Biologically active endophytic *Quambalaria* sp. from *Leptospermum junipae* in Australia

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An endophyte, designated “AV 17-3”, was isolated from a stem sample of an Australian Prickly Tea Tree, *Leptospermum junipae*, growing in the coastal area of the state of Victoria, Australia. Isolation of the partial 18S rDNA sequence and a subsequent search in GenBank revealed high homology to the fungal genus *Quambalaria*. Standard scanning electron microscopy (SEM) as well as environmental SEM of the isolate revealed that it produces widely elliptically shaped spores (4.5–5.5 × 1–2.2 μm) and secondary budding spores (2.0–3.0 × 1.5–1.7 μm) on conidiophores, all showing a close similarity to *Quambalaria pitereka*. The fungus produces bioactive compounds that were inhibitory to all and lethal to some pathogenic fungi, such as *Phytophthora erythroseptica*. It also inhibited or killed other tested bacteria and fungi, including *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Xanthomonas citri*, *Mycosphaerella fijiensis*, *Saccharomyces cerevisiae* and *Bacillus subtilis*. It is well known that *Quambalaria pitereka* is a cause of blight in Australian tree species; however, our isolate of *Quambalaria* produced no evidence of disease in its host plant and exhibited strong antimicrobial activities against a variety of human and plant pathogens. At least one reddish biologically active product was isolated and shown to be polar and labile.

**Keywords:** bioactive endophyte; bioassay tests; fungi; plant pathogens; rDNA

1. Introduction

Endophytes are fungal and bacterial microbes that reside within the living tissues in plants, while causing no symptoms or signs of disease in their hosts (Strobel and Daisy, 2003). Many endophytes produce unusual and valuable bioactive products (Strobel and Daisy, 2003). The search for novel microbes and the bioactive or otherwise useful compounds existing within such endophytes is often referred to as biodiscovery or bioprospecting. There are approximately 300,000 vascular plant species in the world today, but only a few have been studied for their endophytic microbes (Strobel and Daisy, 2003). Thus, a worldwide search is on for novel and useful endophytes.

*Quambalaria pitereka* has been isolated from species of *Corymbia*, *Blakella*, and *Angophora* in Australia. In these tree species, *Q. pitereka* has acted as a significant pathogen causing necrosis, spotting, and distortion of growing leaves and stems. It has also been reported to cause significant shoot blight on spotted gum trees in the region during their preliminary two years of growth (Pegg et al., 2009).

Recently, a unique endophytic fungus, with 98% homology of the ITS sequence to *Quambalaria pitereka* culture BRIP48531 (GenBank accession no. EF427371.1), was isolated from a Prickly Tea Tree, *Leptospermum junipae*, near Otway Harbor in Australia. The organism was singled out from among the tens of other endophytes within this plant due to its unique growth patterns. Compared to most endophytes, this *Quambalaria* isolate appeared to grow at a significantly slower rate with a sooty/powdery cultural characteristic similar to most streptomycetes. Of most interest, however, this organism produced reddish pigments and released them into the agar. Because most endophytes produce no pigments and grow exceedingly fast, often covering an entire Petri dish within a week, this particular organism was examined for its biological, chemical and physiological properties. Studies of this unique fungus revealed that it produces one or more bioactive organic compounds with strong activity against many plant and human pathogens. In addition, a unique bioassay test for fungal antimicrobials has been devised and is described.

2. Methods

2.1. Isolation of endophytes

Several stems of a mature *Leptospermum junipae*, located at 38° 46′ 71″ S and 143° 09′ 798″ E, in the state of Victoria in Australia, were removed and brought to Montana State University-Bozeman. Several small (2–5 inch) portions of the stems were cut and immersed in 70% ethanol for 30 s under a laminar flow hood. Using sterile tweezers,
the specimens were then exposed to a flame until the alcohol was eliminated. The bark was removed using a sterile blade and underlying tissues were excised. Small pieces of the bark and tissue were placed on separate Petri plates, some containing water agar and others with glycerol/arginine medium (GAM) agar. Similar stem samples were placed on both types of agar plates due to the unknown nature of the endophytes residing in the tissues. Water agar and GAM agar each typically support the growth of different endophytes growth to varying degrees. All plates were stored at room temperature, approximately 23 °C. After 14 days, those endophytes that had grown on the media were transferred to separate plates containing potato dextrose agar (PDA) using a sterile, fine-tipped needle. One particular isolate, designated “AV 17-3,” produced prominent red pigments and was chosen for further research based on its unique biological and morphological properties.

