

Comparative Genomic Analysis of *Colletotrichum fructicola* and *Colletotrichum spaethianum* Reveals Contrasting Pathogenic Strategies in *Polygonatum cyrtonema*

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Abstract

Colletotrichum fructicola and *C. spaethianum* cause distinct anthracnose symptoms on *Polygonatum cyrtonema*, but the genomic basis of their divergent pathogenesis remains unclear. Here, we performed a comparative genomic analysis using near-complete genome assemblies and functional annotation across eight databases, revealing a pronounced dichotomy in genomic investment. CfC1 exhibits expansions in cytochrome P450s, secondary metabolite clusters, transcription-associated domains, transporters, and CAZymes, particularly those degrading cellulose, hemicellulose, and pectin—consistent with a generalist aggressive maceration strategy. Conversely, CsY1 possesses a streamlined genome enriched in signal transduction and membrane components, uniquely harboring bacteria-like pathways (two-component system, chemotaxis, flagellar assembly, PTS) that are largely absent in CfC1. CfC1 encodes 46.8% more secreted proteins and 71.4% more effectors, while CsY1 shows a higher proportion of virulence-modulating genes, suggesting fine-tuned rather than redundant virulence. Collectively, CfC1 emerges as a generalist pathogen leveraging detoxification and regulatory expansion, whereas CsY1 is specialized for rapid host sensing and colonization via enhanced signaling and transport. This study provides a genomic framework for understanding niche partitioning within *Colletotrichum* and informs species-specific disease management on *P. cyrtonema*.

Background

Polygonatum cyrtonema Hua (Asparagaceae), known as Duohua Huangjing in Chinese, is a perennial medicinal herb widely cultivated for its rhizomes, which possess immunomodulatory, antiaging, antidiabetic, and antifatigue properties (Chinese Pharmacopoeia Commission 2025). However, increasing market demand has necessitated artificial cultivation, leading to the emergence of anthracnose as a serious threat, causing substantial yield losses and reduced rhizome quality (Zhou et al. 2017; Ma et al. 2021; Dan et al. 2023). Recent surveys across major production areas have confirmed the widespread occurrence of this disease. Between 2022 and 2023, 50 *Colletotrichum* isolates were obtained from diseased leaves collected in three *P. cyrtonema* production areas within the Two Lakes region of China (Hubei and Hunan provinces), with morphological and molecular analyses identifying six *Colletotrichum* species as causative agents: *C. aenigma*, *C. siamense*, *C. gloeosporioides*, *C. spaethianum*, *C. fructicola*, and *C. karsti* (Cai et al. 2025).

The genus *Colletotrichum* comprises one of the most economically important groups of plant pathogenic fungi, exhibiting remarkable variation in host specificity, tissue preference, and infection lifestyles, ranging from biotrophy to necrotrophy (Dean et al. 2012; O'Connell et al. 2012; De Silva et al. 2017; Liu et al. 2022; Usman et al. 2026). In a comprehensive survey of anthracnose on *P. cyrtonema* across four cultivation areas in Nanping, Fujian Province, China, two species emerged as the dominant causal agents: *C. spaethianum* (31 strains, 40.79%) and *C. fructicola* (20 strains, 26.32%). Critically, *C. spaethianum* causes more severe damage to leaves, while *C. fructicola* displays higher virulence on stems (Hu, 2023). This differential pathogenicity on the same host provides a valuable opportunity to investigate the genomic mechanisms underlying host adaptation and tissue-specific virulence.

To investigate the genomic basis of this tissue-specific virulence, we recently obtained near-complete genome assemblies for representative isolates of both species: *C. fructicola* CfC1 and *C. spaethianum* CsY1. The CfC1 assembly is 56.77 Mb in 13 contigs (N50 = 4.94 Mb), whereas the CsY1 assembly is 54.49 Mb in 107 contigs (N50 = 1.60 Mb). Both assemblies show high completeness, with BUSCO scores exceeding 97.6%. Gene prediction identified 16,928 protein-coding genes in CfC1 and 15,753 in CsY1. Among these, Pfam annotated 11,547 (68.21%) and 11,456 (72.72%), GO annotated 6,837 and 6,778, KOG annotated 12,378 and 12,680, KEGG annotated 5,121 and 6,428, PHI-base annotated 3,531 and 3,616, and TCDB annotated 337 and 481 genes, respectively. Annotation against Pfam, GO, KOG, KEGG, PHI-base, P450, TCDB, and CAZy achieved overall rates of 68–80%, providing broad coverage for systematic comparison. Repeat annotation revealed that CsY1 contained more than twice the proportion of repetitive sequences of CfC1, mainly LTR retrotransposons (4.41% vs. 0.43%), whereas CfC1 had a higher proportion of DNA transposons (0.87% vs. 0.27%). Initial functional annotation suggested a pronounced dichotomy in genomic investment: CfC1 is enriched in metabolic detoxification and immune-suppression genes, with an expanded secretome and effector repertoire, whereas CsY1 is biased toward environmental sensing, membrane transport, and tissue maceration (D'Angelo et al. 2025). However, a systematic comparative analysis integrating multiple functional databases has been lacking.

Comparative genomic approaches have proven powerful in deciphering the genetic basis of pathogenicity in *Colletotrichum*, revealing expansions of gene families encoding carbohydrate-active enzymes (CAZymes), secondary metabolite biosynthesis enzymes, cytochrome P450s, and secreted effector proteins that correlate with lifestyle transitions and host range (Baroncelli et al. 2016; Gan et al. 2016; Liang et al. 2018, 2020). Recent genus-wide genomic analyses have further advanced our understanding of *Colletotrichum* evolution and host adaptation. A study sequencing 30 *Colletotrichum* species covering the diversity within the genus provided evidence of independent host jumps from dicots to monocots during evolution, coinciding with a progressive shrinking of the plant cell wall degradative arsenal and expansions in lineage-specific gene families (Baroncelli et al. 2024). Comparative genomics of apple bitter rot pathogens identified extensive redundancy in CAZyme classes and effectors across species, while secondary metabolite gene clusters exhibited the greatest diversity, providing a platform for identifying targets underlying pathogenicity and virulence (Khodadadi et al. 2024). Studies comparing closely related *Colletotrichum* species with distinct pathogenic behaviors have provided valuable insights into virulence evolution (Liu et al. 2020). Within-species comparisons have also proved informative: comparative genomic analysis of *C. lindemuthianum* pathotypes with contrasting virulence levels revealed that large genomes with high transposable element contents have undergone expansions generating intraspecific diversity, with pathotypes differing markedly in secretome size, effector content, and CAZyme repertoire enriched in hemicellulolytic enzymes (Morelos-Martínez et al. 2024). Moreover, genomic resources from seven *Colletotrichum* isolates associated with strawberry anthracnose fruit rot and crown rot in North Carolina revealed that *C. siamense* isolates exhibited the highest numbers of effectors (339–480), secondary metabolic gene clusters (67–90), and CAZymes (800–1,060) (Adhikari et al. 2025). However, most comparative studies have focused on

species within the same species complex; comparisons between distantly related complexes that co-infect the same host are rare and can reveal fundamentally different evolutionary solutions to the same ecological challenge.

