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Research Article

Characterization of Potential Plant Growth Promoting Rhizobacteria Isolated from Maize (*Zea mays* L.) in Central and Northern Benin (West Africa)

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Our study aims to characterize Plant Growth Promoting Rhizobacteria (PGPR) isolated from maize roots in five agroecological zones of central and northern Benin. Sixty samples were collected at the rate of four samples per village and three villages per agroecological zone. Rhizobacteria strains were isolated from these samples and biochemically characterized. These strains were analyzed for some of their PGPR traits like ammonia production and hydrogen cyanide following conventional methods. Microbiological investigation of these samples has shown that maize rhizospheres in central and northern Benin contain a high diversity of microorganisms. A total of nine species of maize Plant Growth Promoting Rhizobacteria were identified. Those PGPR include five *Bacillus* species (*B. polymyxa, B. pantothenticus, B. anthracis, B. thuringiensis*, and *B. circulans*), three *Pseudomonas* species (*P. cichorii, P. putida*, and *P. syringae*), and *Serratia marcescens*. The microbial diversity does not depend on the soil types. The microbial density, generally high, varies according to both soil types and agroecological zones. All *Serratia* strains (100%) have produced ammonia, whereas 80% of *Bacillus* and 77.77% of *Pseudomonas* produced this metabolite. The hydrogen cyanide was produced by all isolates (100%) independent of their genus. These results suggest the possibility to use these rhizobacteria as biological fertilizers to increase maize production.

1. Introduction

The first aim of agriculture was to ensure survival by producing the necessary for feeding. It was subsistence farming. But nowadays, due to continued and worrying growth of world population, this primary objective of agriculture changed completely. Indeed, the world population is estimated around 7 billion people and may reach 8 billion by 2020 [1]. So, it is urgent to considerably increase the agricultural production to reply to the strong food demand to reduce the risk of malnutrition and the increasing of poverty.

Therefore, the new cereal varieties of high yield were developed. In addition, agrochemical products such as chemical fertilizers, herbicides, fungicides, and insecticides were currently improperly and excessively used in order to increase crop yield. The direct consequence of these agrochemical products use is the environment pollution through ground water and crop products contamination by heavy metals that are contained in these agricultural inputs. These heavy metals are known to be a public health problems because, transferred to humans, they are involved in the cancer occurrence [2]. Apart from medical damages, other consequences in agricultural area such as natural ecological nutrient cycling interruption and soil biological communities destruction are frequently reported [3]. Regarding the damages caused by the excessive use of agrochemical products, other research paths are explored worldwide. Among the explored paths, the use of microorganism currently called Plant Growth Promoting Rhizobacteria (PGPR) is in pole position.

Indeed, the PGPR is a group of bacteria capable of colonizing actively plant roots system and improving their growth and yield [4]. The expression "PGPR" was firstly proposed by Kloepper et al. [5] and was used especially for the fluorescent *Pseudomonas* involved in biological control of pathogens and the improvement of plant growth. Later, Kapulnik et al. [6] extended this expression to rhizobacteria capable of promoting directly the plant growth. Nowadays, this expression is used to refer to all bacteria living in the rhizosphere (around roots), improving plant growth by one or several mechanisms [7]. A large range of species belonging to the genus *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, and *Serratia* were reported to be PGPR [8].

Yazdani et al. [9] asserted that the PGPR use can reduce the application of phosphorus to 50% without affecting the maize (*Zea mays* L.) seed yield. Several authors reported the increase of maize yield [10, 11], Tea [12], soybeans [13], alfalfa [14], wheat [15], and onion [16] simply by PGPR inoculation.

In this context, the aim of our study was to isolate and identify the potential PGPR from maize (most cultivated and consumed cereal in Benin) rhizosphere in the central and northern Benin. The medium-dated objective of this study is to propose for farmers the biological fertilizers based on native PGPR for increasing maize production.

2. Materials and Methods

2.1. Geographical Characterization of Study Area. This study was carried in five agroecological zones (I, II, III, IV, and V) located in the central and northern Benin, West Africa (Figure 1). Indeed, Benin is localized in West Africa (south of Sahara), in the tropical zone between Equator and Tropic of Cancer, precisely between the parallel 6° 30′ and 12° 30′ of north latitude and meridians 1° and 30° 40′ of east longitude.

2.2. Collection of Rhizospheric Samples. Three (3) villages were selected by agroecological zone and four fields were chosen in each village. Three maize plants distanced at least 10 meters were dug up in each field. Their roots were cut with soil adheres and mixed in a bucket. Three hundred grams of this mixture was packed in a sterile stomacher bag and labeled correctly to form the sample of the field. A total of 60 samples were collected and immediately transported at 4°C to the laboratory for further analysis. Once they are at the laboratory, the microbiological screening was immediately realized or samples were kept at 4°C until screening.

Several other parameters (climate, soil type, annual pluviometry, and other crops grown except for maize) of the sampling sites were collected during the sampling.

2.3. Isolation of Rhizobacteria. According to Speck [17] method, 10 g of each sample was mixed into Erlenmeyer flask containing 90 mL of tryptone salt. The mixture was vigorously shaken for about 30 s to obtain 10^{-1} dilution. The previous dilution (1 mL) was transferred into 9 mL of tryptone salt to obtain 10^{-2} dilution. This operation was repeated until obtaining 10^{-8} dilution. Each dilution (0.1 mL) was streaked on different specific isolation media. The aerobic mesophilic flora was enumerated on Plate Count Agar as recommended by the French standard V08-051. *Bacillus* sp. and *Serratia* sp. were isolated on nutrient agar after incubation at 37°C for 24 h and 30°C for 48 h, respectively [18, 19]. *Pseudomonas* sp. was isolated on King A and King B agar after incubation at 30°C for 72 h [20].

