

Draft genome annotation of *Talaromyces trachyspermus* isolated from juice fruit using a pipeline specific to fungi.

Sami Kawashita Benkhellat, Andreia Salvador, Diana Sousa, Sónia Silva

Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Abstract: The accurate annotation of fungal genomes is essential for understanding biological traits such as spoilage mechanisms in food systems. This project aims to implement a comprehensive pipeline for the annotation of fungal genomes using short-read RNA-seq data. The workflow will encompass key steps including quality control, genome assembly, repeat masking, gene prediction, and functional annotation. To ensure methodological relevance, a review of existing tools and practices tailored to fungal genome annotation will be conducted. Following tool selection, the pipeline will be implemented and tested on the genome of *Talaromyces trachyspermus* isolated from a fruit juice matrix. Known for their resilience in food processing environments and whose genome remains poorly annotated, provide a relevant case study for evaluating the pipeline's performance. The resulting annotation framework aims to generate high-confidence gene models and enable downstream functional analyses, with implications for food safety and spoilage prevention

Keywords: *Talaromyces trachyspermus*, pipeline, fungi, fruit juice, genome annotation, assembly

1. Introduction

The preservation of pasteurized fruit products is mainly based on a combination of their high acidity, the heat and pressure process applied (Rico-Munoz et al., 2017). Although this is usually sufficient to eliminate and prevent the growth of most spoilage microorganisms and pathogens, it is not sufficient to eliminate the spores of some microorganisms (Dos Santos et al., 2018; Tranquillini et al., 2017). One group of microorganisms that poses a particular challenge are heat-resistant molds (HRMs), which are able to survive the pasteurization process (Rico-Munoz et al., 2017). Some of the main HRMs found in fruit juices are *Neosartorya fumigata*, *Neosartorya fischeri*, and *Byssoschlamys nivea* (Dos Santos et al., 2018; Rico-Munoz et al., 2015; Houbroken et al., 2008). It is important to note that there is a plethora of other HRMs that are not noted, with many being found in the wild in association with fruits (Rico-Munoz et al., 2017).

T. trachyspermus was first identified as a heat-resistant mold (HRM) in 1993, when it was observed growing within a mixed culture isolated from fruit juice after being exposed to 80°C heat for 30 minutes in an autoclave (Enigl et al., 1993). Its classification as an HRM was later reaffirmed by Tranquillini et al. (2017), who investigated the species' D-values.

One of the main types of heat-resistant spores are ascospores, which can be induced by stressful situations (Houbroken et al., 2008). Ascospores are specialized thick-walled spores produced during the sexual cycle of ascomycete fungi, providing them with remarkable resistance to heat and harsh environmental conditions (Houbroken et al., 2008; Rico-Munoz et al., 2017). This sexual cycle involves the fusion of compatible mating types, leading to meiosis and the formation of ascospores within asci (Rico-Munoz et al., 2017). Unlike the asexual cycle, which rapidly propagates fungi through conidia, the sexual cycle enhances genetic diversity and enables the development of stress-resistant structures (Houbroken et al., 2008). The interplay between the asexual and sexual cycles ensures both rapid colonization and long-term survival, making these fungi persistent contaminants in food products, particularly fruit juices (Houbroken et al., 2008; Rico-Munoz et al., 2017).

The *Talaromyces* genus has 97 entries in NCBI (as of 20/3/25) and only a single genome assembly of *T. trachyspermus* (CM035349.1). The isolate was an endophyte of *Withania somnifera* (Sahu et al., 2019). The background of our isolate, coming from a fruit juice matrix of Portuguese origin, is vastly different and most likely of separate strains than the isolate mentioned above. When checking UniProt, although the genus has around 105,000 entries, only 32 entries are of *T. trachyspermus* proteins (Taxonomy ID: 28566) (as of 20/3/25).

Consequently, annotating the genome of *T. trachyspermus* is highly significant, as gaining a deeper understanding of this fungus could lead to effective strategies for eradicating it from food products. This would not only help fruit juice manufacturers minimize spoilage-related waste but also improve consumer safety at the point of purchase. Additionally, other industries that consider this genus a promising source for metabolite production (Lan & Wu, 2020; Zhai et al., 2016) could also reap the benefits of this research.

