

INTRODUCTION

- ❖ **Oxalates** are plant compounds that can combine with metal ions to form insoluble precipitates. Over accumulation of oxalate in the body might eventually lead to the formation of kidney stones.^[1]
- ❖ **Oxalate degrading bacteria (ODB)** are bacteria that can speed up the breakdown of oxalate. ODB can be used for industrial waste paper pulp treatment, or as probiotic treatment for kidney stones.^[2]
- ❖ **Root nodules** are found on the roots of leguminous plants that contain nitrogen-fixing bacteria in which some might be ODB.^[3]

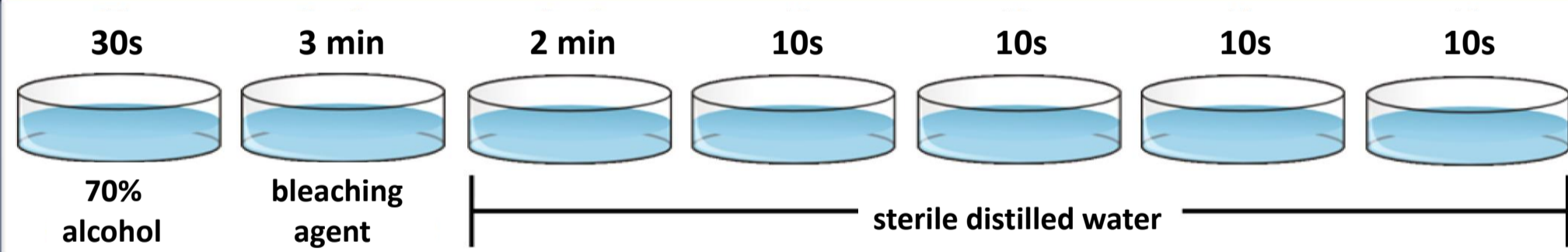
AIMS

- ❖ To develop a protocol for screening and identifying oxalate degrading bacteria.
- ❖ To screen and characterize oxalate degrading bacteria from root nodules of different legumes.

METHODS

Root Nodule Collection

Root Nodule Surface Sterilization



Bacteria Sample Isolation

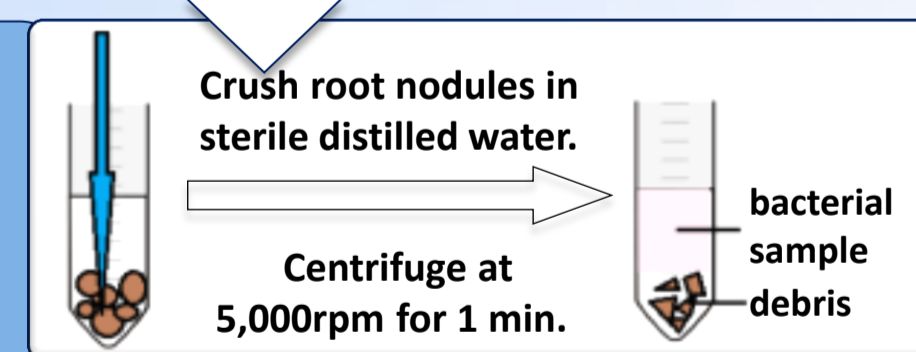


Plate Streaking & Incubation of Yeast-Mannitol (YEM) Agar Plates at 28°C for 2-3 days

Growth of bacteria colonies on YEM agar plates

Purified culture on YEM agar plates & Ammonium Oxalate (AO) agar plates

Gram Staining

Comparison between growth of bacteria in YEM & AO broth

Catalase Test

RESULTS

Table 1: Source of Bacteria Sample and Characterization of Legume Root Nodule Bacteria

Species of Legume	Bacteria Code	Morphology of Bacteria Under Light Microscope		Catalase Activity*
		Gram Nature	Shape	
<i>Calopogonium mucunoides</i>	SRO-1005	Negative	Rod	-
<i>Mucuna bracteata</i>	SRO-1006	Negative	Cocci	-
<i>Mimosa diplotricha</i>	SRO-1007	Negative	Cocci	+
<i>Vigna radiata</i>	SRO-1008	Positive	Rod	++
<i>Crotalaria pallida</i>	SRO-1009	Negative	Cocci	+++
<i>Crotalaria pallida</i>	SRO-1010	Negative	Cocci	-

*Keys : - indicates absence of catalase; + indicates weak catalase activity; ++ indicates medium catalase activity; +++ indicates strong catalase activity.

