Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.2007898

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 9 - Issue 5 : 895-901 / September 2026

ASSESSMENT OF THE ENDOCHITINASE (*ech42*) PROTEIN-CODING GENE AS A DNA BARCODE FOR SPECIES DELIMITATION AND PHYLOGENETIC RECONSTRUCTION IN *Trichoderma*

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Abstract: Accurate species identification is a prerequisite for deciphering the ecological adaptation, evolutionary diversification, and functional potential of *Trichoderma* species. It is providing a foundation for understanding their roles in host interactions, environmental fitness, and biocontrol applications. In this study, the protein-coding gene of endochitinase 42 kDa (*ech42*) was evaluated as a DNA barcode for species delimitation and phylogenetic reconstruction in *Trichoderma* genus. Phylogenetic inferences were drawn from eight local isolates representing four *Trichoderma* species. Sequence alignment of the *ech42* gene generated a dataset of approximately 850 bp, providing sufficient phylogenetically informative characters for species-level discrimination. Phylogenetic analyses revealed that the *ech42* marker consistently resolved all four species, with bootstrap support of 100%. Physicochemical and three-dimensional structural analyses of *ech42* gene revealed that their amino acid numbers ranged from 278 to 283 residues in *Trichoderma* species and *T. virens* exhibited the most hydrophilic protein, with a GRAVY value of −0.303. These findings demonstrate that the *ech42* gene possesses significant potential as a complementary DNA barcode for *Trichoderma* species identification and phylogenetic studies.

Keywords: *Trichoderma guizhouense*, *Trichoderma harzianum*, Amino acid, Structural analysis, GRAVY

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Received: July 31, 2026
Accepted: September 02, 2026
Published: September 15, 2026

Cite as: Korkom, Y. (2026). Assessment of the endochitinase (*ech42*) protein-coding gene as a DNA barcode for species delimitation and phylogenetic reconstruction in *Trichoderma*. *Black Sea Journal of Agriculture*, 9(5), 895-901.

1. Introduction

The genus *Trichoderma* Pers. ex Fr. (Ascomycota) comprises a diverse group in biological control agents (BCAs) that are ubiquitous in soil ecosystems and play important ecological, agricultural, and industrial roles. Species of *Trichoderma* are widely recognized for their ability to colonize soil and plant-associated environments, promote plant growth, enhance nutrient availability, and suppress a broad range of phytopathogens through mechanisms including mycoparasitism, antibiosis, competition, and induction of host resistance (Harman et al., 2004; Sarrocco et al., 2017; González-Pérez et al., 2018). Owing to these beneficial characteristics, *Trichoderma* species have become among the most extensively studied fungal biocontrol agents and are widely used in sustainable crop production systems (Woo et al., 2023). Although most *Trichoderma* species are beneficial, certain species act as economically significant pathogens; for instance, *T. aggressivum* causes green mould disease in *Agaricus bisporus*, resulting in yield losses of 30-100% (Şanver et al., 2025). This dual role further underscores accurate species identification is fundamental for understanding the ecology, evolution, and biotechnological potential of *Trichoderma*. Traditional classification based on morphological and phenotypic characteristics is often unreliable because many species exhibit overlapping traits, phenotypic plasticity, and diversity (Qin and Zhuang, 2016; Bint-e-Zahira et al., 2024). Consequently, molecular approaches have become indispensable for species delimitation and phylogenetic studies within the genus. The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was proposed as the universal DNA barcode for fungi due to its broad applicability and extensive representation in public sequence databases (Schoch et al., 2012). However, ITS frequently lacks sufficient variation to distinguish closely related species of *Trichoderma*, resulting in ambiguous taxonomic assignments and reduced phylogenetic resolution (Jaklitsch and Voglmayr, 2015; Kauserud, 2023). To

