

# Applications of Trichoderma- A Review

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## ABSTRACT

Trichoderma is a filamentous fungus that is widely distributed in the soil, plant material, decaying vegetation, and wood. It belongs to the family Hypocreaceae. They have high potential for colonizing their habitats and have various applications in food industry, agriculture, as a biocontrol agent with mechanism involving antibiosis, competition, mycoparasitism, promotion of plant growth, solubilization and sequestration of inorganic plant nutrients, inducing resistance and inactivating pathogen's enzymes and also as a source of transgene. The major driving force for investigation of biocontrol with Trichoderma is sustainability. As a plant symbiont and effective mycoparasites, numerous species of this genus have the potential to become biofungicides. The extensive studies on Trichoderma, including its diverse physiological traits available, is still progressing and making these fungi versatile model organisms for research on both industrial fermentations as well as natural phenomena.

**KEYWORDS:** *Trichoderma, Hypocreaceae, antibiosis, mycoparasitism, Sequestration, transgene, biofungicides*

## INTRODUCTION

Trichoderma is a genus of fungi in the family Hypocreaceae, that is found in many ecosystems. They are frequently found in soil and growing on wood, bark, which demonstrates their high opportunistic potential and their adaptability to various ecological conditions. Some of them also have been found to grow on other fungi.

The first description of a fungus named *Trichoderma aspergilloides* was made back in 1794 described by Christiaan Hendrik Persoon [1], but the taxonomy has remained difficult to resolve. For a long time, it was considered to consist of only one species, *Trichoderma viride*, named for producing green mould. At present, the International Sub commission has listed around 104 species of *Trichoderma/Hypocrea*, which have been characterized at the molecular level. Around seventy-five species of *Trichoderma* have been identified in temperate Europe [2]. Generally, this fungus has an optimal growth range of 25–30 °C, and they show no growth at higher temperatures.

For culturing the most suitable media are cornmeal dextrose agar, in which the transparent colonies are seen; and potato dextrose agar, which results in white colonies. Into the agar a yellow pigment may be secreted. In case of some species, production of a sweet or coconut odour can be seen.

Many strains of *Trichoderma* have no sexual stage but instead they produce only asexual spores. Teleomorphs (the sexual reproductive stage) of *Trichoderma* are characterized by the formation of fleshy, stromata in shades of light or dark brown, yellow, or orange. Whereas, the ascospores are bicellular and often green in colour [3].

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With respect to various characteristics and applications *Trichoderma* is one of the best studied fungi with the genome of three species currently available, including distribution and phylogeny, defence mechanisms, beneficial as well as deleterious interaction with hosts, enzyme production and secretion, sexual development, and response to environmental conditions such as nutrients and light, which has help us to understand how successful colonizers they are of their habitats, including the efficiency of fighting their competitors. Then they start their potent degradative machinery for decomposition. Thus, they are being used as promising biological fungicides, and also as secondary metabolites with potential applications as novel antibiotics. This review aims to give a broad overview about highlights of the intriguing findings as well as promising applications of the best studied *Trichoderma* species defining its qualities and versatility.

## 1. Applications of Trichoderma

### 1.1. Food Industry

*Trichoderma* have been extensively used in the food industry. Enzymes and metabolites of *Trichoderma* spp. are used as additives. The enzymes obtained from *Trichoderma* spp has various applications [4,5]. Currently, various enzymes obtained from *Trichoderma* spp are  $\beta$ -glucanases used to improve the brewing process, pectinases, cellulases, hemicellulases are used in fruit juice production, xylanases are used as feed additive in livestock farming or as a food source for pets. Cellulases are mainly applied in baking, malting, and grain alcohol production [6]. One of the first products isolated from *T. viride* was a chemical with coconut-like aroma, 6-pentyl- $\alpha$ -pyrone with antibiotic

properties, the production of which was constantly improved to reach concentrations of more than 7 g/L using *T. atroviride* nowadays [7,8]. It was proposed to use *T. harzianum* as a food preservative because of its anti-fungal properties but it was not implemented for commercial application [9].

