Isolation of endophytic fungi from South African plants, and screening for their antimicrobial and extracellular enzymatic activities and presence of type I polyketide synthases

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Highlights

- Six species of endophytic fungi were identified from six South African medicinal plants.
- Endophytes extracts had low levels of antibacterial and antifungal activity.
- Some endophytes produced useful extracellular enzymes and ability to solubilize phosphate.
- Three isolates had polyketide synthase 1 gene, with potential to produce interesting polyketides.

Abstract

Endophytes are bacteria or fungi which live inside the host plant and participate in many biological processes without causing disease or other adverse effects. Endophytes are recognised as a rich source of secondary metabolites with potentially useful pharmacological properties. Many South African medicinal plants are highly under-investigated sources of potentially useful endophytic microbes. In this report six endophytic fungi were obtained from the leaves, stems and roots of South African medicinal plants which are known for their traditional uses and pharmacological properties. The endophytic fungi were isolated from *Cotyledon orbiculata* L., *Psychotria zombamontana* (Kuntze) Petit, *Tecomaria capensis* (Thunb.) Lindl., *Catha edulis* (Vahl) Endl. and *Melianthus comosus* Vahl. The crude extracts of the isolated endophytic fungi were investigated for their antimicrobial potential, extracellular enzymatic activity and phosphate solubilization. Additionally, the present study used genetic screening to assess the ability of the endophytic fungi to synthesize bioactive compounds, indicated by the presence of the polyketide synthase type 1 (PKS 1) gene. In preliminary microbial inhibition screening the fungal extracts had promising antifungal activity against *Cryptococcus neoformans* and *Candida albicans.* Furthermore, the endophytic fungus *Talaromyces funiculosus* displayed extracellular enzymatic activity, namely xylanase and cellulase. Five fungal strains demonstrated ability to solubilize phosphate and three strains demonstrated the presence of polyketide synthase type 1 (PKS 1) gene. It is worth considering further investigation of their bioactive secondary metabolites.

Keywords: South Africa; medicinal plants; endophytes; fungi; antimicrobial activity; enzymatic activity; PKS I

1. Introduction

South Africa has a rich diversity of medicinal plants as well as knowledge of their use (Abdalla and McGaw, 2018). A comprehensive review discussed the potential of South African plants and their traditional uses in terms of discovery of promising endophytic microbes and their secondary metabolites (Abdalla and McGaw, 2018). Extensive research has been done in South Africa to investigate the potency of South African plants and several bioactivities have been discovered including antimicrobial (Omokhua et al., 2018), antioxidant and anti-inflammatory (Motlhatlego et al., 2018), antiviral (Mehrbod et al., 2018) and acaricidal efficacy (Adenubi et al., 2018).

In this report six South African medicinal plants were selected for the isolation of endophytes owing to their traditional uses and remarkable bioactivities. These plants are *Cotyledon orbiculata*, *Psychotria zombamontana*, *Tecomaria capensis*, *Catha edulis* and *Melianthus comosus. C. orbiculata* is a small shrub with fleshy leaves. It is widely distributed in southern Africa, and it is used to manage pain and inflammatory conditions (van Wyk et al., 1997; Watt and Breyer-Brandwijk, 1962). A previous study reported antinociceptive and anti-inflammatory activities, and indicated that the relatively high LD_{50} revealed that *C. orbiculata* is non-toxic to mice (Amabeoku and Kabatende, 2012). *Psychotria zombamontana* is a shrub or small tree 1.5–9 m in height belonging to the Rubiaceae family, found in the understory of evergreen forest, including ravine and kloof forest (Coates-Palgrave, 2002). The Rubiaceae family is characterized by the production of bioactive metabolites with great pharmacological potential including anti-inflammatory, antimicrobial, antimutagenic, anxiolytic, antipyretic and analgesic activity (Nunez et al., 2009, Moraes et al., 2011; Martin and Nunez, 2015). The studies conducted by Aro et al. (2015; 2016; 2019) revealed that the acetone leaf extract of *P. zombamontana* had good antimycobacterial, antioxidant and anti-inflammatory activities as well as immunomodulatory effect.

Tecomaria capensis is a multi-stemmed shrub with orange tubular flowers (Otto, 2015). It is known as a plant of the KwaNibela Peninsula, St Lucia (Corrigan et al., 2011). In traditional medicinal practice, the leaves of *T. capensis* are used for diarrhea and enteritis by the Swazi (Watt and Breyer-Brandwijk, 1962). Additionally, it has toxic characteristics (Wright, 1963). A recent study reported the significant wound healing activity of *T. capensis* (Saini et al., 2012).

