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# Characterization and biocontrol potential of some rhizobacteria against fungal pathogens causing foliar diseases in maize

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**Abstract.** Maize is one of the most consumed cereal crops worldwide, and it is a strategic crop to the attainment of SDG 2 of Zero hunger. Despite its importance, the cultivation of maize has been significantly impaired by fungal pathogens causing foliar diseases. The occurrence of this disease in maize plantations at the Research Farm of the North-West University, Molelwane, Mafikeng, South Africa prompted this investigation. Samples of diseased maize rhizosphere soil were aseptically collected. Bacteria species associated with the rhizosphere were isolated and characterized as *Bacillus siamensis, Enterobacter asburiae, Enterobacter chengduensis, Priestia aryabhattai, Burkholderia* sp., *Priestia megaterium* strain AOA6 and *Priestia megaterium* strain AOA7. The anti-fungicidal potentials of the bacterial species were evaluated against pathogenic fungal species, *Nigrospora sphaerica, Alternaria alternata* and *Fusarium equiseti* in-vitro. The percentage mycelia growths were calculated and the data were subjected to ANOVA using SAS version 9.8. All the seven bacteria isolates tested positive to ammonia production, phosphate solubilization, siderophore production and ACC deaminase tests. The percentage mycelia inhibition showed *Nigrospora sphaerica* (36.29%), *A. alternata* (26.19%) and *F. equiseti* (20.63%) as the order of fungal inhibition by the bacteria species. Furthermore, *E. asburiae* > *P. megatarium* strain AOA7 > *B. siamensis* > *P. aryabhattai* > *E. chengduensis* > *Bulkholderia* sp. were the order of antifungal efficacy of the bacteria species evaluated. In conclusion, the efficacy of the bacteria especially *E. asburiae, P. megatarium* strain AOA7 and *B. siamensis* over various fungal pathogens.

The result obtained, therefore, justifies the further investigation, formulation and deployment of the bacteria species as biofungicide in the management of foliar diseases of maize.

**Keywords:** antifungicidal potential, biofungicide, microbial formulations, rhizosphere, zero hunger.

## Introduction

Maize (*Zea mays* L.) is of immense importance to ensuring food sustainability worldwide. It is an essential crop that plays a strategic role in actualizing the second United Nations Sustainable Development Goal (SDG 2) of attaining zero hunger (Akanmu *et al*., 2023a; Ngoune Tandzi *&* Mutengwa, 2019). This is a result of the nutritional benefits derived from maize by millions of people across the world who depend on it as a staple food (Dlamini *et al*., 2023a). In addition, the relatively high yield potential of maize and the ability to grow in diverse kind of environments ensures its stability in food supply both to human and the livestock (Poole *et al*., 2021). Furthermore, the crop has been reported for its climate resilience which is a unique attribute that makes maize an important crop in regions prone to floods, drought and other weather-related challenges (Agunbiade *&* Babalola, 2023; Prasanna *et al*., 2021).

Despite its resilience, the growth and yield of maize are adversely affected by pests and diseases caused by fungi, viruses, nematodes and bacteria (Akanmu *et al*., 2023b; Dlamini *et al*., 2023b; Gadag *et al*., 2021). The infection of pathogenic organisms has been found to often result in significant yield losses (Akanmu *et al*., 2020; Dlamini *et al*., 2023b; Imade *&* Babalola, 2021), and fungi constitute an important domain of microorganisms infecting the cultivated maize (Babalola *et al*., 2022). Fungi causing the foliar diseases of maize have been reported across the world including grey leaf spot caused by *Cercospora* spp., common rust (*Puccinia sorghi*), leaf spot disease (*Phoma herbarum*), brown spot (*Physoderma maydis*), *Phaeosphaeria* leaf spot (*Phaeosphaeria maydis*), downy mildew (*Peronosclerospora sorghi*), *Bipolaris zeicola* (*Helminthosporium* leaf spot) and a range of foliar and root diseases caused by *Fusarium verticillioides* (Babalola *et al*., 2022; Belisário *et al*., 2022; Korsman *et al*., 2012; Liu *et al*., 2021). The effects of these diseases have been reported to occur from mild to severe cases that are capable of causing an entire yield loss in the field.

Varying management techniques have been employed to salvage maize from fungal diseases. The most commonly employed method among maize growers

is chemical-based fungicides (Padrilah *et al*., 2024). However, the further use of this chemical has been widely discouraged because of its possible contamination of the environment and the associated health risks to humans, which mostly result from inadequate or prolonged chemical usage. Although, some cultural approaches such as crop rotation and minimum tillage practices had yielded progressive results (Degani *et al*., 2022; Sharma *et al*., 2022), but had in most instances, not satisfactorily curtailed the deleterious effect of these fungal pathogens alone. Hence, there is a need to further channel investigation into a sustainable means of fungal disease control, which mimics the natural disease management model, by the use of bioagents with bioprotectant and antagonistic properties (Akanmu *et al*., 2023a; Scortichini, 2022; Wahid *et al*., 2020).

