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ORIGINAL ARTICLE

Bacterial community in apparently healthy and asymptomatic *Eucalyptus* trees and those with symptoms of bacterial wilt

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Abstract

Ralstonia solanacearum and *R. pseudosolanacearum* are well-known bacterial plant pathogens that cause significant losses to both ornamental and agricultural plants. It has been suggested that they are not the primary cause of bacterial wilt in *Eucalyptus* species, but rather are opportunistic, taking advantage of trees predisposed to infection by abiotic and biotic factors. To test this hypothesis, the bacterial community within the vascular tissue of asymptomatic *Eucalyptus grandis x E. urophylla* trees, and those displaying varying stages of infection in China and Indonesia were compared using 16S rRNA profiling. Asymptomatic trees growing in areas where bacterial infections had never previously been reported to occur were included as controls. *Ralstonia* species were found within the vascular tissue of both asymptomatic and symptomatic trees, in high abundance. In the control samples, bacterial diversity within the vascular tissue was high with a low abundance of *Ralstonia* species. The presence of *Ralstonia* species in asymptomatic and control samples supports the hypothesis that these species are latent and/or opportunistic pathogens in *E. grandis x E. urophylla trees.*

Keywords *Ralstonia* · Bacterial community · *Eucalyptus* · Bacterial wilt

Introduction

Bacterial wilt has been reported in 392 plant species and 78 families in over 100 countries (Lowe-Power et al. 2022). It is considered the second most important bacterial plant disease in the world (Mansfield et al. 2012). The causal agent of this disease was known as the "*Ralstonia solanacearum* species complex" for many years (Fegan and Prior 2005). In 2014, the species complex was resolved and three *Ralstonia* species that cause bacterial wilt were formally described as *R. solanacearum, R. pseudosolanacearum* and *R. syzygii* (Safni et al. 2014). However, the phylogrouping as described by Fegan and Prior (2005) is still extensively used, where *R. solanacearum* belongs to phylogroup II, *R. pseudosolanacearum* to phylogroup I and III, and *R. syzgii* to phylogroup IV. The three species have been linked to geographic regions (Safni et al. 2014; Prior et al. 2016; Lowe-Power et al. 2022) with isolates of *R. solanacearum* typically originating from the Americas, with the pandemic potato brown rot lineage being widely distributed (Lowe-Power et al. 2022). *R. pseudosolanacearum* isolates occur mainly in Africa and Asia with several studies now indicating that this species was introduced into the Caribbean and South America (Santiago et al. 2020; Lowe-Power et al. 2022). *Ralstonia syzygii* isolates are known from East and southeast Asia and has recently been reported to occur in East Africa (Lowe-Power et al. 2022).

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The *Ralstonia* species capable of causing bacterial wilt in plants are soilborne and enter their hosts through wounds and/or natural openings on the roots (Vasse et al. 1995). Within the host plants, the bacteria move towards the xylem vessels where they multiply (Ingel et al. 2021) and produce large amounts of exopolysaccharides (EPS) (Denny et al. 1988). They also begin to degrade the cell walls of the vascular tissue and the pit membranes, resulting in a slimy mixture of bacterial cells, EPS and plant cell contents (Yadeta and Thomma 2013; Ingel et al. 2021). It is this mixture that blocks the flow of water to the aerial parts of the host and wilt symptoms are then observed (Vasse et al. 1995; Ingel et al. 2021). These symptoms are typical not only on herbaceous plants but also in trees such as *Eucalyptus* species that were the subject of the present study. However, during the latent phase or when these trees are resistant to infection, wilt symptoms may not be apparent (Swanson et al. 2007; Shi et al. 2023).

The first report of bacterial wilt in *Eucalyptus* was from China in 1982 (Cao 1982). In *Eucalyptus*, this disease is caused by *R. solanacearum* and *R. pseudosolanacearum* (Carstensen et al. 2017; Santiago et al. 2020). They are geographically separated with bacterial wilt of *Eucalyptus* in the Americas caused mainly by *R. solanacearum* and in Africa and Asia by *R. pseudosolanacearum*. These *Ralstonia* species have been shown to be present within asymptomatic *Eucalyptus* seedlings that are grown in nurseries and to be associated with these plants as they become established in plantations where they are subjected to a wide range of stress factors (Coutinho and Wingfield 2017). Difficulties in proving Koch's postulates for bacterial wilt in *Eucalyptus* species (Carstensen et al. 2017) are likely due to these stress factors which are difficult to emulate under greenhouse conditions.

