

Applied Soil Ecology

SUPPLEMENTARY MATERIAL

**Title: Bacteria related to *Bradyrhizobium yuanmingense* from Ghana are effective
groundnut micro-symbionts**

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Table S1

Principal component analysis explaining the variation between variables

	Eigen Value	Percent of Variance	Cumulative Percentage
1	3.36	84.1	84.05
2	0.42	10.5	94.5
3	0.20	5.052	99.6
4	0.016	0.421	100.0

Table S2¹⁵N enrichment and proportion of nitrogen derived from air (%Ndfa) of groundnut and reference plant

Isolate/strain/reference plant	Atom % ¹⁵ N excess	% Ndfa calculated using sorghum as the non-N ₂ - fixing reference plant
KNUST 1001	0.00592 c	91.8 a
KNUST 1002	0.00872 c	87.8 a
KNUST 1003	0.00613 c	91.4 a
KNUST 1004	0.00550 c	92.3 a
KNUST 1005	0.00664 c	90.8 a
KNUST 1006	0.00551 c	92.3 a
KNUST 1007	0.00661 c	90.7 a

Non-inoculated	0.00692 c	90.2 a
BR 10254	0.00594 c	91.7 a
BR 3267	0.00536 c	92.5 a
32H1	0.00601 c	91.5 a
SEMIA 6144	0.00542 c	92.4 a
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Sorghum	0.07125 b	-
NN Soybean	0.18750 a	-
NN Common bean	0.20574 a	-
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CV (%)	48.8	
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Means in the same column followed by the same letter are not significantly different at $P < 0.05$ (Scott Knott Test).

Table S3

Morpho-cultural characteristics of effective isolates

Isolates	Days to single	pH reaction	Colony	Colony transparency/Colour	Mucus
	colony(C)		morphology		produced
	appearance				
KNUST 1001	6	Alkaline	Circular	Opaque/ White	Less
KNUST 1002	7	Alkaline	Circular	Opaque/ White	Less
KNUST 1003	3	Acidic	Circular	Translucent/ Yellow	More
KNUST 1004	7	Alkaline	Irregular	Opaque/ White	Less
KNUST 1005	7	Alkaline	Circular	Opaque/ White	Less
KNUST 1006	7	Alkaline	Circular	Opaque/ White	Less
KNUST 1007	3	Acidic	Circular	Translucent/ Yellow	More

Table S4

Percentage nucleotide similarity at different genetic loci between already described *Bradyrhizobium* strains and isolates from this study. The last column shows values for concatenated sequences of two loci. Similarity values were determined using the Mega 7 software. -: data not available

Strains/ genes	16S rRNA	ITS	<i>nodC</i>	<i>nifH</i>	16S rRNA, ITS
<i>Bradyrhizobium yuanmingense</i> CCBAU 10071 ^T					
KNUST 1001	99.75%	97.52%	89.74%	92.54%	99.05%
KNUST 1002	99.59%	97.88%	89.74%	92.54%	99.16%
KNUST 1004	99.59%	97.89%	90.28%	92.19%	99.16%
KNUST 1005	99.75%	99.83%	94.33%	96.62%	99.78%
KNUST 1006	99.43%	98.59%	90.03%	92.19%	99.33%
<i>Bradyrhizobium yuanmingense</i> BR3267	99.26%	-	89.25%	93.12%	-
<i>Bradyrhizobium japonicum</i> SEMIA 6144 ^T	98.60%	-	86.29%	-	-
<i>Bradyrhizobium</i> sp. BR 10254 ^T	98.68%	-	-	-	-
<i>Bradyrhizobium arachidis</i> CCBAU 051107 ^T	98.85%	98.24%	85.43%	91.88%	98.66%
<i>Bradyrhizobium viridifuturi</i> SEMIA 690 ^T	96.99%	-	-	88.53%	-
<i>Bradyrhizobium embrapense</i> SEMIA 6208 ^T	96.99%	86.61%	78.34%	-	94.15%
<i>Bradyrhizobium subterraneum</i> 58 2-1 ^T	99.51%	97.17%	-	93.09%	98.82%

