Draft genome of *Trichoderma gamsii* strain T035 a promising beneficial fungus in agriculture

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ABSTRACT

Trichoderma gamsii is a filamentous fungus widely recognized for its beneficial roles in agriculture, particularly for its ability to suppress plant pathogens and enhance crop health. However, genomic resources for this species remain scarce, limiting functional and applied studies. Here, we report the high-quality draft genome of *T. gamsii* strain T035, a promising biocontrol strain with strong antibiosis and parasitic activities against diverse pathogens. The assembly consisted of 18 scaffolds, with an N50 value of 7.2 Mb and a total assembly length of 38.8 Mb. This draft genome represents the most complete *T. gamsii* assembly to date and will provide a valuable resource to facilitate the exploration of molecular mechanisms underlying biocontrol and support the development of sustainable plant protection strategies.

Keywords: *Trichoderma*, biocontrol, genome, annotation, Helixer, transposable elements, secondary metabolites

Introduction

The genus *Trichoderma* belongs to the phylum Ascomycota in the family Hypocreaceae. *T. gamsii* is a ubiquitous fungus found in diverse environments, where it can exhibit psychrotolerance and broad pH tolerance (Migheli et al., 2009; Rinu et al., 2014). This species is particularly interesting due to its ability to suppress pathogens through enzymatic activity and the release of volatile organic compounds (Anees et al., 2010; Chen et al., 2016; Galletti et al., 2020; Bedine Boat et al., 2020; Valan Arasu et al., 2023; Marik et al., 2025). Additionally, it promotes plant health by inducing systemic resistance and stimulating plant growth (Anees et al., 2010; Rinu et al., 2014; Chen et al., 2016; Galletti et al., 2020; Valan Arasu et al., 2023). These characteristics highlight the potential of *T. gamsii* for biotechnological applications, underscoring the importance of comprehensive genomic data for further investigation of its biocontrol abilities. To date, the best public genomic sequence available for this species belongs to the strain T6085, which is known for its ability to control *Fusarium* head blight on wheat (Baroncelli et al., 2016).

In this study, we performed *de novo* sequencing of *T. gamsii* T035 genome. This strain exhibits strong antagonistic activity against multiple plant pathogens, making it a promising model to explore *T. gamsii* biocontrol potential. Here, we present a high-quality genome assembly and functional annotation, representing the most contiguous *T. gamsii* genome available to date, which serves as a resource to support future comparative and applied studies.

Figure 1 - Colony morphology of *Trichoderma gamsii* T035 on PDA after 7 days of growth (A). Confrontation assay between T035 and PAT-009 (*Rhizoctonia solani*) (B). Hyphal coiling of *Trichoderma* T035 (Tg) around PAT-009 (Rs) (scale bar = 50 µm) (C).

Material and methods

Sample information

Trichoderma gamsii strain T035 was isolated in 2019 from carrot-cultivated soil in Brain-sur-l'Authion, France (GPS coordinates: latitude 47.473936; longitude −0.390684) (Fig.1). Sampling and isolation were performed following the method described by Chateau et al. (2024). Briefly, 5 g of rhizosphere soil were suspended in 50 mL of sterile water. Serial dilution–extinction was carried out on Potato Dextrose Agar (PDA; Grosseron, 39 g L⁻¹) supplemented with streptomycin (500 mg L⁻¹). Cultures were incubated in darkness at 20 °C for seven days. Strain T035 was subsequently transferred to Malt Agar medium (MA; Grosseron, 20 g L⁻¹ bacteriological malt extract, 15 g L⁻¹ bacteriological agar type A) and maintained under the same growth conditions. For long-term preservation, mycelial explants were stored at −80 °C in 30% glycerol solution as a cryoprotectant.

Trichoderma atroviride strain I1237 was obtained from the commercial product TRI-SOIL® (Agrauxine, Beaucouzé, France).

Pathogenic strains were sourced either from our laboratory fungal collection (IRHS, FungiSem Team, Beaucouzé, France) or from the CIRM-CFBP collection (Beaucouzé, France). A summary of pathogen strains used in this study is provided in Supplementary Table SI.

