



**Rhizobial and non-Rhizobial
nodulators of
*Pueraria phaseoloides***

W. M. M. Wedage and D. Gunawardana

Rubber

- One of the major industrial crops in Sri Lanka
- To realize optimum yield, nitrogen in the growth phase is critical
- Use of leguminous cover crops is a standard practice in rubber plantations
- Leguminous cover crops,
 - Contribute nitrogen to the soil
 - Help in building up of soil organic matter
 - Control weed growth



Rubber

	2008	2011	2013	2015
Total Land Under Cultivation	119 543 Ha	128,119 Ha	132,534 Ha	134,409 Ha
Average Yield Per Hectare	1,246 Kg	1,561 Kg	1290 Kg	776 Kg

Department of Rubber Development, Sri Lanka



Pueraria phaseoloides

- **Known by many names**
 - Puero (Australia)
 - Tropical kudzu (most of the tropics)
 - Centro grande
 - Feuille
- **Legume family**
- **Fodder plant and cover crop**
- **Nitrogen fixers found in nodules**
- **Promiscuous in accommodating multiple nitrogen fixers**



Objectives

- Isolation of nitrogen-fixing microorganisms from root nodules of *Pueraria phaseoloides*
- Characterization using colony and cell morphology
- Investigating plant-microbe communication using flavonoids and proline
- Biochemical characterization – especially secretory enzymes facilitating colonization
- Checking for nodulation by nitrogen fixers through hydroponics
- Identify the nitrogen fixers using molecular biology

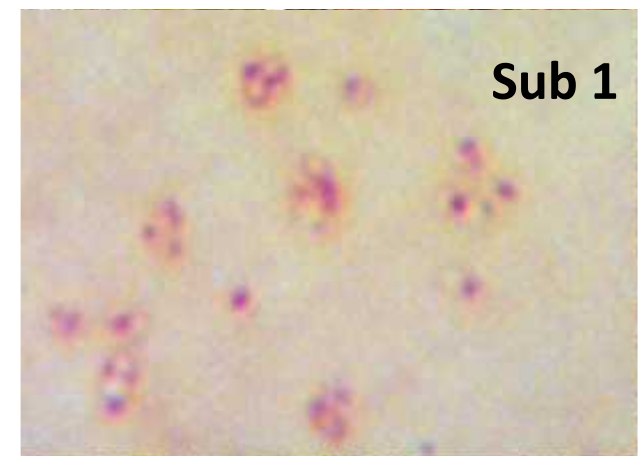
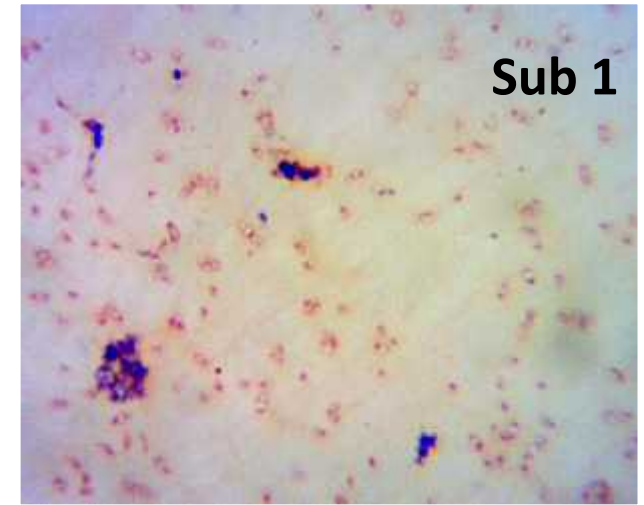


Results – Colony Morphology

Characteristics	Isolates from root nodules			
	Sub 1	Sub 2	Sub 3	Sub 4
Form	Circular	Circular	Irregular	Circular
Elevation	Crateriform	Convex	Crateriform	Convex
Margin	Filiform	Entire	Undulate	Entire
Surface	Smooth	Smooth	Rough	Smooth
Opacity	Translucent	Translucent	opaque	Opaque
Color	Cream	Watery	White	Yellow

Results – Gram Staining

Observed Characteristics	Isolates			
	Sub 1	Sub 2	Sub 3	Sub 4
Grams staining test	Negative	Negative	Negative	Negative
Shape of the cell	Cocobacillus	Bacillus	Bacillus	Bacillus



