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Full Length Research Article

EFFECTS OF PLANT GROWTH PROMOTING BACTERIA (PGPB) RHIZO-INOCULATION ON SOIL PHYSICO-CHEMICAL, BACTERIAL COMMUNITY STRUCTURE AND ROOT COLONIZATION OF RICE (*ORYZA SATIVA* L. VAR. FARO 44) GROWN IN FERRUGINOUS ULTISOL CONDITIONS

*Musa Saheed Ibrahim[1,2] and Beckley Ikhajiagbe[2]

*[1]Department of Biology and Forensic Science, Admiralty University of Nigeria, Delta State, Nigeria
[2]Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria

*Corresponding Author Email Address: musa-biology@adun.edu.ng Phone: +2347031316686

ABSTRACT
Ferruginicity is a special condition when soil became rich in iron. This condition is characterized by high pH and reduced bioavailability of plant limiting nutrients. Unfortunately, this type of soil covered 30% of arable lands in Nigeria. This research aimed at using a native plant growth promoting bacteria (*Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1) with phosphate solubilizing capabilities to remediate iron toxicity and improve soil nutrients, as well as soil micro-biota colonization. Soil physico-chemical properties before rhizo-inoculation (FA), after 16 weeks of rhizo-inoculation with the PGPB into the rhizoid of a growing rice seedling (FB) and after 16 weeks of natural attenuation (FC) were analyzed. Phosphate solubilizing capacity of the PGPB was investigated using a developed Pikovskaya's growth medium. The results showed that FA is rich in iron and deficient in bioavailable phosphorus and nitrogen (200.67 mg/kg, 8.01 mg/kg and 0.20%) respectively, as against the FB soil which showed moderate iron and increased soil nutrients (51.22 mg/kg, 20.21 mg/kg and 0.33%). The presence of acidic exudates, which was indicated by a red litmus paper at the rhizosphere in FB signifies the iron chelation capacity of the PGPBs resulting in iron remediation and soil nutrient improvement. Furthermore, the significant increase in microbial population in the FB soil as against the FA and FC corresponds with the SEM results at the root epidermis. Furthermore, other nitrogen-fixing bacteria such as *Bacillus subtilis* were observed to be active and motile in the FB. This indicated the effectiveness of PGPB with PSB capacities in iron remediation and soil nutrient enhancement.

Keywords: Plant growth promoting bacteria, rhizo-inoculation, iron chelation, root colonization, ferruginois ultisol.

INTRODUCTION

During weathering, iron in the rocks is released into the surficial environments, including soils. In the presence of O_2 and H_2O at a favorable pH range, the released Fe^{3+} that was oxidized from the dissolved Fe^{2+} are immediately hydrolyzed to form "secondary" pedogenic iron minerals, including iron (III) oxides or oxyhydroxides (Cornell and Schwertmann, 2003). The distribution pattern of iron (hydro) oxides in soil varies. For example, in aerobic soils, the Fe^{2+} ions once released from the primary minerals will be immediately oxidized, hydrolyzed and immobilized *in situ*. This form of iron usually forms homogeneous red coloration (Sharma *et al.,* 2013). Fe^{3+} mobilization have been documented by Stevenson (2005) to cause phosphate dynamics in soils, which have a serious negative effect on plants and soil physicochemistry (Musa and Ikhajiagbe, 2021). Phosphates are mineral elements of phosphorus that can be found in nature (Mejia *et al.,* 2016). Phosphorus together with nitrogen and potassium are very important macronutrients that is essential for plant growth (Israel and Yonas, 2021). Therefore, there is need to understand soil chemical dynamics.

