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Isolation and Screening of Maize Growth-Promoting Rhizobacteria for the Production of Biofertilizer: A Case Study of the Hauts Bassins in Burkina Faso

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Abstract

Mineral fertilizers are widely used for maize production in West Africa. However, these fertilizers are not affordable for most of the local farmers. This study was carried out to isolate plant growth-promoting Rhizobacteria (PGPR), which could be used as low-cost biofertilizers. Two farming sites practicing biological agriculture were selected in the "Hauts bassins" region of Burkina Faso. The farming practices were recorded, and rhizospheric soils were sampled. The PGPR strains were isolated from soil and purified using specific media. Furthermore, they were tested for plant growth-promoting capability, including N_2 fixation, HCN, NH_3, exopolysaccharides, and Indole-3-Acetic Acid production. Principal Component Analysis (PCA) was performed to identify the most promising strains, which were tested for the germination of maize variety FBC6. Then, the most promising strains were submitted to presumptive characterization. A total of 235 strains of PGPR were isolated, of which 10 rhizobacteria (493A, 7134A, 341A, 3123A, 59A, 668A, 614B, 3127A, 415A, and 412A) were able to simultaneously produce at least four plant growth-promoting properties. These strains significantly improved the radicle, the coleoptile lengths, the seminal root numbers, and the root biomass of the maize ($p < 0.001$). Root moisture content was significantly ($p < 0.001$) improved by the strains 7143A, 3123A, and 412A. However, the germination rates were not sig-

nificantly increased. The five most promotive strains were affiliated with the genera *Burkholderia*, *Paenibacillus*, and *Sphingomonas*. These promising strains could be used as an alternative to the use of mineral fertilization for maize production.

Keywords

Rhizospheric Soils, NPK-Urea, Maize, Cultivation Practices, PGPR

1. Introduction

Globally, maize cultivation faces several constraints, including climate change and low soil fertility [1]. In Burkina Faso, soil degradation is the main edaphic constraint to maize production [2] because of its low fertility due to its pedogenetic origin [3] [4]. This poor soil quality is further exacerbated by climate change events, unsustainable farming practices, including inadequate fertilization, and the limited financial means of farmers [5]. A range of practices such as mineral or organic fertilization, "zaï" technique, crop rotation, or association, are commonly used as methods to improve soil fertility [2] [6]. For instance, mineral fertilizers are widely used to meet maize plant nutrient demand [2]. However, due to affordability issues, many farmers do not use the recommended doses [2], leading to a decrease in maize productivity [7]. In addition, uncontrolled use of chemical fertilizers can lead to soil degradation and infertility [8] [9]. In this context, it is necessary to find alternative fertilizers for maize.

Previous studies have shown that organic farming promotes the development in rhizospheric soil of Plant Growth Promoting Bacteria (PGPR), which can improve plant productivity [9] [10]. Indeed, some bacteria belonging to the genera *Acinetobacter*, *Azospirillum*, *Klebsiella*, *Pseudomonas*, and *Bacillus* can be used as biofertilizers [11] [12]. These bacteria are known to have the ability of inorganic phosphate solubilization, organic phosphate mineralization, atmospheric nitrogen fixation, Indole-3-Acetic Acid, ammonia, and exopolysaccharides production [11] [12]. Because of their ability to improve plant nutrient uptake and release phytohormones, PGPRs were used as biostimulants and biofertilizers for improving maize productivity. Since bacterial diversity depends on soil type and climatic conditions [13], available strains may not be adapted to the local conditions of Burkina Faso. The current study was performed to contribute to the improvement of maize productivity by isolating the PGPR strains from local maize plants, which could be used as an alternative to mineral fertilizers. For this purpose, bacterial strains were isolated and tested for plant growth-promoting properties.

2. Materials and Methods

2.1. Study Sites

The study was performed in the "Hauts Bassin" region located in the western part

of Burkina Faso. Two sites were selected to assess the impact of cultivation practices on the diversity of plant growth-promoting bacteria. The samples were collected at two sites of the Bama and Bobo Dioulasso districts (11˚21'8.766"N; −4˚23'16.6272"E and 11˚9'6.9948"N; −4˚15'16.236"E, respectively). The climate at both sites is South Sudanian, characterized by an alternating rainy season (May to October) and a dry season from November to April [14]. Maize productivity was particularly higher in these sites. Since soil fertilization management at these sites was mainly based on the use of organic fertilizers, this high productivity could be due to the effect of plant growth-promoting bacteria. Indeed, it has been shown that organic farming promotes the development of plant growth-promoting rhizobacteria [10].

2.2. Farming Practices in the Study Sites

During the sampling, the farming practices in the two study areas were recorded. The main parameters noticed were the cropping system, the fertilization mode, and the pesticides used.

