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Antagonistic potential of endophytic fungal isolates of tomato (*Solanum lycopersicum* L.) fruits against post-harvest disease-causing pathogens of tomatoes: An *in vitro* investigation

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ABSTRACT

Post-harvest decay of fresh agricultural produce is a major threat to food security globally. Synthetic fungicides, commonly used in practice for managing the post-harvest losses, have negative impacts on consumers' health. Studies have reported the effectiveness of fungal isolates from plants as biocontrol agents of post-harvest diseases, although this is still poorly established in tomatoes (*Solanum lycopersicum* L. cv. Jasmine). In this study, 800 endophytic fungi were isolated from mature green and ripe untreated and fungicide-treated tomato fruits grown in open soil and hydroponics systems. Of these, five isolates (*Aureobasidium pullulans* SUG4.1, *Coprinellus micaceus* SUG4.3, *Epicoccum nigrum* SGT8.6, *Fusarium oxysporum* HTR8.4, *Preussia africana* SUG3.1) showed antagonistic properties against selected post-harvest pathogens of tomatoes (*Alternaria alternata*, *Fusarium solani*, *Fusarium oxysporum*, *Geotrichum candidum*, *Rhizopus stolonifera, Rhizoctonia solani*), with *Lactiplantibacillus plantarum* as a positive control. *P. africana* SUG3.1 and *C. micaceus* SUG4.3 significantly inhibited growth of all the pathogens, with antagonistic capabilities comparable to that exhibited by *L. plantarum*. Furthermore, the isolates produced an array of enzymes, including among others, amylase, cellulose and protease; and were able to utilize several carbohydrates (glucose, lactose, maltose, mannitol, sucrose). In conclusion, *P. africana* SUG3.1 and *C. micaceus* SUG4.3 may complement *L. plantarum* as biocontrol agents against post-harvest pathogens of tomatoes.

1. Introduction

South Africa (SA) is a major regional tomato producer in sub-Saharan Africa (Malherbe and Marais, 2015). Tomatoes, with their numerous nutritional and health benefits, are the second most important produce after potatoes, in SA. Unfortunately, post-harvest tomato decay is currently a serious problem in the country. This decay is primarily caused by fungal pathogens, which are ubiquitous in nature. The predominant fungal pathogens responsible for post-harvest losses of tomato include, among others, *Fusarium oxysporum (fusarium wilt), Rhizoctonia solani (collar rot), Geotrichum candidum (sour rot), Altenaria alternata (black rot), Alternaria solani (early blight), Rhizopus stolonifera (bulb rot), Fusarium solani (vascular wilt) and Phytophthora cinnamomic (buckeye rot)*. They also cause diseases such as gray mold, black mold, blue mold, fusarium rot and rhizopus rot on tomato fruits (El-Katatny and Emam 2012; Sharma et al., 2009; Sinno et al., 2020). These fungal pathogens cause tomato fruit rots throughout the tomato production chain: from harvest to transportation, storage, processing, packaging and marketing (Sharma et al., 2009). They are also associated with low fruit yield, crop losses and severe damage to agroecosystems, all of which consequently

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impact the economy negatively (Engelbrecht et al., 2007; Nihorimbere et al., 2010).

Since fungal infestation continues to pose a significant threat to the production of fresh produce, various methods including physical, chemical and biological treatments have been used in the control and management of post-harvest losses of agricultural produce, however, these measures have not succeeded in alleviating food losses in Africa (Buonassisi 2013; Kader, 2003). Examples of physical methods used for managing post-harvest losses include temperature and humidity management (Kader, 2003; Da Vis and Hofman 2006), intermittent warming (Buonassisi 2013; Kader, 2003), modified/controlled atmosphere (Buonassisi 2013; Kader, 2003; Lima et al., 2013) and UV treatment (Buonassisi 2013; Lima et al., 2013). The main disadvantage of physical methods is their possibility to induce damage to produce, making it more susceptible to infection. Furthermore, these treatment methods are relatively expensive, and thus, may not be affordable for smallholder farmers (Arah et al., 2016).

Chemical treatments include the application of fungicides and antibiotics onto the surface tissues of produce, serving as a means to manage infections (Buonassisi 2013; Lima et al., 2013). These chemicals interrupt pathogen development by protecting produce against infections which occur during storage and handling (Buonassisi 2013). Post-harvest fungicides are applied as dips, sprays, fumigants, treated wraps and box liners or in waxes and coatings (Buonassisi 2013). The most commonly applied fungicides are the benzimidazoles (e.g., benomyl and thiabendazole) and the triazoles (e.g., prochloraz and imazalil) (Buonassisi 2013). Chemical treatments are to date the primary method for the control of post-harvest decay of fresh produce, however, some of the chemicals used are not authorized for post-harvest treatment. This has resulted in the removal of numerous chemicals from the market as they pose possible toxicological risks (Puttalingamma and Begum 2012; Wilson and Wisniewski, 1989; Zong et al., 2010). Additionally, the growing public concern about the use of antibiotics and fungicides, the development of antibiotic resistance in several pathogens, the high cost associated with these antibiotics, and the impact of fungicides on human health and the environment has prompted the search for alternative approaches to post-harvest disease control (Puttalingamma and Begum 2012; Wilson and Wisniewski, 1989; Zong et al., 2010). The ideal alternative approach should be safe for the produce, consumer and the environment (Zong et al., 2010).

Biocontrol is the use of nature or controlled microflora and (or) their antimicrobial products to enhance the safety while extending the shelf-life of the food (Lima et al., 2013; Puttalingamma and Begum 2012; Wilson and Wisniewski, 1989; Zong et al., 2010). These include natural plant and animal derived compounds (Chanchaichaovivat et al., 2007), as well as antagonistic microorganisms (Mamphogoro et al., 2021). Fungi, mainly yeasts and yeast-like organisms with antagonistic properties, are used as biological control agents (BCAs) and can be isolated from a variety of sources including, the surface of leaves, fruits and vegetables (Janisiewicz and Korsten 2002; Wilson and Wisniewski, 1989; Wisniewski et al., 1991). Among fungi that live on plants, the fungal endophytes have been attracting a growing amount of attention recently. This is due to the various benefits they offer directly to the plant host through the intimate interactions they establish while colonizing the plant tissues (Schulz and Boyle 2005; Bamisile et al., 2018; McKinnon et al., 2018; Ownley et al., 2010; Saikkonen et al., 2010; Young et al., 2013). Endophytic fungi containing biocontrol properties have the ability to grow inside the plants without causing any symptoms (Stone et al., 2000; Schulz and Boyle 2005). They do this by establishing a plant–fungi association inside the living plant tissue, which can take place within the plant's roots, fruit, stems, and/or leaves (Schulz and Boyle 2005). They do not emerge from the plant tissue until the time of sporulation or upon the senescence of the host (Carroll 1988; Sherwood and Carroll 1974; Stone et al., 2004). The majority of endophytic fungi possessing biocontrol properties, are known to secrete a large number of bioactive secondary metabolites. These metabolites are primarily responsible for the observed beneficial effects, as they can stimulate the plant's defense responses and growth, in addition to exerting a direct antimicrobial or insecticidal effect (Schulz and Boyle 2005; Gouda et al., 2016; Segaran and Sathiavelu, 2019). To this day, the artificial introduction of endophytic fungi has been restricted to a small group of beneficial microbes in tomato as well as in many other horticultural crops. These microbes include species belonging to the genera *Beauveria, Metarhizium, Fusarium, Serendipita* (formerly *Piriformospora*), *Pochonia,* and *Trichoderma* (Akello and Sikora 2012; Akutse et al., 2013; Orole and Adejumoo, 2009). Endophytic fungi have previously been shown to combat the infection caused by fungal pathogens such as *Fusarium oxysporum* f. sp. *lycopersici* (McKinnon et al., 2017), *R. solani* (Azadi et al., 2016), and *Botrytis cinerea* (Tucci et al., 2011; Sare et al., 2021), *Penicillium expansum* (Almadidi et al., 2023; Guerrero et al., 2014; Sare et al., 2021), *Penicillium digitatum*, *Aspergillus tubigensis*, and *Geotrichum citri-aurantii* (Sare et al., 2021). Benefits of endophytic colonization on pathogen control includes disease-suppressing effect, as well as a reduction in the severity of disease symptoms (Sinno et al., 2020).

