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Community structure and plant growth-promoting potential of cultivable bacteria isolated from Cameroon soil

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ABSTRACT

Exploiting native plant growth-promoting rhizobacteria (PGPR) in Cameroonian agro-ecosystems provides a means to improve plant–microbe interactions that may enhance ecosystem sustainability and agricultural productivity in an environmentally eco-friendly way. Consequently, we aimed to investigate the community structure and functional PGPR diversity of maize grown in Cameroon. Native bacteria isolated from Cameroon maize rhizosphere soil were identified by partial 16S rRNA gene sequencing and screened for traits particularly relevant for Cameroon low-fertility soil conditions, such as their abilities to tolerate high concentrations of salt, and their plant growth- promoting potential. Genetic and functional diversity was characterized according to their phylogenetic affiliation. A total of 143 bacteria were identified and assigned to 3 phyla (*Actinobacteria*, *Firmicutes* and *Proteobacteria*), 13 families and 20 genera. *Bacillus* (31.5%), *Arthrobacter* (17.5%), and *Sinomonas* (13.3%) were the most abundant genera identified among all the isolates. Based on their *in vitro* characterization, 88.1% were salt tolerant at 2% NaCl, but only 16.8% could tolerate 8% NaCl, 50.4% solubilized phosphate, 10.5% possessed the *nifH* gene, and 19.6% produced siderophores. Six isolates affiliated to the most abundant genera identified in this work, *Bacillus* and *Arthrobacter,* carrying multiple or only single tested traits were selected to evaluate their growth- promoting potential in an *in vitro* maize germination assay. Three strains possessing multiple traits induced significantly increased hypocotyl and root length of maize seeds compared to non-inoculated control seeds. Our results indicate the potential of selected indigenous Cameroon rhizobacteria to enhance maize growth.

1. Introduction

Maize (*Zea mays* L.) is the most widely-grown staple food crop, occupying nearly 17% of the estimated 200 million ha of cultivated land in sub-Saharan Africa. It is also one of the world's three most important food crops (Johnston-Monje and Raizada, 2011), growing under a wide spectrum of soil and climatic conditions around the world (Farooq et al., 2015). In Cameroon, maize is the most consumed cereal, easily exceeding sorghum, rice, or wheat (Manu et al., 2014). Maize is a strategic crop in terms of food security and economic profitability. The plant is mainly grown by small-scale subsistence farmers (Epule and Bryant, 2014). Although maize is cultivated in all five agro-ecological zones of Cameroon and is the most affordable crop in terms of market price and cost of seeds, grain yields often remain low compared with local food demand. One reason is that Cameroonian soils are generally low in fertility, particularly lacking phosphorus (P) and nitrogen (N) (Fankem et al., 2006). Low soil fertility and other issues such as salinity, soil acidity and water stress are the major limiting factors in Cameroonian agriculture, since nutrient deficient and salt-stressed soils are known to severely suppress plant growth and crop productivity. Considering the low corn yields still pervasive in farmers' fields, increasing maize production is an urgent task to secure food supplies in the country.

In particular, the P fixation capacity of soils is a critical problem that

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leads to low soil fertility. P is a component of key molecules such as nucleic acids, phospholipids, and ATP. It is also involved in controlling key enzyme reactions and regulating metabolic pathways, and consequently, plants cannot grow without a reliable supply of this nutrient (Theodorou and Plaxton, 1993). Like many tropical and subtropical soils, Cameroonian soils are predominantly acidic; a high content of iron and aluminum ions effectively react with P in such soils. Consequently, about 75% of P applied as chemical fertilizer or natural rock phosphate is converted into insoluble complexes (Gyaneshwar et al., 2002), making the P-deficiency in soil difficult to overcome.

Seeking a solution, soil microorganisms could contribute efficiently to improving soil fertility. In the rhizosphere, the volume of soil surrounding and under the influence of plant roots is where plants interact with soil microorganisms (Antoun and Prévost, 2005). Many rhizosphere-inhabiting bacterial species are known to exert beneficial effects upon plant growth. Those bacteria are generally referred to as plant growth-promoting rhizobacteria (PGPR). PGPR use various strategies to promote plant growth, such as improving phosphate uptake by solubilizing phosphate complexes into plant absorbable and usable forms, suppressing plant diseases by competitive colonization or inducing systemic or acquired resistance, and producing phytohormones or vitamins (Glick, 2012; Berger et al., 2015). Besides the other plant growth-promoting (PGP) traits, the ability to solubilize different kinds of synthetic inorganic phosphate and natural rock phosphates is particularly crucial in the selection of suitable bacterial candidates for Cameroonian agriculture. Moreover, seeking PGPR possessing novel traits such as salt tolerance will improve salinity management in these nutrient deficient and salt affected soils.

In the rhizosphere of maize grown in many countries, PGP activities have been reported for a series of bacterial species including *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Bacillus* and *Serratia* (Agbodjato et al., 2015; Zahid et al., 2015; Kuan et al., 2016). Despite the potential benefits of using PGPR to enhance crop productivity and improve crop protection under normal and salt stressed conditions (Ahemad and Kibret, 2014; Sharma et al., 2016), these strategies are still largely untapped in the effort to improve maize production in Africa. Especially in Cameroon, little information is available on the occurrence and use of PGP bacteria, and no research has been devoted so far to studying indigenous PGPR associated with maize grown in the country.

It is important to study native microbial communities associated with plants in order to understand their ecological role in specific environments (Cavaglieri et al., 2009). Studies have shown that to maximally exploit the plant-bacteria association effective bacteria must be selected in plant studies that take specific ecological conditions into consideration, e.g. crop management, soil type and climate (Perez-Montano et al., 2014). Under such conditions, knowledge about the native bacterial populations, their identification and their implications for plant physiology, is required for improving management practices regarding plant nutrition and defense.

Application of bacterial inoculants to reduce the use of chemical fertilizers without compromising plant yield and quality is currently an important challenge in agriculture, microbiology, and biotechnology. Towards a sustainable agricultural vision, interest in the beneficial rhizobacteria associated with cereals in particular has increased recently (Vejan et al., 2016). Making this technology readily accessible to farmers in both developed and developing countries and efforts are being made to exploit diverse PGPR as bio-fertilizers for various economically important crops (Ahemad and Kibret, 2014; Zahid et al., 2015). Many PGPR genera and species have been reported to be present in the rhizosphere of numerous crops (Mehnaz et al., 2010). Moreover, they have been used to successfully improve plant growth of maize, rice, wheat, soybean and other horticultural crops both in the laboratory and in the field under various ecological conditions (Shaharoona et al., 2008; Perez-Montano et al., 2014). The use of indigenous PGPR for creating bacterial inoculants can be an advantage since these organisms easily acclimatize to the respective environmental conditions and may more easily establish the plant-microbe interaction (Verma et al., 2013).

In this study, we aim to isolate and characterize rhizobacteria from maize cultivated in the region of Cameroon with the highest maize cropping density. We hypothesize that the rhizosphere of maize grown in Cameroon harbors a high diversity of cultivable bacteria exhibiting multiple plant growth– promoting and salinity tolerance activities. The main goal of our study was to: (i) isolate a wide range of native cultivable bacterial strains from the maize rhizosphere in Cameroon; (ii) characterize the isolates based on their attributes and phylogenic affiliation (partial 16S rRNA gene sequence); (iii) evaluate their *in vitro* potential for salinity tolerance, synthetic and natural inorganic phosphate solubilization, atmospheric nitrogen (N_2) fixation by searching for the presence of *nifH* gene and siderophore production, and (iv) assess the bacterial *in vitro* effect on maize seedlings at the germination stage.

2. Materials and methods

2.1. Study site and sample collection

Soil samples with the characteristics described in Table 1 were collected from maize rhizospheres at a farm in the Ngaoundal locality (6° 30″ North, 13° 16″ East) in the high Guinea savanna zone II, where the southern plateau raises northward to the grassy, rugged Adamawa Plateau (Fig. 1). This feature stretches from the western mountain area and forms a barrier between the country's north and south. Its average elevation is 1,100 m, and temperatures range from 22 °C to 25 °C with high rainfall. The spot was chosen for sample collection because it is the most cultivated maize region in the country. Rhizosphere soils adhering to maize roots at a depth of 10–20 cm were collected from 20 randomized plant rhizospheres of the farm. The samples were mixed to form a composite sample, then packed in a sterile plastic bag and immediately taken to the laboratory. The soil was passed through a 4 mm sieve to eliminate coarse rock and plant material, thoroughly mixed to ensure uniformity, and stored at 4 °C prior to use. A subsample about 0.5 kg was air dried and passed through a 2 mm sieve for chemical analysis. Soil pH was determined in a suspension of soil in saline solutions of neutral reactivity (calcium chloride/$CaCl_2$) in a ratio of 1–2.5 according to Krey et al. (2011). Available amounts of phosphate (P) and potassium (K) were extracted by the double lactate (dl) method and measured using flow injection analysis (FIA) for P (P_{dl}) and atomic absorption spectrophotometry (AAS) for K (K_{dl}) (Krey et al., 2011). Exchangeable magnesium (Mg) was determined using a $CaCl_2$ solution by ASS at 285.2 nm. Total carbon (C_t) and total nitrogen (N_t) contents were measured using a CHN-O Rapid elemental analyzer (Heraeus, Germany) (Ruppel et al., 2006).

