

*Full Length Research Paper*

# **Biofertilising, plant-stimulating and biocontrol potentials of maize plant growth promoting rhizobacteria isolated in central and northern Benin**

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Plants constantly interact with a multitude of microorganisms that they select among other things through their roots. Some bacteria, known as plant growth promoting rhizobacteria (PGPR), are able to stimulate growth and control plant diseases, thanks to the expression of a wide range of beneficial properties to the plant. The aim of this work was to search for biofertilizing, plant-stimulating and biocontrol potentials in PGPR in central and northern Benin. To achieve this goal, the metabolic properties, especially phosphate solubilization, the production of indole acetic acid, hydrogen cyanide, ammonia, exopolysaccharides, certain enzymes and antifungal activity were investigated on nine rhizobacteria strains: *Bacillus polymyxa*, *Bacillus anthracis*, *Bacillus circulans*, *Bacillus thuringiensis*, *Bacillus pantothenicus*, *Pseudomonas cichorii*, *Pseudomonas putida*, *Pseudomonas syringae* and *Serratia marcescens*. The results reveal that the three genera of rhizobacteria were producers of hydrogen cyanide, indole acetic acid, catalase and solubilized phosphate. All *Pseudomonas* and *Serratia* isolates were producers of exopolysaccharides, protease and lipase while 80% of *Bacillus* strains were lipase producers and 60% produced exopolysaccharides and protease. As regards the production of ammonia by rhizobacteria, 100% by *S. marcescens*, 78% of *Pseudomonas* strains and 80% of *Bacillus* strains produce them. These results show the possibility of using these rhizobacteria as biological fertilizers to stimulate growth, control fungal diseases and improve crop productivity in Benin.

**Key words:** Rhizobacteria, *Bacillus*, *Pseudomonas*, *Serratia*, enzyme production, P-solubilizing bacteria, indole acetic acid (IAA).

## **INTRODUCTION**

After a long dependence on plant protection products and synthetic fertilizers, today's global agriculture is hit by a current trend that favors more sustainable and more environmentally friendly practices. To meet these New

demands, farmers need to turn to the exploitation and profitability of natural resources through agricultural practices that combine performance and crop protection at a lower environmental cost.

In this context, inoculation of plants with rhizobacteria is a very popular technology in organic farming. This biotechnology is gaining attention and is seen as an alternative to reducing the amounts of mineral fertilizers that are used without affecting crop yields; and therefore a component to be assessed in integrated management strategies in agriculture (Adesemoye et al., 2009).

Indeed, plant growth promoting rhizobacteria (PGPR) can increase plant growth. Their secondary metabolites present an unavoidable source of various compounds, involved in several fields: industrial, medical and agricultural (Donadio et al., 2002). Rhizobacteria beneficially influence the plant by stimulating its growth (direct pathway) and/or by protecting it against infections of phytopathogenic agents (indirect pathway) (El Houda, 2011). Directly, they promote the growth of plants by producing phytohormones (auxins and cytokinins), increasing nutrient absorption (phosphates solubilization, fixation of atmospheric nitrogen) and they also promote tolerance to salt stress and drought (Antoun, 2013). Indirectly, they can increase plant growth by inducing resistance in plants, producing anti-pathogenic molecules. They are also able to compete for space and nutrients (Antoun, 2013).

It is in the light of all the above that we undertook to search for biofertilizing, plant-stimulating and biocontrol potentials in PGPR in central and northern Benin, in order to select the best performing ones for the formulation of bio-fertilizer as a substitute for synthetic fertilizers, to overcome the problems of declining fertility and environmental pollution, while improving the productivity of crop.

## MATERIALS AND METHODS

The present work has been performed *in vitro* at the laboratory of biology and molecular typing in microbiology. Nine rhizobacteria strains isolated from the maize rhizosphere of different agroecological zones of central and northern Benin by Agbodjato et al. (2015): *Bacillus polymyxa*, *Bacillus panthothenicus*, *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus circulans*, *Pseudomonas cichorii*, *Pseudomonas putida*, *Pseudomonas syringae* and *Serratia marcescens* were characterized.

Four reference fungal strains: *Aspergillus parasiticus* (CMBB 20), *Aspergillus ochraceus* (CMBB 91), *Aspergillus fumigatus* (CMBB 89) and *Aspergillus clavatus* (NCPT 97); known for their phytopathogenic power were employed for the evaluation of antifungal activity.