Soil Phosphorus dynamics is characterized by physicochemical (sorption-desorption) and biological (immobilization-mineralization) processes. When large amount of phosphate (PO_4^{-3}) enters in to the immobile pools containing Fe^{3+}, the (PO_4^{-3}) is trapped under acidic condition and therefore making phosphorus not bioavailable for plant to use, while iron becomes excess (Gyaneshwar *et al.,* 2002; Hao *et al.,* 2002). This special soil condition is termed ferruginous ultisol (Deubel *et al.,* 2005). Ferruginous soil which is also called red soil are usually poor growing soils with low nutrients and humus levels (Yu *et al.,* 2016). It tends to form in warm, temperate, humid climates and in regions covered with deciduous or mixed forests (Doyou *et al.,* 2017). These special soil landscapes are primarily distributed throughout the tropical and subtropical areas, particularly in South America, Southern North America and Africa (Zhao, 2014). The total area of red soils is approximately 64 million km^2, accounted for 45.2% of the Earth's surface area (Anumalla *et al.,* 2019) and resided by 2.5 billion people, nearly half of the global population (Chandran *et al.,* 2004). In Nigeria, it is predominant in some Southern States such as Edo state, occupying about seven zones, including extreme north and central Benin (Doyou *et al.,* 2017). The excessive presence of iron and insufficient macronutrients in this type of soil has seriously affected food production and security in Nigeria, especially in Edo State. This necessitates sustainable remediation and soil improvement strategies (Zhao *et al.,* 2015; Duan *et al.,* 2016).

Soils can be remediated through the removal of toxic metals and improvement of soil physicochemistry, which can be achieved through bioremediation (Maddela *et al.,* 2017). Bioremediation has become an important area of research, especially for its sustainable approach that does not produce secondary pollution along with low economic costs (Liu *et al.,* 2018). Several bioremediation strategies using either plants, animals or

microorganisms (Musa, 2019) have been suggested by previous researches. Among these cost-effective bioremediation strategies, microbe-assisted bioremediation attracted much attention in recent years (Cavalca *et al.,* 2010; Ghosh *et al.,* 2011; Mallick *et al.,* 2014; Ullah *et al.,* 2015). Many species of microorganisms have been previously used in remediation of different metallic pollution (Musa *et al.,* 2018). These microorganisms are usually present in larger quantities with fast metabolic activities and can improve soil biogeochemistry either as a single living organism or in symbiosis interaction with other microbes and plants rhizoids (Chen *et al.,* 2017; Jing and Kjellerup, 2018). Ferruginous soil has a unique geochemistry therefore, bacteria that can survive acidic condition, with the capacity to mineralize and solubilize phosphate is essential (Deubel *et al.,* 2005), such bacteria are called phosphate solubilizing bacteria (PSB).

PSB are beneficial bacteria that have the capacity to chelate iron-phosphorus bond and make phosphorus bioavailable for plant use (Babalola and Glick, 2012; Bhattacharyya and Jha, 2012). This process is usually achieved through the ability of the bacteria to release low molecular weight metabolites such as organic acids, mainly gluconic and keto-gluconic acids (Deubel *et al.,* 2000) which through their hydroxyl and carboxyl groups chelate the cation (Fe) bound to phosphate and decrease the pH in basic soils (Stevenson, 2005). This converts the phosphorous to soluble form and available by plants. In addition to lowering the pH of rhizosphere, the iron level is reduced and nutrient levels is increased (Sharma *et al.,* 2013). For this purpose, scientist have proposed its use as biofertilizer since 1950s (Kudashev, 1956).

In the current research, plant growth promoting bacteria (*Bacillus cereus* strain GGBSU-1, *Proteus mirabilis* strain TL14-1 and *Klebsiella variicola* strain AUH-KAM-9) that were previously isolated by Musa and Ikhajiagbe (2021) from different soil types in Benin City and then characterized following the 16S rRNA were used. In the previous study by Musa and Ikhajiagbe (2020), the PGP bacteria was able to solubilize phosphorus by forming diameter of holo-zone under Pikovskaya's powdered media (Plate 1). In addition, the *in vitro* growth medium experiment showed that the PGP bacteria can tolerate acidic condition of up to 4 (Table 1) however, *in vivo* field trials for related research have rarely been reported (Chen *et al.,* 2013; Pan *et al.,* 2017). The soil dynamics and abiotic changes have been the major reasons why environmental researchers have decided not to consider *in vivo* experimentation on similar research. Therefore, this research aims at investigating the capabilities of the three PGPB to solubilize phosphate in field experiment (*in vivo*) through rhizo-inoculation, to remediate iron toxicity in ferruginous soil and improve the rhizo-bacteria colonization, as well as the physico-chemical properties of ferruginous ultisol.

Table 1: Tolerance of PSBs at different pH levels in Pikovskaya's medium

PSBs/pH levels	4	5	6	7	8	9	10
EMBF2	+	+	++	+	+	-	-
BCAF1	+	+	+	+	+	-	-
BCAC2	++	++	++	+	+	+	-

- indicates absence of growth in the pikovskya's medium, while "+" indicates present of weak growth and "++" indicates strong growth. Source: Musa and Ikhajiagbe (2020).