Catha edulis (known as Khat) is commonly grown in the horn of Africa. The tender leaves and young buds of *C. edulis* are chewed for generating the sensation of stimulation and euphoria (Wabe, 2011). It has been reported in South African traditional medicine to treat and manage hypertension (Balogun and Ashafa, 2019). In addition to its other uses such as furniture, firewood, fencing poles and as an insect repellent, *C. edulis* has medicinal potential against respiratory diseases and sleeplessness, as well as for relieving fatigue. Furthermore, it is used for stimulating the heart (Hutchings et al., 1996), and as an antidote against asthma, cough, and other chest diseases (Van Wyk et al., 1997). Ondua et al. (2019) reported that *C. orbiculata* and *C. edulis* had very good inhibitory activity against nitric oxide production (99.37 % and 97.88 %, respectively).

Melianthus comosus is an erect shrub, much branched and covered with stellate hairs (Weber, 2017). *M. comosus* was recommended by most people interviewed in the Bredasdorp/Elim region (Western Cape Province of South Africa) to manage wounds and sores (Thring and Weitz, 2006). Additionally, the leaf juice or paste of *M. comosus* is used for the treatment of wounds (Mabona and Van Vuuren, 2013). The ethanol extract of *M. comosus* had moderate activity against methicillin-resistant *Staphylococcus aureus*, and inhibited the growth of *S. aureus* at 0.5 mg/ml (Heyman et al., 2009).

Endophytes are known to be an important resource of various classes of secondary metabolites (Jumpathong et al., 2010; Abdalla and Matasyoh, 2014; Abdalla, 2017). Relatively little is known about endophytes from South African plants. In this report, endophytic fungi isolated from South African medicinal plants were screened for their antimicrobial potential, extracellular enzymatic activity, phosphate solubilization and the presence of polyketide synthase type 1 (PKS 1) gene. It was anticipated that the endophytic fungi may be responsible in part for the antimicrobial activity of the plant species selected. Additionally, further useful properties were investigated, including presence of the extracellular enzymes cellulose and xylanase. Many fungal enzymes are used in food processing. They are known to be more stable than enzymes derived from other sources (Raveendran et al., 2018). Fungal enzymes are used in food preparations to enhance good properties such as taste and texture and flavor. Furthermore, these enzymes are preferred because of their high specificity and low level of by-products (Saxena et al., 2001). Besides the important role of fungal enzymes in food processing, they have substantial uses in

beverages, confectionaries, textiles and leather industries to facilitate the transformation of raw materials (Abdel-Azeem et al., 2014).

Phosphorus plays a crucial role in plant growth and development, so a deficiency of this mineral can inhibit the growth and development of the plant (Kalayu, 2019). Phosphate solubilizing microbes act as an important approach to enhance plant growth (Adhikari and Pandey, 2019). A number of microorganisms display phosphate solubilization activity such as bacteria, fungi, and algae (Sharma et al., 2013). Solubilization of inorganic insoluble phosphate salts by different microbes can be mediated by different mechanisms; one of these mechanisms is release of mineral dissolving compounds such as organic acids in the respective environment (Adhikari and Pandey, 2019). These organic acids reduce the pH of the medium, in order to facilitate the exchange of the metal part of insoluble phosphate either to potassium or sodium, to form soluble phosphate salts (Adhikari and Pandey, 2019). Phosphate solubilizing microbes are known to enhance the soil fertility by decreasing the problem caused by the enzyme phytase, which hydrolyzes both phytate and phytic acid and releases the inorganic phosphate in bound form (Sharma et al., 2013).

Polyketides are a group of natural products produced by many plants, bacteria, fungi and marine organisms (Risdian et al., 2019). They are biosynthesized by polyketide synthases (PKSs), which are large, multifunctional, and multimodular enzyme complexes which catalyze the polymerization of acyl-CoA thioester building blocks (Miller et al., 2012). Polyketides have a wide range of biological activities including antimicrobial, anticancer, immune-suppressing, antiviral, anti-inflammatory, and anti-cholesterol activity (Risdian et al., 2019). Identifying the presence of PKS (Type 1) or the ability of endophytes in South African plants to produce PKSs could provide useful leads for study of these interesting metabolites.

2. Materials and methods

2.1. Plant material collection and isolation of endophytic fungi

The root and stem of *C. orbiculata*, leaves of *M. comosus*, and the root of *T. capensis* were collected from the grounds of the Faculty of Veterinary Science, University of Pretoria in March 2017. The leaves of *P. zombamontana* were collected from the Pretoria National Botanical Garden in April 2017. The leaves of *C. edulis* were collected in Countryview,

Vorna Valley, President Park, Midrand, (Gauteng, South Africa) in February 2017. Voucher specimens were deposited in the HGWJ Schweickerdt Herbarium of the University of Pretoria (PRU).

