Comparative genomics uncovers genetic diversity and synthetic biology of secondary metabolite production of the genus *Trametes*

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Abstract

It's well-established that the CAZyme genes of genus *Trametes* contributed to the degradation processes of polysaccharides, including lignin or crystalline cellulose. However, the comprehensive analysis of the composition of CAZymes and the biosynthetic gene clusters of *Trametes* genus remain unclear. We conducted comparative analysis, detected the CAZyme genes, and predicted the biosynthetic gene clusters for 9 *Trametes* strains. Among 82,053 homologous clusters we obtained for genus *Trametes*, we identified 8,518 core genes, 60,441 accessory genes and 13,094 specific genes. Our results showed that a large proportion of CAZyme genes were catalogued into glycoside hydrolases, glycosyltransferases, and carbohydrate esterases. The predicted BGCs of *Trametes* genus were divided into 6 strategies and the 9 *Trametes* strains harbored 47.78 BGCs on average. Our study uncovers the genus *Trametes* exhibited an open pan-genome structure, provides insights into the genetic diversity and explores the synthetic biology of secondary metabolite production for *Trametes* genus.

Introduction

The genus *Trametes*, one of white-rot basidiomycetes, belonging to the family Polyporaceae and the class Agaricomycetes of fungi commonly grows in the tiled layer of decaying wood [1] and uses deciduous tree [2]. *Trametes* genus currently comprises at least 20 species until April 2018 [3, 4], such as *Trametes* sp., *Trametes trogii* and *Trametes villosa* CCMB561. The genus *Trametes* is known to be decolorize dyes [1] and several species of genus *Trametes* have been used for centuries in traditional medicine in East Asia counties [5]. A previous study has reported that *Trametes* sp. SQ01 has the ability to decolorize azo, anthraquinone, and triphenylmethane dyes [6]. *T. trogii*, which is a worldwide-distributed white-rot fungi and an outstanding laccase producer, degrade all lignocellulosic materials [7]. *T. villosa* CCMB561 was isolated from the decaying wood and a common species in the Brazilian semiarid region [8]. It is well-established that *T. villosa* is a high potential fungus for biotechnological applications due to the fact that it is a good producer of the three important ligninolytic enzymes (laccase, manganese peroxidase and lignin peroxidase) [4]. Moreover, the laccases from the *Trametes* genus have been reported as a biocatalyst in enzymatic biofuel cells [9, 10]. Hence, understanding the genetic diversity of *Trametes* genus is beneficial for its biotechnological applications.

Carbohydrate-active enzymes (known as CAZymes) are involved in the degradation processes of polysaccharides of plant cell wall [11], lignin, cellulose, hemicellulose and pectin [12]. Especially, the CAZyme classes (glycoside hydrolases, carbohydrate esterases, and polysaccharide lyases) play central roles in plant biomass decomposition by bacteria and fungi [13]. It has been reported that the total expression levels of CAZyme genes of *T. versicolor* F21a were almost twice as that of the control group during the algicidal process and the majority of expressed CAZyme genes belonged to glycoside hydrolases family and auxiliary activities [14]. Similarly, 590 genes of *T. villosa* CCMB561 were identified as CAZyme genes [4]. Although the compositions of CAZyme genes of fewer species of the *Trametes* genus have been reported, the comprehensive analysis of the composition of CAZyme genes of *Trametes* genus still remains unclear.

Secondary metabolites (referred to as natural products or specialized metabolites) are the foundation of many drugs [15] and are also important chemicals widely used in agriculture and nutrition [16]. It is well-known that fungi are rich sources of thousands of second metabolites (SMs) and fungal SMs can be grouped into four main chemical types: polyketides (PKS), terpenoids, shikimin acid derived compounds, and non-ribosomal peptides (NRPS) [17]. Secondary metabolites of fungal are crucial players in its development and actively shape interactions with other microbes [18]. A previous study has reported that the number of genes coded for secondary metabolism of ascomycetes is higher than that of basidiomycetes, archeo-ascomycetes, and chytridiomycetes [19]. In recent years, lot of fungal genomes were sequenced and the development of various genome mining software tools, such as antiSMASH [16] and ClusterFinder [20], enabled researchers to analyze the biosynthetic gene clusters (BGCs) of secondary metabolites. It has been reported that the genomes of filamentous fungi contain up to 90 potential BGCs encoding their diverse secondary metabolites [21] and 24 genomes of *Penicillium* genus were mined for BGCs and associated PKS and NRPS BGCs to known pathways [22]. However, there has been no systemically comparative analysis on the identification of BGCs of secondary metabolite in *Trametes* genus.

