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Plant Growth Promotion and Biocontrol Properties of *Aneurinibacillus migulanus* Isolated from Maize Roots (*Zea mays*)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To isolate Plant Growth Promoting Bacillus strain from maize roots, to evaluate its biocontrol potentials and to characterize the isolate using16S rRNA sequencing.
Place and Duration of Study: Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, between February 2019 and March 2020.
Methodology: The isolation of Plant Growth Promoting Rhizobacteria (PGPR) from maize roots was done using Pikovskaya (PVK) agar. Quantitative determination of phosphate was carried out using PVK broth. Evaluations of other plant growth promoting properties were carried out such as IAA, etc. Fusarium and Enterobacter plant pathogens were isolated from diseased maize plants. The in vitro antagonism effects of the PGPR isolates against the pathogens were analyzed using the dual culture plate technique. The pot experiment was carried out in a completely randomized design. Plant characteristics such as plant height, shoot and root weight, chlorophyll content, as well as disease assessment were recorded accordingly. The organisms were identified using phenotypic and molecular methods.
Results: Seven PGPR bacteria were isolated from maize (Zea mays) roots using PVK agar. Aneurinibacillus migulanus gave the highest solubilization index of 4.21 while isolate IS48 gave the

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lowest solubilization index of 1.47. A. migulanus produced IAA, ammonia and cellulase enzyme but no hydrogen cyanide. The organism showed antagonism activity against the two tested phytopathogens. In the pot experiment, A. migulanus treated plants showed a statistically insignificant difference in maize plant height at P=0.05 but gave significant increases in shoot and root wet weights. The organism offered 83.33% and 71.43% protection against Enterobacter and Fusarium pathogens respectively in the pot experiment.

Conclusion: A. migulanus solubilized phosphate in addition to other plant growth promoting properties. It showed biocontrol potentials both in vitro and in vivo and thus can be used as substitute for synthetic agrochemicals.

Keywords: PGPR; Phosphate solubilization; Aneurinibacillus migulanus; Phytopathogens.

1. INTRODUCTION

Plant rhizosphere, the narrow zone of soil surrounding the root system of growing plants, represents a hotspot for microbial activity in the soil [1,2]. The rhizosphere is colonized by a wide range of microorganisms among which bacteria and fungi are predominant [3], exhibiting fundamental ecological functions. The use of efficient plant growth promoting rhizobacteria (PGPR) is deliberated as a suitable substitute for minimizing the use of synthetic agrochemicals in crop production [4,5]. PGPR-mediated growth promotion occurs by the alteration of the whole microbial community in rhizosphere niche, through the production of various substances [6]. Different PGPR strains are capable of increasing crop yields, exhibit biocontrol abilities, enhance resistance to foliar pathogens, promote nodulation in legumes, and enhance the emergence of seedlings [7,8].

Plant diseases critically endanger agricultural resources. In particular, soil-borne pathogens cause dramatic yield and economic losses, with fungi being the most aggressive of all pathogens [9,10]. The use of efficient PGPR as biofertilizers and biological control agents is deliberated as a suitable substitute for minimizing the use of synthetic agrochemicals in crop production [3]. This is necessitated owing to the fact that rampant overuse of synthetic agrochemicals for enhancing crop productivity has deteriorated the biological and physicochemical health of the arable soil, leading to a declining trend in agricultural productivity across the globe, over the past few decades [11]. Microorganisms with antagonistic activity such as fungi, bacteria, and actinomycetes are employed in biological control of plant pathogens. However, the first two have been the most widely used, especially the *Trichoderma* and *Bacillus* genera [12]. Promoting sustainable agriculture with a gradual decrease in the use of synthetic agrochemicals and more prominent utilization of the biowaste-derived substances [13] as well as PGPR is an effective strategy to combat the rapid environmental deterioration, while ensuring high agricultural productivity and better soil health [14]. Agricultural sustainability, food security and energy renewability depends on a healthy and fertile soil.

