# Phenotypic and Phylogenetic Characterization of Micro-symbionts Nodulating Winged Bean (*Psophocarpus tetragonolobus* L. DC.) Landraces

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**Abstract**

Winged bean (*Psophocarpus tetragonolobus* L.) is a potential source of protein for the tropics and almost equivalent to protein content of soybean. However, information on winged bean-bacteria association is still limited. This study was conducted to assess phenotypic and genetic characterization of micro-symbionts that could effectively nodulate winged beans. The greenhouse experiment was performed in a completely randomized design with five accessions of winged bean and five replicates. The micro-symbionts were isolated from the root nodules and subjected to nodulation test on host plants. The effective nodulating isolates were characterized by phenotypic characteristics and 16S rRNA gene sequencing technique. The strains were also assayed for plant growth promotion traits. Thirty micro-symbionts were isolated from root nodules of winged bean plants but only twelve (40.0%) effectively nodulated their host plants. All the effective micro-symbionts were Gram negative, rod-shaped bacteria. Six of the effective rhizobia isolates were slow growers while others were fast growers. The results further showed that four of the isolates could produce ammonia and indole acetic acid, as well as solubilizing phosphate. The phylogenetic analysis revealed that the micro-symbionts were similar to strains of *Rhizobium*, *Bradyrhizobium* and *Mesorhizobium*. The study therefore showed the potential of these micro-symbiont strains in nodulating winged bean.

Keywords: Micro-symbionts, *Psophocarpus tetragonolobus*, nodulation, bioinoculants

## Introduction

Winged bean (*Psophocarpus tetragonolobus* L.), a dicotyledonous vine species belonging to the family *Fabaceae* and subfamily *Papilionoideae*, is cultivated mainly at a subsistence scale in many countries including some African countries (Klu, 2000). It is an underutilized leguminous crop, grown for its green pods, tuberous roots, mature seeds and leaves (Tanzi et al., 2019). Many parts of this plant are consumed, including immature bean pods, leaves and tubers. Locally, immature bean pods are mostly eaten raw or as a cooked vegetable in a wide variety of local dishes (Tuquero and Takai, 2018). In some countries, mature seeds are roasted and boiled. Boiled, steamed, baked, fried or roasted tubers could also be consumed (Tanzi et al., 2019). Nutritionally, matured winged bean seeds contain protein content equivalent to that of soya bean. Fresh, young bean pods contain good amounts of Vitamin C, thiamin pyridoxine (Vitamin B6), niacin and riboflavin as well as other minerals such as iron, copper, manganese, and calcium (Wong et al., 2017; Tuquero and Takai, 2018). Moreover, the crop could play a major role in diet improvement of people, especially in areas where major vegetable crops require high-inputs, limiting consumers' access to vegetables. Furthermore, production of mature seeds could also help to reduce the demand and over-dependence on major crops such as cowpea and soybean for production of value-added products (Tanzi et al., 2019). Besides the nutritional benefits of this crop, winged bean's effective symbiotic associations with several rhizobia strains make it a good nitrogen-fixer for low-input and self-resilient agricultural systems. Its vining nature and ability to store high concentrations of nitrogen in its biomass also makes it usable as cover crop and thus can be incorporated into rotation or intercropping systems (Anugroho et al., 2010; Tanzi et al., 2019).

Winged bean nodulates profusely and fixes atmospheric nitrogen, thereby replenishes soil nitrogen. The crop has a great capacity to survive on a range of tropical soils: from poor acidic clay and loam soils to sandy, swamp peats and heavy clay soils. It could fix atmospheric nitrogen through symbiotic relationship with rhizobia, meeting the nitrogen need of the host plant and thus, directly promoting the growth. At the same time, the host plant provides shelter and energy to rhizobia (Namkeleja et al., 2016). The native rhizobia usually exhibit high capability of nitrogen fixation and plant growth promotion when compared to introduced commercial strains (Onyango et al., 2015). In recent years, many neglected and underutilized crops including winged bean have received much attention in terms of their genetic, economic and nutritional values; information on winged bean-bacteria association is still limited. This study was therefore conducted to assess phenotypic and genetic characterization of micro-symbionts that could effectively nodulate winged beans (*Psophocarpus tetragonolobus*) with the aim of developing the strains as potential bioinoculants for improving winged beans production in Africa.

## Materials And Methods

*Experimental Site*

Pot experiment was conducted in the greenhouse of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta (7°N, 3.5°E), Nigeria. It lies within the rain forest agro-ecological zone of South-west Nigeria in Odeda Local government of Ogun-state. The soil used for planting was obtained from the FADAMA farm, Federal University of Agriculture, Abeokuta. The soil was collected from different sites at 0 – 20 cm depth after removing surface litters and bulked together.

*Source of Winged Bean (Psophocarpus tetragonolobus)*

Seeds of five winged bean accessions [TPt2 (Tropical *Psophocarpus tetragonolobus*), TPt6, TPt11, TPt16 and TPt19] (Plate 1) were obtained from the Genetic Resources Center of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State.