Objectives

In this work study, we plan to establish a pipeline for annotating the genome of the *T. trachyspermus* fungus using data obtained from an isolate sourced from a fruit juice matrix. We will conduct a thorough literature review to identify the most effective tools for each step of the process, including the preprocessing of reads generated by Illumina, the assembly of these reads, and the genome annotation using bioinformatics tools that are particularly suited for a fungus with limited existing information, such as *T. trachyspermus*. Our specific goal is to identify genes associated with mycotoxins and resistance mechanisms, which could later inform strategies for controlling *T. trachyspermus* in fruit juice products.

1.1 Whole-Genome Sequencing (WGS)

Whole-genome sequencing (WGS) has revolutionized fungal research by providing comprehensive genetic insights into thousands of fungal species (Ma & Fedorova, 2010; Fitzpatrick, 2012). As a cost-effective and high-throughput method, WGS enables researchers to decode entire fungal genomes, revealing their evolutionary relationships, pathogenic mechanisms, and metabolic capabilities. The advent of next-generation sequencing (NGS) platforms like Illumina, PacBio, and Oxford Nanopore has made fungal genome sequencing more accessible, leading to fungi becoming some of the most well-sequenced eukaryotes (Haridas et al., 2011; Ma & Fedorova, 2010; Fitzpatrick, 2012). This extensive genomic data has facilitated advancements in fungal taxonomy, biotechnology, and disease management, enhancing our understanding of their roles in ecosystems and industries (Fitzpatrick, 2012).

The Illumina technology was chosen to sequence the genome of the *Talaromyces* strain isolated from the juice fruit matrix. The Illumina sequencing platform relies on a highly accurate short-read technology, starting with DNA extraction and library preparation. The extraction process involves isolating high-quality genomic DNA from a sample, typically using commercial kits or phenol-chloroform methods (Shendure et al., 2008). The extracted DNA is then fragmented into short sequences, usually between 150–300 base pairs, through enzymatic or mechanical shearing. Adapters are ligated to these fragments, allowing them to bind to the sequencing flow cell (Quail et al., 2012). During sequencing, the fragments undergo bridge amplification, creating clusters of identical DNA strands (Bentley et al., 2008). With it being the method used for the isolation of *Talaromyces* strain, it is important to adapt our pipeline to fit such needs.

1.2 Overview of Genome Annotation

Genome annotation involves multiple computational steps to extract biologically meaningful information from raw sequencing data. These steps include quality control, genome or transcriptome assembly, structural gene prediction, and functional annotation. Each phase requires different types of input data, applies distinct computational principles, and outputs intermediate files that feed into subsequent stages.

Preprocessing

Preprocessing focuses on the quality assessment and cleaning of RNA-seq reads, which are typically obtained in FASTQ format from next-generation sequencing platforms like Illumina. Each read contains a nucleotide sequence and an associated Phred quality score that reflects the confidence of each base call. However, raw reads may contain sequencing adapters, low-quality bases, or contaminants.

To address these issues, FastQC (Andrews et al., 2010) can generate graphical summaries of sequencing quality. Including per-base quality, GC content, sequence duplication levels, and overrepresented sequences, which helps to identify problematic regions. Subsequently, Trimmomatic (Bolger et al., 2014) can remove adapter sequences and trim or discard low-quality reads. Trimmomatic is especially valued for its flexibility, supporting single-end and paired-end reads and allowing parameter customization to suit different datasets. The result is a set of cleaned, high-quality reads, in FASTA files, suitable for downstream analyses in.

Genome Assembly

Next comes genome assembly, where the previously cleaned reads are ordered to construct a possible genome stored in a BAM file (Li et al., 2009). A BAM file (Binary Alignment/Map) is a binary, compressed version of a SAM file (Sequence Alignment/Map), which stores alignment information for sequencing reads mapped to a reference genome. Many tools require the BAM format for downstream predictions due to its computational efficiency.

Repetitive elements are particularly abundant in the eukaryotic genome (Yandell and Ence, 2012). There are two main strategies to deal with this problem. Tools such as RepeatModeler2 (Flynn et al., 2020) identify transposable elements and other repeats *de novo*. Whilst RepeatMasker, a procedure embedded in the Maker pipeline, will mask these regions. Masking involves replacing repetitive sequences with placeholder characters to prevent any false alignments. Subsequently, this step is also crucial to avoid false predictions downstream.

There are two main strategies for genome assembly. *De novo* assembly, which predicts genome structure using computational algorithms from scratch, or reference-based assembly, which leverages pre-existing genomic data from related species (Gorman et al., 2023).

