



# The diversity of endophytic bacteria isolated from genetically and non genetically modified maize

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# Introduction

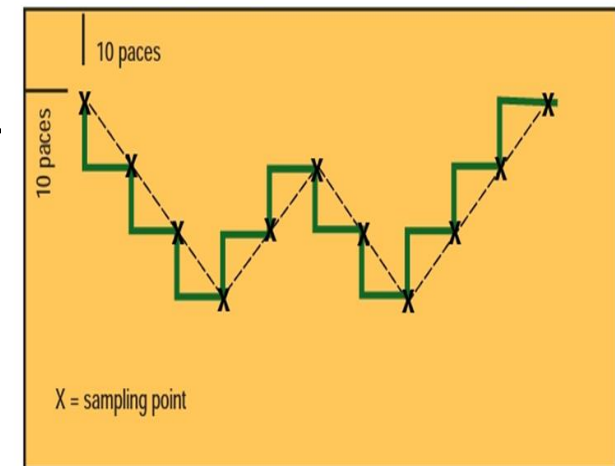
- Endophytes bacteria.
- They are found in many crops, including maize (Gayathri and Muralikrishnan, 2013).
- Maize (*Zea mays* L.)
- Specific maize strains have been genetically modified to display agricultural desired features such as insect resistance and herbicide tolerance (Szilagy-Zecchin, Keda, Hungria, Adamoski and Kava-cordeiro, 2014)



# Materials and methods

## Study area

- The GM maize plants were collected from Curativo North, a research institution in Nelspruit; and the Non-GM maize were collected from the University of Mpumalanga farm, Nelspruit, South Africa.
- Ten plants from each plot ( in a zig zag pattern) (Figure 2).
- Tap water was used to remove any soil
- debris
- Then they were divided into 3 plant part (leaves, roots, and stems)



**Figure 2:** Zig-zag sampling method used for maize collection (Lafitte, 1993)



# Materials and methods cont...

## Sample preparation and initial culture

- The 3 plant parts were sterilized following a method described by Eevers *et al.*, (2015).
- After the final step of sterilization, the specimens were left to air dry for 3 minutes on the laminar flow cabinet.
- The plant parts were plated out on Nutrient agar and incubated at 36°C for 24 hours



## Materials and methods cont...

### **IDENTIFICATION OF ENDOPHYTIC BACTERIA**

The bacteria obtained were identified using morphological, biochemical and molecular methods.

#### CELLULAR MORPHOLOGICAL IDENTIFICATION

- The morphological identification is used to determine character based on the shape and color. shape: Cocci (round) and Bacilli (rod). Color: Pink (Negative) and purple (Positive).
- The gram stain is considered the first stage of identification



# Materials and methods cont...

## BIOCHEMICAL METHOD

The staining is followed by use of various biochemical reagents and tests for identification of bacteria (Salo and Novero, 2020).

Four biochemical tests were used to perform:

1. Oxidase test
2. Catalase test
3. Simon citrus test
4. Dnase test



## Materials and methods cont...

### MOLECULAR METHOD

Bacterial universe primers 27F and 149R were used to amplify bacteria 16S rRNA gene fragment from all the isolates.

### **Specific species**

- All isolates were amplified using genes specific for *Methylobacterium mesophilicum*, *Stenotrophomas*, *Bacillus subtilis* and *Streptomyces*.
- The present of the genes with a 390bp, 1200bp, 1311bp and 1350 bp DNA fragment band confirms that the isolates are for the above genus.
- Extracted DNA amplified using thermal cycle



# Results and Discussion

## Sample processing

- 20 maize samples: 10 Non-GM and 10 GM (Herbicides tolerant and Insecticides tolerant) maize were collected from the farms
- After growth, a total number of 115 isolates were identified

## Gram staining

- Gram staining was performed on all the 115 isolates

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Gram Negative Bacilli	96	83.5	83.5	83.5
	Gram Positive Bacilli	4	3.5	3.5	87.0
	Gram Negative Cocci	8	7.0	7.0	93.9
	Gram Positive Cocci	7	6.1	6.1	100.0
	<b>Total</b>	<b>115</b>	<b>100.0</b>	<b>100.0</b>	





## Results and discussion cont...

### BIOCHEMICAL TESTS

#### Catalase test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	100	87.0	87.0	87.0
	Negative	15	13.0	13.0	96.9
	<b>Total</b>	<b>115</b>	<b>100.0</b>	<b>100.0</b>	



## Results and discussion cont...

### Oxidase test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	39	33.9	33.9	33.9
	Negative	76	66.0	66.0	95.9
	<b>Total</b>	<b>115</b>	<b>100.0</b>	<b>100.0</b>	



## Results and discussion cont...

### MOLECULAR TEST Species Specific (PCR)

Amplicon Size	Gram Negative Bacilli	Gram Positive Bacilli	Gram Positive Cocci
390bp ( <i>Methylobacteria mesophilicum</i> )	47 isolates		
1200bp ( <i>Stenotrophomas</i> )	7 isolates		
1311bp ( <i>Bacillus Subtilis</i> )		4 isolates	
1350bp ( <i>Streptomyces</i> )			1 isolate
Other	43 isolates	9 isolates	4 isolates

Species name	Non GM			% abundance for Non GM	GM			% abundance for GM	Total	Species percentage abundance (%)
	Roots	Leaves	Stems		Roots	Leaves	Stems			
<b>Methylobacteria</b>	6	5	2	43.3%	13	13	8	40%	47	40.8%
<b>Stenoptomycetes</b>	0	0	2	6.7%	2	3	0	5.6%	7	6.1%
<b>Bacillus Substili</b>	0	0	0	0	2	1	1	4.7%	4	3.5%
<b>Streptomycetes</b>	0	0	0	0	1	0	0	1.2%	1	0.9%
<b>Unidentified</b>	6	6	3	50%	15	13	13	48.2%	56	48.7%
<b>Total per plant part</b>	12	11	7	100%	33	30	22	100%	115	
<b>Total per maize cultivar</b>	30				85				115	
<b>Shannon-Weaver Index (H')</b>	0.7	0.7	1.1		1.2	1.1	0.8			
<b>Pielou's Evenness (J)</b>	0.3	0.3	0.6		0.3	0.3	0.3			
<b>Simpson Diversity Index (D)</b>	0.5	0.5	0.2		0.4	0.4	0.5			

Diversity indices



## Conclusion

- **OBJECTIVE 1:** To determine the diversity of the endophytic bacteria isolated on genetically and no genetically modified maize
- In this study: The Shannon wiener index values ranges from 0.7 to 1.1 :Non GM and 0.8-1.2 in GM.
- The Pielous`s eveness index ranges from 0.3-0.6 in Non GM and 0.3 in GM
- The Simpson index ranges from 0.3-0.5 in Non GM and 0.4-0.5 in GM.
- Therefore, based on the values found, we can accept the null hyphothesis and conclude that endophytic bacteria are not diverse in both GM and Non GM.



## Conclusion cont...

**OBJECTIVE 2:** To compare the endophytic bacteria isolated from genetically modified maize with those isolated from non- genetically modified maize

- The endophytic bacteria isolated from genetically modified maize are more compared to the ones isolated from non-genetically modified maize.
- The percentage abundance for GM is more than the percentage abundance of Non-GM.



## References

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# Thank you for your attention!

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