Members of the genus *Bacillus* constitute an important group of PGPR, which improve growth and yield of crops [15] and also, biocontrol agents. The genus represents one of the most abundant and phylogenetically diverse groups of easily cultivable PGPR [16]. *Bacillus* species, as a result of their avid rhizosphere colonization and Plant Growth Promotion characteristics, offer considerable interest for improving crop productivity and yield [17]. These bacteria are favored for commercialization as PGPR owing to their ability to produce heat and desiccation-tolerant endospores. These structures are crucial in maintaining high cell viability and prolonging shelf life in formulations under storage [18]. *Aneurinibacillus migulanus* has been shown to be an effective biocontrol agent (BCA), inhibiting plant pathogens via production of cyclic peptides, particularly gramicidin S [19]. In addition, *A. migulanus* strain *Nagano* produces a biosurfactant which decreases surface tension on plant surfaces, and consequently inhibits spore germination, adding to the action of gramicidin S.

The objective of this study was to isolate *Bacillus* species with biocontrol potentials, evaluate the ability of the organism to promote maize plant growth and protect the plant from pathogens under the green house and field conditionsas well as to characterize the isolates phenotypically and molecularly based on the 16S rRNA gene sequencing.

2. MATERIALS AND METHODS

2.1 Isolation of Phosphate Solubilizing Bacteria

The maize plants were carefully uprooted and immediately transported to the laboratory. The roots were washed thoroughly to remove adhering soil particles and cut into 1 inch length. The isolation was carried out using a heat shock method of Al-Humam, [20]. The roots were heated at 80^{o}C in a water bath for 60 min and subcultured unto Pikovskaya [21] Agar (Yeast Extract 0.50 g, Dextrose 10.00 g, Calcium Phosphate 5.00 g, Ammonium Sulphate 0.50 g, Sodium Chloride 2 mg, Potassium Chloride 0.20 g, Magnesium Sulphate 0.10 g, Agar 15.00 g, distilled water 1000 ml) using the method of Baliah *et al*., [22]. The isolates were purified by further subculturing unto a solid fresh PVK medium until pure colonies were obtained. The organisms were inoculated onto PVK medium and incubated for 7 days. Halozone formation showed the ability to solubilize phosphate. Phosphate solubilization index was evaluated according to the ratio of the halozone diameter (colony diameter + halo zone) and the colony diameter [23].

2.2 Quantitative Determination of Phosphate Solubilization

Quantitation of the amount of soluble phosphate was done according to the methodology of Mehta and Nautiyal [24]. The flasks containing 50ml of pvk broth medium prepared as stated above but without addition of agar were inoculated with 50 microlitre bacterial culture in triplicates and incubated at room temperature for 5 days in the rotary Shaker. Simultaneously, the uninoculated controls were also kept under similar conditions. The cultures were harvested by centrifugation at 10,000 rpm for 10 min and solubilized phosphate determined using photometric chlorostannous reduced phosphomolybdic acid blue method of Jackson [25].

Fifty microlitre of the sample (supernatant) was transferred to 25ml volumetric flask using a micropipette. Chloromolybdic acid (5ml) was added along the sides of the flask and the volume made up to 20ml using distilled water. Chlorostannous acid (0.5ml) was added, mixed and the volume was quickly made up to 25ml using distilled water. Absorbance was read at 600nm wavelength. The total soluble phosphorus was calculated from the regression equation of standard curve. The pH of culture supernatants was also measured using a pH Meter.

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2.3 Evaluation of other Plant Growth Promoting Properties of the Organism

2.2.1 Detection of Indole acetic acid

2.2.1.1 Culture growth conditions

Fifty mililitres of Nutrient broth (NB) containing 0.1% DL tryptophan was inoculated with 500 microlitre of 24h old bacterial culture and incubated with shaking at 180 rpm for 48h. The bacterial culture was centrifuged at 6,000 rpm for 10 min. Estimation of indole-3-acetic acid (IAA) in the supernatants was done using colorimetric assay [26].

2.2.1.2 Colorimetric estimation

One mililitre of supernatant was mixed with 4 ml Salkowski reagent and absorbance of the resultant pink colour was read after 30 min at 535 nm in UV/Visible Spectrophotometer. Appearance of pink colour in test tubes indicated IAA production as described by Gordon and Weber [27].

2.2.2 Assay for NH_3 production

The isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated into 10 ml peptone water in each tube and incubated for 48 h at 30^{o}C. Nessler's reagent (0.5 ml) was then added to each tube. Development of brown to yellow colour showed a positive test for ammonia production [28].

