Genetic diversity of root nodule bacteria isolated from Bambara groundnuts [*Vigna subterranea* (L.) Verdc] in the soils of the drier parts of Lake Victoria basin.

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Grain Legume Production

Impact of Climate Change

DAILY NATION
Wednesday October 16, 2013

Boost food reserves now

If there are people out there who still doubt that climate change is a man-made phenomenon with a deep impact on food security, then it is time those doubts were laid to rest.

These days, the rains are either too much, leading to flooding during which crops are washed away or the rains are too inadequate, leading to crops drying up prematurely.

Climate Smart Agriculture

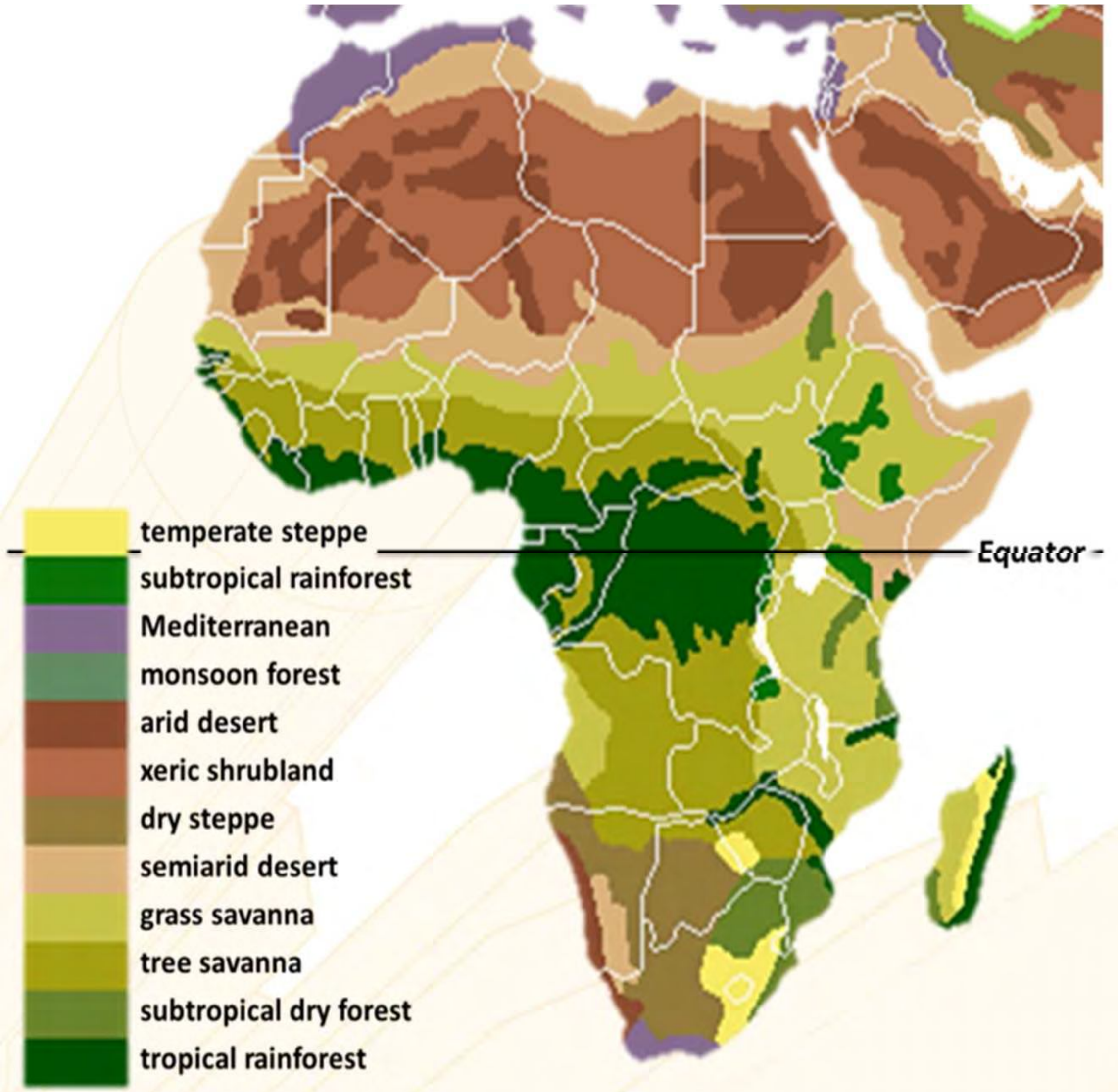
- **Minimal use of inorganic fertilizers**
- **Improved soil health**
- **Minimal tillage**
- **Drought tolerant crops**
- **Intensification agriculture**
- **Legume – cereal intercrop or rotation**

Why bambara groundnuts?

- A highly underutilized exemplar crop (Azam Ali, 2012).
- Drought tolerant and records high yields in poor soils.
- Rich source of important nutrients.
- Forms symbiotic association with root nodule bacteria fixing nitrogen into the soil.




