Phytopathology Research

RESEARCH

Open Access

The first complete genome of *Robbsia andropogonis* reveals its arsenal of virulence system causing leaf spot disease of areca palm

Jingyang Sun[1†], Yonglin Li[2†], Li Zheng[3], Daipeng Chen[3], Xiaofan Zhou[2] and Peng Li[1,2,4*]

Abstract

Robbsia andropogonis is one of the most destructive leaf spot disease pathogens of numerous host plants and causes heavy economic damage. In the present study, the complete genome of *R. andropogonis* strain BLB1, causing the leaf spot disease of areca palm, was generated using a hybrid method combining ONT PromethION long reads and BGISEQ-500 short reads. The resulting genome consists of seven replicons totaling 6,828,120 bp, and 5,808 genes were annotated, including 788 virulence-related genes. Function analysis showed that genes involved in metabolism were the most abundant group. Impressively, the bacteria were well-equipped with four, two, and four sets of type three, four, and six secretion systems, respectively, highlighting the virulence features of *R. andropogonis* BLB1. As the first complete genome sequence of the species of genus *Robbsia*, the BLB1 genome provides a solid foundation for investigation of mechanisms underlying the pathogen virulence and disease control, and will promote further discovery and characterization of the genus *Robbsia*.

Keywords Leaf spot disease, *Burkholderia*, Horizontal gene transfer, Secretion system, Virulence factors

Background

Areca palm (*Areca catechu* L.), known as betel nut or areca nut, is an important tropical medicinal crop and is also used for masticatory and industry purposes in Asia countries (Henderson 2009). The areca palm is a source of alkaloids and flavonoids (Giri et al. 2006); the medicinal properties owned by areca palm have been used to treat leukoderma, worms, anaemia, and nasal ulcers (Manimekalai et al. 2018; Yang et al. 2021), and provide livelihood options to millions of farmers in the Old World tropics.

As the second largest commercial crop in Hainan Province, China, the susceptibility of areca palm to various diseases caused by insects and pathogens poses a great economic threat. The bacterial leaf spot disease, caused by *Robbsia andropogonis* (synonym: *Burkholderia andropogonis*), is one of the most destructive foliar diseases of the areca palm, which causes heavy damage, especially during the rainy season (Tang et al. 2013; Lopes-Santos et al. 2017). In the early stage of the disease, radiating and water-soaking lesions with yellow edges appear on the leaves. The lesions spread and form one-centimeter-length brown stripes with yellow

†Jingyang Sun and Yonglin Li contributed equally to this work.

*Correspondence:
Peng Li
bio_lip@hainnu.edu.cn
[1] Ministry of Education Key Laboratory for Ecology of Tropical Islands, Key Laboratory of Tropical Animal and Plant Ecology of Hainan Province, College of Life Sciences, Hainan Dongzhaigang Mangrove Ecosystem Provincial Observation and Research Station, Hainan Normal University, Haikou 571158, China
[2] Guangdong Province Key Laboratory of Microbial Signals and Disease Control, Integrative Microbiology Research Centre, South China Agricultural University, 483 Wushan Road, Guangzhou 510642, China
[3] Hainan Yazhou Bay Seed Laboratory, Sanya Nanfan Research Institute of Hainan University, Sanya 572025, China
[4] Key Laboratory of Green Prevention and Control of Tropical Plant Diseases and Pests, Ministry of Education and School of Plant Protection, Hainan University, Haikou 570228, China

haloes around them, and several to dozens of these spots can be found per leaf, leading to declined production of areca nut. Recently, the bacterial leaf spot disease caused by *R. andropogonis* has been especially severe in Hainan Province, China, raising great attention to this pathogen and its pathogenicity mechanisms. However, hardly any research progress on its virulence mechanisms and disease control has been reported, and the genome of *R. andropogonis* isolated from areca palm has not been fully sequenced. Notably, *R. andropogonis* can also infect other host plants, such as *Pueraria montana* var. *thomsonii* (Cui et al. 2022), *Sorghum bicolor* (Young et al. 2022), *Bougainvillea* spp. (Morales-Galvan et al. 2022), and *Simmondsia chinensis* (Cother et al. 2004). Thus, *R. andropogonis* is also a broad host range pathogen that can affect many crops worldwide. Due to their global importance as phytopathogens, we are urged to reveal their genomic characteristics and infection mechanisms.

In this work, we isolated *R. andropogonis* strain BLB1 from areca palm, performed genome sequencing using the BGISEQ-500 and ONT PromethION platforms, obtained a complete genome assembly, and conducted systematic genomic analyses. Our results demonstrated that this phytopathogen has powerful and unique virulence systems, such as four type III secretion systems, two type IV secretion systems, and four type VI secretion systems, as well as other genomic characteristics among known *R. andropogonis* strains. Moreover, the draft genomes of the other five available *Robbsia* strains were also analyzed, and their main virulence factors were annotated. So far, the wealth of data reported here provides a robust reference for further research on *R. andropogonis*.

Results

General genomic features of *R. andropogonis* strain BLB1

From the typical leaf spot disease samples infected by *R. andropogonis* (Fig. 1a), we isolated and identified the pathogen strain BLB1 following Koch's postulates. The quality of *R. andropogonis* strain BLB1 genome was assessed by BUSCO analysis with the enterobacterales_odb10 dataset, revealing a near perfect completeness of 99.8%. The resulting complete genome consists of 6,828,120 bp, with an overall GC content of 58.78%. The result of whole-genome sequence-based phylogenetic analysis demonstrated that strain BLB1 had the closest relationship with *R. andropogonis* ICMP 2807 (Fig. 1b). In addition, the average nucleotide identity (ANI) value between BLB1 and the *R. andropogonis* ICMP 2807 was 100%. Here, we also analyzed genomes of strains of *R. andropogonis* (Table 1) and *Robbsia betulipollinis* (isolated from the pollen of *Betula pendula*) (Shi et al. 2023) and found that the BLB1 clustered with the other *R. andropogonis* strains with 100% bootstrap support (Additional file 1: Figure S1). Seven replicons were found in the BLB1 genome. We suggest to name the three larger replicons as Chromosome 1 (3,671,051 bp; GC content 59.75%), Chromosome 2 (1,375,786 bp; 57.96%), and

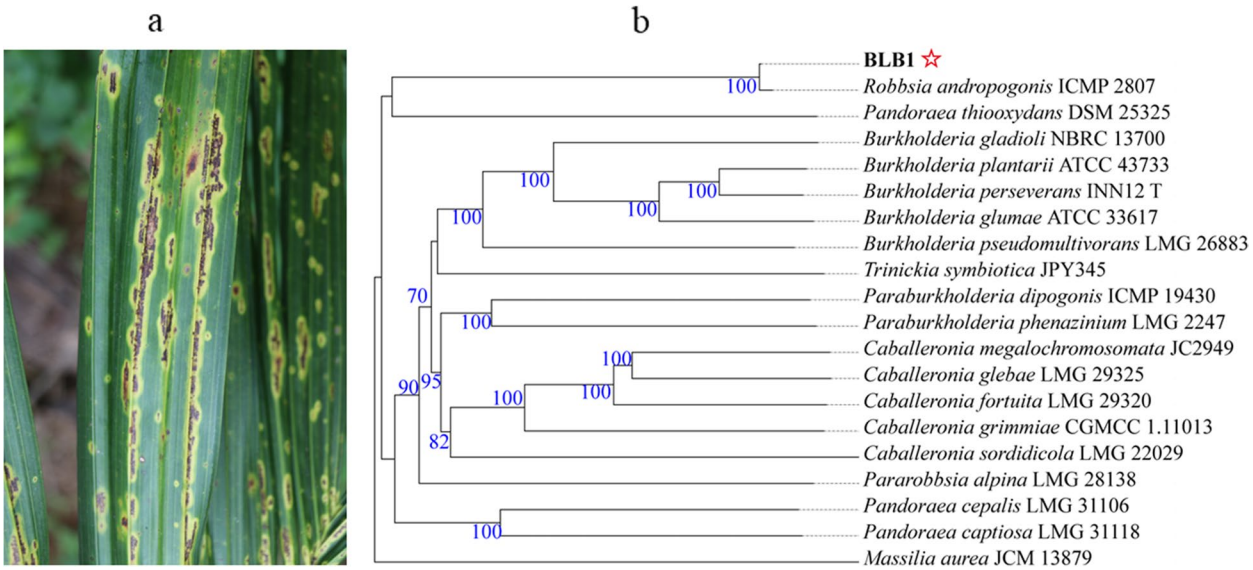

Fig. 1 **a** Typical leaf spot disease symptom of areca palm, **b** genome phylogenic cluster analysis of strain BLB1 with its closely related species. The conserved genes were used to perform phylogenic analysis together with the most closely related species in the Genome Taxonomy Database, and the ANI between genomes of BLB1 and other closely related strains was calculated with pyani v0.2.11 software and online tool GGDC3.0 (http://ggdc.dsmz.de/ggdc.php)

Table 1 The genome statistics of newly sequenced BLB1 and the other five sequenced *R. andropogonis* strains

Strain	GenBank	Size (Mb)	GC%	Gene	Host	Completed
BLB1		6.828	58.78	5808	*Areca catechu*	Yes
LMG2129	GCA902833845.1	6.329	58.90	5475	*Sorghum bicolor*	75 contigs
ICMP2807	GCA000970345.1	6.204	58.90	5498	*Sorghum bicolor*	272 contigs
BRIP72957a	GCA024160665.1	6.429	58.80	5717	*Sorghum bicolor*	305 contigs
BRIP72872a	GCA024160725.1	6.437	58.80	5745	*Sorghum bicolor*	344 contigs
Ba3549	GCA000566705.1	6.179	58.90	5408	*Saccharum officinarum*	301 contigs