Fresh plant leaves, roots and stems were immediately rinsed under running tap water for 5 min to remove soil. Then plant parts were cut into 0.5 × 0.5 cm^2 segments. The following step was the surface sterilization, where different plant segments (roots, stems and leaves) were treated with 70% ethanol for 1 min, followed by a strong surface sterilant (3.5% sodium hypochlorite) for 5 min. The material was washed again with 70% ethanol for 30 s followed by several rinses in sterile distilled water for 1 min to remove residual sterilant (Abdalla and McGaw 2018). The sterilized segments were inoculated onto Potato Dextrose agar (PDA), which was supplemented with penicillin G (30 mg/L) and streptomycin sulfate (30 mg/L). The Petri dishes were incubated 28°C for two weeks. Additionally, a control sample taken from the water washings of the surface-sterilized segments were streaked onto PDA (antibiotic-free) and incubated under the same conditions. The primary growth of the fungi was isolated after 14 days and several sub-culturing steps were performed using PDA and incubated at 28°C for a further 7 days until the fungal strains were purified.

2.2. Identification of the endophytic fungi and phylogenetic analysis

The endophytic fungal isolates were identified by amplification and sequencing of the ITS region (ITS1-5.8S-ITS2). Briefly, the total genomic DNA was extracted from pure mycelia of the recovered endophytes following the protocol of Lee and Taylor (1990). The DNA was subjected to PCR amplification of the ITS region (ITS1-5.8S-ITS2) using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) using a Veriti thermal cycler (Applied Biosystems, Singapore). The 25 µl PCR reaction consists of 1X PCR assay buffer, 1.5 mM $MgCl_2$, 200 µm of each dNTPs, 10 pmols of each primer, 1 U Taq DNA polymerase and 50 ng template DNA. The PCR conditions were as follows: 95°C for 5 min with 35 cycles of denaturation for 1 min at 95°C, annealing at 56°C for 1 min, extension at 72°C for 1 min 20 sec; and 72°C for 10 min.

The sequencing was performed commercially at Sci-Genome Pvt. Ltd. Kochin, India and the obtained sequences were examined by visual assessment of chromatograms using Finch TV v1.40v (http://www.geospiza.com/finchtv). The sequences were subjected to separate nucleotide searches using the NCBI BLASTn algorithm. The endophytic fungi were

identified based on the maximum identity percent obtained from the BLASTn results. The sequences were submitted to the NCBI GenBank to obtain accession numbers. The obtained sequences were subjected to BLAST analysis with the deposited sequences in the NCBI database to find the homology with the closest related organisms. The neighbor-joining phylogenetic trees for ITS rRNA gene were constructed based on Tamura 3-parameter model (T92+G) according to lowest BIC (9123.340) and highest AIC values (8897.908) values using MEGA X (Tamura et al., 2013). The type strains based on similarity identity of BLASTn result were obtained from NCBI database and multiple sequence alignment was carried out using the Clustal W (Thompson et al., 1997). The bootstrap analysis was used up to 1000 replicates.

2.3. Antimicrobial assay

The antimicrobial activity of methanol extracts of cultures of the isolated endophytic fungi was determined against the following bacteria: *Bacillus cereus* (ATCC 21366), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 39183) and fungi: *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (clinical isolate). The antimicrobial activity was determined in terms of minimum inhibitory concentration (MIC) using a rapid broth microdilution technique with p-iodonitrotetrazolium violet (INT) as a growth indicator (Eloff, 1998). Solvents used to resuspend the extracts were included as negative controls, in addition to the broth used to inoculate a colony for overnight culture. Gentamicin and amphotericin B were the positive controls for bacteria and fungi respectively.

2.4. Extracellular enzymatic activity

Endophytic fungi were screened for their ability to produce extracellular cellulose and xylanase on solid media. For screening of cellulase production; the fungal cultures were inoculated on yeast extract peptone medium (peptone 0.5 g; yeast extract 0.1 g; agar 18 g in 1 L of distilled water; pH 5.4) supplemented with 0.5% Na-carboxymethyl cellulose (CMC). After incubation at 28^{o}C for 6 days, the plates were stained with 1% Congo red and destained with 1 M NaCl. The clear zone surrounding the colony indicated the cellulase activity (Maria et al., 2005).