Housseiny and Aboelmagd (2019) research has shown evidence of using naringinase enzyme extracted by *T. longibrachiatum*. Naringinase is an enzyme used for enzymatic debittering. Research showed that nano-encapsulation of naringinase on chitosan exhibits higher stability to temperature and pH (4.0 to 7.0), and better kinetic and operational stability in comparison to free enzyme with retention of 67% of its initial activity even after 13 cycles of use [10]. Also, Kapoor and Iqbal (2013) extracted tannase from *T. Harzianum*. Tannase is used for clarification of fruit juices. Tannase extracted from *T. Harzianum* showed decrease of tannin by 57% [11].

## 1.2. Agriculture

As the human population is increasing day by day, the demand for more agricultural productivity including its quality is also increasing, which has led to an excessive use of chemical fertilizers, creating serious environmental pollution. However, people are now understanding the effects of pollution and thus are using an alternate way i.e. biofertilizers and biopesticides, as it helps in providing a sustainable as well as high production with low ecological impact. The studies have shown that the different soil-borne bacteria and fungi are able to colonize plant roots and they have beneficial effects on the plant, the classic examples are mycorrhizal fungi and *Rhizobium* bacteria.

There are plant-growth-promoting rhizobacteria (PGPR) and fungi such as *Trichoderma* spp. and *Piriformospora indica*, which help in plant growth by suppressing plant diseases [12]. These micro-organisms form associations and interact with other microbes in the rhizosphere, thus help in plant growth and yield as well as provides disease protection. These associations are involved in molecular recognition between the two partners through a signalling network mediated by the plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). The hormones like JA and ET have been found out to work as signal transduction molecules for induced systemic resistance (ISR) due to the effect of beneficial microbes, and the signal transduction pathway through SA accumulation is found in the systemic acquired resistance (SAR) induced by attack by pathogens. A common feature of ISR responses to beneficial microbes is enhancement of defence i.e. defence priming. In defence primed plants, the responses are not activated directly, but by the attack of pathogens they are accelerated which results in faster and stronger resistance to the particular pathogen [13].

Many species in this genus have been characterized as opportunistic avirulent plant symbionts. By inhibiting plant pathogens, some strains can reduce the severity of plant diseases, mainly in the soil or on plant roots, through their high mycoparasitic and antagonistic potential [14].

There are some *Trichoderma* rhizosphere-competent strains which have been shown direct effects on plants. The effects were as follows: increasing their growth potential and

nutrient uptake, stimulation of plant defences against biotic and abiotic damage and fertilizer use efficiency, percentage and rate of seed germination [15]. It has been well established that microbes closely associated with plant roots can directly influence plant growth and development, and this ability has been reported for *Trichoderma* species for many years. For example, there was a report on a significant increase in the height (28%) and weight (8%) of dwarf tomato plants grown in sterile conditions after treatment with *Trichoderma viride* [16]. Similarly, it was also observed that there is enhanced germination, more rapid and increased flowering, and increased heights and fresh weights in a number of plant species, including pepper, periwinkle and chrysanthemum after soil treatment with *Trichoderma harzianum* [17]. Interestingly, a report had previously tested the same isolates for growth promotion and obtained different results indicating a lack of consistency in the expression of the growth promotion effect [18]. A report demonstrated plant growth promotion by *T. harzianum* and *Trichoderma koningii* strains in corn, tomato, tobacco and radish that included increased germination rates, emergence and dry weights [19]. Since then, numerous other reports have demonstrated plant growth promotion on a wide range of plants by different species of *Trichoderma*.

## 1.3. Biological control agent:

*Trichoderma* species is considered as promising biological control agents against numerous phytopathogenic fungi. Despite the substantial research on capability of *Trichoderma* spp. to reduce the occurrence of disease caused by soilborne plant pathogens, the mechanism by which disease control is achieved is not clearly understood. The mechanisms suggested to be involved in bio-control by these fungi are antibiosis, competition, mycoparasitism, promotion of plant growth, solubilization and sequestration of inorganic plant nutrients, inducing resistance and inactivating pathogen's enzymes.

### 1.3.1. Antibiosis

*Trichoderma* has been recommended to produce antibiotics and secondary metabolites during microbial development and sporulation, and are among the most important natural products that inhibits microbial growth. Of these, alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes, and steroids have frequently been associated with biocontrol activity of some species and strains of *Trichoderma*. *Trichoderma harzianum* has shown antibiotic activity against phytopathogens such as *L. maculans* (causative agent for blackleg disease on crucifers), *P. cinnamomi* (causative agent for forest dieback disease), *B. cinerea* (causative agent for various grey mould diseases) [20].

