First report of bacterial endophytes from the leaves of *Pellaea calomelanos* in South Africa

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Bacteria have an endosymbiotic association with plants. Previous studies have identified endophytic bacteria and their importance in biocontrol and drug development. However, most medicinal plants identified have not been assayed for bacterial endophytes. In this study, we characterised and identified bacterial endophytes from surface-sterilised leaves of *Pellaea calomelanos*, a common fern in the Limpopo and Gauteng Provinces, South Africa. Using morphological data and 16S rRNA gene sequencing, we differentiated and identified six putative endophytic bacteria, with *Pantoea* as the dominant genus; the other two identified bacteria belong to genera *Arthrobacter* and *Bacillus*. Data from this study are an addition to the previously less studied phylloplane bacteria. This study is a pilot in cataloguing bacterial endophytes from *Pellaea calomelanos*.

Significance:

- This study provides the first report of six putative bacterial endophytes from *Pellaea calomelanos.*
- Our results will pave the way for exploring the antimicrobial activity of *P. calomelanos* bacterial endophytes and whole genome comparisons between plant bacterial endophytes and plant bacterial pathogens.

Introduction

Plants have a mutualistic relationship with varied endophytes.[1] Endophytes are endosymbiotic; they are often bacterial or fungal species which colonise the plants without causing harm or pathogenic infection[3] and can spend part or their entire life cycle within the plant host[2-4].

Most plant species are known to host one or more endophytic microorganisms.[5] Endophytes form a symbiotic relationship with the plants by providing a biological defence mechanism for the plant host against pathogens,[2-5] through the production of secondary metabolites. These metabolites halt the growth of or attack invading antagonists or lyse-infected plant cells; furthermore, the metabolites can induce plant host defence mechanisms and promote plant growth.[6]

The present study was designed to isolate, identify and characterise endophytic bacteria from *Pellaea calomelanos* obtained in South Africa. Pteridaceae is a family of fern plants with over 45 genera and more than 1000 species.[7] One such genus is *Pellaea* with over 35 described species found growing in arid rocky regions and within narrow open pockets in the soil.[7,8] *P. calomelanos* is a common fern species in the Limpopo and Gauteng Provinces of South Africa, but also grows throughout the rest of the country.[9] Common names of *P. calomelanos* in South Africa include *inkomankomo* (Zulu), *lehorometso* (Sotho), *legogoana* (Tswana), *phalatjane* (Sepedi) and hard fern (English).[9]

P. calomelanos is a multipurpose medicinal plant used for the treatment of headaches, chest colds, asthma, head colds and mouth and nasal ulcers.[9] Like other plants, *Pellaea* species have a mutualistic relationship with endophytes, although, to date, endophytes have been reported in only *P. concolor* and *P. viridis*.[10,11] We thus report here on the occurrence of bacterial endophytes within the leaves of *P. calomelanos*. Ours is the first study to describe the isolation, identification and characterisation of bacterial endophytes from *P. calomelanos* using morphological and phenotypic characteristics and the sequencing of the 16S rRNA gene.

Materials and methods

Collection of plant material

Aerial portions of the plant material were collected from Botlokwa (23°29'34.8'S, 29°42'11.2'E) in the Limpopo Province of South Africa. Whole plants were placed in sterile polyethylene bags and transported to the laboratory under 4 °C. The plant material was collected in March 2017 from a site with sandy loam soil.

Identification of the plant material

The identification of the plant material was carried out at the University of Johannesburg Herbarium (JRAU). A sample specimen of the plant material was deposited in the University of Johannesburg Herbarium (JRAU) with voucher specimen number Serepa-Dlamini 201 and species name *Pellaea calomelanos*. The remaining collected plant material was immediately processed in the laboratory.

Isolation of endophytic bacteria

Immediately after collection of plant material in the lab, plant leaves were washed with running tap water followed by a sequential sterilisation with the following solutions: sterile distilled water for 1 min, 70% ethanol for 1.5 min, 1% sodium hypochlorite for 3 min and finally washed three times in sterile distilled water. The final washing water was then plated as control. The surface-sterilised leaves were ground in 2 mL of saline using a pestle and mortar. Under sterile conditions, the homogenate was streaked onto nutrient agar plates. The plates were incubated for 24–48 h at 28 °C and inspected daily for bacterial colony growth. Isolated colonies were re-cultured on sterile nutrient agar plates until pure colonies with uniform morphology were obtained. For each endophyte bacterial isolate, 35% glycerol (glycerol diluted in sterile distilled water) stock cultures were prepared and stored at -80 °C for future use.

Preliminary identification of endophytic bacterial isolates

Pure colonies were subjected to a Gram reaction test.[12] The Schaeffer–Fulton stain method[13] was conducted to determine if the bacterial endophytes produced endospores. The hanging-drop method adapted from MacFaddin[14] was used to determine motility of the bacterial isolates. All the prepared slides were examined using a bright-field compound light microscope (CX21FS1, Olympus Corporation, Tokyo, Japan) with 400x and 1000x magnification.

