Rhizospheric bacteria from pristine grassland have beneficial traits for plant growth promotion in maize (Zea mays L.)

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Abstract: An emerging strategy in the sustainable production of staple crops such as maize is the use of plant growth-promoting rhizobacteria (PGPR) as inoculants. This study reports the screening and characterization of several rhizobacteria isolated from rhizosphere of pristine grassland in South Africa. The rhizobacteria were screened for their ability to exhibit important plant growth-promoting traits in vitro and under glasshouse conditions on a maize plant. In total, 98 isolates were initially characterized based on their colony morphology on different growth media of which 13 isolates tested positive for the production of siderophores and indole-3-acetic acid (IAA), whereas eight isolates solubilized inorganic phosphate. Screening for growth promotion experiment revealed that the PGPR isolates significantly (P ≤ 0.05) increased plant biomass, root length, and chlorophyll content index (CCI) when compared to uninoculated (control) plants. The best performing isolates were identified using 16S ribosomal RNA sequencing with additional characterization by DNA recombinase (recA) gene analysis. The 16S analysis indicated the effective rhizobial isolates are closely related to bacteria belonging to Pseudomonas, Burkholderia and Bacillus spp. at ≥98% nucleotide similarity and one isolate identified as Enterobacter sp. Most of these isolates exhibited multiple PGPR traits and

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Langutani Sanger Khambani is a PhD student at the Department of Molecular and Cell Biology, the University of Witwaterstrand. The research results presented in this manuscript constitutes a portion of Khambani’s Master’s study in Biotechnology on screening and characterization of plant growth promoting rhizobacteria (PGPR) for use as inoculants to improve crop productivity and quality. Ahmed Idris Hassen, a research scientist in Applied Microbiology (PhD) at the ARC-Plant Health and Protection Institute supervised LS Kambani towards the MSc study and his area of expertise is plant microbe interaction of both free living beneficial rhizobacteria and that of the symbiotic rhizobia for use in sustainable agriculture. Thierry Regnier is a full Professor at the Department of Biotechnology and Food Technology, Tshwane University of Technology and co-supervised LS Khambani. His expertise and research focus include biochemistry of food safety, food products development and plant physiology.

PUBLIC INTEREST STATEMENT
Since the onset of the green revolution until now, improvement in crop yield and management of diseases and pests has been heavily dependent on chemical fertilizers and pesticides. However, such prolonged use of synthetic fertilizers and pesticides resulted in environmental pollution and deterioration of soil health. It also resulted in decreased nutritional quality of foods, and coupled with this, the residual effects could ultimately affect human health. There is thus a huge public concern regarding how such indiscriminate use of chemical fertilizers and pesticides result in environmental damage, jeopardize human health and also result in the development of plant pathogens resistant to chemicals. One of the best strategies to address these concerns is to search for alternative ways of improving crop production in a sustainable way. The use of plant growth-promoting rhizobacteria (PGPR) as inoculants to improve crop yield and manage phytopathogens represents one of the promising sustainable solutions to improve agricultural productivity.
resulted in enhanced growth of maize under glasshouse condition. The data generated provide vital information for use in the development of PGPR inoculants as alternatives to artificial chemical inputs in the cultivation of maize and possibly other crops.

Subjects: Plant Microbe Interaction; Applied Microbiology

Keywords: rhizobacteria; PGPR; siderophore; phosphate solubilization; indole-3-acetic acid

1. Introduction

The use of plant growth-promoting rhizobacteria (PGPR) is a promising alternative method to external chemical inputs to improve crop yield in sustainable agricultural systems (Kloepper, Lifshitz, & Zablotowicz, 1989; Raaijmakers, Paulitz, Steinberg, Alabouvette, & Moenne-Loccoz, 2009; Weller et al., 2007). The rhizosphere constituent of a plant is determined by the synergistic relationship between the soil, plant root, and the microbes present and is influenced by the soil pH, texture, complexity and plant roots exudates mainly composed of amino acids, sugars, and different nutrients (Lakshmanan et al., 2014; Mendes et al., 2013; Moe, 2013). It has been established that many rhizospheric microbes have tremendous effect on the growth and development of plants by making nutrients available, suppressing and controlling diseases, and ultimately improving yield (Mendes et al., 2013).