2.3. Sample Collection

Root samples were collected from the study sites following the method described by Agbodjato *et al.* [15]. At each site, the roots of ten (10) plants at maturity spaced ten (10) meters apart were harvested. A composite sample was made from the collected roots, and then three hundred grams (300 g) were taken and placed in sterile bags. All samples were stored at +4˚C.

2.4. Isolation of the Rhizobacteria

Rhizobacteria were isolated using the method described by Agbodjato *et al.* [15] with modified media. A reconstituted Luria-Bertani (LB) medium supplemented with Agar (15 g/mL) and antifungal Nystatin (25 µg/mL) was used. The inoculated plates were incubated at 30˚C for 24 hours. Then, well-isolated colonies were purified after successive transfers on the same media. The purified colonies were stored at +4˚C in yeast-dextrose-$CaCO_3$ broth supplemented with 20% glycerol [16].

2.5. Evaluation of the Plant Growth-Promoting Properties of the Rhizobacterial Isolates

To evaluate the plant growth-promoting properties of the isolates, several tests were performed, including nitrogen fixation and the production of ammonia, hydrogen cyanide, Indole-3-Acetic Acid (IAA), and exopolysaccharides.

2.5.1. Nitrogen Fixation

The nitrogen fixation test was carried out using the method described by AlAli *et al.* [17], on the nitrogen-free medium (NFDM) broth. Each strain was inoculated in 5 ml of NFDM broth. And the optical density of the inoculated media was measured using a Spectronic 601 spectrophotometer at 600 nm. The measure of

the OD was repeated after an incubation at 30˚C for 7 days. The nitrogen fixation was estimated using the method described by Giroux [18]. The positive media for nitrogen fixation were subcultured on the same culture medium supplemented with agar (15 g/l) and Nystatin (25 µg/ml), to confirm the results obtained.

2.5.2. Ammonia Production

For each strain, one colony was used to inoculate 5 ml of peptone broth. The mixture was incubated at 30˚C for 72 hours. The amount of ammonia produced was assessed by adding 1 ml of Nessler's reagent to the culture. The light yellow color of the mixture was attributed to low ammonia production, while the dark yellow color was considered as to high ammonia production [19].

2.5.3. Hydrogen Cyanide Production

For this test, the nutrient agar media supplemented with glycine (4.4 g/l) was inoculated with each strain. The inoculated plates were then covered with Whatman paper soaked in 5 ml of a mixture of 2% sodium carbonate and 0.5% picric acid, and incubated at 30˚C for 24 hours. The positive production of hydrogen cyanide was evidenced by the color change of the Whatman paper from yellow to reddish-brown [20].

2.5.4. Production of Indole-3-Acetic Acid (IAA)

The ability of the strains to produce Indole-3-Acetic Acid (IAA) was assessed using the modified method described by Al-Kahtani *et al.* [21] using Tryptophan broth as media. Tryptophan broth (10 ml) was inoculated with each isolate and incubated at 30˚C for 24 hours. After 24 hours, the supernatant from each culture obtained after centrifugation at 6000 g for 30 minutes was collected. Then, 2 ml of Salkowski's reagent was added to 1 ml of the supernatant. The appearance of a pink color indicated the production of IAA.

2.5.5. Assessment of the Exopolysaccharides Production

The exopolysaccharide production was evaluated using RVC-Sucrose broth. A quantity of 50 ml of broth was inoculated with each isolate and incubated at 30˚C for 24 hours. The exopolysaccharides were collected by centrifugation at 8000 g for 20 minutes according to the method described by Bajpai *et al.* [22].

2.6. Effect of Selected Bacterial Strains on Maize Seed Germination

After the evaluation of the plant growth-promoting properties of the rhizobacteria, some isolates were selected to determine their real effect on the germination of maize seeds.

2.6.1. Plant Material

The *FBC6* variety developed by the Institute of Environment and Agricultural Research (INERA), Farako-bâ in Burkina Faso, was used. This variety is drought-tolerant, resistant to *Striga*, and can produce up to 5.6 tons/ha of maize grain [23].

2.6.2. Inoculum Preparation

Each colony was inoculated in 50 ml of LB broth and incubated at 30˚C for 24 hours. Then, each mixture was centrifuged at 4000 g and 4˚C to collect the bacterial pellets [24]. Each pellet was diluted with sterile distilled water to obtain a bacterial concentration of 10^8 CFU/mL on the spectrometer at 600 nm according to the McFarland standard.

2.6.3. Inoculation of Maize Seeds with Selected Rhizobacteria

Before the maize seeds were inoculated with the rhizobacterial strains, they were sterilized: the seeds were treated with sodium hypochlorite (2%) for 2 minutes, washed with distilled water, and then with ethanol (95%) for 30 seconds [24]. The sterilized seeds were incubated in the inoculum of each strain for 30 minutes, and placed on cotton wool in Petri dishes at a rate of 10 seeds per dish. Five replicates were performed for each strain and incubated at 25˚C for 7 days. Sterile distilled water was used to water the seeds every day at a rate of 5 ml per Petri dish.