*Planting of Winged Beans*

Pot experiment was carried out in a completely randomized design with five accessions and five replications per accession. Each replicate consisted of a single pot with one plant per pot. Surface sterilization of the winged bean seeds was performed by immersion in 95% ethanol for one minute, followed by 2% sodium hypochlorite solution for 30 seconds and finally rinsed five times in sterile distilled water (Jida and Assefa, 2012). The seeds were air dried at 25±2°C. Then, each plastic pot (20 cm diameter and 25 cm height) filled with soil was sown with two seeds and thinned to one plant per pot at seven days after sowing. All pots were placed in a greenhouse at 25 to 30°C and 12 h photoperiod. The plants were

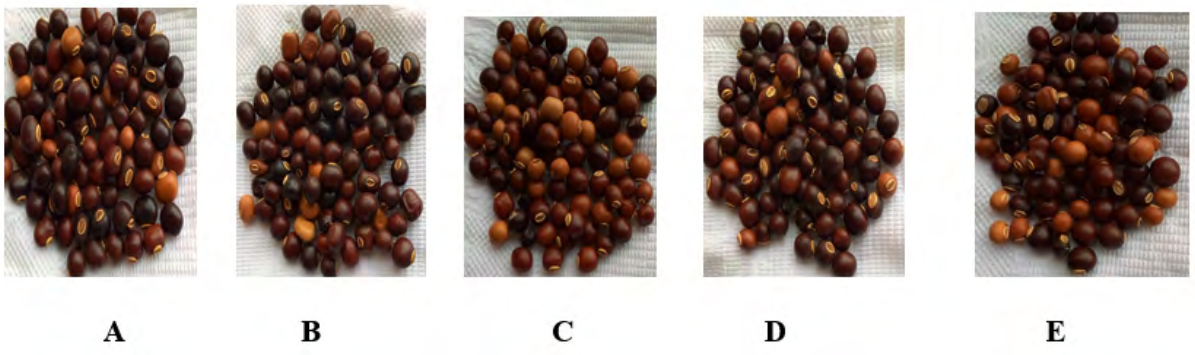


**Plate 1:** Seeds of Winged beans accessions used. A: TPt2, B: TPt6, C: TPt11, D: TPt16 and E: TPt19

watered regularly without applying fertilizer.

*Isolation of Rhizobia Associated with Winged Beans*

The winged bean plants were carefully uprooted at 45 days after planting and the soil adhering to the roots was washed off without detaching the nodules. Well-formed red or pink, healthy and unbroken nodules were then collected from the roots of each plant and used for isolation of rhizobia.

The rhizobia were isolated from the root nodules of winged bean plants adopting the methods described by Onyango et al. (2015) and Koskey et al. (2018) with slight modifications. The nodule samples were washed, surface-sterilized in 96% ethanol for 3 seconds, followed by immersion in 2% sodium hypochlorite solution for 3 minutes and finally, rinsed in five changes of sterile distilled water and air-dried. The nodules were carefully crushed under aseptic conditions and the resulting milky suspension was inoculated (in triplicates) on Yeast extract mannitol agar (YEMA) plates containing yeast extract (1.0 $gL^{-1}$), mannitol (10.0 $gL^{-1}$), $K_2PO_4$ (0.5 $gL^{-1}$), $MgSO_4$ (0.2 $gL^{-1}$), NaCl (0.1 $gL^{-1}$), congo red (25 $mgL^{-1}$) and agar (20.0 $gL^{-1}$), adjusted to pH 6.8 with 0.1M NaOH. The plates were then incubated in the dark at 28 ± 2ºC for 5 days. After incubation, the colonies on the Yeast extract mannitol agar (YEMA) plates were sub-cultured on fresh YEMA plates in order to obtain the pure cultures of rhizobia.

*Authentication of Rhizobia Strains*

Isolates were authenticated using plant nodulation test on their respective host plants as described by Ngo et al. (2015) and Zou et al. (2016) with little modifications. Surface-sterilized winged bean seeds were scarified with concentrated $H_2SO_4$ to soften the thick and hard seed coat, rinsed six times with sterile distilled water and pre-germinated on moistened sterile tissue papers in petri-dishes. The pre-germinated seeds were then sown into pots containing sterilized soil (autoclaved twice at 121ºC for 30 minutes). Seven days after planting, the pots were inoculated with 1.0 ml of YEM broth culture containing $1\times10^8$ cfu/ml of each isolate. Pots inoculated with 1.0 ml of sterile YEM broth served as control. Plants were watered regularly with N-free nutrient solution [$CaCl.2H_2O$ 1.0mM, $KH_2PO_4$ 0.5mM, $MgSO_4.7H_2O$ 0.25mM, $K_2SO_4$ 0.25mM, $MnSO_4.H_2O$ 1.0µM, $H_3BO_3$ 0.3µM, $ZnSO_4.H_2O$ 0.50µM, $CuSO_4.5H_2O$ 0.20µM, $NaMoO_2.H_2O$ 0.01µM, $CoSO_4.7H_2O$ 0.01µM, Fe Citrate 10.0µM](Maingi et al., 2006). The seedlings were then harvested four weeks after inoculation and their roots were assessed for nodulation. The isolates that induced effective nodule formation on the test host plants were considered as true rhizobia. The pure cultures of true rhizobial isolates were maintained on YEMA slants and preserved in the refrigerator at 4ºC.

*Morpho-physiological and Biochemical Characterization of Rhizobia Isolates*

The true rhizobia isolates were streaked on Yeast extract mannitol agar (YEMA) plates and incubated at 28 ± 2ºC. The plates were observed daily for 15 days. The size, colour, texture, elevation, margin and opacity of the bacterial colonies were assessed. The ability of the rhizobia isolates to produce acid or alkali was also determined by streaking the isolates on YEMA medium containing 12.5$mgL^{-1}$ Bromothymol blue (YEMA-BTB). The plates were then incubated at 28±2ºC for 10 days in the dark. Colour change of the medium was observed. The isolates that changed the colour of YEMA-BTB from green to blue were classified as alkaline producers and slow growers, while those that changed the medium to yellow were considered as acid producers and fast growers (Koskey et al., 2018). The isolates were then subjected to Gram staining, motility and series of biochemical tests (catalase, citrate, urease, starch hydrolysis, oxidase, hydrogen sulphide and sugars' fermentation).