MATERIALS AND METHODS

Preparation of soil used in the experiment:
The experiment was carried out at the experimental garden of the Department of Biology and Forensic Science, Admiralty University of Nigeria, Delta State Nigeria. The ferruginous soils used in this study was a composite soil that was previously obtained by Musa and Ikhajiagbe (2020) from Benin City, Edo State of Nigeria. The ferruginous soils were divided into three (FA= Ferruginous soil before rhizo-inoculation, FB= ferruginous soil after 16 weeks rhizo-inoculation, FC= ferruginous soil after 16 weeks natural attenuation) and prepared in experimental bowls (30 x 25 cm) and made in five replicates.

Collection of microbial isolates:
Three bacteria species (*Bacillus cereus* strain GGBSU-1, *Proteus mirabilis* strain TL14-1 and *Klebsiella variicola* strain AUH-KAM-9) with phosphate-solubilizing capabilities that were previously isolated from humus soil in an earlier study by Musa and Ikhajiagbe (2020) in Benin City. The three bacteria were prepared in slant and streak onto petri dishes for quantitative estimation of plant growth promoting properties.

Quantitative estimation of plant growth promoting (PGP) capabilities:
The PGP capabilities of the bacteria isolates (*Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1) were calculated by determining the IAA and Siderophores. The IAA (µg/ml) was quantitatively determined using Luria Bertani broth following Gupta *et al.* (2012), while the siderophore (nmol) production was quantitatively determined using MB medium and incubated for 72 hours following (Balkar, 2013). The absorbance levels of the sample were measured spectrophotometrically at 630 nm and 520 nm respectively following Schwyn and Neilands (1987) and Gordon and Weber (1951).

Preparation of inoculum:
The pure bacteria isolates (*Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1) were prepared by streaking on to agar plates and incubated at 28^{o}C for 48 hours. After 48 hours growth, the isolates were inoculated in Nutrient broth and then prepared into 0.5 McFarland Standard with Cat. No (TM50) to standardize the approximate number of bacteria in the suspension. Following this process 500 mL of each bacteria isolate was prepared to obtain an average microbial suspension of 1.5x10^{8} CFU/mL.

Soil physiochemical parameter:
Soil samples from around the root regions in FA, FB and FC were air-dried at temperature of 22-25^{o}C and then analyzed for soil organic matter levels (SOM), soil available phosphorus, cation exchange capacity (CEC), pH of the soil, total nitrogen, organic carbon (OC), exchangeable acidity (EA), available potassium, available micronutrients such as sodium (Na) and Aluminum (Al), electrical conductivity, soil texture class and maximum water holding capacity following Musa and Ikhajiagbe (2020). The iron levels of the soil were analyzed following the method of Cheng *et al.* (2013) by using concentrated perchloric acid to digest the soil sample and subjecting it to titration with versanate solution.

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Rhizo-inoculation of rice seedlings:
Rice seedling were grown for 10 days in the ferruginous soil to allow root acclimatization, then the prepared McFarland standard (McFarland and Nephelometer, 1944) of 500 mL bacteria inoculum were made in to 10 mL of the mix bacteria (*Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1). Through random selection, 10 seedlings were selected and the calculated inoculum volume was introduced into each of the root region of the growing seedling using 10 mL syringe following Etesami *et al*. (2014). The setup was further observed for 16 weeks using randomized blocked design and wetted with 5 mL distilled water every 3 days.

Biofilm formation and root colonization:
To study the efficiency of the PGPB isolates to form biofilm, a 96-well cell culture plate was used following (Nunclon Delta Surface, Thermo Scientific) after 4 days of rhizo-inoculation in the rice root regions in the ferruginous soil. The biofilm cells attached to each well was investigated using the plate reader at 600 nm. The percentage of cells within the biofilm was calculated by calculating the relation between biofilm cells and the total amount of cells.

Scanning electron microscopic (SEM) analysis of biofilm:
After the biofilm assay, the cells forming biofilms were inoculated on coverslips (1% v/v) kept in Pivoskaya media 24 h at 37 °C without disturbance (Koerdt *et al.,* 2010). The biofilms were fixed following the same protocol as mentioned earlier (Mallick *et al.,* 2014). After complete dehydration, the coverslips were dried and viewed under SEM.