Mutations have been introduced in certain strains that have been expected to be involved in biocontrol activities. Many of these mutants show better biocontrol activity than the parental strains. For example, scientists were able to prepare UV mutants of a strain of *T. harzianum* that produced the antibiotic 6-n-pentyl pyrone but lacked ability to produce isonitrile antibiotics [21]. This is believed to be due to mutation causes expression of those pathways and regulatory processes that had been silenced in the parental organism. Experiments like these strongly suggest that the parental strain have cryptic genes which were not expressed

until their activities were in some way released through the mutation process [22].

### 1.3.2. Mycoparasitism

Mycoparasitism is defined as the interrelationships of a fungus parasite and a fungus host. The term mycoparasite is defined as fungi that has the ability to parasitize other fungi and mycohost means the fungi act as host to be parasitized. Mycoparasitism has long been considered an important mechanism of action of biocontrol by *Trichoderma* spp. This is a process that involves tropic growth of the biocontrol agent toward target fungi, lectin-mediated coiling of attachment of *Trichoderma* hyphae to the pathogen, and finally attack and dissolution of the target fungus' cell wall by the activity of enzymes, which may be associated with physical penetration of the cell wall.

Research by Lifshitz, R it is strongly seen that *Trichoderma* spp has been able to control damping off of pea plants. This was proven when they observed hydrolytic enzymes like beta-1, -3-glucanase and cellulase. Also, *T. harzianum* also causes to control damping off caused by *P. anphanidermatum* was correlated to the level of hydrolytic enzyme produced in soil [23].

### 1.3.3. Competition

Microorganisms that are present in soils and living plant surfaces are frequently available in environment where nutrient is limited. For successful colonization in the phytosphere a microbe must effectively compete for available nutrients. Exudates, leachates, or senesced tissue provide nutrients on the surface of the leaves. Also, nutrients can be obtained from waste products of other organisms such as insects (e.g. aphid honeydew on leaf surface) and the soil. While difficult to prove directly, much indirect evidence suggests that competition between pathogens and non-pathogens for nutrient resources is important for limiting disease incidence and severity [24]. Research by F. Vinale et al. which was to find out factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. It was observed that *T. Harzianum* strain T22 showed a broad spectrum of activity even if applied at low concentrations, while T39 butenolide and harzianolide secreted by strain T39 significantly inhibited pathogens growth only when used at higher concentrations. Not only this confirmed the knowledge of the spectrum of antifungal activity of these secondary metabolites but also proved competition between two pathogens. This is an effect attributed to concentration [25].

### 1.3.4. Tolerance through Biotic and Abiotic stresses through enhanced root development

Another possible mechanism recently gaining attention is tolerance to stress through enhanced root and plant development. The drought tolerance and enhanced nitrogen utilization indicated in are examples of this mechanism. The enhanced rooting also probably also induces tolerance to pests that it does not directly control. Indeed, in several studies, it was found to have no effect on this pathogen. However, growers have indicated that *Phytophthora*-attacked plants were larger in the presence of T-22 than in its absence. It is not directly proven but it is believed enhanced rooting can withstand the damaging effects of the pathogen [26].

### 1.3.5. Solubilization and sequestration of inorganic plant nutrients

In soil, microorganisms can influence the solubility of different insoluble compounds to soluble forms. This is observed in T-22 strain to enhance nitrogen use efficiency in corn. However, this strain can also solubilize a number of poorly soluble nutrients like rock phosphate, Zn metal, Mn<sup>4+</sup>, Fe<sup>3+</sup>, and Cu<sup>2+</sup>; these processes are not due to medium acidification or production of organic acids [27].

### 1.3.6. Induced resistance

*Trichoderma* species induce elicitors that activated the plant defence responses. These responses are introduced due to xylanase of *Trichoderma virdehave* been examined. Xylanses induces plant defence responses including K<sup>+</sup>, H<sup>+</sup>, AND Ca<sup>2+</sup>channelling, pre protein biosynthesis, ethylene biosynthesis, and glycosylation and fatty acylation of phytosterols [28].

Some *Trichoderma* strains are able to induce systemic resistance. The performance of *Trichoderma harzianum*, strain T-22 (T22) to control Cucumber mosaic virus (CMV) in *Solanum lycopersicum* var. *cerasiforme* was studied. The analysis of plant hormones demonstrated that treating with T22 before or at the same time when CMV infection takes place, led to a systemic resistance by jasmonic acid/ethylene and salicylic acid signalling pathways [29].