Here, we present a comprehensive comparative genomic analysis of *C. fruticola* CfC1 and *C. spaethianum* CsY1, using the annotated genome resources described above, to systematically characterize their genomic architecture and pathogenicity-related gene repertoires. By integrating multiple functional annotation databases (Pfam, GO, KEGG, KOG, PHI, P450, TCDB and CAZy), we aimed to identify the genetic determinants that explain their distinct host interactions and tissue-specific virulence. Our findings provide a genomic framework for understanding divergent pathogenic strategies and offer novel targets for disease management and resistance breeding in this economically valuable crop.

Results

Infection Symptoms

In *P. cyrtonema* plantations, *C. spaethianum* infected leaves or leaf margins, causing lesions on leaf tissues. At early stages, the lesions appeared as light reddish-brown circular spots. As the disease progressed, they gradually expanded, turned rust-colored, and became subcircular to irregular in shape, resulting in extensive necrosis, with perforation occurring at later stages (Fig. 1a). Additionally, *C. spaethianum* also infected stems, producing dark brown spots that later coalesced into elongated lesions on stems (Fig. 1b), and nearly circular, dark brown lesions on fruits (Fig. 1c). In contrast, *C. fruticola* infected leaves, with pale yellow spots initially emerging on leaf margins; subsequently, the lesions turned grayish brown, merged into larger patches, and eventually led to leaf perforation (Fig. 1d). *C. fruticola* also infected stems, where small brown spots appeared initially and gradually coalesced into large, irregularly shaped brownish-black lesions, causing stem dieback (Fig. 1e). Furthermore, *C. fruticola* infected fruits, producing black, nearly circular lesions with depressed centers (Fig. 1f).

Figure 1. Symptoms caused by *C. spaethianum* and *C. fruticola* on *Polygonatum cyrtonema*. leaves, stems, and fruits. **a** Early light reddish-brown circular spots and later rust-colored, irregular lesions with perforation on leaves caused by *C. spaethianum*. **b** Dark brown spots coalescing into elongated lesions on stems caused by *C. spaethianum*. **c** Nearly circular, dark brown lesions on fruits caused by *C. spaethianum*. **d** Pale yellow spots turning grayish brown, merging into large patches with perforation on leaves caused by *C. fruticola*. **e** Small brown spots developing into large, irregular brownish-black lesions and stem dieback caused by *C. fruticola*. **f** Black, nearly circular lesions with depressed centers on fruits caused by *C. fruticola*.

Genomic Determinants of the Aggressive Pathogenic Lifestyle in CfC1

Pfam domain analysis revealed that CfC1 possesses substantially expanded repertoires relative to CsY1 across multiple functional categories. Notably, CfC1 exhibits marked expansions in cytochrome P450s (279 vs. 158), secondary metabolite biosynthetic gene clusters (90 vs. 59), and transcription-associated domains, including fungal-specific transcription factors (378 vs. 168) and Zn₂Cys₆ binuclear cluster domains (342 vs. 103) (D'Angelo et al. 2025). Additional expansions were observed in transporter families (major facilitator superfamily: 458 vs. 317; sugar and other transporters: 193 vs. 127), oxidoreductases (short-chain dehydrogenase: 160 vs. 133; zinc-binding dehydrogenase: 100 vs. 76; alcohol dehydrogenase GroES-like domain: 98 vs. 66), protein kinases (125 vs. 94), enoyl-ACP reductase (101 vs. 79), FAD-binding domains (190 vs. 125), methyltransferases (99 vs. 59), heterokaryon incompatibility proteins (HET, 164 vs. 52), and ankyrin repeats (111 vs. 59) (Fig. 2a; Additional file 1: Table S1). Consistent with these Pfam findings, GO annotation indicated that CfC1 is enriched in genes associated with transmembrane transport, transcriptional regulation, zinc binding, and secondary metabolism (e.g., mycotoxin biosynthesis), whereas CsY1 showed greater representation in signal transduction, ATP binding, and membrane components (Fig. 2b; Additional file 2: Table S2).

Figure 2. Domain and GO functional annotation of CfC1 and CsY1. **a** Pfam domain analysis. **b** Gene Ontology (GO) annotation.

KOG classification corroborated these trends, with CfC1 exhibiting significant enrichment in energy production and conversion (626 vs. 559) and secondary metabolism (989 vs. 761) (Fig. 3a; Additional file 3: Table S3). In contrast, KEGG pathway analysis revealed that CfC1 possesses a more limited metabolic and regulatory repertoire, with lower gene counts across nearly all categories and the absence of several pathways present in CsY1—particularly those involved in cell envelope biosynthesis, motility, signal transduction, and host–pathogen interactions (Fig. 3b; Additional file 4: Table S4).

Figure 3. KOG classification and KEGG pathway analysis of CfC1 and CsY1. **a** KOG functional classification. **b** Key differential pathways identified by KEGG

Pathogenicity-related annotations further distinguished the two species. PHI-base analysis showed that CfC1 harbors more genes conferring ‘lethal’ (141 vs. 130) and ‘unaffected pathogenicity’ (1,705 vs. 1,539) phenotypes, while counts for ‘increased’ and ‘reduced’ virulence were lower than in CsY1 (Fig. 4a; Additional file 5: Table S5). Transporter classification via TCDB revealed that several families were more abundant in CfC1 than in CsY1. Specifically, CfC1 exhibited slightly higher copy numbers in the P-ATPase (22 vs. 21), NPC (22 vs. 20), and MC (16 vs. 14) families. More notably, CfC1 possessed three copies of the Arv1 family (lipid intermediate transporter) and single copies of M6PR, Trk, OPT, and CNT, all of which were absent in CsY1 (Fig. 4b; Additional file 6: Table S6). Comparative genomic analysis of P450 genes revealed a substantially larger and more diverse repertoire in CfC1, with expansions in specific families—particularly CYP65, CYP68, CYP526, CYP567, and CYP620—and the presence of multiple lineage-specific P450 families (Fig. 4c; Additional file 7: Table S7).

Figure 4. Comparison of PHI, TCDB, and P450 annotations between CfC1 and CsY1, emphasizing pathogenicity-related gene count differences. **a** PHI annotation. **b** TCDB annotation. **c** P450 annotation.

Furthermore, the CfC1 strain exhibited a notably higher abundance of genes assigned to Glycoside Hydrolases (GH) (427 vs. 371), Auxiliary Activities (AA) (224 vs. 166), and Carbohydrate-Binding Modules (CBM) (40 vs. 20), and slight advantages were also observed for CfC1 in Carbohydrate Esterases (CE) (76 vs. 70) and Polysaccharide Lyases (PL) (51 vs. 39) (Fig. 5a, Additional file 8: Table S8). The most striking discrepancy was identified within the AA7 and AA3 subfamilies, where CfC1 substantially outnumbered CsY1, with 74 vs. 34 genes in AA7 and 52 vs. 37 genes in AA3, respectively. Additionally, CfC1 showed a pronounced advantage in the GH43 (43 vs. 25) and GH3 (30 vs. 30, with a higher relative ratio) subfamilies, as well as in CBM50 (16 vs. 5) and CBM67 (10 vs. 2) (Fig. 5b, Additional file 8: Table S8).