Plant growth enhancement in plants by PGPR takes place in a variety of modes of action such as the production of phytohormones (Arzanlou, Mousavi, Bakhshi, Khakvar, & Bandehagh, 2016), phosphate solubilization (Das, Katiyar, & Goel, 2003), and siderophore production (Kloepper et al., 1989) and production of 1-aminocyclopropane-1-carboxylic (ACC) deaminase (Penrose & Glick, 2003). These modes of actions of PGPR can result in improved root growth as well as increased shoot biomass in a number of agriculturally important crops (Dobbelaere et al., 2001; Walker et al., 2012). The expression of several PGPR traits by any given rhizobacteria can be used as one of the major criteria in the selection of beneficial soil microbes for use as inoculants in sustainable crop production systems.

Maize (Zea mays L.) is a staple food for the majority of South Africans, and its successful production depends on the correct application of production inputs such as fertilization and effective disease control that will sustain the environment and improve crop production (Du Plessis, 2003; Kirsten, Townsend, & Gibson, 1998). The use of artificial fertilizes to replenish soil nitrogen (N) and phosphorus (P) in agricultural systems however results in high costs and increased environmental pollution (Singh, 2013). Inoculation of maize and other economically important crops with various PGPR strains, however could result in significant increases in plant biomass, root and shoot length, leaf surface area and uptake of essential plant nutrients. Improvement in the uptake of essential nutrients such as N and P would ultimately result in increased plant growth and yield (Yazdani, Bahmanyar, Pirdashti, & Esmaili, 2009). There is, however, limited research on the roles and mechanisms of plant growth-promoting rhizobacteria on the growth promotion of economically important crops including maize plants in South Africa.

One of the strategies to isolate PGPR isolates with varying plant growth promotion ability is to isolate from the rhizosphere of grasses (Hassen & Labuschagne, 2010). The rationale behind this is that most grass species are perennials and therefore there is continuous rhizodeposition of carbon from plant roots that results in complex chemical and biological interactions in the soil, resulting in microbial diversity (Singh, Munro, Potts, & Millard, 2007). Isolating bacteria from pristine perennial grasses will therefore increase the chance of selecting a wide range of potential root colonizing and growth-promoting strains. The aim of this study is therefore to isolate rhizobacteria from the
rhizosphere of pristine grassland located at the Nylsvley Nature Reserve in South Africa and to characterize them for their potential in enhancing the growth of a maize crop.

2. Materials and methods

2.1. Soil sample collection and isolation of bacteria

A total of 1 kg of soil samples were randomly collected from the rhizosphere of grass species including Aristida bipartite, Cyperus articulatus, Diplachne fusca, Setaria incassata and Chloris virgate growing in the Nylsvley Nature Reserve (24° 39’ 0” S, 28° 39’ 54.4” E) (Limpopo Province, South Africa). Samples were collected from the upper 5–10 cm layer of the rhizosphere, deposited in sterile plastic bags and transported to the laboratory in a cooler box (5 ± 2°C). The isolation of bacteria was conducted following a standard microbiological procedure using the serial dilution technique (Koch, 1883). Briefly, 9 mL of a saline solution (0.85%) was added to 1 g of soil. A 10-fold serial dilution was made and 0.1 mL aliquot of each 10⁻⁵ – 10⁻⁷ dilution was spread plated on sterile King's B medium (Oxoid, London, UK), Nutrient agar (Biolab, Wadeville, South Africa) and Luria Bertani (LB) agar (Biolab, Wadeville, South Africa).

Plates were prepared in two replicates and were incubated at 28 ± 2°C for 24–48 h after which colonies with different morphological appearances were streaked onto a new plate of the same media to obtain pure colonies. All isolates were maintained at −80°C in 20% glycerol and were assigned a unique code.

2.2. Siderophore production

Siderophore production was detected using the universal chrome Azurol S (CAS) agar assay as described by Schwyn and Neilands (1987). A 2 mm diameter well was prepared in which 60 µL of the supernatants from each culture of the iron-deprived medium was seeded or applied. For the negative control, the same amount of sterile medium was inoculated into the wells of the CAS agar plate. The plates were incubated at room temperature for at least 8 h. Siderophore production in the culture filtrate was confirmed by the formation of yellow halos surrounding the supernatants in the wells of the blue CAS-agar medium. The diameter of the halos formed was measured and recorded.