Some biochemical mechanisms including ACC deaminase, phosphate solubilization, ammonium and siderophore production have been linked to the reduction of both plant pathogenic fungi and deleterious rhizobacteria by Plant Growth-Promoting Rhizobacteria (PGPR) (Enebe and Babalola, 2018; Etesami & Maheshwari, 2018). Therefore PGPR has been reported for its potential to offer alternative and sustainable methods of plant disease management (Benaissa, 2024; Jiao *et al*., 2021). The association of PGPR with the plant roots has been documented to promote plant growth through enhanced mineral nutrition, trigger the production of plant hormones or other molecules that stimulate plant growth and strengthen plant defenses against both biotic and abiotic stresses. The rhizobacteria also protect plants by regulating the abundance and activities of pathogenic microbes (Fadiji *et al*., 2023; Gupta & Pandey, 2023; Lyu *et al*., 2019). As a result of the suitability of the use of PGPR in the same and sustainable agricultural production, this study set out to investigate the potentials of some rhizobacteria associated with maize rhizosphere in the management of fungal diseases in maize.

## Materials and methods

***Source of the fungal species*.** The fungal pathogens used in this study were obtained from the culture collections of the Microbial Biotechnology Laboratory, North West University, South Africa. The consensus nucleotide sequence of the fungal isolates had been earlier deposited in GenBank with accession numbers OP536174, OP536183 and OP536186.

***Sample collection and isolation of bacteria*.** The experimental samples were collected from the rhizosphere region of maize plants at the Teaching and Research Farm of the North-West University, Molelwane (25°47′26.4″ S 25°37′01.6″ E)

in March 2022. A total of eight diseased maize plants were randomly selected and uprooted. The soils that were tightly bound to the plant roots were scraped from each of the collected samples. The samples were transferred to the laboratory in an ice cooler box (-4$^{o}$C). The soil samples were subjected to serial dilution and plated on Nutrient Agar (NA). The plates were incubated at 30°C for 24 hours. The colonies obtained were further subcultures on NA to obtain pure cultures. The isolates were then categorized for morphological characterization using the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

***Screening for plant-growth promoting rhizobacteria (PGPR) properties.*** The following screening was conducted to determine the suitability of the bacteria isolates as PGPR candidates and their potential to inhibit the pathogenic effects of the foliar fungal pathogens of maize.

***Ammonia production.*** The assessment of ammonia production by bacterial isolates was in line with the method described by Islam *et al*. (2009). In each test tube, 10 µl of freshly prepared bacterial cultures with an optical density of 0.2 were introduced into 10 ml of peptone water. After inoculation, the test tubes underwent a seven-day incubation period at 34°C. Post-incubation, 1 ml of Nessler's reagent was introduced into each test tube, and any observable changes in color were documented. A positive outcome, indicating ammonia production, was denoted by a shift in the medium color to brown or yellow. The experimental procedure was replicated three times to ensure reliability and consistency.

***Phosphate solubilization.*** The methodology outlined by Islam *et al.* (2009) was employed to assess the phosphorus solubilization potentials of the seven bacterial isolates. Specifically, 10 µl of freshly prepared culture was spot-inoculated onto Pikovskaya agar plates containing 2% tri-calcium phosphate. Subsequently, these inoculated plates were incubated for 7 days at 37°C, during which the development of a clear zone surrounding the bacterial colonies was monitored. Following the procedure detailed by Farooq and Bano (2013), the solubility index was then determined.

$$Phosphatase\ index = \frac{\text{Clear zone diameter} - \text{Colony diameter}}{\text{Colony diameter}}$$

***Siderophore production.*** The investigation of siderophore production by bacteria isolates involved testing their ability to utilize Chrome Azurol S (CAS) dye (CAS from Merck, SA) as an indicator. Initially, 60.5 mg of CAS was dissolved in 50 ml of distilled water and mixed with 10 ml of iron (III) solution

(1 mM FeCl3. 6H20 in 10 mM HCl). This mixture was gradually added to 72.9 mg of hexadecyltrimethylammonium (HDTMA, Merck, SA) bromide that had been previously dissolved in 40 ml of distilled water, with continuous stirring. The resulting solution was autoclaved for 15 minutes at 121°C. To prepare the Petri plates, 900 ml of sterilized LB broth, pH-adjusted to 6.8, was poured, and 100 ml of the autoclaved mixture was added while stirring. Once solidified, freshly prepared bacterial cultures were spot-inoculated onto Petri plates and incubated for 7 days at 25°C. Successful siderophore production was confirmed by the presence of a yellowish-orange halo surrounding bacterial colonies.

***ACC deaminase activity*.** The investigation into ACC deaminase activity among the recovered bacterial isolates was conducted according to the method described by Ali *et al*. (2014). All bacterial isolates were cultured in 5 milliliters of tryptone-soy broth (TSB, rich medium, Merck, SA) at room temperature for 48 hours. Subsequently, bacterial cells were centrifuged at 5000 g for 5 minutes, washed twice with sterile 0.1 M Tris-HCl (pH 7.5), and then resuspended in 1 ml of the same solution. The cleaned bacterial cells were spot-inoculated on Petri plates containing modified Dworkin and Foster salts minimum media (Dworkin and Foster, 1958). The exclusive nitrogen source in the minimal media utilized for this investigation was 3 mM ACC. Negative control plates comprised Petri dishes with DF minimal salt medium without ACC, while positive control plates contained DF minimal salt medium supplemented with 0.2% (w/v) $(NH_4)_2SO_4$. The inoculated plates were incubated at 30°C for 7 days. The growth of bacteria on DF minimum plates, utilizing ACC as the solitary nitrogen source, was compared to both positive and negative controls. The experiment was replicated three times, and the evaluation of cultured Petri plates was predicated on the growth of bacteria that relied exclusively on ACC for nitrogen (Penrose & Glick, 2003).

