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Using superior plant growth-promoting microorganisms through bioprospecting to create inoculants for peanut (*Arachis hypogaea* L.) farming

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Experiments with pot-grown plants are among the most common in plant research. In this study, we isolated plant growth-promoting bacteria (PGPB) from the rhizosphere soils and root nodules of peanut plants grown under greenhouse conditions, and evaluated their PGP properties to select elite strains as inoculants. The isolates were characterized for the following PGP activities: phosphate (P) solubilization, auxin and siderophore production, fluorescence emission, and nodule formation capacity. Isolates were also identified through 16S rRNA gene sequencing. A total of 90 isolates were obtained and characterized, belonging to five genera: *Burkholderia, Bacillus, Brevundimonas*, *Dyella* and *Leifsonia*. Among these, *B. cepacia* and *B. territorii* were the most abundant species, exhibiting the highest levels of auxin and siderophore production, as well as superior P solubilization. Furthermore, nodule isolates demonstrated more intense PGP activities than free-living isolates from rhizosphere soils. This is the first report documenting the nodulation capacity of the genus *Dyella*, and further studies targeting the nifH gene will be necessary to confirm its nitrogen-fixing ability. The in vitro screening provided sufficient evidence for further in vivo peanut growth-promoting tests of both *Burkholderia* and *Dyella* isolates. Such agricultural applications could enhance peanut yields while reducing environmental pollution.

Key words: Peanut, biofertilizers, promoting rhizobacteria, characterization, soil and root nodule.

INTRODUCTION

Peanut, also known as groundnut (*Arachis hypogaea* L.), is a grain legume native to Latin America and more than

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300 cultivars are cultivated in the tropics and subtropics, including sub-Saharan Africa (Sene et al., 2010; Noba et al., 2014; Jayaprakash et al., 2019; Sene et al., 2023). Peanut products are consumed worldwide for human and animal nutrition (Noba et al., 2014; Alexander et al., 2020; Mekdad et al., 2021). In Senegal, peanut has been a cash crop for more than a century, contributing to 60% of the country's agricultural gross domestic product (GDP) and about 80% of its export earnings (Sene et al., 2010; Noba et al., 2014). It is the most important oil-producing crop, and the four oil factories established in the country form the backbone of the national industrial fabric. After a long period of decline, peanut yields have increased in the last five years. However, factors that determine this increase, that is, soil fertility, have steadily deteriorated, with the reduction in fallow land and low levels of fertilizer use (Sene et al., 2010; 2021). Various agricultural practices, including the use of mineral fertilizers, have been adopted to increase yields and mitigate food shortages. However, the high cost of chemical fertilizer and the need for sustainable alternative sources have increased the strategic importance of harnessing soil microbiota.

Soil microbial communities are involved in several functions in agroecosystems, such as nutrient availability, pathogen control, and resilience to abiotic stresses (Sene et al., 2014; Olanrewaju et al., 2017; Kandasamy and Kathirvel, 2023).

Plant growth promoting bacteria, known as PGPB, are among these soil microorganisms and are one of the most important bacteria capable of forming beneficial associations with crop plants (Hamim et al., 2019; Vandana et al., 2021; Sene et al., 2021, 2023). PGPB are known to reside around plant roots (rhizosphere) and in root tissues, and have no external signs or adverse effects on their host (Vessey, 2003; Collavino et al., 2012; Olanrewaju et al., 2017; Kandasamy and Kathirvel, 2023). The benefits derived from their association with plants depend on both interacting partners. The low molecular weight compounds (e.g. sugars, organic acids and amino acids) exuded by plant roots in sizeable quantities (that is, 5 to 30% of total photosynthetically fixed carbon) are commonly used as food sources by bacteria (Vessey, 2003; Olanrewaju et al., 2017; Ríos-Ruiz et al., 2019; Vandana et al., 2021; Kandasamy and Kathirvel, 2023). In turn, PGPB can promote plant growth by both direct and indirect mechanisms. Direct mechanisms are defined as the use of bacterial properties that result in the direct promotion of plant growth (Olanrewaju et al., 2017). These mechanisms include phosphorus solubilization; auxin, cytokinin and gibberellin production; iron sequestration through bacterial siderophores and nitrogen fixation (Vessey, 2003; Collavino et al., 2012; Olanrewaju et al., 2017; Kandasamy and Kathirvel, 2023). Indirect mechanisms refer to bacterial traits that inhibit the function of one or more plant pathogens, both fungi and bacteria (Olanrewaju et al., 2017). There is a wide range of PGPBs, each with different activities under different environmental and soil conditions. No single organism has the ability to use all the available mechanisms available to promote plant growth.

Phosphorus in particular is one of the most important elements in plant mineral nutrition (Bagyalakshmi et al., 2017; Pande et al., 2017). In particular, phosphorus plays an important role in enhancing nitrogen fixation, but it is also involved in all major metabolic processes occurring in plants, including photosynthesis, energy transfer, macromolecular biosynthesis and respiration (Vessey, 2003; Nath et al., 2017; Hamim et al., 2019; Kandasamy and Kathirvel, 2023). It is required in large quantities for adequate plant growth and development, but its availability to plants is limited in many agricultural soils in West Africa and generally in tropical soils. In these areas, the availability of phosphorus in insoluble, immobilized and precipitated forms makes its use by plants quite limited (Nath et al., 2017; Lotfi et al., 2022; Lu et al., 2023).Together with mycorrhizal fungi, PGPBs provide an alternative to industrial phosphorus fertilizers and a means to maintain or improve soil fertility (Vessey, 2003; Collavino et al., 2012; Kotasthane et al., 2017; Nath et al., 2017; Lotfi et al., 2022). Both mycorrhizal fungi and PGPBs are currently attracting much scientific interest due to their potential to improve nutrient use efficiency (NUE), facilitate nutrient uptake, promote growth (Frank et al., 2017; Hamim et al., 2019) and protect the host from predators through the production of secondary metabolites. Several PGPBs, rhizobia or mycorrhizal fungi have been commercialized either as biocontrol agents or biofertilizers (Vessey, 2003; Lesueur et al., 2016; Mohanty and Swain, 2018). However, the commercialization of microbial inoculants in West Africa has lagged behind expectations. In these areas, commercial inoculants often fail to compete with indigenous soil microorganisms due to strain variation, abundance and composition, as well as many environmental factors (Sene et al., 2010). The goal of the current study was to identify and assess the elite or most productive PGPBs in order to estimate when PGPB biofertilizers will be included into peanut farming. From the root nodules and rhizosphere soils of peanut plants grown in greenhouses, we extracted bacteria and assessed their PGP potential. As selection characteristics of elite PGPBs, we employed phosphate solubilization, indole acetic acid (IAA) and siderophore synthesis, fluorescence emission, and nodule formation capacity. We also used 16S rDNA gene sequencing to identify the isolates.

MATERIALS AND METHODS

Soil sampling and greenhouse experiment

Soil samples were collected at the beginning of the dry season from three sites [Mbousnakh, Department of Thiès (MBS); Sagne

Table 1. Soil physicochemical properties of the three sites along a rainfall gradient in the peanut basin of Senegal.