2.2. Scanning electron microscopy of the endophyte

Carnation leaves were placed on the AV 17-3 colonies to induce spore formation. After a 5-day period of growth, fruiting structures on the carnation leaves were examined by light and stereo microscopy. The samples were also prepared for viewing by scanning electron microscopy (SEM). It was performed on an AV-17 according to procedures described by Castillo et al. (2005). Agar pieces and host plant pieces supporting fungal growth were placed in filter paper packets and then placed in 2% gluteraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2–7.4) with Triton X-100, a wetting agent, aspirated for 5 min and left overnight. The following day, they were washed in six 15-min changes in water buffer 1:1 (v/v), followed by one 15-min change in 10% ethanol, one 15-min change in 30% ethanol, one 15-min change in 50% ethanol, five 15-min changes in 70% ethanol, and were then left overnight or longer in 70% ethanol. They were then rinsed six times for 15 min in 95% and then three 15-min changes in 100% ethanol, followed by three 15-min changes in acetone. The microbial material was critically point dried, gold sputter-coated and images were recorded with an XL30 ESEM FEG in the high vacuum mode using the Everhart–Thornley detector.

Because the spores and other fruiting structures of the fungus appeared fragile and easily subjected to disruption, the organism was subjected to the relatively unique microscopic application that preserves the sporophore intact (spores attached). Thus, fresh or non-treated specimens were examined by environmental scanning microscopy (ESEM) and images were recorded with a Philips XL 30 ESEM FEG (Strobel et al., 2007). The temperature was 4 °C with a chamber pressure that ranged from 5 to 6 Torr providing humidity up to 100% at the sample.

2.3. Plate and other bioassays of the bioactive endophyte

The endophyte of interest, AV 17-3, was observed to release dark red pigments into the PDA after growing for 2–3 weeks. After allowing uninhibited growth of the endophyte on Petri dishes with PDA for 4 weeks, bioassays were performed using a variety of pathogenic test fungi. Small (3 × 3 × 3 mm) plugs of agar containing freshly grown cultures of fungi were placed in the range of 0.5–1.0 cm to the AV 17-3 colony on the PDA plate. The fungal test cultures used were: *Pythium ultimum*, *Pythophthora erythroseptica*, *Trichoderma*, *Cercospora*, *Verticillium dahiae*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Mycosphaerella fijiensis*, the black sigatoka pathogen in bananas, *Fusarium solani*, *P. palmivora*, *Aspergillus fumigatus*, *Geotrichum candidum*, *Colletotrichum lagenarium*, and *Ceratocystis ulmi*. All pathogenic cultures were less than 1 week old at the time of bioassay with AV 17-3 and all test fungal organisms were obtained from the Department of Plant Sciences at Montana State University and the bacteria were from the Department of Microbiology.

An additional plate bioassay test was performed on other 4-week-old cultures of AV 17-3 by placing 3 × 3 × 3 mm plugs of the test fungal colonies on the inverted surface of the AV-17 culture growing on PDA. This was accomplished by removing a 3 × 3-cm section of the AV-17-3 culture and placing it upside down in a sterile Petri plate. Pathogenic fungal test organisms on small plugs of agar were placed upon the bottom surface of the inverted AV 17-3 culture and growth was recorded after 24 h. This method may be especially useful for those endophytes that may be producing powerful antimicrobial compounds but often go unnoticed due to their rapid proliferation on PDA (7–10 days) which precludes doing any in vitro plate assays since bioactive products are usually produced only after 14–20 or more days of incubation have occurred. This situation leaves little opportunity to perform a traditional bioassay in order to analyze the bioactive substances. In this case, AV-17-3 was used as a test case for this method of bioassay analysis for antibiotic-like substances.

Yeast and bacteria pathogens were streaked 1–1.5 cm from the edge of the colony and growth was observed and compared to a control plate after 24 and 48 h. Bacterial cultures tested included: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Xanthomonas citri*, the causal agent of citrus canker, and *Bacillus subtilis*. The yeast cultures used for this bioassay were *Saccharomyces cerevisiae*, and *Candida albicans*. These organisms were selected because they represent a range of plant and human pathogens and are representative of both Gram-positive and Gram-negative bacteria. Test organisms were transferred to PDA after 24, 48 and 72 h of exposure of the test organism to check for viability.
2.4. Isolation of bioactive compounds