Results – Biochemical Characterization

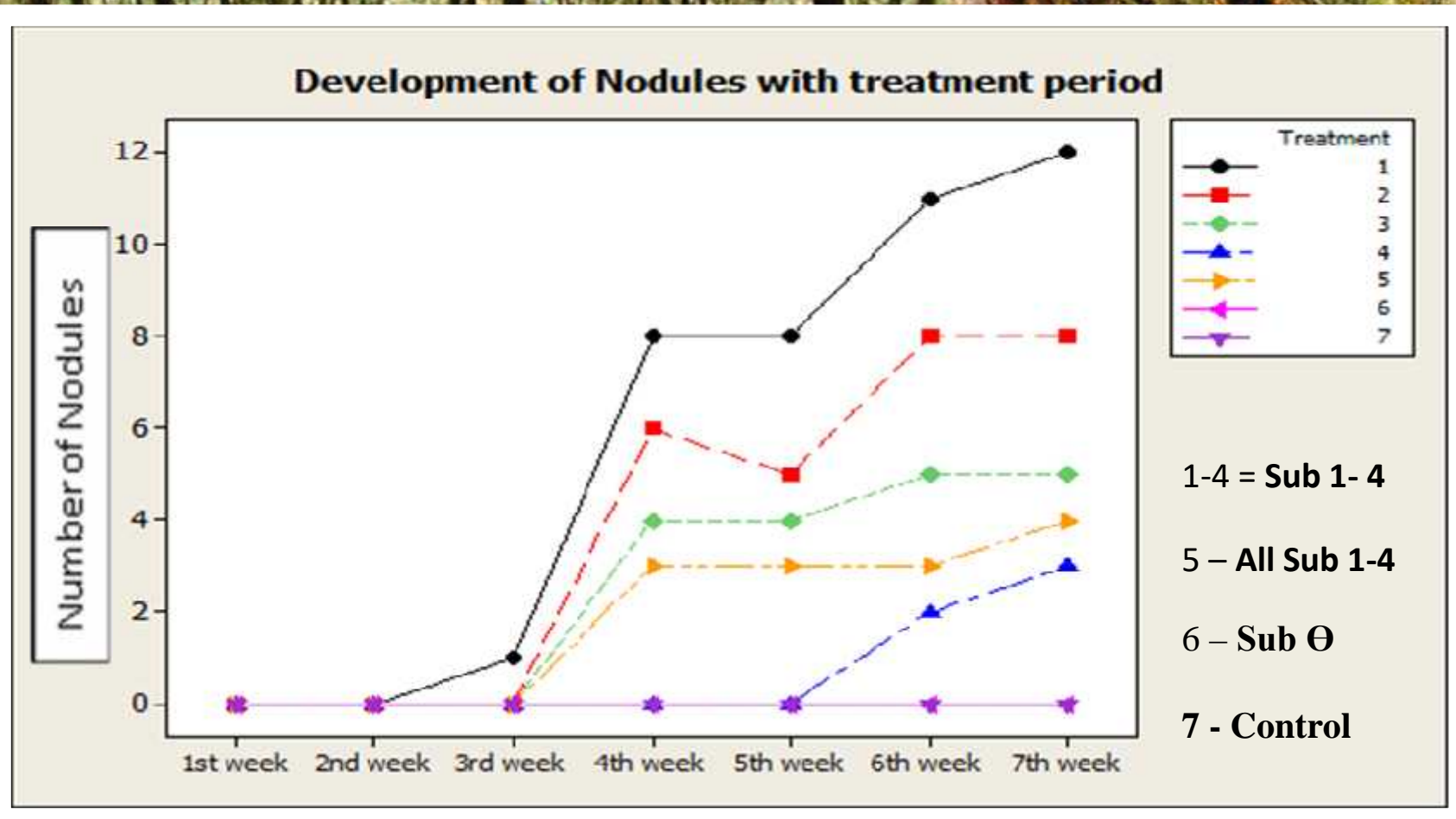
Biochemical	Cellulose	Pectin	Protein
Sub 1	+	-	+
Sub 2	+	+	+
Sub 3	+	+	+
Sub 4	+	+	-