Property	Sampling site		
	MBS	**SAG**	**KAT**
Latitude (N)	14°38'47.21"	14°12'0.43"	13°57'33.38"
Longitude (W)	16°47'33.84"	16°11'4.71"	14°52'7.91"
Clay (%)	6.03 ± 0.72^{a}	5.57 ± 0.58^{a}	5.83 ± 0.72^{a}
Silt (%)	1.61 ± 0.62^{c}	8.83 ± 2.38^{b}	15.87 ± 3.95^{a}
Sand (%)	93.3 ± 0.10^{a}	84.43 ± 1.02^{b}	77.00 ± 0.56^{c}
Texture	**Sandy**	**Sandy loam**	**Sandy loam**
C (g/kg)	2.64 ± 0.23^{b}	6.17 ± 0.46^{a}	3.34 ± 0.53^{b}
C org. (g/kg)	3.29 ± 0.50^{b}	6.38 ± 0.25^{a}	3.61 ± 0.98^{b}
C/N	12.33 ± 0.58^{b}	13.67 ± 1.53^{ab}	15.67 ± 1.53^{a}
pH H_2O	6.11 ± 0.03^{a}	5.56 ± 0.11^{b}	6.00 ± 0.04^{a}
pH KCl	5.24 ± 0.04^{a}	4.65 ± 0.09^{a}	5.11 ± 0.01^{a}
N kj. (mg/kg)	336.43 ± 30.43^{b}	464.30 ± 10.81^{a}	251.21 ± 14.77^{c}
N (NO_3) mg/kg	4.03 ± 0.25^{a}	5.56 ± 1.62^{a}	1.10 ± 0.10^{b}
N (NH_4) mg/kg	3.90 ± 0.70^{b}	5.37 ± 0.47^{a}	4.17 ± 0.55^{b}
Total N (g/kg)	0.220 ± 0.026^{b}	0.447 ± 0.023^{a}	0.217 ± 0.015^{b}
Total P (mg/kg)	90.82 ± 7.79^{a}	58.83 ± 4.90^{b}	62.18 ± 13.59^{b}
Available P (mg/kg)	4.74 ± 0.79^{a}	2.73 ± 0.78^{b}	2.46 ± 0.34^{b}

MBS (Mbousnakh, Department of Thiès); SAG (Sagne Mbambara, Department of Kaolack); KAT (Keur Ablaye Touré, Department of Koungheul); Values (mean ± standard error) are the average of four replicates; means ± standard error within the same line followed by the same superscript letter are not statistically different at the 5% probability according to the Tukey test.

Mbambara, Department of Kaolack (SAG); and Keur Ablaye Touré, Department of Koungheul (KAT)] in the Senegalese peanut basin along a rainfall gradient. The geographical location of each site was recorded by Global Positioning System **(**GPS**)** and is shown in Table 1. Soil samples were taken from the top 25 cm using a 25 cm length and 6.3 cm diameter soil auger. The soil samples were then sieved (< 2 mm), bulked, homogenized and split into two subsamples. The first sub-sample was air-dried for chemical analysis at the Laboratoire des Moyens Analytiques (LAMA), Dakar, Senegal [www.lama.ird.sn] and the second sub-sample was used for bacterial greenhouse trap culture.

Seeds of selected peanut cultivar 55 to 437 were first surface sterilized (to avoid seed-borne diseases) with 5% sodium hypochlorite (NaOCl) for 5 min, 70% ethanol for 3 min and thoroughly rinsed with sterile distilled water. The seeds were then placed on Petri dishes containing moist filter paper for germination under sterile conditions and kept in the dark at 25°C. The germinated seeds were manually transplanted to a depth of 2 to 3 cm into 1.5 L plastic pots disinfected with a solution containing 1.81% calcium hypochlorite and filled with the soil substrates. Two germinated seeds were planted in each pot. The plants were thinned on the 3rd day after planting to one plant per pot. The pots were arranged in randomized blocks, with five replications. The pots were placed at 10 and 40 cm spacing within and between rows. Plants were grown under greenhouse conditions (temperature of 27 to 35ºC, relative humidity of 70 to 80% and 12 h of light) and were watered every two days with chlorine treated water without added nutrients.

Plants were uprooted at the time of flowering. The soil adhering to the roots was collected and used as rhizosphere soil for bacterial isolation. Roots were washed thoroughly with tap water and then nodules were carefully detached from the roots by using sterilized forceps. Intact and undamaged nodules were immersed in 70% ethanol (v/v) for 2 min and surface sterilized in 5% sodium hypochlorite (NaOCl) for 5 min and washed three times with distilled water.

Bacterial isolation and fluorescent pigment assay

Bacterial strains were isolated from the above collected root nodules and rhizosphere soils of peanuts. Sterilized nodules were crushed with a glass rod in a drop of sterile distilled water and the suspension streaked on yeast mannitol agar (YMA) (Vincent, 1970). Plates were incubated at 28ºC for 3 to 7 days. Pure bacterial colonies were obtained by repeated streaking of the individual colony on YMA broth and stored in 30% glycerol (v/v) at -80ºC for further characterization.

Ten grams of each rhizosphere soil sample was suspended in 90 ml of sterile phosphate buffered saline (PBS), mixed thoroughly, and allowed to settle. The samples were then diluted to achieve the appropriate dilutions (10^{-2}, 10^{-3}, 10^{-4}) using the serial dilution technique (Somasegaran and Hoben, 1994). The culture was performed by spreading 50 to 100 µl of each dilution in Petri dishes containing Luria-Bertani (LB) agar medium (Sambrook et al., 1989). Four replicates were made for each dilution. The Petri dishes were incubated in an oven at 28ºC for 24 h. 24 h after incubation; bacterial colonies grown on the medium were purified. Isolates were then selected based on the following morphological characteristics: size, shape, colour, opacity and surface of the colonies.

The isolates were tested for their ability to produce fluorescence with a diffusible yellowish-green pigment in King B medium (King et al., 1954) under ultraviolet light (360 nm).

Plant growth promoting tests

The collection of bacterial isolates from rhizosphere soils and nodules was tested for their plant growth promoting (PGP) abilities

by solubilizing inorganic phosphate, auxin production, siderophore production and fluorescence production under ultraviolet radiation.

Screening for phosphate solubilizing bacteria

The ability of the bacterial strain to solubilize inorganic phosphate was tested on Pikovskaya's a solid medium (PVK) containing 0.5% tri-calcium phosphate ($Ca_3(PO_4)_2$, pH 7) as the sole source of P (Pikovskaya, 1948). The presence of a halo around the colonies indicates the production of phosphorus solubilizing substances (Desai and Amaresan, 2022).

For each strain, aliquots (5 µL) of bacterial cultures were spot inoculated on solid PVK medium with 4 replicates per isolate. After incubation of bacterial cultures at 28ºC for 7 days, the P solubilization index (PSI) was calculated following the formula proposed by Pande et al. (2017).

$$PSI = \frac{\text{Colony diameter} + \text{halo zone diameter}}{Colony\ diameter}$$

Screening for siderophore production

The bacterial isolate was spotted on chrome azurol sulphonate (CAS) agar plates and incubated at 28ºC for 5 days to screen for siderophore production (Schwyn and Neilands, 1987). Colonies exhibiting an orange halo in the dark blue medium were considered positive for siderophore production and the diameter of the halo was measured. Comparison of siderophore production between isolates was evaluated using the halo zone diameter and the ratio of halo to colony diameter (R), determined by the following formula:

$$R = \frac{\text{Halo zone diameter}}{Colony\ diameter}$$

Screening for indole acetic acid (IAA) production

Auxin (IAA) production was measured by a colorimetric method. The assay was performed in the presence or absence of the IAA precursor (l-tryptophan). Purified single colonies of bacterial isolates were added to Erlenmeyer flasks (250 ml) containing Luria-Bertani (LB) broth (100 ml) supplemented with 100 mg/L of l-tryptophan. The cultures were incubated for 48 h at 28°C with continuous shaking, followed by centrifugation at 6,000 x g for 6 min. The collected supernatant was filtered through nylon filters (0.2 µm) and this filtered supernatant (100 µL) was mixed vigorously with Salkowski reagent (100 mL) after 60 min incubation at room temperature. The IAA production was qualitatively assessed by the development of a pink colour. Auxin production was quantified using a standard curve against a known concentration of IAA at λ = 530 nm. The optical density of IAA was estimated at 530 nm by comparison with a standard curve prepared from known concentrations of IAA (Chen et al., 2021).