2.2. Isolation, purification and conservation of bacterial isolates

The isolation of microorganisms was assessed in non-selective nutrient agar (NA) medium (Standard nutrient agar I, Carl Roth, Germany) containing 6 g NaCl, 3 g yeast extract, 15 g peptone, 1 g glucose, 12 g agar–agar L^{-1}, pH 7. Four independent replicates were analyzed as follows: Ten g soil was homogenized in Erlenmeyer flasks in 90 mL of sterile buffer (NaCl, 0.05 M) by shaking at 290 rpm for one

Table 1
Elemental composition of the soil sample (mean values of four replicates).

pH	mg kg^{-1}					C/N ratio
	P_{dl}	K_{dl}	Mg	N_t	C_t	
5.6	49	62	81	2380	27930	11.7

dl = double lactate extractable

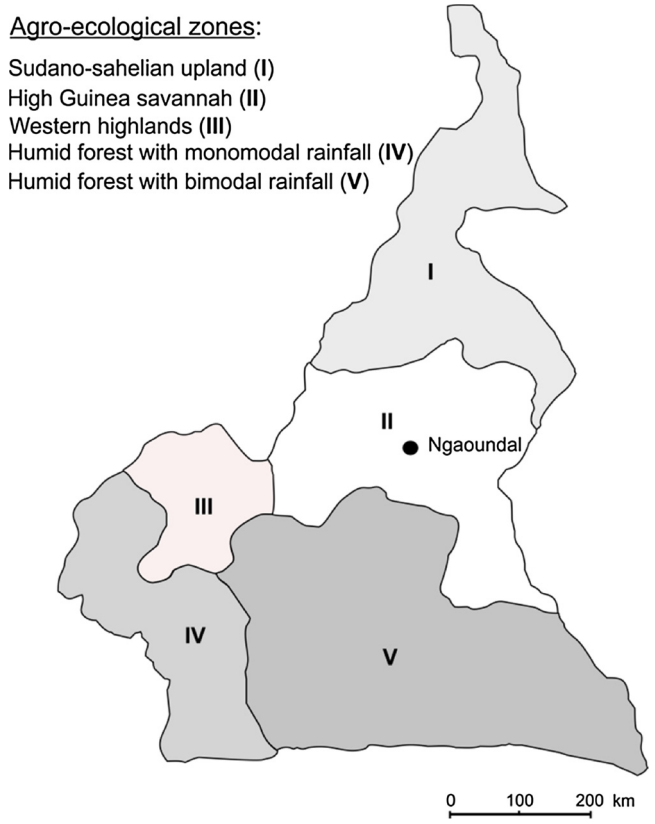

Fig. 1. Location of the sampling site: different agro-ecological zones of Cameroon and the sampling site (Ngaoundal) represented as a black dot in zone II.

hour. This solution was ten-fold diluted (10^{-1} to 10^{-7}) and 0.1 mL aliquots of dilutions 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were plated on nutrient agar media in triplicates. The plates were incubated at 28 °C for five days. Individual colonies were morphological characterized based on their size, shape, color, surface, margin and elevation (Santos et al., 2015), grouped and quantified. Twenty colonies of each morphotype were picked and streaked on the NA media for further purification. Pure strains were stored in 50% sterile glycerol at −80 °C.

2.3. Molecular identification and phylogenetic analysis of bacterial isolates

The extraction of genomic DNA from overnight pure bacterial culture grown in nutrient broth (Standard nutrient broth I, Carl Roth, Germany) at 28 °C was performed using the DNeasy Plant Mini kit (QIAGEN, Germany) by following the manufacturer's instructions. The DNA concentration was determined photometrically at 260 nm and quality was checked by the A_{260}/A_{280} ratio calculation being above 1.9 and the A_{320} measurement nearly 0 using NanoDropR (ND-100 spectrophotometer, Peqlab, Germany). The genomic DNA extracted from all isolates was used for partial 16S rRNA gene amplification using 16S rDNA universal primers: 9bfm (5′ -GAGTTTGATYHTGGCTCAG-3′) and 1512R (5′ -ACGGHTACCTTGTTACGACTT-3′) (Muhling et al., 2008). Amplification reactions were performed in 25 µL reactions containing 12.5 µL Top Taq Mastermix, 5.5 µL PCR water, 2.5 µL of (3.1 pmol µL^{-1}) each primer and 2 µL of template DNA (15 ng µL^{-1}). Amplification of 16S rRNA gene portions was carried out in a thermocycler (Peqstar of Peqlab, Germany). The conditions were as follows: an initial activation step at 96 °C for 4 min, followed by 30 cycles comprising denaturation at 96 °C for 1 min, primer annealing at 56 °C for 1 min, primer extension at 74 °C for 90 s and finally extension at 74 °C for 10 min. All amplified PCR products were confirmed by electrophoresis on 1.5% agarose gels in 1X TAE buffer containing gel red nucleic acid stain (0.025 µL mL^{-1}), using gene ruler 1 kb DNA ladder as a size marker. Amplicons were then purified using a PCR purification kit (QIAGEN, Germany) according to the standard protocol recommended by the manufacturer.

The purified PCR products were sequenced using the DNA sequencing service of Eurofins Genomics, Germany. The bacterial 16S rDNA nucleotide sequences (mean length of 790 bp) were aligned with known sequences in the NCBI (http://blast.ncbi.nlm.nih.gov) and Ribosomal Database Project (RDP) databases using BLASTn. Sequences of all related species were retrieved to derive the nomenclature of the isolates. Multiple sequence alignments with the most closely related bacterial sequences were performed using Muscle (https://www.ebi.ac.uk/Tools/msa/muscle/) and phylogeny was inferred by the Maximum Likelihood approach based on the Tamura 3-parameter model and the neighbor-joining method (Saitou and Nei, 1987), using Mega 7 version 7.0.21 (http://www.megasoftware.net/). Phylogenetic tree topology based on re-sampling 1000 times the neighbor joining data set was evaluated by bootstrap analysis. To better understand and facilitate the visualization of functional diversity between and within genera, Interactive Tree Of Life web-based tool (http://itol.embl.de) was used to display the different traits harbored by each bacterial isolate on the phylogenetic trees (Letunic and Bork, 2016).

2.4. In vitro characterization of bacterial isolates

2.4.1. Bacterial tolerance to salinity

The intrinsic tolerance of bacterial isolates to salinity was evaluated by observing the bacterial growth on Standard I Nutrient agar (Carl Roth, Germany) amended with various concentrations of NaCl (2, 4, 6, and 8% w:v). Control plates were also maintained with 0.05% NaCl (w:v). The isolates were streaked on plates and placed in an incubator at 28 °C. The plates in triplicate for each strain were examined for bacterial growth after 72 h, and the supplemented plates were compared with controls without salt addition (Upadhyay et al., 2009).

2.4.2. Phospate solubilization assay

The ability of isolates to solubilize seven different inorganic phosphate sources (tricalcium phosphate, hydroxyapatite, Malian rock phosphate (RP), Cameroonian RP, Algerian RP, Mexican RP, Moroccan RP) was assessed on plates filled with the National Botanical Research Institute's Phosphate growth medium (NBRIP) (Nautiyal, 1999). The NBRIP was modified as follows, containing per liter of distilled water: 20 g glucose, 5 g $MgCl_2{\cdot}6H_2O$, 0.25 g $MgSO_4{\cdot}7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$ and one phosphate type at 5 g, agar–agar 15 g, plus 0.5% bromocresol green, pH 7.5 (Fankem et al., 2014). A stock solution of 0.5% dye was prepared by dissolving a corresponding weight of bromocresol green into 70% ethanol and the final pH adjusted to 6.5 with 1 M KOH. To remove their soluble P fractions, all phosphate sources were washed 4 times with warm water following the cycle: 1 h–24 h–1 h–24 h. They were then dried at 60 °C until complete evaporation of water, and homogenized before use. 10 µL of each bacterial suspension of adjusted OD to 0.2 at 620 nm were then transferred onto a single point of compartmented Petri dishes. Plates in triplicate for each bacterial isolate were incubated at 28 °C for 7 days and positive isolates recorded through the yellow halo zone surrounding the bacterial colony.

