Original Research Article

Isolation, characterisation, and selection of bacterial endophytes from Soybean (*Glycine max*) nodules and roots in some soils of Cameroon

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ABSTRACT

Aim: The present study aimed to isolate, characterise, and select the most effective bacterial endophytes to enhance the growth, biomass, and yield of soybean.

Methodology: Seven soil samples were collected from the rhizosphere of forage legumes in fields across three agroecological zones of Cameroon. Bacterial endophytes were isolated from soybean roots and nodules and cultured on nutrient agar. The isolates were screened for the tolerance tests, extracellular enzymatic activity, P-solubilisation activity, and IAA production. The best isolates were selected using a two-factor block factorial design. Each treatment was replicated six times.

Results: A total of 85 bacterial endophytes were isolated. Characterisation results of 22 preselected isolates revealed an optimal growth temperature of 37°C and a pH range between 6 and 7. Additionally, all 22 isolates solubilised inorganic phosphate, 7 produced IAA, and 8 exhibited amylase activity. PCR analysis of the *nifH* and *nodC* genes showed that the isolates possessed the *nifH* gene as a nitrogen fixation marker and the *nodC* gene as a nodulation marker. The findings show that, out of the 22 bacterial endophyte isolates, NTT1 and BOSH9 were the most effective in increasing plant height by 26.74% and 31.78%, respectively. Additionally, they resulted in a 94.24% to 120.48% increase in biomass and a 71.59% to 76.70% increase in grain yield compared to the control treatment.

Conclusion: The selected isolates significantly enhance plant growth, increase biomass, and improve soybean grain yield. However, their potential use as biofertilisers in agriculture will require further investigation under real field conditions.

Keywords: Bacterial endophytes; glycine max, nifH gene; nodC gene; nodules; roots; selection.

1. INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] plays crucial roles in food and nutrition security due to its high nutrient contents, while its ability to biologically fix atmospheric nitrogen in symbiosis with nitrogen fixing endophytic bacteria enhances the productivity of agricultural systems. Soybean production in Cameroon has been increasing since 2010, and it is the second most cultivated legume after peanuts, with the rapid development of cultivated areas from 6,705 ha in 2008 to 15,020 ha in 2018 [1,2,3]. Macroeconomic data show that Cameroon imports an average of 20, 000 tons of soybeans worth approximately CFAF 10 billion a year [1] and GMO soybean meal worth CFAF 14 billion [2]. Hence, there is a challenge to increase domestic supply to meet agro-industrial demand, which is indicative of the enthusiasm of farmers for soybean production. According to [4], soybean yield ranged from 448 and 709 kg/ha across the first and second planting seasons, with a significant effect of soil nutrients (especially magnesium content) on soybean yield. In Cameroon, crop production is significantly affected by poor soil fertility, with nitrogen (N) and phosphorus (P) being the primary limiting factors [5, 6, 7]. Soil nutrient deficiencies are typically addressed through the use of chemical NPK fertilisers, which can have harmful effects on the environment and human health when overused. As a result, alternative management practices have been developed to promote crop productivity while maintaining sustainability [8, 9, 10]. Therefore, a promising alternative to increase crop growth and yield is the use of beneficial microbes [11,12], such as nitrogen-fixing endophytic bacteria to enhance nitrogen who play a crucial role in the growth and development of soybean plants, soil fertility, plant nutrition, and protection. Nitrogen is an indispensable component of amino acids, proteins, chlorophyll, and many essential enzymes critical for photosynthesis and plant growth [13]. It is also necessary for partitioning photosynthate, stimulating root growth and enhancing uptake of other nutrients by plants [14]. Bacterial endophytes can be found in various parts of the plant, such as roots, stems, leaves, berries, seeds, and xylem sap [15,16,17,18]. Endophyte population density is higher in roots than in any other plant organ. In the root, the average density is 10^5 cfu per g of fresh weight while average values of 10^4 and 10^3 are indicated for the stem and for the leaf, respectively [19]. Many plants harbour a diverse range of bacterial endophytes in their roots, consisting of hundreds of species (219 in 2006) and almost 100 genera (71 in 2006). The most common genera are Bacillus, Burkholderia, Enterobacter, and Pseudomonas [15, 17, 19]. The aim of this study was to characterise bacterial endophyte isolates after isolation and to select the most effective to promote the growth, biomass, and yield of forage legumes.

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2. MATERIALS AND METHODS

2.1 Description of the study site, sampling procedure, and soil treatment

The study site was conducted in Garoua (Bocklé), located in the north of Cameroon region. The site's latitude and longitude are 9 ° 18′05 ′ North and 13 ° 24′51 ′ East, respectively, and it has an altitude of 249 m above sea level. Garoua has a savannah climate with a dry winter (Aw) according to the Koppen-Geiger classification and an average annual temperature of 800 mm. The climate is tropical and belongs to the sudano-Sahelian category. We randomly picked seven soils from the rhizosphere of forage legumes (soya, peanuts and stylosanthes) in fields across three agroecological zones of Cameroon: Zone 1 (Extreme North and North region), Zone II (Adamaoua region) and Zone V (Centre region). Using an auger, we collected 30 kg of soil from each sampling point at a depth of 3-20 cm from all fields. After collection, we thoroughly mixed the soil samples to form a composite sample. We mixed the soil samples with coarse sand in a ratio of 3:1 to aerate them. Then, we separated particles larger than 2 mm by passing the soil and sand mixture through a coarse sieve with a 2-mm mesh.

2.2 Plant and microbial material

Soybean seeds (Houla 1, Docko, and TGX 1910-14F variety) were purchased from the Institute of Agricultural Research for Development (IRAD) and from the National Veterinary Laboratory (LANAVET). A total of 22 bacterial endophytic isolates (BOSH4, BOSH8, BOSH9, BOSD1, BOSD2, BOSD3, BOSD4, BOSD5, BOSD6, BOSD12, BOT1, BOT2, BOS2, SPT1, SPT2, SPS2, SPS3, YAT2, MBT2, MBS2, MBS3 et NTT1) were obtained from soybean roots and nodules. These isolates were selected for their ability to fix nitrogen and promote nodulation in forage legumes. Two reference bacterial strains, *Bradyrhizobium japonicum* and Phosphorus Solubilising Microorganism (PSM), obtained from the Yaounde I and the National Veterinary Laboratory, were used. Additionally, an Arbuscular Mycorrhizal Fungus (AMF) inoculum was obtained from the GIC Agribiocam and used as microbial material.

2.3 Isolation of bacterial endophytes isolates from soybean roots and nodules

The root fragments, measuring 1-2 cm, were disinfected by soaking them in 2% sodium hypochlorite for 10 minutes, followed by 70% ethanol for 2 minutes. After that, they were rinsed three times in sterile distilled water for 1 minute each. The fragments were then ground separately in sterilised mortars under strict aseptic conditions and allowed to release bacterial endophytes for 15-20 minutes. The juice from the ground root materials was then inoculated into Petri dishes containing culture media [20,21]. A drop of each extract from the ground root material was inoculated in Petri dishes containing culture media, including Nutrient Agar (Meat extract 1 g. L-1; yeast extract 3 g. L-1; peptone 5g. L-1; mannitol 5 g. L-1; Nacl 5 g. L-1; agar 15 g. L-1; and 1 L dis. H_20) and Yeast Extract Mannitol Agar (Mannitol 10 g. L-1; $MgSO_4$ 7(H_2O) 0.2 g. L-1; K_2HPO_4 0.5 g. L-1; Nacl 0.1 g. L-1; yeast extract 0.5 g. L-1; agar 15 g. L-1; and 1 L dis. H_20; pH 6.8). The boxes will be incubated at 35°C until bacterial colonies appear. Then we isolated endophytic bacterial isolates from Glycine max using the method described by [22]. First, we disinfected the harvested root nodules using [23]. Subsequently, sterile nodules were individually crushed in distilled water on a sterile Petri dish. The operation was performed under aseptic conditions. Using a platinum loop heated by the Bunsen burner, the juice extracted from the nodule was

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spread on a Petri dish containing the specific medium: Yeast-Extract-Mannitol-Agar + Red Congo. The inoculation process was then conducted using the four-quadrant technique to obtain isolated colonies that could be easily characterised.

