Phytostimulating Mechanisms and Bioactive Molecules of *Trichoderma* Species: Current Status and Future Prospects

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Abstract

Ever-increasing pressure on the agricultural land due to various biotic and abiotic stresses made agriculture a nonprofitable venture. In order to bring back the lost glory to agriculture, there is an urgent need to reclaim this eroded agriculture with sustainable practices, one among them is the use of plant growth-promoting microorganisms such as rhizosphere-competent *Trichoderma* sp. In this chapter, the major mechanisms and bioactive molecules involved in plant growth promotory activity of *Trichoderma* sp. are described in detail. *Trichoderma* sp. is also known to produce growth-regulating phytohormones and other bioactive molecules which are known to protect them against antimicrobial compounds secreted by plant, but they also help the plants in overcoming various stresses. Various hydrolytic enzymes such as chitinases, glucanases, and proteinases are produced by *Trichoderma* which aid in its mycoparasitic response. The fungus is also able to enhance plant growth through nutrient solubilization and its uptake. It mobilizes phosphates from fixed organic/inorganic phosphorus sources through both enzymatic (phosphatases, phytases) and nonenzymatic mechanisms (production of organic acids and siderophores). *Trichoderma* produces a wide array of secondary metabolites and volatile compounds which are mainly responsible for its biocontrol action. Suppression of fungal plant pathogens through mycoparasitism involves signal transduction and G protein signaling in *Trichoderma*. Secondary metabolites and volatile compounds produced by this fungus are very

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diverse in their occurrence and mode of action against phytopathogens. Recent developments in molecular biology, metabolomics, and proteomics have opened an insight for the use of secondary metabolites as biopesticides rather than the application of whole organisms.

Keywords
Trichoderma • Biocontrol • PGP activity • Secondary metabolites • Plant defense mechanisms

10.1 Introduction

Agriculture is the largest private enterprise in India and will continue to be the lifeline of Indian economy in the future. Present population growth rate together with diversions of fertile land for non-agriculture uses exerts tremendous pressure to expand agriculture. Fast-changing environment, chemical-intensive agricultural practices, and several other soil factors are imposing a paramount pressure on sustaining the agricultural production. Continuous agricultural practices have resulted in depletion of nutrients in soil; moreover, several other abiotic stresses such as drought or water logging, high or low temperature, soil pH, and salinity and biotic (phytopathogens, insects) factors also affect the crop yield (Nagaraj kumar et al. 2005); farmers are, therefore, facing several problems especially scarcity of cultivable land and excessive demand for chemical fertilizers and pesticides. The agrochemicals cannot increase crop yield beyond a threshold level, rather their excessive use not only adversely affects the environment and human health but also disturbs the natural microbial flora of soil. This has resulted in new challenges for agricultural productivity. Hence, during recent years, there has been a growing concern for environmental hazards caused by these agrochemicals. Under these circumstances, focus on eco-friendly, climate-resilient, sustainable organic agricultural practices assumes lot of importance as an alternative to chemical-driven agriculture. The use of microbial inoculants having dual potential for biocontrol of phytopathogens as well as plant growth enhancement is an important approach in this direction (Srivastva et al. 2004). The use of microorganisms as plant growth-promoting agents is not a new concept, since from ancient days microbes are known to play a key role in enhancing plant growth through various mechanisms such as nitrogen fixation, phosphate solubilization, ACC deaminase activity, induction of plant immune response, tolerance to abiotic stress, and suppression of phytopathogens (Shoresh et al. 2010).

Rhizosphere, the most dynamic region of the soil, is well known for its microbial diversity and is colonized with several plant growth-promoting microorganisms (PGPMs) such as mycorrhizal fungi; species of *Rhizobium*, *Pseudomonas*, *Bacillus*, *Azotobacter*, *Trichoderma*, *Aspergillus*; and others which have also been reported to stimulate plant growth by suppressing plant diseases (Wees et al. 2008). These soil bacteria and fungi are known to mediate processes such as nutrient immobilization and mineralization, nitrogen fixation, and denitrification (Rashid et al. 2004). Among the fungi, members belonging to the genus *Trichoderma* are outstanding due to their high adaptability to various ecological conditions and variety of lifestyles. They live in soil interacting with animals and plants and also grow saprophytically on wood, bark, and many other substrates. These fungi can form endophytic associations with plants and also interact with other microbes in the rhizosphere, thereby influencing disease protection, plant growth, and yield. Several *Trichoderma* sp. (e.g., *T. harzianum*, *T. viride*, *T. virens*, *T. atroviride*, *T. koningii*, etc.) have been identified as potential biocontrol agents which are also having other plant growth-enhancing abilities (Harman 2000). Recent progress in molecular biology has opened the door to uncover the vast mechanisms of biocontrol action of *Trichoderma* as well as the responses induced in plants upon its colonization (Shinozaki and Shinozaki 2006). Due to these reasons, today *Trichoderma* is used worldwide as a potential biopesticide. They are also well-known producers of several secondary metabolites and other bioactive molecules, and their role in activating plant defense mechanisms has been studied recently in depth (Vinale et al. 2008). Different types of bioactive molecules such as siderophores, peptaibols, pyrones, antibiotics, volatile organic compounds, and polyketides are synthesized by *Trichoderma* spp. Secondary metabolites, which are produced during the later growth phases, are mainly responsible for various plant growth-promoting and biocontrol abilities of the fungus as well as inducing stress tolerance and immune response of plants. The recent in-depth understanding of the functioning of the bioactive molecules of *Trichoderma* sp., has opened a vast scope to formulate efficient biopesticides and biofertilizers involving secondary metabolites.

10.2 The Genus *Trichoderma*: A Potential Rhizosphere-Competent Fungus

The genus *Trichoderma* consists of asexually reproducing fungi that are commonly found in nearly all types of soils, root ecosystems, and other natural habitats especially those containing high organic matter throughout the world. These are free-living fungi that are highly interacting with other rhizosphere microflora. The fungus grows fast in culture and produces numerous green spores. It is considered as one of the most important soilborne plant growth-promoting fungi. Mycoparasitic activity and antibiotic-producing potential were first demonstrated in *Trichoderma lignorum* by Weindling (1932). One of the most interesting aspects of studies on *Trichoderma* is its potential to employ varied mechanisms for disease control. In general the fungus exhibits a preference for wet soil. They show a high level of genetic diversity and can be used to produce a wide range of products of commercial and ecological interest. *Trichoderma* are effective root colonizers by which they deplete the nutrients and make pathogenic microbes to starve, produce organic acids causing the release of macro- and micronutrients for uptake by plants, release volatile substances and secondary metabolites that act as antimicrobial agents, are capable of producing plant hormones such as zeaxanthin (maize) and gibberellins that accelerate seed germination, trigger the plant immunity, and provide tolerance to plants against abiotic stress.