Bacterial pathogens typically enter a latent period during the infection process where the host is still asymptomatic (Hayward 1974). In some cases, this period can be prolonged and they are thus referred to as latent pathogens. Latent infections by *Ralstonia* species have been reported in various hosts including woody plants (Swanson et al. 2005; Cruz et al. 2014; Du et al. 2017; Tjou-Tam-Sin et al. 2017). The reason they establish latent infections is unclear but various factors have been suggested to play a role in triggering latency. These include inoculum density, age of the host, the nutritional status of the host and plant resistance (Swanson et al. 2005). *Ralstonia* species are also known to occur in a viable but non-culturable state (VBNC) under environmental stress such as low temperature (Grey and Steck 2001). They are able to emerge from this state and cause infection. It is important that in the absence of symptoms, the bacterium may be latent or in a VBNC state. Thus some or all of these factors could be involved in the expression of bacterial wilt on *Eucalyptus.*

Although opportunistic infections are well defined in the medical literature (Amstrong 1993), when opportunistic bacterial plant pathogens are detected, they can be defined in numerous different ways (De Boer 2003; Janse 2005; Charkowski 2016). Currently, opportunism in the case of bacterial plant pathogens refers to cases where the host plants are predisposed to infection due to abiotic or biotic factors (English et al. 1980). In this sense, and for the purpose of the present study, predisposition as defined by Yarwood (1959) is "the tendency of non-genetic factors, acting prior to infection, to affect the susceptiblility of plants to pathogens".

The aim of this study was to investigate whether *Ralstonia* species are present in the vascular tissue of healthy and asymptomatic trees as opposed to those displaying symptoms of bacterial wilt. To answer the question, clonal trees of the hybrid *Eucalyptus grandis x E. urophylla* displaying various symptoms of wilt and those that were asymptomatic were sampled. Healthy trees growing in areas where bacterial wilt has not previously been reported to occur were sampled as controls.

Materials and methods

Sample collection

Core samples (5×5 mm) of the xylem tissue of *Eucalyptus grandis x E. urophylla* clones displaying various stages of wilt reportedly caused by *Ralstonia* species, were collected from plantations in China. Seven trees were sampled of which two had completely wilted (brown/dead leaves), four were dying (wilted, green leaves) and one was asymptomatic (Table 1 and Fig. 1). In Indonesia, four asymptomatic and four symptomatic hedge plants were sampled from a nursery (Table 1). Core samples(one per tree) were also collected from five *Eucalyptus grandis x E. urophylla* trees growing in a South African plantation and were regarded as the healthy controls because bacterial wilt had never been reported in the area (Table 1).

The 5 cm samples collected from each tree in China and South Africa with an increment borer, were broken into 1 mm^2 blocks and placed into 5 ml of RNAlater (Sigma-Aldrich) solution directly after collection in the field. Care was taken to discard the bark from the core samples and only the vascular tissue was placed into the RNAlater solution. The increment borer was sterilized with ethanol prior to collecting each sample. The stem samples from Indonesia were collected by removing the bark from the stems of the hedge plants and then immersing 5 cm of xylem tissue,

Table 1 Origin and disease status of *Eucalyptus* "*urograndis*" trees sampled

Sample	Country	Disease status
China asymptomatic_1	China	Asymptomatic
China symptomatic_1	China	Dying
China symptomatic_2	China	Dying
China symptomatic_3	China	Dying
China symptomatic_4	China	Dying
China wilted (dead)_1	China	Wilted (dead)
China wilted (dead)_2	China	Wilted (dead)
Indonesia asymptomatic_1	Indonesia	Asymptomatic
Indonesia asymptomatic_2	Indonesia	Asymptomatic
Indonesia asymptomatic_3	Indonesia	Asymptomatic
Indonesia asymptomatic_4	Indonesia	Asymptomatic
Indonesia symptomatic_1	Indonesia	Dying
Indonesia symptomatic_2	Indonesia	Dying
Indonesia symptomatic_3	Indonesia	Dying
Indonesia symptomatic_4	Indonesia	Dying
Control (healthy)_1	South Africa	Healthy
Control (healthy)_2	South Africa	Healthy
Control (healthy)_3	South Africa	Healthy
Control (healthy)_4	South Africa	Healthy
Control (healthy)_5	South Africa	Healthy

broken into 1 mm^2 blocks, into 5 ml of RNAlater solution. The RNAlater solution was used to preserve the bacterial DNA for shipment to the laboratory. The samples were stored at −4 °C until they could be processed.

Sample preparation

Three 1 mm^2 blocks of core or stem tissue from each sample collected in China, Indonesia and South Africa were placed in a sterile mortar and ground with a pestle into a fine powder using liquid nitrogen. The resulting vascular tissue was then transferred into a 1.5 ml Eppendorf tube where 500 µl of DNA extraction buffer from the Genomic DNA™ extraction kit (ZymoResearch), 95 µl dH_2O and 5 µl of proteinase K (ZymoResearch) was added to each sample. Once in the buffer solution, the samples were incubated at 55 °C for 10 min and then spun down at 10 000 rpm for 1 min. The supernatant was transferred to a spin column (ZymoResearch). The cell monolayer DNA extraction protocol was then followed according to the manufacturer's instructions (ZymoResearch).