Distribution of bambara



- ➢Found in most of sub-Saharan Africa
- ➢ Has spread to other parts of the world including Malaysia, Indonesia, Thailand, Papua region and South America (Somta *et al*., 2013)
- ➢ It is an emerging food security crop

Nutritional values of bambara

Biochemical composition		**Amino acids ($g/16g\ N_2$)**		**Minerals (mg/100g)**		**Fatty acids (%)**	
Energy	1826KJ	Lysine	6.8	P	345	Palmitic	23.2
Proteins	25.2%	Glutamine	16.2	Ca	66	Stearic	5
Oils	7.9%	Asparagine	11.1	K	1935	Oleic	22.6
Carbohydrates	42.8%	Leucine	7.6	Mg	350	Linoleic	39.0
Fibres	12.8%	Arginine	7.0	Fe	8	Linolenic	3.1
		Phenylalanine	5.6	Mn	15	Arachidic	1.6
		Isoleucine	3.9	Zn	8	Bohenic	4.4
		Threonine	3.5	Na	12	Eicosenoic	0.8
		Tyrosine	3.5	Cu	1		

[**Source: Mahala and Mohamed, (2010)**]

Biological nitrogen fixation

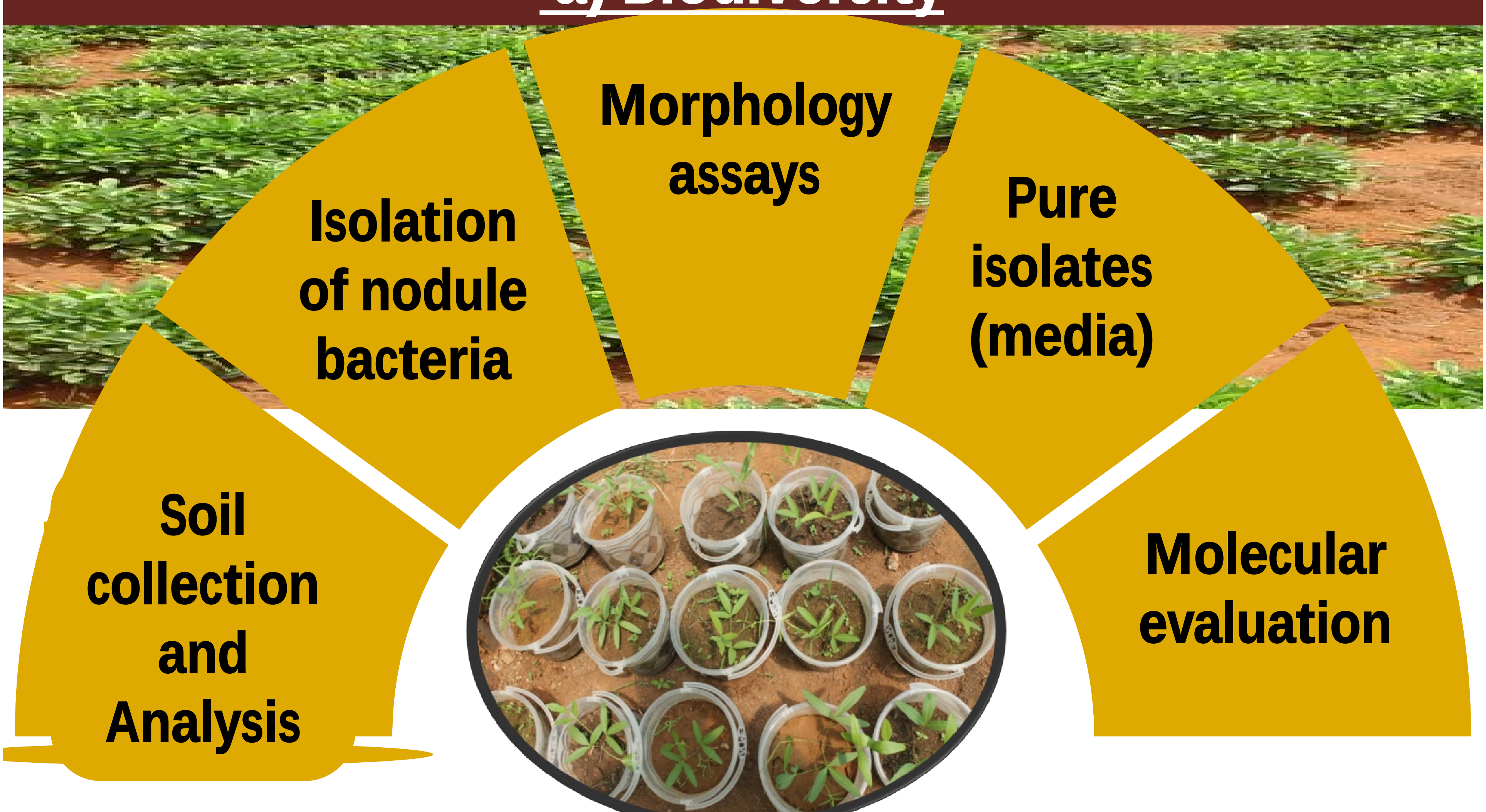
- Bambara groundnut forms N_2-fixing symbioses with soil rhizobia (Sprent, 2009).
- Convert N_2 into NH_3 by infecting and establishing in plant roots (Dakora, 2014).
- Currently, there is little information on the most efficient groups of rhizobia in soils of Lake Victoria basin.

Research strategy

- Isolation of root nodule bacteria
- Identification of isolates
    - Morphological identification
    - Multiple gene sequencing and phylogenetics.
    - 16S rRNA, *nifH*, *nodC* and housekeeping genes *atpD* and *recA*
- Specificity of isolates
    - Evaluate symbiotic functioning and nitrogen fixation.