The genus *Trichoderma* belongs to the phylum *Ascomycetes*, class *Sordariomycetes*, order *Hypocreales*, and family *Hypocreaceae*. The fungus belonging to the genus *Trichoderma* was isolated for the first time from soil and decomposing organic matter and introduced by Persoon in 1794; in 1865, a link to the sexual state of a *Hypocrea* species was suggested (Tulasne and Tulasne 1865). It was difficult to distinguish different species assigned to the genus *Trichoderma/Hypocrea* morphologically, and it took until 1969 that development of a concept for identification was initiated (Rifai 1969; Samuels 2006). Thereafter, numerous new species of *Trichoderma/Hypocrea* were discovered, and by 2006, the genus contained more than 100 phylogenetically defined species (Druzhinina et al. 2006). *Trichoderma*, for the most part, was classified as *imperfect fungi*, in that they produce only asexual spores. The sexual stage, when found, is within the *Ascomycetes* in the genus *Hypocrea* (Harman 2002). These fungi also colonize woody and herbaceous plant materials, in which the sexual teleomorph (genus *Hypocrea*) has most often been found. Rifai (1969) outlined the speciation concept within the genus *Trichoderma* and described nine species aggregates: *T. piluliferum* Webster & Rifai, *T. polysporum* (Link) Rifai, *T. virens* Gidden & Foster, *T. hamatum* (Bon.) Bain. *T. koningii* Oudem. Apud Oudem. Et Koning, *T. aureoviride* Rifai, *T. harzianum* Rifai, *T. longibrachiatum* Rifai, *T. pseudokoningii* Rifai, and *T. viride* Pers. However, with the use of molecular approaches particularly sequence polymorphism with internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (rDNA), several new species have been identified (Hayes et al. 1994). The mycelium is hyaline with septate, profusely branched and smooth-walled hyphae (Fig. 10.1a, b). Chlamydospores are present in most species. The conidiophores are highly ramified and phialides are flask shaped or ovoidal (Hermosa et al. 2000). Safe identification of new species was significantly facilitated in recent years, by development of an oligonucleotide barcode (TrichOKEY) and a customized similarity search tool (TrichoBLAST), both available online (Druzhinina et al. 2011; Kopchinskiy et al. 2005).

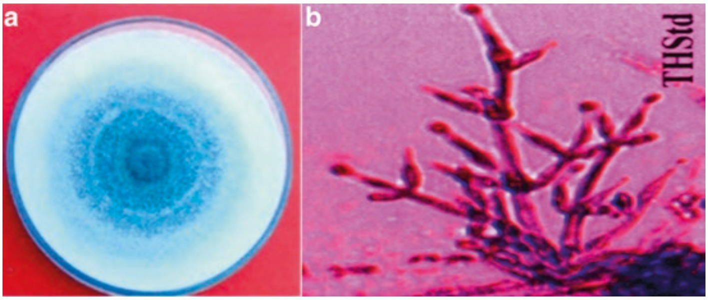


Fig. 10.1 *Trichoderma harzianum*: colony morphology on culture medium (**a**) and microscopic view of mycelial structure with conidiospores (**b**)

10.3 Establishment of Plant-*Trichoderma* Association

Colonization is the principal activity needs to be performed by any beneficial or pathogenic microorganism in order to achieve its goals. Colonization involves the ability to adhere and recognize plant roots, penetrate the plant, and withstand toxic metabolites produced by the plants in response to invasion by a foreign organism whether it is a pathogen or beneficial organism (Brotman et al. 2008). In this regard, *Trichoderma* is the most effective rhizosphere colonizer, which can establish long-lasting relationship with the plants and induce many beneficial responses such as localized or systemic plant resistance responses, nutrient acquisition/mobilization, tolerance to abiotic stress, and deactivation of toxic compounds secreted by phytopathogens, increasing the population of beneficial microflora. These beneficial attributes of *Trichoderma* are due to its unique properties such as resistant to antimicrobial compounds, i.e., phytoalexins, flavonoids, terpenoids, and phenolic derivatives synthesized by plant. *Trichoderma* in turn synthesizes many bioactive molecules which not only react with the antimicrobial products of plant, but they also aid in the plant growth promotion, in various cell-to-cell communication processes, morphogenesis of fungus, adhesion, and hyphal aggregation process. For example, *T. koningii* suppresses the production of phytoalexins during colonization of roots of lotus. Tolerance to antimicrobial compounds by plant is achieved by the presence of ABC transport systems in *Trichoderma* (Ruccco et al. 2009). Some *Trichoderma* strains can colonize only local sites on roots, but rhizosphere-competent strains can colonize entire root surfaces for several weeks or months. *Trichoderma* modifies the rhizosphere by secreting growth-regulating hormones such as auxins that promote the root growth which in turn facilitates colonization by increasing the available surface area. Some of the chemicals are reported to be secreted by *Trichoderma* such as cysteine-rich hydrophobin-like proteins that facilitate anchoring/attachment, Tastdy1 from *T. asperellum* and Qid74 of *T. harzianum*, and expansin-like proteins with cellulose-binding molecules and endopolygalacturonases to facilitate root penetration (Viterbo and Chet 2006). Driving force for colonization, coordination of defense mechanism, and increased rate of photosynthesis are the plant-derived sucrose. Interestingly, *Trichoderma* strains can also colonize leaf surfaces under some conditions, but biocontrol activity might not be dependent on the growth of *Trichoderma* on leaf surfaces (Hermosa et al. 2012). According to recent reports, *Trichoderma* sp. is not confined to outer root tissues only but can also live in the plant as *true endophytes* (Bae et al 2009). Interestingly, most of the endophytic *Trichoderma* discovered are "new species (*T. stromaticum*, *T. amazonicum*, *T. evansii*, *T. martiale*, *T. taxi*, *T. theobromicola*)" (Chaverri et al. 2011). The benefits offered by the endophytic *Trichoderma* species are much better than non-endophytic *Trichoderma*, as they are directly involved in the induction of the transcriptomic changes in plants and protect plants from diseases and abiotic stresses (Bailey et al. 2006, 2009); these endophytes deploy various modes of entry into the plants and form appressorium-like structures. Hence, this interaction is mutually beneficial, but since *Trichoderma* spp. are also capable of living freely in soil, they are considered as opportunistic plant symbionts (Vargas et al. 2009).

10.4 Effect of *Trichoderma* Colonization on Plant Metabolism

Significant changes are observed after the colonization of *Trichoderma* in the plant metabolism, wide range of compounds are released by *Trichoderma* sp. into zone of interaction, and they are known to play a key role in plant growth promotion. Increase in levels of fungal proteins such as xylanases, cellulases, and swollenin by *Trichoderma* induces disease resistance in plants; products of avirulence-like (Avr) genes, peptaibols, also aid in this process. There is also induction of pathogenesis-related (PR) proteins. Mainly systemic acquired resistance (SAR) and induced systemic resistance (ISR) are triggered by *Trichoderma* colonization. SAR is usually triggered by local infection, and ISR is known to result from colonization of roots. Fungal colonization is also known to increase the percentage of germination and photosynthetic capacity of plants; increase dry matter content, starch, and total and soluble sugars; and reduce sugar content in leaves of different parts. The fungal colonization also increases the root proliferation by enhancing the levels of growth-promoting hormones such as auxins and cytokinins and hence provides suitable niche for *Trichoderma*. There is an increased level of antimicrobial compounds upon colonization by *Trichoderma*. In cucumber, root colonization by strain T-203 causes an increase in phenolic glucoside levels; their aglycones (which are phenolic glucosides with the carbohydrate moieties removed) are strongly inhibitory to a range of bacteria and fungi. Thus, root colonization by these fungi induces significant changes in the plant metabolic machinery (Sivan and Chet 1989).