Nodulation and host symbiotic preferences

The isolates were tested for their ability to form nodules on Siratro (*Macroptilium atropurpureum*) and peanut (*A. hypogaea* cultivar 55-437). We included in the tests the *Bradyrhizobium* sp. strain LMG9283, isolated from peanut, as a positive reference control. Scarification and surface sterilization of *M. atropurpureum* seeds were achieved by soaking them in sulfuric acid (H_2SO_4, 96%) for 60 min. Each strain was grown in YM medium at 28°C with rotary shaking at 150 rpm. One week after germination, seedlings were inoculated with approximately log phase 10^8 cells of each isolate.

Plants inoculated using standard methods (Sene et al., 2013) and uninoculated controls were grown in Gibson tubes. Siratro plants were grown in a growth chamber (alternating 20 and 28°C) for 7 weeks. Plant nodulation was recorded at 30 days after inoculation.

Seeds of 55-437 peanut cultivars were first surface sterilized (to avoid seed-borne diseases) with 5% sodium hypochlorite (NaOCl) for 5 min, 70% ethanol for 3 min and thoroughly rinsed with sterile distilled water. The seeds were then placed on Petri dishes containing moist filter paper for germination under sterile conditions and kept in the dark at 25°C. The germinated seeds were manually transferred to 800 ml pots containing 200 g of leached-out loam sterilized at 120°C for 20 min. The pots were previously disinfected with a solution containing 1.81% of calcium hypochlorite. At harvest, whole peanut plants were uprooted. Data on plant height and leaf chlorophyll content were collected 45 days after planting. Plant height (cm) was measured with a ruler from the base of the stem to the apex, while the leaf chlorophyll content was quantified using a SPAD-502Plus chlorophyll meter (Konica-Minolta). The presence of nodules was checked, and the number of nodules was recorded. Shoot and root biomass was determined by weighing sample parts after over-drying to constant weight at 65°C.

Bacterial identification based on 16S rDNA gene

Fresh bacterial cells obtained as described above were subjected to a 16S full-length DNA sequencing. Primers PA (5'-AGAGTTTGATCCTGGCTCAG-3') and PH (5'-AAGGAGGTGATCCAGCCGCA-3') (Edwards et al., 1989) were used for PCR amplification. PCR was performed in a 50 µL reaction volume by mixing 2 µL DNA extract with the polymerase reaction buffer (10 mM Tris-HCl pH 8.3; 50 mM KCl; 0.1% gelatin, 2 mM $MgCl_2$); *Taq* polymerase (Bioprobe); 0.8 µL (5 U/reaction); 2 µL each of the 10 µM dNTPs (dATP, dCTP, dGTP, dITP); 1 µL of each primer (0.05 µM). PCR amplification was performed in a Gene Amp PCR System 2400 thermal cycler adjusted to the following temperature profile: initial denaturation at 94°C for 5 min; 35 amplification cycles (denaturation at 94°C for 1 min, hybridization of primers at 55°C for 1 min and extension at 72°C for 15 min); final extension at 72°C for 5 min. Negative control reactions (without a template) were also performed to check for experimental contamination. Amplification was checked by horizontal agarose (1%, w/v) gel electrophoresis using 10 µL of the PCR product. Amplicons were sequenced at the *Plateforme d'analyses génomiques* (IBIS, Université Laval, Quebec City, Canada).

The resulting forward and reverse DNA sequences were edited using BioEdit Sequence Alignment Editor version 7.7.1 to resolve oligonucleotide ambiguities and generate 16S sequence fragments. Sequences were compared to the National Center for Biotechnology Information GenBank databases using the Basic Local Alignment Search Tool nucleotidic (BLASTn) search program, and the closely related sequences found were included in phylogenetic analyses. Multiple alignments of sequence matrices were processed using ClustalW software implemented in the Mega 11 package (Tamura et al., 2021). Evolutionary distances were computed as described by Jukes and Cantor, and the evolutionary trees were inferred using the neighbor-joining method. Sequences were submitted to the GenBank database and assigned accession numbers, shown in Table 3.

Data analyses

All data were tested for normality and homogeneity using the Shapiro-Wilk and Levene tests, respectively, and statistically analyzed with a one-way analysis of variance (ANOVA) using the R

Table 2. Plant growth-promoting activities of the representative bacteria isolated from peanut nodules and rhizosphere soils.

Isolate	Tested propertie					
	Solubilization of inorganic phosphorus	Fluorescence under UV	Siderophor production	Auxin production	Nodulation with Siratro	Nodulation with peanut*
S6	+	+	+	+	-	-
S8	+	+	+	+	-	-
S13	+	+	+	+	-	-
S14	+	+	+	+	+	-
S16	+	+	+	+	+	-
S17	+	+	+	+	+	-
S19	+	+	+	+	-	-
S20	+	+	+	+	-	-
S22	+	+	+	+	-	-
S25	+	+	+	+	-	-
S26	+	+	+	+	-	-
S27	+	+	+	+	+	-
S29	+	-	+	+	-	-
S31	+	+	+	+	+	+
S33	+	+	+	+	+	+
S34	+	+	+	+	-	-
S35	+	+	+	+	-	-
S36	+	+	+	+	-	-
S37	+	+	+	+	+	+
S38	+	+	+	+	-	-
S41	+	+	+	+	-	-
S43	+	-	-	+	-	-
S44	+	+	-	+	-	-
S49	+	+	+	+	-	-
S50	+	+	+	+	-	-
S55	+	+	+	+	-	-
S56	+	+	+	+	-	-
S57	+	+	+	+	-	-
S58	+	+	+	+	-	-
S59	+	+	+	+	-	-

*The peanut cultivar *55-437* was used for the test.

software v3.4.4 (R Core Team, 2022).

Significantly different means and standard errors were separated using the Tukey (HSD) test at the 5% probability threshold.

RESULTS

Soil properties and bacterial isolation

The physical and chemical characteristics of the sampled sites are shown in Table 1 and differ from each other. Based on the macroscopic characters of bacterial colonies on LB medium, 61 isolates were obtained from soils, corresponding to 20, 15 and 26 isolates from MBS, SAG and KAT, respectively. In addition, 29 isolates were obtained from peanut nodules. Fluorescence emission under ultraviolet light showed that 39.56% of our collection emitted green fluorescence on the King B medium. Furthermore, the results differed according to the source of isolation. Isolates from nodules showed a higher capacity for fluorescence emission with 93% of the isolates tested. In contrast, fewer isolates from rhizospheric soils responded positively to the test, with 15 and 20% of isolates from KAT and MBS, respectively, and no fluorescence emission was detected with isolates from SAG (Figure 4).

Plant growth promoting attributes

In the current study, the strains were screened for their ability to solubilize inorganic phosphate and produce auxin and siderophore. Isolates were also tested for their ability to form nodules on siratro and peanut cultivar 55-437 (Table 2).

Table 3. Effect of inoculation with plant growth promoting bacteria on plant nodulation, leaf chlorophyll content, shoot length and biomass production (above-ground and root biomass). Peanut cultivar *55-437* was used for the test and plants were harvested 45 days after inoculation.