In this study, we collected 9 available genomic data from 8 species of *Trametes* genus and conducted a comparative genomics analysis to investigate the genetic diversity and the synthetic biology of secondary metabolite. We obtained the pan-genome of *Trametes* genus, annotated the pan-genome against COG database and eggNOG database, and detected the CAZyme genes. The biosynthetic gene clusters for 9 *Trametes* strains were also predicted. Our results showed that the genus *Trametes* exhibited an open pan-genome structure and diverse genetic diversity for *Trametes* strains. The different distribution of CAZymes of *Trametes* genus revealed that the difference of ability for utilizing the carbohydrates for *Trametes* strains. The predicted biosynthetic gene clusters with unknown functions in *Trametes* genus suggested that *Trametes* genus has a great potential value for producing secondary metabolite.

Results And Discussions

Pan-genome construction and analysis

To conduct the pan-genome analysis, 9 available genomic data from 8 species within the genus *Trametes* were collected. The assembled genome of *Trametes* genus ranged from 31.62 Mb to 57.98 Mb, while the number of contigs/scaffolds ranged from 13 to 10,327, as well as the number of CDSs/ORFs predicting by Pordigal ranged from 56,735 to 101,817 (Table 1). To characterize the differences of genomic composition among these 9 strains, we detected the orthologs from the 610,421 high-quality proteins of *Trametes* genus. In total, we obtained 82,053 homologous clusters and the accumulation curve of homologous cluster showed that *Trametes* genus exhibited an open pan-genome structure (Figure 1a). The size of pan-genome of *Trametes* genus tended to increase gradually with new strains and was estimated to be 82,053 non-redundant genes within 9 strains (Figure 1a). While the size of core-

genome tended to decrease progressively with new strains and was comprised of 8,518 non-redundant genes within 9 strains of *Trametes* genus (Figure 1a).

In contrast, we obtained 60,441 accessory genes and 13,094 specific genes of *Trametes* genus. The distribution of accessory genes varied from 17,587 to 31,856. Although the size of *T. cinnabarina* FP 104138-Sp is smallest (Table 1) among 9 strains of *Trametes* genus, it had the maximum number (31,856) of accessory genes. *T. hirsute* had the minimum number (17,587) of accessory genes. The number of specific orthologs of 9 *Trametes* strains ranged from 368 to 4,698, in which *T. villosa* CCMB561 clearly stood out possessing the highest number (4,698) of specific genes, whereas *T. cinnabarina* FP 104138-Sp had the minimum number (368) of specific genes (Figure 1b). The distribution of accessory genes and specific genes revealed that the genomes of *Trametes* genus are considerably diverse.

Phylogenetic analysis of *Trametes* pan-genome

It is well-established that the nuclear ribosomal Internal Transcribed Spacer (ITS) region is the primary fungal barcode marker [23] and useful to identify the broadest range of fungi [24]. To analyze the phylogenetic relationships for 9 *Trametes* strains, we built the phylogenetic tree for *Trametes* genus and *Coriolopsis caperata*, which was selected as outgroup to root the topology of the tree, based on their ITS1 and ITS2 sequences, respectively. We observed that the ITS1 and ITS2 sequences have a strong power to differentiate the 9 strains of *Trametes* genus (Figure 1c-1d). Among the phylogenetic trees, we found that *T. pubescens*, *T. versicolor* FP-101664 SS1 and *T. sp.* AH28-2 have closer evolutionary relationship, and *T. cinnabarina* BRFM137 has closer evolutionary relationship with *T. coccinea* BRFM310. However, the evolutionary relationship of *T. hirsuta* was more distant from that of remaining strains of *Trametes* genus (Figure 1c-1d).