2.6.4. Data Collection on Seed Emergence

Seed emergence was observed every 24 hours for 7 days. The lengths of the radicle and coleoptile were measured each day using a cotton thread and a graduated ruler. Dry root biomass was determined by weighing using a RADWAG PS 210 R2 electronic balance (Polish company RADWAG, Poland).

Germination Rate (*GR*) and Elongations (*E*) of radicle and coleoptile were determined using Equations (1) and (2) described by D. Come [25], respectively.

$$GR = \frac{\text{number of seeds germinated}}{\text{number of seeds tested}} \times 100 \quad (1)$$

$$E = \frac{1}{n}\sum_{i=1}^{5} L_i \quad (2)$$

n = total number of radicles or coleoptiles, and L_i = individual length of radicle or coleoptile.

Root moisture content was determined using Equation (3):

$$H(\%) = \frac{M_0 - M}{M_0} \times 100 \quad (3)$$

H = Moisture content; M_0 = Initial mass; M = Mass after drying.

2.7. Biochemical and Physiological Characterization of Most Strains

For the most promising strains, the nature of the wall was observed under a microscope after Gram staining. Sporulation was determined using the heat shock method. Cytochrome oxidase and catalase production were determined using oxidase (OX) discs and hydrogen peroxide (10%), respectively. The use of sugars by the strains was tested on a minimal medium supplemented with agar (15 g/l) and 1% (w/v) of melibiose, glycogen, L-(+)-arabinose, D-(-)-fructose, D-(+)-glucose, and sucrose, respectively [26]. Bromothymol blue (0.25 g/l) was added to the broths to evaluate acid production. Mannitol metabolism and strain motility were

tested using Mannitol Motility Test Agar. Glucose and lactose fermentation were tested on Kliger-Iron Agar. Citrate utilization, urea utilization, and indole production were tested using Simmons Citrate Agar and Urea-Indole Broth, respectively. The temperature effect on the growth of strains was tested at 4˚C, 41˚C, 45˚C, and 65˚C on LB broth supplemented with agar (15 g/l). The growth of strains at pH 5.7 was determined on LB broth supplemented with agar (15 g/l). Finally, the growth of the strains at 7% NaCl was assessed.

2.8. Presumptive Identification of Strains

The physiological and biochemical characteristics of the strains were used to inform the ABIS Online platform, which was used for their presumptive identification. The databases consulted were those for *Pseudomonas* and *Bacillus* databases, 8.23.25-052025 and 4.8.11-032025 versions, respectively. The optimal number of tests for the *Bacillus* database was 18, with a minimum requirement of 9. The optimal number of tests for the *Pseudomonas* database was 24, with a minimum requirement of 9.

2.9. Statistical Analyses

EXCEL-2019 software was used for data recording. Principal Component Analysis (PCA) was used to analyze the plant growth-promoting properties data of the bacterial strains using R software version 4.3.1. The results were visualized in the form of correlation circles and group graphs to identify promising strains. The means of the data were compared using the ANOVA test, Tukey at a probability threshold of $p = 0.05$.

3. Results

3.1. Farming Characteristics of the Study Sites

Different farming practices were applied in the study sites (Table 1). The soil fertility management option in the Bama district was crop association combined with organic fertilization. Maize was grown in association with tomato, combined with chicken droppings and compost made from neem residues at the recommended rate of 6 t/ha. The soil fertility management option in the Bobo Dioulasso area was based on the use of compost made from agricultural residues combined with NPK (14-23-14) and urea (46% N) at doses below those recommended. In addition, on both sites, no synthetic chemicals were used in the crop protection.

Table 1. Cultivation practices at the study sites.

Study Sit	Type of Crop	Fertilization	Phytosanitary
Bama (farm)	Association with Tomato	Chicken droppings, compost (neem cake)	*Neem* oil, *Jatropha* oil
Bobo Dioulasso (market gardening)	no association	Compost (agricultural residues) NPK (100 kg/ha) Urea (100 kg/ha)	*Neem* oil, aqueous *Neem* extract

3.2. Diversity of the Rhizobacterial Isolates

A total of 235 bacterial strains were isolated, of which 168 strains were from Bama and 67 from Bobo Dioulasso district. Figure 1 presents the Principal Component Analysis (PCA) of strain diversity based on their plant growth-promoting properties. All strains were at least positive for one of the five (05) tested plant growth-promoting properties. The Principal Component Analysis (PCA) showed two main axes. The horizontal axis contributed 29.6% and was positively correlated with ammonia production, exopolysaccharides, IAA, and atmospheric nitrogen fixation. The vertical axis contributed 21.8% and was positively correlated with hydrogen cyanide production, exopolysaccharides, IAA, and atmospheric nitrogen fixation.