Fungal BCAs are used to ensure that extended shelf-life and availability of quality fresh produce is achieved (Jiang et al., 2009). Therefore, biocontrol through the use of antagonistic fungi is the method of choice, since no chemical applications are involved. Furthermore, these antagonists are virtually nontoxic to non-target organisms and are largely accepted by 'eco-friendly' consumers (Zong et al., 2010). Despite these desirable attributes of antagonistic fungi, their potential for use in the management of post-harvest losses of tomato, one of the most important horticultural fruit plants, is still sketchy, yet this information is important to sustainably and safely safeguard food and nutritional security on a global scale. Therefore, the objectives of this study were to isolate endophytic fungi from tomato fruits and to screen them for antifungal activity against the common fungal pathogens that cause post-harvest decay of tomatoes. We hypothesize that some endophytic isolates from tomato fruits can significantly inhibit growth of the most common post-harvest pathogens of tomatoes, and that their performance can match that of the already established microbial biocontrol agents.

2. Materials and methods

2.1. Trial establishment and crop management

A tomato variety, *Solanum lycopersicum* L. cv. Jasmine, sourced from Agricultural Research Council-Vegetable, Industrial and Medicinal Plants (ARC-VIMP) was planted under two management regimes, i.e., two growing habitats (hydroponic system and open soil conditions) and two pest control treatments (treated and untreated). Twenty tomato plants were planted under each management regime, 20 replicates of tomato plants were planted at the ARC-VIMP, in Roodeplaat, Pretoria, South Africa (Latitude = 1200, Longitude = 25°590 S; 28°350 E) (www.arc.agric.za/arc-vop). The mean temperature under hydroponic growing conditions was 33 °C day/15 °C night. In the open field, average temperatures of 34.5 °C Day/15 °C night were recorded. Once, at mature green and red stage, plants in the pest control group were treated with the insecticides: Biomectin (60 mL/100 L), Suntap (300 g/100 L) and Closer (25 mL/100 L) for control of leaf miner, thrips, red spider mites and whiteflies, respectively. On the other hand, for control of the fungal pathogens (i.e., powdery mildew, early and late blight in tomato), the fungicides Benomyl (50 g/100 L), Sporekill (100 mL/100 L), CoppercountN (500 mL/100 L), were used.

2.2. Sample collection and processing

Sampling was done across the two management regimes (i.e., two growing habitants and two pest control treatments), but also at two maturity levels (i.e., green and red fruits). Using this strategy, a total of 80 tomato fruits (i.e., 10 hydroponic treated green (HTG), 10

hydroponic treated red (HTR), 10 hydroponic untreated green (HUG), 10 hydroponic untreated red (HUR), 10 soil treated green (STG), 10 soil treated red (STR), 10 soil untreated green (SUG) and 10 soil untreated red (SUR)) were harvested. Only fresh, intact, and healthy fruits were aseptically collected in sterile Ziploc bags, kept at 4 °C in the laboratory (ARC-Animal Production, Pretoria) and were processed within 24 h. Tomatoes were washed in sterilized PBST buffer and then disinfected by placing them in 80 % ethanol for 2 min, followed by 5 % sodium hypochlorite (NaClO, containing 0.01 % Triton X-100) for 10 min, and then rinsed three times with sterilized distilled water. To confirm our disinfection process, 100 μL of the third rinse was inoculated onto potato dextrose agar (PDA) plates, and no microbial growth was observed after several days of incubation at 25 °C.

2.3. Macroscopic and microscopic characterization of endophytic fungi from tomatoes

The 80 tomato samples were separately homogenized using pestle and mortar. Then 100 μL of homogenized tomato fruit was aseptically spread plated onto Potato Dextrose Agar (PDA) plates. The plates were then sealed with parafilm and incubated at ambient temperature (±25 °C) for 7 days. Colonies that grew on PDA plates were sub-cultured promptly onto PDA plates to attain single cultures and incubated at ambient temperature (±25 °C) for 7 days. Macroscopic identification of the colonies growing on PDA plates was conducted by physical detection of colour pigmentation, appearance (as yeast or mould) and the colony form (arrangement of the fungal structures) according to methods of (Ogbonna et al., 2014; Salvamani and Nawawi 2014).

Microscopic examination of fungal structures was also conducted. Briefly, fungi were cultured onto PDA plates and then transferred to a clean glass slide. Fungal characteristic structures were observed by agar block examination under a light microscope (× 40 and × 100 magnification). A drop of lactophenol blue was used to stain and improve the visibility of the cell or specific cellular components under the microscope (Salvamani and Nawawi 2014). Ten colonies with unique morphologies (based on differences in colour, shape, and texture) per plate were selected for further purification (i.e., n = 800, 80 swabs × 10 colonies; Table S1).

2.4. Fungal pathogens of tomato fruits

A total of 8 pathogenic strains (4 pathogenic fungal strains from South Africa and 4 American type culture collection (ATCC) strains) were used in the study. The South African plant pathogenic fungi isolated from tomato plants were acquired from the culture bank of the ARC's Plant Protection Biosystems Laboratories, in Pretoria, South Africa (www.arc.agric.za/arc-ppr). The South African pathogenic strains include: *Fusarium oxysporum* (North West, Brits), *R. solani* (Gauteng, Pretoria), *G. candidum* (Gauteng, Pretoria) and *Alternaria alternata* (Limpopo, Sakata Vegenetics). All the ATCC strains (*Rhizopus stolonifer* (−) ATCC 6227a, *Rhizopus stolonifer* (+) ATCC 6227b, *G. candidum* ATCC 3461 and *Fusarium solani* ATCC 36031) were purchased from Quantum Biotechnology (https://www.quantumbiotech.co.in/). Lyophilized fungal cultures were reconstituted in sterile distilled water and recovered on PDA slants after 5–7 days of incubation at 25 °C. These strains were stored at −20 °C at the Microbiology unit of ARC-AP (www.arc.agric.za/arc-ap) until they were reactivated on PDA agar plates before their use in experiments.

2.5. In vitro antagonistic assay

The antifungal activity of the 800 endophytic fungal strains against known fungal pathogens of tomato fruits was screened *in vitro* using the modified dual-culture plate antagonism described previously (Zhao et al., 2022). Briefly, fungi were cultured on PDA plates at 25 °C for 7 days. Then, 5 mm plugs of each endophyte and fungal pathogens were co-cultured in 90 mm sterile Petri dishes, with approximately 28 mL of PDA yielding a final depth of 5 mm, and incubated at 25 °C for 7 days. The plugs were placed symmetrically (3 cm away from each other) on each side of the Petri dishes. Fungal pathogens alone were inoculated as the control for growth on media. All the plates were incubated at 25 °C for 7 days. The growth radii of pathogens as well as the production of an inhibition zone were recorded at day 7. The barrier (inhibition zone) between the test fungal isolate and the pathogenic fungi indicated antagonistic interactions between them. The experiment was repeated in three independent trials. The endophytes which produced the inhibition zone with widths >2 mm between them and the pathogen were selected for further analysis (Yu et al., 2018). The endophytic activity of the selected isolates from the screening (5/800) was assayed by placing the 5 mm plug of each endophyte was placed in the middle of the PDA plate onto which 0.1 mL (10^6 spores mL^{-1}) of each fungal pathogen was spread plated. The plates were incubated at 25 °C for 7 days. The inhibition zone formed around the endophyte was recorded at day 7.