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overcome these limitations, protein-coding genes represent valuable sources of phylogenetic information because they generally evolve more rapidly than ribosomal DNA regions and contain a greater number of informative nucleotide substitutions. Among these, the translation elongation factor 1-alpha (*tef1*), actin (*act*), calmodulin (*cal*), and the second largest nuclear RNA polymerase subunit (*rpb2*) are considered among the most informative markers for *Trichoderma* identification and have been widely adopted in species recognition studies (Jaklitsch and Voglmayr, 2015; Cai and Druzhinina, 2021). Nevertheless, the exploration of functionally important different genes associated with fungal ecology and antagonistic activity as DNA barcodes remains a current topic. The *ech42* gene encodes a 42-kDa endochitinase involved in chitin degradation and fungal cell-wall hydrolysis during mycoparasitic interactions. Chitinases constitute a key component of the antagonistic machinery of *Trichoderma* species and contribute substantially to their effectiveness as biological control agents (Villanueva et al., 2022; Tang et al., 2026). Previous studies have characterized *ech42* with respect to gene expression, enzymatic activity, and its role in pathogen suppression (Seidl, 2008; Tien et al., 2024); however, its potential application as a molecular barcode for species identification and phylogenetic reconstruction has only recently been evaluated. Therefore, this study evaluated the potential of the *ech42* gene as a DNA barcode for phylogenetic reconstruction within native *Trichoderma* species. The *ech42* gene analysis was conducted using eight isolates representing four locally identified *Trichoderma* species.

2. Materials and Methods

2.1. Local *Trichoderma* Isolates

Local *Trichoderma* isolates were isolated from the soils of different agriculture fields collected in Aydın province, Türkiye (Korkom, 2022). Information on the *Trichoderma* isolates, including their geographic origin, host plant, and sampling date, is provided in Table 1. The species-level identification of these *Trichoderma* isolates was previously established (Korkom, 2022) using a combination of morphological and molecular approaches. Representative isolates of each *Trichoderma* species are maintained in the Mycology Laboratory of Faculty of Agriculture, Aydın Adnan Menderes University, Türkiye.

2.2. Genomic DNA Extraction, PCR Amplification, and Phylogenetic Analysis

For genomic DNA extraction, fresh mycelium of *Trichoderma* isolates were harvested from grown in potato dextrose broth (PDB) on a rotary shaker (150 rpm) at 25 °C for 4 days. Total genomic DNA was extracted using a DNeasy fungal DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The primer set and polymerase chain reaction (PCR) deployed for *ech42* gene is summarized in Table 2. Amplified PCR products were separated on a 1.5% (w/v) agarose gel, stained with SYBR Safe nucleic acid dye (Invitrogen, S33102), and visualized under a UV transilluminator. The resulting 850 bp PCR product was bidirectionally sequenced by Sanger Sequencing at Macrogen Europe (Amsterdam, Netherlands) using the initial amplification primer set. Forward and reverse chromatograms generated in ABI format were assembled into consensus sequences using SeqTrace v0.9.0 (Stucky, 2012). Sequence quality was assessed through manual inspection of chromatograms, and ambiguous nucleotide positions were corrected based on peak morphology and signal intensity. Multiple sequence alignments were generated with GenBank reference sequences and used for phylogenetic analyses in MEGA v11 (Tamura et al., 2021).

Phylogenetic relationships among *Trichoderma* isolates were inferred using the maximum likelihood (ML) method under the best-fit nucleotide substitution model selected according to the Bayesian Information Criterion (BIC). The robustness of inferred phylogenetic relationships was evaluated by Kimura 2-parameter substitution model (Kimura, 1980) with bootstrap analysis (1,000 replicates) (Felsenstein, 1985).