2.4.3. Amplification of the nifH gene

Potential N_2-fixing bacteria were determined by searching for the presence of the *nifH* gene, the marker gene for biological nitrogen fixing ability (Juraeva et al., 2006). Universal primers 19F (5′-GCIWTYTAYGGIAARGGIGG-3′) and 366R (5′-AAICCRCCRCAIACIACRTC-3′) (Juraeva et al., 2006), 50 ng of template DNA, 5.5 µL PCR water, 2.5 µL of (3.1 pmol µL^{-1}) each primer and 12.5 µL of QIAGEN Top Taq Mastermix were used to amplify *nifH* PCR fragments (350 bp). The following PCR reaction conditions were applied: initial activation step at

94 °C for 4 min, followed by 35 cycles comprising denaturation at 94 °C for 30 s, primer annealing at 55 °C for 1 min, primer extension at 72 °C for 75 s and finally extension at 72 °C for 10 min in a thermocycler (Peqstar of Peqlab, Germany). PCR with water was used as a no template control, and the diazotrophic bacterial strain DSM16656 *Kosakonia radicincitans* (Ruppel and Merbach, 1995; Berger et al., 2015) served as a positive control.

2.4.4. Siderophore production assay

Siderophore production by bacterial isolates was determined following the universal assay of Schwyn and Neilands using CAS-blue plates as described by Ji et al. (2014). First bacterial suspensions were adjusted to OD 0.2 at 620 nm, then 10 µL were dropped onto the plate and incubated at 28 °C for 72 h. Color change reaction on the CAS-blue agar plate to orange, indicated the presence of siderophore after chelation of the bound iron. The experiment was performed once with three replicates for each bacterial strain.

2.5. Seed germination bioassay

Six selected bacterial strains (V1, V39, V54, V62, V64, V84) presenting different abilities for the specific traits tested and belonging to *Arthrobacter* and *Bacillus* genera were assessed for their ability to promote maize growth at the germination stage. Maize seeds variety "LUIGI CS" (Caussade, France) were surface sterilized using NaOCl 3% as described earlier by Johnston-Monje and Raizada (2011). Bacterial inoculums were prepared by transferring a single pure colony of each bacterial isolate into 100 mL Erlenmeyer flasks containing 50 mL nutrient broth and grown in flasks on a rotary shaker (180 rpm) at 28 °C for 24 h. Bacterial cells were harvested and washed three times in 0.05 M sterilized NaCl solution after centrifugation (10,000 × *g*) for 15 min at 4 °C and finally suspended in 0.05 M sterilized NaCl solution until the population reached 10^8 colony forming units (CFU) mL^{-1}. Six surface sterilized seeds were dipped in bacterial cultures (microbial treatments) or 0.05 M NaCl (control treatments) for 20 min and then

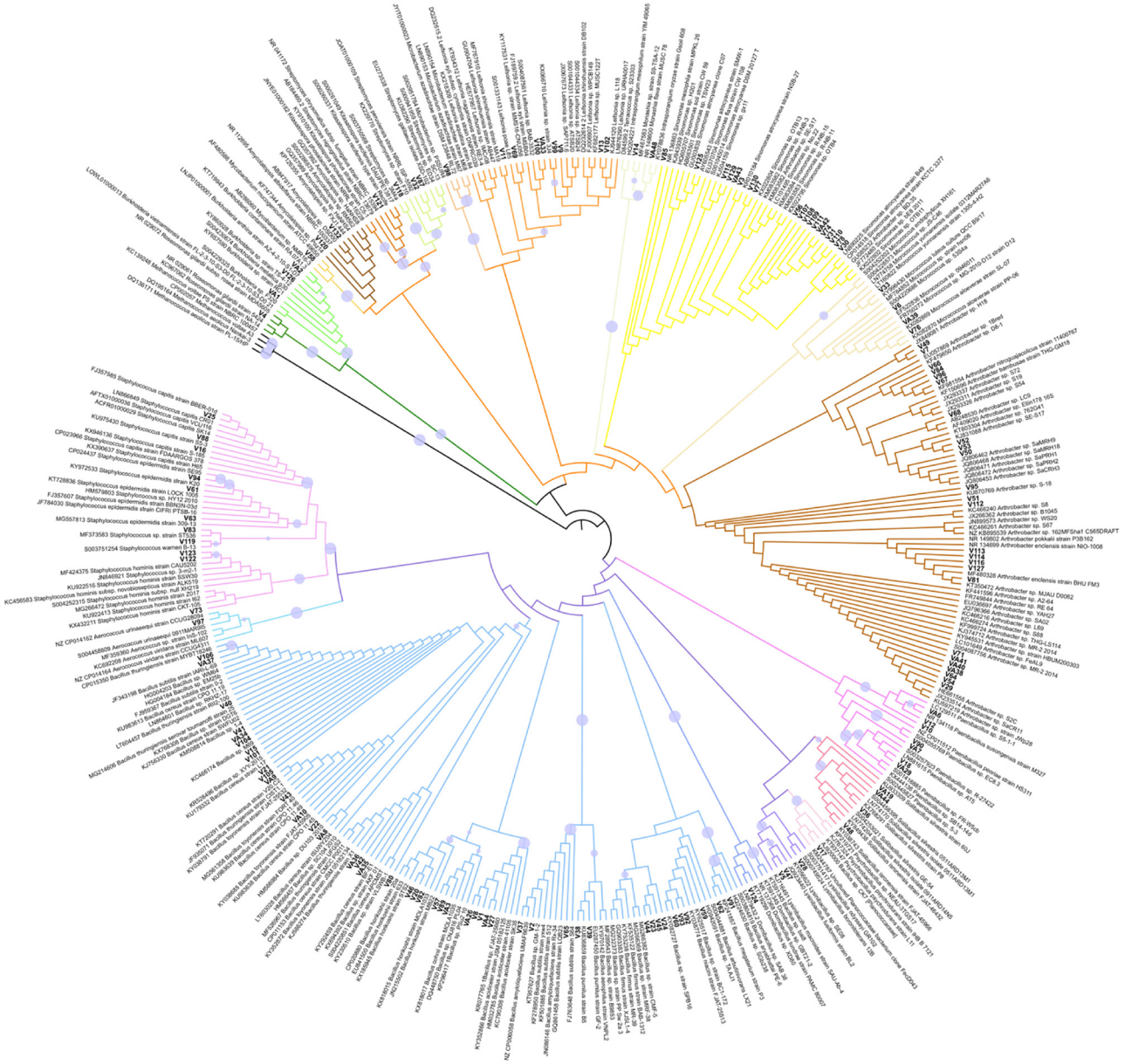

Fig. 2. Bootstrap consensus tree based on 16S rDNA gene sequences revealing phylogenetic classification of the 143 isolates: The colors indicate different bacterial genera. The isolates between sequences are represented in bold. The Maximum Likelihood tree was constructed using the Tamura 3-parameter model and the neighbor joining method, with the bootstrap analyses of 1000 replicates. Only bootstrap values equal and greater than 90% are displayed as circles with increasing size up to 100%. *Methanococcus* ssp. was used as an outgroup.

dried in a laminar flow bench for 1–2 h at room temperature. Seeds were placed in petri dishes lined with sterilized moistened filter paper. The plates were incubated for 5 days at 28 °C. Germinated seeds were counted at day 5 and the germination rate, hypocotyl and root lengths were recorded. This experiment was carried out three times.

2.6. Statistical analyses

Statistical analyses were performed using Sigma plot software version 12.3. Data were subjected to the one-way analysis of variance (ANOVA) to find differences between treatments and the control. Mean comparison between treatments was conducted using the Duncan multiple-range test. Significance was determined at 5% ($p \leq 0.05$) probability level, and significantly different means were indicated by different letters.

3. Results

3.1. Cultivable bacteria inhabiting maize rhizosphere in Cameroon: Actinobacteria, Firmicutes and Proteobacteria

We were able to recover maize rhizosphere soil bacterial isolates on NA medium and grouped them into 31 morphotypes according to their colony characteristics. Four morphotypes represented approximately 10^4, while some could reach values of approximately 10^7 CFU per gram of soil, which comprises a total population captured of 6.64×10^7 CFU per gram of soil. We randomly selected and purified a total of 156 isolates from all the 31 different morphotypes, and designated them by letters and progressive numbers of isolation.

Based on their partial 16S rRNA gene sequencing, 143 isolates provided good-quality sequences (mean length of 790 bp) and were affiliated to bacterial species using phylogenetic classification based on NCBI and RDP databases. We further investigated the phylogenetic assignment of all isolates by constructing a phylogenetic tree using representative sequences obtained from NCBI and RDP databases (Fig. 2; See Fig. 1 in Ref (Tchuisseu et al., 2018)). We classified all the strains into the three Phyla: *Actinobacteria*, *Firmicutes* and *Protebacteria*. We could set up 17 clades from the 143 isolates based on their 20 different genera with high bootstrap values.

Representative strains of ***Actinobacteria*** were placed in 6 major clades (marked in brownish to yellowish colors in Fig. 2). Except for isolates belonging to *Amycolatopsis*, *Mycobacterium*, *Kitasatospora* and *Streptomyces* genera, and to *Leifsonia* and *Microbacterium* genera, which clustered together each forming one clade, all other clades comprised isolates belonging to the same genus. Isolates V76, VA39, VA125, V98 and V9 were respectively affiliated to *Micrococcus yunnanensis*, *Micrococcus aloeverae*, *Sinomanas atrocyaneus*, *Microbacterium azadiractae* and *Leifsonia shinshuensis*.