2.2.3 Hydrogen Cyanide production

Screening of bacterial isolates for hydrogen cyanide (HCN) production was done as per methodology described by Rijavec and Lapanje, [29]. Bacterial cultures were streaked on nutrient agar medium containing 4.4 g per liter of glycine. A Whatman filter paper No. 1 soaked in 0.5% picric acid solution (in 2% sodium carbonate) was placed inside the lid of the plate. Plates were sealed with parafilm and incubated at 30°C for 4 days. Development of light brown to dark brown color indicated HCN production.

2.2.4 Cell wall degrading enzyme production

Colonies were screened for cellulase activity by plating on Carboxymethyl CMC agar 1.88 g, sodium citrate 0.5 g, K_2HPO_4 7.0 g, KH_2PO_4 2.0 g, $(NH_4)_2SO_4$ 1.0 g, $MgS0_4.7H_20$ 0.1 g, Agar 10 g, Congo red 0.20 g, pH 7.0, Distilled water 1000ml. The agar plate was prepared and spot inoculated with test organism and incubated at 30^{o}C for 5 days. Development of halo zone around the colony was considered as positive for cellulase production. To visualize the hydrolysis zone, the plates were flooded with an aqueous solution of 1% (w/v) Congo red for 15 min and discolored with 1 M NaCl for 15 min [30].

2.4 Isolation of Phytopathogens

The isolation of fungal plant pathogen, *Fusarium solani* was made using the method of Fadhal *et al.,* [31] and later identified using 16S rRNA sequencing. Root samples were collected from some diseased maize plants showing typical symptoms of root rot in a farm at Nnamdi Azikiwe University, Awka. The plants were immediately transported to the laboratory for isolation of the fungal pathogen. The roots were washed to remove adhering soil particles, cut into small pieces, sterilized with NaOCl (1%) solution for 2 mins. They were dried using a filter paper to remove excess water and cultured onto Potato Dextrose Agar media, supplemented with chloramphenicol antibiotics at a concentration of 200mg/l. The plates were incubated at room temperature for 5 days.

The isolation of the bacterial pathogen *Enterobacter tabaci* was done using the method of García-González *et al*., [32]. Leaves of symptomatic maize plants were collected from a farm, and then were rinsed with sterile distilled water (SDW), disinfected with 0.3% sodium hypochlorite for 30s and rinsed with SDW for three times. Then leaves were drained and dried before aseptically sectioning. Small (3 × 3 mm) leaf sections were then excised from the edge of the diseased tissue and macerated in 3 ml of SDW for 90 s in a sterile mortar. The suspensions were streaked onto nutrient agar (NA) plates and incubated at 30°C for 24-48 h. Single colonies were isolated and re-streaked at least twice to purify the bacterial strains.

2.5 *In vitro* Antagonism Tests Against Phytopathogens

The *in vitro* antagonism effect of the PGPR isolates on the mycelial growth of *Fusarium* was analyzed on nutrient agar medium using the dual culture plate method [33]. The pathogenic fungi was applied as mycelial plug (10 mm in diameter) from the edge of a pre-cultured colony and are placed onto the center of each nutrient plate (90 mm in diameter). The bacterial isolates (about 10^8 CFU/ml) were equidistantly spot inoculated around the fungal inoculum at a distance of 35 mm at 24h after pathogen inoculation. Nutrient Agar medium inoculated with pathogen alone was used as the control. Plates were incubated at room temperature for 7 days. The *in vitro* antagonism test of the isolates against *Enterobacter sp* was carried out according to the method of Marčić *et al*., [34]. A lawn of the soft rot isolate was made with a loopful of the bacterial suspension (10^8 cfu/ml) prepared using a 0.5 McFarland standard, on a nutrient agar medium. The test isolates were equidistantly inoculated at a distance of 3cm from each other. Plates were incubated at room temperature for 48 hours, and inhibition zones were measured as the distances between the edge of antagonistic bacterial growth and the edge of the *Enterobacter* tested isolates. The isolates which showed the highest degree of antagonism were used for the pot experiment.

2.6 Formulation of PGPR into Biofertilizer

Endospore-containing cultures of the PGPR were used to coat the maize seed (Suwan-1-SR) variety seeds. The PGPR bacterial growth (14-day old) were scraped from twenty petri dishes and suspended in 120ml phosphate buffer saline (PSB), pelleted by centrifugation for 20 min at 10,000rpm, re-suspended in fresh PBS (60ml) obtaining about a McFarland standard of 2, with about 5.83 x 10^8 cfu/ml. The suspension was thickened with 2g Carboxymethyl cellulose CMC + 1g sterile composted saw dust. 200 maize seeds (surface-disinfected in 0.5% NaOCl for 20 min and rinsed in sterile distilled water) were thoroughly mixed in the suspension [35]. The seeds and suspensions were transferred to Petri plates lined with Whatman No.1 filter paper and allowed to air-dry and develop plumules and radicles, before being planted.