Identification of bacterial endophytes by the Biolog MicroPlate system

The following procedure was utilised to identify the putative endophyte bacterial isolates. Under sterile conditions, overnight single colonies of the isolates were sub-cultured in six separate 5 mL of 0.85% saline solution. The Biolog turbidimeter was used to monitor and measure the turbidity of the suspension until a 90–98% transmittance was reached. The suspension (150 μL) was aliquoted into each well of the Biolog MicroPlate (with GEN III MicroPlate™; Biolog Inc., Hayward, CA, USA) and incubated at 26 °C for 24 h. The plates were scanned using the Biolog automatic system and samples identified using the Biolog software.[15]

Scanning electron microscopy

A scanning electron microscope was used to observe and study the shape of the endophyte bacterial isolates. Each glycerol stock of the isolated bacterial endophytes was inoculated into 5 mL nutrient broth and incubated for 48 h at 28 °C. The bacterial suspension was centrifuged at 2935 rcf for 10 min, the supernatant was discarded and bacterial cells were rinsed with sterile distilled water three times with a 5-min interval between each rinse. The bacterial cells were then fixed in 8% glutaraldehyde overnight (glutaraldehyde 25% EM grade diluted in Ringers' solution). Sterile distilled water was used to rinse the cells twice, followed by a series of dehydration with 30%, 50%, 70%, 90%, 95% and 100% ethanol at 10-min intervals and a centrifugation step at 2935 rcf for 10 min. The bacterial pellets were left to dry overnight in open Eppendorf tubes placed in a refrigerator at 4 °C. The cells were then mounted on scanning electron microscope stubs and coated with gold and viewed using the TESCAN VEGA 3 LMH (AVG9731276ZA, Warrendale, PA, USA) scanning electron microscope fitted with a digital camera.

Identification of bacterial endophytes using 16S rRNA

Extraction of genomic DNA

Genomic DNA of each bacterial isolate was extracted from nutrient agar pure colonies using a Quick-DNA™ ZR fungal/bacterial DNA MiniPrep kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. The extracted DNA was quantified using the NanoDrop ND-2000 UV-Vis spectrophotometer (ThermoFisher Scientific, USA); the DNA concentration was 40 ng/μL prior to cleaning with ZR fungal/bacterial DNA clean and concentrator-5 (Zymo Research).

Polymerase chain reaction amplification and sequencing

The 16S rRNA gene of each bacterial isolate was amplified by polymerase chain reaction (PCR) in a 12.5-μL reaction containing 1.5 μL of template DNA, 1.5 μL of each primer 5`-AGAGTTTGATCCTGGCTCAG-3`f and 5`-AAGGAGGTGATCCAAGCCGCA-3`r, 6 μL of One Taq® 2X PCR master mix with standard buffer (20 mM Tris-HCl, 1.8 mM $MgCl_2$, 22 mM NH_4Cl, 22 mM KCl, 0.2 mM dNTPs, 5% glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween® 20, 25 units/mL One Taq® DNA polymerase) and final volume filled up to 12.5 μL with nuclease free water. The PCR cycle was performed using MyCycler™ Thermal Cycler (catalogue number 580BR 08389, BioRad, Hercules, CA, USA) with the following conditions: initial denaturation at 94 °C for 3 min, followed by 35 cycles of amplification: denaturation at 94 °C for 1 min, 55 °C for 1 min (annealing), 72 °C for 2 min (extension), followed by a final extension at 72 °C for 10 min.[16] The PCR products were cleaned with ExoSAP-it™ (ThermoFisher Scientific) following the manufacturer's recommendations and sent for sequencing with primers to Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

Phylogenetic analysis

The 16S rRNA gene sequences of the bacterial isolates were subjected to BLAST (v.2.6.0) analysis against the rRNA sequence database (Bacteria and Archaea) at the National Center for Biotechnology Information (NCBI) to identify the closest related bacterial species. Only bacterial species with 95–100% identity similarity were selected for phylogenetic analysis. The aligned sequences, containing the isolate and closest related bacterial species, were determined by MUSCLE[17] and phylogenetic analysis carried out using the maximum likelihood method based on the Tamura–Nei model[17]. Positions overlapping with gaps and missing nucleotide data were eliminated. All evolutionary analyses were conducted in MEGA 7.[18] *Escherichia coli* ATCC 11775T with GenBank accession number X80725 was used as the outgroup. The phylogenetic trees were reported with bootstrap percentages. The 16S rRNA gene sequences of bacterial isolates identified in the study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with the following accession numbers and names: MF613647 (*Arthrobacter* sp. strain MHSD1), MF613648 (*Pantoea* sp. strain MHSD2), MF613649 (*Bacillus infantis* strain MHSD3), MF613650 (*Pantoea* sp. strain MHSD4), MF613651 (*Pantoea ananatis* strain MHSD5) and MF613652 (*Pantoea* sp. strain MHSD6). The assigned names of the bacterial isolates were based on the BLAST homology percentages as well as phylogenetic results.

Results and discussion

Isolation and identification of endophytic bacteria

Morphological identification

The isolation and enumeration of endophytes from surface-sterilised plant material is the recommended method.[3,4,19-23] In the current study, six different putative bacterial endophytes were isolated from the surface-sterilised leaves of *P. calomelanos* obtained from Botlokwa, Limpopo Province, South Africa. Previous studies on bacterial endophytes have focused on rhizosphere bacterial endophytes because of their major role in nutrient uptake and high diversity in soils.[19-21]

Preliminary morphological observations of the six isolated colonies that included the production of endospores, motility and Gram staining has enabled the grouping of the bacterial cultures into various groups, some with similar characteristics as shown in Table 1. The Gram stain results indicated that all bacteria were rod shaped and two out of the six isolates were Gram positive and the remaining four isolates were Gram negative. Only one of the Gram-positive isolates was an endospore former. All the isolates, except one Gram-positive isolate, exhibited motility. The morphological shapes were confirmed by the scanning electron microscope results (not shown) which showed uniform cells depicting pure cultures of the bacterial isolates.