Chromosome 3 (1,063,849 bp; 58.05%), and the other four replicons as Plasmid 1 (210,877 bp; 55.80%), Plasmid 2 (206,007 bp; 56.98%), Plasmid 3 (176,508 bp; 56.38%), and Plasmid 4 (124,042 bp; 56.96%) (Fig. 2). The origin of seven replicons were analyzed using PlasFlow (https://github.com/smaegol/PlasFlow), BLAST (Basic Local Alignment Search Tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi), and PLSDB (a resource of complete bacterial plasmids, https://ccb-microbe.cs.uni-saarland.de/plsdb/); the results demonstrated that Chromosome 2 and Plasmid 3 have the highest identities with the genome sequences of *Paraburkholderia caribensis* (99.82%) and *Caballeronia* sp. (80.67%), respectively, and all the other replicons were highly similar to the genome sequences of *Burkholderia* spp.. In addition, a total of 12 rRNAs, 56 tRNAs, 48 sRNAs (5926 bp; accounting for 0.087% of the genome), 54 interspersed repeats (3664 bp; 0.06% of the genome), and 33 tandem repeats (7293 bp; 0.13% of the genome) were also identified in the BLB1 genome (Additional file 2: Table S1).

Gene annotation of *R. andropogonis* strain BLB1

The BLB1 genome is predicted to have 5808 protein-coding genes, and 5636 genes can be assigned to the Non-Redundant Protein Database. To better evaluate the functional classification of annotated genes, we conducted functional analyses using databases of Gene Ontology (GO), Clusters of Orthologous Genes (COG), and Kyoto Encyclopedia of Genes and Genome (KEGG). A total of 3549 protein-coding genes (accounting for 61.11%) were functionally annotated using the GO database (Fig. 3), including 1869 genes clustered into ten elements in biological process (BP), 1655 genes into ten elements in cellular component (CC), and 2923 genes into ten elements in molecular function (MF). The top three annotated BPs were the transmembrane transport (GO:0055085), regulation of DNA-templated transcription (GO:0006355), and DNA integration (GO:0015074), which include 106, 105, and 74 genes, respectively. The top three CCs, the integral component of membrane (GO:0016021), plasma membrane (GO:0005886), and cytoplasm (GO:0005737), include 878, 465, and 315 genes, respectively. Notably, there were 336 genes annotated and belonged to GO:0016021 and GO:0005886. Meanwhile, three MFs with the most annotated genes, ATP binding (GO:0005524), DNA binding (GO:0003677), and metal ion binding (GO:0046872), include 367, 346, and 224 genes, respectively.

For COG analysis, 4497 protein-coding genes involving 24 categories were annotated (Fig. 4). Among them, 490 (10.90%) genes were annotated as amino acid transport and metabolism, which was the most abundant category, followed by transcription (405 genes, 9.01%) and cell wall/membrane/envelope biogenesis (383 genes, 8.52%).

Next, KEGG pathway analysis annotated 3090 protein-coding genes, and the main pathways were "global and overview maps" for metabolism, "membrane transport" for environmental information processing, "cellular community-prokaryotes" for cellular process, "translation" for genetic information processing, and "endocrine system" for organismal systems (Fig. 5).

Genomic islands, CRISPRs and prophage sequences prediction

The existence of genomic islands (GIs) in bacteria suggests horizontal origins (Langille et al. 2010). We predicted GIs in the BLB1 genome using both IslandPath-DIMOB and Islander. A total of 37 GIs (772,473 bp, accounting for 11.31% of the genome size, Additional file 3: Table S2) were found, which covered 802 coding genes. Among these, 18, 6, 4, 4, 1, 2, and 2 GIs were found on the Chromosome 1, Chromosome 2, Chromosome 3, Plasmid 1, Plasmid 2, Plasmid 3, and Plasmid 4, respectively. In particular, the largest GI (61,158 bp, GI20 on Chromosome 2, Additional file 3: Table S2) contains 60 genes encoding Hsp20 family protein, LysR family transcriptional regulators, alkene reductase, oxidoreductases, methyl-accepting chemotaxis proteins, MCP four-helix bundle domain-containing protein, DUF2309/DUF1440 domain-containing proteins, sigma-70 family polymerase sigma factor, transposases, two type III effector proteins, enoyl-CoA hydratase-related protein, transporters,

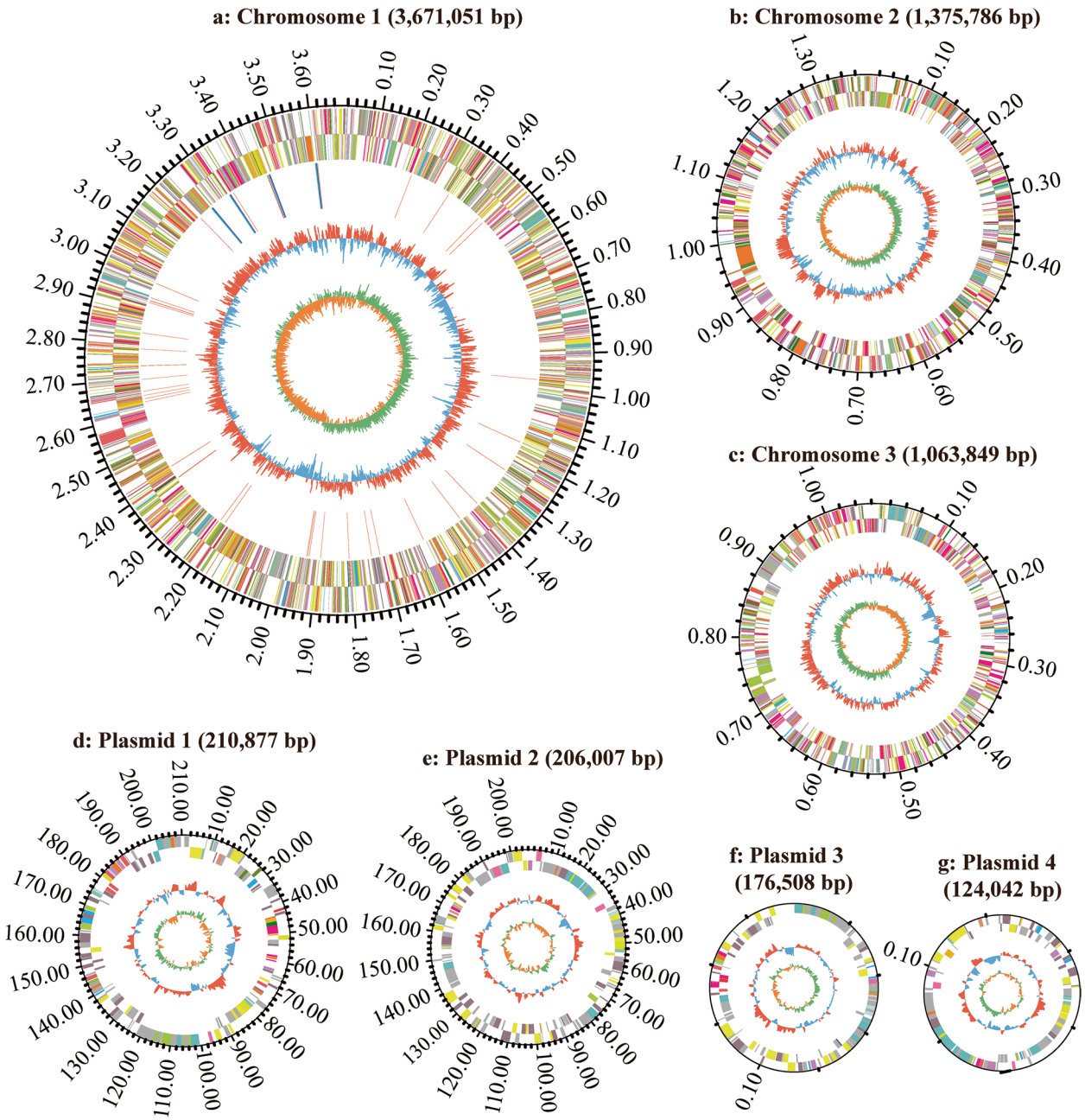

Fig. 2 Circle diagram of *R. andropogonis* strain BLB1 genome. Seven replicons were obtained: **a–g** Chromosome 1 (3,671,051 bp), Chromosome 2 (1,375,786 bp), Chromosome 3 (1,063,849 bp), Plasmid 1 (210,877 bp), Plasmid 2 (206,007 bp), Plasmid 3 (176,508 bp), and Plasmid 4 (124,042 bp). The circles from outer to inner of each replicon present the information on genome size, forward strand genes, reverse strand genes, ncRNAs, repeat sequences, GC percent, and GC-SKEW

porin, hydrolases, oxidases, methyltransferase, and eight hypothetical proteins.

CRISPRs (Clustered Regularly Interspersed Short Palindromic Repeats) can confer resistance to foreign plasmids and phages and exist in approximately 40% of the sequenced bacterial genomes (Barrangou et al. 2007). Here, two putative CRISPR-Cas sequences were found on Chromosome 1 and Chromosome 2 in the BLB1 genome (Additional file 3: Table S2).