2.7 Green House Experiment

A sandy loam soil was used for this experiment and about 5kg portion was distributed into polyethylene bags. PGPR coated maize seeds were used as test plants. The experiment was carried out in a completely randomized design using 5 replicates for each test. These

experiments were employed to test: the plant growth promoting properties of the PGPR isolate; the *in vivo* antagonistic activities of the phytopathogens against maize seedlings; and ability of the PGPR isolates to protect the maize seedlings from the *in vivo* antagonistic activities of the phytopathogens. The treatments were as follows;

1. Control: Maize seed alone.
2. T_1: Maize seed + PGPR
3. T_2: Maize seed+ *Fusarium*
4. T_3: Maize seed + *Enterobacter*
5. T_4: Maize seed + PGPR + *Fusarium*
6. T_5: Maize seed + PGPR + *Enterobacter*

2.8 Inoculation with *Enterobacter* pathogen

The organism was grown for 24hr in nutrient broth on a rotary shaker and harvested by centrifugation for 5min at 5,000rpm. Cell pellets were diluted in sterile distilled water to give a final concentration of 1.9 x 10^8 CFU/ml using a spectrophotometer (0.5 McFarland). Three weeks after sowing, plants were injected with 1ml of the organism [36]

2.9 Inoculation with *Fusarium* pathogen

Twenty eight day-old seedlings were inoculated using a modified root dip method of Güler and Güldür [37]. The spore suspensions were prepared from 7-10 days old isolates cultured on the PDA at room temperature. The radicles of the seedlings were trimmed with a scissor and submerged into beakers containing 100 ml of *Fusarium* spore suspensions (1 x 10^8 spores/ml) for 30 mins before being transplanted into the soil.

After a period of 50 days, plant characteristics such as plant height, shoot and root weight, chlorophyll content, as well as disease assessment were recorded accordingly and compared with respect to control.

$$\text{Disease Incidence } (\%) = \frac{\text{No of Infected Plant}}{\text{Total no. of Plants}} \text{ X } 100$$

Disease incidence data were reused to calculate percentages of disease reduction of each treatment based on the formula;

$$\textbf{Biocontrol Efficacy } (\%) = \frac{[\text{Disease incidence of control} - \text{ Disease inascidence of PGPR Treated Group}]}{[\text{Disease incidence of Control}]} \text{ X } 100$$

Pathogenic strains that caused symptoms in inoculated plants were re-isolated and identified to fulfill Koch's postulate.

2.10 Chlorophyll Content Estimation

The effect of microbial inoculants on the chlorophyll content of maize seedlings was determined using the spectrophotometer according to the method of Li *et al*., [38]. 0.5 grams of fresh leaves were taken and ground in pestle and morter containing 10ml acetone (85%) with some sterile sand, then centrifuged at 3000 rpm for 15min. The supernatant was removed into 50 ml conical flask through whatman No. 1 filter paper, and then the flask was filled to 50 ml mark, using acetone. The absorbance was measured at 663nm and 644nm wave lengths to determine Chl a and Chl b respectively. Then pigment concentrations were calculated in µg/ml according to the following equations [39]:

Chl a (mg /ml) = 12.7A663 – 2.69 A645
Chl b (mg/ml) = 22.9A645 – 4.68A663

2.11Identification of Isolate

The isolate showing the greatest good potentials for plant growth promotion and biocontrol was biochemically characterized and identified using Bergey's manual of systematic bacteriology [40]. Molecular identification was carried out using 16S rRNA gene sequencing. Extraction of DNA was done using CTAB method, the 16S rRNA gene was amplified by PCR using universal primer for bacteria: 16S forward primer (27F TCCTCCGCTTATTGATATGS) and reverse (1525R GGAAGTAAAAGTCGTAACAAGG). The amplified 16S rRNA gene PCR products (gene fragment of 1000 bp length) from this isolate, after purification by 2 M Sodium acetate wash techniques was

directly sequenced on the Gene Sequencer 3130XL genetic analyzer from Applied Biosystems. The 16S rRNA gene fragment (1500 bp length) sequenced in both direction to obtain gene sequence in the form of A, C, T and G was then blasted on http://ncbi.nlm.nih.gov to assess the DNA similarities.