Biolog MicroPlates

The phenotypic assays were performed on the isolates using the Biolog MicroPlates (GEN III MicroPlate) and the results are presented in Table 1. The bacterial isolates can be differentiated based on the utilisation of some of the carbon sources such as dextrin, maltose, sucrose, stachyose and pectin. Most of the isolates appear to hydrolyse these carbon sources, although variations were observed. Some of the results were not determined (ND), such as utilisation of N-acetyl-d-glucosamine, N-acetyl-β-d-mannosamine and N-acetyl neuraminic acid, and thus we cannot rely solely on the phenotypic tests for variation of the bacterial isolates.

The Biolog system can identify bacterial species both at genus and species levels, as well as through Gram stain reaction. The system does so by providing four top-ranked species for identification; Table 2 indicates that isolates 2, 4, 5 and 6 were Gram negative and belonged to the *Enterobacteriaceae* family. Although the species ID levels for these isolates had different species names for each (of the four top-ranked ID species), they all were identified to be of the genus *Pantoea*. The Gram reaction results obtained here correlate with those performed initially in the study. Isolates 1 and 3 were also predicted to be Gram positive; however, the family names as well as the genus and species names differed among each of the four predicted species names. This difference could be because we utilised a different growth medium from the one recommended in the MicroStation™ System/MicroLog User Guide.[15]

Table 1: Summary of phenotypic characteristics of bacterial endophytes isolated from *Pellaea calomelanos*

Characteristic	1	2	3	4	5	6
Gram reaction	+	-	+	-	-	-
Colony morphology	White circular	Yellow circular	Pink circular	Yellow circular	Yellow circular	Yellow circular
Endospore stain	-	-	+	-	-	-
Motility	-	+	+	+	+	+
Dextrin	+	+	+	+	+	+
Maltose	+	+	-	+	+	+
Sucrose	+	+	-	+	+	+
Stachyose	-	-	-	-	-	-
Ph 6	+	+	+	+	+	+
Ph 5	+	+	-	ND	ND	ND
D-melibiose	-	-	+	ND	ND	ND
D-salicin	+	+	-	ND	ND	ND
N-acetyl-d-glucosamine	ND	ND	-	+	+	+
N-acetyl-β-d-mannosamine	+	+	-	ND	ND	ND
N-acetyl-d-galactosamine	ND	ND	-	ND	ND	ND
N-acetyl neuramic acid	-	-	-	ND	ND	ND
1% NaCl	+	+	+	+	+	+
4% NaCl	+	+	+	+	+	+
8% NaCl	-	-	+	-	-	-
D-mannose	+	+	ND	+	+	+
D-fructose	+	+	+	+	+	+
D-galactose	+	+	ND	+	+	+
L-rhamnose	+	+	+	+	+	+
Inosine	+	+	-	+	+	+
% Sodium lactate	+	+	+	+	+	+
Glycerol	+	+	-	+	+	+
Pectin	+	+	+	+	+	+
Tween 40	-	-	-	-	-	-

Species: 1, Arthrobacter *sp. MHSD1; 2,* Pantoea *sp. MHSD2; 3,* Bacillus infantis *strain MHSD3; 4,* Pantoea infantis *strain MHSD4; 5,* Pantoea *sp. MHSD5; 6,* Pantoea *sp. MHSD6.*
+, positive; - negative; ND, not determined

The MicroStation™ System/MicroLog User Guide[15] states that if the top four identified species belong to the same or closely related genera, then the identification can be concluded as a positive result. The other parameters that can be considered from the identified results are: the probability (PROB), similarity (SIM) and distance (DIST). These parameters indicate the approximate degree of matching between the MicroPlate results and the corresponding database. SIM $\geq$ 0.5, DIST $\leq$ 5.0 and PROB close to 1 indicate reliability of the test results. Although the SIM and DIST results of the top ranked ID species for each isolate were greater than 0.5 and less than 5.0, respectively, the PROB for all isolates was at 0.7 or above 0.7, which is relatively close to 1, thus indicating reliable results.

Although the variation of phenotypic tests could not be concluded from the Biolog MicroPlates (GEN III MicroPlate), the system has shown to be reliable when identifying bacterial species to genus level; further identification can be supported by Gram stain reaction and colony morphology. We do, however, recommend that the use of Biolog MicroPlates (GEN III MicroPlate) be complemented with other strain identification methods, despite the current study showing the reliability of identifying bacterial species by use of the Biolog MicroPlates (GEN III MicroPlate). Furthermore, previous studies have identified and examined phenotypic characteristics of bacterial species utilising this system.[24-27]

Table 2: Biolog MicroPlate readings of isolated endophytic bacteria

Bacterial isolate number	Species ID	PROB	SIM	DIST	Organism type
1	*Arthrobacter globiformis*	0.771	0.612	7.145	GP-Rod
	Arthrobacter oxydans	0.056	0.093	8.908	GP-Rod
	Arthrobacter ilicis	0.034	0.047	8.04	GP-Rod
	Arthrobacter cummunsii	0.052	0.037	8.23	GP-Rod
2	*Pantoea agglomerans*	0.814	0.520	5.216	GN-Ent
	Pantoea dispersa	0.104	0.058	6.537	GN-Ent
	Pantoea cypripedii	0.067	0.036	6.815	GN-Ent
	Pantoea eucrina	0.015	0.007	7.798	GN-Ent
3	*Bacillus cereus/thuringiensis*	0.562	0.562	6.418	GP-Rod
	Brevibacterium linens	0.160	0.160	6.720	GP-Rod
	Corynebacterium xerosis	0.104	0.104	7.257	GP-Rod
	Micrococcus luteus D	0.072	0.072	7.721	GP-Coccus
4	*Pantoea agglomerans*	0.610	0.610	5.622	GN-Ent
	Pantoea dispersa	0.089	0.089	6.596	GN-Ent
	Pantoea cypripedii	0.053	0.053	6.907	GN-Ent
	Pantoea eucrina	0.007	0.007	8.155	GN-Ent
5	*Pantoea agglomerans*	0.575	0.575	6.260	GN-Ent
	Pantoea dispersa	0.102	0.102	7.504	GN-Ent
	Pantoea cypripedii	0.101	0.101	7.526	GN-Ent
	Pantoea eucrina	0.047	0.047	8.486	GN-Ent
6	*Pantoea agglomerans*	0.603	0.603	5.744	GN-Ent
	Pantoea dispersa	0.104	0.104	7.118	GN-Ent
	Pantoea cypripedii	0.086	0.086	7.343	GN-Ent
	Pantoea eucrina	0.057	0.057	7.862	GN-Ent

