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The Diversity and Antimicrobial Activity of *Preussia* sp. Endophytes Isolated from Australian Dry Rainforests

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Abstract Limited knowledge currently exists regarding species diversity and antimicrobial activity of endophytic isolates of *Preussia* within Australia. This report describes endophytic *Preussia* species that were identified through molecular analysis of the internal transcribed spacer region. Screening for antimicrobial secondary metabolites was determined by testing crude ethyl acetate (EtOAc) extracts derived from fungal mycelia against a panel of ATCC type strains which included *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, methicillin-resistant *Staphylococcus aureus* (MRSA) and the opportunist yeast pathogen *Candida albicans*. Subsequently, high-performance liquid chromatography generated fractions of bioactive EtOAc extracts which were subject to confirmatory testing using the Clinical and Laboratory Standards Institute reference microdilution antimicrobial activity assay. A total of 18 *Preussia* were isolated from nine host plants with 6/18 having a <97 % sequence similarity to other known species in Genbank, suggesting that they are new species. In preliminary screening, 13/18 *Preussia* isolates revealed antimicrobial activity against at least one of the microbes tested, whilst 6/18 isolates, including 4/6 putative new species showed specific antimicrobial activity against MRSA and *C. albicans.* These results highlight the antimicrobial potential of Australian *Preussia* spp. and also the importance of Australian dry rainforests as an untapped repository of potentially significant bioactive compounds.

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Introduction

The genus *Preussia* is an environmentally diverse but poorly understood taxon. Members of the taxa are predominantly coprophilous, although they have been isolated from wood, soil, dead plant material and occasionally as endophytes [4, 5, 8, 13, 22, 30]. Morphological characteristics for the genus are cleistothecoid ascomata and dark brown phragmospores with elongated germinal slits [4, 5]. Differentiation of *Preussia* from the *Sporormiella* and *Sporormiella* genera based on morphologic examination has been problematic, although supplementation of this data with molecular characterisation of nuclear ribosomal sequences has prompted a general acceptance that new species are now defined as *Preussia* [23]. Several species of *Preussia* have been documented in Australia and include *P. africana*, *P. australis*, *P. minima*, *P. cylindrica* and *P. funiculata* [8, 30]. Furthermore, several species have also been shown to produce bioactive secondary metabolites, particularly the preussomerins which display antimicrobial activity [14, 42, 43]. The spectre of emerging microbial resistance to last line antibiotics has increased the urgency for the discovery and development of new antibiotic formulations [3, 25, 28, 36]. Two strategies have developed to address this challenge. The first is based on combinatorial chemistry which aims at computational design of effective drugs [2, 15], whilst the second is for continued exploration

of the natural biota for novel bioactive metabolites [10, 18, 29, 41]. Fungi have proved to be a rich source of antimicrobial compounds over many years [1, 18, 24, 31, 32, 37]. In this paper, we have explored a largely under-examined group of *Preussia* endophytes isolated from dry rainforests in south-east Queensland, Australia.

Materials and Methods

Sample Collection and Fungal Isolation

A total of 23 plants were sampled across three separate semi-evergreen vine thicket (dry rainforest) sites near Toowoomba in south-east Queensland, Australia. Plants were identified using Harden et al. [21]. Leaves were sampled in duplicate from living plants within each site and processed within 10 h of collection. Leaves were rinsed with water and surface-disinfected by immersion in ethanol and passing through a flame. For test samples, a sterile hole punch was used to remove eight tissue segments per leaf which were placed in Petri dishes containing potato dextrose agar (PDA) with 1.5 mg/ml sterile streptomycin and 1.5 mg/ml tetracycline and cultured at 23 °C under dark conditions.