2.12 Statistical Analyses

The pot experiments were performed in five replicates and the results were subjected to statistical analysis using analysis of Variance (ANOVA) using OriginLab. Means were separated using Tukey's Honest Significance Difference (HSD) and Compact Letter Display.

3. RESULTS AND DISCUSSION

Seven PGPR bacteria were isolated from maize roots (*Zea mays*) based on phosphate solubilization activities on PVK agar. Colonies having clear halos on PVK solid medium were selected. The diameters of the halozones and colonies were measured. The solubilization index was calculated by halozone diameter/colony diameter as shown in Table 1. Isolate IS16 gave the highest solubilization index of 4.21 while isolate IS48 gave the lowest index of 1.47. Phophorus usually form insoluble complexes with cations in the soil solution, making it unavailable for plant use. Therefore, Phosphate solubilizing ability is an important parameter while assessing PGPR [6].

3.1 NB: Sol index- solubilization Index

In the solubilization of insoluble phosphate in the liquid medium, results revealed that the organism coded with IS16 was the best P solubilizer, yielding 66.8µg/ml phosphate. This result corresponds to earlier observations made on solid PVK medium in the solubilization of Tricalcium Phosphate. This contradicts what was reported by Li *et al.,* [41], where he showed that phosphate solubilization activities of organisms on solid PVK medium (Phosphate solubilization index) often contradicts that obtained from liquid medium, deeming it necessary to supplement qualitative method with quantitative measurement of phosphate in order to draw a more reliable inference.

Table 1. Solubilization Index on PVK Agar (72 hrs)

Isolate	Diameter halozone (mm)	Diameter colony (mm)	Sol. Index
IS14	10.50	3.9	2.69
IS16	16.00	3.8	4.21
IS28	12.00	7.0	1.70
IS40	11.67	6.0	1.95
IS45	13.20	7.0	1.89
IS46	10.60	5.0	2.12
IS48	8.80	6.0	1.47

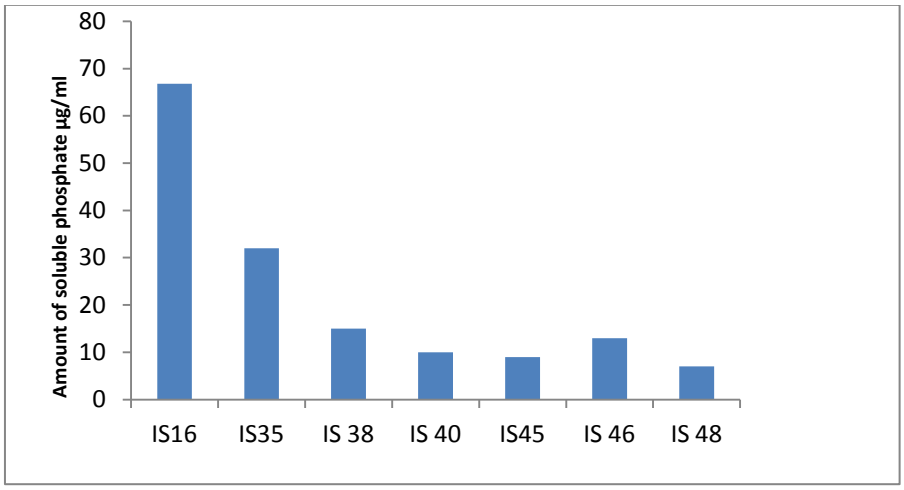


Fig. 1. Solubilization of phosphate in pvk broth

Table 2. Plant Growth Promoting Properties of the Isolate *A. migulanus* (IS16)