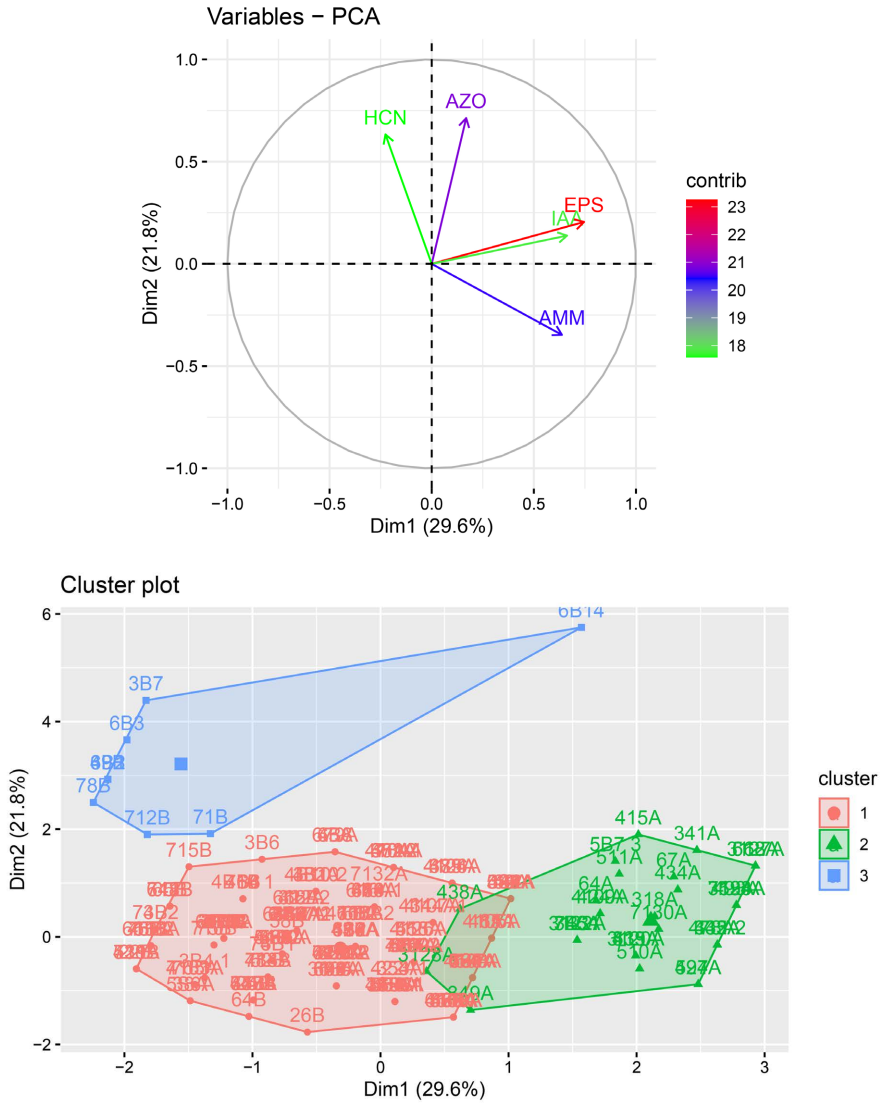

Figure 1. PCA of strain diversity according to plant growth-promoting properties. Variables were HCN: ability to produce hydrogen cyanide; AZO: ability to fix atmospheric nitrogen; EPS: ability to produce exopolysaccharides; AMM: ability to produce ammonia; IAA: ability to produce Indole-3-Acetic Acid.

The cluster graph showed three main clusters. Table 2 presents the percentages of isolates in each group, as well as their distribution by site.

Table 2. Group distribution frequency (%) of the strains per site.

Group	All Sites	Bama Site	Bobo Dioulasso Site
Group 1	81	71	29
Group 2	15	94	06
Group 3	04	00	100

The first group comprised 81% of all strains, with 71% from Bama and 29% from the Bobo Dioulasso site (Table 2). This group is characterized by the production of Indole-3-Acetic Acid, Ammonia, and to fix atmospheric nitrogen, and the absence of exopolysaccharide and hydrogen cyanide production (Table 3).

Table 3. Frequency distribution (%) of combined plant growth-promoting properties expressed by group 1 strains.

Study site	AZO + AMM	AZO + IAA	AMM + IAA	AZO + AMM + IAA	IAA
Bama	04	08	15	44	00
Bobo Dioulasso	06	15	02	05	01
Total	10	23	17	49	01

AZO: N_2 fixation; AMM: NH_3 production; IAA: Indole-3-Acetic Acid production.