Several small blocks of PDA containing AV 17-3 were inoculated in 200 mL PD broth in 2-L Erlenmeyer flasks and incubated for 3 weeks at 23 °C in a shaker. The culture filtrate was extracted with either an equal volume of methylene chloride, ethyl acetate or n-butanol. The solvent extract was pooled and dried using a rotary evaporator at 30 °C. The yield of dry material per liter varied depending on the solvent used. Extraction with n-butanol yielded approximately 57 mg of dry material and it was dissolved in 0.5 ml methanol (MeOH), of which 11 mg were placed on a Petri plate with PDA and dried. A small block of agar containing Pythium ultimum was placed 1.0 cm away from the test spot. This fungus was chosen for the bioassay because of its rapid growth and sensitivity in the plate bioassay test before extraction (Young et al., 1992). Unlike the other solvents used, the n-butanol extract produced the best biological activity against P. ultimum and, therefore, it was the solvent of choice to be used for further study and separation of the fungal extract by HPLC and TLC methods.

2.5. Minimum inhibitory concentrations (MICs)

Microboth (PD broth) dilution assays of pathogenic bacteria and fungi were performed as described in the NCCLS M27A (NCCLS, 1997). The assays were performed in sterile 24-well plates. Each plate contained 0.5 ml PDB along with the bioactive compound tested at concentrations from 20 to 400 μg ml⁻¹. The fungal test organisms were cut into 3 mm² agar blocks and placed in the MIC plates with PD broth. Bacterial test organisms were scraped using a thin wire loop and the bacteria was dissolved into the PD broth. All test organisms were obtained from Montana State University. The plates were incubated for 24 h at 23 °C. The MIC was defined as the minimum concentration of compound resulting in no visible growth of the test organism.

2.6. HPLC tests of AV 17-3 extracts

Approximately 1 g (dry weight) of the n-butanol extract of a 3-week-old liquid broth PD culture was fractionated on a Waters 600E HPLC with a Varian Dynamax column ODS (5 μm, 250 × 10 mm) under conditions of flow: water at 5 ml min⁻¹ for 23 min, methanol at 5 ml min⁻¹ for 20 min and then acetonitrile for 20 min. Detection was at 254 nm and, on the most biologically active product, MIC tests were performed to determine the relative effectiveness of the active compound. Finally, thin layer chromatography was performed on the active fraction using Merck 0.25-mm silica plates, standard solvents and the detection was by short wave length UV-254 nm.

2.7. Isolation of fungal DNA and identification of ITS-5.8S and 18S sequence information

Mycelium were isolated from a culture of AV 17-3 that had been grown on PDA for 7 days, after which mycelium were processed using Qiagen’s DNeasy Plant and Fungi MiniKit according to the manufacturer’s instructions to obtain pure genomic DNA. The ITS (Internal Transcribed Spacer) regions of the fungal genomic DNA were amplified by PCR using the universal ITS primers ITS1 (TCCGTAAGGTGAACCTGGG) and ITS4 (TCTTCCGTTATTTATGC). Presence of the correct PCR products was confirmed and PCR product purification was carried out according to Ezra et al. (2004). Pure DNA from the reaction was sequenced at the W.M. Keck facility at Yale University and then processed using PreGap4 and Gap4 programs to find the region of maximum alignment between the two sequences and correct rare base reading errors in areas of low sequence fidelity. Using the BLAST software, aligned sequences were compared to those stored in GenBank on the NCBI website to gauge the degree of homology among the respective rDNA segments between AV17-3 and the closest match in GenBank. ITS sequences for selected organisms were aligned to AV17-3 with MUSCLE 3.7, and gaps cleaned using Phyutility. After trimming of sequence ends in Jalview, a tree was constructed in MRBAYES using a GTR+G+I nucleotide substitution model, with 1,000,000 generations in each of two runs of four chains (Brown et al., 2009; Edgar, 2004; Ronquist and Huelsenbeck, 2003; Smith et al., 2008). In addition, a separate PCR reaction was performed using NS4 (CTTCCGTCATTCCTTTAAG) and SR7R (TAAAAAGCTCGTAGTTGAAC) primers to amplify a more highly conserved portion of the 18S rDNA. The product was subsequently purified, each strand was sequenced and the information was processed as was done for the ITS sequencing.