***Molecular characterization of the bacteria isolates*.** The Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA) was employed to extract DNA from each bacterial isolate, following the manufacturer's provided instructions. A PCR was carried out to amplify the 16S rRNA gene in the genomic DNA of the extracted bacteria, utilizing the primers 1492R (CGGTTACCTTGTTACGACTT) and 27F (AGAGTTTGATCMTGGCTCAG). The PCR reaction was conducted using a Thermocycler Bio-Rad T100. The reaction mixture consisted of 12.5 µl of 1×NEB OneTaq 2x MasterMix with Standard buffer (Catalogue No. M0482S), 1 µl (10 µM) of each primer, 2 µl of the DNA template, and 9.5 µl of nuclease-free water in a 25 µl reaction volume. The PCR protocol included an initial denaturation stage at 94 °C for 5 minutes, followed by 35 cycles of 30 s at 94 °C, 30 s at 50 °C, and 1 min at 68 °C, with a final 10-minute elongation step at 68 °C.

Subsequent to PCR, ExoSAP-IT from Applied Biosystems, Foster City, CA, USA, was employed for the purification of the resulting PCR products according to the manufacturer's instructions. The separated DNA molecules were visualized through gel electrophoresis using the ChemidocTM imaging system (BIO-RAD Laboratories, Hercules, CA, USA). The sequencing of the amplified and purified DNA was carried out by Inqaba Biotechnology Pty in Pretoria, South Africa.

***Phylogenetic analysis.*** Pairwise comparisons and multiple alignments were carried out using BioEdit software version 7.0.5, which made it easier to evaluate nucleotide variants and similarities. Pairwise affinity values were computed and the maximum likelihood approach was then applied to create phylogenetic trees. For this approach, the Tamura–Nei parameter was used as a substitute model (Tamura & Nei, 1993). MEGA X (v 10.1.7) was used for the implementation (Kumar *et al*., 2018). The bootstrap approach with 1000 repetitions was used to evaluate the internal branches' reliability (Felsenstein, 1985). All the consensus nucleotide sequences in used in this investigation have been deposited in GenBank under the accession numbers OR044417–OR044423 (Tab. 1).

**Table 1**. 16s rRNA gene sequence-based identification of the isolates and their accession numbers

| Code | Isolate name | Accession number | Blasting, closest relative | Relative accession | Similarity % | E value |
|---|---|---|---|---|---|---|
| B1 | *Bacillus siamensis* strain AOA1 | OR044417 | *Bacillus siamensis* | OP904261 | 99.85 | 0.00 |
| B2 | *Enterobacter asburiae* strain AOA2 | OR044418 | *Enterobacter asburiae* | OP986762 | 99.18 | 0.00 |
| B3 | *Enterobacter chengduensis* strain AOA3 | OR044419 | *Enterobacter chengduensis* | OP811866 | 84.60 | 0.00 |
| B4 | *Priestia aryabhattai* strain AOA4 | OR044420 | *Bacillus aryabhattai* | MT453993 | 100.00 | 0.00 |
| B5 | *Burkholderia* sp. strain AOA5 | OR044421 | *Burkholderia* sp. | MW930845 | 99.75 | 0.00 |
| B6 | *Priestia megaterium* strain AOA6 | OR044422 | *Bacillus megaterium* | MT827122 | 99.30 | 0.00 |
| B7 | *Priestia megaterium* strain AOA7 | OR044423 | *Priestia megaterium* | OQ931927 | 99.74 | 0.00 |

***Evaluation of the antifungal potentials of bacteria species.*** A line of bacteria culture was streaked on the solidified Potato Dextrose Agar (PDA) at 3 cm from the edge of 9 cm diameter Petri-dishes. A point inoculation of fungi was made at a distance of 4 cm away from the streaked bacteria (Fig. 1).

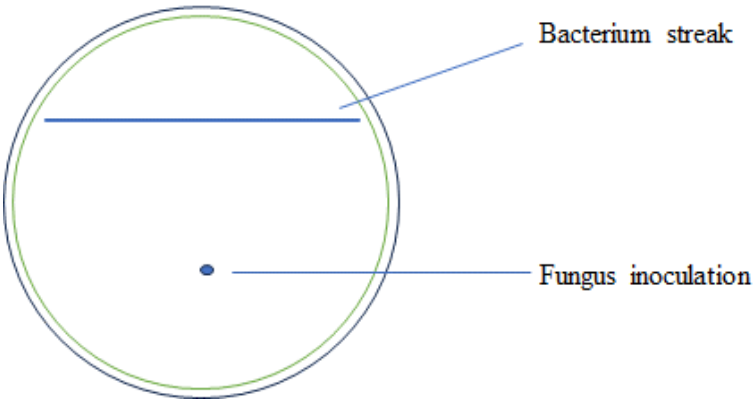


**Figure 1.** Bacteria streak and fungal inoculation on a PDA plate

A total of 7 bacterial isolates were assessed against 3 pathogenic fungal species, with fungal plates serving as the control. The experiment was replicated 3 times and incubated at 25 ± 2 °C for 7 days. The radius of mycelia growths was measured at two days intervals. The percentage rate of mycelia inhibition was calculated as:

$$\%\ Growth = \frac{Rc - Rt}{Rc}\ x\ 100$$

$R_t$ = radius of the fungi when inoculated in a plate with bacteria
$R_c$ = radius of the fungi when inoculated alone (control).

***Statistical analysis.*** The data collected from each replicate were subjected to Analysis of Variance (ANOVA) using Statistical Analysis Software (version 9.8). Post hoc analysis was performed using Duncan's Multiple Range Test to separate mean values. All statistical analyses were executed at a significance level of 5% ($p \leq 0.05$).

## Results

The isolated bacteria species were categorized into seven distinct groups based on their morphological characterization i.e. color, shape and growth patterns. All seven bacteria isolates tested positive for ammonia, phosphate siderophore and ACC (1-aminocyclopropane-1-carboxylate) deaminase tests. Isolate B5 demonstrated higher phosphatase potentials compared to other isolates. More so, isolate B1 showed no growths with $(NH_4)_2SO_4$ treatments in the ACC deaminase test (Tab. 2).

**Table 2**. PGPR screening of bacteria isolates

| Isolate | Ammonia production (mg/L) | Phosphate solubilization (mg/L) | Siderophore production (µg/mL) | ACC deaminase test | | |
|---|---|---|---|---|---|---|
| | | | | ACC (µmol/min / mg protein) | $(NH_4)_2SO_4$ (mg/L) | Control |
| B1 | + | ++ | + | + | - | + |
| B2 | + | ++ | + | + | + | + |
| B3 | + | ++ | + | + | + | + |
| B4 | + | ++ | + | + | + | + |
| B5 | + | +++ | + | + | + | + |
| B6 | + | ++ | + | + | + | + |
| B7 | + | ++ | + | + | + | + |

Note: For phosphate solubilization and siderophore production, the clear zones were: (+) = 0.10 to 0.50 cm; (++) = 0.60 to 1.00 cm; (+++) = 1.10 to 1.50 cm.

Sequences of the isolated bacteria strains were aligned with those earlier documented on the NCBI platform. The phylogenetic relationship tree of the isolates revealed B1, B4, B6 and B7 clustered into a distinct, well-supported clade with *Bacillus siamensis* OP904261, *Priestia arybhattai* OQ519933, *Preistia megatarium* OM033601, and *Priestia megaterium* MT827122 (Fig. 2a). Similarly, the phylogenetic relationship between B2 and B3 was observed in their cluster to a distinct clade with *Enterobacter asburiae* OP986762 and *Enterobacter chengduensis* OP811866 (Fig. 2b), while a separate phylogenetic tree, B5 clustered with *Burkholderia* sp. MW930845 (Fig. 2c).

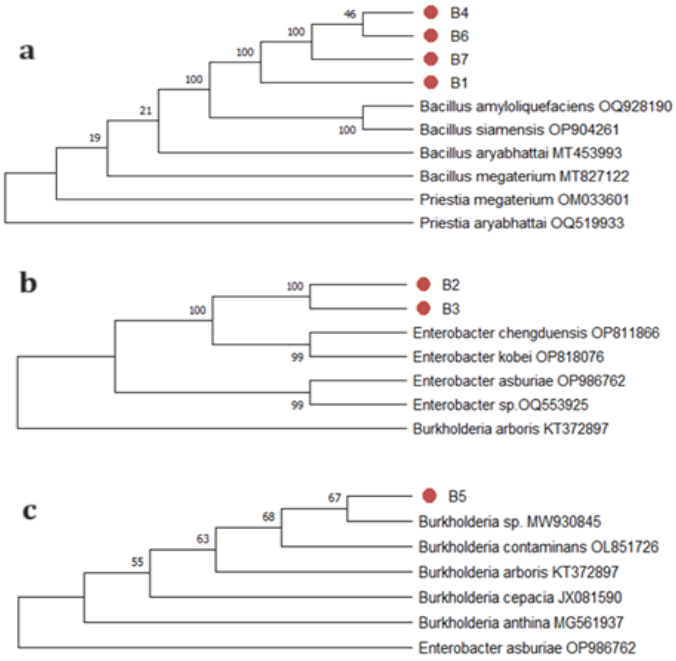


**Figure 2.** The phylogenetic relationships between the bacteria species and selected database relatives on the NCBI generated through transcribed spacer 16s rRNA genes, analyzed via Kimura's two-parameter models. Bootstrap support values higher than 50% from 1000 replicates are shown at the nodes. *Priestia aryabhattai* (OQ519933) (a) *Burkholderia arboris* (KT372897) (b) and *Enterobacter asburiae* OP986762 (c) were used as the out-group.