2.4. Identification of Rhizobacteria. The identification of isolated rhizobacteria consisted firstly in macroscopic (colony morphology, pigmentation, etc.) and microscopic (gram reaction, mobility, cell shape, spores position, etc.) observations. This first identification was followed by several biochemical and enzymatic tests. The performed tests are production of oxidase, catalase, indole, urease and hydrogen sulfide, respiratory type, acid and gas production on glucose agar, citrate and nitrate utilization, hydrolysis of starch, casein, mannitol, gelatin and lecithin, fermentation of glucose and lactose, growth on MacConkey and Cetrimide agar; Voges-Proskaur test, growth at 42, 45, 55, and 65°C, and fluorescence at 360°C [18, 21–23].

2.5. Plant Growth Promoting Properties

2.5.1. Hydrogen Cyanide Production. All isolated rhizobacteria were screened for hydrogen cyanide production following the method described by Lorck [24]. Each rhizobacterium was streaked on nutrient agar medium added with glycine (4.4 g/L). The agar was covered with a Whatman number 1 filter paper previously soaked in a specific solution (0.5% picric acid and 2% sodium carbonate w/v). Plates were sealed with parafilm paper and incubated at 36 ± 2°C for 4 days. The appearance of orange or red color indicates the production of hydrogen cyanide.

2.5.2. Ammonia Production. To research the production of ammonia, each identified rhizobacteria strain was grown in peptone broth (10 mL) and incubated at 36 ± 2°C for 48 to 72 h. After incubation, 0.5 mL of Nessler's reagent was added to bacterial suspension. The development of brown to yellow color indicated ammonia production [25].

2.6. Statistical Analysis. Microsoft Office Excel 2007 had been used to create data base. The different parameters evaluated were submitted to Analysis of Variance (ANOVA)

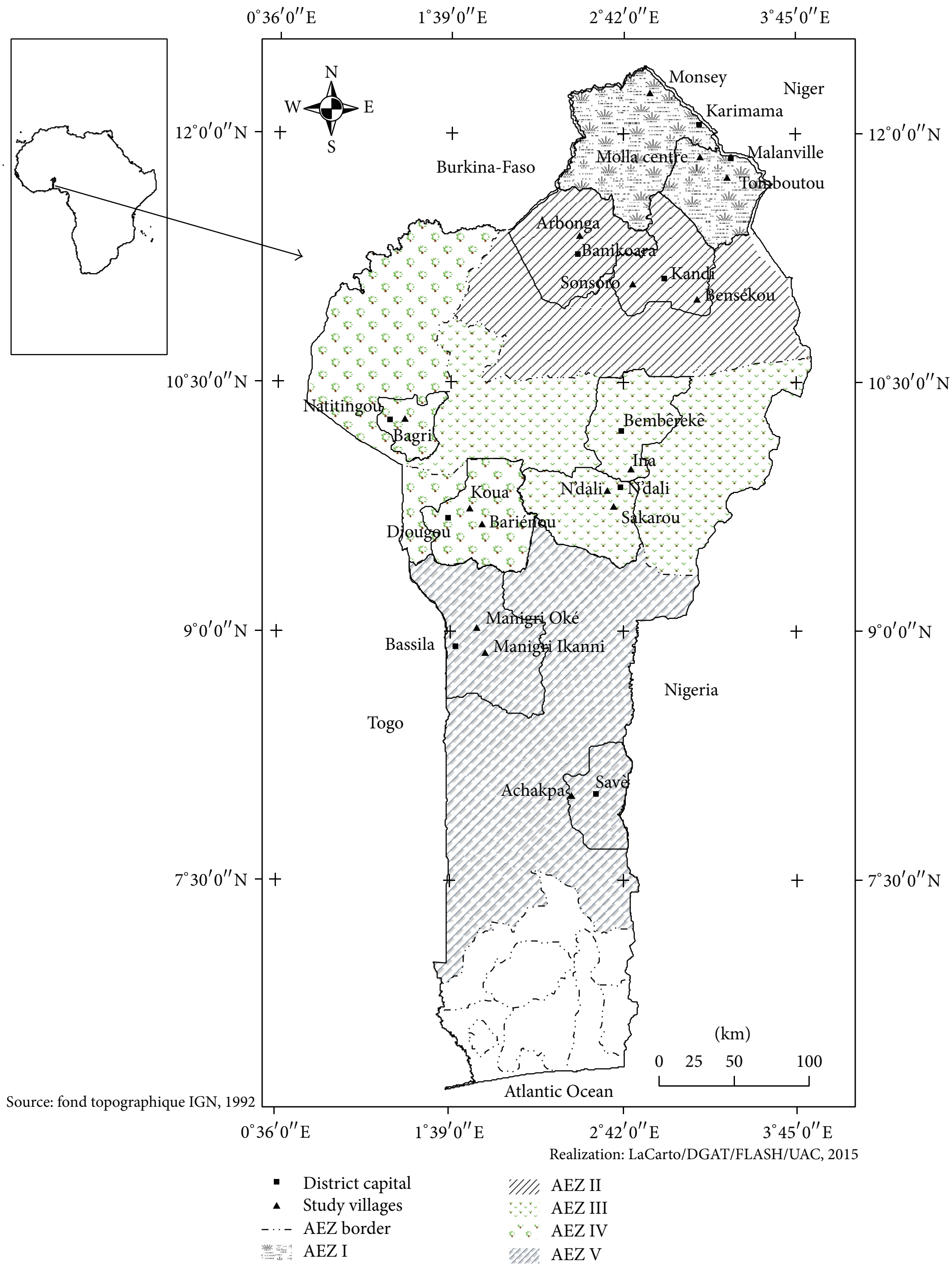

Figure 1: Agroecological zones surveyed. AEZ: agroecological zone; AEZ I = Far North Benin; AEZ II = Cotton zone of North Benin; AEZ III = food-producing zone of South Benin; AEZ IV = West Atacora zone; AEZ V = Cotton zone of Central Benin; AEZ IV = Bar Land zone.

at probability level of 5%, following a mean separation (Student-Newman-Keuls test), by Statistical Analysis System (SAS) software Version 8.1. In this model, soil types and agroecological zones were considered as a fixed factor while replicates were considered as a random factor.