Furthermore, phage-related sequences in bacterial genomes also suggest the occurrence of horizontal gene transfer events, which provide resources for bacterial evolution. Overall, four prophages, which contain 52,683 bp and account for 0.77% of the genome

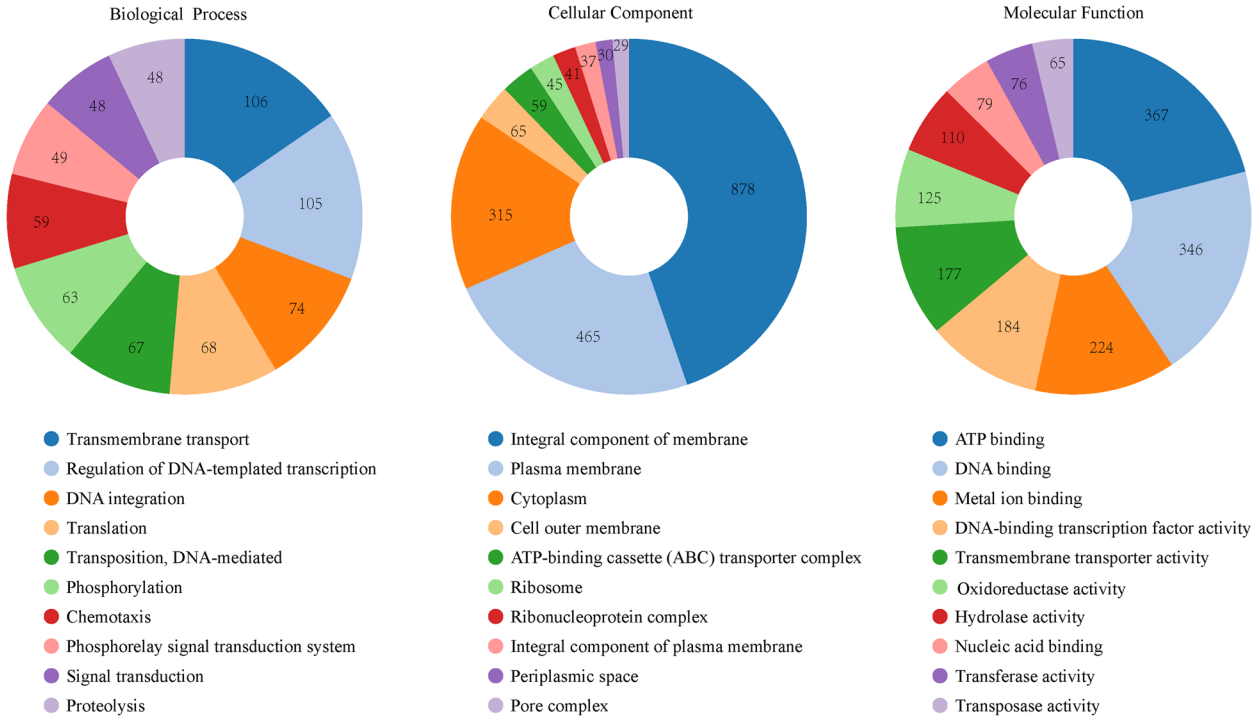

Fig. 3 Gene ontology (GO) functional annotation of the *R. andropogonis* strain BLB1 genome. A total of 3549 protein-coding genes were functionally annotated using the GO database by software blast2go (e-value: ≤ 1e-5), including 1869 genes clustered into ten elements in biological process, 1655 genes clustered into ten elements in cellular component, and 2923 genes clustered into ten elements in molecular function

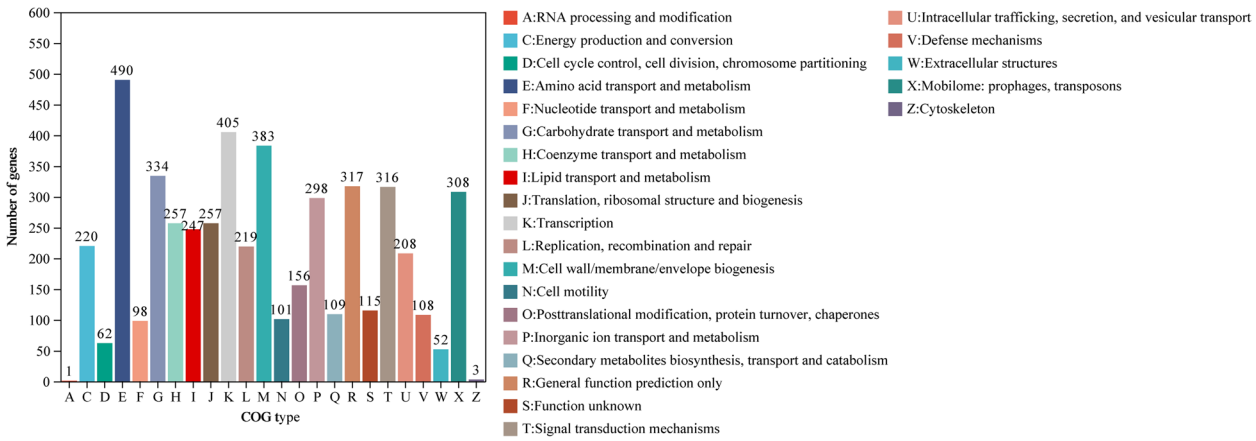

Fig. 4 Clusters of orthologous groups of proteins (COG) functional annotation of the *R. andropogonis* strain BLB1 genome. A total of 4497 protein-coding genes involving 24 categories were annotated based on the eggnog database by Diamond software (e-value: ≤ 1e-5)

size (Additional file 3: Table S2), were identified in the BLB1 genome; their sequence sizes are 24,316, 15,284, 9703, and 3380 bp, which encode 28, 18, 15, and 3 genes, respectively.

Genomic comparison analysis with other *R. andropogonis* strains

To further reveal the genomic characteristics of the available data, we then conducted core- and pan-genome analyses on the strain BLB1 with the other five

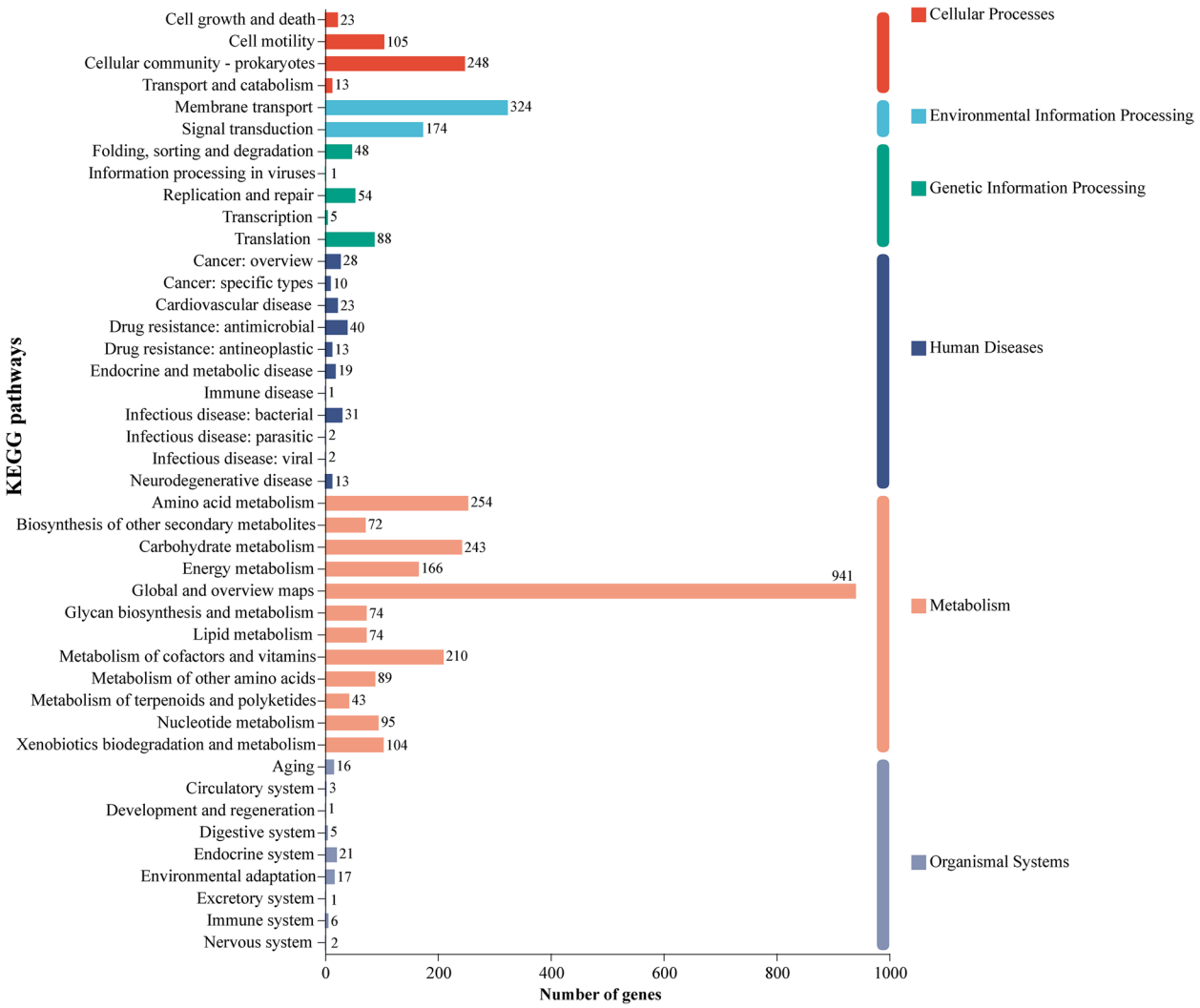

Fig. 5 Kyoto encyclopedia of genes and genome (KEGG) functional annotation of the *R. andropogonis* strain BLB1 genome (3090 protein-coding genes) based on the KEGG database by Diamond software (e-value: ≤ 1e-5)

uncompleted genomes of *R. andropogonis* strains available (Table 1). Among which the strains LMG2129, ICMP2807, BRIP72957a, and BRIP72872a were isolated from diseased leaves of *Sorghum bicolor*, with 75, 272, 305, and 344 contigs of their genome, respectively; the strain Ba3549 was isolated form *Saccharum officinarum* with 301 contigs. In total, 6058 distinct homolog families were identified across the genomes of six *R. andropogonis* strains. The final core genome (the gene families shared by all compared genomes) comprised 4181 gene families, accounting for 69.02% of the pan-genome (Fig. 6).