The majority of strains in group 1 are capable of producing several plant growth-promoting factors at the same time. Indeed, 10% to 49% of the strains exhibit at least two properties simultaneously, while only 1% of the strains produce IAA alone (Table 3). Table 4 presents frequencies (%) of plant growth-promoting property combinations expressed by group n˚ 2 strains. The second group was composed of 15% of all strains with an abundance of 94% at the Bama site (Table 2). The rhizobacteria in this group were mainly unable to produce HCN (Table 4). In this group, only Bama strains, representing 3% of the strains, can fix atmospheric nitrogen, produce IAA, and exopolysaccharides simultaneously (Table 4). In addition, 14% of the bacteria, which were isolated from Bama, could produce exopolysaccharides, ammonia, and Indole-3-Acetic Acid simultaneously (Table 4).

Table 4. Frequencies (%) of plant growth-promoting property combinations expressed by group 2 strains.

Site	AZO + EPS + IAA	AZO + AMM + EPS + IAA	EPS + AMM + IAA
Bama	03	77	14
Bobo Dioulasso	00	6	00
Total	03	83	14

AZO: N_2 fixation; AMM: NH_3 production; IAA: Indole-3-Acetic Acid production; EPS: exopolysaccharide production.

In the second group, 83% of the bacteria were able to fix atmospheric nitrogen and produce IAA, exopolysaccharides, and ammonia concurrently (Table 4).

The third group consists of only 4% of strains, all isolated from the Bobo Dioulasso site (Table 2). The bacterial strains in this group were mainly able to produce HCN (Table 5). The ability to fix atmospheric nitrogen, produce hydrogen cyanide, and IAA was expressed by 56% of the strains in this group (Table 5).

Table 5. Frequencies (%) of plant growth-promoting property combinations expressed by group 3 strains.

Site	AZO + HCN + IAA	AZO + AMM + HCN + IAA	AMM + IAA + HCN	AZO + AMM + EPS + IAA + HCN
Bama	00	00	00	00
Bobo Dioulasso	56	22	11	11
Total	56	22	11	11

AZO: N_2 fixation; AMM: NH_3 production; IAA: Indole-3-Acetic Acid production; EPS: Exopolysaccharide production; HCN: Hydrogen cyanide production.

The ability to fix atmospheric nitrogen and produce hydrogen cyanide, ammonia, and IAA was expressed by 22% of the strains (Table 5). In addition, 11% of the strains can produce ammonia, IAA, and hydrogen cyanide. Globally, 11% of the strains of this group were found to have all of the desired properties.

3.3. Effect of Selected Bacterial Strains on the Germination of Maize Grain

Ten (10) bacterial strains exhibiting the best plant growth-promoting abilities were selected and tested on the germination of maize grain. The selected strains are able to produce IAA, ammonia, and exopolysaccharides, and are able to fix nitrogen (Table 6). In addition, the strain 614B was able to produce hydrogen cyanide. All selected strains except strain 614B originate from groups 2 and 3 and from the Bama site.

Table 6. Growth-promoting characteristics of the selected strains.

Properties	3123A	341A	7134A	59A	493A	412A	668A	415A	3127A	614B
IAA	++	+++	++	++	++	+++	+++	+++	+++	+++
EPS	+++	+++	+++	+++	+++	+++	++++	+++	+++	+++
NH_3	+++	++	+++	+++	+++	+++	+++	++	+++	+++
N_2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
HCN	-	-	-	-	-	-	-	-	-	+++

-: no production; ++: average production; +++: good production; ++++: very good production. EPS = Exopolysaccharide; NH_3 = Ammonia; N_2 = atmospheric nitrogen; HCN = hydrogen cyanide.

The inoculation of the grains with all of the selected strains significantly increased all of the germination parameters ($p < 0.001$) except the germination rate (Table 7). The longest radicle length was obtained with the strain 3127A (Table 7). The inoculated grains showed the highest coleoptile elongation compared to the non-inoculated control ($p < 0.001$). In addition, the number of roots of the inoculated seedlings was significantly higher than that of the control ($p < 0.001$). Indeed, seedlings treated with strains 668A, 3127A, 415A, 412A, and 614B had 6 seminal roots, compared with control seedlings, which had 02 seminal roots (Table 7). Furthermore, the maize grain inoculation with the strains significantly improved ($p < 0.001$) the seedling fresh root biomass (Table 7). The strains 493A and 3127A evidenced the best values with 214.8 mg/plantlet and 268 mg/plantlet obtained, respectively (Table 7). Moreover, the maize grain inoculation with the strains significantly improved the seedlings' root dry biomass ($p < 0.001$), with strain 3127A showing the best value (30.40 mg/plantlet). The strains 7134A, 3123A, and 412A significantly increased the root moisture content of the seedlings ($p < 0.001$), with strain 7134A presenting the highest value of 92.44% of humidity.

Table 7. Effects of selected rhizobacterial strains on maize germination parameters.