METHODS

a) Biodiversity



16S rRNA, nifH, nodC, recA, glnI, atpD genes

Sanger sequencing to identify root nodule bacteria

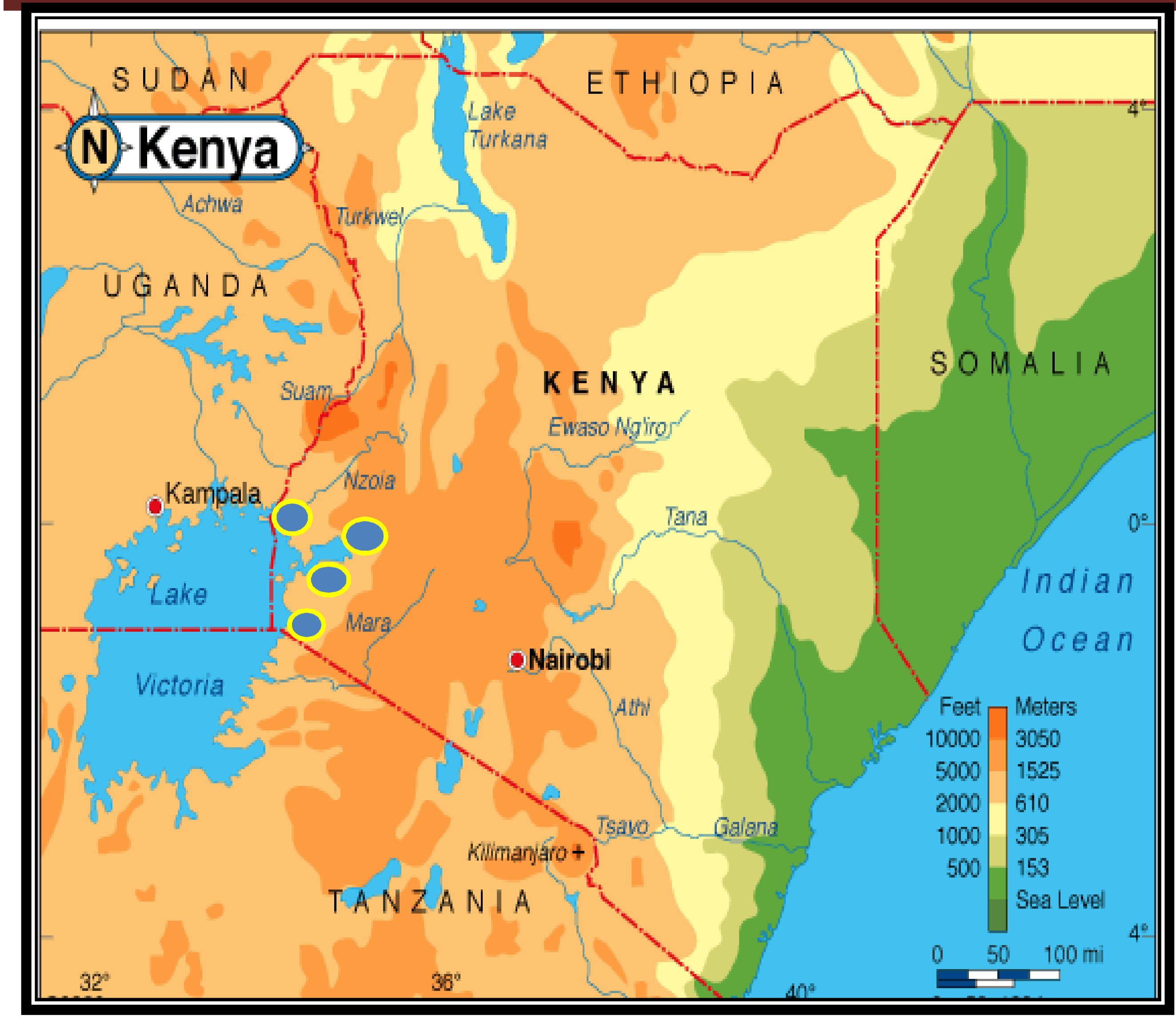
b) Symbiotic efficacy

Pre-germination of bambara seeds

Inoculation with isolates

Nodulation and N-content

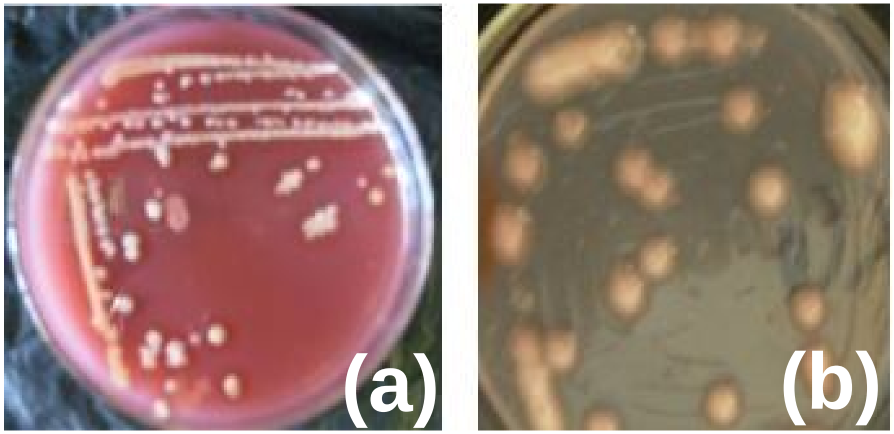
Sample collection sites



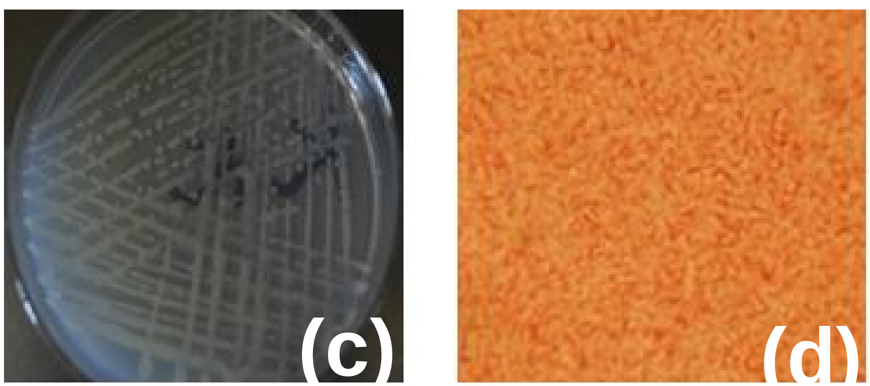
Site	No.
S1 – Port Victoria	35
S2 – Kisumu	40
S3 – Kendu bay	35
S4 – Karungu	40