Genes related to virulence

As a phytopathogenic bacteria, *R. andropogonis* can infect the leaves of many host plants, which should have evolved their special virulence systems for leaf infection. In order to decipher its virulence systems, we performed the prediction of virulence-related genes, and a total of 788 virulence-related genes were annotated (Fig. 7a and Additional file 4: Table S3), which accounted for 13.57% of the total genes. For example, the main virulence gene groups are nutritional/metabolic factor (179 genes), effector delivery system (144 genes), motility (139 genes), immune modulation (91 genes), adherence (60 genes), regulation (57 genes), exotoxin (34 genes), and biofilm (29 genes). Interestingly, although exoenzymes, such as the cell wall-degrading enzymes (CWDEs), can help pathogens break the plant cell wall for invasion, no cellulases, pectinases, and polygalacturonases coding genes were

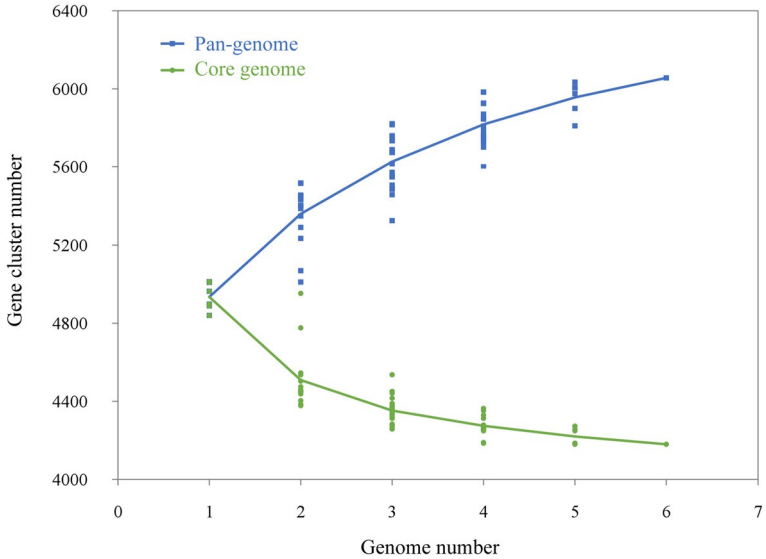

Fig. 6 Pan- and core-genome curves of *R. andropogonis* strains (BLB1, LMG2129, ICMP2807, BRIP72957a, BRIP72872a, and Ba3549) based on PanGP software (http://PanGP.big.ac.cn). For every included genome the size of the pan-genome is increasing, and the core-genome is decreasing

found in the genome, and only two protease genes were identified.

In addition, quorum sensing (QS) is widely distributed in bacteria and has been shown to regulate biofilm formation, secreted products, virulence factors, and extracellular enzymes (Miller et al. 2002; Baltenneck et al. 2021). However, no known QS systems were found in the BLB1 genome, as well as the other five draft genome sequences of *R. andropogonis*. It is important to note that cyclic dinucleotides are highly versatile signaling molecules in prokaryotes. The bis-(3′-5′)-cyclic dimeric GMP (c-di-GMP) is the best-studied example of the intracellular second messenger involved in the control of various important biological processes in bacteria (Jenal et al. 2017). Here, 22 genes encoding c-di-GMP-related proteins were identified in the BLB1 genome, including four proteins with both GGDEF and EAL domain, nine proteins only with EAL domain, eight proteins only with GGDEF domain, and one protein with both EAL and HDOD domains (Additional file 4: Table S3).

Moreover, we compared the groups of virulence genes of BLB1 with those of other three well-studied bacterial phytopathogens *Ralstonia solanacearum* (strain GMI1000) (Peeters et al. 2013), *Pseudomonas syringae* (strain DC3000) (Xin & He 2013), and *Xanthomonas oryzae* (strain PX099A) (NiÑO-Liu et al. 2006) (Fig. 7b). We found that the strain BLB1 possesses a larger number of virulence genes functioning in adherence, antimicrobial activity, exotoxin production, immune modulation, motility, nutritional/metabolic factor, regulation, and stress survival.

Metabolic system analysis

Carbohydrate-active enzymes (CAZyme) are involved in the complex carbohydrates' synthesis, degradation, and recognition (Lombard et al. 2014), and the CAZyme are particularly abundant in plants and plant degrading/saprophytic/pathogenic microbes (Huang et al. 2018). In this study, 162 genes were found to encode CAZymes, including 61 glycosyl transferases, 50 glycoside hydrolases, 29 carbohydrate esterases, and 22 enzymes with auxiliary activities (Additional file 4: Table S3). To compare with the well-known bacterial pathogens, we also listed the number of CAZyme genes of *R. solanacearum* (strain GMI1000), *P. syringae* (strain DC3000), and *X. oryzae* (strain PX099A), and 91, 112, and 121 CAZyme genes were found from their genome annotation, respectively. Thus, the strain BLB1 also has a larger number of CAZyme genes than the three bacterial pathogens.

Furthermore, antiSMASH prediction of secondary metabolite biosynthetic gene clusters (SMBGCs) showed that nine SMBGCs were found, of which six were on Chromosome 1, and three were on Chromosome 2 (Additional file 1: Figure S2). Except for the region 3 on Chromosome 2, which encodes a gramibactin analogue (80% similarity) (Hermenau et al. 2018), all the other eight clusters showed no or low similarity to known SMBGCs.

Secretion systems

It is well known that the secretion systems are also crucial to the virulence of pathogens. Collectively, our results revealed 3, 12, 53, 20, and 43 genes encoding

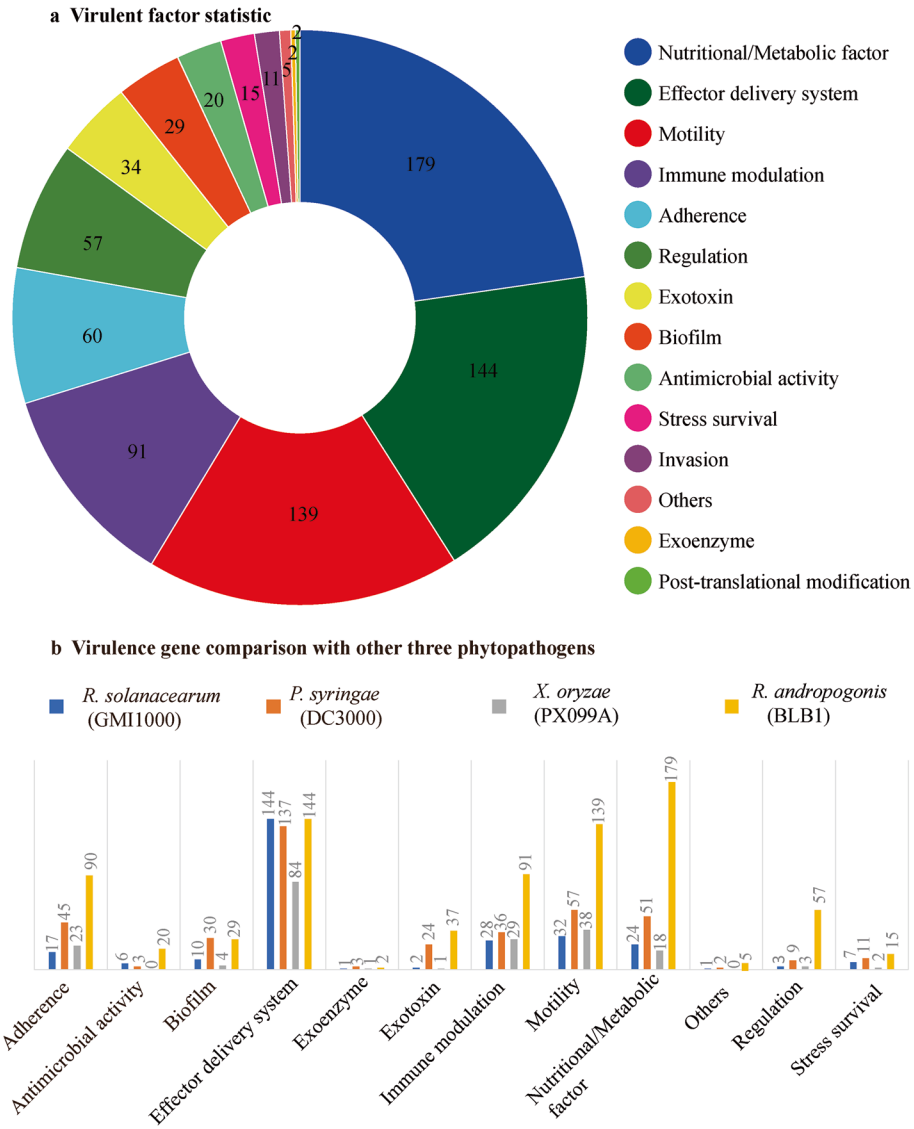

Fig. 7 **a** Virulence-related gene prediction of *R. andropogonis* strain BLB1 based on the VFDB (virulence factor database, http://www.mgc.ac.cn/VFs/) by Diamond software (e-value: ≤ 1e-5). **b** Comparison of the virulence gene groups of BLB1 with the other three well-studied plant bacterial pathogens *R. solanacearum* strain GMI1000, *P. syringae* strain DC3000, and *X. oryzae* strain PX099A