10.5 Plant Growth Promotory Mechanisms

The plants colonized with *Trichoderma* are benefited in many ways such as increased rate of metabolism, i.e., photosynthesis, activation of plant defense mechanism, and deactivation of harmful microbial compounds secreted in and around their occurrence, increase in root growth, accumulation of antimicrobial compounds, increased resistance to the abiotic stress, enhanced nutrient acquisition capacity such as nitrogen use efficiency, phosphorous solubilization, and micronutrient mobilization and uptake (Fig. 10.2).

10.5.1 Direct Mechanisms of Plant Growth Promotion

The rhizosphere-competent fungus *Trichoderma* sp. like other beneficial root-colonizing microorganisms also enhances plant growth and productivity in different ways. Responses to application of *Trichoderma* sp. are characterized by reducing germination time especially in case of vegetables, increased germination percentage, and increased plant development and metabolism and crop yield. These responses may be due to one or more of the attributes like increased uptake and translocation of minerals, solubilization of nutrients and their release from the soil or organic matter, vitamin production or conversion of materials to a form useful to

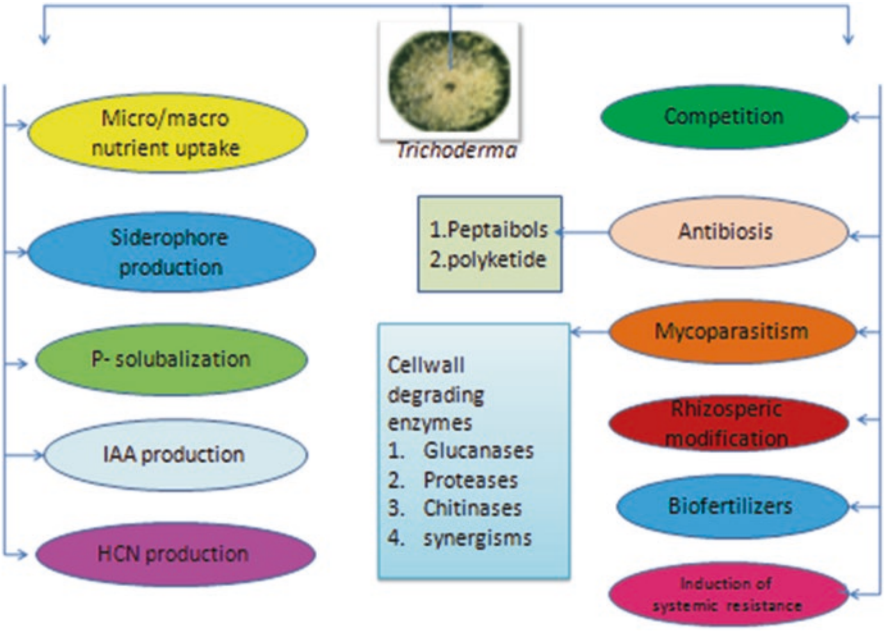


Fig. 10.2 Schematic representation of plant growth-promoting activities of *Trichoderma* sp.

the plants, suppression of deleterious root microflora including those not causing obvious disease, production of growth-stimulating factors (hormones), and synthesis of metal-chelating agents, i.e., ionophores/siderophores. Root colonization by plant growth-enhancing strains of *Trichoderma* results in increased development of root and/or aerial systems and crop yields (Glick 1995).

10.5.1.1 Increase in Nutrient Uptake Efficacy

Recent studies have proved that seed treatment with *Trichoderma* strains results in an increase in nitrogen use efficiency of plants. This effect was first noticed with *T. harzianum* T-22 strain in maize during field trials in the late-1990s; the treated plants were more green and healthy as compared to untreated plants. One of the major threats faced by present-day agriculture is "yield plateauing" frequently observed phenomenon in almost all crop plants. The plants which are engineered in such a manner to respond to high levels of fertilizers and inputs now started showing this phenomenon. To counter this phenomenon, seeds are treated with *Trichoderma* strains which will increase the plant nitrogen use efficiency, and it is a long-term effect that persists for the whole productive lifetime of crop (Yedidia et al. 2001). In the case of maize, the presence of *T. harzianum* T-22, yield plateau was reached with 40–50% less nitrogen fertilizer. This particular mechanism is commercially exploited in the United States, and approximately 0.3 million hectares of wheat are being planted with seeds treated with *T. harzianum* strain T-22 (Porras et al. 2007).

10.5.1.2 Enhancing Nutrient Availability for Plants

Soil is the most complicated dynamic ecosystem in which both micro- and macronutrients undergo a complex dynamic equilibrium of soluble and insoluble forms which is greatly influenced by soil pH and microflora. Soil microbes play an important role in maintaining the equilibrium between soluble and insoluble forms of nutrients by carrying out the processes of mineralization and immobilization in soil. Phosphorus is one of the key macronutrients limiting plant growth and metabolism as approximately 95 to 99% is present in the form of insoluble phosphates in soil and cannot be utilized by plant. Moreover, a major portion (more than 80%) of the phosphatic fertilizers added to soil becomes immobile and unavailable for plant uptake because of adsorption, precipitation, or conversion to insoluble fixed inorganic form (Hiolford 1997). It is generally fixed as tricalcium phosphate (TCP) in alkaline soil (at pH above 7.0) and as ferric phosphate ($FePO_4$) and $AlPO_4$ in acidic soil (at pH ≤ 5.0), which needs to be solubilized where phosphate-solubilizing microorganisms play an important role (Fankem et al. 2006). Numerous soil bacteria and fungi have been reported to mineralize and mobilize nutrients from soil. *Trichoderma* spp. strongly influence the complex transitions of various plant nutrients from insoluble forms to soluble forms, thereby enhancing accessibility and absorption by roots (Saravanan et al. 2007). Several species of *Trichoderma*, e.g., *T. harzianum*, *T. virens*, *T. viride*, and *T. atroviride*, have been reported to solubilize various forms of inorganic plant nutrients and thus play an important role in nutrient management (Fig. 10.3). *Trichoderma* sp. can solubilize and store phosphate in its biomass that is released in readily available form in close proximity of

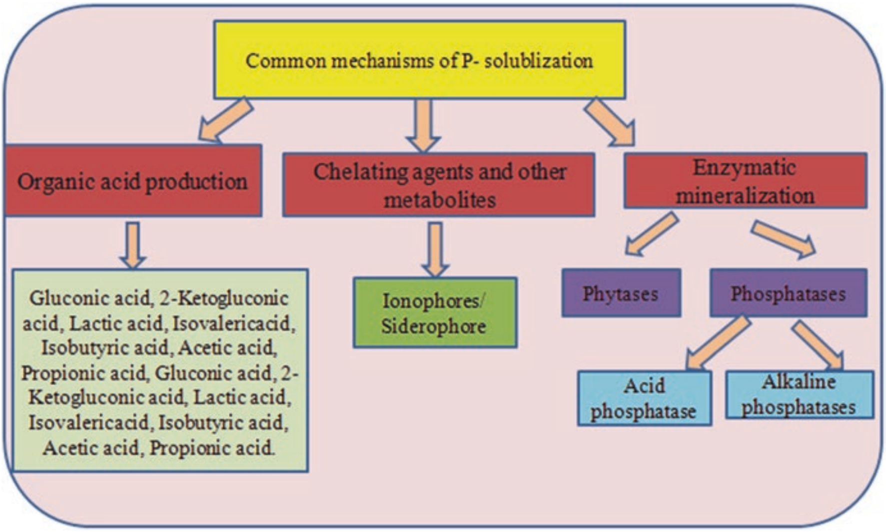


Fig. 10.3 Schematic representation of possible mechanisms of phosphate solubilization adopted by *Trichoderma* spp.

roots after lysis of the mycelium with age. *Trichoderma harzianum* is a potential phosphate solubilizer, but strain variability is always observed (Sakia et al. 2015). It is also capable of solubilizing other nutrients such as iron, MnO_2, and metallic zinc; iron and manganese in particular have been investigated with regard to both solubilization and their influence on plant disease.