PROB, probability; SIM, similarity; DIS, distance between #1 and #2 species

GN, Gram negative; GP, Gram positive. The name appearing after the Gram stain result refers to either family or shape of identified bacteria; Ent, Enterobacteriaceae; Rod, rod shaped; Coccus, spherical or ovoid shaped.

Phylogenetic analysis

The 16S rRNA gene sequence lengths (in base pairs) are shown in Table 3. The NCBI BLAST search results indicated that bacterial endophyte isolate number 1 had a 100% identity similarity with *Arthrobacter* spp., bacterial endophyte isolate 3 had 100% identity similarity with *Bacillus* spp. and bacterial endophyte isolates 2, 4, 5 and 6 had 99% identity similarities with *Pantoea* spp. These results indicate that the dominating endophytic bacteria from *Pellaea calomelanos* belong to the genus *Pantoea*. The homology percentage identity was performed among all the bacterial isolates (results not shown). The lowest homology similarity percentage was observed between *Arthrobacter* vs *Bacillus, Arthrobacter* vs *Pantoea* and *Bacillus* vs *Pantoea* isolates, indicating that these are different species. There was, however, a 99% homology similarity between all the *Pantoea* isolates. These isolates were further resolved with phylogenetic analysis (Figures 1–6).

All the delineated phylogenetic trees for the isolates had polytomy relations with closely related species. Isolate 1 had a polytomy relationship with undescribed *Arthrobacter* species and *Arthrobacter polychromogenes* (Figure 1) with a 47% bootstrap value. Isolate 3 had a polytomy relationship supported by a 98% bootstrap value with undescribed *Bacillus* species and other strains of *Bacillus infantis* (Figure 3). Isolates 2, 4, 5 and 6 had polytomy relationships with closely related *Pantoea* species and all showed varying bootstrap values – the phylogenetic relationships were different for each isolate indicating that these are different *Pantoea* species (Figures 2, 4, 5 and 6). A delineated tree including all the isolated *Pantoea* species showed that *Pantoea* sp. MHSD2 and *Pantoea* sp. MHSD4 had a monophyletic relation supported by a 60% bootstrap value, whereas *Pantoea* sp. MHSD5 and *Pantoea* sp. MHSD6 had a polytomy relationship with *Pantoea eucalypti* and *P. brenneri* species (Figure 7). The 16S rRNA gene sequence is the foremost molecular marker used to identify bacterial species; however, from the results obtained, it is evident that 16S rRNA does not resolve the phylogenetic and evolutionary relationships between closely related bacterial strains. The phylogeny of these closely related bacterial strains can be resolved with the use of multilocus sequence analysis.[28]

Figure 1: Maximum likelihood tree based on the 16S rRNA gene sequences of *Arthrobacter* sp. MHSD1 and its closest phylogenetic neighbours. The numbers on nodes indicate bootstrap values after 1000 replicates expressed in percentages. *Escherichia coli* strain ATCC X80725 was included as an outgroup. T indicates type strains.