### Characterization of the isolated rhizobacteria strains

#### Phosphate solubilization

The phosphate solubilization was studied on Pikovskaya solid medium (PVK). The medium was divided into petri

dishes and seeded by the disc method. The incubation was performed at 28°C for five days, according to Jang (2006) and Harrison et al. (1972). The development of a transparent halo around the colony reflects a positive result. The experiences have been achieved in three repetitions. The solubilization index (SI) was calculated using the following formula used by Shakeela et al. (2017):

$$\text{Solubilization index (\%)} = \frac{\text{Diameter of the halo (mm)} + \text{Diameter of the colony (mm)}}{\text{Diameter of the colony (mm)}} \times 100$$

#### Indole acetic acid production (IAA)

The production of IAA was demonstrated on Luria Bertani liquid medium (LB) supplemented with 0.1% L-tryptophan. Bacterial colonies were cultured on the medium and then incubated at 28°C. After 48 h, 1 mL was recovered by centrifugation of samples at 10000 rpm for 10 min. The obtained supernatant was mixed with the same volume of Salkowski's reagent (1 mL of 0.5 mol/L iron chloride III (FeCl<sub>3</sub>) and 49 mL of perchloric acid (HClO<sub>4</sub>) at 35%) according to Gumiere et al. (2014) and Patten and Glick (1996). The appearance of a pink color after 10 to 30 min reveals the production of IAA. According to the color intensity, three point scale (+ = low intensity; ++ = Average intensity and +++ = high intensity) were used for analyze the results.

#### Production of exopolysaccharides (EPS)

The production of EPS was investigated using the method described by Leveau et al. (1991). The rhizobacteria strains were streaked onto agar hypersaccharosed cast in a Petri dish. After incubation at 37°C for 24 h. The production of exopolysaccharides was manifested by the appearance of large and sticky colonies.

#### Production of compounds with antibiotic effect

##### Volatiles (hydrogen cyanide, ammonia)

The production of hydrogen cyanide (HCN) was demonstrated by the method described by Lorck (1948). On agar nutrient containing glycine (4.4 g/L), cast in a Petri dish, bacterial streaks were performed. The agar was covered with a Wattman No.1 filter paper previously soaked in a 2% sodium carbonate and 0.5% picric acid solution. The Petri dish was then sealed with paraffin paper and incubated at 36 ± 2°C for four days. The appearance of an orange or red color indicates the production of HCN.

The production of ammonia (NH<sub>3</sub>) was investigated according to the method of Cappuccino and Sherman (1992). Fresh bacterial colonies were cultured in 10 mL of peptone water and incubated at 36 ± 2°C for a period of time between 48 and 72 h. After incubation, 0.5 mL of Nessler's reagent was added. The turn from brown to yellow indicates the production of ammonia.

#### Antifungal activity of the characterized rhizobacteria

The antifungal activity of rhizobacteria tested was evaluated by the double cropping method described by Kumar et al. (2002) on four

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**Table 1.** Average solubilization index of PGPR isolates.

Rhizobacteria	Solubilization index (%)	
	m	Cv
<i>Bacillus polymysa</i>	263.28 <sup>f</sup>	9.70
<i>Bacillus anthracis</i>	258.43 <sup>f</sup>	2.68
<i>Bacillus circulans</i>	260.65 <sup>f</sup>	1.07
<i>Bacillus thuringiensis</i>	282.17 <sup>e</sup>	1.42
<i>Bacillus panthothenicus</i>	251.90 <sup>f</sup>	10.09
<i>Pseudomonas cichorii</i>	440.16 <sup>c</sup>	1.71
<i>Pseudomonas putida</i>	483.89 <sup>a</sup>	0.73
<i>Pseudomonas syringae</i>	470.24 <sup>b</sup>	4.85
<i>Serratia marcescens</i>	366.93 <sup>d</sup>	4.19
Probability	0.000	
Meaning	***	

\*\*\* =  $p < 0.001$  (very highly significant). Averages with the same letters in the same column are not significantly different. m = average; cv = coefficient of variation.