In cases where reference genomes are incomplete or absent, as is common for non-model fungi, guided transcriptome assembly is particularly valuable. In this method, RNA-seq reads are first aligned to a draft genome using spliced aligners such as HISAT2, which are optimized to recognize exon-intron boundaries in eukaryotic transcripts (Kim et al., 2015). These alignments guide the assembly of full-length transcripts using Trinity (Grabherr et al., 2011), a robust *de novo* assembler that reconstructs isoforms and transcripts even in species lacking well-annotated genomes. Trinity uses a three-step process involving Inchworm, Chrysalis, and Butterfly modules, which sequentially assemble reads into contigs, cluster them into graphs, and reconstruct alternative splice forms and transcript variants (Haas et al., 2013).

Gene Prediction or Annotation

Gene prediction involves identification of gene structures from the previously assembled genome. In the case of eukaryotes this includes exons, introns, and UTRs. GFF files (also known as GFT files), or Gene Feature Format files, store the structural information gathered from gene prediction for later functional annotation.

Gene annotation can be achieved using two main approaches. *Ab initio* methods rely on statistical models trained on known gene features. like AUGUSTUS, GeneMark-ET, and SNAP. For example, AUGUSTUS uses a generalized hidden Markov model for exon-intron predictions and can function independently or as part of larger pipelines (Stanke et al., 2006). It is particularly effective when complemented by transcriptome evidence, enabling the

utilization of limited information for organisms like *T. trachyspermus*. Evidence-based approaches leverage RNA-seq alignments and homology to known proteins to enhance predictions.

Pipelines like MAKER (Cantarel et al., 2008) and BRAKER2 (Brůna et al., 2021) combine these methods to produce accurate gene models. MAKER integrates repeat masking, aligns transcriptomic and protein data, and incorporates tools like SNAP and AUGUSTUS. Similarly, BRAKER2 incorporates RNA-seq data with *ab initio* tools, like AUGUSTUS and GeneMark-ET, to refine predictions.

Functional Annotation

Once genes are predicted, their biological functions can be inferred using a combination of computational tools. Functional annotation involves multiple steps to assign roles to genes, assess genome quality, and gain insights into metabolism, resistance, and pathogenesis.

Predicted protein sequences are aligned against the NCBI non-redundant (nr) protein database using BLASTp (Altschul et al., 1997). This comparative approach leverages evolutionary conservation by identifying homologous sequences in well-characterized organisms. High-scoring matches with low E-values provide strong candidates for functional assignment, enabling the identification of gene families, protein functions, and enzymatic activities. This approach is particularly valuable for characterizing metabolic pathways and resistance mechanisms in *T. trachyspermus*.

BUSCO (Benchmarking Universal Single-Copy Orthologs) evaluates the completeness of genome assembly and annotation by searching for conserved single-copy orthologous genes (Simão et al., 2015). These orthologs are essential functional components expected to be present in nearly all fungal genomes. Metrics such as the presence, duplication, fragmentation, or absence of these genes provide quantitative insights into genome quality. For *T. trachyspermus*, the fungal lineage dataset (e.g., ascomycota_odb10) ensures phylogenetic relevance in assessing annotation accuracy.

InterProScan integrates multiple signature recognition methods (e.g., Pfam, SMART, TIGRFAMs, SUPERFAMILY) to identify conserved protein domains and classify proteins into families (Jones et al., 2014). By assigning Gene Ontology (GO) terms and detecting structural motifs, it informs on biochemical function, cellular roles, and evolutionary relationships. In fungi, this approach provides insights into enzymes associated with secondary metabolism, cell wall synthesis, and environmental adaptation. These findings can further guide efforts to inhibit the presence of *T. trachyspermus* in undesired contexts.

1.3 Annotating a Poorly Studied Species

When annotating a species with very little prior information, as is the case with *T. trachyspermus*, standard annotation pipelines relying on well-characterized reference genomes may not be suitable. Instead, a combination of de novo gene prediction and RNA-seq evidence is crucial for improving accuracy (Granados-Casas et al., 2023). Since existing gene models may not be applicable, multiple tools should be used to ensure comprehensive prediction, and functional annotation must leverage broader taxonomic databases rather than species-specific ones (Quan et al., 2024).

