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Full Length Article

Biochemical and Molecular Characterization of Bacterial and Fungal Isolates Associated with the Rhizosphere of Healthy and Diseased *Solanum lycopersicum*

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Abstract

Tomato is a significant vegetable grown for its essential vitamins and antioxidant-producing potential. The rhizosphere microbes of plants are involved in promoting plant growth through increased availability of nutrients and improving the health status of the plants. This study was designed to explore the bacteria and fungi dwelling in the rhizospheric soil samples of healthy and diseased tomato plants employing a culture-dependent approach. Bacterial and fungal communities were isolated from the rhizosphere soil obtained from tomato plants by culture-dependent techniques. The biochemical and molecular characterizations were conducted on the isolates using 16S rRNA gene and ITS sequencing for the isolated bacterial and fungal isolates, respectively. The results revealed that various species of *Bacillus* and *Streptomyces* were obtained for ten bacterial isolates while species of *Trichoderma, Purpureocillium, Mortierella, Chaetomidium,* and *Mortierella* were obtained for five fungal isolates. The results further revealed that the microorganisms were greater in healthy tomato plants' soil samples than in the diseased tomato rhizosphere. The prevalence of bacterial and fungal species revealed the importance of these rhizospheric microbes and how they contribute to enhancing the growth and productivity of tomato plants.

Keywords: 16S rRNA; ITS; Microbial isolates; Rhizosphere soil; Tomato

Introduction

The rhizosphere is the area of soil around plant roots where plant roots and soil microbes interact, which is proportionally influenced by root exudates (Mukai *et al.* 2022). This region is regarded as the most probable zone where plants roots and microbes dwell. Plants manufacture carbon compounds (simple and complex sugar) after photosynthesis in which microbes inhabiting the rhizosphere soil are utilized. Microbial communities carry out important roles in the process of biogeochemical cycles of these ecosystems (Koner *et al.* 2022; Rüthi *et al.* 2023). They are involved in significant functions in maintaining plant health as a result of the availability of nutrients that contribute to plant growth and prevent biotic and abiotic stresses affecting plants (Das *et al.* 2022; Koza *et al.* 2022). Therefore, knowing the microbial diversity in the rhizosphere is significant for promoting the production of crops in sustainable agriculture. The equivalence of bacteria, fungi, Archaea and other microbes reveals the abundance of microbes in the soil. The microbes involved in the degradation and decomposition of inorganic and organic compounds in the soil making it available for plants' consumption to improve their growth (Wang *et al.* 2022a). Sun *et al.* (2022) reported how the organic matter was decomposed and the mineralization of nutrients by *Bacillus, Bradyrhizobium, Bryobacter* and *Chujaibacter* species in non-aeration environments. Duan *et al.* (2022) reported how *Leucocalocybe mongolica,* a fungus species promotes the growth of plant. *Trichoderma* spp. and *Penicillium* spp. isolated from the rhizosphere of tomato plants have been reported for their antifungal activities against *Sclerotium rolfsii* causing white rot in tomato plants (Coulibaly *et al.* 2022). In the soil where bacteria are mostly found, the decomposition of organic matter and mineralization of nutrients are speedy (Ling *et al.* 2022). In another way where fungi are abundant in the soil, the rate of transition of nutrients and energy is slow, which is conducive to organic matter storage and nutrient retention (Du *et al.* 2022).

Invasion of powdery mildew diseases on tomato plants may occur at different stages (preharvest or postharvest) when environmental conditions are comfortable for the

multiplication of the disease. The symptoms of powdery mildew disease observed at the primary stage include light green and yellow spots on leaves. In the early 1990s, powdery mildew disease is a significant disease of tomatoes cultivated in the greenhouse and on the field that causes global challenges since the disease was accounted in Asia, Europe, and North and South America (Abubakar *et al.* 2022). This disease chiefly affects the tomato leaves, causing defoliation, drying, necrosis and yellowing of the leaves. The disease is caused by a fungus called *Oidium neolycopersici* in tomatoes (Lebeda *et al.* 2015). Various studies have been reported on the equivalence of bacteria and fungi in the soil, revealing their potential to control the functioning ecosystem, the fertility of the soil, and the abundant production of crops (Jiao *et al.* 2022). Several studies have been conducted on the persistence of bacteria and fungi in the tomato rhizosphere (Karthika *et al.* 2022; Malik *et al.* 2022; Runge *et al.* 2023). Furthermore, studies on bacterial and fungal diversity associated with healthy and powdery diseased tomatoes have been conducted (Adedayo *et al.* 2022a). Based on the various methods, farming systems can modify the nature of the soil soils and the soil microbes inhabiting it. So, it is important to have knowledge of the diversity of bacteria and fungi and their functions in soils. Microbiologists have made a lot of effort to isolate diverse bacterial and fungal taxa employing a culture-dependent approach using various culturing media (Dai *et al.* 2022; Smrhova *et al.* 2022). Various microbes were obtained after being cultured that carried out substantial activities which involved plant growth promotion, disease resistance, antibiotics nature, and biocontrol potential (Elnahal *et al.* 2022). Some reports identified the microbial isolate by either macroscopic (on the plate) or microscopic (under the microscope) method (Afridi *et al.* 2022; Kumar and Prasher 2022; Ruangwong *et al.* 2022) but not the best standard of identification of the organisms.