Identification of Fungal Isolates

DNA was extracted from actively growing cultures using a DNeasy Plant Mini Kit™ (Qiagen, Doncaster, Victoria, Australia). PCR amplification was performed by adding 0.5 µl of the extracted fungal DNA to 14 µl sterile Milli-Q water, 2 µl 10× buffer (Scientifix, Cheltenham, Victoria, Australia), 2 µl 10 mM dNTP (Scientifix) and 0.5 µl of each of the universal fungal primers ITS1F [20] and ITS4 [44] and 0.5 µl of Hot Start DNA polymerase (Scientifix). PCR amplification was conducted at 35 cycles of 94 and 50 °C for 1 min each followed by 72 °C for 1 min and a concluding incubation period of 10 min at 72 °C. Amplicons were resolved by electrophoresis in a 2 % (w/v) agarose gel and observed under UV light. Following purification PCR products were sequenced at the Australian Genome Research Facility (AGRF; Brisbane, QLD, Australia). Sequences were analysed with the NCBI BLAST search program to establish closest sequence matches. Phylogenetic analysis was performed with MEGA version 5 [38]. Sequences were aligned using Clustal W [40] and a neighbour-joining tree [35] was constructed from the alignment file using the Maximum Composite Likelihood method [38] and bootstrapping of 1000 replicates [19]. Following molecular identification *Preussia* isolates were also grouped into morphotypes based on culture morphology and growth rate [6, 7].

Identification of Antimicrobial Activity

Preussia isolates were initially screened for antimicrobial activity using a modification of a method described by Amin et al. [1]. In brief, a 0.5 cm^2 plug of fungal culture was placed in the centre of a Sensitest agar plate (Oxoid Australia, Adelaide, SA, Australia) and incubated at 23 °C. Once cultures were >2 cm in diameter, test microbes were streaked onto the plate beginning at the edge of the fungal colony and continuing outwards. The panel of microbes tested were: *Bacillus cereus* (ATCC 14579), *Escherichia coli* (ATCC 25922), *Enteroccocus faecalis* (ATCC 19433), *Pseudomonas aeruginosa* (ATCC 25668), *Serratia marcescens* (ATCC 14756) and methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 43300) and *Candida albicans* (ATCC 10231). Plates were then incubated overnight at 37 °C and examined for the presence of microbial growth inhibition zones of >1 mm adjacent to fungal hyphae. Following initial screening, biologically active *Preussia* sp. were cultured in 250 ml flasks containing malt extract broth (ME; Oxoid Australia) and incubated under static conditions at 23 °C until vigorous mycelial growth was observed. The temperature was then reduced to 20 °C in order to slow growth and increase secondary metabolite production. After 4 days of growth at 23 °C, a 1 ml mixture of autoclaved MRSA and *C. albicans* cells was aseptically transferred to the cultures to enhance production of antimicrobial compounds [1, 11]. Incubation was then continued for a further 3 weeks, following which the broth and mycelia were extracted using 500 ml of EtOAc. EtOAc extracts were then examined for specific activity against MRSA and *C. albicans* using the reference CLSI microtitre well antimicrobial assay [16]. Wells showing no microbial growth were sub cultured on appropriate agar medium to differentiate between microstatic or microcidal inhibition. Microbial sensitivity to the extracts was recorded as growth inhibition compared to negative (growth medium only) control. Antibacterial standards used were 4 µg/ml oxacillin, 6 µg/ml vancomycin, 3 µg/ml ciprofloxacin, 4 µg/ml tetracycline and 4 µg/ml amphotericin B as the anti-fungal standard.

Analytical HPLC Fractionation of EtOAc Extracts

1–5 mg of each fungal EtOAc extract was dissolved in 200 µl of DMSO of which 100 µl was injected onto an analytical C18 Onyx HPLC column (4.6 mm × 150 mm). Extracts were then fractionated using a methanol/water/0.1 % trifluoroacetic acid gradient, conditions of which have been reported elsewhere [12]. The total run time for each injection was 11 min with five fractions (5 × 1 min) collected between 2 and 7 min. Following fractionation of all eight bioactive extracts, a standard mixture consisting of

methyl 4-hydroxy benzoate, ethyl 4-hydroxy benzoate, benzophenone and uracil (all in 0.125 mg/ml in DMSO) was injected as a positive control for the HPLC process. Following fractionation, solvents were evaporated to dryness, with each fraction being resuspended in 50 or 25 % EtOH, depending on solubility. Fractions were then retested for antimicrobial activity by the CLSI method as described above.