*Phylogenetic Characterization of Rhizobia Isolates*

The phylogenetic characterization of true rhizobia isolates was carried out by 16S

rRNA gene sequencing method. Extraction of genomic DNA was performed using Norgen bacterial genomic DNA extraction kit (Norgen Biotek Corporation, Canada) following the manufacturer's instructions. The quantification of the extracted DNA was done using Nanodrop Spectrophotometer.

The amplification of the 16S rRNA genes of the isolates was conducted using polymerase chain reaction technique with the oligonucleotide primer pair of 27F (5'- AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'- ACG GCT ACC TTG TTA CGA CTT-3'). Each PCR mix was carried out in a total reaction volume of 20.0 µl consisting of 2.0 µl of template DNA, 10.0 µl of 2× PCR master mix (Norgen Biotek Corporation, Canada) containing Taq DNA polymerase, dNTPs, reaction buffer, MgCl2, KCl and PCR enhancer; 1.0 µl of forward primer (2.5µM), 1.0 µl of reverse primer (2.5µM) and 6.0 µl of nuclease-free water. The PCR amplification conditions included initial denaturation for 5 min at 94 °C, followed by 35 amplification cycles of denaturation for 1 min at 94 °C, annealing for 30 sec at 50 °C, extension for 5 min at 72 °C, final extension for 10 min at 72 °C and hold at 4°C. The amplified products were separated on a 1.0% (w/v) agarose gel electrophoresis in 1x TAE buffer at 100V for 1 hour. The amplified products were purified and sequenced in an automated gene sequencer using 27F and 1492R primers. Gene sequences were compared with GenBank database at National Centre for Biotechnology Information (*http://www.ncbi.nlm.nih.gov.*) using BLASTn search tool to identify the isolates.

For phylogenetic analysis, 16S rRNA sequences of *Rhizobium leguminosarium* biovar *viciae*, *Rhizobium etli* strain PhyCEm-90, R. *leguminosarum biovar trifolii* strain ICMP2163, *Bradyrhizobium japonicum* SitBG29, *Bradyrhizobium yuanmingense* strain RC-458-01 and *Mesorhizobium mediterraneum* strain R31 were retrieved from the NCBI database. Along with the sequences of the rhizobia isolates, they were aligned using MEGA 5.2 version. A dendrogram showing the phylogenetic relatedness of the isolates was then constructed by neighbour-joining method.

### *Plant Growth Promotion Assays of Rhizobia Isolates*

#### *Phosphate solubilization*

The ability of the rhizobia isolates to solubilize phosphate was carried out according to the method of Wekesa et al. (2021) with slight modifications. Each isolate, grown in YEM broth for 48 hours, was spot inoculated on phosphate solubilizing medium ($Ca_3PO_4$ 5.0g, glucose 2.5g, $MgSO_4.7H_2O$ 1.0g, agar 20.0g, distilled water 1000ml, pH 6.8) plates and incubated at 28 ± 2°C for 10 days. Formation of halozone (clear zone) surrounding the colonies indicated solubilization of phosphate and the isolates forming clear halos were considered as phosphate solubilizers. The clear zones were then measured and recorded. Phosphate solubilization index (PSI) of each isolate was determined as the ratio of the total diameter to the colony diameter (Pande et al., 2017).

$$\text{Phosphate solubilization index (PSI)} = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

#### *Production of Indole acetic acid (IAA)*

The ability of the rhizobial isolates to produce indole acetic acid was performed by adopting the colorimetric estimation procedure. The isolates were inoculated in duplicate in a tryptophan YEM broth (5.0g of tryptophan per litre of YEM broth) and incubated with shaking for 10 days at 28±2°C. Visually turbid cultures were observed and 5.0 ml of each culture was centrifuged at 10,000 rpm for 15 minutes at 4°C. Then, 1.0 ml of the supernatant was mixed with 2.0 ml of Salkowsky reagent (50.0 ml of 35% Perchloric acid, 1 ml of 0.5M $FeCl_3$ solution), and the mixture was incubated in darkness at room temperature (25 ± 2°C) for 2 hours. Development of pink-red colour after incubation at room temperature indicated IAA production.

*Production of Ammonia*

The rhizobia isolates were tested for the production of ammonia using the method described by Joseph et al. (2007) with slight modifications. Each bacterial culture was inoculated in 10.0 ml YEM broth and incubated at 28±2°C for 10 days on a rotary shaker. After incubation, 0.5 ml of Nessler's reagent (50.0g potassium iodide, 35.0ml saturated mercuric chloride, 400.0ml potassium hydroxide (40%) and 25.0ml distilled water) was added to each tube. The development of a yellow to brown precipitates indicated ammonia production.