Results - Chemotaxis

Bacterial strains	Positive or Negative	Swarming pattern
Sub 1	++	Bull's eye
Sub 2	++	Dendritic
Sub 3	+++++	Featureless
Sub 4	+	Featureless



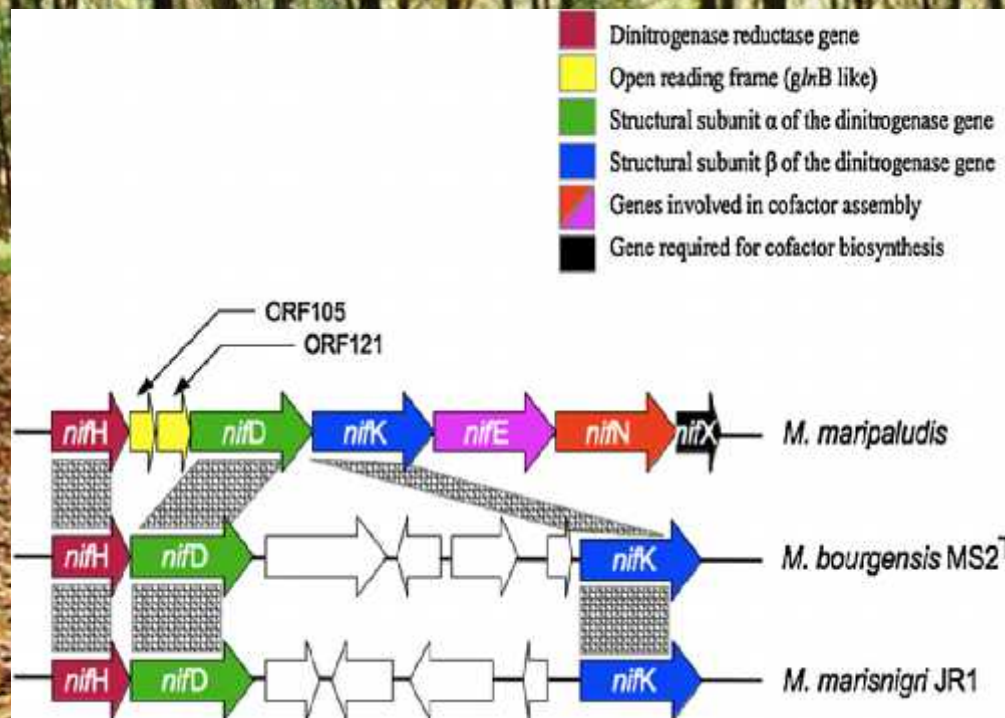
Results - Nodulation



Results – Growth Parameters

Treatment	Shoot length(cm) Mean ± SE	Root length(cm) Mean ± SE	Number of leaflets Mean ± SE	Number of nodules Mean ± SE
Sub 1	10.825 ± 0.788	8.68 ± 0.834	6.25 ± 3.25	3.000 ± 0.816
Sub 2	10.30 ± 1.11	9.60 ± 1.11	11.00 ± 1.15	2.000 ± 0.913
Sub 3	8.975 ± 0.484	8.13 ± 1.76	9.00 ± 1.73	1.250 ± 0.750
Sub 4	9.800 ± 0.725	15.40 ± 1.64	8.500 ± 0.750	0.750 ± 0.479
All 4	7.750 ± 0.253	9.80 ± 2.10	10.00 ± 1.15	1.000 ± 0.707
Sub 0	8.500 ± 0.561	8.000 ± 2.33	8.50 ± 1.04	0.000 ± 0.000
C	8.967 ± 0.567	15.000 ± 0.875	9.250 ± 0.289	0.000 ± 0.000