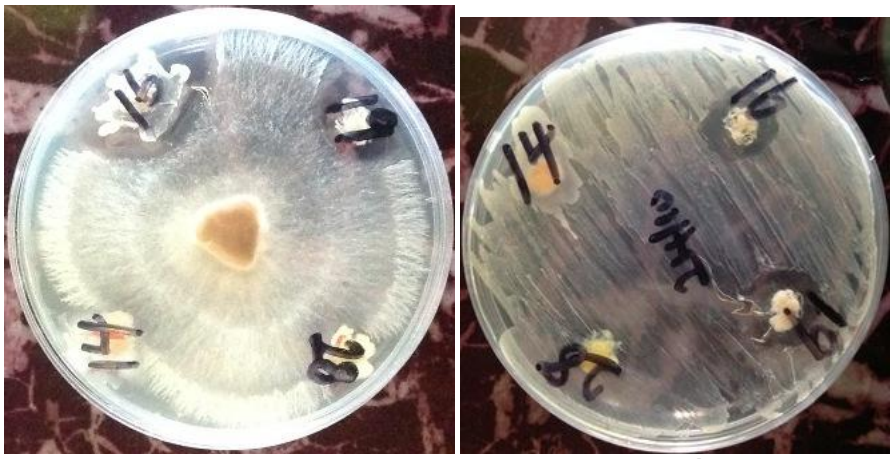
IAA (µg/ml)	NH3	HCN	Cellulase
98.6	+	-	+

Fig. 2. Cellulase production by *A. migulanus* (IS16)

Plant Growth Promoting Rhizobacteria are known to employ series of mechanisms in promoting plant growth. These include the production of phytohormones such as Indole Acetic Acid IAA, secretion of cell wall degrading enzymes such as cellulase, production of various compounds such as Hydrogen Cyanide, Ammonia etc [5,42]. *Aneurinibaclillus migulanus* yielded 98.6 µg/ml of IAA, produced ammonia and cellulase enzyme but no hydrogen cyanide (Table 2).

The *Fusarium* and *Enterobacter* pathogens were isolated in pure culture forms. Members of the genera *Fusarium* [43] are pathogens that infect a wide range of plants, including cereal crops such as maize, leading to huge economic losses. *Fusarium* spp occur in maize worldwide and can cause various diseases in different growth stages of maize, such as root and seedling rot as well as stalk and ear rot [44]. Plant diseases caused by fungi destroy or contaminate a significant proportion of global agricultural production, making fungi the most deleterious class of plant pathogens [45]. On the other hand, the genus *Enterobacter* is considered as phytopathogen for various plants [46]. *Enterobacter* underwent several taxonomic changes [32]. Some of these species were formerly classified as *Erwinia.* Although the *Enterobacter* is recognized mainly as causing harmful diseases affecting humans [47], it has also an important role as a causal agent of several plant diseases [32].

The isolate showed antagonism activity against the two tested phytopathogens as shown in fig 3. Good *invitro* antifungal activities against the phytopathogen *Fusarium,* was established by the strain *A. migulanus* using the dual culture technique. This organism prevented *F.solani*from forming dense hyphal mats (Fig 3a). Similarly, the organism showed high antagonism activities against the bacterial phytopathogen *Enterobacter tabaci* by halting the growth of the organism (Fig 3b). Antagonistic activities among *Bacillus* species had been widely noted in previous studies against diverse fungi [48,49] as well as bacteria [50]. A range of antifungal compounds were produced by the *Bacillus* strains and employed in biocontrol activity [49,51]. Kim *et al*., [52] reported that treating harvested apples with *B.subtilis* suspensions yielded a great reduction in disease severity caused by *Colletotrichum acutatum*.



a b

Fig. 3. *In vitro* antagonism against *Fusarium solani* (a) and *Enterobacter tabaci* (b) by *A. migulanus*

Aneurinibacillus migulanus treated plants did not show a statistically significant difference in maize plant height at *P*=0.05 when compared to the control under green house conditions. In the absence of *A. migulanus*, the infected plants showed rather a decrease in plant height of 120% and 68.7% respectively for *Fusarium solani* and *Enterobacter tabaci* pathogens when compared to the control plant. However, in the presence of *A. migulanus*, the infected plants gave significant increase in plant height when compared to the negative control plants (Table 3). *A. migulanus* showed increase of 2.36% in shoot wet weight (Table 4) and 30% increase in root wet weight (Table 5) under the green house conditions. The maize plants at the end of the pot experiment is as shown in Fig 4.