OUTCOMES

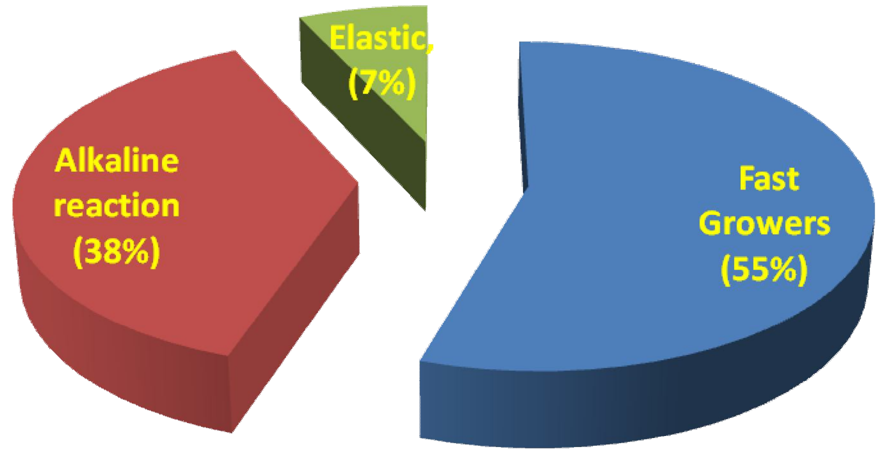
Morphological assays

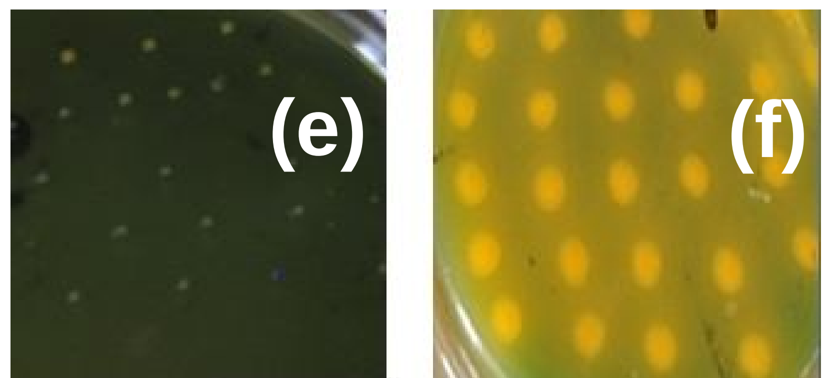


Rhizobia isolates on Congo Red
(a) slow growing rhizobia
(b) fast growing rhizobia



(e) Growth on YEMA at pH 6.8
(f) Gram negative rod cells

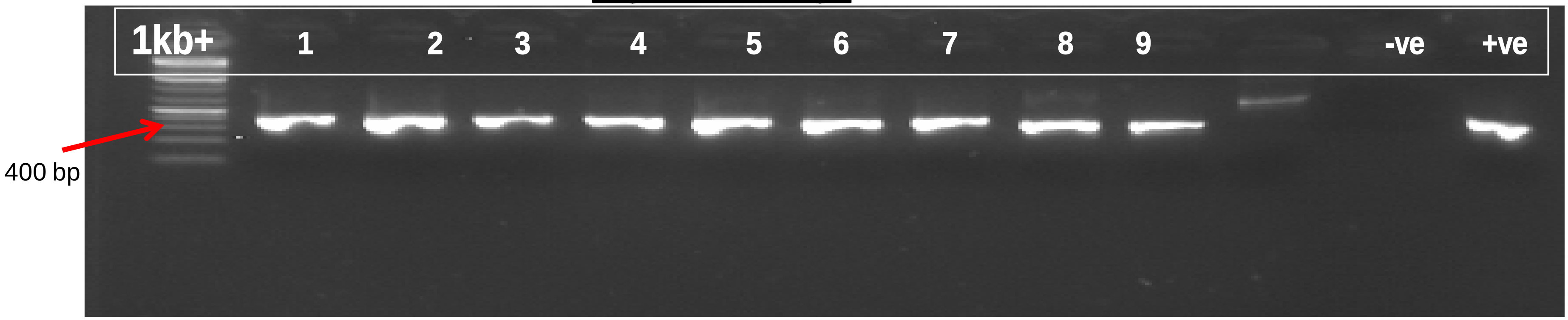




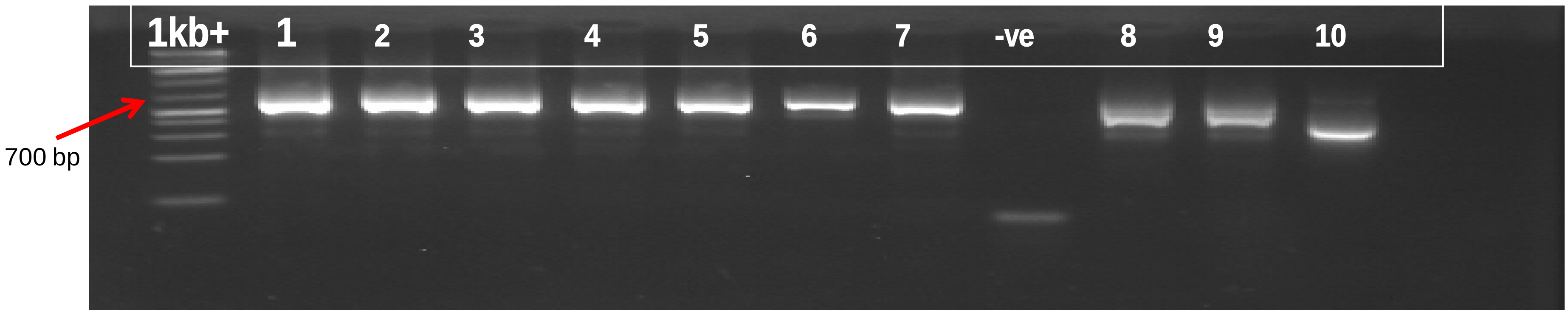
Rhizobia isolates on Bromothymol blue
(c)alkali producing rhizobia
(d) acid producing rhizobia

