Chapter

Distribution and Characterization of the Indigenous Soybean-Nodulating Bradyrhizobia in the Philippines

Maria Luisa Tabing Mason and Yuichi Saeki

Abstract

The research about the indigenous soybean bradyrhizobia in the Philippines is scarce, and this greatly influences the improvement of soybean production in the country. Thus, soil samples were collected from 11 locations in the country and were used to isolate the indigenous bradyrhizobia in the soil. Through the use of polymerase chain reaction—restriction fragment length polymorphism (PCR-RFLP) and sequence analysis of the 16S rRNA gene, 16S-23S rRNA gene internal transcribed spacer (ITS) region and *rpoB* housekeeping gene, the most abundant and dominant indigenous bradyrhizobia in the country were identified. Then, the representative isolates of the most dominant species per location were used to test their symbiotic efficiency and N-fixation ability with the local soybean cultivars. The results showed that among all the tested indigenous strains, the *B. elkanii* IS-2 is the most effective and efficient microsymbiont of the Philippines' local soybean cultivars. This report was able to provide necessary information on the distribution of soybean bradyrhizobia in the Philippines and characterized the symbiotic performance of the indigenous strains.

Keywords: tropical rhizobia, genomic diversity, N-fixation, symbiotic performance

1. Introduction

Soybean (*Glycine max* [L.]) is a leguminous plant that can form a symbiotic relationship with the nitrogen-fixing group of bacteria living in the rhizosphere, which are generally termed as rhizobia. In the Philippines, soybean production has been limited by the poor grain yield which leads to the importation of more than 90% of the country's demand. Thus, it is essential to look for an alternative way to increase the volume of production per unit area.

The research about tropical bradyrhizobia indicated a high diversity of species and their distribution has been reported to be due to several abiotic and biotic factors such as soil acidity [1–3], alkalinity [3, 4], temperature [1, 5–11], climate [12, 13], soil water status [14, 15], soil type [2, 14, 16–18], and soil management or cultural practices [2, 14, 19–22]. In case of the Philippines, the pioneer research that was able to identify the most dominant species of bradyrhizobia in the country reported that *B. elkanii* species was the most abundant, followed by the

B. diazoefficiens, *B. japonicum*, and some yet unclassified *Bradyrhizobium* sp. [14]. In this later study, it was identified that the distribution of these indigenous species of bradyrhizobia were influenced mainly by the water status of the soil, followed by soil pH, nutrient content, and soil type.

Previous studies have reported that aside from the various agro-environmental factors, the competition with the native rhizobia is a hindrance for a successful inoculation [23, 24]. The utilization of inoculants for legumes had shown promising results for the increase in grain yield as evidenced by recent reports [25, 26]. The role of the biological nitrogen fixation (BNF) in providing the N requirement of the plant in a natural way has been deemed necessary especially these times that the soil has become more degraded due to over-fertilization. The indiscriminate use of NPK fertilizer could cause soil pollution and less crop production [27]. Therefore, it is essential to select and evaluate the symbiotic competitiveness of the indigenous strains which are native and existing in high density in the country. The use of different genetic markers to accurately identify the rhizobia for taxonomic purposes has been proposed [28] and so we have used three genetic markers such as the 16S rRNA gene, 16S-23S rRNA gene internal transcribed spacer (ITS) region, and the *rpoB* housekeeping gene.

Thus, this study was formulated with the aim to utilize the recently identified indigenous bradyrhizobia in the Philippines and characterize their symbiotic performance with the local soybean cultivars.

2. Materials and methods

2.1 Collection of soil and soybean cultivation

The soil samples were collected from 11 locations in the Philippines, where some basic information on the sites are listed in **Table 1**. The collection of soil was conducted by first removing the surface litters then, obtaining a bar of soil with a dimension of approximately 20 cm in depth and 3 cm in thickness that weighs about 1 kg. A total of 10 subsamples per location were obtained and were mixed thoroughly until a 1 kg of composite soil sample was taken. A 0.5 kg soil was air-dried for the chemical analyses while the remaining 0.5 kg of the fresh soil was used for the soybean cultivation.

Location	Rep. Isolate	pH[a]	N[a] (%)	P[a] ($mgkg^{-1}$)	K[a] ($mgkg^{-1}$)	CEC[b] (meq)
1. Isabela1 (IS)	IS-2	5.90	0.13	1.86	51.80	17.1
2. Isabela 2 (GI)	GI-4	5.52	0.17	2.30	58.60	27.4
3. Benguet (BA)	BA-24	5.22	0.24	22.22	51.00	29.3
4. Nueva Ecija1 (NE1)	NE1-6	6.21	0.13	6.74	73.90	30.1
5. Nueva Ecija 2 (NE2)	NE2-37	5.81	0.22	21.63	49.40	27.7
6. Sorsogon (SO)	SO-1	5.26	0.22	2.57	55.80	20.3
7. Leyte (LT)	LT-3	5.80	0.15	6.39	174.20	40.6
8. Negros Occ. (NR)	NR-2	5.62	0.07	20.44	74.10	14.1
9. Bohol (BO)	BO-4	5.82	0.06	2.80	47.80	9.7
10. Maguindanao (SK)	SK-5	6.64	0.19	4.53	59.60	40.5
11. South Cotabato (SC)	SC-3	5.52	0.14	31.18	47.20	9.7

[a]*Mason et al., 2018*
[b]*This study*

Table 1.
Result of the soil chemical analysis on the 11 locations in the Philippines.