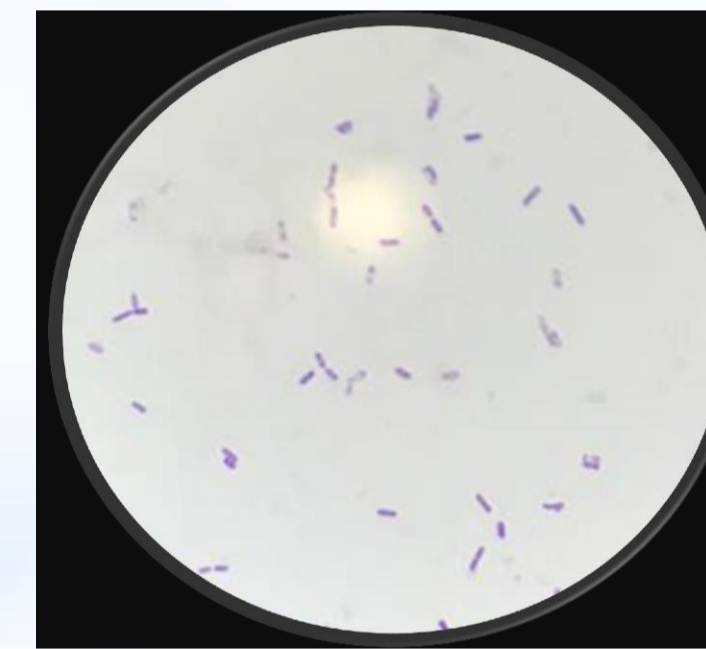


Figure 1: Gram staining of bacteria SRO-1008.
The bacteria are stained in purple (indicates Gram positive bacteria) and bacilli (rod-shaped).

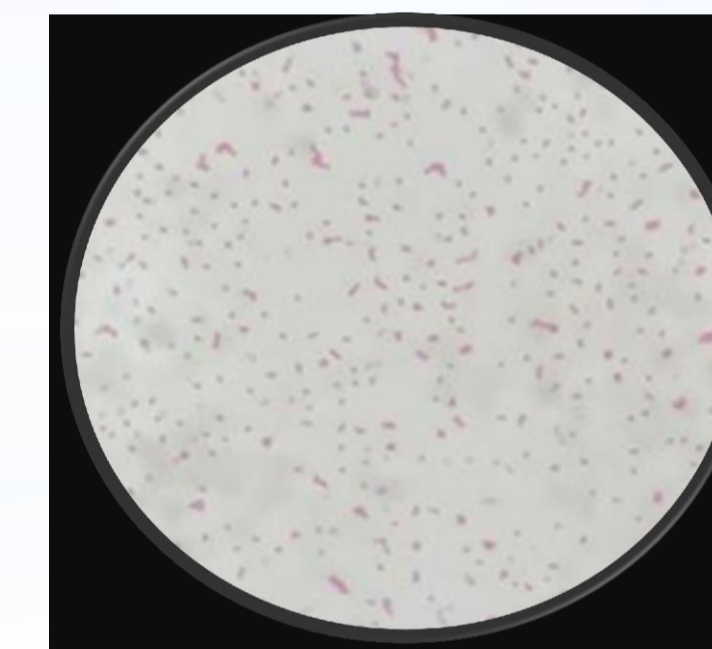


Figure 2: Gram staining of bacteria SRO-1009.
The bacteria are stained in pink (indicates Gram negative bacteria) and cocci (round-shaped).