To gain insights into the evolutionary relationships among the strains of *Trametes* genus, we conducted the phylogenetic tree based on the genes presence and absence in pan-genome. The result showed that *T. villosa* has closer evolutionary relationship with and *T. versicolor* FP-101664 SS1, while *T. sp.* AH28-2, *T. pubescens*, *T. coccinea* BRFM310, and *T. cinnabarina* FP 104138-Sp have closer evolutionary relationship with each other (Figure 1e), which revealed that these strains have similar genetic composition and suggested that these strains might have similar functional metabolism. Meanwhile, the result also revealed that the evolutionary relationship of *T. hirsuta* was distant from that of rest of strains of *Trametes* genus (Figure 1e), which suggested that the functional composition of *T. hirsuta* is different from remaining strains of *Trametes* genus and the functional metabolism of *Trametes* genus is more diverse.

Functional characterization of the *Trametes* pan-genome

To investigate the functional diversity of 9 strains of *Trametes* genus, we annotated the pan-genome of *Trametes* genus against the Cluster of Orthologous Group (COG) to characterize functions of the accessory clusters, core clusters and specific genes. It was obvious that the orthologous genes of the pan-genome were mainly dominant in “general function prediction only” (R, 1,758, 15.47%), “replication, recombination and repair” (L, 998, 8.78%), “amino acid transport and metabolism” (E, 996, 8.77%), “translation, ribosomal structure and biogenesis” (J, 755, 6.64%), and “carbohydrate transport and metabolism” (G, 753, 6.63%, Figure 2a). Moreover, we observed that *Trametes* genus has 442 (3.89%) orthologous genes assigned to “secondary metabolites biosynthesis, transport and catabolism” (Q, Figure 2a). Similarly, the accessory genome was enriched in “general function prediction only” (R, 1,411, 15.29%), “replication, recombination and repair” (L, 810, 8.78%) and “amino acid transport and metabolism” (E, 799, 8.66%, Figure 2a). In addition, the core gene clusters were predominantly composed of genes involved to “general function prediction only” (R, 88, 20.09%), “energy production and conversion” (C, 51, 11.64%), “amino acid transport and metabolism” (E, 38, 8.68%), and “carbohydrate transport and metabolism” (G, 36, 8.21%, Figure 2a). Finally, the specific genes were mainly contributed to the functions involved in “replication, recombination and repair” (L, 610, 28.44%) and “general function prediction only” (R, 259, 12.07%, Figure 2a). We speculated that the high proportions of accessory genes (9,230, 81.23%) and specific genes (2,145, 18.88%) in each strain of *Trametes* genus might lead to a more diverse of functions.

To explore the evolution of function catalogues, we also conducted a GO analysis to characterize genetic functions of the pan-genome. These genes were categorized according to biological process, cellular component and molecular function. The results showed that a great number of genes of pan-genome involved in various enzymes, metabolic pathways and biological processes. Specifically, the enrichment analysis of biological processes showed that many genes were classified into three categories: biosynthetic process (2,265), response to stress (2,186), and small molecule metabolic process (1,882, Figure 2b). In addition, a total of 606 orthologous genes were contributed to the carbohydrate metabolic process (Figure 2b). The classification according to molecular function showed that the majority of genes grouped into binding (such as ion binding, DNA binding, and RNA binding) and enzymes (such as ATPase activity, oxidoreductase activity, and kinase activity), yet various genes also enriched in other categories included transporter activity and enzyme regulator activity (Figure 2b). The enrichment analysis of cellular component showed that the higher proportions of genes were grouped into the functions, including cell (8,492), intracellular (8,386), and cytoplasm (7,363, Figure 2b). Among these categories, we observed that accessory clusters are highly represented in these functions than core and specific genes (Figure 2b). Thus, we speculated that the diversity of accessory clusters might influence their functional diversity, which allows these strains to utilize the resources of surrounding environment and better adapt to the environment.