Isolate	Nodule numbers		Leaf Chlo.	Plant height (cm)		SDW (g)
	Peanut	Siratro	Peanut	Peanut	Siratro	Peanut
S6	0.0±0.0^{d}	0.0±0.0^{d}	20.6±1.6^{c}	45.3±2.0^{d}	16.1±1.3^{e}	0.89±0.24^{d}
S8	0.0±0.0^{d}	0.0±0.0^{d}	34.8±2.9^{b}	54.4±3.2^{bc}	21.4±3.7^{d}	1.42±0.21^{c}
S13	0.0±0.0^{d}	0.0±0.0^{d}	33.7±2.3^{b}	55.7±3.1^{bc}	27.0±3.9^{cd}	1.44±0.27^{c}
S14	0.0±0.0^{d}	4.3±1.26^{c}	35.9±2.5^{b}	56.2±3.2^{bc}	31.4±3.7^{bc}	1.48±0.21^{c}
S16	0.0±0.0^{d}	3.0±0.82^{c}	36.1±2.7^{b}	54.0±3.1^{bc}	36.1±1.3^{b}	1.47±0.21^{c}
S17	0.0±0.0^{d}	4.0±4.00^{c}	34.9±1.6^{b}	57.9±3.8^{ab}	34.6±2.5^{b}	1.46±0.22^{c}
S19	0.0±0.0^{d}	0.0±0.0^{d}	23.1±2.9^{c}	46.9±3.2^{cd}	15.6±3.2^{e}	1.09±0.24^{c}
S20	0.0±0.0^{d}	0.0±0.0^{d}	30.3±2.9^{b}	53.9±3.1^{bc}	29.0±2.7^{bc}	1.44±0.29^{c}
S22	0.0±0.0^{d}	0.0±0.0^{d}	21.5±1.1^{c}	46.5±3.9^{cd}	25.0±1.1^{cd}	1.19±0.25^{cd}
S25	0.0±0.0^{d}	0.0±0.0^{d}	18.9±2.9^{c}	45.9±3.0^{d}	23.8±2.9^{d}	1.12±0.27^{cd}
S26	0.0±0.0^{d}	0.0±0.0^{d}	19.5±2.6^{c}	46.7±3.7^{cd}	23.8±3.9^{d}	1.23±0.33^{cd}
S27	0.0±0.0^{d}	4.3±1.5^{c}	34.2±2.7^{b}	58.6±3.1^{ab}	33.6±3.2^{bc}	1.45±0.23^{c}
S29	0.0±0.0^{d}	0.0±0.0^{d}	21.9±2.5^{c}	48.3±3.6^{cd}	26.3±2.7^{cd}	1.27±0.22^{cd}
S31	92.0±6.6^{b}	16.3±2.7^{a}	44.7±2.8^{a}	62.1±3.4 ^{ab}	40.2±2.3^{a}	3.59±0.34^{b}
S33	40.3±5.5^{c}	14.9±3.8^{ab}	43.1±2.8^{a}	61.7±2.5^{ab}	42.5±2.3^{a}	3.63±0.21^{b}
S34	0.0±0.0^{d}	0.0±0.0^{d}	20.7±2.1^{c}	49.3±3.0^{cd}	15.1±1.5^{e}	1.27±0.23^{cd}
S35	0.0±0.0^{d}	0.0±0.0^{d}	19.8±2.7^{c}	47.9±3.8^{cd}	27.9±1.1^{cd}	1.29±0.24^{cd}
S36	0.0±0.0^{d}	0.0±0.0^{d}	18.6±3.8^{c}	45.5±3.6^{d}	11.6±1.3^{e}	1.22±0.22^{cd}
S37	5.3±0.5^{d}	9.7±1.83^{b}	33.2±2.0^{b}	58.1±3.5^{ab}	35.1±1.5^{b}	1.70±0.22^{c}
S38	0.0±0.0^{d}	0.0±0.0^{d}	34.8±2.3^{b}	53.6±3.5^{bc}	31.2±2.3^{bc}	1.60±0.12^{c}
S41	0.0±0.0^{d}	0.0±0.0^{d}	32.6±2.5^{b}	52.8±3.6^{bc}	25.0±1.1^{cd}	1.54±0.25^{c}
S43	0.0±0.0^{d}	0.0±0.0^{d}	21.3±2.4^{c}	46.8±3.5^{cd}	23.1±2.2^{cd}	1.22±0.27^{cd}
S44	0.0±0.0^{d}	0.0±0.0^{d}	33.9±2.1^{b}	51.7±3.8^{bc}	24.6±2.5^{d}	1.51±0.21^{c}
S49	0.0±0.0^{d}	0.0±0.0^{d}	33.1±2.7^{b}	55.2±3.0^{bc}	25.0±2.3^{cd}	1.54±0.21^{c}
S50	0.0±0.0^{d}	0.0±0.0^{d}	32.5±2.9^{b}	58.8±3.2^{ab}	30.9±3.4^{bc}	1.55±0.27^{c}
S55	0.0±0.0^{d}	0.0±0.0^{d}	34.9±2.6^{b}	54.7±3.1^{bc}	27.1±3.6^{c}	1.47±0.21^{c}
S56	0.0±0.0^{d}	0.0±0.0^{d}	22.4±1.9^{c}	47.2±3.3^{cd}	22.5±2.1^{d}	1.22±0.22^{cd}
S57	0.0±0.0^{d}	0.0±0.0^{d}	33.7±2.5^{b}	53.5±3.0^{c}	26.2±2.7^{c}	1.52±0.26^{c}
S58	0.0±0.0^{d}	0.0±0.0^{d}	24.0±2.8^{c}	48.6±3.0^{cd}	19.9±1.3^{d}	1.25±0.27^{cd}
S59	0.0±0.0^{d}	0.0±0.0^{d}	32.9±2.7^{b}	54.1±3.2^{c}	23.7±2.1^{d}	1.53±0.22^{c}
LMG9283	114.3±7.8^{a}	19.5±2.9^{a}	46.8±2.9^{a}	66.0±4.3^{a}	44.98±3.3^{a}	6.31±1.13^{a}
Control	0.0±0.0^{d}	0.0±0.0^{d}	19.6±2.7^{c}	45.3±3.0^{d}	13.5±2.7^{e}	0.89±0.14^{d}

(SDW) shoot dry weight; (Leaf Chlo.) leaf chlorophyll content; Values (mean ± standard error) are an average of four replications; means ± standard error within the same column followed by the same superscript letter are not statistically different at the 5% probability according to Tukey test.

Phosphate solubilizing bacteria

Pikovskaya solid medium showed phosphate solubilization around the colonies, with PSI index ranging from 0 to 2.9 (Figures 1 and 4). Various bacterial isolates have different phosphate solubilization capacities. No phosphate solubilization activity was found for the 26 isolates from the soil sampled at KAT. Nine of the 20 isolates, representing 45% of the bacteria isolated at MBS, showed a clear halo around the bacterial colony, indicating their phosphate solubilizing activity. These isolates had a PSI index ranging from 1.6 to 2.8. The PSI index varied between 2.3 and 2.8 for isolates from SAG and affected more than half of the total number of isolates. In contrast, all isolates from peanut nodules showed phosphate solubilization ability with a PSI index ranging from 2.4 to 2.9 (Figures 1 and 4).