The cultivation of soybean was performed using a 1-L capacity culture pots (n = 3). Each pot was filled with vermiculite and a N-free solution [29] was added at 40% (vol/vol) water content. The culture pots were sterilized by autoclaving for 20 min at 121°C. Meanwhile, the soybean seeds were surface-sterilized by soaking into a 70% EtOh for 30 s, then by a diluted sodium hypochlorite solution (0.25% available chlorine) for 3 min and followed by washing with sterile distilled water for about 6–8 times. Then, a 2–3 g of soil sample was placed on the vermiculite at a depth of about 2–3 cm, the seeds were sown on the soil and the pot was weighed and recorded. The plants were grown inside a growth chamber for 28 days at 28°C (8 h, night) and 33°C (16 h, day) then were supplied weekly with sterile distilled water until the initial weight of the pot was reached.

2.2 Isolation of soybean rhizobia and DNA extraction

After 28 days, approximately 20 random nodules were collected from the roots of each soybean plants and were sterilized with 70% EtOh and sodium hypochlorite solution as previously described [29]. Each nodule was homogenized with sterile distilled water in a microtube and streaked on to a yeast-extract mannitol agar (YMA) plate [30]. The YMA plate was incubated in the dark at 28°C for about 1 week until a single colony was formed. After then, the single colony was streaked on to a YMA plate containing a 0.002% (wt/wt) bromothymol blue (BTB) [31] and was incubated as above. Repeated streaking was done until a pure single colony was obtained which was cultured for about 3–4 days in a HEPES-MES (HM) broth culture [32, 33] at 28°C in a shaker for 120 rpm. After then, the bacteria cells were collected by centrifugation at 9000×*g* and washed with sterile distilled water. The DNA was extracted by using BL buffer as described [34] from the method reported by Hiraishi et al. [35].

2.3 PCR amplification of the 16S rRNA gene, ITS region, and rpoB gene

For the amplification of the 16S rRNA gene, the primer set: 16S-F: 5′ AGAG TTTGATCCTGGCTCAG-3′ and 16S-R2: 5′- CGGCTACCTTGTTACGACTT-3′ [36]. The PCR tubes were then placed in the PCR Thermal Cycler (TaKaRa Co. Ltd.) with the following conditions: pre-run at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Final extension was set at 72°C for 10 min and indefinite preservation at 4°C.

On the other hand, the PCR amplification of the ITS region was conducted using the following primer set: Bra-ITS-F: 5-GACTGGGGTGAAGTCGTAAC-3′ and Bra-ITS-R1: 5′-ACGTCCTTCATCGCC TC-3′ [6]. The PCR cycle for the ITS region was almost the same with the 16S rRNA gene except for a shorter denaturation and annealing periods which were conducted at 30 s for each step.

For the *rpoB* gene, simplification was done using the following primer sets: *rpo*B83F: 5′-CCTSATCGAGGTTCAC AGAAGGC-3′ and *rpo*B1540R: 5′-AGCTGCGAGGAACCGAAG-3′ [37]. The PCR cycle conditions were as follows: pre-run at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min. Final extension was set at 72°C for 5 min and indefinite preservation at 4°C.

2.4 Restriction fragment length polymorphism (RFLP) of the 16S rRNA gene, ITS region, and rpoB gene

The successfully amplified products were subjected to the RFLP treatment using four restriction enzymes which were *HhaI*, *HaeIII*, *MspI*, and *XspI*. For the rpoB gene, the enzymes that were used for RFLP are *HaeIII*, *MspI*, and *AluI*.

The reference strains that were used in this study are the *Bradyrhizobium* USDA strains (*B. japonicum* USDA 4, 6^T, 38, 62, 115, 122, 123, 124, 125, 127, 129, 135, *B. diazoefficiens* USDA 110^T, *B. elkanii* 31, 46, 61, 76^T, 94, 130, and *B. liaoningense* 3622^T) which were previously described [38]. This was done in a 10-μL reaction mixture containing a 2.5-μL amplified PCR product and was incubated in a 37°C for 16 h. Afterward, a 3–4% agarose gel was used in a submerged gel electrophoresis for about 60 min, stained with ethidium bromide and the patterns were visualized using a Luminiscent Image Analyzer LAS-4000 (FUJIFILM Tokyo, Japan).

2.5 Single-strain inoculation test

After the amplification and the RFLP treatment of the 16S rRNA gene, a single-strain inoculation test was conducted for all the amplified isolates that shared the same restriction enzymes' fragment patterns with the USDA *Bradyrhizobium* reference strains. This was done to confirm the strain's capability to nodulate soybean and was tested on two local varieties which are the PSB-SY2 and Collection 1 which are both commercially available across the country.

The cultivation of soybean was conducted as described above, but without soil. Each isolate was cultured in a YM broth (YMB) [30] at 28°C for about 1 week on a shaker. After then, the cultures were diluted with sterile distilled water at about 10^6 cells mL^{-1} and were inoculated on the cultivated soybean at a rate of 1.0 mL per seed. This was done with three replications. After inoculation, the weight of the pot was recorded and it was placed inside a growth chamber with a condition set to mimic the average temperature in the Philippines at 26°C (8 h, night) and 33°C (16 h, day). The same condition was used for the cultivation of an uninoculated control and a positive control pot that was inoculated with *B. diazoefficiens* USDA110. The pots were kept inside the growth chamber for 28 days and were supplied weekly with sterile distilled water until the initial weight of each pot was reached.