The diversity and abundance of microbial communities have much influence on sustainable productivity obtained from agricultural farmland. The information obtained on the bacterial and fungal taxa associated with rhizosphere is important in choosing a sustainable farming system (Enebe and Babalola 2022). The direct culture approach of these microbes and molecular methods are extensively employed to analyze the microbes dwelling in the rhizosphere soil (Gamalero *et al.* 2022; Mohammadi *et al.* 2022). Using Sanger sequencing (16S rRNA gene and Internal Transcribed Spacer (ITS) for identifying bacterial and fungal diversity will be effective in identifying these microorganisms at the species level because of its high sensitivity (Wang *et al.* 2022b). In this study, we investigated the diversity of bacteria and fungi in the soil samples of healthy and diseased tomato plants and bulk soil employing a culture-dependent approach. Bacterial and fungal abundance and composition were observed following culture-based techniques. We hypothesize that the bacteria and fungi will be greater in healthy rhizosphere soil (HR) compared to diseased rhizosphere soil (DR).

Materials and Methods

Study area and soil sampling

This province of South Africa experiences summer weather with sparing rainfall in October and May. The rainfall experienced annually is 300 to 600 mm, the optimum temperature of the region is 25°C and the highest temperatures range from 38 – 40°C and precipitation of 450 mm every year. During the period of winter, which is experienced between June to August, the temperature falls below 0°C. The cultivated tomato field of the North-West University Research and Teaching Farm (25°47′17.0″ S, 25°37′03.2″E; 25°47′19.1″ S, 25°37′05.1″ E; 26°019′36.9″ S, 26°053′19.0″ E altitude, 159 m) were sampled in this study in March 2021. The farm has a history of tomato cultivation for more than eight years. It also has a history of inorganic (NPK) fertilizer usage to promote soil fertility. A total of 15 soil samples were collected, of which 5 rhizosphere soil samples were collected from healthy Roma tomato plants (HR), 5 rhizosphere soil samples from diseased Roma tomato plants (DR) and 5 bulk (BR) soil (Babalola *et al.* 2022). In each unit, a sample of the rhizospheric soils that is closely attached to the plant roots was collected separately from diseased and healthy samples, while the bulk (control) sample was collected at about 40m away from the tomato field. The rhizosphere soils were collected from the soil around the tomato root at a depth of 4–15 cm using the sterilized auger. The powdery mildew diseased plants were selected based on the bright yellow spot that matures to whitish powder on the leaves and stems of the plant (Istifadah *et al.* 2020). The soil samples were obtained into sterile polyethylene bags, kept in a cold box, and taken to the lab at 4°C. The soil was further sieved with a 2 mm colander and kept at −20°C for further analysis.

Isolation and enumeration of the bacterial and fungal isolates

Isolation of the bacterial and fungal isolates present in the soil sample was carried out by employing the serial dilution plate technique (10^{-4} and 10^{-5}) using nutrient agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi (Cuong *et al.* 2011) respectively. One gram of each soil sample was added to 9 mL of distilled water. One milliliter of the inoculum was picked with the aid of a micropipette from the mixture (10^{-1}) into another 9 mL of distilled water and the procedure was repeated until 10^{-4} fold for bacteria and 10^{-5} for fungi. From 10^{-4} and 10^{-5}, 1 mL of inoculum was picked and then introduced into a sterile plate. Molten NA and PDA were poured on the plates containing the inoculum. The plates were allowed to solidify and incubate at 28°C for 24 h for bacterial isolates and 48 h for fungal isolates. Colonies growing on the agar media with different colors, sizes and shapes were sub-cultured separately on different culture media plates of the same medium and incubated again.