The presence of the extracted DNA was confirmed by running 5 µl of each sample on a 1.5% agarose gel at 80 V for 30 mins. The DNA was mixed with 1 µl of GelRed loading dye (Biotium) before being loaded onto the gel. A 1 kb DNA ladder (Fermentas) was run with the samples. A 16S rRNA gene PCR was also run for each sample to confirm the presence of bacteria. The following primers were used to amplify the 16S rRNA gene: 27F (5' GAG TTT GAT CCT GGC TCA G 3') (modified from Edwards et al. 1989) and 1492R (5' GGT TAC CTT GTT ACG ACT 3') (Lane 1991). Each 25 µl reaction contained 1x Reaction buffer, 1.5 mM $MgCL_2$, 250 µM of each dNTP (dATP, dCTP, dGTP, dTTP), 10 pmol of each primer (forward and reverse), 1.5

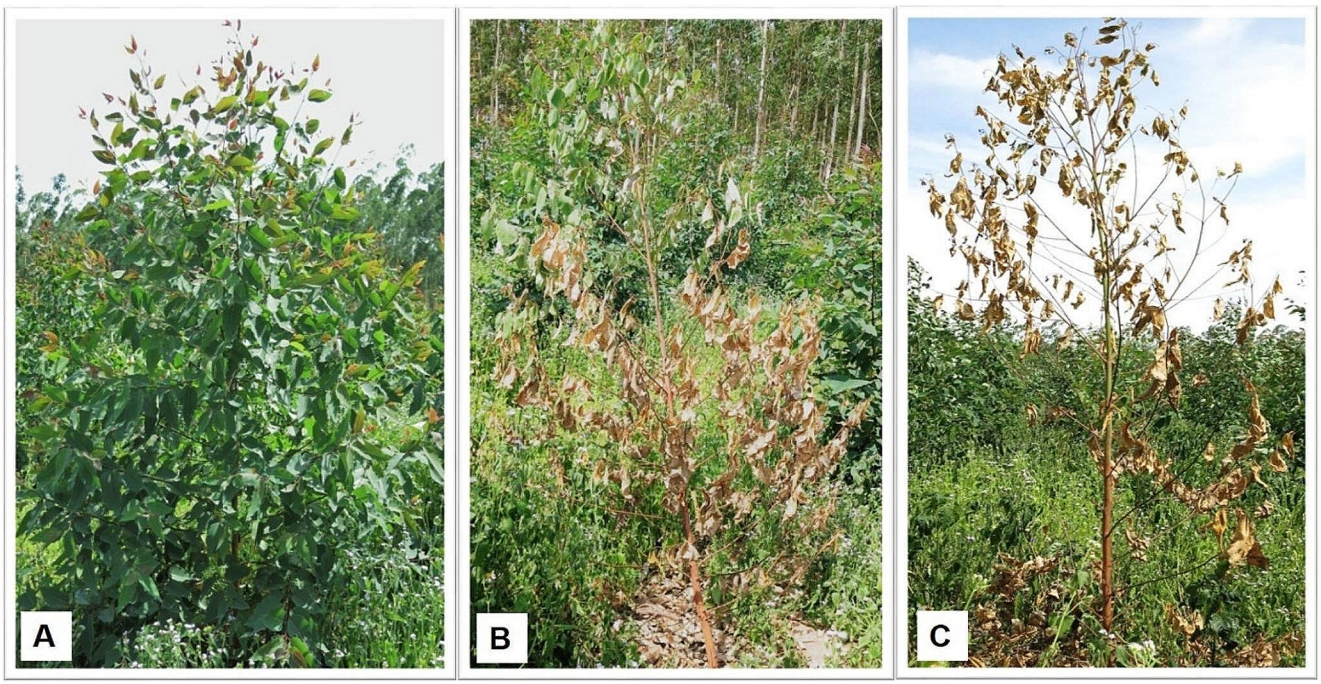

Fig. 1 *Eucalyptus grandis* x *E. urophylla* clones displaying varying symptoms of wilt, growing in China. **A** Asymptomatic tree, **B** Diseased tree and **C** Wilted (dead) tree

U Taq DNA polymerase (Southern Cross Biotechnologies), 1 µl of DNA template and nuclease free water (Promega). Amplification was done in the T100™ Thermal cycler (Bio-Rad). The cycling conditions were as follows: denaturation at 94 °C for 10 min, 30 cycles of denaturation at 94 °C for 1 min, primer binding at 58 °C for 1 min and elongation at 72 °C for 1 min. A final elongation step at 72 °C for 5 min was run before cooling at 4 °C. A negative water control was added to ensure that there was no contamination.

16S rRNA amplicon sequencing and sample data processing

The extracted genomic DNA was sent to the University of Michigan (USA), Medical campus for sequencing. The V4 region of the 16S rRNA gene was sequenced using Illumina MiSeq paired-end sequencing (Kozich et al. 2013). A total of 20 samples were sequenced, seven from China, eight from Indonesia and five from South Africa (Table 1). The raw data was processed using the Mothur software package (v.1.35.1) (Schloss et al. 2009). The method for processing of the raw data was adapted from that used by Kozich et al. (2013). The forward and reverse reads were combined to make contigs. The reads were then quality filtered to remove sequences that contained ambiguous bases or were shorter than 275 bp. The unique sequences were combined to decrease the size of the dataset. The remaining reads were run against the Silva database (Quast et al. 2012) and reads that contained chimeras were removed using UCHIME (Edgar et al. 2011) and were classified using the greengenes database (DeSantis et al. 2006). Any reads classified as Eukaryotic, Archaea, chloroplast, mitochondrial or unknown were subsequently removed. The reads were then grouped into unique representative sequences, also referred to Operational Taxonomic Units (OTUs) with a 97% similarity cut-off after which they were re-classified. Representative OTUs were obtained for each taxonomic group and the relative abundance of each OTU was calculated for each sample for use in downstream analysis.