The use of PGPR in disease suppression and plant growth promotion is a widely adopted strategy. Biocontrol of damping-off of seedlings with bacterial and fungal antagonists, especially *Bacillus* spp. is already established [53]. *Fusarium* root rot disease caused by *Fusarium solani* is widespread and responsible for huge economic losses in agriculture. The disease incidence of plant infected with *Fusarium* was 70% while the disease incidence of plant infected with *Enterobacter* was 60%. This is as shown in table 6. *A. migulanus* offered 83.33% and 71.43% protection against *E. tabaci* and *F. solani* pathogens respectively at the pot experiments. Disease incidence was reduced strongly by the application of the tested antagonist at the time of the planting as seed treatment. *Bacillus subtilis* strain GA1 inhibited mycelial growth of *Botrytis cinerea* by up to 70% [54]. Alenzi [55] provided strong evidence that *Aneurinibacillus migulanus* Nagano is effective in inhibiting growth of a wide range of *Phytophthora species*, suggesting that the Nagano strain could be useful in preventing apple storage rot caused by *Phytophthora* species.

It had been reported previously that PGPR increase chlorophyll content in many plants [56] especially plants grown under abiotic stress condition [57]. Kumar *et al*., [58] reported decrease in chlorophyll content in the leaves of chickpea due to drought stress however; inoculation with PGPR amended the adverse effects of drought on chlorophyll content. The strain *A. migulans* gave 27.24% increase in chlorophyll a content, and 36.7% increase in Chlorophyll b content (Table 7). Previous studies revealed that *Bacillus* speciesare efficient in promoting the N accumulation and, consequently, increasing the chlorophyll content in maize [59].

The bacterial strain IS16 (*A. migulanus*) was a gram-positive endospore forming, motile bacterium with a rod-shaped cell morphology (Table 8). Colonies of this strain on agar plates were creamy and milky white with regular margins. The 16S rRNA gene sequence of the strain was submitted to the NCBI GenBank to get accession number NR115593.1. The results of the BLASTn search showed that the IS16 16S rRNA gene sequence was 99.58% identical to *Aneurinibacillus migulanus* strain (Accession No: NR115593.1). The isolated phytopathogens Fus and Ent were identified as *Fusarium solani* and *Enterobacter tabaci* (Table 9).

Fig. 4. Maize plants at the end of the pot experiment

Table 3. One-way Anova showing variations on plant height in the different soil treatments during pot experiment

Treatment	N Analysis	Mean	Standard Deviations	SE of Mean
Control	5	88.4[a]	2.50998	1.12250
Maize+IS16	5	92.2[a]	2.58844	1.15758
Maize+Fus	5	40.2[d]	3.11448	1.39284
Maize+Ent	5	52.4[c]	5.17687	2.31517
Maize +IS16 +Ent	5	82.4[ab]	2.70185	1.20830
Maize +IS16+Fus	5	76.4[b]	5.36656	2.40000

Means followed by a common letter are not significantly different by the HSD test at the 5% level of significance

Table 4. One-way Anova showing variations on shoot wet weight in the different soil treatments during pot experiment

Treatment	N Analysis	Mean	Standard Deviations	SE of Mean
Control	5	220.6[a]	21.60555	9.6623
Maize+IS16	5	225.8[a]	23.2422	10.39423
Maize+Fus	5	178.0[c]	8.3666	3.74166
Maize+Ent	5	184.0[c]	11.40175	5.09902
Maize +IS16 +Ent	5	207.8[ab]	5.01996	2.24499
Maize +IS16+Fus	5	190.4[b]	8.6487	3.86782

Means followed by a common letter are not significantly different by the HSD test at the 5% level of significance

Table 5. One-way Anova showing variations on root wet weight in the different soil treatments during pot experiment

Treatment	N Analysis	Mean	Standard Deviations	SE of Mean
Control	5	17.60^{ab}	1.14018	0.5099
Maize+IS16	5	22.70^{a}	1.92354	0.86023
Maize+Fus	5	12.48^{c}	1.20499	0.53889
Maize+Ent	5	14.60^{c}	2.94534	1.31719
Maize +IS16 +Ent	5	19.18^{a}	0.74967	0.33526
Maize +IS16+Fus	5	17.80^{ab}	2.58844	1.15758

Table 6. Disease Assessment of treatments under green house condition

Treatment	Disease Incidence %	Biocontrol Efficacy %
Maize+FUS (Negative control)	70	0.00
Maize+ ENT (Negative control)	60	0.00
Maize+IS16+ENT	10	83.33
Maize+ IS16+FUS	20	71.43