The morphological overview of the experimental set-up on day 4 showed varying rates of bacteria inhibition of the fungi mycelia growth, as compared to the control was demonstrated in Fig. 3. The evaluation of the antagonistic potentials of seven bacteria species against the fungal pathogens associated with foliar diseases of maize showed no significant ($p>0.05$) difference in their individual effects on mycelial inhibition of *Enterobacter chengduensis, Priestia aryabhattai, Burkholderia* sp, *Priestia megaterium* strain AOA6 and *Priestia megaterium* strain AOA7 against the fungi; *Nigrospora sphaerica, Alternaria alternata* and *Fusarium equiseti*, as observed at the 3rd, 5th and 7th days of observation. However, *Bacillus siamensis* showed the most significant inhibition of the mycelia growth of *A. alternata* on the 3rd day. In contrast, no significant difference was recorded across the fungal species on the 5th and 7th days. Similarly, *Enterobacter asburiae* recorded a similar level of significance across the fungal species at the 3rd day of evaluation. At the same time, it only showed significant ($p<0.05$) influence on *N. sphaerica* on the 5th and 7th days (Tab. 3).

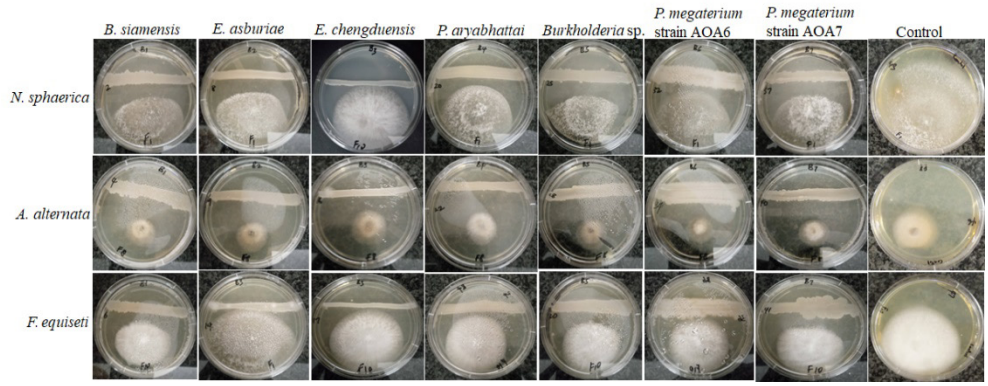


**Figure 3.** Culture plates of three fungi species challenged with seven bacteria species taken at day 4 of the experiment

**Table 3.** Antagonistic potentials of bacteria against *Nigrospora sphaerica*, *Alternaria alternata* and *Fusarium equiseti*

| Bacterial isolates | Days | *Nigrospora sphaerica* | *Alternaria alternata* | *Fusarium equiseti* | LSD |
|---|---|---|---|---|---|
| | 3 | 4.90b ± 3.98 | 25.44a ± 4.29 | 13.97b ± 2.42 | 10.18 |
| *Bacillus siamensis* | 5 | 51.08a ± 18.51 | 25.74a ± 7.01 | 32.01a ± 9.46 | 51.58 |
| | 7 | 66.02a ± 15.78 | 57.53a ± 12.29 | 45.30 a± 11.44 | 29.54 |
| | 3 | 16.78a ± 5.28 | 3.57a ± 2.06 | 9.86a ± 1.76 | 13.65 |
| *Enterobacter asburiae* | 5 | 62.20a ± 17.09 | 16.53b ± 8.21 | 19.60b ± 3.65 | 39.18 |
| | 7 | 67.56a ± 14.71 | 46.31ab ± 3.87 | 29.61ab ± 9.97 | 31.66 |
| | 3 | 3.79a ± 3.79 | 5.63a ± 3.66 | 6.19a ± 3.07 | 13.16 |
| *Enterobacter chengduensis* | 5 | 14.92a ± 5.85 | 5.09a ± 2.25 | 2.59a ± 0.49 | 12.05 |
| | 7 | 24.30a ± 8.22 | 34.06a ± 16.41 | 1.10a ± 0.73 | 33.44 |
| | 3 | 21.26a ± 3.39 | 14.72a ± 7.78 | 11.92a ± 1.95 | 18.26 |
| *Priestia aryabhattai* | 5 | 26.00a ± 15.79 | 18.00a ± 4.64 | 10.75a ± 2.47 | 37.03 |
| | 7 | 30.94a ± 7.71 | 26.64a ± 7.31 | 10.09a ± 3.00 | 21.74 |
| | 3 | 7.65a ± 7.65 | 12.94a ± 8.61 | 8.51a ± 4.17 | 21.81 |
| *Burkholderia* sp. | 5 | 46.64a ± 14.72 | 23.93a ± 10.99 | 31.92a ± 2.99 | 26.28 |
| | 7 | 56.47a ± 11.82 | 63.58a ± 12.66 | 47.55a ± 6.86 | 33.02 |
| *Priestia* | 3 | 11.93a ± 3.61 | 18.13a ± 4.31 | 15.12a ± 2.87 | 14.8 |
| *megaterium* | 5 | 30.02a ± 12.11 | 33.22a ± 4.53 | 16.15a ± 1.11 | 23.43 |
| strain AOA6 | 7 | 38.34a ± 12.12 | 55.88a ± 10.48 | 28.01a ± 4.66 | 33.72 |
| *Priestia* | 3 | 17.78a ± 6.22 | 0.22a ± 2.74 | 13.35a ± 5.43 | 19.71 |
| *megaterium* | 5 | 63.40a ± 19.58 | 20.99a ± 7.55 | 28.50a ± 7.47 | 42.05 |
| strain AOA7 | 7 | 100.00a ± 39.99 | 43.73a ± 5.53 | 51.24a ± 11.67 | 69.40 |