T	GR (%)	RL (cm)	CL (cm)	NSR	FRB (mg)	DRB (mg)	RM (%)
CT	$88.00^{a} \pm 5.83$	$4.75^{h} \pm 0.11$	$3.90^{b} \pm 0.18$	$2.80^{d} \pm 0.20$	$112.20^{f} \pm 1.96$	$13.40^{e} \pm 0.68$	$88.06^{d} \pm 0.52$
493A	$88.00^{a} \pm 3.74$	$7.12^{g} \pm 0.20$	$5.00^{a} \pm 0.00$	$5.20^{bc} \pm 0.20$	$214.8^{a} \pm 2.13$	$21.40^{bcd} \pm 1.78$	$90.02^{abcd} \pm 0.88$
7134A	$90.00^{a} \pm 5.48$	$7.45^{fg} \pm 0.28$	$5.00^{a} \pm 0.00$	$5.00^{c} \pm 0.00$	$222.40^{d} \pm 129$	$16.80^{de} \pm 0.58$	$92.44^{a} \pm 0.27$
341A	$90.00^{a} \pm 4.47$	$9.17^{cde} \pm 0.42$	$5.00^{a} \pm 0.00$	$5.20^{bc} \pm 0.20$	$206.20^{de} \pm 3.00$	$20.00^{cd} \pm 1.30$	$90.29^{abcd} \pm 0.66$
3123A	$90.00^{a} \pm 4.47$	$8.09^{defg} \pm 0.25$	$5.00^{a} \pm 0.00$	$5.40^{bc} \pm 0.24$	$196.80^{e} \pm 2.22$	$16.60^{de} \pm 0.51$	$91.57^{ab} \pm 0.20$
59A	$94.00^{a} \pm 2.45$	$7.57^{efg} \pm 0.61$	$5.00^{a} \pm 0.00$	$5.00^{c} \pm 0.00$	$208.20^{de} \pm 4.45$	$23.40^{bc} \pm 0.87$	$88.76^{cd} \pm 0.32$
668A	$96.00^{a} \pm 4.00$	$9.49^{cd} \pm 0.50$	$5.00^{a} \pm 0.00$	$6.00^{ab} \pm 0.00$	$244.00^{c} \pm 2.45$	$23.40^{bc} \pm 1.33$	$90.40^{abcd} \pm 0.62$
614B	$98.00^{a} \pm 2.00$	$11.22^{b} \pm 0.08$	$5.00^{a} \pm 0.00$	$6.00^{ab} \pm 0.00$	$242.40^{c} \pm 5.39$	$26.40^{ab} \pm 1.41$	$89.08^{bcd} \pm 0.64$
3127A	$98.00^{a} \pm 2.00$	$14.13^{a} \pm 0.36$	$5.10^{a} \pm 0.03$	$6.80^{a} \pm 0.37$	$268.00^{a} \pm 3.74$	$30.40^{a} \pm 0.93$	$88.40^{cd} \pm 0.41$
415A	$100.00^{a} \pm 0.00$	$10.20^{bc} \pm 0.10$	$5.00^{a} \pm 0.00$	$6.00^{ab} \pm 0.00$	$250.00^{bc} \pm 5.48$	$23.60^{bc} \pm 1.36$	$90.59^{abcd} \pm 0.36$
412A	$100.00^{a} \pm 0.00$	$8.99^{cdef} \pm 0.49$	$5.00^{a} \pm 0.00$	$6.00^{ab} \pm 0.00$	$265.60^{ab} \pm 2.32$	$23.40^{bc} \pm 1.63$	$91.40^{abc} \pm 0.60$
p-value	0.11	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

For a given column, values with different letters are significantly different at the 5% threshold (Tukey α = 0.05). T = Treatments; CT = Control; GR = Germination rate; RL = Radicle length; CL = Coleoptile length; FRB = Fresh root biomass; DRB = Dry root biomass; RM = Root moisture content; NSR: Number of seminal roots.

3.4. Physiological and Biochemical Characteristics of the Promising Strains

Table 8 presents the biochemical and physiological characteristics of strains 668A, 3127A, 412A, 415A, and 614B. These strains were chosen because of their ability to significantly increase the number of seminal roots in plants. Since increasing the number of seminal roots improved mineral uptake by maize plants, these strains

could further improve maize growth under natural conditions. All of the tested strains produced cytochrome oxidase, catalase, and indole (Table 8). Except for strain 412A, all strains were urease-negative. None of the strains was able to grow at pH 4. However, all of them are able to grow at the tested NaCl concentrations (Table 8). The strains are mesophilic since no growth was obtained at 4˚C, 45˚C, 55˚C, and 65˚C. However, they did grow at 20˚C and 30˚C. Except for strain 614B, all strains were able to grow at 41˚C. Citrate was metabolized by all strains. Mannitol was metabolized by all strains except strain 614B. However, its metabolism was followed by acid production by strains 3127A, 668A, and 415A (Table 8). All strains were capable of metabolizing Melibiose, glycogen, L-(+)-Arabinose, D-(-)-Fructose, D-(+)-Glucose, and Sucrose (Table 8). However, their metabolism was accompanied by acidification of the medium for strains 3127A, 668A, and 415A. Lactose and glucose were fermented by strains 3127A, 668A, and 415A with gas production (Table 8). None of the strains produced hydrogen sulfide.