<i>Bradyrhizobium betae</i> LMG 21987 ^T	99.01%	94.58%	-	-	97.58%
<i>Bradyrhizobium canariense</i> BTA-1 ^T	99.10%	95.69%	65.71%	83.85%	97.97%
<i>Bradyrhizobium cytisi</i> CTAW11 ^T	99.01%	94.94%	68.72%	83.32%	97.75%
<i>Bradyrhizobium daqingense</i> CCBAU 15774 ^T	99.43%	96.07%	91.57%	91.40%	98.32%
<i>Bradyrhizobium denitrificans</i> LMG 8443 ^T	98.18%	78.59%	-	85.01%	92.44%
<i>Bradyrhizobium elkanii</i> USDA 76 ^T	96.73%	88.28%	80.49%	89.17%	94.45%
<i>Bradyrhizobium erythrophlei</i> CCBAU 53325 ^T	96.99%	89.04%	71.20%	88.04%	94.87%
<i>Bradyrhizobium ferriligni</i> CCBAU 51502 ^T	94.89%	86.82%	81.03%	89.63%	92.67%
<i>Bradyrhizobium ganzhouense</i> RITF806 ^T	99.18%	-	63.98%	85.38%	-
<i>Bradyrhizobium guangdongense</i> CCBAU 51649 ^T	99.26%	-	-	82.43%	-
<i>Bradyrhizobium guangxiense</i> CCBAU 53363 ^T	99.18%	-	-	85.58%	-
<i>Bradyrhizobium huanghuaihaiense</i> CCBAU 23303 ^T	98.68%	97.17%	91.57%	91.40%	98.15%
<i>Bradyrhizobium icense</i> LMTR 13 ^T	96.90%	-	68.99%	85.71%	-
<i>Bradyrhizobium ingae</i> BR 10250 ^T	98.35%	-	63.82%	-	-
<i>Bradyrhizobium iriomotense</i> EK05 ^T	98.35%	95.51%	63.34%	-	97.46%
<i>Bradyrhizobium japonicum</i> USDA 6 ^T	99.43%	95.34%	91.57%	91.40%	98.09%
<i>Bradyrhizobium jicamae</i> PAC 68 ^T	96.65%	88.04%	64.54%	-	94.33%
<i>Bradyrhizobium kavangense</i> 14-3 ^T	98.93%	97.34%	85.72%	-	98.43%

<i>Bradyrhizobium lablabi</i> CCBAU 23086 ^T	96.82%	87.25%	69.66%	85.73%	94.21%
<i>Bradyrhizobium liaoningense</i> LMG 18230 ^T	99.51%	95.51%	59.52%	91.40%	-
<i>Bradyrhizobium manausense</i> BR 3351 ^T	99.18%	96.25%	63.77%	81.61%	98.26%
<i>Bradyrhizobium oligotrophicum</i> LMG 10732 ^T	98.43%	76.86%	-	84.87%	92.25%
<i>Bradyrhizobium ottawaense</i> OO 99 ^T	99.34%	95.69%	91.57%	91.40%	98.15%
<i>Bradyrhizobium pachyrhizi</i> PAC 48 ^T	96.82%	87.43%	81.35%	-	94.27%
<i>Bradyrhizobium paxllaeri</i> LMTR 21 ^T	96.90%	87.01%	69.63%	85.73%	94.21%
<i>Bradyrhizobium retamae</i> Ro 19 ^T	97.24%	86.76%	68.56%	86.14%	94.38%
<i>Bradyrhizobium rifense</i> CTAW 71 ^T	99.10%	94.74%	67.53%	83.38%	97.69%
<i>Bradyrhizobium valentinum</i> LmjM3	96.82%	-	68.85%	86.21%	-
<i>Bradyrhizobium vignae</i> 7-2 ^T	99.01%	97.88%	85.70%	-	-
<i>Bradyrhizobium tropiciagri</i> SEMIA 6148 ^T	96.21%	87.38%	80.58%	88.08%	93.84%
<i>Bradyrhizobium diazoefficiens</i> USDA 110 ^T	99.01%	94.20%	91.57%	91.40%	97.52%
<i>Bradyrhizobium neotropicale</i> BR 10247 ^T	96.39%	95.34%	62.87%	-	-
<i>Microvirga vignae</i> BR 3299 ^T	90.47%	-	-	-	-