## Results

*Mopho-cultural characteristics of Micro-symbionts nodulating Winged beans*

A total of 30 micro-symbionts were isolated from the root nodules of five accessions of winged beans plants but only 40.0% (12 isolates) caused effective nodulation on their host plants and these strains were considered as true rhizobia. The remaining isolates either formed ineffective nodules or failed to nodulate their host plants. Phenotypically, there were variations in the colony characteristics (size, colour, elevation, margin, texture and opacity) of the rhizobia isolates (Figure 1). The colonies were mostly medium (2 – 3 mm) in size, milky white, translucent, flattened with smooth margins. In terms of pH reaction, 50% of the isolates alkalinized the culture medium and considered as slow growers while the remaining 50% were acid producers and regarded as fast growers (Figure 1). The morphological and biochemical characteristics indicated that all the micro-symbionts were Gram negative, rod-shaped, motile bacteria. All the isolates were catalase, urease and oxidase positive. They could also ferment sucrose and hydrolyzed starch (Table 1). All the isolates were able to utilize citrate except WB17. Out of these twelve true rhizobial isolates, two isolates (WB01 and WB05) were obtained from accessions TPt2, two isolates (WB09 and WB12) from TPt6,

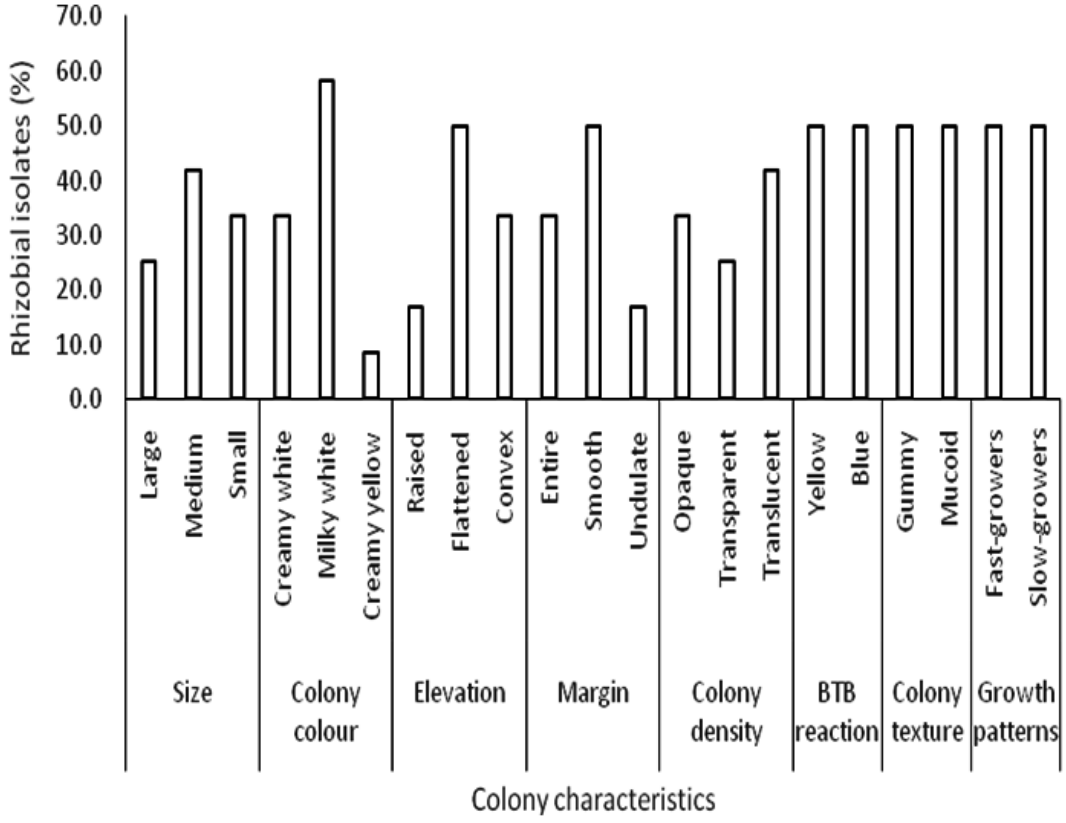


**Figure 1** Morpho-cultural characteristics of rhizobial strains associated with winged beans
Note: BTB reaction Yellow: Acid producers and fast growers;
Blue: Alkaline producers and slow growers

**TABLE 1**
Morphological and biochemical characteristics of rhizobia nodulating winged beans

| Isolates | Accessions | Gram | Cell shape | Glucose | Sucrose | Lactose | $H_2S$ | Catalase | Citrate | Starch hydrolysis | Urease | Oxidasee | Motility | Suspected organisms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WB01 | TPt2 | − | Rod | + | + | + | + | + | + | + | + | + | + | *Rhizobium* spp. |
| WB05 | TPt2 | − | Rod | − | + | + | + | + | + | + | + | + | + | *Bradyrhizobium* spp. |
| WB09 | TPt6 | − | Rod | − | + | + | + | + | + | + | + | + | + | *Bradyrhizobium* spp. |
| WB12 | TPt6 | − | Rod | + | + | + | + | + | + | + | + | + | + | *Rhizobium* spp. |
| WB14 | TPt11 | − | Rod | − | + | + | + | + | + | + | + | + | + | *Bradyrhizobium* spp. |
| WB16 | TPt11 | − | Rod | − | + | + | + | + | + | + | + | + | + | *Bradyrhizobium* spp. |
| WB17 | TPt11 | − | Rod | − | + | − | + | + | − | + | + | + | + | *Mesorhizobium* spp. |
| WB20 | TPt16 | − | Rod | + | + | + | + | + | + | + | + | + | + | *Rhizobium* spp. |
| WB21 | TPt16 | − | Rod | − | + | − | + | + | − | + | + | + | + | *Mesorhizobium* spp. |
| WB23 | TPt16 | − | Rod | − | + | + | + | + | + | + | + | + | + | *Bradyrhizobium* spp. |
| WB26 | TPt19 | − | Rod | − | + | + | + | + | + | + | + | + | + | *Bradyrhizobium* spp. |
| WB30 | TPt19 | − | Rod | + | + | + | + | + | + | + | + | + | + | *Rhizobium* spp. |

Note: +: Positive reaction −: Negative reaction

three isolates (WB14, WB16 and WB17) from accession TPt11, three isolates (WB20, WB21 and WB23) from accession TPt16 and the remaining two isolates (WB26 and WB30) were recovered from accession TPt19.