3. Results

3.1. Agroecological Characteristics of the Villages Surveyed. Table 1 shows agroecological characteristics of villages surveyed by agroecological zone. The Sudanese climate with

Table 1: Agroecological characteristics of villages surveyed.

Agroecological zone	Climate	Annual pluviometry (mm)	Village	Type of soil	Other crops grown
I : Far North Benin	Sudano-Sahelian with one rainy season	700 to 900/years	Tomboutou Monsey Molla centre	Washing and No Concretion Tropical Ferruginous Soil Washing and Hydromorphic Tropical Ferruginous Soil	Rice, sorghum, small millet
II : Cotton zone of North Benin	Sudanese with one rainy season	800 to 900/years	Bensékou Sonsoro Arbonga	Washing and Idurate Tropical Ferruginous Soil Washing and Hydromorphic Tropical Ferruginous Soil	Millet
III: food-producing zone of South Benin	Sudanese with one rainy season	900 to 1300/years	Ina N'dali Sakarou	Washing and Concretion Tropical Ferruginous Soil	Sorghum, cotton, bean, cassava
IV: West Atacora zone	Sudanese with one rainy season	800 to 1300/years	Bariénou Koua Bagri	Few Washing Tropical Ferruginous Soil Few Developed Soil	Sorghum, bean, bambara groundnut, cassava, groundnut, yam
V: Cotton Zone of Central Benin	Sudano-Guinean with two rainy seasons	1100 to 1400/years	Achakpa Manigri Oké Manigri Ikanni	Impoverished Tropical Ferruginous Soil Washing and No Concretion Tropical Ferruginous Soil	Yam, cassava, pimento

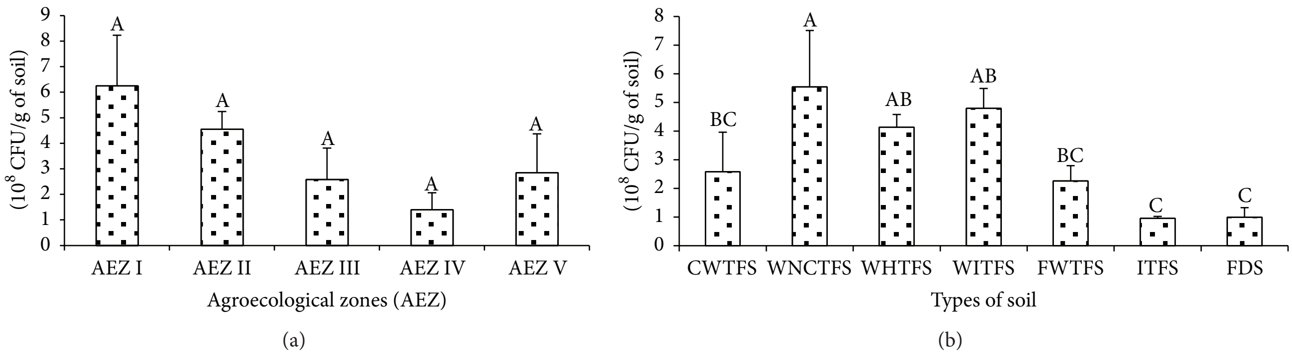

Figure 2: Distribution of aerobic mesophilic flora according to (a) AEZ and (b) type of soil. WCTFS: Washing and Concretion Tropical Ferruginous Soil; WNCTFS: Washing and No Concretion Tropical Ferruginous Soil; WHTFS: Washing and Hydromorphic Tropical Ferruginous Soil; WITFS: Washing and Idurate Tropical Ferruginous Soil; FWTFS: Few Washing Tropical Ferruginous Soil; ITFS: Impoverished Tropical Ferruginous Soil; FDS: Few Developed Soil. The means with different letters are significantly different with probability level of 5% according to Student-Newman-Keuls test.

one rainy season predominates in the five agroecological zones. Annual pluviometry varies from 700 mm/year (zone I) to 1400 mm/year (zone V). Pluviometry increases when we come from northern to southern Benin. The study area is characterized by 7 different types of soil. These soil types are the Washing and Concretion Tropical Ferruginous Soil (WCTFS), the Washing and No Concretion Tropical Ferruginous Soil (WNCTFS), the Washing and Hydromorphic Tropical Ferruginous Soil (WHTFS), the Washing and Idurate Tropical Ferruginous Soil (WITFS), the Few Washing Tropical Ferruginous Soil (FWTFS), the Impoverished Tropical Ferruginous Soil (ITFS), and the Few Developed Soil (FDS). Except for zone III, all the other agroecological zones contain at least two different types of soil. Several other crops were grown by farmers apart from the maize.