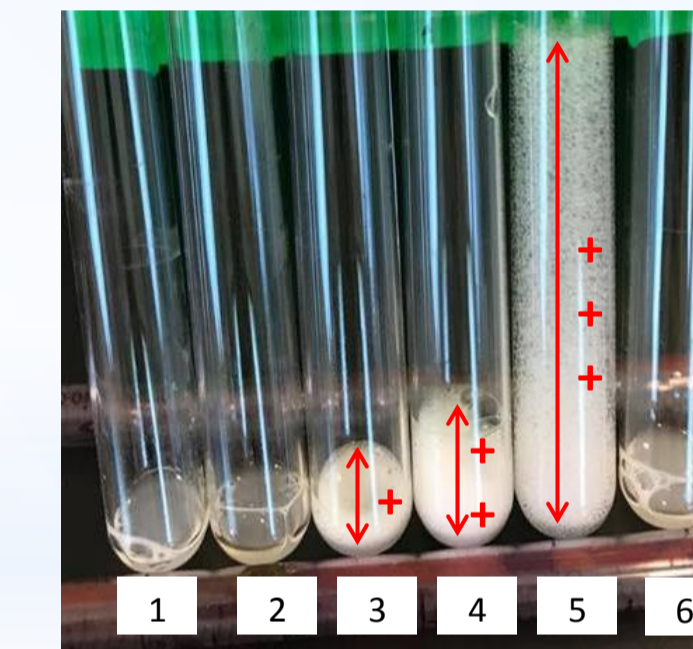


Figure 3: Catalase test results.
Tube 1, 2, 6: No foam formation indicates absence of catalase.
Tube 3, 4, 5: Foam formation indicates presence of catalase.

Table 2: Growth of Legume Root Nodule Bacteria on Agar Plate and Liquid Media

Bacteria Code	Growth of Bacteria on Agar Plate		Growth in the Presence of Ammonium Oxalate in Agar Plate*	Growth of Bacteria in Liquid Media		Growth in the Presence of Ammonium Oxalate in Liquid Media*
	Diameter of Colonies / mm			Number of Bacteria / ml		
	YEM	AO		YEM Broth	AO Broth	
SRO-1005	4.0	1.0	-	8.32E+07	2.23E+08	+
SRO-1006	1.5 - 2.0	1.0	+/-	5.76E+07	1.83E+08	++
SRO-1007	1.0	5.0	+	1.63E+08	3.31E+08	+
SRO-1008	4.0 - 4.5	3.0 - 3.5	+/-	4.64E+07	8.72E+07	+/-
SRO-1009	1.5-2.0	2.5 - 3.0	+/-	5.12E+07	3.96E+08	+++
SRO-1010	3.0	5.0 - 6.0	+	1.44E+08	5.07E+08	++

*Keys : + indicates enhancement; - indicates inhibition; +/- indicates no effects on growth.

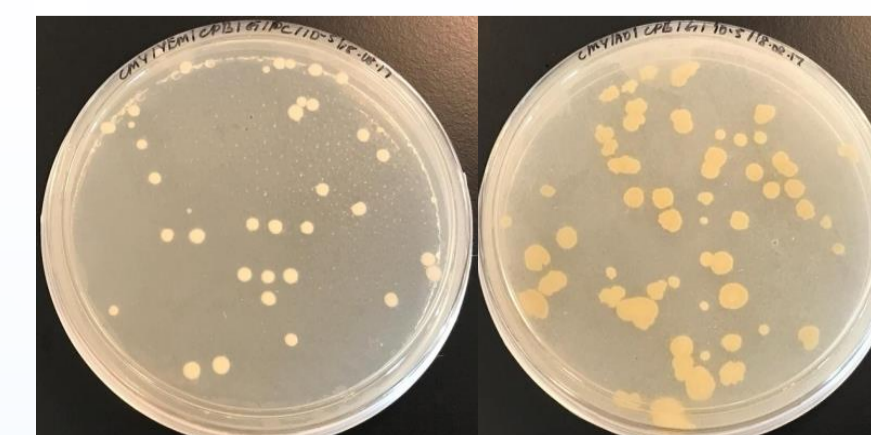


Figure 4: SRO-1010 bacteria colonies on YEM agar plate (left) and on AO agar plate (right).