2.6 Sequence analysis of the 16S rRNA gene and ITS region

According to the similarities of the band patterns through the RFLP treatment, a representative of the most abundant isolates was chosen for each location. In total, there were 11 isolates that were selected to confirm the nucleotide sequence of the 16S rRNA gene and the ITS region. The sequence primers that were used were reported previously [22]. From the PCR amplified product, the samples were purified according to the protocol of the manufacturer (Nucleospin® Gel and PCR Clean-up; Macherey-Nagel, Germany). Then, the samples were sent to the company for the sequence analysis (Eurofins Genomics, Tokyo, Japan).

Then, the Basic Local Alignment Search Tool (BLAST) program in DNA Databank of Japan (DDBJ) was used to determine the nucleotide homology of the isolates. Only the sequences with a similarity of at least 99% for the 16S rRNA and 96% for the ITS region with our isolates were retrieved from the BLAST database. The alignment was performed using the ClustalW and Neighbor-Joining [21] method was used to construct the phylogenetic trees. The genetic distances were computed using the Kimura 2-parameter model [39] in the Molecular Evolutionary Genetic Analysis (MEGA v7) software [40]. Subsequently, the phylogenetic trees were bootstrapped with 1000 replications. All the nucleotide sequences determined in this study were deposited in DDBJ at http://www.ddbj.nig.ac.jp/.

3. Results

3.1 Soil analysis and characterization of the indigenous bradyrhizobia

The soil samples that were used in this study were all slightly to moderately acidic (5.22–6.64) with non-saline condition (0.05–0.20 dS/m), low nutrient status as evidenced by low amounts of NPK and CEC (**Table 1**). These values are generally typical of agricultural soils that are used for crop production all throughout the year. These results showed that the soils used in this study have low fertility status that indicated the need for soil restoration strategies.

The growth morphologies of the pure single colony for each strain of bradyrhizobia were characterized and listed in **Table 2**. All the isolates were slow growers which were able to form single colonies measuring about 2 mm between 5 and 7 days upon streaking on YMA plates and incubation in a dark room. Based on the morphology, the isolates were grouped into three. Group I include the isolates IS-2, NE1–6, NR-2, and BO-4 which were translucent and the colonies are circular

Isolate	No. of isolates	Colony morphology	Formation of acid / alkaline substances
IS-2	40	Translucent, circular, liquid, slightly-convex elevation with entire margin	Alkaline
GI-4	30	Translucent, circular, slightly-mucoid, convex elevation with entire margin	Alkaline
BA-24	31	Translucent, circular, mucoid, convex elevation with entire margin	Alkaline
NE1-6	49	Translucent, circular, liquid, slightly-convex elevation with entire margin	Alkaline
NE2-37	31	Translucent, circular, slightly-mucoid, convex elevation with entire margin	Alkaline
SO-1	44	Translucent, circular, mucoid, convex elevation with entire margin	Alkaline
LT-3	42	Translucent, circular, mucoid, convex elevation with entire margin	Alkaline
NR-2	23	Translucent, circular, liquid, slightly-convex elevation with entire margin	Alkaline
BO-4	24	Translucent, circular, liquid, slightly-convex elevation with entire margin	Alkaline
SK-5	29	Translucent, circular, mucoid, convex elevation with entire margin	Alkaline
SC-3	31	Translucent, circular, mucoid, convex elevation with entire margin	Alkaline
Total	**374**		

Table 2.
Characterization of the morphology of the indigenous bradyrhizobia isolated from Philippines' soil according to their growth on Yeast-Extract Mannitol Agar plate medium [30].

in shape with slightly convex elevation and an entire margin. When they were manipulated with a needle, the colony was liquid. Group II include the isolates BA-24, SO-1, LT-3, and SK-5 were translucent with circular colonies, convex elevation with entire margin. When manipulated with a needle, the colonies have mucoid viscosity. On the other hand, last group (III) are the isolates GI-4 and NE2-37 which have similar growth morphology with Group II except that their viscosity was intermediate between liquid and mucoid. All the isolates produced alkaline substances when grown on YMA plate with BTB which is an indication of the *Bradyrhizobium* genus.