Molecular Biology

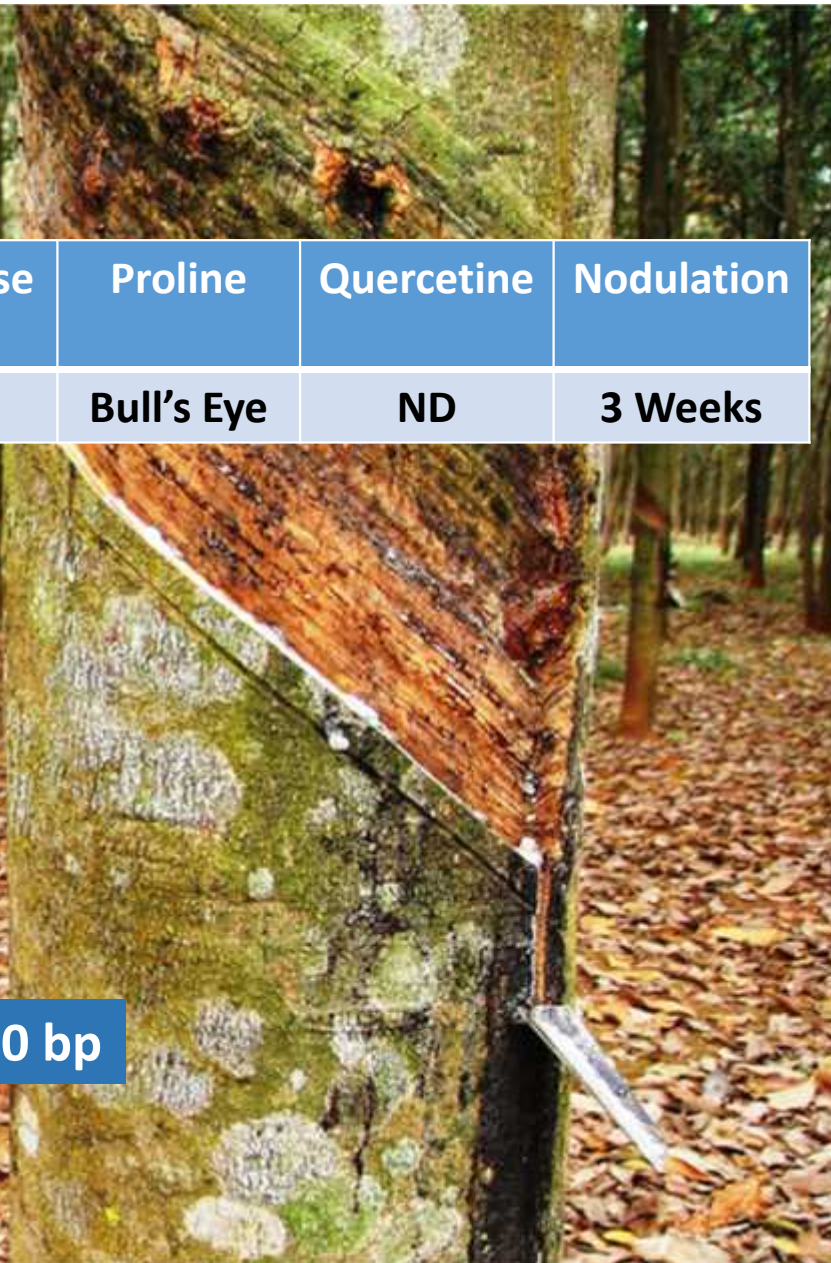


Results – Sub1

Culture	Morphology	Gram Stain	Cellulase	Pectinase	Protease	Proline	Quercetine	Nodulation
Sub1	Cocobacillus	Negative	+	+	-	Bull's Eye	ND	3 Weeks



PCR Product ~ 360 bp



Sub1 - Identity

- Highest alignment with *Pseudoacidovorax intermedius*
- 93% identity in alignment of *nifH* fragment
- β -proteobacteria
- A soil bacterium that so far has not been shown to induce nodulation
- A nitrogen fixer nevertheless