For screening of xylanase activity, the fungal cultures were inoculated on a medium containing 1% oat spelt xylan, 0.1 g yeast extract, 0.1 g peptone and 12 g agar dissolved in 1 L of distilled water (pH 5.4). Post incubation at 28ºC for 6 days, the plates were stained with 1% Congo red and destained with 1 M NaCl. The clear zone surrounding the colony indicated the xylanase activity (Teather and Wood, Asem et al., 2017).

2.5. Phosphate solubilization by endophytic fungi

The identified endophytic fungi associated with medicinal plants were screened for their phosphate solubilizing potential by inoculating a mycelial disc of fungi at the centre of Pikovskaya's medium (Hi-Media) containing calcium phosphate. The formation of a halo zone indicated phosphate solubilizing potential of the isolates (Elias et al., 2016).

2.6. Screening for polyketide synthase type 1 (PKS 1) gene

The identified fungal endophytes were selected to screen for the presence of the PKSI gene to evaluate their ability to produce polyketides. Two sets of degenerate primers, LC1 (GAY CCI MGI TTY TTY AAY ATG), LC2c (GTI CCI GTI CCR TGC ATY TC) and LC3 (GCI GAR CAR ATG GAY CCI CA), LC5c (GTI GAI GTI GCR TGI GCY TC) were used to amplify ketosynthase (KS) domain of the PKS type I gene (Bingle et al., 1999). The 25µl PCR reaction mixture consisted of 50 ng of template DNA, 1X PCR buffer, 4 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol of each primer and 2U of Taq polymerase. The thermal cycler conditions were: 94ºC for 5 min, 35 cycles of denaturation at 94ºC for 1 min, annealing for 1 min 20 sec at 55ºC for both LC1/2 and LC3/5 primers and extension at 72ºC for 3 min followed by 72ºC for 10 min.

3. Results and discussion

Endophytes are an important source of secondary metabolites. By selecting bioactive plant species used in traditional medicine and investigating their endophytes, it might be found that endophytes may contribute to the overall bioactivity of the host plant. Endophytes have the ability to produce several classes of natural products. The present study took advantage of the immense biodiversity and cultural knowledge of South African plants for their traditional medicinal uses to focus on their endophytes. The study screened isolated fungi from selected medicinal plants with known bioactivity for their antimicrobial activity, extracellular enzymatic activity, and phosphate solubilization activity. Additionally, the metabolic potential of the isolated endophytes was tested using PKS genes as a proxy for endophytic production of polyketides. These genes indicate involvement of PKSs in the production of polyketide compounds.

In our study, endophytic fungi isolated and identified from South African medicinal plants are listed in Table 1.

Table 1. South African medicinal plants, their families, plant parts used and the isolated endophytic fungi

Isolate code	SA medicinal plants	Family	Plant part	Endophytic fungi
MA-coty-root2	*Cotyledon orbiculata* L.	Crassulaceae	Root	*Diaporthe* sp.
MA-zom-1	*Psychotria zombamontana* (Kuntze) Petit	Rubiaceae	Leaves	*Talaromyces funiculosus*
MA-zom-2	*Psychotria zombamontana* (Kuntze) Petit	Rubiaceae	Leaves	*Talaromyces funiculosus*
MA- coty- stem-9	*Cotyledon orbiculata* L.	Crassulaceae	Stem	*Penicillium commune*
MA-teco-1	*Tecomaria capensis* (Thunb.) Lindl.	Bignoniaceae	Root	*Cochliobolus* sp.
MA-zoom-3	*Psychotria zombamontana* (Kuntze) Petit	Rubiaceae	Leaves	*Cochliobolus* sp.
MA- coty- stem-2	*Cotyledon orbiculata* L.	Crassulaceae	Stem	*Talaromyces funiculosus*
MA-Ca- 20	*Catha edulis* (Vahl) Forssk. ex Endl.	Celastraceae	Leaves	*Phomopsis* sp.
MA-coty- root-1	*Cotyledon orbiculata* L.	Crassulaceae	Root	*Clonostachys rosea*
MA-coty-root M1	*Cotyledon orbiculata* L.	Crassulaceae	Root	*Talaromyces funiculosus*
MA-me1	*Melianthus comosus* Vahl	Melianthaceae	Leaves	*Talaromyces funiculosus*