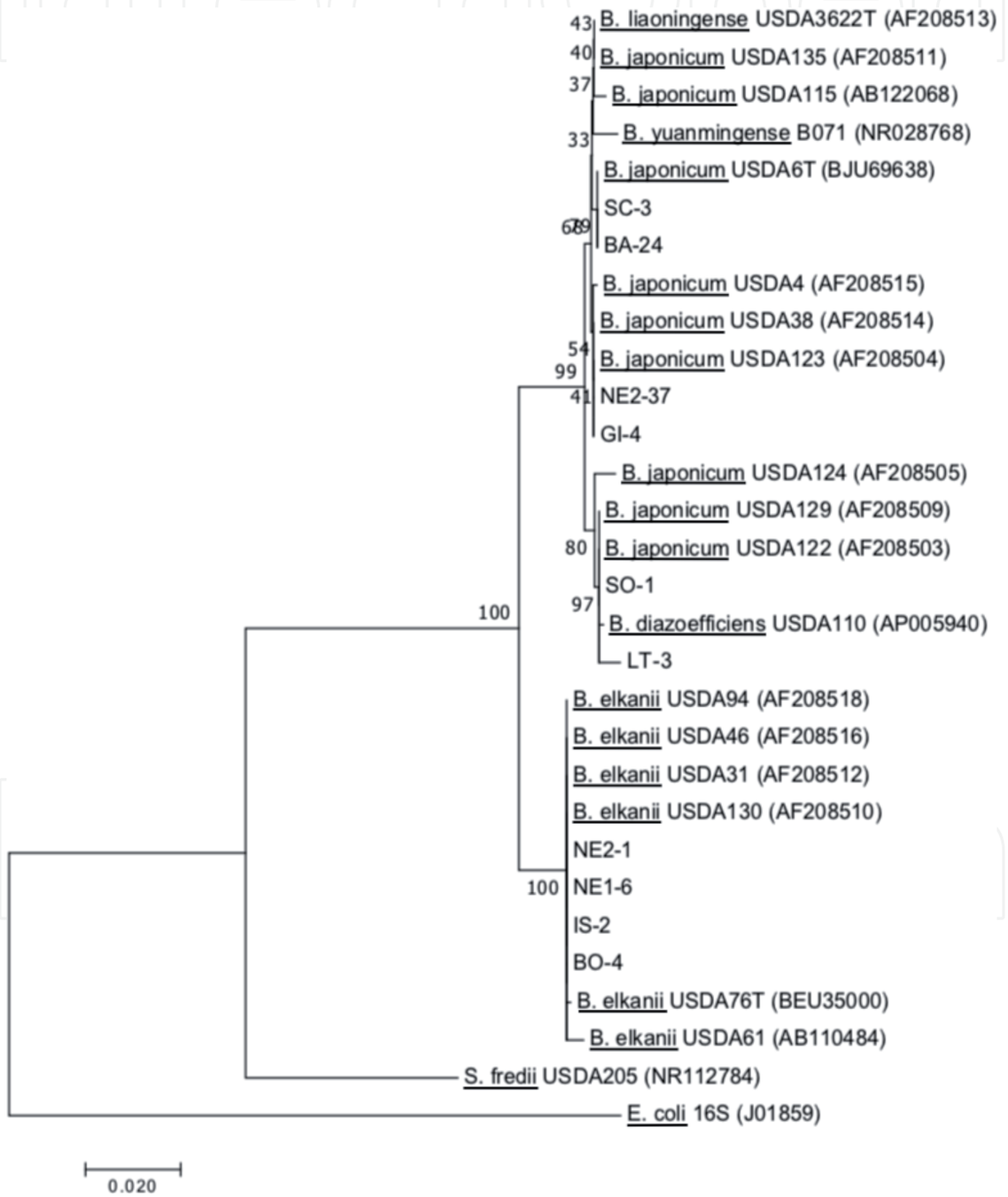

Figure 1.
Phylogenetic tree based on the sequence analysis of the 16S rRNA gene. The tree was constructed using the Neighbor-Joining method with the Kimura 2-parameter (K2P) distance correlation model and 1000 bootstrap replications in MEGA v.7 software. The accession numbers are indicated only for sequences obtained from BLAST. The isolates in this study are indicated with letters and number combinations, for example: BO-4–isolate no. 4 collected from Bohol.

3.2 Distribution of indigenous soybean bradyrhizobia

As seen in **Figure 1**, it is evident that the 11 most abundant indigenous soybean rhizobia in the Philippines are classified under the genus *Bradyrhizobium*, and are separated into its two species, *B. japonicum* and *B. elkanii*, according to the