2. Methodology

In this study, we implemented a pipeline based on FunGAP (Fig 1.) a fungal genome annotation framework. This pipeline was selected because it integrates key steps for genome annotation. Which includes repeat masking, transcriptome-guided assembly, *ab initio* and evidence-based gene prediction, and functional annotation. While at

the same time using tools that are widely adopted and specifically tailored to eukaryotic and fungal genomes (Min et al., 2017). Below, we describe how each step was applied and adapted to annotate the genome of *T.trachyspermus*. The use of various tools, with the addition of a preprocessing step, brings confidence that this model will be suitable for the draft annotation of *T.trachyspermus*.

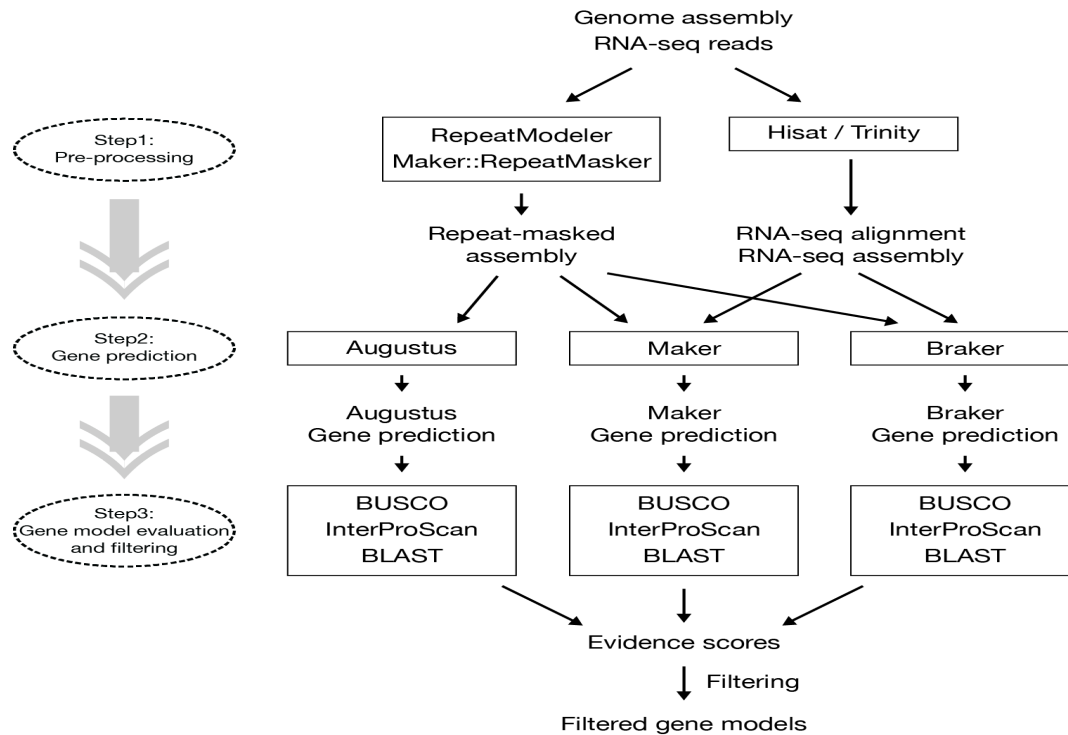


Fig 1. FunGAP pipeline overview (Min B. et al 2017))

Illumina RNA-seq reads will be preprocessed to ensure quality prior to assembly. Quality will be assessed using FastQC. Low quality-reads regions will be removed using Trimmomatic with standard parameters tailored to short RNA-seq reads, generated by Illumina. The resulting clean FASTA files will be used in transcriptome-guided genome assembly.

Repetitive elements in the genome will be identified de novo using RepeatModeler2, and these elements will be masked using RepeatMasker. RNA-seq reads will be aligned to a draft genome using HISAT2, producing splice-aware alignment files in BAM format. These alignments will be used by Trinity in genome-guided mode to assemble transcript sequences. This step aims to provide transcript evidence to guide gene model prediction.

Gene models will be predicted using a combination of *ab initio* and evidence-based tools: MAKER will be used to integrate transcript alignments (from Trinity), protein homology, and repeat-masked genomes. SNAP will be iteratively trained in four rounds using preliminary predictions to refine its gene model accuracy. BRAKER2 will run in parallel, combining RNA-seq alignments with GeneMark-ET and AUGUSTUS for a complementary set of gene predictions. All output will be in GFF files.

The predicted proteins will be annotated functionally using BLASTp, which then searches against the NCBI nr protein database to identify homologs; InterProScan, which identifies conserved protein domains and assigned GO terms; and BUSCO, which assesses annotation completeness using the ascomycota_odb10 dataset.

