

Creation of Trichoderman: From an Idea to Realization

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Abstract

The current world of agriculture suffers from many limitations and limiting factors, and *Trichoderma* fungi as biologically beneficial agents can play considerable roles in soil and crop residue management, biological control of plant diseases and pests even in the niches hardly accessible by chemical pesticides, agricultural environment maintenance, as well as plant growth and yield promotion. Therefore, the creation of *Trichoderma* fungi via genetic transformation of superior isolates by the genetic constructs for the expression and secretion of insect-specific insecticidal peptides (Trichodermans) can improve their effectiveness in the control of insect pest targets.

Keywords: Control; Disease; Pathogen; Peptide; Pest; *Trichoderma*

Current problems in the world of agriculture

The present world of agriculture suffers from multiple of problems. The increasing population of the world means increased demand for agricultural production [1]. Despite of this fact, only about 5% of current agricultural soils are disease suppressive and the remnants are disease conducive [2]. While agricultural soils are exposed to increased rate of erosion and gradual loss of fertility [3], the total area for agricultural activities comes down because of increased constructions and buildings. This situation is more aggravated where the cities are expanded over agricultural regions [1]. Plants are frequently affected by mostly fungal pathogens and insect pests that invade the internal tissues which are not accessible by the pesticides. On the other hand, any increase of pesticide application dose may lead to the poisoned agricultural products which act like reservoirs and can accumulate the applied chemicals [4]. Such products may not be enough consistent to the CODEX regulations kept in developed countries and may lead to various degrees of toxicities and medical problems in consumers in ill-managed countries [5, 6]. With soil-born pests and pathogens, the application of pesticides is not so effective [7]. Pesticide molecules may be fixed to soil colloids and get deactivated, degraded by complex diversity of soil microbes, and or washed out by water movement [8]. The compensation of these losses via increased rates of application has led to increased occurrence of water and soil contamination and consequent medical and veterinary problems in human and livestock [9]. The global warmth beside of leading to increased incidence of regional and floods, threatens the already suffering agriculture via imposture of heat stress to agricultural crops, increased rate of evapotranspiration, drought and salt stress [10] in most areas of the world. The stressed plants get more sensitive to opportunistic pathogens and pests and increased crises occur in agricultural and natural vegetation [11].

Biological Control and the Emergence of Trichoderman

Trichoderma spp. are known as effective biological control agents that are isolated from soil and plants [12]. These mycoparasitic fungi are armed with different types of cell wall degrading enzymes such as chitinases [13], cellulases [14], glucanases [15], and proteases [16]. Also, they produce various volatile (such as 6-Peptenyl Alpha Pyrone, 6-PAP) and involatile antibiotics [17], as well as a range of peptaibiotics [18] and lipases [19] that make them competent disruptors of microbial cell membranes [20] and capable of insect-cutin destruction [21]. This rich arsenal leads to their unique abilities to control a range of

plant pathogenic fungi, oomycetes, gram-positive as well as gram-negative bacteria [22], nematodes [23], and even insect pest [24]. The fungi produce huge amounts of green spores expected to be more UV-resistant than the hyaline spores of the entomopathogenic fungi such as *Beauveria Bassiana* [25]. *Trichoderma* spp. are easily cultivated on simple media are among potent competitors able to fast colonize various substrates [26] and niches on plants [27]. Importantly, these fungi are able to induce plant defensive system through salicylic acid, jasmonic acid and ethylene signaling pathways [28-32]. Unlike chemical inducers of plant resistance that lead to reduced crop yield as the result of matter and energy costs put for the motivation of plant defensive machinery, *Trichoderma* spp. also promote plant growth and yield [33-36] and increase their tolerance to heat, salinity and drought stresses [37]. *Trichoderma* spp. are well-known mycoremediants. Interestingly, *Trichoderma* spp. and other beneficial fungi such as arbuscular mycorrhizal fungi and *Serendipita indica* synergistically impose positive effects on plant growth and yield [26]. These fast growing filamentous fungi produce numerous number of actively secretive hyphal tips and since have extensively been applied for heterologous protein production and secretion [38]. The hyphal structure can penetrate into various solid substrates including plant residues, and the body of invaded (micro) organisms [39].