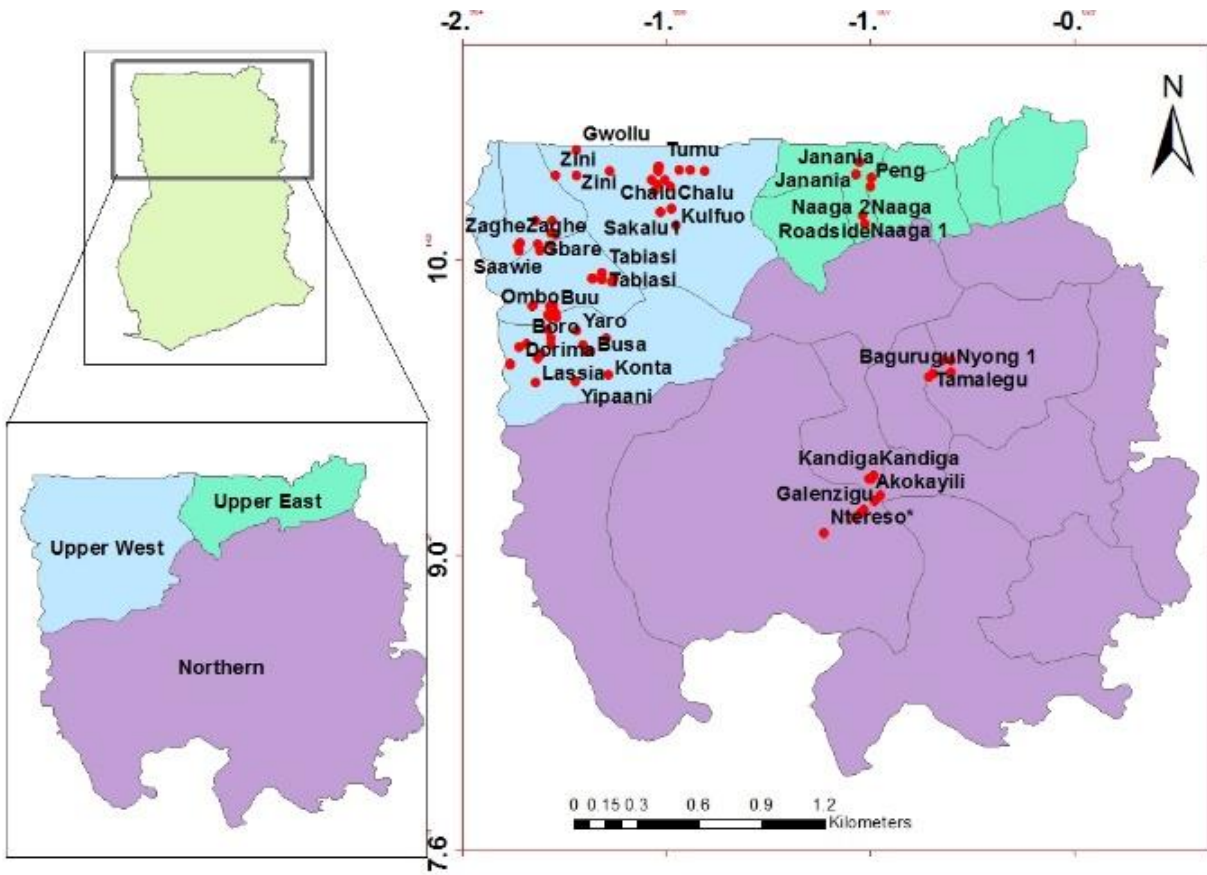


Fig. S1. Locations of cowpea and groundnut nodule sampling sites in northern Ghana representing the major legume growing areas.