3.2. Density of Mesophilic Microflora. The agroecological zones and soil types investigated in this study present a varied microbial density. The rhizosphere of agroecological zone I contains mesophilic microflora (6.25×10^8 CFU/g of soil) clearly abundant compared to the other agroecological zones (Figure 2(a)). The zone IV is the least loaded in mesophilic microflora (1.40×10^8 CFU/g of soil). Our data display that the Washing and No Concretion Tropical Ferruginous Soil (WNCTFS) contains the highest mesophilic microbial

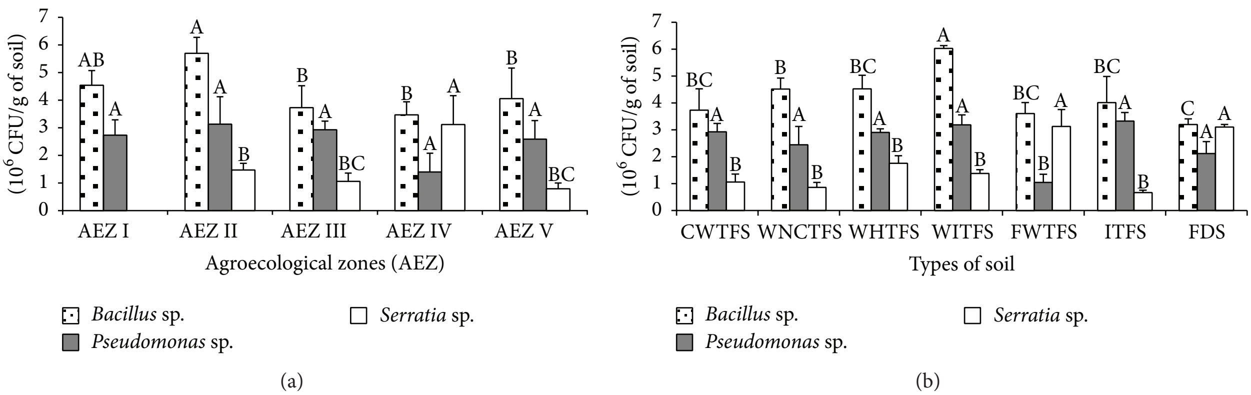

Figure 3: Distribution of rhizobacteria density according to (a) AEZ and (b) type of soil. WCTFS: Washing and Concretion Tropical Ferruginous Soil; WNCTFS: Washing and No Concretion Tropical Ferruginous Soil; WHTFS: Washing and Hydromorphic Tropical Ferruginous Soil; WITFS: Washing and Idurate Tropical Ferruginous Soil; FWTFS: Few Washing Tropical Ferruginous Soil; ITFS: Impoverished Tropical Ferruginous Soil; FDS: Few Developed Soil. The means with different letters are significantly different with probability level of 5% according to Student-Newman-Keuls test.

population (5.54×10^8 CFU/g of soil). The lowest mesophilic microflora charge was recorded with the Impoverished Tropical Ferruginous Soil (ITFS, 0.99×10^8 CFU/g of soil) and Few Developed Soil (FDS, 0.95×10^8 CFU/g of soil) (Figure 2(b)).

3.3. Density of Rhizobacteria Isolated. The density of isolated rhizobacteria according to agroecological zone and soil type is shown in Figure 3. The rhizospheres of agroecological zone II contain the highest density of *Bacillus* sp. (5.70×10^6 CFU/g of soil) and *Pseudomonas* sp. (3.13×10^6 CFU/g of soil). On the contrary, samples of the agroecological zone IV contain the lowest population of *Bacillus* sp. (3.47×10^6 CFU/g of soil) and *Pseudomonas* sp. (1.40×10^6 CFU/g of soil). *Serratia* sp. is not found in rhizosphere of agroecological zone I (Figure 3(a)), but it is abundant in rhizosphere of agroecological zone IV (3.12×10^6 CFU/g of soil).

The density of rhizobacteria strains varies also from a soil type to another (Figure 3(b)). The Washing and Idurate Tropical Ferruginous Soil (WITFS) contains the largest population of *Bacillus* sp. (6.03×10^6 CFU/g of soil) whereas the Few Developed Soil (FDS) contains the least population of *Bacillus* spp. (3.2×10^6 CFU/g of soil). Impoverished Tropical Ferruginous Soil (ITFS) and Few Washing Tropical Ferruginous Soil (FWTFS) contain, respectively, the large populations of *Pseudomonas* sp. and *Serratia* sp. In general, the density of *Bacillus* sp. is higher than *Pseudomonas* sp. and *Serratia* sp. *Serratia* sp. is the least abundant in the majority of soils.

3.4. Rhizobacteria Species Identified. Microbial investigation of samples collected from the 5 agroecological zones showed the presence of several rhizobacterial species. Five *Bacillus* species (*B. polymyxa*, *B. pantothenticus*, *B. anthracis*, *B. thuringiensis*, and *B. circulans*), 3 *Pseudomonas* species (*P. cichorii*, *P putida*, and *P. syringae*), and *Serratia marcescens* were identified. The morphological and biochemical characteristics of these rhizobacteria are shown in Table 2.

3.5. Ammonia and Hydrogen Cyanide Production by Rhizobacteria. The production of ammonia (NH_3) and hydrogen cyanide (HCN) by rhizobacteria isolated from soil samples collected in the northern and central Benin is shown in Table 3. Our data suggested that all the rhizobacteria strains produce hydrogen cyanide. Concerning the production of ammonia, it was observed that all *Serratia* strains produce it against 80% of *Bacillus* sp. and 77.77% of *Pseudomonas* sp. (Table 3).