### 1.3.7. Inactivation of pathogen's enzymes

*Trichoderma* species have an ability to inactivate or reduce the action of enzymes. Two isolates of *Trichoderma harzianum* (T39 a biocontrol agent, and NCIM 1185) were studied and tested for their capacity to reduce the level of hydrolytic enzymes. Out of which T39 was the better inhibitory agent. It reduced enzyme levels of enzymes like cutin esterase, exoPG, endoPG, PME, PL and CMCase, respectively [30].

### 1.4. Source of transgenes

The characteristics of Thkel1 which is a gene that codes for a presumed kelch-repeat protein, were determined after isolation. It was found that when this gene was silenced in *T. harzianum*, it leads to a loss of mycelial growth and glucosidase activity in the presence of abiotic stress environmental conditions. If this particular gene was expressed in *Arabidopsis* the plant tolerance to salt and osmotic stress conditions was improved, followed by a rise in glucosidase activity and abscisic acid levels were decreased when compared to those found in wild-type plants.

The expression of Thkel1 gene in *T. Harzianum* T34 was carried out under various growth conditions, and the maximum transcript levels were observed at 4°C and with the addition of 10% Anclote expression of this gene does not increase due to glucose starvation but gets suppressed if glucose is present. When functional analysis of the Thkel1 gene was carried out by a silencing method in *T. harzianum* T34 it was seen that Thkel1 transformants has less qRT-PCR transcript levels as compared to the wild-type strain, grown under conditions of highest gene expression that is 4°C for 4h.

ThKEL1 shares homology with *Arabidopsis* ESPs which have a major role in the complex regulatory glucosinolate defense

system. Moreover, one of the 6 ESPs present in Arabidopsis has been considered to be the ancestor of proteins with kelch motifs, also found in presumed proteins from *Aspergillus* and *Gibberella*, and has the highest similarity with ThKEL1.

Prominent colony growth variations were seen between wild-type and silenced transformants when salt or mannitol was added in the medium, indicating the involvement of ThKEL1 in tolerance of fungi to salt and osmotic stress conditions.

Some plant glucosidases are related to responses of plants to biotic or abiotic stresses. Hence, taking into consideration the behaviour of Thkel1 in *Trichoderma*, it should not be neglected that the increased expression of Thkel1 in Arabidopsis can enhance plant responses to abiotic stresses [31].

An endochitinase gene from *T. harzianum* was expressed in tobacco and potato at increased levels, and both secretion peptides from fungus and tomato were appropriately cleaved and this led to the accumulation of the transgenic enzyme outside the plant cell. This implied that genes and signal sequences from fungi may be used to secrete and accumulate fungal enzymes in plants or also in media consisting of plant cell suspensions. The introns of the genomic copy (p35S-ThEn42) in *Trichoderma* were spliced correctly in the plant. The level of transcription obtained with the genomic copy was much higher when compared with the original cDNA copy and as high as with the cDNA engineered to enhance the stability of menthe gene expression in plants increases when there is presence of introns and the absence of other noncoding fungal sequences.

It has been observed that disease resistance in various crops gets improved due to transgenic expression in plant of chitinase genes. Expression of ThEn-42 in tobacco and potato produced almost complete resistance to *R. solani*, *A. solani*, *B. cinerea*, and *A. alternata*, was found to be genetically stable and transferred directly to the progeny. This increased level of resistance to both foliar and soilborne pathogens indicates a major improvement in comparison to results from transgenic expression of chitinase genes from plants or bacteria. This improvement is related to the finding that chitinases and other cell wall degrading enzymes from *Trichoderma* have a stronger and wider spectrum of antifungal activity in comparison to corresponding plant and bacterial enzymes. The *Trichoderma* enzyme is highly coordinated with antifungal pathogenesis that is related proteins in plants, such as tobacco osmotin and the *Trichoderma* endochitinase in plant tissues released from the cell wall of invading fungi compounds can bring about the plant defence response [32].