In the study, *Lactiplantibacillus plantarum*, formerly known as *Lactobacillus plantarum* (Zheng et al., 2020), was employed as a positive control strain as it was previously shown to possess antifungal activity (Li et al., 2020). For antifungal activity assays of *L. plantarum* isolates, 5 μL of *L. plantarum* culture was spotted onto MRS agar plate. After 24 h of incubation at 37 °C, the plates were covered with 10 mL of PDA containing 0.1 mL (10^6 spores mL^{-1}) of each fungus and cultured aerobically at 25 °C for 5 days. The diameter (in mm) of the inhibition zone surrounding the *L. plantarum* spot was recorded as its antifungal activity.

2.6. Screening endophytic fungal isolates with antifungal activities for carbohydrate utilization capabilities

The utilisation of carbohydrates (i.e., fructose, glucose, lactose, maltose, starch, sucrose, and mannitol) by the endophytic fungal antagonists was determined by assessing the antagonist's ability to degrade and utilise sugars following the method described by Kali et al. [2015], with minor modifications. Fungal inocula (5 mm) of the endophytic fungal isolates were grown on PDA for 7 days. Subsequently, an inoculating needle was used to stab inoculate each fungal isolate onto the centre of phenol red agar plates containing each carbohydrate as a substrate. The basal phenol red agar was prepared as follows: peptone 10 g L^{-1}, sodium chloride 5 g L^{-1}, dipotassium hydrogen phosphate 3 g L^{-1}, potassium dihydrogen phosphate 1.5 g L^{-1}, beef extract 1 g L^{-1}, phenol red 0.025 g L^{-1} and agar 15 g L^{-1}. Then to determine utilization of each carbohydrate by the fungal isolate, the peptone was replaced with proteose peptone, and then the medium was supplemented with 10 g L^{-1} of the respective carbohydrate, i.e., fructose, glucose, lactose, maltose, starch, sucrose and mannitol. The plates were the incubated at ambient temperature for 7 days. A change in the colour of the agar from red-orange to yellow indicated carbohydrate utilization. The results were captured as "+" for carbohydrate utilization and "–" for lack of carbohydrate utilization.

2.7. DNA extraction, PCR amplification and ITS sequencing

The DNA was extracted from pure cultures of the fungal strains which exhibited antagonistic activity in the *in vitro* assay. The cultures were grown on half strength PDA for 7 days. Extraction of DNA was performed according to the ZR Fungal/Bacterial DNA MiniPrepTM (Zymoresearch, USA) protocol.

Polymerase chain reaction (PCR) was conducted according to the protocol by (Zhao et al., 2022) using synthesized oligonucleotide primers (Inqaba biotech, SA) for ITS analysis. The PCR reaction was performed in a total volume of 50 μL of PCR mixture containing 0.5 μM primer ITS1 (5′-TC CGTAGGTGAACCTGCGG-3′), 0.5 μM primer ITS4 (5′-TCCTCCGCTTAT TGATATGC-3′), MyTaq DNA polymerase, MyTaq buffer, and nuclease free water (Eppendorf, Hamburg, Germany). The PCR cycle conditions were as follows: initial denaturing at 94 °C for 5

min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min; and a final extension step of 10 min at 72 °C. As a negative control, the template DNA was replaced by sterile double-distilled water. Amplified products and their restriction fragments were electrophoresed on 1.5 % agarose gels (Lonza, Rockland, USA), respectively, in 1 x TAE (Tris-acetic acid-EDTA) buffer. Gels were visualized using GelRed (Anatech, SA), and photographed under UV light. Fragment sizes were estimated by comparison against a 100-bp ladder (Lonza, Rockland, USA). The product was cleaned with the Wizard® SV Gel and PCR system (Promega, Madison, Wisconsin) according to the manufacturer's protocol.

Amplicons were then purified with the Zymo Research, ZR-96 DNA Sequencing Clean-up Kit D4053, Irvine, CA, USA. Purified fragments were analysed on the ABI 3500XL Genetic Analyzer with a 50-cm array, using POP7 (Applied Biosystems, ThermoFisher Scientific., Foster City, CA, USA) for each reaction for every sample. The amplicons were sequenced at Inqaba Biotechnical Industries (Pty) Limited (www.inqababiotec.co.za) in the forward and reverse direction, using the Nimagen, BrilliantDyeTM Terminator Cycle Sequencing Kit V3.1, BRD3 100/1000, Nijmegen, Netherlands, following the manufacturer's instructions. The sequence chromatogram generated by the ABI 3500XL Genetic Analyzer were analysed using the FinchT v1.4 software (Applied Biosystem, Foster city, CA, USA) (https://www.softpedia.com/get/Science-CAD/FinchTV.shtml), and the obtained results were compared with the related ITS sequences identified by the Basic Local Alignment Search (BLAST) search program on the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), as well as the National Library of Medicine, USA (Altschul et al., 1997). Sequence alignments were performed using the CLUSTLW algorithm (http://www.ebi.ac.uk/Tools/msa/clustalw2/) in MEGA v6.06 (Tamura et al., 2013), with default settings, and phylogenetic trees were constructed using the Neighbour joining method (Saitou and Nei 1987). Reliability of the phylogenetic tree was evaluated through bootstrap analysis with 1000 re-samplings using a p-distance model, with the numbers on branches indicating percentage level of bootstrap support as described by (Saitou and Nei 1987). The nucleotide sequence accession numbers were deposited in the GenBank database (Table 2).

2.8. Screening endophytic fungal isolates with antifungal activities for production of extracellular enzymes

The potential antifungal mechanism of action of the endophytic fungi was determined by analysing their production of extracellular enzymes: amylase, lipase, urease, gelatinase, cellulase, protease, caseinase and catalase. Fungal fragments (5 mm) of the endophytic fungal cultures were grown in PDA plates for 7 days. They were then transferred to the centre of the Petri dishes containing the solid medium supplemented with the specific substrate for each enzyme.

Determination of amylase production was conducted using a method described by Sunitha et al., (2013). Briefly, each potential fungal antagonist was inoculated onto PDA agar plates supplemented with 0.2 % starch. The plates were incubated at 25 °C for 7 days, then flooded with 1 % iodine solution in 2 % potassium iodide. Clear zones in the agar around the cultures indicated amylase production (recorded as +) and absence of clear zones indicated lack of amylase production (recorded as -).

Lipase production was determined by inoculating the fungal isolate onto PDA agar plates containing 0.01 % $CaCl_2$ and 1 % Tween 80 (w/v). The plates were incubated 25 °C for 7 days. A clear zone around the growth indicated lipase production (Gopinath et al., 2013).

Cellulase production was conducted using PDA agar plates containing 1 % carboxymethylcellulose. Clear zones in the agar around the cultures indicated cellulase production (recorded as +) and absence of clear zones indicated lack of cellulase production (recorded as -) (Bezerra et al., 2012; Loperena et al., 2012).