3.1. Phylogenetic analysis

The phylogenetic tree (Figure 1) showed that all the isolates belonged to the families Diaporthaceae, Trichocomaceae, Pleosporaceae, Valsaceae and Bionectriaceae. The phylogenetic tree was constructed based on the Neighbor-joining method with Tamura 3-parameter model according to lowest BIC (9123.340) and highest AIC values (8897.908) values using Mega X. Gaps were treated by pairwise deletion and the estimated Transition/Transversion bias (R) is 1.04. The phylogenetic tree revealed that all the isolates of the genus *Talaromyces* strain Zom; CS-MZ-MA and CR are closely clustered with representative strains *Talaromyces funiculosus* strain BBWT6 with bootstrap value of 99%. Isolates *Tecomaria* 1 and Zoom were similarly clustered to reference isolates *Cochliobolus* sp. isolate APS1 with 99% bootstrap supported. Moreover, *Penicillium commune* strain CS and *Clonostachys rosea* strain CR1 was similarly clustered to *Penicillium commune* isolate R97185 and *Clonostachys rosea* strain EG99 with bootstrap value of 100% and 92% respectively.

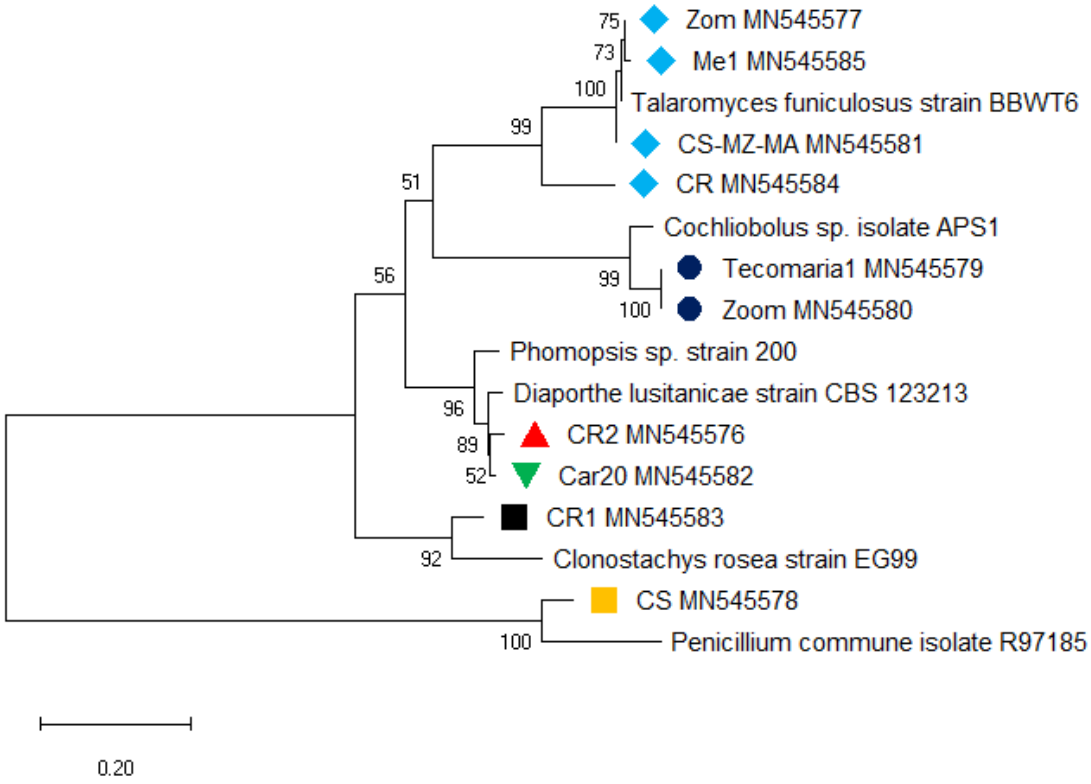

Figure 1. ITS-rRNA phylogenetic tree constructed based on Tamura 3-parameter model using Mega X

3.2. Antimicrobial activity

There is an urgent need for new drug candidates to treat infectious diseases in humans and animal, to combat multidrug-resistance in microbial pathogens (Monciardini et al., 2014). Therefore, the use of endophytes as a source of bioactive compounds may hold promise for the discovery of potential drugs against infectious diseases. The investigation of the antimicrobial activity of isolated fungal endophytes revealed some antifungal activity against *Cryptococcus neoformans* and *Candida albicans.* Two endophytic fungi, named *Talaromyces funiculosus* and *Cochliobolus* sp. obtained from *P. zombamontana*, *T. capensis*, *C. orbiculata* and *M. comosus* showed similar minimum inhibitory concentration (MIC) values of 0.625 to 1.25 mg/ml against *Cryptococcus neoformans* and *Candida albicans*. *Phomopsis* sp. isolated from *C. edulis* afforded activity against *Cryptococcus neoformans* and *Candida albicans* with MIC values in the same range. In the antibacterial assays, the endophyte *Phomopsis* sp. had antibacterial activity against *Staphylococcus aureus* and *Salmonella* Typhimurium with MIC = 1.25 mg/ml. Moreover, it exhibited moderate activity against *Enterococcus faecalis* and *Escherichia coli*. Overall the antimicrobial results were not exceptional.