Community and statistical analysis

The relative abundance values for the bacterial genera present in the control, asymptomatic, symptomatic and wilted (dead) samples were plotted onto percentage stacked bar graphs (Figs. 2 and 3). The bacterial genera indicating a relative abundance value of < 1% were combined and annotated as "other". The Shannon's diversity index values

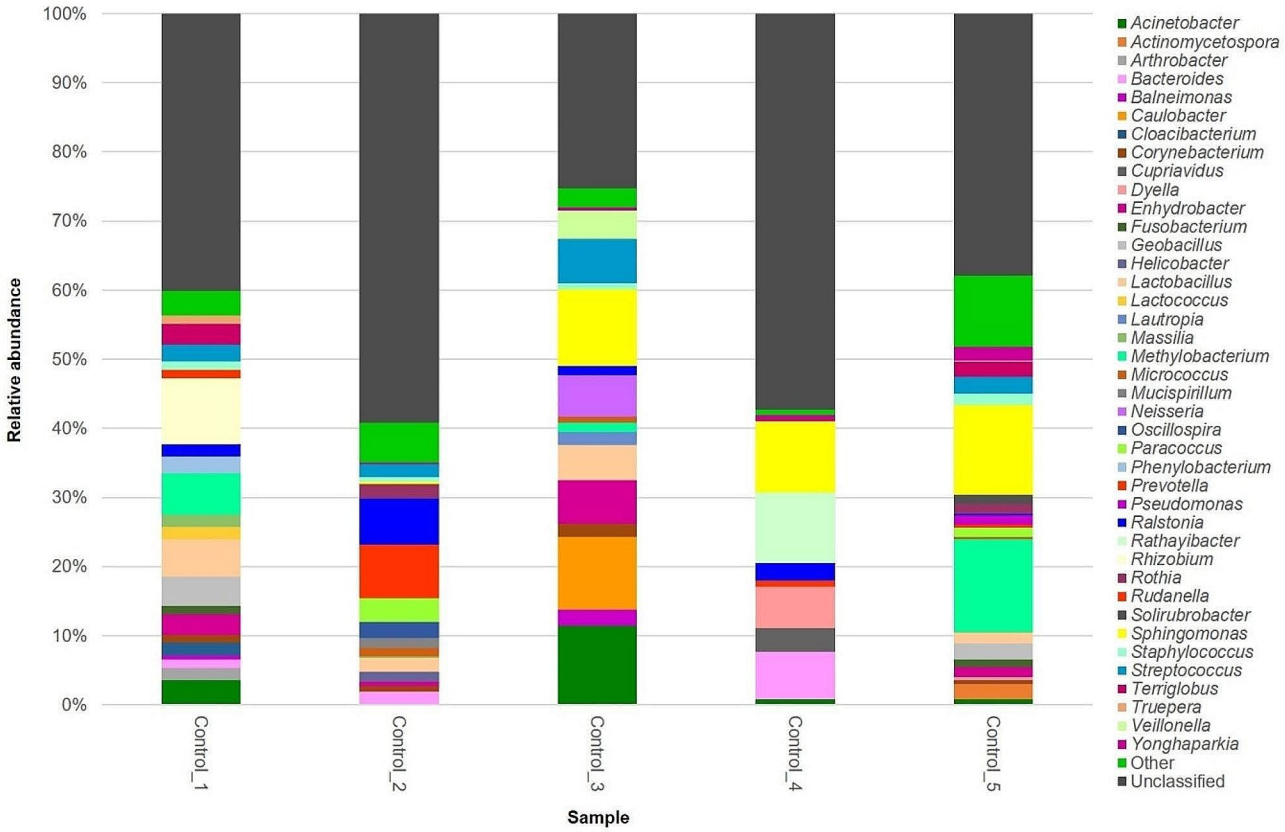

Fig. 2 Graphical representation of the bacterial genera present within each of the control samples obtained from plantations in South Africa where bacterial wilt has not previously been reported to occur