The ***Firmicutes*** phylum comprised 9 clades (marked in purplish to pinkish colors in Fig. 2). Each clade constituted isolates pertaining to the same genus, with the exception of *Bacillus*, *Solibacillus*, unclassified *Planococcaceae* and *Lysinibacillus*. Isolates belonging to the *Bacillus* genus were split into three clades and were affiliated to six different species. Isolates of the three other genera formed one group, even though they do not all belong to the same family. Two isolates, V88 and V119, were almost identical to the type of strains *Staphylococcus capitis* and *Staphylococcus warneri*. V1 was closely related to *Bacillus acidiceler*, VA8 and V21 to *Bacillus cereus*, V39 and V38 to *Bacillus pumilus*, and V62 to *Bacillus megaterium*, while one isolate V28 clustered with *Lysinibacillus odysseyi*. Isolate V17 clustered to uncultured *Planococcaceae* bacteria despite showing high similarity to *Bacillus* sp. CK7, classified as *Planococcaceae* in the RDP database.

For ***Proteobacteria*** (greenish colors in Fig. 2), this phylum was divided into two clades comprising isolates belonging to *Roseomonas* and *Burkholderia* genera. Isolate V4 was close to *Roseomonas gilardi*, while VA2 was similar to *Burkholderia contaminans*.

To summarize the above results, sequences of the bacterial isolates were assigned to 20 distinct genera, distributed in 13 families and 3 Phyla: *Actinobacteria*, *Firmicutes* and *Proteobacteria* (Fig. 3). The most abundant bacterial genera found among isolates were *Bacillus* (45) and *Arthrobacter* (25) representing 31.5% and 17.5% of the total isolates, respectively, followed by *Sinomonas* (19), *Staphylococcus* (11), *Leifsonia*

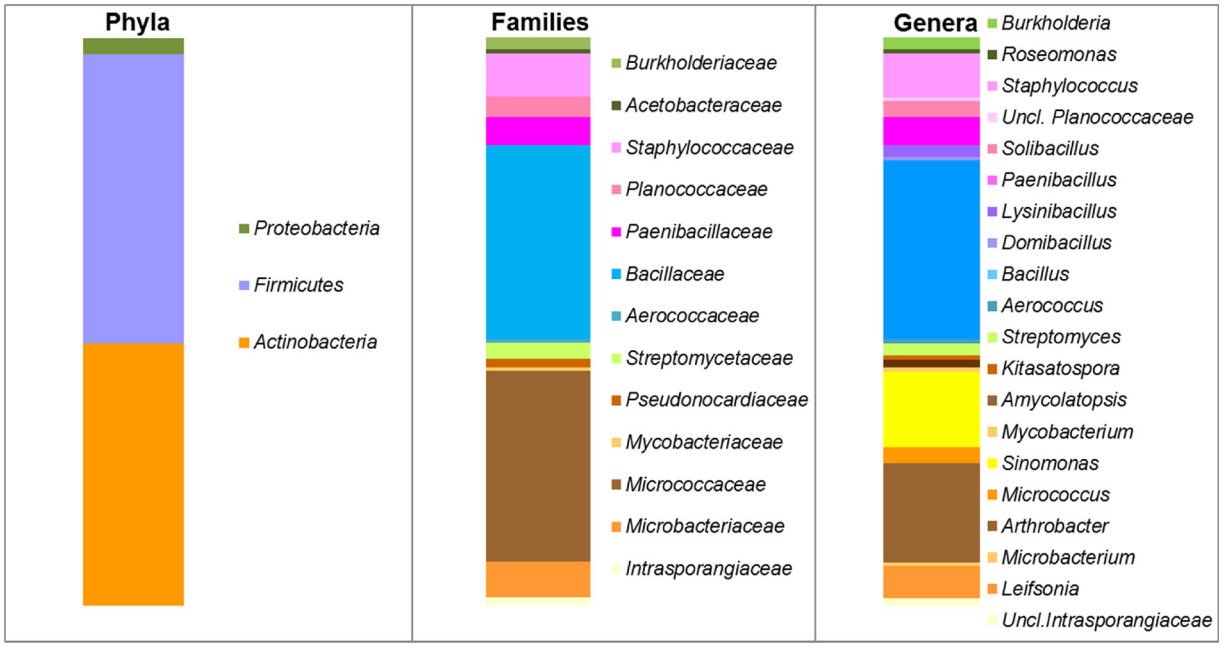

Fig. 3. Taxonomic affiliations based on partial 16S rRNA gene sequencing analysis: the relative abundance of cultivable bacteria inhabiting maize rhizosphere is shown at phylum, family and genus levels. The different color shades represent the dominance of each phylum, each family and each genus at the respective level. Uncl. = unclassified.

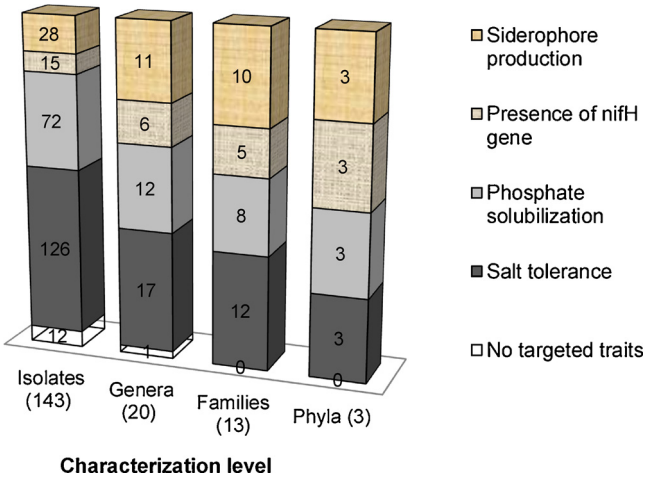

Fig. 4. The most common functional traits: distribution of salt tolerance, phosphate solubilization, presence of *nifH* gene for atmospheric nitrogen fixation and siderophore production activity found within all isolates, families, genera and phyla. The number in parenthesis indicates the total number of each taxonomic unit. The number in the different color shades represents the number of each taxonomical unit possessing each trait among the total number.

(8) and *Paenibacillus* (7) belonging to *Bacillaceae*, *Micrococcaceae*, *Staphylococcaceae*, *Microbacteriaceae* and *Paenibacilaceae* families, respectively. Besides these six genera, the rhizosphere bacterial community analyzed also comprised 12 other gram positive bacterial genera, all together accounting for 16.8% of all isolates, with a total number of representative strains ranging from one to four isolates, including various genera such as *Micrococcus* (4), *Lysinibacillus* (3) and *Domibacillus* (1), *Solibacillus* (3) and unclassified *Planococcaceae (1)*, *Streptomyces* (3) and *Kitasatospora* (1), *Amycolatopsis* (2), unclassified *Intrasporangiaceae* (2), *Aerococcus* (1), *Microbacterium* (1), and *Mycobacterium* (1) members of these respective families: *Micrococcaceae*, *Bacillaceae*, *Planococcaceae*, *Streptomycetaceae*, *Pseudonocardiaceae*, *Intrasporangiaceae*, *Aerococcaceae*, *Microbacteriaceae* and *Mycobacteriaceae*. Gram negative bacteria were represented by four strains: *Burkholderia* (3) and *Roseomonas* (1) genera (Fig. 3) pertaining to *Burkholderiaceae* and *Acetobacteraceae* families.

3.2. Bacterial isolates harbor different functional traits: most common were salinity tolerance and phosphate solubilization

We screened all identified bacterial strains *in vitro* for their ability to tolerate different concentrations of salt (2–8% NaCl); to solubilize seven different inorganic phosphate compounds typically used as fertilizers (tricalcium phosphate, hydroxyapatite, and five rock phosphates from different origins: Mali, Cameroon, Algeria Mexico and Morocco); for atmospheric nitrogen fixation by possessing the *nifH gene*, and to produce siderophore. Out of 143 bacterial isolates, 131 (91.6%) displayed at least one of the targeted traits tested, distributed in 19 out of 20 genera, in all families and in all phyla (Fig. 4). The most dominant trait among the isolates was salinity tolerance with an abundancy of 88.1%, followed by phosphate solubilization (50.4%), siderophore production (19.6%), and the presence of the *nifH* gene (10.5%; Table 2).

Salinity tolerance was the most common trait in all the isolates except members of *Domibacillus*, *Kitasatospora* and *Roseomonas* genera; all other genera (17 out of 20) distributed in 12 families had at least one isolate able to tolerate 2% NaCl (Fig. 4). However, the number of salt-tolerant isolates decreased with increasing NaCl concentrations in the medium. In detail, 64.3% (92/143) of the strains were able to tolerate 4% NaCl, 32.9% (47/143) could tolerate 6%, and only 16.8% (24/143) were able to grow on plates supplemented with 8% NaCl, with most isolates, 10 out of 24, belonging to the *Bacillus* genus (See Table 1 in Ref (Tchuisseu et al., 2018)).