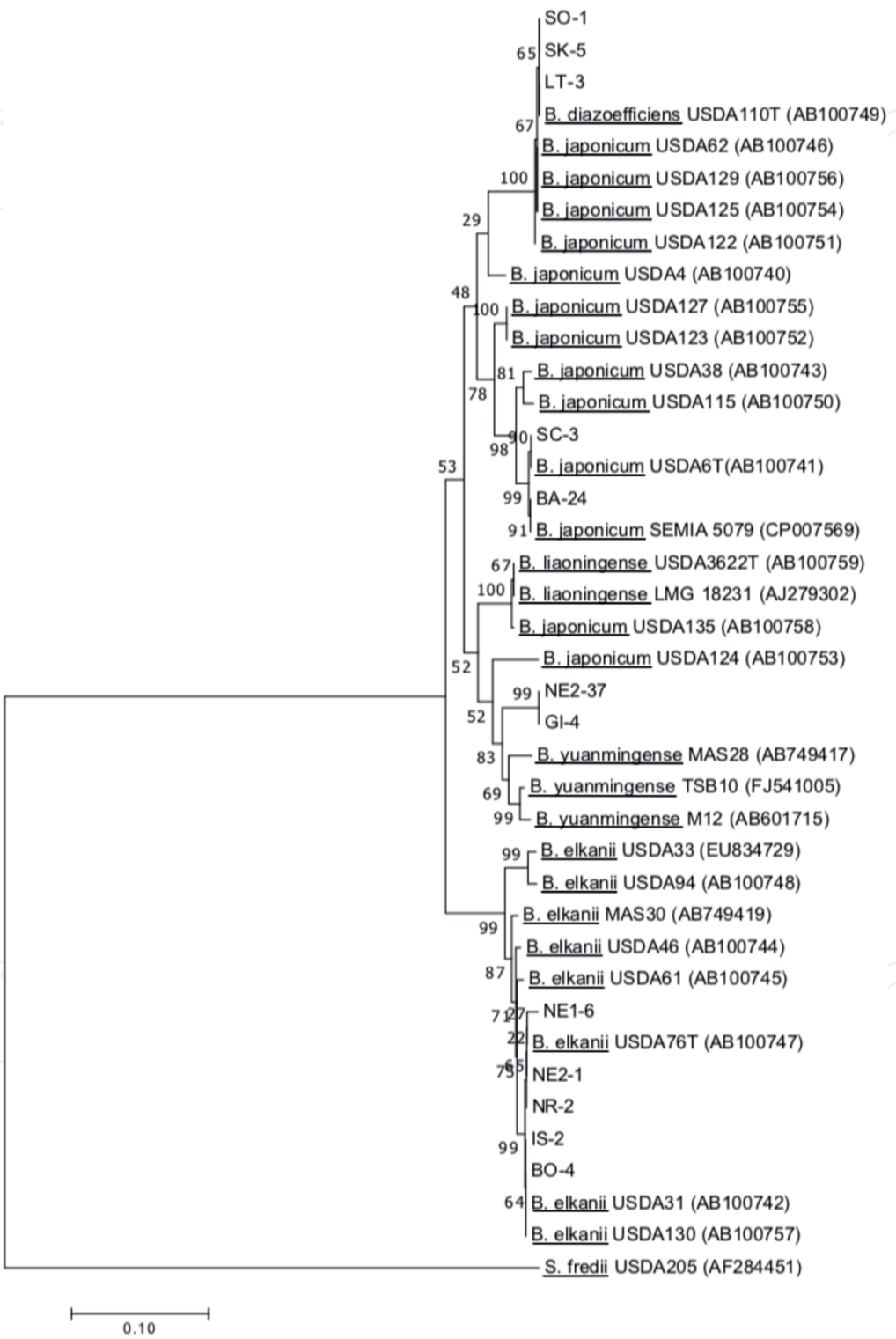

Figure 2.
Phylogenetic tree based on the sequence analysis of the 16S-23S rRNA internal transcribed spacer (ITS) region. The tree was constructed using the Neighbor-Joining method with the Kimura 2-parameter (K2P) distance correlation model and 1000 bootstrap replications in MEGA v.7 software. The accession numbers are indicated only for sequences obtained from BLAST. The isolates in this study are indicated with letters and number combinations, for example: BO-4–isolate no. 4 collected from Bohol.

phylogenetic tree from the sequence analysis of the 16S rRNA gene. To further confirm the classification of the indigenous bradyrhizobia, the phylogenetic trees constructed from the ITS region and the *rpoB* gene are presented in **Figures 2** and **3**, respectively. For the ITS region and the *rpoB* gene, the isolates were distinctly grouped into three species, *B. elkanii*, *B. japonicum*, and *B. diazoefficiens*. Additionally, an independent cluster composed of the representative isolates GI-4