2.3. Indole acetic acid production

For the determination of IAA, a modified colorimetric method was used (Asghar et al., 2000). Bacterial isolates were inoculated in Luria Bertani (LB) broth containing 5 µg/mL of L-tryptophan and incubated at 27 ºC ± 1 for 3 days. After incubation, the cultures were centrifuged at 3 000 rpm for 30 min. The supernatant (2 mL) was mixed with two drops of orthophosphoric acid and 4 mL of Salkowski's reagent (50 mL; 35% perchloric acid and 1 mL of 0.5 M FeCl₃) and incubated at room temperature for 25 min. Development of pink color indicated IAA production, and the absorbance of the solutions were read at 530 nm. For the control experiment, sterile LB broth was used.

2.4. Phosphate solubilization

Bacterial phosphate solubilization ability was determined by spot inoculating the isolates on agar plates containing Pikovskya's (PVK) medium (Pikovskaya, 1948). The isolates forming a clear halo zone around the bacterial colonies were considered phosphate solubilizers. The diameter of zone of clearance (halo) surrounding the bacterial colony was measured after incubation to compare the efficacy of phosphate solubilization by the isolates.

2.5. Glasshouse pot experiment

Yellow maize mix cultivar (US6910) obtained from United Seeds CC South Africa were surface sterilized in 95% ethanol for 30 s and 1% sodium hypochlorite solution for 1 min and finally washed 3 times in sterile water. The seeds were covered with sterile distilled water and kept at 4°C for an overnight to imbibe. The imbibed seeds were placed in 7.5% (w/v) water agar and incubated at 28 ºC for 3–4 days to germinate. Three germinated seedlings were transplanted into ±2 L size pot filled
with sterile loam soil. Bacterial inoculum was prepared as described in Hassen and Labuschagne (2010) except that the final cell concentration was adjusted to $10^8$ cfu/mL using spectrophotometer absorbance reading (OD $600\text{nm}$ of $\geq 0.6$) using 0.85% saline solution. The bacterial inoculum (30 mL) was applied per pot immediately after planting and second inoculation a week apart, while controls were treated with sterile water only. The experiment was arranged in a randomized complete block design (RCBD) with three replications and was repeated once. The trial was monitored in the glasshouse adjusted to a temperature of 27 ºC (day) and 18 ºC (night) with regular watering using non-sterile water. Plants were harvested 6 weeks after the first inoculation to evaluate the growth-promoting ability of the bacterial isolates based on the plant’s fresh weight, dry weight, chlorophyll content and root length.

2.6. Statistical analysis
The analysis of the in vitro and glasshouse data was performed with SAS version 9.3 statistical software by initially subjecting the data to Analyses of Variance (ANOVA) (SAS, 2003).

The standardized residuals were tested for deviations from normality using Shapiro-Wilk’s test and the least significant difference (LSD) was calculated at a $5\%$ significance level to compare means of significant source effects.

2.7. Identification of bacterial isolates
Total genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). The 16S ribosomal RNA (rRNA) gene was amplified by PCR using primer pairs fD1 (5’AGA GTT TGA TCC TGG CTC AG-3’) and rD1 (5’TAG GAG GTG ATC CAG CC3’) corresponding to E. coli positions 8–27 and 1541–1525, respectively (Weisburg, Barns, Pelletier, & Lane, 1991). The amplification was performed in a total reaction mixture of 25 µL containing: 0.5 µL of forward and reverse primers (10 µM), 2.5 µL dNTP’s (10 mM), 0.125 µL Taq G2 flexi DNA polymerase (5 u/µL), 1.5 µL MgCl$_2$ (25 mM), 2.5 µL green go Taq flexi buffer (5X), 2.5 µL of extracted genomic DNA and 16.875 µL of nuclease-free water. Thermal cycling conditions for the PCR amplification were programmed at an initial denaturation of 94 ºC, 4 min followed by 30 cycles of denaturation at 94 ºC, 1 min; annealing at 54 ºC, 30 s; extension at 72 ºC, 2 min and final extension step at 72 ºC, 5 min. The recA (DNA recombinase A) gene was also amplified using primer pairs recA-63F (5ATCGAGCGGTCGTTCGGCAAGGG-3’) recA 504R (5’TGTGCCAGCGCTGGCTCAT-3’) (Gaunt, Turner, Rigottier-Gois, Lloyd-Macgilp, & Young, 2001). The PCR amplification conditions were: initial denaturation at 95 ºC, 5 min and 35 cycles of denaturation at 94 ºC, 45 s, annealing at 57 ºC, 1 min, extension at 72 ºC, 1 min and a final extension step at 72 ºC, 5 min. For both the 16S and recA genes, PCR products were sequenced using Sanger sequencing method (Inqaba Biotech Laboratories, Pretoria, South Africa). The resulting sequences were edited by base calling on Bioedit programs after which gaps were deleted by using MEGA7. Each edited sequence was BLAST searched on the NCBI database library (http://www.ncbi.nlm.nih.gov/BLAST/) and aligned with MUSCLE, a multiple sequence alignment tool on MEGA7 (Edgar, 2004).

