THE SAMUEL ROBERTS NOBLE FOUNDATION

Evaluation and Assessment of Rhizobia Diversity for the Establishment of Annual Medics in Soils at Different pH Levels

Authors: Ruchi Singh, Sindy Interrante, Carolyn Young, and Twain Butler
The Samuel Roberts Noble Foundation, Forage Improvement Division, 2510 Sam Noble Parkway, Ardmore, Oklahoma 73401 USA
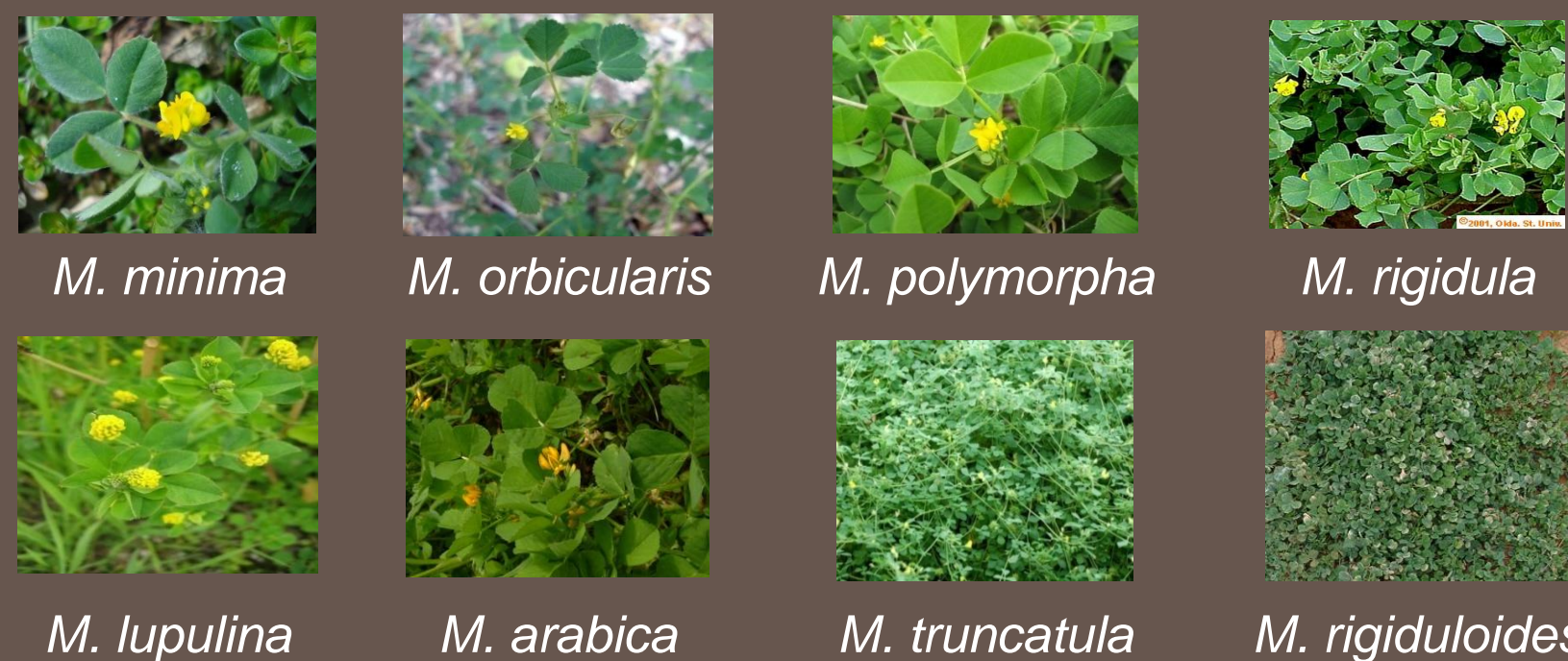
Introduction

New forage legumes are needed to complement warm and cool season grass ecosystems in the Southern Great Plains. While minimal information is available regarding the agronomic management of annual medics, they do possess many of the desired traits such as:

- Adapted to dry areas
- Productive with pH range 6 to 8
- Good seed producers
- Grow vegetatively during the early spring

Objective

Selection of medics better adapted to this region, in association with relevant symbiotic bacterial partner to maximize forage yield and forage quality for eight annual medics
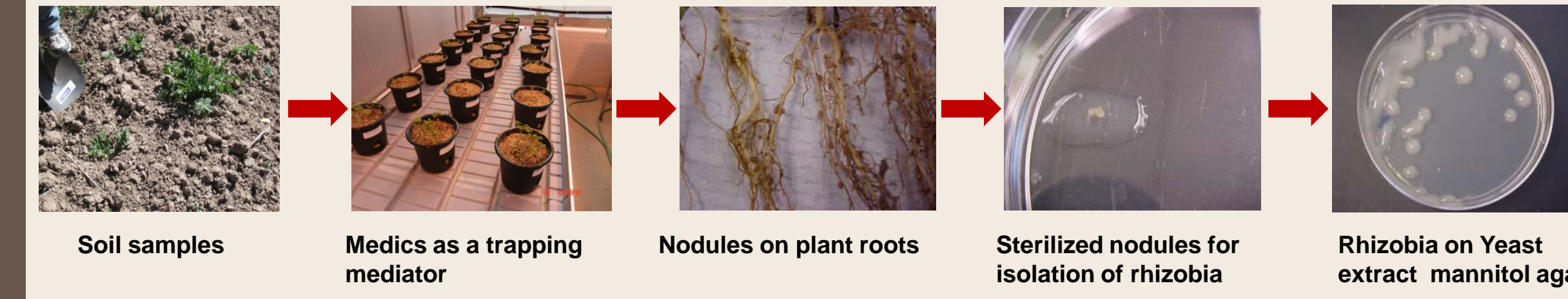
Medic Species

M. minima   *M. orbicularis*   *M. polymorpha*   *M. rigidula*

M. lupulina   *M. arabica*   *M. truncatula*   *M. rigiduloides*

Soil Sources for isolation of rhizobia

(Soil Collection: 3 April 2009)

Field ID	1:1 Soil pH	Nitrate-N ppm N	Potassium ppm K	Mehlich P-III ppm P	Total P ppm	Calcium ppm Ca	Magnesium ppm Mg	Texture
Eufaula soil,OK	7.85	2.65	91.5	36.5	167	536	132	Loamy Sand
Norwood soil,OK	7.8	54.8	513.5	41.5	694	5574	482.5	Clay
Bonti soil,TX	4.7	61.65	105.5	26	222	410	138.5	Sandy Loam
Dale soil, OK	5.2	29	550	230	463	910	331	loam

Isolation Methods

1. **Trapping method** using eight different medic species as trapping mediators

Soil samples   Medics as a trapping mediator   Nodules on plant roots   Sterilized nodules for isolation of rhizobia   Rhizobia on Yeast extract mannitol agar

2. **Direct isolation** from soil by plating on selective media

Soil Serial dilution   Spread plating on selective media   Purified Rhizobia

Trapping method :
- Nodules recovered only from Norwood clay loam soil at pH 8.0 with all eight species
- Nodules formed only on *M. truncatula* in Eufaula loamy sand
- Nodules did not form on plants grown in Bonti or Dale soils

Direct isolation method: Total isolates: 36
7 from Bonti ; 5 from Dale; 11 from Eufaula; 13 from Norwood