2.4 Characterisation of bacterial endophyte isolates

2.4.1 Morphological Characterisation

The bacterial endophyte isolates were morphologically characterised to determine their growth rate (slow or fast), pH changes during growth, and characteristics. The formation of colonies on Yeast-Extract-Mannitol-Agar plates was monitored daily over a 10-day period, and the pH changes were noted on medium containing 0.25 mg/l bromothymol blue (BTB). Cultures were incubated for 10 days at 28 ° C and monitored daily for any colour changes. Isolates that turned the growth medium yellow were classified as fast growers and acid producers, while those that turned it blue were classified as slow growers and alkaline producers. After incubation at 28°C for 2 to 10 days, separate colonies were identified. The isolates were classified based on their dimensions, colour, shape, transparency, borders, and elevation. Dimensions were categorised as very small (1mm), small (less than 2mm), medium (2-4mm), and large (4-5mm). The colour was classified as white/milky or transparent. The shape was classified as round or ellipsoid. Transparency, borders, and elevation were assessed based on whether the isolates were convex, raised, flattened, or umbonate. To assess the isolates' ability to absorb Congo red, a 1% Congo red solution was incorporated into Yeast-Extract-Mannitol-Agar medium. The medium was prepared and autoclaved before poured into a sterile Petri dish [24].

2.4.2 Physiological characterisation

Effect of Temperature on Growth of Bacterial Endophytes isolates: The isolates' growth was assessed by inoculating them in nutrient broth and incubating them at different temperatures (28°C, 37°C, 47°C, and 52°C) for 48 hours [25]. Subsequently, the optical density at 520 nm was measured.

Effect of pH on Growth of Bacterial Endophytes isolates: The growth of isolates across a range of pH values was optimised and standardised by inoculating them onto a nutrient broth medium with pH values ranging from 4 to 9. The inoculated isolates were then incubated at 37°C for 48 hours [26]. Growth was evaluated by measuring the optical density at 520 nm.

Effect of NaCl on Growth of Bacterial Endophytes isolates: The bacterial endophytic isolates growth in nutrient broth medium with varying concentrations (1-5%) of NaCl was examined by inoculating them into the medium. After incubation at 37°C for 48 hours [27], the optical density at 520 nm was measured to determine growth.

2.4.3 Biochemical Characterisation

Catalase test: Catalase activity of bacterial endophyte isolates was detected by transferring the isolated pure colony onto a clean glass slide using a sterile nicrome wire loop. Then, 3% hydrogen peroxide was added to the slide. The presence of oxygen bubbles indicated positive catalase activity, while the absence of gas bubbles indicated negative catalase activity [28].

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Urease activity: The bacterial endophyte isolates were streaked on agar slant in test tubes containing Urea Agar Slant (Pancreatic digest of gelatin 1 g. L-1; dextrose 1 g. L-1; K_2HPO_4 2 g. L-1; Nacl 5 g. L-1; urea 20 g. L-1; phenol red 0.012 g. L-1; agar 15 g. L-1; and 1 L dis. H_20) [29] The tubes were incubated at 37°C for 24 to 48 hours. Development of pink colour in tubes indicated positive urease activity of the isolates [28].

Amylolytic activity: Amylase production was determined by inoculating the isolates on Nutrient Agar with 1% starch and incubating at 37°C for 48 hours. The plates were flooded with Gram's iodine to produce a deep blue coloured starch-iodine complex. Isolates that showed a clear zone in starch agar plates were considered to produce amylase [28].

Phosphate solubilisation: Bacterial endophyte isolates were tested for their ability to solubilise insoluble calcium phosphate in Pikovskaya agar medium, as described by [30]. A bacterial colony was placed in Pikovskaya agar medium plates using a sterile loop and incubated at 30 ° C for 7 days. The phosphate solubilising efficiency was measured based on the halo zones around the colonies, as described by [31,32].

Indole 3-acetic acid (IAA) production: Indole acetic acid (IAA) production by bacterial endophyte isolates was tested in Trypticase Soy Broth at 35 ± 2 ◦C for 72 hours. A control was also prepared using Trypticase Soy Broth without bacterial inoculation. To assess IAA production, 0.3 mL (3 drops) of kovac's reagent was added to test tubes containing Trypticase Soy Broth liquid medium and inoculated with endophytic bacteria. A change in colour to red indicated IAA production [33]. The optical density at 530 nm was measured using a spectrophotometer [34].

2.5 PCR analysis of bacterial endophytes

2.5.1 DNA isolation of bacterial endophyte isolates

PCR analysis was used to identify the most optimal bacterial endophyte isolates. This method can help detect various microbial communities. Genomic DNA was extracted from each isolate's overnight culture using a modified version of the [35] and [36] method and used as a template for PCR amplification.

2.5.2 PCR ***nif*H and *nod*C genes amplification**

DNA obtained from the twenty-two bacterial endophytes isolates was subjected to PCR for the simultaneous duplex detection of the *nif*H and *nod*C genes as an indicator of nitrogen fixation and nodulation. *NifH* amplification was performed using the specific nitrogen fixation primers *nif*HF (TACGGNAARGGSGGNATCGGCAA) and *nif*HI (AGCATGTCYTCSAGYTCNTCCA). For amplification of the *nod*C gene, the specific nodulation primers *nod*CF (AYGTHGTYGAYGACGGTTC) and *nod*CI (CGYGACAGCCANTCKCTATTG) were used, according to [37]. The composition of the duplex PCR reaction mixture was as follows: reaction buffer 1X Taq Polymerase, $MgCl_2$ (2.5 mM), dNTP (1.2 mM), Taq Polymerase (0,3U), primers (1 µM nifH+ 0.6 µM nodC, template DNA (40 ng). The amplification conditions on the thermal cycler were an initial denaturation cycle (94 ° C for 5 min); 35 denaturation

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cycles (1 min at 94 °C), annealing (45 sec at 55 °C) and extension (1 min at 72 °C); and a final extension cycle at 72 ° C for 1 min [38]. Migration on 1.5% agarose gel with a marker of 1 Kb.

2.6 Impact of bacterial isolates on soybean growth in Plastic Bags

2.6.1 Experimental design

The experiment was carried out using a two-factor block factorial design where the main factor was the Houla 1 soybean variety and the secondary factor comprised of treatments (BOSH4, BOSH8, BOSH9, BOSD1, BOSD2, BOSD3, BOSD4, BOSD5, BOSD6, BOSD12, BOT1, BOT2, BOS2, SPT1, SPT2, SPS2, SPS3, YAT2, MBT2, MBS2, MBS3, NTT1, PSM, *Bradyrhizobium japonicum,* and AMF). Each treatment was replicated six times and a control treatment of uninoculated plants was also included.

2.6.2 Culture condition

Soil was collected from an agricultural field in the Bocklé-Garoua, then. air dried, sieved with a 2mm sieve and mixed with sand in a soil to sand ratio of 3:1. The mixture was autoclaved twice for one hour at 121 ° C. The best isolates of preselected and identified bacterial endophyte isolates were inoculated in nutrient broth and incubated at 35± 2°C for 48 hours on a shaker at 180 rpm. Soybean seeds (*Glycine max*, variety Houla 1) were sterilised by surface treatment with 2.5% sodium hypochlorite for 3 minutes, followed by 5 washes in sterile distilled water. Four of these treated seeds were then seeded in each 6 L plastic pot, which was filled with a 5 kg mixture of sterile soil and sand. The plants were grown in a temperature range of 30-37°C and were irrigated with tap water.

2.7 Statistical analyses

The collected data were subjected to analysis of variance (ANOVA) using SPSS software version 25.0. Mean separation was performed using Duncan's test at a 5% level of significance. Bacterial endophyte colonies were scored numerically based on their morphological and cultural characteristics. The obtained data was then subjected to hierarchical cluster analysis using the squared Euclidean distance similarity and between-groups linkage procedures in SPSS software version 25.0.

3. RESULTS AND DISCUSSION

A total of 85 bacterial endophytes were isolated from soybean nodules and roots in seven trapping soils from three agroecological zones of Cameroon. After preselection tests based on morphological, physiological, biochemical and PCR analyses, a collection of 22 bacterial endophyte isolates was compiled. Of these, 81.82% displayed fast growth, while 18.18% were slow-growing on YEMA medium with bromothymol blue (BTB). Fast-growing isolates produced colonies that were either pink, white or pale pink on YEMA medium with Congo red, with regular or complete margins ranging from 2-4 mm in diameter within 24 to 48 hours of incubation (**Table 1**). Slow-growing isolates formed pale pink colonies on YEMA with Congo red. Only one isolate formed a white colony with a diameter of <2-3 mm after incubation for 5-10 days. All 22 bacterial isolates exhibited circular bacilli, cocci, and coccobacillus-type colonies. The pH of the culture medium, indicated by BTB, was acidic for the fast-growing isolates within

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24 to 48 hours of incubation, and a yellow colouration of the culture medium was observed. Based on their ability to change the YEMA medium's colour to yellow (acid-producing) within five days of incubation supplemented with BTB, the 22 bacterial isolates were designated as bacterial endophyte isolates. This indicates that the isolates are fast-growing, as described by [39]. These results agree with the findings of [40], who also noted fast-growing isolates isolated from cowpea plants. The isolates displayed either gram-negative or gram-positive characteristics and had cells that were bacilli, coccobacilli, or cocci in shape. They were all cultured on YEMA medium containing Congo red dye. The isolates' inability to absorb Congo red dye was a distinguishing feature [41].