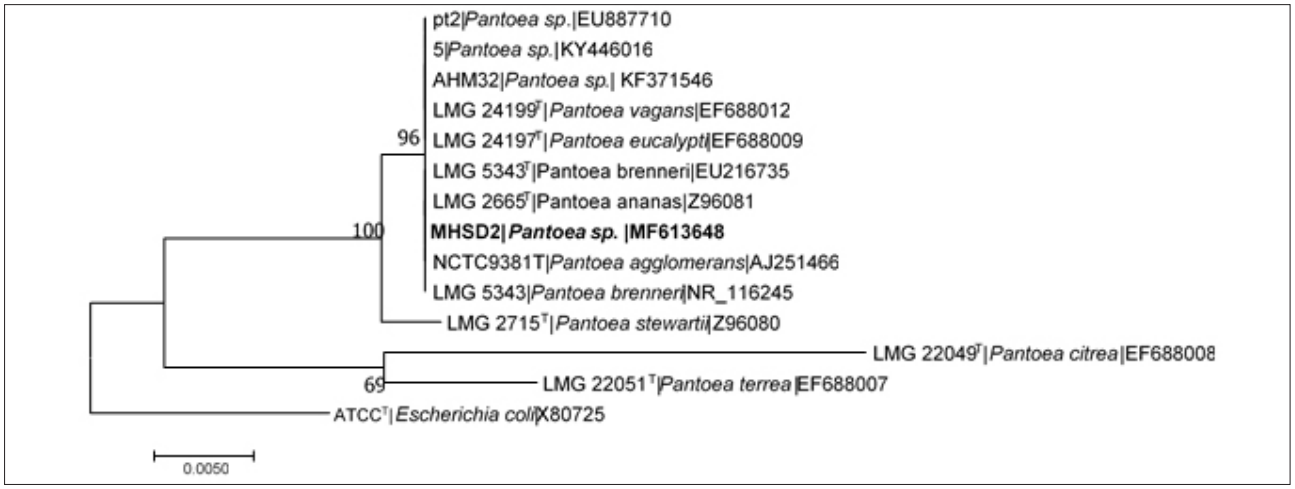

Figure 2: Maximum likelihood tree based on the 16S rRNA gene sequences of *Pantoea* sp. MHSD2 and its closest phylogenetic neighbours. The numbers on nodes indicate bootstrap values after 1000 replicates expressed in percentages. *Escherichia coli* strain ATCC X80725 was included as an outgroup. T indicates type strains.

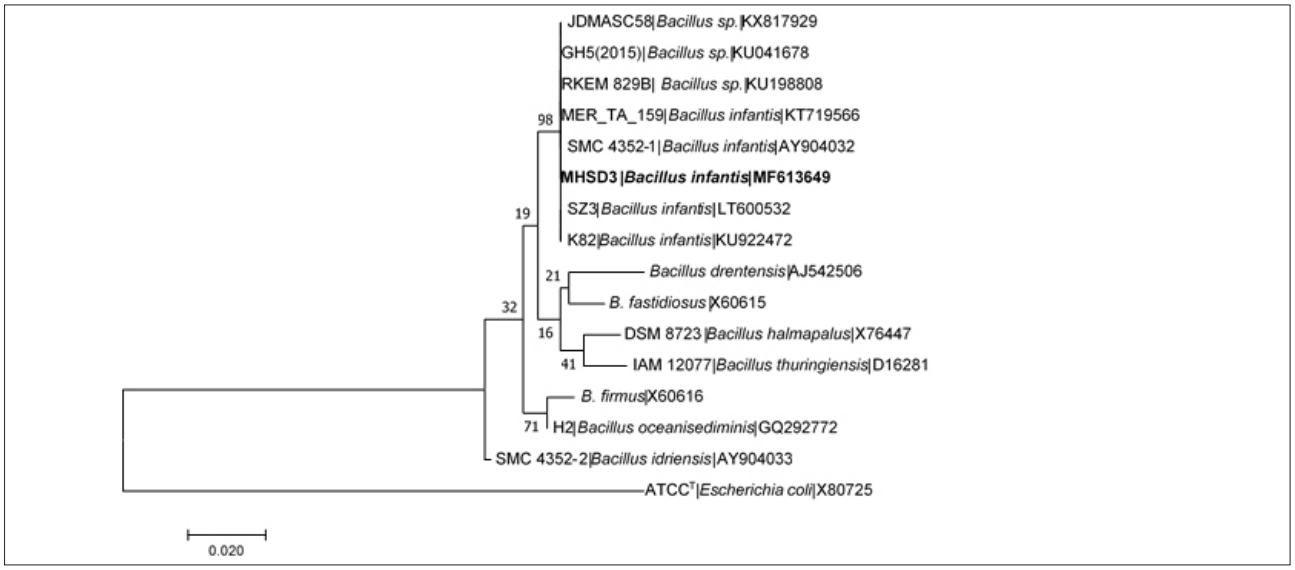

Figure 3: Maximum likelihood tree based on the 16S rRNA gene sequences of *Bacillus infantis* strain MHSD3 and its closest phylogenetic neighbours. The numbers on nodes indicate bootstrap values after 1000 replicates expressed in percentages. *Escherichia coli* strain ATCC X80725 was included as an outgroup. T indicates type strains.