In vitro control of filamentous pathogens

The two *Trichoderma* isolates were evaluated for their mycoparasitic activity against twelve pathogenic fungal isolates using the dual culture assay (Bunbury-Blanchette & Walker, 2019). Square Petri dishes (120 × 120 mm) containing PDA medium were inoculated with two 5 mm mycelial plugs: one obtained from a pathogen colony and the other from a *Trichoderma* colony previously grown on PDA under the conditions described above. The plugs were placed at opposite corners of the plate, with the pathogen inoculated two days before the antagonist. Control plates consisted of single-pathogen plugs grown on PDA alone. Each condition was replicated three times, and the entire experiment was repeated independently three times.

Plates were incubated in darkness at 20 °C for 20 days. Mycoparasitic ability was visually assessed using a scale adapted from Pascouau et al. (2023): 0 = <5% of pathogen colony overgrown by *Trichoderma*; 1 = 5–25% overgrown; 2 = 25–50% overgrown; 3 = 50–75% overgrown; 4 = >75% overgrown (Figure S1).

Statistical analyses were performed in RStudio v2023.12.1. Data were analyzed using a non-parametric analysis of variance (Wilcoxon signed-rank test), and *p*-values were adjusted for multiple testing using the false discovery rate (FDR) method.

In vitro control of bacterial pathogens

Two *Trichoderma* isolates were assessed for their antibiosis capacity against two bacterial isolates following the protocols of Dennis & Webster (1971) and Lee et al. (2012), with modifications. For each *Trichoderma* isolate, 250 mL Erlenmeyer flasks containing 50 mL of Potato Dextrose Broth (PDB, Grosseron, 24 g L⁻¹, pH 5.2) were inoculated with five mycelial plugs (5 mm diameter) taken from 7-day-old PDA cultures. Cultures were incubated at 20 °C on a rotary shaker (100 rpm) for 7 days and subsequently filtered through sterile 0.2 µm syringe filters (ClearLine®, Dutscher) to obtain culture filtrates.

The effect of the filtrates on bacterial growth was evaluated using a spectrophotometric microplate assay (96-well plates, SPECTROstar, BMG Labtech). Autoclaved Lysogeny Broth (LB, Grosseron, 24 g L⁻¹) was inoculated with 1% (v/v) of a bacterial suspension adjusted to OD = 0.05, supplemented with 10% (v/v) of *Trichoderma* culture filtrates, in a final volume of 300 µL per well. Filtrates obtained from non-inoculated PDA disks were incubated under the same conditions and served as controls. Plates were incubated at 25 °C for 33 h with intermittent shaking (5 min at 200 rpm every 10 min), and absorbance at 600 nm was recorded every 10 min.

101   Each condition was tested in triplicate, and three independent experiments were conducted.
102   The area under the growth curve was calculated as described by Joubert et al. (2010) and
103   Warringer & Blomberg (2003). Data were analyzed using analysis of variance (ANOVA) followed
104   by Tukey's multiple comparisons test (α = 0.05).

Trichoderma genome comparison

A global comparison of *Trichoderma* genomes was performed using MashTree v1.2 (Katz et
al., 2019). Genome assemblies of *T. gamsii* and representative assemblies from other
Trichoderma species were retrieved from NCBI RefSeq (accessions listed in Supplementary Table
S2).
Pairwise genomic distances were computed with Mash (genome size parameter set to 40 Mb,
sketch size = 20,000). The distance matrix was used to infer a neighbor-joining tree with MashTree.
The obtained tree was exported in Newick format, and later visualized and annotated using
MEGA12 (Kumar et al., 2024) (Fig.2).

Figure   2:   Mashtree   phylogeny   of   *Trichoderma*   genomes.
The analysis included all reference *Trichoderma* genomes available in NCBI
(yellow) as well as all known *T. gamsii* genomes. The genome obtained in this study
is indicated with an asterisk. Complete genomes are highlighted in orange.