phytopathogenic fungal strains: *A. parasiticus* (CMBB 20), *A. ochraceus* (CMBB 91), *A. fumigatus* (CMBB 89) and *A. clavatus* (NCPT 97). A disk (5 mm diameter) was cut out from a young culture of the fungal pathogen and placed in the middle of a Petri dish of Potato Dextrose Agar (PDA). Ten microliter of a rhizobacteria suspension about  $10^8$  CFU/mL were spotted at approximately 2 cm from and opposite sides of the fungus infected disk. The control of the Petri dishes were realized by monoculture of each pathogen. The Petri dishes were then incubated at  $26 \pm 1^\circ\text{C}$  and checked for zones of inhibition of mycelium growth after seven days when the fungal mycelium had reached the edge of the Petri dish. The experiences have been achieved in three repetitions. When the pathogen grew over the rhizobacteria, we concluded that the rhizobacteria did not have antifungal activity. On the other hand, when fungal growth was restricted by the rhizobacteria, the rhizobacteria's antifungal activity (percentage of inhibition) was calculated by the following formula used by Noumavo et al. (2015):

$$\% \text{ growth inhibition} = \frac{d1 - d2}{d1} \times 100$$

Where: d1 = Growth diameter of the pathogen in monoculture (control) and d2 = Growth diameter of the pathogen in dual culture.

#### Enzyme production by the rhizobacteria tested

The production of catalase was sought according to the method described by Riegel et al. (2006). With a sterile Pasteur pipette, a bacterial colony was dispersed in a drop of hydrogen peroxide previously deposited on a clean dry slide. The positive reaction is shown by an immediate release of oxygen bubbles forming a foam solution.

Proteolytic activity was determined according to the Smibert and Krieg (1994) method by culturing under incubation for two days at  $28^\circ\text{C}$  of the isolated agar skimmed milk. The development of a transparent halo around colonies indicates a positive reaction (Naik and Sakthivel, 2006). The lipase investigation was carried out according to the method described by de Groot et al (1991), by culturing the isolates on nutrient agar supplemented with 1% oleic acid. A positive reaction is characterized by the appearance of a halo around the colonies.

#### Statistical analysis

To determine the most solubilizing rhizobacteria and the rhizobacteria that inhibited more the growth of each mold category, an analysis of variance followed by the student Newman and Keuls test was performed on solubilization index and inhibition rate values of the growth of the molds for all the replicates of the different rhizobacteria. In addition, to determine metabolites production levels per rhizobacteria and specific groups with similar or opposite production levels or of metabolites, a dataset containing rhizobacteria on lines, metabolites and enzymes in columns and the corresponding values established and then subjected to principal component analysis (PCA) in R statistical software with the FactoMineR package (R Core Team 2016).

## RESULTS AND DISCUSSION

#### Phosphate solubilization

One of the important properties of PGPR that is directly influencing plant growth is the solubilization of phosphate. Phosphorous (P), an essential nutrient element is the second most important element after nitrogen. In the soil, phosphate usually exists in insoluble forms, which reduces its availability for plants (Satyaprakash et al., 2017). This is the reason why a bacterium that has a phosphate solubilizing power is important.

In the present study, the nine isolates tested were able to solubilize phosphate. The solubilization index (SI) by its variance analysis results showed that there was a very high significant difference ( $P < 0.001$ ) between the solubilization capacities of the different strains (Table 1). Where the three isolates of *Pseudomonas* was very effective compared to the others, with an SI from 440.16 to 483.89%. *Bacillus* strains presented the lowest solubilizing efficiencies; SI g from 251.90 to 282.17%. As for *S. marcescens* strains was moderately effective with an SI of 366.93%. The good solubilizing efficiency of

**Table 2.** Estimation of the production of some metabolites by the nine rhizobacteria

Rhizobacteria	Qualitative production of metabolites			
	IAA	EPS	HCN	NH <sub>3</sub>
<i>Bacillus polymysa</i>	+++	+	+	+
<i>Bacillus anthra</i>	+	-	++	++
<i>Bacillus circulans</i>	+	-	++	++
<i>Bacillus thuringiensis</i>	++	+	+++	++
<i>Bacillus panthothenicus</i>	+++	+	+	-
<i>Pseudomonas cichorii</i>	+++	++	+++	+
<i>Pseudomonas putida</i>	+++	++	+++	+++
<i>Pseudomonas syringae</i>	+++	++	+	-
<i>Pseudomonas marcescens</i>	++	+++	++	+++

NH<sub>3</sub>: Ammonia; HCN: Hydrogen cyanide; IAA: Indole Acetic Acid; EPS: Exopolysaccharides; + = Low; ++ = average; +++ = Strong, - = no production.