Results

Taxonomic Diversity of *Preussia* Isolates

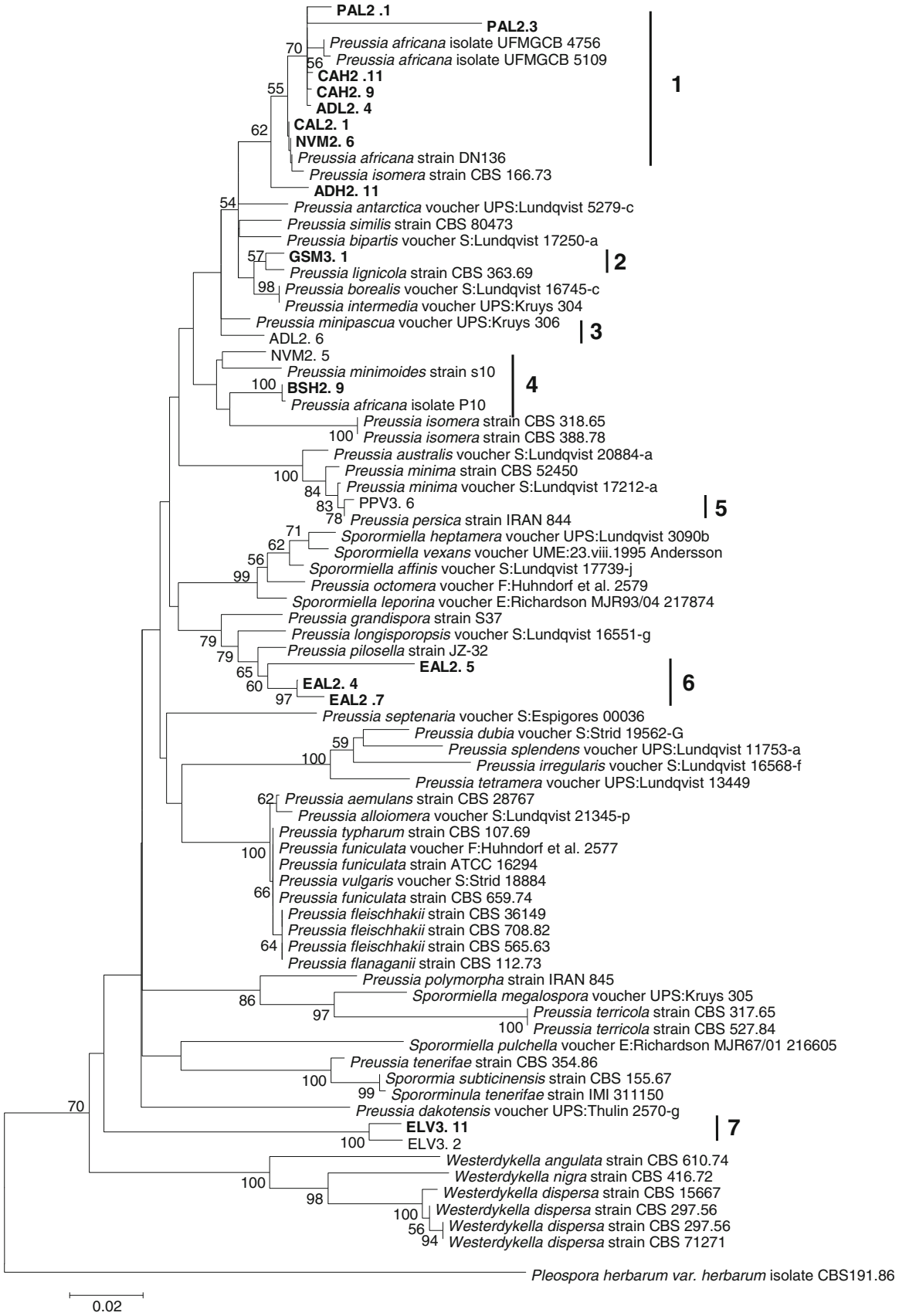
A total of 18 *Preussia* isolates were obtained from nine host plants (Table 1). Eight isolates were uncharacterised *Preussia* taxa having <97 % identity to known sequence when compared against GenBank using the BLAST search option. However, of the eight novel isolates ADM2.11 and NVM2.5 were found to have 98 % similarity over 360 bp and ELV3.11 and ELV3.2 were found to be 99 % similarity over 400 bp, reducing the number of potentially new taxa to six. Of the remaining ten isolates, two had identity to *Preussia africana* (99 % over 507–509 bp). Six isolates had identity to *P. isomera* and two had identity to two separate unnamed *Preussia* spp. (98–99 % over 514–426 bp). Phylogenetic analysis revealed seven separate clades with sufficient bootstrap support for all clades except clades 2 and 3 (Fig. 1). Clade 1 comprised isolates which had closest GenBank matches to *P. isomera* (EU551184.1), with the exception of CAH2.11 whose closest match was a different isolate of *P. isomera* (AY943053.1). Clade 1 also included the PAL2.3 isolate but this is most likely a separate *Preussia* species as it was only 95 % similar to the other members of the clade and had a velvety yellow appearance compared to the orange glossy colonies of the other isolates. When the sequences of clade seven were compared by the Blastn-2 alignment tool all three taxa were found to be less than 97 % similar suggesting they represent different species. Whilst two isolates (BSH2.9, GSM3.1) showed a sequence match of 99 % to *P. africana*, these were different submissions to GenBank and did not group together when phylogenetically analysed.