DNA extraction

High-molecular-weight genomic DNA was extracted from a 7-day-old culture of *T. gamsii* T035 grown on a pectocellulosic membrane placed on PDA medium. Tissues were frozen with liquid nitrogen and bead-milled. DNA extraction followed an adapted Möller protocol: RNase treatment was performed immediately after proteinase K digestion, and DNA was resuspended in 10 mM Tris (pH 8) instead of TE buffer, as EDTA can interfere with Nanopore sequencing (Möller et al., 1992). The A260/280 and A260/230 ratios were 1.85 and 2.3, respectively. DNA concentration was measured using the quBit to 27 ng/µL, and its integrity was confirmed by electrophoresis gel, where the majority of the gDNA was above 10 kilobases (kb) in length.

Oxford Nanopore Technologies (ONT) sequencing

The sequencing library was prepared using the Native Barcoding Kit 96 V14 (SQK-NBD114.96) with 1.5 µg of high-molecular-weight genomic DNA as described in the kit protocol. We performed *de novo* sequencing of *T. gamsii* T035 genome using Oxford Nanopore Technologies' MinION device (Lu et al., 2016).

Genome assembly

Reads were basecalled using the super accuracy (SUP) mode. The long-read assemblies were performed using both Canu v2.2 (Koren et al., 2017) and Flye v2.9.6 (Kolmogorov et al., 2019). The less fragmented assembly was obtained using Flye, with reads filtered before assembly where reads shorter than 1,000 bp or with a quality score below Q10 were removed (De Coster et al., 2018). The assembly underwent three additional polishing steps using Racon (Vaser et al., 2017). The obtained contigs were further assembled in scaffolds using RagTag (Alonge et al., 2022) thanks to the reference genome of *Trichoderma atroviride* (GCF_020647795.1), the phylogenetically closest completed genome to *T. gamsii* (Fig. 2). Assembly quality was later assessed with BUSCO (fungi database) and compared to the reference *T. gamsii* genome (RefSeq: GCF_001481775.2) using QUAST (Mikheenko et al., 2023; Tegenfeldt et al., 2025) (Fig. 3 and Table I). Additionally, k-mer spectra plots were generated from Canu-corrected reads (Koren et al., 2017) using Jellyfish (Marçais & Kingsford, 2011), and Genoscope2 (Ranallo-Benavidez et al., 2020) (Figures S2 - S5).

Table I: Comparison of global genome metrics of *Trichoderma gamsii* genomes. The asterisk indicates the actual reference genome of *T. gamsii* in NCBI.

ASSEMBLY STATISTICS	*TRICHODERMA GAMSII* STRAIN T6085*	*TRICHODERMA GAMSII* STRAIN A5MH	*TRICHODERMA GAMSII* STRAIN G762/22	TRICHODERMA GAMSII STRAIN T035 (THIS STUDY)
SUBMITTED GENBANK ASSEMBLY	GCA_001481775.2	GCA_002894205.1	GCA_049996675.1	
GENOME SIZE	37.9 Mb	38.5 Mb	38 Mb	38.8 Mb
TOTAL UNGAPPED LENGTH	37.9 Mb	38.5 Mb	38 Mb	38.8 Mb
NUMBER OF SCAFFOLDS	172	292	499	18
SCAFFOLD N50	697.4 kb	594.5 kb	285.3 kb	7.2 Mb
SCAFFOLD L50	18	20	44	3
NUMBER OF CONTIGS	216	517	499	30
CONTIG N50	534 kb	299.2 kb	285.3 kb	3.096 Mb
CONTIG L50	23	42	44	5
GC PERCENT	49	48.5	48.5	48.4
GENOME COVERAGE	50x	90x	35x	25.4x
ASSEMBLY LEVEL	Scaffold	Scaffold	Contig	Scaffold

Figure 3: Snailplot generated with BlobToolKit showing assembly statistics for the
Trichoderma gamsii T035 genome. The assembly has a total length of 38,792,561
bp with a maximum scaffold length of 9,818,134 bp (in red). N50 and N90 sequence
lengths are 7,178,191 bp and 4,032,368 bp, indicated by orange and pale-orange
arcs, respectively. The grey spiral represents scaffold lengths in descending order
on a log scale. BUSCO analysis against the fungi_odb10 dataset indicates 98.8%
completeness (top right). The outer ring shows base composition (GC: 48.4%, AT:
51.6%, N: 0.0%). It's important to note that BlobToolKit reports 0.0% N, as the gaps
introduced by RagTag during the scaffolding are synthetic and not associated with
a raw sequence.