2.3. Physicochemical Characterization and Three-dimensional Protein Structure Modeling

Nucleotide sequences were translated into their corresponding amino acid sequences using MEGA v11 (Tamura et al., 2021). The physicochemical properties of the predicted proteins, including molecular weight (MW), theoretical isoelectric point (pI), extinction coefficient (EC), instability index, aliphatic index, and grand average of hydropathicity (GRAVY) were calculated using the ProtParam tool available on the ExPASy server (Gasteiger et al., 2005). Amino acid composition and residue frequencies were further analyzed to assess protein structural characteristics. Three-dimensional (3D) protein structures were predicted using the SWISS-MODEL (https://swissmodel.expasy.org/interactive) automated homology-modeling server (Waterhouse et al., 2018). The most suitable structural templates were selected based on sequence identity, coverage, and model quality parameters. The generated models were subsequently evaluated using the quality metrics provided by SWISS-MODEL, including the Global Model Quality Estimation (GMQE) and Qualitative Model Energy Analysis (QMEAN) scores, to ensure the reliability of the predicted protein structures.

Table 1. Collection information and taxonomic details of the *Trichoderma* isolates used in this study

Trichoderma species	Isolate code	Geographic origin	Host plant	Sampling date
T. afroharzianum	Tr48	Buharkent, Aydın	oak	2018
T. afroharzianum	Tr132	Kuşadası, Aydın	almond	2019
T. guizhouense	Tr49	Buharkent, Aydın	beetroot	2019
T. guizhouense	Tr118	Didim, Aydın	wheat	2019
T. harzianum	Tr124	Kuşadası, Aydın	olive	2022
T. harzianum	Thr15	Sultanhisar, Aydın	strawberry	2015
T. virens	Tvr2	Sultanhisar, Aydın	strawberry	2015
T. virens	Tvr3	Köşk, Aydın	strawberry	2015

Table 2. Primer sequence and PCR configurations utilized for *ech42* gene

Primer name	Sequence (5'→ 3')	PCR components	PCR protocol
Chit42-1a Chit42-2a	GCTYTCCATCGGTGGCTGGAC GGAGTTGGGGTAGCTCAGC (Lieckfeldt et al., 2000)	Total reaction volume of 25 µL; 1 µL genomic DNA, 1 µL primer (forward), 1 µL primer (reverse), 5 µL master mix (PCR buffer, 2 mM $MgCl_2$, dNTP, 0.75 U Taq DNA polymerase) and 17 µL ddH_2O	95°C, 3 min; 35× (95°C, 30 s; 62°C, 30 s; 72°C, 1 min); 72°C, 5 min.

3. Results and Discussion

3.1. Phylogenetic Performance of Endochitinase 42 Gene

The endochitinase 42 *(ech42)* gene was successfully amplified and sequenced from all eight *Trichoderma* isolates, generating an alignment of approximately 850 bp. Phylogenetic reconstruction based on the maximum likelihood (ML) method resolved the four examined *Trichoderma* species into distinct and well-supported clades, with bootstrap support of 100% (Figure 1). The resulting topology was consistent with species boundaries and *ech42* gene resolved all four species in the present dataset. Tr49 (accession number PZ746428) and Tr118 (accession number PZ746429) isolates, both identified as *Trichoderma guizhouense*, formed a distinct and well-supported clade in the phylogenetic analysis. A comprehensive search of the GenBank database revealed no previously deposited *ech42* gene sequences for *T. guizhouense*. Therefore, the present study represents the first characterization of the *ech42* gene in this species, and the newly generated sequences have been submitted to GenBank, thereby expanding the molecular resources available for *T. guizhouense* and facilitating future phylogenetic and functional investigations. The isolates Tr124 (accession number PZ746432) and Thr15 (accession number PZ746433) were found to be related to the species *Trichoderma harzianum* HA1103 (accession number HQ286990) and formed a distinct branch (bootstrap value=100%) in phylogenetic tree. The resulting 850 bp nucleotide sequence of Tr48 (accession number PZ746430) and Tr132 (accession number PZ746431) were 100% sequence identity with *Trichoderma afroharzianum* TBR-4 (accession number OM981156) and *Trichoderma afroharzianum* TRS562 (accession number KP009422) endochitinase 42 gene. The isolates Tvr2 (accession number PZ746434) and Tvr3 (accession number PZ746435) clustered with the reference strain *Trichoderma virens* T2C1.4 (accession number ON492016) and formed a strongly supported (bootstrap value=100%), distinct clade in the phylogenetic reconstruction (Figure 1).