Therefore, *Trichoderma* spp. seem to fulfill most of the requirements in an ecofriendly agricultural system. However, some enzymatic differences have been found in alcohol dehydrogenase, acetyl CoA synthetase, pyruvate decarboxylase, and aldehyde dehydrogenase between *Trichoderma reesei* and the entomopathogenic fungus, *Metarhizium anisopliae* [40]. As the metabolic engineering seems rather difficult and time-consuming, the superior isolates of *Trichoderma* spp. can be genetically engineered in order to produce and secrete a range of insecticidal peptides. I have called these genetically transformed superiors as Trichodermans [25]. These are able to use their signal transduction pathway [41] in order to pursue the parasitic fungi as well as insects, grow toward more humid areas and infect the concealed evils in planta. Different insect-specific insecticidal neurotoxic peptides have been identified in arachnida (such as spiders and scorpions), cnidarians and mollusks that target specific sites on metal ion channels [42, 43]. Although, it is believed that spider insecticidal peptides globose (easy to be secreted) and more stable (resistant to proteases) [44], the co-transformation of superior *T. asperelloides* isolates with a genetic construct for the expression and secretion of μ -agatoxin IV (a neurotoxin from American funnel web spider, *Agelenopsis aperta*) using *Trichoderma harzianum* 42KDa endochitinase signaling peptide was not successful. The reason seemed to be the endoplasmic reticulum stress imposed due to the short length of the peptide (37 aa) and four intramolecular disulfide bonds [45]. Another reason might be that the natural toxin is of an amidated carboxylic end and such an amidation might have not been taken place in *T. asperelloides*. The construct was successfully expressed (as revealed by the bicistronic expression of eGFP), however, fungal conidiation was inhibited possibly due to the ER- and the subsequent secretory-stress inhibited [25].

There are other insecticidal peptides that target different sites on sodium, calcium, and potassium ion channels of insect neurotic system. For example, hainantoxins and huwentoxins specifically target site-1 on voltage-gate sodium channels [46]. Alpha-insect toxins are another group of insect-specific toxins of insect origin that target site-3 on sodium pumps [47]. Interestingly, at least some of the insecticidal peptides are insect group-selective and even species-selective [48]. Making it possible to control deleterious insect pests without harmful impacts on beneficial insects. Anyway, biking toward the realization of Trichoderman can be peddled using other insecticidal peptides.(Figure 1)

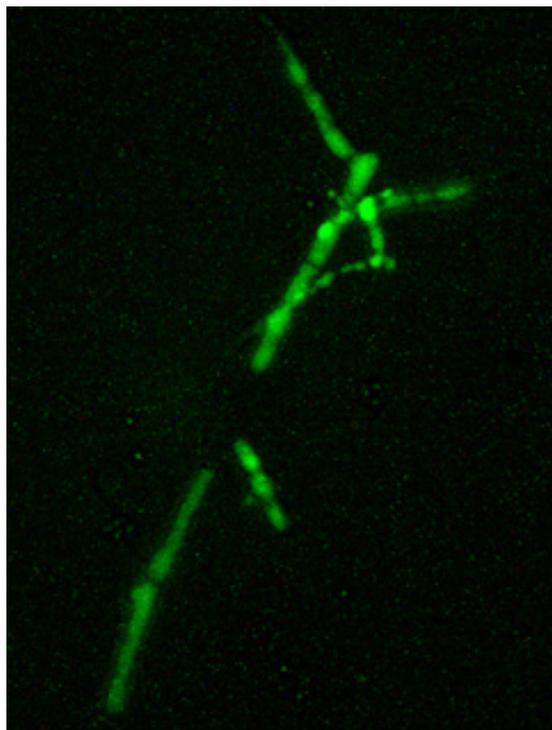


Figure 1: The conidiophore of *Trichoderma asperelloides* genetically transformed to express two separate open reading frames coding for the bicistronic co-expression of eGFP and the insecticidal peptide μ -agatoxin IV under government of a single promoter. So, the intensity of green fluorescence infers to the rate of toxin production, and allows histopathological studies on pests and pathogens due to be tested [25].

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