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Table 1. Collection of Bacterial endophyte isolates

Isolates / Characteristics	BOSH4	BOSH8	BOSH9	BOSD1	BOSD2	BOSD3	BOSD4	BOSD5	BOSD6	BOSD12	BOT1	BOT2	BOS2	SPT1	SPT2	SPS2	SPS3	YAT2	MBT2	MBS2	MBS3	NTT1
Gram	-	+	-	+	+	-	+	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+
Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Type of colony	Bacille	Bacille	Bacille	Bacille	Bacille	Bacille	Bacille	Bacille	Bacille	Bacille	Coccobacille	Coccobacille	Coccobacille	Bacille	Bacille	Coccobacille	Bacille	Bacille	Cocci	Bacille	Coccobacille	Bacille
Colony size	Mean	Small	Mean	Mean	Small	Mean	Mean	Small	Mean	Mean	Small	Mean	Very small	Very small	Mean	Mean	Small	Mean	Small	Small	Very small	Mean
Colony diameter range (mm)	2-4	2	2-4	2-4	2	2-4	2-4	2	2-4	2-4	2	2-4	1	1	2-4	2-4	2	2-4	2	2	1	2-4
Colour	Light pink	White	Light pink	Pink	Pink	Light pink	Light pink	Light pink	Light pink	Light pink	Light pink	Light pink	Light pink	White	Light pink	Light pink	Pink	Light pink	Light pink	Pink	White	Light pink
Colony type of	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Slow	Fast	Slow	Fast	Fast	Slow	Fast	Fast	Fast	Fast	Slow	Fast

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growth																						
Colony growth time (hours)	24h	24h	24h	24h	24h	24h	24h	24h	24h	24h	120h	24h	120h	24h	24h	120h	24h	48h	48h	48h	120h	48h
Regrouping	Individualised	In chain	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	Individualised	In clusters	individualised	Individualised	Individualised
Elevation	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
Margin	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Whole	Regular	Whole	Regular	Whole	Regular	Whole	Regular	Regular	Regular	Regular	Régu
Fresh state	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile	Moble	Mobile	Mobile	Mobile	Mobile

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Physiological Characterisation

All isolates displayed optimal growth in the pH range of 6 to 7 in the nutrient broth medium (Table 2). BOSH9 showed the best growth at pH 7 (1.15), while BOSD3 and SPS3 exhibited the lowest growth values among all isolates, with absorbance values of 0.26 and 0.25 at 520 nm, respectively. These results are similar to those obtained by [42] in their study on the isolation of endophytic bacteria from root and leaf tissues of the *Prosopis cineraria* plant. It was found that the strains grew best at a temperature of 37°C and a pH of 7, which confirms earlier research.

Table 3 shows the growth results of bacterial endophyte isolates at various temperatures. The results indicate that temperature tolerance varies among the isolates and at different temperatures (28°C, 37°C, 47°C, and 52°C). All 22 isolates exhibited good growth at 28°C, very good growth at 37°C, average growth at 47°C, and reduced growth at 52 ° C. BOSH9 and BOS2 showed the best growth at 37°C, with absorbance values of 1.15 and 0.97 at 520 nm, respectively. The majority of isolates showed an optimal growth temperature of 37°C. These results were in agreement with those of [28].

All the isolates in **Table 4** grew at 0.1% NaCl, but only 77% of them were resistant to a salt concentration of 1%. Furthermore, 59% of the isolates were resistant to a salt concentration of 2%, and an additional 23% were resistant to a salt concentration of 3%. BOSD5 and SPT1 were the isolates that exhibited superior growth in salt concentrations higher than 3%, with OD values of 0.31 and 0.39, respectively. These findings contradict the results of [43], who established that 100% of isolates were able to grow in 2% (w/v) sodium chloride, and 65% could do so in 3% NaCl. However, these results agree with the findings of [44] for Rhizobium sp. isolated from Phaseolus vulgaris in Morocco, which can tolerate 3% NaCl. According to [45], the microbiological isolates' ability to tolerate NaCl could be attributed to the presence of osmoprotective molecules like proline in bacteria.

Table 2. Effects of pH on growth of bacterial endophyte isolates

	pH				
Isolate Code	**4**	**5**	**6**	**7**	**8**
BOSH4	0.54	0.64	0.24	0.48	0.42
BOSH8	0.19	0.73	0.51	0.52	0.55
BOSH9	0.25	0.58	0.68	1.15	0.47
BOSD1	0.44	0.42	0.30	0.49	0.21
BOSD2	0.36	0.39	0.47	0.29	0.20
BOSD3	0.27	0.33	0.31	0.26	0.13
BOSD4	0.61	0.62	0.82	0.54	0.44
BOSD5	0.25	0.42	0.43	0.51	0.31
BOSD6	0.34	0.41	0.38	0.40	0.26
BOSD12	0.15	0.48	0.43	0.58	0.14
BOT1	0.76	0.87	0.76	0.69	0.26
BOT2	0.20	0.40	0.39	0.44	0.59
BOS2	0.41	0.67	0.63	0.67	0.13
SPT1	0.55	0.36	0.69	0.35	0.28
SPT2	0.78	0.71	0.49	0.30	0.66
SPS2	0.71	0.84	0.85	0.81	0.65
SPS3	0.08	0.66	0.34	0.25	0.15
YAT2	0.54	0.43	0.23	0.43	0.06
MBT2	0.50	0.74	0.30	0.53	0.14
MBS2	0.08	0.25	0.58	0.39	0.34
MBS3	0.50	0.70	0.60	0.46	0.53
NTT1	0.42	0.62	0.71	0.50	0.83

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Table 3. Effects of temperature on growth of bacterial endophyte isolates

	Temperature (°C)			
Isolate Code	**28**	**37**	**47**	**52**
BOSH4	0.12	0.48	0.33	0
BOSH8	0.17	0.52	0.22	0
BOSH9	0.09	1.15	0.22	0
BOSD1	0.18	0.65	0.16	0
BOSD2	0.18	0.64	0.15	0
BOSD3	0.10	0.29	0.14	0
BOSD4	0.11	0.54	0.30	0
BOSD5	0.15	0.72	0.51	0.19
BOSD6	0.10	0.40	0.07	0
BOSD12	0.14	0.58	0.63	0
BOT1	0.38	0.69	0.48	0.07
BOT2	0.25	0.44	0.51	0.04
BOS2	0.33	0.97	0.95	0.14
SPT1	0.27	0.35	0.54	0.39
SPT2	0.30	0.81	0.66	0
SPS2	0.72	0.95	0.81	0.14
SPS3	0.26	0.68	0.25	0
YAT2	0.31	0.49	0.43	0.14
MBT2	0.32	0.53	0.25	0
MBS2	0.13	0.45	0.39	0.15
MBS3	0.38	0.75	0.46	0.32
NTT1	0.21	0.50	0.45	0

Table 4. Effects of NaCl on growth of bacterial endophyte isolates

	NaCl (%)			
Isolate Code	**0.1**	**1**	**2**	**3**
BOSH4	0.48	0	0	0
BOSH8	0.52	0.03	0	0
BOSH9	1.15	0.38	0	0
BOSD1	0.66	0.01	0	0
BOSD2	0.30	0	0	0
BOSD3	0.26	0	0	0
BOSD4	0.54	0	0	0
BOSD5	0,51	0.42	0.32	0.31
BOSD6	0.40	0	0	0
BOSD12	0.58	0.44	0.01	0
BOT1	0.69	0.43	0.19	0
BOT2	0.44	0.43	0.04	0
BOS2	0.67	0.39	0.07	0
SPT1	0.45	0.44	0.44	0.39
SPT2	0.30	0.16	0.12	0.01
SPS2	0.81	0.31	0.23	0
SPS3	0.25	0.09	0.02	0.02
YAT2	0.43	0.04	0	0
MBT2	0.53	0.23	0.16	0
MBS2	0.39	0.36	0.35	0
MBS3	0.46	0.38	0.05	0
NTT1	0.50	0.15	0.13	0.06

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Biochemical Characterisation

All bacterial endophytes (**Table 5**) tested positive for catalase and 16 out of 22 bacterial isolates were positive for urease. Only 8 isolates (BOSH4, BOSH9, BOSD5, BOSD6, BOSD12, BOT1, BOS2, and MBT2) exhibited amylase activity. Among these, BOSD6 demonstrated the highest amylase activity, measuring 8.75±0.01 mm² in diameter of the clean zone. Bacterial endophytes produce extracellular hydrolytic enzymes that indirectly promote plant growth and protect against pathogens [46, 47]. Catalase activity is crucial for bacteria to reproduce by avoiding cellular toxicity. The 22 tested bacterial endophyte isolates demonstrated positive catalase activity. The urease test was performed to determine the ability of the isolates to break down urea into simple forms of nitrogen that can be rapidly absorbed by plants. Results indicated that sixteen isolates had the potential to degrade urea to nitrogen forms, while six isolates had negative urease tests. [42] also reported high urease activity of *Bacillus subtilis* strain isolated from roots of *Prosopis cineraria*. Our results indicate that eight out of 22 bacterial endophyte isolates tested exhibited amylolytic activity. In addition, [47] isolated endophytic Bacillus from two mangrove species in Brazil that showed extracellular amylase activity.