These findings indicate that *ech42* gene possesses sufficient sequence variability to serve as an informative marker for species delimitation and phylogenetic reconstruction within *Trichoderma*.

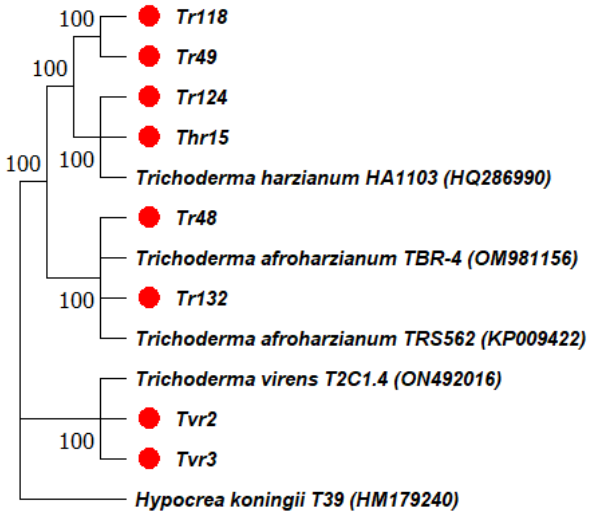


Figure 1. Phylogenetic tree inferred from maximum-likelihood (ML) using *ech42* sequences of *Trichoderma* spp. Bootstrap values are presented at the nodes.

Reliable species identification remains one of the major challenges in *Trichoderma* taxonomy due to the presence of morphologically similar and phylogenetically complex species groups (Samuels, 2006; Jaklitsch and Voglmayr, 2015). Although the ITS region has been formally adopted as the universal fungal barcode (Schoch et al., 2012), its discriminatory power is often insufficient for resolving closely related *Trichoderma* species (Druzhinina et al., 2005; Cai and Druzhinina, 2021). In the present study, the protein-coding gene of *ech42* successfully discriminated all four examined species and

generated well-supported phylogenetic clades with bootstrap support of 100%. Several similar studies were previously identified the endochitinase gene (*ech42*) in *Trichoderma virens* (Kamble et al., 2016), in *T. harzianum* (Sorabh et al., 2020), in *T. afroharzianum*, and in *Trichoderma asperellum* (Luong et al., 2021). The *ech42* can be attributed to the characteristics of protein-coding gene, which generally contain a higher proportion of phylogenetically informative sites than ribosomal DNA regions (Stielow et al., 2015).

3.2. Physicochemical Characterization of Endochitinase 42 Proteins

The physicochemical analyses revealed a high degree of conservation among proteins of endochitinase 42 kDa (*ech42*) gene despite the nucleotide-level variation observed in the corresponding gene sequences (Table 3). The numbers of amino acid ranged from 278 to 283 among the four *Trichoderma* species, while *Trichoderma virens* exhibited the highest amino acid number (n=283). Molecular weights (Mw) ranged from 29.54 to 30.44 kDa, while theoretical isoelectric point (pI) values were varied uniformly (5.15–5.36), indicating a conserved biochemical profile among local *Trichoderma* species. The highest pI value was observed in *Trichoderma virens* (5.36). The extinction coefficient (EC) values showed minimal variation, ranging from 52830 to 58330. This result was suggested a highly conserved aromatic amino acid composition across species. Similarly, the instability index (II) values (from 22.88 to 24.20) remained below the threshold value of 40.00, suggesting that all predicted proteins are stable. The lowest instability index was observed in *T. harzianum* (22.88), whereas *T. afroharzianum* exhibited the highest value (24.20). Grand average of hydropathicity (GRAVY) values varied from −0.227 to −0.303, indicating that all *ech42* proteins are slightly hydrophilic. The most hydrophilic protein was identified in *T. virens* (−0.303), while *T. guizhouense* exhibited the highest GRAVY value (−0.227) (Table 3). The negative GRAVY values and narrow range of physicochemical parameters indicated a predominantly hydrophilic character, which is consistent with the extracellular activity of fungal chitinases involved in cell-wall degradation and mycoparasitism (Seidl, 2008; Cheng et al., 2015; Zhu et al., 2025).