The aligned sequences were used to construct Maximum Likelihood (ML) and Neighbour Joining (NJ) phylogenetic trees to elucidate the taxonomic position and species relatedness of the bacterial isolates. The analysis was made using MEGA 7 program (Kumar, Stecher, & Tamura, 2016). The 16S rRNA sequences were deposited at the NCBI Genebank with accession numbers MF380489—MF380500.

3. Results

3.1. Glasshouse pot experiment
The effect of inoculation with rhizobacterial strains on the growth promotion of maize under glasshouse condition was evaluated based on the growth parameters that include fresh weight, dry weight, root length and chlorophyll content index of the leaves (Figures 1 and 2). It was observed that plants inoculated with the PGPR strains resulted in statistically significant increase ($P \leq 0.05$) in
shoot fresh weight (Figure 1(a)), chlorophyll content (Figure 1(b)), root length (Figure 2(a)) and shoot dry weight (Figure 2(b)). High chlorophyll content was recorded for the treatments inoculated with bacterial isolates lb-fp1/3-1b, na-bw1-1b and na-arc-2a with chlorophyll content index (CCI) that ranges between 8.43 CCI—9.30 CCI, values that were much higher than that of the control treatment (5.50 CCI). The maximum growth-promoting activity in terms of fresh weight was detected for isolate na-fp2/4-1c whereas maximum root length was observed in plants treated with isolate na-arc-1a, na-fp2/4-1b, and na-bw1-1b when compared to the uninoculated maize plants.
3.2. Siderophore production

Of the isolates evaluated for the production of siderophores, 34 isolates showed a positive test as indicated by the formation of an orange/yellow halo on CAS agar media. The orange halos were produced as a result of siderophores in the bacterial culture filtrate which sequester iron from the CAS agar medium. The isolates with a considerable amount of siderophore based on the diameter of the yellow halo zone on the CAS agar plate are na-s15-1a (17 mm), kb-s3-1a (16.67 mm) and na-fp2/4-1b (15.33 mm) (Figure 3(a and b)). The 34 isolates were further evaluated for additional in-vitro modes of action studies including phosphate solubilization and indole-3-acetic acid (IAA) production.
3.3. Phosphate solubilization
Nine isolates, which also tested positive for siderophore production, solubilized phosphate in-vitro by forming clear halo zones around the bacteria colonies when grown on PVK agar. The phosphate solubilization activity among the isolates was compared and evaluated in terms of the diameter (mm) of the clear zone formed around the colonies on PVK medium. Clear zone formation as a result of P-solubilisation differed among the isolates ranging from 5.33 mm to 19.67 mm in diameter, the highest being by isolates kb-s3-1a (19.67 mm), na-fp2/4-1c (18.00 mm) and na-s3-1a (18.33 mm) (Figure 3(c and d)).

3.4. Indole acetic acid production
Among the 34 isolates, 13 isolates tested positive for the production of indole-3-acetic acid (IAA). It was very interesting to observe that only the isolates that tested positive for the production of siderophores and/or solubilize phosphate were able to produce IAA. The amount of IAA produced by these isolates ranged from 20.81 µg/mL to 97.20 µg/mL in LB broth supplemented with L-tryptophan as a precursor of IAA synthesis under laboratory conditions. The highest IAA production was recorded for bacterial isolates lb-fp1/3-1b (97.20 µg/mL) and lb-fp2/4-1b (54.72 µg/mL) (Figure 3(e and f)). The isolates which did not show any of the three PGPR traits tested in this study were discarded and only 13 of the originally tested isolates were further evaluated for the glasshouse inoculation study due to their multiple PGPR traits.
3.5. Identification of bacteria

Nucleotide sequence analysis and BLAST search of the 16S ribosomal RNA for the 13 effective isolates revealed that the majority of the isolates show sequence similarity (≥98%) to *Burkholderia* spp. (5 isolates) and *Pseudomonas* spp. (5 isolates), whereas 2 isolates were identified as *Bacillus* spp. and one isolate belonged to the genus *Enterobacter*.