The induction of plant responses by *Trichoderma* depends on concentration. During the starting interaction between the strain T34 belonging to *Trichoderma asperellum* and plant roots of cucumber, salicylic and jasmonic acid levels and typical antipathogenic peroxidase activity increase in the cotyledons to varied degrees depending on the applied concentration of the fungi. Utilization of 2-DE protein profiling method and also MS analysis led to the

determination of 28 proteins whose expression was influenced in cotyledons after cucumber root colonization by *Trichoderma* applied at high concentrations: 17 were found to be up-regulated and 11 were down-regulated. *Trichoderma* differentially regulated the proteins involved in ROS scavenging, stress response, isoprenoid and ethylene biosynthesis, and in photosynthesis, photorespiration, and carbohydrate metabolism. The analysis of proteome changes gives an explanation of how plants treated with *Trichoderma* eventually become more resistant to attacks by *Trichoderma* by the variation in expression of a group of proteins that are defence-oriented which can either protect the plant directly or cause changes in the metabolism to a defensive and non-assimilatory state [33].

The 42-kDa endochitinase (cht42) gene was obtained from the mycoparasitic fungus, *Trichoderma virens* and is driven by 35S CaMV promoter, was transferred into rice by *Agrobacterium*-mediated transformation. Eight transgenic plants harbouring single copies of complete T-DNA were determined by Southern blot analysis. Using Southern blot analysis homozygous transgenic plants were identified for five lines in the T1 generation. Homozygous T2 plants together accumulated high levels of the cht42 transcript, and 2.4- to 4.6-fold increase in chitinase activity in total leaf extract and 1.6- to 1.8-fold higher chitinase activity in the extracellular fluid was observed. When infection assays were carried out on the homozygous T2 plants with *Rhizoctonia solani*, up to 62% reduction in the sheath blight disease index was found [34].

## 2. Future Prospect

The dominant topics in our everyday now is the sustainable economy and protection of our environment and one alarming report about contaminated landscapes or catastrophes caused by climate change follows another. Today, almost 87% of energy used in the world comes from non-renewable sources like natural gas, oil, and coal [35]. Although in order to decrease the requirement for fossil fuels, biofuel production is now being pushed. In order for the generation of this fuel i.e. second-generation biofuels must be produced from the agricultural waste products by the aid of cellulases and hemicellulases (produced for example by *T. reesei*) and lastly fermentation of the resultant oligosaccharides is done by yeast.

The major driving force for investigation of biocontrol with *Trichoderma* is sustainability. Numerous species of this genus have the potential to become biofungicides as they are plant symbiont and effective mycoparasites.

The fields like green and white biotechnology become increasingly important for environmentally safe production of enzymes and antibiotics. By the studies on molecular physiology and regulatory processes, these industrial applications will also be benefited, which continuously reveal novel and valuable metabolites and enzymes to be modified or adjusted for cost effective high yield production.

Lastly, the extensive studies done on this genus *Trichoderma*, including its diverse physiological traits available, is still progressing and making these fungi versatile model organisms for research on both industrial fermentations as well as natural phenomena.

