Evaluation of Gene Finding Tools When Applied to *Trichoderma* Genomes - Update

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Outline

- Progress update with results
- Progress assessment
- Timeline for completion
- Discussion of next steps

What is *Trichoderma*?

- *Trichoderma* is a genus of filamentous fungi with many species.
- *Trichoderma* species are ubiquitous in soil and play a significant role in nutrient cycling.
- They are also used in biocontrol and as biofertilizers.
- Known for their production of plant cell wall degrading enzymes, and secondary metabolites.
- Further genomic studies can help in understanding their biology and potential applications.

Novel *Trichoderma* Genomes

- *Trichoderma* species are diverse, with many species not yet fully characterized.
- Recent advances in sequencing technology have made it possible to generate high-quality genomes for these species.
- Two novel *Trichoderma* genomes have been sequenced at the Global Institute for food Security
- DC1 and Tsth20, shown to improve drought and salt tolerance when applied to crops, and have been shown to breakdown hydrocarbons in soils.

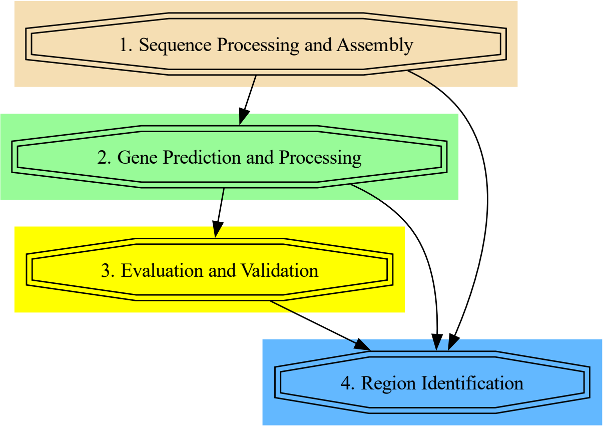
Motivation

- Gene finding is a critical step in understanding *Trichoderma*
- Various tools exist for gene prediction, but their implementations and performance can vary significantly.
- Few studies have compared these tools in fungi, and even fewer in *Trichoderma*.
- In addition, high-quality reference genomes are not available for all species, which makes comparisons difficult.
- These genomes provide an opportunity to evaluate gene finding tools in a comparative context, and contribute to the understanding of *Trichoderma* biology.

Research Objectives

- Assemble and evaluate novel assemblies of DC1 and Tsth20.
- Apply gene finders Braker2 and GeneMark in five *Trichoderma* assemblies.
- Compare gene finding tools based on several criteria, including:
    - Proportions of gene lengths predicted.
    - Presence of functional domains and closely related protein sequences.
    - Presence of genes in AT-rich sequence.
    - Agreement of gene finders on start and stop positions of a gene.

Workflow Overview


1. Sequence Processing and Assembly
2. Gene Prediction and Processing
3. Evaluation and Validation
4. Region Identification

Datasets and Tools

- **Datasets:**
  - Novel *Trichoderma* genomes: DC1 and Tsth20.
  - Reference genomes and annotations: *T. reesei, T. harzianum, T.virens*.
  - RNAseq training data from *T. reesei*.
  - Benchmarking Universal Single-Copy Orthologs (BUSCO) fungal database.
  - Protein sequence queries for tblastn from *T. atroviride, Fusarium graminearum, and Saccharomyces cerevisiae*.
- **Tools:**
  - Sequence processing: FastQC, Trimmomatic, Hisat2.
  - Genome assembly: NextDenovo and NextPolish.
  - Gene finding tools: Braker2 and GeneMark-ES.
  - Evaluation tools: BUSCO, tblastn, InterProScan, and custom scripts.

Assembly Results

Name	Total Contigs	Total Length	Largest Contig	GC%	N50	L50
DC1	8	38.6 Mb	11.49 Mb	47.97	5.69 Mb	3
Tsth20	7	41.58 Mb	8.02 Mb	47.33	6.52 Mb	3
T. harzianum	532	40.98 Mb	4.08 Mb	47.61	2.41 Mb	7
T. virens	93	39.02 Mb	3.45 Mb	49.25	1.83 Mb	8
T. reesei	77	33.39 Mb	3.75 Mb	52.82	1.21 Mb	9

- DC1 and Tsth20 assemblies are of high quality, with few contigs in comparison to other *Trichoderma* assemblies.
- Input sequences and assemblies show a bimodal distribution of GC content.
- AT-rich sequence content may be related to transposable elements and repeat-induced mutations, which may be of interest in secondary metabolite production.