Antimicrobial Activity

During initial fungal colony screening, 13/18 isolates displayed antimicrobial activity against at least one of the microbe type species tested (Table 2). Subsequently, EtOAc extracts and HPLC-derived fractions were restricted to testing for specific activity against MRSA and *C. albicans* (Fig. 2, 3). The reason for this focus was that current therapeutic options against these two microbes are very limited and often toxic, particularly in the treatment of systemic infections, and all isolates had shown activity towards at least one of these (Fig. 2). The EtOAc extracts from 8/18 *Preussia* isolates showed antimicrobial activity against MRSA and/or *C. albicans* (Table 3). All HPLC fractions were re-tested for antimicrobial activity and revealed that the bioactive compounds eluted between 3

Table 1 Host plant and GenBank matches of *Preussia* taxa isolated in this study

Isolate code	Host plant	Closest match	% Similarity	Proposed identity	GenBank No.
ADL2.6	*Alectryon diversifolium*	FJ210518.1 *Preussia* sp.	99	*Preussia* sp.	JN418765
ADL2.4	*Alectryon diversifolium*	EU551184.1 *Sporormiella isomera*	98	*Preussia isomera*	JN418766
ADM2.11	*Alectryon diversifolium*	GQ203763.1 *Preussia isomera*	95	*Preussia* sp.1	JN418767
BSH2.9	*Bursaria spinosa*	EU551195.1 *Preussia africana*	99	*Preussia africana*	JN418768
CAH2.11	*Cassine australis*	AY943053.1 *Sporormiella isomera*	98	*Preussia isomera*	JN566152
CAH2.9	*Cassine australis*	EU551184.1 *Sporormiella isomera*	98	*Preussia isomera*	JN418769
CAL2.1	*Cassine australis*	EU551184.2 *Sporormiella isomera*	99	*Preussia isomera*	JN418770
EAL2.4	*Erythroxylum australe*	HQ637314.1 *Preussia pilosella*	96	*Preussia* sp.2	JN566153
EAL2.5	*Erythroxylum australe*	GU212409.1 *Pezizomycotina* sp.	92	*Preussia* sp.3	JN418771
EAL2.7	*Erythroxylum australe*	DQ468033.1 *Preussia pilosella*	95	*Preussia* sp.4	JN418772
ELV3.2	*Eustrephus latifolius*	HQ602666.1 *Preussia* sp.	89	*Preussia* sp.5	JN418773
ELV3.11	*Eustrephus latifolius*	HQ602666.2 *Preussia* sp.	89	*Preussia* sp.5	JN418774
GSM3.1	*Geijera salicifolia*	DQ865095.1 *Preussia africana*	99	*Preussia lignicola*	JN418775
NVM2.5	*Notelaea venosa*	EU551195.1 *Preussia africana*	95	*Preussia* sp.1	JN418776
NVM2.6	*Notelaea venosa*	EU551184.1 *Sporormiella isomera*	99	*Preussia isomera*	JN418777
PAL2.1	*Pittosporum angustifolium*	EU551184.2 *Sporormiella isomera*	98	*Preussia isomera*	JN418778
PAL2.3	*Pittosporum angustifolium*	HQ130664.1 *Preussia* sp.	95	*Preussia* sp.6	JN418779
PPV3.6	*Pandorea pandorana*	FJ210521.1 *Preussia* sp.	98	*Preussia* sp.	JN418780