Statistical analysis:
Data obtained from the analysis were presented as means and standard errors of five replicates. Data were analyzed following two-way analysis of variance on GENSTAT (8th edition). Significant p-values were obtained, differences between means were separated using Student Newman Keuls Test (Alika, 2006). The ferruginous soil used in the current experiment was homogenized.

RESULTS

Physico-chemical properties of the experimental soil:
Table 2 showed the physical and chemical properties of the ferruginous soils assayed in the research. The FA (ferruginous soil before rhizo-inoculation) was observed to be rich in Fe^{2+}, Mg^{2+} and Na^{2+} compared to the FB (ferruginous soil after 16 weeks rhizo-inoculation) and FC (ferruginous soil after 16 weeks natural attenuation). Low water holding capacity and available phosphorus were also observed in the FA, as against the FB and FC. Furthermore, the FA soil showed to be acidic pH (5.01). Meanwhile, the FB soil was observed to show significantly higher organic matter, more bioavailable phosphorus and reduced iron levels, bringing about a relative soil pH of 5.92. Higher macronutrients such as nitrogen were also observed in the FB compared to the FA and FC however, the FC was observed to have higher macronutrients than the FA.

Table 2: Physical and chemical parameters of the ferruginous soil.

Parameters	FA	FB	FC
Available phosphorus (mg/kg)	8.01 ± 0.04	20.21 ± 0.05	10.01 ± 0.11
Electric conductivity (µS/cm)	301.09 ± 1.22	111.0 ± 1.21	303.0 ± 1.55
pH	5.01 ± 0.21	5.92 ± 0.65	5.43 ± 0.98
Total organic carbon (%)	0.41 ± 0.11	0.72 ± 0.04	0.65 ± 0.10
Soil organic matter (%)	7.31 ± 0.56	17.08 ± 0.09	9.08 ± 0.02
Total Nitrogen (%)	0.20 ± 0.01	0.33 ± 0.11	0.11 ± 0.05
Exchangeable acidity (meq/100g)	0.16 ± 0.13	0.21 ± 0.32	0.15 ± 0.20
Cation exchange capacity (cmol/kg)	1.70 ± 0.02	2.22 ± 0.24	1.22 ± 0.01
Textural class	Loamy-sandy	Loam-silty	Loam-sandy
Clay (%)	10.92 ± 1.42	11.24 ± 0.01	12.24 ± 0.01
Silt (%)	8.72 ± 2.76	40.10 ± 0.11	9.10 ± 0.09
Sand (%)	95.10 ± 0.09	34.65 ± 0.03	92.65 ± 0.11
Fe (mg/kg)	200.67 ± 2.44	51.22 ± 0.90	198.22 ± 0.02
Water holding capacity (%)	68.89 ± 0.12	85.11 ± 0.12	69.11 ± 0.09
Available potassium (mg/kg)	0.02 ± 0.08	0.11 ± 0.11	0.09 ± 0.12
Mg^{2+} (meq/100g)	4.21 ± 1.12	1.63 ± 0.17	1.22 ± 0.04
Na^{+} (meq/100g)	3.09 ± 0.29	1.91 ± 0.22	2.41 ± 0.02
Al (meq/100g)	6.23 ± 1.22	0.74 ± 0.11	5.74 ± 0.05

FA= Ferruginous soil without rhizo-inoculation, FB= Ferruginous soil after 16 weeks PGPB rhizo-inoculation, FC= Ferruginous soil after 16 weeks of natural attenuation. Fe = iron, Al= aluminum, Na= sodium, Mg= magnesium.

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Quantitative estimation of plant growth promoting (PGP) capabilities:
Figure 1 and 2 showed the capabilities of the three bacteria used in the study to show plant-growth promoting properties. The result showed that *Bacillus cereus* strain GGBSU-1 produced the highest indole acetic acid (IAA=11.0 µg/ml) levels, followed by *Klebsiella variicola* strain AUH-KAM-9 (7.0 µg/ml), while *Proteus mirabilis* strain TL14-1 (6.0 µg/ml) synthesized the least IAA. Furthermore, a similar result was obtained in the case of Siderophores, where the *Bacillus cereus* strain GGBSU-1 was found to synthesize highest siderophore (8.0 nmol) as against the *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1 which synthesize equal siderophore level (7.0 nmol).