Figure 5. Comparative analysis of CAZyme families between CfC1 and CsY1. **a** Abundance and category distribution of CAZymes in the predicted secretome. **b** Key CAZyme subfamilies involved in fungal and plant cell wall degradation.

Collectively, the pathogenic strategy of CfC1 is not a blunt increase in virulence but a coordinated program of effector deployment and regulatory fine-tuning that modulates virulence spatiotemporally, evading host immunity and averting premature lysis. This is achieved via convergent expansions of CAZymes, toxins/P450s, and transporters, all integrated under a complex regulatory network.

Genomic Specialization for Signal Perception, Transport, and Motility in CsY1

Pfam domain analysis of CsY1 revealed a functionally streamlined genome, with substantial contractions across multiple functional categories relative to CfC1. Marked reductions were observed in cytochrome P450s (674 vs. 817), secondary metabolite biosynthetic gene clusters (59 vs. 90), and transcription-associated domains, including fungal-specific transcription factors (168 vs. 378) and Zn₂Cys₆ binuclear cluster domains (103 vs. 342) (D'Angelo et al. 2025). Additional contractions were evident in transporter families (major facilitator superfamily: 317 vs. 458; sugar and other transporters: 127 vs. 193), oxidoreductases (short-chain dehydrogenase: 133 vs. 160; zinc-binding dehydrogenase: 76 vs. 100; alcohol dehydrogenase GroES-like domain: 66 vs. 98), protein kinases (94 vs. 125), enoyl-ACP reductase (79 vs. 101), FAD-binding domains (125 vs. 190), methyltransferases (59 vs. 99), heterokaryon incompatibility proteins (HET, 52 vs. 164), and ankyrin repeats (59 vs. 111) (Fig. 2a; Additional file 1: Table S1).

Consistent with these Pfam findings, GO annotation indicated that CsY1 is enriched in genes associated with signal transduction, ATP binding, and membrane components, contrasting with CfC1 which showed greater representation in transmembrane transport, transcriptional regulation, zinc binding, and secondary metabolism (Fig. 2b; Additional file 2: Table S2). KOG classification further corroborated these trends, with CsY1 exhibiting lower representation in energy production and conversion (559 vs. 626) and secondary metabolism (761 vs. 989) (Fig. 3a; Additional file 3: Table S3). In contrast, KEGG pathway analysis revealed that CsY1 possesses a more extensive and diverse metabolic and regulatory

repertoire, with higher gene counts across nearly all categories and the presence of several pathways absent in CfC1—particularly those involved in cell envelope biosynthesis, motility, signal transduction, and host–pathogen interactions. Notably, KEGG uniquely identified bacteria-like pathways in CsY1, including the two-component system (+ 72 KOs), bacterial chemotaxis (+ 11), flagellar assembly (+ 17), and the phosphotransferase system (PTS), all of which were either minimally represented or absent in CfC1 (Fig. 3b; Additional file 4: Table S4).

Pathogenicity-related annotations further distinguished the two species. PHI-base analysis showed that CsY1 harbors more genes associated with ‘increased’ and ‘reduced’ virulence phenotypes than CfC1, while counts for ‘lethal’ (130 vs. 141) and ‘unaffected pathogenicity’ (1,539 vs. 1,705) were lower, suggesting a more finely tuned but less redundant virulence arsenal (Fig. 4a; Additional file 5: Table S5). The most striking differences were observed in the ABC and RND superfamilies. CsY1 had more than double the ABC copies (43 vs. 20), the RND family showed an even greater disparity (17 copies in CsY1 vs. 1 in CfC1), CsY1 also outnumbered CfC1 in MFS (53 vs. 46), DMT (13 vs. 8), and Sec (6 vs. 2), reflecting broader secondary transport and secretion capabilities. Furthermore, COX, MTB, PMO, and OMR were present only in CsY1 (Fig. 4b; Additional file 6: Table S6). Comparative genomic analysis of P450 genes confirmed a substantially smaller and less diverse repertoire in CsY1, with contractions in specific families—particularly CYP53, CYP65, CYP68, and CYP526—and the absence of several lineage-specific P450 families present in CfC1 (Fig. 4c; Additional file 7: Table S7). Furthermore, CsY1 possessed marginally more Glycosyltransferases (GT) genes than CfC1 (134 vs. 123) (Fig. 5a, Additional file 8: Table S8). CsY1 harbored a greater number of genes in specific subfamilies, primarily GT2 (30 vs. 21), GH18 (28 vs. 20), AA9 (34 vs. 28), and CE4 (19 vs. 15) (Fig. 5b, Additional file 8: Table S8).

Overall, CsY1 adopts a non-aggressive pathogenic strategy, with a streamlined genome enriched in signal transduction, bacterial-like two-component systems, and efflux transporters (ABC/RND). Its virulence is finely modulated with low redundancy, supported by cell-wall synthesis (GTs) for host adaptation, reflecting a sensing-and-responding lifestyle distinct from CfC1’s offensive arsenal.

Divergent Pathogenic Toolkits

The pathogenic repertoires of CfC1 and CsY1 reflect a fundamental dichotomy in genomic investment. CfC1 exhibits a markedly expanded secretome (1,299 vs. 885) and effector candidate pool (677 vs. 395), with apoplastic effectors showing the most pronounced expansion (495 vs. 278) (D'Angelo et al. 2025), alongside a higher proportion of PHI-base genes classified as “no effect” on pathogenicity—consistent with a redundant, broad-spectrum strategy. Conversely, CsY1 is enriched in genes modulating virulence (both increased and reduced), indicative of fine-tuned regulation, and specializes in signal transduction, membrane transport, and motility pathways for rapid host sensing and invasion (Fig. 4a, Additional file 5: Table S5). Collectively, these findings position CfC1 as a generalist pathogen leveraging detoxification and transcriptional plasticity, while CsY1 emerges as a specialist optimized for efficient colonization.

Discussion

The genus *Colletotrichum* encompasses hemibiotrophic phytopathogens with remarkable diversity in host range and infection strategies (Liang et al. 2018). Species within this genus have evolved tailored virulence-related gene repertoires reflecting their specific ecological niches (Gan et al. 2016). Our comparative genomic analysis of *C. fructicola* CfC1 and *C. spaethianum* CsY1—two species causing distinct symptoms on *P. cyrtonema*—reveals a pronounced dichotomy in genomic investment that aligns with their respective infection strategies.

Genomic Architecture and Repeat Element Dynamics

The two genomes differ substantially in structural composition. CfC1 possesses a more compact genome with markedly lower repeat content, whereas CsY1 exhibits a more fragmented assembly with over twice the proportion of repetitive sequences, primarily LTR retrotransposons (D'Angelo et al. 2025). This elevated transposable element load in CsY1 may contribute to genomic plasticity and accelerated evolution of lineage-specific genes, potentially facilitating adaptation to its specialized host niche (Liang et al. 2018). Conversely, the higher proportion of DNA transposons in CfC1 suggests alternative mechanisms of genomic diversification. The near-complete assemblies and comprehensive functional annotation provide a robust foundation for interpreting these structural differences (D'Angelo et al. 2025).