Annotation

Structural annotation was performed using the deep-learning tool Helixer (Holst et al., 2023).
This annotation was later confirmed using STAR (Dobin et al., 2013) and public transcriptomic
data (Supplementary Table S3). The location of transposable elements in the assembled genome
was assessed using EDTA (Su et al., 2021) (Supplementary Table S4).
Functional annotation was carried out with EggNOG-mapper (Cantalapiedra et al., 2021)
(Supplementary Table S5), signal peptides were predicted using DeepSig (Savojardo et al., 2018)
(Supplementary Table S6), secondary metabolite clusters were identified using antiSMASH 8.0
(Blin et al., 2025) (Supplementary Table S7), and carbohydrate-active enzymes were identified
with high confidence (confirmed by HMMER, DIAMOND, and dbCAN_sub) using dbCAN3 (Zheng
et al., 2023) (Supplementary Tables S8 and S9). All annotation results are synthesized in the
Supplementary Table S10.

Results

Biocontrol assays

The biocontrol potential of *T. gamsii* strain T035 was assessed through its parasitism and antibiosis capacities. Strain T035 displayed greater parasitic activity than the commercial strain I1237 against eight of the twelve tested filamentous plant pathogens (Fig. 4A). Notably, while I1237 exhibited antibiosis activity against both *Acidovorax valerianellae* and *Xanthomonas campestris*, strain T035 was more than twice as effective (Fig. 4B).

An intriguing pattern emerged in the parasitism assays with *Globisporangium* species: strain T035 was particularly effective against isolate PAT-018, but showed markedly reduced activity against the two other *Globisporangium* strains tested (Fig. 4A).

Figure 4: Evaluation of biocontrol capacities of *T. gamsii* strain T035 compared with a commercial *Trichoderma* strain (I1237). (A) Radar plot showing the parasitic capacity of both strains against twelve isolates from four different filamentous plant pathogens. Scores range from 0 (no colonization) to 4 (complete colonization). Asterisks indicate significant differences between strains (Wilcoxon signed-rank test; p-value 0.05). (B) Boxplot showing the growth of two phytopathogenic bacteria under control conditions (grey) or after exposure to culture filtrates of strain I1237 (pink) and *T. gamsii* T035 (blue). Different letters indicate significant differences according to Tukey's multiple comparisons test (α = 0.05).

Genome sequencing and assembly results

ONT sequencing of *T. gamsii* strain T035 generated 196,604 reads with a N50 read length of 6,669 bp, yielding approximately 965 Mb of sequence data, which corresponds to around 25X genome coverage (Fig. 5). The median read quality and length were respectively 21.8 and 3,217 bp.

Figure 5: Scatterplot of Nanopore reads for *T. gamsii* strain T035, showing read length versus quality score, generated with NanoPlot (kde plot). A total of 196,604 reads were analyzed, representing approximately 965 Mbp of sequence data. The read length N50 is 6,669 bp, with a median read length of 3,217 bp, while the median read quality is 21.8.

The obtained genome of *T. gamsii* T035 consisted of 30 contigs, with a total assembly length of 38.8 Mbp (N50 = 3.1 Mbp and N90 = 1.3 Mbp), a GC content of 48.44% and a largest contig of 7.09 Mbp. Those contigs were then assembled as 18 scaffolds, with a N50 of 7.18 Mb and a N90 of 4.03 Mb (Fig. 3 and Table I), introducing 3.09 N's per 100 kbp. Assembly completeness, assessed with BUSCO, indicated 98.8% complete universal genes (complete and single-copy: 98.3%; complete and duplicated: 0.5%; fragmented: 0.4%; missing: 0.8%) against the fungi_odb10 database (Fig. 3) (Tegenfeldt et al., 2025). The main genome metrics for *T. gamsii* strain T035 are compared with those of other published *T. gamsii* genomes in Table I.