All 22 bacterial endophyte isolates demonstrated a marked capability to solubilise inorganic phosphate, resulting in clear zones on the Pikovskaya medium. The width ranged from 0.07 ± 0.01 to 7.87 ± 0.83 mm³. Seven isolates were shown to be producers of IAA when subjected to Kovac's reagent, as indicated in Table 6. SPT1 showed the highest IAA production at 2.21 μg·mL-1.

Indole-3-acetic acid (IAA) is a phytohormone that can be produced by plants and various microorganisms. IAA promotes plant growth and contributes to plant-microorganism interactions [48]. In this study, seven bacterial endophytic isolates were found to have the ability to produce IAA in the presence of Kovac's reagent and in the absence of tryptophan. While most microorganisms use tryptophan in the synthesis of IAA [49,50], bacterial endophytes have the advantage of producing IAA without the need for tryptophan supplementation. All 22 bacterial endophytes solubilised inorganic phosphate in Petri dishes. These bacteria can help plants in obtaining insoluble forms of phosphate, such as apatite, by releasing protons and organic acids, mainly gluconic acid, making phosphate available for uptake by plants [51,52]. These bacteria can produce enzymes that mineralise organic phosphorus, making it available to plants [51]. Microorganisms can absorb immobile nutrients such as P from soils and transfer them to their host plants, which is one of the main effects of microbial symbiosis.

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Table 5. Extracellular enzymatic activities of bacterial endophyte isolates

Bacterial isolates	Amylase Diameter of Clean Zone (mm^2)	Urease	Catalase
C	0^a	-	-
BOSH4	$2.75±0.00^c$	+	+
BOSH8	0^a	+	+
BOSH9	$2.5±0.01^b$	+	+
BOSD1	0^a	+	+
BOSD2	0^a	+	+
BOSD3	0^a	+	+
BOSD4	0^a	+	+
BOSD5	$5.75±0.02^f$	+	+
BOSD6	$8.75±0.01^h$	+	+
BOSD12	$6.75±0.01^g$	+	+
BOT1	$2.5±0.01^b$	-	+
BOT2	0^a	+	+
BOS2	$3.25±0.03^d$	+	+
SPT1	0^a	+	+
SPT2	0^a	-	+
SPS2	0^a	-	+
SPS3	0^a	-	+
YAT2	0^a	-	+
MBT2	$4.05±0.01^e$	+	+
MBS2	0^a	+	+
MBS3	0^a	-	+
NTT1	0^a	+	+

C: control without bacterial inoculation. Different letters between lines in the same column denote that mean values are significantly different ($p \le 0.05$) by Duncan's test, means ± standard Error. – denotes no enzyme production; + denotes enzyme production.

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Table 6. IAA production and phosphate solubilisation of bacterial endophyte isolates

Bacterial isolates	IAA production	P Solubilisation Diameter of Clean Zone (mm^3)
C	0^a	0^a
BOSH4	0^a	$2.36±0.21^f$
BOSH8	0^a	$7.87±0.83^h$
BOSH9	0^a	$0.33±0.02^{abc}$
BOSD1	0^a	$0.36±0.05^{abc}$
BOSD2	0^a	$1.57±0.25^{de}$
BOSD3	0^a	$0.33±0.03^{abc}$
BOSD4	0^a	$1.43±0.34^d$
BOSD5	0^a	$1.93±0.52^e$
BOSD6	0^a	$0.42±0.07^{abc}$
BOSD12	0^a	$6.99±0.60^g$
BOT1	$1.93±0.00^f$	$0.07±0.01^{ab}$
BOT2	$1.41±0.00^e$	$0.28±0.04^{abc}$
BOS2	$1.09±0.00^d$	$0.07±0.01^{ab}$
SPT1	$2.21±0.00^h$	$0.36±0.04^{abc}$
SPT2	0^a	$0.07±0.01^{ab}$
SPS2	$2.10±0.01^g$	$0.42±0.04^{abc}$
SPS3	0^a	$0.34±0.04^{abc}$
YAT2	$0.16±0.00^b$	$0.14±0.04^{ab}$
MBT2	$1.09±0.00^d$	$0.14±0.01^{ab}$
MBS2	0^a	$0.36±0.04^{abc}$
MBS3	0^a	$0.49±0.04^{bc}$
NTT1	0^a	$0.16±0.02^{ab}$

C: control without bacterial inoculation. Different letters between lines in the same column denote that mean values are significantly different (p ≤ 0.05) by Duncan's test, means ± standard Error.

***nif*H and *nod*C genes detection**

The presence of the *nifHF* and *nifHI* genes was investigated as a marker for nitrogen fixation, which is a crucial part of the nitrogenase system. Additionally, the *nodCF* and *nodCI* genes were investigated as markers for nodulation, using isolates **(Fig 1a)**. PCR analysis showed that the isolates (BOSH4, BOSH9, BOSD3, BOSD4, BOSD6, BOSD12, BOSH8, BOSD1, BOSD2, BOSD4) were able to fix nitrogen and form nodules. This was confirmed by the amplification of a 780-890 bp PCR product corresponding to the *nifH* gene fragment and a 930-1300 bp PCR product corresponding to the *nodC* gene fragment in the duplex-PCR.

All 12 isolates, including BOT1, BOT2, BOS2, SPT1, SPT2, SPS2, SPS3, YAT2, MBT2, MBS2, MBS3 and NTT1 **(Fig 1b),** produce a PCR product of 780-890 bp in duplex-PCR, indicating the presence of nifH that confers the ability to fix nitrogen. In Regard to nodulation, 10 of the 12 isolates tested possess the nodC gene fragment, while isolates SPS2 and SPT1 do not possess.

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Multiplex PCR is a widely used technique in soil and environmental microbiology, particularly in rhizobiology [54]. In their study, the authors identified a 360 bp amplicon corresponding to the *nif*H gene and a 980 bp amplicon corresponding to the *nod*C gene when amplified alone. However, the amplicon of the *nod*C gene was shown to be much larger than 980 bp when amplified in duplex PCR. According to [37], the size of the amplicon for the *nodC* gene varies between 930-1300 bp, while that of the *nifH* gene varies between 780-890 bp across six bacterial genera. The authors who conducted a combined analysis of the *nif* and *nod* genes by RFLP analysis identified 50 symbiotic genotypes (*nod-nif*). The results show that both sets of isolates (BOSH4, BOSH9, BOSD3, BOSD4, BOSD6, BOSD12) and (BOSH8, BOSD1, BOSD2, BOSD4) contain the *nifH* gene, which is a marker for nitrogen fixation and an essential component of nitrogenase (Fig 1a), as well as the *nodC* genes, which are a marker for nodule formation. The results of the duplex-PCR testing indicate that these isolates have the potential for nitrogen fixation, as evidenced by the presence of the *nifH* gene fragment (780-890 bp). Additionally, the isolates have the ability to form nodules, as indicated by the detection of the *nodC* gene fragment (930-1300 bp). Fig 1b shows that ten isolates (BOT1, BOT2, BOS2, SPT2, SPS3, YAT2, MBT2, MBS2, MBS3, and NTT1) amplified a PCR product of 780-890 bp in the duplex-PCR testing.