## References

- [1] Persoon CH. *Disposita methodica fungorum*. Römer's Neues Mag Bot. 1794;1:81–128
- [2] European species of *Hypocrea* Part I. The green-spored species. *Jaklitsch WM Stud Mycol*. 2009; 63(1):1-91.
- [3] Samuels GJ. *Trichoderma*: systematics, the sexual state, and ecology. *Phytopathology*. 2006; 96:195–206.
- [4] Blumenthal CZ. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. *Regul Toxicol Pharmacol*. 2004 Apr; 39(2):214-28. doi: 10.1016/j.yrtph.2003.09.002. PMID: 15041150.
- [5] Nevalainen H, Suominen P, Taimisto K. On the safety of *Trichoderma reesei*. *J Biotechnol*. 1994 Nov 15; 37(3):193-200. doi: 10.1016/0168-1656(94)90126-0. PMID: 7765573.
- [6] Galante YM, Conti A, Monteverdi R. Application of *Trichoderma* enzymes in the food and feed industries. In: Harman GE, Kubicek CP, editors. *Trichoderma and Gliocladium*. London: Taylor and Francis; 1998. pp. 327–342.
- [7] Collins RP, Halim AF. Characterization of the major aroma constituent of the fungus *Trichoderma viride*. *J Agric Food Chem*. 1972; 20:437–438.
- [8] Oda S, Isshiki K, Ohashi S. Production of 6-pentyl-[alpha]-pyrone with *Trichoderma atroviride* and its mutant in a novel extractive liquid-surface immobilization (Ext-LSI) system. *Process Biochem*. 2009;44:625–630
- [9] Fuglsang CC, Johansen C, Christgau S, Adler-Nissen J. Antimicrobial enzymes: applications and future potential in the food industry. *Trends Food Sci Technol*. 1995; 6:390–396.
- [10] Housseiny, M. M., Aboelmagd, H.I., 2019. Nano-encapsulation of naringinase produced by *Trichoderma longibrachiatum* ATCC18648 on thermally stable biopolymers for citrus juice debittering. *J. Microbiol*. 57, 1–11. <https://doi.org/10.1007/s12275-019-8528-6>.
- [11] Kapoor, A., Iqbal, H., 2013. Efficiency of tannase produced by *Trichoderma harzianum* MTCC 10841 in pomegranate juice clarification and natural tannin degradation. *Int. J. Biotechnol. Bioeng Res*. 4 (6), 641–650
- [12] Van Wees, S. C., Van der Ent, S., & Pieterse, C. M. (2008). Plant immune responses triggered by beneficial microbes. *Current Opinion in Plant Biology*, 11(4), 443–448. doi:10.1016/j.pbi.2008.05.005
- [13] Viterbo, A. & Horwitz, B. A. (2010). Mycoparasitism. In *Cellular and Molecular Biology of Filamentous Fungi*, vol. 42, pp. 676–693. Edited by K. A. Borkovich & D. J. Ebbole. Washington: American Society for Microbiology.
- [14] Kubicek, C. P., Herrera-Estrella, A., Seidl-Seiboth, V., Martinez, D. A., Druzhinina, I. S., Thon, M., Zeilinger, S., Casas-Flores, S., Horwitz, B. A. & other authors (2011). Comparative genome sequence analysis underscores mycoparasitism as the ancestral lifestyle of *Trichoderma*. *Genome Biol* 12, R40.
- [15] Shores, M., Yedidia, I. & Chet, I. (2005). Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. *Phytopathology* 95, 76–84.
- [16] Lindsey, D.L., Baker, R., 1967. Effect of certain fungi on dwarf tomatoes grown under gnotobiotic conditions. *Phytopathology* 57, 1262–1263.
- [17] Chang, Y.C., Baker, R., Kleifeld, O., Chet, I., 1986. Increased growth of plants in the presence of the biological-control agent *Trichoderma harzianum*. *Plant Dis*. 70 (2), 145–148.
- [18] Baker, R., Elad, Y., Chet, I., 1984. The controlled experiment in the scientific method with special emphasis on biological control. *Phytopathology* 74 (9), 1019–1021.
- [19] Windham, M. T., Elad, Y., Baker, R., 1986. A mechanism for increased plant-growth induced by *Trichoderma* spp. *Phytopathology* 76 (5), 518–521.
- [20] Sivasithamparam, K., and Ghisalberti, E. L. 1998. Secondary metabolism in *Trichoderma* and *Gliocladium*. Pages 139-191 in: *Trichoderma and Gliocladium*, Vol. 1. C. P. Kubicek and G. E. Harman, eds. Taylor and Francis, London <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1472-765X.2009.02599.x>
- [21] Graeme-Cook, K. A., and Faull, J. L. 1991. Effect of ultraviolet-induced mutants of *Trichoderma harzianum* with altered antibiotic production on selected pathogens in vitro. *Can.J.Microbiol.*37:659-664. <https://www.nrcresearchpress.com/doi/pdf/10.1139/m91-112>
- [22] Woo, S. L., Donzelli, B., Scala, F., Mach, R., Harman, G. E., Kubicek, C. P., Del Sorbo, G., and Lorito, M. 1999. Disruption of the ech42 (endochitinase-encoding) gene affects biocontrol activity in *Trichoderma harzianum* P1. *Mol. Plant-Microbe Interact*. 12:419-429. <https://apsjournals.apsnet.org/doi/pdf/10.1094/MPMI.1999.12.5.419>
- [23] Lifshitz, R., Windham, M. T., and Baker, R. 1986. Mechanism of biological control of preemergence damping-off of pea by seed treatment with *Trichoderma* spp. *Phytopathology* 76:720-725. [https://www.apsnet.org/publications/phytopathology/backissues/Documents/1986Articles/Phyto76n07\\_720.PDF](https://www.apsnet.org/publications/phytopathology/backissues/Documents/1986Articles/Phyto76n07_720.PDF)
- [24] Pal, K. K. and B. McSpadden Gardener, 2006. *Biological Control of Plant Pathogens The Plant Health Instructor*. DOI: 10.1094/PHI-A-2006-1117-02. <https://www.apsnet.org/edcenter/disimpactmngmnt/topc/Pages/BiologicalControl.aspx>
- [25] Elad, Y., David, D. R., Levi, T., Kapat, A., Kirshner, B., Guvrin, E., and Levine, A. 1999. *Trichoderma*

