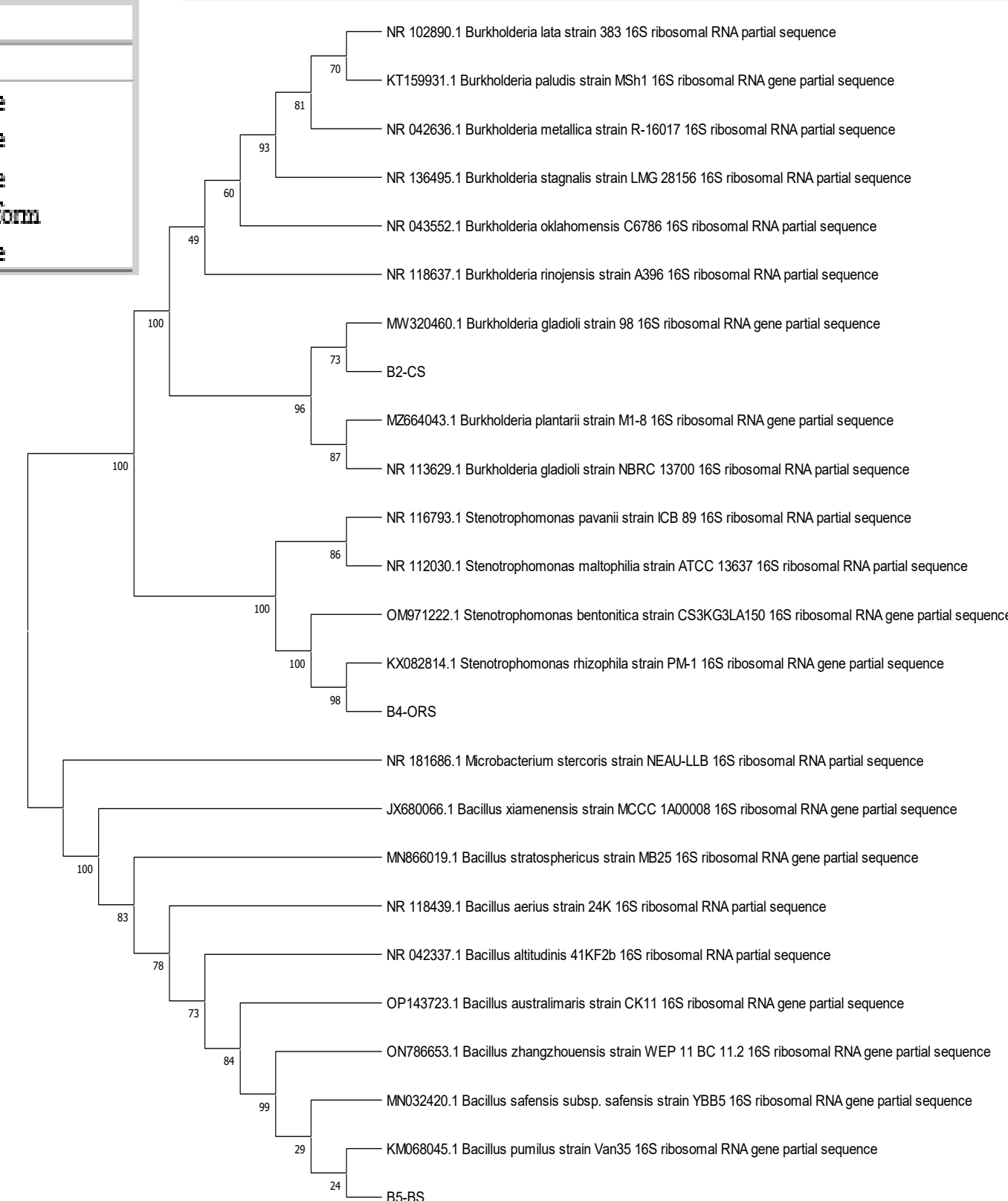


Figure 4. Phylogenetic tree of B2-CS, B4-ORS, and B5-BS species based on their Ribosomal RNA (rRNA) gene sequence. Sequences were aligned through BLAST, and phylogeny was concluded based on sequence similarities.

REFERENCES

- Wang Z., et al. 2019. Diversity of sugarcane root-associated endophytic Bacillus and their activities in enhancing plant growth. J. Appl. Microbiol. 128 814–827. 10.1111/jam.14512
- Laurel et al. 2021. Identification of *Epicoccum sorghinum* and its effect on stalk sugar yield. Sugar Tech, 23(6), 1383-1392