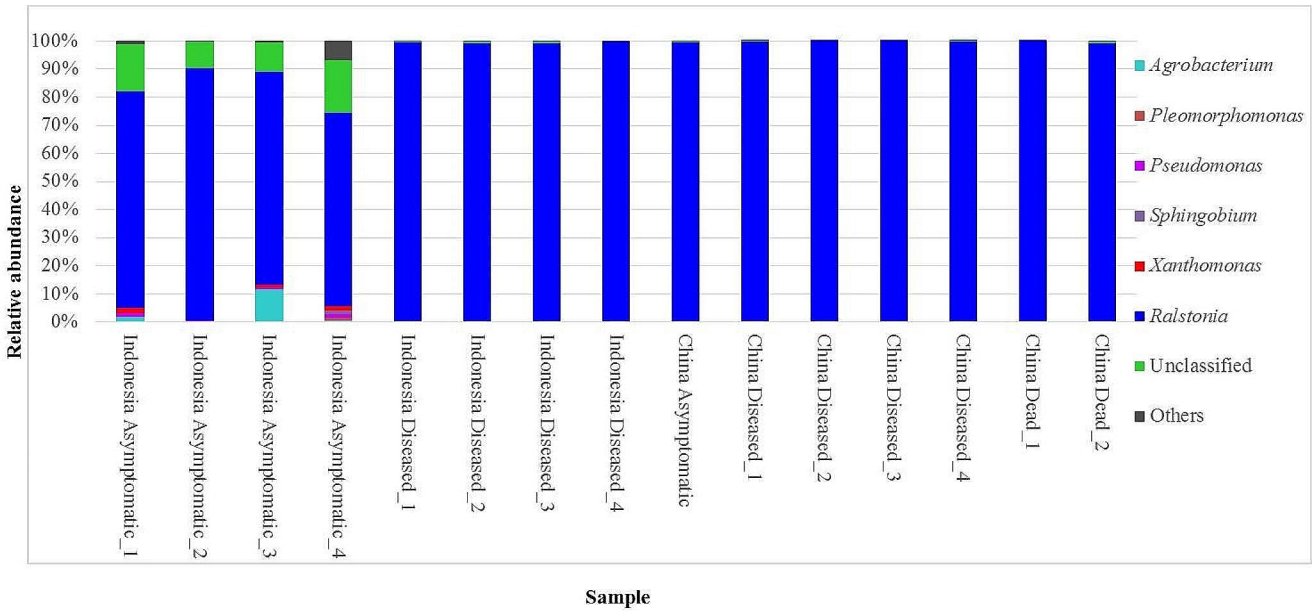

Fig. 3 Graphical representation of the bacterial genera present within the vascular tissue of asymptomatic, diseased (symptomatic) and wilted (dead) trees growing in Indonesia and China

were calculated for each sample using Mothur (Schloss et al. 2009) and plotted in RStudio using ggplot (ver. 2.2.1) (Wickham 2009) (Fig. 4). Nonmetric Multidimentional Scaling (NMDS) ordination was performed in RStudio using Bray-Curtis dissimilarities to identify patterns in the community structures of the asymptomatic, symptomatic and wilted (dead) samples. The "vegan" package in RStudio (ver. 1.9.136) (RStudio Team 2016) was used to construct this plot (Fig. 5). Analysis of Molecular Variance (AMOVA) (Schloss 2008) was run using Mothur (Schloss et al. 2009) to determine the statistical significance between the bacterial endophytic communities within the vascular tissue of the control, asymptomatic and symptomatic samples (Table 2).

Detection of *Ralstonia* species using phylotyping multiplex PCR (mPCR)

To identify the *Ralstonia* species present in the samples, a mPCR (Fegan and Prior 2005) was run for each of the samples. To detect amplification, 5 µl of product from each of the samples was mixed with 1 µl gel red loading dye (Biotium) and run on a 1.5% agarose gel at 5 V/cm. A 1 kb DNA plus ladder (Life Technologies) was run with the samples to determine the size of the amplicons. The gels were viewed under UV light.

Results

16S rRNA amplicon sequencing

A total of 343 operational taxonomic units (OTUs) were identified from the 20 core samples collected from *E. "urograndis"* trees displaying varying symptoms of bacterial wilt and from control (healthy) trees. The dominant phyla within the control trees were Proteobacteria (43–75%), Actinobacteria (3–12%), Bacteroidetes (1–5%), Cyanobacteria (0–24%), Firmicutes (0–20%) and Acidobacteria (0–7%). In the asymptomatic, symptomatic and wilted (dead) trees, Proteobacteria was the most dominant phylum identified in the majority of the samples (96–99%) with Bacteroidetes (0–2%) and Verrucomicrobia (0–1%) being the other phyla present within some of the Indonesian asymptomatic samples.

Community analysis

The relative abundance data for genera present within each sample indicated a higher diversity within the vascular tissue of the control (healthy) samples as opposed to the asymptomatic, symptomatic and wilted (dead) trees (Figs. 2 and 3). The dominant endophytic bacterial genera identified within the control (healthy) samples included *Methylobacterium* (0–13%), *Sphingomonas* (0–12%), *Acinetobacter* (O-11%), *Caulobacter* (0–11%) and *Rhizobium* (0–6%). Within these bacterial communities there were a large number of sOTUs that were unclassified to genus level (25–59%). *Ralstonia*