3. Results and discussion

3.1. Identification of the endophytic fungal isolate- AV 17-3

Although a number of endophytes were isolated from L. junipae, AV 17-3 proved to be of greatest interest. This organism grew best on PDA and released red pigments into the agar after growing for a period of 2–4 weeks (Figure 1). The organism’s cultural morphology resembled a Streptomyces sp. in many respects, especially as related to its powdery surface appearance. The fungus produced slow-growing, erumpent, multi-sectored colonies on PDA. It produced whitish mycelia and, after 3–4 weeks, exuded a water-soluble pigment that diffused into the PDA to give it a distinct red coloration around individual colonies. The organism grew best on PDA followed by nutrient agar (NA) and GAM. Careful microscopic examination
revealed that the microbe was in fact a fungus and not a filamentous bacterial species. An examination of the organism by scanning electron microscopy revealed the presence of numerous solitary or caespitose conidiophores with conidia possessing notable linear striations (4.5–5.5 × 1–2.2 μm) (Figure 2A). Interestingly, there were also secondary conidia (2.0–3.0 × 1.5–1.7 μm) budding from primary conidia and these were best visualized via ESEM (Figure 2B). The conidiophores were hyaline and the spore scars were protruding on small geniculations on the conidiogenous cells. The overall morphology of the organism indicated that it best fit the description of a *Quambalaria* sp. (Simpson, 2000).

### 3.2. BLAST and phylogenetic evidence for identity of AV17-3 as *Quambalaria* sp.

To obtain molecular confirmation of the identity of the organism it was grown on PDA and subsequently its DNA was extracted for sequence analyses. The partial sequences of 18S, ITS1, 5.8S and ITS2 rDNA, as amplified by ITS1 and ITS4 primers, are highly conserved regions of DNA in fungi. Therefore, divergence in the sequences is sufficient to make taxonomic designations about a fungal organism (Mitchell et al., 1995). The sequences corresponding to the aligned portions of the ITS-5.8S and 18S rDNA of AV 17-3, along with a proposed taxonomic identity established based on best homology to the closest ITS match using BLAST, were deposited in GenBank with the accession numbers GQ258351.1 and GQ258352.1, corresponding to the regions sequenced with the ITS and the NS4/SR7R primers, respectively. A BLAST search of the database designated at least 98% sequence homology between the ITS sequence of AV 17-3 and *Quambalaria pitereka* isolate BRIP48531 (GenBank accession no. EF427371.1).

Because the ITS primers are more often used for fungal amplification, the GenBank database did not contain sequence information for the 18S rDNA gene of any *Quambalaria* sp. as would be obtained from amplification and sequencing using NS4 and SR7R primers. Thus, a BLAST search of the database using sequence information from NS4/SR7R primer amplification and sequencing of AV...
17-3 returned a list of isolates from various genera unrelated to *Quambalaria* all with 98% homology to AV 17-3. Furthermore, the ITS sequencing data were processed to generate a phylogenetic tree according to Smith et al. (2008) providing support for the conclusion that AV 17-3 is, indeed, an isolate of *Quambalaria*. ITS sequences of related organisms from the phylogeny of Quambalariaceae family (de Beer et al., 2006) were aligned to ITS of AV17-3 using MUSCLE 3.7 (Edgar, 2004), gaps were cleaned with Phyutility (Smith and Dunn, 2008), and ends trimmed with Jalview (Waterhouse et al., 2009). Using MRBAYES, Markov chain Monte Carlo analysis was done with a GTR+G+I chain nucleotide substitution model with 1,000,000 generations for each of two runs of four chains total. The analysis removed the first 25% of trees as a burn-in. Figtree v1.1.2 was then used to view the tree, which has the *a posteriori* probabilities at the nodes. Scale bar represents branch length for 0.03 substitutions per nucleotide site.

**3.3. Biological activity of *Quambalaria* sp. in antibiotic bioassays**

Cultures of *Quambalaria* sp. that were at least 30 days old, grown on PDA, were tested for their inhibition of plant pathogens. Small plugs of test fungi were placed surrounding the culture and growth was measured after 24 and 48 h. Growth of the fungi was compared to growth of equal sized plugs on a control plate. After 3 days, the test organisms were transferred to a separate plate to determine whether they were still viable or if they had been killed from exposure to *Quambalaria* sp. Bacterial pathogens were streaked next to 30-day-old *Quambalaria* sp. cultures to test its activity against them. Bioassays showed the *Quambalaria* sp. to be remarkably active against all test pathogenic fungi (Table 1), suggesting that *Quambalaria* sp. as an endophyte may play an important role in protecting the host plant from invading pathogens. Certainly, while only a few organisms were tested in the bioassay system, there is a good indication that the bioactivity of *Quambalaria* sp. is broadly based and quite general given that the range of fungal pathogens. However, in most cases only fungal growth inhibition was observed and not killing with the exception of *P. erythroseptica* (Table 1).