PAL2 .1
PAL2.3
Preussia africana isolate UFMGCB 4756
Preussia africana isolate UFMGCB 5109
CAH2 .11
CAH2. 9
ADL2. 4
CAL2. 1
NVM2. 6
Preussia africana strain DN136
Preussia isomera strain CBS 166.73
ADH2. 11
Preussia antarctica voucher UPS:Lundqvist 5279-c
Preussia similis strain CBS 80473
Preussia bipartis voucher S:Lundqvist 17250-a
GSM3. 1
Preussia lignicola strain CBS 363.69
Preussia borealis voucher S:Lundqvist 16745-c
Preussia intermedia voucher UPS:Kruys 304
Preussia minipascua voucher UPS:Kruys 306
ADL2. 6
NVM2. 5
Preussia minimoides strain s10
BSH2. 9
Preussia africana isolate P10
Preussia isomera strain CBS 318.65
Preussia isomera strain CBS 388.78
Preussia australis voucher S:Lundqvist 20884-a
Preussia minima strain CBS 52450
Preussia minima voucher S:Lundqvist 17212-a
PPV3. 6
Preussia persica strain IRAN 844
Sporormiella heptamera voucher UPS:Lundqvist 3090b
Sporormiella vexans voucher UME:23.viii.1995 Andersson
Sporormiella affinis voucher S:Lundqvist 17739-j
Preussia octomera voucher F:Huhndorf et al. 2579
Sporormiella leporina voucher E:Richardson MJR93/04 217874
Preussia grandispora strain S37
Preussia longisporopsis voucher S:Lundqvist 16551-g
Preussia pilosella strain JZ-32
EAL2. 5
EAL2. 4
EAL2 .7
Preussia septenaria voucher S:Espigores 00036
Preussia dubia voucher S:Strid 19562-G
Preussia splendens voucher UPS:Lundqvist 11753-a
Preussia irregularis voucher S:Lundqvist 16568-f
Preussia tetramera voucher UPS:Lundqvist 13449
Preussia aemulans strain CBS 28767
Preussia alloiomera voucher S:Lundqvist 21345-p
Preussia typharum strain CBS 107.69
Preussia funiculata voucher F:Huhndorf et al. 2577
Preussia funiculata strain ATCC 16294
Preussia vulgaris voucher S:Strid 18884
Preussia funiculata strain CBS 659.74
Preussia fleischhakii strain CBS 36149
Preussia fleischhakii strain CBS 708.82
Preussia fleischhakii strain CBS 565.63
Preussia flanaganii strain CBS 112.73
Preussia polymorpha strain IRAN 845
Sporormiella megalospora voucher UPS:Kruys 305
Preussia terricola strain CBS 317.65
Preussia terricola strain CBS 527.84
Sporormiella pulchella voucher E:Richardson MJR67/01 216605
Preussia tenerifae strain CBS 354.86
Sporormia subticinensis strain CBS 155.67
Sporormiella tenerifae strain IMI 311150
Preussia dakotensis voucher UPS:Thulin 2570-g
ELV3. 11
ELV3. 2
Westerdykella angulata strain CBS 610.74
Westerdykella nigra strain CBS 416.72
Westerdykella dispersa strain CBS 15667
Westerdykella dispersa strain CBS 297.56
Westerdykella dispersa strain CBS 297.56
Westerdykella dispersa strain CBS 71271
Pleospora herbarum var. herbarum isolate CBS191.86
1
2
3
4
5
6
7
0.02

◀ **Fig. 1** Neighbour-joining phylogenetic tree of ITS rDNA of Sporormiaceae sequences. Cladal groupings of the *Preussia* spp. isolated in the study are indicated by numbers in *bold*. AF071345 *Pleospora herbarum* (Dothideales) was used as an out-group to root the tree. Isolates showing bioactivity occur in *bold*

and 7 min from both known and presumptive new *Preussia* species (Table 3). Isolates which had similar bioactive profiles also exhibited similar HPLC traces (Online resource A).