The bacterial plates were incubated in the dark for one week at 25°C and 60% relative humidity (Heidelberg *et al.* 1997). After incubation, 200 colonies of bacteria were selected at random and inoculated to fresh plates containing R2A medium with low nutrient medium (Becton-Dickinson, Sparks, MD, USA). 20% (v/v) of glycerol was added to the pure culture plates, and kept at -80°C (Byappanahalli *et al.* 2006). The total number of viable bacterial cells per gram of wet rhizosphere soil was obtained by counting the stained cell preparation under the microscope employing the LIVE or DEAD BacLight bacterial viability kit (Molecular Probes Inc., Eugene, Oreg, USA) according to the instructions on the manual. After a week of incubation of wet rhizosphere soil, the colony-forming units (CFU g^{-1}) were confirmed.

Characterization of bacterial and fungal isolates

From the 24 h old bacterial cultured plate, an inoculum was picked with the aid of a sterile wire loop and introduced on a glass slide to produce a smear. The smear was heat fixed on the glass slide. The Gram staining procedure was carried out on the heat-fixed glass slide following the method of (Bartholomew 1962).

The isolated bacterial and fungal morphological features were examined on PDA (latter) and NA (former) plates employing pure cultures of the isolates which were incubated at 28 ± 2°C for 24 h for bacteria and the same temperature for 48 h for fungi. The physical features of the colonies including the pH, temperature required for their growth, color and pigmentation were obtained after incubation. Biochemical characterization (catalase test, oxidase test, starch hydrolysis, citrate, nitrate reduction test, indole test) was conducted employing standard protocols according to (Clarke and Cowan 1952). Carbon sources including fructose, galactose, glucose, lactose, maltose, sucrose and xylose utilization were confirmed.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA of bacterial and fungal isolates was extracted employing ZR soil Microbe DNA MiniPrep™ (Zymo Research, USA) extraction kit according to the manufacturer's instructions. The partial 16S rRNA gene (v3–v4 region) of the bacterial DNA was amplified in a polymerase chain reaction (PCR) using the universal primer set 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). The amplification reaction was carried out using a PCR machine DYAD Peltier thermal cycler (BioRad, USA) thermal cycler with a total volume of 25 μL containing 11 μL DNA sample, 0.5 μL of each primer (forward and reverse), 0.5 μL *Taq* DNA polymerase (Nuclease free water) and 12.5 μL of master mix with standard buffer (New England, Biolabs Inc. USA). For bacteria samples, the thermocycling conditions employed were initially denatured at 95℃ for 2 min, 30 cycles of denaturation at 95℃ for 30 s, annealing at 55℃ for 30 s, extension at 72℃ for 30 s and final extension of 72℃ for 5 min. The amplicon sizes were observed via electrophoresis employing 1% agarose gel in 1× TBE buffer. The gel was run at 80 V for 60 min and observed with a Gel Doc (BioRad Laboratories, USA). For fungi samples, the thermocycling conditions employed were initially denatured at 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 2 min and final extension of 72°C for 7 min. The amplicon sizes were observed via electrophoresis employing 1% agarose gel in 1× TBE buffer. The gel was run at 80 V for 60 min and observed with a Gel Doc (BioRad Laboratories, USA). The amplicons or PCR products were sequenced at Inqaba Biotechnical Industrial Limited, Pretoria, South Africa, employing ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems, USA).

Phylogenetic analysis

The nucleotide sequences obtained after sequencing were analyzed and edited using Chromax lite 2.4 software (Connell *et al.* 2010) and BioEdit software (Hall 1999). The nucleotide sequences obtained were compared to sequences in the NCBI GenBank database with the BLAST (Altschul *et al.* 1990). Phylogenetic and molecular evolutionary analyses were conducted employing MEGA version 5.2.2 (Tamura *et al.* 2011). Evolutionary distance matrices were generated as described by Jukes and Cantor (1969) and a phylogenetic tree was inferred by the neighbor-joining method (Saitou and Nei 1987). Tree topologies were bootstrapped (Felsenstein 1985) based on 1000 re-samplings of the neighbor-joining data set (Felsenstein 1985).