Note: Each value represents the mean of four replicates ± standard error. Means with different letters across the row are significantly ($p<0.05$) different.

The antagonistic effect of the bacteria species on the fungi *Nigrospora sphaerica*, *Alternaria alternata* and *Fusarium equiseti* showed similar significance levels at the 3rd day of observation. The mycelial growth of *N. sphaerica* (42.04%, 54.82%) was the most significantly inhibited on the 5th day, and together with *A. alternata* (46.53%) on the 7th day recorded a similar level of significance, while *F. equiseti* (30.41%) was the least inhibited (Fig. 4). However, the pooled effects of days of observation revealed *N. sphaerica* (36.29%) as the most significantly inhibited, followed by *A. alternata* (26.19%) and *F. equiseti* (20.63%) (Fig. 5).

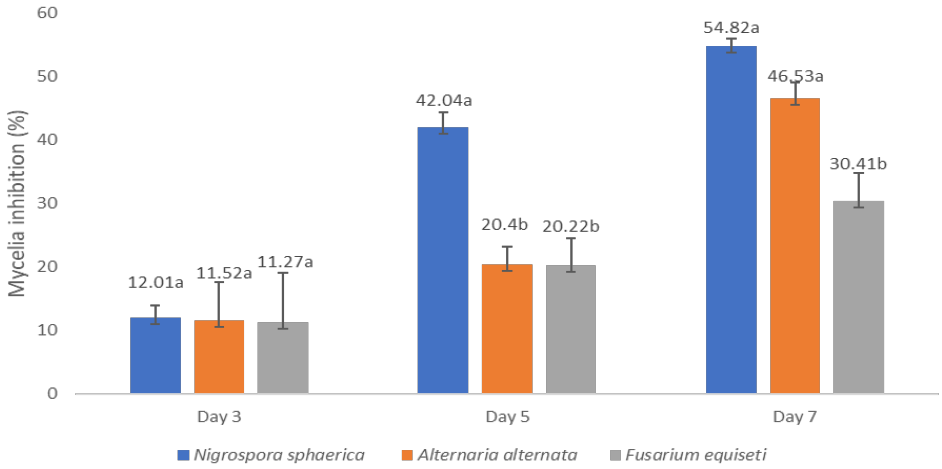


**Figure 4.** The percentage mycelia inhibition across different fungal species. Each value represents the mean of four replicates ± standard deviation

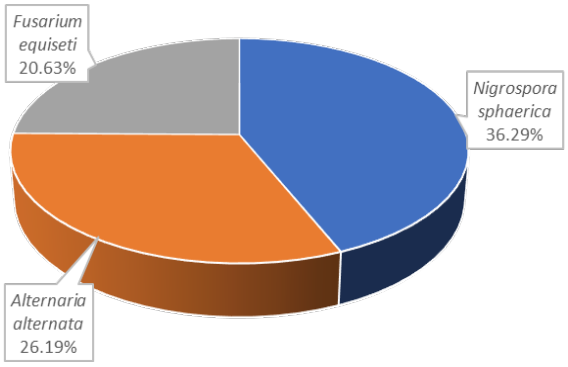


**Figure 5.** The pooled effects of days on the mycelia inhibition by the bacteria species

*Bacillus siamensis* (14.77%), *Priestia aryabhattai* (15.97%) and *Priestia megaterium* strain AOA6 (15.06%), which showed a similar level of significance at $p<0.05$ expressed the mostly inhibition of the fungal species at day 3 of the observation. On the 5th day, *B. siamensis* (36.28%) and *P. megatarium* strain AOA7 (37.63%) showed the most significant inhibition, with *P. megatarium* strain AOA7 (65.02%) consistency as the most significant on the 7th day of observation. However, *Enterobacter chengduensis* (5.20%, 7.53% and 19.82%) recorded the least inhibition on days 3, 5, and 7 of the observation, respectively (Tab. 4). The antagonistic effects of bacteria against the pooled fungal species showed *E. asburiae* (35.96%) and *P. megatarium* strain AOA7 (34.65%), as causing the most significant inhibition of fungal mycelia growth. This result was followed by *B. siamensis* (30.60%), *P. aryabhattai* (27.35%), *E. chengduensis* (23.01%), and *Bulkholderia* sp. (21.23%), while *P. megaterium* strain AOA6 (21.13%) showed the least mycelia inhibition (Fig. 6).