The translated *ech42* sequences by MEGA11 encoded proteins with highly conserved physicochemical characteristics among the four *Trichoderma* species examined (Table 4).

Table 3. Physicochemical characterization of *ech42* in local *Trichoderma* species

Trichoderma species	Number of amino acids	Molecular weight (Mw)	Theoretical pI	Extinction coefficients (Ec)	Instability index (II)	Grand average hydropathicity (GRAVY)
Trichoderma guizhouense	278	29549.00	5.35	52830	23.17	-0.227
Trichoderma afroharzianum	280	29837.22	5.15	52830	24.20	-0.267
Trichoderma harzianum	280	29880.25	5.15	58330	22.88	-0.274
Trichoderma virens	283	30440.90	5.36	58330	24.13	-0.303

Table 4. Amino acid composition of *ech42* in local *Trichoderma* species

Amino acid	Composition							
	T. guizhouense		*T. afroharzianum*		*T. harzianum*		*T. virens*	
Ala (A)	36	12.9%	34	12.1%	35	12.5%	32	11.3%
Arg (R)	5	1.8%	5	1.8%	5	1.8%	7	2.5%
Asn (N)	16	5.8%	17	6.1%	18	6.4%	18	6.4%
Asp (D)	21	7.6%	21	7.5%	21	7.5%	22	7.8%
Cys (C)	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Gln (Q)	7	2.5%	9	3.2%	8	2.9%	7	2.5%
Glu (E)	5	1.8%	5	1.8%	5	1.8%	5	1.8%
Gly (G)	32	11.5%	32	11.5%	32	11.5%	30	10.6%
His (H)	3	1.1%	3	1.1%	3	1.1%	3	1.1%
Ile (I)	13	4.7%	13	4.7%	13	4.7%	14	4.9%
Leu (L)	23	8.3%	23	8.3%	22	7.9%	24	8.5%
Lys (K)	17	6.1%	16	5.7%	16	5.7%	16	5.7%
Met (M)	6	2.2%	6	2.2%	6	2.2%	6	2.2%
Phe (F)	11	4.0%	11	4.0%	11	4.0%	11	4.0%
Pro (P)	12	4.3%	12	4.3%	12	4.3%	12	4.3%
Ser (S)	21	7.6%	22	7.9%	21	7.6%	25	8.8%
Thr (T)	18	6.5%	19	6.8%	19	6.8%	19	6.8%
Trp (W)	5	1.8%	5	1.8%	6	2.1%	6	2.1%
Tyr (Y)	17	6.1%	17	6.1%	17	6.1%	17	6.1%
Val (V)	10	3.6%	10	3.6%	10	3.6%	9	3.2%
Pyl (O)	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Sec (U)	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Amino acid composition analysis showed that alanine (Ala, A) was the most abundant residue in all *Trichoderma* species, accounting for an average of 12.20% of the total amino acids. The highest alanine abundant was observed in *T. guizhouense* (12.9%). Histidine (His, H) occurred at the lowest frequency at 1.1% in local *Trichoderma* species (Table 4). The alanine is very common in composition of fungal mycelia (Watkinson, 2016). Its acts are a nitrogen-carbon source and acting in cell response against salt stress (Ding et al., 2019; Kerkaert et al., 2022).