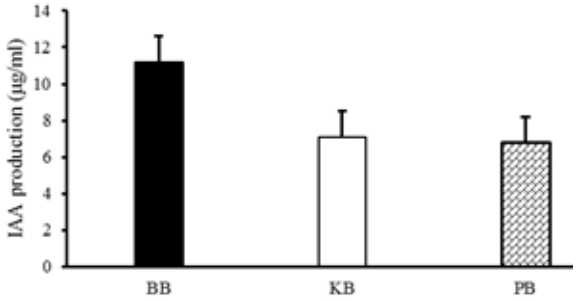


Figure 1: Quantitative analysis of plant growth promoting activities: IAA production (a). Data are the mean of five replications for each bacterium (p = 0.05). BB= *Bacillus cereus* strain GGBSU-1, KB= *Klebsiella variicola* strain AUH-KAM-9, PB= *Proteus mirabilis* strain TL14-1

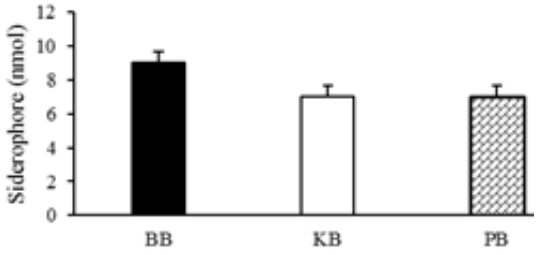


Figure 2: Quantitative analysis of plant growth promoting activities: Siderophores production. Data are the mean of five replications for each bacterium (p = 0.05). BB= *Bacillus cereus* strain GGBSU-1, KB= *Klebsiella variicola* strain AUH-KAM-9, PB= *Proteus mirabilis* strain TL14-1.

Bacteria community structure:
The effects of PGP bacteria on the bacteria community structure around the rice root region were determined (Figure 3). The results showed that the total number of bacteria in the rhizosphere of the test plants was 4 x 10^7 CFU/g before inoculation (FA). Inoculating the rice rhizoid with the PGP bacteria after 16 weeks (FB) increased the number of bacteria in the rhizosphere to 34 x 10^7 CFU/g. In order to see if the natural attenuation could have an effect bacterial community structure, bacteria count after 16 weeks of natural attenuation was observed to show approximately 7 x 10^7 CFU/g of bacteria (FC). Furthermore, high weed abundance per 3 days was observed in the FB compared to the FA and FC soils.

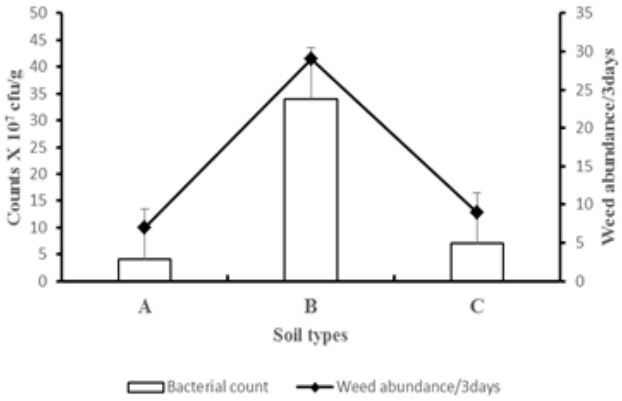
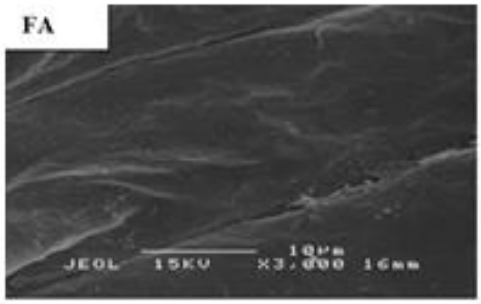
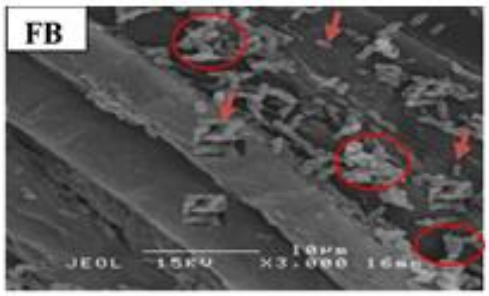


Figure 3: Bacteria community structure and weed abundance analysis of the ferruginous soil.