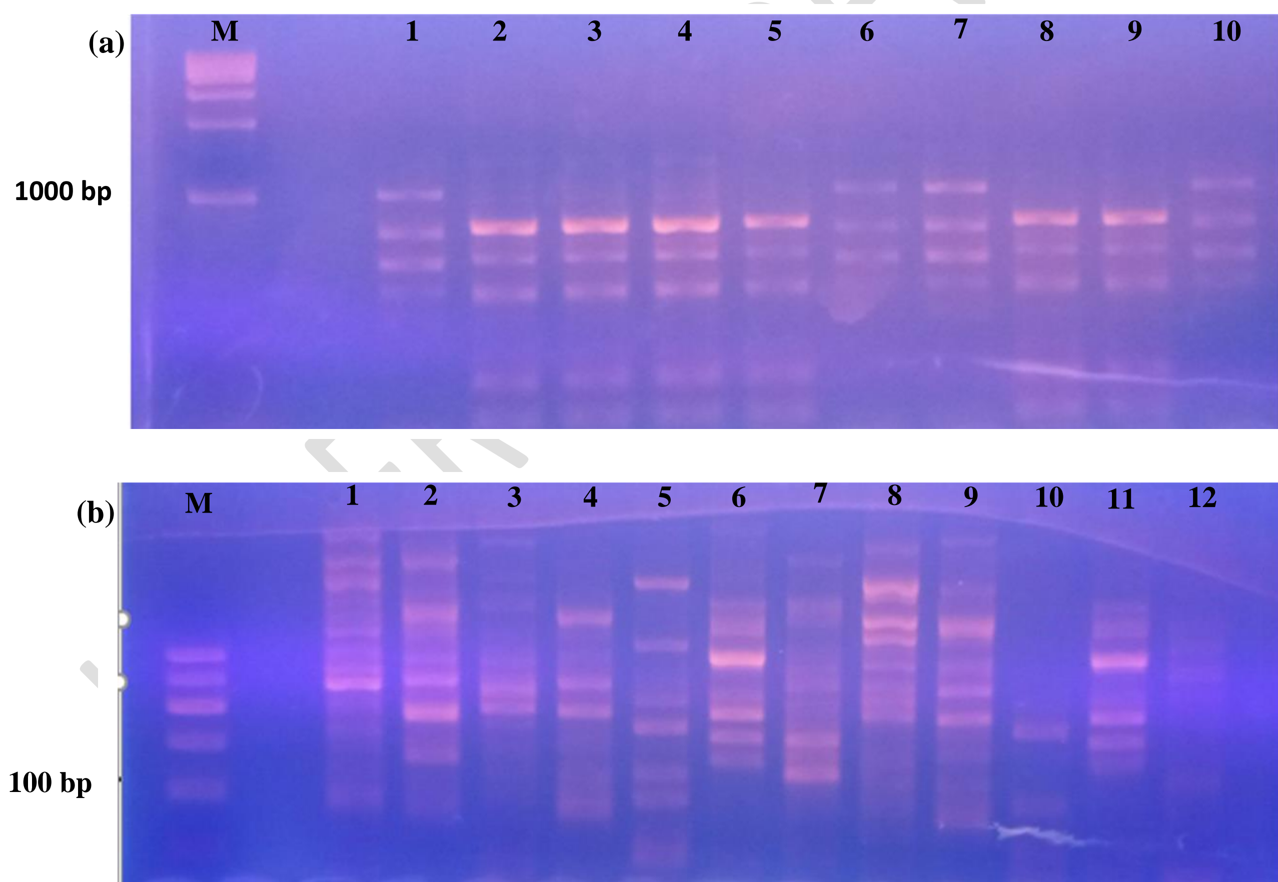

Fig. 1. Duplex-PCR Simultaneous duplex amplification of the *nif*H and *nod*C genes of legume isolates from *G. max* (Houla 1, Docko and TGX 1910-14F variety).

Caption 1a: Agarose gel electrophoresis of duplex-PCR amplification of nifHF, nifHI and nodCF, nodCI with conditions 1.5% agarose gel, 40 ng of DNA template loaded per lane. (Fig 1a) M, 1kb Marker ladder; 1, isolate BOSH4; 2, isolate BOSH8; 3, isolates BOSH9; 4, isolate BOSD1; 5, isolate BOSD2; 6, isolate BOSD3; 7, isolate BOSD4; 8, isolate BOSD5; 9, isolate BOSD6 and 10, isolate BOSD12.

Caption 1b : M, 100 bp Marker ladder ; 1, isolate BOT2 ; 2, isolate SPT2; 3, isolate BOS2; 4, isolate YAT2; 5, isolate BOT1; 6, isolate SPS3; 7, isolate MBS3; 8, isolate NTT1; 9, isolate MBS2; 10, isolate SPS2; 11, isolate MBT2; 12, isolate SPT1.

Phenotypic Characteristic Analysis of bacterial endophyte isolates

The phenotypic characteristics **(Fig. 2)** were compared using mean distance analysis between the groups in a cluster composed of multiple isolates, subdivided into five groups. Group 1 includes fast-growing isolates (BOSH8, BOSD1, BOSD2, BOSD4, BOT2, SPT1, YAT2, MBS2 and NTT1) on yeast extract-mannitol broth medium containing bromothymol blue, with morphological characteristics similar to those of the *Bacillus sp*. reference strain: gram reaction (positive), colony growth type (fast-growing) and regrouping (individualised). Group 2 includes isolates (SPT2, SPS3, BOS2 and BOT1) that are similar in both morphological and biochemical characteristics with the *Pseudomonas sp*. reference strain. These characteristics include a negative gram reaction and rod-shaped colonies. Group 3 contains fast-growing isolates (BOSD6, BOSD12, BOSD3, BOSH9, BOSH4 and BOSD5), which share phenotypic characteristics such as negative gram reaction, colony diameter range of 2-4mm, white colour on yeast extract mannitol agar, and convex elevation compared to the *Rhizobium sp.* reference isolate. Group 4 consists of isolates (SPS2 and MBS3) with morphological characteristics similar to the reference strain of *Paenibacillus sp.* Group 5 represents isolate MBT2 with morphological characteristics similar to the reference strain of *Staphylococcus sp*.

In this study, phenotypic characteristics assigned the 22 isolates to established groups of bacterial endophytic isolates (*Rhizobium spp.*), including microorganisms of the genera: *Bacillus spp., Paenibacillus spp., Staphylococcus sp* and *Pseudomonas spp.* The latter representative genera are not nitrogen-fixing bacteria in the conventional sense [53]. Their ability to fix nitrogen could possibly be attributed to lateral gene transfer. However, the evolutionary impact of lateral gene transfer mechanisms remains poorly understood. The PCR analysis results were confirmed by morphological and biochemical data.

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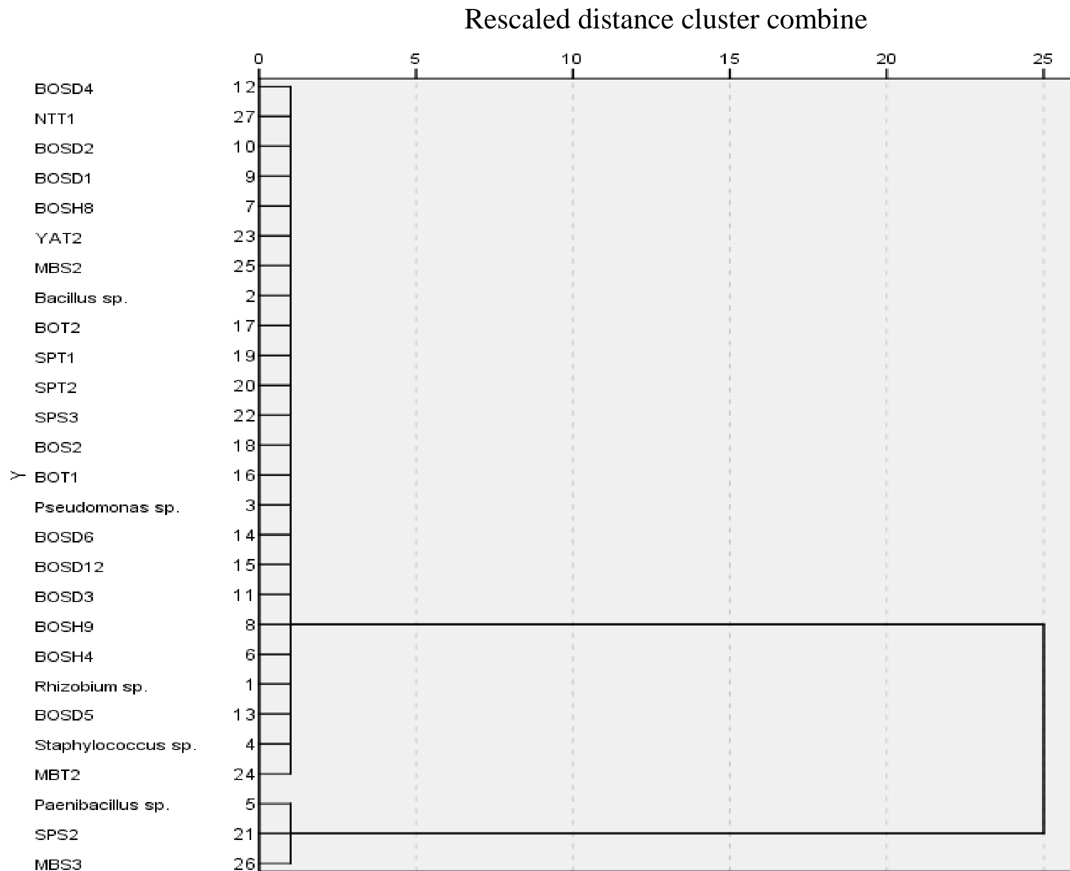