Results

Bacterial CFU counts

From the HR, DR and BR soil samples collected from the agricultural field of North-West University, the total number of viable bacterial cells in 1 g of the soil samples was confirmed with the aid of a microscope and the result obtained is 1.8×10^9 CFU g^{-1} for HR, 1.42×10^9 CFU g^{-1} for DR and 0.9×10^9 CFU g^{-1} for BR. Also in 1 g of rhizosphere soil and bulk soil samples, the average number of CFUs confirmed after a week of incubation on R2A medium was $9.9 \times 10^6 \pm 1.9 \times 10^6$ for HR, $8.7 \times 10^6 \pm 1.5 \times 10^6$ for DR and $6.2 \times 10^6 \pm 1.2 \times 10^6$ are presented in Table 1. The cultivability revealed the percentage of CFUs obtained in comparison to the total number of viable bacterial cells, which was 0.60% of the total. Morphological and biochemical characterization employed in the study of sixteen bacteria were presented in Table 2 and five fungal isolates in Table 3.

Macroscopic and microscopic observation of bacterial and fungal isolate

After the pure culture of isolates have been isolated, bacteria isolates were gram-stained and observed under the microscope at magnification X100 objectives lens (Fig. 1).

Table 1: Colony count of bacteria under a microscope

Site	Amount of the soil sample (g)	Initial colony count ($CFUg^{-1}$)	After a week Colony ($CFUg^{-1}$)
Healthy soil (HR)	1	1.8×10^9	9.9×10^6
Diseased soil (DR)	1	1.42×10^9	8.7×10^6
Bulk soil (BR)	1	0.9×10^9	6.2×10^6

Table 2: Morphological and biochemical characterization of bacterial isolates obtained from tomato rhizosphere

	Bacillus species							*Streptomyces* species					
Morphological features	Bac_1	Bac_2	Bac_6	Bac_8	Bac_10	Bac_11	Bac_12	Bac_13	Bac_15	Bac_16	Bac_3	Bac_5	Bac_14
Endospore	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+	+
pH	5-8	4-7	5-8	5-8	5-8	5-8	5-8	5-8	5-8	5-8	5-8	4-7	5-8
Temperature (°C)	25-38	25-38	25-38	25-38	25-38	25-38	25-38	25-38	25-38	25-38	25-40	25-40	25-40
Pigmentation	-	-	-	-	-	-	-	-	-	-	-	-	-
Color	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	White	White	White
Biochemical test													
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	-	+	-	+	+	+	-	+	-	+	+	-	-
Nitrate	-	-	-	-	+	-	-	-	+	-	-	-	+
Urease	+	+	+	+	+	+	+	+	+	-	+	+	+
Phosphate	+	-	+	-	+	+	-	-	+	-	+	-	+
Indol	+	+	+	+	+	+	-	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	-	+	+	-	+	-	+	+	+	-	+	+	+
Galactose	-	-	-	+	-	-	-	+	+	-	-	-	+
Sucrose	+	-	-	+	-	-	-	+	-	+	-	+	-
Lactose	+	-	-	+	-	-	+	-	+	+	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	+	+	-	+	+	+	-	-	-	+	+	-	-

Table 3: Morphological and biochemical characterization on fungal isolates obtained from tomato rhizosphere

Morphological features	b_fun1	c_fun2	d_fun3	e_fun4	f_fun5
Growth pattern	Mycelia are white and have no formation of conidial	The colonies are oval and bulged	Arachnoid in shape	circulating, curved, curled, wavy, spirally and threadlike	Arachnoid in shape
Texture	Cottony	Cottony	Cottony	Woolly	cottony
Color	Whitish green	Wine	White	Orange	White
Isolate identified	*Trichoderma koningii*	*Purpureocillium lilacinum*	*Mortierella alpine*	*Chaetomidium fimeti*	*Mortierella alpina*

Below are some of the images observed under the microscope. The figure below showed the observation of pure cultured fungi on the PDA plate (Fig. 2).

Bacterial diversity in the soil samples employing a culture-dependent approach

Based on 16S rRNA gene analysis employed to detect bacteria isolate present in the employed soil samples, *Bacillus* species and *Streptomyces* species were obtained after conducting the sequencing. Ten bacterial isolates were identified as *Bacillus* species include; *Peribacillus frigoritolerans, B. amyloliquefaciens*, *Peribacillus simplex*, *B. velezensis*, *B. megaterium*, *B. mojavensis*, *Peribacillus frigoritolerans*, *Priestia aryabhattai*, *Bacillus cereus*, and *B. pseudomycoides* (Fig. 3). These isolates are obtained in HR, DR and BR, of which the isolates were greater in HR because of the healthy status of the soil. The following species were obtained in HR include; *Bacillus cereus* (100%), *Peribacillus simplex* (100%), *Brevibacterium frigoritolerans* (99.7%), *Peribacillus castrilensis* (99.8%), *B. proteolyticus* (99.5%), *B. simplex* (100%), *B. thuringiensis* (99.8%), *B. subtilis* (99.8%), *B. megaterium* (100%), *Priestia aryabhattai* (99.5%), *B. amyloliquefaciens* (99.8%), *B. velezensis* (99.8%), *Peribacillus frigoritolerans* (99.8%), *P. simplex* (99.8%), *B. mojavensis* (99.8%), *B. atrophaeus* (99.8%) and *B. licheniformis* (99.8%). In DR, the following *Bacillus* species were obtained: *B. nitratireducens* (99.8%), *Peribacillus frigoritolerans* (99.8%), *B. pseudomycoides* (99.8%), *B. velezensis* (99.8%), *B. siamensis (*99.8%), *B. zanthoxyli* (99.5%) and *B. subtilis* (99.8%). While *B. ginsengisoli* (99.8%), *B. amyloliquefaciens* (99.8%) and *Priestia aryabhattai* (99.8%) were discovered in BR.