Genome structural annotation and annotation quality control

Structural annotation was performed using the deep-learning tool Helixer (Holst et al., 2023), predicting 13,036 protein-coding genes. Compared to the previously published reference genome of *T. gamsii* T6085, annotated with MAKER2 (Holt & Yandell, 2011), *T. gamsii* T035 annotation identified over 2,000 additional protein-coding genes (Table II).

		Trichoderma gamsii strain T6085 Actual reference genome (Baroncelli et al., 2016)	*Trichoderma gamsii* strain T035 Genome from this study
	Structural annotation	MAKER2	Helixer
	Predicted protein-coding genes	10,944*	13,036
	Signal peptide	1,356*	1,046
Secondary metabolite	Isocyanide	2	2
	NRPS	14	11
	PKS	11	11
	Terpene	10	12
	Isocyanide-nrp	3	3
	PKS/NRPS	4	4
	NRPS/PKS/terpène	1	1
Carbohydrate-	Glucoside hydrolase (GH)	250	257
	Glycosyltransferase (GT)	87	88
	Polysaccharide Lyase (PL)	10	10
	Carbohydrate Esterase (CE)	19	20
	Carbohydrate-Binding Module (CBM)	33	33
	Auxiliary Activity (AA)	45	44

To evaluate annotation quality, we mapped RNA-seq reads from nine public transcriptomic
datasets (Supplementary Table S3) to both the new and the previous annotations using STAR
(Dobin et al. 2013). Mapping to the new annotation resulted in more than a 10% increase (from
64% to 75%) in reads assigned to annotated regions (present in the GTF file), demonstrating a
substantial improvement in gene model accuracy (Table III). Interestingly, depending on the tested
dataset, this increase ranged from 9% up to nearly 20% more reads mapping to annotated regions
in T035 compared to T6085.

		Trichoderma gamsii strain T6085 Actual reference genome (Baroncelli et al., 2016)	*Trichoderma gamsii* strain T035 Genome from this study
	Structural annotation	MAKER2	Helixer
Read mapping using STAR expressed in percentage	Unmapped	16.87	15.57
	Multimapped	0.88	1.62
	No Feature	17.73	5.38
	Ambiguous	0.22	2.42
	Mapped on Feature	64.30	75.02

Transposable element analysis revealed that only 3.3% of the T035 genome is composed of
transposable elements (Table IV and Supplementary Table S4). This proportion is low compared
to published TE contents in some other filamentous fungi, which can exceed 20% (e.g., *Blumeria*
graminis, *Leptosphaeria maculans*, *Fusarium graminearum*) (Ma et al., 2010; Spanu et al., 2010;
Grandaubert et al., 2014). The most abundant superfamilies were LTR retrotransposons of the
Gypsy type (0.51%), helitrons (0.58%), and TIR elements of the CACTA and Mutator families
(0.16% each). Other TE classes, including LINEs and SINEs, were only marginally represented
(<0.1%).

Table IV: Composition of transposable elements in *Trichoderma gamsii* strain T035
as determined with EDTA.

Class		Number of elements	Lenght occupied	% of sequence
LINE	Tad1	51	22715	0.06%
LTR	Gypsy	181	198697	0.51%
	unknown	78	99301	0.26%
SINE	tRNA	17	1754	0.00%
	unknown	15	1694	0.00%
TIR	CACTA	32	63747	0.16%
	Mutator	34	63446	0.16%
	PIF_Harbinger	8	13027	0.03%
	Tc1_Mariner	4	10557	0.03%
	hAT	1	1463	0.00%
nonTIR	helitron	24	224071	0.58%
rDNA	45S	13	6676	0.02%
repeat_fragment	–	797	573520	1.48%