*Pseudomonas* obtained in our study was also reported in the works done by Sulbaran et al. (2008) and Chibani (2017). Messele and Pant (2012) observed improved biological yield and P uptake in chickpea (*Cicer arietinum* L.) by phosphate-solubilizing *Pseudomonas*. In addition, the evaluation of the solubilizing activity by an Iranian team in different Rhizobium strains revealed an SI ranging from 141 to 248% (Alikhani et al., 2006). Rajkumar et al. (2006) indicated that phosphate solubilization by *Pseudomonas* sp. PSA4 and *Bacillus* sp. BA32 stimulated the proliferation of plant roots and improved the absorption of soil minerals such as iron and phosphate by the host plant. Yazdani et al. (2009) explained that the application of phosphate solubilizing bacteria can reduce phosphorus application to 50% without affecting the yield maize seeds. Exploitation of the phosphate solubilizing bacteria as bio inoculants will increase the available P in the soil; therefore, it will minimize the application of P fertilizers, and so, reduce environmental pollution and promote sustainable agriculture (Zennouhi et al., 2018)

### Indole acetic acid production

The synthesis of plant growth hormones, of which IAA is the most effective, is a very common phenomenon with root exudates of rhizosphere bacteria. All tested strains were found to produce IAA at varying rates. The highest yields were observed in all *Pseudomonas* and some of *Bacillus* strains; while mean productions were observed in *B. thuringiensis* and *S. marcescens* and finally the lowest productions in *B. anthracis* and *B. circulans* (Table 2). The production of IAA by PGPR depends on species and strains and is also influenced by the culture conditions, stage of development and availability of substrates in the rhizosphere (Ashrafuzzaman et al., 2009). Indiragandhi et al. (2008) showed that strains of

*Serratia* spp. PRGB11 are capable of producing IAA. As far as Cherif (2014) is concerned, he showed in his works that some *Bacillus* strains synthesize IAA. The work of Abbas et al. (2018) showed that all five selected endophytic bacterial strains produced indole acetic acid. Indeed, IAA functions as an important signal molecule in the regulation of plant development, acting on organogenesis, trophic responses, cellular responses such as cell expansion, division, differentiation and regulation of cells (Ryu and Patten, 2008; Mezaache, 2012). As a result, rhizobacterial IAA is identified as an effector molecule in plant–microbe interactions, both in pathogenesis and phytostimulation (Spaepen and Vanderleyden, 2011).

### Production of exopolysaccharides (EPS)

With the exception of *B. circulans* and *B. anthracis*, all strains tested were found to produce EPS at variable rates (Table 2). The absence of production in some strain can be explained by the fact that the tested rhizobacteria does not have the gene producing the exopolysaccharides. Previous studies showed that bacterial EPS under salt stress can bind sodium ions and reduces its toxicity in the soil (Arora et al., 2010). EPS bind to Na<sup>+</sup> cations and, in particular, decrease its content, thereby helping to reduce salt stress in plants (Ashraf et al., 2004). EPS-producing PGPR strains induce tolerance to soil salinity, promote the growth of soybean (*Glycine max*) plants (Bezzate et al., 2000) and limit the uptake of Na<sup>+</sup> by wheat roots (Ashraf et al., 2004). Sandhya et al. (2009) argue that EPS participate in the formation of bacterial aggregates and consequently improve soil aeration, water infiltration and root growth. In salt stress condition, the EPS chelate cations available in the root zone, thus contributing to reduce the salinity of the rhizosphere. The bacterial EPS in conditions of water

**Table 3.** Estimation of the production of some enzyme by the nine rhizobacteria.

Rhizobacteria	Qualitative production of enzyme		
	Cat	Pro	Lip
<i>Bacillus polymysa</i>	+	-	+
<i>Bacillus anthra</i>	+	++	+
<i>Bacillus circulans</i>	+	+	+
<i>Bacillus thuringiensis</i>	+	-	-
<i>Bacillus panthothenicus</i>	+	++	+
<i>Pseudomonas cichorii</i>	+	++	+++
<i>Pseudomonas putida</i>	+	+	+++
<i>Pseudomonas syringae</i>	+	+	+++
<i>Pseudomonas marcescens</i>	+	+++	++

Cat: catalase, Prot: protease; Lip: lipase; + = Low; ++ = average; +++ = Strong, - = no production.

stress in the soil can limit or delay the middle of desiccation (Heulin and Achouak, 2012). Conversely, in case of excess water (rain, floods), EPS contribute to avoid dispersion of soils clayey (Henao and Mazeau, 2009). According to many authors, PGPRs producing EPS have a selective advantage over other bacteria during biotic stress (Wang et al., 2000) and abiotic stress (Mayak et al., 2004).