3.3. Extracellular enzymatic activity

In this report, the isolated endophytic fungi were screened for extracellular enzyme activity including cellulase and xylanase on solid media. The endophytic fungus *Talaromyces funiculosus* showed positive results for both enzymes. Moreover, *Cochliobolus* sp. displayed positive results for xylanase. Our study suggested that although these fungal isolates may produce cellulase and xylanase, more investigations must be performed to improve their activities and to make their production economically possible.

A previous study emphasized the usefulness of cellulase due to its diverse application in the food industry (Kuhad et al., 2011). Importantly, Beg et al. (2001) indicated that xylanases also have a promising role in baking industries, due to their potential to enhance the specific bread volume. Interestingly, Bhat (2000) stated that both cellulase and xylanase obtained from more than one microbe have better properties when applied to improve the extraction of olive oil, compared to both enzymes produced from a single microorganism. It is worth reporting that commercial enzymes available in the market are usually fungal enzymes such as cellulases and xylanase (Polizeli et al., 2005). Fungal cellulases are less complex and may be obtained in higher quantity in comparison to bacterial cellulases (Behera et al., 2017). It can be assumed that it will then be easier to clone the fungal genes, in order to obtain the

corresponding enzymes by using a recombination method for advanced up-scaling (Archarya and Chaudhary, 2012). The findings of extracellular enzymatic activity are represented in Table 2 and Figure 2.

Table 2. Screening of endophytic fungi for extracellular enzymatic activity, phosphate solubilization, and polyketide synthase type 1 (PKS 1) gene

	Isolate Name	Presence of extracellular xylanase	Presence of extracellular cellulase	Ability to solubilize phosphate	Presence of PKS 1 gene
	Diaporthe sp.	-	-	-	-
	Talaromyces funiculosus	+	+	+	+
	Cochliobolus sp.	+	-	+	+
	Clonostachys rosea	-	-	+	-
	Penicillium commune	-	-	+	+
	Phomopsis sp.	-	-	+	-

Isolate code	Endophytic fungus	Enzyme	Diameter of the colony (cm)	Diameter of the hydrolysis zone (cm)	Hydrolysis capacity
MA-zom-1	*Talaromyces funiculosus*	Xylanase	3.4	0.7	0.20
MA-zom-2	*Talaromyces funiculosus*	Cellulase	3.0	0.9	0.3
MA-teco-1	*Cochliobolus* sp.	Xylanase	2.4	1.4	0.58

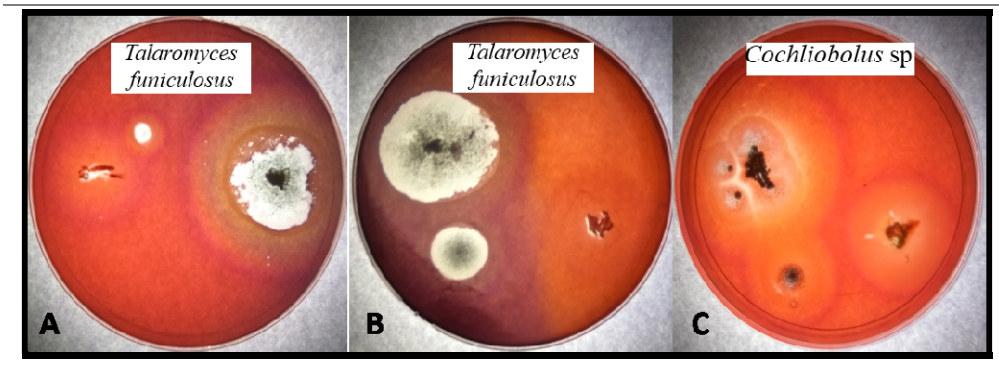

Figure 2. (A) *Talaromyces funiculosus* showing cellulase production (B) *Talaromyces funiculosus* showing xylanase production and (C) *Cochliobolus* sp. showing xylanase production (isolates are MA-zom-1 (A), MA-zom-2 (B), and MA-teco-1 (C).