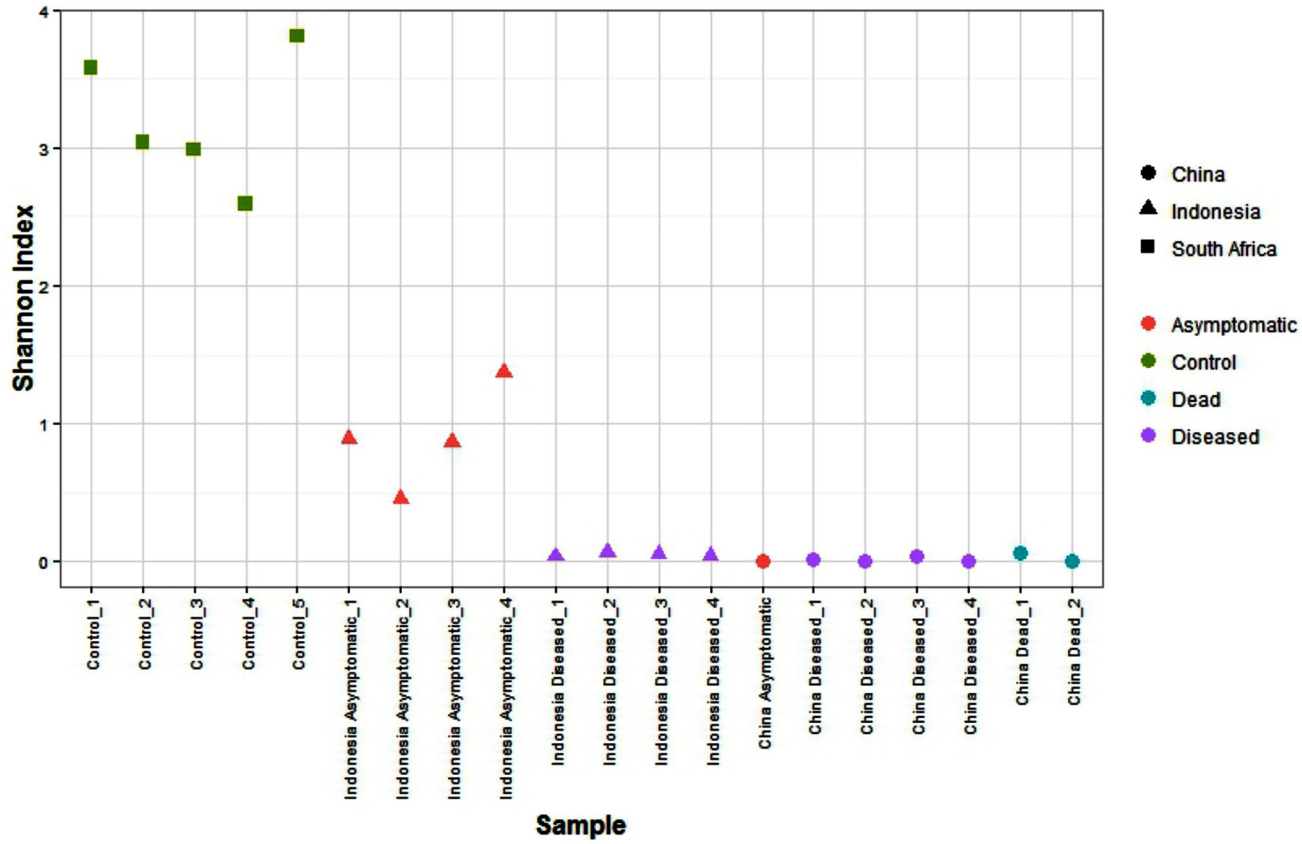

Fig. 4 Shannon Diversity Index of the bacterial communities within the vascular tissue of the control, asymptomatic, diseased (symptomatic) and advanced symptoms (dead) trees. A high species richness is indicated for the control samples, a lower species richness is indicated for the asymptomatic samples from Indonesia and a very low species richness is indicated for those samples originating from the vascular tissue of diseased (symptomatic) trees in Indonesia and all of the samples from China

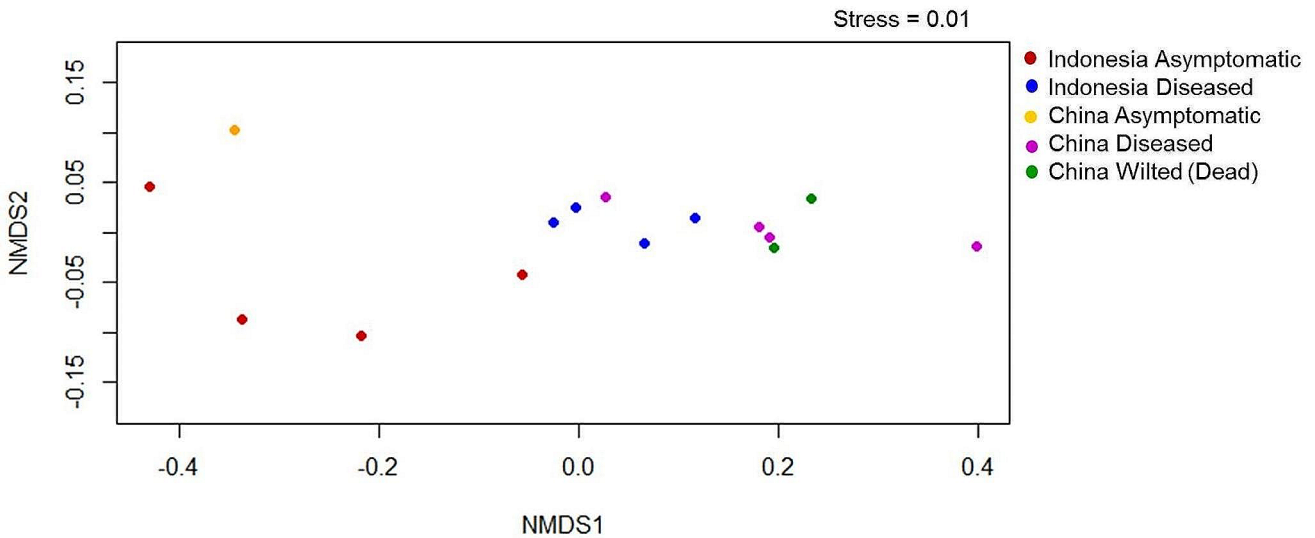

Fig. 5 Non-metric multidimensional scaling (NMDS) ordination of the bacterial communities within the vascular tissue of the asymptomatic, diseased (symptomatic) and wilted (dead) trees from Indonesia and China