The ML phylogenetic tree constructed from the 16S rRNA nucleotides to infer the evolutionary history and relatedness of these isolates with known species from the NCBI data base library also concurs with the blast search result (Figure 4(a)). According to the results, the 13 PGPR isolates were placed into three major clades and one minor clade. Clade I placed isolates na-arc-1a, na-arc-2a, lb-fp2/4-1b, lb-fp1/3b and kb-s3-a with *Pseudomonas* spp. Clade 2 is the minor clade that placed isolate na-s19-b with *Enterobacter* sp. Clade 3 placed isolates na-fp2/4-1b, lb-bw-1a, na-fp 2-4c, na-bw-1b and lb-fp2/2a with *Burkholderia* spp. The fourth clade places isolates na-s-3a and na-s-15a with *Bacillus* spp. The results obtained from the recA analysis partially confirmed the phylogeny of the isolates analyzed by the 16S rRNA gene (Figure 4(b)). Analysis of the recA gene by Neighbour Joining phylogenetic tree supported the placement of isolates na-arc-2a, kb-s3-1a and na-arc-1a with *Pseudomonas* spp. Isolate na-s-15A was placed with *Bacillus* spp. The placement of the isolates na-s19-1b, lb-bw1-1a, lb-fp2/4-1b and na-s3-1a is however not consistent with the blast result and the 16S rRNA phylogenetic tree. This is partly because it was not possible to amplify the expected length of the recA gene for most of the isolates and amplification of partial recA gene sequence was obtained only for 11 out of the 13 isolates tested.

4. Discussion

World food production is at an increasing threat due to the challenges posed by global population increase, climate change and prolonged use of synthetic fertilizers that affects soil health. There is thus a greater demand to promote agricultural production in a sustainable way (Timmusk, Behers, ...
One of the best strategies that helps intensify agricultural production in a sustainable manner is the application and use of plant growth-promoting rhizobacteria (PGPR) as inoculants in the cultivation of several economically important crops. In this study, we report the beneficial effects of plant growth-promoting rhizobacteria (PGPR) isolated from the rhizosphere of pristine grassland in improving the growth of maize (*Zea mays* L.) under glasshouse condition.

Plant growth-promoting rhizobacteria (PGPR) form a beneficial interaction with plants by colonizing either the rhizosphere or internal tissue of many plant species. Such interactions result in several positive effects including plant growth, reduced susceptibility to disease and improve tolerance to abiotic stress (Poupin, Timmermann, Vega, Zuniga, & Gonzalez, 2013).

It is known that gramineaceous plants such as maize have iron (*Fe*³⁺) acquisition system through their own phytosiderophores. However, several microbial siderophores have also been described to have important roles in iron acquisition and reduce chlorosis in the host plant (Sharma & Johri, 2003). Apart from this, bacterial siderophores stimulate plant growth by increasing the iron availability in the rhizosphere (Kloepper et al., 1989). Several strains of rhizobacteria that produce siderophores have been previously reported (Singh & Jha, 2016; Zhao et al., 2013). The strategy we used in this study to screen for beneficial PGPR isolates for enhanced growth promotion in maize was to first evaluate the isolates for the production of siderophores. This is because siderophore production is one of the most important PGPR traits, and many beneficial rhizobacteria are known to possess a high-affinity iron transport system where siderophores are produced and the Fe-siderophore complex is taken up via a specific transport pathway to make it available to plants (Budzikiewicz, 1997; Meyer, 2000). Several studies have previously indicated that most PGPR isolates exhibit multiple PGPR activities (Kalam, Das, Basu, & Podile, 2016). Hence, in the current study, the other criteria we used to screen the best PGPR isolates were to evaluate the isolates for their ability to show at least one additional PGPR trait.