Identification of CAZymes for *Trametes* genus

It is well-established that the white-rot basidiomycetes have ability to decompose lignin most efficiently [25]. The genus *Trametes*, as one of branches of white-rot basidiomycetes, the members of this genus, such as *T. villosa* [25] and *T. gibbosa* BRFM 952 [26], have been reported that these strains have an unexpected high activity on lignin or crystalline cellulose. Previous study has showed that the carbohydrate-active enzymes (CAZymes) play a central role in the degradation process of glycoconjugate, oligo- and polysaccharide [27]. Hence, to obtain systematic understanding of the CAZymes of *Trametes* genus, we systemically identified the pan-genome of *Trametes* genus against the CAZy database and grouped CAZyme genes into different CAZyme families and CBMs based on family-specific HMMs [28]. In total, we identified 280 orthologous genes (Figure 3a) and grouped these genes into 87 CAZyme families. Our results showed that a large proportion of orthologous genes catalogued into glycoside hydrolases (35.36%, GHs), glycosyltransferases (21.07%, GTs), and carbohydrate esterases (13.57%, CEs, Figure 3a). The GH hydrolyze the glycosidic bond between two or more carbohydrates [27] and it contributes the most catalytic enzymes and involves in the degradation of lignocelluloses [29]. Moreover, we observed that the number of CAZymes of accessory clusters are higher than that of core clusters and specific genes (Figure 3a) and the number of genes belonged to different CAZymes in each *Trametes* strain (Figure 3b) are different, which suggested that the ability of strains of *Trametes* genus in the degradation of carbohydrates have significant difference.

To gain insights into the CAZyme composition of 9 strains of *Trametes* genus, we subdivided the 87 CAZyme families based on accessory clusters, core clusters and specific genes (Figure 3c-3e). As to the orthologous genes of accessory clusters, a slice of CAZyme families, such as AA9, CBM1, CE10, CE16, CE4, GH10, GH16, GH18, GH5, GH79, GT2, GT20, GT76 and PL14, were detected in all 9 *Trametes* strains (Figure 3c). Among these CAZyme families, we found that the number of CE10 (ranged from 5 to 20) and GH16 (ranged from 6 to 15) were higher than other CAZyme families (Figure 3c). We found that the counts of AA2 of *T. cinnabarina* BRFM137 (14) and CBM21 of *T. villosa* CCMB561 (24) were higher than that of other strains (Figure 3c). In contrast, the distributions of CAZyme families of core clusters, including AA2, CBM1, CBM14, CBM50, GH18, GH3, GH37 and GT90, in *Trametes* strains were similar (Figure 3d). Additionally, the orthologous genes of specific genes were assigned to different CAZyme families (Figure 3e). For example, 14 CAZyme families, including GH18 and GT4, were dominant in *T. villosa* CCMB561, and CE10 and GH16 were enriched in *T. sp.* AH28-2. Interestingly, we observed that AA7 was only detected in *T. villosa* CCMB561, CE5 was only identified in *T. versicolor* FP-101664 SS1, CH6 only appeared in *T. pubescens*, and CH89 was only detected *T. hirsute*. In a summary, the composition of CAZyme families of 9 *Trametes* strains showed that these strains have the ability to metabolize the same carbohydrates and also have the potential ability to degrade different carbohydrates.

Identification of biosynthetic gene clusters (BGCs) of *Trametes* genus

To obtain a better understanding of the secondary metabolite of *Trametes* genus, we applied antiSMASH to predict the biosynthetic gene clusters (BGCs). Our results showed that the BGCs of *Trametes* genus

were mainly divided into 6 strategies, including putative cluster of unknown type identified with the ClusterFinder algorithm (cf_putative), terpene cluster (terpene), nonribosomal peptide synthetase cluster (nrps), putative fatty acid cluster identified with the ClusterFinder algorithm (cf_fatty_acid), type I PKS cluster (t1pks), and lanthipeptide cluster (lantipeptide, Figure 4). Specifically, the 9 strains of *Trametes* genus harbored 47.78 BGCs on average and the number of BGCs of *Trametes* genus was ranged from 18 (*T. cinnabarina* FP 104138-Sp) to 66 (*T. sp.* AH28-2, Figure 4). Clusters of cf_putative and terpenes represented the majority of predicted specialized metabolite BGCs and contributed to the highest share among the 9 *Trametes* strains (Figure 4). The results showed that the clusters of cf_putative were enriched in the 9 strains of *Trametes* genus (ranged from 3 to 39, on average 25 BGCs, Figure 4), while *T. cinnabarina* FP 104138-Sp harbored only 3 clusters of cf_putative. When comparing the BGCs of terpene, it can be seen that *T. hirsute* harbored 17 BGCs and *T. cinnabarina* BRFM137 and *T. cinnabarina* FP 104138-Sp all harbored 9 BGCs. Moreover, a majority of these identified terpene BGCs could not be linked to known terpene BGCs. In addition, many predicted BGCs were grouped into “other” of these 9 strains.