3.3. Three-dimensional (3D) Protein Structure Analysis of Endochitinase 42 Proteins

Three-dimensional models of *ech42* proteins were constructed using the SWISS-MODEL homology-modeling platform (Figure 2). Structural comparisons demonstrated a remarkably conserved tertiary architecture across the local *Trichoderma* species, indicating strong evolutionary conservation of this enzyme. This combination of conserved protein structure and informative nucleotide polymorphisms is particularly advantageous for DNA barcoding applications.

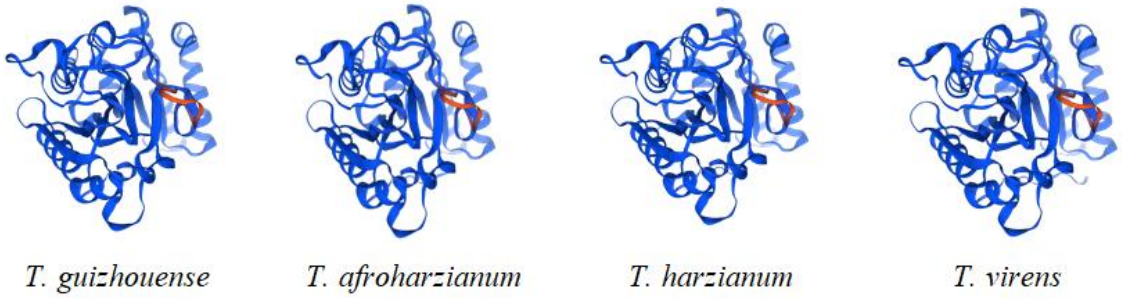


Figure 2. Three-dimensional structural models of *ech42* proteins in local *Trichoderma* species generated using SWISS-MODEL.

4. Conclusion

This study demonstrates that the structural, physicochemical, and phylogenetic analyses collectively support the suitability of *ech42* gene as a DNA barcode for *Trichoderma* species. The *ech42* gene exhibits a balance between functional conservation and evolutionary variability, enabling reliable species discrimination while preserving the structural integrity of the encoded protein. Future studies incorporating larger taxonomic datasets and comparisons with additional markers such as *rpb2*, and *tef1* will be necessary to further assess the universality and taxonomic resolution of *ech42* gene. Nevertheless, the findings presented here support the inclusion of *ech42* gene in future multilocus frameworks and highlight its promise as a complementary marker for *Trichoderma* taxonomy and molecular systematics.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	Y.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

References

Bint-e-Zahira, S., Khalid, A. N., Yousaf, N., Iqbal, M., Anwar, T., Qureshi, H., ... & Ansari, M. J. (2024). Exploring Trichoderma species in industrial wastewater: morphological and molecular insights from isolates. *Life, 14*(6), 750. https://doi.org/10.3390/life14060750

Cai, F., & Druzhinina, I.S. (2021). In honor of John Bissett: authoritative guidelines on molecular identification of Trichoderma In honor of John Bissett: authoritative guidelines on molecular identification of Trichoderma. F*ungal Diversity, 107*, 1–69. https://doi.org/10.1007/s13225-020-00464-4

Cheng, P., Song, W., Gong, X., Liu, Y., Xie, W., Huang, L., & Hong, Y. (2015). Proteomic approaches of Trichoderma hamatum to control Ralstonia solanacearum causing pepper bacterial wilt. *International Journal of Agriculture & Biology, 17*, 1101–1109. https://doi.org/10.17957/IJAB/15.0049

Ding, X., Liu, K., Lu, Y., & Gong, G. (2019). Morphological, transcriptional, and metabolic analyses of osmotic-adapted mechanisms of the halophilic Aspergillus montevidensis ZYD4 under hypersaline conditions. *Applied Microbiology Biotechnology, 103*, 3829–3846. https://doi.org/10.1007/s00253-019-09705-2