For production of proteases, the fungal isolates were assayed for production of caseinase and gelatinase. Production of caseinase was conducted by inoculating the isolate onto PDA containing 1 % milk casein. The plates were incubated at 25 °C for 7 days. Clear zones in the agar around the cultures indicated positive caseinase production (recorded as +) and absence of clear zones indicated lack of caseinase (recorded as -). For gelatinase production, the fungal isolates were inoculated onto PDA agar plates supplemented with 3 % gelatin. The plates were incubated as was done previously. The clear zones in the agar around the cultures indicated gelatinase production (recorded as +) and absence of clear zones indicated lack of gelatinase production (recorded as -) (Bezerra et al., 2012; Loperena et al., 2012).

Catalase production was determined using the method described by Somashekar et al., (1999) with modifications. The fungal isolate was cultured onto PDA agar plates for 7 days and then the plate was flooded with 1 % hydrogen peroxide. The presence of effervescence indicated catalase production.

For determining urease production, the fungal isolate was inoculated onto Christensen's urea agar using the method described by Lerm et al., (2017), with minor modifications. The plates were incubated at 25 °C for 7 days. Urea hydrolysis was indicated by the appearance of a deep pink (purple) colour.

2.9. Statistical analysis

Statistical differences between the isolates and the positive control in suppressing the fungal pathogens were detected using the Tukey's HSD test, using the '*TukeyHSD*' function in the agricolae R package (Mendiburu and Simon, 2015). In order to visualize how the isolates differ in performance (i.e., pathogen suppression) in comparison with the control isolate, a scatter plot was used. Scatter plots were graphed using the 'ggplot' function in the ggplot2 v3.0.0 R package (Wickham et al., 2015).

3. Results

3.1. Macroscopic and microscopic characteristics of fungal endophytes from tomatoes

A total of 800 endophytic fungal strains based on their unique morphologies in terms of colour, shape, and texture, were obtained from the 80 tomato fruit samples (i.e., 10 HTG + 10 HTR + 10 HUG + 10 HUR + 10 STG + 10 STR + 10 SUG + 10 SUR) (Table S1). The macroscopic and microscopic properties of the five endophytic fungal strains which were subsequently used in further experiments are shown in Table 1. These strains were mostly opaque and were brightly coloured. The diversity of features displayed by all the endophytic strains can be observed in Table S1.

3.2. Antagonistic activity

Among the 800 isolated strains, only five exhibited inhibitory effects against the selected fungal pathogens. Of these, two strains, SUG3.1 and SUG4.3 exhibited inhibitory effects against all 8 pathogens (*F. oxysporum*, *R. solani*, *A. alternata*, *G. candidum*, *G. candidum* ATCC 34614, *F. solani* ATCC 36031, *R. stolonifera* (−) ATCC 6227a, and *R. stolonifera* (+) ATCC 6227b). The diameters of the inhibition zones formed by these two strains were generally smaller than those by *L. plantarum* (Table S1 and Fig. 1). However, the sizes of inhibition zones formed by SUG4.3 were much closer to those of *L. plantarum* for *A. alternata*, *G. candidum* and *R. stolonifera* (−) ATCC 6227a. All the test pathogens except *F. solani* ATCC 36031, grew evenly over the surfaces of the PDA plates inoculated with isolates SUG4.1, HTR8.4 and STG8.6, with no discernible inhibition zones (Table S1 and Fig. 1).

Further evaluation of the antagonistic ability of the endophytic fungal isolates revealed some interesting trends. Fig. 2 shows growth inhibition of the test pathogen by the fungal endophytes relative to the

Table 1
Macroscopic and microscopic features of the potential endophytic fungal antagonists, as well as their carbohydrate utilisation capabilities.

Isolate	Micro and macroscopic identification						Carbohydrate utilization						
	Shape	Margin	Elevation	Opacity	Surface	Pigmentation	Fructose	Glucose	Lactose	Maltose	Mannitol	Starch	Sucrose
SUG3.1	Rhizoid	Filiform	Convex	Opaque	Rough	Yellow	+	+	+	+	+	–	+
SUG4.1	Circular	Undulate	Flat	Opaque	Smooth	Peach	+	+	+	+	+	+	+
SUG4.3	Irregular	Undulate	Crateriform	Opaque	Rough	White	+	+	+	+	+	–	+
HTR8.4	Filamentous	Filiform	Umbonate	Opaque	Rough	Purple	+	+	+	+	+	+	+
STG8.6	Filamentous	Undulate	Umbonate	Opaque	Rough	Orange	–	+	+	+	+	+	+

- = clear zone absent; no utilization of the carbohydrate; + = clear zone present; utilization of the carbohydrate.

Table 2
Molecular identification of ITS gene of endophytic fungal strains with *in vitro* antagonistic traits.

Sample number	Strain code[a]	Size (Base pair)[b]	Fungal isolates	GeneBank Accession number[c]	Identity (%)
1	SUG4.1	568 bp	*Aureobasidium pullulans* SUG4.1	OP967911	100
2	SUG4.3	679 bp	*Coprinellus micaceus* SUG4.3	OR835561	100
3	STG8.6	544 bp	*Epicoccum nigrum* STG8.6	OR866191	100
4	HTR8.4	551 bp	*Fusarium oxysporum* HTR8.4	OR866192	100
5	SUG3.1	542 bp	*Preussia africana* SUG3.1	OP967908	100

[a] Code for the selected strains with antagonistic traits.
[b] Fragment length of selected strain.
[c] GeneBank sequence accession numbers of selected isolates.

positive control. There was no significant difference ($p > 0.05$) in the inhibition of *F. oxysporum*, *G. candidum*, and both *R. stolonifera* strains by *L. plantarum* and *Coprinellus micaceus* SUG4.3 (Fig. 2). Similarly, no significant differences were observed in the inhibition of *G. candidum* ATCC 34614, *F. solani* ATCC 36031 and the two *R. stolonifera* strains by *L. plantarum* and *Preussia africana* SUG3.1. However, inhibition of *F. oxysporum*, *R. solani*, *G. candidum* and *A. alternata* by SUG3.1 was significantly lower ($p < 0.05$) than that by *L. plantarum*. This trend was also observed when comparing the inhibition of *R. solani* and *F. solani* ATCC 834614 by *C. micaceus* SUG4.3 with that caused by *L. plantarum*. All endophytes inhibited growth of *F. solani* ATCC 36031. *Aureobasidium pullulans* SUG 4.1, *F. oxysporum* HTR8.4 and STG8.6 inhibited growth of only one of the tested fungal pathogens, *F. solani* ATCC 36031.

3.3. Carbohydrate utilization by endophytes

All the five strains indicated the ability to utilise five of the carbohydrates (glucose, lactose, maltose, mannitol and sucrose) tested in the study. Strain STG8.6 showed inability to utilize fructose, while strains SUG3.1 and SUG4.1 were both unable to utilize starch (Table 1).

3.4. Molecular identification of endophytes with antifungal activity

Sequences of the rRNA genes of the potential fungal antagonists exhibited 100 % similarities to their corresponding reference sequences in the GenBank database at NCBI (Table 2). Briefly, strain SUG3.1 showed highest sequence similarities with *P. africana* MN341248 (100 %), SUG4.1 shared the highest sequence similarity with *Aureobasidium pullulans* MH329613 (100 %), SUG4.3 unveiled closest similarities to *C. micaceus* OM574635, whereas SGT8.6 showed highest identity to *Epicoccum nigrum* OL362040 (100 %), and HTR8.4 displayed highest sequence identity to *Fusarium oxysporum* KT803066. Based on these similarities, the isolates have been coded as follows: SG3.1 as *P. africana* SUG3.1; SUG4.1 as *Aureobasidium pullulans* SUG4.1; SUG4.3 as *C. micaceus* SUG4.3; SGT8.6 as *E. nigrum* STG8.6; and lastly HTR8.4 as *Fusarium oxysporum* HTR8.4 (Table 2). The phylogenetic analysis of the rRNA gene sequences showed that the isolated endophytic fungal antagonistic strains clustered with other species of *P. africana*, *Aureobasidium pullulans*, *C. micaceus*, *E. nigrum*, and *Fusarium oxysporum* (Fig. 3).