4. Discussion

Several studies have reported the benefit of seeds inoculation by Plant Growth Promoting Rhizobacteria. This growth promoting effect is influenced by biotic and abiotic factors including bacterial species and the soil types. It is in this context that this prospective study was realized in prelude of the promotion of microbial biofertilizers based on native rhizobacteria. The agroecological characteristics of sites samples collected were presented in Table 1. Each agroecological zone contains at least two soil types. The soil types encountered are Washing and Concretion Tropical Ferruginous Soil, Washing and No Concretion Tropical Ferruginous Soil, Washing and Hydromorphic Tropical Ferruginous Soil, Washing and Idurate Tropical Ferruginous Soil, Few Washing Tropical Ferruginous Soil, Impoverished Tropical Ferruginous Soil, and Few Developed Soil. This result is different to those obtained in the southern Benin by Adjanohoun et al. [26]. Indeed, our result reflects the large soils diversity in Benin as Adjanohoun et al. [26] reported other types of soil such as Vertisols, Degraded Bar Land, and No Degraded Bar Land.

Apart from maize, many other crops are growing in different villages surveyed in this study. These crops are rice, sorghum, small millet, millet, cotton, bean, cowpea, cassava, bambara groundnut, yam, and pimento. In southern Benin, except maize, Adjanohoun et al. [26] had identified cotton, groundnut, sweet potato, cowpea, and cassava. These cultures were mostly found in northern and central Benin. Firstly,

Table 2: Morphological and biochemical characteristics of rhizobacteria isolated from samples collected in the central and northern Benin.

Test	*Bacillus*					*Pseudomonas*			*Serratia*
	polymyxa	*pantothenticus*	*anthracis*	*thuringiensis*	*circulans*	*cichorii*	*putida*	*syringae*	*marcescens*
Bacteria shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram reaction	+	+	+	+	+	–	–	–	–
Catalase production	+	+	+	+	+	+	+	+	+
Spore position	Central	Terminal	Central	Central	Central	nd	nd	nd	nd
Growth on anaerobic condition	+	+	+	+	+	nd	nd	nd	nd
Acid from glucose	+	+	+	+	+	nd	nd	nd	nd
Gas from glucose	+	–	–	–	–	nd	nd	nd	nd
Mobility	+	+	–	+	–	+	+	+	+
Delay on glucose	–	–	–	–	–	nd	nd	nd	nd
Voges-Proskauer	+	–	+	+	–	nd	nd	nd	nd
Indole production	–	–	–	–	–	nd	nd	nd	–
Citrate utilization	–	–	–	–	–	nd	nd	nd	+
Mannitol utilization	nd	nd	nd	nd	nd	+	+	+	+
Starch hydrolysis	+	+	+	+	+	nd	nd	nd	nd
Casein hydrolysis	+	+	+	+	–	nd	nd	nd	+
Gelatin liquefaction	+	+	+	+	+	nd	nd	nd	+
Lecithin hydrolysis	+	–	+	+	–	+	–	–	nd
Urease hydrolysis	–	–	–	–	–	nd	nd	nd	–
DNAse activity	nd	nd	nd	nd	nd	nd	nd	nd	+
Nitrate reduction	+	+	+	+	–	nd	nd	nd	nd
Growth at 45°C	+	+	–	+	+	nd	nd	nd	nd
Growth at 55°C	–	–	–	–	–	nd	nd	nd	nd
Growth at 65°C	–	–	–	–	–	nd	nd	nd	nd
Fluorescence à 360 nm	nd	nd	nd	nd	nd	+	+	+	nd
Colony on nutrient agar	nd	nd	nd	nd	nd	Whitish-Shiny	Whit-Shiny	Whitish-Shiny	nd
Oxidase production	nd	nd	nd	nd	nd	+	+	–	nd
Glucose fermentation	nd	nd	nd	nd	nd	–	–	–	+
Lactose fermentation	nd	nd	nd	nd	nd	–	–	–	–
Gas production	nd	nd	nd	nd	nd	–	–	–	–
H_2S production	nd	nd	nd	nd	nd	–	–	–	–

Table 2: Continued.

Test	*Bacillus*					*Pseudomonas*			*Serratia*
	polymyxa	*pantothenticus*	*anthracis*	*thuringiensis*	*circulans*	*cichorii*	*putida*	*syringae*	*marcescens*
Growth on Cétrimide (37°C)	nd	nd	nd	nd	nd	−	+	+	nd
Growth on Cétrimide (42°C)	nd	nd	nd	nd	nd	−	+	−	nd
Growth on MacConkey	nd	nd	nd	nd	nd	nd	nd	nd	+
Pigment production	nd	nd	nd	nd	nd	nd	nd	nd	Red

+ = positive; − = negative; nd = no determined.

Table 3: Microbial production of NH_3 and HCN.

Rhizobacteria	Production of NH_3 (%)	Production of HCN (%)
Bacillus sp.	80	100
Pseudomonas sp.	77, 77	100
Serratia sp.	100	100

we can think there are more crops associated with maize in northern and central Benin than in southern Benin. But it is important to indicate that Adjanohoun et al. [26] had noted the crops sown in fields before maize sowing, while this study registered the crops sown in the study zone during the sampling.

The soil aerobic mesophilic microflora has greatly varied from an agroecological zone to another (Figure 2(a)), but this difference is not significant ($p > 0.05$) at probability level 5%. This result can be explained by the large variability of microbial density in a same agroecological zone due to the soil heterogeneity existing in each zone. On the contrary, the density of aerobic mesophilic microflora has also greatly varied from a soil type to another, but the difference is highly significant ($p < 0.001$) at probability level 5% (Figure 2(b)). The variability of microbial density is probably due to physicochemical properties of the different soil types, which certainly impact the microbial activity in rhizosphere. These results are similar to those obtained by Adjanohoun et al. [26] in southern Benin when they observed a large difference of microbial density between Vertisols, Degraded Bar Land, and Not Degraded Bar Land. Indeed, Schoenborn et al. [27] reported that rhizosphere contains a great microbial population between 10^8 and 10^9 CFU/g of soil. This microbial abundance is explained by the richness of rhizosphere in nutrients such as sugars, amino acids, organic acids, hormones, and other small molecules derived from root exudates [28]. The microorganisms find in rhizosphere the energy substrates required for their metabolism [29]. Conversely, in stressed ecosystem the microorganism population can be less than 10^4 CFU/g of soil [30]. So in spite of the different environmental stress (climate change), the maize rhizosphere in central and northern Benin still contains an abundant microbial population.