Characterization on the basis of 16S rDNA

The 16S rDNA sequencing was used for characterization of all the isolates. On the basis of sequence similarity, when compared to sequences within GenBank and rhizobase we found all rhizobia recovered from the root nodules belong to *Sinorhizobium* group.
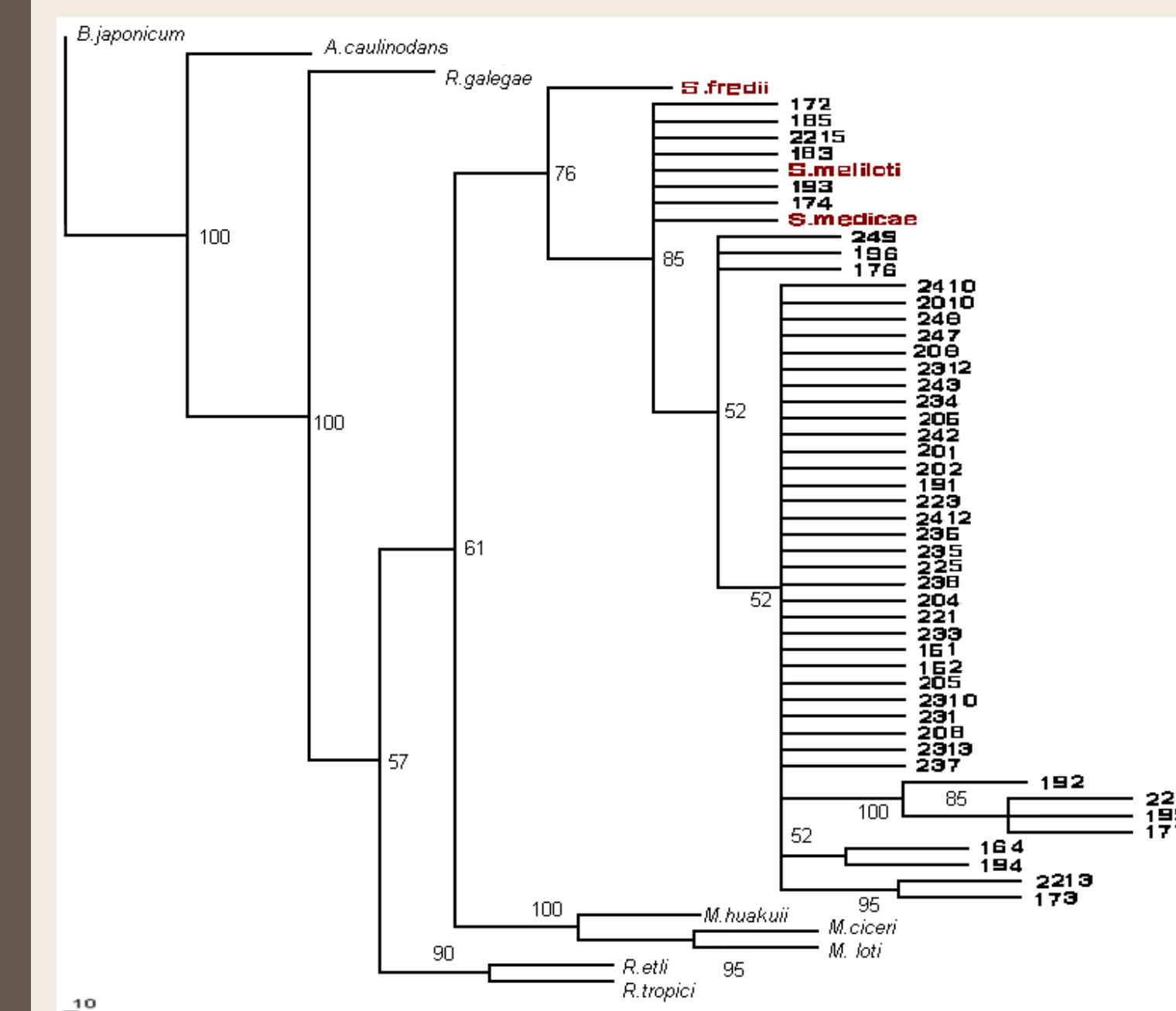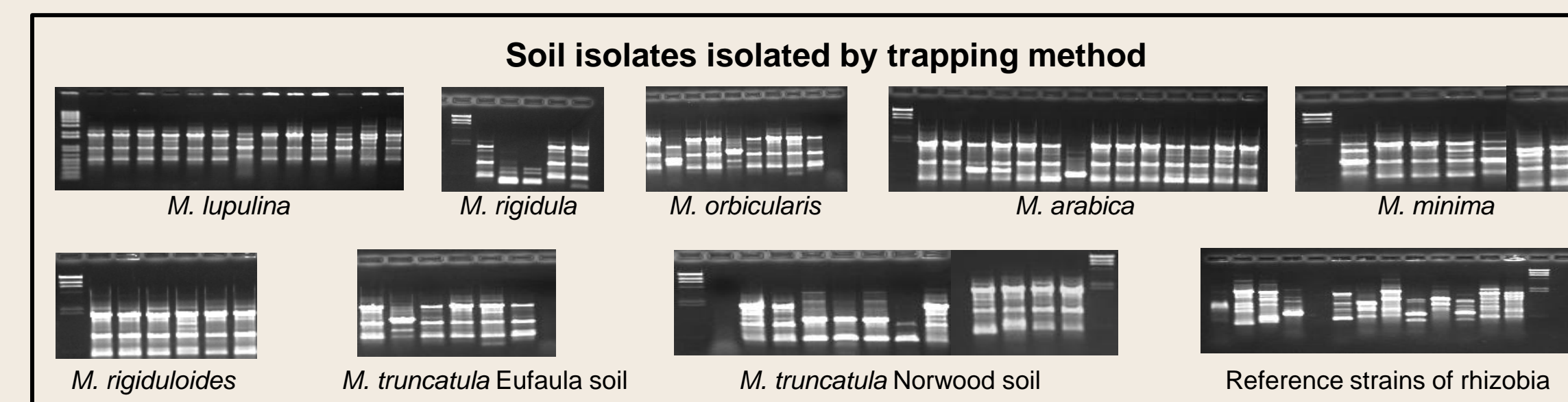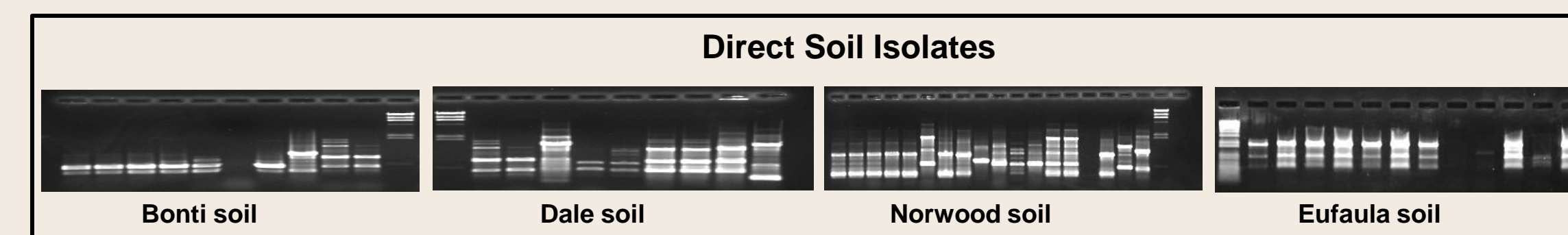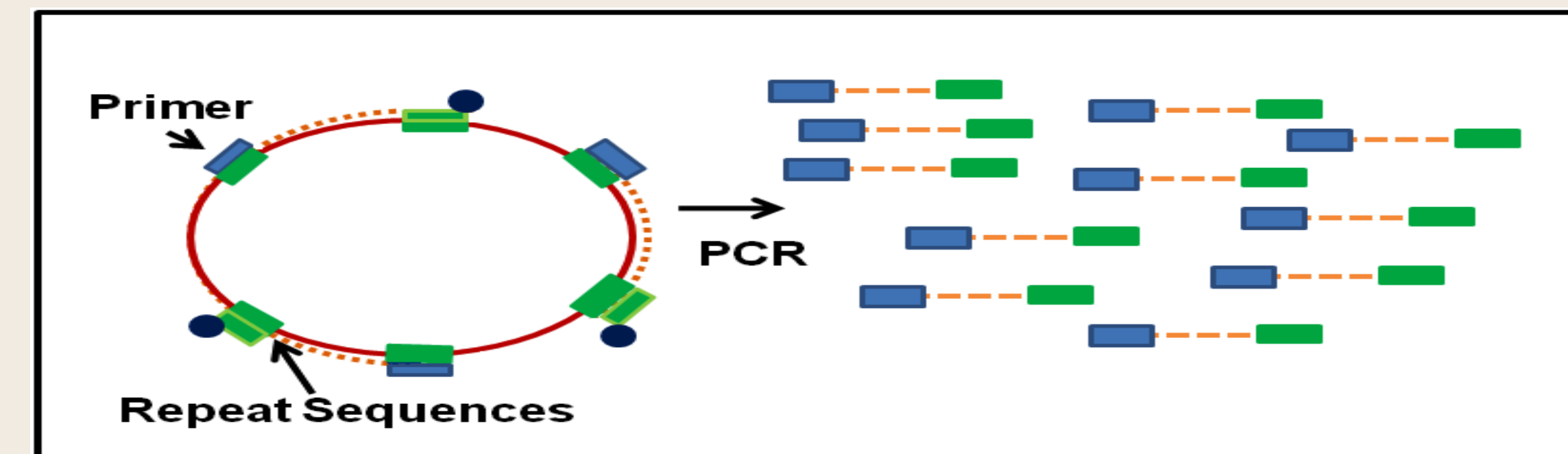