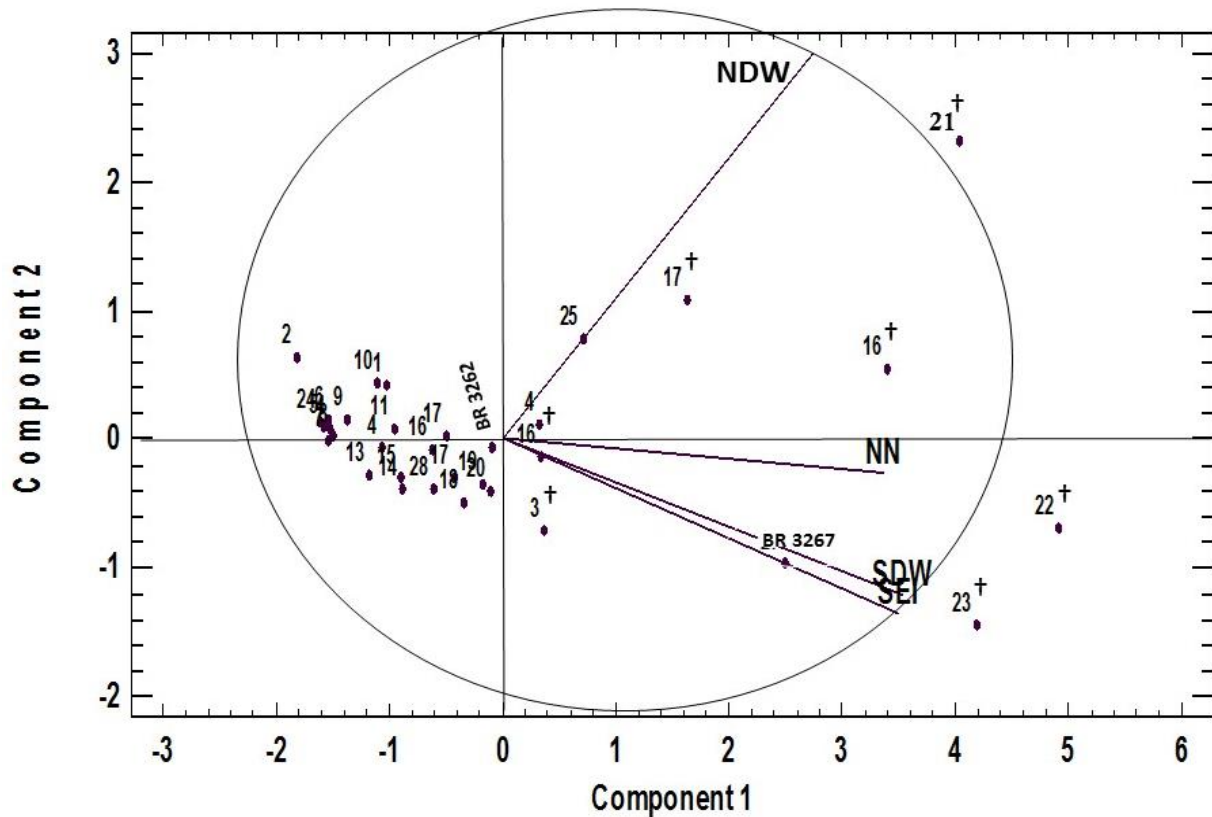


Fig. S2. Principal component analysis for the relationships among sampling sites as sources of effective or ineffective isolates. Reference strains *Bradyrhizobium yuanmingense* strain BR 3267 and *Bradyrhizobium pachyrhizi* strain BR 3262 represent sources of good performing strains. Numbers represent sampling sites: 1- Tamale; 2- Gbare; 3- Akokayilli; 4- Kandiga; 5- Naaga; 6- Pishegu; 7- Chiatanga; 8- Punyoro; 9- Dorima; 10- Varimpere; 11- Tabiasi; 12- Kandiga; 13- Tabiasi4 14- Varimpere 2; 15- Kuncheni; 16- Kandiga 2; 17- Boro; 18- Zaguo daryiri; 19- Zaguo daryiri; 20- Saawie; 21- Punyoro kb; 22- Konta; 23- Yipaani; 24- Tabiasi 2; 25- Kpalga.

† Isolates selected for second experiment. SDW: shoot dry weight, NN: nodule number, NDW: nodule dry weight, SEI: symbiotic effectiveness index

Fig S3a.