A subsequent bioassay was performed by placing test pathogenic fungi on the direct underside of a 4-week-old *Quambalaria* sp. colony and showed similarly high levels of inhibition. Growth and viability were measured after a period of 24 h (Table 2). This bioassay revealed that the pathogenic-inhibitory compounds produced by *Quambalaria* sp. are present on the direct underside of the colony on agar. The underside bioassay tests that were performed on *Quambalaria* sp. suggest that antimicrobial compounds have diffused to the surrounding agar and expressed bioactivity. While the activities of this assay...
Table 1. Biological activity of *Quambalaria* sp. against various fungi and bacteria. “Dead” indicates that no growth occurred in the test period and the organism was not recoverable. Numbers represent the average percentage inhibition ± standard deviations over the control in three separate tests. The test organism, in each of these cases, remained alive. “Inhibited” indicates that the culture did not grow as fast or as well as the control but no linear measurements could be made.

| Test organism                        | Inhibition by *Quambalaria* sp. after 24 h (%) | Inhibition by *Quambalaria* sp. after 48 h (%) | Viability after 3 days |
|--------------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------|
| *Aspergillus fumigatus*              | 37 ± 6                                        | 45 ± 6                                        | Alive                  |
| *Bacillus subtilis*                  | 100                                           | Inhibited                                    | Dead                   |
| *Botrytis cinerea*                   | 20 ± 5                                        | 20 ± 7                                       | Alive                  |
| *Candida albicans*                   | Inhibited                                     | Inhibited                                    | Dead                   |
| *Ceratocystis ulmi*                  | 27 ± 2                                        | 45 ± 6                                       | Alive                  |
| *Cercospora*                         | 24 ± 8                                        | 29 ± 5                                       | Alive                  |
| *Colletotrichum lagenarium*          | 36 ± 7                                        | Inhibited                                    | Alive                  |
| *Escherichia coli*                   | Inhibited                                     | Inhibited                                    | Dead                   |
| *Fusarium solani*                    | 50 ± 7                                        | 75 ± 3                                       | Alive                  |
| *Geotrichum candidum*                | 57 ± 6                                        | 78 ± 1                                       | Alive                  |
| *Mycosphaerella fijiensis*           | 29 ± 7 (after 8 days)                         | 65 (after 22 days)                           | Alive                  |
| *Phytophthora erythroseptica*        | 51 ± 5                                        | 69 ± 3                                       | Dead                   |
| *Phytophthora palmivora*             | 61 ± 5                                        | 36 ± 4                                       | Alive                  |
| *Pseudomonas aeruginosa*             | 100                                           | Inhibited                                    | Inhibited              |
| *Pythium ultimum*                   | 81 ± 3                                        | 92 ± 1.5                                     | Alive                  |
| *Saccharomyces cerevisiae*           | 100                                           | Inhibited                                    | Inhibited              |
| *Salmonella typhimurium*             | 100                                           | Inhibited                                    | Inhibited              |
| *Sclerotinia sclerotiorum*           | 54 ± 5                                        | 79 ± 2                                       | Alive                  |
| *Staphylococcus aureus*              | 100                                           | Inhibited                                    | Dead                   |
| *Trichoderma viride*                 | 82 ± 2                                        | 88 ± 1                                       | Alive                  |
| *Verticillium dahliae*               | 45 ± 7                                        | 65 ± 5                                       | Alive                  |
| *Xanthomonas citri*                  | 100                                           | Inhibited                                    | Inhibited              |

Table 2. Biological activities of the underside of a *Quambalaria* sp. culture. “Alive” indicates that the organism was recoverable after removed from the presence of *Quambalaria* sp. The viability test was done by placing the original inoculum plug on a Petri plate containing potato dextrose agar (PDA). The numbers represent the average percentage inhibition ± standard deviations over the control in three separate tests.

| Test organism                        | Inhibition by *Quambalaria* sp. after 24 h (%) | Viability after 1 day |
|--------------------------------------|-----------------------------------------------|-----------------------|
| *Aspergillus fumigatus*              | 30 ± 9                                        | Alive                 |
| *Ceratocystis ulmi*                  | 24 ± 9                                        | Alive                 |
| *Fusarium solani*                    | 62 ± 2                                        | Alive                 |
| *Geotrichum candidum*                | 55 ± 4                                        | Alive                 |
| *Phytophthora erythroseptica*        | 54 ± 4                                        | Alive                 |
| *Pythium ultimum*                    | 86 ± 1                                        | Alive                 |
| *Trichoderma viride*                 | 77 ± 1                                        | Alive                 |

were comparable to that described above it was noticed that *P. erythroseptica* was not killed in the reverse side assay test. Nevertheless, the method has the potential of identifying the bioactivity of a great variety of fast-growing active endophytes by allowing them to be tested even after colony growth has overtaken the entire plate (Table 2).