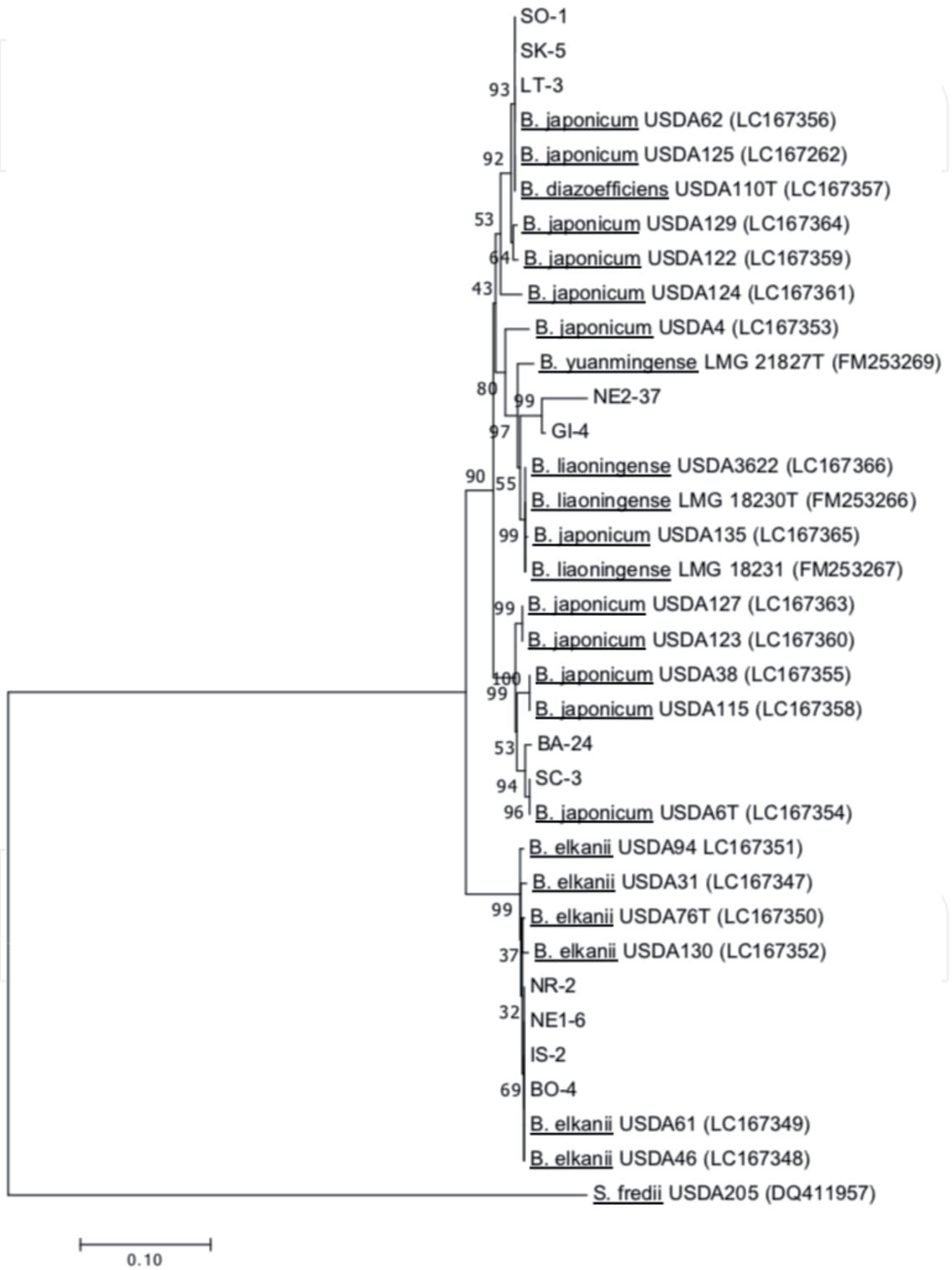

Figure 3.
Phylogenetic tree based on the sequence analysis of the rpoB housekeeping gene. The tree was constructed using the Neighbor-Joining method with the Kimura 2-parameter (K2P) distance correlation model and 1000 bootstrap replications in MEGA v.7 software. The accession numbers are indicated only for sequences obtained from BLAST. The isolates in this study are indicated with letters and number combinations, for example: BO-4–isolate no.4 collected from Bohol.

and NE2–37 that are seen in the ITS region and *rpoB* phylogenetic trees were treated as *Bradyrhizobium* sp. due to their nucleotide divergence with the known species from the BLAST engine.

Meanwhile, the distribution of the most abundant soybean bradyrhizobia in the country is shown in **Table 3**, which was classified according to the results of the sequence analysis of the three genetic markers used in this study. From here, it can be seen that 4 of the 11 locations were dominated with *B. elkanii* species (37.74%), 3 locations were dominated by the isolates under the *B. diazoefficiens* (28.54%), whereas 2 locations each were dominated by the species of *B. japonicum* (16.98% and *Bradyrhizobium* sp. (16.74%). This indicated that in the Philippines, the species of *B. elkanii* is the most prevalent in terms of population and the most widespread in terms of location as its presence was detected even in minor populations on all the locations except for one, which was Sorsogon.

3.3 Symbiotic efficiency and N-fixation ability of the indigenous bradyrhizobia

Upon classification, it is important to determine the capability of the indigenous bradyrhizobia for their symbiotic performance and N-fixation ability. As can be seen in **Figure 4A**, although USDA110 strain has the highest N-fixation ability, it should be noted that the amount of N that was fixed by *B. elkanii* IS-2 is the highest among all the indigenous bradyrhizobia isolated from the Philippines' soil on Rj_4 plants. However, the N-fixation ability of IS-2 was comparably similar with other strains (GI-4, NE2–37, and SK-5) with the non-*Rj* plants. The lowest N-fixation ability was observed from the strain LT-3 which was classified under the *B. diazoefficiens* species. This suggested that the process of biological N-fixation is a mutual relationship that is influenced by both the plant and the rhizobia and that the plant-rhizobia compatibility should be taken into consideration for inoculation strategies.

Presented in **Figure 4B** is the nodulation test performed on the strains and it can be seen for Rj_4 plants, there was not much significant difference in the nodulation

Location	*B. elkanii*	*B. diazoefficiens*	*B. japonicum*	*Bradyrhizobium* sp.	No. isolates
Isabela1 (IS)	100.0	-	-	-	40
Isabela 2 (GI)	16.7	-	-	83.3	36
Benguet (BA)	3.0	-	97.0	-	33
Nueva Ecija1 (NE1)	87.5	1.8	-	10.7	56
Nueva Ecija 2 (NE2)	8.6	-	2.9	88.6	35
Sorsogon (SO)	-	100	-	-	44
Leyte (LT)	2.3	97.7	-	-	43
Negros Occ. (NR)	74.2	3.2	22.6	-	31
Bohol (BO)	82.8	13.8	3.4	-	29
Maguindanao (SK)	23.2	67.4	-	9.3	43
South Cotabato (SC)	8.8	-	91.2	-	34
Total isolates	**160**	**121**	**72**	**71**	**424**
Percentage (%)	**37.74**	**28.54**	**16.98**	**16.74**	

Table 3.
Percentage distribution of the dominant Bradyrhizobium species in the Philippines as identified from the sequence analysis of the 16S rRNA gene, 16S-23S internal transcribed spacer (ITS) region, and rpoB housekeeping gene.