*Genotypic Characterization and Phylogenetic Relatedness of Micro-symbionts nodulating Winged beans*

Comparison of the 16S rRNA sequences of rhizobia isolates at NCBI GenBank showed 95% to 100% similarities to strains of *Bradyrhizobium*, *Rhizobium* and *Mesorhizobium* (Table 2). Also, Figure 2 shows a Neighbor joining dendrogram of rhizobia strains nodulating winged bean. From the phylogenetic tree, the rhizobia isolates clustered into three main groups. The first group comprised of *Rhizobium leguminosarum* biovar *trifolii* and *R. etli* clustered with winged bean isolates WB01, WB12, WB20 and WB30, indicating their close genetic relationship. The second group comprised of isolates WB17 and WB21 clustering together with *Mesorhizobium mediterraneum* while in third group, isolates WB05, WB09, WB14,

**TABLE 2**
Genotypic identification of micro-symbionts associated with different accessions of winged bean

| **Isolate** | **Genotypic identification** | **% Similarity at NCBI** |
|---|---|---|
| WB01 | Rhizobium leguminosarum biovar trifolii | 95.0 |
| WB05 | Bradyrhizobium japonicum | 96.0 |
| WB09 | Bradyrhizobium yuanmingense | 100.0 |
| WB12 | Rhizobium etli | 95.0 |
| WB14 | Bradyrhizobium yuanmingense | 97.0 |
| WB16 | Bradyrhizobium japonicum | 98.0 |
| WB17 | Mesorhizobium mediterraneum | 95.0 |
| WB20 | Rhizobium leguminosarum biovar trifolii | 98.0 |
| WB21 | Mesorhizobium mediterraneum | 95.0 |
| WB23 | Bradyrhizobium japonicum | 100.0 |
| WB26 | Bradyrhizobium japonicum | 95.0 |
| WB30 | Rhizobium etli | 98.0 |

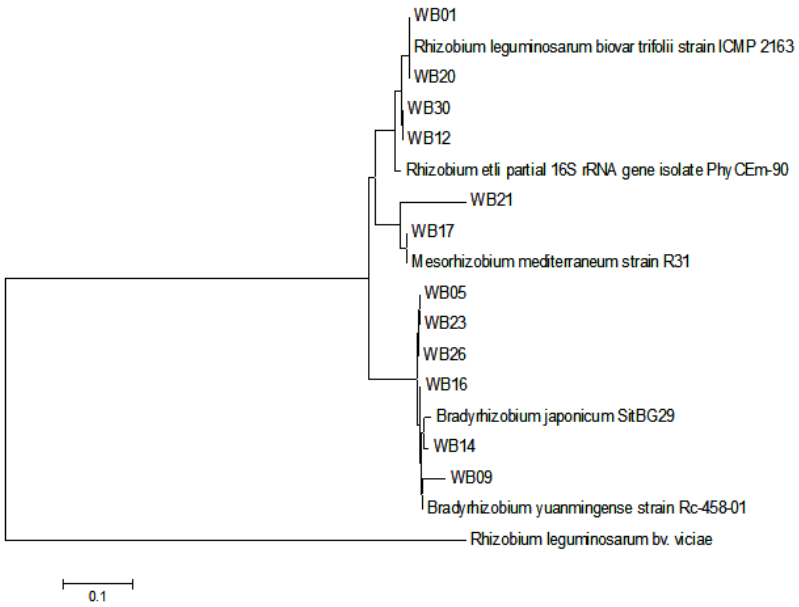


**Figure 2** A Neighbor Joining Dendrgram of micro-symbionts associated with different accessions of winged bean

WB16, WB23 and WB26 clustered together with strains of *Bradyrhizobium*, indicating their close genetic relatedness. In terms of abundance, *Bradyrhizobium* species were the most dominant micro-symbionts of Winged beans accounting for about 50% of the isolates (Figure 3).

*Growth promotion traits of Rhizobia isolated from Winged beans*

Table 3 shows the growth promotion traits of rhizobia strains isolated from root nodules of Winged beans. Out of twelve true rhizobia, 5 (41.7%) isolates solubilized insoluble phosphate on phosphate solubilizing agar plates as revealed by clear halo zones around the colonies. Isolate WB01 exhibited significantly higher phosphate solubilization index than other isolates. Also, 7 (58.3%) isolates were found to produce indole acetic acid (IAA) in tryptophan-supplemented medium while all the isolates produced ammonia. Among these strains, isolates WB01, WB14, WB16 and WB30 exhibited all the assayed growth promotion traits and therefore, they could be adopted as potential biofertilizers.