components of type I, II, III, IV, and VI secretion systems, respectively, and 11 and 3 genes were found to encode the Sec-signal recognition particle (Sec-SRP) secretion system and twin arginine targeting (Tat) systems, respectively (Fig. 8 and Additional file 4: Table S3). The haemolysins, leukotoxins, bacteriocins, and other effectors could be secreted via the type I secretion system (T1SS) and contribute to the pathogenesis during infection of host organisms, as well as be involved in nutrient acquisition (Kanonenberg et al. 2013). In the BLB1 genome, three T1SS genes encoding outer membrane protein (tolC), membrane fusion protein (hlyD), and ATP-binding cassette protein (hlyB) were found on Chromosome 1 (Fig. 8). No T1SS-related gene has been reported so far in *R. andropogonis*, and we did not find T1SS-related genes in the strains LMG2129 and ICMP2807 (Additional file 5: Table S4).

The type II secretion system (T2SS) can secret a broad repertoire of substrates contributing to bacteria adaption, which affords the bacteria a fundamental importance in inducing local and global modifications of their external environment (Shaliutina-Loginova et al. 2023). In addition to the T2SS gene cluster consisting of eleven genes on Chromosome 1 (Fig. 8), we

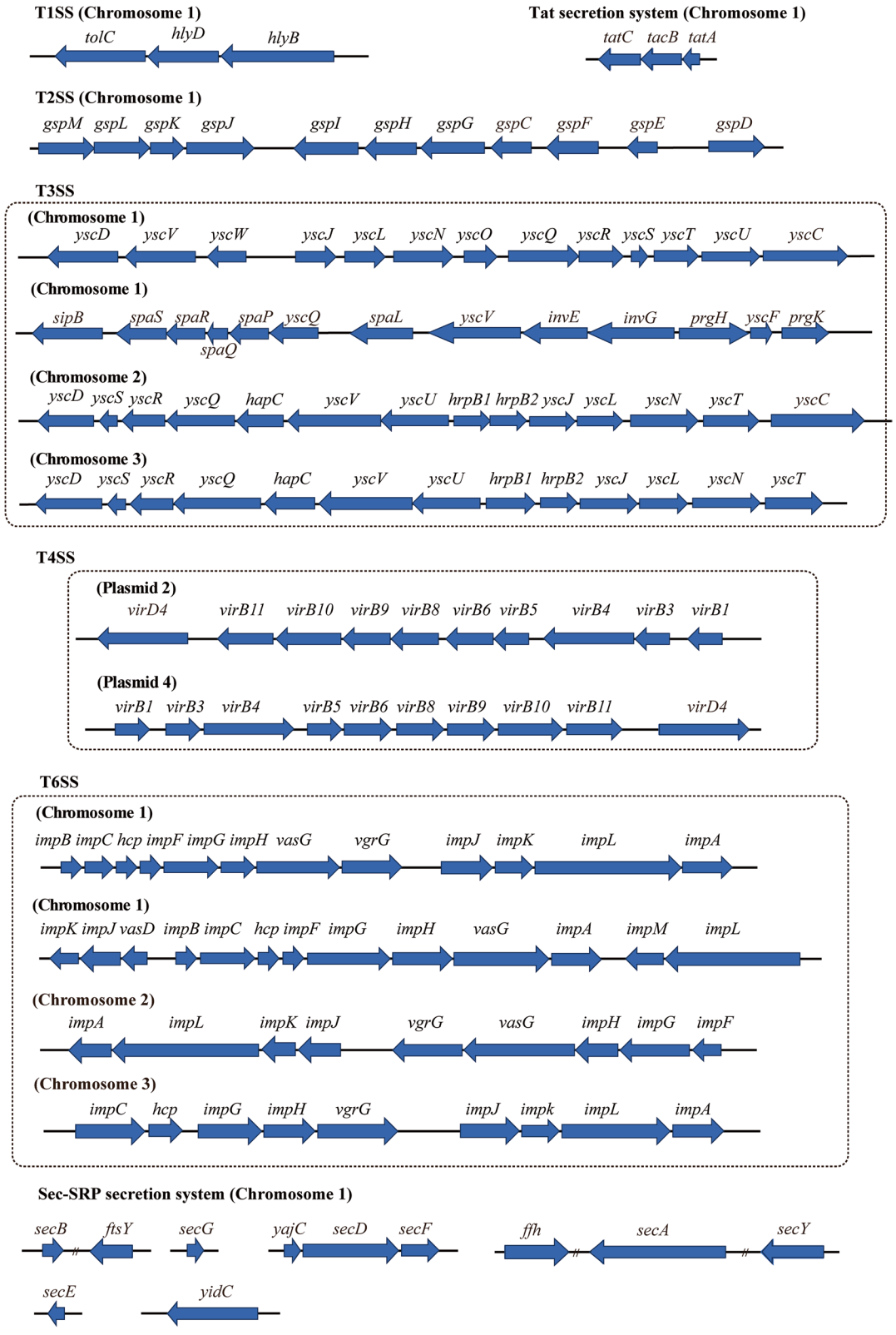

Fig. 8 Gene clusters of different secretion systems of *R. andropogonis* strain BLB1

also found a homologous gene (*gspE*) on Chromosome 3, but the T2SS gene cluster was not identified in the strain Ba3549.

Type III effectors (T3Es) secreted via the Type III secretion system (T3SS) are key virulence factors underpinning the infection strategy of many clinically and economically important Gram-negative pathogens (Sanchez-Garrido et al. 2022). Here, four T3SS gene clusters were found on Chromosome 1 (two sets), Chromosome 2, and Chromosome 3 (Fig. 8). The T3SS gene clusters on Chromosome 2 and Chromosome 3 contain major T3SS regulators hrpB1 and hrpB2, and are more conserved at both levels of sequence identity (50.9%) and gene order; the sequence identities among other T3SS gene clusters ranged from 31.7% to 38.9%. We searched for the T3SS gene clusters in the genomes of the other five *R. andropogonis* strains, and results showed that the strain LMG2129, ICMP2807, BRIP72957a, BRIP72872a, and Ba3549 had four, three, three, three, and two sets of T3SSs, respectively (Additional file 5: Table S4). Meanwhile, 62, 60, 61, 63, 64, and 58 T3Es were identified in strains BLB1, Ba3549, ICMP2807, BRIP72957a, BRIP72872a, and LMG2129, respectively (data from the NCBI genome annotation). Indeed, although the *Robbsia* species have yet to be well studied, the fact that multiple diverse T3SS sets exist in their genomes indicates that the *Robbsia* species should have great potential to infect numerous hosts.

The type IV secretion system (T4SS) can deliver DNA, protein, or other macromolecules to bacterial or eukaryotic cell targets (Costa et al. 2021). In the BLB1 genome, one T4SS gene cluster was identified in Plasmid 2, and another one was in Plasmid 4 (Fig. 8), but sequences of both gene clusters were highly conserved with 97.6% identity. We also identified 2, 0, 1, 1, and 1 T4SS in the strains LMG2129, ICMP2807, BRIP72957a, BRIP72872a, and Ba3549, respectively.

The type VI secretion system (T6SS) is a potent anti-competitor weapon and determines whether a strain can invade or defend its niche in both environmental and host-associated microbial communities (Smith et al. 2020). We identified four T6SS gene clusters in the genome of BLB1, two of which were on Chromosome 1, and one was on each of Chromosome 2 and Chromosome 3 (Fig. 8). The two T6SS sets on Chromosome 1 were complete T6SS gene clusters, and they share 52.6% sequence identity. However, each genome of the other five *Robbsia* strains only has one T6SS gene cluster (Additional file 5: Table S4).

Additionally, eleven genes of the Sec-SRP secretion system were identified in the BLB1 strain, including seven genes of Sec pathways (*secA*, *secB*, *secD*, *secE*, *secF*, *secG*, and *secY*) and four genes of SRP pathway (*ftsY*, *yajC*, *ffh*, and *yidC*). The Sec-SRP section system could translocate unfolded proteins across the cytoplasmic membrane, while, the twin-arginine translocation (Tat) secretion system could transport folded proteins across biological membranes (Natale et al. 2008). Here, three key functional genes (*tatA*, *tatB*, *tatC*) were identified in the BLB1 strain (Fig. 8).