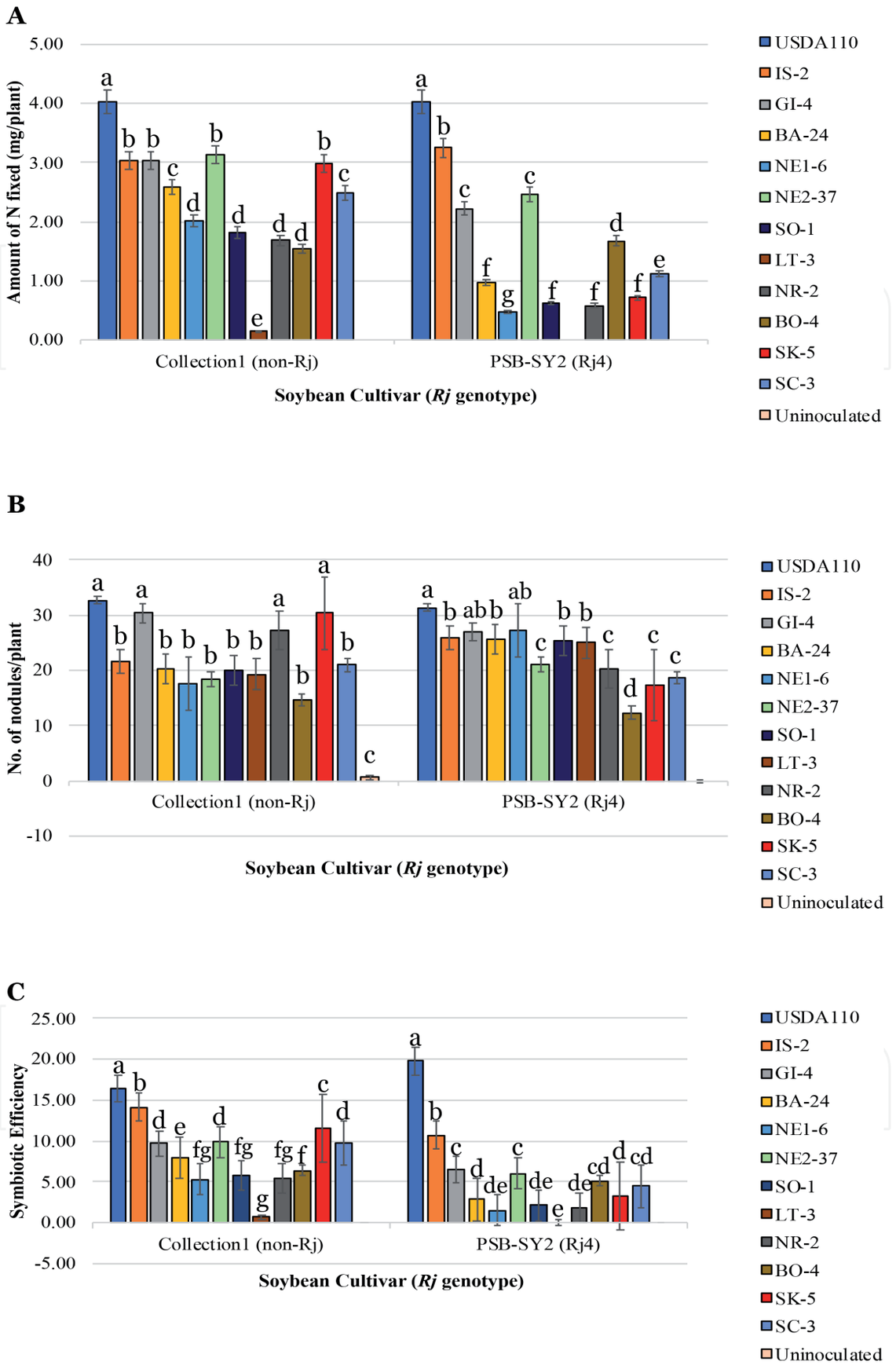

Figure 4.
Characterization of the dominant indigenous Bradyrhizobium strains isolated from the 11 locations in the Philippines based on the (A) amount of Nitrogen fixed (B) nodulation ability and (C) symbiotic efficiency as influenced by the single-strain inoculation test against the reference strain B. diazoefficiens USDA110 for the two soybean cultivars from the Philippines. Different letters indicate a significant difference by Tukey's test at $p > 0.05$, n=3, bar=SE.

ability of the strains, except for the low nodulation ability that was observed for the BO-4. In contrast, a significant difference in the nodulation ability was detected on the strains upon inoculation on the non-*Rj* plants. Although all the strains were able to form nodules on both soybean cultivars, the strains GI-4, NR-2, and SK-5 obtained the highest number of nodules for the non-*Rj* plants.

On the other hand, the symbiotic efficiency of the strains used in this study is presented in **Figure 4C**. Similar with the N-fixation ability, the USDA110 still possesses the highest symbiotic efficiency. But among all the indigenous bradyrhizobia, the strain IS-2 obtained the highest efficiency regardless of the *Rj* genotype of the soybean plants. As with the N-fixation, LT-3 obtained the least efficiency for symbiosis. This result indicated that the symbiotic efficiency of the rhizobia might not be directly influenced by the *Rj* genotype of the plant.