3.5. Extracellular enzyme production

Table 3 shows the different extracellular enzymes produced by the endophytic isolates with antifungal activities. All isolates produced catalase and cellulase, with these being the only enzymes produced by *A. pullulans* SUG4.1 and *E. nigrum* SGT8.6. Compared to the other isolates, *C. micaceous* SUG4.3 and *A. pullulans* SUG4.1 produced catalase in abundance. *P. africana* SUG3.1 is the only isolate which produced caseinase and urease. Moreover, amylase was only produced by *P. africana* SUG3.1 and *C. micaceous* SUG4.3 while lipase was produced by *P. africana* SUG3.1 and *F. oxysporum* HTR8.4. Gelatinase was produced solely by *C. micaceous* SUG4.3 (Table 3).

In summary, none of the isolates produced all the test enzymes while *P. Africana* SUG3.1 was the isolate that produced most of the enzymes.

4. Discussion

Endophytic fungi occur ubiquitously in plants, and their capacity to defend the host from pathogens has for several years being investigated (El-Katatny and Emam 2012 and Grebka et al., 2022). The secondary metabolites produced by these fungi have the potential for bioprospecting applications in the agriculture industry. These metabolites exhibit antagonistic activities against a variety of plant pathogens, positioning them as possible alternative biocontrol agents for control of such pathogens. In comparison to other biocontrol agents, the use of endophytic fungi for control of plant disease provides potential advantages since these fungi colonize the plant from the inside, keeping the plant safe from environmental pressures and changes (Grabka et al., 2022).

The present study depicted that strains *P. africana* SUG3.1, *A. pullulans* SUG4.1 and *C. micaceus* SUG4.3 have antagonistic activities against *A. alternata*, *F. solani* ATCC 36031, *G. candidum* and *G. candidum* ATCC 34614 comparable to those of *L. plantarum*. This study has, for the first time, demonstrated the potential of *P. africana* SUG3.1, *C. micaceus* SUG4.3 and *E. nigrum* SGT8.6 as biological control agents against tomato post-harvest fungal pathogens *in vitro*.

P. africana SUG3.1 which was isolated from untreated, mature green tomatoes of *S. lycopersicum* L. cv. Jasmine plants grown on open soil, had cultural and morphological characteristics resembling those of *Preussia* species as previously described by Arenal et al. (2004, 2007). Interestingly, this strain displayed antagonistic effects against *F. oxysporum, R. solani*, *A. alternata*, *G. candidum*, *G. candidum* ATCC 34614, *F. solani* ATCC 36031, *R. stolonifera* (−) ATCC 6227a and *R. stolonifera* (+) ATCC 6227b (Figs. 1 and 2). There is a dearth of data on *Preussia* species as biocontrol agents, considering that even a few studies reporting the antagonistic activity of genus *Preussia* do not specify the actual species names. Tane et al. [2019] reported that *Preussia* species isolated from

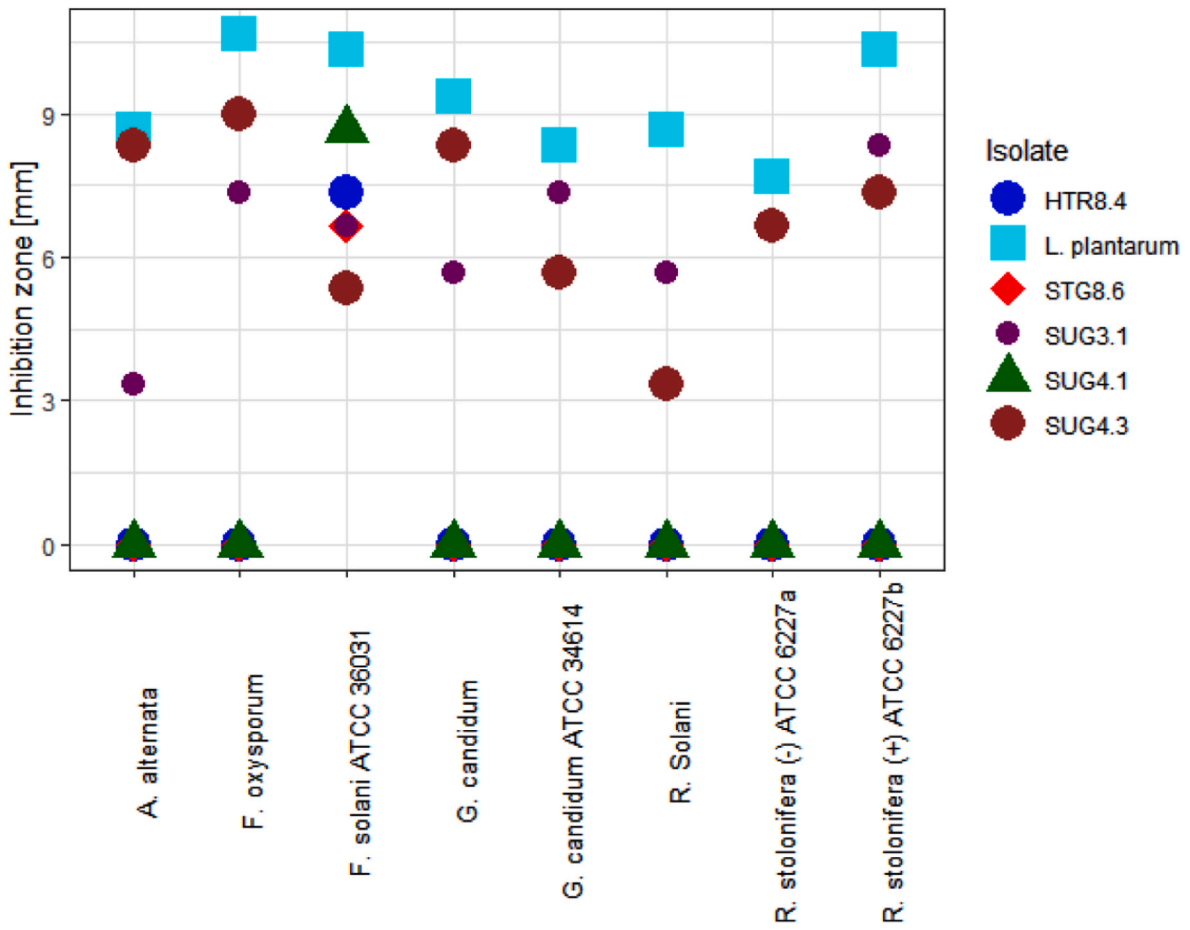

Fig. 1. A scatter plot showing mean inhibition zones from of the three replicates for antagonistic isolates (SUG3.1, SUG4.1, SUG4.3, HTR8.4 and STG 8.6) against *F. oxysporum*, *R. solani*, *G. candidum*, *A. alternata*, *G. candidum* ATCC 34614, *F. solani* ATCC 36031, *R. stolonifera* (−) ATCC 6227a, *R. stolonifera* (+) ATCC 6227b, with *L. plantarum* as a positive control.