Nutrient-solubilizing processes. Three possible mechanisms for in vitro solubilization of some insoluble or sparingly soluble minerals by *T. harzianum* (Rifai) have been proposed: (a) acidification of the medium through production of organic acids, (b) production of chelating metabolites such as ionophores or siderophores, and (c) redox activity.

Acidic Solubilization

Production of organic acids as a mechanism for phosphate solubilization by many PSMs has been reported by many workers (Rodriguez and Fraga 1999; Nautiyal et al. 2000); it results in lowering of pH which ultimately solubilizes the insoluble phosphorous source (Gaur and Sachar 1980; Gaind and Gaur 1991; Gaur 1990; IIlmer et al. 1995; Puente et al. 2004). The most common organic acid produced by gram-negative bacteria is gluconic acid; the bacteria oxidizes glucose from the medium (environment) to gluconic acid resulting in the acidification of the medium and solubilization of insoluble phosphate (Goldstein 1996). Phosphate-solubilizing microorganisms have been shown to produce monocarboxylic acid (acetic, formic); monocarboxylic hydroxy acids (lactic, gluconic, glycolic); monocarboxylic keto acids (2-keto gluconic); dicarboxylic acid (oxalic, succinic); dicarboxylic hydroxy acids (malic, maleic); and tricarboxylic hydroxyl acids (citric) in liquid media from simple carbohydrates (Goldstein 1986; Iyamurimye et al. 1996; Gyaneshwar et al. 1998; Kim et al. 1998; Vinay kumar 2003; Puente et al. 2004). Therefore, release of organic acids that sequester cations and acidify the microenvironment near root zone is thought to be a major mechanism of solubilization of nutrients such as phosphorous, manganese, iron, and zinc by several phosphate-solubilizing microorganisms (PSM).

Enzymatic Solubilization

The term “phosphatases” has been used broadly to describe a wide group of enzymes that hydrolyze organic phosphorus (P) compounds, pyrophosphates, metaphosphates, and inorganic polyphosphates that occur in plenty amounts in soil. Three different groups of enzymes are involved in solubilization of insoluble phosphate sources, namely, phosphatases, phytases, and phosphonatases (C-P lyases). As acid phosphatases and phytases are dominant in soil, therefore, they are mainly responsible for solubilization of fixed phosphorous present in organic matter of soil. The phosphatases attack the phospho-ester or phospho-anhydride bond of organic matter, causing its dephosphorylation. Phytases work particularly on phytate/phytic acid (inositol hexakisphosphate), while C-P lyases perform C-P cleavage in organo-phosphonates.

- **Phosphatases** (**phosphohydrolase**: **esterases**) are the enzymes that hydrolyze phosphoric acid monoesters from substrate by cleaving phosphor-ester bond and release phosphate ions. Due to their wide potential for biotechnological applications, phosphatases have gained the attention of the present era (Rodríguez et al. 2006). On the basis of pH optima, phosphatases may be divided into two broad groups: alkaline phosphatases and acid phosphatases. Both acid and alkaline phosphatases exist in soil and are distinguished on the basis of pH ranges at which they are active (Malcolm 1983). Phosphatases have been reported to be secreted in response to signals of the absence of soluble phosphates (Peleg et al. 1996).
- **Alkaline phosphatases** or basic phosphatases are the enzymes having pH optima greater than 7.0; that means, they work well in alkaline or basic environments (Tamas et al. 2002). The enzyme alkaline phosphatase (Alp, EC 3.1.3.1.) that catalyzes the cleavage of monophosphate groups from inorganic or organic backbones is frequently used in soil ecology as a marker for microbial activities (Kuperman and Carreiro 1997). The enzyme is homodimeric metalloenzyme with molecular weight of 86,000 kDa. To each monomer, one magnesium (Mg) and two zinc (Zn) ions are attached. Bacterial alkaline phosphatases are highly resistant to several environmental adversities as they are present in the periplasmic space in gram-negative bacteria. The enzyme dephosphorylates many molecules like sugar phosphates, phenols, alkaloids, etc. with the help of some accepter molecule; it can transphosphorylate alcohols. Bacterial alkaline phosphatases are highly active with several applications as in epitope mapping, immunoblotting, expression, analysis of mutants, etc. Induced expression of alkaline phosphatase in *Trichoderma* sp. in the presence of insoluble phosphorus source (tricalcium phosphate) has been well reported (Kapri and Tewari 2010). *Trichoderma* spp. can also retain its phosphate-solubilizing potential under abiotic stress conditions, such as under phosphorus-deficient conditions and in the presence of heavy metals (Rawat and Tewari 2011).
- **Acid phosphatase**: Acid phosphatases have been described in several bacteria, fungi, and yeast. Several fungi, e.g., *Aspergillus* and *Penicillium* spp., have the ability to synthesize acid phosphatase enzyme, but there is scarcity of literature on acid phosphatase activity of *Trichoderma* strains. These are the phosphatase enzymes that work in acidic pH range, i.e., pH < 6.0. They are dominant in soil solutions; thus major P solubilization in soil is performed by acid phosphatases. Many genes for acid phosphatases have been isolated from different species of gram-negative bacteria such as acpA gene for acid phosphatase with pH optima at 6 which having a wide range of substrate has been isolated from *Francisella tularensis*. Three molecular families (class A, class B, and class C) form the nonspecific acid phosphatases in bacteria. They are located inside the cell and thus contribute to hydrolyze organic phosphor-ester bond of nucleotide, phosphate sugars, etc. (Rodríguez et al. 2006).

- **Phytase (*myo*-inositol hexakisphosphate phosphohydrolase) enzyme**: The enzyme phytase catalyzes hydrolysis of *myo*-inositol hexakisphosphate (phytic acid) to inorganic monophosphate and lower *myo*-inositol phosphates and in some cases to free *myo*-inositol. The Enzyme Nomenclature Committee of the International Union of Biochemistry distinguishes two types of phytases: 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26). This classification is based on the first phosphate group attacked by the enzyme. Thus, bioavailability of inositol phosphate depends on their mineralization by both types of extracellular phytases, which have many biological sources. Most phytases come under high molecular weight acid phosphatases. Microbial phytase activity is most frequently detected in fungi, particularly in *Aspergillus* species. Shieh and Ware (1968) screened over 2000 microorganisms isolated from soil for phytase production. Most of the positive isolates produced only intracellular phytase. Extracellular phytase activity was observed only in 30 isolates. All extracellular phytase producers were filamentous fungi. Twenty-eight belonged to the genus *Aspergillus*, one to *Penicillium*, and one to *Mucor* (Aowson and Davis 1983). Generally, phytate is the primary source of inositol, and in the plant seeds, and pollen is found as the major stored form of phosphate. Phytases that are optimal for improving animal nutrition have been in focus of many genetic engineering studies. Another attractive property of these enzymes that is not currently utilized is solubilization of soil organic phosphorus through phytate degradation. Phytate is the main component of organic forms of P in soil (Rodríguez et al. 2006). Plants are not able to obtain phosphorus directly from phytate efficiently. The mechanisms employed by the *Trichoderma* in P solubilization are almost similar to the mechanisms employed by the general P-solubilizing rhizobacteria. Some of the mechanisms employed by *Trichoderma* are acidification of the medium, production of chelating metabolites, and redox activity. *T. asperellum* has been shown to enhance the availability of P and Fe to plants with significant increases in dry weight, shoot length, and leaf area (Yedidia et al. 2001).