Discussion

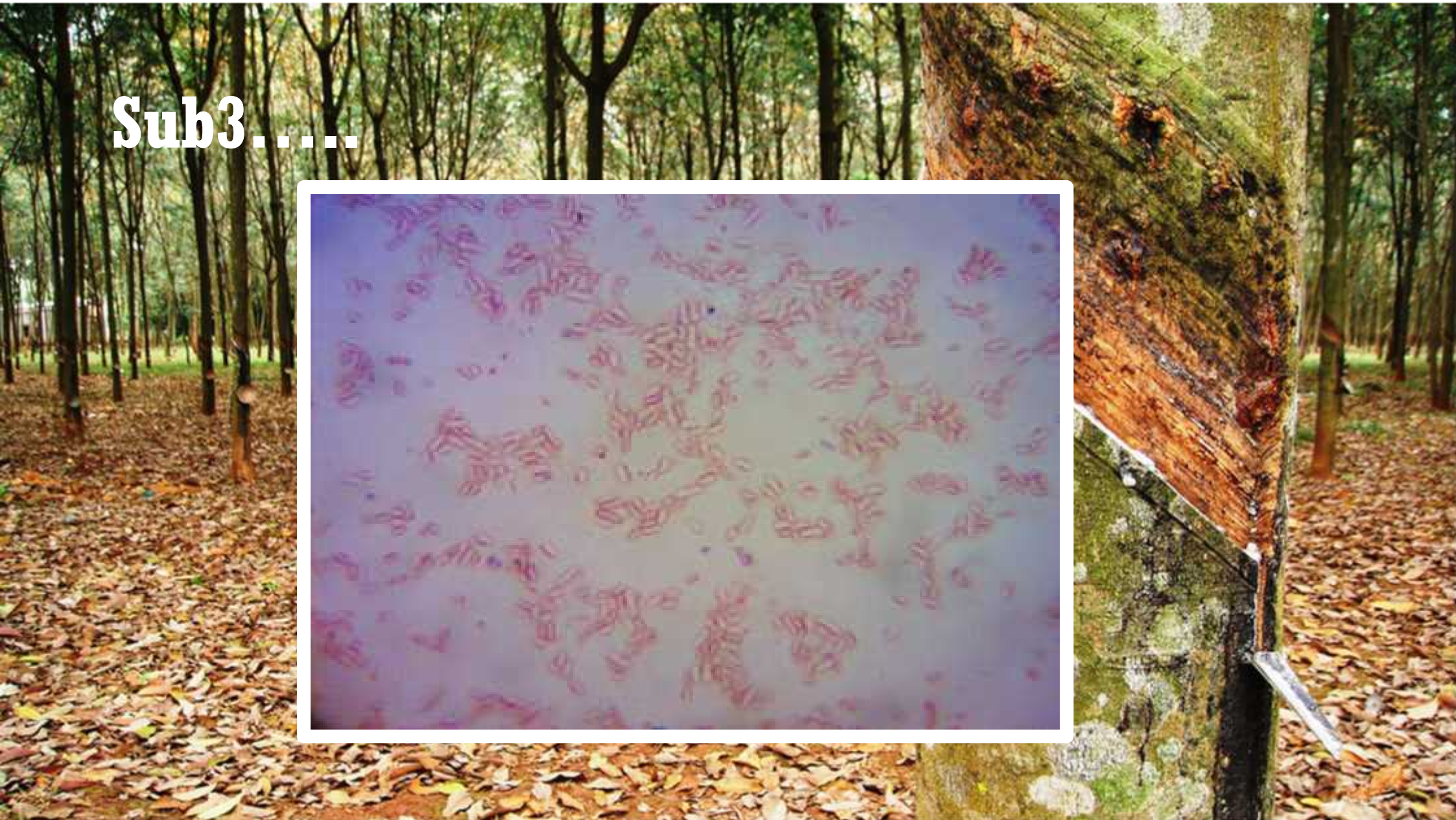
- So far, only a few β -proteobacteria have been shown to inhabit legume nodules and a very low number have been shown to induce nodulation
- Shows clearly that other genera, outside of *Rhizobium* can nodulate legumes
- Very little information on plant-microbe interaction on non-Rhizobial nodulators
- We will be starting a research program on the plant-microbe crosstalk in the infection and nodulation processes of Sub1 – funding has been secured



Conclusions

- We have unearthed here 4 bacteria capable of nodulating *Pueraria phaseoloides*
- Sub1 is a potent nodulator of *Pueraria phaseoloides*
- We have unveiled here a relatively rare non-rhizobial nodulator in Sub1
- We have not identified Sub2, Sub3 and Sub4 yet
- Even Sub3 is not of a conventional rod shape which points to a Non-rhizobial identity
- Perhaps there is a far bigger diversity in legume nodulation than science has revealed this far

Sub3.....



Acknowledgements

- Ms Methsala Wedage for her commitment and dedication to the undergraduate project
- Members of the academic and non-academic staff - Department of Botany, University of Sri Jayewardenepura, Sri Lanka



спасибо 谢谢
GRACIAS 谢谢
THANK YOU

ありがとうございました MERCI

DANKE धन्यवाद

شُكْرًا **OBRIGADO**