### Production of compounds with antibiotic effect

Volatile substances are also involved in the suppression of different pathogens. Hydrocyanic acid is one of those metabolites synthesized by certain rhizobacteria as a means of avoiding predation or competition (Heydari et al., 2008). All strains produced HCN at varying rates where *B. thuringiensis*, *P. cichorii* and *P. putida* strains recorded the highest yields; *B. anthracis*, *B. circulans*, and *S. marcescens* produced moderate HCN, and *B. polymysa*, *B. panthothenicus* and *P. syringae* produced only a small amount (Table 3). Contrary to the work done by Cherif, (2014), this work has shown that the majority of these isolated strains do not have the capacity to produce HCN. HCN production is a common activity in *Bacillus* (50%) in rhizosphere soils (Ahmad et al., 2008). *Pseudomonas* strains producing HCN are used in biological control against bacterial canker of tomato (Lanteigne et al., 2012). HCN production by the *P. fluorescens* CHA0 strain reduces the pathogenicity of fungi such as *Thielaviopsis basicola*, a black rot agent in tobacco (Mercado-Blanco and Bakker, 2007).

Another secondary metabolite produced by some rhizobacteria which indirectly influences plant growth is ammonia (NH<sub>3</sub>). The results obtained in our study show that *Pseudomonas* and *Bacillus* isolates and *S. marcescens* produced ammonia at 78, 80, and 100%, respectively. Strains of *P. putida* and *S. marcescens* produced high amounts of ammonia, whereas *B.*

*anthracis*, *B. circulans* and *B. thuringiensis* recorded average ammonia production. In contrast to *P. cichorii* and *P. syringae*, which had low ammonia production, *B. polymysa* and *B. panthothenicus* did not produce ammonia (Table 2). These ammonia production rates are lower than the 95% and 94% obtained by *Bacillus* and *Pseudomonas* isolates respectively was agree in the work done by Joseph et al. (2007). Yadav et al. (2010) where they also found higher rates of ammonia production by *Bacillus* spp. and by *Pseudomonas* spp on chickpea (*Cicer arietinum*) in India.

### Antifungal activity

Regarding to the results of variance analysis on inhibition levels of mycelial growth by the rhizobacteria tested, there were showed highly ( $p < 0.01$ ) or extremely significant ( $p < 0.001$ ) differences between the rhizobacteria (Table 4) for all the reference molds. With the exception of *P. cichorii*, the other *Pseudomonas* isolates did not express their potentiality to inhibit fungus growth. For of *Serratia* and *Bacillus* strains, the inhibitory activity was on the other hand very remarkable. Indeed, the rhizobacteria *B. polymysa*, *B. panthothenicus* and *S. marcescens* were the ones which showed more inhibitory effect on the mycelial growth of *A. parasiticus* at average inhibition rates of 75.55, 73.33 and 70.73% respectively. *B. anthracis* had the lowest average rate of inhibition (60%). For the *A. ochraceus* mold, *B. panthothenicus*, *B. anthracis* and *B. circulans* rhizobacteria strongly inhibited mycelial growth with mean inhibition rates of 70.50, 68.85 and 67.14% respectively and were significantly different. *P. cichorii* is the rhizobacterium that showed the least inhibitory effect on the growth of *A. ochraceus* with an average rate of 58.57%. As for the *A. fumigatus* and *A. clavatus* molds, the *B. polymysa* rhizobacteria followed by *B. panthothenicus* showed more inhibitory effect on their mycelial growth. The results also show that *B.*