Discussion

The complete genome of *R. andropogonis* provides a valuable resource to reveal the highlights of its genomic characteristics

The *R. andropogonis* was first isolated and identified as *Bacterium andropogonis* (1911), *Pseudomonas andropogonis* (1925), and *Burkholderia andropogonis* (1955), and its taxonomic position was reassessed to *Robbsia andropogonis* based on the calculated results of 16S rRNA gene sequences, multilocus sequence analysis, average nucleotide identity, tetranucleotide frequency, and percentage of conserved proteins (Lopes-Santos et al. 2017). Although the *R. andropogonis* species cause leaf spots, streaks, or stripes in a broad range of host plants including *Zea mays* (Ullstrup 1960), *Coffea arabica* (Rodrigues et al. 1981), *Citrus* sp. (Duan et al. 2009), *Simmondsia chinensis* (Cother et al. 2004), *Ruscus* sp. (Irene et al. 2009), and *Bougainvillea* sp. (Li & De Boer 2005) with extensive geographical distribution, only five highly fragmented uncompleted genomes of *R. andropogonis* were available in GenBank before our research. The low quality of the draft genomes reduces the reliability of gene annotation and limits further studies of gene function, comparative genomics, and population genomics. By taking a hybrid assembly of both long and short-read sequencing data, we finally obtained a high-quality genome of *R. andropogonis*. Thus, the strategy of integrating the data from long and short-read sequencing platforms is helpful for obtaining the complete genome, especially for the species with multiple replicons. The primary goal of this study was to assemble the first complete genome of the species of genus *Robbsia* and to characterize the virulence genes at the genome level, which is an important addition to the genomic resources of *Robbsia* species and is favorable for further studies of pathogenic mechanisms.

The genus *Burkholderia*, from which the genus *Robbsia* was separated, is a large bacterial genus that englobes at least 31 distinct species (Coenye & Vandamme 2003). The *Burkholderia* species commonly contain two chromosomes and several plasmids, whereas *R. andropogonis* BLB1, as the closely related species, has multiple replicons. The origin analyses of seven replicons of the BLB1 strain have shown that Chromosome 1 and 3, Plasmid 1, 2, and 4 were closely related to the genome sequence

from *Burkholderia*, but Chromosome 2 and Plasmid 3 have the highest identities with the genome of *Paraburkholderia caribensis* (99.82%) and *Caballeronia* sp. (80.667%), respectively. A plausible explanation is that the obtaining of new replicons contributes to the genomic distinction between *Burkholderia* and *Robbsia*, and it may be a common feature for members of *R. andropogonis* to own multiple replicons in their genomes. Next, all the rRNA and tRNA coding sequences are distributed on Chromosome 1, which further indicates that Chromosome 1 may be the conserved replicon. Though most genomes of *R. andropogonis* species are uncompleted, core- and pan-genome analyses of *R. andropogonis* species showed that the percentage of gene families belonging to the core-genome is 69.02%, indicating these *Robbsia* species are experiencing highly dynamic genome evolution, which likely has great importance in the adaptation of *R. andropogonis* species to new host plants and different environmental conditions. Moreover, genome islands (GIs) and prophages are major drivers of genome evolution (Dobrindt et al. 2004). Our results showed that the 37 identified GIs and four prophages account for 12% of the gene content (Additional file 3: Table S2), which is particularly interesting since they often provide adaptive traits that enhance the fitness of bacteria and archaea within a niche.

Genome sequencing reveals the powerful and unique virulence mechanisms of *R. andropogonis* for leaf infection

The prediction results showed that the BLB1 strain has a large number of genes (788/5808, 13.57%) encoding virulence factors (Additional file 4: Table S3). However, unlike most phytopathogenic bacteria that infect root tissues in the soil, *R. andropogonis* commonly infects the leaves of host plants and causes leaf spot disease. Our results demonstrated that *R. andropogonis* BLB1 bears a series of interesting characteristics for leaf adaption and infection. For example, cellulase, pectinase, or polygalacturonase coding genes were absent from its genome. Instead, the BLB1 strain has a higher number of main groups of virulence genes encoding nutritional and metabolic factors, effector delivery system, motility, immune modulation, and adherence than the *R. solanacearum*, *P. syringae*, and *X. oryzae* (Fig. 7). Thus, our results indicated that *R. andropogonis* has a great potential to infect numerous plants though it has yet to draw the attention broadly.

Results of GO, COG, and KEGG annotations (Figs. 2, 4, and 5) all revealed abundant metabolism-related genes, suggesting that excellent nutrition and metabolism ability might have contributed to the BLB1 survival on and infection of host leaves. This notion was also supported by a higher number of CAZyme genes found in the BLB1 genome (Additional file 4: Table S3) than in *R. solanacearum*, *P. syringae*, and *X. oryzae*. Similarly, analysis of the other five *Robbsia* strains also revealed powerful metabolism abilities and genes related to adherence, antimicrobial ability, biofilm, effector delivery systems, exotoxin, immune modulation, motility, nutritional factors, regulation, stressful survival, and multiple secretion systems (Additional file 5: Table S4).

Moreover, it has been extensively reported that in the *Burkholderia* spp. possess CepRI (Lewenza et al. 1999) and CciIR (Baldwin et al. 2004) N-acyl homoserine lactones (AHLs) QS systems and BDSF-dependent QS system (Bi et al. 2012), which can regulate the biofilm formation, as well as the expression of virulence factors and secondary metabolites. However, no known QS signal synthase gene and signal receptor gene was found in the BLB1 genome. Interestingly, at least 22 c-di-GMP-related genes were identified in the BLB1 genome. It is well known that c-di-GMP regulates various biological functions such as motility, biofilm formation, and virulence by a variety of mechanisms (Yang et al. 2017), and BDSF-QS system receptor RpfR links QS signal perception with the regulation of virulence through c-di-GMP (Deng et al. 2012). Thus, we can predict that the c-di-GMP signaling systems play roles in regulating virulence-related genes, either alone or in cooperation with other virulence regulators. Meanwhile, whether the eight SMBGCs predicted in BLB1 with unknown products (Additional file 1: Figure S1) have the functions in signaling or competition with other environmental microbes is still worth investigating in the future.

Complex secretion systems highlight the sophisticated virulence mechanism

The secretion system is one of the essential components of pathogenicity for many pathogens, playing roles in promoting bacterial virulence, enhancing attachment to eukaryotic cells, scavenging resources in environmental niche, and intoxicating and disrupting host cells (Green & Mecsas 2016). The Sec and Tat secretion systems are general secretion systems, commonly conserved and used to transport proteins, which are important for physiology and survival of both pathogenic and nonpathogenic bacteria. Our results showed that Sec-SPR and Tat secretion systems of BLB1 are conserved and exist on Chromosome 1; the genome data will lay a valid foundation for further research of the function of both systems. The T1SS resembles a large family of ATP-binding cassette (ABC) transporters to export antibiotics and toxins out of the cell (Symmons et al. 2009), which contribute to virulence in many pathogens. In the BLB1 genome, the T1SS gene cluster contains the *hly* genes encoding transporters which may secrete the substrates hemolysin

A proteins (HlyA) that can cause the host cells to rupture (Welch et al. 1981). T2SSs are capable of secreting a diverse array of substrates outside of the cell, some of which contribute to the virulence of pathogens (Korotkov et al. 2012), or play a key role in phage infection (Xavier et al. 2022), which is conserved among the *R. andropogonis* (Additional file 5: Table S4). Undoubtedly, the *R. andropogonis* BLB1 may use the T2SS to secret enzymes that help it adapt to its environment.

The T3SS has a core of nine conserved proteins but are typically horizontally acquired; thus, the evolutionarily distant bacteria may have closely related systems and vice versa (Troisfontaines & Cornelis 2005). Notably, there were four T3SS gene clusters in BLB1 genome (Fig. 8), and each of the other five *R. andropogonis* strains also has two to four T3SS gene clusters (Additional file 5: Table S4). Thus, multiple T3SSs should be one of the typical characteristics of this group of bacteria. Here, at least 62 T3Es were predicted, nearly equaling the number of T3Es and effector delivery systems (Fig. 7b) in the *Ralstonia solanacearum* species complex, which has been well-studied as one of the most notorious agricultural phytopathogens. We infer that the *R. andropogonis* strains have acquired multiple T3SSs through horizontal gene transfer, which may facilitate their infection on multiple hosts. However, little is known about the molecular basis of the interaction between *R. andropogonis* and the host plants. Therefore, the detailed function of these T3SSs and T3Es is still worthy of elucidation.

Impressively, two highly conserved T4SSs were identified in Plasmid 2 and Plasmid 4 (97.6% identity) of strain BLB1. One T4SS gene cluster was found in the *R. andropogonis* strains Ba3549, BRIP72957a, and BRIP72972a; two T4SSs existed in the strain LMG2129, while T4SS was absent in the strain ICMP2807 (Additional file 5: Table S4). T4SSs can transfer both DNA and proteins and can serve a variety of functions, including DNA uptake and release and translocation of effector proteins (Cascales & Christie 2003). Recent results also highlight the T4SS and T4Es function in quorum quenching (Liao et al. 2023), bacterial killing, biofilm invasion, and biocontrol (Purtschert-Montenegro et al. 2022). Hence, further research on the function and mechanisms of the two highly conserved T4SSs in BLB1 will enhance our understanding of host–pathogen interactions and may lead to novel insights into environment adaption.

Furthermore, four T6SS gene clusters were found in the BLB1 genome, but each of the other five *R. andropogonis* strains has only one T6SS gene cluster (Additional file 5: Table S4). T6SSs are capable of transporting effector proteins in a contact-dependent manner and are believed to play vital roles in bacterial communication and competition (Wang et al. 2020; Sa-Pessoa et al. 2023). Thus, the T6SSs of *R. andropogonis* may contribute to the virulence, delivery of effectors to host cells, and secreting proteins into neighboring bacteria thereby competing for a specific host niche.