**Table 4.** Pooled percentage mycelia inhibition by each bacteria species

| Bacteria | Day 3 | Day 5 | Day 7 |
|---|---|---|---|
| *Bacillus siamensis* | 14.77a ± 3.17 | 36.28a ± 7.37 | 56.28ab ± 7.41 |
| *Enterobacter asburiae* | 10.07ab ± 2.42 | 32.78ab ± 8.57 | 47.83ab ± 7.21 |
| *Enterobacter chengduensis* | 5.20b ± 1.86 | 7.53c ± 2.49 | 19.82d ± 6.93 |
| *Priestia aryabhattai* | 15.97a ± 2.90 | 18.25bc ± 5.36 | 21.87cd ± 4.25 |
| *Burkholderia* sp. | 9.68ab ± 3.12 | 34.16ab ± 6.29 | 55.87ab ± 5.96 |
| *Priestia megaterium* strain AOA6 | 15.06a ± 2.05 | 26.46ab ± 4.51 | 40.74bc ± 6.11 |
| *Priestia megaterium* strain AOA7 | 10.45ab ± 3.45 | 37.63a ± 8.73 | 65.02a ± 14.74 |
| LSD | 7.80 | 15.43 | 19.71 |

Note: Each value represents the mean of four replicates ± standard error. Means with the different letters across the column are significantly ($p<0.05$) different.

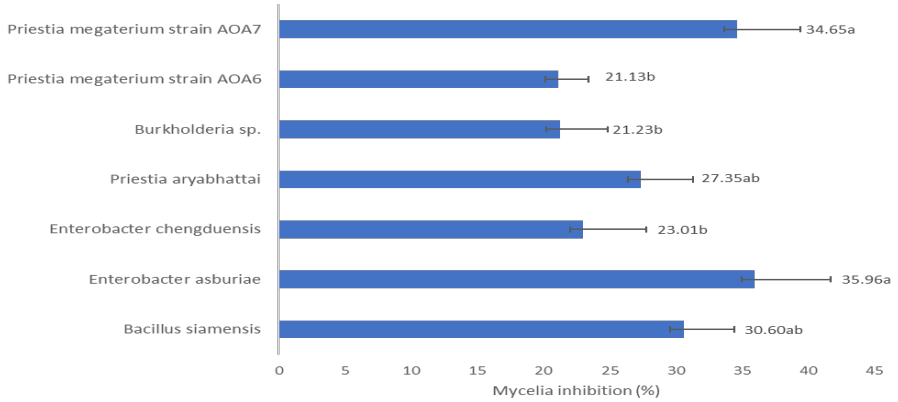


**Figure 6**. The antagonistic potentials of each bacterium against the mycelia growth of fungal species (LSD = 10.23)

## Discussion

The positive tests of the seven bacteria isolates in this study to ammonium, ACC deaminase, phosphate and siderophore production indicate the capability of each of the isolates to suppress the soilborne fungal pathogens of maize. The isolates demonstrated the potential to sequestrate iron thereby limiting the nutrient availability to fungal pathogens as revealed by the siderophore production (Tripathi *et al*., 2020). Bacteria species that solubilize phosphate also produces secondary metabolites with antifungal properties, thereby enhancing plant defense responses (Fasusi *et al*., 2021; Wei *et al*., 2024). Also, the ACC results indicated the potential of bacteria in ethylene modulation which aids in the suppression of fungal pathogenicity (Jha *et al*., 2021). Furthermore, the ammonium production recorded revealed the potential of the bacteria to compete for nitrogen sources in the surrounding environment, and limiting its availability to fungal pathogens (Lau *et al*., 2020).

The BLAST search on the NCBI based on the seven bacteria species found associated with diseased maize rhizosphere and characterized in this study using 16S rRNA gene, revealed the organisms as *Bacillus siamensis* strain AOA1 (B1), *Enterobacter asburiae* strain AOA2 (B2), *Enterobacter chengduensis* strain AOA3 (B3), *Priestia aryabhattai* strain AOA4 (B4), *Burkholderia* sp. strain AOA5 (B5), *Priestia megaterium* strain AOA6 (B6) and *Priestia megaterium* strain AOA7 (B7). This validates the earlier claim that bacteria are major microbiome domain that is commonly associated with plant rhizosphere (Babalola, 2010; Saqib *et al*., 2020). Bacteria species have been reported to perform varying roles, especially in plant–microbe relationships, where they aid in plant growth promotions, health, stress and drought management, as well as phytoremediation (Dlamini *et al*., 2022; Fadiji *et al*., 2022b; Kabeer *et al*., 2022), among many others. *Bacillus siamensis,* is a gram-positive bacterium in the phylum Firmicutes and class Bacilli. It is commonly isolated from soil, especially in the tropical regions. The organism has been found to produce enzymes such as amylases, proteases, and lipases, which contribute to its ability to degrade complex organic compounds. Furthermore, as a result of the antimicrobial compounds it secretes, it has been found effective against plant pathogens, as evident in its significant inhibition of mycelia growths of some pathogenic fungi i.e. *Rhizoctonia solani* and *Botrytis cinerea* (Jeong *et al*., 2012). It has also been reported as effective against the brown spot disease of tobacco caused by *Alternaria alternata* (Xie *et al*., 2021), *Pestalotiopsis versicolour* causing bayberry twig blight of sweet potato (Ali *et al*., 2020) and soybean root rot disease (He *et al*., 2023) among many studies.