**Table 4.** Antifungal activity of rhizobacteria on fungal plant pathogens.

Rhizobacteria	Inhibition of mycelial growth (%)							
	<i>A. parasiticus</i> CMBB 20		<i>A. ochraceus</i> CMBB 91	<i>A. fumigatus</i> CMBB 89		<i>A. clavatus</i> NCPT 97		
	cv	cv	m	cv	m	cv	m	cv
<i>Bacillus polymyxa</i>	75.55 <sup>a</sup>	6.02	66.00 <sup>abc</sup>	7.39	68.79 <sup>a</sup>	3.83	70.71 <sup>a</sup>	1.01
<i>Bacillus anthracis</i>	60.00 <sup>d</sup>	1.86	68.85 <sup>ab</sup>	2.28	-	-	-	-
<i>Bacillus circulans</i>	68.33 <sup>bc</sup>	2.44	67.14 <sup>ab</sup>	2.13	-	-	-	-
<i>Bacillus thuringiensis</i>	65.00 <sup>cd</sup>	2.56	63.14 <sup>abc</sup>	4.07	-	-	-	-
<i>Bacillus panthothenicus</i>	73.33 <sup>ab</sup>	3.68	70.50 <sup>a</sup>	1.32	57.86 <sup>b</sup>	10.97	68.57 <sup>a</sup>	3.08
<i>Pseudomonas cichorii</i>	63.89 <sup>cd</sup>	4.35	58.57 <sup>c</sup>	4.28	52.86 <sup>b</sup>	7.02	53.57 <sup>b</sup>	3.87
<i>Pseudomonas putida</i>	-	-	-	-	-	-	-	-
<i>Pseudomonas syringae</i>	-	-	-	-	-	-	-	-
<i>Serratia marcescens</i>	70.73 <sup>ab</sup>	1.93	60.35 <sup>bc</sup>	10.14	54.39 <sup>b</sup>	7.17	55.65 <sup>b</sup>	6.65
Probability	0.000		0.005		0.008		0.000	
Meaning	***		**		**		***	

\*\* =  $p < 0.01$  (highly significant); \*\*\* =  $p < 0.001$  (very highly significant). Averages with the same letters in the same column are not significantly different. - = no inhibition, m= average; cv= coefficient of variation.

*polymyxa* had the highest inhibitory effect on the mycelial growth of all molds. No antagonism was observed between the rhizobacteria *P. putida* and *P. syringae* and the four fungal strains. It is also to be noted that all the rhizobacteria strains that showed antagonistic effect to *A. parasiticus* also had same effect on *A. ochraceus*. Saranya and Sowndaram (2014) revealed a complete inhibition of mycelia growth of *Rhizotonia solani* (85%) and partial inhibition of *Sarocladium oryzae* (45%) against two rhizobacteria as Singh et al. (2017), whose showed in their results that *Bacillus* spp. possess antifungal activity against the spore forming fungi of *Alternaria* spp., *Fusarium* spp., *Bipolaris* spp. and *P. fluorescens* did not show any inhibition against the spores of the phytopathogenic fungi.