Genome functional annotation

Functional annotation using EggNOG-mapper (Cantalapiedra et al., 2021) assigned functional
categories to 11,073 out of 13,036 predicted genes.
Secretome prediction identified 1,046 proteins with signal peptides using DeepSig (Savojardo
et al., 2018) (Table II). For comparison, 1,356 signal peptide-containing proteins were identified
with SignalP (Petersen et al., 2011) and previously reported in strain T6085 (Baroncelli et al.,
2016).
Secondary metabolite biosynthetic gene clusters were predicted in both *T. gamsii* strains T035
and T6085, with 44 and 45 clusters identified, respectively (Table II). The clusters comprised a
globally similar distribution of types, including NRPS, PKS, terpene, isocyanide, and mixed
NRPS/PKS clusters, reflecting comparable secondary metabolic potential in both strains.
Finally, carbohydrate-active enzymes (CAZymes) were identified with high confidence
(validated by HMMER, DIAMOND, and dbCAN_sub), resulting in the identification of 412 and 403
CAZymes in T035 and T6085, respectively (Table II).
275
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Discussion

In this study, we generated the most contiguous genome assembly of the biocontrol fungus *T. gamsii* to date, with scaffold N50 and L50 values of 7.18 Mb and 3, respectively, compared to the previously best available assembly, which had an N50 of 697.4 kb and an L50 of 18 (Table I, Fig. 3). The high quality of the assembly and annotation was further supported by BUSCO analysis (Fig. 3) and by the consistency between predicted gene models and experimental transcriptomic data (Table III).

Trichoderma gamsii T035 exhibits strong biocontrol potential, outperforming a commercial *Trichoderma* strain in direct antagonism assays. We demonstrated that T035 secretes highly effective antibiotic compounds into its culture medium (Fig. 4B). Consistently, the genome harbors a rich repertoire of secondary metabolite clusters and carbohydrate-active enzymes, providing a molecular basis for these activities and offering prospects for future functional characterization.

Interestingly, strain T035 displayed strong activity, particularly against the Basidiomycete *R. solani* and the Ascomycete *Phoma* spp. However, this strain showed little to no activity against wood-decay fungi. In the case of *Globisporangium*, the situation appears more complex, as the activity levels are isolate-dependent (Fig. 4A). Such specificity recalls mechanisms described in plant-pathogenic fungi producing host-selective toxins, which are typically low-molecular-weight secondary metabolites (Hatta et al., 2002; Courtial et al., 2022), and/or deploying effectors, small cysteine-rich secreted proteins, that suppress host immunity (Ayukawa et al., 2021).While such mechanisms are well documented in phytopathogens, their occurrence in mycoparasitic interactions remains unclear. Nevertheless, we cannot exclude that similar principles might underlie the observed specificity of T035. In line with this idea, studies on *Trichoderma* suggest that its antagonistic activity can involve pathogen-dependent regulatory pathways (Sharma et al., 2017). We therefore speculate that the observed specificity of T035 against *Globisporangium* spp. could involve specialized attack pathways, possibly mediated by host-specific toxins and/or effectors (Ramírez-Valdespino et al., 2019), or alternatively reflect differences in host defense mechanisms. Confirming this hypothesis will require further investigations of the identified repertoire of secondary metabolite clusters, as well as the predicted set of secreted proteins.

Overall, this study provides a high-quality genome assembly of *T. gamsii* T035, including detailed annotation of protein-coding genes, secondary metabolite clusters, and carbohydrate-active enzymes. The *T. gamsii* T035 genome was estimated to have 13,036 protein-coding genes, while the actual public reference genome of *T. gamsii* (for the T6085 strain) contained 10,944 predicted genes. The draft genome sequence has been deposited in NCBI and is available in the BioProject PRJNA1327094. This genomic resource, together with the biological assays, will support future research aimed at elucidating the molecular basis of *T. gamsii* biocontrol activity and its potential applications in sustainable agriculture.

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Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

Data, scripts, code, and supplementary information availability

Data, supplementary figure and tables, scripts, and code are available online on GitHub and stored with Zenodo: https://doi.org/10.5281/zenodo.17293674 (Colou, 2025).

T035 genomic data are available in NCBI BioProject PRJNA1327094.

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