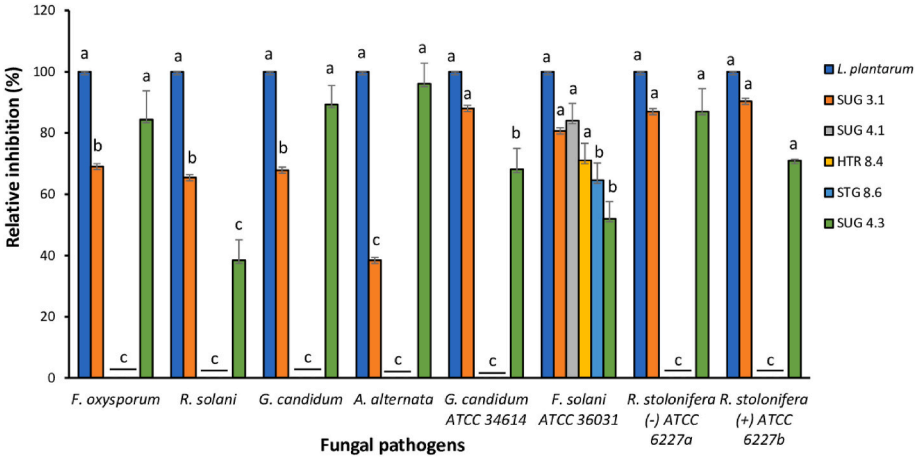

Fig. 2. Growth inhibition of the fungal pathogens by fungal endophytes. Inhibition of the pathogens by the positive control (*L. plantarum*) was presented as 100 % and then percentage inhibition by the test pathogens was calculated relative to that. Data are averages of the three independent experiment. Error bars are standard deviations of the averages of the three independent experiments. Bars marked with different letter indicate significant difference at $p < 0.05$.

Artemisia thuscula was an effective biocontrol agent against *Alternaria* species of tomato seedlings. In another study, Gherbawy and Elhariry (2016) reported that *Preussia* species isolated from twigs of *Juniperus procera* antagonised the plant pathogens, *F. solani* and *Candida albicans*. Likewise, Matlagh and Usefipoor (2016) reported on *Preussia* species from rice plants with antagonistic activity against *Pyricularia grisea*, the causal agent of rice blast disease. Therefore, possession of the antagonistic activity by *P. africana* SUG3.1 against pathogens causing post-harvest losses of tomatoes does not come as a complete surprise. However, unlike most of the previous studies, our current study reports specifically on a given species of *Preussia*.

The cultural and morphological characteristics of *E. nigrum* SGT8.6

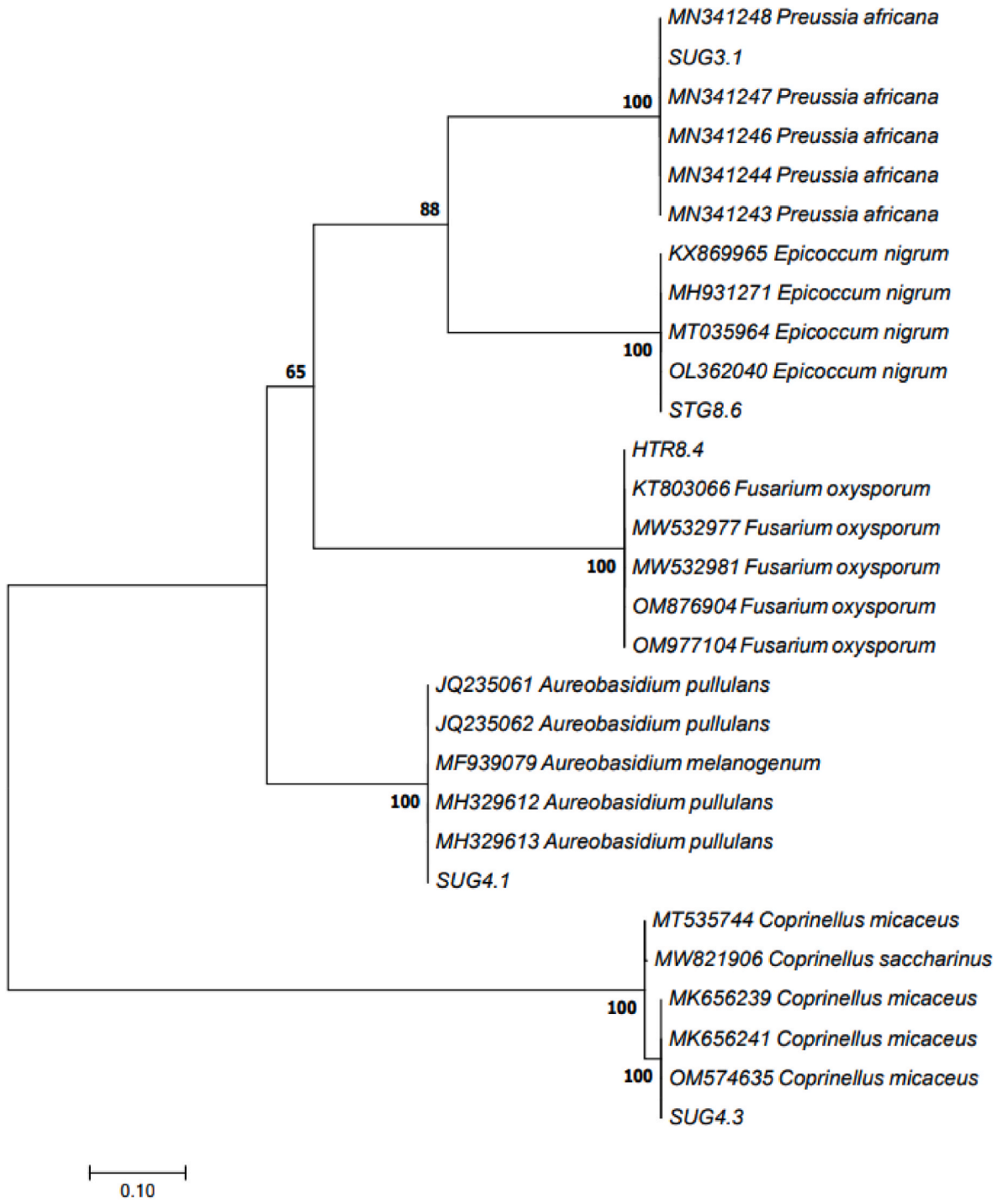

Fig. 3. Neighbour-joining phylogenetic tree based on rRNA gene sequences of potential fungal antagonistic strains showing the relationship to the closest type strain sequences. The phylogenetic tree was constructed using the Neighbour-joining algorithm method (1000 bootstrap replications). Bootstrap values (65–100 %) are indicated at the relevant nodes.

as described in this study, resemble those of other *Epicoccum* species (Taguiam et al., 2020). Our study demonstrated for the first time that *E. nigrum* SGT8.6 isolated from tomato samples collected from hydroponic, treated, mature red tomatoes have antagonistic effects against *F. solani* ATCC 36031 (Figs. 1 and 2) and thus is a potential biocontrol agent for this pathogen on tomatoes. *E. nigrum* has been previously reported as an effective inhibitor against a wide range of *Fusarium* species, including *Fusarium avenaceum*, *Fusarium graminearum* and *F. oxysporum* (Jensen et al., 2016; Ogorek and Plaskowska, 2011). Also, *E. nigrum* P16 isolated from sugarcane produced compounds that inhibit the growth of sugarcane pathogens, *Fusarium verticillioides*, *Colletotrichum falcatum*, *Ceratocystis paradoxa*, and *Xanthomomas albilineans* (de Fávaro et al., 2012). Koutb and Ali [2010] reported that *E. nigrum* 5615AUMC reduced root discoloration of black-eyed peas (*Vigna unguiculata)* caused by *Pythium irregulare*. *E. nigrum* XF1 has been demonstrated to inhibit the leaf blight and late blight caused by *Phytophthora infestans* (Li et al., 2013). Furthermore, *Epicoccum layuense* E24 and *Epicoccum mezzettii*, demonstrated antifungal activity against *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum,* the causal agents of esca disease complex on grapes (Del Frari et al., 2019).