HPLC Fractions of *Preussia* aff. *africana* Isolate

Preliminary chemical analysis of the bioactive HPLC fractions from isolate BSH2.9 (*Preussia* aff. *africana*) suggests that there are up to six new polyketide-derived compounds present in the later eluting fractions (Fig. 3). Chemical analysis consisted of proton nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), followed by literature searching using SciFinder (http://www.cas.org/products/scifinder) and Dictionary of Natural Products [39]. Whilst the polyketide structure class is evident from the initial data analysis, additional chromatographic work is currently underway in order to purify larger quantities of the new compounds. This will enable structure determination studies to be undertaken via interpretation of 1D and 2D NMR data. Initial results indicate that HPLC fractions 2–4 contained secondary metabolites with antimicrobial activity against MRSA, but not *C. albicans* (Table 3).

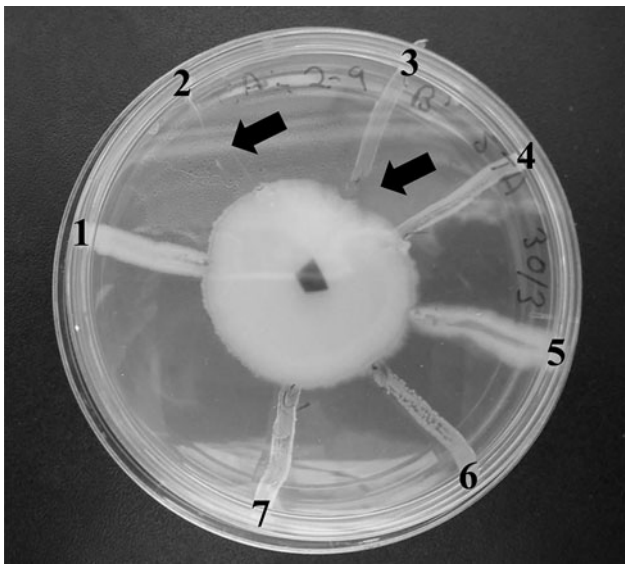


Fig. 2 Example of primary screening of *Preussia* isolate for antimicrobial activity. Microbes used:(*1*) *Bacillus cereus*, (*2*) *Candida albicans*, (*3*) MRSA, (*4*) *E. coli*, (*5*) *P. aeruginosa*, (*6*) *Serratia marsescens*, (*7*) *E. faecalis*. *Arrows* denote zones of inhibition for MRSA and *C. albicans*

Discussion

Species Diversity

The diversity of *Preussia* spp. present in dry rainforest plants of south-east Queensland is surprising given that

Table 2 Antimicrobial activity of *Preussia* isolates

Isolate code	Proposed identity	*B. cereus*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*S. marcescens*	MRSA	*C. albicans*
ADL2.4	*Preussia* sp.	+	–	++	–	–	++	–
ADL2.6	*Preussia isomera*	–	–	–	–	–	–	–
ADM2.11*	*Preussia* sp.1	++	–	++	–	–	+	++
BSH2.9*	*Preussia africana*	+	–	+	–	–	++	–
CAL2.1	*Preussia isomera*	+	–	+	–	–	++	–
CAH2.9*	*Preussia isomera*	–	–	+	–	–	++	++
CAH2.11*	*Preussia isomera*	++	–	+	–	–	++	++
EAL2.4*	*Preussia* sp.2	–	–	+	–	–	++	–
EAL2.5	*Preussia* sp.3	–	–	+	–	–	+	–
EAL2.7*	*Preussia* sp.4	+	–	+	–	–	+	–
ELV3.2	*Preussia* sp.5	–	–	–	–	–	–	–
ELV3.11*	*Preussia* sp.5	–	–	–	–	–	+	–
GSM3.1	*Preussia lignicola*	–	–	–	–	–	–	–
NVM2.5	*Preussia* sp.1	–	–	–	–	–	–	–
NVM2.6	*Preussia isomera*	–	–	+	–	–	++	–
PAL2.1	*Preussia isomera*	–	–	+	–	–	–	–
PAL2.3*	*Preussia* sp.6	–	–	++	–	–	++	++
PPV3.6	*Preussia* sp.	–	–	–	–	–	–	–
Total		6	0	12	0	0	12	4