10.5.1.3 Enhancing Micronutrient Availability

Iron availability to plants is a unique kind of phenomenon; it is neither assimilated by bacteria nor plants in aerobic soils, because ferric ion, which is the predominant form in nature, is only sparingly soluble so that the amount of iron availability for assimilation by living organisms is extremely low. Under these critical circumstances, microorganisms have evolved special mechanisms for the assimilation of iron, such as the production of low-molecular-weight compounds known as siderophores (the iron-specific ionophores), which transport this element into their cells. Siderophores are divided into three main families depending on the characteristic functional group, i.e., hydroxamate, catecholates, and carboxylates. At present more than 500 different types of siderophores are known, of which 270 have been structurally characterized (Ali and Vidhale 2013). Interestingly, siderophores play a key role in both direct and indirect enhancement of plant growth. Fungal siderophores mainly are fusarinins, coprogens, and ferrichromes that all belong to the group of hydroxamate type of siderophores that share the structural unit N-5-acyl-N-5 hydroxy ornithine (Renshaw et al. 2002). Coprogen, Coprogen B, and ferricrocin were excreted from all the six *Trichoderma* species tested under iron-deficient conditions by Anke et al. (1991).

10.5.1.4 Secretion of Phytohormones

Highly effective molecules, which are known to influence the most essential stages of plant growth and development, are produced by plant in minute quantities, which is insufficient for meeting the needs of large requirement of plants; several plant growth-promoting rhizospheric microorganisms are able to synthesize phytohormones such as auxins (indole acetic acid), cytokinins, gibberellins, abscisic acid, and ethylene and thus enhance the plant growth. The first report on discovery of gibberellins was made from the fungus *Gibberella fujikuroi*. Indole acetic acid (IAA) is also produced by *Pseudomonas fluorescens*. Also, an increase of IAA, gibberellin, and cytokinin level was observed in *G. fasciculatum*-inoculated *Prosopis juliflora*. However, these mechanisms are not well studied in the case of *Trichoderma*. *Trichoderma* strains are capable of enhancing plant biomass production, promoting lateral root growth and development through an auxin-dependent mechanism or able to produce indole-3-acetic acid or auxin analogues (Contreras-Cornejo et al. 2009); an auxin-like effect has been observed in etiolated pea stems treated with harzianolide and 6-pentyl-a-pyrone, the major secondary metabolite produced by different *Trichoderma* strains (Vinale et al. 2008). The growth-promoting activity of *T. atroviride* on tomato seedlings has been suggested to be associated with the reduced ethylene production resulting from a decrease in its precursor l-amino cyclopropane-1-carboxylic acid (ACC) through the microbial degradation of IAA in the rhizosphere or through the ACC deaminase (ACCD) activity present in the microorganisms (Gravel et al. 2007).

10.5.2 Indirect Mechanisms (Biocontrol Action)

Trichoderma spp. is well known today for its biocontrol potential against soilborne fungal phytopathogens such as *Fusarium*, *Pythium*, *Sclerotium*, *Rhizoctonia*, *Sclerotinia*, *Macrophomina* sp., etc. which are the major wilt-causing pathogens. Among various *Trichoderma* sp., the most widely reported and commonly used biocontrol species are *T. harzianum*, *T. viride*, and *T. virens* (Cook and Baker 1983). They are known to produce a wide range of antibiotic substances and parasitize fungal phytopathogens. They also compete with other soil microorganisms for space, nutrients, and key exudates from seed and roots that stimulate the germination of propagules of plant pathogenic fungi in soil. *T. harzianum* has high potential to control sheath blight of rice by antagonizing the pathogen *Rhizoctonia solani* (Tewari and Bhanu 2004). Today, *Trichoderma* strains are used for biological control, either alone or in combination with other microbes or chemical adjuvants. They are also known to produce certain lytic enzymes that degrade the cell wall of the pathogen. These versatile fungi are highly efficient producers of many extracellular enzymes like cellulases, chitinases, glucanases, proteases, etc. *Trichoderma* sp. is the most extensively used fungal biocontrol agent for the management of plant pathogens affecting seed, root, and aerial plant parts. *Trichoderma* uses a variety of mechanisms to provide protection against several plant pathogens and/or plant diseases and enhance plant growth, such that it may (1) directly kill the pathogen by mycoparasitism and/or antibiosis; (2) adversely affect the growth and development of the pathogen by competing for the nutrients, oxygen, or space; (3) alter fitness of the pathogen; (4) induce systemic plant resistance; (5) enhance plant growth and its tolerance to stress; (6) metabolize plant exudates supporting pathogen; (7) inactivate enzymes produced by the pathogens; and (8) synthesize cell wall-degrading enzymes (lytic enzymes) that degrade the cell wall of pathogen. Some of the most common mechanisms involved in biocontrol of fungal phytopathogens by *Trichoderma* spp. are described below:

10.5.2.1 Mycoparasitism

Mycoparasitism is a complex process that involves tropic growth of biocontrol agent toward the target (pathogen) organism and finally attack and dissolution of the pathogen's cell wall by the activity of various enzymes, which may be associated with physical penetration of cell wall (Rawat and Tewari 2010). Thus, in this process, the antagonist exists in intimate association with the other target fungi from which it derives some or all its nutrients. Mycoparasitism is a well-known phenomenon in biocontrol action of *Trichoderma*. In general, the overall process of mycoparasitization of fungal pathogen involves four steps: (1) The first stage is the chemotropic growth of the biocontrol fungus toward the target fungi that produce chemical stimuli, for example, a volatile or water-soluble substance produced by the host fungus serves as a chemoattractant for parasite. (2) The next step is recognition of the target pathogen, in which lectins of the host (pathogen) and carbohydrate receptors on the surface of the biocontrol fungus might be involved. (3) The third step is attachment, secretion of lytic enzymes, and cell wall degradation. Mycoparasites can usually either coil around host hyphae or grow alongside it and produce cell wall-degrading enzymes to attack the target fungus, and (4) the final step is penetration of the biocontrol agent into host by forming appressorium-like structures to penetrate the target fungus cell wall. A large number of mycoparasitic *Trichoderma*-based formulations are commercially available in the market as a promising alternative to chemical pesticides for the use of farmers in countries like the United States, India, Israel, New Zealand, and Sweden (Howell 2003).