Three isolates were *Streptomyces* species namely *S. anulatus, S. pratensis*, and *S. globisporus*, (Fig. 4). In HR, the following *Streptomyces* species were obtained; *S. anulatus* (100%), *S. cavourensis* (99.8%), *S. globisporus* (99.8%), *S. pratensis* (99.8%), *S. parvus* (99.8%), *S. rhizosphaericola* (99.8%), and *S. griseus* (99.8%). As for DR, *Streptomyces* species obtained are; *S. badius* (99.8%), *S. pluricolorescens* (99.7%) and *Kitasatospora albolonga* (99.5%) while *S. pratensis* (100%) was obtained in BR.

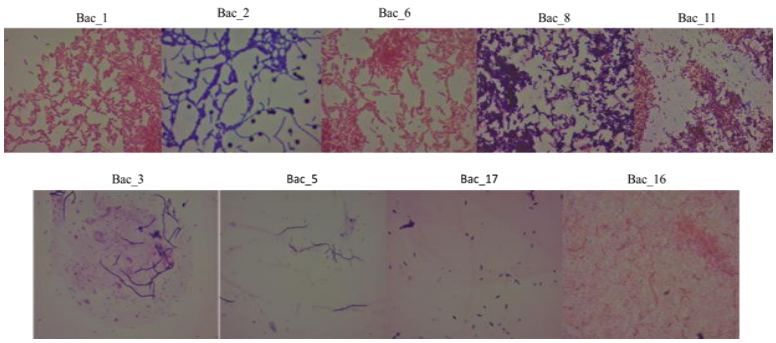

Fig. 1: Microscopic observation of bacteria isolates under a microscope

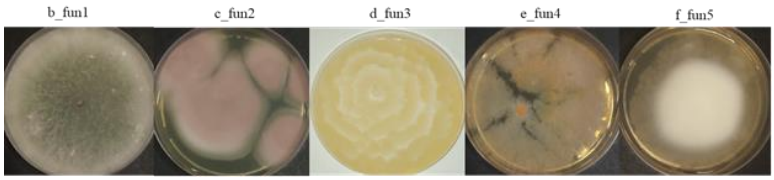

Fig. 2: Macroscopic observation of fungi isolate on plate

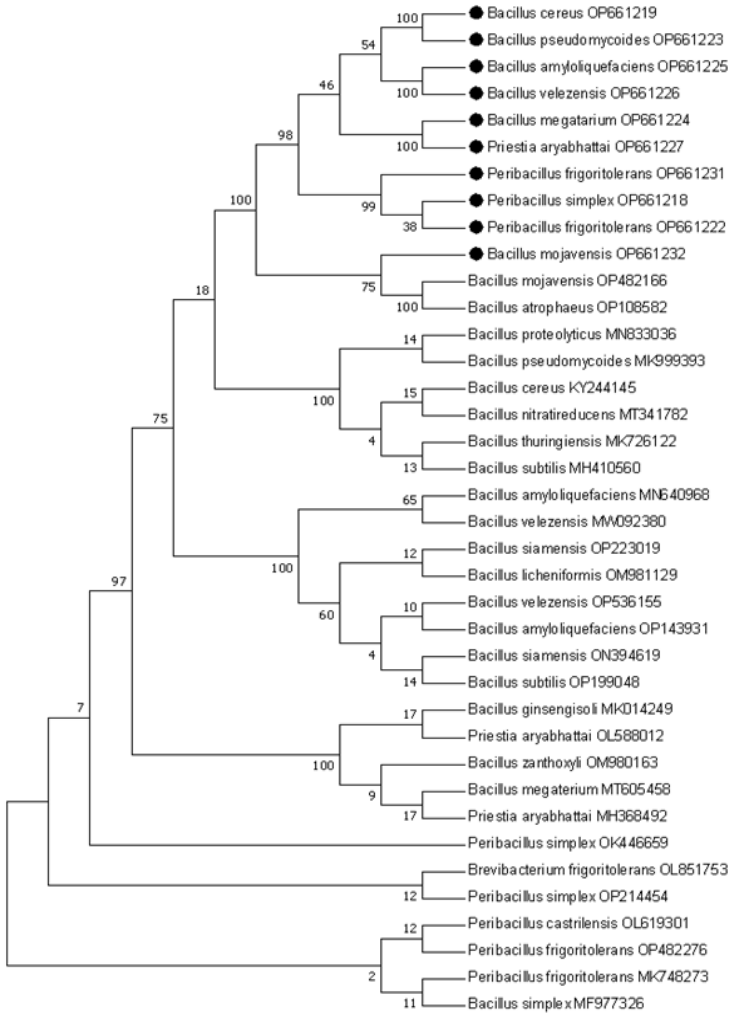

Fig. 3: Molecular Phylogenetic showing *Bacillus* spp. dwelling in the rhizosphere soil of tomato plants

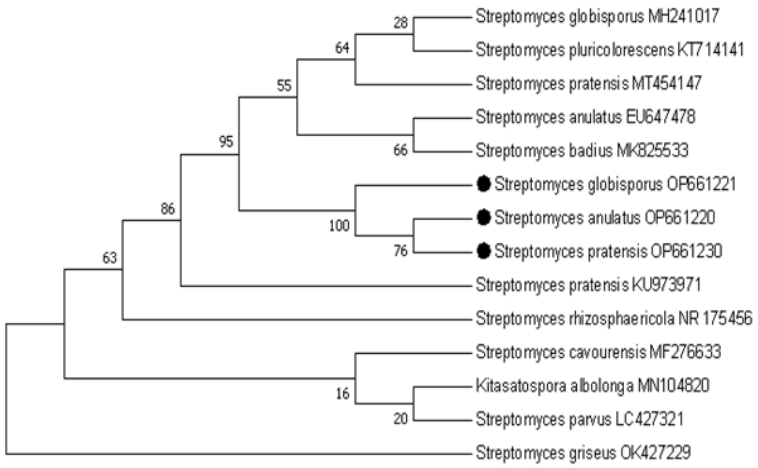

Fig. 4: Molecular Phylogenetic tree showing *Streptomyces* spp. dwelling in the rhizosphere of the soil

Table 4: Identification of pure fungal strains based on ITS

Isolate ID	Accession Number of the isolates	The scientific name of the isolate	Accession number and similarity (%) in the databank
b_fun1	OP669338	*Trichoderma koningii*	MG940968 (100)
c_fun2	OP669339	*Purpureocillium lilacinum*	MH483732 (99.63)
d_fun3	OP669340	*Mortierella alpina*	MK014152 (100)
e_fun4	OP669341	*Chaetomidium fimeti*	JN709488 (100)
f_fun5	OP669342	*Mortierella alpina*	MT447479 (96.68)

Fungal diversity in the soil samples employing a culture-dependent approach

Based on the internal transcribed spacer (ITS) analysis employed to detect fungi isolate present in the employed soil samples, Table 4 revealed the fungal isolates obtained. Various species included *Trichoderma koningii, Purpureocillium lilacinum, Mortierella alpina,* and *Chaetomidium fimeti.* In the HR soil sample, the fungal isolates were greater including *Trichoderma koningii, Purpureocillium lilacinum, Mortierella alpina* while *Mortierella alpina* and *Chaetomidium fimeti* were obtained in DR.