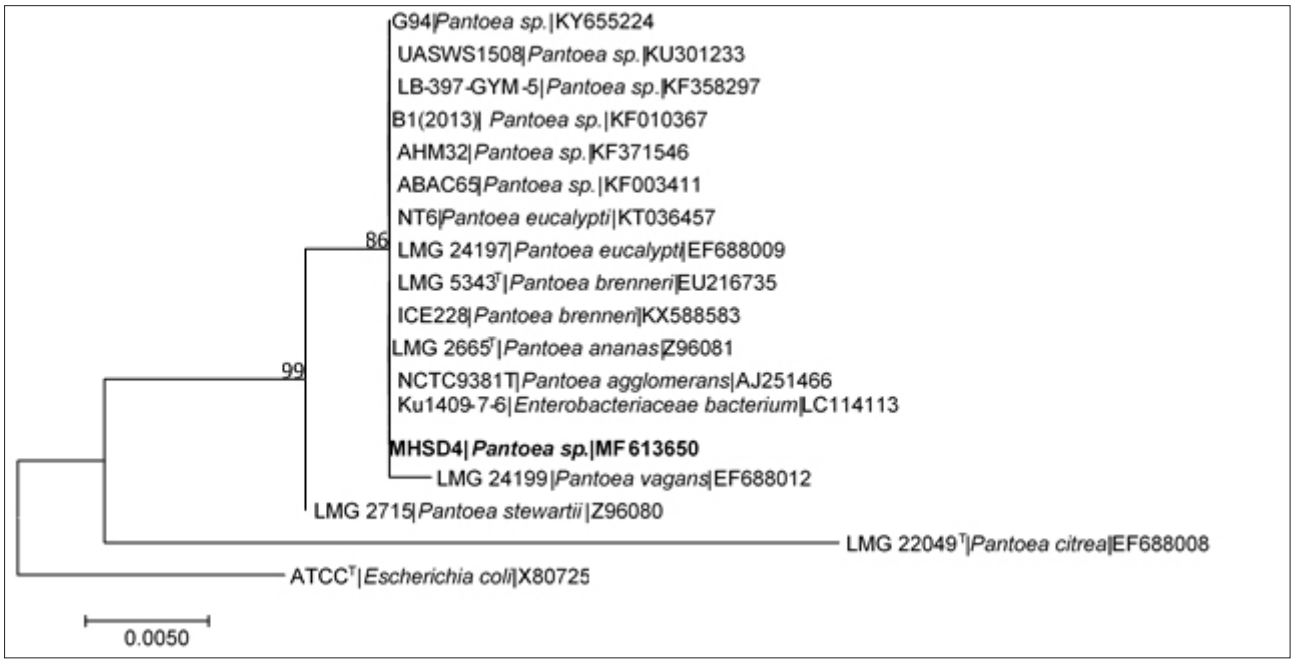

Figure 4: Maximum likelihood tree based on the 16S rRNA gene sequences of *Pantoea* sp. strain MHSD4 and its closest phylogenetic neighbours. The numbers on nodes indicate bootstrap values after 1000 replicates expressed in percentages. *Escherichia coli* strain ATCC X80725 was included as an outgroup. T indicates type strains.

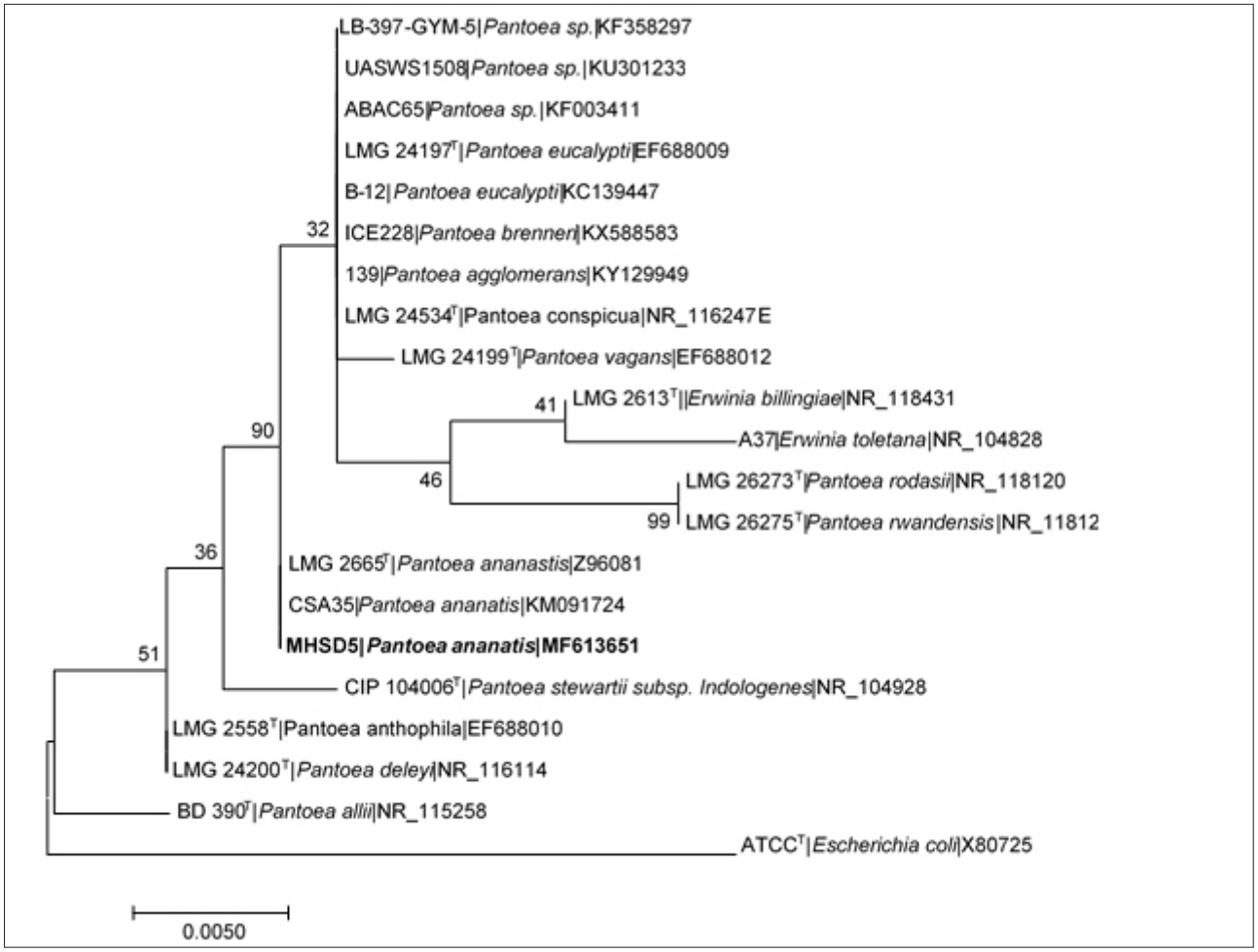

Figure 5: Maximum likelihood tree based on the 16S rRNA gene sequences of *Pantoea ananatis* strain MHSD5 and its closest phylogenetic neighbours. The numbers on nodes indicate bootstrap values after 1000 replicates expressed in percentages. *Escherichia coli* strain ATCC X80725 was included as an outgroup. T indicates type strains.