The ability to solubilize phosphate was the second most common feature of the isolates, regardless of the type of phosphate source, and occurred in isolates of 12 genera (*Amycolatopsis*, *Arthrobacter*, *Bacillus*, *Kitasatospora*, *Leifsonia*, *Microbacterium*, *Micrococcus*, *Paenibacillus*, *Roseomonas*, *Sinomonas*, *Solibacillus*, *Staphylococcus* and *Streptomyces*), grouped into eight families (Fig. 4; Table 2). Tricalcium phosphate with 46.2% (66/143) was the phosphate source most easily solubilized among the different inorganic phosphate sources tested, followed by Malian RP 43.4% (62/143), Cameroonian RP and hydroxyapatite 29.4% (42/143). In contrast, Moroccan RP with only 2.8% (4/143) was the most recalcitrant phosphate source (See Table 2 in Ref (Tchuisseu et al., 2018)). Isolates identified as phosphate solubilizers mainly belonged to the genera *Bacillus* 27.8% (20/72) and *Arthrobacter* 26.4% (19/72; Table 2).

The presence of the *nifH* gene and siderophore production were the

Table 2
Occurrence and characterization of bacterial isolates for salinity tolerance, phosphate solubilization, atmospheric nitrogen fixation and siderophore production at the genus level.

Genus	Total number of isolates	Number of isolates with at least one tested trait	Salt Tolerance (≧2% NaCl)	Total P Solubilization	*nifH* gene presence	Siderophore production
Aerococcus	1	1	1	0	0	0
Amycolatopsis	2	1	1	1	0	0
Arthrobacter	25	25	24	19	7	2
Bacillus	45	39	38	20	3	13
Burkholderia	3	3	2	0	0	1
Domibacillus	1	0	0	0	0	0
Kitasatospora	1	1	0	1	0	1
Leifsonia	8	7	7	3	1	1
Lysinibacillus	3	3	3	0	1	0
Microbacterium	1	1	1	1	0	0
Micrococcus	4	4	4	3	0	1
Mycobacterium	1	1	1	0	0	1
Paenibacillus	7	7	7	7	2	1
Roseomonas	1	1	0	1	0	1
Sinomonas	19	19	19	12	0	0
Solibacillus	4	4	4	0	0	0
Staphylococcus	11	11	11	3	1	5
Streptomyces	3	1	1	1	0	0
Uncultured intrasporangiaceae	2	1	1	0	0	1
Uncultured Planococcaceae	1	1	1	0	0	0
Total 20	143	131	126	72	15	28
Percentage (%)		(91.6)	(88.1)	(50.4)	(10.5)	(19.6)

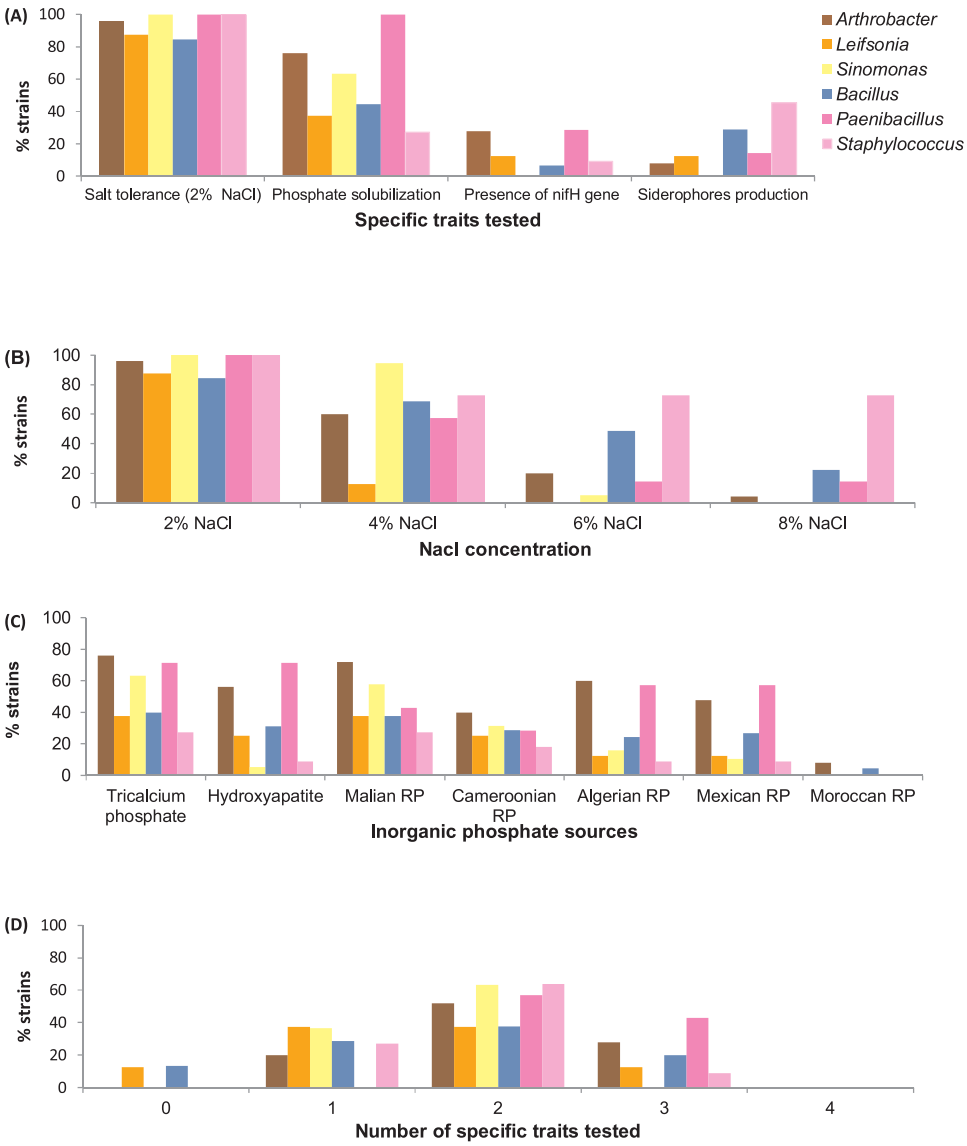

Fig. 5. Percentage of isolates of the six most abundant genera displaying the different traits tested: *Arthrobacter* (maroon bars), *Leifsonia* (orange bars), *Sinomonas* (yellow bars), *Bacillus* (blue bars), *Paenibacillus* (fuchsia bars), *Staphylococcus* (light pink bars); (A) percentage of bacterial strains displaying salinity tolerance and (PGP) traits within each genus, (B) percentage of bacterial strains showing tolerance to different concentrations of NaCl within each genus, (C) percentage of bacterial strains solubilizing different inorganic phosphate compounds within each genus; RP = rock phosphate, (D) percentage of bacterial strains showing certain numbers of specific traits tested (from 0 = no trait, to 4 = all tested traits detected) within each genus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rarest traits among the isolates. *nifH* gene could be detected in isolates of six genera: *Arthrobacter*, *Bacillus*, *Leifsonia*, *Lysinibacillus*, *Micrococcus* and *Paenibacillus* pertaining to five families (*Bacillaceae*, *Microbacteriaceae*, *Micrococcaceae*, *Paenibacillaceae* and *Streptomycetaceae*), and the ability to produce siderophores was found in isolates of 10 genera (*Arthrobacter*, *Bacillus, Burkholderia*, *Kitasatospora*, *Leifsonia, Micrococcus, Mycobacterium*, *Paenibacillus*, *Roseomonas* and *Staphylococcus*) members of ten families. While the *Arthrobacter* genus presented the greatest number of isolates 46.7% (7/15) among potential N_2- fixing bacteria, the *Bacillus* genus had the most isolates with siderophore production capacities 46.4% (13/28; Table 2).

We also assessed the variability of the bacterial isolates in displaying the different traits tested by focusing only on the six most abundant genera found among the isolates. The different bacterial genera showed different efficiencies in strains expressing single traits (Fig. 5). For example, the percentage of salinity tolerance at 2% NaCl varied from 84.4% in *Bacillus* to 100% in *Paenibacillus*, *Sinomonas* and *Staphylococcus* strains. Concerning the PGP traits, strains showing phosphate solubilization ranged from 27.3% in *Staphylococcus* to 100% in *Paenibacillus*, while *nifH* gene was detected in 6.7% of *Bacillus*, 28% of *Arthrobacter* and 28.6% of *Paenibacillus* strains. Finally, siderophores were produced by 45.5% of *Staphylococcus*, 28.8% of *Bacillus* and only 8% of *Arthrobacter* strains. No *Sinomonas* strain possessed *nifH* gene and siderophore producing activity (Fig. 5A). Only 1 out of 38 *Arthrobacter* strains and 10 out of 48 *Bacillus* strains could tolerate high salt concentration (8% NaCl) vs. 8 out of 11 *Staphylococcus* strains. Neither *Leifsonia* nor *Sinomonas* strains showed tolerance to 8% of NaCl (Fig. 5B). Likewise, except *Arthrobacter* and *Bacillus* strains, no isolate belonging to other genera was able to solubilize Moroccan rock phosphate (Fig. 5C). The percentage of strains displaying two to three functional traits tested was about 78% in *Staphylococcus*, 67% in *Arthrobacter*, 63% in *Paenibacillus*, 57% in *Micrococcus*, 54% in *Bacillus* and 50% in *Leifsonia* strains. No genera possessed a strain displaying all the four traits tested. Interestingly, all *Arthrobacter, Paenibacillus, Sinomonas and Staphylococcus* strains displayed at least one functional trait (Fig. 5D).