Discussion

Studies of bacterial and fungal communities and their diversity in the tomato plant's rhizosphere is essential as these microorganisms are reported in various studies to carry out beneficial interaction with tomato plants thereby improving to the growth of the plants. The microbes were cultured *in vitro* and the culture revealed the molecular procedures that assist in in-depth metabolic, physiological, and genomic characterization for the potential to acquire a better understanding of the microbes' functions in the rhizosphere of tomato. Although, some bacterial and fungal species inhabiting the soil that cannot be cultured in the laboratory because the nutrient required are not in the medium, some microbes growing on that medium produce certain substance that suppress the growth of other microbes, or the culture media re toxic for the microbial growth (Stanley *et al.* 2016). Our study reported the CFU counts of the bacteria which agrees with previous research that reported CFU counts of bacteria isolated from soil samples from other regions (Pascual *et al.* 2016; Youseif Sameh *et al.* 2021). However, this study presented the result of some culturable bacteria and fungi that were identified through molecular means of Sanger sequencing. The culturable microbial community assists in the determination of the structural abundance of viable and valuable bacteria and fungi that can be employed as inoculum to promote tomato plant health. Only a few studies have reported the diversity of bacteria and fungi in the tomato rhizosphere (Adedayo *et al.* 2022b; Anzalone *et al.* 2022). *Bacillus* spp. dominated the culturable isolates obtained from the rhizosphere soil of the tomato followed by *Streptomyces* spp. Moreover, five fungi isolate were obtained, among which *Trichoderma, Purpureocillium, Mortierella* and *Chaetomidium* were observed. The population of both bacteria and fungi was greater in HR compared to DR and BR.