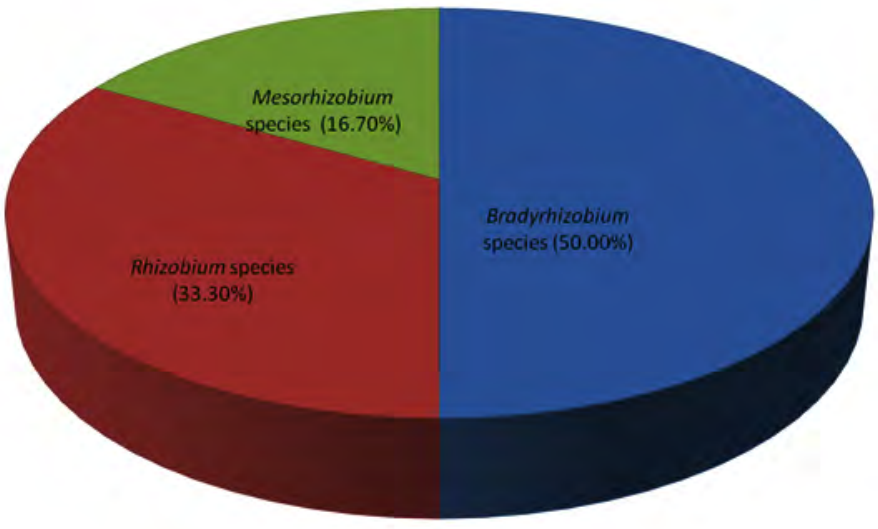


**Figure 3** Diversity of micro-symbionts associated with different accessions of winged bean

**TABLE 3**
Growth promotion traits of rhizobia isolated from winged beans

| Rhizobial isolates | $PO_4$ solubilization (%PSI) | $NH_3$ | IAA |
|---|---|---|---|
| WB01 | 5.00[a] | + | + |
| WB05 | 0.00[c] | + | − |
| WB09 | 0.00[c] | + | − |
| WB12 | 0.00[c] | + | − |
| WB14 | 3.50[b] | + | + |
| WB16 | 3.00[b] | + | + |
| WB17 | 0.00[c] | + | + |
| WB20 | 3.00[b] | + | − |
| WB21 | 0.00[c] | + | + |
| WB23 | 0.00[c] | + | + |
| WB26 | 0.00[c] | + | − |
| WB30 | 4.50[a] | + | + |

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ using Duncan's Multiple Range Test (DMRT).
PSI: Phosphate solubilization index; IAA: Indole acetic acid; $NH_3$: Ammonia production
+: Produced; −: Not produced

## Discussion

Biological nitrogen fixation contributes to the development of the sustainable agriculture by diminishing input of the hazardous chemical fertilizer in the field (Temam and Alemayu, 2017). In the present study, 30 bacterial strains were isolated from the root nodules of five accessions of Winged bean, but only 12 isolates (40.0%) were found to induce effective nodulation on their host plants and therefore, considered as true rhizobia. Similar study conducted by Ngo et al. (2015) obtained 66.7% true rhizobia from root nodules of Bambara groundnuts while Osei et al. (2018) obtained 20% true rhizobia from groundnuts. The effective nodulation was observed to produce pink/red nodules on the roots indicating the presence of high concentration of leghaemoglobin (Dekak et al., 2018). The colony characteristics exhibited by the bacterial isolates are typical features of rhizobia nodulating leguminous plants and these isolates could be categorized as winged bean rhizobia. These colony appearances are similar to the characteristics of rhizobia nodulating winged bean, pea, gram, lentil and moong as reported by Kumari et al. (2018). Similar colony characteristics were also found in native rhizobia nodulating several leguminous crops including chicken pea (Jida and Assefa, 2012), common bean (Kawaka et al., 2014) and Faba bean (Melak et al., 2018). Furthermore, the absorption of bromothymol blue could also be used as a criterion for rhizobia-identification strategy (Nyaga and Njeru, 2020). In this study, 50% of the isolates (WB01, WB12, WB17, WB20, WB21 and WB30) were acid producers and fast-growers, and they could be probably classified under the genera *Rhizobium* and *Mesorhizobium* while other isolates (WB05, WB09, WB14, WB16, WB23 and WB26) were alkaline – producing and slow – growing rhizobia, and they could be classified under the genus *Bradyrhizobium* as previously reported by Al-Mujahidy et al. (2013) and Koskey et al. (2018).

In addition, the morphological and biochemical characteristics of the isolates are consistent with the results of Panwar et al. (2012), Datta et al. (2015) and Wekesa et al. (2021).

The results of phenotypic and 16S rRNA gene sequences of the bacterial isolates obtained from the root nodules of Winged beans confirmed the micro-symbionts belonged to the genera *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*. Isolation of *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium* species from

the root nodules of Winged bean plants agrees with several studies that have reported *Bradyrhizobium* and *Rhizobium* species as effective micro-symbionts of Winged bean (Kumari et al., 2018), Bambara groundnuts (Puozaa et al., 2017; Guei et al., 2019; Ibny et al., 2019), groundnuts (Osei et al., 2018) and common bean (Wekesa et al., 2021). Phylogenetic analysis using neighbour joining shows that the rhizobia strains were closely related and they may have common ancestral origin. This agrees with the report of Berrada et al. (2012). It could also possibly be as a result of the highly conserved nature of the 16S rRNA genes, which could not discriminate between closely related bacterial species as reported by Koskey et al. (2018).