* Denotes those selected for further analysis

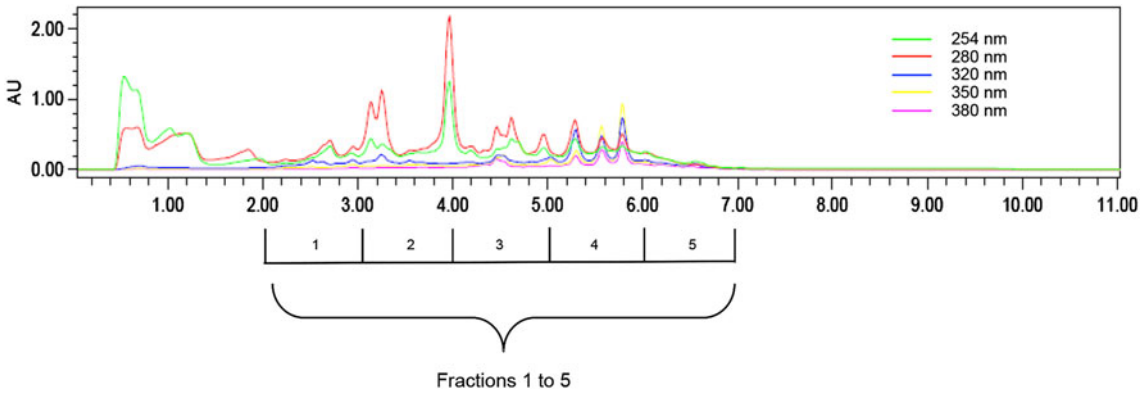


Fig. 3 Analytical HPLC trace of BSH2.9 isolate extract showing fractions collected for initial antimicrobial screening

Table 3 Analytical HPLC fractions from *Preussia* isolates with antimicrobial activity against MRSA and *C. albicans*

Isolate code	Proposed identity	HPLC fractions and MRSA activity					HPLC fractions and *C. albicans* activity				
		1	2	3	4	5	1	2	3	4	5
ADM2.11	*Preussia* sp. (presumptive new taxa 1)	–	–	–	–	–	–	–	–	–	++
BSH2.9	*Preussia* aff. *africana*	–	+	+	+	–	–	–	–	–	–
CAH2.9	*Preussia isomera*	–	+	–	+	–	++	–	++	++	–
CAH2.11	*Preussia isomera*	–	–	–	+	–	++	++	–	++	++
EAL2.4	*Preussia* sp. (presumptive new taxa 2)	–	–	–	+	–	–	–	–	–	–
EAL2.7	*Preussia* sp. (presumptive new taxa 4)	–	+	+	–	–	++	–	–	++	++
ELV3.11	*Preussia* sp. (presumptive new taxa 5)	–	+	+	–	–	–	–	–	++	++
PAL2.3	*Preussia* sp. (presumptive new taxa 6)	–	–	–	–	–	–	++	++	++	–

Five fractions (1–5) were collected from the HPLC separation of each isolate extract