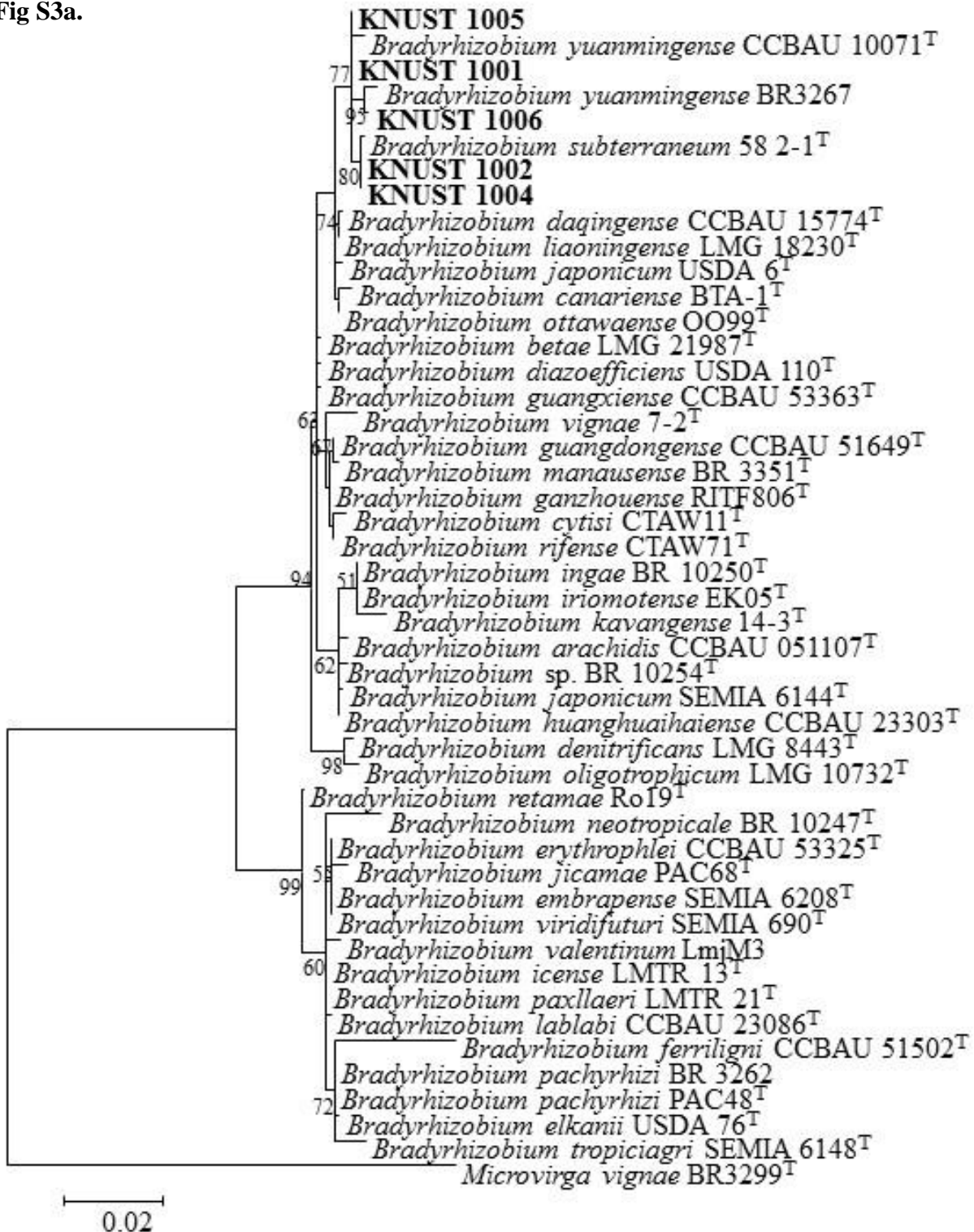


Fig S3b.