Several reports have revealed how *Bacillus* spp. has shown the potential of plant growth-promoting traits (PGPT) in the rhizosphere of healthy plants (Ahmed *et al.* 2022; Sawant *et al.* 2022; Wang *et al.* 2023), thereby improving the growth of such crop plants. In HR, the following *Bacillus* species were reported in this study, and their activities are discussed below; According to Gashash *et al.* (2022), *B. subtilis* and *B. amyloliquefaciens* are potential plant growth-promoting rhizobacteria (PGPR) contributing to the growth of tomato plants. *B. megaterium, B. thuringiensis, B. cereus,*

and B. subtilis act as an antagonist as reported by Engelbrecht *et al.* (2022) for the immobility of nematocidal diseases caused by *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* infecting soybean. *B. substilis* prevent the invasion of diseases and induce systemic resistance against *Botrytis cinerea*in tomato plants (Zhou *et al.* 2021). *B. velezensis* has been reported to biologically control *F. oxysporum* causing *Fusarium* wilt of tomatoes (Hwang *et al.* 2022). It is also regarded as PGPR contributing to the growth of tomato plants. *Peribacillus frigoritolerans, P. castrilensis*, and *P. simplex* have displayed PGPR trait and biocontrol qualities in wild rice (Rodríguez *et al.* 2022; Yao *et al.* 2022). *B. mojavensis* and *B. atrophaeus* endeavor to the tolerance of salt stress and improve plant growth (Shultana *et al.* 2022). The following *Bacilus* spp; *B. proteolyticus, B. pseudomycoides, B. velezensis, B. siamensis, B. zanthoxyli, B. cereus, B. subtilis, B. licheniformis, B. ginsengisoli, B. amyloliquefaciens*, and *B. nitratireducens* portrays arsenic resistance and plant growth promoting activities in agricultural soils (Magar *et al.* 2022). *Priestia aryabhattai* has also displayed plant growth-promoting potential with Wheat (*Triticum turgidum*) in Mexico (Ortega-Urquieta *et al.* 2022). *B. velezensis* and *S. griseus* have been notified to produce bacterial volatile compounds (BVCs) for conferring immunity to the tomato plant and eliciting systemic resistance in tomato plants in the greenhouse experiment (Riu *et al.* 2022).

Other bacteria species reported in this study are *Streptomyces* spp., which are greater in HR compared to DR and BR. As a result of their abundance, they contribute to the health status of tomato plants. *S. globisporus* has been reported by Ebrahimi-Zarandi *et al.* (2021) to be an effective PGPR and biocontrol agent for many phytopathogens (*Rhizoctonia solani*) infecting tomatoes thereby inhibiting disease invasion. These bacteria can activate jasmonic acid and phenyl propanoid signaling pathways to induce systemic resistance. The biochemical characterization of the bacteria strains revealed that they can produce siderophores. *Streptomyces anulatus,* has been isolated from the rhizosphere of *S. lycopersicum* according to Djebaili *et al.* (2020) contributing to plant-promoting growth potential. *Pythium aphanidermatum* the causative agent of the tomato plant root rot was reported to be inhibited by *S. pratensis S. badius and S. flavogriseus* (root symbiont) (Hassanisaadi *et al.* 2021). Anusha *et al.* (2019) explained how *Streptomyces parvus* and *Bacillus* spp. biologically control the invasion of *Fusarium* wilt in *Cicer arietinum. Streptomyces cavourensis* and *Streptomyces griseofuscus* revealed their biocontrol potential against *Macrophomina phaseolina* and *Fusarium oxysporum*, in invitro or a dual culture assay in pulses and pigeon pea. The bacteria inhibit and eradicate the growth of the phytopathogen as reported by Manikandan *et al.* (2022). The rhizobacteria also displayed plant growth-promoting traits aside from producing non-volatile metabolites, lytic enzymes, and volatile organic carbon compounds with antifungal activities. *Streptomyces pluricolorescens* has proven a lot of potential on *Lycopersicon esculentum*. According to Fialho de Oliveira *et al.* (2010), *S. pluricolorescens* is an outstanding biocontrol agent by controlling phytopathogens. Siderophore production, antimicrobial activity, phosphate solubilization, and indoleacetic acid production were among the PGPR potentiality on tomato plants.