The Generalist Offensive Strategy of *C. fructicola*

CfC1 exhibits a genomic architecture consistent with a generalist pathogen capable of exploiting diverse hosts and environments. The substantial expansion of cytochrome P450 genes and secondary metabolite biosynthetic gene clusters endows CfC1 with enhanced detoxification capabilities against plant antimicrobial compounds and an expanded capacity for virulence-associated secondary metabolite biosynthesis (D'Angelo et al. 2025). A broad CYPome has been suggested to play a critical role in optimizing colonization and virulence (Ortiz-Álvarez et al. 2024). Notably, CfC1 harbors three accessory genes within its P450 and NRPS repertoires that are essential for pathogenicity in *C. fructicola* causing Glomerella leaf spot on apple (Liang et al. 2024), highlighting the functional significance of these expansions in mediating host-specific virulence.

The expanded CAZyme repertoire of CfC1—considerably larger than that of CsY1—further supports its offensive strategy, with significant amplifications in families involved in cellulose, hemicellulose, and pectin degradation (GH28, PL1, GH43). This enhanced capacity for plant cell wall degradation enables efficient tissue maceration across multiple host tissues, consistent with the aggressive lesion expansion observed during *C. fructicola* infection.

The enrichment of transcription-associated domains—including fungal-specific transcription factors and Zn₂Cys₆ binuclear cluster domains—indicates a sophisticated regulatory network capable of fine-tuning gene expression in response to diverse host environments (D'Angelo et al. 2025). This regulatory

complexity, combined with expanded transporter families (e.g., major facilitator superfamily), equips CfC1 with broad metabolic adaptability and efficient nutrient acquisition.

The expanded effector arsenal of CfC1 (677 vs. 395 candidates) reflects substantial genomic investment in host immune suppression. Recent functional studies have validated the adaptive significance of such expansions. For instance, the secreted xylanase CfXyn11A from *C. fructicola* not only degrades plant cell wall polymers but also directly suppresses host immune responses in the apoplast (Han et al. 2025). The functional redundancy within CfC1's effector arsenal—evidenced by its high proportion of “reduced virulence” (rather than “loss of virulence”) genes in PHI-base—provides considerable adaptive flexibility, allowing the pathogen to overcome host resistance and maintain a broad host range (Vleeshouwers and Oliver 2014). These genomic attributes align with previous reports that generalist *Colletotrichum* species exhibit expansions of pathogenicity-related gene families (Baroncelli et al. 2016; Liang et al. 2018).

The Specialist Sensing-and-Invasion Strategy of *C. spaethianum*

The genomic configuration of CsY1 embodies a distinct evolutionary philosophy—prioritizing environmental perception and rapid physiological response over broad enzymatic versatility. CsY1 has undergone a marked contraction in its CAZyme inventory, particularly among families responsible for pectin backbone cleavage (GH28, PL1) and hemicellulose modification (CE5, GH43). However, the selective retention and expansion of auxiliary activity family AA9 and glycosyltransferase family GT2 point to a specialized strategy favoring localized oxidative disruption of crystalline cellulose and cell wall synthesis modulation over wholesale polysaccharide hydrolysis—consistent with a pathogen that relies on rapid entry and nutrient scavenging rather than sustained enzymatic maceration (Wang et al. 2024).

The most compelling signature of CsY1's specialization lies in what has been amplified and acquired. The 6.8-fold enrichment in phosphorelay signal transduction genes relative to CfC1 underscores an extraordinary capacity to perceive and transduce extracellular signals. More strikingly, KEGG-based identification of complete or near-complete pathways for bacterial chemotaxis, flagellar assembly, and the phosphotransferase system (PTS) in CsY1—alongside their near-absence in CfC1—hints at a remarkable evolutionary innovation. These pathways, classically associated with prokaryotic motility and carbon uptake, are virtually unprecedented in filamentous ascomycetes, raising the possibility of horizontal gene transfer from bacterial sources (Liang et al. 2018). Such prokaryotic-like signaling modules may have been co-opted to govern polarized hyphal growth, directional sensing of host surface topography, or rapid metabolic reprogramming upon host contact.

Complementing this enhanced sensory apparatus is an expanded membrane transporter repertoire, particularly within the MFS and ABC families. In necrotrophic pathogens, the ability to rapidly import host-derived nutrients and efflux plant-derived antimicrobial compounds is paramount to successful colonization (Coleman and Mylonakis 2009; Buiate et al. 2017). The elevated transporter counts in CsY1 reflect a physiological prioritization of resource competition and toxin tolerance, enabling the fungus to thrive in the chemically hostile environment of necrotic leaf tissue. This transporter-centric strategy

aligns with the observed disease phenotype: rapidly expanding, rust-colored lesions on leaves that coalesce and perforate.

PHI-base analysis revealed that CsY1 harbors a higher proportion of genes associated with increased or reduced virulence phenotypes, whereas CfC1 contains more genes with no effect on pathogenicity. This pattern suggests that CsY1's virulence relies on a more critical, non-redundant set of effectors, rendering the pathogen potentially more vulnerable to resistance breeding strategies targeting these essential factors. At the same time, the PHI-base profile implies that CsY1's pathogenic success is not primarily dependent on prolonged immune suppression, but rather on the capacity to rapidly induce and exploit host cell death—an aggressive necrotrophic strategy particularly advantageous on leaf tissues (De Silva et al. 2017).

Recent comparative mitogenome analyses have revealed that the *C. spaethianum* and *C. gloeosporioides* complexes have diverged substantially in genomic organization and adaptive strategies (Wu et al. 2025). The observation of niche partitioning among *Colletotrichum* species on the same host is not unique to *P. cyrtoneura*; studies on mango anthracnose have revealed contrasting roles of *C. siamense* on leaves and *C. asianum* on fruits (Meng et al. 2026), paralleling our finding that CfC1 and CsY1 have partitioned stem and leaf niches through fundamentally different genomic strategies.

Genotype–Phenotype Links

The contrasting genomic architectures provide a compelling mechanistic explanation for the distinct symptom types and tissue preferences observed in the field. On stems—characterized by thickened cell walls, abundant lignin and cellulose, and diverse antimicrobial secondary metabolites—CfC1's expanded cytochrome P450 repertoire (particularly CYP53, CYP65, CYP68) is well-suited to detoxify host defense compounds (Jawallapersand et al. 2014; Fraser et al. 2002). Concurrently, the large arsenal of apoplastic effectors enables CfC1 to suppress pattern-triggered immunity during the early biotrophic phase, critical for establishing infection in immunologically vigilant stem tissues. The abundance of transcription factors and Zn₂Cys₆ regulators further suggests a sophisticated ability to modulate gene expression in response to the stem's chemical environment.

In contrast, CsY1's preferential virulence on leaves aligns with its genomic specialization for rapid sensing, nutrient acquisition, and aggressive tissue maceration. The dramatic expansion of two-component signal transduction systems and the unique presence of bacterial-like chemotaxis and flagellar assembly pathways suggest an extraordinary capacity for surface perception and directional growth toward infection sites. Recent studies have elucidated the molecular basis of such perception: the Ras2 homologue Cgras2 regulates conidial germination polarity, appressorium formation, and virulence in *C. gloeosporioides* (Xiao et al. 2025), while the G protein alpha subunit CgrGa3 governs vegetative growth, stress responses, and pathogenicity in *C. graminicola* (Wei et al. 2025). Once established, expanded MFS and ABC transporters facilitate efficient nutrient uptake from damaged cells and efflux of plant-derived toxins, supporting necrotrophic growth (Oliveira et al. 2022; Li et al. 2021).