Rhizobacteria colonization:
To confirm the effective rhizobacteria colonization by the three PGPB, the epidermal section of rice root in FA, FB and FC were viewed using SEM. Images of the epidermal sections (Figure 4) viewed under 3000× magnification showed that the three PGPB (*Bacillus cereus strain GGBSU-1*, *Proteus mirabilis* strain TL14-1 and *Klebsiella variicola* strain AUH-KAM-9) has successfully colonized and proliferate the epidermal sections of rice roots within the 16 weeks after rhizo-inoculation (FB). However, the FA and FC soil showed no signs of microbial colonization.





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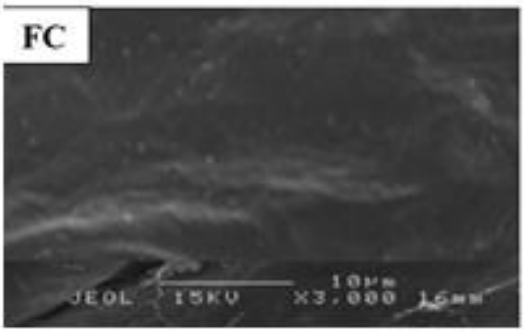

Figure 4: Images of rice root epidermis showing in situ rhizo-colonization by PGPB (indicated by oval) under SEM. FA= Ferruginous rhizoid without inoculation, FB= Ferruginous rhizoid with PGPB inoculation after 16 weeks, FC= Ferruginous rhizoid under 16 weeks natural attenuation.

Furthermore, signs of root exudation (Figure 5) from the rice rhizoid were predicted when the root region of the FB turned blue litmus paper to red after 16 weeks of study as against the root in FA and FC. *Bacillus subtilis*, *Nitrosomonas* spp., *Pseudomonas* spp, *Pseudomonas fluorescens* and *Azoarcus* spp were the notable bacteria found in the rice rhizosphere. All bacteria at the rhizosphere proved to be motile.

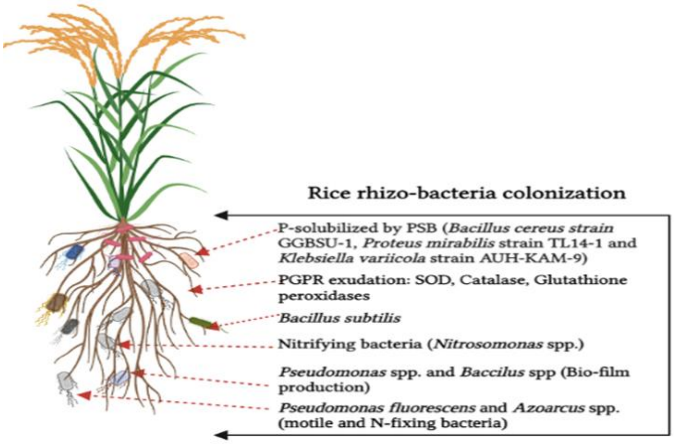

Figure 5: Schematic description of rice rhizo-bacteria colonization at 16th week after rhizo-inoculation of PGPB

DISCUSSION

The low bioavailable phosphorus and nitrogen, as well as the high iron observed in the FA soil indicated the low micronutrient level of the soil. According to Musa *et al.* (2018), the best soil type for growing crops are the ones that are rich in soil micronutrients such as nitrogen and phosphorus. The bioavailability of soil micronutrient encourages nutrient cycling in soil as well as beneficial bacterial proliferation in soil. Furthermore, the increased water holding capacity observed in the FB showed the influence of the PGPB inoculum in encouraging soil nutrient balance and organic carbon increase, which helps in improving water retention in soils. This improved water retention levels after 16 weeks inoculation with PGPB may be the reason for the increase in relative soil pH. According to Ikhajiagbe *et al.* (2020), one of the yardsticks used in predicting the nutrient level of soil is its pH. Soils that are strongly acidic (as observed in the FA-5.01) are known not to encourage bacterial growth and thereby inhibit nutrient recycling and balancing in the soil (Israel and Yonas, 2021). On the other hand, moderately acidic soil (as observed in FB) has been documented to be an average soil condition for optimum plant growth as well as rhizobacteria proliferation (Maddela *et al.,* 2017; Musa *et al.,* 2018). Considering the optimum pH level of the FB soil, as well as the water holding capacity and the micronutrients bioavailability, we can easily juxtapose it as the reason why high weed abundance was observed in the FB soil as against the FA soil.