Neighbor joining dendrogram derived from a partial 16S rDNA sequence distance matrix (HKY85) of the rhizobia isolates.

*nif*H and *nod*C gene amplification

| Isolates from	CF/C1	Cfu/C1	CF2/C1	CF4/C1	CFn/C1	nifHF/nifHR
M. lupulina	-	-	+	-	+	+
M. arabica	-	-	+	-	+	+
M. minima	-	-	+	-	+	+
M. rigiduloides	-	-	+	-	+	+
M. orbicularis	-	-	+	-	+	+
M. rigidula	-	-	+	-	+	+
M. truncatula	-	-	+	-	+	+

We evaluated by PCR the presence of *nod*C and *nif*H, known to be required for nodulation and nitrogen fixation, respectively. Five sets of degenerate primers to *nod*C and one set of species specific primers to *nif*H were designed. PCR amplification products were produced with two primer combinations of *nod*C, CF2/C1 and CFn/C1, and the *nif*H primers with genomic DNA from the rhizobia isolates. These results confirmed all isolates belonged to the rhizobiaceae family.

Characterization of Isolates by *rep* PCR

Direct Soil Isolates

Bonti soil   Dale soil   Norwood soil   Eufaula soil

Soil isolates isolated by trapping method

M. lupulina   *M. rigidula*   *M. orbicularis*   *M. arabica*   *M. minima*

M. rigiduloides   *M. truncatula* Eufaula soil   *M. truncatula* Norwood soil   Reference strains of rhizobia

The diversity among the *Sinorhizobium* group was studied on the basis of BOX profile using rep PCR. Significant diversity was observed among isolates directly isolated from the soil. These isolates exhibited 3 patterns, while the nodule isolates predominantly exhibited one pattern. These results imply that only one type of *Sinorhizobium* population is able to nodulate the eight medic species