This genotype–phenotype concordance underscores that the two species have evolved coherent pathogenic strategies in which gene family expansions and contractions are directly aligned with the physical and biochemical challenges of their preferred infection sites.

Evolutionary and Management Implications

The observed divergence reflects the distinct evolutionary trajectories of the *C. gloeosporioides* and *C. spaethianum* complexes. The former typically adopts a hemibiotrophic lifestyle with prolonged biotrophy, while the latter transitions more rapidly to necrotrophy (O'Connell et al. 2012; Damm et al. 2009). While both CfC1 and CsY1 are capable of infecting multiple tissues of *P. cyrtonema*, quantitative differences in virulence are evident: CsY1 exhibits stronger pathogenicity on leaves, whereas CfC1 demonstrates greater virulence on stems (Hu 2023). This pattern suggests that both species have retained the capacity to colonize diverse tissues but have evolved specialized adaptations conferring differential fitness advantages on specific tissue types.

From a disease management perspective, the unique bacteria-like pathways in CsY1 represent novel targets for fungicide development; inhibitors targeting bacterial two-component systems could be adapted for antifungal strategies (Bem et al. 2015). Furthermore, the expanded transporter repertoire in CsY1 suggests that efflux pump inhibitors could be particularly effective against this species. For resistance breeding, the functional redundancy in CfC1's effector arsenal suggests that developing broad-spectrum resistance may be challenging, as the pathogen can likely compensate for the loss of individual effectors. In contrast, the more streamlined virulence dependency of CsY1 suggests that deploying resistance genes recognizing its critical effectors could be more readily achieved (Vleeshouwers and Oliver 2014). Recent advances in phylogenetic discrimination within the *C. gloeosporioides* species complex (Tan et al. 2025) will facilitate future population-level studies and the development of molecular diagnostics for species-level identification in *P. cyrtonema* production systems.

Conclusions

Comparative genomic analysis revealed fundamentally divergent pathogenic strategies in *C. fructicola* CfC1 and *C. spaethianum* CsY1 on *P. cyrtonema*. CfC1 employs a generalist offensive strategy, with expanded cytochrome P450s, secondary metabolite biosynthetic gene clusters, carbohydrate-active enzymes, transcriptional regulators, and effectors, supporting detoxification, chemical suppression, cell wall degradation, and broad tissue colonization, especially stems. In contrast, CsY1 adopts a specialist sensing and invasion strategy, with streamlined enzymatic and regulatory machinery but enriched signal transduction, membrane transporters, bacteria-like pathways, virulence-associated genes, and glycosyltransferases, enabling rapid host perception, nutrient acquisition, and tissue maceration, especially leaves. These contrasting architectures explain their distinct symptoms and tissue preferences—prolonged hemibiotrophic stem colonization by CfC1 versus rapid necrotrophic leaf

progression by CsY1—and highlight the genomic plasticity underlying niche partitioning within a single host.

Methods

Fungal Isolates

Colletotrichum fructicola CfC1 (C1_DY2_B) and *Colletotrichum spaethianum* CsY1 (Y1_DY3_A) were isolated in 2021 from diseased leaves of *Polygonatum cyrtoneura* exhibiting typical anthracnose symptoms; CfC1 was obtained from Chongren, and CsY1 from Huaqiao, Nanping City, Fujian Province, China. Species identities were confirmed by morphological characterization and multilocus phylogenetic analysis. Both strains are deposited at the College of Plant Protection, Fujian Agriculture and Forestry University. Their genome assemblies were first reported in the IMA Genome-F20 initiative (D'Angelo et al. 2025).

Genome Sequencing, Assembly, Annotation and Data Visualization

Detailed methods for DNA extraction, genome sequencing, assembly, and annotation were described in D'Angelo et al. (2025). Briefly, genomic DNA was extracted using a rapid fungal genomic DNA isolation kit (Sangon Biotech, Shanghai, China). PacBio RSII and Illumina HiSeq 3000 sequencing were performed at Beijing Novogene Bioinformatics Technology Co. Ltd. Genome size was estimated using GenomeScope 2.0 (Ranallo-Benavidez et al. 2020). De novo assembly was performed using Flye (v2.4.2) (Kolmogorov et al. 2019) followed by error correction with NextPolish (v1.4.0) (Hu et al. 2020). Assembly completeness was assessed using BUSCO (v5.3.0) (Manni et al. 2021) with the fungi_odb10 and ascomycota_odb10 lineages. Repeat elements were annotated using RepeatModeler (v2.0.2) (Flynn et al. 2020) and RepeatMasker (v4.1.2) (Tarailo-Graovac and Chen 2009). Protein-coding genes were predicted using BRAKER (v2.1.6) (Bruna et al. 2021).

Functional annotation of predicted proteins was performed using multiple databases. EggNOG-mapper (v2) (Cantalapiedra et al. 2021) was used for annotation against the Pfam, GO, KEGG, and KOG databases. Diamond BLASTP (v2.0.11.149) (Buchfink et al. 2021) was used against the NR and Swiss-Prot databases. InterProScan (v5.52-86.0) (Jones et al. 2014) was used for domain identification.

CAZymes were identified using dbCAN3 (Zheng et al. 2023). Cytochrome P450 enzymes were annotated by BLASTP search against the Fungal Cytochrome P450 Database (Moktali et al. 2012). Secondary metabolite biosynthesis-related gene clusters were predicted using antiSMASH (v6.1.1) (Blin et al. 2021). Secreted proteins were predicted using SignalP (v5.0) (Almagro Armenteros et al. 2019) and TMHMM (v2.0) (Krogh et al. 2001). Effector proteins were identified using EffectorP (v3.0) (Sperschneider and Dodds 2022). Transport proteins were annotated by searching against the

Transporter Classification Database (Saier et al. 2021). Pathogen– interaction genes were identified by BLASTP search against PHI-base (v4.14) (Urban et al. 2022). Origin 2024 was used for data processing and graph generation (OriginPro 2024, OriginLab Corporation, Northampton, MA, USA).

Abbreviations

AA

Auxiliary Activities

ABC

ATP-binding Cassette

APC

Amino Acid-Polyamine-Organocation

BGCs

Biosynthesis-related Gene Clusters

CAZymes

Carbohydrate-Active enZymes

CBM

Carbohydrate-Binding Modules

CE

Carbohydrate Esterases

DMT

Drug/Metabolite Transporter

F-ATPase

F-type ATPase

GH

Glycoside Hydrolases

GO

Gene Ontology

GT

Glycosyl Transferases

KEGG

Kyoto Encyclopedia of Genes and Genomes

KO

KEGG Orthology

KOG

Eukaryotic Orthologous Groups

LTR

Long Terminal Repeat

MC

Mitochondrial Carrier

MFS
Major Facilitator Superfamily
NDH
NADH dehydrogenase
NPC
Nucleoside-phosphate transporter
NRPS
Non-Ribosomal Peptide Synthetase
P-ATPase
P-type ATPase
PHI
Pathogen-Host Interaction
PKS
Polyketide Synthase
PL
Polysaccharide Lyases
PTS
Phosphotransferase System
RND
Resistance-Nodulation-Division
TCDB
Transporter Classification Database

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Competing interests:

The authors declare no competing interests.