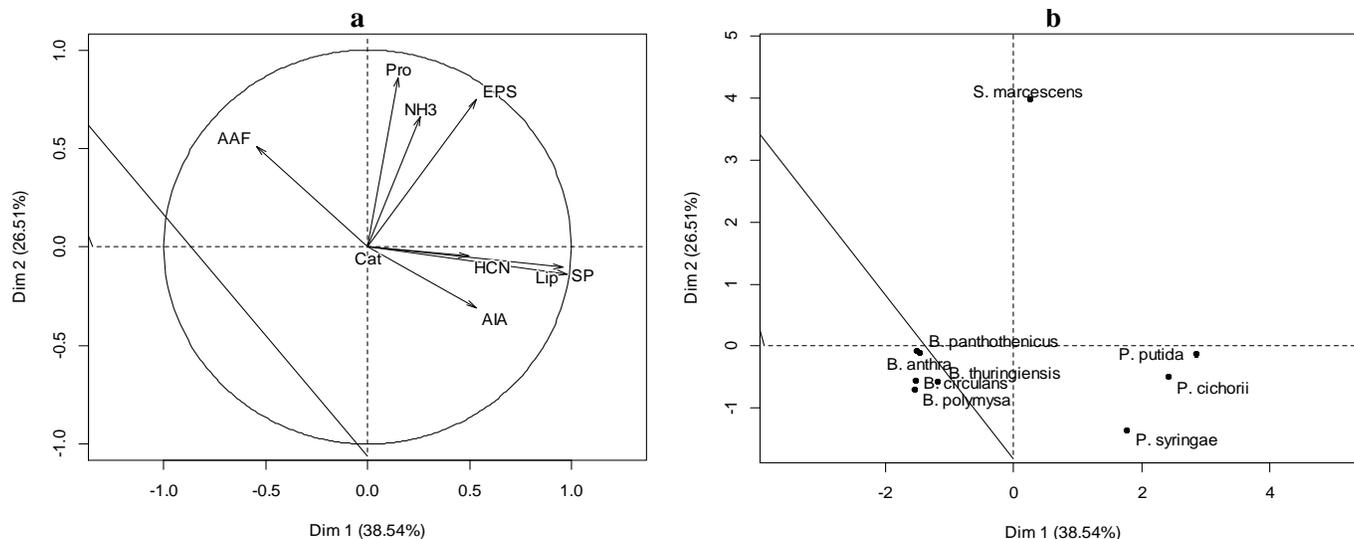
### Hydrolytic enzyme production

Some PGPR strains have the ability to degrade fungal cell walls through the production of hydrolytic enzymes. All the strains tested produced catalase. Protease production was high in *S. marcescens*, while a medium production was recorded in *P. cichorii*, *B. panthothenicus* and *B. anthracis*. The *B. polymyxa* and *B. thuringiensis* strains did not produce the protease. All strains of *Pseudomonas* had a high lipase production, while this production was average in *S. marcescens* and it was low in *Bacillus* strains with the exception of *B. thuringiensis* which did not produce lipase (Table 3). Similar results were observed by Noumavo et al. (2015) as well as Preeti et al. (2012) on the production of catalase. The bacterial strains producing catalase are potentially very

advantageous because of their resistance to environmental, mechanical and chemical stresses (Preeti et al., 2012). *Bacillus* species are catalase positive and able to form endospores that allow them to survive for long periods under adverse environmental conditions (Alizadeh and Ordoorkhani, 2011).

### Relationship between the different metabolites and the nine tested rhizobacteria

Estimation of the production of different metabolites by rhizobacteria was analyzed through a principal component analysis (Figure 1). The main results obtained indicated that the first two main components account at 65.04% for all the variability linked to the production of metabolites by rhizobacteria. The results showed that the different rhizobacteria do not have the same production potential of the metabolites evaluated. The projection of metabolites and rhizobacteria in the factorial axis plane formed by the two main components shows that *P. cichorii*, *P. syringae* and *P. putida* solubilized more phosphate, produced more indole acetic acid, exopolysaccharides, hydrogen cyanide, ammonia and lipase. In contrast *B. anthracis*, *B. circulans*, *B. thuringiensis*, *B. panthothenicus* and *B. polymyxa* have a strong antifungal activity (AAF) and produce less ammonia, hydrogen cyanide indole acetic acid, exopolysaccharides, lipase and solubilize less phosphate. *S. marcescens* produced more proteases, exopolysaccharides and ammonia with strong antifungal activity (AAF) and in contrast *B. polymyxa*, *B. circulans* and *P. syringae* produce more indole acetic acid and vice



**Figure 1.** (a): Projection of metabolites in the correlation circle formed by the first two major components; (b): Projection of rhizobacteria in the axis plane formed by the first two principal components.

NH<sub>3</sub>: ammonia ; HCN : Hydrogen cyanide; AIA : Indole Acetic Acid; EPS : Exopolysaccharides ; SP : Phosphate solubilization, Cat : catalase, Prot : protease ; Lip : lipase ; AAF : Antifungal activity. *B.*= *Bacillus* ; *P.*= *Pseudomonas* ; *S.*= *Serratia*.

versa. In addition, all rhizobacteria produced catalase in high proportion.

The results of this study showed that isolates were heterogeneous; some strains have remarkable phytostimulatory and biofertilising capacities. Indeed, the solubilization of phosphates and the simultaneous production of IAA has been demonstrated (Weller and Thomashow, 1994). While others had exclusive biocontrol abilities (antifungal activity). According to many authors, a correlation exists between these different activities (Mehta et al, 2010).

## Conclusion

The responses of isolated bacteria to the various tests inherent to the promotion of plant growth allow the demonstration of the natural potentialities of each strain. The present study is interested in determining properties with direct beneficial effects on plants through the provision of nutrients or indirect mechanisms by way of protection against plant pathogens. Indeed, the search for pro-PGPR properties on the different rhizobacteria under study, revealed that all *Pseudomonas* species solubilized more phosphate, produced more Indole acetic acid, exopolysaccharides, hydrogen cyanide, ammonia and lipase, while all species of *Bacillus* had a strong antifungal activity, As *S. marcescens* produced more proteases, these results could exploit these PGPR strains as beneficial multi-effect bioinoculants to increase productivity and improve plant and soil health, which will likely reduce the problems associated with the use of toxic fertilizers in agricultural practices.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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