Signal Transduction Pathways Involved in Mycoparasitism

Environmental signaling plays an important role in cellular organisms. Understanding of the mechanisms of cell signaling in *Trichoderma* spp. is limited compared to "model" fungi like *Neurospora crassa*, but there has been significant progress based on genetic approaches. The seven transmembrane G protein-coupled receptor Gpr1 of *Trichoderma* is involved in sensing the fungal prey; silencing of the gpr1 gene in *T. atroviride* rendered the mycoparasite unable to respond to the presence of the host fungus (Omann et al. 2012). Binding of a ligand to such receptors leads to

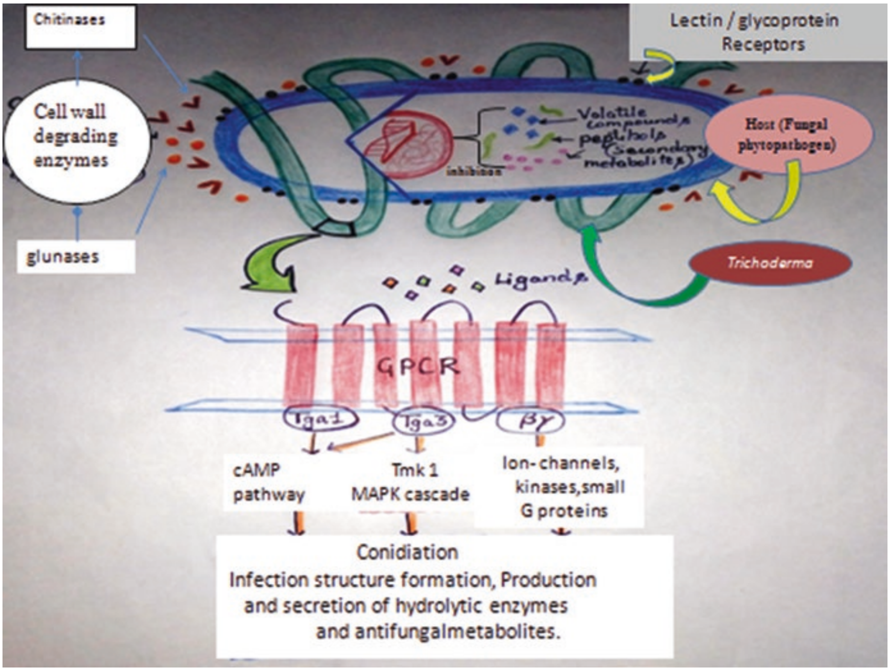


Fig. 10.4 Overall process of mycoparasitization of fungal phytopathogen by *Trichoderma* spp. showing involvement of various compounds and G protein signaling (Source: modified from Omann and Zeilinger 2010)

downstream signaling events via activation of G protein cascades. The overall process of mycoparasitism of fungal pathogen by Trichoderma sp. is illustrated in Fig. 10.4. Indeed, deletion of the Tga3 Gα protein-encoding gene affected the mycoparasitic abilities of *T. atroviride* in a similar way to loss of Gpr1 (Zeilinger et al. 2005). Deletion of the adenylate cyclase gene tac1 severely impaired growth and mycoparasitic abilities of *T. virens* (Mukherjee et al. 2007). Like most other filamentous fungi, *Trichoderma* spp. have three MAP kinase cascades comprising MAPKKK, MAPKK, and MAPK (Schmoll 2008), and MAPK pathways may act in mycoparasitism and biocontrol (Kumar et al. 2010). These reports imply important functions of signaling cascades in mycoparasitism and related biocontrol properties (Mukherjee et al. 2012).

The Role of G Protein Signaling in Biocontrol Action of *Trichoderma*

The heterotrimeric G protein signaling in *Trichoderma* sp. consisted of three parts: a G protein-coupled receptor (GPCR), a heterotrimeric G protein (α, ß, γ subunits), and an effector molecule (Neer 1995). More than 1000 GPCR-encoding genes have been identified and characterized from different organisms; most of them were of vertebrate origin (Kolakowski 1994). All these receptor proteins have seven transmembrane domains and have the N-terminus outside and the C-terminus inside the cytoplasm. When ligand binds to the receptor, it changes the conformation that leads to the release of the G proteins and exchange of GDP for GTP on the Gα subunit. GTP bound α dissociates from its ß γ partner, allowing both signaling units to regulate the activities of downstream effectors (Kaziro et al. 1991; Gutkind 1998). Highly conserved heterotrimeric G proteins act as signal transducers that couple cell surface receptors to cytoplasmic effector proteins. G proteins are necessary during sexual and pathogenic development and during secondary metabolism. In fungi, they are part of the pheromone signaling cascade and also affect a number of developmental and morphogenetic processes which determine the virulence of fungi and plant-fungal pathogen interactions (Bölker 1998). Rocha-Ramirez et al. (2002) reported that *T. atroviride* subgroup I Gα subunit Tga1 is involved in both coiling and conidiation. This has been shown by overexpression of tga1 gene and by tga1 gene silencing. These results were confirmed by tga1 gene deletion mutant study (Reithner et al. 2005).

10.5.2.2 Antibiosis

Antibiosis is one of the most powerful mechanisms employed by *Trichoderma* sp. as its biocontrol strategy against fungal pathogens. Antibiosis occurs during interactions involving low-molecular-weight diffusible compounds or antibiotics produced by *Trichoderma* strains that inhibit the growth of other microorganisms. There is a wide diversity of antibiotics produced by both bacteria and fungi which are known to have profound effect on pathogens. *Trichoderma* produces wide variety of secondary metabolites, which play predominant role in biocontrol activity. It releases more than 43 substances that have antibiotic activity. Most *Trichoderma* strains produce volatile and nonvolatile toxic metabolites that impede colonization by antagonized microorganisms. Secondary metabolites are chemically different natural compounds that play an important role in regulating interactions between organisms, such as phytotoxins (secondary metabolites produced by fungi that attack plants), mycotoxins (toxins produced by fungi that colonize crops capable of causing diseases and death in humans and other animals), pigments (colored compounds also with antioxidant activity), and antibiotics (natural products capable of inhibiting or killing microbial competitors. Weindling (1934) characterized the “lethal principal” excreted by a strain of *T. lignorum* into the medium as “gliotoxin” and demonstrated that it was toxic to both *R. solani* and *Sclerotinia americana*. At present *Trichoderma* species are reported to produce a number of antibiotics, such as gliotoxin, gliovirin, glioviridin, viridin, alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, and sesquiterpenes, and some steroids.

10.5.2.3 Production of Hydrolytic Enzymes

Attachment of *Trichoderma* to the host (pathogen) is followed by a series of degenerating events and degradation of cell wall of the pathogen by synthesizing various cell wall-degrading enzymes (CWDEs); among them, chitinases, glucanases, and proteinases are the major ones. *Trichoderma* are good producers of hydrolytic enzymes, and the most intensively studied of these belong to the chitinolytic system (chitinases and NAGase), glucanases followed by proteinases (de Almeida et al. 2010).