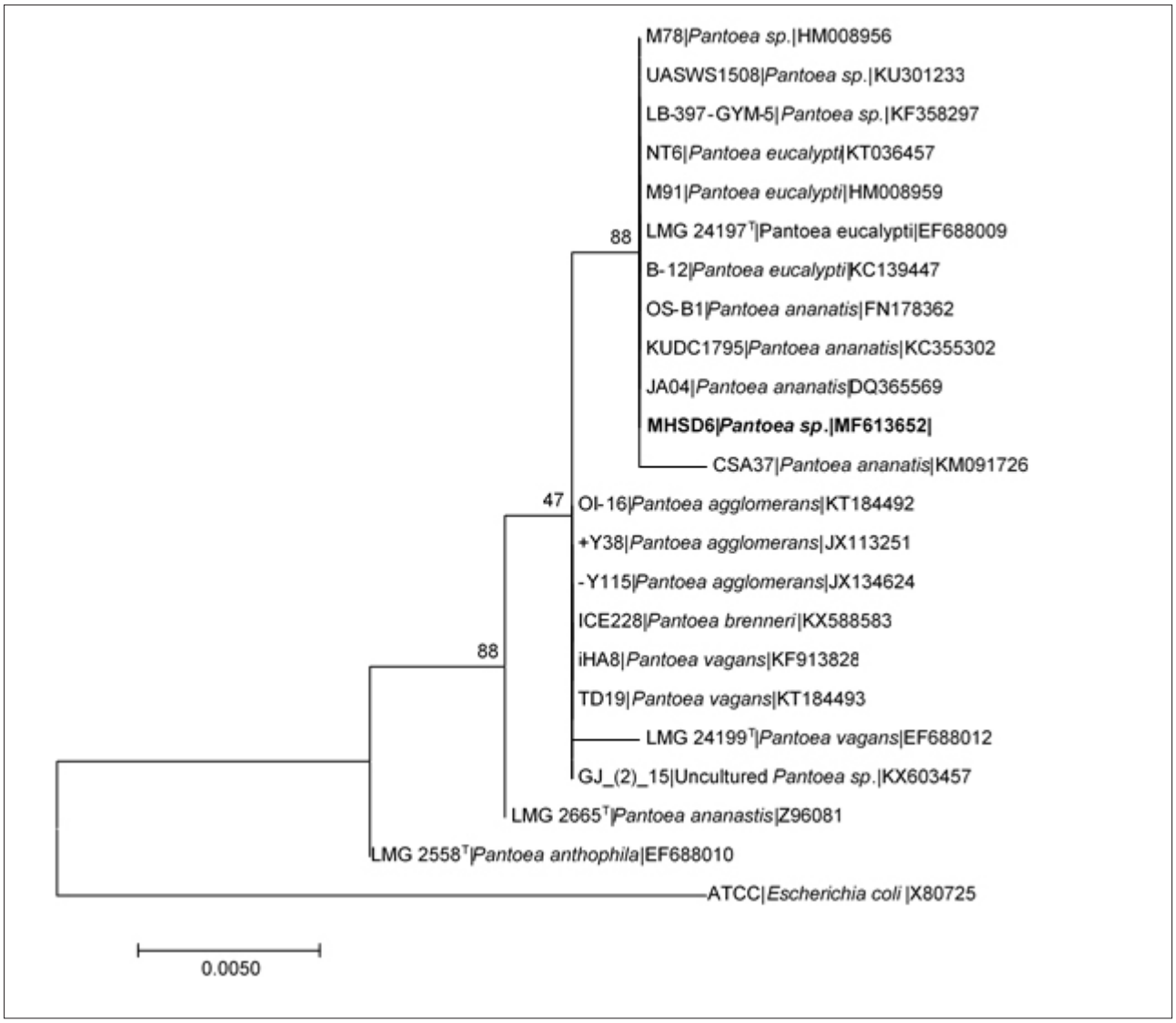

Figure 6: Maximum likelihood tree based on the 16S rRNA gene sequences of *Pantoea* sp. MHSD6 and its closest phylogenetic neighbours. The numbers on nodes indicate bootstrap values after 1000 replicates expressed in percentages. *Escherichia coli* strain ATCC X80725 was included as an outgroup. T indicates type strains.

Table 3: Identification of endophytic bacterial isolates

Bacterial isolate number	Size of 16S rRNA gene (base pairs)	NCBI BLAST hit results				Assigned bacterial isolate/strain name	Assigned accession number
		Dominant bacteria genus	% Query cover	E-value	% Identity		
1	629	*Arthrobacter*	100	0	100	*Arthrobacter* sp. strain MHSD1	MF613647
2	604	*Pantoea*	100	0	99	*Pantoea* sp. strain MHSD2	MF613648
3	600	*Bacillus*	100	0	100	*Bacillus infantis* strain MHSD3	MF613649
4	551	*Pantoea*	100	0	99	*Pantoea* sp. strain MHSD4	MF613650
5	557	*Pantoea*	100	0	99	*Pantoea ananatis* strain MHSD5	MF613651
6	549	*Pantoea*	100	0	99	*Pantoea* sp. strain MHSD6	MF613652