In the same vein, possession of growth promotion traits such as nitrogen fixation, production of phytohormones like indole acetic acid, phosphate solubilization, production of ammonia and siderophore production could also be an essential feature for rhizobia application as biofertilizers. However, it has been reported that rhizobia produce significant levels of IAA both in free living conditions, and symbiotically in nodules. In this study, about 58.3% rhizobial strains were capable of producing IAA when grown on YEMA medium supplemented with L-tryptophan, and this contrasts the study of Halda-Aliza (2003) who reported that 74% of rhizobia strains associated with different crops could produce IAA. Jida and Assefa (2011) also found that 36.7% of rhizobia strains isolated from lentil were able to produce IAA. Indole acetic acid could help in the production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake (Kumari et al., 2018). IAA is also involved in multiple processes including cell division, differentiation and vascular bundle formation which are essential for nodule formation. Auxin production by rhizobia is therefore considered to improve growth and nitrogen fixation in many legumes (Zafar-ul-Hye et al., 2013). Other plant growth promotion traits exhibited by some of the rhizobia isolates include production of ammonia and solubilization of phosphate.

## Conclusion

The study shows that *Bradyrhizobium*, *Rhizobium* and *Mesorhizobium* species are micro-symbionts inducing effective nodulation of Winged beans and these strains could be considered as potential bioinoculants for sustainable cultivation of winged bean. However, field experiments need to be conducted to ascertain the applicability of these strains.

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## References

**Al-Mujahidy, S. J., Hassan, M., Rahman, M. and Mamun-Or-Rashid, A. N. M.** 2013. Isolation and characterization of *Rhizobium* spp. and determination of their potency for growth factor production. *International Research Journal of Biotechnology*, **4(7)**:117–123.

**Anugroho, F., Kitou, M., Kinjo, K. and Kobashigawa, N.** 2010. Growth and nutrient accumulation of winged bean and velvet bean as cover crops in a subtropical region. *Plant Production Science*, **13 (4)**:360 –366.

**Berrada, H., Nouioui, I., Houssaini, M. I., El Ghachtouli, N., Gtari, M., and Benbrahim, K. F.** 2012. Phenotypic and genotypic characterizations of rhizobia isolated from root nodules of multiple legume species native of Fez, Morocco. *African Journal of Microbiology Research*, **6(25)**:5314 – 5324.

**Datta, A., Singh, R.K. and Tabassum, S.** 2015. Isolation, characterization and

growth of *Rhizobium* strains under optimum conditions for effective biofertilizer production. *International Journal of Pharmaceutical Sciences Review and Research*, **32(1)**:199-208.

**Dekak, A., Chabi, R., Menasria, T. and Benhizia, Y.** 2018. Phenotypic characterization of rhizobia nodulating legumes *Genista microcephala* and *Argyrolobium uniflorum* growing under arid conditions. *Journal of Advanced Research*, **14**:35-42.

**Guei, N. K. R., Konate, I., Bakayoko, S., Ouattara, A., Amani, K., Tidou, A. S. and Filali-Maltouf, A.** 2019. Nodulation and agronomic performance of indigenous Rhizobia isolated from Bambara groundnut (*Vigna subterranean* L.) nodules in Dalao, Côte d'Ivoire. *International Journal of Research Studies in Microbiology and Biotechnology*, **5(2)**:13-18.

**Halda-Aliza, L.** 2003. Identification of indole-3-acetic acid producing freshwater wetland rhizosphere bacteria associated with *Juncus effuses* (L.). *Canadian Journal of Microbiology*, **49(12)**:781-787.

**Ibny, F. Y. I., Jaiswal, S. K., Mohammed, M. and Dakora, F. D.** 2019. Symbiotic effectiveness and ecologically adaptive traits of native rhizobial symbionts of Bambara groundnut (*Vigna subterranea* L. Verdc.) in Africa and their relationship with phylogeny. *Scientific Reports*, **9**:1-17.

**Jida, M. and Assefa, F.** 2011. Phenotypic and plant growth promoting characteristics of *Rhizobium leguminosarum bv. viciae* from lentil growing areas of Ethiopia. *African Journal of Microbiology Research*, **5(24)**:4133-4142.

**Jida, M. and Assefa, F.** 2012. Phenotypic diversity and plant growth promoting characteristics of *Mesorhizobium* species isolated from chickpea (*Cicer arietinum* L.) growing areas of Ethiopia. *African Journal of Biotechnology*, **11(29)**: 7483-7493.

**Tuquero, J. and Takai, G.** 2018. Winged bean (*Psophocarpus tetragonolobus*) varieties for Guam. *Food Plant Production*, FPP-06:1-6.

**Joseph, B., Patra, R. R. and Lawrence, R.** 2007. Characterization of plant growth-promoting rhizobacteria associated with chickpea (*Cicer aerietinum* L.). *International Journal of Plant Production*, **2**:141-152.

**Kawaka, F., Dida, M. M., Opala, P. A., Ombori, O., Maingi, J., Osoro, N., Muthini, M., Amoding, A., Mukaminega, D. and Muoma, J.** 2014. Symbiotic efficiency of native rhizobia nodulating common bean (*Phaseolus vulgaris* L.) in soils of Western Kenya. *International Scholarly Research Notices*, **2014**:1–8.

**Koskey, G., Mburu, S. W., Kimiti, J. M., Ombori, O., Maingi, J. M. and Njeru, E. M.** 2018. Genetic characterization and diversity of *Rhizobium* isolated from root nodules of mid-altitude climbing bean (*Phaseolus vulgaris* L.) varieties. *Frontiers in Microbiology*, **9**:1 – 12.