Together, the existence of complex and multiple secretion systems afforded *R. andropogonis* the potential for infecting their host plants. In addition, the detailed mechanism of *R. andropogonis* operating the multiple T3SSs, T4SSs, and T6SSs during infection of plants still needs to be discovered. The illustration of these mechanisms should certainly contribute to the design of effective and sustainable control measures against this underestimated phytopathogen.

Conclusion

The first complete genome sequence of *Robbsia* species presented here and comparison results with the other five available genomes of *Robbsia* strains provide a solid foundation for further investigation of its virulence mechanisms and represent a valuable resource for the comparative genomics of genus *Robbsia*. The assembly consists of seven replicons totaling 6,828,120 bp, and a total of 5808 genes were annotated, including 788 virulence-related genes. Function analysis showed that genes of metabolism were enriched significantly and have evolved to suit leaf infection. Moreover, secretion systems were well equipped in their genomes, especially the T3SS, T4SS, and T6SS, with more than one set, which highlights the virulence features of *R. andropogonis*.

Methods

Robbsia andropogonis culture and DNA isolation

R. andropogonis strain BLB1 was isolated from the infected leaves of areca palm and confirmed as the causal pathogen following Koch's postulates. *R. andropogonis* strain BLB1 was subsequently cultured in Luria–Bertani (LB, peptone 10 g/L, yeast extract 5 g/L, sodium chloride 10 g/L) medium at 30°C, 150 rpm for 12 h, and cells were collected for further application or genomic DNA extraction.

DNA extraction and sequencing

DNA was extracted using the Tiangen DNA Isolation kit (TIANGEN BIOTECH, Beijing) following the manufacturer´s protocol. The harvested DNA was detected by the agarose gel electrophoresis and quantified by Qubit R 2.0 Fluorometer (Thermo Scientific, Waltham, MA, United States). The genome was sequenced using the BGISEQ-500 and ONT PromethION platforms at the Nextomics Biosciences Co., Ltd., China.

DNA assembly and function annotation

The resulting assembly was polished with both long and short reads data using NextPolish v1.4.1, then followed by a hybrid assembly of both long and short read sequencing data using the Unicycler 0.4.8 (Wick et al. 2017). The completeness of the genome assembly was assessed with the Firmicutes BUSCO lineage (Seppey et al. 2019). The circular visualization of BLB1 genome characteristics was created by Circos (Krzywinski et al. 2009), and the plasmids were predicted by PlasFlow (Krawczyk et al. 2018). The gene annotation was performed by using DIAMOND (Buchfink et al. 2021), rRNAs were predicted using RNAmmer (Lagesen et al. 2007), tRNA were predicted by tRNAscan-SE (Lowe and Eddy 1997), and sRNA were predicted by comparing the Rfam database V14 (Kalvari et al. 2021). The tandem repeats (TR) were predicted using Tandem repeats finder (Benson 1999). The interspersed repeats (IR) were predicted using RepeatMasker (Smit et al. 2022).

The gene ontology (GO) was performed by taking blast2go with GO database (http://www.geneontology.org/) with e-value ≤ 1e-5, including cellular component, molecular function, and biological process; the clusters of orthologous groups of proteins (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed by taking DIAMOND analysis with eggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups, http://eggnog.embl.de/) and KEGG database (http://www.genome.jp/kegg/) with e-value ≤ 1e-5, respectively. The genome island (GI), CRISPR_Cas, and prophage prediction were analyzed using IslandVierer 4 (Bertelli et al. 2017), MinCED (Skennerton et al. 2021), and Phigaro (Starikova et al. 2020), respectively.

Virulence factors were predicted by PathoFact v1.0 with default settings (de Nies et al. 2021), and only the predictions with high confidence levels (i.e., "1: Secreted Virulence factor" and "2: Non-secreted Virulence factor") were considered. In addition, the carbohydrate-active enzymes and secondary metabolisms were predicted by blasting in the CAZy DB (http://www.cazy.org/) and antiSMASH v6.0 (Blin et al. 2021), respectively.

Genome phylogenic analysis and the core- and pan-genome analyses

The genome sequence was analyzed with GTDB-tkv1.6.0 software, and the conserved genes were chosen and used to perform phylogenic analysis together with the most closely related species in the Genome Taxonomy Database. Meanwhile, the ANI between BLB1 and other closely related strains' genomes was calculated with pyani v0.2.11 software and the online tool GGDC3.0 (http://ggdc.dsmz.de/ggdc.php). The core- and pan-genome comparison analysis referenced the previously reported method (Zhao et al. 2014).

Abbreviations

ANI	Average nucleotide identity
BP	Biological process
CAZyme	Carbohydrate-active enzymes
CC	Cellular Component
c-di-GMP	Cyclic dimeric guanosine monophosphate
COG	Clusters of orthologous groups
CRISPR	Clustered Regularly Interspersed Short Palindromic Repeat
GI	Gene Island
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
MF	Molecular Function
QS	Quorum sensing
T1/2/3/4/6SS	Type I/II/III/IV/VI secretion system
Tat	Twin-arginine translocation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00269-2.

Additional file 1: Figure S1. Genome phylogenic cluster analysis of strain BLB1 with five *R. andropogonis* strains and one *R. betulipollinis* strain. **Figure S2.** Identification results of secondary metabolite synthesis region by the antiSMASH v6.0 software.

Additional file 2: Table S1. a rRNA, **b** tRNA, **c** sRNA, **d** interspersed repeat sequences, **e** tandem repeat sequences prediction and statistics of *R. andropogonis* strain BLB1.

Additional file 3: Table S2. a Genome islands, **b** CRISPR_Cas, and **c** prophages prediction of *R. andropogonis* strain BLB1.

Additional file 4: Table S3. a Virulence related genes, **b** c-di-GMP, **c** CAZyme, **d** secretion system prediction of *R. andropogonis* BLB1 genome.

Additional file 5: Table S4. a COG, **b** GO, **c** secretion system prediction, **d** virulence factors prediction, and **e** KEGG annotation of the other five genomes of *R. andropogonis* strains.

Acknowledgements

Not applicable.

Authors' contributions

PL and LZ designed the research; DC, PL, LZ, and JS prepared the materials, PL, YL, XZ, and JS analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by Hainan Province key R&D Project (ZDYF2022XDNY242), and the specific research fund of the Innovation Platform for Academicians of Hainan Province (YSPTZX202130).

Availability of data and materials

The datasets generated during the current study are available in the NCBI repository (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_034047095.1/). The GenBank accession numbers of the seven replicons are CP139164-CP139170. The strain BLB1 is stored in the Ministry of Education Key Laboratory for Ecology of Tropical Islands, Hainan Normal University, People's Republic of China.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 21 November 2023 Accepted: 25 June 2024
Published online: 08 October 2024