Scoring and Filtration of Gene Models

To refine and select the most accurate gene predictions, the FunGAP pipeline applies a scoring function that integrates multiple sources of evidence (Min B. et al 2017). Each predicted gene model is evaluated based on support from protein homology (BLAST), conserved single copy orthologs (BUSCO), and protein domain presence (Pfam). The evidence score is calculated using the following formula:

$$\text{Evidence score} = (\text{BLAST bit score} \times \text{query coverage} \times \text{target coverage}) + \text{BUSCO score} + \text{Pfam score}$$

This scoring function ensures that each gene model is assessed not only by sequence similarity but also by how well it is conserved and functionally characterized. Following the scoring process, FunGAP performs a filtration step to determine the final set of annotated genes. Gene models that overlap by at least one base pair are grouped into “gene blocks.” For each block, all possible combinations of gene models are assessed, and the combination with the highest cumulative evidence score is selected as the final annotation for that region. Minimal overlaps between coding sequences (less than 10% of the CDS length) are permitted to avoid exclusion of valid adjacent models. This systematic approach enhances the reliability of the final gene predictions by prioritizing biologically supported annotations (Min B. et al 2017).

References

1. Min, B., Grigoriev, I. V., & Choi, I. G. (2017). FunGAP: Fungal genome annotation pipeline using evidence-based gene model evaluation. *Bioinformatics*, 33(18), 2936–2937. <https://doi.org/10.1093/bioinformatics/btx354>
2. Rico-Munoz, E. (2017). Heat resistant molds in foods and beverages: Recent advances on assessment and prevention. *Current Opinion in Food Science*, 17, 75–83. <https://doi.org/10.1016/j.cofs.2017.08.006>
3. Tranquillini, R., Scaramuzza, N., & Berni, E. (2017). Occurrence and ecological distribution of heat resistant moulds spores (HRMS) in raw materials used by food industry and thermal characterization of two *Talaromyces* isolates. *International Journal of Food Microbiology*, 242, 116–123. <https://doi.org/10.1016/j.ijfoodmicro.2016.11.024>
4. Dos Santos, J. L. P., Samapundo, S., Biyikli, A., Van Impe, J., Akkermans, S., Höfte, M., ... & Devlieghere, F. (2018). Occurrence, distribution and contamination levels of heat-resistant moulds throughout the processing of pasteurized high-acid fruit products. *International Journal of Food Microbiology*, 281, 72–81. <https://doi.org/10.1016/j.ijfoodmicro.2018.05.012>
5. Houbraken, J., Varga, J., Rico-Munoz, E., Johnson, S., & Samson, R. A. (2008). Sexual reproduction as the cause of heat resistance in the food spoilage fungus *Byssoschlamys spectabilis* (anamorph *Paecilomyces variotii*). *Applied and Environmental Microbiology*, 74(5), 1613–1619. <https://doi.org/10.1128/AEM.01708-07>
6. Rico-Munoz, E., Samson, R. A., & Houbraken, J. (2019). Mould spoilage of foods and beverages: Using the right methodology. *Food Microbiology*, 81, 51–62. <https://doi.org/10.1016/j.fm.2018.12.003>
7. Enigl, D. C., King Jr, A. D., & Török, T. (1993). *Talaromyces trachyspermus*, a heat-resistant mold isolated from fruit juice. *Journal of Food Protection*, 56(12), 1039–1042. <https://doi.org/10.4315/0362-028X-56.12.1039>
8. Lan, X., & Wu, Q. (2020). Spiculispore acid production by *Talaromyces trachyspermus*: A promising approach for industrial metabolite production. *Bioresources and Bioprocessing*, 8(1), 1–10. <https://doi.org/10.1186/s40643-021-00414-1>
9. Zhai, M., Li, Q., Gu, Q., & Lin, Y. (2016). Bioactive secondary metabolites from *Talaromyces* species: Chemical diversity and biological activity. *Natural Product Reports*, 33(3), 337–359. <https://doi.org/10.1039/C5NP00106C>
10. Ma, L. J., & Fedorova, N. D. (2010). A practical guide to fungal genome projects: Strategy, technology, cost and completion. *Mycology*, 1(1), 9–24. <https://doi.org/10.1080/21501201003735530>