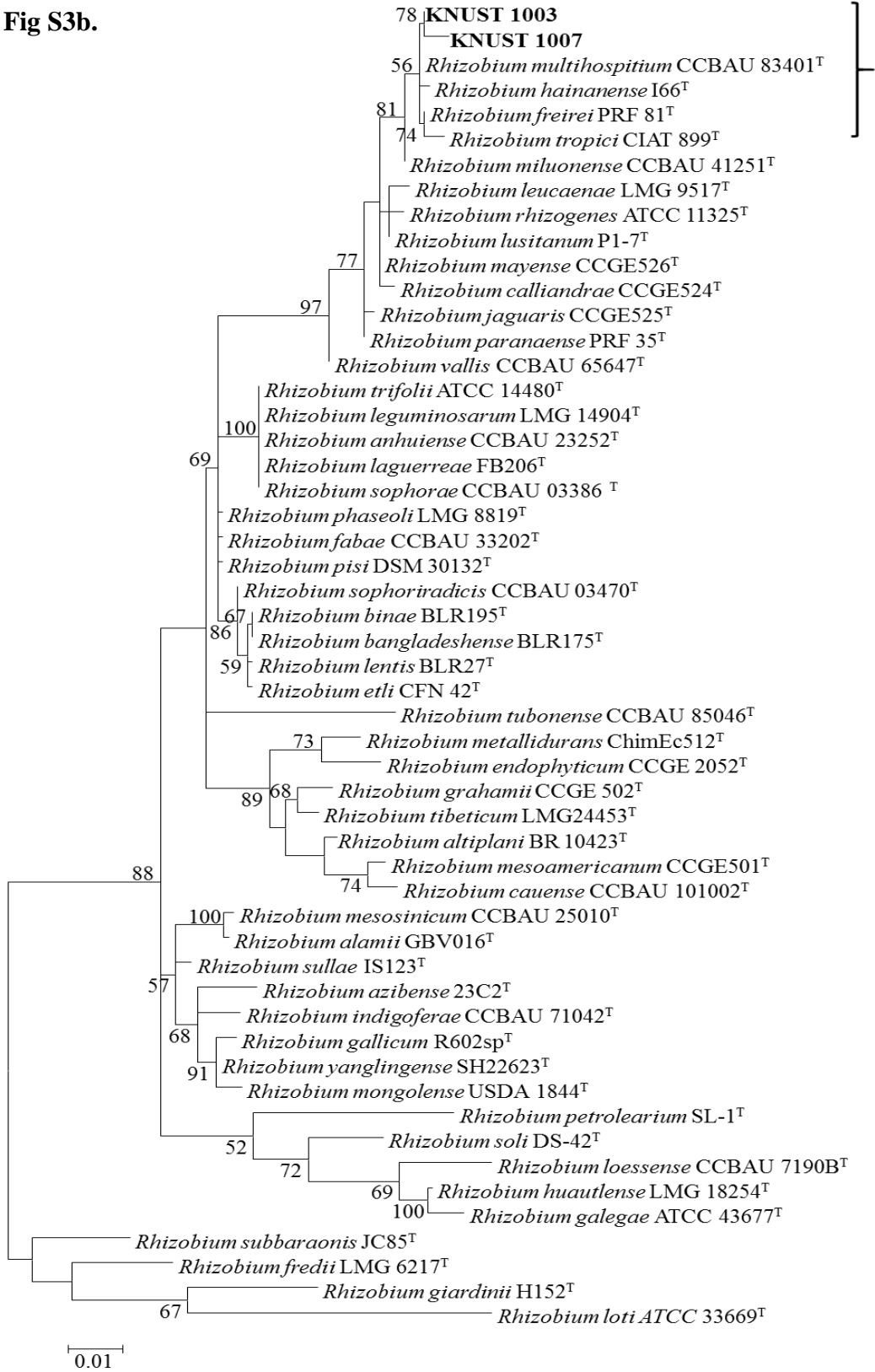


Fig. S3. Maximum likelihood phylogenetic tree based on 16S rRNA gene showing relationships between isolates, type species (^T) and reference strains of the genera *Bradyrhizobium* (a) and *Rhizobium* (b). The phylogenetic tree was built based on the Tamura 3-parameter model. Bootstrap values were inferred from 500 replicates and are indicated at the tree nodes when $\geq 50\%$. The bar represents two or one estimated substitutions per 100 nucleotide positions.