Siderophore production

According to the results of this experiment, the production of the orange halo indicates that the strains are capable of producing siderophores. The ratio of halo to colony

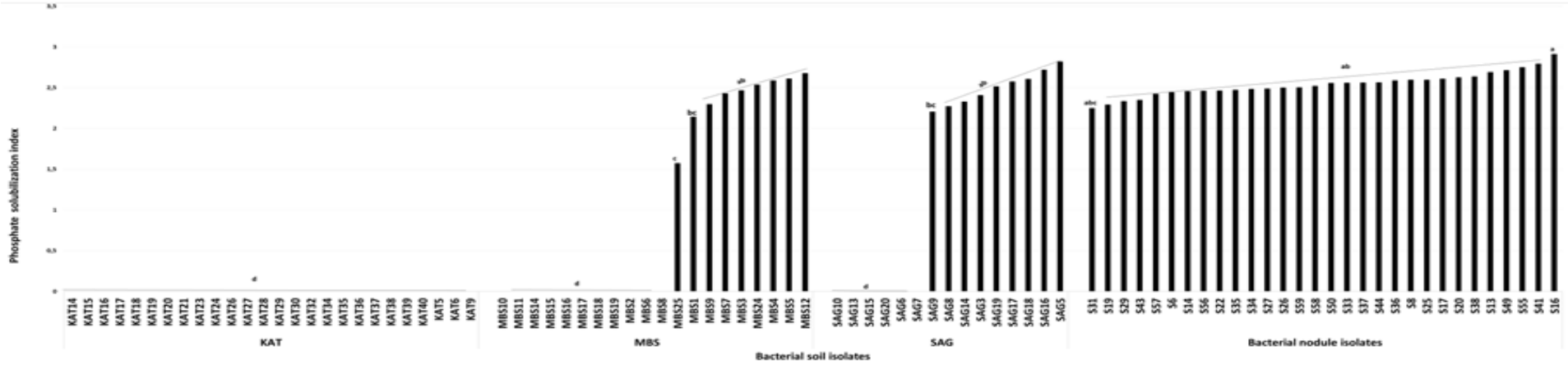

Figure 1. Comparison of the phosphate solubilization index (PSI) of the bacteria isolated from peanut nodules and rhizosphere soils. MBS (Mbousnakh, Department of Thiès); SAG (Sagne Mbambara, Department of Kaolack); KAT (Keur Ablaye Touré, Department of Koungheul); Values are the average of four replicates; bars with the same letter are not statistically different at the 5% probability according to the Tukey test.

diameter varied between 0 and 2.10 for different strains. The highest ratio of halo (2.1 cm) to colony diameter belonged to the strain isolated from peanut nodules, for which 93.4% of the isolates showed siderophore production activity. The bacterial isolates from rhizospheric soils produced halos with small diameters and the ratio of halo to colony diameter was 0 in 73.07, 75 and 93.33% of the isolates from KAT, MBs and SAG, respectively (Figures 2 and 4).

Indole acetic acid (IAA) production

The results showed that all isolates were able to produce auxin in the presence of L-tryptophan (Figure 3). However, there were significant differences among isolates. Auxin production was lower for isolates from rhizospheric soils and ranged from 100 to 400 ng/ml. The highest auxin production was recorded for isolates from nodules and ranged from 200 to 900 ng/mL, with the majority (60%) producing more than 600 ng/mL (Figures 3 and 4). All isolates tested showed the ability to produce auxin.

Nodulation ability and host symbiotic preferences

Seven isolates were able to form nodules with *M. atropurpureum* and three of them formed nodules with the peanut cultivar 55-437 (Tables 2 and 3). The results showed that PGPBs formed a weak nodulation compared to those of the reference strain LMG9283, isolated from peanut. Our comparison showed that treatments S31 and S33 produced very small nodules with a green colour, which were inefficient compared to LMG9283. Shoot dry biomass was significantly higher in plants inoculated with the reference strain LMG9283, while leaf colour and chlorophyll content were not different in this later treatment compared to the S31 and S33 treatments (Table 3). The other non-nodulated plants and the controls showed lower leaf chlorophyll content. In addition, plant height was significantly higher in the S31, S33 and LMG9283 treatments than in the control plants, but no significant difference was found compared to inoculation with S17, S27, S37 and S50, despite the absence of nodulation in these treatments (Table 3).

Bacterial identification using the 16S rDNA gene

A total of twenty-nine representative isolates were successfully sequenced for the PCR 16S rDNA region. The Blastn analysis of these sequences

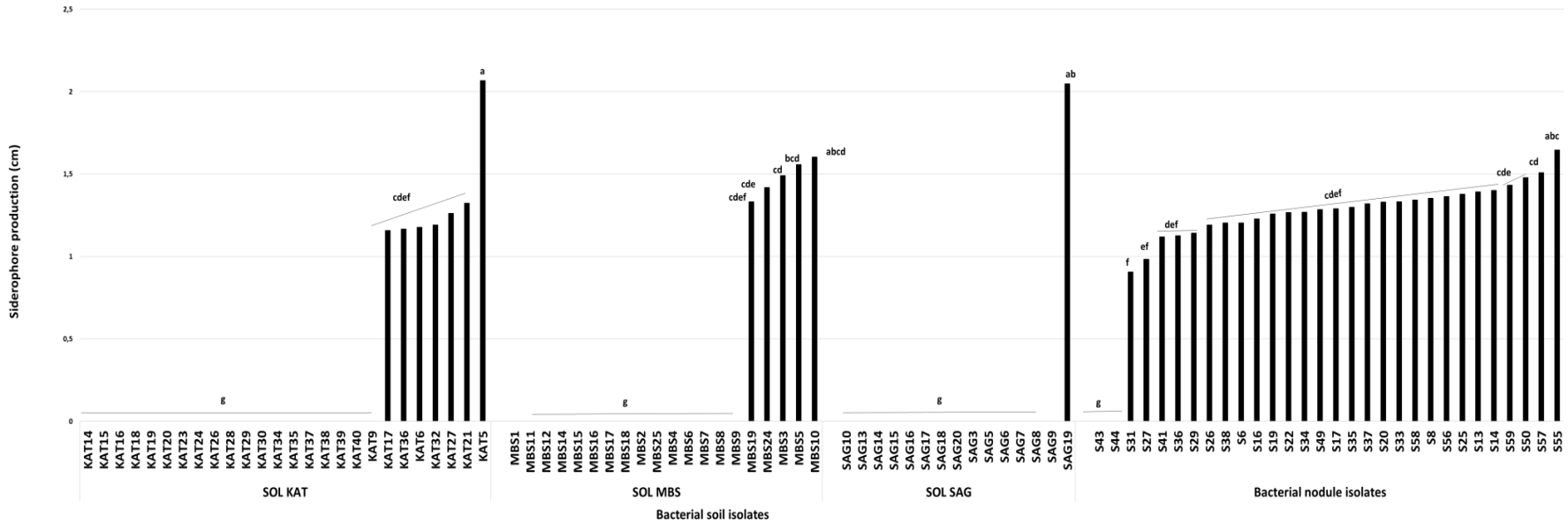

Figure 2. Comparison of siderophore production (diameter of halo around the colonies) by bacteria isolated from peanut nodules and rhizosphere soils. MBS (Mbousnakh, Department of Thiès); SAG (Sagne Mbambara, Department of Kaolack); KAT (Keur Ablaye Touré, Department of Koungheul); Values are the mean of four replicates; bars with the same letter are not statistically different at the 5% probability according to the Tukey test.

indicated that most of them (26 isolates) belonged to the genus *Burkholderia* (Table 4). The remaining isolates were most closely related to members of the genera *Bacillus*, *Brevundimonas* and *Leifsonia* (Figure 5). All of these genera have been previously reported to show PGP abilities. Among the *Burkholderia* isolates, *B. cepacia*, *B. cenocepacia* and *B. territorii* were the most representative species. The sequences were submitted to the GenBank database under accession numbers from OR642252 to OR642301 (Table 4).