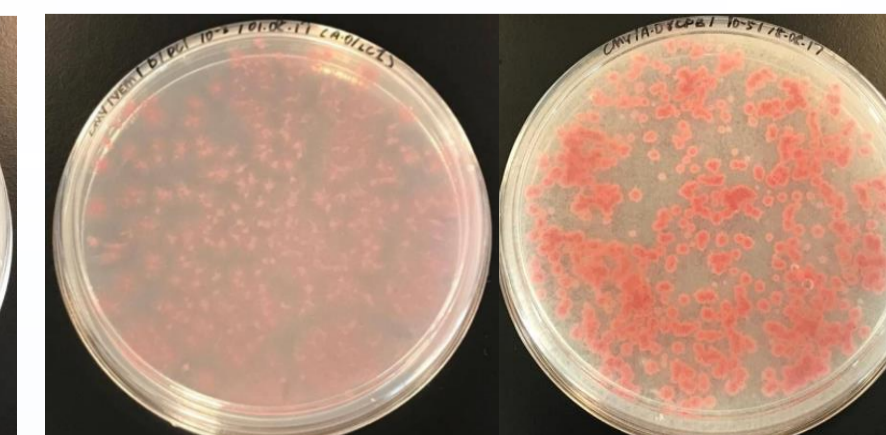


Figure 5: SRO-1009 bacteria colonies on YEM agar plate (left) and on AO agar plate (right).

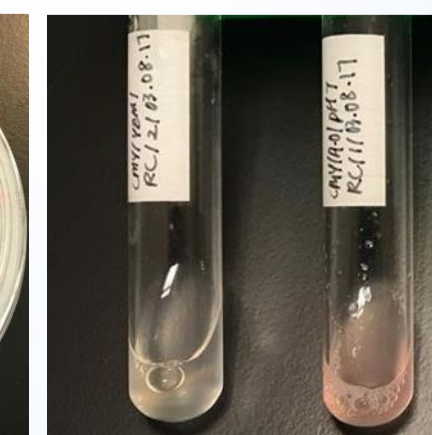


Figure 6: SRO-1009 bacteria in YEM broth (left) and AO broth (right).

DISCUSSION

- ❖ One Gram positive bacteria and five Gram negative bacteria were detected from legume root nodules.
- ❖ Three catalase-positive bacteria with range of catalase activity were detected from legume root nodules.
- ❖ Five strains of legume root nodule bacteria with increased growth in AO broth were observed.
- ❖ The growth of legume root nodule bacteria were enhanced in the presence of ammonium oxalate in liquid media. This suggests that the bacteria might have the ability to utilize ammonium oxalate for growth.
- ❖ Further functional characterization to identify the types of oxalate degrading enzyme present in the bacteria, followed by PCR and DNA sequencing to identify the gene coding for the enzyme could be conducted in future.

CONCLUSION

- ❖ The protocol for isolating and characterizing legume root nodule bacteria has been developed. However, protocol for screening oxalate degrading bacteria is still underdeveloped.
- ❖ Five strains of legume root nodule bacteria with increased growth in the presence of ammonium oxalate were observed with preliminary characterization.

ACKNOWLEDGEMENT

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REFERENCES

1. The George Mateljan Foundation. Can you tell me about oxalates, including the foods that contain them and how are they related to nutrition and health? : The George Mateljan Foundation; [16 September 2017]. Available from: <http://whfoods.org/genpage.php?tname=george&dbid=48>
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