Fig. 2. Dendrogram showing the phenotypic relationships generated between bacterial endophyte isolates from agricultural soils of Bocklé, Jalingo, Mbangassina, Ngaounderé, Ntui, Sanguéré paul, Yagoua in Cameroon

caption 2: Dendrogram of mean distance analysis between the groups in a cluster comprised of multiple isolates. (Fig 2), Group 1 (Bacillus spp.); BOSH8, BOSD1, BOSD2, BOSD4, BOT2, SPT1, YAT2, MBS2, and NTT1, Group 2 (Pseudomonas spp.); SPT2, SPS3, BOS2, and BOT1, Group 3 (Rhizobium spp.); BOSD6, BOSD12, BOSD3, BOSH9, BOSH4, and BOSD5, Group 4 (Paenibacillus spp.); SPS2 and MBS3, Group 5 (Staphylococcus sp.); MBT2.

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Evaluation of the impact of bacterial endophyte isolate application on the growth, biomass and yield of soybeans cultivated in plastic bags

Table 7 presents the results that demonstrate the effects of bacterial endophytes isolates on the growth, biomass, and grain yield of soybean in plastic bags. Out of the 22 isolates inoculated into soybean plants, 6 isolates (BOSH9, BOSD5, BOT1, BOS2, SPT1, and NTT1) had a greater impact than the others. The plant treated with isolates BOSH9 showed a 30.21% increase in the number of leaves. BOSD5 and BOS2 showed increases of 11.23% and 15.77% respectively. Additionally, BOT1 showed a 16.84% increase, SPT1 showed a 10.16% increase, and NTT1 had an increase of 24.60% compared to the control treatment. These results indicate a significant improvement. It is worth noting that the plants' height under the BOS2 treatment (26.66 ± 2.95) is greater. The isolate significantly increased the

plant height by +34.51% compared to the control, by +7.93% compared to (AMF), by +11% compared to (Brady j), and by +6.61% compared to (MSP) treatment. The BOSH9 isolate (+49.36) produced the greatest increase in plant leaf area compared to the control treatment. Additionally, inoculating the plants with the isolates resulted in a significant increase in the number of flowers: +169.52% (BOSH9), +83.76% (BOSD5), +119.45% (BOT1), +94.48% (BOS2), +91.42% (SPT1), and +129.70% (NTT1) compared to the control. The top-performing isolate, BOSH9, increased plant biomass by 120.48% and grain yield by 76.70% compared to the control treatment. Inoculating soybean plants with bacterial endophytic isolates in this study also significantly increased the number of leaves, plant height, leaf area, number of flowers, biomass, and grain yield, as reported by [12-55]. The increase in soybean growth and yield is consistent with the role of inoculated Plant Growth-Promoting Bacteria (PGPB), specifically certain strains of Bacillus, in solubilising nutrients and producing siderophores. In addition, the inoculated bacteria may have released ammonia or produced nitrogenase via their *nifH* genes to fix N_2 and uptake by plant roots to enhance growth and yield as reported for *Bacillus spp.* [56,57,58]. Microbial activity also plays a role in the rhizosphere, as indicated by the presence of acid phosphatase, and supported by research conducted by [59]. The superior performance of soybean plants that were inoculated with the BOSH9 isolate is supported by [60]. They reported improved soybean growth and N_2 fixation, and grain yield after inoculation with bacterial endophytic strains such as *Bradyrhizobium*.

Table 7. Effect of bacterial endophytes isolates on the growth, biomass and yield of soybean

Treatments	Number of leaves /plant	Plant height (cm)	Leaf area (cm^2)	Flowers number/ plant	Total weight of dried biomass (g/plant)	Seeds weight (g/plant)
C	3.74 ± 0.59^{a}	19.82 ± 0.80^{a}	17.36 ± 0.83^{ab}	6.53 ± 0.80^{a}	8.69 ± 0.46^{bc}	7,04 ± 0,32^{g}
AMF	4.24 ± 0.93^{a}	24.20 ± 1.84^{bcd}	19.61 ± 2.45^{abcde}	12.39 ± 0.07^{k}	12.29 ± 0.97^{hij}	3,81 ± 0,14^{bc}
Brady J	3.54 ± 0.75^{a}	23.53 ± 2.19^{abcd}	17.56 ± 0.79^{abc}	9.36 ± 0.02^{fh}	12.54 ± 0.97^{hij}	2,89 ± 0,08^{a}
PSM	3.78 ± 0.59^{a}	24.50 ± 2.15^{abcd}	21.16 ± 1.59^{abcdef}	9.06 ± 0.09^{efh}	12.26 ± 0.88^{hij}	7,65 ± 0,16^{h}
BOSH 4	4.24 ± 0.95^{a}	23.87 ± 2.43^{abcd}	20.71 ± 1.54^{cdefg}	12.87 ± 0.08^{k}	14.07 ± 0.44^{kl}	7,57 ± 0,21^{h}
BOSH 8	3.83 ± 0.62^{a}	25.29 ± 3.21^{bcd}	21.39 ± 1.25^{efg}	11.16 ± 0.12^{j}	11.00 ± 0.71^{efghi}	4,87 ± 0,09^{de}
BOSH 9	4.87 ± 0.29^{a}	26.12 ± 3.80^{cd}	25.93 ± 2.26^{h}	17.6 ± 0.08^{o}	19.16 ± 1.09^{o}	12,44 ± 0,24^{l}
BOSD 1	4.16 ± 0.95^{a}	23.87 ± 2.23^{abcd}	20.02 ± 1.68^{bcdef}	8 ± 0.12^{bcd}	11.27 ± 1.08^{fghi}	10,66 ± 0,94^{k}
BOSD 2	3.41 ± 0.29^{a}	23.28 ± 1.35^{abcd}	20.25 ± 1.49^{bcdefg}	7.33 ± 0.08^{abc}	7.44 ± 1.12^{ab}	4,96 ± 0,10^{de}
BOSD 3	3.74 ± 0.47^{a}	21.07 ± 2.51^{abc}	17.25 ± 1.53^{ab}	8.16 ± 0.08^{cde}	11.30 ± 0.91^{fghi}	4,08 ± 0,01^{c}
BOSD 4	3.95 ± 0.41^{a}	19.45 ± 2.56^{a}	16.55 ± 1.53^{a}	9.5 ± 0.93^{h}	9.33 ± 0.36^{cde}	6,13 ± 0,12^{f}
BOSD 5	4.16 ± 0.51^{a}	25.87 ± 1.37^{cd}	21.45 ± 1.65^{efg}	12 ± 0.93^{k}	14.56 ± 0.58^{lm}	4,77 ± 0,21^{de}
BOSD 6	3.91 ± 0.99^{a}	24.75 ± 2.38^{abcd}	17.83 ± 2.21^{abcd}	8.85 ± 0.93^{defh}	10.06 ± 0.88^{cdefg}	8,51 ± 0,24^{i}
BOSD 12	3.91 ± 0.89^{a}	25.12 ± 2.77^{bcd}	23.03 ± 0.95^{fgh}	14 ± 0.93^{l}	11.07 ± 0.64^{efghi}	9,59 ± 0,16^{j}
BOT 1	4.37 ± 0.79^{a}	25.33 ± 3.60^{bcd}	23.39 ± 2.37^{gh}	14.33 ± 0.93^{lm}	16.79 ± 0.14^{n}	8,92 ± 0,09^{i}
BOT 2	4.08 ± 0.97^{a}	25.03 ± 3.61^{bcd}	20.32 ± 0.94^{bcdef}	14 ± 0.93^{l}	11.78 ± 0.55^{ghi}	4,44 ± 0,19^{cd}
BOS 2	4.33 ± 0.91^{a}	26.66 ± 2.95^{d}	21.04 ± 2.02^{defg}	12.7 ± 0.93^{k}	17.21 ± 1.29^{n}	8,82 ± 0,10^{i}
SPT 1	4.12 ± 0.97^{a}	25.70 ± 1.87^{cd}	20.96 ± 0.89^{defg}	12.5 ± 0.93^{k}	15.75 ± 0.67^{mn}	6,74 ± 0,18^{fg}
SPT 2	4 ± 0.70^{a}	24.49 ± 2.82^{abcd}	20.30 ± 1.24^{bcdefg}	12.16 ± 0.93^{k}	14.11 ± 0.59^{lm}	6,76 ± 0,17^{fg}
SPS 2	3.54 ± 0.49^{a}	23.24 ± 2.33^{abcd}	19.09 ± 0.69^{abcde}	8.5 ± 0.93^{def}	9.74 ± 0.91^{cdef}	3,39 ± 0,26^{ab}
SPS 3	4.16 ± 0.71^{a}	23.78 ± 2.90^{abcd}	19.45 ± 0.69^{abcde}	10.33 ± 0.93^{i}	10.69 ± 0.27^{defghi}	5,20 ± 0,16^{de}
YAT 2	3.54 ± 0.57^{a}	20.08 ± 3.76^{ab}	18.98 ± 0.69^{abcdef}	7.16 ± 0.93^{ab}	8.90 ± 0.77^{bcd}	3,06 ± 0,04^{a}
MBT 2	4.08 ± 0.69^{a}	24.37 ± 2.79^{abcd}	19.87 ± 0.69^{abcdef}	8.53 ± 0.93^{def}	8.75 ± 0.33^{bc}	8,65 ± 0,92^{i}
MBS 2	3.87 ± 0.53^{a}	21.70 ± 2.62^{abcd}	17.05 ± 0.69^{a}	8.5 ± 0.93^{def}	6.59 ± 0.86^{a}	3,81 ± 0,07^{bc}
MBS 3	3.58 ± 0.62^{a}	23.26 ± 2.21^{abcd}	19.10 ± 0.69^{acde}	6.66 ± 0.93^{a}	10.74 ± 0.42^{efghi}	7,17 ± 0,30^{gh}
NTT 1	4.66 ± 0.78^{a}	25.12 ± 3.27^{bcd}	23.37 ± 0.69^{gh}	15 ± 0.93^{n}	16.88 ± 1.30^{n}	12,08 ± 0,24^{l}