The density of isolated rhizobacteria has varied according to the agroecological zone and the soil types (Figure 3). The density difference between the soil types is significant for *Bacillus* sp. ($p > 0.05$) and highly significant ($p < 0.001$) for *Pseudomonas* sp. and *Serratia* sp. The microbial density varied from 3.2 to 6.03 × 10^6 CFU/g of soil (*Pseudomonas* sp.), 1.05 to 3.33 × 10^6 CFU/g of soil (*Bacillus* sp.), and 0.67 to 3.13 × 10^6 CFU/g of soil (*Serratia* spp.). These microbial densities are inferior to those obtained by Joseph et al. [31] on chickpea (*Cicer arietinum L.*) in India. During their work, these authors counted about 0.5 to 2.1 × 10^9 CFU/g and 1.1 to 2.1 × 10^9 CFU/g of soil for *Bacillus* sp. and *Pseudomonas* sp., respectively. In our study, *Bacillus* sp. population is most abundant than *Pseudomonas* sp. and *Serratia* sp. This remark was earlier done by Saharan and Nehra [8] when they asserted that *Bacillus* sp. is the most abundant genus in their studied rhizosphere. In addition, Garbeva et al. [32] had concluded that a majority of soil gram positive bacteria (95%) are member of the genus *Bacillus* (*B. mycoides*, *B. pumilus*, *B. megaterium*, *B. thuringiensis*, and *B. firmus*, etc.) similar to *Paenibacillus*.

Several rhizobacteria species were isolated, namely, *B. polymyxa*, *B. pantothenticus*, *B. anthracis*, *B. thuringiensis*, *B. circulans*, *P. cichorii*, *P. putida*, *P. syringae*, and *Serratia marcescens* (Table 2). In southern Benin, Adjanohoun et al. [26] isolated from maize rhizosphere *B. coagulans*, *B. thuringiensis*, *B. pumilus*, *B. polymyxa*, *B. licheniformis*, *B. lentus*, *B. circulans*, *B. firmus*, *P. fluorescens*, *P. aeruginosa*, *P. putida*, *S. hygroscopicus*, *S. rimosus*, *S. fasciculatus*, and *A. lipoferum*. These results still indicate the large microbial diversity of maize rhizosphere in Benin.

In order to identify Plant Growth Promoting Rhizobacteria among isolated rhizobacteria in the central and northern Benin, we have screened all the strains for ammonia and hydrogen cyanide production. Thus, all the *Serratia* strains followed by 80% of *Bacillus* and 77.77% of *Pseudomonas* produced ammonia. These rates of ammonia production are lower than the 95% and 94% obtained, respectively, for *Bacillus* sp. and *Pseudomonas* sp. by Joseph et al. [31]. Likewise, all *Bacillus* and *Pseudomonas* isolated by Yadav et al. [33] from chickpea rhizosphere in India have also produced ammonia. Ammonia production is an important characteristic of PGPR, which indirectly influences plants growth [33].

All strains produced hydrogen cyanide (100%). Our results seem higher than the 75% of hydrogen cyanide production by *Bacillus* sp. strains isolated from rice rhizosphere [34] and 40% of bacteria (*Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Acinetobacter* sp., and *Micrococcus* sp.) isolated from the beans rhizosphere [35] in India. Indeed, the hydrogen cyanide is part of powerful antifungal compounds produced by PGPR and involved in pathogens biological control [36].

5. Conclusion

The maize rhizospheres in central and northern Benin contain high diversity of microorganisms. The bacterial density is generally high and varies according to both the agroecological zones and the type of soils. Nine species of potentially maize plants growth promoting rhizobacteria (*B. polymyxa*, *B. pantothenticus*, *B. anthracis*, *B. thuringiensis*, *B. circulans*, *P. cichorii*, *P putida*, *P. syringae*, and *Serratia marcescens*) were identified during this study. All isolates have produced hydrogen cyanide, while 86.66% of them produced ammonia. In perspective, these rhizobacteria will be assessed to promote maize seeds germination and plant growth.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] B. R. Glick, "Plant growth-promoting bacteria: mechanisms and applications," *Scientifica*, vol. 2012, Article ID 963401, 15 pages, 2012.

[2] S.-Y. Koo and K.-S. Cho, "Isolation and characterization of a plant growth-promoting rhizobacterium, *Serratia* sp. SY5," *Journal of Microbiology and Biotechnology*, vol. 19, no. 11, pp. 1431–1438, 2009.

[3] P. Karuppiah and S. Rajaram, "Exploring the potential of chromium reducing *Bacillus sp*. and there plant growth promoting activities," *Journal of Microbiology Research*, vol. 1, no. 1, pp. 17–23, 2011.

[4] S. C. Wu, Z. H. Cao, Z. G. Li, K. C. Cheung, and M. H. Wong, "Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial," *Geoderma*, vol. 125, no. 1-2, pp. 155–166, 2005.

[5] J. W. Kloepper, M. N. Schroth, and T. D. Miller, "Effects of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield," *Ecology and Epidemiology*, vol. 70, pp. 1078–1082, 1980.