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Author Contribution

XZ and HH conceived and designed the study. XZ, YH, and HH collected diseased samples and took photographs. YH, BS and JZ performed fungal isolation, DNA extraction, and genome assembly. XZ, YH, BS, and HH performed functional annotation of the genome. XZ and HH performed comparative genomic analyses and drafted the manuscript. All authors have read and approved the final manuscript.

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Data Availability

Genome assemblies and raw sequencing data are available in the Genome Warehouse (GWH) under accession numbers GWHEQUI000000000 and GWHEQURO000000000. Raw reads are accessible at the Genome Sequence Archive under accession CRA013791 (BioProject PRJCA021822). Detailed gene functional annotation data are provided in Additional files 1–8.

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58. Additional. file legends.
59. Additional file 1. Table S1. a Pfam domain comparison, b Pfam summary of CfC1, c Pfam annotation CfC1, d Pfam summary of CsY1, e Pfam annotation.of CsY1.
60. Additional file 2. Table S2. a GO summary of CfC1, b GO summary of CsY1, c GO annotation of CfC1, d GO annotation of CsY1.
61. Additional file 3. Table S3. a KOG comparison, b KOG annotation of CfC1, c KOG annotation of CsY1.
62. Additional file 4. Table S4. a KEGG pathway of CfC1, b KEGG annotation of CfC1, c KEGG pathway of CsY1, d KEGG annotation of CsY1.
63. Additional file 5. Table S5. PHI comparison between CfC1 and CsY1.
64. Additional file 6. Table S6. a TCDB comparison, b TCDB summary of CfC1, c TCDB summary of CsY1.
65. Additional file 7. Table S7. a P450 annotations, b P450 Subfamily Statistics.
66. Additional file 8. Table S8. a CAZymes Comparison, b CAZymes annotation of CfC1, c CAZymes annotation of CsY1.

Figures

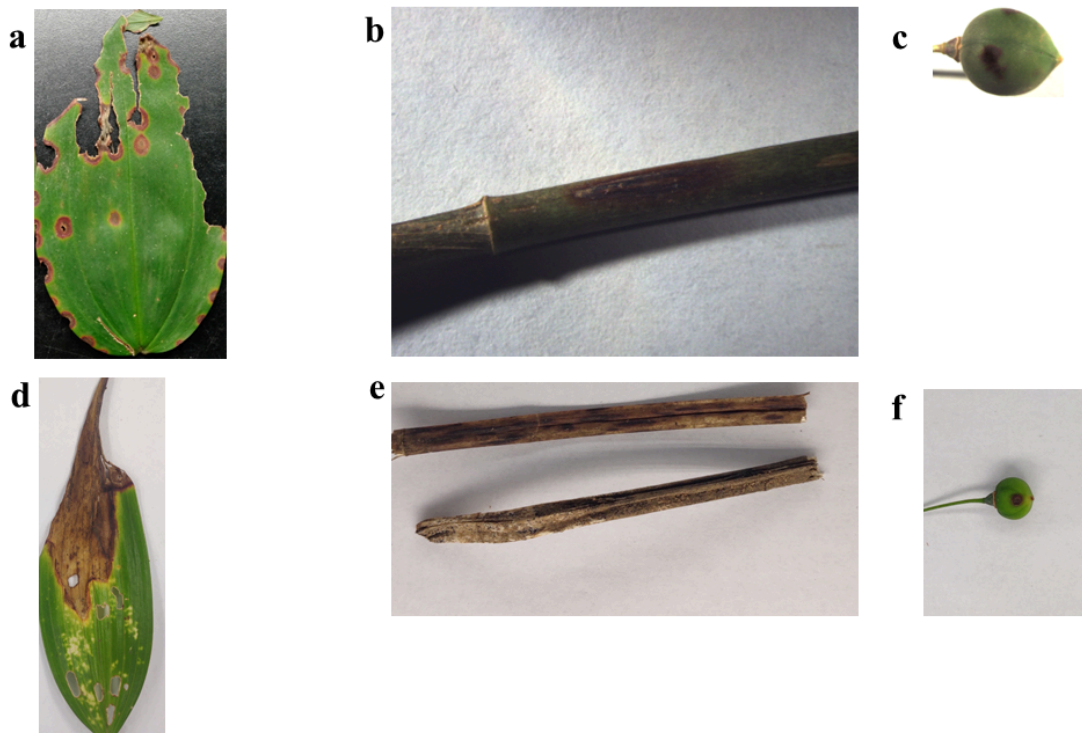


Figure 1

Symptoms caused by *C. spaethianum* and *C. fructicola* on *Polygonatum cyrtonema*. leaves, stems, and fruits. **a** Early light reddish-brown circular spots and later rust-colored, irregular lesions with perforation on leaves caused by *C. spaethianum*. **b** Dark brown spots coalescing into elongated lesions on stems caused by *C. spaethianum*. **c** Nearly circular, dark brown lesions on fruits caused by *C. spaethianum*. **d** Pale yellow spots turning grayish brown, merging into large patches with perforation on leaves caused by *C. fructicola*. **e** Small brown spots developing into large, irregular brownish-black lesions and stem dieback caused by *C. fructicola*. **f** Black, nearly circular lesions with depressed centers on fruits caused by *C. fructicola*.