Two strains of *Prestia megaterium,* which is a new separate genus from *Bacillus,* previously known as *Bacillus megaterium* (Liu *et al*., 2023). This ubiquitous environmental bacterium was initially identified by Anton De Bary in 1884. It has been reported as important in biotechnology for the synthesis of enzymes, recombinant proteins, and vitamins as well as for bioremediation activities (Vary *et al*., 2007). Its role from simple soil bacterium to industrial protein production host has been further enumerated (Biedendieck *et al*., 2021; Vary *et al*., 2007). Also, *P. megaterium* has been isolated from different plants, including pepper, cucumber, wheat, Alfalfa, carrot, black, clover etc. (Rajan *et al*., 2021). *P. megaterium* is categorized as a potential biocontrol agent for plant diseases due to its antimicrobial activities (Jayakumar *et al*., 2021). As indicated in previous investigations, three main mechanisms by which *P. megaterium* promotes plant growth include the secretion of organic acids which provides the template for phosphate solubilization. The organisms also cause variations in the concentration of phytohormone and other plant growth regulators, while they also can function as a biocontrol or biopesticide agent (Liu *et al*., 2023). However, *Priestia aryabhattai,* which is another *Priestia* specie isolated in this study, has earlier been described as a stress-tolerant bacterium with the capability of enhancing salt tolerance in wheat (Shahid *et al*., 2022), the organisms similarly showed the potential of degrading aromatic compounds, and as well antagonized both the fungal and bacterial phytopathogens (Esikova *et al*., 2021).

In addition, the isolated *Enterobacter* species are Gram-negative bacteria in the family *Enterobacteriaceae.* Some genera within the family *Enterobacteriaceae*, such as *Enterobacter, Pantoea, Serratia* and certain species of *Klebsiella,* have been classified as Plant Growth Promoting Bacteria (PGPB). However, some of the genera have also been found to contain species, which are plant pathogens causing varying diseases of crops such as wilting, galls, necrosis and soft rot diseases (Walterson & Stavrinides, 2015). *Enterobacter asburiae* and *Enterobacter chengduensis* were isolated from diseased maize rhizosphere in this study, while earlier research had isolated a strain of *E. chengduensis* from blood in China (Wu *et al*., 2019), indicating the diverse host of this member, as including plants, animals, humans and water (Fadiji *et al*., 2022a). Furthermore, *E. asburiae* has been implicated with rice bacterial blight disease in China (Xue *et al*., 2021), while another strain of *E. asburiae* was reported as a plant growth-promoting rhizobacteria in the study conducted by Saikia *et al*. (2023). Similarly to *Enterobacter*, *Burkholderia* are also versatile microorganisms that have been recovered from a wide range of ecological niches. Many of the species have been effective in plant growth promotion, biocontrol of plant pathogens and bioremediation purposes (Coenye & Vandamme, 2003). However, some *Burkholderia* species demonstrate

a twist of activities, such as the case of *Burkholderia cepacia*, which is a known plant pathogen and multiresistant pathogen in patients suffering from cystic fibrosis, yet this organism is still an efficient biological fertilizer and pesticide of a range of plant pathogens, including bacteria, fungi, and nematodes (Govan *et al*., 1996; Parke and Gurian-Sherman, 2001; Vandamme *et al*., 2003).

The fungi species; *Nigrospora sphaerica, Alternaria alternata,* and *Fusarium equiseti* are some of the commonly reported pathogens of maize (Akanmu *et al*., 2023b; Aveling *et al*., 2020). This study revealed *N. sphaerica* (36.29%) as the most inhibited by the antagonist bacteria species, followed by *A. alternata* (26.19%) and *F. equiseti* (20.63%). Thereby validating the earlier report on the antifungal potentials of bacteria species in the management of fungal diseases of maize as earlier reported (Fasusi *et al*., 2021; Orole *et al*., 2023; Tagele *et al*., 2019). Furthermore, *E. asburiae* > *P. megatarium* strain AOA7 > *B. siamensis* > *P. aryabhattai* > *E. chengduensis* > *Bulkholderia* sp. were the order of the antifungal efficacy of the bacteria species evaluated. This result is consistent with the earlier characteristics of each of the isolates discussed. Thus, the presence of bacteria with biocontrol potentials in the rhizosphere soil of maize plants examined in this study signifies the natural potential of plant root architecture and exudation to modulate the rhizosphere microbiome, as to function based on the plant's requirement (Dlamini *et al*., 2023a; Marco *et al*., 2022).

## Conclusion

In conclusion, *F. equiseti* (20.63%), *A. alternata* (26.19%) and *N. sphaerica* (36.29%) were the order of susceptibility of the pathogenic fungi to the biocontrol potentials of the bacteria evaluated. More so, the efficacy of *E. asburiae, P. megatarium* strain AOA7, and *B. siamensis* over a range of fungal pathogens justifies their further investigation, formulation and deployment as biofungicide in the management of foliar diseases of maize.

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