BOSH4, BOSH8, BOSH9, BOSD1, BOSD2, BOSD3, BOSD4, BOSD5, BOSD6, BOSD12, BOT1, BOT2, BOS2, SPT1, SPT2, SPS2, SPS3, YAT2, MBT2, MBS2, MBS3 and NTT1 are potential bacterial endophyte isolates for increasing the growth, biomass and seed yield of the soybeans in plastic bags; C (control without bacterial inoculation); AMF (control with Arbuscular Mycorrhizal Fungus); Brady j (control with Bradyrhizobium japonicum); PSM (control with Phosphorus Solubilising Microorganism).

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Different letters between lines in the same column denote that means are significantly different ($p \leq 0.05$) by Duncan's test, means ± standard Error.

4. CONCLUSION

In this study, we obtained 85 bacterial endophyte isolates from nodules and roots of three soybean varieties grown in seven different soils in Cameroon. Characterisation of the bacterial endophyte isolates enabled us to preselect 22 out of 85. The characterisation focused on tolerance tests for pH, temperature, and NaCl, as well as extracellular enzymatic activity, P-solubilisation activity, IAA production, and detection of nitrogen fixation and nodulation genes. The two best isolates, BOSH9 and NTT1, significantly promoted plant growth, increasing plant height, number of leaves, number of flowers, biomass, and soybean grain yield. However, to demonstrate the beneficial role of bacterial endophytes (BOSH9 and NTT1) in promoting plant growth, especially under real field conditions, further investigation is required to determine their potential use as biofertilizers in agriculture.

REFERENCES

1. WWF, The Growth of Soy: Impacts and Solutions. Gland: WWF International; 2014.
2. Nyahnone TJ. Le Développement De La Culture Du Soja Dans Les Fronts D'extensions Cotonniers du Nord-Cameroun : Le cas du Mayo-Rey. Mémoire De Master De Géographie, Université de Ngaoundéré, Yaounde. 2017; 135.
3. Nzossié EJ, Bring C. Soybean (Glycine max (L.) Merr.) Production in the Cameroonian Cotton Basin between the Dynamics of Structuring an Agricultural Value Chain and Sustainability Issues. London : Intechopen. 2020; 93981.
4. Wendt J, Atemkeng M. Soybean, cowpea, groundnut, and pigeonpea response to soils, rainfall, and cropping season in the forest margins of Cameroon. Plant Soil. 2004; 263 :121–132. doi: 10.1023/B: PLSO.0000047731.35 668.e0
5. Tening AS, Foba-Tendo JN, Yakum-Ntaw SY, Tchuenteu F. Phosphorus fixing capacity of a volcanic soil on the slope of mount Cameroon. Agriculture and Biology Journal of North America. 2013; 4 :166–174. doi: 10.5251/abjna.2013.4.3.166.174
6. Ngosong C, Bongkisheri V, Tanyi CB, Nanganoa LT, Tening AS. Optimizing nitrogen fertilization regimes for sustainable maize (Zea mays L.) production on the volcanic soils of Buea Cameroon. Advances in Agricuture 2019. 4681825. doi: 10.1155/2019/4681825
7. Nanganoa LT, Ngome FA, Suh C, Basga SD. Assessing soil nutrients variability and adequacy for the cultivation of maize, cassava, and sorghum in selected agroecological zones of Cameroon. International Journal of Agronomy 2020. 8887318. doi: 10.1155/2020/8887318
8. Ntambo MS, Isaac SC, Taruvinga A, Hafeez S, Anwar T, Sharif R. The effect of Rhizobium inoculation with nitrogen fertilizer on growth and yield of soybeans (Glycine max L.). International Journal of Biosciences. 2017; 10 :163–172. doi: 10.12692/ijb/10. 3.163-172
9. Mahmud K, Makaju S, Ibrahim R, Missaoui A. Current progress in nitrogen fixing plants and microbiome research. Plants. 2020; 9: 97. doi: 10.3390/ plants9010097

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10. Mndzebele B, Ncube B, Fessehazion M, Mabhaudh T, Amoo S, Plooy. Effects of cowpea-amaranth intercropping and fertiliser application on soil phosphatase activities, available soil phosphorus, and crop growth response. Agronomy. 2020; 10 : 79. doi: 10.3390/agronomy10010079
11. Korir H, Mungai NW, Thuita M, Hamba Y, Masso C. Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Frontiers in Plant Science. 2017; 8 :141. doi:10.3389/fpls.2017.00141
12. Tchakounté VT, Berger G, Patz S, Becker M, Ková VT, Novák O. The response of maize to inoculation with Arthrobacter sp. and Bacillus sp. in phosphorus-deficient, salinity-affected soil. Microorganisms. 2020; 8 :1005. doi: 10.3390/microorganisms8071005
13. Tairo EV, Ndakidemi PA. Bradyrhizobium japonicum Inoculation and Phosphorus Supplementation on Growth and Chlorophyll Accumulation in Soybean (Glycine max L.). American Journal of Plant Sciences. 2013; 4 :2281-2289.
14. Nget R, Aguilar EA, Cruz PC, Reano CE, Sanchez PB, Reyes MR. Responses of Soybean Genotypes to Different Nitrogen and Phosphorus Sources: Impacts on Yield Components, Seed Yield, and Seed Protein. Plants. 2022; 11: 298.
15. Rosenblueth M, Martinez-Romero E. Bacterial endophytes and their interactions with hosts. Molecular Plant- Microbe Interactions. 2006; 19: 827–837.
16. Mercado-Blanco J, Bakker PAHM. Interactions between plants and beneficial Pseudomonas spp.: exploiting bacterial traits for crop protection. Antonie Van Leeuwenhoek. 2007; 92: 367-89.
17. Weyens N, Van der Lelie D, Taghavi S, Vangronsveld J. Phytoremediation: plant-endophyte partnerships take the challenge. Current Opinion Biotechnology. 2009; 20: 248-254.
18. Malfanova N, Lugtenberg B, Berg G. Bacterial endophytes: who and where, and what are they doing there? In: Molecular Microbial Ecology of the Rhizosphere; de Bruijn F J, Ed. ch 36, WileyBlackwell, Hoboken, NJ, USA, 2013; 393-403.
19. Hallmann J, Berg G. Spectrum and population dynamics of bacterial root endophytes. In: Microbial root endophytes; Schulz B, Boyle C, Sieber T, Eds. Springer-Verlag, Berlin Heidelberg. 2006; 15-31.
20. Krimi Z, Alim D, Djellout H, Tafifet L, Mahmoud M, Raio. Bacterial endophytes of weeds are effective biocontrol agents of Agrobacterium spp., Pectobacterium spp., and promote growth of tomato plants. Phytopathologia Mediterranea. 2016; 55 (2) :184 -196.
21. Wegrzyn A, Ewa F. Isolation of Bacterial Endophytes from Phalaris arundinacea and their Potential in Diclofenac and Sulfamethoxazole Degradation. Polish Journal of Microbiology. 2018; 67 (3): 321-331.
22. Vincent. A manual for the practical study of root- nodule bacteria. Blackwell-scientific publications limited, oxford, England; 1970.
23. Somasegaran P, Hoben H. Collecting Nodules and Isolating Rhizobia. In Handbook for Rhizobia, Springer, New York, NY, USA; 1994.
24. Damaris-Ondieki K, Evans-Nyaboga N, John-Wagacha M, francis-Mwaura B. Morphological and Genetic Diversity of Rhizobia Nodulating Cowpea (Vigna unguiculataL.) from Agricultural Soil of Lower Eastern Kenya. International Journal of Microbiology. 2017: 9.
25. Jalgaonwala RE, Mahajan RT. Isolation and Characterization of endophytic bacterial flora from some Indian medicinal plants. Asian Journal of Research Chemistry. 2011; 42: 296-300.