Table 3
Extracellular enzyme produced by endophytic fungal strains with antifungal capabilities.

Isolates	Amylase	Catalase	Caseinase	Cellulase	Gelatinase	Lipase	Urease
Preussia africana SUG3.1	+	+	+	+	–	+	+
Aureobasidium pullulans SUG4.1	–	+++	–	+	–	–	–
Coprinellus micaceus SUG4.3	+	+++	–	+	+	–	–
Epicoccum nigrum SGT8.6	–	+	–	+	–	–	–
Fusarium oxysporum HTR8.4	–	+	–	+	–	+	–

- = enzyme not produced; + = diameter of yellow zone due to the production of extracellular enzyme ≤0.5 cm; +++ = diameter of yellow zone due to the production of extracellular enzyme ≥1.5 cm.

Fusarium oxysporum HTR8.4 isolated from mature green tomatoes cultivated on treated soil showed inhibitory activity against *F. solani* ATCC 36031 (Figs. 1 and 2). This finding correlates with reports from other studies where non-pathogenic *Fusarium* strains were reported to antagonise pathogenic *Fusarium* strains of plants and fresh produce (Fravel et al., 2003; Srinivas et al., 2019; Kaur et al., 2010; Sajeena et al., 2020). Non-pathogenic *F. oxysporum* F. o. T5 suppressed the pathogenic strain *F. oxysporum* F. o. T2, the causal agent of tomato vascular wilt. Other studies highlighted the potential of the non-pathogenic *Fusarium* isolate Fo47 isolated from fusarium wilt suppressive soils in regulating the vascular wilt disease of vegetable and floral crops caused by *F. oxysporum* (Alabouvette and Couteaudier 1992; Alabouvette et al., 1993). Forsyth et al. [2006] reported the discovery of an endophytic, non-pathogenic *F. oxysporum* (BRIP 29089) strain isolated from banana roots, which reduced the severity of the wilt disease in bananas. Two non-pathogenic *F. oxysporum* strains A37 and A 38, reportedly reduced root rot and petiole blight of strawberry (Bicevska et al., 2014). Moreover, *F. oxysporum* strains inhibit *Pythium ultimatum* (Park 1963); *Phytophthora erythroseptica* (Benhamou et al., 2002) and *Sclerotinia sclerotiorum* (Zazzerini 1985). Cultural and morphological characteristics of *F. oxysporum* STG8.6 as described in this study, resemble those of *Fusarium* species (Hafizi et al., 2013).

Aureobasidium pullulans SUG4.1 isolated from mature green tomatoes grown on untreated open soil exhibited inhibitory abilities against *F. solani* ATCC 36031 (Figs. 1 and 2). Due to the high efficacy of *A. pullulans* against several postharvest pathogens, numerous studies have reported *A. pullulans* as a possible active biocontrol agent of various diseases infecting other fresh produce. In a study conducted by di Francesco et al. [2017], two strains, *A. pullulans* L1 and L8, isolated from surfaces of peach fruits, inhibited *P. infestans*, the casual-agent of tomato late blight. *Aureobacidium pullulans* strain Ach1-1 from apple fruits, was reported as an effective inhibitor of *Penicillium expansum,* the causal agent of blue mould of apples (Bencheqroun et al., 2007). Additionally, *A. pullulans* obtained from the flesh of fresh cherries was effective against botrytis rot and monilinia rot of grapes and cherries, caused by *Botrytis cinerea* and *Monilinia laxa,* respectively (Schena et al. 1999, 2003). Moreover, *A. pullulans* has been also been reported to effectively control *Monilinia laxa* of banana (Wittig et al., 1997), penicillium rots (*Penicillium* spp.) of citrus (Wilson and Wisniewski, 1989), soft rot (*Monilinia laxa*) of grapes (Barkai-Golan 2001), gray and blue moulds of apples and grapes (Barkai-Golan 2001; Ippolito et al., 2000), and rhizopus rot of grapes (Castoria et al., 2001). Hence, the antifungal activity of *A. pullulans* SUG4.1 against *F. solani* observed in this study correlates with these previous reports. The cultural and morphological characteristics of *A. pullulans* SUG4.1 as described in this study, resemble those of *Aureobacidium* species (Prasongsuk et al., 2018).

C. micaceus SUG4.3 which was isolated from tomato samples collected from soil, untreated, mature green tomatoes demonstrated antagonistic effects as biocontrol agent against *F. oxysporum, R. solani*, *G. candidum*, *G. candidum* ATCC 34614, *F. solani* ATCC 36031, *R. stolonifera* (−) ATCC 6227a and *R. stolonifera* (+) ATCC 6227b (Figs. 1 and 2). Even though there are no comparable studies available in the literature reporting on inhibitory effect of *C. micaceus* against fungal pathogens, a study by Zahid et al. (2006), however, has reported on natural bioactive compounds extracted from *C. micaceus* isolated from marine organisms. Their study showed that methanolic extracts of *C. micaceus* exhibited antibacterial activity against *Coryanebacterium xeroides*. The cultural and morphological characteristics of *C. micaceus* SUG4.3 as described in this study resemble those of *Coprinellus* species (Badalyan 2018).

Endophytic fungi utilise carbohydrates as nutritious substances for growth (Zhao et al., 2016). In this study, all the endophytic fungal isolates with antifungal activities utilized glucose, lactose, maltose, mannitol and sucrose (Table 1). The ability of *P. africana* SUG3.1 and *C. micaceus* SUG4.3 to utilize most of the carbohydrates tested coincided with their somewhat broad inhibitory capabilities against the tested fungal pathogens of tomatoes. The ability of these two strains to utilise the carbohydrates would contribute to their effectiveness as biocontrol agents for fungal pathogens of tomatoes. Macıas-Rodrıguez et al. [2018] conducted a similar study and reported that the inhibitory effect of *Trichoderma atroviride* against *Phytophthora cinnamomi,* the causative agent of tomato root and crown rot, stem canker, leaf blight and fruit rot (Engelbrecht et al., 2017; Ioannou and Grogan 1984) was associated with its carbohydrate utilization, and, that these carbohydrates provided a nutritional source for *T. atroviride*. Even though the carbohydrate utilization has been associated with the inhibitory effects of biocontrol agents, it is worth acknowledging that possibly, the enzymes involved might also breakdown the sugars and other polymers in the tomatoes, thereby influencing their organoleptic properties. The biochemical changes occurring during tomato ripening increases the activity of the cell wall degrading enzymes (Gebregziabher et al., 2021). Cellulase has been reported to not be a major factor controlling softening of tomato fruit during ripening (Hobson, 1968). Taking all these into consideration, it becomes apparent that the ability of the enzymes produced by the antagonistic fungal isolates from this study on the organoleptic properties of the tomato during different stages of ripening has to be investigated.