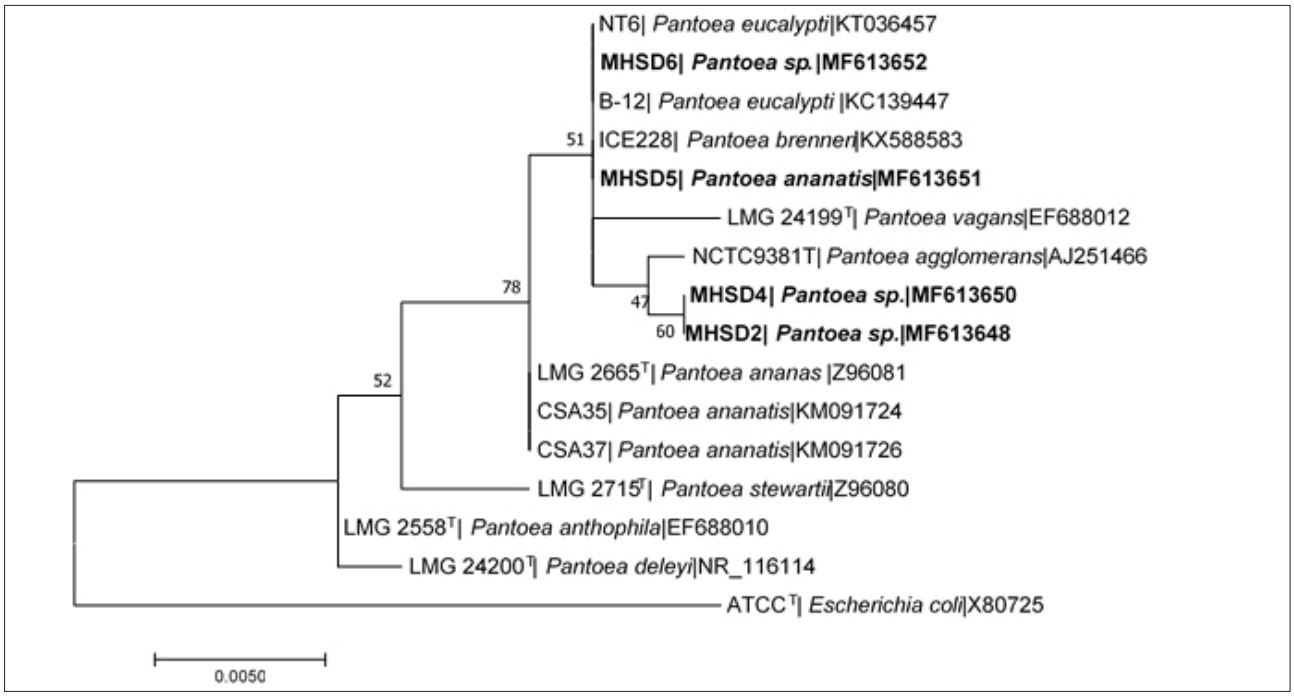

Figure 7: Maximum likelihood tree based on the 16S rRNA gene sequences of *Pantoea* spp. isolated in this study and their closest phylogenetic neighbours. The numbers on nodes indicate bootstrap values after 1000 replicates expressed in percentages. *Escherichia coli* strain ATCC X80725 was included as an outgroup. T indicates type strains.

We isolated bacterial endophytes from only the leaves and we believe the results obtained here will be additional to the minimally reported number of phylloplane bacterial endophytes.[4] Braithwaite et al.[29] have reported on the antimicrobial activity of *P. calomelanos* against bacterial pathogens and yeast. Thus, in exploring the antimicrobial activity and other potential applications of bacterial endophytes from this plant, we had to identify and understand its associated bacterial endophytes.

Pantoea spp., *Bacillus* spp. and *Arthrobacter* spp. are common soil bacteria. Furthermore, these bacterial species have been previously isolated from maize, rice and medicinal plants as bacterial endophytes.[30-33] Although they are considered to be plant endophytes, bacteria from the same genera as the reported bacterial endophytes have also been isolated from diseased plants and clinical samples.[27,34] From these reports and observations, there is a need to understand the evolutionary relationships of these bacterial species, and also the occurrences and differences between bacterial endophytes as plant endophytes and as phytopathogens. Therefore, we recommend that whole genome sequencing and comparisons can provide more comprehensive insights to resolve the evolutionary relationships and identify the bacterial groups to strain level, as well as to identify genetic components that prompt the occurrence of bacterial species as bacterial endophytes.

Soil type[35], season[36,37] and host type[38,39] have been reported to affect the diversity and seasonal fluctuations of bacterial endophytes. These factors could explain the low number of bacterial endophytes isolated from the current study. Furthermore, the *Pantoea* genus was dominant, with four identified *Pantoea* species, and we strongly believe that more bacterial endophytes are likely associated with *P. calomelanos*. Further studies are underway to isolate and identify bacterial endophytes from *P. calomelanos* collected in different seasons and from different soil types. Methods which are culture independent can also be employed in identifying bacterial endophytes.

Bacterial endophytes produce the same or similar metabolites as their hosts. Therefore, because *P. calomelanos* has antibacterial and antifungal activities, the ability of bacterial endophytes from this plant to produce metabolites which (1) have therapeutic activity, (2) are similar to those produced by *P. calomelanos* and (3) have other possible potential applications, needs to be studied.

Conclusion

Bacterial endophytes from *P. calomelanos* are poorly studied. The current study provides information on the isolation and diversity of bacterial endophytes from *P. calomelanos.* This study is a pilot to ongoing research on *P. calomelanos* obtained in South Africa, on its secondary metabolites and bacterial endophytes and potential application of the secondary metabolites and bacterial endophytes. Furthermore, whole genomic studies are underway to understand the evolutionary relationships between bacteria that occur as plant endophytes and plant pathogens. The genomic components that drive the symbiosis between *P. calomelanos* and its bacterial endophytes will also form part of future studies.

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Authors' contributions

M.H.S-D. conceptualised the research; S.G.M. conducted the experiments; both authors analysed the data and contributed to the writing and editing of the manuscript.

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