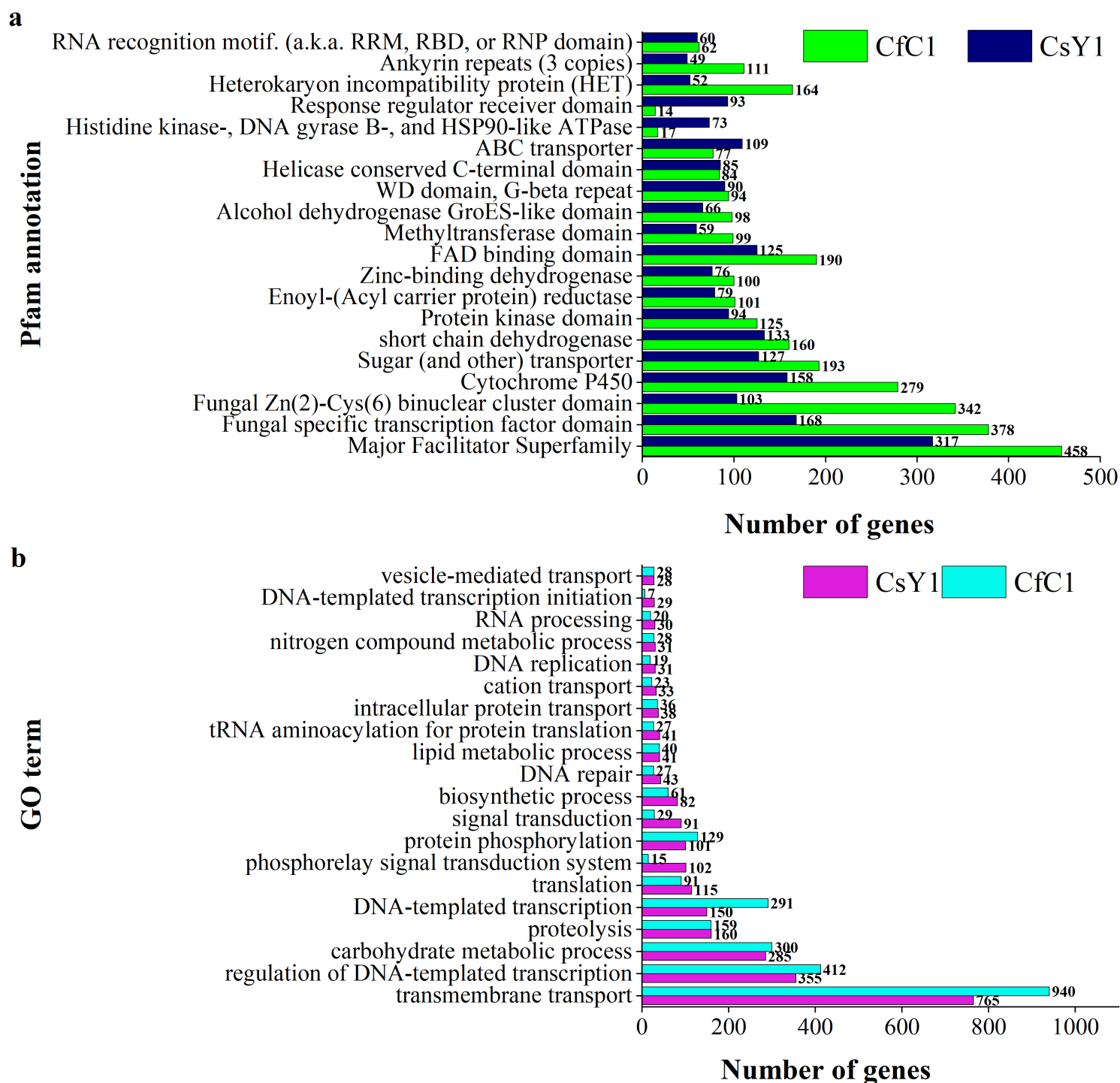


Figure 2

Domain and GO functional annotation of CfC1 and CsY1. **a** Pfam domain analysis. **b** Gene Ontology (GO) annotation.

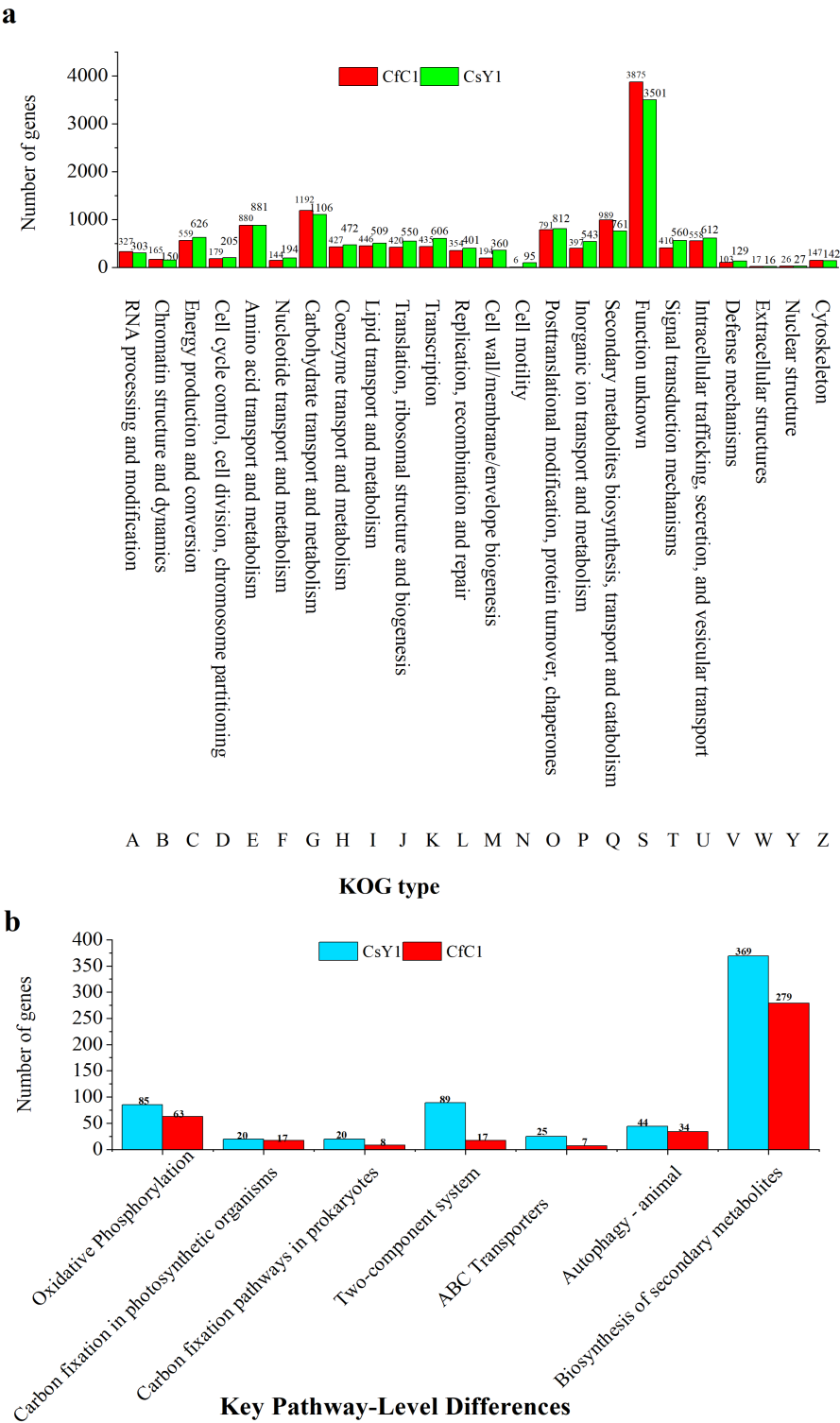


Figure 3

KOG classification and KEGG pathway analysis of CfC1 and CsY1. **a** KOG functional classification. **b**Key differential pathways identified by KEGG