- harzianum T39-mechanisms of biocontrol of foliar pathogens. Pages 459-467 in: Modern Fungicides and Antifungal Compounds II. H. Lyr, ed. Intercept Ltd., Andover, Hampshire, UK. <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1472-765X.2009.02599.x>
- [26] Smith, V. L., Wilcox, W. F., and Harman, G. E. 1990. Potential for biological control of Phytophthora root and crown rots of apple by Trichoderma and Gliocladium spp. *Phytopathology* 80:880-885. [https://www.apsnet.org/publications/phytopathology/backissues/Documents/1990Articles/Phyto80n09\\_880.PDF](https://www.apsnet.org/publications/phytopathology/backissues/Documents/1990Articles/Phyto80n09_880.PDF)
- [27] Altomare, C., Norvell, W. A., Björkman, T., and Harman, G. E. 1999. Solubilization of phosphates and micronutrients by the plantgrowth promoting and biocontrol fungus Trichoderma harzianum Rifai 1295-22. *Appl. Environ. Microbiol.* 65:2926-2933. <https://aem.asm.org/content/65/7/2926>
- [28] Direct effects of Trichoderma and Gliocladium on plant growth and resistance to pathogens. Pages 185-204 in: Trichoderma and Gliocladium, Vol. 2. G. E. Harman and C. P. Kubicek, eds. Taylor and Francis, London.
- [29] Vitti A, Pellegrini E, Nali C, et al. Trichoderma harzianum T-22 Induces Systemic Resistance in Tomato Infected by Cucumber mosaic virus. *Front Plant Sci.* 2016; 7:1520. Published 2016 Oct 10. doi:10.3389/fpls.2016.01520 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5056173/>
- [30] Kapat, Arnab & Zimand, G. & Elad, Yigal. (1998). Effect of two isolates of Trichoderma harzianum on the activity of hydrolytic enzymes produced by Botrytis cinerea. *Physiological and Molecular Plant Pathology - PHYSIOL MOLEC PLANT PATHOL.* 52. 127-137. 10.1006/pmpp.1997.0140. [https://www.researchgate.net/publication/248573834\\_Effect\\_of\\_two\\_isolates\\_of\\_Trichoderma\\_harzianum\\_on\\_the\\_activity\\_of\\_hydrolytic\\_enzymes\\_produced\\_by\\_Botrytis\\_cinerea](https://www.researchgate.net/publication/248573834_Effect_of_two_isolates_of_Trichoderma_harzianum_on_the_activity_of_hydrolytic_enzymes_produced_by_Botrytis_cinerea)
- [31] Hermosa, R., Botella, L., Keck, E., Jimenez, J. A., Montero-Barrientos, M., Arbona, V., Gómez-Cadenas, A., Monte, E. & Nicola's, C. (2011). The overexpression in Arabidopsis thaliana of a Trichoderma harzianum gene that modulates glucosidase activity, and enhances tolerance to salt and osmotic stresses. *J Plant Physiol* 168,1295–1302.
- [32] Lorito, M., Woo, S. L., García, I., Colucci, G., Harman, G. E., Pintor-Toro, J. A., Filippone, E., Muccifora, S., Lawrence, C. B. & other authors (1998). Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. *Proc Natl Acad Sci USA* 95, 7860–7865
- [33] Segarra, G., Casanova, E., Bellido, D., Odena, M. A., Oliveira, E. & Trillas, I. (2007). Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with Trichoderma asperellum strain T34. *Proteomics* 7, 3943–3952.
- [34] Shah, J. M., Raghupathy, V. & Veluthambi, K. (2009). Enhanced sheath blight resistance in transgenic rice expressing an endochitinase gene from Trichoderma virens. *Biotechnol Lett* 31, 239–244.
- [35] Progress and challenges in enzyme development for biomass utilization. Merino ST, Cherry J *Adv Biochem Eng Biotechnol.* 2007; 108():95-120.