Table 7. Effect of Plant growth Promoting Rhizobacteria on the Chlorophyll Content of the Maize leaves under green house condition

Treatments (mg/ml)(mg/ml)	Chlorophyll a	Chlorophyll b
Control	5.91	9.28
Maize+IS16	7.52	12.50
Maize+Fus	0.75	1.30
Maize+Ent	4.26	3.45
Maize +IS16 +Ent	5.95	4.39
Maize +IS16+Fus	1.89	4.75

NB: Values are means of three replicates

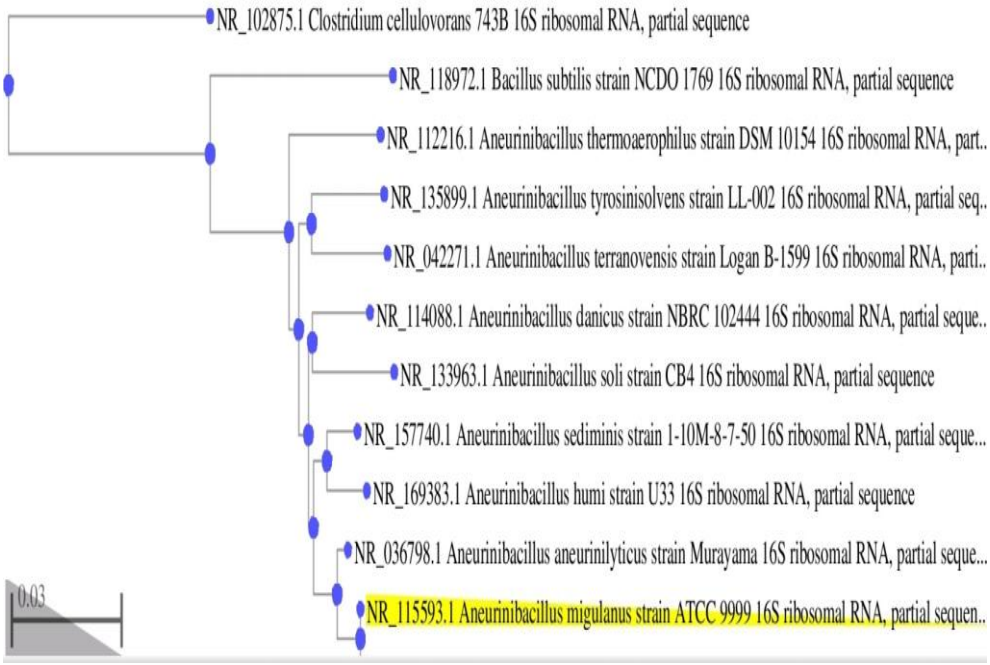


Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of *Aneurinibacillus migulanus* strain ATCC9999

Table 8. Biochemical Characterization of the PGPR isolates

Isolate	GR	Shape	Cat	SH	Cit	SS	Glu	Lac	Fru	Rib	Mal	Man	MR	Indole-test	Urease test	Mot	SI
IS16	+	Rod	+	-	-	+	+	+	+	+	-	-	-	-	-	+	*Bacillus*
Ent	-	Rod	+	-	+	+	+	-	+	+	+	+	-	-	-	+	*Enterobacter*

Key:GR-gram reaction, Cat- catalase, SH- starch hydrolysis, Cit- Citrate, SS- spore staining, Glu-glucose, Lac-lactose, Fru-fructose, Rib-ribose, Mal-maltose, man-mannose, MR-methyl red, Mot-motility, SI-suspected isolate,

Table 9. Molecular Identification of Isolates based on 16S rRNA gene sequence similarity

Isolate code	Accession	Identity Percentage %	Closely related taxa
IS16	NR115593.1	99.58	*Aneurinibacillus migulanus*
Ent	MH465164.1	94.94	*Enterobacter tabaci*
Fus	KY978584.1	99.43	*Fusarium solani*

4. CONCLUSION

The PGPR isolate has multifunctional activities for plant growth promotion. Promoting sustainable agriculture with a gradual decrease in the use of synthetic agrochemicals and prominent utilization of PGPR is an effective strategy to combat the rapid environmental deterioration while ensuring high agricultural productivity. However, more research is needed to ascertain the mechanism of action of *A. migulanus* in plant growth promotion and disease suppression.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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