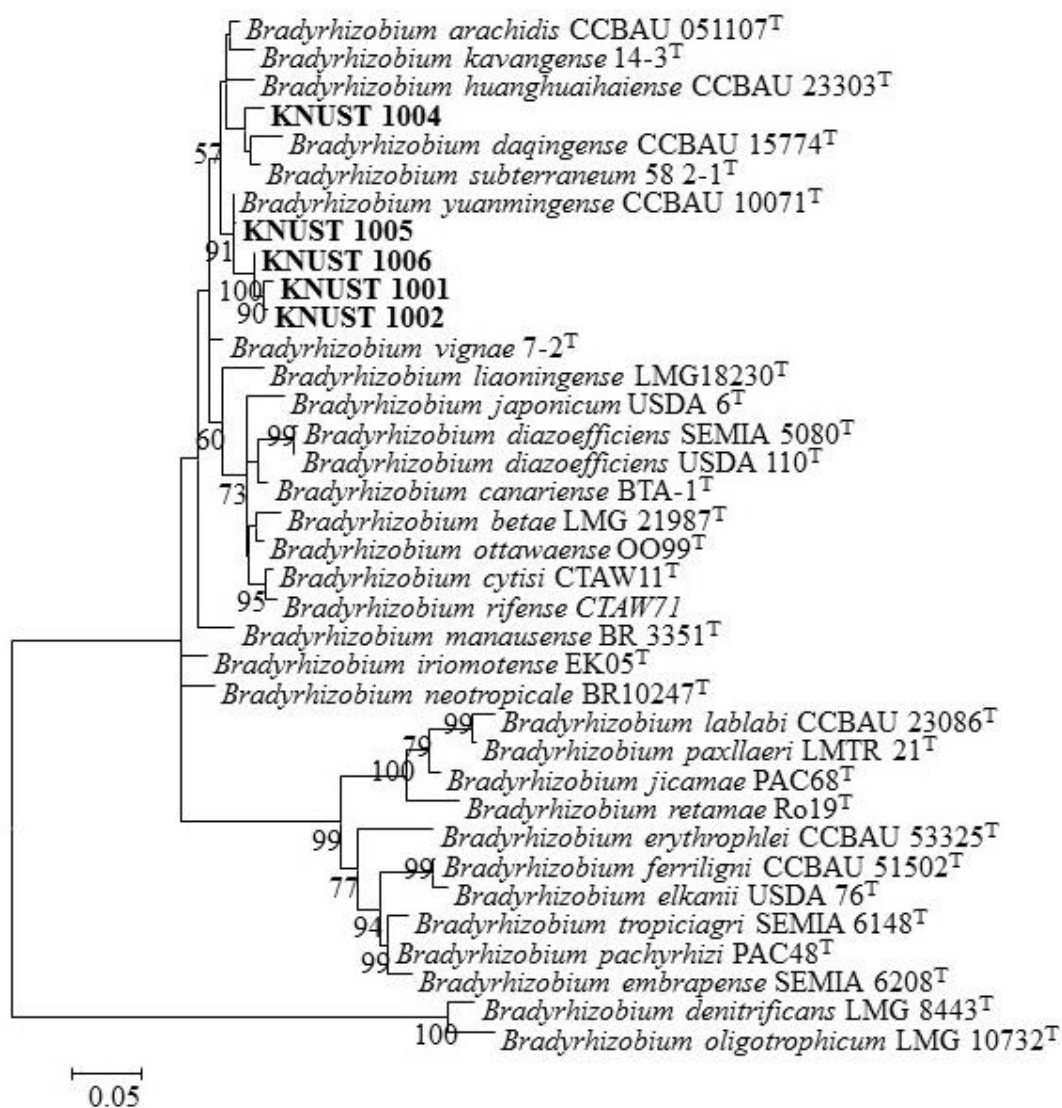


Fig. S4. Maximum likelihood phylogenetic tree based on ITS region showing relationships between isolates, type species (^T) and reference strains of the genus *Bradyrhizobium*. The phylogenetic tree was built based on the Kimura 2-parameter model. Bootstrap values were inferred from 500 replicates and are indicated at the tree nodes when $\geq 50\%$. The bar represents five or two estimated substitutions per 100 nucleotide positions.