3.4. Phosphate solubilization by endophytic fungal isolates

The endophytic fungal isolates *Talaromyces funiculosus*, *Cochliobolus* sp., *Clonostachys rosea*, *Penicillium commune*, and *Phomopsis* sp. showed different abilities to solubilize insoluble phosphates (Figure 3). The fungal mycelium is the vegetative part of a fungus consisting of several branched hyphae. The qualitative screening for phosphate solubilizing activity of fungi was carried out by spot inoculation on Pikovskaya's media (HiMedia) followed by incubation at 28°C for 5 days. The formation of a halo zone indicated the phosphate solubilizing potential of endophytic fungal isolates. The solubilization index (SI) can be calculated by subtracting colony diameter from total diameter (colony diameter + halo zone diameter). However, due to the uneven growth of the fungi on the media, it was not possible to accurately calculate the solubilization index and hence it is difficult to describe one particular isolate as the best phosphate solubilizing fungal organism.

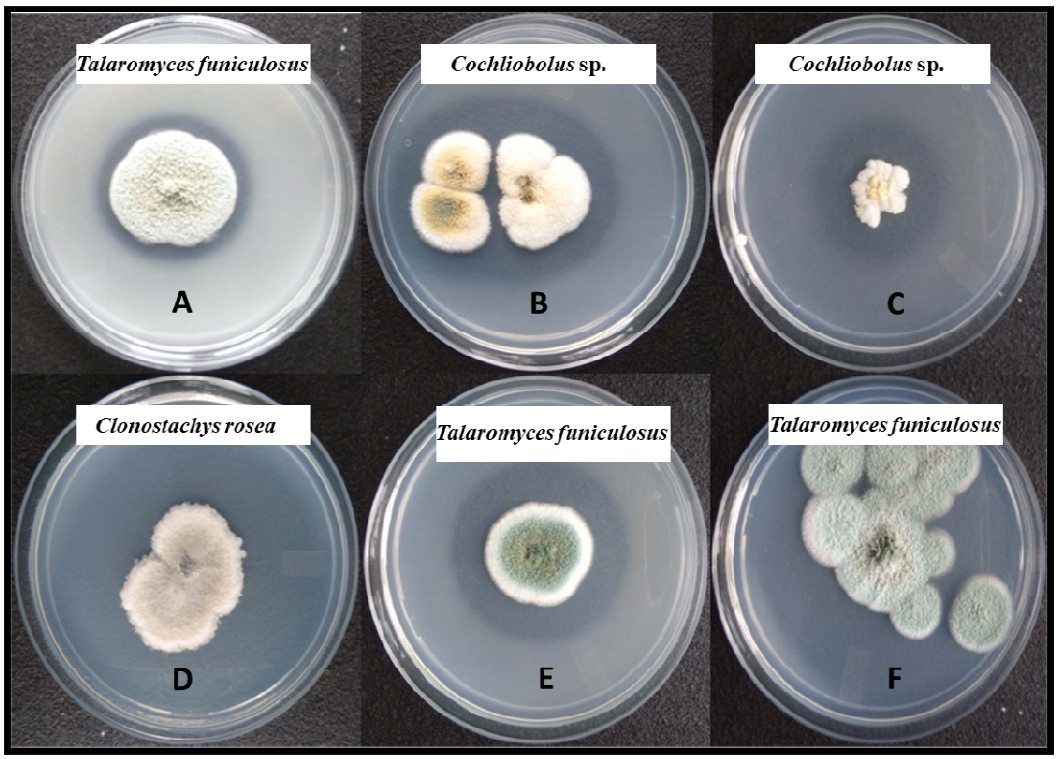

Figure 3. Screening for phosphate solubilization by fungal endophytes (isolates are: MA-zom-1 (A), MA-teco-1 (B), MA-zom-3 (C), MA-coty- root-1 (D), MA- coty- stem-2 (E), and MA-me1 (F).

A previous study isolated two phosphate-solubilizing fungi from rhizosphere soil of moso bamboo and identified them as *Talaromyces aurantiacus* and *Aspergillus neoniger*. The authors found that both fungi had the ability to produce soluble P by decomposing recalcitrant P-bearing metabolites. The study suggested that the two fungi have considerable potential as environment-friendly biofertilizers in subtropical bamboo ecosystems (Zhang et al., 2018).

3.5. Screening for polyketide synthase type 1 (PKS 1) gene

Three PCR screens were successful in targeting fungal PKS1 in three fungal isolates: *Penicillium commune*, *Talaromyces funiculosus* and *Cochliobolus* sp. These results demonstrated the biosynthetic potential of the isolated endophytic fungi for producing potential polyketides. The results of screening for the polyketide synthase type 1 (PKS 1) gene are provided in Table 2 and Figure 4.