Although the secondary metabolites of predicted BGCs are unknown to us, we identified quite a few enzymes as the core biosynthetic enzymes, such as cembrene C synthase, pristinol synthase, (+)-epi-cubebol synthase, and epi-isozizaene synthase, in several BGCs in most strains of *Trametes* genus. To gain insights of BGCs of *Trametes* genus, we chose three BGCs of *T. pubescens* as examples (Figure 5). Specifically, the BGCs of No. 13 was detected by the ClusterFinder algorithm built-in antiSMASH with 81.47% probability and the homologous gene clusters were identified from *T. versicolor* FP-101664 SS1 with 75% of genes show similarity and *Dichomitus squalens* LYAD-421 SS1 with 25% genes show similarity (Figure 5a). In addition, the monomer of predicted core structure of substrate was ala-ala ($C_6H_{12}O_2N_2$). The BGCs of No. 15 was detected as other unknown cluster with 83.63% probability and the homologous gene clusters were identified from *T. cinnabarina* BRFM137 with 66% of genes show similarity and *T. versicolor* FP-101664 SS1 with 66% of genes show similarity (Figure 5b), while the BGCs of No. 20 was also detected as other unknown cluster and the homologous gene clusters were identified from *T. versicolor* FP-101664 SS1 with 93% of genes show similarity and *T. cinnabarina* BRFM 137 with 56% of genes show similarity (Figure 5c). In summary, these results revealed that many BGCs of *Trametes* genus were undiscovered and the *Trametes* genus has a great potential value for producing secondary metabolite.

Conclusions

In conclusion, we systemically comparative analyzed the pan-genome of *Trametes* genus, one of important branches of white-rot basidiomycetes, based on the available genome of 9 *Trametes* strains and we explored the functional composition of 9 strains of *Trametes* genus, including COG annotation, GO annotation and CAZyme families. Furthermore, we predicted the biosynthetic gene clusters for each strain of *Trametes* genus. Our comparative analysis revealed that the *Trametes* genus exhibited an open pan-genome structure and tremendous diversity in both the number and variety of genes. The results of comparative analysis of pan-genome of *Trametes* genus also showed that the differences in the

composition of functional metabolisms, as well as the evolutionary relationships among the 9 strains of *Trametes* genus. Additionally, we observed that the high proportion of accessory genes and specific genes annotated with different COG annotations and GO annotations showed that the diversity of the functions among the *Trametes* strains of *Trametes* genus. Importantly, our results showed that the CAZyme families, such as GH, GT and CE, enriched in the *Trametes* strains, while the number of CAZymes of accessory clusters are higher than that of core clusters and specific genes and the number of genes belonged to different CAZymes in each *Trametes* strain suggested that the potential ability to degrade different carbohydrates for different strains of *Trametes* genus. Finally, we predicted many biosynthetic gene clusters with unknown functions in *Trametes* genus, which suggested that *Trametes* genus has a great potential value for producing secondary metabolite. Together, we uncover the genetic diversity and synthetic biology of secondary metabolite production of *Trametes* genus in this work. Our results provide a broader understanding of *Trametes* genus and importantly shed the lights on the composition of CAZyme families and the biosynthetic gene clusters of *Trametes* genus.

Materials And Methods

Genomic data collection of genus *Trametes*

We used 'Trametes' as the keyword and searched in genome database of National Center for Biotechnology Information (NCBI) on Sep 14th, 2018. We found that the genus *Trametes* comprising 8 species and containing 9 full genomes, such as *T. cinnabarina* BRFM137, *T. cinnabarina* FP 104138-Sp, *T. coccinea* BRFM310, *T. hirsuta*, *T. polyzona*, *T. pubescens*, *T. sp.* AH28-2, *T. versicolor* FP-101664 SS1 and *T. villosa*. Thus, we downloaded the genomic data (draft) of these strains for comparative genomics analysis.