In our results, isolates na-fp2/4-1b, kb-s3-1a and na-s15-1a, which were identified as *Burkholderia* sp, *Pseudomonas* sp and *Bacillus* sp., respectively, showed maximum production of siderophores based on the diameter of the yellow halos formed on the CAS agar media. The result concurs with other similar studies as several strains from both *Burkholderia* and *Pseudomonas* spp. have previously been reported to have the ability to produce siderophores. Boukhalfa & Crumblis (2002) for instance reported that members of the genus *Pseudomonas* produce as many as 50 different types of pyoverdines siderophores whereas some strains of *Burkholderia* spp. have been reported as efficient siderophore producers resulting in enhanced plant growth promotion in the rhizosphere of rice and sugar cane (Meyer et al., 1995; Luvizotto et al., 2010). *Bacillus* is also reported as a potential biofertilizer due to its ability to possess multiple plant growth promotion traits, including siderophore production (Bjelic, Marinkovic, Tintor, & Mrkovacki, 2018). Recently Grobelak and Hiller (2017) isolated siderophore producing isolates from the rhizosphere of roots that mainly belong to *Bacillus* and *Pseudomonas* spp. It is very interesting to observe that all the plants inoculated with the siderophore producing isolates in our study had significantly higher amounts of biomass (p ≤ 0.05) and were less chlorotic in comparison with the uninoculated controls. The screening of siderophore producing strains is essential particularly with reference to plant iron (*Fe*³⁺) nutrition which is required as a cofactor for many metabolic pathways and for proper plant development (Zuo & Zhang, 2011).

Most of the isolates that tested positive for siderophore production and which resulted in maize growth promotion in the gnotobiotic study were equally effective in their phosphate solubilization activity as the second important PGPR trait demonstrated by these isolates. We detected three *Bacillus*, five *Pseudomonas* and four *Burkholderia* spp. that showed significant phosphate-solubilizing activity on Pikovskaya agar medium. Members of these three genera have been previously reported as having efficient phosphate solubilization properties by several researchers (Ahmad et al., 2008; Caballero-Mellado et al., 2007; Peix et al., 2004). The gnotobiotic test conducted using the same
bacterial strains also verified the in-vitro phosphate solubilization test results in which significant increase was detected in shoot biomass and root length (Figures 1&2). Several strains of PGPR belonging to different genera such as Bacillus, Pseudomonas and Serratia spp. have been previously isolated from the roots and rhizosphere soil of various crops with phosphate-solubilizing activities (Frey-klett et al., 2005; Granada, Costa, Lisboa, Vargas, & Passaglia, 2013; Hamidi, Ghalavand, Dehghan-shoar, Malakuti, & Asgharzadeh, 2008). The primary mechanism involved in phosphate solubilization by PGPR is acidification due to organic acid production (Puente, Li, & Bashan, 2004). Report on the PGPR properties of Burkholderia sp. by Linu, Stephen, and Jisha (2009) demonstrated that phosphate-solubilizing Burkholderia strains resulted in improved growth of cowpea with additional PGPR properties. Species belonging to Burkholderia and Herbaspirillum genera promote maize and sugarcane growth (Da Silva et al., 2016; Pereira, Do Amaral, Dall’Asta, Brod, & Arisi, 2014). Dos Santos et al. (2017) also observed a significant increase in grass and sorghum biomass inoculated with Burkholderia spp. This validates the finding in this study where we detected five phosphate-solubilizing Burkholderia strains, which also showed significant growth enhancement of the maize plants under glasshouse condition.

Another important PGPR property displayed by the isolates in this study was the production of indole-3-acetic acid (IAA) which is an important mechanism of plant growth promotion that improves root development and uptake of nutrients. Like several other investigations that reported the production of IAA by 80% of the bacteria isolated from rhizospheric soil (Zahid, Abbasi, Hameed, & Rahim, 2015), all of the rhizosphere isolates in the current study (100%) were capable of producing IAA. Among the strains that were predominantly isolated with their IAA producing ability in the past include strains of Bacillus, Pseudomonas, Azotobacter and Azospirillum spp. (Shobha & Kumudini, 2012; Wahyudi, Astuti, Widyawati, Meryandini, & Nawangsih, 2011). In this study, isolate lb-fp1/3-1b that showed a high level of IAA production was identified as Pseudomonas sp. which concurs with similar other studies (Ahmad et al., 2008; Kumar et al., 2012). These researchers reported the isolation of siderophore producing Pseudomonas spp. which also produced higher amounts of IAA. Most of these IAA producing bacteria reported earlier are Gram negative (Lindow et al., 1998) which again supports our results in which out of the 13 isolates that showed IAA production, 11 were Gram-negative PGPR. Only two isolates belonging to the Gram-positive Bacillus sp. showed the ability to produce IAA in the current study. This result also resembles another report by Wahyudi et al. (2011) where only few Gram-positive strains of Bacillus were capable of producing IAA. Rhizobacterial IAA secreted in the root rhizosphere enables plants to develop highly organized root systems that facilitate efficient uptake of nutrients (Ribeiro & Cardoso, 2012) and also plays an important role in cell elongation, division and enlargement (Khan, Bano, & Zandi, 2018). Root growth induced by bacterial inoculation in plants enhances water and nutrient (N, P and K) uptake by the plant, which results in plant growth promotion (Mantelin & Touraine 2004). According to Gopalakrishnan et al. (2015) and Sreevidya, Gopalakrishnan, Kudapa, and Varshney (2016), root development and the ability to promote nutrient availability are key to the functions of PGPR.