+ bacteriostatic activity observed, ++ fungicidal activity

only a small number of *Preussia* spp. have been previously described in Australia. Peterson et al. [30], for example, reported finding *P. africana*, *P. australis*, *P. minima* and *P. isomera* in the dung of Koalas whilst Bell [8] described *P. cylindrica* and *P. funiculata,* in a study of Australian coprophilous ascomycetes. Bell also identified nine Australian *Sporormiella* spp. all of which have corresponding sequences in GenBank, but none of these correlate with our samples. Samples ADL2.4, CAL2.1, CAH2.9, NVM2.6 and PAL2.1 all returned the same closest match in GenBank (EU551184.1/EU551184.2 *Sporormiella isomera* [syn. *Preussia isomera*]), displayed similar morphological characteristics and grouped together when phylogenetically analysed. From this, it may be concluded that they are different isolates of *P. isomera*. Given that sample PAL2.3 had a divergent ITS sequence and differing colony morphology, it is possible that this isolate is a related, but different *Preussia* species. Samples GSM3.1 and BSH2.9 both returned closest matches to *P. africana;* however, when the samples were phylogenetically analysed, they did not group together. Furthermore, when the samples were compared to each other, they only had a 92 % sequence similarity which would suggest that they are also different species. Moreover, GSM3.1 clustered more closely with *P. lignicola*. From this, it is possible that GSM3.1 is an isolate of *P. lignicola* whilst BSH2.9 is potentially *P. africana.* Further characterisation of the two unnamed species in GenBank, *Preussia* sp. (FJ210518) and *Preussia* sp. (FJ210521), together with the six new species obtained from this study will add to the understanding of species found in Australia for this fungal genus. Current convention is to use both molecular taxonomic and morphological characters for the description of new fungal taxa [27]. *Preussia* spp. are typically distinguished via differences in ascospore morphology [13]. We are endeavouring to induce sporulation in the fungal cultures and will combine morphological descriptions with the ITS data outlined here to name these new species. Phylogenetic analysis of the samples in this study and those in GenBank showed *Sporomiella*, *Spororominula* and *Sporormia* clustered within other known *Preussia* spp. Kruys and Wedin [23] argued for the merging of the genera *Sporormiella*, *Spororominula* and potentially *Sporormia* into the genus *Preussia* [23], and the results of this study would concur with this perspective.

Antimicrobial Activity in Secondary Metabolites

The search for novel classes of antimicrobial compounds is a critical strategy in addressing the emergence of antimicrobial resistance in medically important infections [9, 17]. For example, vancomycin is the antibiotic of choice for treating MRSA infections; however, the drug is renally toxic requiring constant dose monitoring and its effectiveness is being potentially compromised by the emergence of vancomycin-resistant enterococci [3, 41]. Similarly, amphotericin B, which is often the only effective antimycotic for treatment of systemic fungal infections (including *C. albicans*) is similarly limited due to severe infusion-related nephrotoxicity and numerous other side effects [25]. Consequently, we have explored the under exploited genus *Preussia* for potential drug development. Interest in examining endophytic species as a source of novel antimicrobial natural products has been heightened by reports which have shown that when endophyte-colonised plants are challenged by microbial pathogens they activate host defences more efficiently than non-symbiotic plants [29, 33, 34]. As indicated above, initial antimicrobial activity was demonstrated for 13/18 *Preussia* isolates. The activity of EtOAc extracts in eight of these isolates indicated that some of the bioactive compounds are lipid soluble and more specifically, HPLC fractions contained secondary metabolites that inhibited the growth of MRSA and/or *C. albicans*. Interestingly, isolates that had similar ITS regions showed differing bioactivity. In particular, isolate ADM2.11 was found to have a 99 % sequence similarity over 350 bp with NVM2.5. However, when these isolates were tested for bioactivity, sample ADM2.11 showed both antifungal and antibacterial activity whilst NVM2.5 did not. Variable *Preussia* isolate biochemistry has been described by Peterson et al. [30], who found that the two *P. africana* isolates produced such differing enzymatic activity that it was suggested they were potentially different species. However, differing antimicrobial activity among isolates of the same species has not been previously reported for the genus *Preussia*, although they have been reported for many other fungal species [26, 31]. The observance of several isolates that showed either a loss or gain of bioactivity at the HPLC stage is most likely due to concentration issues when testing fractions. Of particular interest was the bioactivity present in HPLC fractions from isolate BSH2.9 (*Preussia* aff. *africana*). Fractions collected at 3, 4 and 5 min each contained mixtures of compounds that inhibited MRSA. Thus far our chemical investigations have suggested the presence of at least six new compounds in these bioactive fractions. Additional HPLC work is currently underway in an attempt to purify all these new compounds.

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