UNDER PEER REVIEW

26. Nair DN, Padmavathy S. Impact of endophytic microorganisms on plants, environment and humans. Science World Journal. 2014; 1-11. Article ID: 250693.1-11.
27. Girmaye K, Fassil A, Mussie YH. Phenotypic and genotypic characteristics of cowpea rhizobia from soils of Ethiopia. African Journal of Biotechnology. 2018; 17 (42) :1299-1312.
28. Bind M, Nema S. Isolation and Molecular Characterization of Endophytic Bacteria From Pigeon Pea Along With Antimicrobial Evaluation against Fusarium udum. Journal of Applied Microbiology Open Access. 2019; 5:163.
29. Christensen W.B. Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. Journal of Bacteriology. 1946; 52: 461-466.
30. Pikovskaya R. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya. 1948; 17:362-370.
31. Qureshi M, Ahmad Z, Akhtar N. Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth. Journal of Animal Plant Science. 2012; 22: 204-210.
32. Jasim B, John C, Lyothis M, Radhakrishnan EK. Plant growth promoting potential of endophytic bacteria isolated from Piper nigrum. Plant Growth Regulation. 2013; 71: 1-11.
33. Singh P, Kumar V, Agrawal S. Evaluation of phytase producing bacteria for their plant growth promoting activities. Internationtinal Journal of Microbiology. 2014; 426-483.
34. Marag P, Suman A. Growth stage and tissue specific colonization of endophytic bacteria having plant growth promoting traits in hybrid and compositive maize (Zea mays L.). Microbiology Research Journal International. 2018; 214 :101-113.
35. Weisburg W.G., Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology. 1991; 173:697–703. https://doi.org/10.1128/jb.173.2.697-703.1991
36. Miller DN, Bryant JE, Madsen EL, Ghiorse WC. Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Applied and Environmental Microbiology. 1999; 65.
37. Laguerre G, Nour SM, Macheret V, Sanjuan J, Drouin P, Amarger N. Classification of rhizobia based on nodC and nifH gene analysis reveals a close phylogenetic relationship among Phaseolus vulgaris symbionts. Microbiology. 2001; 147, 981-993. https://doi.org/10.1099/00221287-147-4-981
38. Maria do Carmo CP, Dolores ASF, Maria luiza Ribeiro BS, Rosemberg VB, Vinicius dos Santos GS, Ferreira da Silva A. Diversity of rhizobia isolated from nodules of indigenous tree legume from the Brazilian dry forest. Acta agron. 2019; 68: 47-55. https://doi.org/10.15446/acag.v68n1.61243
39. Jida M, Assefa F. Phenotypic and plant growth promoting characteristics of Rhizobium leguminosarum bv.viciae from lentil growing areas of Ehiopia. African Journal of Microbiology Research. 2011; 5 (24):4133-4142.
40. Zhang WT, Yang JK, Yuan TY, Zhou JC. Genetic diversity and phylogeny of indigenous rhizobia from cowpea [Vigna unguiculate (L.) Walp.] Biology and Fertility of Soils. 2007; 44 (1):201-210.
41. Somasegaran P, Hoben H. Handbook for rhizobia: Methods in legume Rhizobium Technology, Springer-Verlag, New York; 1994. http://dx.doi.org/10.1007/978-1-4613-8375-8
UNDER PEER REVIEW

42. Gupta RM, Kale PS, Rathi ML, Jadhav N.N. Isolation, characterization and identification of endophytic bacteria by 16S rRNA partial sequencing technique from roots and leaves of Prosopis cineraria plant. Asian Journal of Plant Science and Research. 2015; 5 (6):36-43.
43. Cevheri C, Kuçuk CD, çetin E. Fungicide, antibiotic, heavy metal resistance and salt tolerance of root nodule isolates from Vicia palaestina. African Journal of Biotechnology. 2011; 10 (13): 2423-2429.
44. Faghire M, Mandri B, Oufdou K, Bargaz A, Ghoulam C, Ramírez-Bahena MH. Identification at species and symbiovar levels of strains nodulating Phaseolus vulgaris in saline soils of the Marrakech (Morocco) and analysis of otsA gene putatively involved in osmotolérance. Systematic and Applied Microbiology Journal. 2012; 35:156-164.
45. Nour SM, Fernandez MP, Cleyet Marcel JC. Rhizobium cicero SP. consisting of strains that nodulate chickpeas (Cicer arietinum L.). International Journal of Systematic Bacteriology. 1994; 44: 511-522.
46. Glick BR. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica. 2012; 1-15.
47. Castro RA, Quecine MC, Lacava PT, Batista BD, Luvizotto DM, Marcon J. Isolation and enzyme bioprospection of endophytic bacteria associated with plants of Brazilian mangrove ecosystem. SpringerPlus. 2014; 3:382.
48. Lin L, Zhengyi L, Chunjin H, Zhang X, Chang S, Yang L. Plant growth-promoting nitrogen-fixing Enterobacteria are in association with sugarcane plants growing in Guangxi, China. Microbes and Environment. 2012; 27: 391-392.
49. Fouda A, Hassan SE, Eid AM, Ewais EE. Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinacia (Bioss). Annals of Agricultural Sciences. 2015; 60:95-104.
50. Hassan SE, Fouda A, Radwan AA, Salem SS, Barghoth MG, Awad MA. Endophytic actinomycetes Streptomyces spp mediated biosynthesis of copper oxide nanoparticles as a promising tool for biotechnological applications. JBIC. 2019; 24:377-393.
51. Khan N, Bona A, Babar MA. The root growth of wheat plants, the water conservation and fertility status of sandy soils influenced by plant growth promoting rhizobacteria. Symbiosis. 2017; 72:195-205.
52. Nassem H, Ahsan M, Shahid MA, Khan N. Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance. Journal of Basic Microbiology. 2018; 58:1009-1022.
53. Liu H, Zhang L, Meng A, Zhang J, Xie M, Qin Y. Isolation and molecular identification of endophytic diazotrophs from seeds and stems of three cereal crops. Journal of PLoS ONE. 2017; 12 (10): 11.
54. Fernandes JP, Morgante C, Gava C, Santos C, Cunha J, Martins L. Duplex PCR para a Amplificação Simultânea de Fragmentos dos Genes nifH e nodC em Bactérias Isoladas de Nódulos de Leguminosas. Petrolina: Embrapa Semiárido 2013. http://dx.doi.org/10.13140/RG.2.1.2726.9605
55. Tchakounté T, Berger B, Patz S. Community structure and plant growth-promoting potential of cultivable bacteria isolated from Cameroun soil. Microbiological Research. 2018; 214:47-59.
56. Ding Y, Wang J, Liu Y. Isolation and identification of nitrogen-fixing bacilli from plant rhizospheres in Beijing region. Journal of Applied Microbiology. 2005; 99:1271-1281.
57. Hayat R, Ali S, Amara U. Soil beneficial bacteria and their role in plant growth promotion: a review. Annals of Microbiology. 2010; 60:579-598.
UNDER PEER REVIEW

58. Kuan K, Othman R, Rahim R. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. PLoS One. 2016; 11.
59. Lamptey S, Ahiabor B, Yeboah S. Response of soybean (Glycine max) to Rhizobial inoculation and phosphorus application. Journal of Experimental Biology and Agricultural Science. 2014; 2: 73-77.
60. Leggett M, Diaz-Zarito M, Koivunen Ahiabor S, Yeboah S. Soybean response to inoculation with Bradyrhizobium japonicum in the United States and Argentina. Agronomy Journal. 2017; 109: 1031-1038.

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