Because *Quambalaria* sp. cultures were remarkably active against an array of plant-pathogenic fungi, bioassays were conducted to test it against a variety of human and plant-pathogenic bacteria and yeast as well. Both Gram-positive and Gram-negative bacteria species were tested and all were inhibited or killed by *Quambalaria* sp. (Table 1).

### 3.4. Attempts at product purification and characterization

The results of these bioassays led to attempts to isolate and characterize the active component(s) of *Quambalaria* sp. The results showed the n-butanol extract to be most active against the fungal pathogen, *P. ultimum*. Neither the ethyl acetate nor methylene chloride extract showed significant inhibition against this fungal pathogen, suggesting that the active compound(s) in *Quambalaria* sp. has a polar chemical nature.

Separation of 1.0 g of the n-butanol extract of *Quambalaria* sp. by HPLC revealed the presence of one major reddish component having biological activity with a retention time of 27.6 min. The MICs of this component against some pathogens such as *S. sclerotiorum* and *B. cinerea* were as low as 2.0 μg ml⁻¹, indicating the strong inhibitory activity of this component against their growth. In contrast, the MIC against *P. ultimum* was 75 μg ml⁻¹ and that against *F. solani* was 150 μg ml⁻¹.

Evaluation of the HPLC product by thin layer chromatography on Merck silica-gel plates (0.25 μm) revealed that its R_f in Solvent A = ethyl acetate/methanol/water...
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(6:1:1, v/v) = 0.25 and in Solvent B = toluene/methanol/water (5:2.5:2.5, v/v) = 0.44 (Figure 4). Unfortunately, under all conditions of HPLC and preparative TLC, the bioactivity of the product eventually became reduced after 24 h. Other technologies will be needed to eventually isolate and characterize the compound. Overall, it is suspected to be polar and aromatic in nature. Also, because it is pigmented, it may be related to javanicin, a naphthquinone with anti-bacterial activities (Kharwar et al, 2008).

3.5. Conclusions and anticipated applications of Quambalaria sp.

It remains unknown why a particular _L. junipae_ in Australia harbored an unusually active endophyte with inhibitory powers against a wide range of pathogens, while nearby plants did not reveal similar endophytes. Perhaps, _Quambalaria_ sp. provides enhanced survival ability to the host plant by protecting it from a wide variety of pathogens, many of which have yet to be identified. In addition, this particular _L. junipae_ tree may have offered an especially suitable condition for the growth and survival of this _Quambalaria_ isolate. Furthermore, it may be the case that specific non-pathogenic isolates of this fungus exists primarily to survive in an endophytic form.

Each bioassay test revealed that _Quambalaria_ sp. possesses bioactive compounds with the ability to kill or inhibit the growth of a wide variety of plant and human pathogenic fungi and bacteria. An interesting observation regarding this organism was its inability to kill most pathogens, while producing strong inhibition against them. This may be because the powerful inhibitory effects are typically enough to ward off most pathogens from limiting the growth of the endophyte or harming its host plant. When exposed to the _Quambalaria_ sp. isolate, the pathogens appear to go into a state of inactivity, but retain the ability to resume normal growth and proliferation patterns once removed from the presence of _Quambalaria_ sp. (Table 1). The isolation and characterization of the specific bioactive substances produced by this _Quambalaria_ isolate may offer greater insight into its molecular mechanisms involved especially as they relate to the biology of this endophyte.

The precise biological role of this _Quambalaria_ isolate in its host, _Leptospernum junipae_, remains a mystery. However, measures of its anti-pathogenic activity offer important clues regarding its growth and proliferation in its native environment. The organism clearly produces one or more powerful products, some pigmented, which cause severe growth inhibition and even death in a large variety of plant and human pathogens. The plant pathogens tested in the bioassays represent a variety of genera including many that likely attack plant species in Victoria, Australia. Some pathogens, such as _Pythium_, _Sclerotinia_, and _Phytophthora_, affect a broad range of plant species around the world.

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