Six endophytic fungal isolates tested positive for the presence of the PKS1 gene using PCR amplification using two sets of degenerate primers (LC1/2c and LC3/5c). The gel picture (Figure 4) reveals the presence of a few faint nonspecific PCR products along with the desired bright PCR products. The bright bands were excised, purified and sequenced commercially. The obtained sequences were analyzed using NCBI ORF Finder and a total of four sequences were matched to PKS1 gene sequences in the NCBI database using BLASTx and BLASTn searches. The details of isolates which were screened positive for the presence of the PKSI gene are mentioned in Table 3.

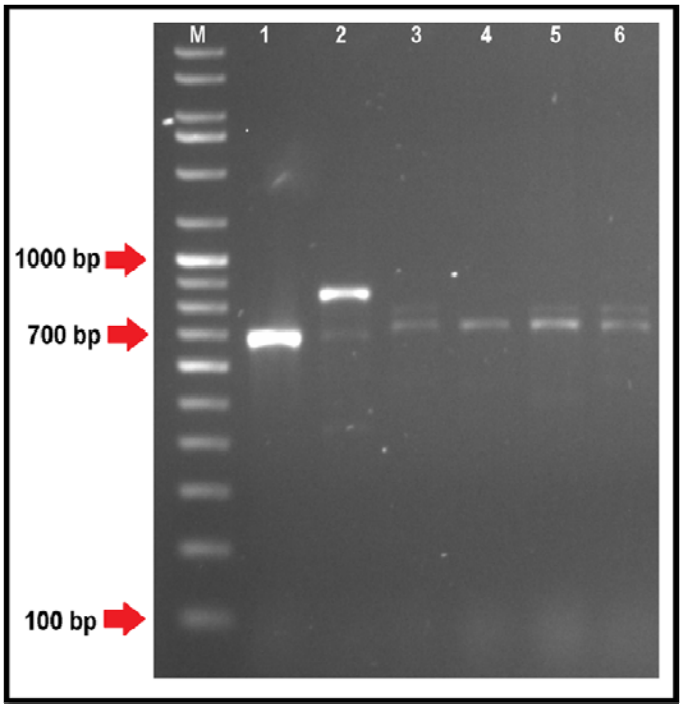

Figure 4. Detection of the PKS1 gene in endophytic fungal isolates. Lanes (1) and (2) denotes amplified PCR product by using LC3/5 series primers whereas, lanes (3), (4), (5) and (6) denote amplified PCR product by using LC1/2 series primer.

Table 3. Fungal endophytes isolates containing the PKS1 gene

Lane code	**Isolate Code**	**PCR products**	**Size (Base pairs)**	**Primers**	**Identification using NCBI BLASTx/BLASTn**
1	MA-teco-1	Single bright fragment	700	LC3/5 series primers	-
2	MA- coty-stem-2	Top (bright) Bottom (faint)	850 700	LC3/5 series primers	*Diaporthe helianthi*
3	MA-zom-1	Top (faint) Bottom (bright)	800 700	LC1/2 series primers	*Talaromyces funiculosus*
4	MA-teco-1	Single fragment	700	LC1/2 series primers	*Penicillium chrysogenum*

5	MA-zoom-3	Top (faint) Bottom (bright)	750 700	LC1/2 series primer	-
6	MA- coty-stem-9	Top (faint) Bottom (bright)	750 700	LC1/2 series primer	*Talaromyces funiculosus*

The degenerate PCR approach was therefore a useful tool for determining the presence of the PKSI gene and, with the ability of this gene to produce polyketides, this could be used to screen fungi with potential for producing bioactive natural products.

4. Conclusion

In this study, six species of endophytic fungi were identified from important South African medicinal plants with promising biological activities, and their extracts tested for antimicrobial activity. Two endophytic fungal isolates showed antimicrobial potential but generally little activity was found. Other uses of the endophytes such as secretion of extracellular enzymes and ability to solubilize phosphate were then investigated with more promising results. *Talaromyces funiculosus* showed positive results for both enzymes and *Cochliobolus* sp. displayed positive results for xylanase but these results need to be compared with production of these useful enzymes with those cultures already in commercial use. Five isolates exhibited phosphate solubilization activity, indicating their potential as fertilizing agents by making insoluble phosphate available for plant uptake. Additionally, three of the isolates were shown to contain the polyketide synthase type 1 (PKS1) gene, indicating their potential to produce interesting polyketides with useful bioactivities under appropriate conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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