Table 2 Analysis of molecular variance (AMOVA) *p*-values for the bacterial communities within the vascular tissue of the control, asymptomatic, diseased and wilted (dead) trees

	Control	Asymptomatic 'healthy'	Diseased	Dead
Control	–	0.008*	0.001*	0.043*
Asymptomatic		–	0.002*	0.040*
Diseased (symptomatic)			–	0.312
Wilted (dead)				–

*p-value < 0.05 indicating significant difference between the bacterial communities

species were detected within the vascular tissue of all give control (healthy) trees, the relative abundance of which ranged from 0.2 to 7%.

The bacterial communities in the vascular tissues of the asymptomatic trees from Indonesia were less diverse than that seen in the control (healthy) trees. Other bacterial genera present within these asymptomatic samples included *Agrobacterium* (0–12%), *Pleomorphomonas* (0–1%), *Sphingobium* (0–1%), *Pseudomonas* (0–1%) and *Xanthomonas* (0–2%). The unclassified bacterial genera within these samples ranged from 1 to 19% relative abundance. The relative abundance of *Ralstonia* species in the vascular tissue of asymptomatic trees from China (100%) was found to be high compared to those asymptomatic trees sampled in Indonesia (69–88%). The vascular tissue of the symptomatic and wilted (dead) trees showed a very high relative abundance of *Ralstonia* species (99–100%). Other bacterial genera identified within these symptomatic and wilted (dead) samples were found to be less than 1% relative abundance.

The Shannon Diversity Index (Fig. 4) indicated high species richness in all of the control (healthy) samples. The symptomatic samples from Indonesia along with the asymptomatic, symptomatic and wilted (dead) samples from China had low species richness. The asymptomatic samples collected in Indonesia had a lower species richness value compared to the control (healthy) samples, but not as low as in the symptomatic samples. The Nonmetric Multidimentional Scaling (NMDS) ordination (Fig. 5) of the bacterial communities within the vascular tissue of the asymptomatic, symptomatic and wilted (dead) trees indicated that the communities within the asymptomatic trees from Indonesia were different to those bacterial communities in the vascular tissue of the symptomatic and wilted (dead) trees. The Analysis of Molecular Variance (AMOVA) results indicated that there was a significant difference (p-value < 0.05) between the bacterial communities in the vascular tissue of the control (healthy) trees, the asymptomatic trees and the symptomatic and wilted (dead) trees (Table 2).

Detection of *Ralstonia* species using phylotyping multiplex PCR (mPCR)

Amplification of the respective gene regions of the mPCR was seen only in the asymptomatic and symptomatic samples obtained from China and Indonesia. The samples grouped in phylogroup I, which indicated that *R. pseudosolanacearum* was present. No amplification was seen in the control (healthy) samples from South Africa.

Discussion

Ralstonia species were present in all trees sampled in this study. These included trees showing various stages of wilt, asymptomatic trees in areas where wilt occurred and in asymptomatic trees where bacterial wilt had never previously been recorded. The relative abundance of this bacterium in the vascular tissue differed depending on the external symptoms of the sampled trees. In the case of asymptomatic and healthy trees, other bacterial species in addition to *Ralstonia* were detected in the sampled vascular tissue. Utilisation of the multiplex PCR also showed that the *Ralstonia* species present in the samples obtained from Indonesia and China were phylogroup I strains, which indicated that they were *R. pseudosolanacearum* (Safni et al. 2014; Prior et al. 2016). This result is consistent with the fact that this *Ralstonia* species is known to be associated with bacterial wilt in *E. grandis x E. urophylla"s*" trees growing in China, Indonesia and Africa (Carstensen et al. 2017). The control (healthy) samples from South Africa most likely harboured *Ralstonia* species cell numbers that were inordinately low for detection using mPCR, although the species present in those trees would most likely be *R. pseudosolanacearum* based on the origin of the species. In a similar study by Klair (2022), on *Casuarina quisetifolia* subsp. *equisetifolia* (ironwood), in healthy and asymptomatic trees testing negative for the bacterial wilt pathogen using immunostrips, endpoint PCR and qPCR, the relative abundance of *Ralstonia* in woody tissue ranged from 1.09 to 4.06% in asymptomatic trees to 99.36% in a single healthy tree root.