DISCUSSION

Several useful plant growth promoting rhizobacteria (PGPR) can improve plant growth and yields (Vandana et al., 2021; Kandasamy and Kathirvel, 2023; Sene et al., 2021, 2023). These microorganisms are being promoted as biofertilizers to improve crop yields and minimize dependence on chemical fertilizers (Ríos-Ruiz et al., 2019; Kandasamy and Kathirvel, 2023; Sene et al., 2021, 2023). The present work was carried out to isolate bacteria from peanut nodules and rhizosphere soils and to evaluate the isolated bacteria for their PGP abilities.

A total of thirty representative isolates were successfully sequenced for the PCR 16S rDNA region. Among the nodule isolates, the genus *Burkholderia* was the most represented, followed by the genera *Bacillus*, *Brevundimonas*, *Dyella* and *Leifsonia*. All of these genera reported in our study (*Burkholderia*, *Bacillus*, *Brevundimonas*, *Dyella* and *Leifsonia*) have been previously shown to process PGP abilities (Busby et al., 2016; Ríos-Ruiz et al., 2019; Naqqash et al., 2020; Kandasamy and Kathirvel, 2023; Lu et al., 2023; Sene et al., 2021, 2023). Furthermore, *B. cepacia*, *B. cenocepacia* and *B. territorii* were the most abundant species isolated from nodules. It has been previously reported that *B. cepacia* and *B. territorii* species have great potential as plant growth promoting bacteria (Bagyalakshmi et al., 2017; Nath et al., 2017; Tapia-García et al., 2020; Júnior et al., 2020; Lau et al., 2020), which was consistent with our results. *Burkholderia*, *Bacillus* and *Leifsonia* have previously been reported as putative PGPBs in Senegal (Khbaya et al., 1998; Diouf et al., 2007), but this is the first time that

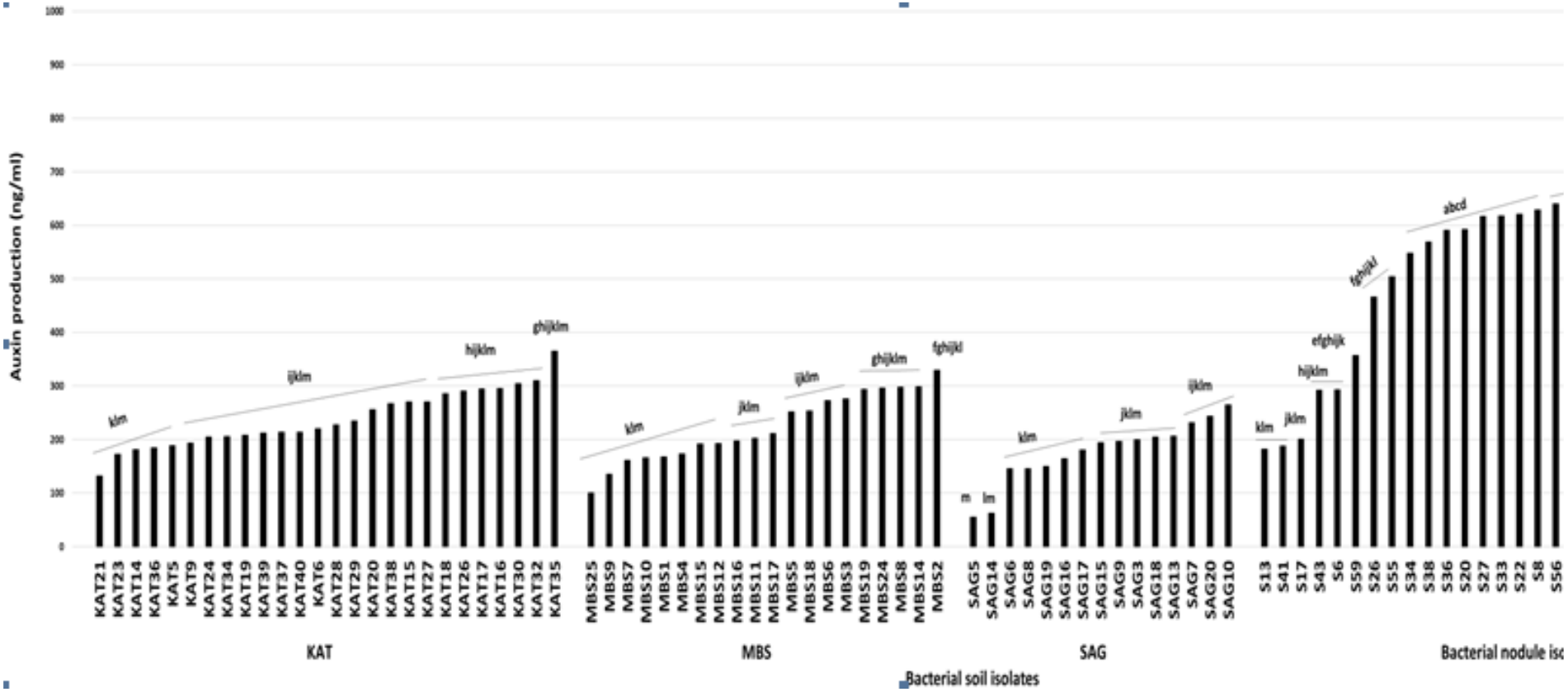

Figure 3. Screening and quantification of auxin production from bacteria isolates of peanut nodules and rhizosphere soils. MBS (Mbousnakh, Department of Thiès); SAG (Sagne Mbambara, Department of Kaolack); KAT (Keur Ablaye Touré, Department of Koungheul); Values are the mean of four replicates; bars with the same letter are not statistically different at the 5% probability according to the Tukey test.

Brevundimonas and *Dyella* are PGPBs and, for the latter, to nodulate peanut and siratro.

Screening bacterial isolates for PGP activities is important for selecting strains that can be used as bio-inoculants (Olanrewaju et al., 2017; Kandasamy and Kathirvel, 2023), increasing the likelihood of a more sustainable agricultural strategy. For instance, the ability of bacterial isolates to solubilize inorganic phosphate in the soil is highly beneficial as it can increase the availability of P to the plant (Kandasamy and Kathirvel, 2023), thereby increasing plant growth.

Fluorescent pigments are also important siderophores (Nath et al., 2017; Kandasamy and Kathirvel, 2023). Furthermore, the Fe chelating property of siderophores can greatly enhance plant Fe uptake and hence plant growth, particularly in calcareous soils where Fe deficiency is common (Collavino et al., 2012; Lotfi et al., 2022) or avoid Fe toxicity in soils with high Fe concentrations (Harish et al., 2023). On the other