Extracellular enzymes are proteins that catalyze the chemical reactions, thereby, reducing complex substances to their simpler components (El-Gendi et al., 2022). The enzymes reported in this study are hydrolytic enzymes, the most comprehensively studied class of enzymes produced by among other organisms, fungi (El-Gendi et al., 2022). As reported in this study, the antagonistic endophytic isolates, *P. africana* SUG3.1, *A. pullulans* SUG4.1, *C. micaceus* SUG4.3, *E. nigrum* SGT8.6 and *F. oxysporum* HTR8.4, produced hydrolytic enzymes, catalase and cellulase (Table 3). Likewise, Zhu et al. (2020) also reported on *Aspergillus flavus* that produced catalase, which acted as a virulent factor against pathogenic fungi. Lipase production was also demonstrated by *P. africana* SUG3.1 and *F. oxysporum* HTR8.4. Lipases are a class of hydrolytic enzymes which are responsible for hydrolysis of triacyl-glycerol resulting in the release of fatty acid and glycerol. Biocontrol-wise, fungi which produce lipases have the capability to break down complex cellulose material of the pathogenic strains (Palanisamy et al., 2015; Várnai et al., 2014). Furthermore, amylases and proteases were produced *P. africana* SUG3.1 and *C. micaceus* SUG4.3 can antagonise pathogens by hydrolyzing 1,4-glycosidic bonds in the carbohydrate chain, releasing a single maltose unit (Amin et al., 2008; Oseni 2014). Proteases are crucial to fungal physiology as they are used in both the digestion of large

extracellular peptides and in defence against adhering pathogens (Yike 2011). *Trichordema* species, *Aspergillus orycae* and *A. flavus* were reported to produce protease enzymes which had biocontrol activity against *R. solani*, *F. oxysporum* and other pathogenic fungi (El-Gendi et al., 2022; Zanutto-Elgui et al., 2019). Additional studies by El-Gendi et al. [2022], Viterbo et al. (2002) and Liu et al. [2020] also reported on different fungi which produced amylases, cellulases and proteases, which served as antimicrobial agents against pathogenic organisms, by agar diffusion assay. Caseinase and urease were produced by *P. africana* SUG3.1 while, gelatinase was produced by *C. micaceus* SUG4.3. Similarly, studies by El-Gendi et al. [2022], Yike [2011], Zanutto-Elgui et al. [2019] and Lerm et al. [2017] also reported production of proteases and ureases by different fungi, and further mentioned that these enzymes served as antimicrobial agents against pathogenic microorganisms *in vitro*. The production and use of hydrolytic enzymes by the endophytic fungal isolates can be one of the mechanisms by which these fungi are able to antagonise growth of the pathogenic fungi affecting tomatoes. Alimadadi et al. [2023] also attributed the antagonistic activity produced by yeasts strains against *P. expansum*, to their production of diffusible enzymes.

Endophytic fungi employ an array of modes of action including among others, competitive exclusion, production of lytic enzymes, induction of resistance, antibiosis and mycoparasitism, to inhibit growth of pathogens (Adeleke et al., 2022; Guerrero et al., 2014; Latz et al., 2018). Taking into consideration the study findings, we deduce that another mechanism of action used by the endophytic isolates tested in our study for inhibition of fungal pathogens is antibiosis. Antibiosis is a process whereby the endophyte produces metabolites which inhibit growth of a phytopathogen (Adeleke et al., 2022). Thus, fungal antibiosis is the capacity of fungal BCAs to generate secondary metabolites that possess antimicrobial characteristics, serving as a defence mechanism against infections. The metabolites responsible for antibiosis include alkaloids, steroids, flavonoids, peptides, quinones, terpenoid, phenols and polyketides (Akram et al., 2023; dos Santos et al., 2015; Latz et al., 2018). Additionally, antibiotics, lytic enzymes, volatile organic compounds (VOCs), and a range of toxins (Carmona-Hernandez et al., 2019; Borras and Aguilar, 1990; Friel et al., 2007) are also responsible for antibiosis. Of these metabolites, production of antibiotics is regarded as the second most important mechanism by which fungal BCAs suppress diseases (Raaijmakers et al., 2002; Sharma et al., 2009). The metabolites produced by fungal BCAs affect the metabolic activity of pathogens, inhibit their growth and can ultimately kill the pathogens by endolysis and cytoplasmic disintegration (Roshan et al., 2013; Carmona-Hernandez et al., 2019). The efficacy or the metabolite or the enzymes to inhibit pathogen growth will be influenced by its concentration and its extent of diffusion into the medium (Alimadadi et al., 2023). Researchers are however currently placing more emphasis on the development of non-antibiotic producing fungal antagonists for the control of post-harvest diseases of fresh produce due to the negative effects of the antibiotics on the health of the consumer and the environment.

5. Conclusions

The isolates reported in this study (i.e., *P. africana* SUG3.1, *A. pullulans* SUG4.1, *C. micaceus* SUG4.3, *F. oxysporum* HTR8.4 and *E. nigrum* STG8.6) demonstrated potential to serve as future biopreservatives. *P. africana* SUG3.1 and *C. micaceus* SUG4.3 have demonstrated a strong and broad-spectrum antifungal activity as compared to other antagonist in the study. Potentially, these endophytic fungi can be used in combination with *L. plantarum* for protecting tomatoes against fungal pathogens.

Therefore, it is imperative for researchers to examine the overall activities of the generated enzymes on fungal pathogens. Furthermore, the efficacy of these antagonists in both the open field and post-harvest storage facilities has to be evaluated. It is also suggested that studies be carried out to ascertain microbial biomass and the overall activities of the enzymes on fungal pathogens. A thorough understanding of their mechanisms of action is also required in their mutualistic association with plants, both to optimize their efficacy and for registration as plant protection products. It is also of utmost importance for future studies to explore further on the mining of the genes responsible for the antagonistic activities of these fungi.

Supplementary Materials: Table S1: The 80 morphologically distinct isolates from tomato fruit of cultivar Jasmine with 40 green and red tomato fruit samples, grown under hydroponic and open field conditions, fungicide-treated and untreated at the ARC-Vegetables and Ornamental Centre in South Africa, during the 2014–2015 autumn and summer season where negative means incapable of suppressing the pathogen and positive means capable of suppressing the pathogen; Table S2: Mean inhibition zones of the SUG3.1, SUG4.1, SUG4.3, HTR8.4 and STG8.6 antagonists isolates against *F. oxysporum*, *R. solani*, *G. candidum*, *A. alternata*, *G. candidum* ATCC 34614, *F. solani* ATCC 36031, *R. stolonifera* (−) ATCC 6227a, *R. stolonifera* (+) ATCC 6227b and the positive control, *L. plantarum*; and Table S3: Tukey's HSD mean comparisons of potential fungal antagonistic isolates among themselves and *L. plantarum* against *F. oxysporum, R. solani, G. candidum, A. alternata, G. candidum* ATCC 34614, *F. solani* ATCC 36031, *R. stolonifera* (−) ATCC 6227a and *R. stolonifera* (+) ATCC 6227b (Excel file). Table S2: Mean inhibition zones of the endophytic isolates (SUG3.1, SUG4.1, SUG4.3, HTR8.4 and STG 8.6) against *F. oxysporum, R. solani, G. candidum, A. alternata, G. candidum* ATCC 34614, *F. solani* ATCC 36031, *R. stolonifera* (−) ATCC 6227a, *R. stolonifera* (+) ATCC 6227b and a positive control strain, *L. plantarum*.

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Institutional review board statement

Not applicable.

Data availability statement

The data presented in this study are available in the article and in the supplementary materials.

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Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2024.05.006.

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