Gene Finding Results

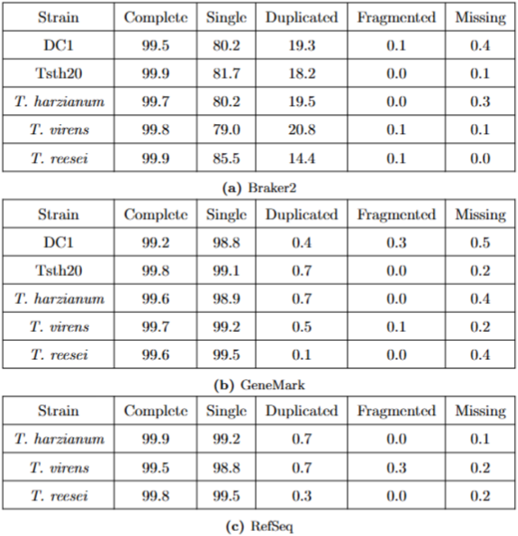
Assembly	Braker2		GeneMark		RefSeq	
	Genes	CDS	Genes	CDS	Genes	CDS
DC1	8546	8637	11353	11353	N/A	N/A
Tsth20	8784	8858	12362	12362	N/A	N/A
T. reesei	9659	10175	9196	9196	9109	9118
T. harzianum	8314	8385	12164	12164	14269	14090
T. virens	7801	7863	11866	11866	12405	12406

- Braker2 predicts more genes and coding sequences than GeneMark and RefSeq in *T. ressei*, but fewer in other assemblies.
- GeneMark and RefSeq predictions are similar, except in *T. harzianum*, where RefSeq predicts 17% more genes.
- GeneMark only predicts one coding sequence per gene, while Braker2 and RefSeq predict multiple coding sequences.

Examining Coding Sequence Lengths

Genome	Tool #1	Tool #2	*P*-value
DC1	Braker2	GeneMark	0.999
Tsth20	Braker2	GeneMark	0.965
T. reesei	Braker2	GeneMark	$9.481 * 10^{-07}$
T. reesei	GeneMark	RefSeq	0.002
T. reesei	Braker2	RefSeq	$1.340 * 10^{-07}$
T. harzianum	Braker2	GeneMark	0.863
T. harzianum	GeneMark	RefSeq	$4.313 * 10^{-52}$
T. harzianum	Braker2	RefSeq	$4.674 * 10^{-55}$
T. virens	Braker2	GeneMark	0.635
T. virens	GeneMark	RefSeq	$7.352 * 10^{-12}$
T. virens	Braker2	RefSeq	$1.794 * 10^{-09}$

BUSCO Results



Strain	Complete	Single	Duplicated	Fragmented	Missing
DC1	99.5	80.2	19.3	0.1	0.4
Tsth20	99.9	81.7	18.2	0.0	0.1
T. harzianum	99.7	80.2	19.5	0.0	0.3
T. virens	99.8	79.0	20.8	0.1	0.1
T. reesei	99.9	85.5	14.4	0.1	0.0

(a) Braker2

Strain	Complete	Single	Duplicated	Fragmented	Missing
DC1	99.2	98.8	0.4	0.3	0.5
Tsth20	99.8	99.1	0.7	0.0	0.2
T. harzianum	99.6	98.9	0.7	0.0	0.4
T. virens	99.7	99.2	0.5	0.1	0.2
T. reesei	99.6	99.5	0.1	0.0	0.4

(b) GeneMark

Strain	Complete	Single	Duplicated	Fragmented	Missing
T. harzianum	99.9	99.2	0.7	0.0	0.1
T. virens	99.5	98.8	0.7	0.3	0.2
T. reesei	99.8	99.5	0.3	0.0	0.2

(c) RefSeq

Agreement of Gene Predictions

- In DC1 and Tsth20, Braker2 and GeneMark tend to agree on the start and stop positions of genes when they predict the same gene. Both gene finders have a large portion of singleton predictions.

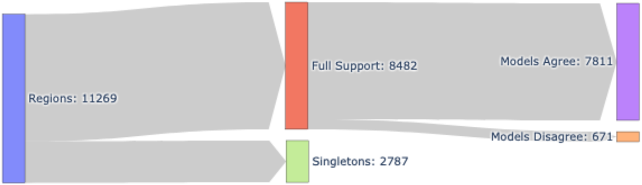


Figure: DC1

- In *T. reesei*, Braker2, GeneMark and RefSeq tend to disagree more on the start and stop positions of genes when they predict the same gene.
- Disagreement is more pronounced in *T. harzianum* and *T. virens*, where there are fewer genes with supportinng predictions from each gene finder.