Table 8. Biochemical and physiological characteristics of selected strains.

Test	412A	3127A	668A	415A	614B
Mobility	+	+	+	+	+
Gram	−	+	+	−	−
Sporulation	−	+	+	−	−
Catalase	+	+	+	+	+
Oxydase	+	+	+	+	+
Urease	+	−	−	−	−
Indole	+	+	+	+	+
Usual medium	+	+	+	+	+
pH 5.7	+	+	+	+	+
NaCl 7%	+	+	+	+	+
4˚C	−	−	−	−	−
41˚C	+	+	+	+	−
45˚C	−	−	−	−	−
65˚C	−	−	−	−	−
Citrate	+	+	+	+	+
Mannitol	+	+ a	+ a	+ a	−
Melibiose	+	+ a	+ a	+ a	+
Glycogen	+	+ a	+ a	+ a	+
L-(+)-Arabinose	+	+ a	+ a	+ a	+
D-(-)-Fructose	+	+ a	+ a	+ a	+
D-(+)-Glucose	+	+ a	+ a	+ a	+
Saccharose	+	+ a	+ a	+ a	+

Continued

	Fermentation				
Glucose	−	+	+	+	−
Lactose	−	+	+	+	−
Gaz	−	+	+	+	−
H_2S	−	−	−	−	−

+: positive test; −: negative test; + a: with acid production.

Table 9 shows the presumptive identification of the strains. Based on these physiological and biochemical characteristics and using the *ABIS online* platform, the strains 3127A, 668A, and 415A could be affiliated with the *Paenibacillus* genus (Table 9). The strain 614B could be affiliated with the *Sphingomonas* genus with a membership probability of 80.6% (Table 9). The strain 412A could be affiliated with the genus *Burkholderia* with a membership probability of 64.4% (Table 9).

Table 9. ABIS identification of the strain.

Strain	Affiliated species	Similarity (%)	Probability (%)	Matrix integrity (%)
412A	*Burkholderia cenocepacia*	85	64.4	100
3127A	*Paenibacillus glucanolyticus*	82.7	92.1	100
668A	*Paenibacillus glucanolyticus*	82.7	92.1	100
415A	*Paenibacillus glucanolyticus*	78	88.8	100
614B	*Sphingomonas paucimobilis*	89.4	80.6	100

4. Discussion

The study showed that two different farming practices were used. The Bama farmer practiced organic farming (compost) as fertilizer, while the Bobo Dioulasso farmer used mineral fertilizer (NPK and urea) in addition to compost (Table 1). In addition, the number of PGPR isolated from Bama (168 strains) was higher than that from Bobo Dioulasso (67 strains). These results could be explained by the use of chemical fertilizers in the site of Bobo Dioulasso. Indeed, [9] showed that the abundance of the soil PGPR decreases with increasing doses of mineral fertilizers. However, organic fertilizers as a source of nutrients are used to improve soil health, biology, fertility, and crop productivity [10]. Indeed, the compost can release hormones, nitrogen, and enzymes into the plant rhizosphere, increasing its plant growth [10]. Moreover, fertilizers are usually applied without preliminary control of the soil content. NPK and urea were used at the recommended dose of 50% and 67%, at the Bobo Dioulasso site. Indeed, the uncontrolled use of mineral fertilizers at the Bobo Dioulasso site could explain the difference in the PGPR number between the two study sites.

There is considerable diversity among strains in terms of plant growth-promoting properties and site, as revealed by the PCA analysis. This diversity could be explained by the different cropping practices. Indeed, Wang *et al.* [9] reported that the combined application of organic fertilizer and chemical fertilizer increased the bacteria abundance and diversity in the rhizospheric soil. However, other unmeasured agronomic factors could also contribute to this diversity.

The group graph showed two groups (group n˚ 2 and n˚ 3), which had strains that showed promise for improving most of our soils. A total of 10 strains, including 9 from group n˚ 2 and 1 (614B) from group n˚ 3, were selected as the best plant growth promoters.

The analysis revealed the presence of 10 promising rhizobacterial strains capable of fixing atmospheric nitrogen. Previous studies have reported the presence of PGPR able to fix the atmospheric nitrogen in the maize plant rhizosphere [27]-[29]. Atmospheric nitrogen fixation, as a nitrogen source, improves the soil's agronomic quality. In fact, nitrogen-fixing bacteria can convert atmospheric nitrogen into plant-available ammonia and nitrate [30]. In addition, in rice fields, [31] demonstrated that atmospheric nitrogen-fixing bacteria can stimulate urease, phosphatase, and catalase activities.

All selected strains were capable of ammonia generation. Indeed, previous studies reported the presence of ammonia-producing bacteria in the maize plant rhizosphere [27] [32]. Ammonia production through mineralization of organic matter by strains could improve maize production.