Molecular Analysis

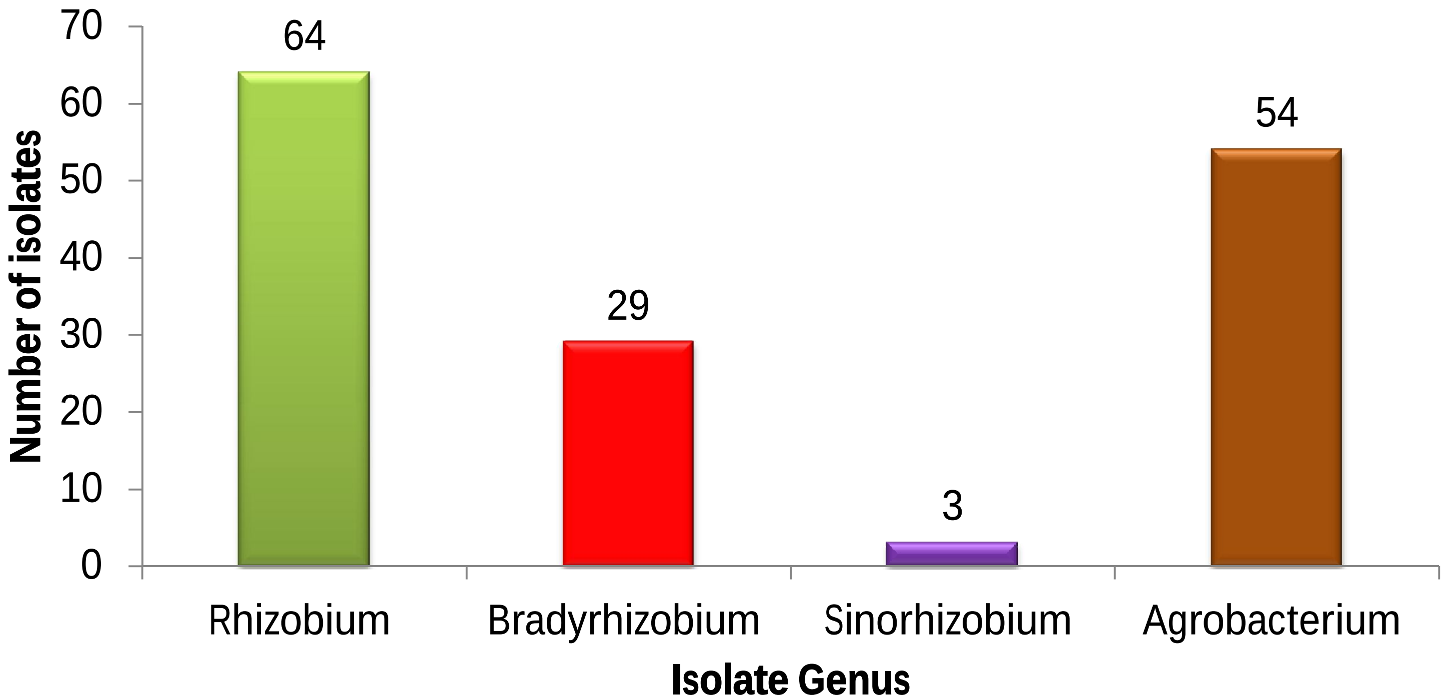
***nifH* Gel Image**



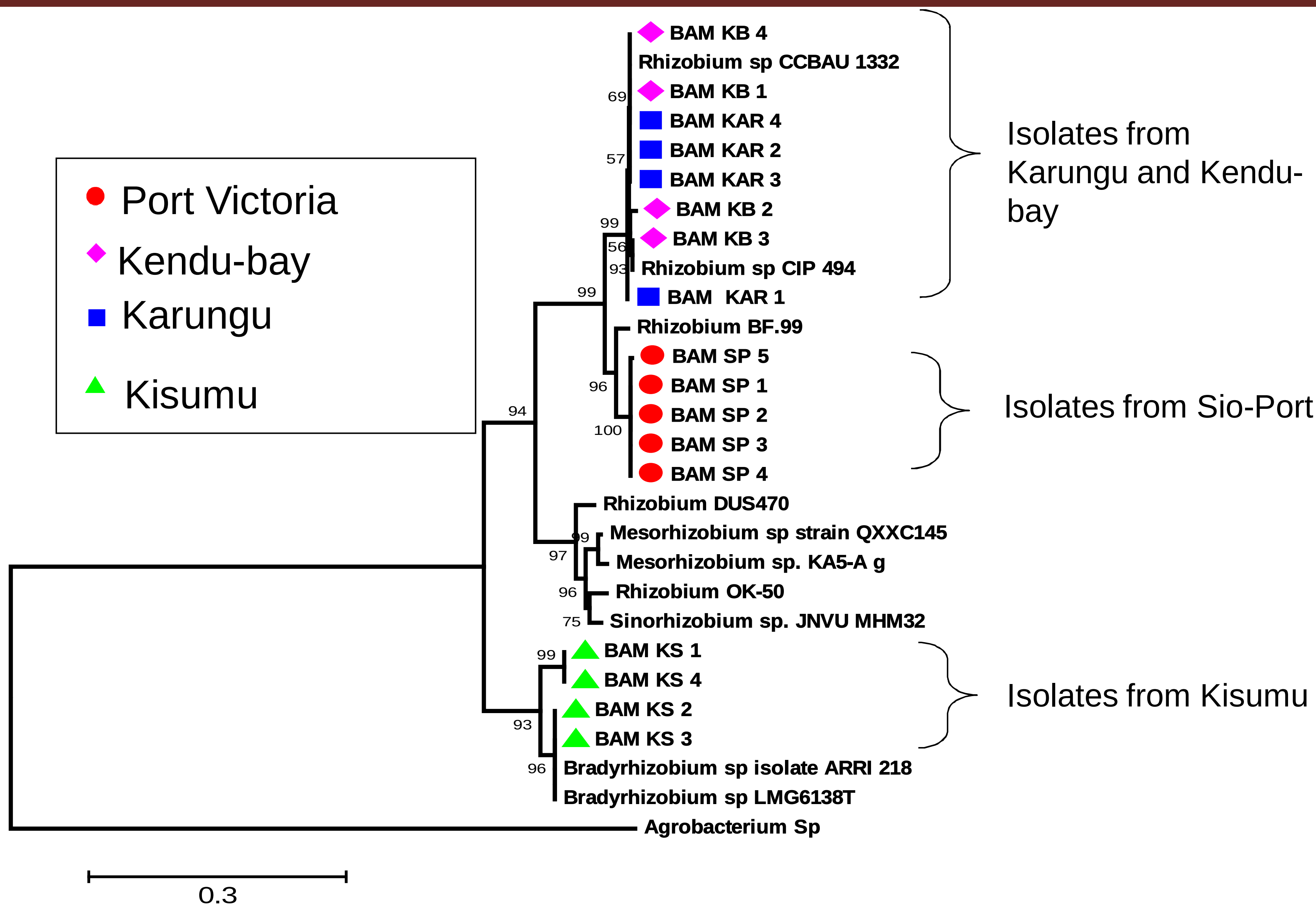
***nodC* Gel Image**



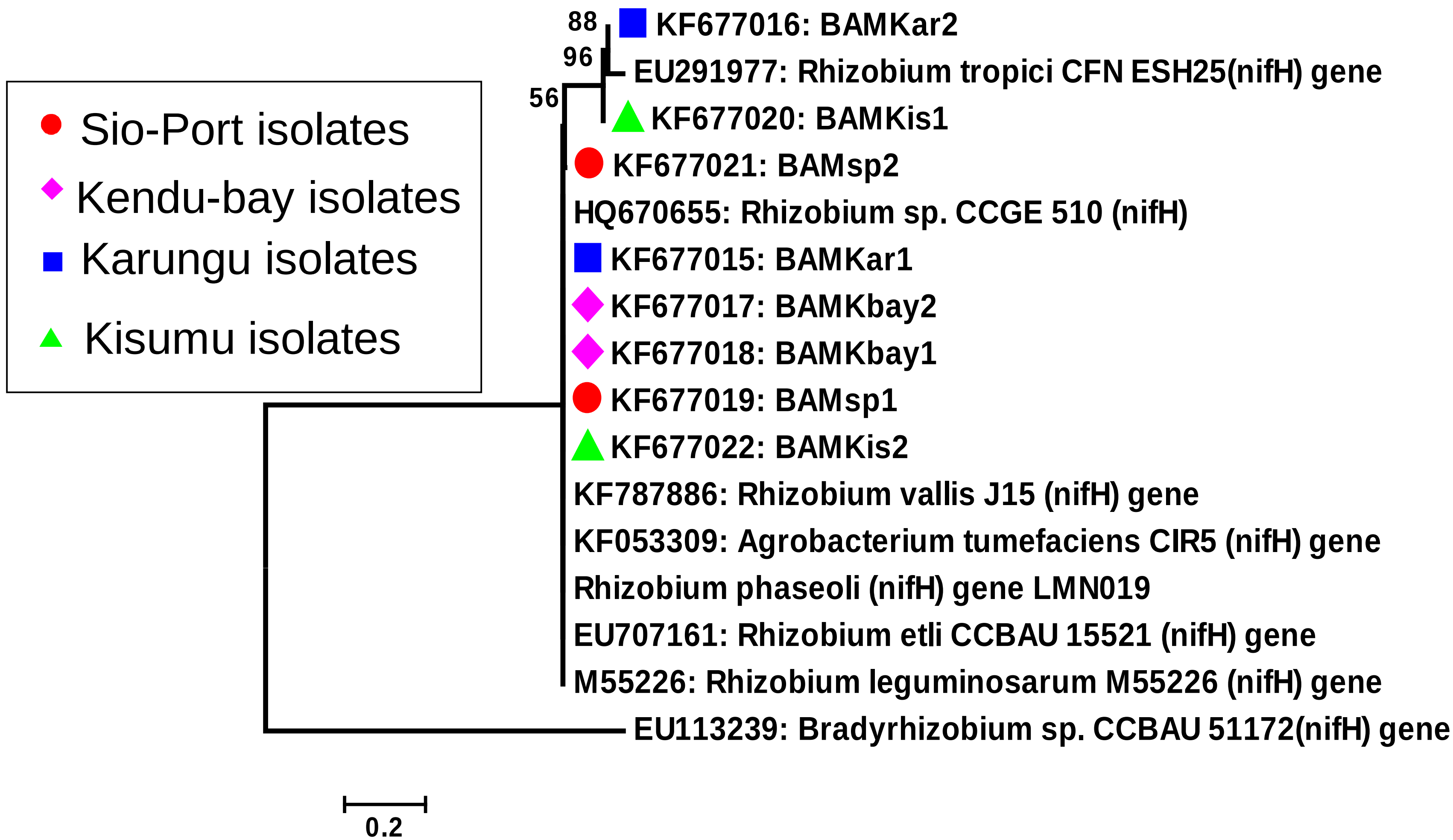
Diversity based on 16S rRNA gene



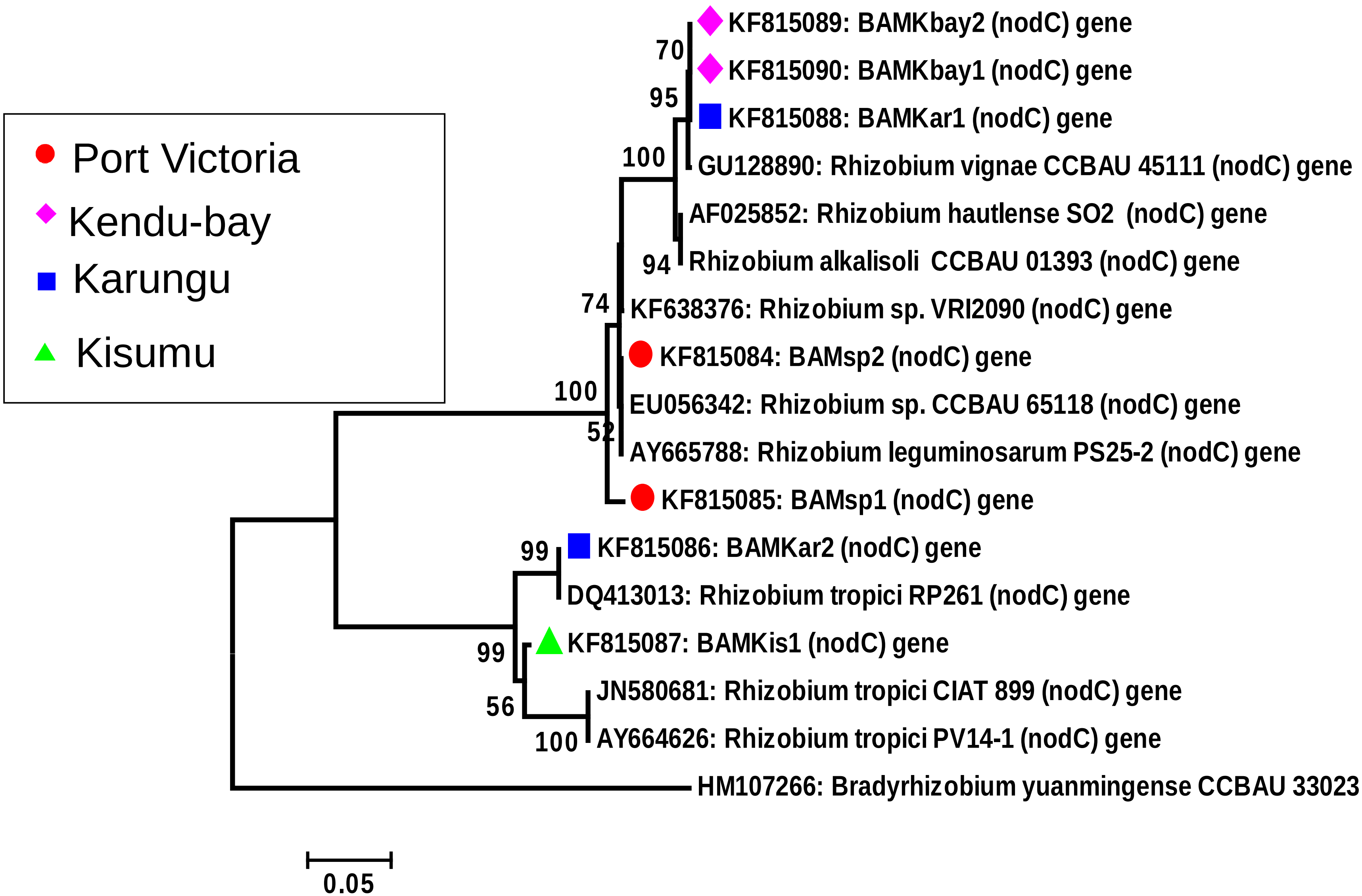
16S rRNA Phylogenetic tree



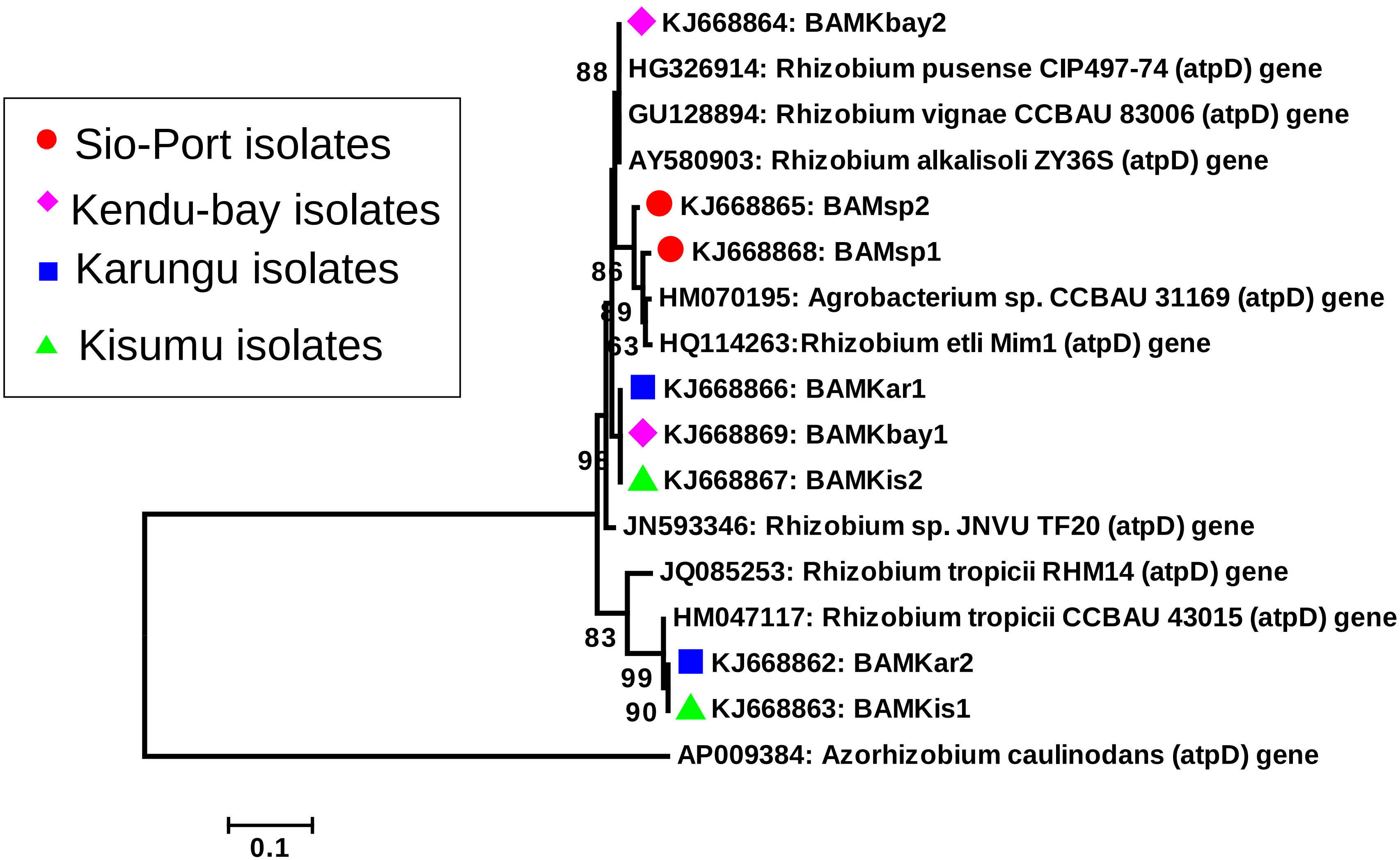
*nif*H gene phylogenetic tree



nodC gene phylogenetic tree



atpD gene phylogenetic tree



Symbiotic efficacy of isolates

Strain	Nodulation: Nodule no. plant $^{-1}$	Nodulation: Nodule fresh wt. (g)	Dry matter weight (g. Plant $^{-1}$): Shoot	Root	Whole plant	Shoot/ Root ratio