[6] Y. Kapulnik, Y. Okon, J. Kigel, I. Nur, and Y. Henis, "Effect of temperature, nitrogen fertilization and plant age on nitrogen fixation by Setaria italica inoculated with *Azospirillum brasilense* (strain cd)," *Plant Physiology*, vol. 68, no. 2, pp. 340–343, 1981.

[7] B. J. Haghighi, O. Alizadeh, and A. H. Firoozabadi, "The role of plant growth promoting rhizobacteria (PGPR) in sustainable agriculture," *Advances in Environmental Biology*, vol. 5, no. 10, pp. 3079–3083, 2011.

[8] B. S. Saharan and V. Nehra, "Plant growth promoting rhizobacteria: a critical review," *Life Sciences and Medicine Research*, vol. 21, pp. 1–30, 2011.

[9] M. Yazdani, M. A. Bahmanyar, H. Pirdashti, and M. A. Esmaili, "Effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn (*Zea mays* L.)," *World Academy of Science, Engineering and Technology*, vol. 49, pp. 90–92, 2009.

[10] P. A. Noumavo, E. Kochoni, Y. O. Didagbé et al., "Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development," *American Journal of Plant Sciences*, vol. 4, no. 5, pp. 1013–1021, 2013.

[11] A. Adjanohoun, P. Noumavo, R. Sikirou et al., "Effets des rhizobactéries PGPR sur le rendement et les teneurs en macroéléments du maïs sur sol ferralitique non dégradé au Sud-Bénin," *International Journal of Biological and Chemical Sciences*, vol. 6, pp. 279–288, 2012.

[12] U. Chakraborty, B. N. Chakraborty, P. R. Chowdhury, C. Tongden, and M. Basnet, "Investigation on plant growth promoting rhizobacteria of tea rhizosphere," in *Proceedings of the 6th International Workshop on Plant Growth Promoting Rhizobacteria (PGPR '06)*, pp. 78–82, IISR, Calicut, India, 2006.

[13] M. H. Abd-Alla, "Use of organic phosphorus by *Rhizobium leguminosarum* biovar*viceae* phosphatases," *Biology and Fertility of Soils*, vol. 18, no. 3, pp. 216–218, 1994.

[14] H. Rodríguez and R. Fraga, "Phosphate solubilizing bacteria and their role in plant growth promotion," *Biotechnology Advances*, vol. 17, no. 4-5, pp. 319–339, 1999.

[15] M. A. Whitelaw, T. J. Harden, and G. L. Bender, "Plant growth promotion of wheat inoculated with *Penicillium radicum* sp. nov.," *Australian Journal of Soil Research*, vol. 35, no. 2, pp. 291–300, 1997.

[16] N. Vassilev, M. Toro, M. Vassileva, R. Azcon, and J. M. Barea, "Rock phosphate solubilization by immobilized cells of *Enterobacter* sp. in fermentation and soil conditions," *Bioresource Technology*, vol. 61, no. 1, pp. 29–32, 1997.

[17] M. L. Speck, *Compedium of Methods for Examination of Food Microbiological*, American Public Health Association, Washington, DC, USA, 1976.

[18] Y. Aparna and J. Sarada, "Molecular characterization and Phylogenetic analysis of Serratia sp-YAJS—an extracellular Dnase producer isolated from rhizosphere soil," *Science Research Reporter*, vol. 2, no. 1, pp. 104–109, 2012.

[19] A. T. Wahyudi, R. P. Astuti, A. Widyawati, A. Meryandini, and A. A. Nawangsih, "Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting rhizobacteria," *Journal of Microbiology and Antimicrobials*, vol. 3, pp. 34–40, 2011.

[20] E. O. King, M. K. Ward, and D. E. Raney, "Two simple media for the demonstration of pyocyanin and fluorescein," *The Journal of Laboratory and Clinical Medicine*, vol. 44, pp. 301–307, 1954.

[21] J. Guiraud and P. Galzy, *L'analyse microbiologique dans les industries alimentaires*, L'Usine Nouvelle, Paris, France, 1980.

[22] D. Clauss and R. C. W. Berkeley, "Genus *Bacillus*," in *Bergey's Manual of Determinative Bacteriology*, Williams & Wilkins, Baltimore, Md, USA, 1986.

[23] F. M. Olajuyigbe and J. O. Ajele, "Production dynamics of extracellular protease from *Bacillus* species," *African Journal of Biotechnology*, vol. 4, no. 8, pp. 776–779, 2005.

[24] H. Lorck, "Production of hydrocyanic acid by bacteria," *Physiologia Plantarum*, vol. 1, no. 2, pp. 142–146, 1948.

[25] J. C. Cappuccino and N. Sherman, *Microbiology: "A Laboratory Manual"*, Benjamin/Cummings, New York, NY, USA, 1992.

[26] A. Adjanohoun, L. Baba-MouSSA, R. Kakai et al., "Caractérisation des rhizobactéries potentiellement promotrices de la croissance végétative du maïs dans différents agrosystèmes du Sud-Bénin," *International Journal of Biological and Chemical Sciences*, vol. 5, no. 2, pp. 433–444, 2011.

[27] L. Schoenborn, P. S. Yates, B. E. Grinton, P. Hugenholtz, and P. H. Janssen, "Liquid serial dilution is inferior to solid media for isolation of cultures representative of the phylum-level diversity of soil bacteria," *Applied and Environmental Microbiology*, vol. 70, no. 7, pp. 4363–4366, 2004.

[28] D. V. Badri, T. L. Weir, D. van der Lelie, and J. M. Vivanco, "Rhizosphere chemical dialogues: plant-microbe interactions," *Current Opinion in Biotechnology*, vol. 20, no. 6, pp. 642–650, 2009.