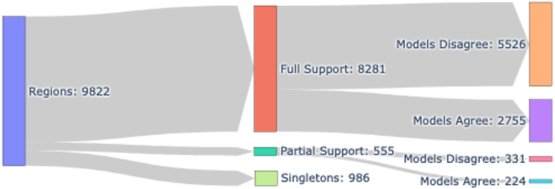


Figure: *T. reesei*

InterProScan Results

Assembly	Braker2	GeneMark	RefSeq
DC1	10676/14479	8416/11354	N/A
Tsth20	11389/15546	9168/12373	N/A
T. reesei	8471/11704	6990/9196	6964/9111
T. harzianum	11370/15408	9061/12164	9293/14065
T. virens	11249/15062	8871/11866	9062/12383

- I need to look into this. Braker2 numbers strange

BLAST Results

Reference	Ref. Proteins	DC1	Tsth20	*T. reesei*	*T. harzianum*	*T. virens*
Trichoderma atroviride	11807	11552	11080	10601	11081	11078
Fusarium granminarium	13312	10327	10429	10064	10434	10490
Saccharomyces cerevisiae	6014	3537	3517	3445	3509	3500

- The *T. atroviride* and *Fusarium* datasets are well respresented in the tblastn searches, while the *S. cerevisiae* dataset is less well represented.

Subject	Query	Braker2	GeneMark	RefSeq
DC1	*T. atroviride*	5902	4679	N/A
DC1	*F. graminarium*	4955	4114	N/A
DC1	*S. cerevisiae*	2105	1850	N/A
T. reesei	*T. atroviride*	5072	5174	4989
T. reesei	*F. graminarium*	4577	4685	4529
T. reesei	*S. cerevisiae*	2055	2114	2022
T. harzianum	*T. atroviride*	6363	4611	6835
T. harzianum	*F. graminarium*	5659	4198	5982
T. harzianum	*S. cerevisiae*	2424	1963	2560

- Regions with Braker2 and RefSeq gene predictions consistently have more tblastn hits than GeneMark, with the exception of *T. reesei*.

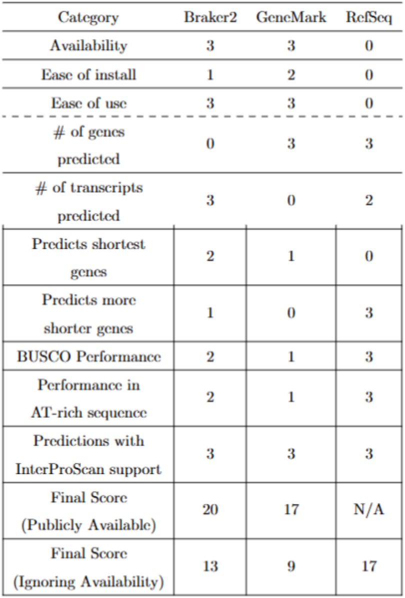
Gene Predictions in AT-rich Sequence

Assembly	Braker2	GeneMark	RefSeq
DC1	31	11	N/A
Tsth20	11	2	N/A
T. reesei	39	48	107
T. harzianum	81	30	154
T.virens	21	8	20

- Very few genes are predicted in AT-rich regions of DC1, Tsth20 and *T. virens* assemblies in comparison to the *T. reesei* and *T. harzianum* assemblies.
- Possibly due to higher quality assemblies? Although *T. virens* is still fragmented.
- A two-sided binomial test confirms that gene finders do not predict the same proportion of genes in AT-rich sequence as they do in normal genomic sequence.

Conclusions

- In terms of gene finding performance based on several criteria, RefSeq predictions are typically the best, while GeneMark performs the worst.
- Braker2 performs well in *T. reesei*, but not as well in the other assemblies. Users should be careful with the training data selected for Braker2 training, as it can have a significant impact on the results.
- RefSeq predictions are not always available, so Braker2 can be used if appropriate training is available, but GeneMark can be used as a fallback.



Category	Braker2	GeneMark	RefSeq
Availability	3	3	0
Ease of install	1	2	0
Ease of use	3	3	0
# of genes predicted	0	3	3
# of transcripts predicted	3	0	2
Predicts shortest genes	2	1	0
Predicts more shorter genes	1	0	3
BUSCO Performance	2	1	3
Performance in AT-rich sequence	2	1	3
Predictions with InterProScan support	3	3	3
Final Score (Publicly Available)	20	17	N/A
Final Score (Ignoring Availability)	13	9	17

Future Work

- **Explore the landscape of secondary metabolite gene clusters in *Trichoderma* genomes further.**
- Extend the analysis to include more *Trichoderma* species, more gene finding tools, and more datasets.
- Investigate further methods for validation of gene predictions.
- Continue to analyze the newly assembled genomes of DC1 and Tsth20.

Timeline for Completion

- Initial draft of thesis complete - end of August 2025
- Revisions and changes - Sept./Oct. 2025
- Submit thesis to committee - Early November 2025
- Schedule defence for December 2025 or January 2026
- Time for extra revisions post-defence - January/early February 2026
- Completion - End of February 2026

Current Status

- Introduction - 70%
- Background - 85%
- Methods - 90%
- Research Questions - 85%
- Results and Discussion - 80(?)%
- Conclusions and Future Work - 75%
- What to focus on next?