We evaluated isolates individually for their ability and efficiency in expressing each functional trait (Fig. 6A) and to solubilize each inorganic phosphate (Fig. 6B) in relationship to their phylogenetic affiliation. Interestingly, no bacterial isolate, over all detected phyla, exhibited all the four specific traits tested in this study. However, a large number of strains were able to express multiple functional potentialities. Those displaying three traits were V2, V20, V22, V38, V39, V43, V65, VA9 and VA 35 belonging to *Bacillus*, V49, V50, V52, V53, V64, V71 and V127 belonging to *Arthrobacter*, three to *Paenibacillus* (V12, V18 and VA7), and one to *Staphylococcus* (V83), *Micrococcus*

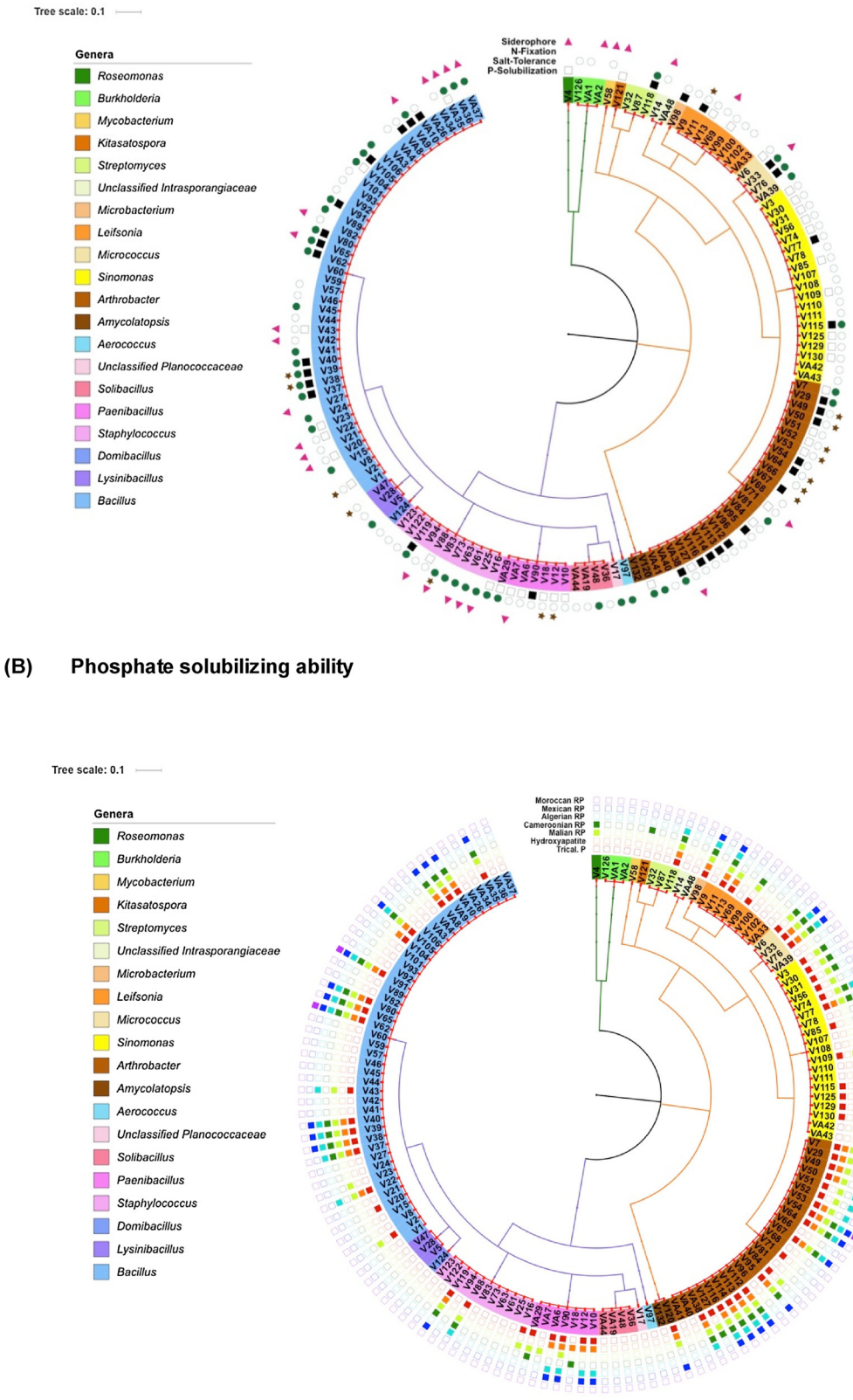

Fig. 6. Bacterial isolates associated with the maize rhizophere displaying specific traits tested according to their taxonomic affiliation: (A) ability of each bacterial isolate in expressing salinity tolerance, phosphate solubilizing, atmospheric nitrogen fixation, and siderophore production activities. Phosphate solubilization: 1–4 phosphates sources (□), 5–7 phosphate sources (■); salt tolerance: 2–4% NaCl (○), 6–8% NaCl (●); detection of *nifH* gene responsible for N_2-fixation: presence (★); siderophore production: presence (▲); if unfilled, no activity. (B) Ability of each bacterial isolate to solubilize different phosphate sources. Positive: trical. P (■), hydroxyapatite (■), Malian RP (■), Cameroonian RP (■), Algerian RP (■), Mexican RP (■) and Morroccan RP (■); if unfilled, it is not able to solubilize; trical. P = tricalcium phosphate and RP = rock phosphate. The Interactive Tree of Life web-based tool was used to display the different traits that each bacterial isolate harbors on the trees.

(V33) and *Leifsonia* (V11). For phosphate solubilization, only three bacterial isolates, the *Bacillus* strains V62 and V91, and the *Arthrobacter* strain V54, showed the ability to solubilize all seven inorganic phosphate sources tested. In general, bacterial isolates exhibiting two to three abilities concurrently were mostly represented by strains belonging to *Bacillus* (26/143) and *Arthrobacter* (20/143) genera.

Based on the above results, we chose six strains, two *Arthrobacter* (V54 and V64) and two *Bacillus* (V39 and V62) with high PGP and