Druzhinina, I.S., Kopchinskiy, A.G., Komoń-Zelazowska, M., Bissett, J., Szakacs, G., & Kubicek, C.P. (2005). An oligonucleotide barcode for species identification in Trichoderma and Hypocrea. *Fungal Genetics and Biology, 42*(10), 813–828. https://doi.org/10.1016/j.fgb.2005.06.007

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution, 39*(4), 783-791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S.E., Wilkins, M.R., Appel, R.D., & Bairoch, A. (2005). *Protein identification and analysis tools on the ExPASy server*. Humana press. https://doi.org/10.1385/1-59259-890-0:571

González-Pérez, E., Ortega-Amaro, M. A., Salazar-Badillo, F. B., Bautista, E., Douterlungne, D., & Jiménez-Bremont, J. F. (2018). The Arabidopsis-Trichoderma interaction reveals that the fungal growth medium is an important factor in plant growth induction. *Scientific Reports, 8*(1), 16427. https://doi.org/10.1038/s41598-018-34500-w

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., & Lorito, M. (2004). Trichoderma species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology, 2*, 43–56. https://doi.org/10.1038/nrmicro797

Jaklitsch, W.M., & Voglmayr, H. (2015). Biodiversity of Trichoderma (Hypocreaceae) in Southern Europe and Macaronesia. *Studies in Mycology, 80*, 1–87. https://doi.org/10.1016/j.simyco.2014.11.001

Kamble, S., Mukherjee, P.K. & Eapen, S. (2016). Expression of an endochitinase gene from Trichoderma virens confers enhanced tolerance to Alternaria blight in transgenic Brassica juncea (L.) czern and coss lines. *Physiology and Molecular Biology Plants, 22*, 69–76. https://doi.org/10.1007/s12298-016-0340-8

Kauserud, H. (2023). ITS alchemy: On the use of ITS as a DNA marker in fungal ecology. *Fungal Ecology, 65*, 101274. https://doi.org/10.1016/j.funeco.2023.101274

Kerkaert, J.D., Le Mauff, F., Wucher, B.R., Beattie, S.R., Vesely, E.M., Sheppard, D.C., Nadell, C.D., Cramer, R.A. (2022). An alanine aminotransferase is required for biofilm-specific resistance of Aspergillus fumigatus to echinocandin treatment. *MBio, 13*(2), e02933-21. https://doi.org/10.1128/MBIO.02933-21

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*(2), 111-120. https://doi.org/10.1007/BF01731581

Korkom, Y. (2022). Determination of the effects of Trichoderma spp., obtained from agricultural areas in Aydin province, on charcoal rot disease in strawberry and plant growth (PhD Thesis, Aydın Adnan Menderes University).

Lieckfeldt, E., Cavignac, Y., Fekete, C., & Börner, T. (2000). Endochitinase gene-based phylogenetic analysis of Trichoderma. *Microbiological Research, 155*(1), 7-15. https://doi.org/10.1016/S0944-5013(00)80016-6

Luong, N. N., Tien, N. Q. D., Huy, N. X., Tue, N. H., Man, L. Q., Sinh, D. D. H., ... & Loc, N. H. (2021). Expression of 42 kDa chitinase of Trichoderma asperellum (Ta-CHI42) from a synthetic gene in Escherichia coli. *FEMS Microbiology Letters, 368*(16), fnab110. https://doi.org/10.1093/femsle/fnab110

Qin, W. T., & Zhuang, W. Y. (2016). Seven wood-inhabiting new species of the genus Trichoderma (Fungi, Ascomycota) in Viride clade. *Scientific Reports, 6*(1), 27074. https://doi.org/10.1038/srep27074

Samuels, G.J. (2006). Trichoderma: Systematics, the sexual state, and ecology. *Phytopathology, 96*(2), 195–206. https://doi.org/10.1094/PHYTO-96-0195