References

Baldwin A, Sokol PA, Parkhill J, Mahenthiralingam E. The *Burkholderia cepacia* epidemic strain marker is part of a novel genomic island encoding both virulence and metabolism-associated genes in *Burkholderia cenocepacia*. Infect Immun. 2004;72:1537–47.
Baltenneck J, Reverchon S, Hommais F. Quorum sensing regulation in phytopathogenic bacteria. Microorganisms. 2021;9:239.
Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315:1709–12.
Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 1999;27:573–80.
Bertelli C, Laird MR, Williams KP, Lau BY, Hoad G, Winsor GL, et al. IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. Nucleic Acids Res. 2017;45:W30–5.
Bi H, Christensen QH, Feng Y, Wang H, Cronan JE. The *Burkholderia cenocepacia* BDSF quorum sensing fatty acid is synthesized by a bifunctional crotonase homologue having both dehydratase and *thioesterase* activities. Mol Microbiol. 2012;83:840–55.
Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, et al. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res. 2021;49:W29–35.
Buchfink B, Reuter K, Drost HG. Sensitive protein alignments at tree-of-life scale using DIAMOND. Nat Methods. 2021;18:366–8.
Cascales E, Christie PJ. The versatile bacterial type IV secretion systems. Nat Rev Microbiol. 2003;1:137–49.
Coenye T, Vandamme P. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. Environ Microbiol. 2003;5:719–29.
Costa TRD, Harb L, Khara P, Zeng L, Hu B, Christie PJ. Type IV secretion systems: advances in structure, function, and activation. Mol Microbiol. 2021;115:436–52.
Cother EJ, Noble D, Peters BJ, Albiston A, Ash GJ. A new bacterial disease of jojoba (*Simmondsia chinensis*) caused by *Burkholderia andropogonis*. Plant Pathol. 2004;53:129–35.
Cui X, Cai Y, Chen R, Liu Q. First report of bacterial leaf spot disease on Pueraria montana var. thomsonii caused by Robbsia andropogonis in China. Plant Dis. 2022;106:2985.
Deng Y, Schmid N, Wang C, Wang J, Pessi G, Wu D, et al. Cis-2-dodecenoic acid receptor RpfR links quorum-sensing signal perception with regulation of virulence through cyclic dimeric guanosine monophosphate turnover. Proc Natl Acad Sci U S A. 2012;109:15479–84.
de Nies L, Lopes S, Busi SB, Galata V, Heintz-Buschart A, Laczny CC, et al. PathoFact: a pipeline for the prediction of virulence factors and antimicrobial resistance genes in metagenomic data. Microbiome. 2021;9:49.
Dobrindt U, Hochhut B, Hentschel U, Hacker J. Genomic islands in pathogenic and environmental microorganisms. Nat Rev Microbiol. 2004;2:414–24.
Duan YP, Sun X, Zhou LJ, Gabriel DW, Benyon LS, Gottwald T. Bacterial brown leaf spot of citrus, a new disease caused by *Burkholderia andropogonis*. Plant Dis. 2009;93:607–14.
Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, Gonzalez FJ. A metabolomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. Chem Res Toxicol. 2006;19:818–27.
Green ER, Mecsas J. Bacterial secretion systems: An overview. Microbiol Spectr. 2016;4. https://doi.org/10.1128/microbiolspec.
Henderson A. The palms of Southern Asia. Princeton University Press; 2009.
Hermenau R, Ishida K, Gama S, Hoffmann B, Pfeifer-Leeg M, Plass W, et al. Gramibactin is a bacterial siderophore with a diazeniumdiolate ligand system. Nat Chem Biol. 2018;14:841–3.
Huang L, Zhang H, Wu P, Entwistle S, Li X, Yohe T, et al. dbCAN-seq: a database of carbohydrate-active enzyme (CAZyme) sequence and annotation. Nucleic Acids Res. 2018;46:D516–21.
Irene MG, Almeida LOS, Beriam AM, Sannazzaro NJR. Mancha bacteriana em *Ruscus* sp. causada por *Burkholderiaandropogonis* no Brasil. Trop Plant Pathol. 2009;34:339–42.
Jenal U, Reinders A, Lori C. Cyclic di-GMP: second messenger extraordinaire. Nat Rev Microbiol. 2017;15:271–84.
Kalvari I, Nawrocki EP, Ontiveros-Palacios N, Argasinska J, Lamkiewicz K, Marz M, et al. Rfam 14: expanded coverage of metagenomic, viral and microRNA families. Nucleic Acids Res. 2021;49:192–200.
Kanonenberg K, Schwarz CK, Schmitt L. Type I secretion systems - a story of appendices. Res Microbiol. 2013;164:596–604.
Korotkov KV, Sandkvist M, Hol WG. The type II secretion system: biogenesis, molecular architecture and mechanism. Nat Rev Microbiol. 2012;10:336–51.
Krawczyk PS, Lipinski L, Dziembowski A. PlasFlow: predicting plasmid sequences in metagenomic data using genome signatures. Nucleic Acids Res. 2018;46:e35.
Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19:1639–45.
Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 2007;35:3100–8.
Langille MGI, Hsiao WWL, Brinkman FSL. Detecting genomic islands using bioinformatics approaches. Nat Rev Microbiol. 2010;8:372–82.
Lewenza S, Conway B, Greenberg EP, Sokol PA. Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. J Bacteriol. 1999;181:748–56.
Li X, De Boer SH. First report of *Burkholderiaandropogonis* causing leaf spots of *Bougainvillea* sp. in Hong Kong and clover in Canada. Plant Dis. 2005;89:1132.
Liao J, Li Z, Xiong D, Shen D, Wang L, Lin L, et al. Quorum quenching by a type IVA secretion system effector. ISME J. 2023;17:1564–77.
Lombard V, Golaconda RH, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 2014;42:D490-495.
Lopes-Santos L, Castro DBA, Ferreira-Tonin M, Corrêa DBA, Weir B, Park D, et al. Reassessment of the taxonomic position of *Burkholderia andropogonis* and description of *Robbsia andropogonis* gen. nov., comb. nov. Antonie van Leeuwenhoek. 2017;110:727–36.
Lowe TM, Eddy SR. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 1997;25:955–64.
Manimekalai R, Nair S, Naganeeswaran A, Karun A, Malhotra S, Hubbali V. Transcriptome sequencing and *de novo* assembly in arecanut, *Areca catechu* L elucidates the secondary metabolite pathway genes. Biotechnol Rep (amst). 2018;17:63–9.
Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL. Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. Cell. 2002;110:303–14.
Morales-Galvan O, Nigam D, Young AJ, Ignatov AN, Mejia-Sanchez D, Flores-Lopez LF. Molecular, phenotypical, and host-range characterization of *Robbsiaandropogonis* strains isolated from *Bougainvillea* spp. in Mexico. Plant Dis. 2022;106:603–11.
Natale P, Bruser T, Driessen AJ. Sec- and Tat-mediated protein secretion across the bacterial cytoplasmic membrane–distinct translocases and mechanisms. Biochim Biophys Acta. 2008;1778:1735–56.
Niño-Liu DO, Ronald PC, Bogdanove AJ. Xanthomonas oryzae pathovars: model pathogens of a model crop. Mol Plant Pathol. 2006;7:303–24.
Peeters N, Carrère S, Anisimova M, Plener L, Cazalé AC, Genin S. Repertoire, unified nomenclature and evolution of the Type III effector gene set in the *Ralstonia solanacearum* species complex. BMC Genomics. 2013;14:859.
Purtschert-Montenegro G, Carcamo-Oyarce G, Pinto-Carbo M, Agnoli K, Bailly A, Eberl L. *Pseudomonas putida* mediates bacterial killing, biofilm invasion and biocontrol with a type IVB secretion system. Nat Microbiol. 2022;7:1547–57.
Rodrigues NJ, Figueiredo P, Mariotto PRFRC. *Pseudomons andropogonis* (Smith 1911) Stapp 1928, agente causal da "'mancha bacteriana escura"' em folhas de cafeeiro (*Coffea arabica* L.). Instituto Biolo´gico. 1981;48:31–3.

Sanchez-Garrido J, Ruano-Gallego D, Choudhary JS, Frankel G. The type III secretion system effector network hypothesis. Trends Microbiol. 2022;30:524–33.
Sa-Pessoa J, Lopez-Montesino S, Przybyszewska K, Rodriguez-Escudero I, Marshall H, Ova A, et al. A trans-kingdom T6SS effector induces the fragmentation of the mitochondrial network and activates innate immune receptor NLRX1 to promote infection. Nat Commun. 2023;14:871.
Seppey M, Manni M, Zdobnov EM. In: Kollmar M, editor. Gene Prediction: Methods and Protocols. New York: Springer New York; 2019. p. 227–24.
Shaliutina-Loginova A, Francetic O, Doležal P. Bacterial type II secretion system and its mitochondrial counterpart. mBio. 2023;14:e03145-03122.
Shi H, Ambika Manirajan B, Ratering S, Geissler-Plaum R, Schnell S. Robbsia betulipollinis sp. nov., Isolated from Pollen of Birch (Betula pendula). Curr Microbiol. 2023;80:234.
Skennerton CT, Soranzo NFA. MinCED. https://github.com/ctSkennerton/minced . Accessed 31 Mar 2021.
Smit AFA, Hubley RPG. RepeatMasker Open-3.0. 1996–2010. Available online: http://www.repeatmasker.org. Accessed on 26 Feb 2022.
Smith WPJ, Brodmann M, Unterweger D, Davit Y, Comstock LE, Basler M, et al. The evolution of tit-for-tat in bacteria via the type VI secretion system. Nat Commun. 2020;11:5395.
Starikova EV, Tikhonova PO, Prianichnikov NA, Rands CM, Zdobnov EM, Ilina EN, et al. Phigaro: high-throughput prophage sequence annotation. Bioinformatics. 2020;36:3882–4.
Symmons MF, Bokma E, Koronakis E, Hughes C, Koronakis V. The assembled structure of a complete tripartite bacterial multidrug efflux pump. Proc Natl Acad Sci U S A. 2009;106:7173–8.
Tang QH, Yu FY, Zhang SQ, Niu XQ, Zhu H, Song WW, et al. First report of *Burkholderia andropogonis* causing bacterial leaf spot of betel palm in Hainan Province. China Plant Dis. 2013;97:1654–1654.
Troisfontaines P, Cornelis GR. Type III secretion: more systems than you think. Physiology. 2005;20:326–39.
Ullstrup AJ. Bacterial stripe of corn. Phytopathology. 1960;50:906–10.
Wang T, Hu Z, Du X, Shi Y, Dang J, Lee M, et al. A type VI secretion system delivers a cell wall amidase to target bacterial competitors. Mol Microbiol. 2020;114:308–21.
Welch RA, Dellinger EP, Minshew B, Falkow S. Haemolysin contributes to virulence of extra-intestinal *E. coli* infections. Nature. 1981;294:665–7.
Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017;13:e1005595.
Xavier ADS, de Melo AG, Hendrich CG, Tremblay DM, Rousseau GM, Plante PL, et al. In through the out door: A functional virulence factor secretion system is necessary for phage infection in *Ralstonia solanacearum*. mBio. 2022;13:e0147522.
Xin X, He S. Pseudomonas syringae pv. tomato DC3000: a model pathogen for probing disease susceptibility and hormone signaling in plants. Annu Rev Phytopathol. 2013;51:473–98.
Yang C, Cui C, Ye Q, Kan J, Fu S, Song S, et al. *Burkholderia cenocepacia* integrates cis-2-dodecenoic acid and cyclic dimeric guanosine monophosphate signals to control virulence. Proc Natl Acad Sci U S A. 2017;114:13006–11.
Yang Y, Huang L, Xu C, Qi L, Wu Z, Li J, et al. Chromosome-scale genome assembly of areca palm (*Areca catechu*). Mol Ecol Resour. 2021;21:2504–19.
Young AJ, Birt HWG, Hoelzle RD, Dennis PG. Draft Genome sequences of *Robbsia andropogonis* isolated from *Sorghum bicolor* exhibiting bacterial leaf stripe in Australia. Microbiol Resour Announc. 2022;11:e0024722.
Zhao Y, Jia X, Yang J, Ling Y, Zhang Z, Yu J, et al. PanGP: a tool for quickly analyzing bacterial pan-genome profile. Bioinformatics. 2014;30:1297–9.