Gene prediction and orthologs identification

We conducted the genome annotation for 9 strains using Pordigal [30] (version: 2.6). Specifically, to predict the genes and proteins for 9 genomic data of *Trametes* genus, we applied the Pordigal with default setting to recognize open reading frames (ORFs) and protein sequences [31]. We identified the protein orthologs of *Trametes* genus by using OrthMCL [32] (version: 2.0.9) with e-value < 1e-5 and inflation parameter of 1.5. We divided the homologous clusters into three groups: core, accessory and specific groups. The core genes or proteins represent the genes or proteins shared in all 9 genome of *Trametes* genus used in our study, while the accessory genes or proteins comprised genes or proteins shared by at least two strains but not all 9 strains of *Trametes* genus. The rest of genes or proteins only occurred in one strain were clustered into specific groups (strain-specific genes or proteins). We obtained the protein sequences of homologous clusters for *Trametes* genus.

Phylogeny analysis

The nuclear ribosomal Internal Transcribed Spacer (ITS) region has been reported as the primary fungal barcode marker [23] and ITS1 or ITS2 was widely used to identify the broadest range of fungi [24]. Hence, we applied ITSx [33], which is an open source software utility to extract the highly variable ITS1 and ITS2 subregions from ITS sequences, to extract the ITS1 and ITS2 sequences from the genomic data of 9 strains of *Trametes* genus. Meanwhile, we chose the ITS1 and ITS2 sequences of *Corioloropsis caperata* (accession number: AB158316) as outgroup. In our work, due to the fact that the ITS1 and ITS2 sequences of *Trametes coccinea* were not extracted, we selected the ITS1 and ITS2 sequences of *Pycnoporus coccineus* strain MUCL 38523 [34] (one strain of *Trametes coccinea*, accession number: FJ873395) to represent *Trametes coccinea* and construct the phylogenetic tree. The ITS1 and ITS2 sequences were aligned using the MUSCLE (version: 3.8.31) [35] with default setting, respectively. The multiple sequences alignment result was eliminated by using Gblocks (version: 0.91b) [36] to remove the regions that were divergent, misaligned, or with a larger number of gaps. Then we used PHYLIP (version: 3.696) [37] with 100 bootstrap iterations to construct a maximum likelihood tree based on the concatenated multiple sequences alignment result. Moreover, we built a pan-genome tree by MEGA software (version: 5) [38] based on the composition of orthologous. Specifically, we calculated the pairwise Manhattan distance between each strain based on the presence and absence of orthologs proteins.

Functional annotation

To obtain the COG functions, the protein sequences of homologous clusters for *Trametes* genus were aligned to individual COG proteins [39], which downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/COG/>) using BLSATP with e-value < 1e-4 and the top one of the result was chosen as the best annotation of each protein. The annotations of the protein sequences of homologous clusters were assigned to 25 functional categories. We also annotated the protein sequences of homologous clusters against eggNOG annotation database by using the eggnog-mapper [40] and we divided the GO annotations of protein sequences of homologous clusters into three groups, including biological process, molecular function and cellular component. We annotated these protein sequences against CAZyme database to investigate the functional composition related to carbohydrate metabolism. Specifically, we downloaded the CAZy database from dbCAN [41] (<http://csbl.bmb.uga.edu/dbCAN/>). According to the manual of dbCAN CAZyme annotation, protein sequences were annotated by running hmmscan from the HMMER (version: 3.1b1) [27, 42] with default setting and the annotated results was summarized into glycosyltransferase (GT), glycoside hydrolase (GH), carbohydrate-binding module (CBM), auxiliary activity (AA), polysaccharide lyase (PL) and carbohydrate esterase (CE).

Biosynthetic gene clusters detection

To detect the biosynthetic pathways of secondary metabolites for *Trametes* genus, antiSMASH (version: 4.0) [16] was used to identify the biosynthetic gene clusters (BGCs). A set of 9 genome sequences of

Trametes genus was used as the input data for predicting the BGCs by using antiSMASH with the mode of fungi and other default setting. The BGCs were determined to their subtypes (e.g., type I polyketide, terpenoid).

Declarations

Authors' contributions

This study was designed by YZ. YZ collected and downloaded the data. YZ and YJC analyzed the data. YZ, JJW, and ZDX wrote the initial draft of the manuscript. All authors revised the manuscript.