Fast-growers						
Un-inoculated	0.0	0.0	0.5	0.1	0.6	3.0
BAMsp-1 KF677015 (*R. tropicii*)	24.0	0.4	0.3	0.1	0.4	3.3
BAMkis-5 KF677019 (*R. pusense*)	12.3	0.3	0.5	0.2	0.7	2.2
BAMkar-7 KF677021 (*R. grahaminii*)	11.7	0.2	1.1	0.5	1.6	2.1
BAMkbay-3 KF677031 (*R. etli*)	18	0.5	0.7	0.1	0.4	2.2
Slow-growers						
Uninoculated	0.0	0.0	0.5	0.1	0.6	0.3
BAMsp-3 KF677011 (*Bradyrhizobium sp*)	88.0	1.1	1.8	0.4	2.1	4.9
BAMkis- 8 KF677017 (*Ensifer adherens*)	67.3	1.0	1.2	0.2	0.8	2.3
BAMkar- 31 KF677018(*Bradyrhizobium sp*)	42.7	1.2	0.9	0.3	1.2	3.6
BAMkbay-25 KF677016 (*Bradyrhizobium sp*)	46.7	0.6	1.1	0.4	1.5	2.7

Bambara Vs other legumes

Legume	Plant density (no. m^{-2})	Ndfa (%)	N-fixed (kg hac^{-1})
Karungu			
Bambara	6.9[c]	33[a]	16.8[b]
Cowpeas	14.2[a]	25[b]	44.2[a]
Groundnuts	11.3[ab]	21[c]	8.3[bc]
Common beans	9.7[b]	37[a]	14.6[b]
Kisumu			
Bambara	13.4[ab]	42[b]	38.8[b]
Cowpeas	9.2b[c]	50[a]	54.5[a]
Groundnuts	18.4[a]	27[c]	11.2[d]
Common beans	10.7[b]	45[ab]	18.6[c]
Sio-Port			
Bambara	15.2[b]	71[a]	62.1[a]
Cowpeas	20.0[a]	41[c]	43.8[b]
Groundnuts	13.8[bc]	35[d]	21.5[d]
Common beans	11.4[cd]	62[b]	26.7[cd]
Kendu-bay			
Bambara	7.4[b]	37a	21.3[b]
Cowpeas	16.8[a]	24b	34.9[a]
Groundnuts	15.2[a]	18c	7.6[d]
Common beans	9.6[b]	39a	16.8[c]

NB: Means followed by same letter along a column are not significantly different at $LSD_{0.05}$

Conclusions

- **The results confirm diverse groups of rhizobium bacteria in Lake Victoria basin soils. *Rhizobium sp.* is the most dominant with *Bradyrhizobium* and *Sinorhizobium* less dominant.**
- **12 strains were effective on bambara with *B. japonicum, R. tropicii* and *R. etli* producing the highest number of nodules,nitrogen content of leaves and yield biomass.**

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……….AND THE CONFERENCE ORGANIZERS!

Thank you for listening