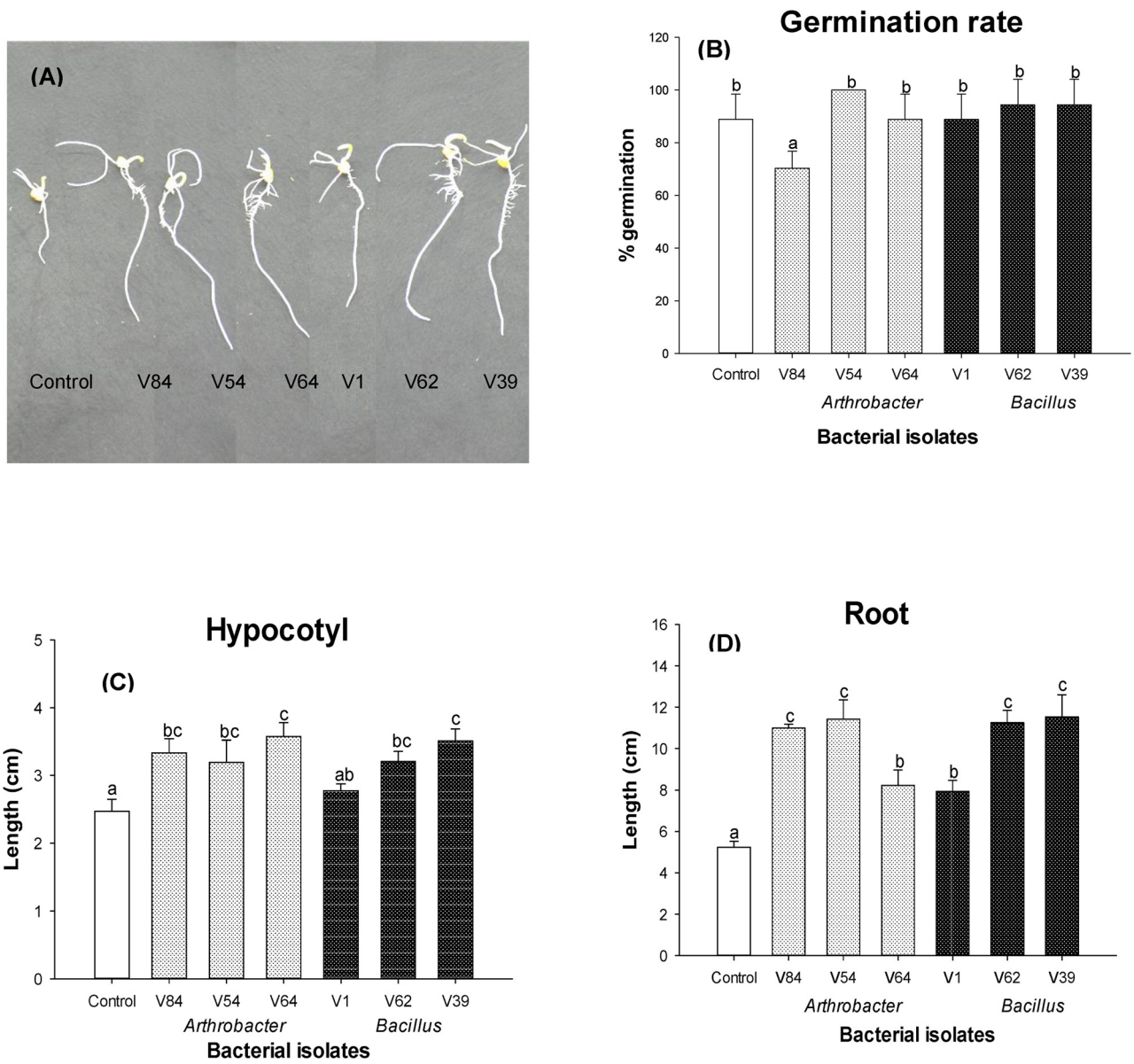

Fig. 7. Effect of selected bacterial isolates on maize seedlings: (A) seed germination assay, (B)germination rate, (C) hypocotyl length, (D) root length of 18 maize seeds *(Zea mays* L. var. LUIGI CS) either inoculated with 0.05 M NaCl buffer (control) or 10^8CFU mL^{-1} of bacterial inoculum V84 (*Arthrobacter sp.*), V54 (*Arthrobacter sp.*), V64 (*Arthrobacter* sp.), V1 (*Bacillus* sp.), V62 (*Bacillus* sp.) or V39 (*Bacillus* sp.). Different letters indicate significant difference between treatments ($p < 0.05$) using Duncańs test. CFU = colony forming unit.

salinity tolerance potential, as well as V84 *(Arthrobacter* strain) and V1 (*Bacillus* strain) with less or no PGP and salinity tolerance potential, for further *in vivo* plant experiments. Among the selected isolates, V54 was the only *Arthrobacter* strain solubilizing all seven phosphate sources tested. V64 was closely related to V54, solubilizing six phosphate sources and additionally is a potential N_2-fixing strain. We randomly choose V84, not closely related to the two others, as a less efficient strain. Regarding *Bacillus* strains, V62 was selected as one of the two strains solubilizing all the seven phosphate sources. V39 was one of the two isolates (V39 and V38) displaying high phosphate solubilizing ability and additionally is a potential N_2-fixing strain. We arbitrarily selected V1, which is not closely related to others among the isolates without any trait.

3.3. Arthrobacter sp. and Bacillus sp. from maize rhizosphere accelerate seed germination

Selected bacterial strains, V84, V54 and V64 (*Arthrobacter* sp.) and V1, V62 and V39 (*Bacillus* sp.), were inoculated and tested against non-inoculated controls to evaluate their potential as PGPR in maize at the germination stage. Results clearly showed that these six bacterial strains had variable effects on hypocotyl and root lengths of maize seeds (Fig. 7). Bacterial inoculation significantly enhanced both hypocotyl and root lengths ($p \leq 0.05$) except for the effect of V1 (*Bacillus* strain) on hypocotyl length (Fig. 7C, 7D). Except for V84 *(Arthrobacter* strain), all bacterial treatments supported a germination rate superior or equal to the control (Fig. 7B). Bacterial strains V64 and V39 with an increase of 44.5% and 41.1% were the most effective inoculants for hypocotyl length, while the best root growth was induced by V39 (120.4%) and V54 (118.3%) (Fig. 7C, 7D). In all cases, V1 had the lowest effect compared to the other isolates tested.

4. Discussion

The benefit PGP bacteria exert to plant growth and yield is well known. However, the growth-promoting effect depends mainly on

native biotic and abiotic factors including bacterial species and the soil types. To improve the selection of climate and environmental adapted efficient PGP bacteria from Cameroonian soil, we used a combination of molecular/bioinformatics tools and *in vitro* studies.

Partial 16S rDNA sequencing analysis assigned our isolates to 20 genera, belonging to 13 families categorized in three phyla: *Actinobacteria, Firmicutes and Proteobacteria*. The prevalence of bacteria, and members of these three phyla has already been observed in the rhizosphere of maize plants cultivated in a Mediterranean carbonate-rich soil (pH 8.5) in Spain and in different areas of the Rio Grande do Saul State in Brazil (Arruda et al., 2013; Garcia-Salamanca et al., 2013). Although differences between the geographic localizations, climatic conditions and soil properties exist, these phyla appear to be common in maize rhizosphere. This suggests that these bacteria colonize maize rhizosphere irrespectively of the soil type or geographic location and might be used as maize fertilizers worldwide. The total number of bacterial genera (20) found in this study was higher than that obtained in recent studies also based on culture-dependent methods, which recorded numbers of 8, 7 and 6 bacterial genera (Pereira et al., 2011; Montañez et al., 2012; Abiala et al., 2015). This reveals a high diversity in the bacterial community linked to maize crops in Cameroon.

More than 48% of our isolates from Cameroonian soil were members of *Bacillus* and *Arthrobacter* genera, which clearly indicate abundance among isolates in the present work that could be representative within these genera living in association with maize in the soil studied. The dominance of *Bacillus* and *Arthrobacter* in maize rhizosphere is comparable to that reported by numerous studies on the microbial community associated with maize in Argentina and in tropical soil of Brazil (Gomes et al., 2001; Pereira et al., 2011). Bacteria of these two genera are widely distributed in various ecological niches and are common in the rhizospheres of a variety of plants such as wheat, sugarcane, rice, apple, grapevine and signalgrass (*Bracharria* sp.) (Upadhyay et al., 2009; Marasco et al., 2013; dos Passos et al., 2014; Rodrigues et al., 2016; Mutai et al., 2017). Other genera we isolated from Cameroonian soil samples, such as *Burkholderia*, *Lysinibacillus*, *Microbacterium*, *Micrococcus*, *Paenibacillus* and *Staphylococcus*, were also frequently isolated from maize rhizosphere populations grown in Argentina (Pereira et al., 2011; Lopez-Reyes et al., 2015). Some less frequently isolated genera such as *Amycolatopsis*, *Sinomonas, Kitasatospora*, *Solibacillus* and *Streptomyces* have been reported by recent studies in Italy, Pakistan, Brazil and China (Oliveira et al., 2009; Ahmad et al., 2013; Gomes et al., 2014; Zhao et al., 2014; Pathan et al., 2015). Interestingly, despite extensive work on the microorganism residents of maize, *Aerococcus*, *Leifsonia*, *Roseomonas* and *Domibacillus* have not been found previously as maize associated bacteria. For the first time, our results show the occurrence of these bacteria in the maize rhizosphere using culture-dependent methods. However, some work revealed the presence of strains of these genera, possessing PGP activities in the rhizosphere of seagrass, bitter gourd, tomato, tuber and root crop like radish, carrot and potato (Jose et al., 2014; Ahmad et al., 2016; Kalam et al., 2017; Yanxing et al., 2017), although they are less known as PGPR.

Phylogenetic analysis based on the 16S rRNA gene sequence could not differentiate between strains belonging to *Bacillus cereus* and *Bacillus thuringiensis* in the *Firmicutes* group, nor *Arthrobacter* and *Sinomonas* strains in the *Actinobacteria* group. Difficulties in discriminating between bacterial strains belonging to the *B. cereus* group (*B. anthracis, B. thuringiensis* and *B. cereus*) via 16S rDNA sequence analysis have also been reported recently by other groups (Abaid-Ullah et al., 2015). Moreover, some strains reported as *Arthrobacter atrocyaneus* (Yamada and Komagata, 1972) have been reclassified as *Sinomonas atrocyanea* (Zhou et al., 2009). This is in agreement with the fact that the accuracy of 16S rRNA gene sequencing is often limited for identifying bacteria at the species or even genera level (Kumar et al., 2014; Abaid-Ullah et al., 2015).

In the present study, we found in the explored conditions that the total population of isolated bacteria from the rhizosphere of maize grown in Cameroon is 6.64×10^7 CFU per gram of soil. Our result confirm those found by a recent study reporting a population size range from $2 \times 10^6 - 8.2 \times 10^8$ CFU per g of soil of indigenous bacteria isolated from maize rhizospheres in different regions of Pakistan (Zahid et al., 2015). In contrast, a bacterial population ranging from $1.5 \times 10^4 – 8.5 \times 10^6$ CFU per g of soil was obtained in the rhizosphere of avocado trees from different regions of southern California (Nadeem et al., 2012). This variability in bacterial population sizes may be related to the soil type, plant species, farming practices, and climatic factors, which are the main determinants of composition in bacterial communities (Vieira and Nahas, 2005; Cavaglieri et al., 2009; Lopez-Reyes et al., 2015; Schrey et al., 2015). However, due to the high number of species present, as well as to the fact that most bacteria are viable but not cultivable (Barriuso et al., 2008), microorganisms that can be cultured in laboratory conditions occupied only a small fraction of these populations (< 1%) (Yang et al., 2017). Thus, several authors used molecular techniques (Illumina pyrosequencing and molecular fingerprinting techniques) to decipher the dynamics and complexity of microbiota associated with plants (Pereira et al., 2011; Correa-Galeote et al., 2016; Yang et al., 2017). Nevertheless, the isolation and purification of beneficial bacterial strains remains essential in order to integrate knowledge about rhizosphere-associated bacterial communities obtained through molecular studies with data on their functional properties. This perspective opens new ways for targeted management of beneficial bacteria in sustainable food production systems (Battini et al., 2016).