The results of the glasshouse pot experiment in this study demonstrate significant increases in root length and fresh weight biomass by isolates na-bw-1a (Burkholderia) and na-arc-1a (Pseudomonas). These two isolates exhibited statistically significant improvement in terms of increase in root length and fresh weight compared to the other isolates and the control. Maize plants generally require a high rate of fertilizer inputs, particularly fertilizer-N for maximum crop yield and profitability that requires the application of fertiliser-N to ensure adequate nitrogen supply to promote crop growth. In doing this, only 30–50% of the fertilizer N applied is absorbed by plants, the rest is either rendered unavailable as adsorbed soil organic-N or leached into the environment (Hodge et al., 2000). However, application of PGPR isolated from plant rhizosphere can not decrease chemical fertiliser-N use and increase plagrowth and yield when associated with plant roots and other plant parts (Lugtenberg & Kamilova 2009).
The role of PGPR in increasing growth and yield of crops such as maize and many others has been reported in the past (Berge, Heulin, & Balandreau, 1991). There are several other reports of improved plant biomass when maize seeds were inoculated with a strain of *Pseudomonas* spp. (Sandhya et al., 2010). In another study, an increase in plant height, plant biomass, and root length was observed when maize was inoculated with *Burkholderia* sp. strain CC-Al74 (Young et al., 2013). The isolates effective in enhancing maize growth in this study were identified as *Pseudomonas, Bacillus* and *Burkholderia* spp. in which the maximum growth-promoting activity in terms of fresh weight was detected for isolate na-fp2/4-1c identified as *Burkholderia* sp. Similar finding by Chiarini, Bevivino, Tabacchioni, and Dalmastri (1998) and Bevivino et al. (2000) revealed that some members of the genus *Burkholderia* can directly stimulate plant growth. In a related experiment, inoculating crops such as maize and sorghum with *Burkholderia* spp. resulted in increased root and shoot biomass (Govindarajan, Balandreau, Muthukumarasamy, Revathi, & Lakshminarasimhan, 2006). The fact that the isolation and identification of one of the effective isolates, na-s19-1b as *Enterobacter* sp., in this study is indicative of the beneficial properties of certain *Enterobacter* isolates for their growth promotion activities in the rhizosphere. Our result is supported by the previous report that *Enterobacter* strains promoted nutrient uptake and yield when inoculated alone or in combination with other PGPR strains (Kumar, Maurya1, Raghuwanshi, Meena1, & Islam, 2017). Additional evidence is presented by Goudaa et al. (2018) that *Enterobacter* spp. are very efficient phosphate solubilizers and plant growth promoters.

5. Conclusion and summary

The plant growth promotion activity facilitated by the PGPR strains in maize plants in the current study is mainly a direct mechanism as most of the isolates produced siderophores, indole acetic acid (IAA) and solubilized phosphate. Generally, the inoculation of maize with the rhizobacterial strains capable of showing the above PGPR traits resulted in increased plant growth parameters including plant biomass, root length and chlorophyll content index when compared to the uninoculated controls. To our knowledge, this is the first report that rhizobacteria isolated from the rhizosphere of pristine grassland have a role in the growth promotion of maize (*Zea mays*) under the gnotobiotic condition in South Africa. Due to the multiple PGPR traits of the strains detected in the current study, there is a high potential of these strains to be equally effective in enhancing plant growth in other crops as well. Further investigation on additional plant growth-promoting traits coupled with field screening trials of the isolates is recommended as a major prerequisite step in developing these PGPR strains for future commercial use.

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Competing interests
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This article has been republished with minor changes. These changes do not impact the academic content of the article.

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