Trichoderma strains generally produce β-1, 3 and β-1, 6 glucanases that hydrolyze the glucan polymer of the cell wall of the pathogen. *T. harzianum* produces at least four β-1, 3-glucanase isoenzymes under different in vitro culture conditions. Some *Trichoderma* strains also secrete β-1, 6 glucanases which are also involved in cell lysis, along with chitinase and proteinase activity. The enzyme chitinases act on chitin, a major component of fungal cell wall. Fungal cell wall contains chitin and/or β-glucan fibrils that are embedded in protein matrix. Thus, extracellular proteases, synthesized by *Trichoderma*, hydrolyze these proteins present in pathogens' cell walls and play a significant role in mycoparasitism. Extracellular hydrolytic enzymes of *Trichoderma*, e.g., *T. harzianum*, act synergistically as shown by in vitro studies (Harman 2000).

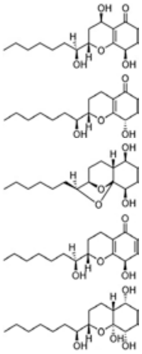
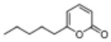
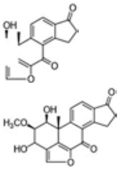
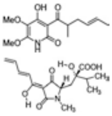
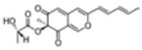
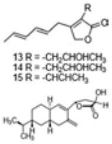
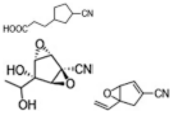
10.5.2.4 Production of Secondary Metabolites and Volatile Compounds

Trichoderma sp. produces different types of volatile compounds and secondary metabolites as means of its biocontrol activity. The production of secondary metabolites by *Trichoderma* sp. is strain dependent and includes antifungal substances belonging to a variety of chemical compounds (Table 10.1). They have been classified into three categories: (1) volatile antibiotics, i.e., 6-pentyl-a-pyrone (6PP), and most of the isocyanide derivatives; (2) water-soluble compounds, i.e., heptelidic acid or koningic acid; and (3) peptaibols, which are linear oligopeptides of 12–22 amino acids rich in a-aminoisobutyric acid, N-acetylated at the N-terminus and containing an amino alcohol at the C-terminus (Howell 2003). The production of low-molecular-weight, nonpolar, volatile compounds (i.e., 6PP) results in a high concentration of antibiotics in the soil environment that have a relatively long-distance range of influence on the microbial community, while a short distance effect may be due to the polar antibiotics and peptaibols acting in close proximity to the producing hyphae. Although the role and the effects of peptaibols are clear, the mode of action of other *Trichoderma* secondary metabolites (i.e., pyrones) and their possible synergisms with other compounds are yet to be elucidated. Viride pyrone showed antagonistic activity against *Sclerotium rolfsii*, whereas δ-decanolactone was found to control *B. cinerea*, *Phytophthora* spp., *Aspergillus niger*, and *Candida albicans* (Hill et al. 1995; Kishimoto et al. 2005). Plant growth promotion activity of *Trichoderma* is a result of combined activities of primary and secondary metabolites, but the role of secondary metabolites is largely appreciated because they exhibit several biological functions and play an important role in regulating interactions between organisms.

10.5.2.5 Competitive Inhibition of Pathogen

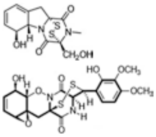
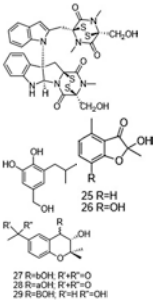
Competition seems to be an important mechanism of biocontrol, but it is difficult to assess its actual contribution in biological control. Competition is considered as a "classical" mechanism of biological control that involves competition between antagonist and plant pathogen for space and nutrients (Chet 1987). The omnipresence of *Trichoderma* in agricultural and natural soils throughout the world proves that it is an excellent competitor for space and nutritional resources. Neither

Table 10.1 List of secondary metabolites produced by *Trichoderma* sp.

Secondary metabolites	Synthesizing organism	Molecular structure	Effective against	References
1. **Koninginins**	*T. koningii* and *T. harzianum*		*Rhizoctonia solani*, *Phytophthora cinnamomi*, *Pythium middletonii*, *Fusarium oxysporum*, and *Bipolaris sorokiniana*	Almassi et al. (1991) and Ghisalberti and Rowland (1993)
2. Pyrones	*T. atroviride* and *T. harzianum*		*Rhizoctonia solani*, *Fusarium oxysporum*, and some bacteria	Scarselletti and Faull (1994) and Worasatit et al. (1994)
3. Viridins	*T. viride* and *T. virens*		*Botrytis allii*, *Colletotrichum lini*, *Fusarium caeruleum*, *Penicillium expansum*, *Aspergillus niger*, and *Stachybotrys atra*	Reino et al. (2008) and Brian et al. (1944)
4. Nitrogen heterocyclic compounds	*T. harzianum*		*Botrytis cinerea*, *R. solani*, *G. graminis* var. *tritici*, and *Pythium ultimum*	Dickinson et al. (1989) and Vinale et al. (2006)
5. Azaphilones	*T. harzianum*		*R. solani*, *P. ultimum*, and *G. graminis* var. *tritici*	Vinale et al. (2006)
6. Butenolides and hydroxy-lactones	*T. harzianum*		*P. ultimum*, *R. solani, and B. cinerea*	Almassi et al. (1991) and Vinale et al. (2006)
7. Isocyano metabolites	*T. hamatum*, *T. viride*, and *T. koningii*		*Phytophthora* sp.	Tamura et al. (1975)

(continued)

Table 10.1 (continued)

Secondary metabolites	Synthesizing organism	Molecular structure	Effective against	References
8. Diketo-piperazines	*T. virens*		*R. solani*, *P. ultimum*	Howell (1998)
9. Peptaibols	*T. harzianum*		*P. ultimum*, *R. solani*	Daniel and Filho (2007)

antibiosis nor mycoparasitism is mainly involved in biocontrol of seedling disease in cotton, but competition is the main mechanism in this case. Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phytopathogens. Indirect mechanisms in plant growth promotion include the competition for nutrient (Fe^{+3}), in which the siderophore secreted by *Trichoderma* scavenges iron from the environment thus making it unavailable for competing microorganisms, and it is described as one of the key factors in antagonism of *T. asperellum* against *F. oxysporum* f.sp. lycopersici (Segarra et al. 2010). A recent study further reported the detection of an average 12–14 siderophores by isotope-assisted screening of *T. atroviride*, *T. asperellum*, *T. gamsii*, *T. hamatum*, *T. virens*, *T. harzianum*, *T. polysporum*, and *T. reesei* with dimerum acid, coprogren, fusigen, fusarinine A, and the intracellular siderophore ferricrocin being produced by all examined species (Lehner et al. 2013).