Growth characteristics

$y = 0.8235x - 7.0386$
$R^2 = 0.9938$

$y = 1.0449x - 9.9635$
$R^2 = 0.976$
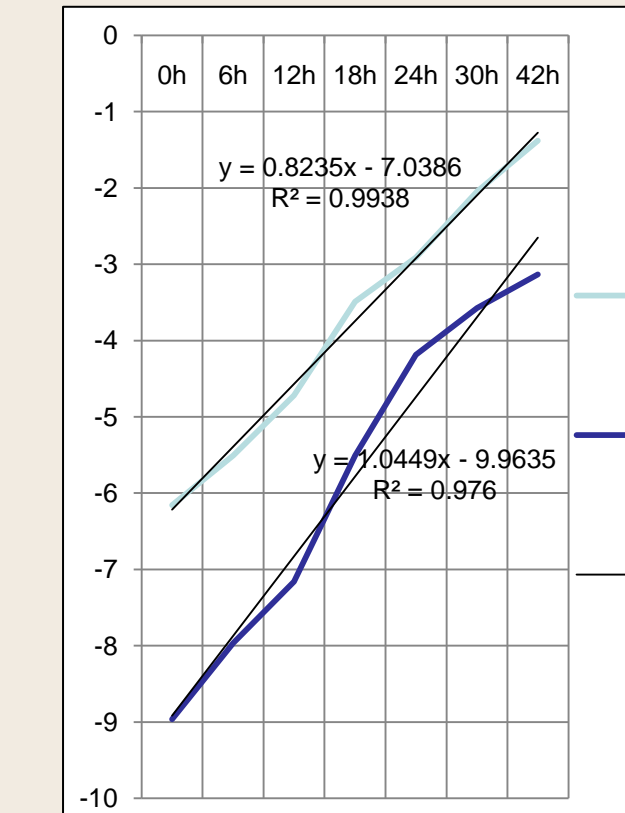
Rhizobia Isolate # (plant host)	Maximum Growth after 36 h	Doubling time (h)
191 (*M. rigiduloides*)	0.20	1.3
183 (*M. rigidula*)	0.26	1.4
176 (*M. minima*)	0.20	1.2
161 (*M. truncatula*; Efaula soil)	0.13	1.1
247 (*M. truncatula*)	0.21	1.1
2313 (*M. arabica*)	0.19	1.0
208 (*M. orbicularis*)	0.27	1.2
221 (*M. lupulina*)	0.38	1.4
S. fredii USDA205	0.12	1.1
S. meliloti USDA1002	0.15	4.2
S. medicae USDA1037	0.23	1.1

Isolates (host)	Growth on different pH						Growth on different salt concentrations					
	pH 7.0	pH 3.8	pH 4.0	pH 4.3	pH 4.8	pH 5.0	pH 6.5	0.20%	0.25%	0.30%	0.35%	0.40% 0.10%
191 (*M. rigiduloides*)	0.2	0.03	0.13	0.13	0.13	0.14	0.37	0.22	0.22	0.25	0.19	0.14 0.19
183 (*M. rigidula*)	0.26	0.05	0.1	0.12	0.16	0.17	0.26	0.24	0.25	0.3	0.29	0.19 0.25
176 (*M. minima*)	0.2	0.05	0.07	0.07	0.15	0.14	0.22	0.24	0.2	0.21	0.19	0.14 0.19
161 (*M. truncatula*) Efaula	0.13	0.01	0.02	0.04	0.1	0.09	0.16	0.12	0.13	0.16	0.14	0.14 0.12
247 (*M. truncatula*)	0.21	0.06	0.03	0.04	0.06	0.07	0.21	0.12	0.13	0.16	0.16	0.08 0.2
2313 (*M. arabica*)	0.19	0.2	0.07	0.07	0.12	0.09	0.18	0.13	0.15	0.18	0.18	0.16 0.19
208 (*M. orbicularis*)	0.27	0.16	0.09	0.09	0.2	0.18	0.28	0.12	0.2	0.2	0.22	0.16 0.27
221 (*M. lupulina*)	0.38	0.09	0.05	0.16	0.29	0.35	0.38	0.26	0.32	0.3	0.29	0.26 0.36
S. fredii USDA205	0.12	0.05	0.08	0.05	0.12	0.16	0.16	0.01	0.02	0.05	0.04	0.03 0.11
S. meliloti USDA1002	0.15	0.011	0.14	0.02	0.14	0.15	0.16	0.1	0.11	0.16	0.14	0.11 0.15
S. medicae USDA1037	0.23	0.001	0.1	0.06	0.18	0.19	0.21	0.2	0.23	0.2	0.21	0.16 0.23

Conclusions

- *Sinorhizobium sp.* was able to form nodules in all eight Medics.
- Most favorable pH for the isolates is 6.5, they can tolerate as low as pH 4.8.
- Optimum salt concentration of the isolates is 0.3%

Future plans

- Cross inoculation test
- Plant growth promoting activities of indigenous rhizobia
- Green house experiments for the evaluation of the indigenous rhizobia in different conditions such as acidic and drought
- Comparative study of commercially available rhizobia vs. indigenous rhizobia