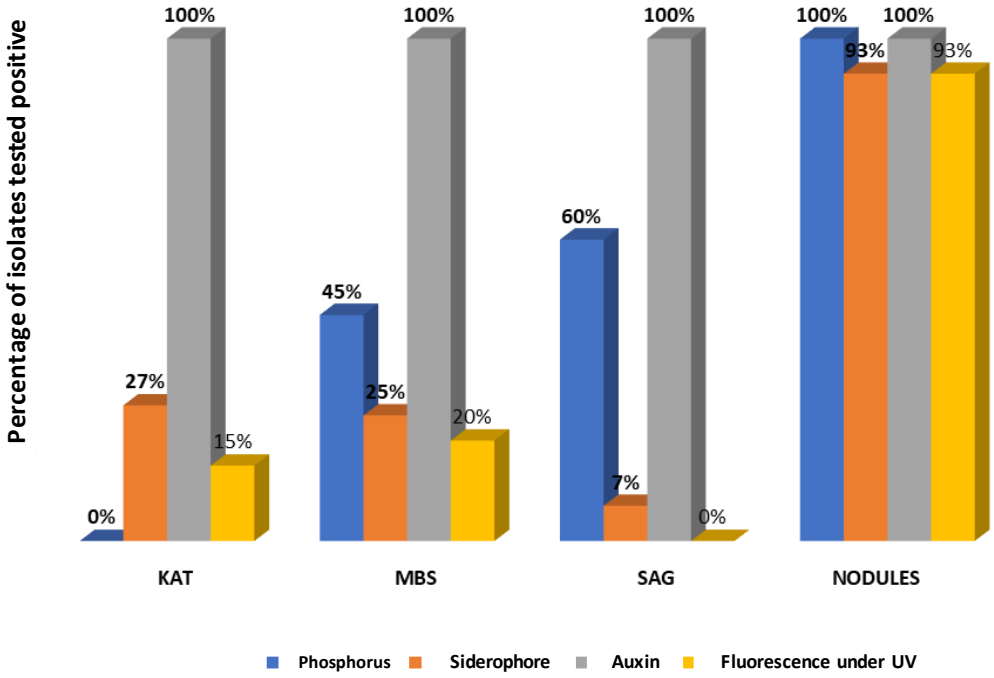

Figure 4. Percentage of isolates positive for PGP characteristics according to sample type (rhizosphere soils or nodules) and soil origin (MBS, SAG and KAT). MBS (Mbousnakh, department of Thiès); SAG (Sagne Mbambara, department of Kaolack); KAT (Keur Ablaye Touré, department of Koungheul).

hand, auxin is an important phytohormone involved in many important physiological processes in plants, including cell growth and division and tissue differentiation, thereby promoting the growth of plant roots and shoots (Chen et al., 2021). With these considerations in mind, we evaluated our bacterial collections to identify strains with PGP functions. A subset of isolates from each category (nodule and rhizosphere soil) exhibited these traits, indicating their potential to promote plant growth. Fluorescence emission under ultraviolet light was found in 39.56% of our collection and all isolates were able to produce auxin, which appears to be a general feature of PGP bacteria. Numerous previous studies have also reported that auxin production is a trait common to PGPBs (Olanrewaju et al., 2017; Hamim et al., 2019; Kandasamy and Kathirvel, 2023), in contrast to other PGP attributes. Overall, about 52% of the isolates showed phosphate solubilizing capacity and 46.47% produced siderophores. Although we have not yet tested the PGPBs under field conditions, their potential as valuable biological resources for enhancing growth and biomass production effects when interacting with peanut and siratro plants under the tested growth chamber conditions is evident. Thus, the isolates have the potential for future biotechnological applications through the development of biologicals for sustainable crop improvement.

The comparison between isolates from rhizosphere soils and those from nodules showed striking differences in their PGP performance. In particular, we found that nodule isolates were more responsive to phosphorus and siderophore PGP assays. For instance, all isolates from peanut nodules showed the ability to solubilize phosphate and produce siderophores, whereas only a few isolates from rhizospheric soils responded positively. Endophytic bacteria that reside intercellularly or intracellularly in host tissues have an advantage over free-living bacteria in that they are protected from environmental stress and microbial competition (Vandana et al., 2021; Kandasamy and Kathirvel, 2023). This may explain why nodule isolates exhibited more intense PGP activities than free-living bacteria from rhizospheric soils.

Therefore, it could be recommended to use nodules instead of soils for the isolation of peanut PGPB. On the other hand, amplification of peanut roots using primers targeting the gyrB gene (Barret et al., 2015) resulted in very weak bands on the gel, whereas the AMV4ngs/AMDGRngs primer pair targeting small subunit rRNA (SSU, 18S) (Lumini et al., 2010) showed clear bands in the same protocol (data not shown). This suggests that endophytic bacteria associated with peanut plants may preferentially coexist within root nodules and are probably less present in root tissues.

Bradyrhizobium spp. strains were also isolated together with PGP bacteria, suggesting that both coexist in peanut nodules. Other studies have confirmed the existence of a

Table 4. Phylogenetic affinity between the 16S gene sequences of the representative PGPR isolates from the peanut root nodules and the sequences published so far in GenBank.

Isolates	GenBank Accession no.	Most related species in GenBank	Strain	GenBank accession no.	% Identity
S6	OR642297	*Burkholderia cepacia*	G63	EU597838.1	97.19
S8	OR642298	*Burkholderia cepacia*	TR2	OK428691.1	98.55
S13	OR642299	*Burkholderia territorii*	KBB5	MN032407.1	100
S14	OR642285	*Burkholderia metallica*	BAB-6777	MF319855.1	99.58
S16	OR642261	*Burkholderia cepacia*	G63	EU597838.1	99.65
S17	OR642262	*Burkholderia cepacia*	G63	EU597838.1	99.58
S19	-	*Burkholderia cenocepacia*	BCC90	LT158018.1	85.20
S20	-	*Burkholderia metallica*	BAB-6777	MF319855.1	99.37
S22	OR642286	*Burkholderia metallica*	BAB-6777	MF319855.1	99.79
S25	OR642287	*Burkholderia seminalis*	IHB B 15122	KM817204.1	99.86
S26	OR642288	*Burkholderia cepacia*	JCM 5508	LC462130.1	99.93
S27	OR642265	*Burkholderia cepacia*	ORS 3307	EF054874.1	99.44
S29	OR642289	*Burkholderia cepacia*	ORS 3307	EF054874.1	99.65
S31	OR642268	*Burkholderia cepacia*	ATCC 25610	AY741341.1	100
S33	OR642270	*Dyella telluris*	G9	NR_175549.1	99.18
S34	-	*Burkholderia sp.*	JSA5	LC682223.1	99.74
S35	OR642300	*Burkholderia metallica*	BAB-6777	MF319855.1	99.53
S36	OR642301	*Burkholderia cepacia*	ORS 3307	EF054874.1	99.37
S37	OR642271	*Burkholderia territorii*	KBB5	MN032407.1	99.72
S38	-	*Burkholderia cepacia*	ORS 3307	EF054874.1	99.58
S41	OR642290	*Burkholderia cenocepacia*	FC1395	MG871242.1	99.65
S43	-	*Bacillus piscis*	CGNE-105	OL697806.1	89.20
S44	-	*Brevundimonas sp.*	PP_Gw_2a_4	MG232361.1	98.98
S49	OR642291	*Burkholderia metallica*	BAB-6777	MF319855.1	99.72
S50	OR642292	*Burkholderia cepacia*	G63	EU597838.1	97.86
S55	OR642293	*Burkholderia metallica*	BAB-6777	MF319855.1	99.38
S56	-	*Leifsonia sp.*	J1	MG417098.1	95.50
S57	OR642294	*Burkholderia metallica*	BAB-6777	MF319855.1	97.52
S58	OR642295	*Burkholderia cepacia*	G63	EU597838.1	99.52
S59	OR642296	*Burkholderia metallica*	BAB-6777	MF319855.1	97.10