According to Gustavo *et al.* (2021), a number of bacterial species have been successfully tried for soil improvements, among which the best is those with plant growth promoting capabilities (PGP). The major PGP properties are the ability to synthesize indole acetic acid (IAA) and siderophores among others (Compant *et al.,* 2019). The high concentration of IAA and siderophores synthesized from the three bacteria used in this study further buttress the PGP capabilities of the bacteria, leading to improved soil properties by balancing soil nutrients. Siderophores and IAA are low-molecular-weight secondary metabolites that are produced majorly by PGPB in iron toxic soils such as ferruginous soil. In these soils, the high iron levels tend to trap soil phosphorus and therefore making it not available for organisms to use (Orttiz-Galeana *et al.,* 2018; Phour *et al.,* 2020). When siderophore and IAA are released by PGPB, the organic acid chelates the Fe-P bond and therefore making phosphorus bioavailable in the soil. This situation also improves the bioavailability of other soil micronutrients, thereby improving soil properties (Dimkpa, 2016). Phosphorus is one of the limiting nutrients for agricultural soils. Most soils contain a large amount of phosphorus (Zhang *et al.,* 2019), but only low amount is bioavailable for microbial and plant use, especially in iron toxic soils.

Furthermore, the higher levels of bacteria count observed in the FB soil may be as a result of the improved physico-chemical properties of the soil, providing conducive environment for bacteria to proliferate and rapidly grow. The soil conditions observed in the FB soil such as improved water holding capacity, improved organic matter and improved bioavailability of nutrients as a result of the released secondary metabolites by PGPB may be responsible for the increased bacterial count witnessed in the FB soil as against the FA and FC soils. According to Zhang *et al.* (2019), microbes breed rapidly at favorable condition such as moisture, optimum pH and nutrients availability.

The effective rhizobacteria colonization observed in the epidermal section of FB soil through SEM indicated the ability of the PGPB to form biofilm. Biofilms are extracellular matrices that can play important roles in root tip colonization, as demonstrated by Dekkers *et al.* (1998) in Pseudomonas sp. The ability of Bacillus species in this study to show effective colonization is consistent with the work of (Danhorn and Fuqua, 2007) who observed bacteria of the genera Bacillus and Pseudomonas as the genera most commonly used to study the process of biofilm production. Furthermore, the colonization capacity of the PGPB observed in the FB indicated the ability of the PGPBs to proliferate and grow rapidly, thereby improving soil properties.

Furthermore, the ability of the plant rhizoid in the FB soil to turn blue litmus paper to a red one indicated the secretion of some acidic exudates (chemotaxis) from the biofilms. Chemotaxis is the ability of bacteria to receive a chemical stimulus (low molecular weight organic acid) and coordinate movement towards a stimulus with the help of cellular organelle such as flagella or pili. Bacterial flagella and pili allow the motility of bacteria, including those that inhabit the rhizosphere, mainly because of the chemical attraction exhibited by root exudates (Bakker *et al.,* 2010). Motility is a key trait for the colonization of the rhizosphere by various rhizospheric species. This study is consistent with the work of (Barahna *et al.,* 2021; Fernandez-Llamosas *et al.,* 2021) who observed that PGPB that are motile can easily release chemotaxis that can be used in improving bacteria colonization. In the present study, the organic acid released has brought about the chelation of the iron-phosphorus bonding, thereby making phosphorus and other micronutrients bioavailable for soil improvement (Sachdev *et al.,* 2009). The *Bacillus subtilis*, *Nitrosomonas* spp., *Pseudomonas* spp, *Pseudomonas fluorescens* and *Azoarcus* spp that were notable at the rhizoid in FB soil indicated the conducive nature of the soil, thereby encouraging the proliferation and growth of bacteria.

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Declaration of interest statement:
No competing interest is recorded. Consent is given for publication of this manuscript when accepted.

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