The fungal species identified in this species were identified first on morphological characters and then their identification was confirmed on molecular basis. Identification of fungi on molecular basis has gained a lot of importance in the recent years especially for those fungi whose identification is difficult on morphological characters (Khan *et al.* 2021; Khan and Javaid 2022; 2023). The fungi revealed in this study have been reported in various studies to carry out plant promoting-growth and biologically control the emergence of infections in crop plants, especially tomatoes. They can be collectively called plant growth-promoting fungi (PGPF). *Trichoderma koningii* has been reported to show tolerance against thermal or heat stress by controlling the production of highly fatal reactive oxygen species (ROS) metabolism in tomato plants (Tripathi *et al.* 2021; Adedayo and Babalola 2023). Many *Trichoderma* spp. have been reported to possess the ability to control a number of economically important soil-borne plant pathogens including *Fusarium oxysporum* (Akhtar and Javaid 2018), *Macrophomina phaseolina* (Khan and Javaid 2020), *Sclerotium rolfsii* (Javaid *et al.* 2021) and others. The PGPF has also been reported to biologically control the soil-borne bacterium *Ralstonia solanacearum* causing the disease of tomato *in vitro* and *in vivo* and contributing to the healthy growth of the tomato plant (Guo *et al.* 2021). *Purpureocillium lilacinum* is filamentous in nature. *P. lilacinum* possesses the ability to biocontrol root-knot disease of tomatoes caused by a nematode *Meloidogyne incognita*. *Phytophthora* blight disease of pepper can be controlled by the combination of *P. lilacinum* and arbuscular mycorrhizal (AM) and further promote the growth of the pepper plants (Hu *et al.* 2020). *Mortierella alpina* is a soil fungus associated with plants in the rhizosphere soil improving plant health. With their potential, they are known as PGPF. Wang *et al.* (2022a) explained how *M. alpina* was isolated from the rhizosphere soil of the ginseng plant and employed to observe its activities on plant disease. The result showed that the fungi produced high indoleacetic acid. The fungus was reported to have shown effectiveness to the soil-borne pathogen (*Fusarium oxysporum*) by reducing the invasion rate of the disease caused by *Fusarium oxysporum.* Although, the prevalence of *M. alpina* was inhibited by some PGPR (*Bacillus, Brevibacterium, Streptomyces*, and *Trichoderma*) despite its ability to inhibit phytopathogens as reported by Wang *et al.* (2022b). The introduction of *Trichoderma longibrachiatum* and *Mortierella alpina* has an antagonistic effect on root-knot nematode, *Meloidogyne javanica* on tomatoes. The fungi also promote plant growth, provide nutritional elements and stimulate the systemic resistance in

plants against disease invasion under greenhouse conditions (Al-Shammari *et al.* 2013). *Chaetomidium fimeti* was reported by Huang *et al.* (2020) as a prominent *Rhizoctonia* root rot disease suppressive fungi and as well better growth-promoting strain on cucumber plants.

Conclusion

This study reports an array of bacterial and fungal isolates dwelling in the rhizosphere of healthy and diseased tomato plants. The results obtained after isolation and identification revealed that the diversity of bacteria and fungi was greater in the HR compared to DR. The identified bacterial and fungal species have been reported in various studies to be involved in plant growth promotion, biological control of disease invasion and other activities. Further investigations should be conducted on the plant-microbe interactions to explore more beneficial microbes and investigate how they contribute to the growth of tomato plants.

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Author Contributions

AAA handled the literature findings, conducted the laboratory and fieldwork, executed all necessary analyses, interpreted the results, and prepared the manuscript. AEF provided technical input and assisted in result analyses and interpretations. OOB initiated the next-generation sequence research, supervised AAA and AEF, helped shape the research, verified the analytical methods, secured funds for the study and commented on the manuscript at all stages.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Data is publicly available on the NCBI database with the nucleotide accession number OP661218: OP661233[accn] for bacteria and OP669338: OP669342[accn] for fungi.

Ethics Approval

Not applicable

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