[29] A. Adam, *Elicitation de la résistance systémique induite chez la tomate et le concombre et activation de la voie de la lipoxygénase par des rhizobactéries non-pathogènes [Thèse de Doctorat]*, Université de Liège, Liège, Belgium, 2008.

[30] S. Timmusk, V. Paalme, T. Pavlicek et al., "Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates," *PLoS ONE*, vol. 6, no. 3, Article ID e17968, 2011.

[31] B. Joseph, R. Ranjan Patra, and R. Lawrence, "Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.)," *International Journal of Plant Production*, vol. 2, pp. 141–152, 2007.

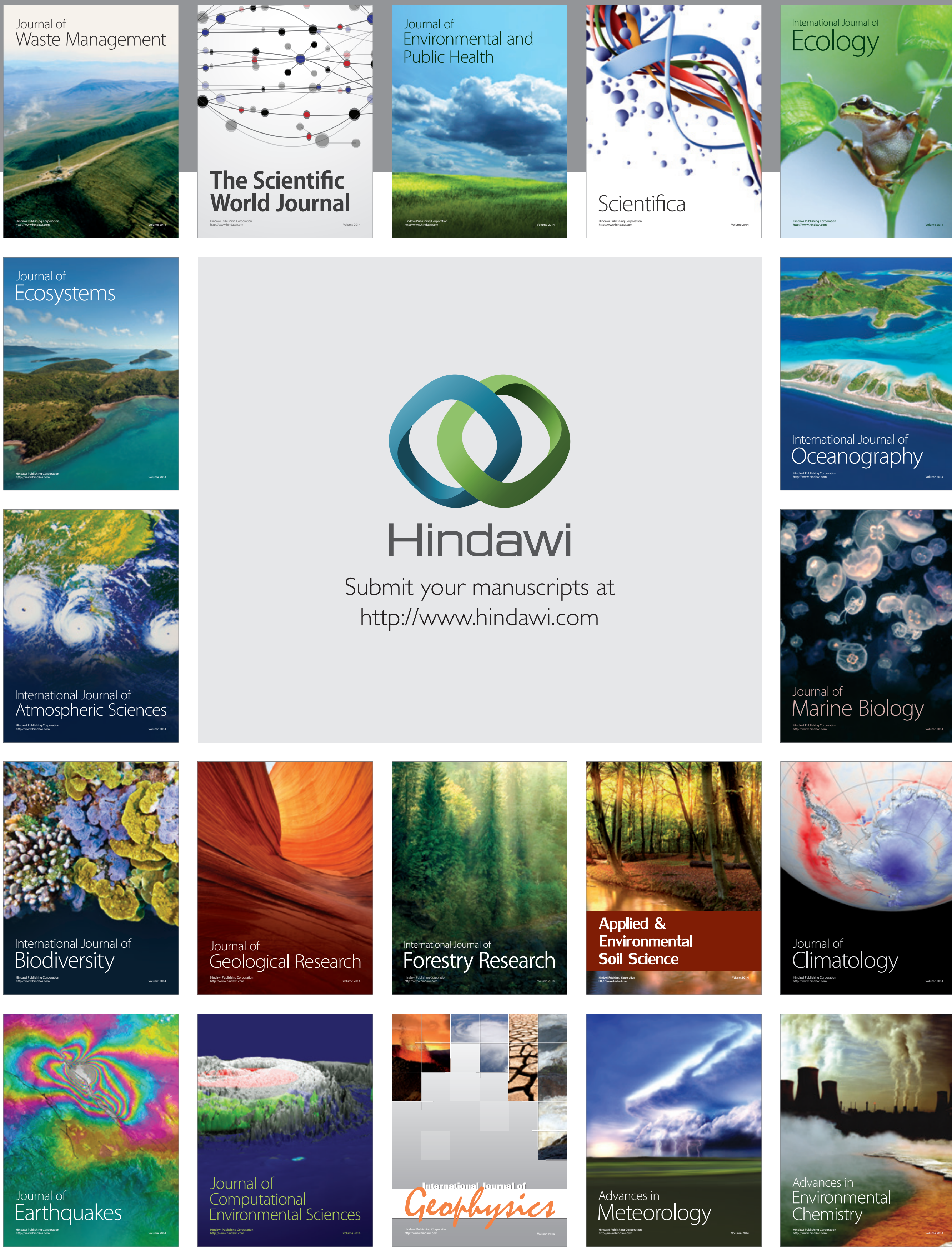
[32] P. Garbeva, J. A. Van Veen, and J. D. Van Elsas, "Predominant *Bacillus spp*. in agricultural soil under different management regimes detected via PCR-DGGE," *Microbial Ecology*, vol. 45, no. 3, pp. 302–316, 2003.

[33] J. Yadav, J. P. Verma, and K. N. Tiwari, "Effect of plant growth promoting Rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum* L.) under *in vitro* conditions," *Biological Forum*, vol. 2, no. 2, pp. 15–18, 2010.

[34] P. Karuppiah and S. Rajaram, "Exploring the potential of chromium reducing *Bacillus* sp. and there plant growth promoting activities," *Journal of Microbiology Research*, vol. 1, no. 1, pp. 17–23, 2011.

[35] A. Kumar, A. Kumar, S. Devi, S. Patil, C. Payal, and S. Negi, "Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study," *Recent Research in Science and Technology*, vol. 4, no. 1, pp. 1–5, 2012.

[36] D. Haas and G. Défago, "Biological control of soil-borne pathogens by fluorescent pseudomonads," *Nature Reviews Microbiology*, vol. 3, no. 4, pp. 307–319, 2005.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