The ability to produce IAA was observed in all selected strains. Several studies reported IAA-producing bacteria in maize plant rhizosphere [27] [28] [33]. The production of indole-3-acetic acid by bacteria can occur in the absence or presence of L-tryptophan [34] [35]. However, L-tryptophan-dependent synthesis of indole-3-acetic acid stimulates plant growth more effectively [35]. IAA is a plant growth hormone [27] [32], but its exogenous accumulation can inhibit root growth. This inhibitory effect can be mitigated by some of the PGPR through the degradation of the excessive amounts [9].

The selected strains were capable of producing exopolysaccharides. [36] demonstrated that water stress can stimulate exopolysaccharide production. [37] showed that the bacterial exopolysaccharides production can improve the soil porosity, moisture, and microbial activity. Furthermore, improving rhizospheric soil porosity could indirectly enhance atmospheric nitrogen fixation by bacteria [38]. In addition, exopolysaccharides may adsorb mineral elements from the soil, such as Mn, Cu, Fe, and Zn, increasing nutrient availability for plants [37].

From the selected strains, only 614B was capable of producing hydrogen cyanide. Indeed, unlike other properties, HCN production ability by maize rhizobacteria is scarce [27]. Hydrogen cyanide is involved in chemical processes, such as metal chelation [39] and the inhibition of the growth of several predators, such as nematodes [40].

The study showed that the maize seed inoculation with the strains did not sig-

nificantly improve the germination rate of the FBC6 variety. However, previous studies demonstrated the ability of PGPR to stimulate the germination of maize kernels [33] [41]. There are a few studies on the mechanisms used by the PGPR to stimulate grain germination. A recent study demonstrated their ability to stimulate wheat grains via the gibberellic acid pathway [42].

However, the result showed that all strains significantly increased the radicle, coleoptile, and seminal root lengths of seedlings. In addition, a significant increase in fresh and dry root biomass and moisture content was observed in inoculated plants. These characteristics are important parameters of the development of the plants. Previous studies showed that PGPR can increase the aerial and root length, the biomass, and the moisture content of maize roots [33] [41]. The improved agronomic parameters of the seedlings could be due to IAA production, which is a plant growth hormone [33] [35].

In addition to their ability to fix atmospheric nitrogen and produce ammonia, exopolysaccharides, indole-3-acetic acid, and hydrogen cyanide, strains 668A, 3127A, 412A, 415A, and 614B had obtained high values for the number of seminal roots. Given that an increase in the number of seminal roots improves mineral absorption by plants, and that soils in Burkina Faso are not very fertile, these indigenous strains could be the most suitable, as bacterial diversity depends on climatic conditions and soil type. The use of these strains could also help reduce the use of synthetic chemical fertilizers, which can pollute water if not used properly. It should be noted that the evaluation of plant growth-promoting properties was qualitative. Quantitative tests should be carried out for optimal use of the strains.

These strains were catalase-positive. Previous studies showed that catalase production by bacteria can reduce the accumulation of hydrogen peroxide, which oxidizes nucleic acids, proteins, and lipids in root cells. In addition, catalase accumulation in the rhizosphere could mitigate damage due to water stress [43]. The study showed that the selected bacteria were mobile and could use the tested sugars for their growth. The interactions between the maize plant and the rhizobacterial isolates could be enhanced by the mobility and the bacteria's ability to use the key root exudates, such as sugars.

The strains 3127A, 668A, and 415A could be affiliated with the genus *Paenibacillus*. This genus contains species that improve plant growth [44] [45]. For example, the species *Paenibacillus polymyxa* YF improved the root growth of *Codonopsis pilosula* by inhibiting *Fusarium oxysporum*, which causes root rot [46].

The strain 614B could be affiliated with the genus *Sphingomonas*. Species of this genus, such as *Sphingomonas panaciterrae* PB20 and *Sphingomonas* sp Cra20, have been shown to improve plant growth and nutrient assimilation [46] [47].

Strain 412A is affiliated with the genus *Burkholderia*. Several studies reported the improved growth of *Arabidopsis thaliana* under saline stress conditions by *Burkholderia* sp. BK01 [48] [49]. However, *Burkholderia cenocepacia* is an opportunistic pathogen in humans, which means that this information must be taken into account when using it as a biofertilizer. To confirm the identification of these

strains, molecular characterization is required, including sequencing of the 16S rRNA gene.

5. Conclusion

Strains promoting plant growth were isolated at two sites according to cultivation practices. A difference in the diversity of these strains was observed between the two sites. This difference could be explained by differences in fertilization. The rhizospheric soils studied contained very few cyanide-producing bacteria (4%). The study identified five promising strains that could be used as biofertilizers. As a perspective, a study should be conducted to evaluate the effect of the selected strains on maize growth under semi-real conditions, particularly through pot trials.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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