In this study, *Ralstonia* species were found in high relative abundance in the wilted (dead), symptomatic and asymptomatic trees from China as well as in the diseased plants from Indonesia (Fig. 3). This suggests that symptoms of bacterial wilt are seen in *Eucalyptus* trees when the bacterial numbers become sufficient to occlude the vascular tissue. This has been reported for herbaceous hosts where high bacterial numbers and large quantities of EPS block the flow of water to the aerial parts of the plants, thus resulting in wilting symptoms (Vasse et al. 1995; Bittner et al. 2016; Ingel et al. 2021). However, blockage of xylem vessels does

not always result in wilting, as shown in resistant/tolerant plants (Grimault and Prior 1993) or in asymptomatic plants (Weibel et al. 2016). In our study, other bacterial genera present in the samples were found to be at very low relative abundance, which was similar to the results obtained by Klair (2022) in *C. quisetifolia* subsp. *equisetifolia*. This suggests that by the time wilt symptoms have developed in these trees, the other bacteria that were present in the vascular tissue had been out-competed by *Ralstonia* species.

Statistically, the bacterial community in the vascular tissue of the Indonesian asymptomatic samples was different from that in the symptomatic and wilted (dead) samples from China and Indonesia. The trend was supported by the Nonmetric Multidimensional Scaling (NMDS) plot. These results again suggest that bacterial wilt symptoms appear only in *Eucalyptus* trees when the *Ralstonia* population is sufficiently high to block the vascular tissues. This would be consistent with the fact that bacterial numbers have been recorded to be as high a 4×10^7 CFU/g of stem tissue when symptoms of bacterial wilt are seen in susceptible tomato plants (Denny 2007).

In the vascular tissue of the asymptomatic *Eucalyptus* trees samples in Indonesia, the OTUs identified as *Ralstonia* species were not dominant as was seen in the symptomatic and wilted (dead) trees. The asymptomatic state of the trees was apparently either due to a latent infection or the fact that the trees were growing vigorously and able to outcompete the bacteria. In the case of latency, the bacteria may have reached high cell densities within the vascular tissue, but the environmental conditions were not favourable for them to reach sufficiently high numbers to occlude the xylem vessels and cause disease (Hayward 1974; Swanson et al. 2005; Ingel et al. 2021). The health status of the trees in terms of the presence or absence of additional abiotic or biotic factors is also an important consideration. In the study by Klair (2022) on *C. quisetifolia* subsp. *equisetifolia*, in samples from woody tissue and roots there was a high abundance of *Ralstonia* in trees where root-infecting fungi (Ganodermataceae) and/or termite activity was prevalent. *Ralstonia* was present in 71% of trees where Ganodermataceae were present and/or where they were damaged by termite activity. There was a high abundance of *Ralstonia* in 7% of trees that had a disease severity score of zero. In a similar study by Ayin et al. (2019), R. *solanacearum* and fungi in the *G. australe* species complex were found to be significant "predictors" of ironwood decline in Guam.

The overall bacterial community structure in the vascular tissue of the control (healthy) trees was more diverse than that seen in the asymptomatic, symptomatic or wilted (dead) trees, a finding supported by the Shannon Diversity Index. Similar observations were made by Klair (2022) in *C. quisetifolia* subsp. *equisetifolia* samples. *Ralstonia* was detected at very low relative abundance in the vascular tissue of the control (healthy) samples. In one root sample classified as coming from an asymptomatic tree and with a disease severity score of zero, the relative abundance of *Ralstonia* was calculated at 99.36%. It is not uncommon for *Ralstonia* to be detected in healthy trees (Campisano et al. 2014; Hardoim et al. 2015; Shen and Fulthorpe 2015), however, the identity of the *Ralstonia* species present in these trees was not investigated. Our results and those of Klair (2022) support the view that during disease development in *Eucalyptus* and *C. quisetifolia* subsp. *equisetifolia*, symptoms of bacterial wilt are seen only when the bacterial numbers of *Ralstonia* are very high and the trees are stressed.

The presence of *Ralstonia* in the control (healthy) samples suggests opportunism (Coutinho and Wingfield 2017) and/or latency (Swanson et al. 2005) due to a lack of symptoms or the presence of the disease in the area sampled. The term "opportunistic plant pathogen" is one not commonly used in plant pathology (Charkowski 2016). It has, however, been used in the literature to refer to those microoorganisms that take advantage of a host with a weakened immune system and hence disease development includes the state of the host, i.e. nutritional status and fitness, the environmental conditions and the virulence of the pathogen (Yadeka and Thomma 2013). Following this definition, it is probable that *Ralstonia* causes disease in *Eucalyptus* only when the immune system of the host is compromised. This has significant implications in *Eucalyptus* plantation forestry where tree health arising from, for example, poor planting practice, should not be attributed solely to pathogens such as *Ralstonia*.

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Author contributions T.C., S.V. and M.J.W. conceived and designed the experiments and led the project. C.G., S.C. and M.T. collected the samples. C.G. performed the laboratory work and wrote a first draft of the manuscript. T.A.C., S.V. and M.J.W. revised the manuscript. All authors read and approved the final manuscript.

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Data availability All sequencing data are available from the corresponding author.

Declarations

Ethical approval The authors declare that the research complies with ethical standards.

Conflict of interest All authors declare no conflict of interest.

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