diversity of bacteria coexisting with rhizobia in root nodules (Sene et al., 2013; Ríos-Ruiz et al., 2019; Hossain et al., 2023; Lu et al., 2023). In general, most of these coexisting PGPs are unlikely to be involved in nodule formation per se. Diouf et al. (2007) reported the coexistence of phytopathogenic *Agrobacterium* and *Burkholderia* strains in the root nodules of *Vachellia seyal* Del. (formerly: *Acacia seyal*) plants with symbiotic rhizobia. Non-nitrogen fixing bacteria have also been isolated from the root nodules of many legume species in Africa (Khbaya et al., 1998; de Lajudie et al., 1999; Diouf et al., 2007). However, in our case study, the cross-inoculation test showed that seven isolates were able to form nodules with *M. atropurpureum*, and three of these (*Burkholderia* and *Dyella*) induced root nodule formation with *A. hypogaea* cultivar 55-437. This ability to nodulate siratro and peanut legumes suggests that these bacteria may have acquired the genes responsible for nodulation in Senegalese soils, probably by lateral transfer, as previously reported by Tapia-García et al. (2020). We did not directly measure gene expression or N_2-fixation activity in these nodules, but the examination of several nodules revealed small nodules with a light pink/ or green colour, suggesting ineffective nodules. Previous reports also suggest that small nodules (<1.0 mm) with a light red/ or pink colour may have less N_2-fixing activity than medium-sized (1.5 to 2.0 mm) nodules (Tajima et al., 2007). Further studies targeting the nifH gene (encoding the nitrogenase reductase subunit) (Gaby, and Buckley, 2012) are required to confirm the nitrogen fixing abilities of these isolates. The rates of nitrogen fixation have been previously reported to be associated with both nifH abundance (Reed et al., 2010) and nifH diversity (Hsu and Buckley, 2009). Furthermore, despite their immense potential in various aspects of promoting plant growth, many of the *Burkholderia* species, especially *B. cepacia* and *B. territorii*, are opportunistic human and plant pathogens (Diouf et al., 2007; Ríos-Ruiz et al., 2019),

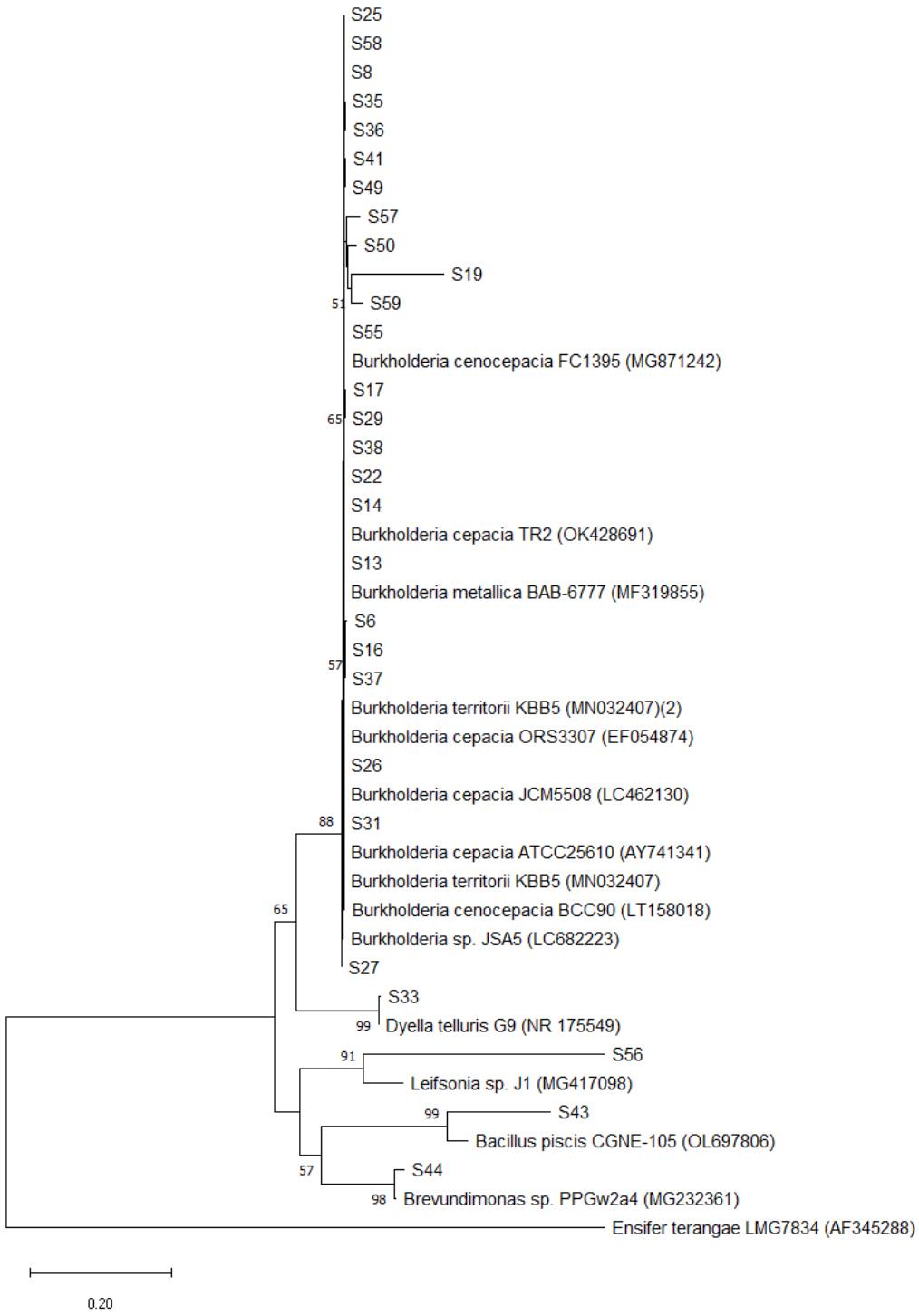

Figure 5. Phylogenetic tree based on partial 16S rRNA gene sequences for the isolates obtained in this study and related bacteria retrieved from GenBank. The tree was constructed by using the neighbor-joining method with the Kimura 2-parameter distance correlation model. Numbers at the nodes are bootstrap percentages for 1,000 resamplings; values below 50% are not shown. The accession numbers of the reference strains are given in parentheses.

thus raising pathogenicity problems in their agricultural applications. It is therefore equally important to determine the potential pathogenicity of *Burkholderia* isolates prior to any environmental application. We are also carrying out Illumina-MiSeq sequencing studies to disentangle this complex relationship between different bacteria within

peanut nodules and to improve our understanding of their occurrence into legume-bacteria associations.

Conclusion

As agriculture moves toward ecological intensification practices, plant symbionts, such as plant growth-promoting rhizobacteria (PGPR), will likely play a larger and more important role in sustainable agriculture. The objectives of this study were to isolate PGP bacteria from peanut nodules and rhizosphere soils and to evaluate the isolated bacteria for their PGP functions. We obtained 90 isolates from soils and root nodules collected along a rainfall gradient in the Senegalese peanut basin. The isolates were identified through 16S rRNA sequencing. The results showed that the isolates belonged to the genera *Burkholderia, Bacillus, Brevundimonas, Dyella*, and *Leifsonia*. All isolates screened were positive for at least one of the evaluated traits; however, isolates belonging to *B. cepacia* and *B. territorii* were the most abundant, producing the highest levels of auxin and siderophores while demonstrating superior performance in phosphate solubilization. Additionally, the strains with the best plant growth-promoting abilities were isolated from peanut root nodules. Our results suggest that diverse endophytic bacteria preferentially coexist with bradyrhizobia within peanut nodules and are likely to be less abundant in root tissues. These isolates have potential for future biotechnological applications through the development of biologicals for sustainable crop improvement. However, further research is needed to evaluate the in vivo PGP performance of these microorganisms on peanut plants to produce safe and beneficial inoculants. Additionally, it is crucial to assess the potential pathogenicity of *Burkholderia* spp. isolates before any environmental applications. We found that seven isolates were capable of inducing nodule formation on siratro plants, and three of them also induced nodule formation on peanuts. Furthermore, this report demonstrates for the first time the nodulation capacity of the genus *Dyella*. Thus, further studies are required to confirm the nitrogen-fixing abilities of these PGPB isolates, particularly by targeting the nifH gene, which encodes the nitrogenase reductase subunit.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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