**Klu, G.Y.** 2000. Induced mutations for accelerated domestication - a case study of winged bean (*Psophocarpus tetragonolobus* L.). *West African Journal of Applied Ecology*, **1**: 47–52.

**Kumari, S., Yadav, R. C. and Jha, M. N.** 2018. Evaluation of physiological properties of winged bean *Rhizobium* and its comparison with other rhizobia. *International Journal of Current Microbiology and Applied Sciences,* **7(04)**:3254-3264.

**Maingi, J.M., Gitonga, N.M., Shisanya, C.A., Hornetz, B. and Muluvi, G.M.** 2006. Population levels of indigenous *Bradyrhizobia* nodulating promiscuous soybean in two Kenyan soils of the semi-arid and semi-humid agroecological zones. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, **107(2)**: 149–159

**Melak, W., Degefu, T., Welde-Meskel, E. and Yilma, S.** 2018. Morphophysiological and symbiotic characteristics of Rhizobia nodulating Faba bean (*Vicia faba* L.) from Bale, Ethiopia. *Journal of Biology, Agriculture and Healthcare*, **8(15)**:51 – 61.

**Namkeleja, Y., Mtei, K. and Ndakidemi, P.** 2016. Isolation and molecular characterization of elite indigenous rhizobia nodulating *Phaseolus* bean (*Phaseolus*

*vulgaris* L.). **American Journal of Plant Sciences**, **7(14)**: 1905-1920.

**Ngo, N. L., Ngo, B. M., Fankem H., Adamou, S., Kamguia, K., Ngakou, A., Nwaga, D. and Etoa, F.** 2015. Isolation and screening of indigenous Bambara groundnut (*Vigna Subterranea*) nodulating bacteria for their tolerance to some environmental stresses. *American Journal of Microbiological Research*, **3(2)**:65–75.

**Nyaga, J. W. and Njeru, E. M.** 2020. Potential of native rhizobia to improve cowpea growth and production in semi-arid regions of Kenya. *Frontiers in Agronomy*, **2(28)**:1-12.

**Onyango, B., Anyango, B., Nyunja, R., Koech, P. K., Robert, A. S. and Stomeo, F.** 2015. Morphological, genetic and symbiotic characterization of root nodule bacteria isolated from Bambara groundnuts (*Vigna subterranea* L. Verdc) from soils of Lake Victoria basin, Western Kenya. *Journal of Applied Biology and Biotechnology*, 3(1): 1-10.

**Osei, O., Abaidoo, R. C., Ahiabor, B. D. K., Boddey, R. M., and Rouws, L. F. M.** 2018. Bacteria related to *Bradyrhizobium yuanmingense* from Ghana are effective groundnut micro-symbionts. *Applied Soil Ecology*, **127**:41–50.

**Pande, A., Pandey, P., Mehra, S., Singh, M. and Kaushik, S.** 2017. Phenotypic and genotypic characterization of phosphate solubilising bacteria and their efficiency on the growth of maize. *Journal of Genetic Engineering and Biotechnology*, **15**: 379-391.

**Panwar, A., Choudhary, S., Sharma, M., Shrama, Y. K., Meena, R. S., Malhotra, S. K., Mehta, R. S. and Aishwath, O. P.** 2012. Morphological and biochemical characterization of *Rhizobium* isolates obtained from Fenugreek (*Trigonella foenum*). *Seed Research*, **40(2)**: 196-200.

**Puozaa, D. K., Jaiswal, S. K. and Dakora, F. D.** 2017. African origin of *Bradyrhizobium* populations nodulating Bambara groundnut (*Vigna subterranean* L. Verdc) in Ghanaian and South African soils. *PLoS ONE*, **12(9)**: e0184943.

**Tanzi, A, S., Eagleton, G. E., Ho, W. K., Wong, Q. N., Mayes, S. and Massawe, F.** 2019. winged bean (*Psophocarpus tetragonolobus* (L.) DC.) for food and nutritional security: synthesis of past research and future direction. *Planta*, **250**: 911-931.

**Temam, A. H. and Alemayehu, L. A.** 2017. Isolation and characterization of rhizobia from rhizosphere and root nodule of cowpea, elephant and labLab plants. *International Journal of Novel Research in Interdisciplinary Studies*, **4(4)**:1-7.

**Wekesa, C. S., Furch, A. C. U. and Oelmüller, R.** 2021. Isolation and characterization of high-efficiency rhizobia from Western Kenya nodulating with common bean. *Frontiers in Microbiology*, **12**:1-13.

**Wong, Q.N., Tanzi, A.S., Ho, W.K., Malla, S., Blythe, M., Karunaratne, A., Massawe, F. and Mayes, S.** 2017. Development of gene-based SSR markers in winged bean (*Psophocarpus tetragonolobus* (L.) DC.) for diversity assessment. *Genes*, **8(100)**:1-12.

**Zafar-ul-Hye, M., Ahmad, M. and Shahzad, S.M.** 2013. Synergistic effect of rhizobia and plant growth promoting rhizobacteria on the growth and nodulation of Lentil seedlings under axenic conditions. *Plant, Soil and Environment*, **32(1)**:79-86.

**Zou, L., Chen, Y. X., Penttinen, P., Lan, Q., Wang, K., Liu, M., Peng, D., Zhang, X., Chen, Q., Zhao, K., Zeng, X., and Xu, K. W.** 2016. Genetic diversity and symbiotic efficiency of nodulating rhizobia isolated from root nodules of Faba bean in one field. *PLoS ONE*, **11(12)**: e0167804.