Sarrocco, S., Matarese, F., Baroncelli, R., Vannacci, G., Seidl-Seiboth, V., Kubicek, C. P., & Vergara, M. (2017). The constitutive endopolygalacturonase TvPG2 regulates the induction of plant systemic resistance by Trichoderma virens. *Phytopathology, 107*(5), 537-544. https://doi.org/10.1094/PHYTO-03-16-0139-R

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *PNAS, 109*, 6241–6246. https://doi.org/10.1073/pnas.1117018109

Seidl, V. (2008). Chitinases of filamentous fungi: a large group of diverse proteins with multiple physiological functions. *Fungal Biology Reviews, 22*, 36–42. https://doi.org/10.1016/j.fbr.2008.03.002

Sorabh, C., Sushma, S., Mukesh, K., Mehi, L., Vinay, K., & Akash, T. (2020). Molecular cloning, characterization and semi-quantitative expression of endochitinase gene from the mycoparasitic isolate of Trichoderma harzianum. *Research Journal of Biotechnology, 15*(4), 40–56.

Stielow, J.B., Lévesque, C.A., Seifert, K.A., Meyer, W., Irinyi, L., Smits, D., Renfurm, R., Verkley, G.J.M., Groenewald, M., Chaduli, D., et al. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia, 35*, 242–263. https://doi.org/10.3767/003158515X689135

Stucky, B.J. (2012). SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. *Journal of Biomolecular Techniques, 23*(3), 90–93. https://doi.org/10.7171/jbt.12-2303-004

Şanver, U., Çetin, M., Güneş, N., Atila, F., Eren, E., & Özaktan, H. (2025). Indigenous bacterial endophytes as sustainable alternatives for management of green mould disease in Agaricus bisporus. *Diversity, 17*(11), 757. https://doi.org/10.3390/d17110757

Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution, 38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tang, Y. Y., Zhou, Y. M., Lin, W. K., Zhi, J. R., & Zou, X. (2026). The role of fungal chitinases in the tripartite interactions among insects, plants and entomopathogenic fungi. *Pest Management Science, 82*(9), 8120-8131. https://doi.org/10.1002/ps.70955

Tien, N. Q. D., Van Thanh, D., Ha, H. T. T., Tue, N. H., Phuc, N. H., Hoa, P. T. B., ... & Loc, N. H. (2024). Improving the thermostability of 42 kDa chitinase from Trichoderma asperellum SH16 expressed in Nicotiana benthamiana via site-directed mutagenesis. *Plant Cell, Tissue and Organ Culture (PCTOC), 159*(1), 10. https://doi.org/10.1007/s11240-024-02870-x

Villanueva, M. E., Da Silva, M. A., Barra, J. L., Montich, G. G., Bianco, I. D., & Salinas, S. R. (2022). Biophysical characterization of the recombinant chitinase chi18-5 with potential biotechnological interest. *Applied Microbiology and Biotechnology, 106*(3), 1185-1197. https://doi.org/10.1007/s00253-022-11782-9

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., ... & Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research, 46*(W1), W296-W303. https://doi.org/10.1093/nar/gky427

Watkinson, S. C. (2016). Physiology and adaptation. In *The fungi* (pp. 141-187). Academic Press. https://doi.org/10.1016/B978-0-12-382034-1.00005-0

Woo, S. L., Hermosa, R., Lorito, M., & Monte, E. (2023). Trichoderma: a multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. *Nature Reviews Microbiology, 21*(5), 312-326. https://doi.org/10.1038/s41579-022-00819-5

Zhu, T., Hussain, M., Ning, J., Chen, X., Shi, C., Yang, D., ... & Wu, G. (2025). Genome sequence, comparative genome analysis, and expression profiling of the chitinase GH18 gene family in Cordyceps javanica Bd01. *International Journal of Molecular Sciences, 26*(5), 2031. https://doi.org/10.3390/ijms26052031