10.5.2.6 Stimulation of Plant Immune Responses

Another very important indirect means of plant growth promotion is the induction of host resistance; plants are known to respond to variety of environmental stimuli, including gravity, light, temperature, physical stress, water, and nutrient availability; and they also respond to the chemical stimuli produced by soil-/plant-associated microbes. Those stimuli are known to induce the resistance in plants against wide range of pathogens. This resistance in plants is achieved by various mechanisms such as systemic acquired resistance (SAR); is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection; and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins include a variety of enzymes, some of which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death and another mechanism known as induced systemic resistance (ISR) that is mediated by jasmonic acid (JA) and ethylene, which are produced following applications of some nonpathogenic rhizobacteria (Kloepper et al. 1992). The defense responses may also include the physical thickening of cell wall by lignifications, deposition of callose, accumulation of antimicrobial low-molecular-weight substance (e.g., phytoalexins), and synthesis of various proteins (e.g., chitinases, glucanases, peroxidases). The ISR triggered by *Trichoderma* occurs through the JA/ET signaling pathway similarly to PGPR ISR. Recent studies have shown the colonization of Arabidopsis roots by *T. atroviride* that induces a delayed and overlapping expression of the defense-related genes of the SA and JA/ET pathways against biotrophic and necrotrophic phytopathogens, both locally and systemically (Salas-Marina et al. 2011). *Trichoderma* is able to trigger a long-lasting upregulation of SA gene markers in plants unchallenged by pathogens, although when plants are infected by a pathogen such as *B. cinerea*, the pretreatment with *Trichoderma* may modulate the SA-dependent gene expression, and, soon after infection, the expression of defense genes induced through the JA signal transduction pathway occurs, causing ISR to increase over time (Tucci et al. 2011).

- *Role of bioactive* Neither antibiosis nor mycoparasitism is mainly involved in biocontrol of seedling disease in cotton, but competition is the main mechanism in this case. Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phytopathogens.

10.5.2.7 Elicitors in Plant Stress Response

Various abiotic stresses such as drought, low/high temperatures, salinity and acidic conditions, light intensity, submergence, anaerobiosis, and nutrient starvation are the main factors that are impacting agricultural production. Speaking numerically, water deficient (drought) has affected 64% of the land area, flood (anoxia) 13%, salinity 6%, mineral deficiency 9%, acidic soils 15%, and cold 57%. But any accurate estimation of agricultural loss in terms of ecological disturbances due to abiotic stress could not be made. Recently, several PGPR have been shown to efficiently help plants to overcome abiotic stress such as salinity and drought in both field crops and trees (Contreras-Cornejo et al. 2014). The role played by *Trichoderma* in mitigating the problems caused by abiotic stress is very significant. *T. harzianum* when treated to seeds (tomato) or soil treatment (Arabidopsis) largely improved the germination at osmotic potentials of up to 0.3 MPa (Mastouri et al. 2010, Harman 1991). *T. harzianum* treated to maize seeds showed the enhanced deep rooting ability thus surviving under water-deficit conditions. Further in *Trichoderma*-inoculated cacao seedlings, drought-induced changes such as stomatal closure and reduction of net photosynthesis were delayed under drought compared with non-inoculated plants; *Trichoderma*-treated squash plants showed higher fresh weight compared to untreated seeds. Salt stress is known to cause other inhibitory effects in plant growth such as reduced uptake of potassium ions; as potassium is a compatible solute, its uptake is essential for osmotic adaptation of plants; and it also plays an important role in the closure of stomata; hence trichodermal treatment can ameliorate the salt-induced multiple growth inhibition. *Trichoderma* inoculation also increased calcium content under salinity compared with nonsaline condition. *Trichoderma harzianum* has recently been shown to improve resistance to heat and cold (seedlings of tomato were imbibed at 25 °C for 1 day, then exposed either 10 °C or 35 °C, and then returned to 25 °C). Seedlings were much less damaged by the temperature extremes in the presence of T *harzianum* (Hermosa et al. 2011).

10.6 *Trichoderma*-Based Commercial Products

It is estimated that 90% of all antagonistic fungi used in plant protection belong to the genus *Trichoderma* (Benitez et al. 2004). The success of *Trichoderma* as a biocontrol agent is due to the ability of the fungus to produce plethora of secondary metabolites *Trichoderma* interacts with other microorganisms but mainly with pathogenic fungi. Today several Trichoderma-based commercial products are available in the market that can be used as biopesticides and biofertilizer in green and sustainable agriculture (Lorito et al. 2010) (Table 10.2).

Table 10.2 List of some of the commercial *Trichoderma*-based biopesticides

Organism name	Trade name	Mode of action	Effective against
T. harzianum + *T. viride*	Trichodex	Mycoparasitic	Effective against *Armillaria* and *Botryosphaeria* and others
	Trichopel		
	Trichojet Trichodowels		
Trichoderma sp.	Trichodry	Mycoparasitic	Suppresses root pathogens
	Trichoflow Trichogrow		
	Trichopel		
Trichoderma viride	Ecosom TV	Mycoparasitic	Effective against rot diseases
	Tricon		
Trichoderma harzianum	Root shield	Mycoparasitic antagonistic	Effective against variety of soil pathogens and wound pathogens
	BioTrek 22 g		
	Supresivit		
Trichoderma viride	Bioderma	Mycoparasitic	*Fusarium* wilt and *Verticillium* wilt and all types of leaf spot and leaf blight
Trichoderma harzianum	Bioderma-H	Mycoparasitic	*Pythium*, *Rhizoctonia*, *Schlerotinia*, *Fusarium*, and *Verticillium* wilt, all types of leaf spot and leaf blight

10.7 Biotechnological Applications of *Trichoderma* Bioactive Molecules

1. Various compounds produced by *Trichoderma* such as secondary metabolites, volatile compounds, and antimicrobial compounds are known to have great application in the field of agriculture and industry such as drug and cosmetics.
2. *Trichoderma* are known to produce wide range of hydrolytic enzymes which are great application in industry and agriculture.
3. Trichodermal mycoparasitism provides valuable biotechnological tools to understand the basic process and in vitro biocontrol studies.
4. Various volatile and secondary metabolites produced by *Trichoderma* serve as a starting material for synthesis of chemicals which are effect against phytopathogens.

10.8 Conclusion

Increased pressure on agriculture with abiotic and biotic stress and the use of *Trichoderma* as phytostimulant reduce the pressure on the use of the chemicals. *Trichoderma* provide various direct and indirect mechanisms of plant growth promotion and hence can be used as phytostimulant in various crops as it offers a sustainable alternative to chemical agriculture. *Trichoderma* are known to produce a wide range of antimicrobial compounds which do not benefit them, but they also induce plant resistance to pathogens. *Trichoderma* are known to synthesize secondary metabolites, and volatile compounds are directly responsible for antagonistic properties against phytopathogens, and they are also responsible for various plant growth promotion activities. Recent studies have concluded that secondary metabolites from *Trichoderma* offer a wide range of application in biocontrol. In fact, treatment with *Trichoderma* metabolites produces extensive changes of the plant expressome, proteome, and metabolome by acting on specific pathways involved in synthesis of major hormones resistance to biotic and abiotic stresses and nutrient uptake.

10.9 Future Prospects

With this current understanding about *Trichoderma* and its various plant growth-promoting characters, there is a need to understand these mechanisms at molecular levels to gain deeper insights in these mechanisms. It is necessary to elucidate the mechanisms of mycoparasitism in detail such as how biotic and abiotic interactions affect the mycoparasitic activity and how it can give us information regarding the biocontrol activity of the *Trichoderma*. So far our knowledge concerning mycoparasitism's genomics is based on single genome per species, and there is a need to address the long-standing axiom of strain variation of biocontrol relevant mycoparasitic traits. Recent breakthroughs also point out the importance of assessing and delineating the ecological niche and life histories of mycoparasites to better interpret data emerging from comparative genomics and to allow highly targeted application of respective strains. Study of secondary metabolites produced by *Trichoderma* as biopesticides rather than whole organism needs special attention, and it can revolutionize the biopesticide industry.

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