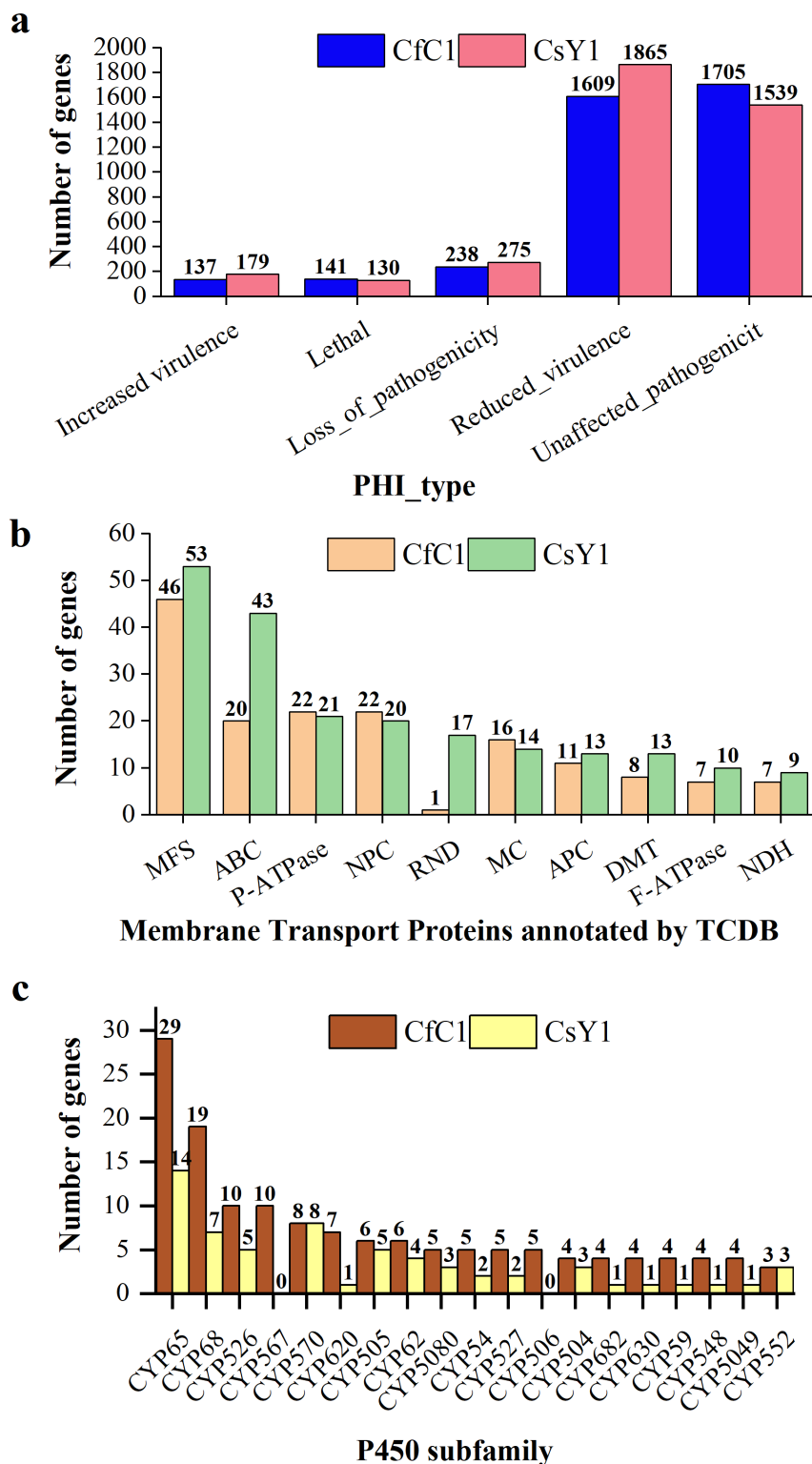


Figure 4

Comparison of PHI, TCDB, and P450 annotations between CfC1 and CsY1, emphasizing pathogenicity-related gene count differences. **a** PHI annotation. **b** TCDB annotation. **c** P450 annotation.

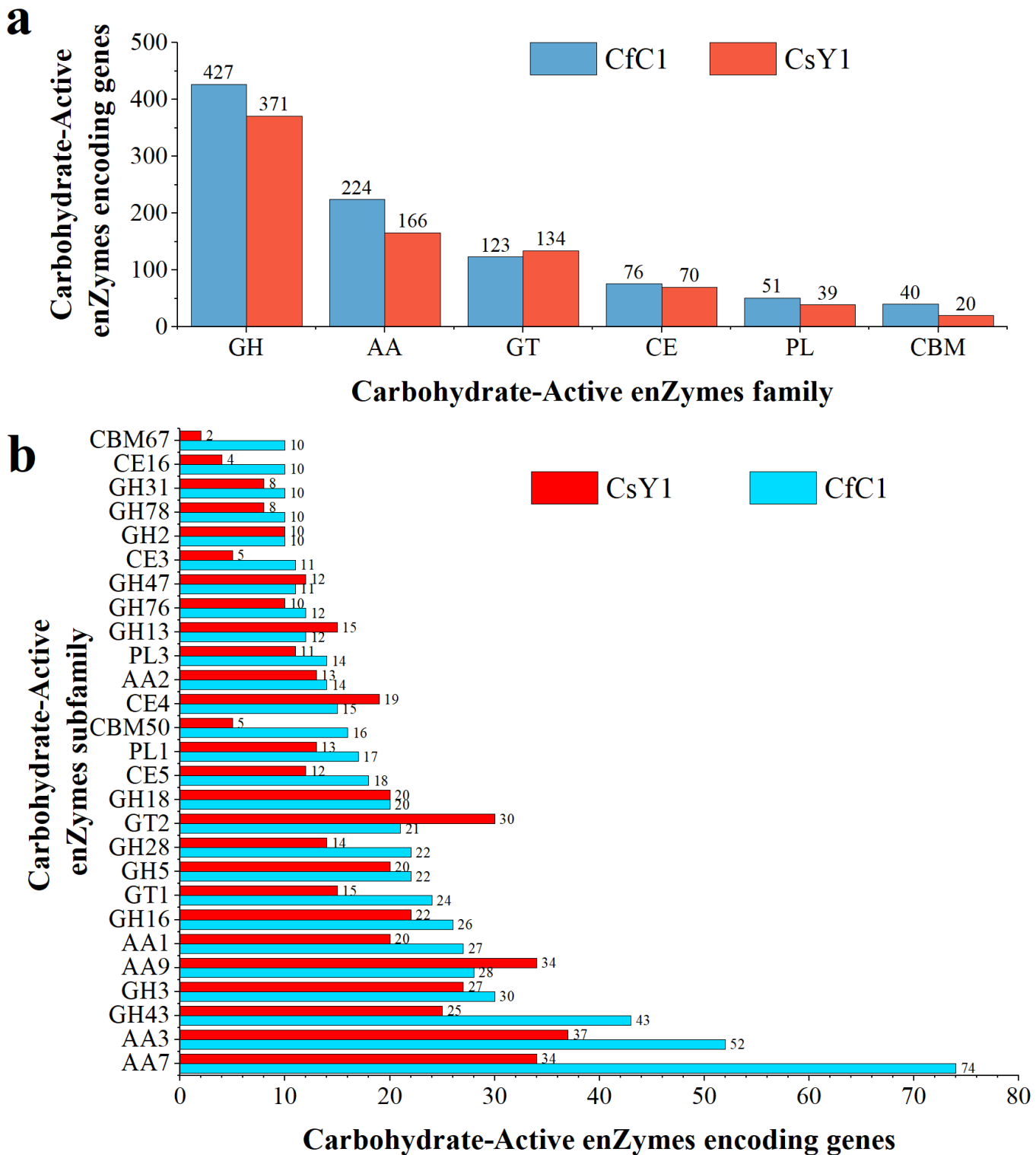


Figure 5

Comparative analysis of CAZyme families between CfC1 and CsY1. **a** Abundance and category distribution of CAZymes in the predicted secretome. **b** Key CAZyme subfamilies involved in fungal and plant cell wall degradation.

Supplementary Files

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