Several studies have reported plant growth promotion and protection of PGPR by using bacteria possessing traits such as phytohormone production, pathogen suppression, ethylene production suppression, and heavy metal detoxifying potential (Nadeem et al., 2012; Ribeiro and Cardoso, 2012; Ahemad and Kibret, 2014; Ji et al., 2014; Abiala et al., 2015). However, our objective was to identify bacterial strains able to improve maize productivity under the nutrient deficient and saline conditions found in Cameroonian soils; this was the basis for selecting the different functional traits tested in this study. The potential of the isolated bacterial strains to tolerate salt and promote plant growth was first assessed *in vitro*. The *in vitro* screening for different traits is considered an effective tool for investigating microorganisms that can be used as bio-fertilizers. These tests are extremely important because they allow the selection of microorganisms with better agronomic potential before testing them *in planta* (Szilagyi-Zecchin et al., 2014; Rodrigues et al., 2016). An interesting feature of the bacteria studied here was their ability to tolerate salt.

Although direct plant growth-promoting mechanisms are highly important when selecting a potential strain for a biological preparation, the presence of traits such as resistance to abiotic stress helps the microorganisms to establish in the plant (Hayat et al., 2010; Rodrigues et al., 2016). Like in many soils of arid and semiarid areas, the problem of soil nutrient deficiency is generally associated with salinity in Cameroonian soils. Consequently, even a bacterial strain harboring high PGP abilities will not be able to colonize a plant root and promote plant growth if it cannot propagate in a saline environment. About 17% of the isolates were found to tolerate up to 8% NaCl, predominantly *Staphylococcus* and *Bacillus* strains. Other studies confirmed the high salinity tolerance of *Staphylococcus* (Parfentjev and Catelli, 1964; Tsai et al., 2011) and *Bacillus* (Siddikee et al., 2011; Nadeem et al., 2012) species. The halotolerant bacteria are able to withstand high salt concentrations because of their capability to accumulate compatible osmolytes to maintain intracellular osmotic balance. Use of halophilic or salt tolerant PGPR is an effective approach that has been employed successfully in various crops to improve their growth and tolerance under salt stress condition (Sharma et al., 2016). Tolerance to high salt concentrations could serve as a criterion for selecting strains for soil inoculation to improve crop salinity tolerance and crop production in Cameroon.

The capacity to solubilize different inorganic phosphate compounds

is a promising attribute for selecting bacteria capable of increasing P availability in the rhizosphere. It is assumed that 1–50% of bacteria isolated from soil have the ability to solubilize P (Sharma et al., 2013). Mutai et al. (2017) analyzing the community of bacteria associated with *Brachiaria* grasses in Kenya, isolated 84 bacterial isolates in total, of which 56% were phosphate solubilizers. In agreement with these previous surveys, phosphate solubilizing activity was observed in 72 (50.34%) isolates regardless of the type of phosphates, although tricalcium phosphate was the one solubilized best. It is well known that tricalcium phosphate is more soluble in water than other hardly soluble phosphate sources, and it is commonly used as a universal factor for selecting phosphate solubilizing microorganisms. However, many isolates tested positive for solubilizing tricalcium phosphate fail when they are further tested for directly contributing to phosphorus nutrition in the plant. Therefore, multiple sources of insoluble phosphates are recommended for selecting efficient phosphate solubilizing bacteria (Sharma et al., 2013). Gomes et al. (2014) identified *Bacillus* and *Burkholderia* isolates as the most efficient to solubilize two natural phosphates extracted from Brazil mines. Some of our isolates belonging to *Arthrobacter* and *Bacillus* spp. were able to solubilize all the seven inorganic phosphate compounds tested, but interestingly, none of our isolated *Burkholderia* strains could solubilize any phosphate source. Phosphate solubilizing ability clearly depends on the phosphate source used.

With other direct PGP traits tested, about ten percent of bacterial strains isolated in this study revealed the 350 bp amplicon of the N_2-fixing marker gene *nifH*, predominantly *Arthrobacter* strains. Such data confirm previous findings reporting the detection of the *nifH* gene in only two *Arthrobacter* strains among bacteria isolated from the wheat rhizosphere in India (Upadhyay et al., 2009). So, the existence of the *nifH* gene is used as an indirect evidence for a potential nitrogenase activity of these strains. The capability to fix atmospheric nitrogen is widespread among many bacterial genera such as *Bacillus*, *Burkholderia*, *Pantoea*, *Enterobacter* and *Erwinia*, isolated from the maize rhizosphere (Montañez et al., 2012; Zahid et al., 2015). By mediating the acquisition of nitrogen from the air and delivering it to the plant, these bacteria may be used as bio-fertilizers to improve crop productivity and reduce synthetic nitrogen fertilizer application (Ribeiro and Cardoso, 2012).

Siderophores are low molecular weight iron-chelating agents secreted by bacteria under iron-limiting conditions to help them scavenge iron from the environment (Neilands, 1981). Microorganisms producing siderophore can complex Fe^{3+} ions and stimulate plant growth by depriving plant pathogens of iron, which inhibits pathogen growth, and also by making iron available to the plants. More than half (51%) of the bacteria isolated from Kenyan soil tested positive for siderophore production (Mutai et al., 2017). However, in our study, out of 143 isolates only 28 (19.58%) could produce siderophore. We found in the *Bacillus* group that some bacterial exhibiting the same functional traits, clustered together in the phylogenetic tree. For instance, bacterial strains with the highest phosphate solubilizing potential (V62 and V91) belong to the same clade of *Bacillus megaterium*. Likewise, those with high salinity tolerance, high phosphate solubilizing potential and N_2-fixing ability (V38 and V39) formed the clade of *Bacillus pumilus*. Therefore, our observations support the fact that closely related species often possess similar ecological features and functional capabilities (Morrissey et al., 2016).

We observed that rhizobacteria isolated in this study display multiple traits. We also noted a large variation among isolates of different genera with respect to the different combinations of traits they carried. About 15% of isolates expressed more than two of the studied traits, mostly belong to *Bacillus*, *Arthrobacter* and *Paenibacillus* genera. Other researchers have likewise reported that indigenous rhizobacteria commonly possess a variety of functional traits, alone or in combination, including N_2 fixation, phosphorus solubilization, siderophore production, and salinity tolerance (Upadhyay et al., 2009; Montañez et al., 2012; Nadeem et al., 2012; Ji et al., 2014; Rodrigues et al., 2016; Silva et al., 2016). Multiple traits are expected to be an advantage for seedling growth under multiple types of adverse conditions (Abiala et al., 2015). Indeed, upon inoculating six bacteria displaying different combinations of tested traits we saw much greater maize seedling growth stimulation when different traits were present in one strain.

It has previously been shown that indigenous Cameroonian bacterial isolates from palm tree rhizospheres in Cameroon also improved plant growth (Fankem et al., 2014). Although bacterial inoculation did not significantly increase the germination rate in our study, three strains, mainly the multiple PGP ones, significantly enhanced the hypocotyl and root length of maize seeds compared to the non-inoculated control. This finding is consistent with a recent study reporting that maize growth was caused by a sum of factors and not by individual values obtained *in vitro*, thus suggesting the application of microorganisms possessing different growth promotion factors for *in vivo* plant tests (Rodrigues et al., 2016). However, the net effects of the simultaneous expression of different combinations of different traits on plant growth are still not well understood, and need to be elucidated under *in vivo* conditions (Baez-Rogelio et al., 2017).

5. Conclusion

For the first time we provide a comprehensive phylogenetic affiliation of cultivable bacterial communities associated with maize grown in Cameroon in relationship to their potential plant growth-promoting abilities. Our findings confirm our hypothesis that the rhizosphere of maize grown in Cameroon harbors a high diversity of cultivable bacteria exhibiting multiple plant growth-promoting and salinity tolerance activities, ideal for seedling establishment and growth. The findings demonstrate the potential of selected indigenous bacteria from Cameroon soil to enhance maize growth and productivity. Our approach using *in silico* selection procedures provides a time saving and cost efficient method to detect such bacteria. Further work will focus on the impact of simultaneous expression of different functional traits on plant growth under specific local conditions. Developing such microbial-based, low input tools may enhance the sustainability of crop production in sub-Saharan Africa.

Declarations of interest

None.

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