4. Discussion

4.1 Distribution and characterization of bradyrhizobia in the Philippines

The distribution of the most dominant and abundant species of soybean bradyrhizobia in the Philippines are reported in this study along with the characterization of their growth morphology. According to our earlier reports, we have elucidated that the Philippines was dominated by the soybean-nodulating bradyrhizobia that were classified under the *B. elkanii* species and the most important agro-environmental factors that affected their diversity and prevalence in the country was the similarity of soil pH, salinity, and temperature in the study locations [5, 14]. Our observation that there are abundant and high diversity of indigenous bradyrhizobia in the Philippines is similar with previous reports in other sub-tropical and tropical regions [12, 25, 41–44]. The temperate regions of Japan and USA were studied in the past and were reported to be dominated by species of *B. japonicum* and *B. diazoefficiens* [6, 9–11, 13, 45]. Our report showed that the distribution of bradyrhizobia in a tropical region like the Philippines seemed to be different from those of temperate regions.

Meanwhile, it was included in a recent report that the distribution and abundance of *B. diazoefficiens* and *B. japonicum* at specific locations were due to the longer period of flooding conditions [14]. The effect of nutrient content and soil type were also correlated with the abundance of these two species. In a report by Shiina et al. [17], it was stated that the predominance of *B. diazoefficiens* was observed on more anaerobic condition; whereas, *B. japonicum* was predominant on aerobic soils which was supported by another study [18]. Additionally, it was reported that *B. diazoefficiens* becomes predominant with enhanced flooding condition [15]. These results confirmed that our observations for the abundant of *B. diazoefficiens*, followed by *B. japonicum* and *Bradyrhizobium* sp. on flooded areas in the country which were usually used for planting rice.

4.2 Symbiotic and N-fixation ability of indigenous bradyrhizobia

In this report, the symbiotic performance, N-fixation and nodulation ability of the indigenous soybean bradyrhizobia form the Philippines were evaluated against that of the *B. diazoefficiens* USDA110 strain. The USDA110 has been extensively used in the world as a model strain for soybean inoculation due to its high ability for N-fixation and symbiotic efficiency [25, 46, 47]. Additionally, its possession of a complete set of denitrification genes that allows the release of N_2 back into the atmosphere makes it an ideal strain also for climate change mitigation studies [17, 48–50].

Therefore, we hypothesized that the indigenous isolates SO-1, LT-3, and SK-5, which were phylogenetically clustered under the USDA110 would also prove to be as effective N-fixer and efficient microsymbiont of soybean cultivars from the Philippines. However, our results indicated that the N-fixation ability and symbiotic efficiency of LT-3 and SO-1 were very low in comparison to the other indigenous isolates. For the low performance of these two isolates, it is hypothesized that the inherent ability of these strains to fix N and establish a symbiotic relationship with soybean is low. This could be explained by the fact that their nodulation ability was comparably similar with the other strains which possess higher N-fixation ability and symbiotic efficiency. In contrast, the isolate IS-2, which was clustered under the *B. elkanii* species, showed the highest symbiotic efficiency for both *Rj*-genotypes of the soybean cultivar used and the highest N-fixation ability for Rj_4 plants. In a previous report, the *Rj* genes that could restrict the nodulation of soybean by some strains of bradyrhizobia was summarized [51] but in case of our present report, all the strains in this study were not restricted by the two *Rj*-plants that were used. This led us to consider that the low N-fixation and symbiotic performance of some strains were not due to the restrictions from the *Rj*-genotypes of the plants but could be attributed to the strains' intrinsic capabilities. These observations might explain the reason for low yield of soybean in the Philippines. It was reported that many strains of *B. elkanii* were relatively inefficient microsymbionts of soybean and can induce chlorosis in soybean plants [52]. In a previous report [53], the high temperatures in tropical regions can limit the nodulation which could explain the low soybean yield.

It was expected that the strains which were classified as *B. diazoefficiens* could provide a better symbiotic performance than the other strains that were collected. However, the data showed that *B. elkanii* might establish a better symbiosis with local soybean cultivars in the Philippines. This result is crucial in order to devise strategies on how to increase the local production of soybean by inoculation with the indigenous strains.

Upon considering these results with the N-fixation and symbiotic performance ability of the strains, the number of nodules that can be formed from the single-strain inoculation does not seem to influence the amount of N that each strain can fix nor their symbiotic ability.

5. Conclusion

In this report, we have revealed that the distribution of tropical soybean bradyrhizobia seemed to be different than those of temperate bradyrhizobia in terms of population dominance of *B. elkanii* on higher temperature region like the Philippines. Additionally, it is proposed that for the Philippines, the most efficient N-fixer and symbiotically efficient species of bradyrhizobia would be *B. elkanii*. Yet, our results were made under the laboratory conditions only, so the results that were obtained here might not be as expected when done in field condition. For future research, utilization of more local soybean varieties with different soil types both in a controlled environment and on natural field condition would be beneficial to target the development of a site-specific and useful potential soybean inoculant. The data generated in this report would be beneficial for the augmentation of inoculation strategies in the country.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Maria Luisa Tabing Mason[1] and Yuichi Saeki[2]*

1 College of Agriculture, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

2 Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

*Address all correspondence to: yt-saeki@cc.miyazaki-u.ac.jp

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