11. Fitzpatrick, D. A. (2012). Horizontal gene transfer in fungi. *FEMS Microbiology Letters*, 329(1), 1–8.
<https://doi.org/10.1111/j.1574-6968.2011.02465.x>
12. Haridas, S., Albert, R., Binder, M., Bloem, J., LaButti, K., Salamov, A., ... & Grigoriev, I. V. (2011). 101 Dothideomycetes genomes: A test case for predicting lifestyles and emergence of pathogens. *Studies in Mycology*, 96, 141–153. <https://doi.org/10.3114/sim.2011.96.06>
13. Bentley, D. R., Balasubramanian, S., Swerdlow, H. P., Smith, G. P., Milton, J., Brown, C. G., ... & Smith, A. J. (2008). Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*, 456(7218), 53–59.
<https://doi.org/10.1038/nature07517>
14. Shendure, J., & Ji, H. (2008). Next-generation DNA sequencing. *Nature Biotechnology*, 26(10), 1135–1145.
<https://doi.org/10.1038/nbt1486>
15. Quail, M. A., Smith, M., Coupland, P., Otto, T. D., Harris, S. R., Connor, T. R., ... & Turner, D. J. (2012). A tale of three next-generation sequencing platforms: Comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics*, 13(1), 341. <https://doi.org/10.1186/1471-2164-13-341>
16. Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*.
<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>
17. Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
18. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079.
<https://doi.org/10.1093/bioinformatics/btp352>
19. Yandell, M., & Ence, D. (2012). A beginner's guide to eukaryotic genome annotation. *Nature Reviews Genetics*, 13(5), 329–342. <https://doi.org/10.1038/nrg3174>
20. Flynn, J. M., Hubley, R., Goubert, C., Rosen, J., Clark, A. G., Feschotte, C., & Smit, A. F. (2020). RepeatModeler2 for automated genomic discovery of transposable element families. *Proceedings of the National Academy of Sciences*, 117(17), 9451–9457. <https://doi.org/10.1073/pnas.1921046117>
21. Gorman, B. J., Smith, L. M., & Johnson, M. T. (2023). Advances in reference-based genome assembly for non-model organisms. *Genome Biology and Evolution*, 15(1), evad001. <https://doi.org/10.1093/gbe/evad001>
22. Kim, D., Langmead, B., & Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, 12(4), 357–360. <https://doi.org/10.1038/nmeth.3317>
23. Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., ... & Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, 29(7), 644–652.
<https://doi.org/10.1038/nbt.1883>
24. Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., ... & Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, 8(8), 1494–1512. <https://doi.org/10.1038/nprot.2013.084>
25. Stanke, M., Keller, O., Gunduz, I., Hayes, A., Waack, S., & Morgenstern, B. (2006). AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Research*, 34(Web Server issue), W435–W439.
<https://doi.org/10.1093/nar/gkl200>
26. Cantarel, B. L., Korf, I., Robb, S. M. C., Parra, G., Ross, E., Moore, B., ... & Yandell, M. (2008). MAKER: An easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Research*, 18(1), 188–196.
<https://doi.org/10.1101/gr.6743907>
27. Brůna, T., Hoff, K. J., Lomsadze, A., Stanke, M., & Borodovsky, M. (2021). BRAKER2: Automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database. *NAR Genomics and Bioinformatics*, 3(1), lqaa108. <https://doi.org/10.1093/nargab/lqaa108>
28. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, 25(17), 3389–3402. <https://doi.org/10.1093/nar/25.17.3389>
29. Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, 31(19), 3210–3212.
<https://doi.org/10.1093/bioinformatics/btv351>
30. Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., ... & Hunter, S. (2014). InterProScan 5: Genome-scale protein function classification. *Bioinformatics*, 30(9), 1236–1240.
<https://doi.org/10.1093/bioinformatics/btu031>

31. Granados-Casas, A. O., Sastoque, A. P., Stchigel, A. M., Fernández-Bravo, A., & Cano-Lira, J. F. (2023). Hybrid de novo whole-genome assembly, annotation, and identification of secondary metabolite gene clusters in the ex-type strain of *Chrysosporium keratinophilum*. *Journal of Fungi*, 9(4), 389. <https://doi.org/10.3390/jof9040389>
32. Quan, Y., Zhang, Y., Wang, X., Li, J., & Liu, Z. (2024). Genome sequencing and comparative genome analysis of *Rhizoctonia solani* AG-3 strains causing tobacco target spot disease. *Frontiers in Microbiology*, 15, 1360524. <https://doi.org/10.3389/fmicb.2024.1360524>