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Additional Information

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Table

Table 1 Comparison of the genomic features of 9 strains in *Trametes* genus.

Organism	DB accession number	Contigs/ Scaffolds	Genome size(bp)	CDSs/ ORFs*	Reference
<i>T. cinnabarina</i> BRFM137	CCBP010000001	776	33,668,918	59,163	[43, 44]
<i>T. cinnabarina</i> FP 104138-Sp	JSYY010000001	9,648	31,621,284	61,560	[45]
<i>T. coccinea</i> BRFM310	KZ084086	222	32,757,859	56,735	[46]
<i>T. hirsuta</i>	CP019371	13	37,433,966	61,150	[47]
<i>T. polyzona</i>	MKKQ010000001	5,321	36,629,716	65,446	[48]
<i>T. pubescens</i>	MNAD010000001	1,731	39,739,821	67,916	[49]
<i>T. sp.</i> AH28-2	LJJJ010000001	306	38,904,488	65,191	[50]
<i>T. versicolor</i> FP-101664 SS1	NW_007360321	283	44,794,008	71,443	[51]
<i>T. villosa</i> CCMB561	PUDQ010000001	10,327	57,987,392	101,817	[4]

Note: * represent the CDSs/ORFs were predicted by Pordigal.

Figures

Figure 1

Genetic diversity of strains in *Trametes* genus. a. The size of pan-genome (red) and core genome (blue) shared by different genomes, respectively. b. Core and specific genes families of 9 strains of *Trametes* genus. The number core genome shared by all strains is in the center (8,518). The number of non-overlapping portions of each oval represents the size of specific families. c. The phylogenetic tree based on the ITS1 sequences of 9 strains of *Trametes* genus and *Coriolopsis caperata*. d. The phylogenetic tree based on the ITS2 sequences of 9 strains of *Trametes* genus and *Coriolopsis caperata*. *C. caperata* was selected as outgroup to root the topology of phylogenetic tree. e. The UPGMA tree of Manhattan distance based on the pan-genome composition of 9 strains of *Trametes* genus.

Figure 2

COG and GO annotation of gene families. a. The distribution of COG annotation of pan-genome that assigned in 25 functional categories. b. The distribution of GO annotation of pan-genome in biological process, molecular function and cellular component. Accessory clusters (pink), core clusters (green), and specific genes (wathet).

Figure 3

The distributions of CAZymes in 9 *Trametes* strains of *Trametes* genus. a. The distribution of CAZymes in pan-genome of *Trametes* genus. b. The distribution of CAZymes of pan-genome in each *Trametes* strain grouped by accessory clusters, core clusters and specific genes. The numbers shown in subgraph represents the orthologous genes assigned by CAZymes and grouped by accessory clusters (pink), core clusters (green), and specific genes (wathet). c. The distribution of CAZymes of accessory clusters in each *Trametes* strain. d. The distribution of CAZymes of core clusters in each *Trametes* strain. e. The distribution of CAZymes of specific genes in each *Trametes* strain. The numbers shown in subgraphs represent the orthologous genes assigned by CAZymes. GT, glycosyltransferase; GH, glycoside hydrolase; CE, carbohydrate esterase; CBM, carbohydrate-binding molecule; AA, auxiliary activities; PL, polysaccharide lyases.

Figure 4

The distribution of biosynthetic gene clusters in 9 *Trametes* strains of *Trametes* genus. The distribution of biosynthetic gene clusters in each *Trametes* strain. The numbers shown in graph represent the counts of different types of biosynthetic gene clusters predicted by antiSMASH for each *Trametes* strain. Note: cf_putative, putative cluster of unknown type identified with the ClusterFinder algorithm; terpene, terpene cluster; nrps, nonribosomal peptide synthetase cluster; cf_fatty_acid, putative fatty acid cluster identified with the ClusterFinder algorithm; t1pks, type I PKS cluster; lantipeptide, lanthipeptide cluster; other, cluster containing a secondary metabolite-related protein that does not fit into any other category.

Figure 5

The probability and homologous gene clusters for three putative BGCs of *T. pubescens*. The probability and homologous gene clusters for the putative BGCs of a: No. 13, b: No. 15 and c: No. 20. In the subgraph, the same colors of the boxes with arrows are similar genes.